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Abstract

Chromosomal polymorphisms detected with the stain quinacrine dihydrochloride have been examined in a sample of people resident in County Durham. Attempts have been made to correlate the frequencies of these polymorphisms with certain demographic variables, information about which was collected from the participants using questionnaires. Other genetic markers, detectable from blood samples, have also been studied, with a view to comparing the patterns of variability shown by these in the population, with those of the less well studied chromosome variants.

Demographic information was collected from each person concerning his or her age, sex, occupation and geographic origins. Parental ages and birth order data were also collected in the case of newborn infants. No consistent correlations were observed between any of the chromosome variants and any of the demographic factors. However, there were indications of an association of the total number of variants found per individual karyotype with sex and with age.

The results obtained in this study have been compared with those of published reports of chromosome variability. The conclusion drawn from this comparison is that, although there is evidence that a degree of similarity of frequency exists between populations, the extent of such similarity is extremely difficult to quantify objectively.

Information concerning the molecular nature of chromosomes, and their polymorphisms, has been reviewed with the intention of revealing any theoretical basis there may be for an adaptive significance of the variants. The incidence of other chromosomal variations in human populations has also been described, in order to detect any evidence that may exist for a biological or evolutionary significance of any type of chromosomal variability.

No compelling evidence has been found to indicate that chromosomal polymorphisms have an adaptive importance in present-day human populations, nor has any information which would indicate a theoretical basis for such an observation.

CHROMOSOMAL AND OTHER GENETIC POLYMORPHISMS IN A COUNTY DURHAM POPULATION

Barbara Linda Hudson

Thesis submitted for the degree of
Doctor of Philosophy
Department of Anthropology
University of Durham
1980

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P R E F A C E

The discovery in 1970 of a method of staining chromosomes to make possible the unequivocal identification of each, immediately revealed a new type of genetic polymorphism in the human species. This involved both quantitative and qualitative differences between individuals with respect to considerable proportions of their genomes. These differences do not appear to be correlated with any aspect of the phenotype. It seems to be the case that the portion of the genome involved in these variations does not consist of structural DNA; that is, it does not carry information about the amino acid sequences of enzymes and proteins.

Despite the ignorance which exists concerning the nature and possible function of this variable part of the genome, its variability leads one to ask the same questions that one asks about the distributions of other polymorphic genetic markers. For example, one would wish to know which evolutionary processes influence their distribution in the population.

Evolutionary processes in present day human populations may be demonstrated by correlating demographic information about those factors known to affect the genetic structure of populations with the observed pattern of genetic variability. This procedure may be used to reveal whether a particular genetic marker is subject to natural selection, for instance, or to other systematic or dispersive forces.

In this study the following questions are posed:
1. Do the chromosomal variants detected with methods of differential staining appear to be subject to any influences which affect the distributions of other (structural) genetic markers in the population?
2. Does the pattern of their distribution within the population give any indication of the nature of the evolutionary processes which may affect these particular polymorphisms? Specifically, is there evidence that these chromosomal variants are differentially associated with survival of their carriers?

Another aim of this research was to discover to what extent it was possible to overcome the practical difficulties inherent in the methods employed in the collection of data about this type of chromosome variability, and to examine the findings of similar studies to determine how far these are comparable with the findings of the present study.

As will be seen in the body of this thesis, the examination of variants of the intensity of fluorescence of chromosomes involves certain methodological difficulties which makes one ask whether an adequate research protocol can be devised, so that the study of such variants in different populations and by different researchers will produce results which may be usefully compared.

The introductory chapter relates the historical development of human cytogenetics and shows the impact that the methods of differentially staining chromosomes had in many areas of inquiry. There follows a descriptive account of the physical and biochemical structure of the chromosome, and of the molecular mechanisms involved in the production of chromosome bands. These accounts provide information which might be used to assess the biological significance of the variants examined in this study. Chapter 3 reviews the extent of chromosomal variability within human populations, and also within certain other related species, again with the intention of indicating any possible biological or evolutionary importance of such variability.

The composition of the sample studied, the methods employed in the present study and a statistical analysis of the results obtained are described in chapters 4, 5 and 6. A detailed comparison was made between the results of this study and those of previously published comparable studies. The results of this comparison are given in chapter 7.

The concluding chapter summarises the evidence that chromosome fluorescence intensity variants might be subject to various evolutionary forces occurring in human populations; and considers the usefulness of these variants in studies concerned with the genetic structure of populations.

Chapter 1: THE HISTORICAL DEVELOPMENT OF THE TECHNIQUES OF CYTOGENETIC ANALYSIS.

Chromosomes and their behaviour at cell division were being observed, named and meticulously described at a time when generally the world was still in ignorance of Mendel's paper on the transmission of hereditary factors. Although the idea of the hereditary continuity of the chromosomes had already been suggested by van Beneden (1883-4), their study was fairly well advanced before any associations between chromosomes and the processes of transmission of hereditary characteristics was convincingly demonstrated in the early 1900s.

Chromosomes first appeared in the drawings of plant cells in 1848, and in ones of animal cells a short time later (German 1870). The use of stains for better visualisation of cytological preparations was introduced as a standard procedure by Waldeyer and Beneke in 1863. Chromosomes could then be observed in the tissues of a variety of organisms and the first attempts to enumerate them were made. In 1875 O. Hertwig observed, in sea urchins, the doubling of the chromosome number which occurs with the fusion of nuclei at fertilisation, and Strasburger outlined the principles of somatic cell division. This process was named mitosis in 1882 by Flemming, who had shown in 1880 that the longitudinal splitting of the chromosomes resulted in each half thus formed being passed on to one of the two daughter cells. Also in 1882 Flemming stated that the number of chromosomes per cell was constant in organisms of the same species. That the material of the chromosomes was not lost, but only lost to view, during interphase was demonstrated by Rabl three years later. Chromosomes were eventually named as such in 1888 by Waldeyer. Human chromosomes were first seen in tumour cells by Arnold in 1879, but attempts to count them did not meet with any success until 1912 (Ford 1973).

Sufficient recognition of the unique characteristics of

chromosomes had occurred in the nineteenth century to enable E.B.Wilson to write in the 2nd edition of his standard textbook on the subject "the remarkable fact has now been established with high probability that every species of plant or animal has a fixed and characteristic number of chromosomes, which regularly occurs in the division of all its cells, and in forms arising by sexual reproduction the number is even." (Wilson 1900, quoted in German 1970).

In retrospect, the door to the new science of cytogenetics seems, by this time, to have been wide open. The theoretical need for some structure or organelle with hereditary continuity (which logically follows the acceptance of Darwinian ideas of evolution) had meanwhile been taxing the minds of several people. Whether or not adaptation to environmental conditions was to be by the inheritance of characteristics acquired by the parents during their life, or as a result of natural selection, some physical means was required for the transmission of parental characteristics, so that organisms would resemble more closely their parents than other members of the species. Darwin himself suggested the theory of 'pangenesis' whereby 'gemmules' embodying the characteristics of the various organs and tissues were thrown off by these structures, transported to and stored in the germ cells, thence to control development of the embryo after fertilisation. There was, of course, no evidence for the existence of these gemmules, and the theory did not gain general acceptance. In 1880, Nussbaum had drawn attention to the generational continuity of the germ cells and this idea was later developed by Weismann who suggested that the determining factors of inherited characteristics were transmitted from germ cell to germ cell without the involvement of the somatic, or body, cells.

That chromosomes might in some way be concerned with hereditary processes was first proposed by Roux in 1883. Weismann, realising that any substance concerned with the transmission of heredity would have to be divided and distributed in a controlled and accurate manner, was able by 1887 to construct a theory of inheritance which stressed the

importance of the nucleus (and more especially nuclear chromatin, which, he postulated, contained certain ultra-microscopical units, named 'determinants') for control over cellular development; of mitosis, for the production of new cells each endowed with identical amounts of this chromatin; and which predicted meiosis as a special reduction division leading to the production of eggs and sperm containing only half the usual amounts of genetic material, so that the correct quantity could be restored at fertilisation.

By the early 1900s renewed attention was being given to Mendel's paper, independently by de Vries, Correns and Tschermak. These workers, together with Bateson, were arriving almost simultaneously at similar conclusions: that certain characteristics of living organisms were determined by discrete 'factors' (Mendel's term) which were passed on from generation to generation in unchanged form.

Thus the rediscovery, and independent confirmation, of Mendel's results coincided with the recognition that there were within the cell, physical structures which behaved in a manner exactly like that predicted for the Mendelian factors. The "two and two" were explicitly put together by Sutton in 1903 and by Boveri in 1904 to produce the chromosome theory of inheritance, which postulated the chromosomes as the carriers of genetic determinants and therefore provided a cytological explanation of segregation and independent assortment. Thus was born the science of cytogenetics. Further developments of the theory came rapidly. That there were many more genes (as the hereditary factors had been named by Bateson) than chromosomes was quickly realised by Sutton, and this led to the idea of chromosomes consisting of, or carrying, groups of genes arranged in a linear sequence. Correns, in 1900, had already demonstrated that genes were linked together in some way. In 1901 McClung noted an unpaired chromosome at mitosis and concluded that this was involved in sex determination. Sex chromosomes were recognised in Man by Guyer in 1910, but not until 1917 did Wiemann correctly interpret the male sex chromosome constitution as XY and the

female as XX. Other important advances which seem to have come in very quick succession were discoveries of abnormal chromosome complements, the demonstration by Morgan in 1910 that the number of linkage groups was the same as the diploid chromosome number in Drosophila, the phenomena of crossing over and chromosome breakage and the distinction between heterochromatin and euchromatin.

After this fairly rapid increase in genetic and cytogenetic knowledge, there followed, concerning the human species, a period of relatively slow advance, not to say stagnation. Remarkable discoveries were being made in other areas of genetics, and concerning other species, but as far as human chromosomes were concerned, attempts simply to enumerate them were notoriously unsuccessful for many years. By 1923, Painter had settled on a diploid number of 48, but this was not generally accepted as a satisfactory conclusion. The confusion regarding the number, and also the morphology, of human chromosomes was caused by the inadequate techniques of cell preparation that were available in the early decades of this century.

The first major technical improvement to come to human cytogenetics appeared in the 1950s when Tjio and Levan turned from the traditional methods of cell preparation by means of fixation, dehydration, paraffin embedding, sectioning and staining (altogether a very time-consuming, and not particularly fruitful procedure) to the use of soft, for example, embryological, tissues which could be smeared on to a glass mount, to produce a single layer of cells which could be fixed and stained in one operation. Monolayers of dividing cells were also produced by <u>in vitro</u> methods of growing clones of human cells developed by Puck and his co-workers. (Glass 1978). Visualisation of the chromosomes within these cells was greatly improved by treatment, prior to fixation, with a hypotonic solution; a procedure developed accidentally in Hsu's laboratory in 1951 when monolayers of mammalian cells were washed with such a solution instead of the more usual balanced salt solution (Hsu and Pomerat 1953).

The first of the mitotic spindle inhibitors to gain general use, colchicine, was also included as one of the steps in cell preparation at about this time. Tjio and Levan in 1956 published a paper showing the clearest human chromosomes ever seen, produced by means which incorporated these new technical developments; and they announced the first major finding in the field for many years: that the usual diploid number of human embryonic fibroblasts was 46. This number had actually been noticed as regularly occurring in embryonic liver cells a little earlier but those responsible for this work had discontinued the study because of the failure to find all 48 chromosomes! Ford and Hamerton (1956) confirmed the number the same year after examining testicular material from three men and observing regularly 23 pairs of bivalents. Following this new impetus, further discoveries once again fell thick and fast. In 1959 the first report of Trisomy 21 as the aetiologic factor in Down's syndrome was published (Lejeune et al. 1959), to be followed very closely by reports of the chromosome constitution of Turner's syndrome (45,XO) (Ford et al. 1959a) and Klinefelter's syndrome (47,XXY) (Jacobs and Strong 1959). Mosaicism, that is, the presence of more than one cell line, each derived from the same fertilised egg after an abnormal mitotic division, was reported for the first time (Ford et al. 1959b, Court Brown et al. 1960).

In 1960, another of those fortuitous laboratory accidents occurred. Phytohaemagglutinin (PHA) had been used for many years to aid the separation of erythrocytes from whole blood. Nowell noticed that after such a separation the white cells (lymphocytes) had been induced to divide. This discovery made possible the culturing of cells from a readily available human tissue - peripheral blood. (Moorhead et al. 1960). Further cytogenetic investigations could take place on a much wider scale and within a very few years population surveys were reported investigating the incidences of various unusual karyotypes.

With improvements in chemically defined cell culture media and refinements in all stages of cell preparation cytogenetic investigations proliferated. Many new chromosomal syndromes were reported; for instance, XYY (Sandberg et al. 1961), cri-du-chat, (involving a deletion of the short arm of chromosome 5 (Lejeune et al. 1963), and a variety of sex chromosome abnormalities of the Klinefelter type. In 1961, Penrose and Delhanty identified the triploid condition in the cells of aborted foetal material.

Within about ten years the limitations of these cytogenetic techniques were realised and reached. The staining methods used were based on the binding of staining agents to carbohydrate, or more commonly, to the phosphate groups of the DNA (Caspersson et al. 1972a). The appearance of the chromosomes was very clear and certain morphological features were obvious, but the following inadequacies of technique became apparent:

1. Very few human chromosomes could be unequivocally identified; they could only be grouped according to size.
2. The loss of small amounts of material could not be recognised.
3. Inversion of material within the chromosome arm (paracentric) was not detectable.
4. Reciprocal translocations of approximately equal or small amounts of material could not be detected.
5. Complex rearrangements involving more than two chromosomes could not be resolved.

Several attempts at a more precise identification of chromosomes were made in the 1960s by a variety of methods:

1. By the more accurate measurement of arm ratios.
2. By autoradiography.
3. By differential staining of the chromosomes.

Despite early reports (Tjio and Puck 1958) that all chromosomes in the human complement could be identified on the basis of length and arm ratio, recent opinion has it that automatic (computer-mediated) measurement analyses

show that it is impossible to identify all human chromosomes by measurement alone, however accurate the measurements might be (Harris et al. 1973).

In fact, this situation is not unusual. It is uncommon to find a species of plant or animal in which each chromosome may be unequivocally identified by morphological characteristics (such as size, centromere position, presence or absence of secondary constrictions and/or satellites) alone. Crepis fuliganosa has a low diploid number (2N=6) and each pair of homologous chromosomes has a unique appearance (Hsu 1973). However, in Drosophila melanogaster which also has a low diploid number (2N=8), two pairs of homologues are almost indistinguishable using morphological criteria, and in both mouse and pig it is impossible to identify any of the chromosomes in the karyotype with any certainty using morphological criteria (Caspersson and Zech 1972). The distinct appearance of heterochromatin has long been known, as has the fact that it is distributed in special regions of the chromosomes, but this has not facilitated identification of the chromosomes because both heterochromatin and euchromatin are in a highly condensed state during the stage of cell division (metaphase) in which the chromosomes are most easily observed.

The first attempts to identify segments of chromosomes and chromosome arms came with the use of autoradiography after the incorporation of tritiated thymidine into newly synthesised DNA (German 1967). By the use of this technique regions of the chromosomes could be recognised by the timing of their replication in the S phase of the cell cycle. Various chromosome regions can best be characterised by the time in which they complete replication. Using these techniques individual B,D and E group chromosomes may be identified in the human karyotype, as well as the X chromosomes. However, the method is of little use for distinguishing between the numerous C group chromosomes.

In the late 1960s Caspersson began his attempts to identify individual chromosomes by recognition of the different sequences of DNA contained within them. The first venture was to measure the distribution of DNA along the chromosomes.

This parameter was found to be of little use as DNA is distributed fairly uniformly along the chromosomes (Caspersson and Zech 1972, Caspersson et al. 1972a). The next attempt was by means of a systematic search for a dye which would bind preferentially to one of the four bases contained within the DNA double helix. Caspersson reasoned that, as the base sequence of any gene is not a random one, then the sequences of bases along any particular chromosome would not be random either. Therefore by using any dye which binds preferentially to one of the bases one should be able to detect regions of higher or lower concentration of this base in the DNA sequence. The order and size of such regions should be unique to each chromosome.

Caspersson was particularly interested in fluorescent dyes because of the increased resolution possible under an ultra-violet light system of optics. Several compounds were found which would stain chromosomes differentially, but most gave patterns which were too faint to be of practical use. The exception was quinacrine mustard. In 1968 Caspersson and his co-workers reported that the chromosomes of Vicia faba could be stained with this compound to produce a banded appearance along their length. The brightest bands were about three times as bright as the dullest. The pattern of the bands on each chromosome was unique to that chromosome, and could be used to identify the chromosome as it showed inter-cell stability.

Human chromosomes when similarly stained, also showed distinctive individual patterns (Caspersson et al. 1971a). The basic patterns were consistent from one individual to another, and again from one type of tissue to another. The types examined included meiotic material (Caspersson et al. 1971b), embryonic, malignant and various types of somatic tissues (Caspersson and Zech 1972). The method was simpler, quicker and much more informative than autoradiographic techniques and identified not only whole chromosomes but also portions of them. Quinacrine dihydrochloride was later shown to produce the same banding effect as quinacrine mustard (Alfi et al. 1971) and is now more commonly used.

It was originally thought (Caspersson et al. 1970a) that it would prove difficult or even impossible to analyse these patterns by eye because:

1. the patterns were too complex to memorise, and
2. the human eye is not sufficiently sensitive to the gradations in intensity.

Therefore methods of automatically analysing the patterns by means of fluorometric curves of the chromosomes were developed. Fortunately (as the instrumentation required for these latter procedures is prohibitively expensive for large scale use) neither of these restrictions seems to apply in practice. In order to identify the chromosomes and assemble a karyotype it is necessary only to recognise one or two distinctive features of each chromosome, and processes of elimination are useful as long as awareness remains of the possibility of abnormality.

Occurring almost simultaneously with these developments was the independent discovery by several people that similar patterns of bands could be produced by staining with non-specific Romanovsky dyes such as Giemsa, after one of a variety of pretreatments. These pretreatments included incubation in a warm salt solution (Drets and Shaw 1971, Schmeel 1971, Bhasin and Foerster 1972, Bosman and Schaberg 1973), mild proteolytic digestion (Dutrillaux et al. 1971, Seabright 1971, Finaz and de Grouchy 1972, Wang and Federoff 1972, and others), oxidising agents (Utakoji 1972, 1973), protein denaturants (Shiraishi and Yosida 1971), variations in pH (Patil et al. 1971) and cation concentration (Chaudari et al. 1971, Lomholt and Mohr 1971). For the production of these so-called G-bands every proteolytic agent, no matter what its specific action seems to be efficient (Dutrillaux and Lejeune 1975). These methods stain human chromosomes to produce banding patterns which were virtually identical with those produced by the fluorescent stains (bright bands with quinacrine were dark with the Giemsa methods.)

Several of the early methods were very time-consuming and unreliable. The techniques were constantly being modified and improved. Generally, it seems that enzyme (particularly trypsin) pretreatments lead to the production of very clear and detailed bands (Hsu 1973). Other methods result in more uneven staining which are resolved into the banding patterns with more difficulty. There now exist methods of producing G-bands which incorporate the features of the pretreatment in the staining stage (Sperling and Wiesner 1972, Sun et al. 1973, Walther et al. 1974.).

In 1970, Pardue and Gall reported a method by which dark staining material was revealed at the centromere of mouse chromosomes. These darkly staining regions were the sites of hybridisation between satellite DNA transcribed *in vitro* from RNA also transcribed *in vitro*, this time from satellie DNA, and the homologous DNA on the chromosomes. The same staining pattern was later produced with the omission of the hybridisation steps, and was believed to reveal DNA which had been denatured and then differentially allowed to reanneal. The method was applied to human chromosomes by Arrighi and Hsu (1971). Prominent blocks of darkly staining material appeared on the long arm of the Y chromosome, and the secondary constrictions of chromosomes 1, 9 and 16, and variably on the short arms and satellites of the acrocentrics, as well as the centromeres of each chromosome. Variants of the procedure also were soon published which improved both the quality and speed of preparation, and the consistency of the results (Summer 1972, Alfi and Menon 1973, Madan 1973).

The patterns (known as C-bands) revealed by these methods are not generally useful for the identification of chromosomes, either between or within species, but they do show up a very variable aspect of human chromosomes, namely, the size of the stained blocks, particularly those on chromosomes 1, 9 and 16.

Numerous other procedures for producing patterns of differential staining of chromosomes have been published in recent years. Patterns which are the reverse of those obtained by staining with quinacrine and the various Giemsa methods mentioned above may be produced by staining with Giemsa under altered conditions or by staining with acridine orange after incubation in Phosphate buffer (Bobrow 1974). This technique has special advantages when the visualisation of the telomere regions is important. Such regions usually stain palely with the previous methods, and consequently are dark and easily delimited in these reverse patterns. Staining with acridine orange shows bichromatic differentiation of the chromatids. Those bands which stain brightly with quinacrine negative regions are a dull orange-red in colour, and quinacrine negative regions are a bright yellow-green (Bobrow and Madan 1973).

The fluorescent stain ethidium bromide may be used to produce a reverse banding pattern in the chromosomes of some species, for example, some plants (Vosa 1970a), and in a marsupial (Pearson et al. 1971), but this technique has not been successfully applied to human chromosomes (Bobrow 1973).

Standardisation In Human Cytogenetics

The Paris Conference (1971) on standardisation in human cytogenetics was organised with a view to systematizing the nomenclature of the chromosomes and the newly-revealed unique patterns which could be seen with these staining methods. The chromosomes were first arranged according to size and centromere index into the groups accepted using conventional, uniform staining methods. Those which had been numbered using autoradiography retained their numbers. The patterns produced by quinacrine mustard or its derivatives were named Q-bands. Similar patterns produced by staining with Giemsa or related stains after various pretreatments were named G-bands. Patterns produced by staining with Giemsa after more severe pretreatments such that only blocks of chromatin near the centromeres were stained, were named C-bands. Patterns which were the reverse of the Q- and G-bands were named R-bands.

By such collation of the available data it became possible to see that whichever technique was employed, patterns were revealed which could not be artefacts but which must reflect some consistent aspect of the structure of each chromosome.

The Paris Conference (1971), Supplement (1975) suggested more detailed abbreviations for the type of banding pattern being observed, the purpose of which was to make reference to the type of procedure used to obtain the pattern. For example, the type of band observed in this study, Q-bands by fluorescence using quinacrine were designated QFQ-bands. The manner to be used to refer to each region of each chromosome, and to describe any variation on the normal pattern was also defined and later amended by these reports.

Other Methods of Differentially Staining Human Chromosomes.

This renewed interest in the staining of chromosomes has led in the last ten years or so to the development of numerous staining techniques which are of value, not only for the identification of chromosomes, but also to shed light on aspects of chromosome structure and composition. Some of these techniques will be mentioned below.

1. Other fluorescent stains.

As mentioned above ethidium bromide may be used to produce patterns which are the reverse of those obtained with quinacrine in the chromosomes of some species. Hollander et al. (1976) used this stain to enhance the patterns obtained with quinacrine mustard. Background fluorescence was suppressed and a colour contrast introduced (the background was pale green and the stained chromosomes orange).

Moscetti et al. (1971) reported that very clear pictures of human chromosomes could be obtained using Acrini (A-staining). Areas which stain very brightly with quinacrine stained brightly with this stain. It is not clear whether the less

bright bands were also produced.

The stain Hoechst 33258 has recently been used extensively in cytogenetics. Hilwig and Gropp (1972) found that it was relatively specific to constitutive heterochromatin in mouse cells but did not show good banding in the chromosome arms. Raposa and Natarajan (1974) reported that with this stain constitutive heterochromatin (C-band material) fluoresces brightly in human chromosomes, as well as in those of many other species.

Jalal (1975) managed to produce Q-bands with this stain and suggested that the age of the slides was important (3 to 7 days old for good Q-bands). However, Chen (1977) reported that fresh slides gave Q-bands and those a few days older gave C-bands. The staining method appears then to be very susceptible to experimental conditions and possibly, different slides respond differently to subsequent treatments. It appears that this stain will produce both Q- and C-bands on human chromosomes but the optimum conditions for each vary.

Hoechst 33258 has also been used in conjunction with BrdU (a derivative of the base uridine) and Giemsa to detect sister chromatid exchanges. BrdU, when added during S phase, is incorporated into the DNA. Such DNA is less condensed and stains less darkly than usual. If such chromosomes are stained firstly with Heochst 33258, exposed to light, and subsequently stained with Giemsa, chromosomes having a 'harlequin' appearance may be visualised - showing the sites at which sister-chromatid exchange has occurred (Perry and Wolff 1974). The basic mechanism of this reaction was considered by Goto et al. (1978) to be that photolysis of the BrdU-substituted DNA occurred, with the photosensitive Hoechst 33258 playing a role as sensitiser.

N-bands and the silver-staining of nucleolar organiser regions.

Matsui and Sasaki (1973) reported a method which resulted in the selective staining by Giemsa of the nucleolar

organiser regions of human chromosomes. Funaki et a. (1975) demonstrated these so-called R-bands in a variety of species, both plant and animal, and found that in most cases they were clearly located in certain specific regions of the chromosomes, for example, in secondary constrictions, satellites, centromeres, telomeres, heterochromatic segments.

Goodpasture and Bloom (1975) described a method later to be referred to as the Ag-As staining method which differentially stained with silver compounds the chromosomal locations of ribosomal DNA (that is, the NORs) in certain mammalian species. These areas appeared as black, spherical bodies on yellow-brown chromosome arms. Using these techniques, Matsui and Sasaki (1973) placed the NORs in the satellites of the acrocentric chromosomes. This view was shared by Howell et al. (1975) and by Denton et al. (1976). However, the work of Goodpasture et al. (1976) shows that the NORs are found in the secondary constrictions (stalks) of the acrocentrics, and not in the satellites.

Other differential staining methods which will not be given in detail are:

T-bands. -specific staining of the telomeres (Dutrillaux 1973).

D-bands. -using daunomycin and adriamycin, gives a similar appearance to Q-bands, but is more stable (Lin and van der Sande 1975).

Cd-staining. this shows the centromeres as double dots rather than as a single band. The dots are of the same size on all 46 human chromosomes, and are thought to represent centromeric organelles (Eiberg 1974).

CT-bands. Simultaneous staining of C and T bands (Scheres 1974, 1976, Chamla and Ruffie 1976).

CR-bands. Simultaneous staining of C and R bands (Kanda 1976).

GC-bands. Simultaneous staining of G and C bands (de la Maza and Sanchez 1976).

Caesium chloride banding. used by Geraedts and Pearson (1973) to stain specifically the secondary constriction of chromosome 1.

A-bands. Bands produced by routine G-banding methods on slides which are at least three months old, and which are similar but not identical to G-bands (Crosson 1972, 1974).

Contour staining. Only the contour or periphery of the chromosome is stained (Barnett et al. 1973).

LBA staining. Late replicating bands are detected by staining with Acridine orange after the incorporation of BrdU during S phase (Nakagome et al. 1977b; Oka et al. 1977). This method detects size variants in the stained regions, and is less susceptible to technical variations than the more usual C-banding methods.

DIPI and DAPI staining. This method produces a pattern similar to Q-banding, with the exception that the secondary constrictions of chromosomes 1, 9 and 16 are bright. (Schmedl et al. 1977).

Pre-fixation techniques.

Several compounds appear to induce differential uptake of stain by different chromosome regions when added to lymphocyte cultures for some period prior to harvesting of the cells. Shafer (1973) found that adding AMD to human lymphocyte cultures a few hours before harvesting induced the appearance of G-bands on subsequent staining. Hsu et al. (1973) extended the list of compounds having this effect on human lymphocytes and Chinese Hamster fibroblasts to include Azure B, ethidium bromide, nogalomycin, daunomycin and cytosan. The patterns of bands induced in this way was not always clear and appeared to resemble the reverse pattern more often than the G-band pattern.

Each type of banding pattern described above gives information concerning some aspect of human chromosome structure and variability, but none separately gives an indication of the full extent of easily-detected variability. Certain banding patterns serve merely to identify particular chromosomes, others reflect differing chemical compositions,

and still others demonstrate quantitative variations. Several techniques have been devised to stain any given cell sequentially by two or more methods, in order to gain a more complete picture of particular chromosomes. (Techniques allowing the simultaneous appearance of more than one banding pattern have been given in the previous section). Examples of sequences which combine a technique which identifies chromosomes with one which detects variations (qualitative or quantitative) in the composition of chromosomes are:
G followed by C bands (Merrick et al. 1973, Marshall 1975).
G followed by Q bands (Evans et al. 1971).
Q followed by C bands (Chen 1974).
Q followed by R bands (RFA) (Niikawa and Kajii 1975).
Q followed by G, and by C bands, and
Q followed by G and by R bands (Lubs et al. 1973, Verma and Lubs 1976a).
Q followed by R, then by C bands (Rubenstein et al. 1978).

The many techniques available for the production of Q-, G- and R-bands all result in broadly similar patterns of bands. Depending upon the experimental conditions, any particular band may or may not take up the stain, but the sequence and size of the bands along any chromosome arm is identical with all techniques (Dutrillaux et al. 1972). Generally, bands which stain brightly with Q-band methods are dark G-bands and pale R-bands. Regions of the chromosomes which do not show this simple relationship are those consisting of centromeric heterochromatin (centromeres, and secondary constrictions of chromosomes 1, 9 and 16), short arms and satellites of the acrocentric chromosomes and the distal part of the long arm of the Y chromosome. The secondary constriction of chromosome 9 is not stained by any of these methods. The distal part of Yq° is very bright with quinacrine, and banded with the G-band methods. Other areas such as the short arms and satellites of the acrocentrics have a variable appearance with all methods.

° q refers to the long arm of a chromosome, p to the short arm, and h to a secondary constriction.

Important factors that seem to determine which type of banding pattern will be produced by any method appear to be temperature, pH, and the strength and composition of any incubation solutions, and the length of the incubation time (Biberg 1973). The initial condition of the slides (such as their age, and the method of their preparation) seems also to be important.

Which method of staining chromosomes is to be used obviously depends upon the type of information which is being sought. Q-banding seems to be the most reproducible, and is the least sensitive to variations in slide preparations (possibly owing to the fact that it is the simplest method and requires no pretreatment of the slides prior to staining.). For other methods it is important to use fairly freshly prepared slides (Uchida and Lin 1974). However, G-banded slides are permanent and therefore can be stored for analysis later.

Hoechst 33258 has been suggested as a more stable alternative to quinacrine (Raposa and Natarajan 1974), but the type of banding pattern produced with this compound seems to vary (see above page 16).

Several of the techniques are not suitable for chromosome identification but are useful in specific situations. For example, R-bands are useful for the investigation of abnormalitie involving the telomeric regions. Verma et al. (1976), compared G-, Q- and R-banding patterns (using acridine orange) and found R-bands particularly useful for the analysis of breakpoints because of the colour difference between adjacent bands. G and R patterns used sequentially seemed the best combination, as the R pattern gave more precise information than the G-band pattern. C-bands are particularly useful for the locations of centromeres in meiotic configurations.

Several of the banding methods are important because they disclose regions of the chromosomes which show inter-individual variations with no apparent effect on the phenotype (see below, page 66).

These new techniques have been developed, broadly, and with much overlap, along two lines:

1. Techniques for the identification of chromosomes and chromosome regions. These have become more reliable, more detailed, and less time-consuming; and
2. Techniques which render visible different aspects of chromosome structure and differentiation. These have become more specific to certain components of the chromosome, and interrelationships between the various methods have been important in the elucidation of chromosome organisation.

Some Important Achievements in Human Cytogenetics Made Possible by Methods of Differential Staining of Chromosomes.

1. Previously known chromosome trisomies of groups D, E and G have been identified as involving chromosomes 13, 18 and 21 respectively (Caspersson and Zech 1972, Dutrillaux and Lejeune 1975).
2. A new trisomy syndrome has been identified, involving chromosome 8 (Kakati et al. 1973).
3. The major abnormalities associated with other trisomies (for example, trisomy 20) have been identified (Pan et al. 1976).
4. The frequency of a paternal and maternal origin of the extra chromosome in trisomy 21 has been estimated (Mikelsen et al. 1976, Wagenbuchler et al. 1976).
5. The exact position of the site of breakage may be identified in translocations and other rearrangements of chromosomal material (Breg et al. 1972, Franke 1972, Seabright 1973, Jacobs et al. 1974a, Nielsen and Rasmussen 1976). There has been much disagreement as to whether there is a non-random involvement of chromosome regions in structural rearrangements (Jacobs et al. 1974a) or whether the breakpoints occur at random with respect to chromosome length (Nielsen and Rasmussen 1976). Breakpoints seem to occur more

frequently in Q negative bands than in Q positive bands (Seabright 1973, Aymé et al. 1976).

6. More complex translocations involving three or more chromosomes may be resolved (Alderdice et al. 1971, Caspersson and Zech 1972).
7. Generally, a better separation of different chromosome abnormalities is possible, leading to a more specific correlation of these with phenotypic effects, and the identification of non-specific phenotypic effects (Lewandowski and Yunis 1975). Borgaonkar (1975) has stated that not a single chromosome in the human karyotype has been found to be free of involvement in some kind of abnormality.
8. The frequencies and sites of breakages of the chromosomes under various conditions such as cell culturing, X-irradiation, Fanconi's anaemia etc., have been analysed (San Roman and Bobrow 1973, Aula and von Koskull 1976, Aymé et al. 1976).
9. The non-random involvement of the acrocentric chromosomes in Robertsonian translocations has been shown (Mikelsen 1973). For example, most D/D translocations are of the 13/14 type, with 13/13 and 14/14 occurring more rarely, most D/G instances are of the 14/21 type, followed by 13/21, and most G/G translocations involves two chromosomes 21, with 21/22 and 22/22 occurring more rarely (Dutrillaux and Lejeune 1975). This analysis has very important consequences for genetic counselling (van der Hagen et al. 1971).
10. The banding studies of Daniel and Lam-po-tang (1976) have suggested that all Robertsonian translocations could be interpreted as being stable dicentrics. This finding has very important implications for the role and functioning of the centromeres, and suggest that somehow the acrocentrics are different from the metacentric chromosomes with respect to centromere behaviour.
11. The Ph' chromosome fragment found in some leukaemia patients has been shown to be a deleted chromosome 22, (Caspersson et al. 1970c), the missing material being translocated on to the long arm of chromosome 9 (Whang-Peng et al. 1974).

12. Tumour and cell line studies have shown that each cell-line appears to have specific marker chromosomes with distinctive banding patterns. Thus marker chromosomes for specific tumours may be identified and cell cultures may be monitored for cytogenetic change (Miller et al. 1973).
13. Somatic cell hybridisation has been used in conjunction with chromosome identification techniques to locate gene loci. If a cell continues to show certain characteristically human biochemical activities during the progressive loss of chromosomes in cell culture, then the genes responsible may be localised to particular chromosomes (Bootsma et al. 1973).
14. The male sex-determining factors have been localised on the proximal part of Yq. (Siebers and Vogel 1973, Tiepolo and Zuffardi 1976).
15. Pre-natal sex determination is possible by the detection of the brilliant Y chromosome in amniotic fluid cells (Cervenka et al 1971).
16. By using this sexing technique Fialkow et al. (1971) demonstrated that the cells of a healthy donor had undergone leukaemic transformation in the recipient.
17. Chromosome fluorescence intensity variants have been successfully used to determine the outcome of a paternity dispute (Jonasson et al. 1972).
18. Comparative studies of related species of plants and animals have led to knowledge of the sorts of karyotypic changes which accompany evolution.

The Heritability of Banding Patterns and their Variants.

There is an obvious need to establish the heritability of the banding patterns and their variants before they can be used in comparative (between group, population or species) studies, and before conclusions can be drawn about their implications for ideas on chromosome structure and composition. Variants of the patterns have been used to assign genetic loci to particular chromosomes and to establish the orders of loci in linkage groups (Magenis et al. (1977). The validity of such procedures depends on the knowledge that these variants are true genetic polymorphisms.

That the Q-band patterns and variants seemed to be inherited in a simple Mendelian fashion was noted very early (Caspersson and Zech 1972). This has been independently confirmed several times (Pearson et al. 1973, Alfi et al. 1975, Robinson et al. 1976).

Alfi et al. (1975) examined 109 families (each consisting of a child and both parents) and found no inconsistencies with the hypothesis of Mendelian segregation. Robinson et al. (1976) examined the transmission of both Q- and C- variants in 32 families. For all chromosome regions variants were scored as brilliant (B), intense (I) or normal (N). The results of matings of types BNxBN and BBxBN agreed with Mendelian expectations for all chromosome regions studied. However, matings of the type BNxNN appeared to produce an excess of the BN type in 6 of the 8 regions studied. This excess was significant if the results of all regions, or of all the regions on the acrocentric chromosomes were summed. The excess of BN types seemed to occur in daughters of BN fathers, and sons of BN mothers. No obvious explanation for this phenomenon was suggested. The authors postulated some kind of scoring error as being responsible.

The same group (Robinson et al. (1978) has also reported a possible mutation of a Q-band variant. A child of 21s type NN was born to parents of types II (mother) and IN (father). All other genetic markers investigated indicated that there

was no doubt about the maternity of the child.

Sekhon and Sly (1975) examined the Q- and C-band segregation in 9 unrelated families consisting of 69 individuals in total. All Q-band polymorphisms segregated in a simple Mendelian manner.

Phillips (1977) examined the inheritance of Q- and C-band variants amongst 36 individuals of 3 unrelated families and found no deviations from a simple Mendelian pattern of inheritance of Q-band polymorphisms, and no evidence for any distortion of segregation ratios.

Therefore it seems that Q-band variants may be considered to be genetic polymorphisms which follow the usual patterns of segregation and inheritance. The occasional occurrence of a mutation from one fluorescence intensity to another is not unexpected, especially when it is remembered that the relationship between the reaction to the stain of these variable regions and their structure and composition is as yet unknown. Variants must arise somehow; the evidence from Robinson et al. (1978) suggests that they may arise by a single step process in a single generation rather than by gradual accumulative changes in the material of the variable regions.

That other types of chromosomal polymorphisms are inherited has also been amply demonstrated. Several authors have noted the Mendelian segregation of C-band variants (Craig-Holmes et al. 1975, Carnevale et al. 1976, Robinson et al. 1976, Magenis et al. 1977, Phillips 1977). Many of these authors have reported some distortion of segregation ratios, and some inconsistencies in the variants between parents and child, but in all cases these anomalies are exceptional and possibly indicate a higher frequency of occurrence of unequal crossing-over or de novo duplication within the C-band regions than in other chromosome regions (Sekhon and Sly 1975, Phillip 1977).

Verma and Lubs (1976b) found that RFA variants were inherited in Mendelian fashion in the members of 3 families, no inconsistency being detected.

Banding Patterns in Other Species.

Differential staining with quinacrine and related stains and the various G-banding methods has been observed in the chromosomes of a wide variety of animals and plants. In the case of this latter group of organisms, however, it appears that differential staining with either fluorochromes or with Giemsa reveals only the differences between euchromatin and constitutive heterochromatin (Greilhuber 1977). No banding pattern has been observed within the euchromatic parts of mitotic plant chromosomes. In certain animal groups also, G-banding methods result in C-band patterns (for example in the Urodeles (amphibians) and Orthoptera (insects). It is probable that the lack of G-band patterns in these organisms is due to the particularly large amounts of, and a high degree of condensation of, DNA in each individual chromosome Geilhuber (1977).

As well as allowing the identification of previously unidentifiable chromosomes in several organisms, for example the mouse and pig, (Caspersson and Zech 1972), and the ox (Bostock and Sumner 1978) banding patterns of chromosomes have allowed great advances to be made in the field of comparative cytogenetics. Inter-species, -genus, -family, -order, and even inter-class comparisons may be made with respect to the chromosomes and karyotypic changes involved in, or associated with, evolutionary developments may be elucidated. Phylogenies have been constructed which in several cases show relationships very similar to those obtained by other means.

The general picture seems to be that most karyotypes, or at least their G-band patterns, are virtually homologous in related animal species. Chromosomes of identical shape and banding pattern have been found throughout an order of mammals (Wurster-Hill and Gray 1975) and even throughout an entire class of birds (Stock and Mengden 1975). Many examples have been found of related species having identical

banding patterns in the euchromatic regions and showing differences only in the heterochromatic regions of their chromsomes (for example, in Peromyscus (rodent) (Pathak et al. 1973) and in two groups of insects (Nankivell 1976, Rees et al. 1976).

A very impressive example of the conservative nature of banding patterns is the constancy of the form and patterns of the X chromosome throughout the mammals (Ohno 1973)

In rats there is a uniformity of G-bands in all species and subspecies, despite a high variability in the C-bands (Yosida and Sagai 1975), though characteristic rearrangements of the chromosome material appear and may be identified in different geographical races. The chromosomes of sheep, goat and ox are extremely similar with respect to their banding patterns, and precise matching of homologues across genera is possible (Dover 1977).

The major karyotypic differences which commonly occur between related species and genera (as revealed by differential staining) are:

1. Rearrangement of the chromosome material by means of inversions (paracentric or, apparently more commonly, pericentric) and translocations, for example in the great apes and Man (Dutrillaux et al. 1975, a, b).
2. Alterations in the chromosome number by fusion (for instance, by Robertsonian translocation, first noticed in 1916 in the Orthoptera) and possibly by fission, although this latter is not so easy to demonstrate or to explain and is statistically less likely (Imai and Maruyama 1978). For example, the metacentric chromosomes of the tobacco mouse (Caspersson and Zech 1972).
3. An increase in the amount of chromosome material in some species brought about by unequal crossing over or by duplication of some part of the genome, for example in the rodent genus Peromyscus (Pathak et al. 1973) and in

the ox and goat (Stolla 1972, Sumner and Buckland (1976).

4. Differences in the compositions and amounts of highly repetitive sequences in the constitutive heterochromatin, for example in the primates (Jones 1976).

It seems as though the important chromosomal changes within evolution are Robertsonian translocations and pericentric inversions (de Grouchy et al. 1975). Reciprocal translocation is thought to be less important (Bodmer 1975).

Chromosome Banding Patterns of the Higher Primates.

Karyotypic phylogenies have been examined frequently and in great detail among the species comprising the higher primates, including Man. Throughout the primates, there are some chromosomes which seem to have remained unaltered during evolution (Stock and Hsu 1973). This is in addition to the X chromosome which for various reasons is expected to be more conservative than others. (Ohno 1974). This suggests that there is a need for the retention of certain genetic sequences in all primates (Pearson 1977). The homology of chromosome 1 among different primate species is particularly noticeable. The evolution of this chromosome can be traced back to the common catarrhine ancestor of the higher primates, baboon and cercopithecus. (de Grouchy et al. 1977). The implication is that this chromosome has retained its sequence of bands, and therefore of genetic material, over a period of 50 million years or so.

Altogether 17 human chromosomes seem to have direct counterparts in the karyotype of the chimpanzee. The most important structural differences between individual human chromosomes and those of the great apes are caused by pericentric inversions; at least 8 have occurred to cause differences between the human and chimpanzee karyotypes (Turleau and de Grouchy 1973). Dutrillaux (1975) has stated that the majority of these inversions arose in the direct ancestor of Man.

Translocations have occurred and been fixed only

rarely in the evolution of the Hominoidea. It seems clear that chromosome 2 of Man has resulted from the fusion of two acrocentric chromosomes found in the karyotypes of all the great apes which have no obvious counterpart in the human karyotype. It has been suggested that this fusion is of the Robertsonian type (Turleau and de Grouchy 1972, 1973, Egozcue et al. 1973), but Lejeune et al. (1973) have shown that the mechanism was of end-to-end fusion of the telomeres. This is a fairly unusual type of chromosome rearrangement and is not reported as occurring nearly as often as Robertsonian translocations in present-day human populations. Translocations generally seem to have been more important during the lower orders of primates than in those forming the closest ancestors to Man, for example between the African green monkey and the rhesus monkey (Stock and Hsu 1973).

Comparisons between the karyotypes of Man, chimpanzee, gorilla and the orang utan suggest that the karyotype of the last species is closest to the probable common ancestor of the Pongidae and Man (Dutrillaux et al. 1978). An estimated 99% of the bands are common to all four species indicating that the rearrangements which have occurred have been balanced, that is, they have not involved the loss or gain of chromosomal material (Dutrillaux et al. 1975b). The homology that exists between the banding patterns has been shown to reflect a homology in gene content among these species (de Grouchy et al. 1978).

It is also suggested from studies of chromosome banding that the higher primates diverged from the 'stem line' in the order orang utan, gorilla, chimpanzee and Man. (Dutrillaux et al. 1975b). This more or less confirms previous ideas based on morphological information.

Comings (1973) has suggested that the pairs of chromosomes predicted by Ohno's theory of a polyploid event occurring some time during the evolution of the

mammals can be detected in the karyotypes of the higher primates indicating that there has been relatively little alteration in some banding patterns over 200-300 million years.

Chapter 2: CHROMOSOME STRUCTURE AND COMPOSITION AND THE PRODUCTION OF CHROMOSOME BANDS.

The eukaryotic chromosome, the organelle comprising the genetic material in its most easily observable form, is a transient structure and does not exist in the majority of somatic cells after the early stages of cell formation, and certainly does not exist during most of the period of expression and activity of the genetic material. The chromosomes are structures which become discrete and microscopically visible only during a certain phase (metaphase) of cell division. This division does not normally occur in most differentiated somatic cells. Therefore the chromosome variables examined in this research are a feature of the morphology of chromosomes, but possibly not a feature of genetically active cells.

One can only presume, however, that something becomes of these variable regions during the metabolic period of the cell; and it is possible that, whatever the activity or non-activity of the material is, it varies either in kind or degree according to its composition, and this composition is to some extent reflected in the intensity of fluorescence observed when the fixed metaphase chromosome is stained with the dye, quinacrine dihydrochloride. If these regions are to be treated as genetic variants it is of interest to consider the structure of the metaphase chromosomes, the biochemical explanation(s) of differential staining with certain dyes, the nature of the variants and the manner in which they vary, and their possible function during any phase of the cell cycle. The treatment of these topics, below, will be fairly brief, and, perhaps, not all-embracing, but one hopes essentially correct. Charles Darwin's statement that "the whole subject, however, treated as it necessarily here is with much brevity, is rather perplexing." (Darwin 1859) is applicable here also.

CHROMOSOME STRUCTURE

The cells of eukaryotic organisms contain relatively huge amounts of DNA. For example, the diploid human nucleus contains approximately 5.6 picograms of DNA, which, if extended, would form a molecule approximately 174 cm in length (Lewin 1974). This DNA is divided amongst 46 chromosomes, the smallest of which contains 0.046pg of DNA (equivalent to a molecule of length 1.4cm), and the largest having 0.235pg (7.3cm). The actual lengths of these two chromosomes seems to average out at about 2μm for the shortest and 11μm for the longest. It is obvious that the major requirement of any model of chromosome structure is to provide an explanation of how an enormous degree of compaction of the DNA might occur in a controlled manner.

1. Components of the Chromosome.

Most investigations of the metaphase chromosome involve isolating the chromosome from other cellular and nuclear materials before analysis. Many uncertainties exist regarding the effect of these isolation procedures on the eventual composition and organisation of the resulting chromosome (Bostock and Sumner 1978). Therefore, inexact measurements and analyses only are possible, but where similar results are achieved by a variety of means, then naturally the results must be of some significance, even where they do not reflect precisely the situation in the living cell. The dry mass of any given chromosome may vary quite considerably (the upper limit being about 2.5 times the lower) from one sample to another (Bahr and Golomb 1971). Such variability may indicate genuine variations in the chromosome composition from one mitosis to another (implying that the differences must be due to the amounts of non-histone proteins in the chromosome, see below), or may be technical variations in the preparation of the chromosome for analysis. The dry mass of the chromosomes in a given

metaphase plate seems to vary in proportion to the DNA content of the chromosomes, suggesting that real variation in composition does occur at least to some extent. This type of variation also occurs between tissues (Du Praw 1973).

The chromosome is not then a structure having a fixed composition - certain elements reflect a particular functional and/or structural state of the chromosome at the time of the analysis. The major component of the chromosome, though not the most abundant, is the genetic material, the DNA. Most of the remaining material is protein. Chromosomal proteins are of several types which can very broadly be placed into two categories by virtue of their average acidity or basicity (indicated by the isoelectric point of the molecule). The most basic proteins are the arginine- and lysine- rich histones, of which there are five major types (Delange and Smith 1971). The other proteins are more acidic, but have a wide range of pH points and are a much more heterogenous group, generally referred to as the non-histone proteins.

The histones are closely bound to the DNA molecule within the chromosome and occur in very similar proportions and amounts in a variety of eukaryotic organisms. The DNA: histone weight ratio appears to remain fairly stable at 1.0 in both metaphase and interphase preparations (Sadgopal and Bonner 1970). It had been suggested that the histones molecules lie in the major groove of the DNA double helix (Comings 1972), but recent evidence indicates that the histone molecules form small spherical cores around which the DNA molecule is wound (Olins and Olins 1974). These histone/DNA complexes have been referred to as nu bodies (Latt 1976) or nucleosomes (Evans 1977) and are now accepted as a fundamental feature of eukaryotic chromosomes.

There are about 50 to 100 different kinds of non-histone proteins associated with the metaphase chromosome (Latt 1976). Some are closely bound to the DNA, others have

a more transient presence (Bostock and Sumner 1978). The non-histone proteins vary according to the phase of the cell cycle (Bhorjee and Pederson 1972), and also from one mitosis to another (see above). Moreover, certain non-histone proteins appear to be specific to mitosis (Stein and Farber 1972) and others specific to certain tissues (Dewair and Matthaei 1976).

A certain amount of RNA is also usually found associated with metaphase chromosomes. Some of this represents transcribed material not yet released from the structure (Prescott 1970, Moyne and Gazrido 1976), although much of it is ribosomal (Bostock and Sumner 1978).

Lipids have been reported as a constituent of chromosomes but it is generally thought that those detected are derived from the cell membrane (Jackson et al. 1968).

The permanent invariant materials of the chromosomes are the DNA and histone components. These two components show relatively little variance in their amounts and types (histones) over a wide range of organisms and cell types and thus may be considered to be the material of the chromosome, whilst the other more variable substances are less fundamental to the basic structure of the chromosome, and possibly function during the genetically active stages of the cell cycle.

Relative composition of the chromosome (from Prescott 1970):

DNA 10-28% (Average 20%)

RNA 5-29% (Average 15%)

Proteins 46-77% (Average 65%) - of this 60% is non-histone.

2. Morphology of the Chromosome.

The size, number and shape of metaphase chromosomes varies enormously amongst eukaryotic organisms, but certain features seem to be common to all.

With conventional uniform staining under light microscopy eukaryotic chromosomes appear characteristically

to consist of two identical strands or chromatids held together at some point, known as the centromere. The position of the centromere can vary along the chromatid arms, but is fixed for a given chromosome. There is some dispute as to whether it may occur exactly at the end of the chromatid arm (that is, to form a telocentric chromosome) or whether there is always some chromatid material, however short, at both sides of the centromere. Occasionally the individual chromatids may be constricted at sites also characteristic for the particular chromosome. Such secondary constrictions occur in the human chromosomes on the long arms of chromosomes 1, 9 and 16, in all cases near the centromere region, and in the short arms of all the acrocentric chromosomes. The material at the distal end (furthest from the centromeres) of these latter chromosomes is variable in size (and, indeed, in presence), and forms structures known as satellites. Other human chromosomes (for example chromosome 17) (Priest et al. 1970) have been reported as having satellites, but not consistently.

With electron microscopy, further morphological details of the chromosome become apparent. Thin sections of fixed chromosomes show a mass of electron-dense filaments which probably represent cross-sections of chromosomal fibres. Human metaphase chromosomes appear to have in cross-section 50-100 fibres at most sites, about 100 at the telomeres, and about 40 in the centromere region (Lewin 1974).

In spite of the greater magnification, electron micrographs of whole mount chromosomes show the same general appearance as under light microscopy, but with irregular loops of fibres visible at the edges of the specimen (Du Praw 1966). No 'free' end of these fibres are seen and they often have a 'bumpy' appearance which suggests that they are twisted in an irregular manner.

Estimates of the diameter of these fibres are very consistent from one preparation to another :30nm (Du Praw 1966), 24$\pm$5nm (Abuelo and Moore 1969), 25nm (Wolfe 1965)

25nm (Filip et al. 1975) and 20-30 nm(Lampert and Lampert 1970). Similar fibres are seen in preparations of interphase chromatin having a smaller diameter-23nm (Du Praw 1966). Electron micrographs of chromosomes which have been somehow dispersed show that the fibres occur throughout the chromosome, often in a parallel configuration (Comings and Okada 1970). Fibres in the centromere region appear to be continuous with those in the chromosome arms (Lewin 1974). These chromosomal fibres have been measured (Du Praw and Bahr 1969). The smallest and largest human chromosomes were found to have fibre lengths of 135mm and 723mm respectively, compared with overall metaphase lengths of 2μm and 11μm of the chromosomes themselves. No other basic structural element has ever been shown in chromosomes by electron microscopy (Bostock and Sumner 1978).

3. The Chromosome Fibre.

The discovery of a basic structural element common to all metaphase chromosomes helps one to resolve the problem of chromosome structure into a few manageable questions, such as:

How many fibres are there in each chromosome?
How many molecules of DNA are there per fibre?
How is (are) the fibre(s) packed into the chromosome, and the DNA molecule(s) into the fibre?
What is the role of the histones in the fibre construction? (This last question has to some extent been answered by the discovery of the nucleosome, see above).

There is strong evidence from several sources that only one fibre is found in each chromosome:

(i) Autoradiographical studies show that chromosomes replicate in a semi-conservative fashion (Taylor 1957, 1963). When new chromosomal material is produced from the old, all constituents of the old are retained in the old structure, whilst the new structures comprise entirely new components. Obviously this phenomenon becomes more unlikely the more units there are of old components, and in this case, mechanisms for the regulation of segregation of the new material must be postulated (Prescott 1970).

(ii) Lampbrush chromosomes are specialised chromosomes found in the oocytes of certain amphibians. Loops of the chromosome can be seen in active transcription. The integrity of these chromosomes is destroyed only by DNAase (Callan and MacGregor 1958), but if the chromosome consisted of more than one DNA-containing fibre then presumably these could be separated by some other means.

(ii) The smallest unit apparently involved in X-ray induced abberrations is the chromatid. Damage which seems to be of a subchromatid nature affects both chromatids after one round of replication of the damaged chromatid. If the aberration genuinely affected only half the chromatid then it would appear as a full but single chromatid aberration after replication. Thus, the chromatid seems to be the smallest unit susceptible to this sort of damage, suggesting that it contains only one fibre.

Although it is usually possible to see several parallel chromatin fibres in even the narrowest parts of the chromosomes by electron microscopy, this does not necessarily imply that there are several fibres per chromosome; this appearance could be resolved by postulating longitudinal folding of a single chromosomal fibre (see below p. 40).

It is apparent from the above that chromosomal fibres behave in ways reminiscent of DNA molecules, that is, by showing semi-conservative replication, and being destroyed by DNAase and for this reason it seems probable that in fact there is only one DNA molecule per chromosomal fibre. This view has been challenged from the standpoint that the dimensions of the fibre are such that there are probably two DNA double helixes per strand (Stubblefield 1973). However, estimations of the effects of coiling on the dimensions of the DNA molecules are compatible with there being only one DNA molecule per fibre.

Cairns (1966) has found direct evidence that DNA

molecules in eukaryotic chromosomes are of considerable lengths and Sasaki and Norman (1966) have found DNA molecules of lengths up to 2cm. This would be sufficient to account for the DNA of small human chromosomes, thus supporting the view that each chromosome contains only one chromosonal fibre, which in turn contains only one molecule of DNA.

With dispersed chromatin preparations chromosomal fibres thinner than the 25-30nm diameter fibre may be observed in both interphase and metaphase material (Ris 1967). The diameter of these narrow fibres is of the order of 5-10nm (Pardon et al. 1967, Du Praw and Bahr 1969). The two thicknesses may alternate along the length of one fibre. The broad fibre seems to unwind into the narrow fibre, which may then wind up into the broad structure again. Thus it is easy to imagine the broad fibre as being a super-coiled version of the narrow fibre (Lampert 1971). Treatment of the fibres with trypsin appears to expose thin patches of very thin filaments (2.5-5nm diameter) which is of the order of size of the DNA double helix (Ris 1967, Abuelo and Moore 1969). The fibres may be completely degraded with trypsin to produce a resistant core with a diameter of 2.3nm (Du Praw 1965) which is only fragmented by treatment with DNAase. The average diameter of the DNA double helix has been estimated on theoretical grounds as around 2.5nm by Fuller (1967).

Taking into account the diameters of these various fibres it has been suggested that each broad fibre (25-30nm) contains 2 associated narrow (5-10nm) fibres (Bostock and Sumner 1978) and/or that each narrow fibre contains two DNA double helices. However, when the lengths of the various units are taken into account further evidence is found for there being only single smaller sized fibres in the larger sized ones.

Du Praw and Bahr (1969) have found fibres (25-30nm size) with lengths of 135nm and 723 nm respectively from the smallest and largest chromosomes. The estimated lengths of the DNA molecules of these chromosomes (assuming one molecule

per chromosome) are 1.4cm and 7.3cm, respectively (Lewin 1974). Thus, it appears that the DNA contained within a fibre is packed in such a way as to decrease its length by a factor of approximately 100. The narrow fibres have a dry mass/micrometre ratio which is approximately 10-20% of that of the broad fibres. This implies that the narrow fibre is formed by a ten-fold packing of the DNA molecules (probably by means of interactions between the associated histone molecules (Hamkalo et al. 1973), that is, by supercoiling of the coiled DNA molecule, and that the broad fibre is formed by supercoiling of the supercoil, also causing a 10-fold decrease in the length of the fibre. The narrow fibre has a higher density, suggesting a greater proportion of DNA, than the broad fibre (Lewin 1974). Thus additional protein molecules may be involved in producing and maintaining the higher levels of coiling.

Du Praw (1970,1973) has calculated the packing ratios of DNA in chromosome fibres by means of dry mass estimations and obtained results which confirm the above suggestions, and which have shown considerable constancy over different tissues.

As has been stated earlier, there is no real evidence for any kind of core or backbone in the structure of the metaphase eukaryotic chromosome. The only component of the chromosome is a complex of histone and DNA coiled and supercoiled at several levels, the higher levels being maintained by interactions between histone molecules, and also other non-histone proteins. The simplest model for the structure of the chromosome taking account of these facts is the folded fibre model of Du Praw (1966). This model is incompatable in its initial form with recent developments in chromosome banding, but with modifications still seems to be the most satisfactory.

Du Praw (1966) originally suggested that the chromosomal fibres were tightly folded in an entirely haphazard manner within the chromsome. The centromeres were interpreted as constituting unreplicated portions of the fibre holding the two chromatids together (Du Praw 1970). Folding in a longitudinal as well as a transverse direction was proposed, the former type explaining the phenomenon of 'isolabelling' the apparently simultaneous replication of two chromatid arms (or parts thereof) during autoradiography, and the latter providing a means by which sister chromatid exchanges may be explained.

The constancy of chromosome banding patterns, both between homologues, and within the same chromosome in different cell types, or within different individuals, implies that the folding and packing of the chromosomal fibre in the chromosome must in fact be very consistent and controlled for each chromosome, and section of chromosome. The simplest way to resolve this would be to propose super-coiling of the already many times supercoiled fibre, but variations must be included in order to account for isolabelling. (Does this only occur near the telomeres, and if so is it here only that the fibre is folded back on itself?) Evidence for this proposal comes from the fact that huge coils can be induced in metaphase chromosomes by certain treatments.

However, as already mentioned, the composition of the metaphase chromosome is not constant from mitosis to mitosis and the dry mass shows considerable variation. Therefore the method of packing the fibre into the chromosome, and the molecules present while this is occurring, are not constant features of the chromosomes, though they do appear to be common to all the chromosomes in the cell in any given metaphase. As the banding patterns appear to be constant in different metaphases, it would appear that the differences in stucture which may occur are perhaps differences in the degree of packing, or tightness of packing of the fibre, rather than of the overall arrangement of the fibre within the chromosome.

4. Interphase Chromatin.

Both narrow and broad types of chromosomal fibre may be found in interphase preparations, though the diameter of the latter is smaller than that found in metaphase preparations (Du Praw 1966). This suggests that the basic association between DNA and histones, and the configurations of this complex, remains unchanged during the cell cycle, but that probably the 'tightness' of the packing of the DNA within the narrow fibre, and of the narrow fibre within the broad, changes. Certainly, the other components (for example, the non-histone proteins) show both qualitative and quantitative differences at different stages of the cell cycle. The protein content of interphase chromatin is only about half that of metaphase, and non-histone proteins seem to be responsible for the difference (Lewin 1974).

5. Heterogeneity of the DNA Base Sequences.

Eukaryotic organisms contain within their cells far more DNA than would be required for the provision of the estimated 3×10^4 (Ohta and Kimura 1971) genes thought to be necessary for the control of their metabolic functions. Generally, the more complex the organisms, the higher is its c-value (DNA content) (Yunis et al. 1977a), but this relationship is not simple. Large differences in c-values exist amongst some quite closely related organisms, and the lung-fish has 20-40 times as much DNA per cell as Man (Pederson 1971). Amongst the mammals the DNA varies little, however, and most species have a value similar to that of Man (Fredga 1977). DNA content correlates inversely with specialisation in many different groups of organisms. Those members of the group with low c-values tend to be the more specialised, those with a higher DNA content usually being the more primitive and slowly evolving members of the group (Hinegardner 1976).

DNA base sequences exhibit heterogeneity with respect to several properties, for example, base composition, degree

of repetition of particular sequences and buoyant densities, (when centrifuged in a caesium chloride gradient). These properties have influence on each other. (Comings 1972). For example, both the composition of a particular sequence and the number of times it is replicated obviously have an effect on its molecular weight. This latter quantity is indicated by the buoyant density of the DNA. Centrifugation of DNA in a caesium chloride gradient results in the formation of a band of DNA (the main band) containing most of the DNA around a peak of buoyant density, smaller peaks of various densities occur either heavier or lighter than the main band density (depending upon the base content of the DNA in them). These separate bands have been named 'satellite' DNA. These satellites consist of short sequences of DNA repeated many times (Waring and Britten 1969), thus leading to the formation of blocks of DNA with relative base ratios different from those of the non-repeated sequences.

Only a portion of the DNA, then, occurs in sequences of which there is only one copy (the condition which might be expected if all the DNA present were to code for the amino acid sequences of proteins). In mammals unique sequences of DNA comprise about 60-70% of the genome, the rest comprising repeated sequences (Yunis et al. 1977). This latter fraction may be subdivided according to the degree of repetition of the sequence into:

1. Satellite DNA, which contains short sequences of DNA repeated many times, often having a buoyant density different from that of the rest of the genome. In Man, four types of satellite comprise about 0.5 to 2.0% of the genome each (Jones 1973), a total of 6% of the genome (Ginelli and Corneo 1976).
2. Moderately repeated sequences. As the term suggests these sequences are repeated to a lesser degree than the satellite sequences. The sequences involved tend to be longer. This type of DNA comprises about 30% of the genome (Ginelli and Corneo 1976).
3. Inverted repeated sequences, hairpin or foldback DNA. This type of sequence comprises about 3-6% of the genome

in Man (Yunis et al. 1977).

All these types of sequences seem to be distributed throughout most of the genome. A major portion of the human genome (at least 52%) consists of short unique sequences interspersed with shorter repeated sequences, and a second portion consists of longer unique sequences interspersed with repeated sequences. Together these two patterns of distribution account for 80% at least of the genome (Schmid and Deininger 1975). The inverted sequences are widely distributed throughout the genome (Yunis et al. 1977). The satellite DNAs tend to be clustered in blocks mainly within the pericentromeric heterochromatin (Ginelli and Corneo 1976), but also in the long arm of the Y chromosome and also in the secondary constriction regions of chromosomes 1, 9 and 16 (that is all areas which consist of constitutive heterochromatin).

Four major satellite DNAs have so far been identified in the human genome (Corneo et al. 1973). These have been numbered I, II, III and IV and comprise 0.5%, 2.0%, 1.5% and 2.0% of the genome respectively. Highly repetitive DNA may be found in main band DNA also (Corneo 1976). This author states that there are two types of rapidly renaturing DNA (suggesting a highly repetitive nature) in main band DNA. The first is clustered in the genome, the second being more finely distributed. It is thought that satellite DNA comprises less than half the total highly repetitive DNA present (Schwarzacher 1976).

The various satellite DNAs have been located in the human and other genomes by hybridising radioactive RNA prepared from purified satellite DNA in vitro with denatured DNA in situ (Pardue and Gall 1970, Jones 1970). There are apparently no exclusive locations for any known satellite, and all the C-band regions and Y heterochromatin seem to be composed of more than one satellite (Jones 1977). However, the concentration of a particular sequence may predominate in a given location.

Satellite I — is concentrated on the Y chromosomes, with minor amounts on several other chromosomes, particularly chromosome 3 (Jones et al. 1974)

Satellite II — is concentrated on chromosomes 1 and 16, with lesser though still major concentrations on chromosome 9 (Jones and Purdom 1975).

Satellite III — is concentrated on chromosome 9, and is also present on the A, B and D group chromosomes (Jones et al. 1973a).

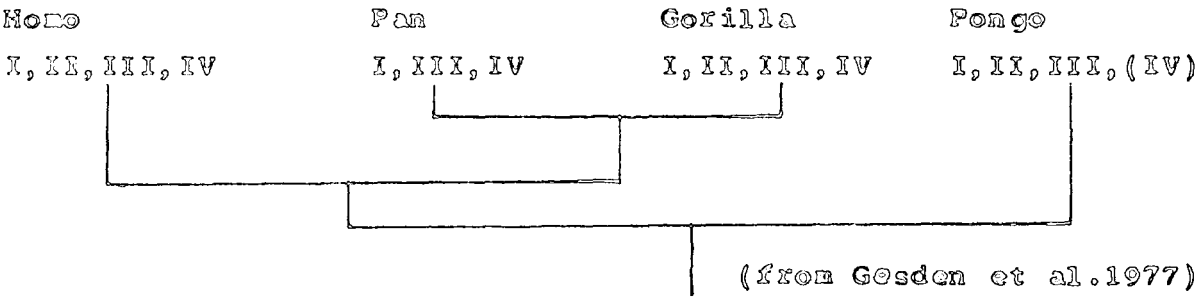
Satellite IV — has a major location on the Y chromosome and has a minor location on chromosome 1 (Evans et al. 1974).

Human Satellite DNAs in the Higher Primates.

RNAs transcribed from all four human satellites may be hybridised with the genomes of three species of higher primates, namely, Pan troglodytes, Gorilla gorilla, and Pongo pygmaeus. Most sites of hybridisation are located on homologous chromosomes in all four species (Gosden et al. 1977), but there are several disparities. For example, certain sites have different satellites in different species. These authors have suggested that the fundamental sequences of all four human satellites were present in the genome of the common ancestor of man and the higher primates, on several, or all chromosomes. During evolution the different sequences would have been amplified to varying extents in the different subgroups or species. Jones (1976) has suggested that the production of new DNA by means of repeated replication of existing sequences would only occur or be tolerated during phases of speciation, so as to be included in all the members of a new species. At first sight this suggestion appears to fit well the observed distribution of different satellite DNAs among the group of related species which form the higher primates, but it is difficult to form a conception of a phase of speciation which is sufficiently well-defined to ensure that all members would from the beginning have this new component in their genomes.

When the efficiency of hybridisation of the four satellites in the four species is examined it is found that some show greater divergence of base sequence than others - implying that those with greater sequence divergence arose earlier than those with the more similar sequences. The time of origin of the satellites has been estimated by measuring the similarity of sequences in the different species.

The distribution of the four human satellite DNAs amongst the higher primates.



(from Gosden et al.1977)

It is possible that the rate of substitution is different, probably faster, in this type of DNA than in unique-copy DNA, because of the operation of different selective forces. Assuming a rate of three to four times faster than for unique-copy DNA, Jones et al. (1973b), has suggested that the maximum value of the age of human satellite III is 25 to 30 m.years (thus arising some time in the Oligocene). Satellites I and II have been estimated to be 20 to 25m and 9 to 12m years old respectively (Early Miocene and Pliocene).

It has been suggested that Homo sapiens and the Pan genus diverged some 30m years ago (by considering the divergence of the amino acid sequence of certain proteins), and this would imply that satellite III originated before this divergence, but that satellites I and II were formed afterwards. However, this would require that these latter two satellites had separate origins in the by now diverged lineages, and this would presumably confound the estimates

of the time of divergence, or of the age of the satellites.

As the hominids and pongids diverged from the cercopithecoids an estimated 36m years ago, it would be unlikely for a satellite resembling human satellite III to be found in these latter animals. There are indications that this is the case. Satellite III is not found in the gibbon (Jones and Purdon 1975) which, it is suggested, diverged about 30 to 40m years ago.

Jones (1976) suggests that evolutionary alterations in satellite DNAs may be relics of important speciation events (see above), and that therefore the different estimated ages of the two satellites I and II are indications that two such events occurred in the hominid lineage after its divergence from the pongid lineage, both involving the emergence of populations from small isolates.

This situation of homology of satellites between different species seems unusual. In the majority of species examined, any satellite DNAs which are present are usually exclusive to the species concerned, though there may be some degree of relatedness of satellites among related species (Jones and Purdon 1975). Among the rodents, differences between species involve both amounts and composition of satellites, but between varieties or sub-species only differences in amount occur (Dover 1977).

The usual lack of homology of satellite DNAs between related species has been suggested by Walker (1971) to be due to a lower efficiency of sequences which have undergone a certain degree of base substitution in performing whatever function satellite DNA has. Therefore one would expect there to be selection in favour of a rapid turnover of satellite DNA (that is a loss of inefficient sequences and the production of new ones) whenever possible.

Possible functions of the different classes of DNA base sequences.

Obviously a major part of the unique copy DNA exists to carry the genetic information for the many proteins and enzymes involved in the metabolic processes of the body. However, as stated earlier, it seems that there is an excess even of this type of DNA over and above the estimated amount needed. There is recent evidence that the bases coding for any given protein do not occur as a single continuous sequence within the genome, but that sections of the base sequence are separated by other sequences which, though they may be transcribed, do not code for the amino acids in the protein (Gilbert 1978). Some of these interspersing sequences may be of the repetitive type, but some may also be of the unique-copy type, thus accounting to some extent for the excess of this latter type of DNA base sequence in eukaryotic genomes above the structural gene requirements.

Interspersed repetitive sequences:

At least some structural genes occur in eukaryotic genomes in multiple copies, for example the rRNA genes (which may comprise up to 2% of the genome (Yunis et al. 1977)), tRNA genes and genes coding for the various histone proteins (Jones and Purdom 1975). It is probable that other repeated structural genes also occur (Hood et al. 1975). However, the characteristics of repetitive DNA indicate that such repetition of structural genes could not possibly account for the bulk of the repetitive DNA which commonly occurs (Bostock and Sumner 1978).

Ginelli and Corneo (1976) recognised two types of repetitive DNA, fast and slow reassociation types. The fast, they suggest, is probably not, or is very poorly transcribed *in vivo*, whereas the more finely interspersed slow repetitive DNA is transcribed to a great extent.

This corroborates other evidence that initial transcription of the DNA leads to the production of heterogeneous nuclear RNA, which does include interspersed repetitive and non-repetitive sequences in the same molecule (Galau et al. 1976). The repetitive sequences are excised and do not occur in the mature mRNA used in protein synthesis. Thus it seems likely that there are two different types of moderately repetitive DNA in the genome, and that the two types have separate and distinct functions.

Davidson et al. (1975) have shown that some structural genes in the sea urchin which are active in embryogenesis are located very close to interspersed repetitive sequences and suggest that these latter sequences are in some way concerned with the regulation of the activity of the structural genes. However, as only a proportion (estimated at 10%) of unique sequences carry structural information, it seems reasonable that only an equivalent amount of repetitive DNA would be situated sufficiently close to be involved in such regulation (Galau et al. 1976).

Several hypotheses have been suggested to explain the mechanism of eukaryotic gene regulation, and several of these require patterns of unique-copy and repetitive DNA distributions which are commonly found in eukaryotic genomes (Britten and Davidson 1969, Georgiev 1969). But, there are discrepancies. For instance, some (about 20%) of the unique-copy DNA is not interspersed with repetitive sequences, and one would not expect such huge differences as are found in the c-values of related organisms if all the DNA was concerned with either coding for proteins, or regulating the expression of those that were (Bostock and Sumner 1978).

It has been suggested that moderately repeated sequences of DNA merely represent very old and consequently extremely diverged satellite or satellite-like DNA sequences and have no special function (Bostock and Sumner 1978).

Parts of the genome which are not actively transcribed *in vivo*, which includes unique-copy as well as moderately repetitive sequences (Yunis 1973), may be involved in the maintenance of the organisation of chromosomes and may influence chromosome evolution and behaviour rather than be involved in fine genetic regulation (Yunis et al. 1977).

Satellite DNA:

The hypotheses suggested most often concerning the possible function(s) of satellite DNA is that this material is in some way involved with aspects of chromosome behaviour, for example, in the pairing and recombination of homologous chromosomes at meiosis.

Walker (1971) has suggested that the types and amounts of satellite DNA at the centromere of a chromosome influence the likelihood of that chromosome entering the egg cell rather than one of the polar bodies during oogenesis, and he has said that there is evidence for such a mechanisms in maize. Evidence has long been available to show that in Drosophila the amount and kind of heterochromatin present at the centromere could affect the firmness by which the chromosome is attached to the spindle fibres - thus influencing the occurrence of non-disjunction of the chromosomes (Lindsley and Novitski 1958).

If it were the case that accumulated base sequences in 'old' satellite sequences reduced the efficiency of the DNA in performing any of these functions, there would be selection pressure for the loss of old sequences and the production of new sequences, leading to the situation that is observed, namely that each species has its own particular satellite sequences. (Though it could be argued that this could just as easily, or more easily, lead to selection for the conservation of old satellite sequences, just as histone amino acid sequences have been conserved unchanged for very many years. Walker (1971) recognises that the situation

could arise where chromosomes were favoured by selection by virtue of their satellite sequences, whilst carrying genes which would otherwise be selected against.

It has also been suggested that satellite DNA may influence the occurrence of crossing over, and lead to the conservation particularly of genes lying close to it (Yunis et al. 1977). The presence of these sequences at the centromere may predispose those chromosomes to fusion (Yunis and Yasmineh 1971).

Yamomato and Miklos (1978) have concluded that the most important functional attribute of satellite DNA is the effect of its variance in amount of meiotic recombination.

Gosden et al. (1977) point out that satellites are not distributed evenly throughout the karyotype of a given organism. Particularly in the human species, several chromosomes exist which do not have a major concentration of any of the identified satellite sequences. Therefore, it is unlikely that the satellite DNA is involved in any functions which necessitate the presence of substantial amounts on each chromosome. However, the very fact that these sequences have been conserved to the extent that they have during the period of primate evolution suggests that there is some selective advantage attached to their presence - that they have some influence on chromosomes and their behaviour which is not negligible. However, there might be differences in satellites not detectable with the methods used, hybridisation might not be a sufficiently sensitive technique, and how much base substitution would be expected during this time anyway?.

It has been suggested that satellites arise during phases of speciation (Jones 1976); Gosden et al. (1977) suggest that it might be during these periods also that satellites have their major influence.

The Relationship between the Different Classes of DNA Base Sequences and the Differential Banding Patterns of Chromosomes.

The locations of satellite DNA sequences in the human karyotype are regions which generally have a uniform reaction to the stain used during the production of banding patterns, but this uniform reaction is not necessarily the same for all regions. For example, the secondary constriction of chromosome 9 is generally pale and unbanded with both Q- and G- banding, but the long arm of the Y chromosome has a very positive reaction with Q-staining and is banded with G-banding. All these regions of satellite DNA stain darkly with C-banding.

The most significant finding about the relationship of the moderately repeated and unique sequences to both Q- and G- banding is that the bulk of the former type of sequence is localised in the positive bands (Sanchez and Yunis 1974, Schmedl 1978). This distribution offers an explanation for the observation that human trisomics of chromosomes having a higher proportion of positive bands are associated with a greater compatability with live-birth and lower disturbance of the phenotype than trisomics of chromosomes with higher proportions of negative bands (Ganner and Evans 1971, Hoehn 1975). For instance, chromosomes 13 and 18 can be seen to have larger areas of positive bands than other chromosomes of similar size and shape, and can be shown to have disproportionately large amounts of moderately repeated sequences (Yunis et al. 1977). These authors also have direct evidence from the hybridisation of tritiated DNA complementary to mRNA that negative G-bands have higher concentrations of DNA complementary to mRNA than G-positive bands. This implies that they have lower amounts of unique structural gene sequences and explains the lower severity of their effect in the trisomic condition.

That negative bands are transcriptionally active is indicated by studies of Homogeneously Staining Regions (HSRs)

which occur in certain chromosomes of cultured cell lines (including those derived from malignant human neuroblastoma cells). These cell lines apparently have an excessive production of one or more proteins which are specific to the malignant neuronal cells (Biedler and Spengler 1976). It is suggested that the HSRs represent areas where there has been amplification of the structural DNA, which in turn leads to the overproduction of the proteins concerned. These uniformly staining regions stain negatively (Evans 1977).

MOLECULAR MECHANISMS OF CHROMOSOME BANDING.

Mode of Action of the Dye.

Although the subject of this thesis is those chromosome variants seen under the action of the fluorescent dye, quinacrine dihydrochloride, information regarding the molecular causes of the banded appearance will be drawn from studies concerned with the dye Giemsa also, as the near-identical pattern produced by staining with this dye after various pretreatments must be indicative of some basic feature of the chromosome organisation which is common to both reactions.

Both these stains appear to attach themselves to the DNA by means of two separate binding sites. In the case of Giemsa this occurs after the formation *in situ* of a new compound composed of two molecules of methylene blue and one molecule of eosin. Therefore, both dyes are sensitive to conformational changes in the DNA, and they can only bind where the required attachment sites are the correct distance apart(Summer and Evans 1973). In apparent contradiction to this, Comings (1975) says that methylene blue, or any of the azure components of Giemsa, alone gives good banding, that thianin, with no methyl group, gives poor banding, and the eosin does not bind to DNA and is not essential for the production of chromosome bands. It is not clear to the present author whether this means that two binding sites are not required, or whether it is simply the case that compounds in the Giemsa bind directly at two sites.

It is probable, if two sites are required, that the dye attaches to the DNA by fixing to two longitudinally adjacent binding sites and that therefore, factors which influence the formation of a banding pattern will be those factors which affect the conformation and accessibility of the DNA molecule within the chromosome. In the case of quinacrine, it appears that once the dye is bound to the DNA it is further subject to enhancement or quenching of its fluorescence under ultra violet light.

The binding and subsequent fluorescence of the dyes appear to be influenced by many aspects of chromosomal organisation and composition. Interactions between all these at various levels seem to be of major importance, but for the sake of simplicity and clarity, individual influences will be dealt with in turn.

DNA concentration.

Differences in the concentration of DNA along the length of the chromosome do occur but they are notsufficient to account for the distinct banding patterns (Caspersson and Zech 1972). However, the slight variations that do occur seem to follow the pattern of Q-banding (Latt 1976) which suggests that the pattern of fluorescence might result from the sensitivity of the dye to factors which amplify, but nevertheless reflect, fluctuations in DNA concentration. The major factor determining the observed pattern may vary in different regions of the chromosomes, or under different conditions.

It has been suggested that the pretreatments of G-banding procedures result in the selective loss of DNA from the negative bands (Ahnström and Natarayan 1973), but this mechanism could not account for Q-banding, which requires no pretreatment of the chromosome preparation before staining. Other authors have found that DNA is not lost from the chromosome during G-banding pretreatments, but only during C-banding procedures (Pathak and Arrighi 1973, Schiamdy et al. 1975).

There is no information about the amounts of dye bound along the length of the chromosome, but Hatfield et al. (1975) have presented evidence that the increased fluorescence of the Y chromosome is not due to an increased binding of quinacrine to this chromosome compared with other chromosomes. Sumner (1977a) says that quinacrine is bound

uniformly along the chromosome, because the banding pattern is suppressed by mounting of the preparations in organic mounting media, which would alter only the non-uniform distribution of chemical groups such as proteins.

Heterochromatic properties of the Positive Bands.

As described above, moderately repeated DNA base sequences have been localised to positive Q-bands. Regions containing predominantly such sequences may have several physical and chemical characteristics which differ from those of regions containing predominantly unique base sequences, and which may be responsible for the production of a banded appearance with certain staining techniques.

Positive Q-bands show several properties of heterochromatin which may be responsible for the banded appearance. For instance, they contain DNA which tends to be replicated late in the replication cycle (Calderon and Schnedl 1973, Zacharov et al. 1974, Grzeschuck et al. 1975), and there is indirect evidence that they are inactive and relatively condensed during interphase (Bostock and Sumner 1978). Dutrillaux et al. (1976) using BrdU incorporation methods, found that the most intense Q-bands replicated latest in the replication cycle, and the widest R-bands (equivalent to Q negative bands) replicated earliest. Okada and Comings (1974) have found that G-bands are similar in position to meiotic chromomeres, and suggest that both represent regions of intercalary heterochromatin which are more condensed in both meiosis and mitosis than the rest of the chromatin.

It is often suggested that the preferential uptake of stain (in the case of G-bands) and the enhanced fluorescence of the bound stain (in the case of Q-bands) is directly due to one or more consequences of the heterochromatic nature of the regions containing moderately repeated DNA base sequences:

(i) DNA base composition.

Opinions differ as to whether quinacrine shows differences in the capacity with which it binds to the various bases in the DNA molecule (Caspersson (1970b) O'Brien et al. (1966), but there do seem to be differences in the degree to which the fluorescence of the dye is affected by these bases. The pattern of differential staining observed does not altogether agree with the expected differences in dye binding along the chromosome, if the first of these factors was important. DNA having high concentrations of adenine-thymine (AT) base pairs seems to have an enhanced fluorescence of quinacrine, compared with the quenching effect produced by high concentration of guanine-cytosine (GC) base pairs. (Pachmann and Rigler 1972, Weisblun and de Haseth 1972). However, these observations were made using fairly dilute DNA in solution, and therefore may not reflect the behaviour of the dye when bound to fixed chromosome preparations (Bostock and Sumner 1978). Latt (1977) expresses the opinion, though, that at the higher dye/DNA saturations which might exist in cytological chromosome preparations stained with fairly high concentrations of quinacrine, the formation of 'energy sinks' adjacent to GC base pairs could exaggerate the quenching effect. Jalal et al. (1974) have found a general correlation in C-bands between GC-rich satellites and dull fluorescence, and AT-rich satellites and bright fluorescence in three mammalian species. Comings and Drets (1976) conclude that the variation in base content along the chromosome is sufficient to account for most Q-banding, with the exception only of the extremes of quinacrine fluorescence, and that an 11.42% difference in GC content in the DNA leads to a 50% alteration in the amount of fluorescence.

The centromeric regions of chromosomes of Mus musculus contain AT-rich DNA but exhibit dull fluorescence with quinacrine. This has been explained by suggesting that

regularly interspersed GC pairs are sufficient to quench the fluorescence despite the presence of the AT pairs (Latt 1976); this implies that it is not just the types of bases present which is important but also their spacing in the sequence, and suggests that the repetition of sequences is important, as it would lead to some degree of regularity of spacing of the bases. (Weisblum and de Haseth 1971).

When fluorescent antibodies to adenine are bound to the chromosome a pattern very like Q-banding results, giving further evidence that Q-positive bands are AT-rich (Hsu 1973), but this technique has been criticised on the grounds that the results of immunofluorescence depend upon the method used to denature the chromosomes, and may reflect the accessibility of the bases rather than their distribution (Bostock and Sumner 1978). It has also been shown that although Q-positive regions tend to be late replicating, they do not have a different AT content from early replicating regions, as the latter also can be pulse-labelled with tritiated thymidine, though early in the replication cycle (Grzeschuck et al. 1975). Also, the same pattern of late-labelling regions can be obtained using tritiated cytidine (Schnedl 1973). In conclusion, it seems that although the two kinds of base pairs present in the DNA do have an effect on the fluorescence of quinacrine, their spacing in the molecule is probably a more important factor than their abundance in determining the eventual pattern of fluorescence of the chromosome.

(ii) Degree of condensation of the chromatin.

It has been suggested that tandemly repeated DNA base sequences might form more condensed, regular stable structures because of interactions with specific proteins (Bostock and Sumner 1978). Zakharov et al. (1974) postulate that the chemical and structural events leading to differential condensation of these regions in the interphase nucleus are

also responsible for the differential banding pattern produced in the metaphase chromosomes. Takayama (1976) says that G-bands are produced by methods which induce compaction of the chromosomal material in the positive bands. In some interphase nuclei, the inactive X chromosome which forms a densely staining Barr body may show very intense quinacrine fluorescence. As it has the same base composition as the active X chromosome, this indicates that it is the state in which the DNA is compacted by proteins that is important in the production of the intense fluorescence (Evans 1977). Seabright et al. (1975) show that bands constantly change in relative size during mitosis suggesting that a degree of condensation has an effect on reaction to stain.

(iii) Base of denaturation and reannealment of the DNA double helix.

Early hypotheses regarding the mechanism of differential staining of chromosomes postulated that the pretreatments required for producing banding patterns were those which caused denaturation of the DNA double helix, and were followed by the selective renaturation of certain regions (Drets and Shaw (1971) Schnedl (1971) Sumner et al. (1971) MacKay (1973)). Bobrow and Madan (1973) maintain that it is just such a mechanism which lies behind the differential staining behaviour of the acridine orange techniques.

While a double-stranded condition of the positive bands and a single-stranded condition of the negative bands no longer seems to exist during all staining procedures (Comings 1972), there is still the suggestion that positive bands are those regions which somehow denature and reanneal more easily than others (Comings et al. 1973). This property seems to have more importance in the staining of the large centromeric blocks of constitutive heterochromatin during C-banding (Sumner et al. 1971, de la Chapelle et al 1973).

(iv) Protein disulphide bridges.

Utakoji (1973) has induced G-bands by using cupric sulphite in the pretreatment to staining. One action of this chemical is to break the disulphide bonds of protein molecules, thus indicating that the existence of the bonds may be relevant to the production of the chromosome bands. These bonds have been shown to be more concentrated in condensed chromatin in both interphase and metaphase and thus by implication in the positive G-bands (Sumner 1973). In the pale negative bands sulphydryl bonds predominate. The existence of this kind of bonding between protein molecules is thought to influence the binding of the Giemsa dye molecules, but would not explain the phenomenon of quinacrine banding.

Histones.

The degree and pattern of the loss of histones from the chromosomes during fixation techniques seems to be considerably variable. Bustin et al. (1976) have shown that several histones are removed after only 5 seconds fixation with a methanol-acetic acid mixture, and that the type (Histone I) most likely to be involved in the production of chromosome bands is the one most readily removed (Comings 1978). Incubation for 4 hours in hydrochloric acid to remove histones does not seem to affect the production of G and C bands (Comings and Avelino 1974).

Non-histone proteins.

The idea of proteins being involved in the production of chromosome bands seems irresistible; many of the conditions of the pretreatments to G-banding are conditions to which proteins are sensitive, and differentially responsive, for example, temperature, pH, treatment with mild proteolyctic agents such as trypsin. Comings (1971) concluded that as the bands did not reflect differences in the distributions along the chromosomes of base composition,

highly repetitive sequences or histones, then by elimination non-histone proteins must be important. Bram (1971), suggested that any differences in the secondary structure of AT-rich DNA as compared with GC-rich DNA might well affect the composition of the overlying proteins. Also, there is now evidence that the proteolytic activity of trypsin is important for the production of chromosome bands (Korf et al. 1976), despite earlier reports to the contrary (Sehested 1973). Kato and Moriwaki (1972) suggest that solubilization or extraction of some chromosomal proteins, probably acidic, is the primary cause of the appearance of bands. However, as in the case of histone proteins, removal of non-histones during fixation procedures seems to be variable. Also, more importantly, there is no information available about the distributions of these proteins along the chromosomes, and specifically, whether or not they occur in the clusters which would be expected if they were responsible for the appearance of banding patterns. Vogel et al. (1974) say that there does exist a base-specific attachment of qualitatively different non-histone proteins to the DNA, but this varies between tissues, which the banding patterns do not.

RNA

Although RNA does exist as a component of the metaphase chromosomes, there is no evidence that it is involved in the production of chromosome bands (Sumner and Evans 1973).

Culturing procedures.

The use of culturing media of different chemical compositions has not been shown to affect the production of chromosome bands (Lubs et al. 1973). It seems to be important to use potassium chloride in the hypotonic solution (see below, page 113) and distilled water alone is not effective (Crossen 1972). Brooke et al. (1962) reported long ago the use of KCl to uncoil and diassociate chromosomes.

Fixation procedures.

Banding patterns and particularly the fluorescence intensity variants, are phenomena observed in chromosomes which have been fixed in some mixture containing acetic acid (Bostock and Sumner 1978), but there are indications that some underlying differentiation along the chromosome arms can be seen in unfixed chromosomes (McKay 1973). The effect of fixation procedures on the distribution and condition of many of the chromosome components is imprecise, and yet the banding patterns often show great clarity. Such findings seem to point to the importance of factors not disturbed greatly by fixation procedures, for instance the DNA, as being important in the production of banding patterns.

Conclusion.

The precise differences which give rise to the appearance of chromosome bands after various staining techniques are likely to occur above the level of the narrow-type (see above, p. 38) of chromosomal fibre, as this fibre seems to occur in both euchromatin and heterochromatin, and such factors as the degree of repetition of the bases in any given DNA sequence do not seem to have a distinguishable affect on the morphology of this fibre.

Several lines of evidence indicate that the degree of repetition of the base sequence of the DNA affects either the uptake of the stain or the degree of enhancement or quenching of the fluorescence, in the case of quinacrine. When the staining procedures are considered, however, it appears that generally the pretreatments which lead to the production of G-bands are those which probably influence the condition of the chromosomal proteins and their association with DNA.

This does not imply necessarily that banding patterns are artefactual, and only observable after such pretreatments. Phase contrast microscopy (Wang and Federoff, 1972, Dutrillaux and Lejeune 1975) and electron microscopy (Sanchez and Yunis 1973, Goradia and Davis 1977) of unfixed chromosomes reveal an underlying differentiation along the chromosomes which corresponds to the stained banding patterns. This shows that the banding patterns reflect normal morphological differences along the chromosome which occur at a fairly crude level. Quinacrine stains with a banded appearance on fixed chromosomes with no pretreatment. Thus it would appear that the pretreatments accentuate an underlying phenomenon which is already present, and allow the Giemsa to reveal this. It would appear that proteins and their interactions with DNA are important for these processes.

The necessary variation in protein composition, association and interactions must be intimately related to aspects of DNA composition. The morphology of the chromosome fibres seems to depend upon interactions between both types of molecules; thus one may conclude that probably the resultant banded appearance is due to interaction between the dye molecules and specific aspects of the chromosome, and chromosome fibre composition. These interactions may be different for each staining technique used, but as all such aspects of the chromosome are intimately related to each other, identical, or near-identical patterns would be expected from any treatment and dye combination which reflected some of the interlinked aspects of chromosome morphology, such as;

(i) Base composition and repetition;
(ii) Protein association with the DNA;
(iii) Degree of condensation of the chromosome fibre.

Banding patterns can reflect differences in the first of these without necessarily being a direct chemical consequence of it.

Breg et al. (1972) reported that the bright satellites of one chromosome 14 were labelled more heavily with tritiated thymidine after replication than the less bright satellites of the homologue; this indicated a higher AT content of the brightly fluorescing material in comparison with the dull.

Pearson et al. (1973) have shown that intensely fluorescent material on the acrocentric chromosomes is always C-band positive (indicating that it is rich in highly repetitive DNA), but the less bright (normal or negative) material may be either positive or negative with C-band staining. This demonstrates that the chromatin of these polymorphic regions is not always rich in highly repetitive sequences, and that even when it is, these repetitive sequences may show qualitative differences (to which the Q-staining but not the C-staining method is sensitive).

Different chromosomes in the karyotype, whilst having highly repetitive satellite sequences in the centromeric regions, have different satellites as their major component (see above). It is possible that this sort of difference exists between homologous regions of chromosomes which show different reactions to quinacrine staining.

Schnedl (1978) has said that the DNA in the short arms of the acrocentric chromosomes (on the centromeric side of the stalk or NOR) is GC-rich, whilst that in the satellites is AT-rich. As both regions are polymorphic with quinacrine staining it is difficult to know what to make of this.

There seems to be several unanswered questions concerning the molecular compositions of negatively, intensely, and brilliantly fluorescent variants:

1. Does all chromatin showing a similar response to the

stain have a similar composition?

2. It is usually felt that the various categories to which polymorphic regions are assigned represent arbitrary divisions in a continuous range of intensity of fluorescence. If this is the case, do the regions vary in the kinds of base present, or in the degree of repetition of the bases?

3. Do the differences in intensity of fluorescence exhibited by different non-polymorphic regions of the chromosomes reflect the same types of differences that exist between different variants of the same region?

Chapter 3: THE EXTENT OF CHROMOSOME VARIABILITY IN HUMAN POPULATIONS.

Variations of the normal, or most usual human chromosome complement fall into two broad categories:

(i) major abnormalities, including numerical abnormalities and structural rearrangements and disturbances of various kinds;

(ii) minor variants, including Q-, C- and RFA polymorphisms and several size variations seen with uniform conventional staining.

The frequencies and distributions of variations of both categories amongst particular sections of the population have been the subject of numerous reports. Samples comprising unselected consecutive newborn infants probably give the best estimate of the frequency of the variations within the human population, as those variations which have a damaging effect on the development and consequent phenotype of the carrier, even if they do not lead to early death, often lead to physical separation of such individuals from the general population. Thus samples of the general adult population will exclude many individuals carrying chromosome variants.

Other groups which have been sampled quite frequently consist of mentally subnormal and behaviourally 'abnormal' individuals (including prison populations), infants dying during the perinatal period and persons suffering reproductive failure for a variety of reasons. Several series of spontaneous abortions have also been examined. None of these latter groups gives an accurate estimate of the frequency or the distribution of chromosome variations in any given population, but they do provide information concerning the possible phenotypic effects of the different variants.

The term 'major abnormality' with respect to the human chromosome complement refers to any serious disturbance

of the chromosomal material, especially those affecting the structural genetic component of the chromosomes. Such disturbances include:

(i) Numerical abnormalities, for example, X-monosomy, trisomies of the autosomes and of the sex chromosomes, triploids and tetraploids, and the presence of supernumary or 'marker' chromosomes.

(ii) Structural rearrangements, for example, reciprocal and Robertsonian translocations, paracentric and pericentric inversions, and deletions, insertions and duplications of genetic material.

The term 'minor variant' refers to those inter-individual differences in human chromosomes which have no obvious phenotypic effect and which probably involve quantitative and possibly qualitative differences in the heterochromatic component of the chromosomes. These include:

(i) Q-band intensity variants.

(ii) C-band size variants, particularly of regions 1qh, 9qh and 16qh. These variants had often been noticed as overall size variations with conventional staining.

(iii) Acrocentric chromosome short arm and satellite size variants also revealed by both C-banding and conventional staining.

(iv) RFA-band variants. The short arms and satellites of the acrocentrics show both size and colour variations with this stain.

(v) N-band variants. The size of these bands varies considerably (Hayata et al. 1977). With the silver-staining procedures, the following features may vary, apparently normally:

the modal number of stained chromosomes, the range of the number stained, and the proportion of D group chromosomes stained as a percentage of the total. These parameters are characteristic of each individual (Varley 1977).

(vi) LBA-staining. This method shows that the size of

late and early replicating sections of the chromosomes varies in many of the chromosomes, including those which do not vary with other methods (Nakagome et al. 1977b, Oka et al. 1977).

(vii) G-bands. Crossen (1975) has shown variation in the position and size of the centromeric bands of chromosome 19 after G-banding although usually the pattern is fairly consistent between individuals.

As stated earlier, estimates of the frequencies of all these types of chromosomal variation are derived in the main from samples of unselected newborn infants. For the quantification of the major abnormalities, several large series of consecutive live-born babies have been examined; from Edinburgh, U.K.(N=11 680) (Jacobs et al. 1974b), Arhus, Denmark (N=11 148)(Nielsen and Sillesen 1975), Ontario, Canada (N=2081) (Sergovich et al. 1969), Winnipeg, Canada (N=13 969) (Hamerton et al. 1975), Toronto, Canada (N=72 739) (Bell and Corey 1974, sex chromatin determination only), Boston U.S.A. (N=13 751) (Walzer and Gerald 1977), New Haven U.S.A. (N=4 353)(Lubs and Ruddle 1970a,b) and Moscow,U.S.S.R. (N=2 500)(Bochkov et al. 1974). These surveys were all conducted, or at least begun, before the use of banding techniques became widespread and therefore the information obtained from them is to some extent limited. It is often not known, for example, which chromosome is involved in any particular anomaly, and, furthermore, many rearrangements of the chromosomal material probably go undetected. Cohen et al. (1975) have published results of an examination of banded chromosome preparations from 500 normal healthy newborn infants in Jerusalem.

The results from Edinburgh, Arhus, Ontario, Winnipeg, Boston (using a reduced sample of 9048 children), New Haven and Moscow have been collated and the overall frequencies of chromosome aberrations calculated by Nielsen and Sillesen (1975). The frequencies of the various chromosome anomalies

found in the Edinburgh study (the only such series from the United Kingdom) and in the seven series mentioned above, combined together, are set out in table 3.1. The total incidence of chromosome abnormalities in the seven studies comprising 54 749 children is 6.03 per thousand: 3.87 per thousand involved the autosomes and 2.16 per thousand were abnormalities of the sex chromosomes (Nielsen and Sillesen 1975). Comparable figures for the Edinburgh series are:
total abnormality incidence = 6.59 per thousand.
autosomal aberrations = 3.94 per thousand.
sex chromosome aberrations = 2.65 per thousand.

The incidence of sex chromosome abnormalities was very similar in all seven studies. (Nielsen and Sillesen 1975). The most common types of autosomal aberrations were the trisomies (especially of the G group chromosomes), and the translocations. The incidence of autosomal trisomies was comparable in the different series (Jacobs et al. 1974b). Reciprocal and Robertsonian translocations appeared with about equal frequency in all the large series, their combined incidence being about $1\frac{1}{3}$ times as high as the incidence of the autosomal trisomies.

Aneuploid structural rearrangements (that is, those resulting in an inbalance in the genetic material) were in all series much rarer than euploid rearrangements. A total of 5 (0.04%) were found in the 11 680 infants of the Edinburgh series. 21 (0.05%) were found in the 43 558 infants comprising 6 series reviewed by Jacobs et al (1974b). Of these, 5 were mosaics and "only 8 (0.02%) had abnormalities of the phenotype recognisable at birth and attributable to the chromosomal imbalance."

In a series of 72 739 newborn infants Bell and Corey (1974) found 63 infants with sex chromosome abnormalities and 9 mosaics by determining the sex chromatin composition of their cells. Thus the incidence is 0.99 per thousand, which

TABLE 3.1: Frequencies of chromosome aberrations in series of newborn infants. (adapted from Nielsen and Sillesen (1975) and Jacobs et al. (1974b))

Karyotypes	Edinburgh study		7 series combined	
	No.	rate/1000	No.	rate/1000
(i) Autosomal				
+13			3	0.05
+18	2	0.17	8	0.15
+21	17	1.46	63	1.15
+mar			12	0.22
+mar,mosaics	1	0.08	5	0.09
deletions			5	0.09
inversions	2	0.17	7	0.13
D/D translocations	6	0.51	43	0.79
D/G translocations	4	0.34	11	0.20
reciprocal translocations	10	0.86	47	0.85
unbalanced Y/autosomal translocations			6	0.11
others	4	0.34	2	0.04
Total	46	3.94	212	3.87
(ii) Sex chromosomal				
47,XYY	10	1.27	28	0.81
47,XYY mosaics	2	0.25	7	0.20
47,XXY	9	1.15	33	0.96
47,XXY mosaics	2	0.25	6	0.17
46,XX male	1	0.13	2	0.06
45,X/46,XY male	1	0.13	1	0.03
46,Xinv(Y)			9	0.26
45,X			2	0.10
45,X mosaics	1	0.26	6	0.29
47,XXX	5	0.64	20	0.98
47,XXX mosaics			4	0.20
Total	31	2.65	118	2.16
Total abnormalities	77	6.59	330	6.03

is somewhat less than the figures obtained by the full karyotype analysis by other authors. This same study found 9 sex chromosome anomalies in 3660 newborn males examined for Y bodies. This gives an incidence of 2.46 per thousand, which is quite similar to that obtained from the previously mentioned studies.

Cohen et al. (1977) examined 500 healthy newborn infants in Jerusalem, using banded karyotypes for 163 (33%) of them. They detected no numerical anomalies, and 6 inherited structural abnormalities (12 per thousand). This higher incidence probably reflects the greater resolution of the more modern techniques. Two of the abnormalities were deletions, one of the long arm of the Y chromosome, and one of the short arm of chromosome 22. It is unlikely that these would have been recognised or scored as abnormalities in the earlier surveys. The other 4 abnormalities were inversions, one in chromosome 9 and three in chromosome 2.

Patil et al. (1977) have examined the karyotypes of 4342 7 and 8 year old children using banding procedures. The children were included in the survey during the pregnancy of their mothers and should be representative of the population as far as their chromosome constitution is concerned. 21 chromosome abnormalities were found (4.84 per thousand), 11 involving the autosomes and 10 the sex chromosomes. These results are very similar in many respects to those of the newborn infant surveys, with the exception that this series had a much lower incidence of autosomal trisomies (0.04% as compared with 0.14% (Nielsen and Sillesen 1975)). This is probably due to the death of affected children before the age of 7 or their confinement in institutions (Patil et al. 1977).

A series of 998 adults "with no known bias for increased aberration frequency" has been examined by Court Brown and

Smith (1969). 7 cases of chromosome anomalies were found, two involving the sex chromosomes and five the autosomes.

Zeuthen et al. (1973) examined 3840 men for military service and detected five XYY persons (1.3 per thousand). Other aberrations were not recorded. The frequency of this type of aberration in the seven series of newborn infants was 1.02 per thousand (including mosaics) (Nielsen and Sillesen 1975).

The incidence of Q-band intensity variants amongst various populations is dealt with in detail elsewhere (Chapter 7).

C-band variants.

The extent of variation of the secondary constriction regions of chromosomes 1, 9 and 16 and that of the short arms and satellites of the acrocentrics has been investigated in several series of newborn infants and unselected adults. The secondary constriction regions vary in size and, particularly in the cases of chromosomes 1 and 9, also in position. They can be either on the short arm or the long arm or both.

Classification of these variants has proved difficult and inter-laboratory comparability seems virtually impossible to achieve.

Müller and Klinger (1976) group the C-band regions of chromosomes 1, 9 and 16 into five size classes based on the size of the long arm of chromosome 21, for a series of 316 newborn infants. The second and third size classes were by far the largest for chromosomes 1 and 9. For chromosome 16 only the first three classes were used, the second class being the most common, followed by the first. The position of the C-band material was also recorded. For all three chromosomes in at least half the cases there was no extension

of the C-chromatin on to the short arm, and in the case of chromosome 16, this proportion was as high as 86.5%. Only in the case of two chromosomes 9 was there more material on the short arm than on the long.

Buckton et al. (1976) examined C-band variants in three samples of individuals (see below page 342). These consisted of newborn infants (N=467), fourteen-year old children (N=101) resident in the Edinburgh region, and all persons aged 65 or over and living on the island of Barra (N=210). The C-bands of chromosomes 1, 9 and 16 were classified into four categories: very large (VL), large (L), medium (M) and small (S). For each chromosome and each series more than 90% of the regions were classified as medium. The rest were about equally divided between large and small. Variants of size 'very large' were extremely rare.

The position of the C-bands was also recorded. "Total inversions" were cases where all the material was on the short arm. "Partial inversions" were, as the name implies, cases where only some of the C-band material was on the short arm. Total inversions were only seen on chromosome 9 (nine examples in all, less than 1%). Partial inversions were found on both chromosomes 1 and 9 (0.5-1.4% for chromosome 1, 0.5-3.7% for chromosome 9).

Lubs et al.(1977a,b) examined the C-band regions of a series of seven and eight year old children. The C-band regions of chromosomes 1, 9 and 16 were placed in five categories based on the size of the short arm of chromosome 16. The centromere regions of the other autosomes and the X-chromosomes were also assessed, and graded either small, normal or large. The incidence of small and large variants was low for all chromosomes. The children were classified by race as black or white, and large C-band regions were found to be more common among black children than among white. No such "racial" difference in frequency was observed

for the small variants.

For chromosomes 1, 9 and 16 most cases again were in classes 2, 3 and 4; fewer than 4%, and usually fewer than 1%, of chromosomes being placed in the extreme classes. No racial differences were found for classes 1 and 5.

Other studies have classified C-band regions of chromosomes 1, 9 and 16 as (+) variants, (-) variants or normal (McKenzie and Lubs 1975, Craig-Holmes 1977), using smaller series. High proportions of variants have been found (55 variants in 35 adults (Craig-Holmes 1977) and 166 variants in 77 infants (McKenzie and Lubs 1975)). Such studies amply demonstrate that these regions are highly variable in size, and to a lesser extent in position, but quantification of this variability seems difficult. Opinions differ as to whether the variations are continuous (Buckton et al. 1976) or discontinuous.

Minor variants shown with conventional staining procedures.

With uniform staining of the chromosomes only those variants which lead to a fairly marked change in the morphology and/or size of certain chromosome regions may be identified. Where such alterations may affect several similar chromosomes, as in the case of the satellites of acrocentric chromosomes, it is impossible to specify the chromosome concerned. Those variants which are visible and detectable using conventional stains are probably the size variants observed with C-banding (Chen and Ruddle,1971). However, in the case of chromosomes 1, 9 and 16 only the effect of variation in the size of the C-band region on the overall size of the chromosome may be observed.

Nielsen and Sillesen (1975) observed the incidence of minor chromosome variants among a sample of 11 148 newborn infants and found an overall frequency of 1.68%, the most

common variants being those affecting the short arm and satellites of the acrocentric chromosomes and the length of the long arm of the Y chromosome. Their results were compared with those of Hamerton et al. (1975) in Canada and Bochkov et al. (1974) in the U.S.S.R. No significant differences were found with respect to any variant except the length of Yq. Bochkov et al. had found 38 people out of 2500 (1.52%) having chromosome variants with no phenotypic manifestation.

Zankl and Zang (1971) examined 280 phenotypically normal newborn infants and found 38 minor variants amongst 32 children. The most common variant was an increased size of the short arm of the D group chromosomes. This occurred in 15 individuals (5.4%). These variants were twice as common among male infants, as compared with female. Such a sex difference had already been reported in other studies (Court Brown 1967, Walzer et al. 1969).

Ferguson-Smith (1973) reported a total of 173 (7.5%) minor variants amongst 2291 adults referred to a chromosome diagnostic service. The most common variants were those affecting the D group chromosomes (2.4%), the G group (1.7%) and chromosome 9 (1.1%). A smaller series (N=367) was examined using banded preparations. In this series a much higher frequency (31.6%) of variants was recorded, for example, 10.4% of chromosomes 1 had variants (compared with 0.5% unbanded) and 8.2% 9qh+ were found and 1.1% inversions of the C-band region on chromosome 9.

RFA-colour variants.

Verma et al. (1977) have examined the frequency of colour variants with RFA-staining in 100 healthy Caucasians (both sexes) between the ages of 25 and 65. The short arms and satellites of all acrocentric chromosomes vary in their possible reaction to the stain. The variants have been classified as red, red-orange, orange-yellow, pale yellow,

bright yellow and pale green. Considerable variability of all regions was observed among the 100 individuals. The most common variant was orange-yellow for all regions examined, pale yellow and pale green accounting for the majority of the remainder. As these regions also vary in their reaction to staining with quinacrine, the same persons were also examined with regard to their Q-variants. No consistent relationship was found between negative or brilliant Q variants and the several colours of the RFA variants.

Chromosome Polymorphisms in the Higher Primates.

There are, as stated earlier, basically two common types of variants in human chromosome complements. There are those regions which vary in size (regions of constitutive heterochromatin) and regions which vary in their response to stains (presumably reflecting qualitative chemical differences). There is the possibility that the regions which fluoresce brilliantly with quinacrine are not all chemically the same. The distal part of the long arm of the Y chromosome shows very brilliant fluorescence always, but other regions which react in this way do not do so throughout the population.

Within the higher primates only the gorilla has the Y-type of brilliantly fluorescent material. (The chimpanzee which has a very small Y chromosome, does not.) (Pearson 1977). However, such brilliant fluorescence is seen in other specific regions in both the chimpanzee and the gorilla (Pearson et al. 1971). This material does not seem to be present in the karyotypes of the other primates nor in other mammalian species (Bobrow 1975). This implies a fairly recent origin of the material (probably not more than 20 or 30 million years ago(Pearson et al. 1973).

In the chimpanzee the acrocentric chromosomes are satellited, and these regions show a variable level of fluorescence as they do in Man. These regions are not seen on the chimpanzee equivalent of chromosome 15 (Bobrow and Madan 1973). The frequency of the brilliantly-fluorescing satellites appears to be higher than the average frequency in human populations (Pearson et al. 1973). but the numbers examined have been small. Lin et al. (1973) have suggested that all the chimpanzee acrocentric chromosomes are satellited but this is disputed by Pearson et al. (1973) and Bobrow and Madan (1973). Intensely fluorescent bands may also be variably found on the telomeres of the chimpanzee equivalents of the F and G group chromosomes (de Grouchy et al. 1978).

Fluorescent satellites may also be see on the acrocentric chromosomes of the gorilla karyotype (Pearson et al. 1971. Miller et al. 1974). Also in the gorilla karyotype, chromosome 3 (the equivalent of human chromosome 4) may have an intensely fluorescent band adjacent to the centromere (Pearson et al. 1973, Miller et al. 1974). This region is also polymorphic in human populations.

Lin et al. (1973) have reported that the variable fluorescence of the acrocentric chromosomes occurs mostly in the short arm region of the chimpanzee chromosomes, but in the satellites of the gorillas.

The higher primates also show variations in the size of heterochromatic regions as revealed by C-banding techniques, (Miller et al. 1974) although the extended secondary constrictions of chromosomes 1, 9 and 16 in the human seem to be absent from the chimpanzee chromosomes (Pearson et al. 1973)

Gorillas have small accessory chromosomes which are only variably present, and which presumably consist of constitutive heterochromatin; they are not associated with any abnormality of the phenotype (de Grouchy et al. 1973).

Chromosome Polymorphisms in Other Species.

(i) The presence of brightly fluorescing material.

Brightly fluorescing material is seen in heterochromatic regions of some Drosophila chromosomes (Vosa 1970b). Certain regions are species-specific (Adkisson et al. 1971), and may be seen in both mitotic and polytene chromosomes. However, in at least one species (Drosophila virilis) there seems to be little relationship between the locations of the bright material in these two types of chromosomes (Adkisson et al. 1971). These reports made no specific mention of a

possible variable appearance of the bright regions.

In Vicia faba, the chromosomes stain fairly uniformly with quinacrine, having four clearly enhanced fluorescent bands (Vosa 1976). It appears that the only regions to fluoresce brightly in these chromosomes are heterochromatic regions, and that any variability that exists is with respect to size rather than to staining intensity (Vosa 1976).

Brilliantly fluorescing material with quinacrine staining is not widespread in the animal kingdom. Very intensely fluorescent chromatin has been observed in Samoaia leonensis, and has been shown to consist of highly AT-rich DNA (Ellison and Barr 1972).

(ii) Chromosome variability in other species.

The existence of polymorphic inverted sequences in the Drosophila karyotypes is very well documented, from studies of their polytene chromosomes. While banding patterns have been produced in mitotic chromosomes of a large variety of animal species (see above, page 26) the extent of polymorphic variability is not well known.

Gallimore and Richardson (1973) reported the variable presence of two G-bands on the long arm of a small subterminal autosome in two laboratory strains of rat. Polymorphism for the presence or absence ofsatellites on this chromosome was also noted.

Miller et al. (1976) reported differences in the number of chromosomes with secondary constrictions and in the size of C-band regions on certain chromosomes among several inbred strains of Mus musculus. The constancy of the C-bands over several generations was also noted.

Yosida and Sagai (1975) have noted a high degree of polymorphic variability in the C-band size of chromosomes of all species and subspecies of rat.

Accessory chromosomes or B chromosomes are quite widespread throughout the plant kingdom. These chromosomes consist of constitutive heterochromatin and are polymorphic as far as their number per individual is concerned.

Vosa (1973) has shown that in at least one species of plant (Scilla sibirica) there is a high degree of polymorphism with regard to the presence or size of heterochromatic bands on almost all the chromosomes.

Marks (1976) has found variation in the presence or absence of G-bands (which in plants are thought to be equivalent to the C-bands of animals) in Anemone blanda L. The karyotypic pattern is constant for each individual plant.

The Phenotypic or Adaptive Significance of Chromosomal Variations

The frequency of many types of chromosomal variations in human populations is sufficient to question whether or not there might be any genetic or adaptive significance in their existence. Major imbalances in the chromosomal material are often associated with obviously deleterious conditions, but evidence concerning the effects on the phenotype of structural rearrangements which are balanced, or of the 'minor variants' is not conclusive.

(i) The evidence for a (deleterious) effect of chromosome abnormalities.

Numerical disorders and structural disturbances of the chromosomes which occur with any measurable frequency (more than sporadically) in the human populations appear generally to be associated with recognisable syndromes involving both

physical and mental abnormalities. (For example, Trisomy 21 leads to Down's syndrome, and 47, XXY to Klinefelter's). The only numerical abnormality which does not as yet appear to fit this generality is the 47,XYY karyotype, which seems to involve an increase in height and which may also have an effect on the behaviour and personality of the carrier; though the evidence for this is somewhat contradictory (Jacobs et al. 1965, Court Brown et al 1968, Kessler and Moos 1970, Zeuthen et al 1973, and many others).

The severity of the effect of a particular chromosomal imbalance on the phenotype appears to be associated with both the size of the chromosome, or chromosome segment, involved, and the amount of genetic information within that chromosome or segment (Hoehn 1975). Thus those trisomies which are compatible with survival until at least birth are those involving chromosomes with fairly high proportions of positive G-bands, that is , fairly large heterochromatic segments. (Hoehn 1975).

Trisomies of almost all autosomes have been detected in conceptuses which have been spontaneously aborted (Boué et al. 1976, Creasey et al 1976) at quite high frequencies, for example, 15% of 941 singleton abortuses were trisomic, another 15% either monosomic or polyploid (Creasey et al. 1976); Boué et al. (1976) identified trisomies involving chromosomes 7,8,9,10,12,13,14,15,16,18,20,21 and 22 amongst a series of spontaneous abortions. Trisomies of chromosomes 2,3 and 4 (Creasey et al. 1976), 6 and 11 (Kajii et al. 1973), and 19 (Carr 1975) have been reported. Only chromosomes 1, 5 and 17 have yet to be reported in the trisomic state. These last three conditions occur in all probability, but abortion occurs earlier than can be detected.

Chromosome 21 has the least severe effect on the phenotype in the trisomic state, compared with other chromosomes, but

even in this case it is estimated that 65% of all conceptuses with trisomy 21 are aborted spontaneously (Creasy and Crolla 1974).

Groups of individuals selected for some disturbance in their physical and/or mental development show higher frequencies of trisomy 21 than are found among unselected newborn infants (Sutherland et al. 1976, Newton et al. 1972, Speed et al. 1976). The last authors examined the total population of mentally subnormal persons in N.E. Scotland (N=2770) and found 297 to be chromosomally abnormal. 250 (9%) had trisomy 21. Chromosome aberrations may also lead to an increased risk of developing phenotypic abnormalities not restricted to the syndrome; for example, Stewart et al. (1958) reported that the incidence of leukaemia was significantly increased in children with Down's syndrome, compared with other healthy children. An increased frequency of breast cancer has also been reported in this group but not confirmed (Harnden et al. 1969).

Tharapel and Summit (1977) compared the incidence of major chromosomal aberrations in 200 mentally retarded individuals and in a control group of the same size. 65 such aberrations were found in the first group, but none in the second. Several authors have examined the karyotypes of low birth weight infants and of infants dying in the perinatal period. Such groups also show a raised frequency of chromosome aberrations compared with healthy newborn infants (Chen et al. 1974, Machin and Crolla 1974, Kuleshov 1976).

The harmfulness of balanced structural rearrangements has been questioned. Jacobs et al. (1978) found 5 (1.0%) among 475 mentally retarded patients but only 22 (0.19%) amongst 11 680 newborn infants (Jacobs et al. 1974b). Sutherland et al. (1976) found 12 (2.04%) autosomal abnormalities, excluding Down's syndrome, among 588 mentally retarded patients. Yet Turpin and Lejeune (1969) have said "the great majority of centric fusions have no visible

disadvantage on the heterozygous carrier".

Morton et al. (1975) reported significant effects on fertility, survival, and generation time within a large sample of diverse chromosomal aberrations including Robertsonian and reciprocal translocations, inversions and extreme size variations of the C-bands. With the exception of the last type, all the aberrations represent balanced rearrangements of the chromosome material. In this study it was estimated that the reduced relative fitness experienced by the surviving carriers of chromosome aberrations was .769±.039. However, Jacobs (1975) reminds us that translocations should result in a 50% loss in fitness if expected patterns of segregation occur. The loss associated with crossing over within, and segregation of inverted chromosome segments depends upon factors such as the size of the inverted segment and the frequency of crossing over within it, but it is suggested that a larger loss in fitness is expected theoretically than is observed in practice (Jacobs 1975).

Jacobs (1975) is of the opinion that balanced chromosome abnormalities may carry an advantage in that they both increase the birth interval and reduce the number of offspring that survive with the minimum of morbidity and mortality to the mother, that is, such chromosome abnormalities serve the purpose of regulating reproduction-spacing in a species with long periods of parental care, and no other limitations on the breeding season. This limiting action is most effective when the deleterious effect of the abnormality is expressed early in the pregnancy - thus causing little damage to the health of the mother. In this way, possession of the chromosome abnormality is of advantage not to the carrier but to the healthy members of the offspring. The advantage to these individuals would decline with the survival of the chromosomally abnormal members of the sibship. However, an argument against this reasoning is that there does not seem to be any means by which the healthy

rearrangement-carrying members of the sibship could be conferred with an advantage over the chromosomally normal members. Therefore, Jacob's reasoning provides no mechanism by which the decline of the population frequency of the rearrangement would be halted. In addition to this, her initial contention that a genetic variant resulting in below average family size will be favoured in the population, must be viewed with caution.

Moorhead (1976) points out that in most reports on chromosome aberrations (specifically inversions) the ascertainment of the condition is most usually due to clinical defects in the carriex, or in his or her near relatives. Such defects may be due to small duplications and deletions not detected with the cytological techniques in use rather than to the aberrations identified. He suggests that this fact, together with the fact that there is no theoretical expectation of an effect of the inversion on the phenotype of the carrier (except for position effect, and reduced fertility) indicate that inversions in humans may be viewed as polymorphisms which only rarely contribute to phenotypic disorders. When phenotypic abnormalities and the supposedly causal inversion occur together in a pedigree their occurrence is not always absolutely correlated in other members of the family (that is, some normal carriers and some abnormal non-carriers may occur) (Moorhead 1976).

Soudek (1975) examined carriers of small inversions on chromosomes 1, 3, 9 and 10 and found that most persons were normal and that mental and/or physical abnormalities could usually be explained by coincidence. A lower frequency of inversions of chromosome 3 was found among a group of normal persons than in a group of mentally retarded individuals but the author concluded that this fact alone did not demonstrate a harmful effect of the inversion.

In conclusion, then, the majority of human chromosome abnormalities are lethal before birth, and of the remainder, unbalanced rearrangements and autosomal trisomies usually

lead to severe mental and physical disability and thus are effectively genetically lethal (Jacobs 1975).

The phenotypic and/or genetic disadvantages associated with balanced structural rearrangements are not so clearly defined or quantified. The degree of disturbance of the karyotype, that is the size of the chromosome segment involved is probably of importance.

(ii) The minor variants.

a. Q-intensity variants:

Robinson and Newton (1977) report that the frequency of positive (intense and brilliant) satellites of chromosome 21 is significantly higher among a group of Down's syndrome patients than among a group of controls. Bott et al. (1977) also report this association.

Mikelsaar et al. (1975) in a similar comparison found no association between 'marker brilliant' satellites of chromosome 21 and Down's syndrome - but as the scoring of these variants involved assessment of the size as well as the intensity of fluorescence of the satellite, any association between the latter characteristic and the chromosome abnormality may have been confounded.

b. C-band variants

There has been much speculation that the variants of C-band regions, particularly the extremely large sized variants consisting as they do of heterochromatin, may have influence on the expression of genes (presumably located in the euchromatic material)(Halbrecht and Shabtay 1976), and may increase the risk of nondisjunction and consequently of chromosome aberration (Alimena et al. 1977).

Halbrecht and Shabtay (1976) report a study which finds several families with the 1qh+ variant (increased size of

the C-band region of chromosome 1) in which the incidence of otherwise rare malformations is surprisingly high. They suggest that interactions between all C-band (heterochromatic) regions are of pathogenic significance. These variants do not appear to be the direct cause of the malformations, as they may occur in the same combinations in persons of normal phenotype. Gardner et al. (1974) suggest that the potential for harm of heterochromatin depends upon the genetic background.

Different C-band variants appear to affect the phenotype in different ways. C-band regions of the acrocentric chromosomes may affect CNS malformations (Halbrecht and Shabtay 1976). Two reports mention a possible association between 1qh+ variants and a Patau-Maekel syndrome-like phenotype. This type of variant has also been suggested as being associated with malignant disease (Atkin 1977, Shabtai and Halbrecht 1979). Such an association suggests that perhaps the deleterious effect of the large C-band variants is to increase susceptibility to chromosome breakages and their consequences.

Tharapel and Summit (1978) compared the frequency of certain C-band variants, namely the secondary constriction regions of chromosomes 1, 9 and 16, the length of the long arm of the Y chromosome, and variations in the size of the short arm and satellites of the acrocentric chromosomes in a group of mentally retarded persons and a group of normal controls (200 people in each). No significant differences were found with regard to any variant.

Funderburk et al. (1978), using conventional staining methods, examined the minor chromosomal variants of 1289 child psychiatric patients (of whom 75% had congenital abnormalities and more severe mental disorders). There was no evidence for an effect on development of any of the minor variants including C-band variants. As a consequence of the interest in the possible effects of the 47, XYY karyotype on behaviour (see above, page 80) special attention has been

focused on a possible influence on behaviour and mental development of variants of the Y chromosome. The evidence for or against any such association is contradictory. Nielsen and Nordland (1975), Akesson and Wahlstrom (1977) and Brøgger et al.(1977) report a lack of association between behaviour and the length of the fluorescent segment of the Y chromosome (this segment coincides with the C-band region, and its variation accounts for almost all the variation in length of the Y chromosome); Soudek and Laraya (1974) find that the Y chromosomes of 84 male patients were significantly longer than those of 38 staff men at a psychiatric hospital (the fluorescent and non-fluorescent sections both being involved in the length increase), and Nielsen and Friedrich (1972) report that the mean length of the Y chromosome is greater in 407 criminal males as compared with 140 newborn males (randomly chosen from a larger sample of 1400).

It is difficult to imagine why such an association should exist as the long arm of the Y chromosome is apparently genetically inert. However, some interference of cellular processes by excessive amounts of heterochromatin may possibly be responsible (see above page 47).

SUMMARY

There are two categories of human chromosome variations: major and minor.

The former type are fairly uncommon amongst series of unselected newborn infants, but more frequent amongst groups of individuals having some mental or physical abnormality. This indicates then, a detrimental effect of major chromosomal aberrations, whether of the balanced or unbalanced type.

Very little evidence exists to point to a detrimental, or other, effect on the phenotype of the qualitatively different Q-band variants, but there are several suggestions that quantitative variations in heterochromatic regions of the karyotype (as indicated by C-band staining) have some influence on development, though no variant has been shown to be consistently detrimental in any respect.

Chapter 4: THE POPULATION STUDIED AND METHODS OF SAMPLING.

1. Constraints upon the sample.

Several fairly restricting factors determined the final make-up of the sample. The laboratory techniques used limit to quite a large extent the numbers of blood specimens that can be handled at any one time. For optimum use of the available time they require that the supply of specimens is fairly regular and not too heavy. Red cell grouping, cell culturing, slide preparation and microscope analysis all have to be performed on reasonably fresh samples and therefore it is impracticable to receive large numbers sporadically. These logistic difficulties make it virtually impossible to find a means of sampling rigorously the population while yet collecting sufficient numbers to make statistical analysis of the results worthwhile. With this in mind, it is still hoped, however, that the group of individuals eventually studied is genetically representative of the population from which it derives.

A major problem was not only finding sufficient numbers of volunteers from the general population but also arranging to have venous blood samples taken from them by a suitably qualified and insured individual. I received help in overcoming these difficulties from many quarters, as can be appreciated from Table 4.1 which lists the various sources of the subjects. Dr. D.R.R.Williams took blood from various University employees and students, Dr. L.Weaver from members of the Durham Federation of Young Farmers' Clubs, and Miss Fitch and her fellow medical students at Dryburn hospital from the various hospital employees. Specimens from hospital patients were taken during routine hospital testing by Drs. Bell, Cartwright and Robertson, and by several house officers on the orthopaedic wards.

The purpose was to investigate the chromosomal

TABLE 4.1: Sources of the subjects.

	Male	Female	Total
	(No. and % of total)		
Dryburn hospital maternity ward	124 (17.5)	137 (19.3)	261 (36.8)
Dryburn hospital orthopaedic ward	25 (3.5)	19 (2.7)	44 (6.2)
Durham University and Colleges students	24 (3.4)	18 (2.5)	42 (5.9)
Durham University scientific technical staff	33 (4.6)	7 (1.0)	40 (5.6)
Durham University library staff	4 (0.6)	10 (1.4)	14 (2.0)
Durham University computer operators and card punchers	0	8 (1.1)	8 (1.1)
Durham University secretarial staff and telephonists	0	13 (1.8)	13 (1.8)
Durham University-other non-academic staff	6 (0.8)	4 (0.6)	10 (1.4)
Dryburn hospital physiotherapists	2 (0.3)	6 (0.8)	8 (1.1)
Dryburn hospital laboratory technicians	2 (0.3)	7 (1.0)	9 (1.3)
Dryburn hospital radiographers	4 (0.6)	9 (1.3)	13 (1.8)
Dryburn hospital nursing staff	1 (0.1)	19 (2.7)	20 (2.8)
Members of County Durham Federation of Young Farmers Clubs	38 (5.4)	7 (1.0)	45 (6.3)
Other residents of County Durham	3 (0.4)	0	3 (0.4)
Dryburn hospital medical wards	24 (3.4)	30 (4.2)	54 (7.6)
St. Margaret's hospital	29 (4.1)	97 (13.7)	126 (17.7)
Summary			
Newborn infants	124 (17.5)	137 (19.3)	261 (36.8)
Orthapaedic patients	25 (3.5)	19 (2.7)	44 (6.2)
'Normal' adults	117 (16.5)	108 (15.2)	225 (31.7)
Geriatric patients	53 (7.5)	127 (17.9)	180 (25.4)
Total	319 (44.9)	391 (55.1)	710

variability of the above sample, and to relate it to demographic information as well as to the variability exhibited by other genetic markers. Obvious factors considered when deciding which markers to study were:

(i) How easy is it to collect the data?

(ii) With what reliability may the phenotypes be classified in a manner which indicates the genotype?

(iii) What information has already been published for comparative purposes?

Point (i) is especially important. It is desirable to collect this genetic information with as little inconvenience to the individual as possible. This is particularly true when the individual concerned is already stressed (as in the case of hospital patients) and/or likely to reconsider his or her willingness to participate in the project.

2. Sources of the Subjects.

The individuals sampled can be placed into three categories:

I. Newborn infants.

II. Adults resident in the Durham area.

III. Elderly patients in local hospitals.

I. Newborn infants.

Umbilical cord blood specimens were obtained from a series of newborn infants between the beginning of March and the end of December 1977 at Dryburn Hospital, Durham. Signed permission to use individual specimens was granted on form 1.1 (Appendix 1) which was also used to record demographic information.

II. Adults resident in the Durham area.

Specimens of venous blood were collected from adult volunteers who had been contacted in a number of different

ways. It was hoped that the sample would represent a 'local' population and so academic staff of the University and medical staff of the hospital were not asked to participate. It was thought that too many from these two groups would be more highly mobile than usual because of the nature of their careers, and thus less likely either to originate in, or to contribute to the population of the area.

(i) University staff.

a. The chief technicians in all science departments were approached and asked to recruit volunteers among their technical staff. Arrangements were made to visit the departments at a particular time (usually a coffee- or tea-break) when the blood specimens were taken. At the same time demographic information about the individual was collected using forms 1.2 (Appendix 1). Similar forms were filled in by all adults (with the exception of those in category III). Examples of these forms may be found in Appendix 1.

b. Library staff, computer operators and card punchers were contacted and sampled in a similar way.

c. Copies of form 1.3 were sent to all other members of the non-academic staff of the University. They were asked to participate in the survey by completing the form and taking it along to the Student Health Centre at the University where arrangements had been made with the nursing staff for blood to be taken out of term-time. The main response to this request came from secretarial staff and telephonists.

d. A miscellaneous collection of other volunteers was obtained usually after accidental encounters with them whilst sampling other groups.

(ii) Staff of Dryburn Hospital.

With the cooperation of Dr. Mowbray (Senior Consultant), several groups of individuals were approached and arrangements made to collect blood specimens. Volunteers

were obtained from amongst:

a. Laboratory technicians.
b. Physiotherapists.
c. Radiographers.
d. Nursing staff.

(iii) Durham Federation of Young Farmers Clubs.

Following a suggestion from a member of this association, the county organisation was contacted and the research project was explained to the members at one of their half-yearly general meetings. A number of members agreed to take part and have blood taken from them immediately after the next meeting of this kind, and also further volunteers were obtained after one of the local meetings of the Sedgefield club.

(iv). Dryburn Hospital orthopaedic patients.

A series of trauma patients from the orthopaedic wards had been sampled as part of the control population in an earlier study conducted in this laboratory (Williams 1977). It was intended that when this earlier study ended the sampling of the patients could be continued. Copies of form 1.4 were left in the ward office, together with sterile heparinised bottles of the type used in this study. The several House Officers were requested to give a copy of the form to each new admission and with his or her consent to take a sample of blood at the same time as collecting a specimen for routine hospital tests. However, the venture was largely unsuccessful, and was discontinued after a fairly short time. Most of the individuals from this source were actually sampled as part of the earlier survey.

In comparisons of gene frequencies, for example, with this latter survey (Williams 1977) individuals have been included only once.

Generally, they were included in the present series if the cell cultures were successful and data of chromosomal variability were obtained, and in the diabetic control group if not.

(v). Students.

Students of Durham University and Colleges whose home addresses were within County Durham before the 1974 local government reorganisations were contacted via their colleges and asked to participate in the survey. Most were contacted by Mr. Paul Converse while he was collecting samples for his study on the relationship between HLA frequencies and longevity (Converse 1977, Converse and Williams 1979).

III. Elderly patients at local hospitals.

Venous blood samples were collected from a number of patients in St. Margaret's Hospital, Durham during the course of routine haemoglobin level testing. The dates of birth of the patients were obtained from hospital records, and a few of the patients were asked for their place of birth. A much smaller number of specimens was obtained from patients aged over sixty years newly admitted to the medical wards of Dryburn Hospital. The reason for admission to hospital was recorded in every case. (Table 4.2)

TABLE 4.2: Reasons for admission of the elderly patients to Dryburn and St. Margaret's hospitals.

		No.	%
I Degenerative diseases:			
Arteries	i. Hypertension	1	0.6
	ii. Congestive cardiac failure	12	7.6
	iii. Myocardial infarction	11	7.0
	iv. Ischaemic heart disease	4	2.5
Brain	i. Cerebral ischaemia	2	1.3
	ii. Cerebro-vascular accident	43	27.4
	iii. Parkinson's disease	8	5.1
	iv. Senile dementia	35	22.3
Pancreas	i. Diabetes	4	2.5
Bones/joints	i. Disc lesion	1	0.6
	ii. Paraplegia	1	0.6
	iii. Quadraplegia	2	1.3
Lungs	i. Chronic bronchitis	2	1.3
Bowel	i. Gall bladder	1	0.6
	ii. Diverticulis	1	0.6
Bone	i. Osteo-arthritis	13	8.3
Kidney	i. Renal failure	1	0.6
II Accident:			
	i. Fracture	3	1.9
III Autoimmune:			
	i. Rheumatoid arthritis	5	3.2
	ii. Pernicious anaemia	1	0.6
IV Neurological:			
	i. Epilepsy	1	0.6
	ii. Multiple sclerosis	2	1.3
V Unknown/grouped type:			
	i. Schizophrenia	1	0.6
	ii. Malabsorption	1	0.6
VI Neoplasia:			
	i. Large bowel cancer	1	0.6

Characteristics of the sample inferred from the demographic data.

Details of the following demographic variables were obtained for each member of the sample, with the exception of the third series mentioned above:

1. date of birth.
2. sex
3. place of birth.
4. places of birth of both parents, where known.
5. places of birth of all grandparents, where known.
6. occupation of either the subject, or, in the case of the infants and the students, his or her father.

In the case of the newborn infants the following information was also obtained:

1. number and sexes of siblings.
2. ages of both parents.

The data are summarised in tables 4.3 to 4.10

The ages of the individuals used in the analysis are their ages on 1st June 1977. During the analysis of the genetic data several methods of grouping the subjects by age were used. These are also given.

Demographic data are incomplete and therefore the totals are not the same in tables 4.3 to 4.10.

TABLE 4.3: Age distribution of the sample.

Age(years)	Male		Female		Total	
	No.	%	No.	%	No.	%
1. 5-year intervals.						
0	124	(39.2)	137	(19.4)	261	(37.0)
15 - 19	23	(7.3)	18	(4.6)	41	(5.8)
20 - 24	43	(13.6)	34	(8.7)	77	(10.9)
25 - 29	23	(7.3)	17	(4.4)	40	(5.7)
30 - 34	16	(5.1)	10	(2.6)	26	(3.7)
35 - 39	7	(2.2)	6	(1.5)	13	(1.8)
40 - 44	9	(2.8)	10	(2.6)	19	(2.7)
45 - 49	7	(2.2)	7	(1.8)	14	(2.0)
50 - 54	4	(1.3)	6	(1.5)	10	(1.4)
55 - 59	1	(0.3)	5	(1.3)	6	(0.9)
60 - 64	7	(2.2)	8	(2.1)	15	(2.1)
65 - 69	13	(4.1)	8	(2.1)	21	(3.0)
70 - 74	15	(4.7)	22	(5.7)	37	(5.2)
75 - 79	9	(2.8)	24	(6.2)	33	(4.7)
80 - 84	4	(1.3)	38	(9.8)	42	(6.0)
85 - 89	7	(2.2)	26	(6.7)	33	(4.7)
90 - 94	3	(0.9)	13	(3.3)	16	(2.3)
95+	1	(0.3)	0		1	(0.1)
Total	316		389		705	
2. 20-year intervals.						
0	124	(39.2)	137	(35.2)	261	(37.0)
1 - 19	23	(7.3)	18	(4.6)	41	(5.8)
20 - 39	89	(28.2)	67	(17.2)	156	(22.1)
40 - 59	21	(6.6)	28	(7.2)	49	(7.0)
60 - 79	44	(13.9)	62	(15.9)	106	(15.0)
80+	15	(4.7)	77	(19.8)	92	(13.1)
Total	316		389		705	

TABLE 4.3 contd.:

Age(years)	Male		Female		Total	
	No.	%	No.	%	No.	%
3. 20-year intervals (adults beginning at 15 years).						
0	124	(39.2)	137	(35.2)	261	(36.9)
15 - 34	105	(33.2)	79	(20.3)	184	(26.1)
35 - 54	27	(8.5)	29	(7.5)	56	(7.9)
55 - 74	36	(11.4)	43	(11.1)	79	(11.2)
75+	24	(7.6)	101	(26.0)	125	(17.7)
Total	316		389		705	
4. 30-year intervals.						
0	124	(39.0)	137	(35.2)	261	(36.9)
1 - 29	89	(28.0)	69	(17.7)	158	(22.3)
30 - 59	44	(13.8)	44	(11.3)	88	(12.4)
60+	61	(19.2)	139	(35.7)	200	(28.3)
Total	318		389		707	

5.

	mean age (years)	s.d.	range(years)
Total sample	32.30	32.43	0 - 98.58
Total adults	51.29	26.37	16.18 - 98.58
Male adults	42.71	23.64	16.18 - 98.58
Female adults	57.83	26.50	17.03 - 94.27

TABLE 4.4: Occupational classes of the sample.

Class	I		II		III non-manual		III manual		IV		V		Total
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
Male	28	(12.1)	78	(33.8)	16	(6.9)	58	(25.1)	48	(20.8)	3	(1.3)	231
Female	36	(15.3)	89	(37.9)	30	(12.8)	49	(20.9)	28	(11.9)	3	(1.3)	235
Total	64	(13.7)	167	(35.8)	46	(9.9)	107	(23.0)	76	(16.3)	6	(1.3)	466

TABLE 4.5: Newborn infants subdivided according to age of mother.

Age of mother(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+	
	No.	%	No.	%	No.	%	No.	%	No.	%
Males	10	(8.8)	26	(23.0)	49	(43.4)	21	(18.6)	7	(6.2)
Females	7	(5.4)	47	(36.2)	48	(36.9)	25	(19.2)	3	(2.3)
Total	17	(7.0)	73	(30.0)	97	(39.9)	46	(18.9)	10	(4.1)

mean age of mother = 26.33 years s.d. = 4.65

range = 16 - 42 years

TABLE 4.6: Newborn infants subdivided according to age of father.

Age of father(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+	
	No.	%	No.	%	No.	%	No.	%	No.	%
Males	2	(2.0)	13	(13.3)	40	(40.8)	29	(29.6)	14	(14.3)
Females	5	(4.2)	26	(21.8)	43	(36.1)	37	(31.2)	8	(6.7)
Total	7	(3.2)	39	(18.0)	83	(38.2)	66	(30.4)	22	(10.1)

mean age of father = 28.47 years s.d. = 5.10

range = 17 - 51 years

TABLE 4.7: Newborn infants subdivided according to order of birth.

Order of birth	1		2		3		4		5	
	No.	%	No.	%	No.	%	No.	%	No.	%
Males	43	(42.2)	46	(45.1)	8	(7.8)	3	(2.9)	2	(2.0)
Females	67	(55.4)	39	(32.2)	8	(6.6)	5	(4.1)	2	(1.7)
Total	110	(49.3)	85	(35.1)	16	(7.2)	8	(3.6)	4	(1.8)

TABLE 4.8: Subdivision of the sample according to place of birth.

	No.	%
Within County Durham	457	72.0
Within Tyne and Wear	90	14.2
Elsewhere in the U.K.	88	13.9
Total	635	

TABLE 4.9: Subdivision of the sample according to place of birth of parents.

	No.	%
Both parents from County Durham	181	39.1
One parent from County Durham / other from Tyne and Wear	51	11.0
One parent from County Durham / other from elsewhere in the U.K.	62	13.4
Both parents from Tyne and Wear	59	12.7
One parent from Tyne and Wear / other from elsewhere in the U.K.	39	8.5
Both parents from elsewhere in the U.K.	71	15.3
Total	463	

TABLE 4.10: Subdivision of the sample according to place of birth of grandparents (where at least 3 grandparents are from the same region).

	No.	%
Within County Durham	156	55.1
Within Tyne and Wear	41	14.5
Elsewhere in the U.K.	86	30.4
Total	283	

3. The Adequacy of the Sample.

In the first part of this chapter a number of reasons was given to explain the difficulties encountered in trying to obtain a sample that might be considered representative of the population of the area. It is thought that, on balance, these problems did not have too serious an effect on the validity of the study. The considerations which led to this optimistic conclusion are given below; the three series which make up the sample being dealt with in turn.

I. Newborn infants.

In order to decide whether or not the group of specimens collected was a random sample of the total children born in the region during this period, two assumptions have to be considered. Firstly, it has been assumed that those babies born in the hospital do not differ genetically from the other babies born in the area. Secondly, it has been assumed that the sample is representative of the total born in the hospital.

There is no reason to assume that the first assumption is a false one, except that possibly more first-born children are included in the hospital population than occur in the general population. In 1969, 95% of Edinburgh mothers had babies in hospital (P.A. Jacobs et al. 1974b) and if there is a similar proportion here then there is little bias.

For the second assumption, the following may be considered:

a. Cord blood specimens were obtained from only a proportion of the babies born. Whether or not a specimen was obtained from any particular infant was due almost entirely to the work-load of the hospital staff at the time of delivery, (that is, the number of deliveries they were involved in at any particular time), and also to how

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familiar the several members of the staff were with the fact that the survey was taking place.

b. That no deliberate selection of infants for inclusion in the survey should take place was agreed by hospital staff after discussions which took place before the survey began. However, as far as I am aware, only healthy individuals were sampled. If any infant required any special immediate post-natal care it was highly unlikely that a sample of blood would be collected for the survey.

Cord blood samples were only taken routinely by the hospital in the case of babies born to Rhesus-negative mothers. This often meant that these babies were not included in this survey and therefore might be under-represented in the series.

c. There was a higher rate of culture failure from the cord blood specimens than from the venous blood samples. It is not thought that this is particularly suggestive of any abnormality of the lymphocytes occurring in a higher proportion of newborns than adults. It is more likely to be due to the fact that:

(i) the newborn series was started earlier than the others, at a time when the success rate was not as high as it later became for all the series.

(ii) Similarly, even in the case of successful cultures results were sometimes not obtained because of the greater age of the slides when they were finally examined.

(iii) For a number of reasons it is more difficult to obtain a successful cell culture from a cord blood sample. The red cells do not settle as rapidly and therefore it was virtually always impossible to set up macro-cultures. Sometimes the blood had clotted before it was placed in the heparinised bottle. The lymphocyte composition may differ from that in

venous blood or there may not be an optimal proportion of those cells susceptible to stimulation by PHA. Hatcher and Hook (1976) reported that response to PHA differed in cord blood lymphocytes.

2. Adult residents of County Durham.

As can be seen from Table 4.1, these individuals come from diverse walks of life, and in fact are very likely to be representative of the population. The main criticism to be levelled against the sample is that the method of contacting the subjects depended almost entirely on the fact that they were employed outside the home. Whether or not this would lead to any genetic bias in the sample is unknown.

3. Hospital patients.

a. Orthopaedic patients.

As stated earlier, these individuals were for the most part sampled as part of the control group for the diabetic survey. Justification of their inclusion in that study has been given in Williams (1977) and they have, with the same reservations, been accepted as suitable for inclusion here. In fact they comprise only a small proportion of the total adult sample examined here, and do not differ significantly from the group of non-hospitalised residents in the frequencies of the various genetic markers. (Table 4.11).

b. Patients from St. Margaret's Hospital and Dryburn Hospital medical wards.

This group includes most of the persons aged over 65 years in the sample. Inspection of Table 4.2 will show that almost all were suffering from some condition that is prevalent in the general population of that age group, i.e. most were suffering from some kind of degenerative disease.

TABLE 4.11: The results of a comparison of the blood group and isoenzyme phenotype frequencies of (i) 'normal' adults and (ii) orthopaedic patients.

	sample size (i)	sample size (ii)	X^2	d.f.	P
ABO	225	44	11.20	3	.011
A_1A_2BO	225	44	11.75	4	.019
Rhesus(D)	225	44	2.53	1	.112
MN	209	44	7.83	2	.020
S	216	39	1.87	2	.393
Duffy	220	41	3.62	2	.163
Kell	225	44	1.97	1	.161
Penney	225	44	0.00	1	.988
P_1	224	44	1.43	1	.232
Haptoglobin	216	20	5.57	2	.062
Phosphoglucomutase	215	26	2.15	2	.341
Adenylate kinase	223	26	0.05	1	.819
Acid phosphatase	215	26	3.92	3	.270
Esterase-D	215	25	0.14	1	.708

It is recognised, of course, that many of these conditions may have at least a partly genetic aetiology, but this does not mean that the individuals are not representative of the population from which they derive. Not represented in the sample are those individuals of this age group who are never admitted into hospital. From discussions with the hospital staff it appears that the prolonged stay in hospital of many of the patients does not necessarily reflect the seriousness of their physical condition but is often at least partly due to their personal circumstances.

A more scientific justification for including this group in the sample comes from a comparison of their blood group and enzyme phenotype frequencies with those of a similarly aged subgroup of the diabetic controls. There were only two instances of a significant difference in these frequencies, namely in the Rhesus (D) and Acid phosphatase results. In the case of Acid phosphatase this difference was observed when the two total samples were compared, and therefore is very unlikely to be indicative of anything out of the ordinary about the over-65's. No explanation is offered in the case of the Rhesus (D) results, but it must be remembered that significant findings are expected by chance alone when multiple comparisons are made. (Table 4.12).

TABLE 4.12: A comparison of blood group and isoenzyme phenotype frequencies in (A) elderly hospital patients, (B) total sample of over-60 year olds, and (C) over-60 years old subgroup of control group in diabetic study (Williams 1977).

	A		B		C	
	No.	%	No.	%	No.	%
1. A_1A_2BO						
A_1	45	(25.0)	53	(26.5)	44	(27.5)
A_2	15	(8.3)	17	(8.5)	12	(7.5)
O	86	(47.8)	91	(45.5)	87	(54.4)
B	28	(15.6)	32	(16.0)	14	(8.8)
A_1B	5	(2.8)	6	(3.0)	3	(1.9)
A_2B	1	(0.6)	1	(0.5)	0	
Total	180		200		160	
2. Rhesus(D)						
D+ve	154	(85.6)	172	(86.0)	121	(75.6)
D-ve	26	(14.4)	28	(14.0)	39	(24.4)
Total	180		200		160	
3. MN						
M	59	(32.8)	64	(32.0)	36	(25.7)
MN	96	(53.3)	110	(55.0)	89	(63.5)
N	25	(13.9)	26	(13.0)	25	(17.8)
Total	180		200		140	
4. S						
S	26	(15.1)	27	(14.3)	8	(10.1)
Ss	82	(47.7)	92	(48.7)	39	(49.4)
s	64	(37.2)	70	(37.2)	32	(40.5)
Total	172		189		79	
5. MNS						
MS	12	(7.0)	13	(6.9)	5	(6.4)
MSs	30	(17.4)	33	(17.5)	6	(7.7)
Ms	13	(7.6)	13	(6.9)	7	(9.0)
MNS	14	(8.1)	14	(7.4)	3	(3.8)
MNSs	46	(26.7)	53	(28.6)	28	(35.9)
MNs	34	(19.8)	40	(21.2)	17	(21.8)
NS	0		0		0	
NSs	6	(3.5)	6	(3.2)	5	(6.4)
Ns	17	(9.9)	17	(9.0)	7	(9.0)
Total	172		189		78	
6. Duffy						
Fy^a	31	(18.2)	37	(19.8)	8	(11.3)
Fy^aFy^b	81	(47.6)	86	(46.0)	35	(49.3)
Fy^b	58	(34.1)	64	(34.2)	28	(39.4)
Total	170		187		71	
7. Duffy (tested with anti-Fy^a only)						
Fy^a+ve	112	(65.8)	123	(65.8)	96	(62.7)
Fy^a-ve	58	(34.1)	64	(34.2)	57	(37.3)
Total	170		187		153	
8. Kell (tested with anti-K only)						
K+ve	10	(5.6)	10	(5.0)	13	(8.2)
K-ve	170	(94.4)	190	(95.0)	146	(91.8)
Total	180		200		159	

	χ^2	d.f.	P
1. A_1A_2BO			
A v. C	4.86	4	.302
B v. C	6.02	4	.198
2. Rhesus(D)			
A v. C	4.78	1	.029
B v. C	5.65	1	.017
3. MN			
A v. C	3.54	2	.170
B v. C	3.21	2	.201
4. S			
A v. C	1.18	2	.554
B v. C	0.92	2	.631
5. MNS			
A v. C	7.87	7	.344
B v. C	7.59	7	.371
6. Duffy			
A v. C	1.93	2	.381
B v. C	2.65	2	.265
7. Duffy (anti-Fy^a only)			
A v. C	0.22	1	.637
B v. C	0.22	1	.641

TABLE 4.12 contd.:

	A No.	A %	B No.	B %	C No.	C %
9. Penney (tested with anti-Kp^a only)						
Kp^a+ve	0		1	(0.6)	2	(1.8)
Kp^a-ve	154	(100.0)	173	(99.4)	109	(98.2)
Total	154		174		111	
10. Haptoglobin						
1 - 1	20	(11.3)	21	(11.3)	8	(25.8)
2 - 1	90	(50.8)	96	(51.6)	16	(51.6)
2 - 2	67	(37.9)	69	(37.1)	7	(22.6)
Total	177		186		31	
11. Adenylate kinase						
1 - 1	168	(93.9)	175	(93.1)	125	(91.2)
2 - 1	11	(6.1)	13	(6.9)	12	(8.8)
Total	179		188		137	
12. Acid phosphatase						
A	19	(10.6)	20	(10.6)	13	(20.3)
BA	63	(35.2)	67	(35.6)	31	(48.4)
B	85	(47.5)	88	(46.8)	17	(26.6)
BC	9	(5.0)	10	(5.3)	2	(3.1)
CA	3	(1.7)	3	(1.6)	1	(1.6)
Total	179		188		64	
13. Phosphoglucomutase						
1 - 1	124	(70.9)	130	(70.7)	25	(61.0)
2 - 1	49	(28.0)	51	(27.7)	14	(34.1)
2 - 2	2	(1.1)	3	(1.6)	2	(4.9)
Total	175		184		41	
14. Esterase-D						
1 - 1	140	(79.5)	148	(80.0)	109	(80.7)
2 - 1	31	(17.6)	32	(17.3)	23	(17.0)
2 - 2	5	(2.8)	5	(2.7)	3	(2.2)
Total	176		185		135	

	χ^2	d.f.	P
8. Kell			
A v. C	0.55	1	.459
B v. C	1.01	1	.316
9. Penney			
A v. C	0.91	1	.341
B v. C	0.16	1	.693
10. Haptoglobin			
A v. C	5.86	2	.053
B v. C	5.79	2	.055
11. Adenylate kinase			
A v. C	0.45	1	.504
B v. C	0.16	1	.685
12. Acid phosphatase			
A v. C	10.73	3	.013
B v. C	10.49	3	.015
13 Phosphoglucomutase			
A v. C	3.70	2	.182
B v. C	1.91	2	.384
14. Esterase-D			
A v. C	0.14	2	.932
B v. C	0.08	2	.961

Special ethical considerations concerning the method of collection of blood specimens from newborn infants and hospital patients.

It is a very simple matter to explain the need for, and to obtain permission for, the taking of a sample of venous blood from an adult before that sample is taken. However, the need for prior permission to take a sample caused problems in the case of the cord blood samples. In many cases the mother entered hospital only a short time before actual delivery of the child, and therefore the blood specimen was collected before an opportunity to request permission could arise. It was suggested by the hospital staff that they should collect the specimen as soon as possible after birth of the child, and that I should obtain permission to use the specimen from the mother at the same time as I collected the demographic information from her. In this way, although some cord blood would be collected from the afterbirth, it would not be passed on to me for my use without the agreement of the parent. Despite this arrangement, it did happen that in a small proportion of cases I was unable to see the mother on the day in which I collected the blood specimen, and therefore had to establish cell cultures without her knowledge. In almost every instance the parent appreciated the difficulties, and all agreed to my continuing use of the specimen.

It often seemed to be a fairly unsatisfactory situation, and perhaps the possibilities for obtaining prior permission from the parents should have been looked into more seriously.

It is possible that the immediate post-natal period can be a somewhat worrying and stressful time for many mothers. One could question whether or not approaching individuals ignorant of normal genetic

variability, with a view to performing a series of tests on the blood of their approximately day-old offspring was causing unnecessary concern to them. Most mothers appeared to be satisfied with the short description of the research given on the form that they were asked to complete, and usually it was only necessary to repeat this information in answer to their questions. Occasionally, I was asked for a further explanation as it was obvious that not all the infants born at the hospital were being studied. As far as I could tell, all those who asked about this were satisfied with the explanation that inclusion of particular infants in the study did not depend upon any characteristic of those infants.

No parent expressed any interest in the results of the inquiry. Only one abnormal chromosome constitution was detected, that of a 47, XYY boy. In this case the paediatrician was informed, but as far as I am aware, no particular note was taken of the fact.

As previously stated, blood specimens from the group of elderly patients were taken at the same time as specimens were taken for routine hospital tests. Permission was not asked of the individuals for this to be done. The concensus of opinion of all involved in the operation was that the chances of explaining the purposes of the research to even a small number of the patients, and being understood, were only slight. Therefore responsibility for this permission was taken by Dr. Yell (Senior Medical Consultant at Dryburn hospital). This procedure was accepted and approved by the Durham Area Health Authority Ethical Committee (as were all other procedures concerning the collection of blood samples from hospital patients).

LABORATORY TECHNIQUES.

1. Collection of Blood Specimens.

(i) Cord blood specimens. As soon as possible after delivery of the child, a member of the nursing staff transferred 5 to 20 ml of blood from the umbilical cord to a sterile glass Universal bottle containing approximately 2 drops of heparin without preservative. The blood was then stored in a refrigerator at 4°C until it was collected the following morning. Cultures were established almost always within an hour or an hour and a half of the blood being removed from the refrigerator. If this was not possible, the blood was again stored, in the laboratory, at 4°C.

The specimens were collected from the hospital only once a day and therefore they were sometimes nearly 24 hours old before they were used either for cell cultures or for red cell grouping. As far as could be discerned, this does not seem to have had a detrimental effect on either of these two processes.

Because of the method of collecting the blood, there was a fairly high risk of contamination, but this was not often encountered, and was not noticeably more often in the cord blood than in the venous blood.

Shortly after she had given birth the mother had been given a form by a member of the nursing staff explaining the research project (Form 1.1, Appendix 1 (see above page 90)). The completed forms were collected each morning together with the blood specimens.

(ii) Venous blood specimens.

These were collected in the same type of bottle as mentioned above. About 10ml of blood was taken. This provided sufficient material for two types of cell culture, red cell

grouping, and for samples of the serum and a red cell lysate to be deep frozen for later use.

Generally, these samples were much fresher than the cord blood samples when cultures were set up, as it was more easily arranged for the blood to be taken at convenient times.

All individuals, with the exception of patients at St. Margaret's hospital and those from the medical wards of Dryburn hospital, were asked to sign a form granting permission for the blood sample to be used in scientific research, and they were also asked to provide certain demographic information about themselves.

2. Culturing the Lymphocytes.

The culture methods used were modifications of the usual lymphocyte and whole blood methods given by Moorhead et al.(1960) and Hungerford (1965).

Three blood cultures were established from each specimen, at least one being a whole blood culture. The number of lymphocyte cultures established depended on:

(i) the amount of blood available, and
(ii) the rate of settling of the lymphocytes.

From the cord blood samples only whole blood cultures were set up. The cells were cultured in the same type of sterile Universal bottle as was used when collecting the specimen.

The procedure followed for culturing the lymphocytes is as follows:
1ml of penicillin/streptomycin mixture is added to the contents of a 100ml bottle of culture medium. 22ml of this is then drawn up into a 20ml syringe. 8ml is placed in each of two Universal bottles and the remaining 6ml in a

in a third. 1ml of foetal calf serum is added to this latter bottle and, optionally, to one of the others. 0.3ml phytohaemagglutinin(PHA) is added to each bottle.

The plasma from the settled blood specimen is drawn up slowly using a syringe and wide-bore needle (19G). The needle is skimmed over the surface of the red blood cells, as the lymphocytes tend to settle at this interface.

The needle is removed from the syringe and half the plasma is added to each of the two lymphocyte cultures.

A clean needle is used on the same syringe to draw up about 5ml of whole blood. 10 drops of this (6-8 in the case of cord blood) are added to the third bottle. The rest of this blood is placed in a centrifuge tube and centrifuged at 2000rpm for 10 minutes. The serum is removed and stored immediately at -20°C. The remaining red cells are washed twice in cold(4°C) normal saline. A small quantity of these are resuspended in saline to be used for red cell grouping. The rest are deep-frozen and from them haemolysates are prepared by the carbon tetrachloride method at a later date.

The whole blood which remains in the original Universal bottle is stored at 4°C until the outcome of the cultures is known three days later. It is used to establish repeat cultures if these are necessary. When repeat cultures are set up they are always of the whole blood type, as there is usually little blood available.

The cultures are incubated at 37°C in a water-bath for 68-72 hours, and shaken twice a day. 0.3ml Colcemid or colchicine is added to each culture for the last hour and a half of this period.

3. Harvesting the Cell Cultures.

The cultures are centrifuged at approximately 3000rpm for 5 minutes in the Universal bottles. The supernatant is

removed and approximately 10 ml warmed (37°C) 0.3% KCl solution is added to each. The bottle is shaken and replaced in the water-bath at 37°C for 15 to 20 minutes.

At the end of this time, the cell suspension is transferred to a glass conical centifruge tube and centifruged for 5 minutes at 1000rpm.

The supernatant is removed and approximately 1.5 ml chilled freshly-prepared fixative is added slowly to the cell suspension, whilst this is being agitated.

The centifruging and adding of fixative is repeated three times more. These cell suspensions are then ready for use. Slides may be prepared immediately, or the fixed cells may be stored for up to 24 hours at 4°C.

4. Slide Preparation.

In this study slides were prepared by both the flame-drying and air-drying techniques. Usually, one slide was made up from each of the three cultures from each blood specimen. These were then examined under phase-contrast to determine which of the three cultures had had the most successful growth. Further slides were then prepared only from successful cultures.

The slides were stored in a metal cabinet at room temperature usually for a period of 1 to 2 weeks before being stained. The earliest slides, however, were stored for up to 3 months before being examined. Several of these had to be discarded because of deterioration, but some were found still to be useable.

5. Microscopic Analysis and the Criteria for Scoring the Variants.

The slides were stained for at least 15 minutes in a 0.3% solution of quinacrine dihydrochloride (Atebrin) in

methanol, rinsed in distilled water, and mounted in distilled water using a glass coverslip which was sealed with clear nail varnish.

The fluorescence of the stained chromosomes was observed using a Vickers M17 microscope fitted with a 50W mercury vapour light source and appropriate filters. The photographic equipment was by Nikon.

For photography of Atebrin-stained preparations Kodak Tri-X film was used with an exposure time of 30 to 40s.

Cells were examined to determine the level of fluorescence of 24(2x12) regions in the karyotype. These were:

chromosome 3. A band adjacent to the centromere found almost always on the long arm, occasionally on the short;

chromosome 4. The centromere region;

chromosome 13. The centric region and the satellites;

chromosomes 14,15,21 and 22. The short arms and the satellites.

(Following the convention set by the Paris Conference (1971) the variable region near the centromere of chromosome 13 will be referred to in the presentation of results as the short arm.)

The Paris Conference of 1971 recognised 5 levels of fluorescence intensity of chromosome bands after staining with quinacrine dihydrochloride. These 5 levels are in fact arbitrary subdivisions of a continuous range of intensity. In practice, it is very difficult to distinguish between the lower levels of intensity by eye. In this study such distinction was not attempted; the three lower levels (negative, pale and medium) being grouped together as 'negative'. Thus, the variant regions were classified as being negative, intense or brilliant.

Analysis of the chromosomes was based on intra-cellular

comparisons of several regions with each other. The criteria used to define the levels of intensity were (following the recommendations of the Paris Conference):

(i) A region is judged to be intense if it fluoresces as brightly as the distal half of the long arm of chromosome 13, but not as brightly as the distal part of the Y chromosome.

(ii) A region is judged to be brilliant if it fluoresces as brightly as the distal part of the long arm of the Y chromosome.

(iii) Paler bands are grouped together as negative.

At least ten cells were examined per individual. The procedure was to record from the first cell examined the level of intensity of as many of the variable regions as could be distinguished. With this staining method, fading of the fluorescence occurs fairly rapidly after 3 to 4 minutes and it is almost always impossible to make a decision about all regions from the same cell. Each classification was confirmed in at least three other cells before it was accepted as definite. Where there was indecision about the classification of a particular region, more cells were examined until such a decision could confidently be made. The number of cells analysed, and the amount of attention paid to any particular region was very variable, as fluorescence is influenced greatly by the quality of many aspects of slide preparation.

The cells chosen for analysis were, if possible, those showing the 'B features' as defined by the Paris Conference. That is, the chromosomes were extended, straight, with well-defined bands visible on all chromosome arms. Generally, the poorer the quality of the cell (or 'spread'), the more cells had to be examined before a final decision could be made about all variant regions. McKenzie and Lubs (1975) found a higher frequency of fluorescence polymorphisms in good cultures than in poorer ones.

At least one cell per individual was photographed, providing a permanent record of all fluorescent patterns, and also providing a means of checking the consistency of standards of scoring throughout the year.

The Success Rate of Cell Cultures.

Blood specimens from 710 individuals were collected. Of these 710, 670 eventually provided sufficient metaphase cells of the quality required for fluorescence analysis. Most of the culture failures occurred in the cord blood series (237 successful cultures out of 261 samples collected).

There may have been several reasons for the comparative lack of success in this series:

(i) The presence in the cord blood of quantities of Wharton's jelly which may interfere with the growth of the lymphocytes.

(ii) Unfavourable proportions of lymphocytes susceptible to stimulation by PHA.

(iii) The cultures may have been left too long before harvesting. Cultures from all sources were left to grow for approximately 68-72 hours in this study. However, there is evidence to suggest that growth rates in cord blood lymphocytes and venous blood lymphocytes are not the same. Hatcher and Hook (1976) have reported that in the case of cord blood samples the highest mitotic index was to be found in cultures harvested after only 54 hours and that by 72 hours 37% ±20.03% of cells were in their 3rd mitotic division. In adults venous blood samples the highest mitotic index occurred after 60 hours growth.

6. Red Cell Grouping.

The methods used for determining the phenotype of the various red cell antigen systems have been given in sufficient

detail elsewhere (Mitchell 1974, Williams 1977). The particular serological technique employed by any system varied with the type of antisera available: that is, whether the antibodies were complete or incomplete, and what were the optimum conditions for agglutination. The antisera were obtained from a variety of sources. The following techniques were used:

Saline, tile, room temperature.	for example, for A and B.
" " $4^{o}C$	A_1 and P_1.
" tube, room temperature.	M and N.
Indirect Coombes	Fy, Kell, Kp and S.
Albumin, tube	all Rhesus antigens
Papain, tube	e and C^w.

7. Starch Gel Electrophoresis.

The methods used were again the same as those used at other times in this laboratory (Williams 1977). There was one change made to the methodology - a different staining technique was used for Haptoglobin. Details of this are given below.

Staining Method for Haptoglobin

100 ml glacial acetic
150 ml H_2O
1 gm leuco-malachite green
1 hand full of zinc dust powdered.

Boil the above in a 1 litre beaker until the green colour of the leuco-malachite green has disappeared. This is then stored in the cold room until required.

The above mixture is poured over the sliced gel and left for 5 mins. This is then poured off and 10 ml of $\frac{1}{10}$ 20 vols H_2O_2* is poured on the gel. The bands then appear after a few minutes.

* dilute 20 vols H_2O_2 1 part H_2O_2 9 parts H_2O

Details of reageants and equipment used in cell culture.

The following materials were obtained from Gibco Biocult Ltd:

1. Penicillin/Streptomycin mixture. 5000 units Penicillin and 5000g streptomycin per ml. in normal saline.
2. Culture medium.
   S-MEM Minimum Essential Medium (Eagle) with Earle's salts, with L-glutamine.
3. Phytohaemagglutinin (M.form). Lyopholized.
4. Colcemid. 10 g/ml in Hank's balanced salt solution.
5. Foetal calf serum.

Other materials used were:

1. Heparin. 1000 units/ml from Weddel Pharmaceuticals.
2. Phytohaemagglutinin (Reagent grade) from Wellcome Reagents Ltd.
3. Colchicine from Searle Diagnostics (Gurr Products).
4. Sterile water from Evans (Medical) Ltd.
5. Hypotonic solution. A 0.3% solution of KCl.
6. Fixative. A mixture of 3 parts methanol with 1 part glacial acetic acid.

The following equipment was used:

1. Disposable syringes (Sabre) sizes 20 ml, 10 ml, 5 ml and 1 ml.
2. Disposable needles (Sabre Gillette) sizes 19G, 21G and 25G.
3. Glassware. Universal bottles with the rubber cap liners replaced by silicone wads and conical centifruge tubes.

All glassware is soaked in concentrated nitric acid and washed before being used for the first time. The cleaning procedure subsequently is to soak all glassware for at least 24 hours in the biological detergent Haemosol, rinse each item approximately 20 times in running tap water and soak for half an hour in each of two changes of distilled water and one change of deionised water.

The glassware is then dried in a drying oven and the bottles are sterilised in a steam autoclave.

4. All sterile procedures were carried out at a Hepaire Lamina Flow work-station.

Stains.

The stains used were of two types:

1. Atebrin from Searle Diagnostics (Gurr Products)
2. Quinacrine dihydrochloride from Sigma Chemical Co.

Preparation of the Data for Analysis by Computer.

The bulk of the analysis of these data was carried out using the N.U.M.A.C. facilities available at the Durham University Computer Unit.

Information regarding (a) demography, (b) chromosomal variability, (c) red cell antigens and (d) isozyme phenotypes was recorded on two separate data sheets, the first containing (a) and (b) and the second containing (c) and (d).

The design of the first sheet was a fairly standard one in this department, the various details being entered in as simple a coded form as possible. The second sheet was specially designed so that blood group results could be recorded directly, thereby saving both time and paper. There was space on this sheet for electrophoresis results also to be recorded, but in the event, these were usually added later by editing the file. The format of this second sheet made it possible to use codes for particular blood group phenotypes which had self-evident meaning. This greatly reduced the effort required to code the information and at the same time reduced the likelihood of making errors in coding.

Examples of the data sheets and codes used for coding the information are given in Appendix 2.

Cards were punched from these sheets by the Computer Unit Punching Service. Errors in punching were located by inspection of a list of the file for obvious errors in format, and by running sample jobs such as S.P.S.S. 'frequencies' to detect any unusual or impossible values for a given variable.

Obviously, by this method of checking, any wrongful replacement of a figure by another 'legal' value will go undetected, but it is thought that errors of this type will be fairly rare. Cards are checked by the punching service, and probably the effort required manually to ensure that each card exactly copied its data sheet was out of proportion to the effect on the final results of such errors as would have been found.

To insure against the serious consequences of accidental loss or corruption of the date file, the file was also stored on magnetic tape.

Virtually all the computer analysis was done using the Statistical Package for the Social Sciences (S.P.S.S.), versons 6 and 7.

Chapter 5: RESULTS: I. THE RELIABILITY OF THE RESULTS AND THE CONCLUSIONS DRAWN FROM THEM.

1. The Objectiveness of the Methods of Scoring Chromosomal and Other Genetic Markers.

The methodology of studies designed to determine the extent and possible significance of chromosomal variations is critical, especially when it is remembered that these variations are to be scored somewhat subjectively as arbitrary cut-off points in a continuous range of variability. Some control over technical variables is of great importance in ensuring that different workers are dealing with comparable material. There is a considerable chance that similar material will be interpreted differently by different workers. In the absence of a totally objective standardised method of scoring these variants that is not dependent on a fairly narrow range of quality of the material the most that can be hoped for is that consistency within the laboratory is maintained.

The ability to assess the significance of differences in the frequencies of various genotypes in human populations relies on the possibility of comparing a set of results with those obtained by other workers. It has to be assumed that the method of scoring any particular individual is objective and repeatable. In the case of, for instance, blood group and isoenzyme determination, it is possible to make some check that the required standards are being adhered to by, for example, repeating the technique if rare or unexpected results are obtained, and by using control material.

When making comparisons between different sets of data it is worth bearing in mind Selander's finding on estimates of heterozygosity, determined by gel electrophoresis in different species of Drosophila, that "some of the observed species differences may be real, but the major determinant of the span of variation in estimates of polymorphisms is

the laboratory in which the survey was conducted."(Selander 1976). And this is after using one of the most objective techniques available to population geneticists.

As far as blood group results are concerned, if controls are used throughout and the antisera are of acceptable quality, then the results obtained should be quite accurate. Red cell antigens are not, however, direct gene products and sometimes physiological interactions may lead to inaccurate typing. For example, in the MN system, the N antigen is a precursor of the M. MM individuals convert most, but not all, N to M. If sufficient N remains to be detected the frequency of MN individuals will be over-estimated (Mourant et al. 1976).

The P_1 antigen seems to be particularly labile when compared with other red cell antigens. The P system is therefore more susceptible to mistyping than other blood group systems. The antisera available, also, show great differences in their specificites. It is difficult, therefore, to achieve consistency of results within a sample. Even the use of the same batch of antisera throughout the study is not a foolproof method; deterioration with time may occur and it is virtually impossible to ensure that the results obtained in different laboratories are comparable.

When analysing the chromosomal polymorphisms many difficulties were encountered initially in maintaining a sufficiently objective method of scoring. These were caused by the technical variability of the slide preparations, and as the underlying biochemical basis for the staining reaction is still unknown, it was very difficult to make allowance for the effects of variations in the several stages of preparation of the slides. An attempt was made to alleviate at least some of these problems by the use of intracellular comparisons for assessing the fluorescence of particular regions. For example, deterioration of the preparations in storage, low quality of preparations and inter+intra

slide variations in staining intensity should not, by this method, be a source of bias in the results.

Other problems that were less open to solution by this means, and which possibly became a source of subjectivity in the scoring were as follows:

1. The distinction between the intense and brilliant levels of fluorescence was initially more difficult to make in females, without the presence of the Y chromosome for comparison.

2. Most of the regions which varied in intensity of fluorescence also showed quite considerable variations in size. These were not measured in this study but they may have had some influence on scoring. For example, a particularly small intense region was more likely to go unnoticed than one of medium size. Conversely, a particularly large intense region could be in danger of being scored as brilliant because of its obviousness, especially if there were no other brilliant regions in the cell.

3. The intensity of the bands immediately adjacent to the variable region affects the apparent intensity of the variable region. For instance, an intense region which lies adjacent to a dull region is much more likely to be recognised as intense than one lying adjacent to a region which is itself fairly bright. For example, it was very difficult to decide about the intensity of short arms of chromosome 22, as bands showing any fluorescence at all were brighter than adjacent regions.

4. Satellites in conventionally stained chromosome preparations seems to show variability in their presence from cell to cell. Whether this is true variability in presence or merely a reflection of the physical state of the satellite material is unknown. Bostock and Sumner (1978), when describing chromosome morphology, state that if a

secondary constriction is subterminal it results in the appearance of a satellite. This seems to imply that satellites are composed of material that is much like that of the rest of the chromosome. If this is true it is difficult to see why they show much more morphological variability than other parts of the chromosomes. Unlike the Y chromosome, which also shows much variability in size, even the largest satellites do not appear to be banded. As far as scoring the intensity of the satellites is concerned, the question arises as to whether or not satellites should be considered intense if, for as yet undetermined reasons, they are not observed in all cells examined.

5. When analysing the chromosomes, allowance is made, as mentioned above, for differences between cells in overall staining intensity. It is assumed that staining within the cell is uniform over all the chromosomes. The fluorescence fades after a few minutes of exposure to ultra-violet light. It is not know whether or not the rate of fading is uniform over all the chromosomes within the cell, or for all the original levels of fluorescence in the cell. That is, are the differences between negative and intense, and intense and brilliant maintained during this fading? Such questions have an obvious effect on the accuracy of the scoring.

6. A similar problem is caused by the possibility that the differences between levels of fluorescence are affected by the changes occurring during mitosis. This difficulty should be overcome by choosing for examination only cells that are in a similar stage of contraction, etc.. This is not always possible, however, as some cultures may consist entirely of cells which are not in the optimum state for analysis.

7. It was found (see below, page 152) when comparing the 'phenotype' frequencies of the chromosome variants with those expected from Hardy-Weinberg predictions, that the more common variants showed an excess of heterozygotes more often

than the rarer ones. The question obviously arises as to whether this may have been caused by errors in scoring. The method used to score the variants (by comparing different chromosome regions in the cell) may have led to overscoring of heterozygotes. It is possible that awareness of small differences between two homologues that should both in fact have been placed in the intense category, has led to only one of the homologues being so scored.

It was intended that some of the more difficult cases would be resolved by consulting the photographs that were taken of at least one cell per individual before fading of the fluorescence was too marked. However, it was found that distinctions between different levels of intensity were made more easily by direct observation of the slide; the photographs sometimes showing as much, but never more detail than had already been noticed.

An unavoidable source of error in the final frequencies that could not be compensated for is that naturally, the first slides were examined by a very inexperienced cytogeneticist, whereas the later slides were examined by a person who had devoted many hours to distinguishing between intense and brilliant bands. All series were examined concurrently, and therefore this type of error should affect all subgroups of the sample equally, and a second opinion from another observer was called for when there was difficulty in these earlier slides.

Mosaicism.

It is quite common for persons having some abnormality of the chromosomes in fact to have tissues composed of more than one cell-line. (Jacobs 1972, Bochkov et al. 1974). This might indicate that mosaicism is fairly common in the human population generally (Nielsen 1975), but also probably it is a reflection of the fact that persons having had some

sort of chromosomal abnormality are more likely to survive (and be later examined) if only some of their cells are affected. Additonally, the abnormality could have arisen at some stage later than the first division of the zygote, in which case the original cell-line may still be present. The proportion of cells from different cell-lines in the various tissues and organs probably varies quite considerably, some consisting entirely of one cell-line, others of another, depending on such factors as time of differentiation of the tissue in question, and also on whether or not selective elimination of particular cells takes place during differentiation of organs and tissues.

In this study, as in most studies on chromosomal variability, only one tissue was studied - the lymphocytes of peripheral blood. It is not known if this is representative of the total cell-line composition of the body.

The possibility that any of the individuals in this study were mosaics was not considered here for the following reasons:

1. Though a score could have been attached to each variant to indicate in what percentage of cells examined it was observed, it was felt that in most cases failure to detect a variant in all cells was probably due to technically-caused variations in the quality of cell preparations.

2. The number of cells which would need to have been examined in order to eliminate the possibility of various levels of mosaicism was prohibitively large - especially when the target sample size was considered. For example, in order to exclude mosaicism of say, 10% with 95% confidence, 29 cells per individual would need to be analysed (Hook 1977).

2. Analysis of the Data and the Statistical Tests Used.

(i) Calculation of gene frequencies.

With no real evidence to support the assumption that any given region of a chromosome showing similar fluorescent behaviour as the same region on the homologous chromosome is qualitatively identical with this latter region, the assumption is nonetheless made, and the three different levels of fluorescence recognised here are treated as three 'alleles' throughout the analysis. In fact, regions showing the same level of fluorescence but found in different parts of the karyotype, are considered to have something in common such that it is sensible to consider them together for some aspects of the analysis. This is perhaps a fair assumption to make as different sequences of DNA are possibly similar if not identical, if they show a similar response to a particular stain.

Frequencies of chromosome variants, and of other genetic systems in which all alleles could be detected (MN, S, Duffy, Kell, Penney and all isozyme systems) were calculated by gene-counting methods. Rhesus gene frequencies were estimated using a 'least-square' method computer program. ABO and MNS gene fequencies were calculated by assuming that the genotypes are in the proportions expected under Hardy-Weimberg equilibrium, using formulae given in Mourant et al. (1976).

(ii) Some comments of the possibility of drawing any biologically meaningful inferences from these data.

In this study chromosomal variability may be described, and with appropiate statistical techniques, differences in the various subsamples with regard to the extent and type of this variablity may be examined. In such a study tendencies which exist may or may not be observed for statistical reasons, but the real underlying biological causes and effects will not be detected with this limited amount of information.

The biological complexity of an individual is not indicated by the genotypes at a few loci, and yet it is this entire biological make-up which is open to the effects of the evolutionary processes that we are attempting to comprehend. Lewontin (1974) has referred to an 'epistemological paradox' which confronts anyone involved in the significance of the genetic variability of populations, that is, "What we can measure is by definition uninteresting and what we are interested in is by definition unmeasurable."

Whether or not variations in chromosome content examined in this study are as, or more, interesting than the individual genetic loci usually available for analysis remains unknown until some idea of their possible function and influence on cell physiology comes to light.

(iii) Some comments on the statistical tests used and their validity.

Various statistical tests were available for use in detecting the magnitude and direction, and significance of these, of the association between variables. Each test has certain advantages and disadvantages. In the analysis of these data there were the following requirements:

a. Some measure of the association between the distribution of frequencies of values of one variable with the distribution of frequencies of another.

b. Some method of determining whether such an association was directional or simply 'nonrandom'.

c. That such tests be independant of the distribution of the variables.

d. Some measure of correlation that depended only on the ability to rank the values of the variables, and not on the variables being, even theoretically, continuous.

e. An ability to assign confidence limits to all correlation and association scores.

Initially deviations from chance expectations caused

by any particular factor such as age, sex or presence of another genetic marker, were tested for by using the X^2 * test. This test fulfils many of the requirements given above, but gives no indication of the strength or direction of any association, and also has the disadvantage that rare classes must be pooled. The arbitrary decisions that need to be made when pooling classes have an uncertain effect on the ability to make predictions from the results obtained, and to make analyses over many populations (Hill 1976). Usually classes are pooled simply by virtue of their being individually of low frequency, though it is sometimes possible to devise a manner of pooling which makes a certain amount of common sense.

For those characters where the different classes could be ranked (for example, the number of intense bands on a particular homologue pair, the total number of satellites present in a karyotype or age) a nonparametric correlation coefficient (Spearmann's rho or Kendall's tau was employed, as neither the chromosomal nor the demographic variables satisfied sufficiently the distribution requirements of the more usual parametric statistics.

In some cases the t-test was applied (when required) as it has been said that this test is sufficiently robust to accommodate a certin amount of deviation from normality of distribution of values (Williams 1978).

The Mann-Whitney U test was applied to the same type of data as the t-test, special note being taken when the t-test gave a probability value which lay on the borderline of conventional significance.

The data were not transformed, as it seems (Williams 1978) that although this action removes a source of error

* In this thesis the typed symbol X^2 is used throughout to indicate "chi-squared."

from the calculations and improves the probability estimate, application of a non-parametric test takes the process a step further and is yet another improvement. (That is, the probability estimate arrived at by a t-test on transformed data lies between the estimate obtained from a t-test on untransformed data and an estimate from the Mann-Whitney U test.) Therefore the latter was applied when it was felt that the t-test was inappropriate.

Analysis for trends across age classes was performed using the method suggested by Cox, cited and described in Williams (1977).

For all analyses of the effect of a demographic variable, the sample was divided into subsamples according to other variables which might be thought to confuse any relationship. For example, in the detection of any trends with age, analysis was performed on each of the two sexes separately, as well as on the sample as a whole. Similarly, any sex differences were investigated in different age strata of the sample.

For interpretation of the results of analysis of association and correlation between variables it is necessary to assign significance levels to the value of any statistics obtained. It is common in studies of this nature to accept a statistic as indicative of biological significance if its probability of occurring by chance alone is less than or equal to 0.05. However, as the number of independent comparisons made between subsamples rises, so does the chance occurrence of association between two variables which is significant at the confidence level previously decided upon (Wiener 1970). Therefore it must be remembered to allow for this and adjust P values accordingly.

RESULTS.II. PHENOTYPE AND GENE FREQUENCIES.

Introduction.

Table 5.2 shows the phenotype and gene frequencies of all the genetic markers investigated in this study. Also shown are the results of various statistical analyses of the data. All phenotype frequencies were tested for agreement with Hardy-Weinberg expectations, except where gene frequency calculations were based on the assumption that such equilibrium exists.

Genetic marker frequencies are not reported for each series which comprised the total sample, and these subdivisions were not used for later analyses involving demographic variables, because, as was shown earlier (page 103) groups of individuals of similar age and sex in the different series were not found to be genetically distinct.

Blood Groups, Serum Proteins and Isoenzymes.

Blood group and isoenzyme phenotype frequencies were examined with a view to determining the "representativeness" of the sample studied. The existence of the Williams (1977) report was particularly valuable for this purpose, as the control group therein was drawn from the same geographical area as the present study sample.

Other studies used for comparison purposes were those which sample populations geographically as close as possible to this one. There were available reports of populations which overlapped that of the present study (Kopeć 1970) and others from adjacent regions (Cartwright 1973, Cartwright et al. 1976, Papiha 1973, 1974; Mitchell 1974). For those markers for which no such reports were available, comparisons were made with samples drawn from further afield. However,

as the populations were quite diverse, geographically-related genetic differences might be expected to occur. These series are listed in table 5.1.

Many of these samples consist entirely, or partly, of blood donors. Fears have often been expressed that the use of this group in genetic studies may introduce bias to the results. Some evidence that these fears are justified exists (Mitchell 1974), but Kopeć (1970) found no difference between the blood group frequencies of air force men and those of blood donors. In the present study the adults were found to have a higher frequency of blood group B than a sample of blood donors drawn from a similar area.

The extent of the variability of blood groups and isoenzymes within and between different subgroups of the sample was examined with a view to contrasting this with the patterns of within - and between - subgroup chromosomal variability.

A. Blood Groups.

1. The ABO System.

The results are given with and without subdivisions of group A.

(i) With subdivision:

The adults of the present study were found to have similar frequencies to all the comparative series, two of which were composed of blood donors.

(ii) Without subdivision:

This sample was found to have similar frequencies to those found by Williams (1977), but significantly different frequencies from those found by Kopeć (1970). The main difference lay in a higher frequency of the B gene in the present sample. This gene frequency was also

TABLE 5.1: Key to reports quoted in table 5.2

Series	Reference	Population	No.
Williams	Williams (1977)	'Normal' adult control group in Diabetic survey. County Durham.	462
Kopeć A	Kopeć (1970)	Adult blood donors. New County Durham.	6520
Kopeć B	Kopeć (1970)	Adult blood donors. Old County Durham.	20030
Mitchell	Mitchell (1974)	Adult blood donors and school-children. Cumbria.	521
Sanger	Sanger and Race (1951)	English.	229
Race	Race et al. (1948)	English.	1073
Thomas	Thomas and Hewitt (1939)	Hertfordshire controls in health and mental disease study.	900
Wendt	Wendt and Theile (1963)	German.	108
Cleghorn A	Cleghorn (1960)	English blood donors.(south east)	1000
Cleghorn B	Cleghorn (1965)	English (south east).	656
Giles.	Giles (1964)	British and European.	359
Ikin A	Ikin et al. (1952)	English.	1166
Ikin B	Ikin et al. (1954)	English.	1166
Dichupa	Dichupa et al. (1969)	Blood donors from Manitoba, Canada ('nearly all white')	11239
Shreffler	Shreffler et al. (1971)	Whites of west European origin in Michigan, U.S.A.	8443
Papiha A	Papiha (1973)	Adults. Northumberland.	549
Papiha B	Papiha (1974)	Adults. North-east England.	761
Cartwright A	Cartwright (1973)	Adults. Northern England.	206
Cartwright B	Cartwright et al. (1976)	Newborn infants. Hartlepool.	464
Harris	Harris et al. (1959)	English.	179
Hopkinson A	Hopkinson and Harris (1966)	English.	2110
Hopkinson B	Hopkinson and Harris (1968)	English (south).	880
Hopkinson C	Hopkinson et al. (1973)	British (European).	867
Rapley	Rapley et al. (1967)	British.	1187

TABLE 5.2: Blood group and isoenzyme phenotype and gene frequencies, and results of comparisons with published reports.

1. ABO phenotypes.

	a. Infants		b. Adults		c. Total		d. Williams		e. Kopeć A		f. Kopeć B	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A	108	(41.7)	164	(36.5)	272	(38.4)	172	(37.2)	2528	(38.8)	7916	(39.5)
O	120	(46.3)	215	(47.9)	335	(47.3)	237	(51.3)	3201	(49.1)	9698	(48.4)
B	27	(10.4)	57	(12.7)	84	(11.9)	39	(8.4)	568	(8.7)	1784	(8.9)
AB	4	(1.5)	13	(2.9)	17	(2.4)	14	(3.1)	223	(3.4)	632	(3.2)
Total	259		449		708		462		6520		20030	

Series	X^2	d.f.	P
a v. HW	1.71	1	.19
b v. HW	0.49	1	.48
c v. HW	1.75	1	.19
d v. HW	0.14	1	.71
e v. HW	3.40	1	.07
f v. HW	0.83	1	.36
b v. d	4.49	3	.21
b v. e	8.45	3	.04
b v. f	8.13	3	.04
c v. d	4.54	3	.21
c v. e	9.47	3	.02
c v. f	8.27	3	.04

2. ABO gene frequencies ±1.96 S.E.

A	.2480 ±.0401	.2222 ±.0291	.2316 ±.0236	.2268 ±.0289	.2393 ±.0079	.2428 ±.0045
B	.0621 ±.0211	.0851 ±.0183	.0743 ±.0139	.0590 ±.0154	.0625 ±.0042	.0622 ±.0024
O	.6899 ±.0579	.6963 ±.0441	.6941 ±.0351	.7142 ±.0437	.6981 ±.0116	.6950 ±.0066

3. A_1A_2BO phenotype frequencies.

	g. Adults		h. Williams		i. Mitchell		j. Sanger	
	No.	%	No.	%	No.	%	No.	%
A_1	127	(28.3)	142	(30.7)	160	(30.7)	66	(28.8)
A_2	37	(8.2)	30	(6.5)	65	(12.5)	19	(8.3)
O	215	(47.9)	237	(51.3)	235	(45.1)	115	(50.2)
B	57	(12.7)	39	(8.4)	48	(9.2)	18	(7.9)
A_1B	12	(2.7)	10	(2.2)	12	(2.3)	9	(3.9)
A_2B	1	(0.2)	4	(0.9)	1	(0.2)	2	(0.9)
Total	449		462		521		229	

Series	X^2	d.f.	P
g v. h	5.87	4	.21
g v. i	7.84	4	.10
g v. j	4.95	4	.29

4. A_1A_2BO gene frequencies

	Adults	Williams	Mitchell	Sanger
A_1	.1699	.1806	.1820	.1793
A_2	.0673	.0462	.0816	.0573
B	.0816	.0590	.0606	.0650
O	.6812	.7142	.6758	.6984

numerically but not significantly higher than that found by Williams (1977) which itself was not higher that that found by Kopeć (1970). It is perhaps the smaller size of the Williams sample which leads to the lack of significant differences being found when that sample is compared with both the present sample and the Kopeć sample. The fact that the adult series of the present study shows a higher frequency of the B gene than the total sample suggests that it is not the presence of cord blood samples (the only obvious difference between this sample and the Williams sample) that causes the difference.

It is surprising, perhaps, that this sample is found to differ in ABO frequencies from a sample of blood donors drawn from the same geographical area, when no such differences were found for the A_1A_2BO frequencies between this sample and and a sample of blood donors from a different area of the country. One reason for such differences could be that, in assembling the comparison group for the ABO frequencies results from each postal area were included without weighting to allow for the different proportions of individuals in the present study coming from these areas. Another explanation could be that the sizes of the samples being compared for A_1A_2BO frequencies were insufficient to render any differences which exist significant.

2. <u>The P System</u>.

Doubt has been cast on the technical reliability of subdividing the positive category of the P_1 blood group reactions according to the strength of agglutination (Williams 1977), (see above page 122). In this study, only the positive and negative categories will be considered. Significant or almost significant (at the 5% level) differences were found in the frequencies of P_1-positive individuals in comparisons of the present sample with both the control group of Williams (1977) and a group of

TABLE 5.2 contd.:

5. P_1 phenotype frequencies.

	a. Adults No.	%	b. Williams No.	%	c. Ikin B No.	%
P_1 +ve	310	(69.2)	341	(74.8)	893	(76.6)
P_1 -ve	138	(30.8)	115	(25.2)	273	(23.4)
Total	448		456		1166	

Series	X^2	d.f.	P
a v. b	3.23	1	.07
a v. c	8.93	1	.003

6. P_1 gene frequencies ±1.96 S.E.

	a. Adults	b. Williams	c. Ikin B
P_1	.4550	.4978	.5161
P_2 + p	.5550	.5022	.4839
	±.0456	±.0459	±.0287

7. Rhesus phenotype frequencies.

Reaction C	c	D	E	e		d. Infants No.	%	e. Adults No.	%	f. Total No.	%	g. Williams No.	%	h. Race No.	%
-	+	-	-	+	r r	47	(18.4)	57	(13.8)	104	(15.5)	77	(16.7)	170	(15.8)
+	+	+	-	+	R_1r	88	(34.5)	139	(33.6)	227	(33.9)	167	(36.1)	363	(33.8)
+	-	+	-	+	R_1R_1	39	(15.3)	88	(21.3)	127	(19.0)	77	(16.7)	190	(17.7)
-	+	+	-	+	R_0r	0		12	(2.9)	12	(1.8)	4	(0.9)	19	(1.8)
-	+	+	+	+	R_2r	15	(5.9)	25	(6.0)	40	(6.0)	50	(10.8)	137	(12.8)
-	+	+	+	-	R_2R_2	4	(1.6)	8	(1.9)	12	(1.8)	8	(1.7)	29	(2.7)
+	+	+	+	+	R_1R_2	55	(21.6)	70	(16.9)	125	(18.7)	70	(15.1)	144	(13.4)
+	+	-	-	+	r'r	0		0		0		5	(1.0)	10	(0.9)
+	-	+	+	+	R_zR_1	1	(0.4)	2	(0.5)	3	(0.4)	0		4	(0.4)
+	+	-	+	+	r"r'	1	(0.4)	1	(0.2)	2	(0.3)	0		0	
-	+	-	+	+	r"r	4	(1.6)	4	(1.0)	8	(1.2)	2	(0.4)	7	(0.7)
+	+	+	+	-	R_zR_2	1	(0.4)	7	(1.7)	8	(1.2)	2	(0.4)	0	
-	+	-	+	-	r"r"	0		1	(0.2)	1	(0.1)	0		0	
+	-	-	-	+	r'r'	0		0		0		0		0	
+	-	-	+	+	$r'r^y$	0		0		0		0		0	
+	-	+	+	-	R_zR_z	0		0		0		0		0	
+	-	-	+	-	r^yr^y	0		0		0		0		0	
+	+	-	+	-	$r^yr"$	0		0		0		0		0	
					Total	255		414		669		462		1073	

8. Rhesus gene frequencies.

	d. Infants	e. Adults	f. Total	g. Williams	h. Race
r	.3994	.3399	.3633	.4073	.3973
r'	.0000	.0000	.0000	.0131	.0114
r"	.0202	.0212	.0208	.0050	.0081
r^y	.0020	.0012	.0015	.0022	.0000
R_0	.0000	.0339	.0202	.0105	.0262
R_1	.4320	.4644	.4521	.4155	.4066
R_2	.1393	.1255	.1308	.1466	.1513
R_z	.0072	.0139	.0113	.0000	.0037

Series	X^2	d.f.	P
e v. g	17.54	7	.01
e v. h	23.76	7	.001
f v. g	14.91	7	.04
f v. h	30.89	7	.000

English persons (Ikin 1954). As Williams (1977) has shown that mistyping can occur across the positive/negative demarcation with an uncomfortable frequency, little meaning can be attached to these observed differences.

3. The Rhesus System.

Even after amalgamation of fairly rare phenotypes into one class, comparison by means of the X^2-test revealed highly significant differences between this sample and a sample of English subjects with regard to the rhesus phenotype frequencies. Differences were also found, though not so highly significant, when comparisons were made with the Williams control sample. Williams (1977) reported no significant difference between his group and the group of English subjects.

When the 'D' gene alone is considered, in several comparisons the only frequency difference to reach a significant level is that between the adults of the present study and a sample of blood donors drawn from a similar area. Again, Williams (1977) did not find such a difference, but his blood donor comparison group was drawn from a slightly different area.

The difference in frequency referred to above is that the blood donor sample shows a raised frequency of the negative (dd) phenotype. This finding then, agrees with Saugstad's report (Saugstad 1975) that blood donors, especially females, show a significant excess of Rhesus negative individuals when compared with persons grouped for paternity cases. Mourant, also, has asserted that self-selection for the Rhesus negative phenotype occurs amongst blood donors (Mourant pers.comm.). Kopeć (1970) found no significant difference between the Rhesus phenotypes of blood donors and Air Force recruits, (though of course this latter group also may not be a random sample of the general population.)

TABLE 5.2 contd.:

9. Rhesus(D) phenotype frequencies.

	a. Infants		b. Adults		c. Total		d. Williams		e. Race		f. Kopeć A		g. Kopeć B	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
D +ve	207	(79.9)	380	(84.6)	587	(82.9)	378	(81.8)	886	(82.6)	5269	(80.8)	16316	(81.5)
D -ve	52	(20.1)	69	(15.4)	121	(17.1)	84	(18.2)	187	(18.2)	1251	(19.2)	3714	(18.5)
Total	259		449		708		462		1073		6520		20030	

Series	X^2	d.f.	P
b v. d	1.10	1	.13
b v. e	0.82	1	.37
b v. f	3.75	1	.05
b v. g	2.73	1	.10
c v. d	0.16	1	.69
c v. e	0.01	1	.90
c v. f	1.69	1	.19
c v. g	0.86	1	.35

10. Rhesus(D) gene frequencies ±1.96 S.E.

	a. Infants	b. Adults	c. Total	d. Williams	e. Race	f. Kopeć A	g. Kopeć B
D	.5519	.6080	.5866	.5736	.5825	.5620	.5694
d	.4481	.3920	.4134	.4264	.4175	.4380	.4306
	±.0544	±.0425	±.0335	±.0412	±.0272	±.0109	±.0062

4. The MNSs System.

(i) The MN locus.

No differences were noted between the phenotype frequencies in this sample and the frequencies in comparative groups from County Durham (Williams 1977), Cumbria (Mitchell 1974), and Hertfordshire (Thomas and Hewitt 1939). In all series, except that from County Durham, the phenotypic frequencies agreed with Hardy-Weinberg expected proportions.

(ii) The S locus.

Again no significant differences were found between the phenotype frequencies observed in this sample and those in comparison groups. The largest differences (though still not significant) occur in comparisons with the German series (Wendt and Theile 1963). In this latter sample the phenotype frequencies do not agree with Hardy-Weinberg expectations and, in fact, the gene frequencies are very similar to those found in the other series examined. It is probably this lack of agreement which leads to the between-sample differences observed.

S phenotype frequencies after testing with only one antiserum, were also compared, for the sake of obtaining larger sized comparison groups. No significant differences were observed at the 5% level, but a nearly significant difference was found between the present sample and the Cumbrian sample (Mitchell 1974). Of the four series compared, this latter series had the highest frequency of the s gene.

(iii) The MNS Complex.

The usual linkage disequilibrium was observed between the MN and Ss loci, with the NS combination being

TABLE 5.2 contd.:

11. MN phenotype frequencies.

	a. Infants		b. Adults		c. Total		d.Williams		e.Mitchell		f. Thomas	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
M	80	(32.8)	135	(31.2)	215	(31.8)	120	(27.5)	156	(30.3)	279	(31.0)
MN	125	(51.2)	218	(50.3)	343	(50.7)	237	(54.4)	244	(47.4)	436	(48.4)
N	39	(16.0)	80	(18.5)	119	(17.6)	79	(18.1)	115	(22.3)	185	(20.6)
Total	244		433		677		436		515		900	

12. MN gene frequencies ±1.96 S.E.

	a. Infants	b. Adults	c. Total	d.Williams	e.Mitchell	f. Thomas
M	.5840	.5635	.5709	.5470	.5398	.5522
N	.4160	.4365	.4291	.4530	.4602	.4478
	±.0619	±.0466	±.0372	±.0466	±.0430	±.0325

13. S phenotype frequencies.

	g. Infants		h. Adults		i. Total		j.Williams		k.Mitchell		l. Wendt	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
S	33	(13.2)	46	(10.8)	79	(11.7)	26	(11.1)	26	(10.4)	18	(16.7)
Ss	111	(44.4)	201	(47.1)	312	(46.1)	98	(41.9)	102	(40.8)	39	(36.1)
s	106	(42.4)	180	(42.2)	286	(42.2)	110	(47.0)	122	(48.8)	51	(47.2)
Total	250		427		677		234		250		108	

14. S gene frequencies ±1.96 S.E.

	g. Infants	h. Adults	i. Total	j.Williams	k.Mitchell	l. Wendt
S	.3540	.3431	.3471	.3205	.3080	.3472
s	.6460	.6569	.6529	.6795	.6920	.6528
	±.0592	±.0451	±.0359	±.0598	±.0572	±.0898

Series	X^2	d.f.	P
a v. HW	0.72	1	.39
b v. HW	0.24	1	.63
c v HW	0.79	1	.37
d v. HW	4.07	1	.04
e v. HW	1.11	1	.29
f v. HW	0.38	1	.54
b v. d	0.70	2	.70
b v. e	2.18	2	.34
b v. f	0.85	2	.65
c v. d	1.67	2	.43
c v. e	4.21	2	.12
c v. f	2.23	2	.33

Series	X^2	d.f.	P
g v. HW	0.21	1	.64
h v. HW	0.84	1	.36
i v. HW	0.19	1	.66
j v. HW	0.35	1	.56
k v. HW	0.46	1	.50
l v. HW	4.47	1	.03
h v. j	1.73	2	.42
h v. k	2.97	2	.23
h v. l	5.32	2	.07
i v. j	1.64	2	.44
i v. k	3.18	2	.20
i v. l	4.49	2	.11

TABLE 5.2 contd.:

15. S phenotype frequencies (tested with anti-S only).

	a. Infants No.	%	b. Adults No.	%	c. Total No.	%	d. Williams No.	%	e. Mitchell No.	%	f. Giles No.	%
S +ve	149	(57.6)	253	(57.3)	402	(57.3)	184	(51.8)	255	(51.8)	201	(56.0)
S -ve	110	(42.4)	189	(42.7)	299	(42.7)	171	(48.2)	237	(48.2)	158	(44.0)
Total	259		442		701		355		492		359	

Series	χ^2	d.f.	P
b v. d	2.11	1	.15
b v. e	2.53	1	.11
b v. f	0.08	1	.78
c v. d	2.68	1	.10
c v. e	3.34	1	.07
c v. f	0.13	1	.72

16. MNS phenotype frequencies.

	g. Infants No.	%	h. Adults No.	%	i. Total No.	%	j. Williams No.	%	k. Mitchell No.	%	l. Cleghorn A No.	%
MS	17	(7.2)	25	(6.1)	42	(6.5)	14	(6.0)	16	(6.4)	57	(5.7)
MSs	39	(16.6)	68	(16.5)	107	(16.6)	26	(11.2)	40	(16.0)	140	(14.0)
Ms	17	(7.2)	34	(8.3)	51	(7.9)	22	(9.4)	22	(8.8)	101	(10.1)
MNS	14	(6.0)	20	(4.9)	34	(5.3)	11	(4.7)	10	(4.0)	39	(3.9)
MNSs	51	(21.7)	106	(25.8)	157	(24.3)	65	(27.9)	48	(19.2)	224	(22.4)
MNs	58	(24.7)	83	(20.2)	141	(21.8)	57	(24.5)	55	(22.0)	226	(22.6)
NS	2	(0.9)	0		2	(0.3)	1	(0.2)	0		3	(0.3)
NSs	12	(5.1)	20	(4.9)	32	(5.0)	7	(3.0)	14	(5.6)	54	(5.4)
Ns	25	(10.6)	55	(13.4)	80	(12.4)	30	(12.9)	45	(18.0)	156	(15.6)
Total	235		411		646		233		250		1000	

Series	χ^2	d.f.	P
h v. j	4.85	7	.68
h v. k	5.86	7	.56
h v. l	5.98	7	.54
i v. j	6.82	7	.45
i v. k	7.36	7	.39
i v. l	9.47	7	.22

17. MNS gene frequencies.

	g. Infants	h. Adults	i. Total	j. Williams	k. Mitchell	l. Cleghorn A
MS	.2736	.2826	.2214	.2636	.2523	.2409
Ms	.2987	.2807	.3452	.2879	.2857	.3016
NS	.0838	.0629	.0560	.0583	.0557	.0671
Ns	.3439	.3728	.3774	.3902	.4063	.3904

much less common than expected. No significant differences were observed between the phenotype frequencies in this sample and those from County Durham (Williams 1977), Cumbria (Mitchell 1974), and S.E. England (Cleghorn 1960).

When results from testing with three antisera only are compared, a significant difference is noticed between the phenotype frequencies in this sample and those from a group of English subjects (Ikin 1950). These differences are not so large when only the adults of the present study are included in the comparison. The explanation lies in a difference between the newborn infants and the adults of the present study. (see below, page 205).

5. The Duffy System.

No significant differences were found in comparisons between this sample and the various other series, both after testing with two antisera and after testing with only one antiserum. No series was found with phenotype frequencies differing from Hardy-Weinberg expectations.

6. The Kell-Kp (Penney) System.

There are perhaps lower expectations of finding differences in phenotype frequencies in comparisons between various series for this blood group system as the rarer classes are usually sufficiently small to require amalgamation before such comparisons are made. For the Kell blood group no significant differences were demonstrated, but for the Penney blood group, the frequency of the Kp^a allele was found to be significantly higher in a group of Canadian blood donors (Dichupa et al. 1969) than in the present sample. No difference was found between the present sample and a group of Michigan blood donors (Shreffler et al. 1971) after tests with 4 antisera.

TABLE 5.2 contd.:

18. MNS phenotype frequencies (tested with 3 anti-sera only).

	a. Infants No.	%	b. Adults No.	%	c. Total No.	%	d. Williams No.	%	e. Mitchell No.	%	f. Ikin A No.	%
MS+ve	60	(24.6)	96	(22.5)	156	(23.3)	60	(17.5)	102	(20.7)	230	(19.7)
MNS+ve	66	(27.0)	129	(30.3)	195	(29.1)	103	(30.1)	126	(25.6)	303	(26.0)
NS+ve	14	(5.7)	20	(4.7)	34	(5.1)	17	(5.0)	27	(5.5)	56	(4.8)
MS-ve	20	(8.2)	36	(8.5)	56	(8.4)	32	(9.4)	51	(10.4)	113	(9.7)
MNS-ve	59	(24.2)	87	(20.4)	146	(21.8)	87	(25.4)	103	(20.9)	264	(22.6)
NS-ve	25	(10.2)	58	(13.6)	83	(12.4)	43	(12.6)	83	(16.9)	200	(17.1)
Total	244		426		670		342		492		1166	

Series	X^2	d.f.	P
b v. d	4.80	5	.44
b v. e	4.91	5	.54
b v. f	6.95	5	.22
c v. d	5.15	5	.40
c v. e	7.51	5	.19
c v. f	11.38	5	.04

19. Duffy phenotype frequencies.

	g. Infants No.	%	h. Adults No.	%	i. Total No.	%	j. Williams No.	%	k. Cleghorn B No.	%
Fy^a	41	(16.1)	87	(20.2)	128	(18.7)	41	(17.0)	130	(19.8)
Fy^aFy^b	114	(44.9)	198	(45.9)	312	(45.5)	105	(43.6)	321	(48.9)
Fy^b	99	(39.0)	146	(33.9)	245	(35.8)	95	(39.4)	205	(31.3)
Total	254		431		685		241		656	

Series	X^2	d.f.	P
g v. HW	0.71	1	.40
h v. HW	1.75	1	.19
i v. HW	2.61	1	.11
j v. HW	1.64	1	.20
k v. HW	0.05	1	.83
h v. j	2.33	2	.31
h v. k	1.06	2	.59
i v. j	1.08	2	.58
i v. k	3.07	2	.22

20. Duffy gene frequencies ±1.96 S.E.

	g. Infants	h. Adults	i. Total	j. Williams	k. Cleghorn B
Fy^a	.3858	.4316	.4146	.3880	.4428
Fy^b	.6142	.5684	.5854	.6120	.5572
	±.0598	±.0468	±.0368	±.0615	±.0380

TABLE 5.2 contd.:

21. Kell phenotype frequencies.

	a. Infants No.	%	b. Adults No.	%	c. Total No.	%	d. Williams No.	%	e. Mitchell No.	%	f. Ikin B No.	%
K	0		2	(0.4)	2	(0.3)	1	(0.2)	0		1	(0.1)
Kk	21	(8.1)	32	(7.1)	53	(7.5)	32	(6.9)	10	(9.8)	89	(7.6)
k	238	(91.9)	415	(92.4)	653	(92.2)	428	(92.9)	92	(90.2)	1076	(92.3)
Total	259		449		708		461		102		1166	

22. Kell gene frequencies ±1.96 S.E.

	a. Infants	b. Adults	c. Total	d. Williams	e. Mitchell	f. Ikin B
K	.0405	.0401	.0403	.0369	.0490	.0390
k	.9595	.9599	.9597	.9631	.9510	.9610
	±.0239	±.0182	±.0145	±.0172	±.0419	±.0111

Series	X^2	d.f.	P
a v. HW	0.46	1	.50
b v. HW	2.46	1	.12
c v. HW	0.69	1	.41
d v. HW	0.24	1	.62
e v. HW	0.27	1	.60
f v. HW	0.37	1	.54
b v. d	0.01	1	.91
b v. e	0.30	1	.58
b v. f	0.00	1	.99
c v. d	0.07	1	.78
c v. e	0.26	1	.61
c v. f	0.00	1	.96

23. Penney phenotype frequencies.

	g. Infants No.	%	h. Adults No.	%	i. Total No.	%	j. Williams No.	%	k. Dichupa No.	%
Kp^a	0		0		0		0		5	(0.0)
Kp^aKp^b	5	(2.0)	3	(0.7)	8	(1.2)	6	(1.6)	269	(2.4)
Kp^b	246	(98.0)	420	(99.3)	666	(98.8)	365	(98.3)	10965	(97.6)
Total	251		423		674		371		11239	

24. Penney gene frequencies ±1.96 S.E.

	g. Infants	h. Adults	i. Total	j. Williams	k. Dichupa
Kp^a	.0100	.0035	.0059	.0081	.0124
Kp^b	.9900	.9965	.9941	.9919	.9876
	±.0121	±.0057	±.0057	±.0092	±.0020

Series	X^2	d.f.	P
h v. j	0.83	1	.36
h v. k	4.53	1	.03
i v. j	0.09	1	.77
i v. k	3.82	1	.05

TABLE 5.2 contd.:

25. Kell-Penney phenotype frequencies.

	a. Infants No.	%	b. Adults No.	%	c. Total No.	%	d. Williams No.	%	e. Shreffler No.	%
KKp^b	0		2	(0.5)	2	(0.3)	1	(0.3)	6	(0.1)
$KkKp^aKp^b$	1	(0.4)	1	(0.2)	2	(0.3)	0		5	(0.1)
$KkKp^b$	19	(7.6)	31	(7.3)	50	(7.4)	25	(6.8)	589	(7.0)
kKp^a	0		0		0		0		1	(0.0)
kKp^aKp^b	4	(1.6)	2	(0.5)	6	(0.9)	6	(1.7)	125	(1.5)
kKp^b	227	(90.4)	387	(91.5)	614	(91.1)	339	(91.4)	7717	(91.4)
Total	251		423		674		371		8443	

Series	X^2	d.f.	P
b v. d	1.61	2	.45
b v. e	2.21	2	.33
c v. d	0.49	2	.78
c v. e	0.94	2	.62

26. Haptoglobin (Hp) phenotype frequencies.

	f. Adults No.	%	g. Williams No.	%	h. Papiha B No.	%	i. Cartwright A No.	%	j. Harris No.	%
1 - 1	61	(14.8)	16	(18.6)	112	(14.7)	33	(16.0)	33	(18.4)
2 - 1	198	(47.9)	44	(51.2)	374	(49.2)	104	(50.5)	88	(49.2)
2 - 2	154	(37.3)	26	(30.2)	275	(36.1)	69	(33.5)	58	(32.4)
Total	413		86		761		206		179	

	X^2	d.f.	P
f v. HW	0.04	1	.84
g v. HW	0.12	1	.73
h v. HW	0.69	1	.41
i v. HW	0.36	1	.55
j v. HW	0.00	1	.97
f v. g	1.81	2	.40
f v. h	0.18	2	.92
f v. i	0.87	2	.65
f v. j	1.93	2	.38

27. Haptoglobin gene frequencies ±1.96 S.E.

	f. Adults	g. Williams	h. Papiha B	i. Cartwright A	j. Harris
Hp^1	.3874	.4419	.3929	.4126	.4302
Hp^2	.6126	.5581	.6071	.5874	.5698
	±.0470	±.1051	±.0347	±.0672	±.0725

Blood Groups: A Few Conclusions.

Data regarding world-wide blood group frequency distributions is abundant (although for Britain, the data are not so extensive) (Mourant et al. 1976). The data presented here seems to fit the general pattern that the ABO blood groups show variation between samples; inconsistencies which may well reflect genetic differences between the populations sampled. This is to a lesser extent true of the Rhesus system also. However, other blood groups examined, for example, the MNSs system, and the Duffy blood groups, whilst being shown to be highly polymorphic, do not seem to show this between-population variation, particularly within different regions of the British Isles. One can speculate that the frequencies of the various alleles of these latter blood group systems are geographically homogeneous because of the action of fairly uniform selective forces.

Conversely, it would seem that the ABO and Rhesus blood groups are more influenced by relatively local circumstances, either in response to local variations in selection intensity, or as a result of local random fluctuations.

B. The Serum Proteins and Isoenzymes.

The documentation of world-wide distributions of the alleles of polymorphic proteins and enzymes is at present still in a very incomplete stage. Mourant et al. (1976) give sparse information about some systems, and no information about most. It is therefore difficult to present a very accurate interpretation of the allele frequencies outlined here. It is fortunate that many of the genetic markers studies have been examined in an earlier study which took place in the same laboratory, so some comparisons can be made. However, with only small quantities of data from elsewhere for many of the markers, it is impossible to interpret any discrepancies which may occur

between the two series (present study and Williams 1977) from this laboratory.

Generally, phenotype frequencies of these markers seem to vary as much, or as little, between the two studies from County Durham as they do between one of these studies and a series from elsewhere. It is perhaps worth remembering again (see above, page 121), when considering these results, Selander's comment that the major factor having effect on the allele frequencies of polymorphic enzymes studied in various populations of Drosophila was the laboratory in which the analysis took place. (Selander 1976).

1. Haptoglobin.

Haptoglobin results are available only for the adults in the sample, as the phenotypes of this protein cannot be distinguished in cord blood. The phenotype frequencies show no differences from those in series from Durham (Williams 1977), Northumberland, Cartwright (1973), N.E. England, Papiha(1974) or S.E. England, Harris et al. (1959).

2. Phosphoglucomutase.

The PGM phenotype frequencies of the total sample give a value when tested against Hardy-Weinberg expectations which is just significant at the 5% level. This deviation from equilibrium was not observed when the newborn infants and adults were examined separately. It also did not occur in the other series from this area (Williams 1977). The phenotype frequencies were not shown to be different from another series from an adjacent region (Papiha1973), but were significantly different from the group of English persons examined by Hopkinson and Harris (1966). This difference (an elevated frquency of the PGM1 allele) was also noted by Williams (1977).

TABLE 5.2 contd.:

28. Phosphoglucomutase (PGM) phenotype frequencies.

	a. Infants		b. Adults		c. Total		d. Williams		e. Hopkinson A		f. Papiha A	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1 - 1	142	(58.4)	273	(65.6)	415	(63.0)	79	(68.7)	1238	(58.7)	327	(59.6)
2 - 1	82	(33.7)	123	(29.6)	205	(31.1)	32	(27.8)	754	(35.7)	186	(33.9)
2 - 2	19	(7.8)	20	(4.8)	39	(5.9)	4	(3.5)	118	(5.8)	36	(6.5)
Total	243		416		659		115		2110		549	

29. Phosphoglucomutase gene frequencies ±1.96 S.E.

	a. Infants	b. Adults	c. Total	d. Williams	e. Hopkinson A	f. Papiha A
PGM_1^1	.7531	.8041	.7853	.8261	.7654	.7650
PGM_1^2	.2469	.1959	.2147	.1739	.2346	.2350
	±.0543	±.0382	±.0314	±.0692	±.0181	±.0355

30. Esterase-D (ESD) phenotype frequencies.

	g. Infants		h. Adults		i. Total		j. Williams		k. Cartwright B		l. Hopkinson C	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1 - 1	177	(73.4)	330	(79.3)	507	(77.2)	304	(77.9)	376	(81.0)	705	(81.3)
2 - 1	57	(23.7)	77	(18.5)	134	(20.4)	82	(21.0)	85	(18.3)	154	(17.8)
2 - 2	7	(2.9)	9	(2.2)	16	(2.4)	4	(1.0)	3	(0.7)	8	(0.9)
Total	241		416		657		390		464		867	

31. Esterase-D gene frequencies ±1.96 S.E.

	g. Infants	h. Adults	i. Total	j. Williams	k. Cartwright B	l. Hopkinson C
ESD^1	.8527	.8858	.8737	.8846	.9019	.9020
ESD^2	.1473	.1142	.1263	.1154	.0981	.0980
	±.0447	±.0306	±.0247	±.0318	±.0271	±.0198

Series	X^2	d.f.	P
a v. HW	2.08	1	.15
b v. HW	1.58	1	.21
c v. HW	3.96	1	.05
d v. HW	0.11	1	.73
e v. HW	0.05	1	.82
f v. HW	1.82	1	.18
b v. d	0.57	2	.75
b v. e	7.01	2	.03
b v. f	4.02	2	.13
c v. d	1.90	2	.39
c v. e	4.75	2	.09
c v. f	1.48	2	.48
g v. HW	0.82	1	.36
h v. HW	3.01	1	.08
i v. HW	3.80	1	.05
j v. HW	0.35	1	.55
k v. HW	0.59	1	.44
l v. HW	0.02	1	.90
g v. k	9.11	2	.01
h v. j	2.31	2	.32
h v. l	3.49	2	.17
i v. j	2.61	2	.27
i v. l	7.61	2	.02

3. Esterase D.

The phenotype frequencies of the total sample (but not of the newborn infants or adults when considered separately) were significantly different from those expected in a Hardy-Weinberg equilibrium. The frequencies found in the cord blood series were significantly different from those found in a similar series from Hartlepool (Cartwright et al. 1976).

The total sample differed from the frequencies obtained by Hopkinson et al. (1973) in the south of England, but this difference was not seen when adults alone were compared. Of course, this latter finding may be due to the smaller samples involved in this comparison.

4. Adenylate kinase.

Phenotype frequencies were in the proportions expected under Hardy-Weinberg equilibrium. No differences were found between the present sample and the comparison groups from County Durham, Northumberland and Britain (Williams (1977) Paphia(1973), Rapley et al. 1967).

5. Red Cell Acid phosphatase.

Phenotype frequencies of the total present sample, and of the newborn infant and adult series separately, were in the proportions expected under a Hardy-Weinberg equilibrium. The frequencies showed highly significant differences when compared with a sample from the south of England (Hopkinson and Harris 1968). A similar difference was also found by Williams (1977). A significant difference (but not so great) was also found when comparing the present sample with the other series from County Durham (Williams 1977). No significant differences were found between the present sample and another from Northumberland (Papiha 1973).

TABLE 5.2 contd.:

32. Adenylate kinase (AK) phenotype frequencies.

	a. Infants		b. Adults		c. Total		d.Williams		e.Papiha A		f. Rapley	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1 - 1	232	(95.1)	407	(92.5)	628	(93.5)	363	(91.9)	524	(95.5)	1720	(91.1)
2 - 1	12	(4.9)	33	(7.5)	44	(6.5)	30	(7.6)	25	(4.5)	165	(8.7)
2 - 2	0		0		0		2	(0.5)	0		2	(0.1)
Total	244		440		672		395		549		1887	

33. Adenylate kinase gene frequencies ±1.96 S.E.

	a. Infants	b. Adults	c. Total	d.Williams	e.Papiha A	f. Rapley
AK^1	.9754	.9626	.9673	.9570	.9772	.9552
AK^2	.0246	.0374	.0327	.0430	.0228	.0448
	±.0194	±.0180	±.0135	±.0200	±.0125	±.0094

34. Acid phosphatase (AP) phenotype frequencies.

	g. Infants		h. Adults		i. Total		j.Williams		k.Papiha A		l.Hopkinson B	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A	33	(13.6)	47	(11.2)	80	(12.1)	24	(10.5)	64	(11.7)	119	(13.5)
BA	102	(42.1)	139	(33.1)	241	(36.4)	111	(48.5)	221	(40.3)	379	(43.1)
B	89	(36.2)	191	(45.5)	280	(42.3)	80	(34.9)	208	(37.9)	282	(32.1)
BC	11	(4.5)	28	(6.7)	39	(5.9)	10	(4.4)	40	(7.3)	39	(6.9)
CA	7	(2.9)	13	(3.1)	20	(3.0)	4	(1.7)	16	(2.9)	39	(6.9)
C	0		2	(0.5)	2	(0.3)	0		0		0	
Total	242		420		662		229		549		880	

Series	X^2	d.f.	P
a v HW	0.16	1	.69
b v HW	0.65	1	.42
c v. HW	0.77	1	.38
d v. HW	2.40	1	.12
e.v. HW	0.30	1	.59
f v. HW	0.92	1	.34
b v. d	0.04	1	.84
b v. e	3.33	1	.07
b v. f	0.66	1	.41
c v. d	0.69	1	.41
c v. e	1.89	1	.17
c v. f	3.17	1	.07
g v. HW	0.11	3	.99
h v. HW	3.40	3	.33
i v. HW	2.84	3	.42
j v. HW	1.46	3	.69
k v. HW	0.54	3	.91
l v. HW	4.55	3	.21
h v. j	8.66	4	.07
h v. k	6.70	4	.15
h v. l	27.61	4	.00
i v. j	16.00	4	.00
i v. k	3.21	4	.55
i v. f	19.89	4	.00

TABLE 5.2 contd.:

35. Acid phosphatase gene frequencies ±1.96 S.E.

	g. Infants	h. Adults	i. Total	j. Williams	k. Papiha A	l. Hopkinson B
p^a	.3616 ±.0485	.2927 ±.0338	.3180 ±.0279	.3559 ±.0495	.3324 ±.0311	.3727 ±.0257
p^b	.6012 ±.0578	.6537 ±.0449	.6344 ±.0355	.6135 ±.0597	.6166 ±.0386	.5705 ±.0298
p^c	.0372 ±.0170	.0536 ±.0154	.0476 ±.0116	.0306 ±.0159	.0510 ±.0132	.0568 ±.0110

It is difficult to account simply for these findings. It seems unlikely that only geographically-related genetic differences between populations are involved. Williams (1977) speculated that technical errors of mistyping may have led to an excess of the BA phenotype, and a deficiency of CA in his sample. In the present sample it appears that there are fewer in all classes of heterozygotes, compared with the Hopkinson and Harris sample. In fact this latter sample is the one which agrees least well with Hardy-Weinberg expectations (although the discrepancy is not significant; P= .21).

C. Chromosome variants.

In this, and the following sections, unless it is otherwise indicated, the term "band" refers to those bands which show a variable reaction to the fluorescent stain, and not to those bands found along the length of each chromosome which may be used for identification of the chromosome. The term "variant" or "variant band" refers to non-negatively staining states of the variable regions.

The frequencies of the chromosome variants within individuals are displayed in tables 5.4 and 5.5. The results after combining together certain classes of variants (for example, intense and brilliant variants at the same position, all intense D group satellites, etc.,) are also presented, in tables 5.6 to 5.8.

It can be seen that, generally, those regions whose variant types do not appear in the proportions expected under a Hardy-Weinberg equilibrium are those with the highest frequencies of intense and brilliant variants. It could be that there is a real excess (for almost always this is the direction of the deviation) of heterozygotes, but it is suggested that the explanation is more likely to be due to errors in scoring (see above, page 125).

TABLE 5.3: A key to the series used in the present study.

Series	Composition	No. (Chromosome variants)
1	Total sample	670
2	Newborn infants	237
3	'Normal' adults	224
4	Adults aged 65+ years	174
5	Total adults	433

TABLE 5.4: Phenotype frequencies of chromosome variants.

	chromosome											
	3		4		13p		13s		14p		14s	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Series												
1 BB	3	(0.4)	0		3	(0.4)	0		0		0	
1 BI	49	(7.3)	0		28	(4.2)	3	(0.4)	0		5	(0.7)
1 II	86	(12.8)	0		157	(23.4)	0		0		9	(1.3)
1 IN	281	(41.9)	57	(8.5)	369	(55.1)	121	(18.1)	19	(2.8)	225	(33.6)
1 BN	85	(12.7)	1	(0.1)	8	(1.2)	19	(2.8)	0		22	(3.3)
1 NN	166	(24.8)	612	(91.3)	105	(15.7)	527	(78.7)	651	(97.2)	409	(61.0)
X^2(HW)	4.63		0.00		16.21*		2.74		0.02		8.19*	
2 BB	1	(0.4)	0		2	(0.8)	0		0		0	
2 BI	17	(7.2)	0		9	(3.8)	0		0		2	(0.8)
2 II	25	(10.5)	0		57	(24.1)	0		0		2	(0.8)
2 IN	105	(44.3)	16	(6.8)	137	(57.8)	35	(14.8)	5	(2.1)	78	(32.9)
2 BN	35	(14.8)	0		4	(1.7)	6	(2.5)	0		7	(3.0)
2 NN	54	(22.8)	221	(93.2)	28	(11.8)	196	(82.7)	232	(97.9)	148	(62.4)
X^2(HW)	4.79		0.02		9.54*		0.27		0.09		4.01	
3 BB	1	(0.4)	0		1	(0.4)	0		0		0	
3 BI	18	(8.0)	0		9	(4.0)	1	(0.4)	0		2	(0.9)
3 II	35	(15.6)	0		54	(24.1)	0		0		7	(3.1)
3 IN	85	(37.9)	25	(11.2)	125	(55.8)	47	(21.0)	4	(1.8)	76	(33.1)
3 BN	24	(10.7)	1	(0.4)	1	(0.4)	9	(4.0)	0		12	(5.4)
3 NN	61	(27.2)	198	(88.4)	34	(15.2)	167	(74.6)	220	(98.2)	127	(56.7)
X^2(HW)	0.65		0.02		7.22		2.36		0.12		0.91	
4 BB	0		0		0		0		0		0	
4 BI	11	(6.3)	0		9	(5.2)	3	(1.7)	0		1	(0.6)
4 II	24	(13.8)	0		37	(21.3)	0		0		0	
4 IN	79	(45.4)	16	(9.2)	91	(52.3)	35	(20.1)	10	(5.7)	60	(34.5)
4 BN	21	(12.1)	0		2	(1.1)	3	(1.7)	0		4	(2.3)
4 NN	33	(22.4)	158	(90.8)	35	(20.1)	133	(76.4)	164	(94.3)	109	(62.6)
X^2(HW)	3.66		0.02		3.33		0.66		0.04		4.97	
5 BB	2	(0.5)	0		1	(0.2)	0		0		0	
5 BI	32	(7.4)	0		19	(4.4)	3	(0.7)	0		3	(0.7)
5 II	61	(14.1)	0		100	(23.1)	0		0		7	(1.6)
5 IN	176	(40.6)	41	(9.5)	232	(53.6)	86	(19.9)	14	(3.2)	147	(33.9)
5 BN	50	(11.5)	1	(0.2)	4	(0.9)	13	(3.0)	0		15	(3.5)
5 NN	112	(25.9)	391	(90.3)	77	(17.8)	331	(76.4)	419	(96.8)	261	(60.3)
X^2(HW)	1.32		0.00		8.60*		1.65		0.03		4.56	

* significant at the 5% level

TABLE 5.4 contd.:

		chromosome 15p No.	%	15s No.	%	21p No.	%	21s No.	%	22p No.	%	22s No.	%
Series													
	BB	0		0		0		1	(0.1)	0		1	(0.1)
	BI	0		12	(1.8)	0		5	(0.7)	0		13	(1.9)
1	II	1	(0.1)	16	(2.4)	0		14	(2.1)	8	(1.2)	21	(3.1)
–	IN	23	(3.4)	258	(38.5)	12	(1.8)	207	(28.5)	132	(19.7)	257	(38.4)
	BN	0		25	(3.7)	2	(0.3)	12	(1.8)	2	(0.3)	38	(5.7)
	NN	646	(96.4)	359	(53.6)	656	(97.9)	431	(64.3)	528	(78.8)	340	(50.7)
X^2(HW)		0.00		9.58*		0.00		3.10		0.00		6.28*	
	BB	0		0		0		0		0		0	
	BI	0		1	(0.4)	0		0		0		9	(3.8)
2	II	0		4	(1.7)	0		2	(0.8)	1	(0.4)	8	(3.4)
–	IN	4	(1.7)	93	(39.2)	5	(2.1)	56	(23.6)	44	(18.6)	90	(38.0)
	BN	0		4	(1.7)	0		3	(1.3)	0		9	(3.8)
	NN	233	(98.3)	135	(57.0)	232	(97.9)	176	(74.3)	192	(81.0)	121	(51.1)
X^2(HW)		0.12		4.87		0.09		0.97		0.00		3.99	
	BB	0		0		0		0		0		1	(0.4)
	BI	0		6	(2.7)	0		4	(1.8)	0		1	(0.4)
3	II	1	(0.4)	6	(2.7)	0		6	(2.7)	4	(1.8)	9	(4.0)
–	IN	7	(3.1)	83	(37.1)	4	(1.8)	78	(34.8)	51	(22.8)	88	(39.3)
	BN	0		12	(5.4)	1	(0.4)	4	(1.8)	1	(0.4)	11	(4.9)
	NN	216	(96.4)	117	(52.2)	219	(97.8)	132	(58.9)	168	(75.0)	114	(50.9)
X^2(HW)		0.00		2.53		0.09		2.49		0.00		1.84	
	BB	0		0		0		1	(0.6)	0		0	
	BI	0		5	(2.9)	0		0		0		1	(0.6)
4	II	0		6	(3.4)	0		5	(2.9)	3	(1.7)	3	(1.7)
–	IN	9	(5.2)	71	(40.8)	3	(1.7)	60	(34.5)	31	(17.8)	66	(37.9)
	BN	0		9	(5.2)	0		2	(1.1)	0		16	(9.2)
	NN	165	(94.8)	83	(47.7)	171	(98.3)	106	(60.9)	140	(80.5)	88	(50.6)
X^2(HW)		0.05		2.41		0.16		0.73		0.28		5.71	
	BB	0		0		0		1	(0.2)	0		1	(0.2)
	BI	0		11	(2.6)	0		5	(1.2)	0		4	(0.9)
5	II	1	(0.2)	12	(2.8)	0		12	(2.8)	7	(1.6)	13	(3.0)
–	IN	19	(4.4)	165	(38.1)	7	(1.6)	151	(34.9)	88	(20.3)	167	(38.6)
	BN	0		21	(4.8)	2	(0.5)	9	(2.1)	2	(0.5)	29	(6.7)
	NN	413	(95.4)	224	(51.7)	424	(97.9)	255	(58.9)	336	(77.6)	219	(50.6)
X^2(HW)		0.00		5.37		0.00		3.19		0.09		5.74	

* significant at the 5% level

TABLE 5.5: Frequencies of chromsome variants ±1.96 S.E.

Series	chromosome 3 B	3 I	3 N	4 B	4 I	4 N	13p B	13p I	13p N
1	.1045 ±.0169	.3746 ±.0295	.5207 ±.0332	.0007 ±.0016	.0425 ±.0109	.9567 ±.0378	.0313 ±.0094	.5306 ±.0334	.4381 ±.0313
2	.1139 ±.0295	.3629 ±.0491	.5232 ±.0560	.0000	.0338 ±.0230	.9662 ±.0230	.0359 ±.0169	.5485 ±.0568	.4156 ±.0517
3	.0982 ±.0283	.3862 ±.0517	.5156 ±.0573	.0022 ±.0043	.0558 ±.0216	.9120 ±.0652	.0268 ±.0151	.5402 ±.0581	.4330 ±.0539
4	.0919 ±.0311	.3966 ±.0592	.5115 ±.0648	.0000	.0466 ±.0311	.9540 ±.0311	.0316 ±.0185	.5000 ±.0643	.4684 ±.0629
5	.0993 ±.0205	.3811 ±.0370	.5196 ±.0413	.0012 ±.0023	.0473 ±.0143	.9515 ±.0470	.0289 ±.0112	.5208 ±.0413	.4503 ±.0393

Series	13s B	13s I	13s N	14p B	14p I	14p N	14s B	14s I	14s N
1	.0164 ±.0068	.0925 ±.0159	.8911 ±.0376	.0000	.0142 ±.0090	.9858 ±.0090	.0201 ±.0076	.1851 ±.0219	.7948 ±.0371
2	.0127 ±.0101	.0738 ±.0240	.9135 ±.0634	.0000	.0105 ±.0130	.9895 ±.0130	.0190 ±.0124	.1772 ±.0362	.8083 ±.0624
3	.0223 ±.0138	.1071 ±.0295	.8075 ±.0643	.0000	.0089 ±.0087	.9911 ±.0087	.0313 ±.0163	.2054 ±.0398	.7634 ±.0636
4	.0172 ±.0137	.1092 ±.0338	.8736 ±.0737	.0000	.0287 ±.0248	.9713 ±.0248	.0144 ±.0126	.1753 ±.0420	.8103 ±.0729
5	.0185 ±.0090	.1028 ±.0208	.8788 ±.0467	.0000	.0162 ±.0119	.9838 ±.0119	.0208 ±.0096	.1894 ±.0276	.7898 ±.0460

Series	15p B	15p I	15p N	15s B	15s I	15s N	21p B	21p I	21p N
1	.0000	.0187 ±.0103	.9813 ±.0103	.0276 ±.0088	.2254 ±.0239	.7470 ±.0366	.0015 ±.0021	.0090 ±.0051	.9895 ±.0379
2	.0000	.0084 ±.0116	.9916 ±.0116	.0105 ±.0092	.2152 ±.0395	.7743 ±.0620	.0000	.0105 ±.0130	.9895 ±.0130
3	.0000	.0201 ±.0131	.9799 ±.0131	.0402 ±.0184	.2254 ±.0414	.7344 ±.0631	.0022 ±.0043	.0089 ±.0087	.9888 ±.0655
4	.0000	.0259 ±.0236	.9741 ±.0236	.0402 ±.0209	.2529 ±.0494	.7069 ±.0710	.0000	.0086 ±.0137	.9914 ±.0137
5	.0000	.0242 ±.0145	.9758 ±.0145	.0370 ±.0127	.2309 ±.0301	.7321 ±.0454	.0023 ±.0032	.0081 ±.0060	.9896 ±.0471

TABLE 5.5 contd.:

Series	chromosome 21s B	21s I	21s N	22p B	22p I	22p N	22s B	22s I	22s N
1	.0142 ±.0064	.1791 ±.0216	.8067 ±.0371	.0015 ±.0021	.1104 ±.0173	.8881 ±.0376	.0396 ±.0105	.2328 ±.0243	.7276 ±.0364
2	.0063 ±.0071	.1266 ±.0310	.8671 ±.0631	.0000	.0970 ±.0377	.9030 ±.0377	.0380 ±.0174	.2426 ±.0416	.7194 ±.0611
3	.0179 ±.0123	.2098 ±.0401	.7723 ±.0638	.0022 ±.0043	.1317 ±.0325	.8661 ±.0649	.0313 ±.0163	.2388 ±.0425	.7299 ±.0630
4	.0115 ±.0112	.2011 ±.0449	.7874 ±.0726	.0000	.1063 ±.0458	.8937 ±.0458	.0488 ±.0229	.2098 ±.0455	.7414 ±.0718
5	.0185 ±.0090	.2079 ±.0287	.7737 ±.0459	.0023 ±.0032	.1178 ±.0222	.8799 ±.0468	.0404 ±.0133	.2275 ±.0299	.7321 ±.0454

TABLE 5.6: Phenotype frequencies of chromosome variants (intense and brilliant levels of fluorescence combined).

		chromosome 3		4		13p		13s		14p		14s	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Series													
	II	138	(20.6)	0		188	(28.1)	3	(0.4)	0		14	(2.1)
1	IN	366	(54.6)	58	(8.7)	377	(56.3)	140	(20.9)	19	(2.8)	247	(36.9)
—	NN	166	(24.8)	612	(91.3)	105	(15.7)	527	(78.7)	651	(97.2)	409	(61.0)
X^2(HW)		5.98*				13.68*		3.88*				11.34*	
	II	43	(18.1)	0		68	(28.7)	0		0		4	(1.7)
2	IN	140	(59.1)	16	(6.8)	141	(59.5)	41	(17.3)	5	(2.1)	85	(35.9)
—	NN	54	(22.8)	221	(93.2)	28	(11.8)	196	(82.7)	232	(97.9)	148	(62.4)
X^2(HW)		8.02*				11.97*						4.45*	
	II	54	(24.1)	0		64	(28.6)	1	(0.4)	0		9	(4.0)
3	IN	109	(48.7)	26	(11.6)	126	(56.3)	56	(25.0)	4	(1.8)	88	(39.3)
—	NN	61	(27.2)	198	(88.4)	34	(15.2)	167	(74.6)	220	(98.2)	127	(56.7)
X^2(HW)		0.15				4.75		2.67				1.71	
	II	35	(20.1)	0		46	(26.4)	3	(1.7)	0		1	(0.6)
4	IN	100	(57.5)	16	(9.2)	93	(53.4)	38	(21.8)	10	(5.7)	64	(36.8)
—	NN	39	(22.4)	158	(90.8)	35	(20.1)	133	(76.4)	164	(94.3)	109	(62.6)
X^2(HW)		3.92				0.93		0.22				6.73*	
	II	95	(21.9)	0		120	(27.7)	3	(0.7)	0		10	(2.3)
5	IN	226	(52.2)	42	(9.7)	236	(54.5)	99	(22.9)	14	(3.2)	162	(37.4)
—	NN	112	(25.9)	391	(90.3)	77	(17.8)	331	(76.4)	419	(96.8)	261	(60.3)
X^2(HW)		0.90				4.41*		2.30				6.98*	

		15p		15s		21p		21s		22p		22s	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	II	1	(0.1)	28	(4.2)	0		20	(3.0)	8	(1.2)	35	(5.2)
1	IN	23	(3.4)	283	(42.2)	14	(2.1)	219	(32.7)	134	(20.0)	295	(44.0)
—	NN	646	(96.4)	359	(53.6)	656	(97.9)	431	(64.3)	528	(78.8)	340	(50.7)
X^2(HW)		2.62		9.25*				1.55		0.02		8.22*	
	II	0		5	(2.1)	0		2	(0.8)	1	(0.4)	17	(7.2)
2	IN	4	(1.7)	97	(40.9)	5	(2.1)	59	(24.9)	44	(18.6)	99	(41.8)
—	NN	233	(98.3)	135	(57.0)	232	(97.9)	176	(74.3)	192	(81.0)	121	(51.1)
X^2(HW)				6.91*				1.52		0.84		0.29	
	II	1	(0.4)	12	(5.4)	0		10	(4.5)	4	(1.8)	11	(4.9)
3	IN	7	(3.1)	95	(42.4)	5	(2.2)	82	(36.6)	52	(23.2)	99	(44.2)
—	NN	216	(96.4)	117	(52.2)	219	(97.8)	132	(58.9)	168	(75.0)	114	(50.9)
X^2(HW)		9.53*		1.70				0.37		0.00		3.28	
	II	0		11	(6.3)	0		6	(3.4)	3	(1.7)	4	(2.3)
4	IN	9	(5.2)	80	(46.0)	3	(1.7)	62	(35.6)	31	(17.8)	82	(47.1)
—	NN	165	(94.8)	83	(47.7)	171	(98.3)	106	(60.9)	140	(80.5)	88	(50.6)
X^2(HW)				2.09				0.72		0.68		9.12*	
	II	1	(0.2)	23	(5.3)	0		18	(4.2)	7	(1.6)	18	(4.2)
5	IN	19	(4.4)	186	(43.0)	9	(2.1)	160	(37.0)	90	(20.8)	196	(45.3)
—	NN	413	(95.4)	224	(51.7)	424	(97.9)	255	(58.9)	336	(77.6)	219	(50.6)
X^2(HW)		2.29		3.92*				1.32		0.12		10.26*	

* significant at 5% level

TABLE 5.7: Frequencies of chromosome variants (intense and brilliant levels of fluorescence combined) ±1.96S.E.

Series		chromosome 3	4	13p	13s	14p	14s	15p	15s	21p	21s	22p	22s
1	I	.4791	.0433	.5619	.1090	.0142	.2052	.0187	.2503	.0105	.1933	.1119	.2724
	N	.5209	.9567	.4381	.8910	.9858	.7948	.9813	.7470	.9895	.8067	.8881	.7276
		±.0378	±.0155	±.0376	±.0235	±.0090	±.0306	±.0102	±.0329	±.0077	±.0300	±.0339	±.0337
2	I	.4768	.0338	.5844	.0865	.0105	.1962	.0084	.2257	.0105	.1329	.0970	.2806
	N	.5232	.9662	.4156	.9135	.9895	.8038	.9916	.7743	.9895	.8671	.9030	.7194
		±.0635	±.0229	±.0627	±.0359	±.0129	±.0506	±.0116	±.0533	±.0129	±.0433	±.0376	±.0572
3	I	.4843	.0580	.5670	.1295	.0089	.2366	.0201	.2656	.0112	.2277	.1339	.2701
	N	.5156	.9420	.4330	.8705	.9911	.7634	.9799	.7344	.9888	.7723	.8661	.7299
		±.0654	±.0306	±.0649	±.0440	± .0123	±.0557	±.0184	±.0578	±.0138	±.0549	±.0446	±.0581
4	I	.4885	.0406	.5316	.1264	.0287	.1897	.0259	.2931	.0087	.2126	.1063	.2586
	N	.5115	.9540	.4684	.8736	.9713	.8103	.9741	.7069	.9914	.7874	.8937	.7414
		±.0743	±.0311	±.0741	±.0494	±.0248	±.0583	±.0236	± .0676	±.0138	±.0608	±.0458	±.0651
5	I	.4804	.0485	.5497	.1212	.0162	.2102	.0242	.2679	.0104	.2263	.1201	.2679
	N	.5196	.9515	.4503	.8788	.9838	.7898	.9758	.7321	.9896	.7737	.8799	.7321
		±.0470	±.0202	±.0468	±.0308	± .0120	±.0384	±.0145	±.0417	±.0096	±.0384	±.0306	±.0417

An interesting feature of the results is that in all cases except that of chromosome 13p, the combined frequency of variant bands never exceeds 50%. That is, the negative class is almost invariably the 'normal' class. The highest frequency that a non-negative variant reaches is .5306$\pm$.034 (for chromosome 13p (intense) in the total sample), and the highest frequency of the combined (intense+brilliant) non-negative variant is again that of chromosome 13p (.5619$\pm$.0376 in the total sample). Even these two figures are only a little above 50%. This may obviously reflect some kind of disadvantage associated with carrying these variants, or may indicate a more recent appearance of the more brightly-staining material. (see above page 76).

Measurements of the sizes of the variable regions were not made in this study, but an idea of the amount of intensely and brilliantly-staining material present in each karyotype was obtained by grouping together certain of the variable regions. The groupings made were:

1. Satellites of D group chromosomes (that is numbers 13,14,15).
2. Satellites of the G group chromosomes (numbers 21 and 22).
3. Satellites of all acrocentric chromosomes (D & G groups).
4. All variable regions.

The distributions of these grouped-variables are given in figure 5.1. Statistics concerning these distributions are given in table 5.8. The most noticeable feature of the histograms is that the distributions are usually skewed. This is a consequence of the fact that intensely- and brilliantly-staining material is always less common in each variant region than the negatively-staining material. It does not necessarily reflect a non-random distribution of the intensely- and brilliantly-staining material.

Figure 5.1: Distributions of grouped chromosome variants.

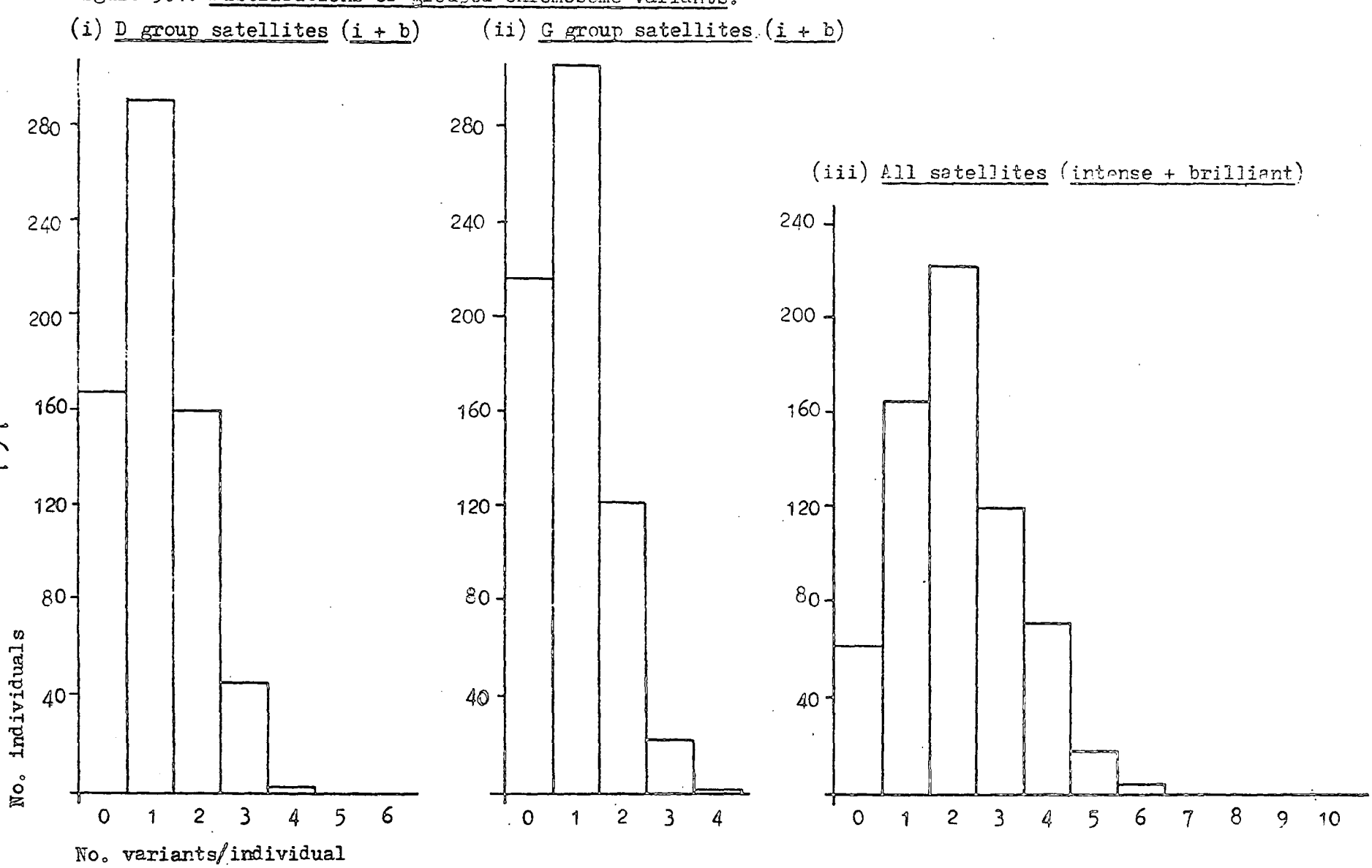


Figure 5.1 contd.:

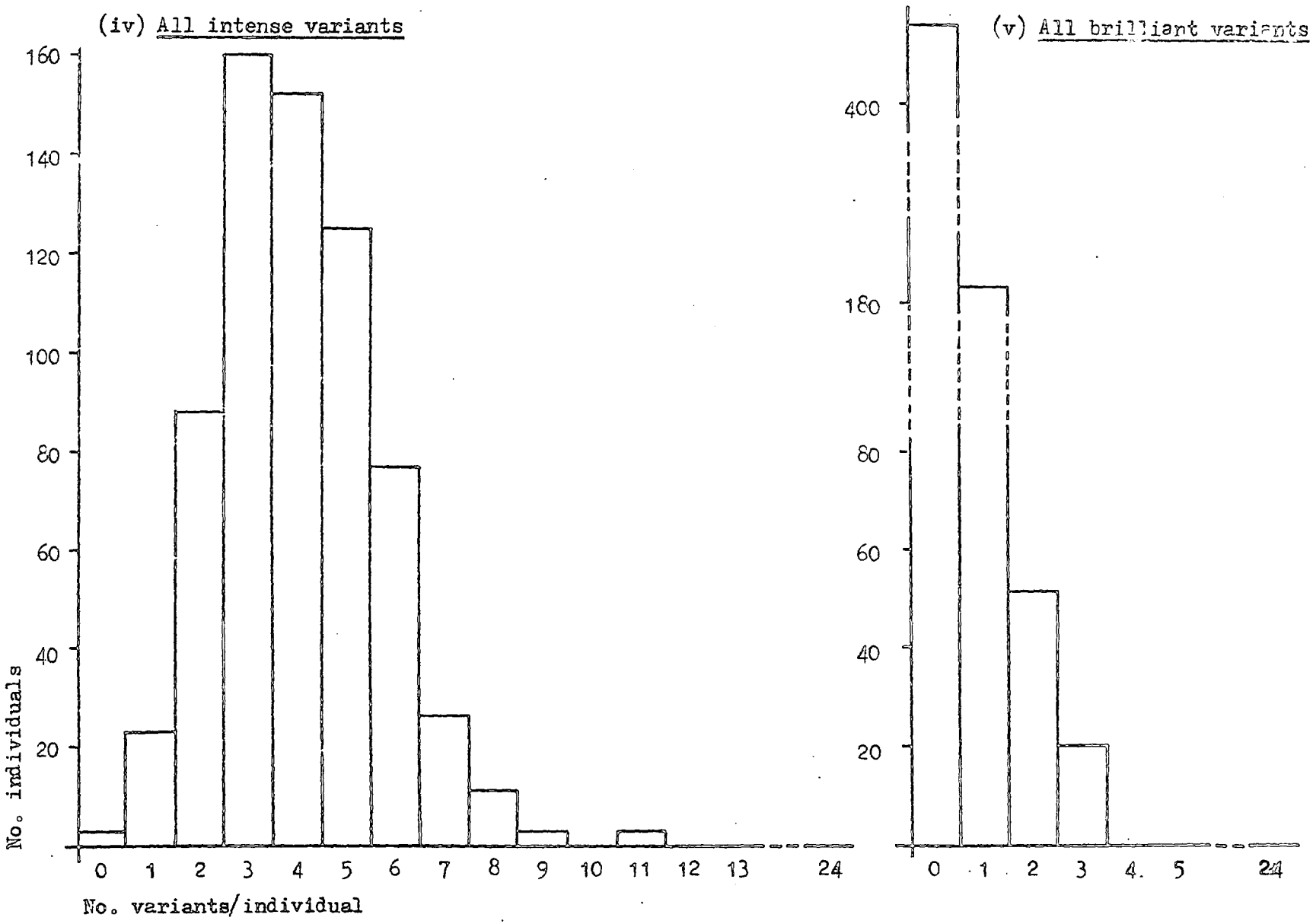


Figure 5.1 contd.:

(vi) All variant bands

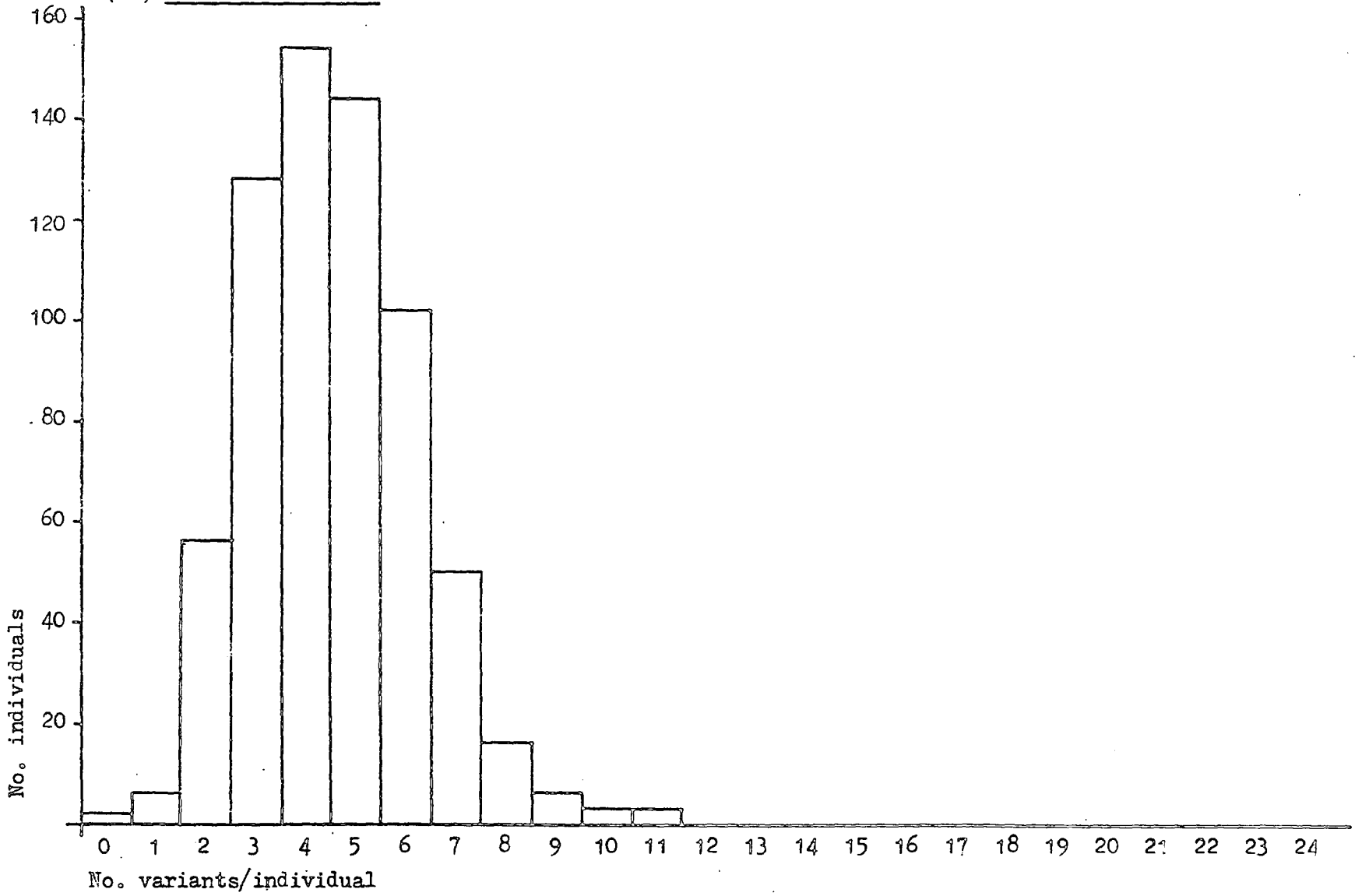


TABLE 5.8: Frequencies of grouped chromosome variables.

	D group satellites			G group satellites			All satellites			All variant bands		
	intense	brilliant	i + b	intense	brilliant	i + b	intense	brilliant	i + b	intense	brilliant	i + b
Possible maximum	6	6	6	4	4	4	10	10	10	24	24	24
Observed maximum	4	2	4	4	2	4	6	3	6	11	3	11
minimum	0	0	0	0	0	0	0	0	0	0	0	0
mean	1.006	0.128	1.134	0.824	0.107	0.931	1.830	0.236	2.066	4.030	0.515	4.545
S.E.	0.032	0.013	0.034	0.030	0.013	0.031	0.045	0.019	0.048	0.064	0.029	0.065
S.D.	0.827	0.344	0.882	0.769	0.333	0.808	1.169	0.499	1.244	1.655	0.763	1.686
variance	0.685	0.118	0.777	0.591	0.111	0.653	1.367	0.249	1.547	2.738	0.582	2.843
mode	1	0	1	1	0	1	2	0	2	3	0	4
median	0.958	0.072	1.068	0.781	0.056	0.884	1.758	0.127	1.970	3.904	0.305	4.429
skewness	0.481	2.447	0.444	0.728	3.131	0.586	0.452	2.112	0.445	0.566	1.479	0.525
kurtosis	-0.279	4.785	-0.360	0.328	9.663	-0.070	-0.022	4.100	-0.052	0.879	1.663	0.659

In all cases except the G group satellites (intense and brilliant combined, for which there is a low possible maximum number) the largest observed number of positive variants in each group falls short of the maximum number of regions at which the positively-staining material could occur.

Coincidence of the mean, mode and median indicates that the distribution is symmetrical. Skewness and kurtosis values indicate its normality. For several of the grouped-variables this coincidence exists, but in many cases the low frequency of the individual variables leads to skewness in their distribution. That is, the fact that the mean has a fairly low value when compared with the maximum value, means that the slope on the positive side of the mean is much more gradual than on the negative side.

Evidence for the random distribution of the positively-staining material between individuals, despite the statistical demonstration in many cases of a non-normal distribution, comes from the fact that the larger the number of regions included in the grouped-variable, the more normal is the distribution. This gives support to the idea that the variants are, in fact, distributed randomly, but that their individual rarity makes this difficult to demonstrate.

The Uniqueness of Combinations of Chromosome Variables.

As might be expected when comparing individual combinations of the twelve variable regions, many unique and very few non-unique combinations occurred. The rarity of individual combinations of conventional blood group phenotypes is often put to practical use in, for example, the resolution of paternity disputes. It was thought interesting to compare the usefulness of the chromosome variables with that of the blood groups, for this type of investigation.

In the total sample, 567 persons were typed for all the following blood groups:

ABO, MNSs, Rhesus, Duffy, Kell, and Penney.

There were 288 unique combinations:-

178 occurred once	4 occurred seven times
49 occurred twice	3 occurred eight times
22 occurred three times	2 occurred nine times
16 occurred four times	1 occurred ten times
9 occurred five times	1 occurred eleven times
2 occurred six times	1 occurred thirteen times.

The most common combination of phenotypes was:

O, MNSs, R_1r, Fy^aFy^b, k, Kp^b

Similarly, in the total sample 670 persons were analysed with respect to their chromosome variables. With a distinction between intense and brilliant levels of fluorescence being made, there were 507 unique combinations.

430 occurred once	2 occurred six times
40 occurred twice	2 occurred seven times
17 occurred three times	1 occurred ten times
10 occurred four times	1 occurred thirteen times
4 occurred five times	

The most common combination of phenotypes was:

3(IN), 4(NN), 13p(IN), 13s(NN) 14p(NN) 14s(NN) 15p(NN) 15s(IN) 21p(NN), 21s(NN), 22p(NN),22s(NN).

With no distinction being made between intense and brilliant levels of fluorescence there were 410 unique combinations.

302 occurred once	4 occurred seven times
52 occurred twice	4 occurred eight times
28 occurred three times	1 occurred ten times
8 occurred four times	1 occurred eleven times
3 occurred five times	1 occurred sixteen times
6 occurred six times	

The most common combination of phenotypes was again: 3 (IN), 4(NN),13p(IN), 13s(NN), 14p(NN), 14s(NN), 15p(NN), 15s(IN), 21p(NN), 21s(NN), 22p(NN), 22s(NN).

This it would seem that the chromosome variables provide an alternative means of characterising an individual genetically. For purposes such as resolving paternity disputes many of the problems of inaccurate scoring encountered in population studies do not arise. This is because the aim of such an investigation is not to classify variants into classes determined by arbitrary cut-off points in the intensity of fluorescence, but to identify chromosome regions which are identical with respect to their fluorescence intensity. Obviously technical variations in slide preparation may influence the ease with which this will be possible, but this would be the only source of difficulty. The variations in size and shape of variant regions hinder a population study, such as the present one, which is concerned only with the staining intensity of the variable region, but aid the identification of identical regions in family studies.

McCracken et al. (1978), using chromosomal polymorphisms to determine the zygosity of sets of twins confirmed the stability and heritability of these variants (see above, page 24), and demonstrated that in fact the identification of chromosome variants represents a more sensitive tool than blood group typing for discriminating zygosity. They determined the phenotypes for six blood group systems. The mean antigen concordance value (for all twins) was $0.87 \pm .14$, and the mean chromosomal concordance value $0.56 \pm .13$. These means were found to be significantly ($P=.001$) different using Student's t-test. That is, twins were more often alike for their blood group antigens than for their chromosome variants (though both these types of variants were identical in monozygotic twins). Thus the

chromosomal variables give a clearer distinction between the monozygotic and dizygotic twins.

Other studies on chromosomal variability have mentioned the uniqueness of each combination of variants found. Müller et al. (1975) found entirely unique combinations in a sample of 376 newborn infants, when either only Q-bands or only C-bands were considered. In Müller's study the Q-bands were not subdivided into the intense and brilliant classes, but the length of the Q-bands was considered. When only the intensity of the Q-bands was considered, 255 unique combinations of variants were found in the 376 individuals. This can be compared with the 410 combinations found in 670 individuals in the present study.

Van Dyke et al. (1977) found 56 unique combinations of chromosome variants in a sample of 67 genetically unique individuals in a study of chromosomal variability in twins. The 5 combinations that occurred twice were found in 5 pairs of dizygotic twins.

Alfi et al. (1975) reported the use of chromosome polymorphism analysis for distinguishing between maternal and foetal cells in amniotic fluid cultures. Chromosome variants (Q-bands) were examined from peripheral blood cultures of both parents when an amniocentesis was to be performed. In 109 families studied it was found that the maternal karyotype could be distinguished from that of the foetus in 101 cases.

Nakagome et al. (1977a) have reported an instance where a child carried a chromosome variant which was not found in either parent, but the variant was of a C-band (enlarged satellite of chromosome 22) and the carrier was phenotypically abnormal. Cases such as this should be noted, but they do not really detract from the value of chromosome analysis which obtains in the above-mentioned studies.

A possible mutation in fluorescent chromosome variants has also been reported (Robinson et al. 1978), but again there are many more reports of the stability of the variants (see above, page 24).

Associations Between Chromosome Variants, and Between Chromosome Variants and Other Genetic Markers.

1. Associations Between Chromosome Variants.

The 'phenotype' frequencies of the chromosome variants (combining intense and brilliant levels of fluorescence) were examined by means of the X^2 test and by calculation of Kendall's tau (a nonparametric correlation coefficient involving ranking of the possible phenotypes, viz., NN, NI, II) to determine whether or not there was any association between the various chromosomal and genetic markers studied. A similar analysis was reported in the papers by Müller et al. (1975) and McCracken et al. (1978); both reported no associations between any pairs of chromosomal variables.

In the present study, comparisons between individual chromosome variants gave about four significant (at the 5% level) X^2 values. It must be remembered that repeated analysis of the data requires that not every value giving a P value of .05 or less will be 'significant' as a proportion of low P values is expected by chance, and a lower than usual significance level must be reached before importance is attached to any particular value.

Table 5.9 gives the P values resulting from comparisons between chromosome variables by means of the X^2-test and by calculating Kendall's tau. It can be seen that there is a fairly close agreement between the results of these two tests, with only a few instances of a nonrandom association not reflecting a consistent trend, (that is, a significant X^2 value occurring together with a non-significant value for Kendall's tau).

TABLE 5.9: (i) Associations between chromosome variants. P values of (A) X^2 and (B) Kendall's tau after crosstabulation of phenotype frequencies (intense and brilliant levels of fluorescence combined).

			chromosome 4	13p	13s	14p	14s	15p	15s	21p	21s	22p	22s
chromosome 3		A	.927	.388	.132	.638	.485	.337	.170	.931	.842	.554	.453
		B	.965	.515	.180	.601	.195	.375	.052	.965	.996	.678	.596
4		A	.612	.295	.479	.726	.073	.156	.028	.161	.660	.040	
		B	.946	.273	.479	.684	.049	.284	.028	.072	.694	.113	
13p		A	.077	.807	.449	.149	.619	.663	.129	.025	.600		
		B	.188	.737	.508	.059	.303	.718	.896	.007	.437		
13s		A	.752	.263	.752	.832	.748	.062	.677	.133			
		B	.729	.144	.603	.796	.978	.072	.672	.064			
14p		A	.667	.305	.751	.867	.071	.186	.499				
		B	.545	.182	.814	.867	.079	.290	.412				
14s		A	.650	.058	.979	.220	.814	.705					
		B	.480	.059	.925	.629	.941	.496					
15p		A	.301	.999	.645	.220	.097						
		B	.554	.715	.486	.168	.036						
15s		A	.125	.004	.618	.507							
		B	.970	.439	.516	.325							
21p		A	.839	.311	.023								
		B	.952	.256	.312								
21s		A	.461	.704									
		B	.643	.992									
22p		A	.531										
		B	.960										

(ii) Associations between grouped chromosome variants. A and B as in (i).

		D group satellites intense	brilliant	i + b
G group satellites intense	A	.446	.614	.513
	B	.103	.669	.222
G group satellites brilliant	A	.803	.020	.688
	B	.587	.020	.521
G group satellites i + b	A	.675	.108	.316
	B	.206	.142	.151

When associations (whether considered significant or not) were detected in these comparisons, it was almost always the case that the presence of an intense band in one region was associated with the presence (rather than absence) of an intense band in the other region. Exceptions to this generalisation are the 13p/13s and 14s/15s comparisons. It is perhaps true that these positive associations are actually a reflection of the quality of slide preparation and staining rather than a real genetic association. That is, there is a better contrast between bright and dull bands in some slides, which leads to an increased or decreased chance of scoring variant bands as intense, say, for all chromosome regions.

If an explanation is required for the two cases where the above generalisation does not apply, perhaps it is that these two comparisons (between 13p and 13s, and between 14s and 15s) are more susceptible to finer distinctions being made between levels of fluorescence than are other comparisons between regions that do not lie so close together, nor on positions so similar in appearance. (That is, closer comparison is possible between 14s and 15s than, say, between 3 or 13p and either 14s or 15s.)

Of particular interest are comparisons between frequencies of variants on the short arms and satellites of each acrocentric chromosome. No significant associations were found in any chromosome. Some associations between the satellites of different chromosomes were found. For example, between 13s and 21s (a positive association, not quite acceptable as significant) 14s and 15s (a negative association, see above) and 15s and 21s (no consistent association, i.e. χ^2 value is significant, Kendall's tau is not).

Other associations which were found to be 'linear' and significant or nearly significant, were between chromosomes (i) 4 and 21p, (ii) 4 and 15p, (iii) 13p and 22p, (iv) 14p and 21s, and (v) 15p and 22s. In view of

the lack of association found in many comparisons it seems unlikely that such sporadic associations reflect a real example of linkage disequilibrium between the variant regions. Consideration of the distribution of the variables when they are grouped into various categories (see above, page 160), prompts the same conclusion.

When the variables were grouped (Table 5.9 (ii)) the only significant association found was between brilliant D group satellites and brilliant G group satellites. The association was a positive one. If this finding is merely a result of scoring methods it is very difficult to see why the association was not found also with intense satellites.

2. Associations Between Chromosome Variables and Other Genetic Markers.

Table 5.10 shows the results of an analysis to detect any associations between the chromosome variants and the other genetic markers studied. The P values assigned to the X^2 values and Kendall's taus (blood group and isoenzyme phenotypes were ranked where possible, for example, MM, MN, NN) are given. The ABO blood groups seem to be associated with chromosome variants more often than would be expected by chance. These associations are with chromosomes 4, 14s, and 15s.

The Kell blood groups also show more associations than would be expected; in this case the correlation coefficients are also significant, indicating that the association is a linear one. In both cases (Kell with chromosomes 4 and 22s) the k allele is associated with the negative variant.

None of the proteins or isoenzymes shows any association which is significant in both the X^2 test and the rank correlation tests. None is then considered to be important. The ESD locus is on chromosome 13. No associations were found

TABLE 5.10: Associations between chromosome variants and other genetic markers. A and B as in table 5.9.

			blood groups and isoenzymes												
			ABO	Rh(D)	MN	Ss	MNSs	Fy	Kell	Kp	P_1*	Hp*	AP	AK	PGM	ESD
chromosomes	3	A	.779	.580	.548	.652	.872	.255	.813	.615	.132	.892	.292	.583	.013	.438
		B		.408	.134	.468		.460	.538	.753	.311	.392		.791	.284	.408
	4	A	.056	.868	.675	.570	.602	.448	.040	.797	.868	.200	.698	.218	.433	.505
		B		.868	.998	.917		.533	.021	.377	.869	.843		.219	.409	.408
	13p	A	.466	.558	.493	.521	.424	.553	.527	.173	.841	.331	.034	.729	.592	.523
		B		.312	.716	.787		.427	.957	.509	.892	.766		.812	.551	.852
	13s	A	.547	.223	.923	.094	.577	.460	.397	.863	.510	.222	.073	.224	.418	.934
		B		.753	.845	.127		.695	.315	.541	.327	.203		.201	.665	.776
	14p	A	.691	.897	.131	.596		.910	.397	.557	.205	.739	.124	.482	.471	.459
		B		.897	.311	.418		.673	.200	.627	.206	.730		.482	.275	.320
	14s	A	.054	.931	.810	.708	.103	.668	.414	.933	.364	.972	.930	.068	.473	.962
		B		.937	.290	.181		.377	.228	.896	.330	.894		.289	.711	.682
	15p	A	.725	.145	.835	.429		.947	.778	.639	.728	.547	.498	.948	.904	.886
		B		.111	.701	.194		.740	.921	.599	.633	.390		.783	.684	.912
	15s	A	.056	.137	.698	.889	.732	.380	.992	.567	.686	.152	.729	.792	.552	.887
		B		.293	.326	.598		.261	.953	.156	.820	.010		.962	.092	.656
	21p	A	.935	.957	.670	.820		.288	.554	.398	.347	.901	.399	.647	.320	.712
		B		.957	.389	.885		.924	.273	.036	.347	.648		.647	.304	.638
* Adults only	21s	A	.093	.579	.361	.295	.554	.195	.464	.778	.151	.783	.847	.958	.063	.197
		B		.866	.651	.039		.663	.772	.766	.158	.802		.977	.595	.738
	22p	A	.213	.934	.393	.681	.664	.139	.214	.871	.961	.066	.612	.909	.218	.810
		B		.919	.623	.759		.016	.146	.544	.831	.578		.871	.127	.585
	22s	A	.296	.290	.832	.618	.782	.381	.074	.572	.379	.181	.519	.169	.699	.354
		B		.465	.378	.325		.997	.022	.375	.418	.127		.075	.711	.161

between either allele of this system and any of the variants on the short arm or satellite of this chromosome.

No significant rank correlation coefficients were found for comparisons between the number of positive variants in an individual's chromosome complement and the number of antigens detected on the red cell surface.

Chapter 6: RESULTS III. ANALYSIS OF THE DATA WITH REGARD TO CERTAIN DEMOGRAPHIC INFORMATION.

1. Sex.

It is very difficult to disentangle the effects of sex from those of age and phenotype frequency differences within different sub-groups of a population. It seems that ageing processes do not occur at the same rates in males as in females (or at least, that for whatever reasons, life expectancy is greater at any particular age for women than men. There is approximately a five-year difference in life expectancies for the two sexes throughout life until about the age of 60, when the difference begins to decline, and it disappears around the age of 77 years. The life expectancy of males does not exceed that of females until very late in life (about 95 years)).

Therefore, any genetic differences found between the two sexes, even when matched for age, will perhaps reflect an effect of ageing and ageing processes on the phenotype frequencies rather than a fundamentally sex-influenced effect.

Unless we are considering genes or gene products which only influence the physiology and genetic fitness of one of the sexes, it is possible that the term 'sex' is to some extent simply a short-hand way of referring to the rate of ageing, and the use of this variable may be to divide the population sample into two groups having different average rates or states of ageing. We can then only conclude that in any instance one can either refer to genetic differences found between individuals of different sexes in the same age-group as an influence of sex, or as an influence of the rate of ageing. The influence of these two factors, sex and age, might then be confused, particularly when the influence is not large. Large effects of either factor would probably not be easily mistaken for effects of the other. It is not enough to match individuals for chronological age when

attempting to isolate some influence of sex which is not related to ageing; individuals should be matched in some way for their physiological or developmental age.

In the older age-groups, when the effects of differential ageing rates might be expected to have evened out to some extent, the proportions of individuals in the two sexes has so altered, as a result of these differential ageing rates, that it is practically difficult to reveal an effect of sex because of the actual small number of males present in any but very large samples.

Inspection of table 6.1 shows that the only significant difference between the sexes in blood group phenotype frequencies is in the case of the MNSs system in the newborn infants. This difference seems to be caused by an excess of the N phenotypes (in all S groups) that is not significant when only the MN groups are considered ($P=.081$). In particular, a disproportionate number of NSs individuals are male. As the actual numbers involved are only ten male and two female, it is not thought that any meaningful conclusions can be drawn from this finding.

Fisher and Fraser Roberts (1943) reported a sex difference in the frequencies of the ABO blood groups, but reanalysis of the data (Fraser Roberts 1948; Allan 1953) has shown that the age distributions of the samples were an important influence.

A significant difference was found in the phenotype frequencies of Esterase-D in the adult series. An excess of the 1-1 phenotype was observed in the females. Further subdivision of the adults into 30-year age-groups revealed a significant difference according to sex in the under-30 year-olds, but not so in the 30 to 60, or over-60 year age-groups. The same sex difference was observed in these latter two age-groups, but was not significant. When using other methods of subdivision by age, this difference in the younger adults of the sample was always present. For example,

TABLE 6.1: Blood group and isoenzyme phenotype frequencies subdivided according to sex.

	Male		Female		Total	
	No.	%	No.	%	No.	%
1. ABO						
A	122	(38.5)	150	(38.4)	272	(38.4)
O	153	(48.3)	182	(46.5)	335	(47.3)
B	33	(10.4)	51	(13.0)	84	(11.9)
AB	9	(2.8)	8	(2.0)	17	(2.4)
Total	317		391		708	
2. A_1A_2BO						
A_1	53	(27.2)	74	(29.1)	127	(28.3)
A_2	16	(8.2)	21	(8.3)	37	(8.2)
O	97	(49.7)	118	(46.5)	215	(47.9)
B	22	(11.3)	35	(13.8)	57	(12.7)
A_1B	6	(3.1)	6	(2.4)	12	(2.7)
A_2B	1	(0.5)	0		1	(0.2)
Total	195		254		449	
3. Rhesus						
r r	49	(16.1)	55	(15.1)	104	(15.5)
R_1r	113	(37.0)	114	(31.3)	227	(33.9)
R_1R_1	58	(19.0)	69	(19.0)	127	(19.0)
R_0r	2	(0.7)	10	(2.7)	12	(1.8)
R_2r	15	(4.9)	25	(6.9)	40	(6.0)
R_2R_2	6	(2.0)	6	(1.6)	12	(1.8)
R_1R_2	54	(17.7)	71	(19.5)	125	(18.7)
R_zR_1	1	(0.3)	2	(0.5)	3	(0.4)
r"r'	1	(0.3)	1	(0.3)	2	(0.3)
r"r	4	(1.3)	4	(1.1)	8	(1.2)
R_zR_2	2	(0.7)	6	(1.6)	8	(1.2)
r"r"	0		1	(0.3)	1	(0.1)
Total	305		364		669	
4. Rhesus(D)						
D+ve	261	(82.3)	326	(83.4)	587	(82.9)
D-ve	56	(17.7)	65	(15.6)	121	(17.1)
Total	317		391		708	
5. MN						
M	83	(28.3)	132	(34.4)	215	(31.8)
MN	149	(50.9)	194	(50.5)	343	(50.7)
N	61	(20.8)	58	(15.1)	119	(17.6)
Total	293		384		677	
6. S						
S	34	(11.3)	45	(12.0)	79	(11.7)
Ss	136	(45.2)	176	(46.8)	312	(46.1)
s	131	(43.5)	155	(41.2)	286	(42.2)
Total	301		376		677	
7. MNS						
MS	15	(5.4)	27	(7.3)	42	(6.5)
MSs	43	(15.5)	64	(17.3)	107	(16.6)
Ms	19	(6.9)	32	(8.7)	51	(7.9)
MNS	17	(6.1)	17	(4.6)	34	(5.3)
MNSs	64	(23.1)	93	(25.2)	157	(24.3)
MNs	62	(22.4)	79	(21.4)	141	(21.8)
NS	1	(0.4)	1	(0.3)	2	(0.3)
NSs	16	(5.8)	16	(4.3)	32	(5.0)
Ns	40	(14.4)	40	(10.8)	80	(12.4)
Total	277		369		646	

Series	X^2	d.f.	P
1. ABO			
Infants	0.63	3	.890
Adults	1.49	3	.685
Total	1.59	3	.661
2. A_1A_2BO			
Adults	1.51	4	.824
3. Rhesus			
Infants	2.78	5	.734
Adults	11.69	6	.069
Total	8.52	7	.289
4. Rhesus(D)			
Infants	0.87	1	.352
Adults	1.43	1	.231
Total	0.07	1	.791
5. MN			
Infants	5.62	2	.081
Adults	1.62	2	.446
Total	5.01	2	.082
6. S			
Infants	2.57	2	.277
Adults	0.26	2	.876
Total	0.37	2	.831
7. MNS			
Infants	14.67	7	.040
Adults	7.07	7	.422
Total	5.27	7	.626

TABLE 6.1 contd.:

	Male		Female		Total	
	No.	%	No.	%	No.	%
8. Duffy						
Fy^a	61	(20.0)	67	(17.6)	128	(18.7)
Fy^aFy^b	130	(42.6)	182	(47.9)	312	(45.5)
Fy^b	114	(37.4)	131	(34.5)	245	(35.8)
Total	305		380		685	
9. Kell						
K	0		2	(0.5)	2	(0.3)
Kk	21	(6.6)	32	(8.2)	53	(7.5)
k	296	(93.4)	357	(91.3)	653	(92.2)
Total	317		391		708	
10. P_1						
P_1+ve	137	(70.3)	173	(68.4)	310	(69.2)
P_1-ve	58	(29.7)	80	(31.6)	138	(30.8)
Total	195		253		448	
11. Haptoglobin						
1 - 1	24	(13.7)	37	(15.5)	61	(14.8)
2 - 1	78	(44.6)	120	(50.4)	198	(47.9)
2 - 2	73	(41.7)	81	(34.0)	154	(37.3)
Total	175		238		413	
12. Phosphoglucomutase						
1 - 1	177	(59.4)	238	(65.9)	415	(63.0)
2 - 1	101	(33.9)	104	(28.8)	205	(31.1)
2 - 2	20	(6.7)	19	(5.3)	39	(5.9)
Total	298		361		659	

	Male		Female		Total	
	No.	%	No.	%	No.	%
13. Esterase-D						
1 - 1	216	(73.2)	291	(80.4)	507	(77.2)
2 - 1	71	(24.1)	63	(17.4)	134	(20.4)
2 - 2	8	(2.7)	8	(2.2)	16	(2.4)
Total	295		362		657	
14. Adenylate kinase						
1 - 1	285	(94.1)	343	(93.0)	628	(93.5)
2 - 1	18	(5.9)	26	(7.0)	44	(6.5)
Total	303		369		672	
15. Acid phosphatase						
A	37	(12.4)	43	(11.8)	80	(12.1)
BA	105	(35.1)	136	(37.5)	241	(36.4)
B	129	(43.1)	151	(41.6)	280	(42.3)
BC	17	(5.7)	22	(6.1)	39	(5.9)
CA	10	(3.3)	10	(2.8)	20	(3.0)
C	1	(0.3)	1	(0.3)	2	(0.3)
Total	299		363		662	

Series	X^2	d.f.	P
8. Duffy			
Infants	0.82	2	.663
Adults	1.93	2	.381
Total	1.94	2	.379
9. Kell			
Infants	2.39	1	.122
Adults	0.01	1	.924
Total	0.78	1	.377
10. P_1			
Adults	0.10	1	.746
11. Haptoglobin			
Adults	2.54	2	.280
12. Phosphoglucomutase			
Infants	0.84	2	.656
Adults	3.84	2	.146
Total	3.04	2	.219
13. Esterase-D			
Infants	0.14	2	.931
Adults	6.68	2	.036
Total	4.79	2	.091
14. Adenylate kinase			
Infants	0.01	1	.903
Adults	0.30	1	.583
Total	0.18	1	.675
15. Acid phosphatase			
Infants	2.40	4	.662
Adults	2.74	4	.602
Total	0.63	4	.960

in a sample of adults aged between 20 and 40 years the ESD frequencies had a sex difference which gave a X^2 value of 5.796, which is a significant between the 1% and 2% levels. In fact, only in a group of over-eighty year-olds was an excess of females with the 1.1 phenotype not seen, but there are very few males in this age-group.

No difference according to sex was found in the ESD frequencies of a sample of adults from County Durham examined by Williams (1977).

It seems true that this sex difference does exist in the present sample, but it may be the one significant finding which is expected by chance in the 20 tests made using the 0.05 significance level.

Tables 6.2 to 6.4 give the chromosome variant frequencies subdivided according to sex, and the results of statistical analysis of these data. No significant sex differences were observed between the frequencies of individual variants in either the newborn infant or the adult sample. However, it was noticed when examining the variant frequency distribution between sexes in the different age-groups, that the trend for a decreasing proportion of males from the NN type, to NI, to II was about twice as common as the trend in the opposite direction. (Because of the low frequency of the brilliant variants, this analysis is of combined (intense+brilliant) frequencies). If this trend is 'real' then it ought to lead to sex differences in the distributions of the grouped-variables (see below). In no case was the trend significant at the 5% level, and there were many examples where no apparent trend could be detected.

It may be that the decrease in the proportion of males in types NI and II, compared with NN, indicates a reluctance to classify variant regions as intense or brilliant when the

TABLE 6.2: Chromosome variant phenotype frequencies subdivided according to sex.

	Male		Female		Total	
	No.	%	No.	%	No.	%
1. Chromosome 3						
BB	3	(1.0)	0		3	(0.4)
BI	20	(6.6)	29	(7.9)	49	(7.3)
II	33	(10.9)	53	(14.4)	86	(12.8)
IN	130	(42.9)	151	(41.1)	281	(41.9)
BN	40	(13.2)	45	(12.3)	85	(12.7)
NN	77	(25.4)	89	(24.3)	166	(24.8)
Total	303		367		670	
2. Chromosome 13p						
BB	1	(0.3)	2	(0.5)	3	(0.4)
BI	11	(3.6)	17	(4.6)	28	(4.2)
II	74	(24.4)	83	(22.6)	157	(23.4)
IN	160	(52.8)	209	(56.9)	369	(55.1)
BN	4	(1.3)	4	(1.1)	8	(1.2)
NN	53	(17.5)	52	(14.2)	105	(15.7)
Total	303		367		670	
3. Chromosome 13s						
BI	1	(0.3)	2	(0.5)	3	(0.4)
IN	53	(17.5)	68	(18.5)	121	(18.1)
BN	5	(1.7)	14	(3.8)	19	(2.8)
NN	244	(80.5)	283	(77.1)	527	(78.7)
Total	303		367		670	
4. Chromosome 14s						
BI	1	(0.3)	4	(1.1)	5	(0.7)
II	7	(2.3)	2	(0.5)	9	(1.3)
IN	91	(30.0)	134	(36.5)	225	(33.6)
BN	12	(4.0)	10	(2.7)	22	(3.3)
NN	192	(63.4)	217	(59.1)	409	(61.0)
Total	303		367		670	
5. Chromosome 15s						
BI	7	(2.3)	5	(1.4)	12	(1.8)
II	4	(1.3)	12	(3.3)	16	(2.4)
IN	112	(37.0)	146	(39.8)	258	(38.5)
BN	10	(3.3)	15	(4.1)	25	(3.7)
NN	170	(56.1)	189	(51.5)	359	(53.6)
Total	303		367		670	
6. Chromosome 21s						
BB	0		1	(0.3)	1	(0.1)
BI	3	(1.0)	2	(0.5)	5	(0.7)
II	8	(2.6)	6	(1.6)	14	(2.1)
IN	93	(30.7)	114	(41.1)	207	(30.9)
BN	7	(2.3)	5	(1.4)	12	(1.8)
NN	192	(63.4)	239	(65.1)	431	(64.3)
Total	303		367		670	
7. Chromosome 22s						
BB	0		1	(0.3)	1	(0.1)
BI	8	(2.6)	5	(1.4)	13	(1.9)
II	5	(1.7)	16	(4.4)	21	(3.1)
IN	110	(36.3)	147	(40.1)	257	(38.4)
BN	19	(6.3)	19	(3.2)	38	(5.7)
NN	161	(53.1)	179	(48.8)	340	(50.7)
Total	303		367		670	

Series	X^2	d.f.	P
1. Chromosome 3			
Infants	6.80	4	.147
Adults	3.76	4	.440
Total	1.98	4	.740
2. Chromosome 13p			
Infants	1.82	3	.612
Adults	3.71	4	.447
Total	2.52	4	.641
3. Chromosome 13s			
Infants	2.62	1	.106
Adults	0.24	3	.971
Total	3.13	2	.209
4. Chromosome 14s			
Infants	1.97	2	.374
Adults	2.47	3	.480
Total	4.14	3	.247
5. Chromosome 15s			
Infants	1.05	2	.592
Adults	4.95	4	.293
Total	4.75	4	.314
6. Chromosome 21s			
Infants	0.36	2	.840
Adults	1.64	4	.802
Total	1.67	3	.645
7. Chromosome 22s			
Infants	3.03	3	.388
Adults	5.95	3	.114
Total	6.27	4	.180

TABLE 6.3: Chromosome variant phenotype frequencies subdivided according to sex (intense and brilliant levels of fluorescence combined).

	Male		Female		Total	
	No.	%	No.	%	No.	%
1. Chromosome 3						
II	56	(18.5)	82	(22.3)	138	(20.6)
IN	170	(56.1)	196	(53.4)	366	(54.6)
NN	77	(25.4)	89	(24.3)	166	(24.8)
Total	303		367		670	
2. Chromosome 4						
IN	26	(8.6)	32	(8.7)	58	(8.7)
NN	277	(91.4)	335	(91.3)	612	(91.3)
Total	303		367		670	
3. Chromosome 13p						
II	85	(28.1)	102	(27.8)	187	(27.9)
IN	165	(54.5)	213	(58.0)	378	(56.4)
NN	53	(17.5)	52	(14.2)	105	(15.7)
Total	303		367		670	
4. Chromosome 13s						
II	2	(0.7)	2	(0.5)	4	(0.6)
IN	57	(18.8)	82	(22.3)	139	(20.7)
NN	244	(80.5)	283	(77.1)	527	(78.7)
Total	303		367		670	
5. Chromosome 14p						
IN	7	(2.3)	12	(3.3)	19	(2.8)
NN	296	(97.7)	355	(96.7)	651	(97.2)
Total	303		367		670	
6. Chromosome 14s						
II	8	(2.6)	6	(1.6)	14	(2.1)
IN	103	(34.0)	144	(39.2)	247	(36.9)
NN	192	(63.4)	217	(59.1)	409	(61.0)
Total	303		367		670	
7. Chromosome 15p						
II	1	(0.3)	0		1	(0.1)
IN	10	(3.3)	13	(3.5)	23	(3.4)
NN	292	(96.4)	354	(96.5)	646	(96.4)
Total	303		367		670	
8. Chromosome 15s						
II	11	(3.6)	17	(4.6)	28	(4.2)
IN	122	(40.3)	161	(43.9)	283	(42.2)
NN	170	(56.1)	189	(51.5)	359	(53.6)
Total	303		367		670	
9. Chromosome 21p						
IN	6	(2.0)	8	(2.2)	14	(2.1)
NN	297	(98.0)	359	(97.8)	656	(97.9)
Total	303		367		670	
10. Chromosome 21s						
II	11	(3.6)	9	(2.5)	20	(3.0)
IN	100	(33.0)	119	(32.4)	219	(32.7)
NN	192	(63.4)	239	(65.1)	431	(64.3)
Total	303		367		670	
11. Chromosome 22p						
II	4	(1.3)	4	(1.1)	8	(1.2)
IN	58	(19.1)	76	(20.7)	134	(20.0)
NN	241	(79.5)	287	(78.2)	528	(78.8)
Total	303		367		670	
12. Chromosome 22s						
II	13	(4.3)	22	(6.0)	35	(5.2)
IN	129	(42.6)	166	(45.2)	295	(44.0)
NN	161	(53.1)	179	(48.8)	340	(50.7)
Total	303		367		670	

TABLE 6.4: X^2 values of the crosstabulations in table 6.3

Series	X^2	d.f.	P
1. Chromosome 3			
Infants	5.23	2	.073
Adults	0.57	2	.745
Total	1.51	2	.469
2. Chromosome 4			
Infants	0.27	1	.602
Adults	0.13	1	.713
Total	0.01	1	.941
3. Chromosome 13p			
Infants	1.48	2	.476
Adults	2.36	2	.307
Total	1.55	2	.461
4. Chromosome 13s			
Infants	2.62	1	.106
Adults	0.06	2	.970
Total	1.28	2	.527
5. Chromosome 14p			
Infants	1.10	1	.294
Adults	2.19	1	.139
Total	0.26	1	.609
6. Chromosome 14s			
Infants	1.72	2	.422
Adults	1.41	2	.494
Total	2.53	2	.282
7. Chromosome 15p			
Infants	0.14	1	.706
Adults	0.03	1	.865
Total	0.02	1	.883
8. Chromosome 15s			
Infants	1.06	2	.588
Adults	1.38	2	.502
Total	1.57	2	.457
9. Chromosome 21p			
Infants	0.02	1	.886
Adults	0.11	1	.739
Total	0.01	1	.927
10. Chromosome 21s			
Infants	0.02	2	.989
Adults	1.10	2	.578
Total	0.87	2	.678
11. Chromosome 22p			
Infants	1.41	1	.235
Adults	0.57	2	.754
Total	0.31	2	.854
12. Chromosome 22s			
Infants	2.23	2	.328
Adults	5.53	2	.063
Total	1.81	2	.404

Y chromosome is present (and, of course, very bright) and comparison between the variant region and this chromosome is possible.

Tables 6.5 to 6.7 give the frequencies of grouped-variables subdivided according to sex, together with the results of statistical analysis of the data. The difference anticipated from the above findings was found, and shown to be significant by the χ^2 test. The rank correlation coefficient Spearman's rho, had a significant value, and the mean number of intense bands in females (4.150 S.D.1.608) was found to be significantly higher by both the t-test and the Mann-Whitney U test, than that in the males (3.884 S.D.1.700). These differences were found when the total intense bands and the total positive bands were examined. The differences were found only in the adult sample, not in the newborn infants, but when these two series were combined the findings were the same in the total sample as they had been in the adults. These differences between males and females were noticed but were not significant when the satellites alone were considered.

TABLE 6.5: Grouped chromosome variants frequencies subdivided according to sex.

	Male		Female		Total	
	No.	%	No.	%	No.	%
1. D group satellites (intense)						
0	98	(32.3)	99	(27.0)	197	(29.4)
1	132	(43.6)	169	(46.0)	301	(44.9)
2	64	(21.1)	80	(21.8)	144	(21.5)
⩾3	9	(3.0)	19	(5.2)	28	(4.2)
Total	303		367		670	
2. D group satellites (brilliant)						
0	266	(87.8)	319	(86.9)	585	(87.3)
⩾1	37	(12.2)	48	(13.1)	85	(12.7)
Total	303		367		670	
3. D group satellites (all)						
0	86	(28.4)	83	(22.6)	169	(25.2)
1	129	(42.6)	163	(44.4)	292	(43.6)
2	69	(22.8)	91	(24.8)	160	(23.9)
⩾3	19	(6.3)	30	(8.2)	49	(7.3)
Total	303		367		670	
4. G group satellites (intense)						
0	120	(39.6)	127	(34.6)	247	(36.9)
1	133	(43.9)	180	(49.0)	313	(46.7)
2	43	(14.2)	49	(13.4)	92	(13.7)
⩾3	7	(2.3)	11	(3.0)	18	(2.7)
Total	303		367		670	
5. G group satellites (brilliant)						
0	268	(88.4)	335	(91.3)	603	(90.0)
⩾1	35	(11.6)	32	(8.7)	67	(10.0)
Total	303		367		670	

	Male		Female		Total	
	No.	%	No.	%	No.	%
6. G group satellites (all)						
0	102	(33.7)	115	(31.3)	217	(32.4)
1	135	(44.6)	172	(46.9)	307	(45.8)
2	56	(18.5)	66	(18.0)	122	(18.2)
⩾3	10	(3.3)	14	(3.8)	24	(3.6)
Total	303		367		670	
7. Total intense satellites						
0	42	(13.9)	38	(10.4)	80	(11.9)
1	95	(31.4)	104	(28.3)	199	(29.7)
2	91	(30.0)	126	(34.3)	217	(32.4)
3	54	(17.8)	61	(16.6)	115	(17.2)
⩾4	21	(6.9)	38	(10.4)	59	(8.8)
Total	303		367		670	
8. Total brilliant satellites						
0	238	(78.5)	295	(80.4)	533	(79.6)
⩾1	65	(21.5)	72	(19.6)	137	(20.4)
Total	303		367		670	
9. Total satellites						
0	29	(9.6)	32	(8.7)	61	(9.1)
1	82	(27.1)	83	(22.6)	165	(24.6)
2	102	(33.7)	130	(35.4)	232	(34.6)
3	52	(17.2)	66	(18.0)	118	(17.6)
⩾4	38	(12.5)	56	(15.3)	94	(14.0)
Total	303		367		670	

	Male		Female		Total	
	No.	%	No.	%	No.	%
10. Total intense bands						
<3	60	(19.8)	57	(15.7)	117	(17.5)
3	71	(23.4)	89	(24.3)	160	(23.9)
4	78	(25.7)	73	(19.9)	151	(22.5)
5	45	(14.9)	80	(21.8)	125	(18.7)
6	31	(10.2)	46	(12.5)	77	(11.5)
⩾7	18	(5.9)	25	(6.8)	43	(6.4)
Total	303		367		670	
11. Total brilliant bands						
0	182	(60.1)	234	(63.8)	416	(62.1)
1	93	(30.7)	90	(24.5)	183	(27.3)
⩾2	28	(9.2)	43	(11.7)	71	(10.6)
Total	303		367		670	
12. Total variant bands						
<3	33	(10.9)	31	(8.4)	64	(9.6)
3	64	(21.1)	64	(17.4)	128	(19.1)
4	69	(22.8)	85	(23.2)	154	(23.0)
5	62	(20.5)	82	(22.3)	144	(21.5)
6	46	(15.2)	56	(15.3)	102	(15.2)
⩾7	29	(9.6)	49	(13.4)	78	(11.6)
Total	303		367		670	

TABLE 6.6: Statistical data for Table 6.5

Series	X^2	d.f.	P	Spearman's rho	P
1. D group satellites (intense)					
Infants	1.99	3	.575	.0790	.225
Adults	3.21	3	.360	.0490	.309
Total	3.82	3	.281	.0598	.122
2. D group satellites (brilliant)					
Infants	0.04	1	.848	.0290	.657
Adults	0.00	1	.950	.0043	.929
Total	0.05	1	.826	.0141	.717
3. D group satellites (all)					
Infants	2.09	3	.555	.0875	.179
Adults	1.84	3	.607	.0532	.269
Total	3.42	3	.331	.0664	.086
4. G group satellites (intense)					
Infants	1.65	3	.649	-.0181	.782
Adults	6.36	3	.095	.0646	.179
Total	2.45	3	.485	.0395	.307
5. G group satellites (brilliant)					
Infants	2.15	1	.142	-.1114	.087
Adults	0.04	1	.838	-.0159	.742
Total	1.18	1	.277	-.0463	.231
6. G group satellites (all)					
Infants	3.64	3	.303	-.0599	.359
Adults	3.94	3	.268	.0524	.277
Total	0.62	3	.893	.0182	.639
7. Total intense satellites					
Infants	1.80	4	.772	.0446	.495
Adults	8.21	4	.084	.0749	.120
Total	5.51	4	.239	.0672	.082
8. Total brilliant satellites					
Infants	0.92	1	.339	-.0711	.276
Adults	0.00	1	.948	.0003	.995
Total	0.24	1	.625	-.0197	.611
9. Total satellites					
Infants	4.33	4	.363	.0191	.770
Adults	6.63	4	.157	.0707	.142
Total	2.55	4	.636	.0569	.141
10. Total intense bands					
Infants	5.55	5	.352	.0417	.523
Adults	9.38	5	.095	.1069	.026
Total	10.35	5	.066	.0856	.027
11. Total brilliant bands					
Infants	0.25	2	.882	-.0053	.936
Adults	4.03	2	.133	-.0358	.457
Total	3.64	2	.162	-.0243	.530
12. Total variant bands					
Infants	4.14	5	.529	.0342	.600
Adults	5.93	5	.313	.0897	.062
Total	4.54	5	.475	.0732	.058

TABLE 6.7: A comparison of the mean numbers of grouped chromosome variants per individual in males and females.

	Series	mean no. in males	mean no. in females	t	P	Mann-Whitney U	z	P
1. D group satellites (intense)	Infants	0.8739	0.9841	-1.10	.274	6401.5	-1.214	.225
	Adults	0.9896	1.0913	-1.23	.219	21808.5	-1.018	.309
	Total	0.9472	1.0545	-1.67	.095	51998.0	-1.545	.122
2. D group satellites (brilliant)	Infants	0.0721	0.0952	-0.61	.545	6882.5	-0.446	.656
	Adults	0.1510	0.1577	-0.19	.853	23064.0	-0.089	.929
	Total	0.1221	0.1362	-0.53	.598	55081.0	-0.361	.718
3. D group satellites (all)	Infants	0.9459	1.0794	-1.25	.214	6333.0	-1.345	.179
	Adults	1.1406	1.2490	-1.23	.219	21782.5	-1.106	.269
	Total	1.0693	1.1907	-1.77	.077	51572.0	-1.715	.086
4. G group satellites (intense)	Infants	0.7387	0.7381	0.01	.995	6859.5	-0.278	.781
	Adults	0.8229	0.9087	-1.12	.261	21533.5	-1.343	.179
	Total	0.7921	0.8501	-0.97	.331	53262.5	-1.020	.308
5. G group satellites (brilliant)	Infants	0.1261	0.0556	1.82	.070	6559.0	-1.711	.087
	Adults	0.1198	0.1162	0.11	.915	22905.5	-0.331	.741
	Total	0.1221	0.0954	1.03	.301	54046.5	-1.199	.231
6. G group satellites (all)	Infants	0.8649	0.7937	0.71	.479	6547.5	-0.920	.358
	Adults	0.9427	1.0249	-1.03	.303	21821.0	-1.089	.276
	Total	0.9142	0.9455	-0.50	.618	54515.0	-0.468	.640

TABLE 6.7 contd.:

	Series	mean no. in males	mean no. in females	t	P	Mann-Whitney U	z	P
7. Total intense satellites	Infants	1.6126	1.7222	-0.77	.441	6645.0	-0.685	.493
	Adults	1.8125	2.0000	-1.61	.107	21190.5	-1.557	.119
	Total	1.7393	1.9046	-1.83	.068	51415.5	-1.738	.082
8. Total brilliant satellites	Infants	0.1982	0.1508	0.84	.400	6635.0	-1.092	.275
	Adults	0.2708	0.2739	-0.06	.953	23130.5	-0.006	.995
	Total	0.2442	0.2316	0.32	.745	54709.0	-0.510	.610
9. Total variant satellites	Infants	1.8108	1.8730	-0.41	.680	6844.5	-0.293	.770
	Adults	2.0833	2.2739	-1.54	.123	21294.0	-1.469	.142
	Total	1.9835	2.1362	-1.58	.114	52052.0	-1.471	.141
10. Total intense bands	Infants	3.7658	3.8571	-0.49	.622	6664.0	-0.640	.522
	Adults	3.9531	4.3029	-1.06	.040	20305.5	-2.222	.026
	Total	3.8845	4.1499	-2.07	.039	50181.5	-1.213	.027
11. Total brilliant bands	Infants	0.4685	0.4782	-0.08	.935	6957.0	-0.081	.936
	Adults	0.5521	0.5270	0.33	.740	22300.5	-0.744	.457
	Total	0.5215	0.5095	0.20	.841	54246.5	-0.631	.528
12. Total variant bands	Infants	4.2342	4.3333	-0.55	.584	6723.0	-0.525	.599
	Adults	4.5052	4.8299	-1.85	.065	20757.5	-1.865	.062
	Total	4.4059	4.6594	-1.94	.053	50964.0	-1.891	.059

2. Age.

The data of the present study have been analysed with a view to revealing any differential survival of any of the phenotypes examined. It must be admitted that the chances of detecting any such advantage are slim. It is generally thought that selection coefficients rarely exceed a few percent (Lewontin and Krakauer, 1973) and that the ability to detect selection pressure of this magnitude by observing differences on longevity of particular genotypes is limited, given the low sample sizes which are usually available for study.

It is well known that genetic factors play a very important part in determining life expectancy (Curtis et al. 1966), and there is much evidence that the role of these genetic factors is to influence the age of onset and progress of ageing processes (Burch 1968, Curtis 1971).

The identification of particular markers which might be associated with longevity and survival is not well advanced. There are relatively few studies which report the phenotype frequencies of particular genetic markers in different age strata of a population, and, more understandably because of methodological difficulties, none which reports a cohort of a population being studied through life with respect to the frequencies of polymorphic genetic markers.

Of the studies that have been published, most have been concerned with the ABO blood groups, and the results that have been obtained are in some cases contradictory. Fraser Roberts (1948) examined the age distributions of a series of blood donors from the S.W. of England and found no significant difference (for either sex) between the mean age of sets of individuals with the four blood groups of the ABO system. He concluded that there were no differences in survival value among the four groups, or that if there was

it was either too small to be detected in this sample, or it took effect outside the age range of the sample (approximately 18 to 60 years).

Bennett and Walker (1956) found no heterogeneity of of the ABO blood group frequencies in different age-groups of married female blood donors from East Anglia, but did find a significant trend for an increasing A/(O+A) ratio with age. However, it had been found that amongst these donors, those with blood group O responded more frequently to the questions put by the researchers than did those of other blood groups. The above trend was not found amongst unmarried female donors.

Buckwalter and Knowler (1958) found no significant differences in the ABO blood groups frequencies in various age-groups (when the data were divided into five or ten year intervals) of a series of blood donors (aged 18 to 60) from Iowa, U.S.A. The authors suggested that selective forces acting against individuals of a particular blood group by, for instance, rendering them more susceptible to certain diseases, were balanced by similar forces acting against individuals of the other blood groups.

Jorgensen and Schwarz (1968) reported the results of a study of ABO blood group frequencies amongst groups of individuals of different ages and different average physical fitnesses (estimated from their general health, and their participation in physically active occupations). They found that possession of blood group O seemed to confer an increased general fitness when comparing, for example, healthy blood donors with healthy over 75 year - olds, older with younger athletes, active with reserve soldiers, and healthy over 75 year- olds with surgically-treated over 75 year- olds.

Van Houte and Kestleoot (1972) reported results which agreed with the findings of the previously mentioned study. Older (55 to 60 year old) male soldiers had a higher frequency of blood group O than younger; and significantly more parents of individuals with blood group A or AB in the 50 to 55 year age-group died before the age of 60 (irrespective of cause) than parents of individuals with blood groups B and O. The authors suggested that part of this differential mortality might be due to the finding that the serum cholesterol level was higher in subjects with blood group A than in those with blood group O in all age-groups, thus causing individuals with blood group A to have a higher risk of suffering ischaemic heart disease, but they also stated that it seemed improbable that the whole of the difference could be explained in this way.

Williams (1977) reported trends in the ABO phenotype frequencies with age in his control series which agreed with the findings of the above two studies, but which were not significant. A similar tendency (towards lower frequency of blood group A in older age groups) was not found among a group of diabetic individuals. It was suggested that the disease processes to which people of blood group A are relatively more susceptible affect all diabetics regardless of their ABO phenotype. This study also reports a suggested relationship with age of the phenotype distributions of the Rhesus and MNS blood groups and the isoenzymes acid phosphatase and phosphoglucomutase.

Genotypes which contribute to an increase in the rate of ageing should be more frequent in persons with such conditions as might be regarded as manifestations of this phenomenon than in healthy persons of the same age. It is widely accepted that degenerative diseases are manifestations of ageing processes. There is an abundance of data concerning associations between genetic markers (particularly

the blood groups) and many degenerative diseases (Mourant et al. 1978), but unfortunately few studies have age-matched control subjects. Despite this omission, Mourant et al. (1978) state that "there is no doubt that a large number of diseases of many kinds are associated with the ABO system, whereas far fewer are known to be associated with the Rhesus or MN systems."

A few examples of reported associations between blood groups and degenerative (ageing) conditions which may influence phenotype frequencies in populations such as that of the present study, are the following:-

(i) cancers in general tend to be associated with blood group A rather than blood group O;
(ii) there are indications of a high A/O ratio in young as compared with older men suffering from coronary thrombosis;
(iii) there are raised frequencies of blood groups A and B in patients suffering from arteriosclerosis;
(iv) there is a deficiency of Rhesus negative individuals among senile dementia patients ((i) to (iv) Mourant et al. 1978);
(v) an association has been reported between cardiac infarction and blood group A (Van Houte and Kesteloot 1972);

Chromosomal analyses of different age strata of populations have revealed a tendency towards increased aneuploidy (usually hypodiploidy) with age, which tends to result in the loss of the sex chromosomes, and which appears to be more marked in females than in males (Galloway and Buckton 1978). Most studies of the subject report an increased frequency of aneuploidy in older age groups, but a steady increase in the rate of aneuploidy in younger persons has not been demonstrated.

Court Brown et al. (1966) indicate that the increase in aneuploidy is restricted to the latter part of adult life, and begins earlier and is greater in women. These authors concluded that the rise in the proportion of hypodiploid cells with increasing age is not a basic factor in, but rather a concomitant of, the ageing process. However, Curtis et al. (1966) considered the same type of findings and concluded from the relationship between aneuploidy and ageing that chromosome stability is genetically controlled and forms an important part of the genetic component of longevity.

Nielsen (1968) after comparing senile dementia patients with healthy persons concluded "Such an accumulation of aneuploid cells with loss of chromosomal material may very well be one of the main causes of the ageing process, and as suggested by Jarvik (1963) one might expect that the loss of chromosomal material would ultimately reach a level when it would interfere with metabolism to an extent that would be incompatible with life."

Levitan and Montagu (1977) suggest that abnormalities in chromosome number are a much more likely result of ageing processes than are anomalies in chromosome structure, for reasons similar to those which lead to an association between numerical chromosomal aberrations and advancing maternal age (see below, page 268).

Several authors have suggested that the loss of chromosomal material from ageing cells has survival value. Sandberg and Sakurai (1973) suggest that the loss of the Y chromosome from bone marrow may protect against leukaemia and other cancers; Jarvik et al. (1974) postulate that "if an accumulation of 'errors' in the genetic material over the life-span of an individual is a significant factor in the process of ageing, then the loss of chromosomes containing such errors would be advantageous." This

suggestion would appear to be true only if such errors tended to accumulate on the sex chromosomes, as it appears that these are lost most frequently from ageing cells.

Relationships between other aspects of chromosomal variability and longevity are unknown. Autosomal abnormalities cause an enormous decrease in life expectancy. Penrose (1947) estimated the life expectancy of patients with Down's syndrome to be 12 years. Collmann and Stoller (1962)give a more recent estimate of 18 years for Australian children with the condition. Abnormalities of the sex chromosome have a less severe effect.

There is some evidence that variant C-bands may be associated with ageing processes (C-band variants of chromosome 1 have been associated with malignancies, Shabtai and Halbrecht 1979; Atkin 1977), but no attempts to enumerate the frequencies of these variants in different age strata of populations has yet been published.

If a particular allele, or chromosome variant, were to have a beneficial effect on its carrier then the time of life at which this adaptive value became effective is important. That is, factors with early beneficial effects have more chance of remaining in a population than those which confer advantage on the carrier later in life (Frazetta 1975). It is important, therefore, to monitor gene and phenotype frequencies over the early period of life. This is rarely possible in human populations. Perhaps one of the most useful comparisons which can be made with the data of the present study, is between phenotype frequencies of genetic markers in the series of newborn infants and those in the adult series. However, there are a few reservations which must be borne in mind about the validity of the conclusions drawn from such comparisons. These have been discussed in an earlier section (see above, page 101).

Most of the elderly persons in the sample used in the present study were patients at the local geriatric hospital. The reasons for their admittance to hospital (diagnoses) were given earlier (page 93 , table 4.2), although, as was mentioned, these conditions were not necessarily the reasons for their prolonged stay in the hospital. However, the majority of the diseases fall into the classes of senile psychoses and cardiovascular diseases, both of which are widely accepted as being manifestations of ageing. Even amongst the other diagnoses, by far the largest number falls into the category of degenerative diseases (in total 90.4%). The second largest category (3.8%) is auto-immune diseases (4.4% if malignant diseases are included in this category). Several authors have suggested that autoimmunity might provide an explanation of certain aspects of ageing (Burch et al. 1971). Thus, 94.8% of the elderly hospital patients were admitted to hospital because of age-dependent degenerative conditions which tend to occur in genetically predisposed persons (Burch 1968).

Therefore, it might be expected that if any of the markers studied here were associated with any of these diseases then age-strata comparisons would reveal them. The difficulty in interpretation of results is in deciding whether an increased frequency of a particular allele or variant in the older age-groups was associated with development of a degenerative disease or with survival. Many of the geriatric patients were sampled long after their illnesses or conditions were diagnosed. It is probable that, especially amongst the oldest group, an elevated frequency of a genetic marker indicates that this marker is associated with survival.

Factors other than age which might lead to erroneous conclusions by creating genetic differences between age-groups are:

(i) Sex. The proportion of females increases with age,

especially in the older age groups in the present sample. Possibly this is due to a sex difference in susceptibility to aetiological factors in ageing processes (see above page 175).

(ii) Geographical origin. No information was collected regarding the birth places of the geriatric patients. Those genetic markers which show geographical variations in frequency may differ between generations if migration has altered significantly over the generations.

(iii) Social Class. Apparent effects of age will be noticed if the individuals of different age groups show important differences in occupational class for those markers which differ in their distributions between social classes. Table 6.67 shows that significant differences were found in the distributions of occupational class in the infants and adults. It was not thought that this particularly showed that the two series sampled different populations (see below, page 318).

The data presented here were analysed in a number of ways in order to determine whether or not phenotype frequencies differed with respect to age. The adult series was subdivided into age-groups by three different methods (table 6.8) in an attempt to obtain age-groups of approximately equal sizes for each interval. Associations between phenotype frequencies and age were tested for by means of the χ^2 test, by calculation of the rank correlation coefficient (Spearman's rho) and by comparison of the mean ages of different phenotype classes of each marker by means of the Student's t-test and the Mann Whitney U test. The date were analysed for trends in the manner described above (see page 130).

As the age-groups used in the above tests spanned quite large intervals and involved the establishment of arbitrary

TABLE 6.8: A key to the methods of subdivision by age and parental age used in the analyses of chapter 6.

I Methods of subdivision by age:

A = newborn infants and all adults

B = newborn infants and adults in 30 year age groups (beginning 1 year)

C = newborn infants and adults in 20 year age groups (beginning 1 year)

D = newborn infants and adults in 20 year age groups (beginning 15 years)

II Methods of subdivision by parental age:

E = 5 year age groups (beginning at 15 years)

F = 6 year age groups (beginning at 15 years)

cut-off points, effects of age were also examined using a system of "moving average age" to subdivide the sample. The adult series was divided into five-year age classes. Groups of four of these age-classes were taken which overlapped with the successive and preceding group, so that three age-classes were common to adjacent groups of four. The average phenotype frequency in each group of four was recorded, and the results displayed in a graph. These are given in Figure 6.1 for those systems which seemed to show variation in phenotype frequency with age.

A. Blood groups, serum proteins and isoenzymes.

Tables 6.9, 6.10, and 6.11 show the phenotype frequencies of these markers subdivided according to age and the results of statistical analysis of these data.

(i) ABO system.

No significant differences are found for any method of subdivision by age in ABO phenotype frequencies. Table 6.9 shows that there is an increase in the frequency of the O blood group with age until the age group of 60 years and over. When it is remembered that many of the over-60 year olds had some kind of degenerative condition, this could be considered to be in agreement with the findings of other authors (Jorgensen and Schwarz 1968, Williams 1977), that blood group O generally confers an advantage on the carrier. However, as no significant differences are found between this group of over-60 year-olds and another sample of the same age group, but not of geriatric patients, this conclusion is not compelling.

(ii) Rhesus system.

The Rhesus phenotypes, when subdivided according to age, are shown to be in distributions which differ significantly from those expected by chance. These differences are observed when both the adults and the total sample are examined, and also when the adults are compared with the newborn infants. If the fairly common phenotypes are examined the only consistent trend which occurs seems to be a decline in the frequency of the rr phenotype with age. This trend appears to be a part of a general decline in the frequency of dd individuals which is noticed when only the D phenotype is considered. There is no significant deviation from chance when the Rhesus (D) phenotypes are analysed by the X^2 test, but the rank correlation coefficient is nearly significant. This apparent trend is in contradiction to

TABLE 6.9: Blood group and isoenzyme phenotype frequencies subdivided according to age.

Age(yrs)	0		1 - 29		30 - 59		60+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. ABO										
A	108	(41.7)	59	(37.3)	33	(37.5)	70	(35.0)	270	(38.3)
O	120	(46.3)	79	(50.0)	45	(51.1)	91	(45.5)	335	(47.5)
B	27	(10.4)	17	(10.8)	7	(8.0)	32	(16.0)	83	(11.8)
AB	4	(1.5)	3	(1.9)	3	(3.4)	7	(3.5)	17	(2.4)
Total	259		158		88		200		705	
2. Rhesus										
r r	47	(18.4)	24	(15.2)	12	(13.6)	21	(12.7)	104	(15.6)
R_1r	88	(34.5)	50	(31.6)	33	(37.5)	55	(33.3)	226	(33.9)
R_1R_1	39	(15.3)	34	(21.5)	20	(22.7)	33	(20.0)	126	(18.9)
R_or	0		4	(2.5)	4	(4.5)	4	(2.4)	12	(1.8)
R_2r	15	(5.9)	7	(4.4)	3	(3.4)	14	(8.5)	39	(5.9)
R_2R_2	4	(1.6)	5	(3.2)	0		3	(1.9)	12	(1.8)
R_1R_2	55	(21.6)	30	(19.0)	11	(12.5)	29	(17.6)	125	(18.8)
R_zR_1	1	(0.4)	0		1	(1.1)	1	(0.6)	3	(0.5)
r"r'	1	(0.4)	0		1	(1.1)	0		2	(0.3)
r"r	4	(1.6)	4	(2.5)	0		0		8	(1.2)
R_zR_2	1	(0.4)	0		3	(3.4)	4	(2.4)	8	(1.2)
r"r"	0		0		0		1	(0.6)	1	(0.2)
Total	255		158		88		165		666	
3. Rhesus(D)										
D+ve	207	(79.9)	130	(82.3)	75	(85.2)	172	(86.0)	584	(82.8)
D-ve	52	(20.1)	28	(17.7)	13	(14.8)	28	(14.0)	121	(17.2)
Total	259		158		88		200		705	
4. MN										
M	80	(32.8)	38	(26.6)	32	(36.8)	64	(32.0)	214	(31.8)
MN	125	(51.2)	70	(49.0)	36	(41.4)	110	(55.0)	341	(50.6)
N	39	(16.0)	35	(24.5)	19	(21.8)	26	(13.0)	119	(17.7)
Total	244		143		87		200		674	
5. S										
S	33	(13.2)	11	(7.4)	7	(8.0)	27	(14.3)	78	(11.6)
Ss	111	(44.4)	70	(47.3)	37	(42.5)	92	(48.7)	310	(46.0)
s	106	(42.4)	67	(45.3)	43	(49.4)	70	(37.0)	286	(42.4)
Total	250		148		87		189		674	
6. MNS										
MS	17	(7.2)	8	(6.0)	4	(4.7)	13	(6.9)	42	(6.5)
MSs	39	(16.6)	14	(10.5)	20	(23.3)	33	(17.5)	106	(16.5)
Ms	17	(7.2)	13	(9.8)	8	(9.3)	13	(6.9)	51	(7.9)
MNS	14	(6.0)	2	(1.5)	3	(3.5)	14	(7.4)	33	(5.1)
MNSs	51	(21.7)	41	(30.8)	11	(12.8)	53	(28.0)	156	(24.3)
MNs	58	(24.7)	22	(16.5)	21	(24.4)	40	(21.2)	141	(21.9)
NS	2	(0.9)	0		0		0		2	(0.3)
NSs	12	(5.1)	8	(6.0)	6	(7.0)	6	(3.2)	32	(5.0)
Ns	25	(10.6)	25	(18.8)	13	(15.1)	17	(9.0)	80	(12.4)
Total	235		133		86		189		643	

TABLE 6.9 contd.:

Age(yrs)	0		1 - 29		30 - 59		60+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
7. Duffy										
Fy^a	41	(16.1)	34	(22.2)	14	(15.9)	37	(19.8)	126	(18.5)
Fy^aFy^b	114	(44.9)	70	(45.8)	41	(46.6)	86	(46.0)	311	(45.6)
Fy^b	99	(39.0)	49	(32.0)	33	(37.5)	64	(34.2)	245	(35.9)
Total	254		153		88		187		682	
8. Kell										
K	0		2	(1.3)	0		0		2	(0.3)
Kk	21	(8.1)	13	(8.2)	9	(10.2)	10	(5.0)	53	(7.5)
k	238	(91.9)	143	(90.5)	79	(89.8)	190	(95.0)	650	(92.2)
Total	259		158		88		200		705	
9. P_1										
P_1+ve			109	(69.4)	72	(91.8)	126	(63.0)	307	(69.0)
P_1-ve			48	(30.6)	16	(8.2)	74	(37.0)	138	(31.0)
Total			157		88		200		445	
10. Haptoglobin										
1 - 1			24	(16.6)	16	(20.3)	21	(11.3)	61	(14.9)
2 - 1			67	(46.2)	33	(41.8)	96	(51.6)	196	(47.8)
2 - 2			54	(37.2)	30	(38.0)	69	(37.1)	153	(37.3)
Total			145		79		186		410	
11. Phosphoglucomutase										
1 - 1	142	(58.4)	98	(64.9)	44	(55.7)	130	(70.7)	414	(63.0)
2 - 1	82	(33.7)	45	(29.8)	27	(34.2)	51	(27.7)	205	(31.2)
2 - 2	19	(7.8)	8	(5.3)	8	(10.1)	3	(1.6)	38	(5.8)
Total	243		151		79		184		657	
12. Esterase-D										
1 - 1	177	(73.4)	111	(74.5)	69	(87.3)	148	(80.0)	505	(77.2)
2 - 1	57	(23.7)	34	(22.8)	10	(12.7)	32	(17.3)	133	(20.3)
2 - 2	7	(2.9)	4	(2.7)	0		5	(2.7)	16	(2.4)
Total	241		149		79		185		654	
13. Acid phosphatase										
A	33	(13.6)	20	(13.4)	7	(8.8)	20	(10.6)	80	(12.1)
BA	102	(42.1)	43	(28.9)	28	(35.0)	67	(35.6)	240	(36.4)
B	89	(36.8)	67	(45.0)	34	(42.5)	88	(46.8)	278	(42.2)
BC	11	(4.5)	10	(6.7)	8	(10.0)	10	(5.3)	39	(5.9)
CA	7	(2.9)	7	(4.7)	3	(3.8)	3	(1.6)	20	(3.0)
C	0		2	(1.3)	0		0		2	(0.3)
Total	242		149		80		188		659	
14. Adenylate kinase										
1 - 1	232	(95.1)	146	(93.6)	72	(88.9)	175	(93.1)	625	(93.4)
2 - 1	12	(4.9)	10	(6.4)	9	(11.1)	13	(6.9)	44	(6.6)
Total	244		156		81		188		669	

TABLE 6.10: Statistical data for Table 6.9.

Series	Method of subdivision	X^2	d.f.	P	Spearman's rho	P
1. ABO						
Total	A	3.19	3	.363		
Total	B	0.09	9	.429		
Adults	B	5.39	6	.495		
Total	C	17.72	15	.278		
Adults	C	12.98	12	.371		
Total	D	11.70	12	.470		
Adults	D	7.51	9	.585		
2. Rhesus						
Total	A	14.92	7	.037		
Total	B	19.92	18	.338		
Adults	B	7.03	12	.856		
Total	C	48.17	25	.034		
Adults	C	29.22	20	.034		
Total	D	33.66	20	.029		
Adults	D	22.90	15	.086		
3. Rhesus(D)						
Total	A	2.25	1	.134	.0604	.108
Total	B	3.34	3	.342	.0681	.071
Adults	B	0.98	2	.614	.0418	.345
Total	C	4.04	5	.543	.0704	.062
Adults	C	1.82	4	.768	.0527	.268
Total	D	2.91	4	.573	.0600	.112
Adults	D	0.59	3	.898	.0254	.593
4. MN						
Total	A	0.70	2	.704	.0276	.473
Total	B	11.78	6	.067	.0412	.712
Adults	B	10.72	4	.030	.0991	.040
Total	C	19.69	10	.032	.0063	.871
Adults	C	18.49	8	.018	.0749	.122
Total	D	16.17	8	.040	.0079	.837
Adults	D	14.90	6	.021	.0797	.100
5. S						
Total	A	1.04	2	.594	.0110	.776
Total	B	8.20	6	.224	.0265	.492
Adults	B	7.42	4	.115	.1022	.035
Total	C	18.19	10	.052	.0215	.578
Adults	C	17.87	8	.022	.0894	.067
Total	D	15.26	8	.054	.0150	.698
Adults	D	15.08	6	.020	.0739	.130
6. MNS						
Total	A	4.85	7	.678		
Total	B	34.91	21	.029		
Adults	B	30.00	14	.008		
Total	C	52.74	35	.028		
Adults	C	42.62	28	.038		
Total	D	40.63	28	.058		
Adults	D	37.06	21	.017		

TABLE 6.10 contd.:

Series	Method of subdivision	X^2	d.f.	P	Spearman's rho	P
7. Duffy						
Total	A	2.60	2	.272	-.0610	.111
Total	B	3.97	6	.681	.0413	.282
Adults	B	1.62	4	.805	-.0241	.620
Total	C	5.60	10	.847	.0486	.206
Adults	C	3.23	8	.919	-.0063	.896
Total	D	6.70	8	.570	.0502	.191
Adults	D	4.40	6	.623	-.0027	.955
8. Kell						
Total	A	0.01	1	.912	.0091	.810
Total	B	3.57	3	.312	-.0379	.315
Adults	B	3.59	2	.166	-.0805	.090
Total	C	4.69	5	.455	-.0471	.213
Adults	C	4.74	4	.315	-.1029	.030
Total	D	5.01	4	.286	-.0402	.288
Adults	D	5.07	3	.167	-.0867	.068
9. P_1						
Adults	B	10.14	2	.006	-.0872	.067
Adults	C	6.66	4	.155	-.0739	.120
Adults	D	6.65	3	.084	-.0819	.085
10. Haptoglobin						
Adults	B	4.67	4	.323	.0291	.557
Adults	C	9.72	8	.286	-.0126	.799
Adults	D	4.26	6	.641	.0189	.703
11. Phosphoglucomutase						
Total	A	4.47	2	.107	-.0775	.047
Total	B	14.67	6	.023	-.0962	.014
Adults	B	11.64	4	.020	-.0712	.148
Total	C	17.68	10	.061	-.0963	.014
Adults	C	15.13	8	.057	-.0694	.160
Total	D	18.27	8	.019	-.0942	.015
Adults	D	15.52	6	.017	-.0650	.188
12. Esterase-D						
Total	A	3.01	2	.222	-.0675	.084
Total	B	7.99	3	.046	-.0790	.043
Adults	B	5.28	2	.071	-.0513	.298
Total	C	12.50	5	.029	-.0890	.023
Adults	C	9.99	4	.041	-.0738	.135
Total	D	5.34	4	.254	-.0770	.048
Adults	D	2.27	3	.519	-.0433	.381
13. Acid phosphatase						
Total	A	8.66	4	.070		
Total	B	14.95	9	.092		
Adults	B	9.03	8	.340		
Total	C	25.05	15	.049		
Adults	C	17.02	12	.149		
Total	D	20.13	12	.065		
Adults	D	11.89	9	.220		

TABLE 6.10 contd.:

Series	Method of subdivision	X^2	d.f.	P	Spearman's rho	P
14. Adenylate kinase						
Total	A	1.31	1	.253	.0497	.198
Total	B	3.85	3	.279	.0460	.235
Adults	B	1.88	2	.392	.0041	.934
Total	C	5.55	5	.353	.0489	.207
Adults	C	3.34	4	.503	.0105	.829
Total	D	4.87	4	.301	.0483	.213
Adults	D	2.74	3	.433	.0088	.857

TABLE 6.11: A comparison of the mean age of certain phenotypes (x) with that of the rest (not - x), in a number of genetic systems; with an analysis for trends in phenotype frequencies with age.

	Series	x	mean age : x	mean age : not - x	t*	P	Mann - Whitney U	z	P	Analysis for trends: z	Analysis for trends: P
ABO	Total	O	32.3334	32.8055	0.19	.848	60364.5	-0.099	.921		
	Adults	O	50.4643	51.9210	0.58	.563	23887.5	-0.379	.705	0.761	.242
	Total	A	30.2683	34.0260	1.49	.136	54784.0	-1.407	.159		
	Adults	A	50.5724	51.7003	0.43	.665	22319.0	-0.356	.722	0.472	.319
Rhesus(D)	Total	+ve	33.3061	28.0092	1.64	.102	31794.5	1.725	.085		
	Adults	+ve	51.5911	49.1177	0.74	.457	12129.5	0.825	.409	0.519	.302
MN	Total	N	31.0701	33.2635	0.66	.507	33404.0	-0.081	.936		
	Adults	N	46.2168	53.7554	2.33	.020	12011.5	01.948	.051	2.112	.017
P_1	Adults	+ve	49.9748	51.3886	1.63	.104	19247.5	-1.376	.169	-1.808	.035
PGM	Total	1 - 1	34.1725	28.0948	2.33	.020	45133.0	-2.126	.034		
	Adults	1 - 1	52.0784	48.2195	1.40	.161	17940.0	-1.016	.309	1.164	.123
	Total	2 - 2	19.1022	32.7166	2.53	.012	9181.0	-2.305	.021		
	Adults	2 - 2	38.2043	51.3643	2.98	.007	2797.0	-1.847	.065	2.056	.020
ESD	Total	1 - 1	33.6394	27.1444	2.15	.032	32704.5	-2.339	.019		
	Adults	1 - 1	51.8479	47.8258	1.24	.215	12172.5	-1.608	.108	-0.792	.215

* sign ignored

the findings of Williams (1977) which showed an increase in the frequency of Rhesus negative individuals with age in a County Durham population.

The mean ages of the Rhesus positive and negative individuals are not significantly different, although that of Rhesus positive individuals is the higher.

(iii) MNSs system.

a. MN

A significant deviation from chance distributions is found by all methods of subdivision, in both the adult and the total sample for this blood group. It appears that these differences are caused by a decline in the frequency of the N phenotype with age in the adult sample. This decline is also shown by the analysis for trends, and by the differences between the mean ages of N individuals and the rest of the sample. However, the frequency of the N phenotype in the newborn infants is almost as low as that found in the oldest adults. When the sexes are examined separately, these differences appear to occur in the females, rather than in the males.

b. S.

Significant X^2 values are found for both the total and adult sample, but the rank correlation coefficient generally is not significant and no consistent trends with age can be detected for any phenotype. The s phenotype appears to increase in frequency with age, except in the very oldest age-group. When the sexes are examined separately it is found that the s phenotype appears to decline in frequency with age in the males, but this decline does not reach a significant level. In the females, however, X^2 values are nearly significant but no consistent trend in phenotype frequencies with age is obvious.

c. MNS complex.

Significant X^2 values are found in the distributions of MNSs phenotypes by all methods of subdivision

by age, except when the comparison is between newborn infants and the adults. These differences appear to result from deviations of the MSs, MNS, MNSs and Ns phenotypes. Only the latter phenotype seems to show a detectable trend, that of declining with age in the adults.

(iv) Duffy system.

No significant effects of age are found in the phenotype frequencies of this blood group system by any of the methods of analysis.

(v) Kell system.

Although the X^2 test never gives a significant result the rank correlation coefficient appears to be significant or nearly significant in the adult series with each method of subdivision. The apparent reason for this is the marked increase in the k phenotype amongst the very oldest adults.

(vi) P_1 system.

P_1 results are only available for the adult series. Two of three methods of subdivision by age give a significant X^2 value, and also the analysis for trends gives a significant result. In two cases the rank correlation coefficient is nearly significant. The mean age of P_1 positive individuals is lower than that of P_1 negative persons, but the difference is not significant. There appears to be no consistent trend for either phenotype. The frequency of P_1 negative individuals declined markedly in the middle age-groups but rises in the oldest age-groups to reach a level higher than that of the youngest age-groups.

(vii) Haptoglobin.

No effects of age are found in the distributions

of phenotypes of this serum protein by any of the methods of analysis.

(viii) Phosphoglucomutase.

Significant X^2 values are found for all methods of subdivision of the sample by age, in both the total and adult series for the distribution of PGM phenotypes. The rank correlation coefficient is also significant in the total sample. There is a somewhat erratic decline in the frequency of the 2-2 phenotype with age. The mean age of 2-2 individuals is significantly lower than that of 1-1 and 2-1 individuals together. The trend for a declining frequency of the 2-2 phenotype is significant. In the total sample the mean age of 1-1 individuals was significantly higher than that of the rest of the sample, but this difference, although present, is not significant when only the adults are considered. The mean age of 2-1 individuals is only a little lower than that of the rest of the sample. These results do not agree with those of Williams (1977) which indicates a significant increase in the frequency of 2-1 phenotypes with age. This latter sample is too small to indicate any trends in the frequency of the 2-2 phenotype.

(ix) Esterase-D.

Significant values of X^2 are found in both the total and adult sample. Generally, for each method of subdivision the X^2 value is more significant in the total sample than in the adults, and in the total sample the rank correlation coefficient is also significant. There appears to be a not-quite linear increase in the frequency of the 1-1 phenotype, and consequent decline in the 2-1 phenotype. (The 2-2 phenotype is fairly uncommon in all age classes.) In the total sample the mean age of the 1-1 phenotype is significantly higher than that of the 2-1 and 2-2 phenotypes

together. The analysis for trends gives a non-significant result for the adult series.

(x) Acid phosphatase.

The X^2 test gives significant or nearly significant result in the total sample for each method of subdivision but not in the adults alone. It appears that the main difference is between the newborn infants and the total adults and is that the frequency of the BA phenotype is higher in the infants, and the frequency of the B phenotype lower. These results do not agree with those of Williams (1977) which indicated an increase in the frequency of the A phenotype with age. In fact, in the present sample the frequency of this phenotype appears to decline with age, although it is as high in the young adults as in the newborn infants.

(xi) Adenylate kinase.

No effects of age are found in the distributions of the phenotypes of this isoenzyme by any of the methods of analysis.

Figure 6.1 shows the changes in frequency of the phenotypes of some of the genetic markers mentioned above, by the method of 'moving average age class'.

Figure 6.1: The change in frequency of blood group and isoenzyme phenotypes with age, shown by the method of 'moving average ageclass'.

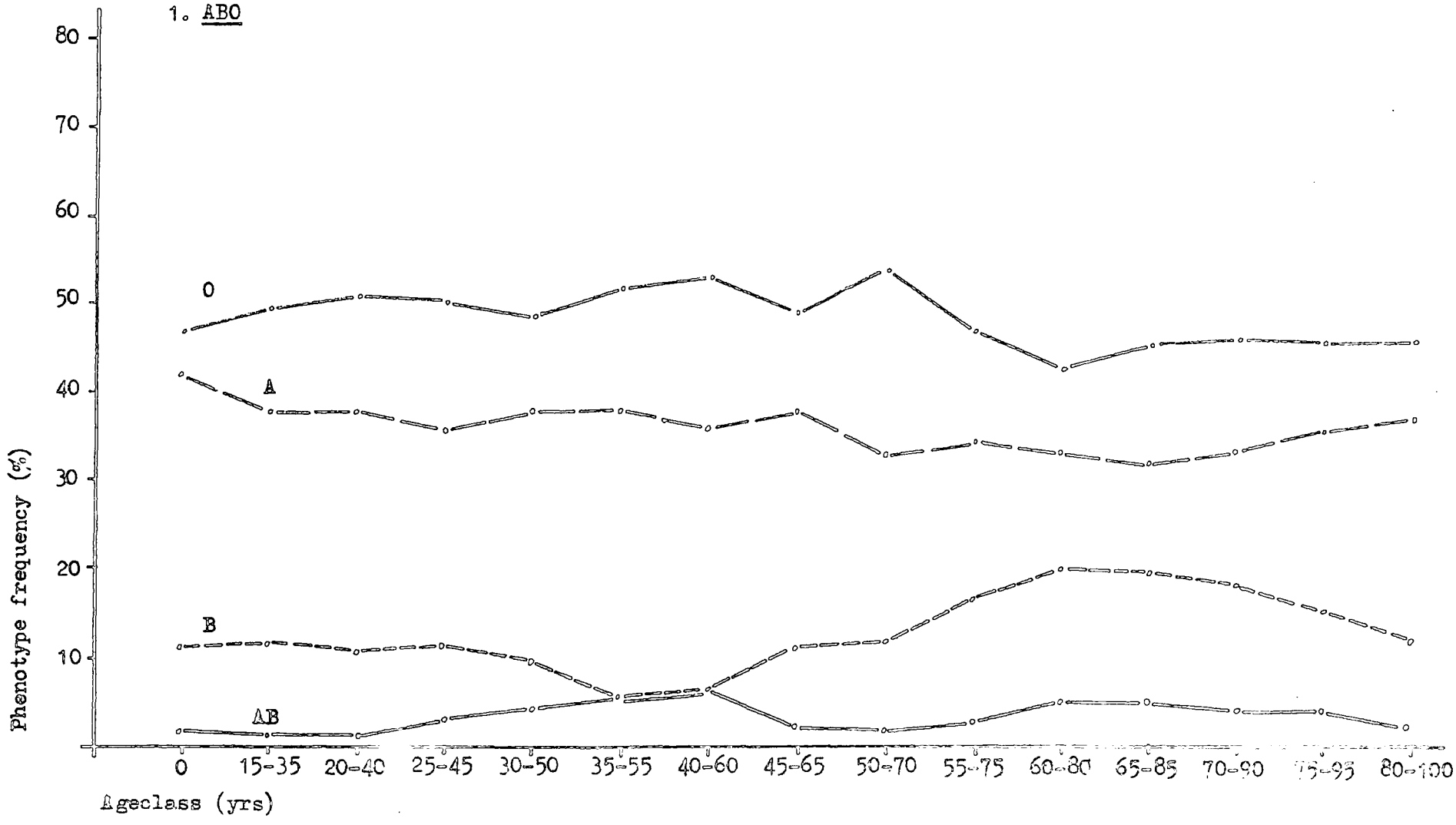


Figure 6.1 contd.:

2. Rhesus(D)

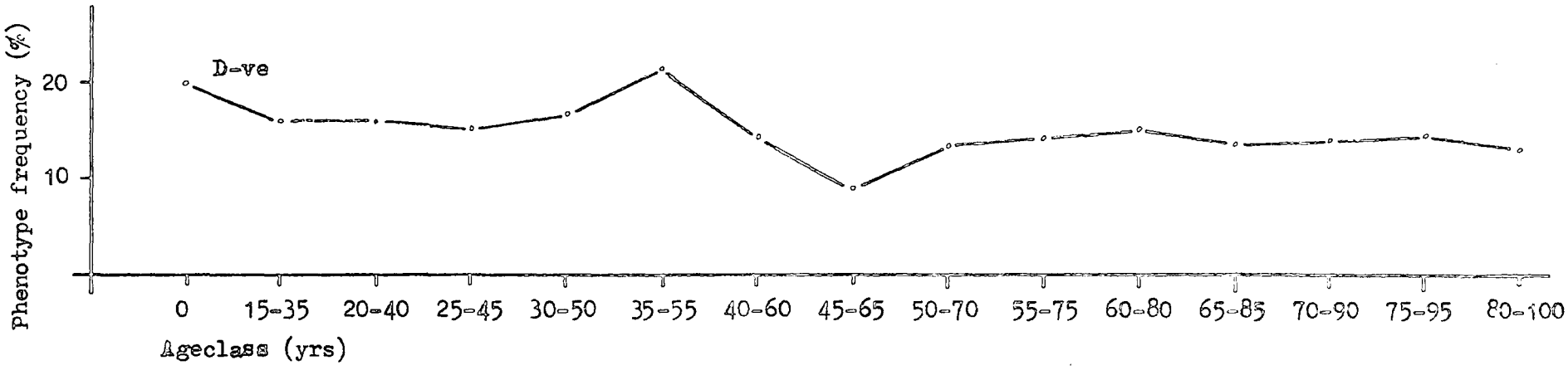


Figure 6.1 contd.:

3. MN

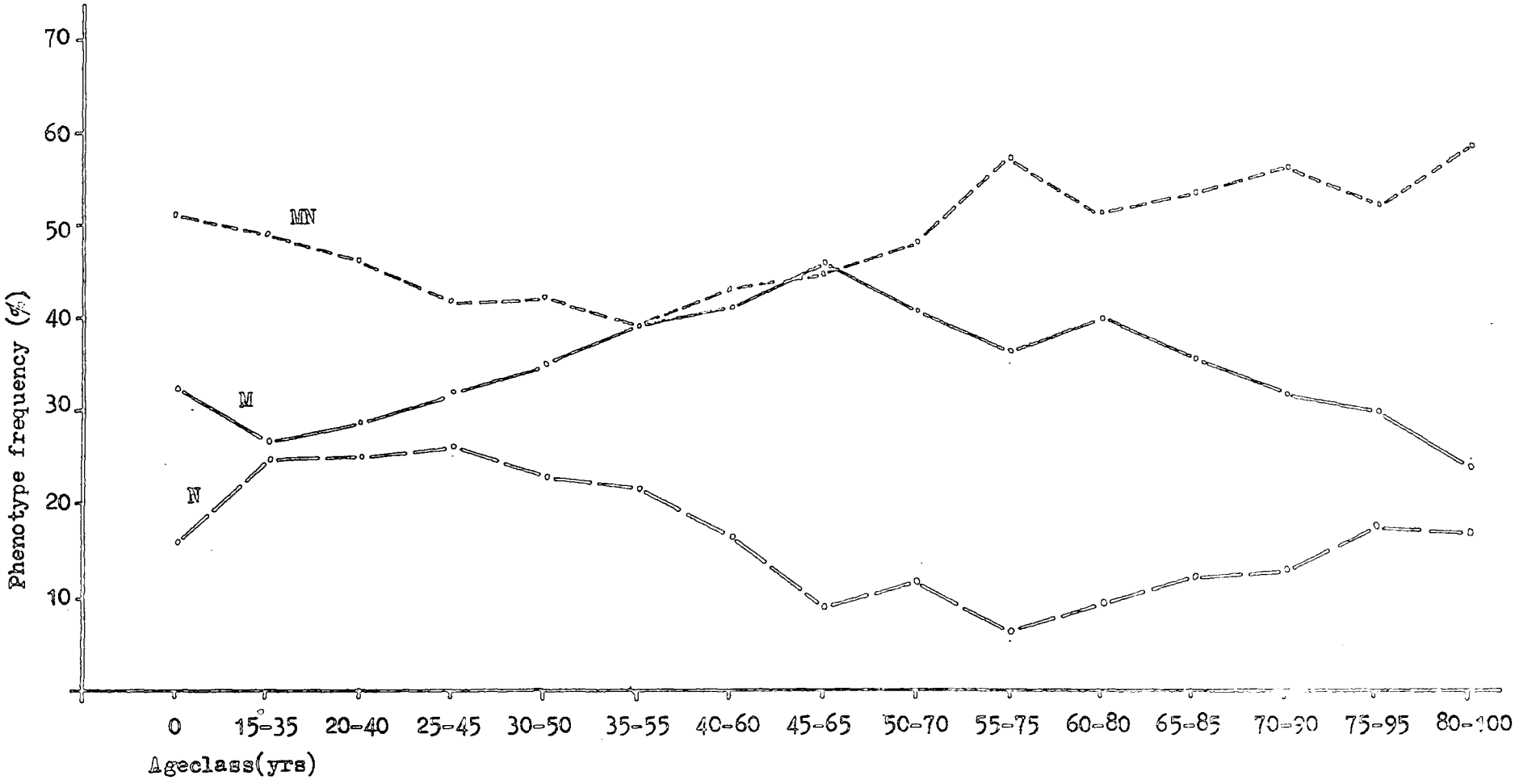


Figure 6.1 contd.:

4. S

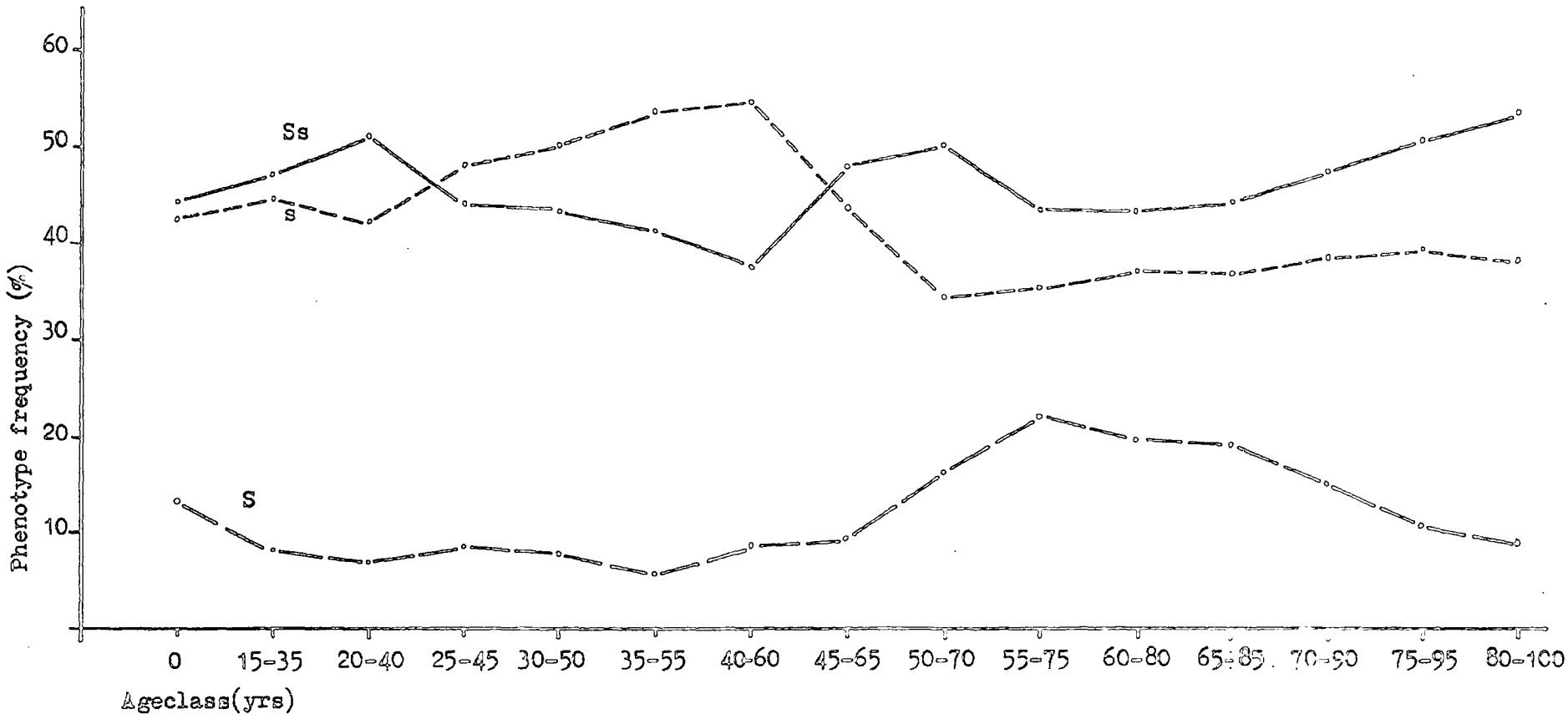


Figure 6.1 contd.:

5. $\underline{P}_1$

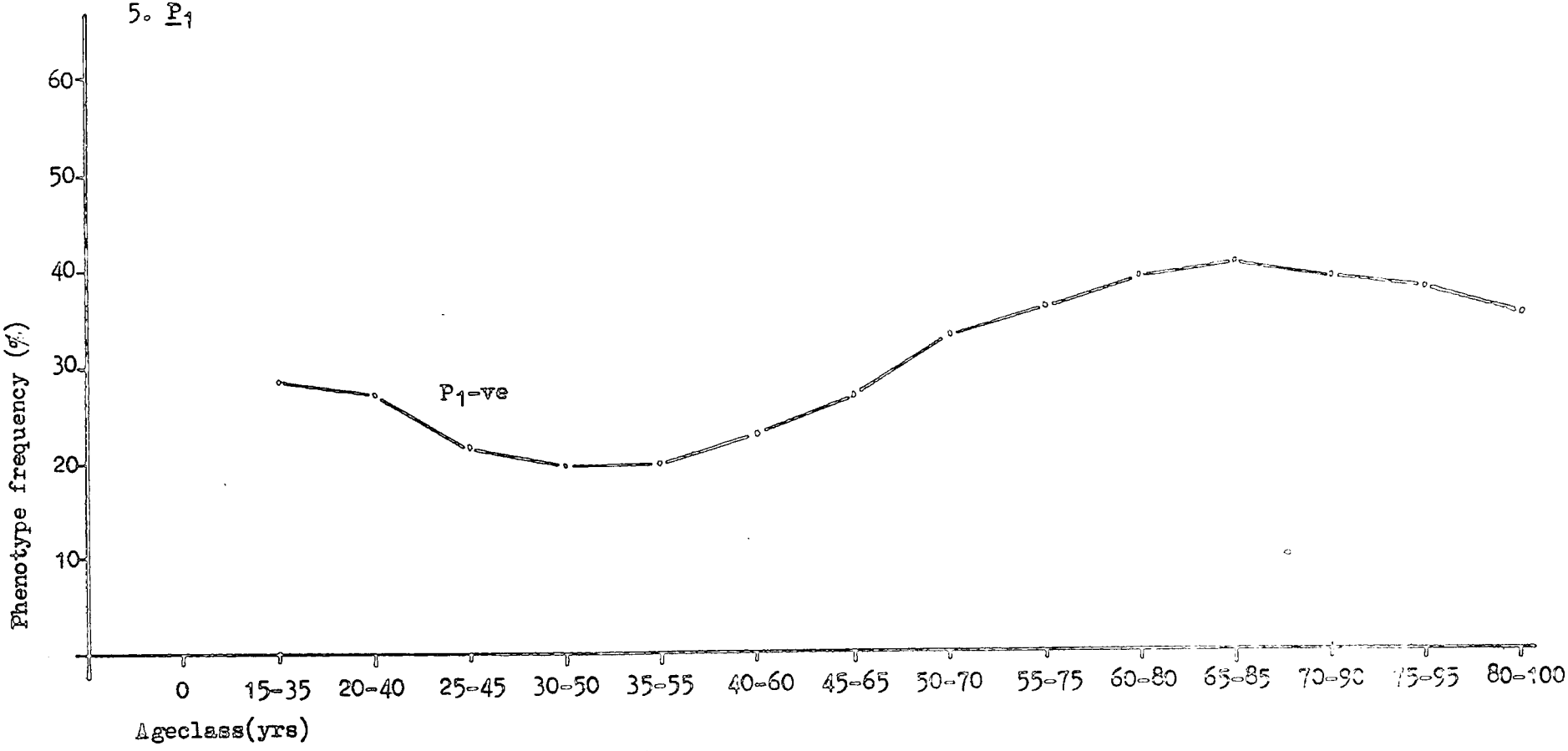


Figure 6.1 contd.:

6. Phosphoglucomutase

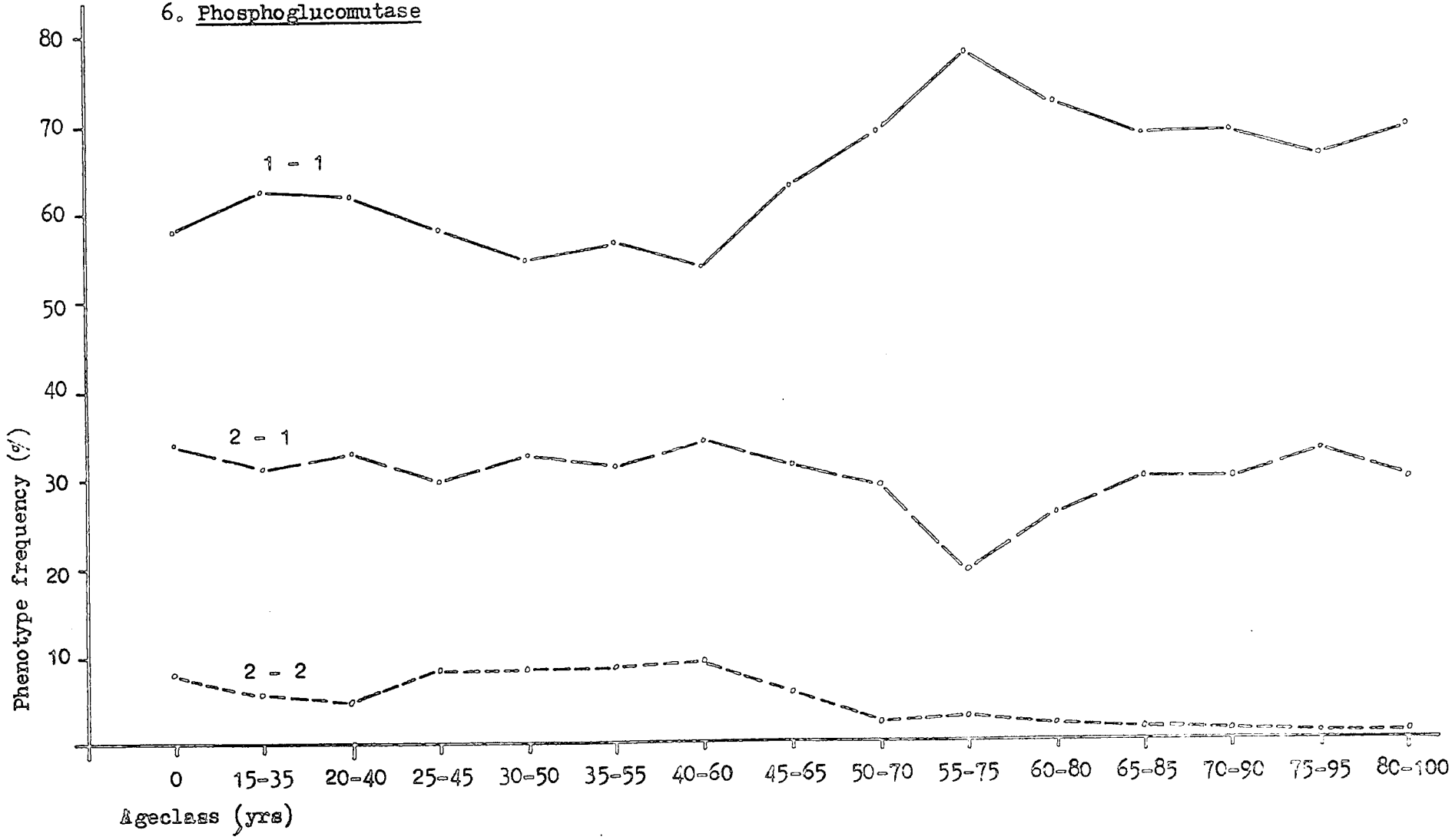
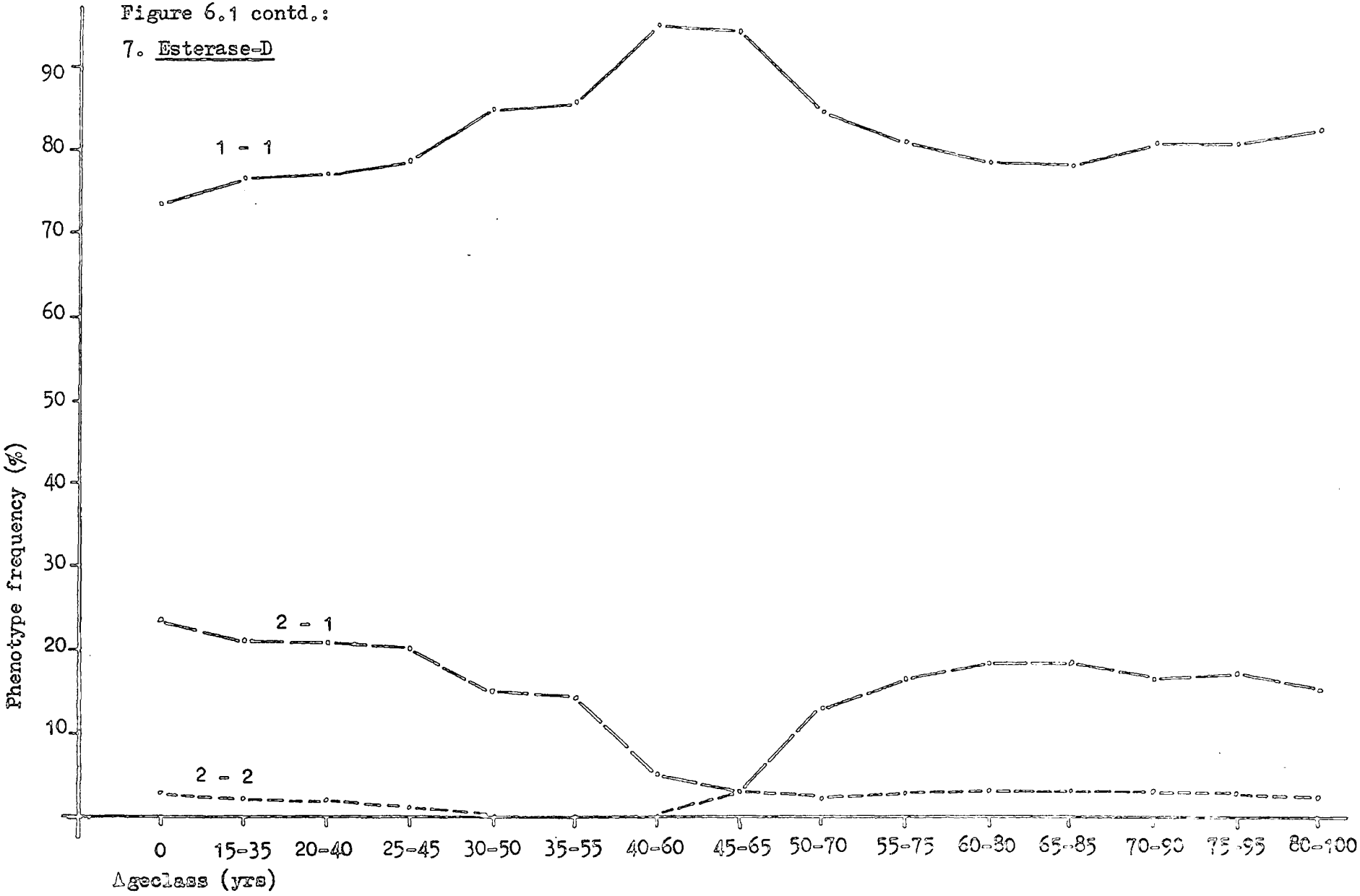


Figure 6.1 contd.:

7. Esterase-D



B. Chromosome variants.

Table 6.12 shows the distribution of chromosome variant phenotype frequencies in different age classes, and table 6.13 shows the results of a χ^2 analysis of these data using the methods of subdivision by age given in table 6.8. The phenotype frequencies of chromosome 4 and the short arms of the acrocentric chromosomes (except chromosome 13) are not analysed here, as the variants in these regions are fairly uncommon. The frequencies of the chromosome 14s variant show some tendency to differ according to age. By one method of subdivision a significant χ^2 value is obtained in the adults, and by another method a nearly significant value, also in the adults. There are no obvious consistent trends for any of the phenotypes. The II type increases with age until the oldest group of adults, and the frequency of the NN type declines, also until the oldest group. However, if this oldest group is omitted, comparisons within the adults, and between the adults and the newborn infants do not show any significant differences with age.

Large differences occur in the frequencies of the variants of satellites on chromosome 21 between the newborn infants and the adults. The χ^2 values are significant in all the analyses of the total sample, but not of the adults alone. The difference appears to be that all types of variant phenotype are less common in the infants, that is the frequency of the NN type is higher in this group than in any of the older groups of adults, amongst which the frequencies are fairly uniform.

Table 6.14 shows the combined chromosome variant frequencies subdivided according to age. Table 6.15 gives the results of analysis of these data. Table 6.16 compares the mean ages of each variant type with those of the rest

TABLE 6.12: Chromosome variant phenotype frequencies subdivided according to age.

Age(yrs)	0		1 - 29		30 - 59		60+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. Chromosome 3										
BB	1	(0.4)	1	(0.6)	0		1	(0.5)	3	(0.4)
BI	17	(7.2)	9	(5.7)	12	(13.8)	11	(5.9)	49	(7.3)
II	25	(10.5)	24	(15.2)	12	(13.8)	25	(13.5)	86	(12.9)
IN	105	(44.3)	62	(39.2)	27	(31.0)	84	(45.4)	278	(41.7)
BN	35	(14.8)	17	(10.8)	9	(10.3)	24	(13.0)	85	(12.7)
NN	54	(22.8)	45	(28.5)	27	(31.0)	40	(21.6)	166	(24.9)
Total	237		158		87		185		667	
2. Chromosome 13p										
BB	2	(0.8)	0		1	(1.1)	0		3	(0.4)
BI	9	(3.8)	7	(4.4)	2	(2.3)	10	(5.4)	28	(4.2)
II	57	(24.1)	36	(22.8)	23	(26.4)	41	(22.2)	157	(23.5)
IN	137	(57.8)	91	(57.6)	44	(50.6)	94	(50.8)	366	(54.9)
BN	4	(1.7)	1	(0.6)	1	(1.1)	2	(1.1)	8	(1.2)
NN	28	(11.8)	23	(14.6)	16	(18.4)	38	(20.5)	105	(15.7)
Total	237		158		87		185		667	
3. Chromosome 13s										
BI	0		1	(0.6)	0		2	(1.1)	3	(0.4)
IN	35	(14.8)	30	(19.0)	20	(23.0)	36	(19.5)	121	(18.1)
BN	6	(2.5)	7	(4.4)	3	(3.4)	3	(1.6)	19	(2.8)
NN	196	(82.7)	120	(75.9)	64	(73.6)	144	(77.8)	524	(78.6)
Total	237		158		87		185		667	
4. Chromosome 14s										
BI	2	(0.8)	0		2	(2.3)	1	(0.5)	5	(0.7)
II	2	(0.8)	4	(2.5)	3	(3.4)	0		9	(1.3)
IN	78	(32.9)	49	(31.0)	34	(39.1)	63	(34.1)	224	(33.6)
BN	7	(3.0)	8	(5.1)	3	(3.4)	3	(1.6)	21	(3.1)
NN	148	(62.4)	97	(61.4)	45	(51.7)	118	(63.8)	408	(61.2)
Total	237		158		87		185		667	
5. Chromosome 15s										
BI	1	(0.4)	2	(1.3)	4	(4.6)	5	(2.7)	12	(1.8)
II	4	(1.7)	3	(1.9)	3	(3.4)	6	(3.2)	16	(2.4)
IN	93	(39.2)	60	(38.0)	30	(34.5)	72	(38.9)	255	(38.2)
BN	4	(1.7)	8	(5.1)	4	(4.6)	9	(4.9)	25	(3.7)
NN	135	(57.0)	85	(53.8)	46	(52.9)	93	(50.3)	359	(53.8)
Total	237		158		87		185		667	
6. Chromosome 21s										
BB	0		0		0		1	(0.5)	1	(0.1)
BI	0		4	(2.5)	0		1	(0.5)	5	(0.7)
II	2	(0.8)	4	(2.5)	3	(3.4)	5	(2.7)	14	(2.1)
IN	56	(23.6)	53	(33.5)	33	(37.9)	63	(34.1)	205	(30.7)
BN	3	(1.3)	3	(1.9)	3	(3.4)	3	(1.6)	12	(1.8)
NN	176	(74.3)	94	(59.5)	48	(55.2)	112	(60.5)	430	(64.5)
Total	237		158		87		185		667	
7. Chromosome 22s										
BB	0		1	(0.6)	0		0		1	(0.1)
BI	9	(3.8)	2	(1.3)	1	(1.1)	1	(0.5)	13	(1.9)
II	8	(3.4)	6	(3.8)	4	(4.6)	3	(1.6)	21	(3.1)
IN	90	(38.0)	64	(40.5)	34	(39.1)	69	(37.3)	257	(38.5)
BN	9	(3.8)	9	(5.7)	2	(2.3)	18	(9.7)	38	(5.7)
NN	121	(51.1)	76	(48.1)	46	(52.9)	94	(50.8)	337	(50.5)
Total	237		158		87		185		667	

TABLE 6.13: Statistical data for Table 6.12.

Series	Method of subdivision	X^2	d.f.	P
1. Chromosome 3				
Total	A	3.84	4	.429
Total	B	15.24	12	.229
Adults	B	11.16	8	.193
Total	C	23.70	20	.256
Adults	C	20.22	16	.211
Total	D	17.69	16	.343
Adults	D	13.68	12	.321
2. Chromosome 13p				
Total	A	4.79	4	.310
Total	B	8.14	9	.520
Adults	B	3.24	6	.778
Total	C	9.07	15	.874
Adults	C	3.92	12	.985
Total	D	11.15	12	.516
Adults	D	6.30	9	.709
3. Chromosome 13s				
Total	A	3.59	2	.166
Total	B	5.92	6	.433
Adults	B	1.50	4	.826
Total	C	9.24	10	.510
Adults	C	5.37	8	.717
Total	D	7.89	8	.444
Adults	D	3.61	6	.729
4. Chromosome 14s				
Total	A	0.57	3	.903
Total	B	10.44	6	.107
Adults	B	9.59	4	.048
Total	C	11.05	10	.354
Adults	C	10.04	8	.262
Total	D	12.72	8	.122
Adults	D	11.68	6	.070
5. Chromosome 15s				
Total	A	8.70	3	.034
Total	B	11.58	9	.283
Adults	B	3.27	6	.774
Total	C	18.02	15	.262
Adults	C	7.44	12	.827
Total	D	10.17	8	.253
Adults	D	4.59	6	.597
6. Chromosome 21s				
Total	A	17.35	2	.000
Total	B	18.91	9	.026
Adults	B	1.55	6	.956
Total	C	22.16	10	.014
Adults	C	4.31	8	.829
Total	D	18.89	8	.016
Adults	D	2.18	6	.902

TABLE 6.13 contd.:

Series	Method of subdivision	X^2	d.f.	P
7. Chromosome 22s				
Total	A	4.96	3	.175
Total	B	14.23	9	.114
Adults	B	8.93	6	.177
Total	C	7.68	10	.660
Adults	C	17.33	12	.138
Total	D	16.85	12	.155
Adults	D	12.00	9	.213

of the sample, for both the total and the adult sample, by means of Student's t-test and the Mann Whitney U test. It also shows the results of the analysis for trends.

For chromosome 3 the X^2 value is nearly significant when the total sample, but not when the adult series alone, is considered. When the sexes are examined separately the difference occurs and is significant (P=.008) in the males, but does not occur in the females. There is no obvious trend across all age-groups. Among the adults the frequency of the IN type increases with age and this trend is significant. However, in the newborn infants the frequency of this type is as high as in the oldest adults.

No effects of age on the frequencies of variants of chromosome 4 are found.

In the case of the short arm of chromosome 13, no significant X^2 values or rank correlation coefficients are found when the phenotype frequencies are examined in all methods of subdivision by age. However, the difference between the mean age of the NN type in the total sample is significantly higher than the mean age of the rest of the sample, and the analysis for trends gives a nearly significant result for the NN type increasing in frequency with age in the adults.

The greater frequency of the NN type of chromosome 13s is almost significant in the adults. The difference is also seen when only under-60 year old adults are included in the comparison, but when the over-60 year olds who are not geriatric patients are included, the difference is less marked. Therefore, it seems as though the results might indicate that the NN type of chromosome 13s tends to increase in frequency in an ageing healthy population.

The number of the IN type of chromosome 14p are small

TABLE 6.14: Chromosome variant phenotype frequencies subdivided according to age (intense and brilliant levels of fluorescence combined).

Age(yrs)	0		1 - 29		30 - 59		60+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. Chromosome 3										
II	43	(18.1)	34	(21.5)	24	(27.6)	37	(20.0)	138	(20.7)
IN	140	(59.1)	79	(50.0)	36	(41.4)	108	(58.4)	363	(54.4)
NN	54	(22.8)	45	(28.5)	27	(31.0)	40	(21.6)	166	(24.9)
Total	237		158		87		185		667	
2. Chromosome 4										
IN	16	(6.8)	15	(9.5)	10	(11.5)	16	(8.6)	57	(8.5)
NN	221	(93.2)	143	(90.5)	77	(88.5)	169	(91.4)	610	(91.5)
Total	237		158		87		185		667	
3. Chromosome 13p										
II	68	(28.7)	43	(27.2)	26	(29.9)	51	(27.6)	188	(28.2)
IN	141	(59.5)	92	(58.2)	45	(51.7)	96	(51.9)	374	(56.1)
NN	28	(11.8)	23	(14.6)	16	(18.4)	38	(20.5)	105	(15.7)
Total	237		158		87		185		667	
4. Chromosome 13s										
II	0		1	(0.6)	0		2	(1.1)	3	(0.4)
IN	41	(17.3)	37	(23.4)	23	(26.4)	39	(21.1)	140	(21.0)
NN	196	(82.7)	120	(75.9)	64	(73.6)	144	(77.8)	524	(78.6)
Total	237		158		87		185		667	
5. Chromosome 14p										
IN	5	(2.1)	3	(1.9)	1	(1.1)	10	(5.4)	19	(2.8)
NN	232	(97.9)	155	(98.1)	86	(98.9)	175	(94.6)	648	(97.2)
Total	237		158		87		185		667	
6. Chromosome 14s										
II	4	(1.7)	4	(2.5)	5	(5.7)	1	(0.5)	14	(2.1)
IN	85	(35.9)	57	(36.1)	37	(42.5)	66	(35.7)	245	(36.7)
NN	148	(62.4)	97	(61.4)	45	(51.7)	118	(63.8)	408	(61.2)
Total	237		158		87		185		667	
7. Chromosome 15p										
II	0		0		1	(1.1)	0		1	(0.1)
IN	4	(1.7)	5	(3.2)	5	(5.7)	9	(4.9)	23	(3.4)
NN	233	(98.3)	153	(96.8)	81	(93.1)	176	(93.1)	643	(96.4)
Total	237		158		87		185		667	
8. Chromosome 15s										
II	5	(2.1)	5	(3.2)	7	(8.0)	11	(5.9)	28	(4.2)
IN	97	(40.9)	68	(43.0)	34	(39.1)	81	(43.8)	280	(42.0)
NN	135	(57.0)	85	(53.8)	46	(52.9)	93	(50.3)	359	(53.8)
Total	237		158		87		185		667	
9. Chromosome 21p										
IN	5	(2.1)	4	(2.5)	2	(2.3)	3	(1.6)	14	(2.1)
NN	232	(97.9)	154	(97.5)	85	(97.7)	182	(98.4)	653	(97.9)
Total	237		158		87		185		667	

TABLE 6.14 contd.:

Age(yrs)	0		1 - 29		30 - 59		60+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
10. Chromosome 21s										
II	2	(0.8)	8	(5.1)	3	(3.4)	7	(3.8)	20	(3.0)
IN	59	(24.9)	56	(35.4)	36	(41.4)	66	(35.7)	217	(32.5)
NN	176	(74.3)	94	(59.5)	48	(55.2)	112	(60.5)	430	(64.5)
Total	237		158		87		185		667	
11. Chromosome 22p										
II	1	(0.4)	3	(1.9)	1	(1.1)	3	(1.6)	8	(1.2)
IN	44	(18.6)	27	(17.1)	29	(33.3)	34	(18.4)	134	(20.1)
NN	192	(81.0)	128	(81.0)	57	(65.5)	148	(80.0)	525	(78.7)
Total	237		158		87		185		667	
12. Chromosome 22s										
II	17	(7.2)	9	(5.7)	5	(5.7)	4	(2.2)	35	(5.2)
IN	99	(41.8)	73	(46.2)	36	(41.4)	87	(47.0)	295	(44.2)
NN	121	(51.1)	76	(48.1)	46	(52.9)	94	(50.8)	337	(50.5)
Total	237		158		87		185		667	

TABLE 6.15: Statistical data for Table 6.14.

Series	Method of subdivision	X^2	d.f.	P	Spearman's rho	P
1. Chromosome 3						
Total	A	2.98	2	.225	.0039	.921
Total	B	10.95	6	.090	.0178	.646
Adults	B	7.62	4	.106	.0355	.463
Total	C	16.60	10	.084	.0233	.549
Adults	C	13.14	8	.107	.0445	.359
Total	D	10.70	8	.219	.0196	.613
Adults	D	7.35	6	.289	.0363	.454
2. Chromosome 4						
Total	A	1.33	1	.249	.0503	.194
Total	B	2.13	3	.546	.0350	.366
Adults	B	0.57	2	.757	-.0148	.760
Total	C	4.68	5	.456	.0259	.505
Adults	C	2.83	4	.587	-.0360	.457
Total	D	2.42	4	.659	.0289	.458
Adults	D	0.78	3	.854	-.0301	.535
3. Chromosome 13p						
Total	A	4.21	2	.122	-.0494	.202
Total	B	7.24	6	.299	-.0579	.135
Adults	B	2.58	4	.630	-.0391	.418
Total	C	8.22	10	.607	-.0587	.131
Adults	C	3.53	8	.897	-.0419	.388
Total	D	9.84	8	.277	-.0562	.148
Adults	D	4.84	6	.565	-.0360	.458
4. Chromosome 13s						
Total	A	3.21	1	.073	.0749	.053
Total	B	4.40	3	.222	.0584	.132
Adults	B	0.61	2	.736	-.0188	.698
Total	C	6.12	5	.295	.0498	.199
Adults	C	2.11	4	.716	-.0396	.414
Total	D	6.23	4	.183	.0600	.122
Adults	D	2.21	3	.530	-.0168	.728
5. Chromosome 14p						
Total	A	0.35	1	.552	.0324	.403
Total	B	6.26	3	.100	.0679	.080
Adults	B	4.86	2	.088	.0913	.058
Total	C	6.65	5	.248	.0720	.064
Adults	C	5.19	4	.269	.0983	.042
Total	D	8.81	4	.066	.0783	.044
Adults	D	7.08	3	.070	.1138	.018
6. Chromosome 14s						
Total	A	0.50	2	.778	.0231	.551
Total	B	10.45	6	.107	.0047	.903
Adults	B	9.24	4	.056	-.0341	.480
Total	C	2.83	5	.726	.0049	.900
Adults	C	2.51	4	.642	-.0364	.453
Total	D	4.54	4	.337	.0088	.820
Adults	D	4.21	3	.239	-.0275	.571

TABLE 6.15 contd.:

Series	Method of subdivision	χ^2	d.f.	P	Spearman's rho	P
7. Chromosome 15p						
Total	A	3.01	1	.083	.0756	.051
Total	B	6.16	3	.104	.0809	.037
Adults	B	1.80	2	.408	.0326	.500
Total	C	4.53	5	.476	.0722	.063
Adults	C	0.49	4	.975	.0134	.783
Total	D	5.50	4	.240	.0734	.058
Adults	D	1.24	3	.743	.0156	.747
8. Chromosome 15s						
Total	A	4.69	2	.096	.0613	.113
Total	B	8.58	6	.198	.0668	.085
Adults	B	3.25	4	.516	.0404	.404
Total	C	11.19	10	.343	.0596	.124
Adults	C	5.54	8	.699	.0234	.629
Total	D	9.97	8	.267	.0675	.082
Adults	D	4.39	6	.625	.0424	.382
9. Chromosome 21p						
Total	A	0.07	1	.798	-.0010	.980
Total	B	0.37	3	.947	-.0120	.757
Adults	B	0.37	2	.832	-.0287	.553
Total	C	8.63	5	.125	-.0353	.364
Adults	C	8.01	4	.071	-.0851	.079
Total	D	3.32	4	.505	-.0256	.510
Adults	D	3.33	3	.344	-.0627	.195
10. Chromosome 21s						
Total	A	18.07	2	.000	.1594	.001
Total	B	19.41	6	.004	.1304	.001
Adults	B	1.41	4	.842	-.0157	.746
Total	C	18.83	5	.002	.1196	.002
Adults	C	7.05	8	.531	-.0319	.511
Total	D	21.80	8	.005	.1228	.002
Adults	D	3.89	6	.692	-.0268	.581
11. Chromosome 22p						
Total	A	2.43	2	.296	.0424	.273
Total	B	10.47	3	.015	.0390	.315
Adults	B	10.29	4	.036	.0017	.972
Total	C	7.83	5	.166	.0278	.474
Adults	C	6.63	4	.157	-.0148	.760
Total	D	7.36	4	.118	.0236	.544
Adults	D	6.18	3	.103	-.0257	.596
12. Chromosome 22s						
Total	A	3.10	2	.212	-.0096	.804
Total	B	6.38	6	.382	-.0203	.601
Adults	B	3.89	4	.421	-.0370	.445
Total	C	13.97	10	.174	-.0084	.829
Adults	C	12.43	8	.133	-.0027	.956
Total	D	9.75	8	.238	-.0088	.821
Adults	D	7.51	6	.276	-.0037	.938

(in the total sample only 19), and therefore any findings from this analysis must be treated with caution. The most noticeable aspect of the distributions according to age is the large increase in the frequency of the IN type in the oldest group of adults. This increase leads to nearly significant values of X^2 being obtained. The rank correlation coefficient is significant or nearly significant in all comparisons of both the total and the adult samples, except in the comparison between the newborn infant and the adult series. The mean age of the IN is not surprisingly, then, significantly higher than that of the NN type, and the analysis for trends also shows a significant tendency for increasing frequency of the IN type with age. When the newborn infants are compared with the adults under-60 years no difference is found at all in the variant frequencies of this chromosome region.

There is no apparent effect of age on the frequencies of the variant types of chromosome 14s.

The frequency of the IN type of chromosome 15p appears to increase with age. X^2 values for all methods of subdivision are nearly significant in the total sample, but not in the adults. Also, in the total sample, the mean age of the IN type is higher than the rest of the sample. This difference is nearly significant by both the t-test and the Mann Whitney U test. Therefore, this difference is shown by whatever method of analysis is applied to the data of the total sample but never quite reaches a significant level with any test.

No apparent effects of age are noted in the variant frequencies of chromosomes 15s or 21p.

The large differences described in the previous section (see above, page 216) are found between the frequencies of the variant types of chromosome 21s in the newborn infants and the adults. It seems even clearer from this comparison that the incidence of the variant (either brilliant or intense) satellites on this chromosome is much higher in the adults

TABLE 6.16: A comparison of the mean age of certain phenotypes (x) with that of the rest (not - x), in a number of chromosome variants; with an analysis for trends in phenotype frequencies with age.

Series	x	mean age : x	mean age : not - x	t*	P	Mann - Whitney U	z	P	Analysis for trends: z	Analysis for trends: P
Chromosome 3										
Total	IN	31.8065	33.0045	0.48	.631	52058.0	-1.155	.248		
Adults	IN	51.8646	48.5454	1.31	.191	21998.0	-0.679	.497	1.744	.041
Chromosome 4										
Total	NN	32.1143	34.8895	0.63	.531	16175.0	-0.851	.395		
Adults	NN	50.4534	48.5049	0.45	.651	7652.0	-0.374	.709	0.507	.305
Chromosome 13p										
Total	IN	30.5376	34.6705	1.66	.098	50551.0	-1.626	.104		
Adults	IN	49.0971	51.6520	1.01	.315	21511.0	-0.961	.337	0.998	.159
Total	NN	40.0977	30.8999	2.72	.007	24772.5	-2.622	.009		
Adults	NN	54.6787	49.2990	1.64	.103	12018.5	-1.521	.128	1.403	.081
Chromosome 13s										
Total	IN	34.1030	31.8896	0.73	.468	34509.0	-1.041	.298		
Adults	IN	48.3707	50.8301	0.82	.415	15379.0	-0.736	.462	0.523	.302
Chromosome 14p										
Total	NN	31.9120	47.3134	2.08	.038	4764.5	-1.702	.089		
Adults	NN	49.7951	64.2110	2.03	.043	2114.5	-1.721	.085	2.281	.011
Chromosome 14s										
Total	IN	32.6832	32.1593	0.20	.839	50591.5	-0.368	.713		
Adults	IN	50.0462	50.3990	0.13	.893	21351.5	-0.071	.943	0.078	.468
Chromosome 15p										
Total	IN	43.5317	31.9516	1.71	.088	5766.0	-1.828	.068		
Adults	IN	52.6963	50.1538	0.41	.680	3664.0	-0.420	.674	0.412	.341

* sign ignored

TABLE 6.16 contd.:

Series	x	mean age : x	mean age : not - x	t*	P	Mann - Whitney U	z	P	Analysis for trends: z	Analysis for trends: P
Chromosome 15s										
Total	II	45.7513	31.7631	2.27	.023	6656.5	-2.326	.020		
Adults	II	55.6973	49.9583	1.02	.307	4051.5	-1.050	.294	1.205	.112
Total	IN	32.9286	31.9358	0.40	.693	53037.5	-0.339	.735		
Adults	IN	50.4785	50.1107	0.14	.886	22385.5	-0.000	1.000	-0.008	.496
Chromosome 21p										
Total	NN	32.5052	25.2322	0.84	.400	3922.5	-0.913	.361		
Adults	NN	50.5033	39.2501	1.28	.203	1254.5	-1.719	.086	1.067	.142
Chromosome 21s										
Total	IN	36.0700	30.5639	1.09	.037	43408.0	-2.243	.025		
Adults	IN	49.6250	50.6391	0.39	.700	20379.0	-0.725	.468	0.091	.464
Total	NN	30.1449	36.3916	2.42	.016	44334.5	-2.675	.007		
Adults	NN	51.0327	49.1495	0.73	.466	20723.5	-1.094	.274	0.458	.323
Chromosome 22p										
Total	IN	33.0626	32.1747	0.29	.775	34158.0	-0.630	.529		
Adults	IN	49.4082	50.4924	0.35	.729	14996.0	-0.086	.931	0.494	.312
Chromosome 22s										
Total	II	21.5193	32.9529	2.07	.039	8770.0	-2.086	.037		
Adults	II	41.8431	50.6365	1.40	.164	2940.0	-1.460	.144	1.430	.076
Total	IN	33.7937	31.2171	1.03	.302	51850.0	-1.102	.271		
Adults	IN	51.0389	49.6273	0.55	.579	21853.5	-0.663	.507	0.811	.209

* sign ignored

than in the newborn infants. This difference occurs in both sexes when the two are examined separately.

The only differences found with age in the frequencies of variants of chromosome 22p is a drop in the frequency of the NN type in the 'middle' group of adults. As the frequency is the same, approximately, in the infants, the youngest adults and the oldest adults it seems probable that the difference can be explained by the smaller size of the middle age-groups.

In the case of chromosome 22s, the II type appears to decline in frequency with age (a trend which almost reached a significant level), but neither of the other two types appears to show a corresponding increase. None of the rank correlation coefficients are significant.

Figure 6.2 shows the phenotype frequencies of some of the variants determined by the method of moving average age class.

Table 6.17 shows the frequencies of the chromosome variants grouped as described earlier (see above, page 160) and subdivided according to age. Table 6.18 gives the results of statistical analysis of these distributions, and table 6.19 gives the results of a comparison between the newborn infants and the adults with regard to these grouped variants.

The frequency of intense satellites on the D group chromosomes does not appear to be affected by age in any consistent manner. However, the mean number found in the adults is slightly (but not quite significantly) higher than that in the newborn infants. Brilliant satellites on D group chromosomes show larger differences in frequencies when adults and infants are compared. Again, more are found in the adults. This time the difference is reflected in significant X^2 values and rank correlation coefficients

Figure 6.2: The change in frequency of chromosome variant phenotypes with age, shown by the method of 'moving average ageclass'.

1. Chromosome 3

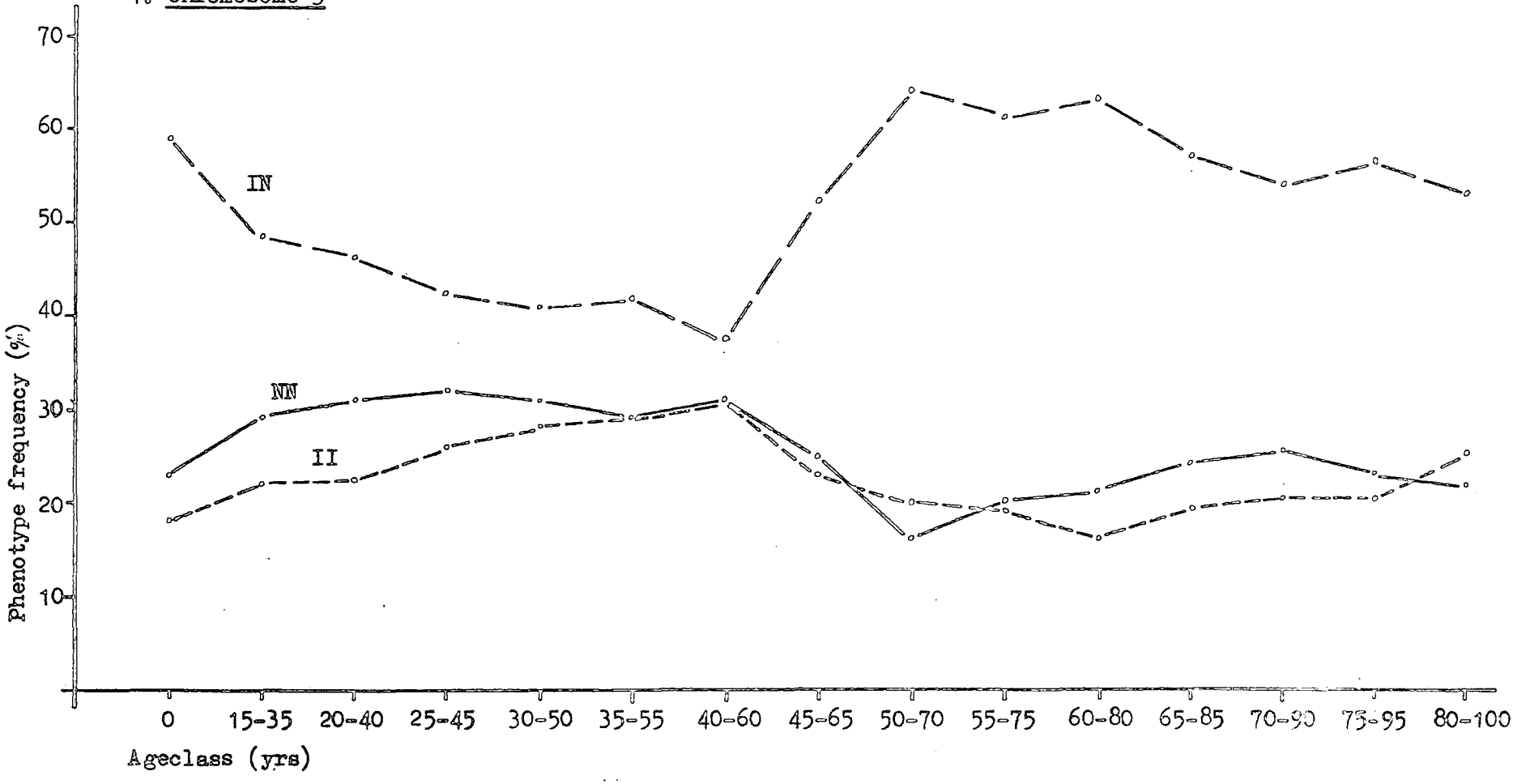


Figure 6.2 contd.:

2. Chromosome 13p

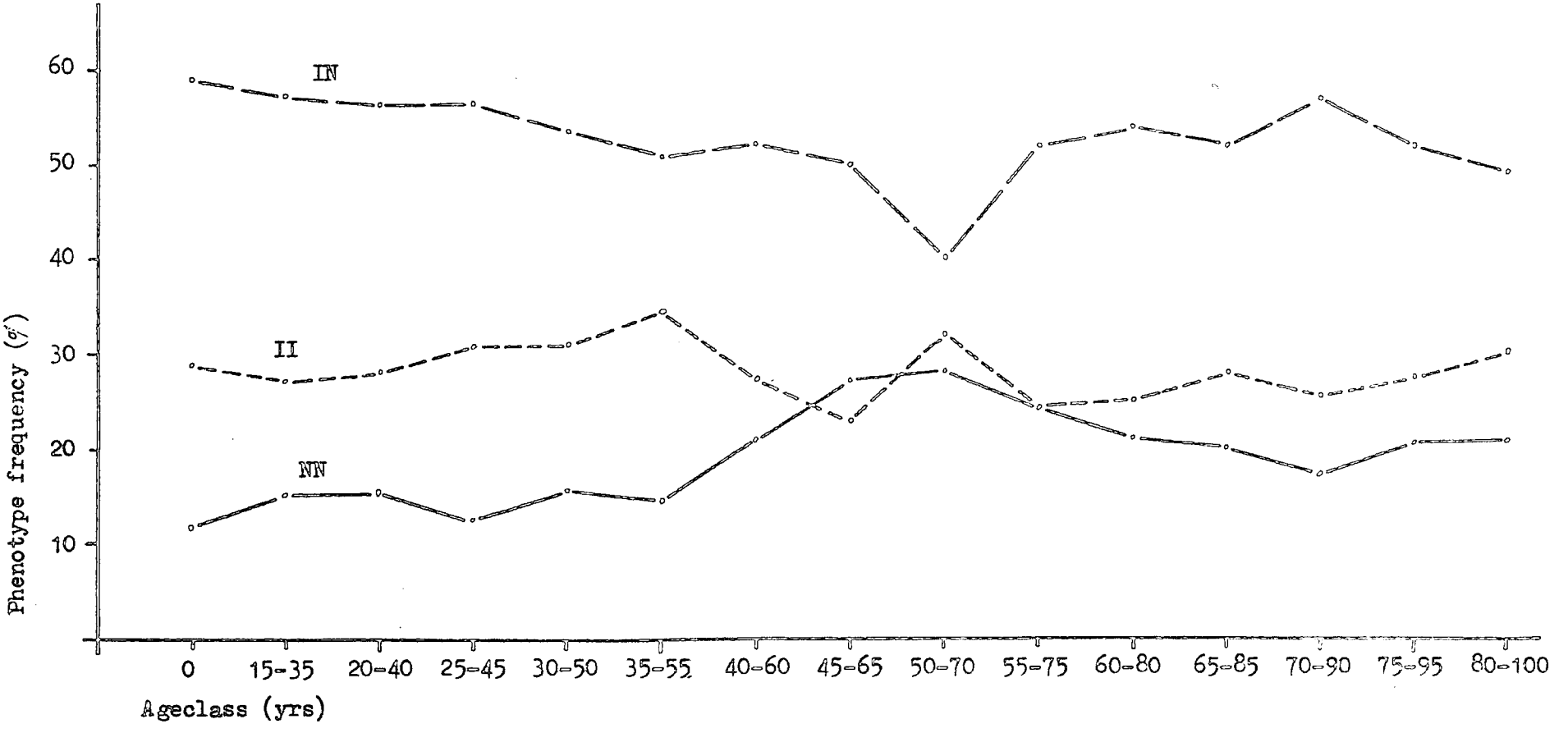


Figure 6.2 contd.:

3. Chromosome 13s

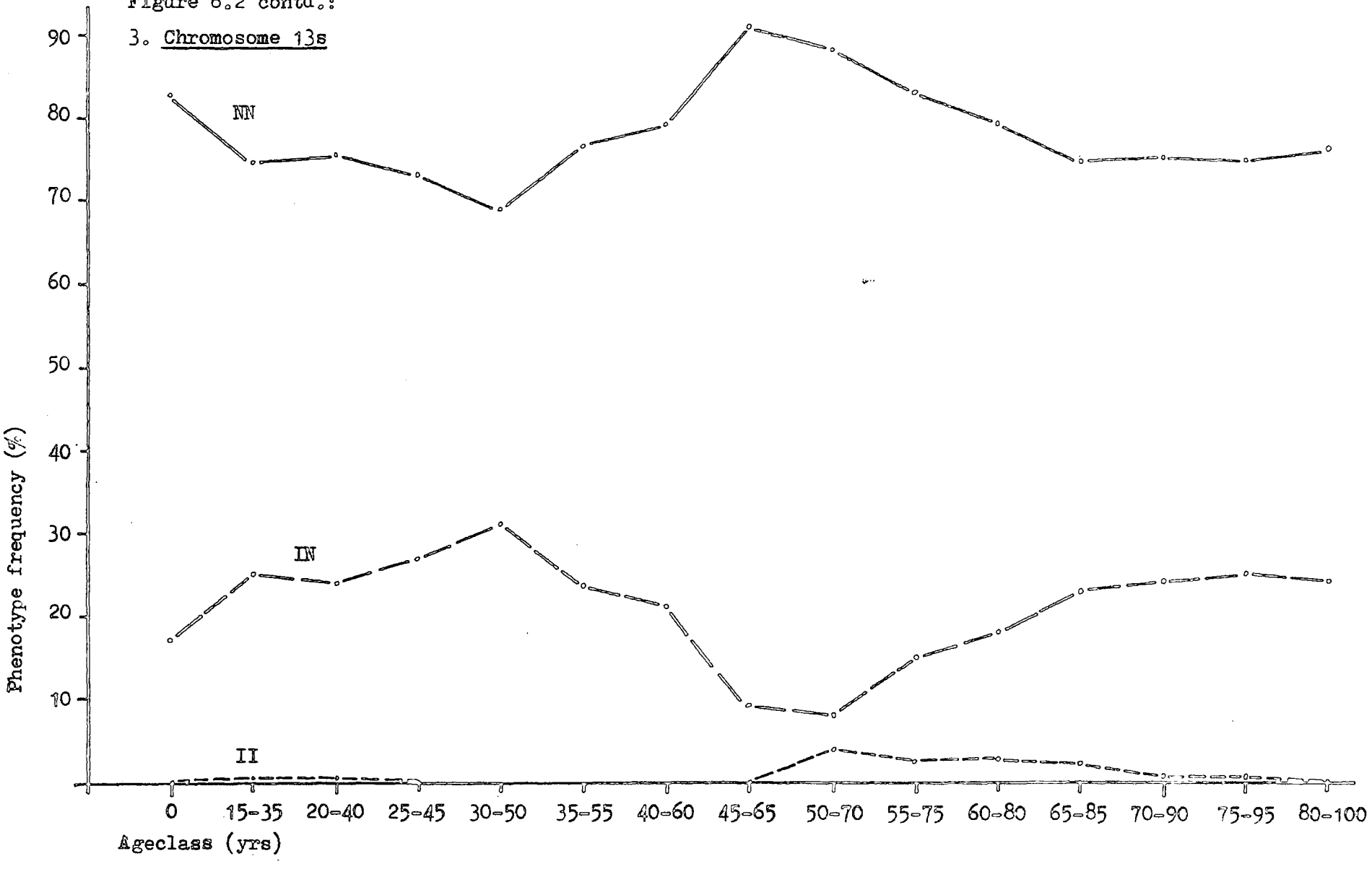


Figure 6.2 contd.:

4. Chromosome 14s

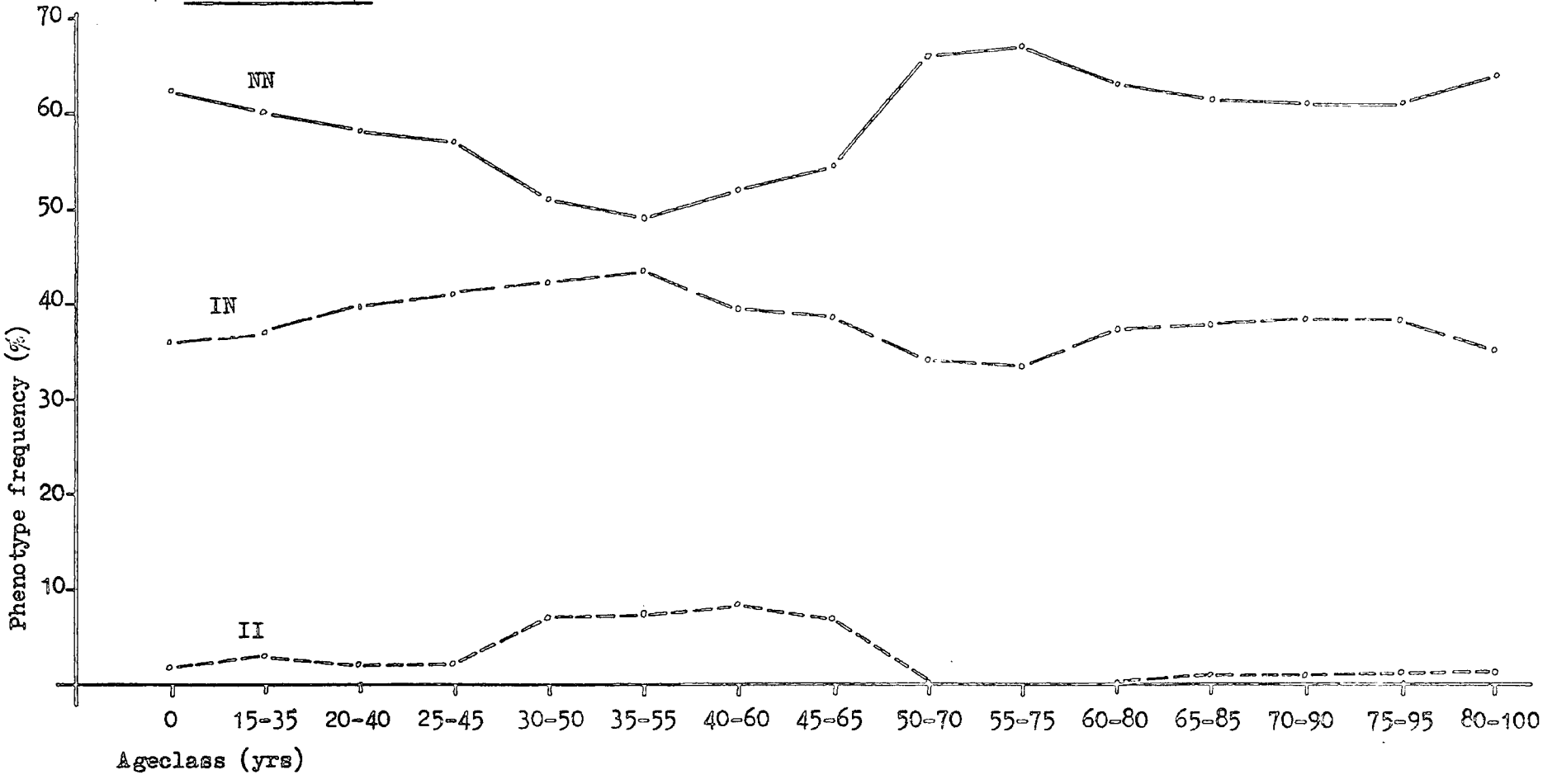


Figure 6.2 contd.:

5. Chromosome 15s

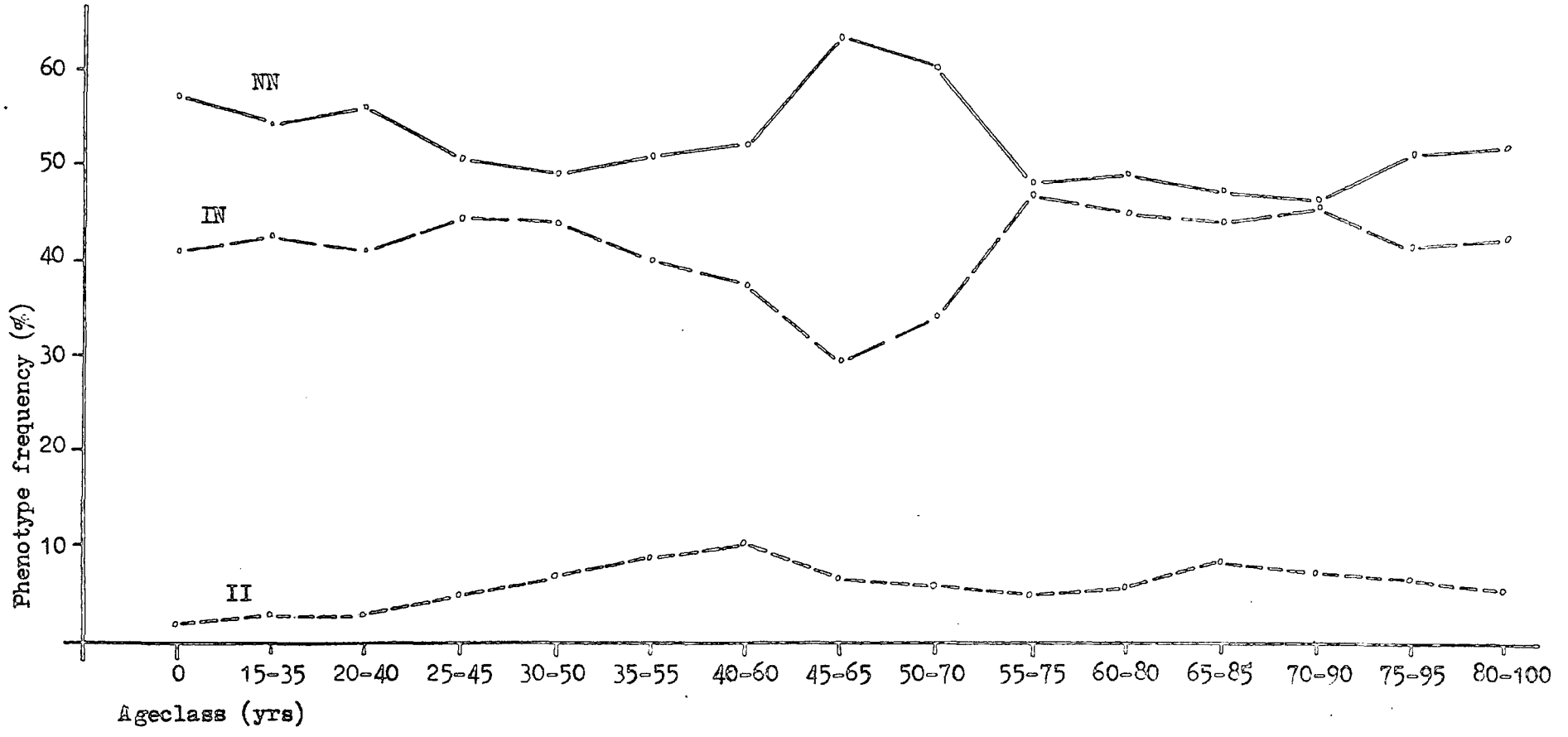


Figure 6.2 contd.:

6. Chromosome 21s

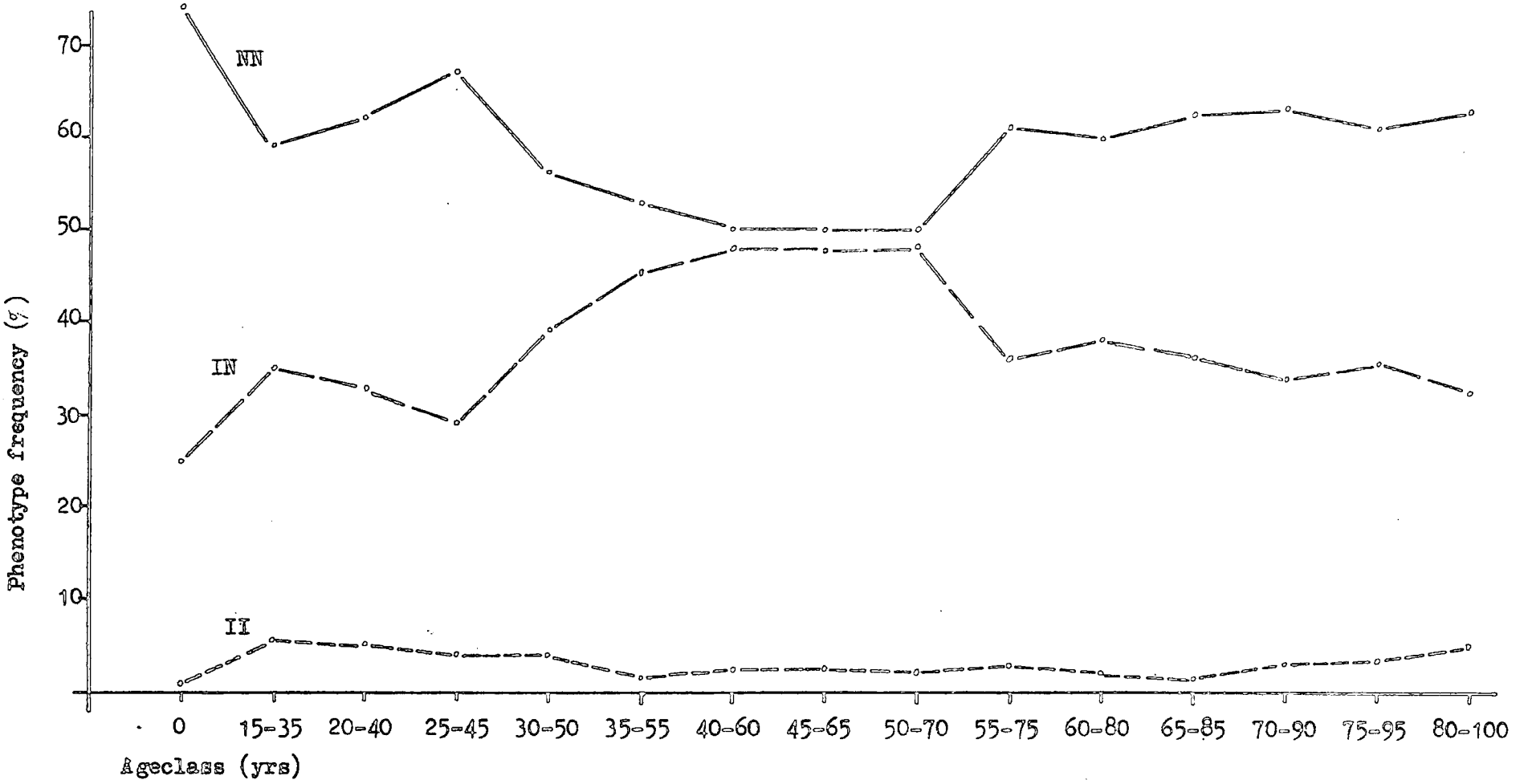


Figure 6.2 contd.:

7. Chromosome 22p

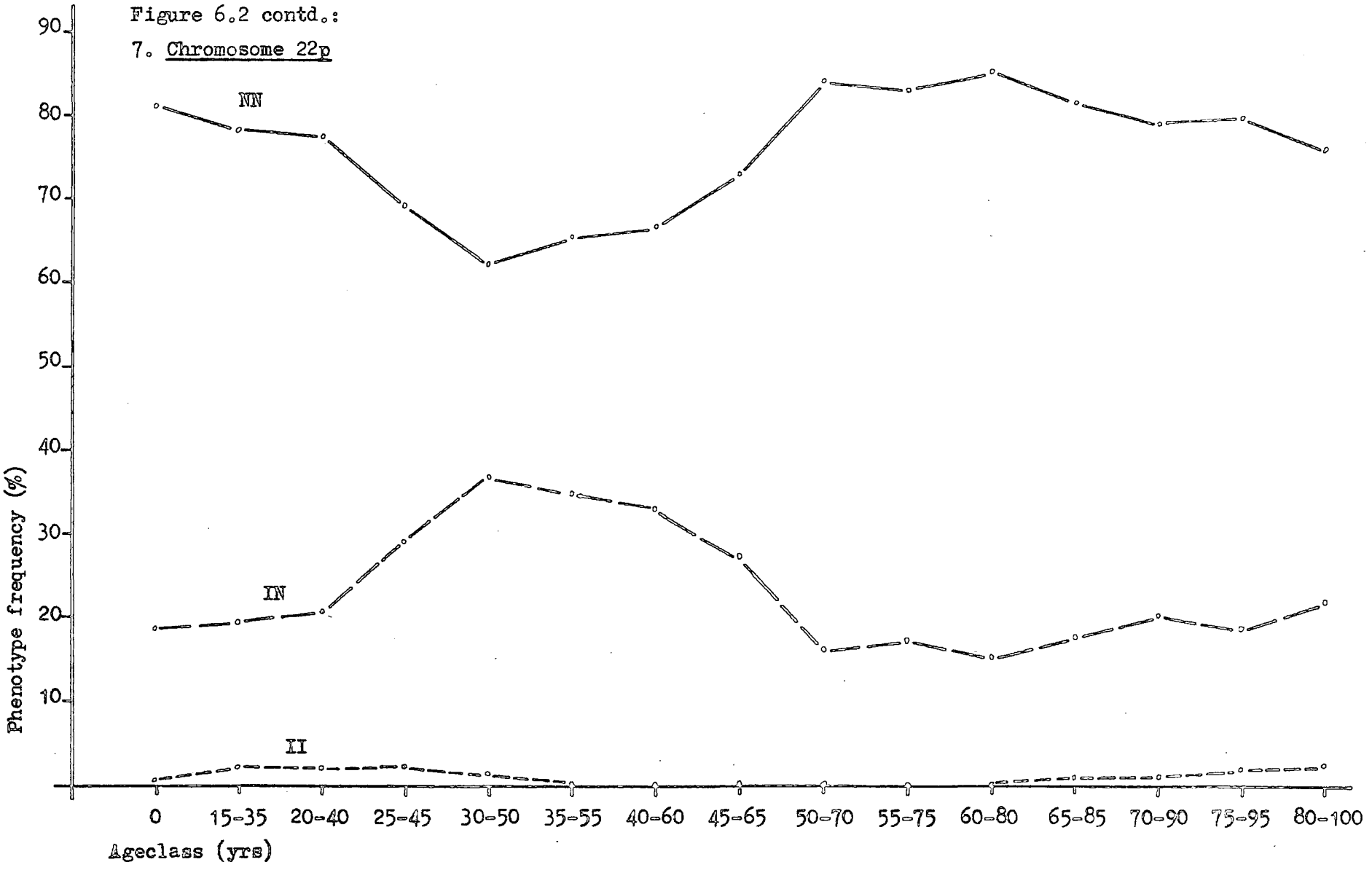


Figure 6.2 contd.:

8. Chromosome 22s

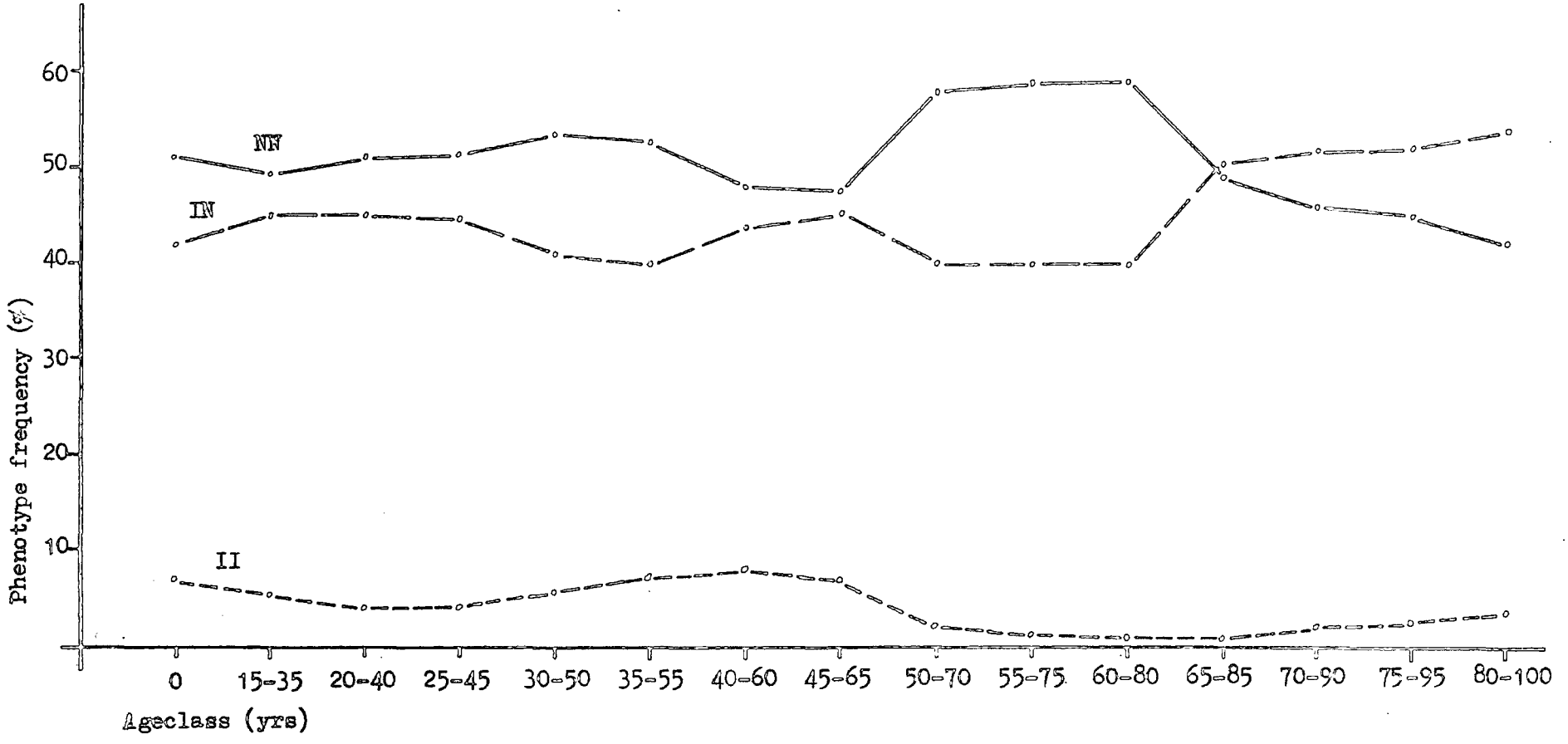


TABLE 6.17: Frequencies of grouped chromosome variants subdivided according to age.

Age(yrs)	0		1 - 29		30 - 59		60+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. D group satellites (intense)										
0	73	(30.8)	46	(29.1)	18	(20.7)	60	(32.4)	197	(29.5)
1	113	(47.7)	71	(44.9)	42	(48.3)	73	(39.5)	299	(44.8)
2	45	(19.0)	38	(24.1)	21	(24.1)	39	(21.1)	143	(21.4)
⩾3	6	(2.5)	3	(1.9)	6	(6.9)	13	(7.0)	28	(4.2)
Total	237		158		87		185		667	
2. D group satellites (brilliant)										
0	218	(92.0)	133	(84.2)	71	(81.6)	161	(87.0)	583	(87.4)
⩾1	19	(8.0)	25	(15.8)	16	(18.4)	24	(13.0)	84	(12.6)
Total	237		158		87		185		667	
3. D group satellites (all)										
0	67	(28.3)	35	(22.2)	14	(16.1)	53	(28.6)	169	(25.3)
1	110	(46.4)	72	(45.6)	38	(43.7)	71	(38.4)	291	(43.6)
2	49	(20.7)	43	(27.2)	25	(28.7)	41	(22.2)	158	(23.7)
⩾3	11	(4.6)	8	(5.1)	10	(11.5)	20	(10.8)	49	(7.3)
Total	237		158		87		185		667	
4. G group satellites (intense)										
0	97	(40.9)	53	(33.5)	31	(35.6)	65	(35.1)	246	(36.9)
1	109	(46.0)	72	(45.6)	35	(40.2)	95	(51.4)	311	(46.6)
2	27	(11.4)	28	(17.7)	16	(18.4)	21	(11.4)	92	(13.8)
⩾3	4	(1.7)	5	(3.2)	5	(5.7)	4	(2.2)	18	(2.7)
Total	237		158		87		185		667	
5. G group satellites (brilliant)										
0	217	(91.6)	139	(88.0)	82	(94.3)	162	(87.6)	600	(90.0)
⩾1	20	(8.4)	19	(12.0)	5	(5.7)	23	(12.4)	67	(10.0)
Total	237		158		87		185		667	
6. G group satellites (all)										
0	87	(36.7)	45	(28.5)	29	(33.3)	55	(29.7)	216	(32.4)
1	111	(46.8)	71	(44.9)	33	(37.9)	90	(48.6)	305	(45.7)
2	32	(13.5)	34	(21.5)	20	(23.0)	36	(19.5)	122	(18.3)
⩾3	7	(3.0)	8	(5.1)	5	(5.7)	4	(2.2)	24	(3.6)
Total	237		158		87		185		667	
7. Total intense satellites										
0	35	(14.8)	16	(10.1)	5	(5.7)	24	(13.0)	80	(12.0)
1	75	(31.6)	43	(27.2)	27	(31.0)	53	(28.6)	198	(29.7)
2	72	(30.4)	56	(35.4)	26	(29.9)	62	(33.5)	216	(32.4)
3	43	(18.1)	30	(19.0)	16	(18.4)	25	(13.5)	114	(17.1)
⩾4	12	(5.1)	13	(8.2)	13	(14.9)	21	(11.4)	59	(8.8)
Total	237		158		87		185		667	
8. Total brilliant satellites										
0	201	(84.8)	118	(74.7)	67	(77.0)	145	(78.4)	531	(79.6)
⩾1	36	(15.2)	40	(25.3)	20	(23.0)	40	(21.6)	136	(20.4)
Total	237		158		87		185		667	

TABLE 6.17 contd.:

Age(yrs)	0		1 - 29		30 - 59		60+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
9. Total variant satellites										
0	29	(12.2)	10	(6.3)	3	(3.4)	19	(10.3)	61	(9.1)
1	63	(26.6)	34	(21.5)	24	(27.6)	43	(23.2)	164	(24.6)
2	86	(36.3)	56	(35.4)	25	(28.7)	65	(35.1)	232	(34.8)
3	38	(16.0)	39	(24.7)	15	(17.2)	24	(13.0)	116	(17.4)
⩾4	21	(8.9)	19	(12.0)	20	(23.0)	34	(18.4)	94	(14.1)
Total	237		158		87		185		667	
10. Total intense bands										
⩽1	9	(3.8)	7	(4.4)	2	(2.3)	8	(4.3)	26	(3.9)
2	29	(12.2)	20	(12.7)	12	(13.8)	27	(14.6)	88	(13.2)
3	65	(27.4)	37	(23.4)	15	(17.2)	42	(22.7)	159	(23.8)
4	64	(27.0)	30	(19.0)	14	(16.1)	42	(22.7)	150	(22.5)
5	42	(17.7)	32	(20.3)	21	(24.1)	30	(16.2)	125	(18.7)
6	20	(8.4)	22	(13.9)	15	(17.2)	19	(10.3)	76	(11.4)
⩾7	8	(3.4)	10	(6.3)	8	(9.2)	17	(9.2)	43	(6.4)
Total	237		158		87		185		667	
11. Total brilliant bands										
0	153	(64.6)	95	(60.1)	48	(55.2)	118	(63.8)	414	(62.1)
1	61	(25.7)	46	(29.1)	30	(34.5)	45	(24.3)	182	(27.3)
⩾2	23	(9.7)	17	(10.8)	9	(10.3)	22	(11.9)	71	(10.6)
Total	237		158		87		185		667	
12. Total variant bands										
⩽2	17	(7.2)	13	(8.2)	11	(12.6)	23	(12.4)	64	(9.6)
3	57	(24.1)	31	(19.6)	11	(12.6)	28	(15.1)	127	(19.0)
4	63	(26.6)	33	(20.9)	12	(13.8)	46	(24.9)	154	(23.1)
5	55	(23.2)	35	(22.2)	17	(19.5)	36	(19.5)	143	(21.4)
6	32	(13.5)	26	(16.5)	17	(19.5)	26	(14.1)	101	(15.1)
⩾7	13	(5.5)	20	(12.7)	19	(21.8)	26	(14.1)	78	(11.7)
Total	237		158		87		185		667	

TABLE 6.18: Statistical data for Table 6.17.

Series	Method of subdivision	X^2	d.f.	P	Spearman's rho	P
1. D group satellites (intense)						
Total	A	4.32	3	.229	.0580	.134
Total	B	14.89	9	.094	.0479	.217
Adults	B	9.54	6	.146	-.0007	.989
Total	C	14.29	15	.503	.0400	.303
Adults	C	9.30	12	.677	-.0216	.656
Total	D	18.01	12	.115	.0499	.199
Adults	D	12.17	9	.204	.0014	.977
2. D group satellites (brilliant)						
Total	A	6.58	1	.010	.1031	.008
Total	B	8.69	3	.034	.0711	.066
Adults	B	1.45	2	.484	-.0391	.418
Total	C	11.99	5	.035	.0682	.079
Adults	C	4.18	4	.382	-.0449	.355
Total	D	9.14	4	.058	.0762	.050
Adults	D	1.75	3	.627	-.0283	.559
3. D group satellites (all)						
Total	A	7.18	3	.066	.0941	.015
Total	B	18.30	9	.032	.0701	.070
Adults	B	10.71	6	.098	-.0198	.682
Total	C	23.15	15	.081	.0617	.112
Adults	C	15.86	12	.198	-.0413	.394
Total	D	19.91	12	.069	.0738	.057
Adults	D	12.13	9	.206	-.0138	.776
4. G group satellites (intense)						
Total	A	4.51	3	.212	.0778	.044
Total	B	12.54	9	.185	.0472	.223
Adults	B	7.16	6	.306	-.0522	.280
Total	C	18.55	15	.235	.0485	.211
Adults	C	13.30	12	.348	-.0421	.385
Total	D	15.45	12	.218	.0484	.213
Adults	D	10.31	9	.326	-.0442	.362
5. G group satellites (brilliant)						
Total	A	0.74	1	.389	.0390	.313
Total	B	4.31	3	.230	.0395	.308
Adults	B	3.02	2	.221	.0113	.815
Total	C	2.96	5	.707	.0417	.283
Adults	C	1.91	4	.753	.0222	.647
Total	D	3.13	4	.537	.0479	.217
Adults	D	2.07	3	.559	.0369	.446
6. G group satellites (all)						
Total	A	7.06	3	.070	.0957	.013
Total	B	12.58	9	.183	.0679	.080
Adults	B	5.05	6	.537	-.0407	.400
Total	C	19.13	15	.208	.0707	.069
Adults	C	11.73	12	.468	-.0253	.601
Total	D	18.57	12	.099	.0723	.062
Adults	D	11.17	9	.264	-.0231	.634

TABLE 6.18 contd.:

Series	Method of subdivision	X^2	d.f.	P	Spearman's rho	P
7. Total intense satellites						
Total	A	9.42	4	.052	.0870	.024
Total	B	17.53	12	.131	.0600	.122
Adults	B	7.93	8	.440	-.0363	.453
Total	C	22.84	20	.297	.0544	.161
Adults	C	13.71	16	.621	-.0461	.342
Total	D	25.15	16	.067	.0613	.114
Adults	D	15.65	12	.208	-.0311	.521
8. Total brilliant satellites						
Total	A	5.74	1	.017	.0971	.012
Total	B	6.84	3	.077	.0683	.078
Adults	B	0.66	2	.720	-.0353	.465
Total	C	10.59	5	.060	.0669	.085
Adults	C	4.23	4	.376	-.0337	.486
Total	D	7.15	4	.128	.0737	.057
Adults	D	1.10	3	.777	-.0188	.698
9. Total variant satellites						
Total	A	12.31	4	.015	.1231	.001
Total	B	29.27	12	.004	.0866	.025
Adults	B	16.72	8	.033	-.0449	.353
Total	C	36.19	20	.015	.0817	.035
Adults	C	23.85	16	.093	-.0505	.297
Total	D	35.68	16	.003	.0913	.019
Adults	D	23.09	12	.027	-.0295	.542
10. Total intense bands						
Total	A	13.85	6	.031	.0803	.038
Total	B	23.33	18	.178	.0578	.136
Adults	B	8.91	12	.711	-.0257	.596
Total	C	44.38	30	.044	.0534	.169
Adults	C	28.17	24	.253	-.0331	.494
Total	D	41.55	24	.015	.0553	.154
Adults	D	25.80	18	.105	-.0293	.545
11. Total brilliant bands						
Total	A	0.97	2	.616	.0390	.313
Total	B	4.21	6	.648	.0233	.548
Adults	B	3.15	4	.532	-.0267	.581
Total	C	25.31	10	.005	.0170	.661
Adults	C	23.72	8	.003	-.0397	.413
Total	D	14.12	8	.079	.0242	.533
Adults	D	12.79	6	.047	-.0231	.633
12. Total variant bands						
Total	A	22.09	5	.001	.1014	.009
Total	B	34.95	15	.003	.0774	.046
Adults	B	11.90	10	.292	-.0229	.636
Total	C	42.63	25	.015	.0718	.064
Adults	C	19.02	20	.521	-.0320	.509
Total	D	40.70	20	.004	.0772	.047
Adults	D	17.43	15	.294	-.0209	.666

TABLE 6.19: A comparison of the mean numbers of grouped chromosome variants per individual in the newborn infants and the adults of the sample.

	mean no. in : Infants	Adults	t	P	Mann - Whitney U	z	P
D group satellites							
intense	0.9325	1.0462	-1.75	.080	47956.5	-1.498	.134
brilliant	0.0844	0.1547	-2.70	.007	47626.5	-2.667	.008
all	1.0169	1.2009	-2.66	.008	45825.5	-2.431	.015
G group satellites							
intense	0.7384	0.8707	-2.14	.033	46880.5	-2.011	.044
brilliant	0.0886	0.1178	-1.13	.257	50054.5	-1.009	.313
all	0.8270	0.9885	-2.48	.013	45803.0	-2.474	.013
All satellites							
intense	1.6709	1.9169	-2.61	.009	46104.5	-2.250	.024
brilliant	0.1730	0.2725	-2.63	.009	47098.0	-2.509	.012
all	1.8439	2.1894	-3.56	.000	43930.5	-3.184	.001
All variant bands							
intense	3.8143	4.1478	-2.66	.008	46430.0	-2.074	.038
brilliant	0.4726	0.5381	-1.06	.288	49230.0	-1.006	.314
all	4.2869	4.6859	-3.18	.002	45137.5	-2.621	.009

for the total sample, but not for the adults alone. When the intense and brilliant satellites of the D group chromosomes are considered together, the difference between newborn infants and adults is almost as clearly indicated as when brilliant satellites alone are considered.

In the case of the G group satellites no trends with age are noticed for either the intense or brilliant types, but there is a significant difference between the mean number of intense satellites in the newborn infants and in the adults. The difference is in the same direction, but is not significant, for the brilliant satellites. This finding can probably be explained by the increased frequency of satellites on chromosome 21 noted earlier (see above, page 216).

When all the satellites are considered together, there are more of both the intense and brilliant types in the adults than in the newborn infants. These differences are very highly significant and become more significant as more types of satellites are included in the comparison.

This difference does not exist with respect to the other variant regions, that is, chromosomes 3 and 4 and the short arms of the acrocentric chromosomes.

When all the variant bands are considered together there appear to be no obvious trends for the number of bands, either intense or brilliant or both, to increase in older age-groups, but there does seem to be a definite difference between adults and newborn infants. In all cases these differences are not distorted by the presence of geriatric patients in the sample. The results are very similar when newborn infants are compared with adults under 60 years of age.

Heterozygosity and Age.

The importance of heterozygosity in recent evolutionary thought has varied considerably. Notwithstanding the long familiar observation of hybrid vigour in agriculture and animal husbandry, the classical view of genetic variability in natural populations held that organisms were homozygous at almost all their loci, and most heterozygous genotypes arose by mutation, often deleterious. However, empirical observations in certain species that heterozygotes had a better survival value than homozygotes under a greater diversity of environments were made in the nineteen fifties. For example, heterozygotes for an inversion of a section of chromosome II in Drosophila pseudoobscura have a higher overall viability in most environments and show a greater sensitivity to environmental fluctuations at both the micro- and macro- level than homozygotes (Dobzhansky and Levene 1955). Such observations led to the theoretical generalisation that heterozygotes were capable of a more efficient buffering against environmental fluctuations, possibly by means of their greater biochemical diversity (Robertson and Reeve 1952; Lerner 1954). Following on from this, the "Balanced hypothesis" of genetic variability states that the adaptive norm is for the individual to be heterozygous at many genetic loci (Dobzhansky 1955).

The development of electrophoretic techniques in the mid-1960s and their application to the study of genetically determined isoenzymes in a variety of natural populations revealed that a large fraction of genes in most sexually reproducing organisms (including the human species) are indeed polymorphic. Estimates of the average heterozygosity in the human species have been derived from electrophoretic and blood group data. About 30% of loci studied have been shown to be polymorphic, the average heterozygosity for each locus being about 10% (Harris 1966).

In spite of both the revelations of large amounts of heterozygosity and the theoretical knowledge of mechanisms which could maintain it in populations, it had proved remarkably difficult to demonstrate that such mechanisms are the cause of balanced polymorphisms at individual loci. This has been the case particularly in human polymorphisms, where only the haemoglobinopathies have been convincingly shown to be maintained polymorphic by heterozygote advantage. It is this lack of evidence to support selection for heterozygotes, much more than the rapid exchanges of the arguments about segregational load (Lewontin and Hubby 1966, Milkman 1967, Sved, Reed and Bodmer 1967) which has gained support for the idea that much polymorphism is due to the random accumulation of selectively neutral alleles (Lewontin 1974).

In the present case a simple model using longevity to imply a component of Darwinian fitness will be used to determine whether heterozygosity at individual loci, or more generally across the genome is favoured by selection.

In almost all organisms, except the human species, an increase in longevity leads to an increase in reproductive life-span; usually organisms do not age very much beyond reproductive life. The identification of the genetic factors involved in longevity may lie in the estimation of degrees of heterozygosity in persons in different age strata of the population. If these increase with increasing age then it can be concluded that individuals with higher levels of heterozygosity at their genetic loci probably have a higher genetic fitness. Whether or not such increase be maintained beyond reproductive life, that is into the higher age strata of the sample used in the present study would depend on, for instance, whether or not the selection pressures acting upon the individuals were the same during reproductive life as afterwards, that is, whether or not

causes of death were similar (or the genetic effects of death similar).

Of the gene loci readily available for study in human populations the HLA loci are perhaps more likely than many to influence the likelihood of survival to advanced age as the HLA system is an immunogenetic and histocompatability system, and it is believed by many that immune mechanisms play a significant role in ageing (Walford 1969). There have been reports of changing levels of heterozygosity at the HLA loci in different age-groups in human populations. Bender et al. (1972) found that persons heterozygous at the loci were more common in older age-groups. Gerkins et al. (1974) found increased levels of heterozygosity at these loci when comparing healthy aged individuals with younger persons, and healthy aged individuals with persons with neoplasms who were matched for age. Bender et al. (1976), using larger samples, failed to repeat the results of their 1972 study, and found no significant differences in the degree of heterozygosity in different age-groups. Williams (1977) found a significant trend for heterozygosity at the B locus to increase with age amongst the control group used in a diabetic survey.

The genetic markers investigated in the present study play no such obviously direct part in ageing processes. It is not known whether or not the well-documented chromosomal loss in white blood cells is a cause of ageing, or merely coincident with it (Galloway and Buckton 1978). It is also unknown (of course) whether or not the presence of any of the chromosome variables examined in this study has any influence on the increase in aneuploidy often observed in aged individuals.

Heterozygote advantage has been demonstrated to be effective in maintaining the polymorphic haemoglobin systems (Allison 1954) and has been suggested as an important factor

in the maintenance of the Rhesus polymorphisms (Cavalli Sforza and Bodmer 1971). Beardmore (pers. comm.) has shown a relationship between heterozygosity and growth and survival rates in a series of newborn infants in Cardiff, though this effect was not associated with any particular locus studied.

In this study, a possible relationship between heterozygosity and ageing and longevity was examined firstly by comparing the age distributions of homozygotes and heterozygotes for all genetic markers for which these qualities could be determined (the MN, S, Duffy, Kell, Penney and Rhesus blood groups, haptoglobin and all the isoenzymes, and all chromosome variants); and secondly, by examining the age distributions of grouped variables, such as, the number of homozygous blood groups per individual.

Tables 6.20, 6.23 and 6.26 show the homozygotes and heterozygotes of each genetic and chromosomal marker (see table 6.14 for chromosomes 4, 14p and 21p) subdivided according to age. Results of analysis by means of the K^2 test are given in tables 6.21, 6.24 and 6.27.

Differences between the mean ages of heterozygotes and homozygotes were tested by means of Student's t-test and the Mann Whitney U test. The results of this analysis and those of the analysis for trends can be found in table 6.22 (for blood groups, serum protein and isoenzymes), 6.25 (for chromosome variants), and 6.16 (for chromosome variants, intense and brilliant levels of fluorescence combined).

No consistent trends for an increase or decrease in homozygosity with age were found for any of the blood groups or polymorphic proteins. Of the eleven systems examined, in six the mean age of the heterozygote was higher

TABLE 6.20: Homozygous and heterozygous blood group and isoenzyme frequencies subdivided according to age.

Age(yrs)	0		1 - 29		30 - 59		60+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. MN										
Homozyg.	119	(48.8)	73	(51.0)	51	(58.6)	90	(45.0)	333	(49.4)
Heterozyg.	125	(51.2)	70	(49.0)	36	(41.4)	110	(55.0)	341	(50.6)
Total	244		143		87		200		674	
2. S										
Homozyg.	139	(55.6)	78	(52.7)	50	(57.5)	97	(51.3)	364	(54.0)
Heterozyg.	111	(44.4)	70	(47.3)	37	(42.5)	92	(48.7)	310	(46.0)
Total	250		148		87		189		674	
3. MNS										
Homozyg.	61	(26.0)	46	(34.6)	25	(29.1)	43	(22.8)	175	(27.2)
Heterozyg.	174	(74.0)	87	(65.4)	61	(70.9)	146	(77.2)	468	(72.8)
Total	235		133		86		189		643	
4. Rhesus										
Homozyg.	90	(35.3)	63	(39.9)	32	(36.4)	58	(35.2)	243	(36.5)
Heterozyg.	165	(64.7)	95	(60.1)	56	(63.6)	107	(64.8)	423	(63.5)
Total	255		158		88		165		666	
5. Duffy										
Homozyg.	140	(55.1)	83	(54.2)	47	(53.4)	101	(54.0)	371	(54.4)
Heterozyg.	114	(44.9)	70	(45.8)	41	(46.6)	86	(46.0)	311	(45.6)
Total	254		153		88		187		682	
6. Kell										
Homozyg.	238	(91.9)	145	(91.8)	79	(89.8)	190	(95.0)	652	(92.5)
Heterozyg.	21	(8.9)	13	(8.2)	9	(10.2)	10	(5.0)	53	(7.5)
Total	259		158		88		200		705	
7. Haptoglobin										
Homozyg.			78	(53.8)	46	(58.2)	90	(48.4)	214	(52.2)
Heterozyg.			67	(46.2)	33	(41.8)	96	(51.6)	196	(47.8)
Total			145		79		186		410	
8. Phosphoglucomutase										
Homozyg.	161	(66.3)	106	(70.2)	52	(65.8)	133	(72.3)	452	(68.8)
Heterozyg.	82	(33.7)	45	(29.8)	27	(34.2)	51	(27.7)	205	(31.2)
Total	243		151		79		184		657	
9. Esterase-D										
Homozyg.	184	(76.3)	115	(77.2)	69	(87.3)	153	(82.7)	521	(79.7)
Heterozyg.	57	(23.7)	34	(22.8)	10	(12.7)	32	(17.3)	133	(20.3)
Total	241		149		79		185		654	
10. Acid phosphatase										
Homozyg.	122	(46.7)	89	(56.3)	41	(46.6)	108	(54.0)	360	(50.9)
Heterozyg.	139	(53.3)	69	(43.7)	47	(53.4)	92	(46.0)	347	(49.1)
Total	261		158		88		200		707	

TABLE 6.21: Statistical data for Table 6.20.

	Series	Method of subdivision	X^2	d.f.	P
1. MN					
	Total	A	0.02	1	.888
	Total	B	4.70	3	.195
	Adults	B	4.64	2	.098
	Total	C	4.28	5	.450
	Adults	C	4.63	4	.327
	Total	D	4.49	4	.343
	Adults	D	4.40	3	.222
2. S					
	Total	A	0.35	1	.553
	Total	B	1.33	3	.723
	Adults	B	0.92	2	.632
	Total	C	7.51	5	.185
	Adults	C	7.17	4	.127
	Total	D	2.23	4	.694
	Adults	D	1.90	3	.594
3. MNS					
	Total	A	6.30	1	.012
	Total	B	5.89	3	.117
	Adults	B	5.50	2	.064
	Total	C	6.65	5	.265
	Adults	C	6.00	4	.199
	Total	D	4.60	4	.331
	Adults	D	4.18	3	.242
4. Rhesus					
	Total	A	0.18	1	.674
	Total	B	1.07	3	.785
	Adults	B	0.81	2	.668
	Total	C	4.82	5	.438
	Adults	C	4.55	4	.337
	Total	D	2.09	4	.719
	Adults	D	1.84	3	.607
5. Duffy					
	Total	A	0.04	1	.850
	Total	B	0.10	3	.992
	Adults	B	0.02	2	.992
	Total	C	2.30	5	.806
	Adults	C	2.21	4	.696
	Total	D	4.09	4	.394
	Adults	D	4.00	3	.261
6. Kell					
	Total	A	0.11	1	.742
	Total	B	3.00	3	.392
	Adults	B	2.91	2	.233
	Total	C	3.45	5	.632
	Adults	C	3.39	4	.494
	Total	D	4.60	4	.330
	Adults	D	4.60	3	.204

TABLE 6.21 contd.:

Series	Method of subdivision	X^2	d.f.	P
7. Haptoglobin				
Adults	B	2.38	2	.304
Adults	C	2.81	4	.590
Adults	D	2.54	3	.469
8. Phosphoglucomutase				
Total	A	1.06	1	.303
Total	B	2.24	3	.525
Adults	B	1.11	2	.575
Total	C	3.97	5	.554
Adults	C	2.83	4	.587
Total	D	5.50	4	.240
Adults	D	4.41	3	.221
9. Esterase-D				
Total	A	2.18	1	.140
Total	B	6.13	3	.105
Adults	B	3.82	2	.148
Total	C	11.04	5	.051
Adults	C	8.98	4	.062
Total	D	4.46	4	.348
Adults	D	1.85	3	.604
10. Acid phosphatase				
Total	A	2.63	1	.105
Total	B	5.09	3	.165
Adults	B	2.21	2	.331
Total	C	11.10	5	.049
Adults	C	8.24	4	.083
Total	D	7.20	4	.126
Adults	D	4.33	3	.228

TABLE 6.22: A comparison of the mean ages of heterozygotes and homozygotes of the blood groups and isoenzymes.

	Series	mean age of : Homozygote	heterozygote	t	P	Mann - Whitney U	z	P	Analysis for trends: z	P
MN	Total	32.7171	34.0522	-0.53	.598	55904.5	-0.220	.826		
	Adults	50.9103	53.9426	-1.20	.231	21717.0	-0.923	.356	0.915	.179
S	Total	31.5051	33.1230	-0.64	.520	54332.0	-0.706	.480		
	Adults	50.9682	51.9682	-0.32	.750	21642.5	-0.416	.677	0.463	.322
MNS	Total	31.5341	33.9408	-0.83	.408	39770.5	-0.493	.622		
	Adults	48.4076	54.1658	-2.01	.046	14627.5	-1.898	.058	1.938	.026
Rhesus	Total	29.7233	30.3224	-0.24	.813	50422.5	-0.277	.782		
	Adults	47.7902	49.7902	-0.93	.354	17917.5	-1.398	.162	0.716	.236
Duffy	Total	31.5329	32.0652	-0.22	.830	56734.0	-0.248	.804		
	Adults	50.7269	50.7153	0.00	.996	22534.0	-0.005	.996	-0.005	.496
Kell	Total	32.8301	27.0493	1.25	.212	15852.5	-0.991	.322		
	Adults	51.7951	44.8004	1.45	.148	5737.5	-1.222	.222	1.437	.075
Hp	Adults	49.8939	53.1858	-1.25	.211	19657.0	-1.019	.308	1.224	.111
PGM	Total	32.9027	29.7700	1.15	.251	43704.0	-1.052	.293		
	Adults	51.1694	49.7793	0.49	.627	17461.0	-0.208	.836	0.223	.413
ESD	Total	33.4933	26.9325	2.08	.038	30175.5	-2.201	.028		
	Adults	51.8349	47.4012	1.31	.190	11131.5	-1.579	.114	0.952	.171
AP	Total	33.5329	31.0262	1.03	.305	59308.0	-1.063	.288		
	Adults	50.7945	51.8601	-0.42	.671	23484.0	-0.775	.438	0.367	.356
AK	Total	31.8000	37.3063	-1.09	.275	12077.5	-1.352	.176		
	Adults	50.6685	51.2962	0.13	.898	5993.5	-0.395	.693	0.006	.496

TABLE 6.23: Homozygous and heterozygous chromosome variant phenotype frequencies subdivided according to age.

Age(yrs)	0		1 - 29		30 - 59		60+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. Chromosome 3										
Homozyg.	80	(33.8)	70	(44.3)	39	(44.8)	66	(25.9)	255	(38.2)
Heterozyg.	157	(66.2)	88	(55.7)	48	(55.2)	119	(64.3)	412	(61.8)
Total	237		158		87		185		667	
2. Chromosome 13p*										
Homozyg.	87	(36.7)	59	(37.3)	40	(46.0)	79	(42.7)	265	(39.7)
Heterozyg.	150	(63.3)	99	(62.7)	47	(54.0)	106	(57.3)	402	(60.3)
Total	237		158		87		185		667	

TABLE 6.24: Statistical data for Table 6.23.

Series	Method of subdivision	X^2	d.f.	P
1. Chromosome 3				
Total	A	2.83	1	.092
Total	B	6.59	3	.086
Adults	B	3.40	2	.183
Total	C	9.49	5	.091
Adults	C	6.27	4	.180
Total	D	6.49	4	.165
Adults	D	3.35	3	.341
2. Chromosome 13p *				
Total	A	1.21	1	.271
Total	B	3.38	3	.337
Adults	B	1.95	2	.377
Total	C	2.84	5	.724
Adults	C	1.31	4	.860
Total	D	2.83	4	.586
Adults	D	1.30	3	.729

* Results for other chromosome regions are very similar to those given in Tables 6.26 and 6.27.

than that of the homozygote, in four lower, and in one (the Duffy blood group) the mean ages were the same. None of these differences was significant, but when the total sample was considered the mean age of ESD homozygotes was significantly higher than that of the heterozygotes. In both the MN and S blood groups the heterozygotes had a higher mean age than the homozygotes. This difference was significant (P=.046) when the MNS complex was examined. This result agrees with the findings of Williams (1977).

No significant associations were found between heterozygosity of the chromosome variants and age. The mean age of heterozygotes of chromosome 21s was significantly higher than that of the homozygotes in the total sample, but this merely reflects the large differences found in the frequencies of variants of this region in newborn infants and adults described earlier.

Table 6.27 shows that there is an apparent effect of age on the distribution of heterozygotes and homozygotes of chromosome 3 when intense and brilliant levels of fluorescence are considered together. The frequency of homozygotes tends to rise with age until the oldest age-groups, in which the frequency falls again. Among the adults the mean age of the heterozygotes is higher than that of homozygotes, but the difference is not significant.

The higher mean age of heterozygotes of chromosome 21s, described above, was also found in this comparison. The X^2-test results show a tendency for age to affect the frequencies of homozygotes of chromosome 22p. Table 6.26 shows that the frequency of homozygotes drops in the middle group of adults, as does the sample size. The significant and nearly significant results are probably caused by sampling effects.

TABLE 6.25: A comparison of the mean ages of homozygotes and heterozygotes of the chromosome variants.

Series	mean age of: homozygote	heterozygote	t	P	Mann - Whitney U	z	P	Analysis for trends: z	P
Chromosome 3									
Total	33.0939	31.8941	0.47	.638	49716.0	-1.055	.291		
Adults	48.3095	51.6081	-1.28	.201	21080.0	-0.810	.418	1.626	.052
Chromosome 13p									
Total	34.6232	30.8479	1.49	.136	49364.0	-1.534	.125		
Adults	51.5458	49.3566	0.85	.395	21089.0	-0.920	.357	0.854	.198
Chromosome 13s									
Total	31.6855	34.7862	-1.03	.304	34724.0	-1.307	.191		
Adults	50.7357	48.7690	0.66	.509	15912.0	-0.655	.513	0.347	.363
Chromosome 14s									
Total	32.1273	32.7260	-0.23	.815	50926.5	-0.405	.686		
Adults	50.3125	50.1932	0.05	.964	21346.5	-0.202	.840	-0.014	.496
Chromosome 15s									
Total	31.3444	33.6478	-0.92	.357	52281.0	-0.889	.374		
Adults	49.8843	50.7332	-0.33	.739	22440.0	-0.187	.852	0.295	.382
Chromosome 21s									
Total	30.5832	35.9066	-2.03	.043	44009.0	-2.216	.027		
Adults	51.0487	48.9837	0.79	.430	20028.0	-1.223	.221	0.437	.330
Chromosome 22s									
Total	31.8443	32.9484	-0.44	.657	53832.5	-0.454	.650		
Adults	49.7048	50.9202	-0.48	.633	22026.5	-0.583	.560	0.725	.233

TABLE 6.26: Homozygous and heterozygous chromosome variant phenotype frequencies (intense and brilliant levels of fluorescence combined) subdivided according to age.

Age(yrs)	0		1 - 29		30 - 59		60+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. Chromosome 3										
Homozyg.	97	(40.9)	79	(50.0)	51	(58.6)	77	(41.6)	304	(45.6)
Heterozyg.	140	(59.1)	79	(50.0)	36	(41.4)	108	(58.4)	363	(54.4)
Total	237		158		87		185		667	
2. Chromosome 13p										
Homozyg.	96	(40.5)	66	(41.8)	42	(48.3)	88	(47.6)	292	(43.8)
Heterozyg.	141	(59.5)	92	(58.2)	45	(51.7)	97	(52.4)	375	(56.2)
Total	237		158		87		185		667	
3. Chromosome 13s										
Homozyg.	196	(82.7)	121	(76.6)	64	(73.6)	147	(79.5)	528	(79.2)
Heterozyg.	41	(29.5)	37	(23.4)	23	(26.4)	38	(20.5)	139	(20.8)
Total	237		158		87		185		667	
4. Chromosome 14s										
Homozyg.	152	(64.1)	101	(63.9)	50	(57.5)	119	(64.3)	422	(63.3)
Heterozyg.	85	(35.9)	57	(36.1)	37	(42.5)	66	(35.7)	245	(36.7)
Total	237		158		87		185		667	
5. Chromosome 15p										
Homozyg.	233	(98.3)	153	(96.8)	82	(94.3)	176	(95.1)	644	(96.6)
Heterozyg.	4	(1.7)	5	(3.2)	5	(5.7)	9	(4.9)	23	(3.4)
Total	237		158		87		185		667	
6. Chromosome 15s										
Homozyg.	140	(59.1)	90	(57.0)	53	(60.9)	104	(56.2)	387	(58.0)
Heterozyg.	97	(40.9)	68	(43.0)	34	(39.1)	81	(43.8)	280	(42.0)
Total	237		158		87		185		667	
7. Chromosome 21s										
Homozyg.	178	(75.1)	102	(64.6)	51	(58.6)	119	(64.3)	450	(67.5)
Heterozyg.	59	(24.9)	56	(35.4)	36	(41.4)	66	(35.7)	217	(32.5)
Total	237		158		87		185		667	
8. Chromosome 22p										
Homozyg.	193	(81.4)	131	(82.9)	58	(66.7)	151	(81.6)	533	(79.9)
Heterozyg.	44	(18.6)	27	(17.1)	29	(33.3)	34	(18.4)	134	(20.1)
Total	237		158		87		185		667	
9. Chromosome 22s										
Homozyg.	138	(58.2)	85	(53.8)	51	(58.6)	98	(53.0)	372	(55.8)
Heterozyg.	99	(41.3)	73	(46.2)	36	(41.4)	87	(47.0)	295	(44.2)
Total	237		158		87		185		667	

TABLE 6.27: Statistical data for Table 6.26.

Series	Method of subdivision	X^2	d.f.	P
1. Chromosome 3				
Total	A	2.92	1	.088
Total	B	10.45	3	.015
Adults	B	7.20	2	.027
Total	C	14.58	5	.012
Adults	C	11.32	4	.023
Total	D	9.89	4	.042
Adults	D	6.65	3	.084
2. Chromosome 13p				
Total	A	1.40	1	.237
Total	B	3.08	3	.379
Adults	B	1.47	2	.479
Total	C	3.22	5	.666
Adults	C	1.48	4	.831
Total	D	3.27	4	.514
Adults	D	1.52	3	.677
3. Chromosome 13s				
Total	A	2.47	1	.116
Total	B	4.10	3	.251
Adults	B	1.22	2	.542
Total	C	6.53	5	.258
Adults	C	3.40	4	.493
Total	D	6.67	4	.155
Adults	D	3.53	3	.316
4. Chromosome 14s				
Total	A	0.07	1	.794
Total	B	1.45	3	.693
Adults	B	1.33	2	.515
Total	C	1.41	5	.923
Adults	C	1.25	4	.869
Total	D	1.62	4	.805
Adults	D	1.46	3	.691
5. Chromosome 15p				
Total	A	2.65	1	.104
Total	B	4.74	3	.192
Adults	B	1.04	2	.595
Total	C	4.34	5	.502
Adults	C	0.69	4	.952
Total	D	5.73	4	.221
Adults	D	1.79	3	.618
6. Chromosome 15s				
Total	A	0.11	1	.744
Total	B	0.73	3	.867
Adults	B	0.56	2	.756
Total	C	1.72	5	.818
Adults	C	1.55	4	.818
Total	D	0.91	4	.923
Adults	D	0.75	3	.862

TABLE 6.27 contd.:

Series	Method of subdivision	X^2	d.f.	P
7. Chromosome 21s				
Total	A	9.24	1	.002
Total	B	10.84	3	.013
Adults	B	1.01	2	.604
Total	C	15.27	5	.009
Adults	C	5.29	4	.259
Total	D	11.90	4	.018
Adults	D	2.11	3	.550
8. Chromosome 22p				
Total	A	0.40	1	.530
Total	B	11.07	3	.011
Adults	B	10.22	2	.006
Total	C	7.38	5	.194
Adults	C	6.71	4	.152
Total	D	8.11	4	.081
Adults	D	7.42	3	.060
9. Chromosome 22s				
Total	A	0.75	1	.386
Total	B	1.70	3	.636
Adults	B	0.80	2	.670
Total	C	4.69	5	.455
Adults	C	3.89	4	.422
Total	D	4.19	4	.381
Adults	D	3.39	3	.335

For six of the twelve chromosome variant regions examined the mean age of homozygotes was higher than that of heterozygotes, for four the mean age of heterozygotes was higher, and for two they were the same. Only in the case of chromosome 14p (heterozygote higher) was the difference significant (see table 6.16).

Figure 6.3 shows the numbers of homozygous chromosomes, blood groups and isoenzymes per individual and the total number of homozygous markers (genetic and chromosomal) per individual. The frequencies of these grouped variables have been subdivided according to age using the four methods of subdivision given in table 6.8 and presented in table 6.28. Results of statistical analysis are given in table 6.29.

The only apparent effect is an increase in the number of homozygous isoenzymes with age. X^2 values and Spearman's rho values were both significant in comparisons in the total sample. The difference seems to be confined to a comparison between the newborn infants and the adults.

In order to determine whether or not the 'healthiness' of the subjects has confounded any effects of age on the degree of homozygosity of individuals, the following comparisons were made with respect to the above-mentioned grouped variables:

1. Newborn infants versus healthy adults.
2. Healthy adults versus all hospital patients.
3. Healthy adults versus geriatric patients.
4. Newborn infants versus geriatric patients.

These data are given in table 6.30, and the results of statistical analysis in table 6.31. In all these comparisons the only significant values for X^2 and Kendall's tau were for the distribution of numbers of homozygous enzymes in the fourth comparison (between newborn infants and geriatric patients. No difference was found between the healthy adults and either the infants or the geriatric patients with respect

Figure 6.3: Distributions of homozygous genetic and chromosomal markers.

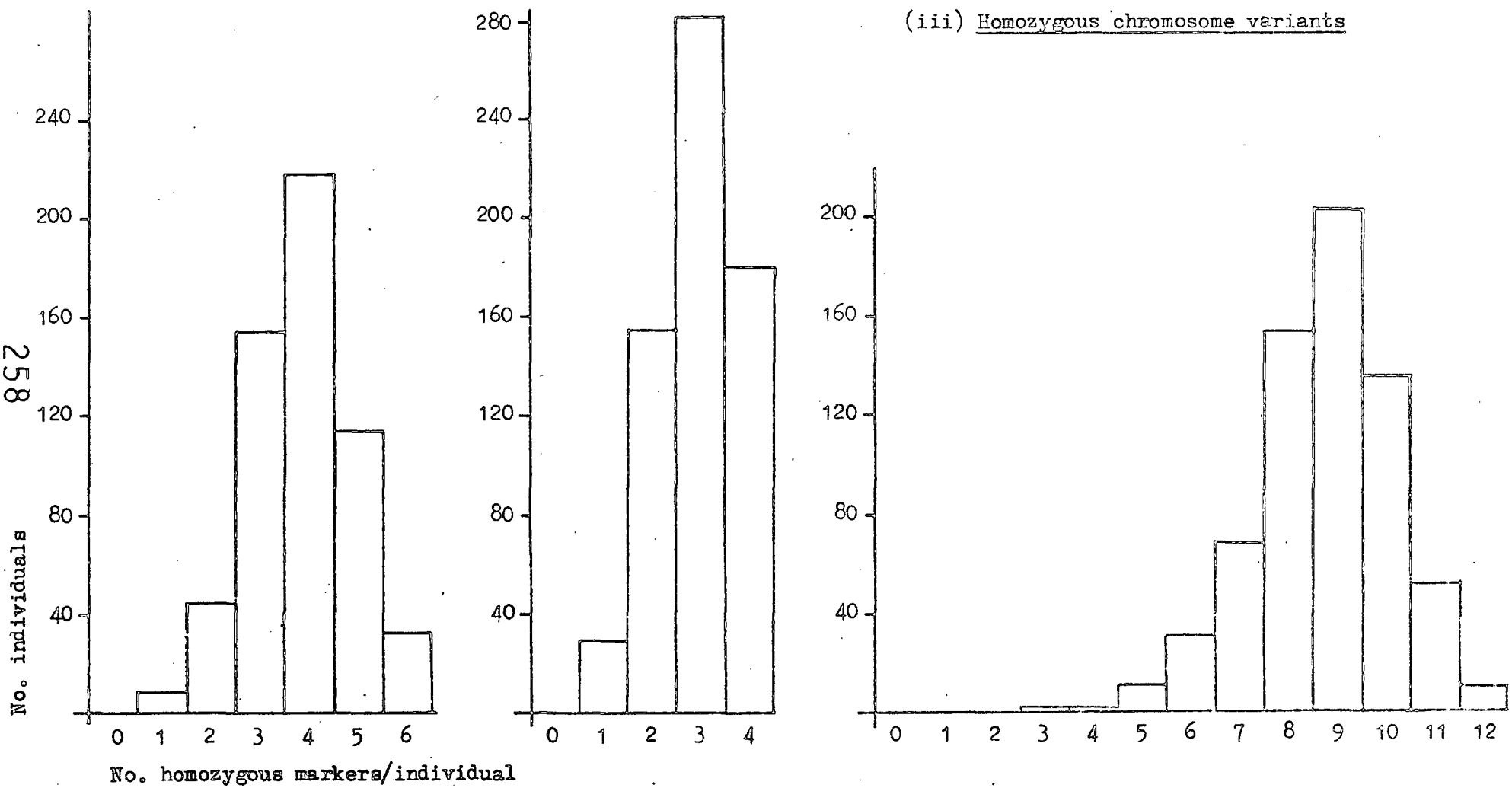


Figure 6.3 contd.:

(iv) Total homozygous markers in the whole sample

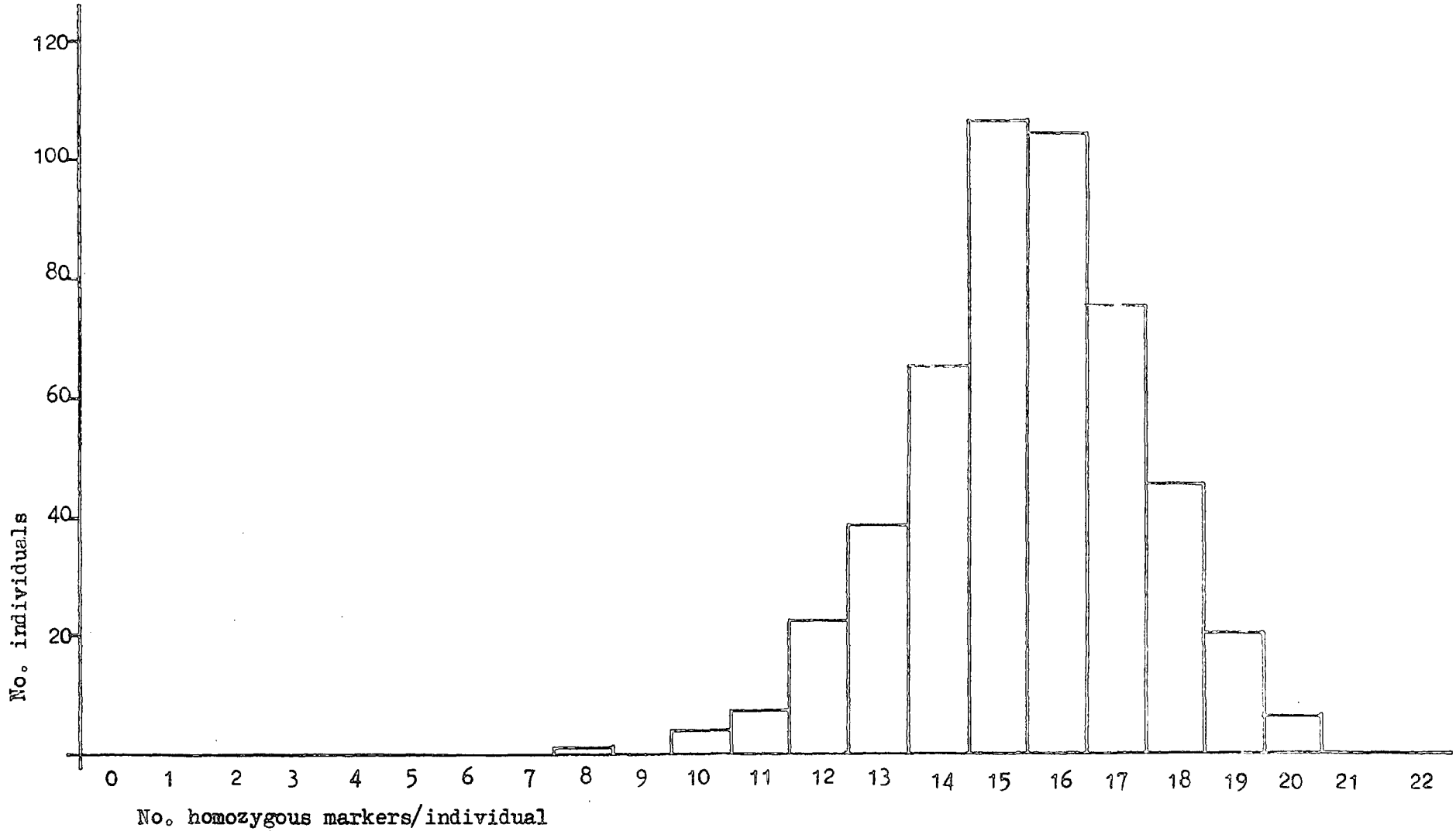


Figure 6.3 contd.:

(v) Total homozygous markers in the adult series

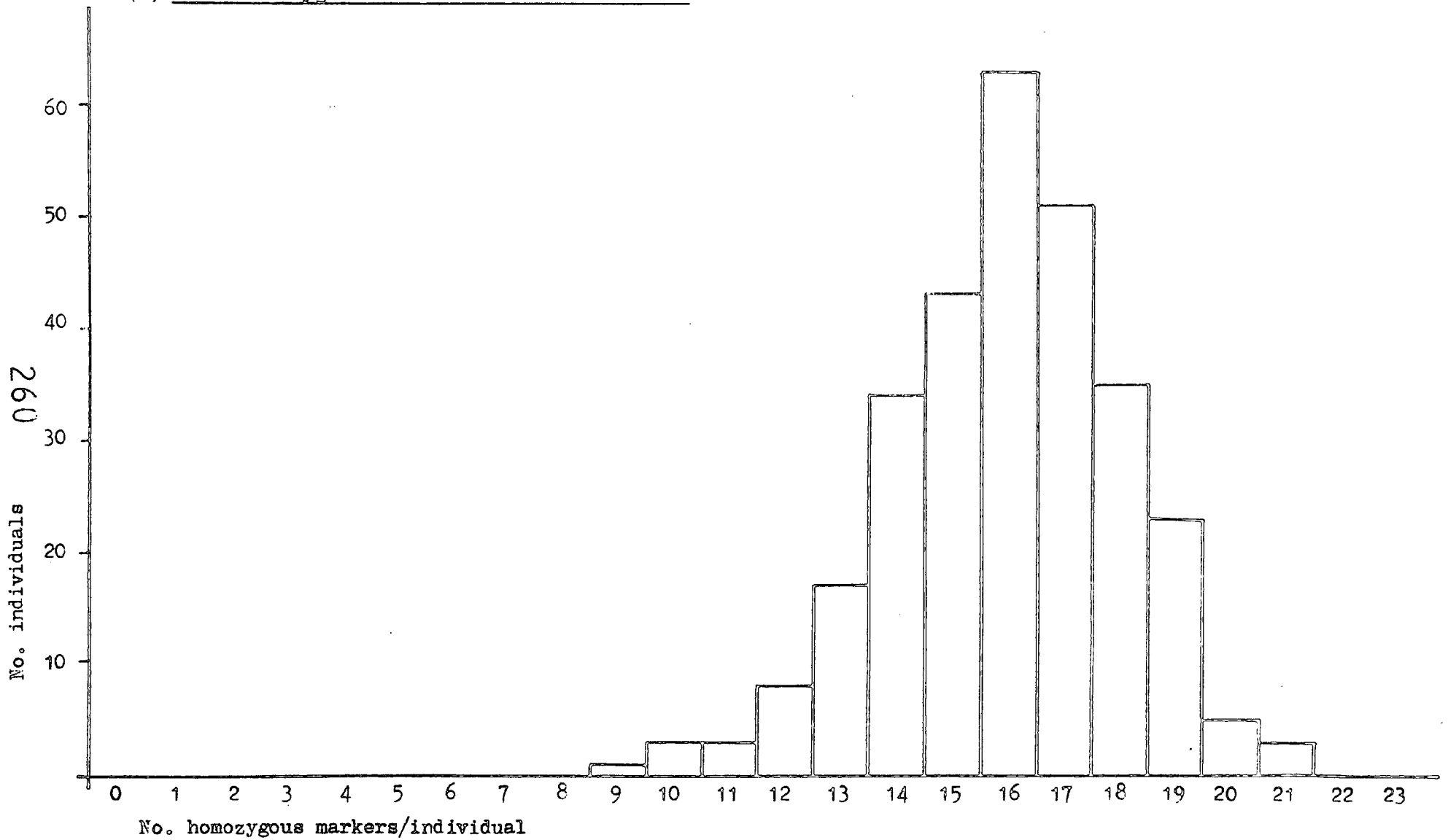


TABLE 6.28: Total numbers of homozygous genetic and chromosomal markers per individual subdivided according to age.

Age(yrs)	0		1 - 29		30 - 59		60+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. Total homozygous chromosomes										
≤6	10	(4.2)	7	(4.4)	9	(10.3)	17	(9.2)	43	(6.4)
7	22	(9.3)	16	(10.1)	9	(10.3)	21	(11.4)	68	(10.2)
8	56	(23.6)	39	(24.7)	17	(19.5)	42	(22.7)	154	(23.1)
9	76	(32.1)	56	(35.4)	24	(27.6)	46	(24.9)	202	(30.3)
10	44	(18.6)	29	(18.4)	21	(18.4)	42	(22.7)	136	(20.4)
≥11	29	(12.2)	11	(7.0)	7	(8.0)	17	(9.2)	64	(9.6)
Total	237		158		87		185		667	
2. Total homozygous blood groups										
≤2	22	(9.9)	16	(12.3)	2	(2.3)	12	(9.6)	52	(9.2)
3	57	(25.6)	30	(23.1)	28	(32.6)	37	(29.6)	152	(27.0)
4	89	(39.9)	44	(33.8)	32	(37.2)	50	(40.0)	215	(38.1)
5	42	(18.8)	31	(23.8)	18	(20.9)	22	(17.6)	113	(20.0)
6	13	(5.8)	9	(6.9)	6	(7.0)	4	(3.2)	32	(5.7)
Total	223		130		86		125		564	
3. Total homozygous enzymes										
≤1	8	(3.3)	9	(6.3)	3	(3.9)	9	(4.9)	29	(4.5)
2	69	(28.8)	30	(21.0)	20	(26.0)	35	(19.2)	154	(24.0)
3	108	(45.0)	60	(42.0)	37	(48.1)	74	(40.7)	279	(43.5)
4.	55	(22.9)	44	(30.8)	17	(22.1)	64	(35.2)	180	(28.0)
Total	240		143		77		182		642	
4. Total homozygous enzymes + serum protein										
≤2			21	(15.7)	14	(18.7)	28	(15.6)	63	(16.2)
3			43	(32.1)	22	(29.3)	52	(28.9)	117	(30.1)
4			42	(31.3)	28	(37.3)	67	(37.2)	137	(35.2)
5			28	(20.9)	11	(14.7)	33	(18.3)	72	(18.5)
Total			134		75		180		389	
5. Total homozygous markers (except serum protein)										
≤12	15	(7.7)	7	(6.0)	6	(7.9)	6	(5.7)	34	(6.9)
13	7	(3.6)	11	(9.5)	6	(7.9)	14	(13.3)	38	(7.7)
14	25	(12.9)	13	(11.2)	8	(10.5)	18	(17.1)	64	(13.0)
15	47	(24.2)	25	(21.6)	16	(21.1)	18	(17.1)	106	(21.6)
16	41	(21.1)	23	(19.8)	20	(26.3)	19	(18.1)	103	(21.0)
17	29	(14.9)	19	(16.4)	11	(14.5)	16	(15.2)	75	(15.3)
≥18	30	(15.5)	18	(15.5)	9	(11.8)	14	(13.3)	71	(14.5)
Total	194		116		76		105		491	
6. Total homozygous markers										
≤13			11	(10.1)	8	(10.8)	13	(12.5)	32	(11.1)
14			11	(10.1)	5	(6.8)	18	(17.3)	34	(11.8)
15			17	(15.6)	11	(14.9)	14	(13.5)	42	(14.6)
16			22	(20.2)	21	(28.4)	19	(18.3)	62	(21.6)
17			22	(20.2)	13	(17.6)	16	(15.4)	51	(17.8)
18			12	(11.0)	11	(14.9)	12	(11.5)	35	(12.2)
≥19			14	(12.8)	5	(6.8)	12	(11.5)	31	(10.8)
Total			109		74		104		287	

TABLE 6.29: Statistical data for Table 6.28.

Series	Method of subdivision	X^2	d.f.	P	Spearman's rho	P
1. Total homozygous chromosomes						
Total	A	7.63	7	.366		
Total	B	16.71	15	.337	-.040	.209
Adults	B	9.33	10	.501	-.007	.882
Total	C	22.63	25	.599	-.028	.362
Adults	C	15.57	20	.743	.012	.686
Total	D	16.14	20	.708	-.039	.221
Adults	D	8.88	15	.884	-.008	.863
2. Total homozygous blood groups						
Total	A	5.21	5	.391		
Total	B	12.54	12	.404	-.014	.684
Adults	B	11.73	8	.164	-.063	.250
Total	C	15.72	20	.734	-.014	.700
Adults	C	14.64	16	.551	-.064	.242
Total	D	11.81	16	.757	-.003	.928
Adults	D	10.69	12	.556	-.030	.583
3. Total homozygous enzymes						
Total	A	8.54	3	.036		
Total	B	14.34	9	.111	.079	.020
Total	C	20.47	15	.155	.077	.021
Total	D	23.96	12	.021	.076	.025
4. Total homozygous enzymes + serum protein						
Adults	B	2.52	6	.867	.011	.829
Adults	C	11.18	12	.513	.002	.976
Adults	D	7.60	9	.575	.004	.931
5. Total homozygous markers (except serum protein)						
Total	A	11.05	8	.199		
Total	B	15.59	18	.622	-.047	.198
Total	C	26.14	30	.668	-.036	.316
Total	D	20.18	24	.686	-.036	.318
6. Total homozygous markers						
Adults	B	10.07	12	.610	-.064	.279
Adults	C	22.43	24	.554	-.047	.432
Adults	D	13.18	18	.781	-.039	.515

TABLE 6.30: Total numbers of homozygous genetic and chromosomal markers per individual, subdivided according to ascertainment group.

Group I = newborn infants
Group II = healthy adults
Group III = orthopaedic patients
Group IV = geriatric patients

Group	I		II		III		IV		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. Homozygous chromosomes										
≤5	2	(0.8)	5	(2.2)	1	(2.4)	5	(3.0)	13	(1.9)
6	8	(3.4)	12	(5.4)	1	(2.4)	10	(6.0)	31	(4.6)
7	22	(9.3)	21	(9.4)	7	(17.1)	19	(11.3)	69	(10.3)
8	56	(23.6)	52	(23.2)	7	(17.1)	39	(23.2)	154	(23.0)
9	76	(32.1)	76	(33.9)	9	(22.0)	42	(25.0)	203	(30.3)
10	44	(18.6)	41	(18.3)	13	(31.7)	38	(22.6)	136	(20.3)
≥11	29	(12.2)	17	(7.5)	3	(7.3)	15	(9.0)	64	(9.5)
Total	237		224		41		168		670	
2. Homozygous blood groups										
≤2	22	(9.9)	16	(8.1)	3	(8.3)	11	(9.9)	52	(9.2)
3	57	(25.6)	52	(26.9)	10	(27.8)	33	(29.7)	153	(27.0)
4	89	(39.9)	69	(35.0)	18	(50.0)	41	(36.9)	217	(38.3)
5	42	(18.8)	46	(23.4)	3	(8.3)	22	(19.8)	113	(19.9)
6	13	(5.8)	13	(6.6)	2	(5.6)	4	(3.6)	32	(5.6)
Total	223		197		36		111		567	
3. Homozygous enzymes										
≤1	8	(3.3)	11	(5.3)	1	(4.0)	9	(5.2)	29	(4.5)
2	69	(28.8)	46	(22.3)	7	(28.0)	32	(18.5)	154	(23.9)
3	108	(45.0)	93	(45.1)	10	(40.0)	70	(40.5)	281	(43.6)
4	55	(22.9)	56	(27.2)	7	(28.0)	62	(35.8)	180	(28.0)
Total	240		206		25		173		644	
4. Homozygous enzymes + serum protein										
≤2			34	(17.0)	4	(20.0)	25	(14.6)	63	(16.1)
3			59	(29.5)	9	(45.0)	50	(29.2)	118	(30.2)
4			70	(35.0)	4	(20.0)	64	(37.4)	138	(35.3)
5			37	(18.5)	3	(15.0)	32	(18.7)	72	(18.4)
Total			200		20		171		391	
5. Total homozygous markers (except serum protein)										
≤12	15	(7.8)	13	(7.3)	2	(8.4)	4	(4.1)	34	(6.9)
13	7	(3.6)	15	(8.4)	3	(12.5)	13	(13.5)	38	(7.7)
14	25	(12.9)	18	(10.1)	5	(20.8)	17	(17.7)	65	(13.2)
15	47	(24.2)	40	(22.3)	1	(4.2)	18	(18.8)	106	(21.5)
16	41	(21.1)	40	(22.3)	6	(25.0)	17	(17.7)	104	(21.1)
17	29	(14.9)	28	(15.6)	3	(12.5)	15	(15.6)	75	(15.2)
18	16	(8.2)	17	(9.5)	4	(16.7)	8	(8.3)	45	(9.1)
≥19	14	(7.2)	8	(4.5)	0		4	(4.2)	26	(5.3)
Total	194		179		24		96		493	

TABLE 6.30 contd.:

Group	I		II		III		IV		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
6. Total homozygous markers										
12			11	(6.3)	1	(5.3)	3	(3.2)	15	(5.2)
13			8	(4.6)	2	(10.5)	7	(7.4)	17	(5.9)
14			14	(8.0)	3	(15.8)	17	(17.9)	34	(11.8)
15			27	(15.4)	2	(10.5)	14	(14.7)	43	(14.9)
16			42	(24.0)	4	(21.1)	17	(17.9)	63	(21.8)
17			30	(17.1)	5	(26.3)	16	(16.8)	51	(17.6)
18			24	(13.7)	0		11	(11.6)	35	(12.1)
19			14	(8.0)	2	(10.5)	7	(7.4)	23	(8.0)
≥20			5	(2.9)	0		3	(3.2)	8	(2.8)
Total			175		19		95		289	

TABLE 6.31: Statistical data for Table 6.30 (pair-wise comparisons of ascertainment groups).

Groups	X^2	d.f.	P	Kendall's tau	P
1. Homozygous chromosomes					
I v. II	4.86	5	.433	-.072	.173
II v. III + IV	9.51	7	.218	-.014	.799
II v. IV	6.92	7	.438	.002	.976
I v. IV	8.43	7	.296	-.068	.226
2. Homozygous blood groups					
I v. II	2.21	4	.698	.042	.454
II v. III + IV	3.55	4	.470	.086	.155
II v. IV	2.09	4	.719	-.076	.224
I v. IV	1.41	4	.843	-.036	.545
3. Homozygous enzymes					
I v. II	3.63	3	.304	.055	.297
I v. IV	11.58	3	.009	.140	.009
4. Homozygous enzymes + serum protein					
II v. III + IV	0.37	4	.985	.009	.884
II v. IV	0.87	4	.934	.027	.649
5. Total homozygous markers (except serum protein)					
I v. II	5.94	8	.654	-.021	.730
I v. IV	13.76	8	.088	-.083	.194
6. Total homozygous markers					
II v. III + IV	9.78	9	.369	.079	.232
II v. IV	8.72	9	.463	-.066	.324

to the number of homozygous enzymes.

The frequency of persons with all four isoenzymes homozygous was higher in the geriatric patients than in the infants or the healthy adults. Therefore, although the results of the analyses are not always significant by the X^2 test, or by calculation of Kendall's tau, it would seem that there might be a disadvantage associated with maximum homozygosity of the isoenzymes studied here, although one would be more convinced of this if the frequency in the infants lay between that of the geriatric patients and that of the entirely healthy adults.

Comparable results were not found when the frequency of persons with all blood groups systems homozygous were examined in the infants, healthy adults and geriatric patients, but it must be remembered that some blood groups were omitted from this analysis, for example the ABO system. Table 6.32 gives the mean number of homozygous variables for each system of variables, in each of the four ascertainment groups compared above. These values were compared using the t-test and also the data were analysed with the Mann Whitney U test. Again, the results (table 6.33) show significant differences only between the numbers of homozygous isoenzymes in newborn infants and geriatric patients. The number is higher in the latter group. The mean number of homozygous enzymes in the healthy adults falls between these two groups. These tests confirm the findings of the previous analysis.

TABLE 6.32: Mean numbers of homozygous genetic and chromosomal markers per individual, in different ascertainment groups.

Group	Chromosomes	Blood Groups	Enzymes	Enzymes + Serum Pr.	All (except Ser. Pr.)	All markers
I Infants	8.8945	3.8251	2.8750		15.5928	
II Healthy adults	8.6563	3.9239	2.9417	3.5200	15.4693	16.0514
III + IV All hospital patients	8.6986	3.7687	3.0505	3.5393	15.3167	15.8421
IV Geriatric patients	8.6726	3.7748	3.0694	3.5673	15.3333	15.8947

TABLE 6.33: Statistical data for Table 6.32 (pair-wise comparisons of ascertainment groups).

Groups	t*	P	Mann-Whitney U	z	P
1. Homozygous chromosomes					
I v. II	1.86	.064	20484.5	-0.907	.364
II v. III + IV	0.30	.763	3561.5	-0.467	.640
II v. IV	0.11	.912	10712.5	-0.036	.972
I v. IV	1.52	.130	13221.5	-0.857	.392
2. Homozygous blood groups					
I v. II	0.94	.350	17370.5	-1.183	.237
II v. III + IV	1.38	.169	2620.0	-1.259	.208
II v. IV	1.20	.230	5196.0	-2.054	.040
I v. IV	0.41	.682	7499.5	-1.231	.218
3. Homozygous enzymes					
I v. II	0.86	.391	18569.5	-1.137	.256
I v. IV	2.36	.019	12277.5	-2.433	.015
4. Homozygous enzymes + serum protein					
II v. III + IV	0.18	.854	1319.5	-1.289	.197
II v. IV	0.44	.662	9276.5	-0.165	.869
5. All homozygous markers (except serum protein)					
I v. II	0.61	.540	14319.5	-0.149	.882
I v. IV	1.09	.277	5458.0	-1.291	.197
6. All homozygous markers					
II v. III + IV	0.84	.404	1130.5	-1.287	.198
II v. IV	0.59	.556	3892.0	-1.546	.122

*sign ignored

3. Parental age and birth order.

The mother's and father's age at birth of a child and the birth order of the individual child within the family are factors which may affect the chances of that child having a particular genetic or chromosomal marker, but all three factors are typically very highly correlated with each other. It is, therefore, a difficult task to disentangle their effects. In the present study information regarding parental ages and birth order was obtained only for the series of newborn infants, and unfortunately, not for the whole of this. Thus, the size of the sample is probably too small to allow the necessary separation of influences.

A possible source of error in the interpretation of an association found between the incidence of any genetic marker and maternal age is that this latter factor is influenced very much by past reproductive history, and tends to be higher if complications have occurred in previous pregnancies (Beardmore pers. comm.). Thus a genetic marker which is in fact associated with the cause of such complications, may appear to be associated with advanced maternal age.

Parental age has been shown to affect the rates of mutation at a number of genetic loci (Cavalli Sforza and Bodmer 1971). These authors have derived an equation to quantify the relationship between the mean parental age of individuals with mutated genotypes and that of the rest of the population:

$$\bar{x}_m = \frac{\text{var}(x)}{\bar{x}} + \bar{x}$$

They have shown, therefore, that the effect of parental age on the mutation rate depends upon the variance in the population of parental age. It seems therefore, that as this quantity (var(x)) is never equal to zero in a human population, the paternal or maternal age of mutation-carrying

individuals is always going to be different from those of other non-mutated individuals.

Cavalli Sforza and Bodmer (1971) suggest that because of the different processes of spermatogenesis and oogenesis, parental age effects might bias mutation rates in males and make them higher than those in females.

If the presence of intensely or brilliantly staining material in the cell affects mutation rates, then it might be speculated that a relationship will be seen between the incidence of these variants and paternal age. If presence of the variant regions leads to an increase in the mutation rate then we might expect them to occur at a low frequency at high parental ages. This would be because a combination of the deleterious effects of the presence of variant bands and parental age might lead to an increase in terminated pregnancies amongst carriers of the variant regions. Conversely if the presence of the variant regions is associated with a decrease in the rates of mutation, their presence would have a protective effect against the deleterious effects of high parental age, and an increased frequency of variant bands might be observed among the offspring of older parents.

The effect of parental age, especially maternal age, on the incidence of various types of chromosomal abnormalities is very well documented. Maternal age appears to affect the incidence of numerical chromosome anomalies especially. Machin and Crolla (1974) found that only 23% of 500 infants dying during the perinatal period were born to mothers over the age of 30, but 43% of the infants with chromosome abnormalities and, more specifically, 71% of the trisomic infants were born to mothers of that age range. 35% of the infants born to women over the age of 40 were chromosomally abnormal.

Penrose and Smith (1966) showed that the incidence of trisomy 21 is very much influenced by maternal age. The risk of having an infant with trisomy 21 is about 1 in 2000 for women under 25, but this risk rises to about 1 in 100 for women over 40, and 1 in 50 for women over 45 years old. However, the incidence of Down's syndrome caused by structural abnormalities of the chromosome (for example by D/G or G/G translocations) does not appear to be related to maternal or paternal age. Most authors state that paternal age has no effect on the incidence of trisomy 21. Levitan and Montagu (1977) state that "recent data suggest a paternal age factor as well" but give no references to these data.

Similar effects of maternal age on the incidence of Edward's syndrome (trisomy 13) and Patau's syndrome (trisomy 18) have been noted. (Ford 1973). In these two conditions, also, there are indications of maternal age-dependent and maternal age-independent groups.

Hamerton et al. (1975) found a higher mean maternal age in female newborn infants with sex chromosome abnormalities. The mean maternal age appears also to be raised for Klinefelter's syndrome (XXY). The incidence of Turner's syndrome (XO), however, appears not to be affected by maternal age (Ford 1973).

Patil et al. (1977) found that in both black and white Americans the lowest frequency of chromosomal abnormalities was found in children born to women in their middle reproductive years. (whites: 30 to 35 years, and blacks: 25 to 34 years). They suggest that a possible explanation for this phenomenon is that there are two maternal age effects:-

(i) the above-mentioned trend towards a greater incidence of chromosome abnormalities in children of older mothers, and

(ii) a similar effect in very young mothers which acts

primarily on the X chromosome.

Nielsen (1969) compared the maternal age at birth of older XXY males in the Danish population with the mean maternal age in the years of births of these persons, and showed that the lowest risk occurred to mothers in the 30-34 year group, the highest in mothers over 40, and comparable intermediate risks in the under-20 and 35-39 year groups. Similar findings have been reported by Court Brown et al. (1969) who found that the mean maternal age of mothers having sons with the 47, XXY chromosome constitution was significantly higher than that of all mothers. The mean maternal age of mothers of 47, XXX daughters was also raised significantly above that of the control population.

As mentioned above, parental, especially maternal age affects the incidence of numerical rather than structural chromosome anomalies. An explanation for this, suggested by Levitan and Montagu (1977) is that these numerical anomalies occur for reasons similar to those which cause increasing aneuploidy of blood cells in aged persons (especially females). These authors suggest that ageing processes cause ageing effects in cell physiology which produce abnormalities in the movements of chromosomes, with resulting abnormalities in their representation in somatic and gonadal tissues.

In view of the above findings it is possible to speculate about the meaning of any association with parental age of the incidence of fluorescent chromosome variants. If possession of these variants has some effect on the likelihood of the carriers suffering irregularities of chromosome movement at cell division, then it might be expected that this influence of the variant bands will become more detrimental with increasing parental, especially maternal, age.

When it is remembered that all the infants examined in the present study were normal and healthy, and also that there is evidence that only a proportion of conceptions with chromosomal abnormalities of all types fail to abort, then it might be expected that the incidence of variant bands would decrease in infants born to older parents, if the supposed effects of the variants occurs.

There is some evidence for an association between a fluorescence marker and Down's syndrome (Robinson and Newton 1977). These authors found that the frequency of positively fluorescent satellites of chromosome 21 was higher in a series of Down's syndrome patients than in a series of controls, and speculated that this particular polymorphic type might predispose towards meiotic non-disjunction.

A. Maternal age.

Effects of maternal age on the phenotype frequencies of all markers investigated in this study were examined by means of the X^2 test, by calculation of Spearman's rho (where possible), by comparing the mean maternal age of homozygotes with that of heterozygotes (where these can be determined), and by the analysis for trends described earlier (see above page 130).

(i) Blood groups and isoenzymes.

Tables 6.34, 6.35 and 6.36 show the frequencies of phenotypes of these markers subdivided according to maternal age, and the results of statistical analysis of these data. No difference in ABO phenotypes was found between age-groups. These data, then, do not confirm Beardmore's (pers. comm)finding that there was a highly significant increase in the frequency of blood Group B with increasing maternal age.

An effect of maternal age was detected only in the case of the MNSs blood group system. The rank correlation coefficients for the MN and Ss blood groups were nearly

TABLE 6.34: Blood group and isoenzyme phenotype frequencies subdivided according to age of mother.

Age(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. ABO												
A	8	(47.1)	29	(39.7)	44	(45.8)	19	(42.2)	3	(30.0)	103	(42.7)
O	7	(41.2)	33	(45.2)	44	(45.8)	21	(46.7)	5	(50.0)	110	(45.6)
B	2	(11.8)	10	(13.7)	6	(6.3)	4	(8.9)	2	(20.0)	24	(10.0)
AB	0		1	(1.4)	2	(2.1)	1	(2.2)	0		4	(1.7)
Total	17		73		96		45		10		241	
2. Rhesus												
r r	3	(18.8)	18	(25.0)	12	(12.6)	10	(22.2)	2	(22.2)	45	(19.0)
R_1r	3	(18.8)	23	(31.9)	40	(42.1)	14	(31.1)	2	(22.2)	82	(34.6)
R_1R_1	4	(25.0)	13	(18.1)	12	(12.6)	5	(11.1)	1	(11.1)	35	(14.8)
R_2r	1	(6.7)	2	(2.8)	6	(6.3)	4	(8.9)	1	(11.1)	14	(5.9)
R_2R_2	0		0		3	(3.2)	0		0		3	(1.3)
R_1R_2	2	(12.5)	15	(20.8)	20	(21.1)	12	(26.7)	3	(33.3)	52	(21.9)
r"r'	0		1	(1.4)	0		0		0		1	(0.4)
r"r	2	(12.5)	0		2	(2.1)	0		0		4	(1.7)
R_zR_2	1	(6.7)	0		0		0		0		1	(0.4)
Total	16		72		95		45		9		237	
3. Rhesus(D)												
D+ve	12	(70.6)	54	(74.0)	82	(85.4)	35	(77.8)	8	(80.0)	191	(79.3)
D-ve	5	(29.4)	19	(26.0)	14	(14.6)	10	(22.2)	2	(20.0)	50	(20.7)
Total	17		73		96		45		10		241	
4. MN												
M	4	(26.7)	19	(27.1)	30	(34.5)	12	(27.3)	4	(40.0)	69	(30.5)
MN	7	(46.7)	35	(50.0)	43	(49.4)	29	(65.9)	5	(50.0)	119	(52.7)
N	4	(26.7)	16	(22.9)	14	(16.1)	3	(6.8)	1	(10.0)	38	(16.8)
Total	15		70		87		44		10		226	
5. S												
S	2	(12.5)	13	(18.6)	11	(12.0)	2	(4.4)	2	(22.2)	30	(12.9)
Ss	7	(43.8)	33	(47.1)	38	(41.3)	21	(46.7)	3	(33.3)	102	(44.0)
s	7	(43.8)	24	(34.3)	43	(46.7)	22	(48.9)	4	(44.4)	100	(43.1)
Total	16		70		92		45		9		232	
6. MNS												
MS	1	(7.1)	5	(7.5)	6	(7.2)	1	(2.3)	1	(11.1)	14	(6.5)
MSs	1	(7.1)	7	(10.4)	17	(20.5)	7	(15.9)	1	(11.1)	33	(15.2)
Ms	1	(7.1)	4	(6.0)	5	(6.0)	4	(9.1)	1	(11.1)	15	(6.9)
MNS	1	(7.1)	7	(10.4)	4	(4.8)	1	(2.3)	1	(11.1)	14	(6.5)
MNSs	2	(14.3)	21	(31.3)	12	(14.5)	12	(27.3)	1	(11.1)	48	(22.1)
MNs	4	(28.6)	7	(10.4)	25	(30.1)	16	(36.4)	3	(33.3)	55	(25.3)
NS	0		1	(1.5)	1	(1.2)	0		0		2	(0.9)
NSs	2	(14.3)	3	(4.5)	4	(4.8)	2	(4.5)	1	(11.1)	12	(5.5)
Ns	2	(14.3)	12	(17.9)	9	(10.8)	1	(2.3)	0		24	(11.1)
Total	14		67		83		44		9		217	

TABLE 6.34 contd.:

Age(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
7. Duffy												
Fy^a	3	(17.6)	9	(12.7)	17	(18.3)	8	(17.8)	0		37	(15.7)
Fy^aFy^b	7	(41.2)	33	(46.5)	43	(46.2)	23	(51.1)	5	(50.0)	111	(47.0)
Fy^b	7	(41.2)	29	(40.8)	33	(35.5)	14	(31.1)	5	(50.0)	88	(37.3)
Total	17		71		93		45		10		236	
8. Koll												
Kk	2	(11.8)	6	(8.2)	7	(7.3)	3	(6.7)	1	(10.0)	19	(7.9)
k	15	(88.2)	67	(91.8)	89	(92.7)	42	(93.3)	9	(90.0)	222	(92.1)
Total	17		73		96		45		10		241	
9. Phosphoglucomutase												
1 - 1	9	(60.0)	39	(56.5)	53	(59.6)	26	(60.5)	6	(60.0)	133	(58.8)
2 - 1	5	(33.3)	26	(37.7)	29	(32.6)	13	(30.2)	2	(20.0)	75	(33.2)
2 - 2	1	(6.7)	4	(5.8)	7	(7.9)	4	(9.3)	2	(20.0)	18	(8.0)
Total	15		69		89		43		10		226	
10. Esterase-D												
1 - 1	13	(86.7)	49	(71.0)	63	(73.3)	33	(75.0)	9	(90.0)	167	(74.6)
2 - 1	1	(6.7)	19	(27.5)	19	(22.1)	11	(25.0)	1	(10.0)	51	(22.8)
2 - 2	1	(6.7)	1	(1.4)	4	(4.7)	0		0		6	(2.7)
Total	15		69		86		44		10		224	
11. Acid phosphatase												
A	0		10	(4.7)	15	(16.9)	6	(14.0)	2	(20.0)	33	(14.7)
BA	6	(40.0)	29	(42.6)	30	(33.7)	23	(53.5)	6	(60.0)	94	(41.8)
B	8	(53.3)	25	(36.8)	37	(41.6)	11	(25.6)	1	(10.0)	82	(36.4)
BC	0		2	(2.9)	6	(6.7)	1	(2.3)	1	(10.0)	10	(4.4)
CA	1	(6.7)	2	(2.9)	1	(1.1)	2	(4.7)	0		6	(2.7)
Total	15		68		89		43		10		225	
12. Adenylate kinase												
1 - 1	13	(86.7)	68	(98.6)	85	(95.5)	41	(93.2)	8	(80.0)	215	(94.7)
2 - 1	2	(13.3)	1	(1.4)	4	(4.5)	3	(6.8)	2	(20.0)	12	(5.3)
Total	15		69		89		44		10		227	

TABLE 6.35: Statistical data for Table 6.34.

	Method of subdivision*	X^2	d.f.	P	Spearman's rho	P
1. ABO						
	E	2.35	6	.885		
	F	2.40	6	.879		
2. Rhesus						
	E	8.55	8	.382		
	F	5.35	8	.719		
3. Rhesus(D)						
	E	4.30	4	.368	.0739	.293
	F	1.04	3	.791	.0379	.540
4. MN						
	E	7.51	6	.276	-.1099	.099
	F	3.23	6	.779	-.1009	.131
5. S						
	E	5.31	6	.505	.1119	.089
	F	2.73	6	.842	.0681	.302
6. MNS						
	E	25.93	14	.026		
	F	17.22	7	.016		
7. Duffy						
	E	1.72	6	.944	-.0411	.530
	F	9.02	6	.172	-.0690	.291
8. Kell						
	E	0.20	2	.905	.0289	.655
	F	0.62	3	.892	.0182	.779
9. Phosphoglucomutase						
	E	2.10	6	.911	-.0042	.950
	F	0.41	3	.939	.0195	.770
10. Esterase-D						
	E	1.99	3	.575	-.0250	.710
	F	0.72	3	.868	.0081	.904
11. Acid phosphatase						
	E	8.09	6	.232		
	F	5.07	9	.828		
12. Adenylate kinase						
	E	2.31	2	.315	.0776	.244
	F	4.60	2	.100	.0852	.201

* see Table 6.8

TABLE 6.36: A comparison of the mean maternal ages of homozygotes and heterozygotes of the blood groups and isoenzymes.

	mean maternal age : heterozygote	homozygote	t	P	Mann-Whitney U	z	P	Analysis for trends: z	P
MN	26.6639	26.0934	0.92	.361	5773.5	-1.211	.226	0.371	.356
S	26.2059	26.3923	-0.31	.759	6511.0	-0.235	.814	0.204	.232
MNS	26.7654	25.3091	2.05	.041	3542.5	-2.274	.024	1.740	.041
Rhesus	26.5584	25.8072	1.21	.229	5636.5	-1.502	.133	0.341	.367
Duffy	26.6396	26.0880	0.90	.368	6574.0	-0.696	.486	1.169	.121
Kell	25.8421	26.3513	-0.46	.648	1944.5	-0.566	.572	0.028	.488
PGM	25.8267	26.6291	-1.21	.229	5143.5	-1.124	.261	1.120	.131
ESD	26.2745	26.4162	-0.19	.852	4360.0	-0.127	.899	0.118	.452
AP	26.6641	25.9652	1.17	.243	6752.0	-1.114	.265	0.653	.258
AK	28.1667	26.2839	0.89	.392	985.0	-1.381	.167	1.074	.142

significant, but in neither case was the X^2 value indicative of any other than random distribution of phenotypes. The distribution of MNSs blood group phenotypes gave significant X^2 values. The mean maternal age of heterozygotes was higher than that of homozygotes; a difference which was found to be significant in both the t-test and the Mann Whitney U test. The analysis for trends also gave a significant result, and indicated an increase in heterozygosity with rising maternal age.

(ii) Chromosome variants.

Tables 6.37, 6.38 and 6.39 show the frequencies of the chromosome variants subdivided according to maternal age and the results of statistical analysis of these data. An effect of maternal age was found in the frequency of variants of the short arm of chromosome 13. The X^2 test gave an almost significant value (P=.080) when maternal age was divided into six-year age groups, but a non-significant value (P=.147) when five-year intervals were used. The mean maternal age of homozygotes was higher than that of heterozygotes; a difference which was found to be significant by both the t-test and the Mann Whitney U test.

The analysis for trends on these data also gave a very significant results (P=.007) and indicated an increase of homozygosity for chromosome 13p with increasing maternal age.

Table 6.40 shows the distribution of chromosome variant frequencies (intense and brilliant levels of fluorescence combined) subdivided according to maternal age. Tables 6.41 and 6.42 give the results of statistical analysis of these data. The only effect of maternal age detected by these means is of a higher mean maternal age of homozygotes for chromosome 13p compared with the heterozygotes. In this case the rank correlation coefficient could be calculated but it was not significant, despite the significant result of the analysis for trends.

TABLE 6.37: Chromosome variant phenotype frequencies subdivided according to mother's age.

Age(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. Chromosome 3												
BB	0		0		1	(1.1)	0		0		1	(0.5)
BI	0		5	(7.7)	7	(8.0)	2	(4.9)	2	(20.0)	16	(7.3)
II	1	(6.7)	9	(13.8)	9	(10.2)	6	(14.6)	0		25	(11.4)
IN	7	(46.7)	37	(56.9)	33	(37.5)	18	(43.9)	4	(40.0)	99	(45.2)
BN	5	(33.3)	4	(6.2)	15	(17.0)	7	(17.1)	2	(20.0)	33	(15.1)
NN	2	(13.3)	10	(15.4)	23	(26.1)	8	(19.5)	2	(20.0)	45	(20.5)
Total	15		65		88		41		10		219	
2. Chromosome 13p												
BB	0		0		1	(1.1)	1	(2.4)	0		2	(0.9)
BI	2	(13.3)	2	(3.1)	2	(2.3)	2	(4.9)	0		8	(3.7)
II	4	(26.7)	10	(15.4)	25	(28.4)	10	(24.4)	3	(30.0)	52	(23.7)
IN	8	(53.8)	47	(72.3)	45	(51.1)	21	(51.2)	5	(50.0)	126	(57.5)
BN	0		2	(3.1)	1	(1.1)	1	(2.4)	0		4	(1.8)
NN	1	(6.7)	4	(6.2)	14	(15.9)	6	(14.6)	2	(20.0)	27	(12.3)
Total	15		65		88		41		10		219	
3. Chromosome 13s												
IN	2	(13.3)	8	(12.3)	17	(19.3)	6	(14.6)	2	(20.0)	35	(16.0)
BN	0		2	(3.1)	2	(2.3)	1	(2.4)	1	(10.0)	6	(2.7)
NN	13	(86.7)	55	(84.6)	69	(78.4)	34	(82.9)	7	(70.0)	178	(81.3)
Total	15		65		88		41		10		219	
4. Chromosome 14s												
BI	0		1	(1.5)	0		1	(2.4)	0		2	(0.9)
II	0		1	(1.5)	0		0		1	(10.0)	2	(0.9)
IN	7	(46.7)	20	(30.8)	32	(36.4)	12	(29.3)	3	(30.0)	74	(33.8)
BN	0		2	(3.1)	1	(1.1)	3	(7.3)	1	(10.0)	7	(3.2)
NN	8	(53.3)	41	(63.1)	55	(62.5)	25	(61.0)	5	(50.0)	134	(61.2)
Total	15		65		88		41		10		219	
5. Chromosome 15s												
BI	0		1	(1.5)	0		0		0		1	(0.5)
II	0		0		2	(2.3)	1	(2.4)	0		3	(1.4)
IN	8	(53.3)	24	(36.9)	33	(37.5)	14	(34.1)	6	(60.0)	85	(38.8)
BN	0		0		1	(1.1)	3	(7.3)	0		4	(1.8)
NN	7	(46.7)	40	(61.5)	52	(59.1)	23	(56.1)	4	(40.0)	126	(57.5)
Total	15		65		88		41		10		219	
6. Chromosome 21s												
II	1	(6.7)	1	(1.5)	0		0		0		2	(0.9)
IN	2	(13.3)	11	(16.9)	23	(26.1)	12	(29.3)	2	(20.0)	50	(22.8)
BN	1	(6.7)	1	(1.5)	1	(1.1)	0		0		3	(1.4)
NN	11	(73.3)	52	(80.0)	64	(72.7)	29	(70.7)	8	(80.0)	164	(74.9)
Total	15		65		88		41		10		219	
7. Chromosome 22s												
BI	1	(6.7)	0		3	(3.4)	2	(4.9)	1	(10.0)	7	(3.2)
II	1	(6.7)	2	(3.1)	4	(4.5)	1	(2.4)	0		8	(3.7)
IN	8	(53.8)	23	(35.4)	34	(38.6)	16	(39.0)	3	(30.0)	84	(38.4)
BN	1	(6.7)	2	(3.1)	5	(5.7)	1	(2.4)	0		9	(4.1)
NN	4	(26.7)	38	(58.5)	42	(47.7)	21	(51.2)	6	(60.0)	111	(50.7)
Total	15		65		88		41		10		219	

TABLE 6.38: Statistical data for Table 6.37.

	Method of subdivision	X^2	d.f.	P
1. Chromosome 3				
	E	7.32	8	.503
	F	3.89	6	.692
2. Chromosome 13p				
	E	9.51	6	.147
	F	11.29	6	.080
3. Chromosome 13s				
	E	1.26	3	.738
	F	1.61	3	.657
4. Chromosome 14s				
	E	1.08	4	.896
	F	1.24	2	.538
5. Chromosome 15s				
	E	2.53	4	.639
	F	5.51	2	.064
6. Chromosome 21s	E	1.78	4	.776
	F	1.88	2	.391
7. Chromosome 22s				
	E	1.27	4	.866
	F	1.60	6	.953

TABLE 6.39: A comparison of the mean maternal ages of homozygotes and heterozygotes of the chromosome variants.

	mean maternal age: heterozygote	mean maternal age: homozygote	t	P	Mann - Whitney U	z	P	Analysis for trends: z	Analysis for trends: P
Chromosome 3	26.2838	26.6479	-0.58	.561	4871.0	-0.875	.382	0.263	.207
Chromosome 13p	25.7971	27.4321	-2.55	.012	4314.0	-2.824	.005	2.455	.007
Chromosome 13s	26.8049	26.3090	0.62	.539	3504.5	-0.396	.692	-0.089	.464
Chromosome 14s	26.5783	26.2941	0.44	.661	5391.5	-0.557	.578	0.681	.248
Chromosome 15s	26.3111	26.4651	-0.24	.810	5625.0	-0.391	.696	0.248	.401
Chromosome 21s	26.9245	26.2349	0.94	.348	3993.5	-1.012	.311	0.164	.436
Chromosome 22s	26.4800	26.3361	0.23	.820	5680.0	-0.580	.562	0.505	.305

TABLE 6.40: Chromosome variant phenotype frequencies (intense and brilliant levels of fluorescence combined) subdivided according to age of mother.

Age(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. Chromosome 3												
II	1	(6.7)	14	(21.5)	17	(19.3)	8	(19.5)	2	(20.0)	42	(19.2)
IN	12	(80.0)	41	(63.1)	48	(54.5)	25	(61.0)	6	(60.0)	132	(60.3)
NN	2	(13.3)	10	(15.4)	23	(26.1)	8	(19.5)	2	(20.0)	45	(20.5)
Total	15		65		88		41		10		219	
2. Chromosome 4												
IN	1	(6.7)	4	(6.2)	6	(6.8)	3	(7.3)	0		14	(6.4)
NN	14	(93.3)	61	(93.8)	82	(93.2)	38	(92.7)	10	(100.0)	205	(93.6)
Total	15		65		88		41		10		219	
3. Chromosome 13p												
II	6	(40.0)	12	(18.5)	28	(31.8)	13	(31.7)	3	(30.0)	62	(28.3)
IN	8	(53.3)	49	(75.4)	46	(52.3)	22	(53.7)	5	(50.0)	130	(59.4)
NN	1	(6.7)	4	(6.2)	14	(15.9)	6	(14.6)	2	(20.0)	27	(12.3)
Total	15		65		88		41		10		219	
4. Chromosome 13s												
IN	2	(13.3)	10	(15.4)	19	(21.6)	7	(17.1)	3	(30.0)	41	(18.7)
NN	13	(86.7)	55	(84.6)	69	(78.4)	34	(82.9)	7	(70.0)	178	(81.3)
Total	15		65		88		41		10		219	
5. Chromosome 14p												
IN	1	(6.7)	0		3	(3.4)	1	(2.4)	0		5	(2.3)
NN	14	(93.3)	65	(100.0)	85	(96.6)	40	(97.6)	10	(100.0)	214	(97.7)
Total	15		65		88		41		10		219	
6. Chromosome 14s												
II	0		2	(3.1)	0		1	(2.4)	1	(10.0)	4	(1.8)
IN	7	(46.7)	22	(33.8)	33	(37.5)	15	(36.6)	4	(40.0)	81	(37.0)
NN	8	(53.3)	41	(63.1)	55	(62.5)	25	(61.0)	5	(50.0)	134	(61.2)
Total	15		65		88		41		10		219	
7. Chromosome 15p												
IN	0		0		2	(2.3)	1	(2.4)	0		3	(1.4)
NN	15	(100.0)	65	(100.0)	86	(97.7)	40	(97.6)	10	(100.0)	216	(98.6)
Total	15		65		88		41		10		219	
8. Chromosome 15s												
II	0		1	(1.5)	2	(2.3)	1	(2.4)	0		4	(1.8)
IN	8	(53.3)	24	(36.9)	34	(38.6)	17	(41.5)	6	(60.0)	89	(40.6)
NN	7	(46.7)	40	(61.5)	52	(59.1)	23	(56.1)	4	(40.0)	126	(57.5)
Total	15		65		88		41		10		219	
9. Chromosome 21p												
IN	0		3	(4.6)	1	(1.1)	0		1	(10.0)	5	(2.3)
NN	15	(100.0)	62	(95.4)	87	(98.9)	41	(100.0)	9	(90.0)	214	(97.7)
Total	15		65		88		41		10		219	

TABLE 6.40 contd.:

Age(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
10. Chromosome 21s												
II	1	(6.7)	1	(1.5)	0		0		0		2	(0.9)
IN	3	(20.0)	12	(18.5)	24	(27.3)	12	(29.3)	2	(20.0)	53	(24.2)
NN	11	(73.3)	52	(80.0)	64	(72.7)	29	(70.7)	8	(80.0)	164	(74.9)
Total	15		65		88		41		10		219	
11. Chromosome 22p												
II	0		0		1	(1.1)	0		0		1	(0.5)
IN	3	(20.0)	15	(23.1)	14	(15.9)	8	(19.5)	1	(10.0)	41	(18.7)
NN	12	(80.0)	50	(76.9)	73	(83.0)	33	(80.5)	9	(90.0)	177	(80.8)
Total	15		65		88		41		10		219	
12. Chromosome 22s												
II	2	(13.3)	2	(3.1)	7	(8.0)	3	(7.3)	1	(10.0)	15	(6.8)
IN	9	(60.0)	25	(38.5)	39	(44.3)	17	(41.5)	3	(30.0)	93	(42.5)
NN	4	(26.7)	38	(58.5)	42	(47.7)	21	(51.2)	6	(60.0)	111	(50.7)
Total	15		65		88		41		10		219	

TABLE 6.41: Statistical data for Table 6.40

Method of subdivision	X^2	d.f.	P	Spearman's rho	P
1. Chromosome 3					
E	3.27	4	.514	-.0286	.674
F	5.21	4	.266	-.1017	.134
2. Chromosome 4					
E	0.05	2	.975	-.0081	.905
F	0.40	2	.819	.0100	.883
3. Chromosome 13p					
E	8.29	4	.082	-.0063	.926
F	9.70	4	.046	.0207	.761
4. Chromosome 13s					
E	1.26	3	.738	.0620	.361
F	1.61	3	.657	-.0137	.841
5. Chromosome 14p					
E	0.09	1	.759	.0104	.878
F	0.01	1	.943	.0010	.988
6. Chromosome 14s					
E	1.08	4	.898	.0132	.846
F	1.19	3	.755	.0709	.296
7. Chromosome 15p					
E	0.07	1	.785	.0707	.298
F	1.49	1	.223	.0954	.164
8. Chromosome 15s					
E	2.53	4	.639	.0338	.619
F	2.79	3	.425	-.0025	.971
9. Chromosome 21p					
E	0.40	1	.527	-.0341	.616
F	0.01	1	.943	.0115	.866
10. Chromosome 21s					
E	1.78	4	.776	.0433	.524
F	0.23	3	.973	-.0100	.884
11. Chromosome 22p					
E	0.98	3	.806	-.0537	.429
F	1.06	3	.786	-.0455	.503
12. Chromosome 22s					
E	1.08	4	.897	-.0097	.887
F	2.23	6	.898	.0325	.632

TABLE 6.42: A comparison of the mean maternal ages of homozygotes and heterozygotes of chromosome variants (intense and brilliant levels of fluorescence combined).

	mean maternal age: homozygote	mean maternal age: heterozygote	t	P	Mann - Whitney U	z	P	Analysis for trends: z	Analysis for trends: P
Chromosome 3	26.7011	26.2045	0.77	.440	5357.0	-0.841	.400	0.348	.363
Chromosome 4	26.4049	26.3571	0.04	.970	1419.0	-0.070	.944	-0.007	.496
Chromosome 13p	27.1798	25.8692	2.07	.040	4642.0	-2.488	.013	1.935	.026
Chromosome 13s	26.3090	26.8049	-0.62	.539	3504.5	-0.396	.692	-0.089	.464
Chromosome 14p	26.3878	27.0000	-0.29	.772	458.5	-0.548	.584	-0.099	.460
Chromosome 14s	26.3116	26.5555	-0.37	.708	5373.0	-0.478	.632	0.673	.251
Chromosome 15p	26.3565	29.6667	-1.23	.221	164.0	-1.472	.141	-.816	.206
Chromosome 15s	26.4308	26.3595	0.11	.912	5677.0	-0.235	.814	0.167	.429
Chromosome 21p	26.3972	26.6000	-0.10	.923	496.0	-0.279	.780	0.099	.460
Chromosome 21s	26.2349	26.9245	-0.94	.348	3993.5	-1.012	.311	0.164	.436
Chromosome 22p	26.5056	25.9512	0.69	.492	3408.5	-0.659	.510	0.352	.363
Chromosome 22s	26.4682	26.3118	0.25	.806	5826.0	-0.071	.943	0.032	.488

Table 6.43 shows the distributions of grouped chromosome variants subdivided according to maternal age. Table 6.44 shows the results of statistical analysis of these data. Brilliant satellites of group D chromosomes apparently occur more frequently with increasing maternal age. No other consistent trends were observed. The total intense satellites gave a significant (P=.018) χ^2 value when subdivided into five-year intervals, but the rank correlation coefficient was non-significant. No importance is, therefore, attached to this result.

B. Paternal age.

(i) Blood groups and isoenzymes.

Tables 6.45, 6.46 and 6.47 give the distributions of the phenotype frequences of these genetic markers subdivided according to paternal age, and the results of statistical analyses of these data.

A significant difference was found in phenotype frequencies of the MNSs system, when the method of subdivision was by six-year intervals. The paternal age of the heterozygotes was higher than that of the homozygotes. This difference was demonstrated to be significant by the Mann Whitney U test and nearly significant by the t-test. There was no significant trend for increased heterozygosity with paternal age. These findings are interesting because they complement the trends observed with regard to heterozygosity of the MNSs system and maternal age. As the correlation seems to be closer and more definite with the latter variable (maternal age) it seems probable that the rather weaker association with paternal age is a result of the close correlation between paternal and maternal ages. Inspection of the data for the MN system in table 6.45 shows that, although the result of the analysis for trends was not significant, the MN phenotype does increase in frequency with paternal age.

TABLE 6.43: Frequencies of grouped chromosome variants subdivided according to age of mother.

Age(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. D group satellites (intense)												
0	4	(26.7)	22	(33.8)	23	(26.1)	15	(36.6)	3	(30.0)	67	(30.6)
1	7	(46.7)	32	(49.2)	46	(52.3)	17	(41.5)	1	(10.0)	103	(47.0)
2	2	(13.3)	9	(13.8)	17	(19.3)	9	(22.0)	6	(60.0)	43	(19.6)
⩾3	2	(13.3)	2	(3.1)	2	(2.3)	0		0		6	(2.7)
Total	15		65		88		41		10		219	
2. D group satellites (brilliant)												
0	15	(100.0)	59	(90.8)	84	(95.5)	34	(82.9)	8	(80.0)	200	(91.3)
⩾1	0		6	(9.2)	4	(4.5)	7	(17.1)	2	(20.0)	19	(8.7)
Total	15		65		88		41		10		219	
3. D group satellites (all)												
0	4	(26.7)	20	(30.8)	22	(25.0)	12	(29.3)	3	(30.0)	61	(27.9)
1	7	(46.7)	31	(47.7)	44	(50.0)	17	(41.5)	1	(10.0)	100	(45.7)
2	2	(13.3)	11	(16.9)	20	(22.7)	10	(24.4)	4	(40.0)	47	(21.5)
⩾3	2	(13.3)	3	(4.6)	2	(2.3)	2	(4.9)	2	(20.0)	11	(5.0)
Total	15		65		88		41		10		219	
4. G group satellites (intense)												
0	5	(33.3)	31	(47.7)	34	(38.6)	15	(16.6)	4	(40.0)	89	(40.6)
1	6	(40.0)	30	(46.2)	41	(46.6)	20	(48.8)	6	(60.0)	103	(47.0)
2	3	(20.0)	2	(3.1)	12	(13.6)	6	(14.6)	0		23	(10.5)
⩾3	1	(6.7)	2	(3.1)	1	(1.1)	0		0		4	(1.8)
Total	15		65		88		41		10		219	
5. G group satellites (brilliant)												
0	13	(86.7)	62	(95.4)	79	(89.8)	38	(92.7)	9	(90.0)	201	(91.8)
⩾2	2	(13.3)	3	(4.6)	9	(10.2)	3	(7.3)	1	(10.0)	18	(8.2)
Total	15		65		88		41		10		219	
6. G group satellites (all)												
0	4	(26.7)	28	(43.1)	29	(33.0)	14	(34.1)	4	(40.0)	79	(36.1)
1	5	(33.3)	33	(50.8)	43	(48.9)	19	(46.3)	5	(50.0)	105	(47.9)
2	5	(33.3)	2	(3.1)	14	(15.9)	8	(19.5)	1	(10.0)	30	(13.7)
⩾3	1	(6.7)	2	(3.1)	2	(2.3)	0		0		5	(2.3)
Total	15		65		88		41		10		219	
7. Total intense satellites												
0	2	(13.3)	16	(24.6)	8	(9.1)	4	(9.8)	1	(10.0)	31	(14.2)
1	2	(13.3)	17	(26.2)	28	(31.8)	20	(48.8)	3	(30.0)	70	(32.0)
2	6	(40.0)	21	(32.3)	32	(36.4)	7	(17.1)	2	(20.0)	68	(31.1)
3	2	(13.3)	7	(10.8)	18	(20.5)	7	(17.1)	4	(40.0)	38	(17.4)
⩾4	3	(20.0)	4	(6.2)	2	(2.3)	3	(7.3)	0		12	(5.5)
Total	15		65		88		41		10		219	
8. Total brilliant satellites												
0	13	(86.7)	57	(87.7)	77	(87.5)	31	(75.6)	7	(70.0)	185	(84.5)
⩾1	2	(13.3)	8	(12.3)	11	(12.5)	10	(24.4)	3	(30.0)	34	(15.5)
Total	15		65		88		41		10		219	

TABLE 6.43 contd.:

Age(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
9. Total variant satellites												
0	1	(6.7)	13	(20.0)	7	(8.0)	3	(7.3)	1	(10.0)	25	(11.4)
1	2	(13.3)	18	(27.7)	21	(23.9)	15	(36.6)	2	(20.0)	58	(26.5)
2	7	(46.7)	21	(32.3)	39	(44.3)	12	(29.3)	3	(30.0)	82	(37.4)
3	2	(13.3)	7	(10.8)	17	(19.3)	7	(17.1)	2	(20.0)	35	(16.0)
⩾4	3	(20.0)	6	(9.2)	4	(4.5)	4	(9.8)	2	(20.0)	19	(8.7)
Total	15		65		88		41		10		219	
10. Total intense bands												
⩽2	1	(6.7)	12	(18.5)	14	(15.9)	6	(14.6)	1	(10.0)	34	(15.5)
3	3	(20.0)	13	(20.0)	25	(28.4)	14	(34.1)	3	(30.0)	58	(26.5)
4	4	(26.7)	22	(33.8)	25	(28.4)	9	(22.0)	3	(30.0)	63	(28.8)
5	5	(33.3)	12	(18.5)	12	(13.6)	6	(14.6)	3	(30.0)	38	(17.4)
6	2	(13.3)	3	(4.6)	8	(9.1)	5	(12.2)	0		18	(8.2)
⩾7	0		3	(4.6)	4	(4.5)	1	(2.4)	0		8	(3.7)
Total	15		65		88		41		10		219	
11. Total brilliant bands												
0	8	(53.3)	46	(70.8)	58	(65.9)	25	(61.0)	4	(40.0)	141	(64.4)
1	4	(26.7)	16	(24.6)	22	(25.0)	8	(19.5)	5	(50.0)	55	(25.1)
⩾2	3	(20.0)	3	(4.6)	8	(9.1)	8	(19.5)	1	(10.0)	23	(10.5)
Total	15		65		88		41		10		219	
12. Total variant bands												
⩽2	0		7	(10.8)	3	(3.4)	3	(7.3)	1	(10.0)	14	(6.4)
3	2	(13.3)	15	(23.1)	22	(25.0)	10	(24.4)	2	(20.0)	51	(23.3)
4	3	(20.0)	19	(29.2)	28	(31.8)	9	(22.0)	1	(10.0)	60	(27.4)
5	5	(33.3)	14	(21.5)	21	(23.9)	10	(24.4)	3	(30.0)	53	(24.2)
6	4	(26.7)	6	(9.2)	9	(10.2)	7	(17.1)	3	(30.0)	29	(13.2)
⩾7	1	(6.7)	4	(6.2)	5	(5.7)	2	(4.9)	0		12	(5.5)
Total	15		65		88		41		10		219	

TABLE 6.44: Statistical data for Table 6.43

Method of subdivision	X^2	d.f.	P	Spearman's rho	P
1. D group satellites (intense)					
E	12.46	8	.132	.0335	.622
F	8.61	6	.197	.0228	.737
2. D group satellites (brilliant)					
E	7.22	2	.027	.1223	.071
F	1.82	2	.404	.1062	.117
3. D group satellites (all)					
E	4.30	6	.596	.0657	.333
F	6.32	6	.388	.0495	.467
4. G group satellites (intense)					
E	6.52	6	.368	.0394	.562
F	2.89	6	.823	.0294	.665
5. G group satellites (brilliant)					
E	0.89	2	.640	.0203	.765
F	1.57	2	.456	.0602	.376
6. G group satellites (all)					
E	12.55	8	.128	.0292	.668
F	5.66	6	.462	.0382	.574
7. Total intense satellites					
E	15.35	6	.018	.0199	.769
F	7.94	9	.541	.0036	.958
8. Total brilliant satellites					
E	5.04	3	.169	.1135	.094
F	4.33	2	.115	.1312	.053
9. Total variant satellites					
E	12.13	8	.146	.0498	.463
F	11.83	9	.223	.0468	.490
10. Total intense bands					
E	5.93	10	.821	-.0531	.434
F	11.55	12	.482	-.0433	.524
11. Total brilliant bands					
E	7.28	6	.296	.0814	.230
F	7.18	6	.304	.0561	.409
12. Total variant bands					
E	6.66	10	.757	.0045	.948
F	5.02	9	.832	.0002	.998

TABLE 6.45: Blood group and isoenzyme phenotype frequencies subdivided according to age of father.

Age(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. ABO												
A	5	(71.4)	10	(25.6)	40	(48.2)	28	(43.1)	10	(45.5)	93	(43.1)
O	2	(28.6)	23	(59.0)	35	(42.2)	30	(46.2)	9	(40.9)	99	(45.8)
B	0		5	(12.8)	6	(7.2)	6	(9.2)	3	(13.6)	20	(9.3)
AB	0		1	(2.6)	2	(2.4)	1	(1.5)	0		4	(1.9)
Total	7		39		83		65		22		216	
2. Rhesus												
r r	2	(28.6)	7	(18.4)	16	(19.5)	12	(18.8)	3	(14.3)	40	(18.9)
R_1r	0		10	(26.3)	31	(37.8)	27	(42.2)	8	(38.1)	76	(35.8)
R_1R_1	1	(14.3)	8	(21.1)	13	(15.9)	7	(10.9)	4	(18.2)	33	(15.6)
R_2r	0		0		3	(3.7)	6	(9.4)	2	(9.1)	11	(5.2)
R_2R_2	0		0		2	(2.4)	1	(1.6)	0		3	(1.4)
R_1R_2	2	(28.6)	12	(31.6)	16	(19.5)	10	(15.6)	4	(18.2)	44	(20.8)
r"r'	0		1	(2.6)	0		0		0		1	(0.5)
r"r	1	(14.3)	0		1	(1.2)	1	(1.6)	0		3	(1.4)
R_zR_2	1	(14.3)	0		0		0		0		1	(0.5)
Total	7		38		82		64		21		212	
3. Rhesus(D)												
D+ve	4	(57.1)	31	(79.5)	66	(79.5)	52	(80.0)	19	(86.4)	172	(79.6)
D-ve	3	(42.9)	8	(20.5)	17	(20.5)	13	(20.0)	3	(13.6)	44	(20.4)
Total	7		39		83		65		22		216	
4. MN												
M	3	(50.0)	10	(25.6)	27	(34.6)	18	(28.6)	5	(23.8)	63	(30.4)
MN	3	(50.0)	18	(46.2)	38	(48.7)	38	(60.3)	14	(66.7)	111	(53.6)
N	0		11	(28.2)	13	(16.7)	7	(11.1)	2	(9.5)	33	(15.9)
Total	6		39		78		63		21		207	
5. S												
S	0		4	(10.8)	13	(16.3)	8	(12.5)	3	(14.3)	28	(13.4)
Ss	4	(57.1)	19	(51.4)	36	(45.0)	29	(45.3)	8	(38.1)	96	(45.9)
s	3	(42.9)	14	(37.8)	31	(38.8)	27	(42.2)	10	(47.6)	85	(40.7)
Total	7		37		80		64		21		209	
6. MNS												
MS	0		3	(8.1)	5	(6.7)	4	(6.5)	1	(5.0)	13	(6.5)
MSs	1	(16.7)	3	(8.1)	15	(20.0)	12	(19.4)	1	(5.0)	32	(16.0)
Ms	2	(33.3)	2	(5.4)	5	(6.7)	2	(3.2)	2	(10.0)	13	(16.5)
MNS	0		1	(2.7)	7	(9.3)	3	(4.8)	2	(10.0)	13	(16.5)
MNSs	2	(33.3)	11	(29.7)	16	(21.3)	14	(22.6)	5	(25.0)	48	(24.0)
MNs	1	(16.7)	6	(16.2)	14	(18.7)	20	(32.3)	7	(35.0)	48	(24.0)
NS	0		0		1	(1.3)	1	(1.6)	0		2	(1.0)
NSs	0		5	(13.5)	2	(2.7)	3	(4.8)	2	(10.0)	12	(6.0)
Ns	0		6	(16.2)	10	(13.3)	3	(4.8)	0		19	(9.5)
Total	6		37		75		62		19		200	

TABLE 6.45 contd.:

Age(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
7. Duffy												
Fy^a	0		7	(18.9)	11	(13.4)	13	(20.0)	2	(9.5)	33	(15.6)
Fy^aFy^b	5	(71.4)	16	(43.2)	35	(42.7)	30	(46.2)	13	(61.9)	99	(46.7)
Fy^b	2	(28.6)	14	(37.8)	36	(43.9)	22	(33.8)	6	(28.6)	80	(37.7)
Total	7		37		82		65		21		212	
8. Kell												
Nk	0		6	(15.4)	3	(3.6)	4	(6.2)	2	(9.1)	15	(6.9)
k	7	(100.0)	33	(84.6)	80	(96.4)	61	(93.8)	20	(90.9)	201	(93.1)
Total	7		39		83		65		22		216	
9. Phosphoglucomutase												
1 - 1	5	(71.4)	22	(57.9)	46	(59.7)	36	(57.1)	13	(65.0)	122	(59.5)
2 - 1	1	(14.3)	13	(34.2)	26	(33.8)	20	(31.7)	6	(30.0)	66	(32.2)
2 - 2	1	(14.3)	3	(7.9)	5	(6.5)	7	(11.1)	1	(5.0)	17	(8.3)
Total	7		38		77		63		20		205	
10. Esterase-D												
1 - 1	5	(71.4)	25	(65.8)	58	(78.4)	49	(76.6)	15	(75.0)	152	(74.9)
2 - 1	1	(14.3)	13	(34.2)	14	(18.9)	14	(21.9)	5	(25.0)	47	(25.0)
2 - 2	1	(14.3)	0		2	(2.7)	1	(1.6)	0		4	(2.0)
Total	7		38		74		64		20		203	
11. Acid phosphatase												
A	0		5	(13.5)	9	(11.7)	9	(14.3)	7	(35.0)	30	(14.7)
BA	3	(42.9)	14	(37.8)	30	(39.0)	34	(54.0)	8	(40.0)	89	(43.6)
B	3	(42.9)	17	(45.9)	31	(40.3)	17	(27.0)	4	(20.0)	72	(35.3)
BC	0		1	(2.7)	4	(5.2)	2	(3.2)	1	(5.0)	8	(3.9)
CA	1	(14.3)	0		3	(3.9)	1	(1.6)	0		5	(2.5)
Total	7		37		77		63		20		204	
12. Adenylate kinase												
1 - 1	5	(71.4)	38	(100.0)	75	(97.4)	60	(93.8)	16	(80.0)	194	(94.2)
2 - 1	2	(28.6)	0		2	(2.6)	4	(6.3)	4	(20.0)	12	(5.8)
Total	7		38		77		64		20		206	

TABLE 6.46: Statistical data for Table 6.45.

	Method of subdivision	X^2	d.f.	P	Spearman's rho	P
1. ABO						
	E	3.24	6	.779		
	F	4.49	6	.610		
2. Rhesus						
	E	9.80	8	.279		
	F	5.21	8	.735		
3. Rhesus(D)						
	E	0.98	3	.808	.0633	.354
	F	0.39	3	.942	.0286	.676
4. MN						
	E	6.34	6	.386	-.0336	.631
	F	4.28	4	.370	-.0991	.155
5. S						
	E	2.24	6	.897	.0287	.680
	F	1.60	6	.953	.0523	.452
6. MNS						
	E	20.53	14	.114		
	F	13.54	7	.060		
7. Duffy						
	E	4.47	6	.613	-.0540	.434
	F	2.35	6	.885	-.0309	.655
8. Kell						
	E	4.07	2	.131	.0437	.523
	F	1.05	2	.592	.0052	.940
9. Phosphoglucomutase						
	E	1.46	6	.962	.0039	.955
	F	1.53	4	.822	.0150	.831
10. Esterase-D						
	E	2.19	3	.534	-.0618	.381
	F	2.27	2	.322	-.0910	.197
11. Acid phosphatase						
	E	13.34	6	.038		
	F	13.61	6	.034		
12. Adenylate kinase						
	E	3.71	2	.156	.1235	.077
	F	3.16	2	.206	.0968	.166

TABLE 6.47: A comparison of the mean paternal ages of homozygotes and heterozygotes of the blood groups and isoenzymes.

	mean paternal age: heterozygote	mean paternal age: homozygote	t	P	Mann - Whitney U	z	P	Analysis for trends: z	Analysis for trends: P
MN	28.9099	27.9271	1.38	.170	4695.0	-1.476	.140	0.639	.261
S	28.0938	28.7345	-0.92	.360	5133.0	-0.669	.503	0.575	.281
MNS	28.7908	27.2553	1.83	.068	2869.0	-2.098	.036	1.219	.111
Rhesus	28.6250	28.1053	0.72	.475	4627.0	-1.266	.205	0.947	.171
Duffy	28.5050	28.4867	0.03	.979	5527.5	-0.148	.882	0.344	.367
Kell	27.7333	28.5174	-0.57	.568	1407.0	-0.431	.666	0.055	.476
PGM	28.3030	28.4964	-0.25	.802	4528.0	-0.149	.882	-0.051	.480
ESD	28.0851	28.5641	-0.56	.579	3403.0	-0.747	.455	0.908	.181
AP	28.7304	28.1765	0.80	.426	5330.0	-1.162	.245	0.908	.159
AK	31.1667	28.2732	1.12	.285	863.0	-1.506	.132	0.875	.195

The X^2 value for comparisons of acid phosphatase phenotype frequencies in different paternal age-groups was found to be significant. The frequency of the B phenotype appeared to decline with paternal age, and the frequency of the BA to rise; at least within the age groups which were numerically fairly large. These findings were not reflected in any difference between the mean paternal ages of the homozygotes and heterozygotes of this system.

(ii) Chromosome variants.

Tables 6.48, 6.49 and 6.50 give the distributions of chromosome variant frequencies subdivided according to paternal age, and the results of statistical analysis of these data. The results were very similar to those obtained in the analysis for effects of maternal age on chromosome variant frequencies. That is, the homozygosity of the variants of the short arm of chromosome 13 appears to be associated with increasing paternal age. As in the case of the MNSs blood group system it is impossible to be certain whether this association is only with maternal or paternal age, or both. The greater significance of the difference between homozygotes and heterozygotes in the case of maternal age may mean that the association with this factor is closer, but may simply be a result of sampling effects.

Tables 6.51, 6.52 and 6.53 give the distribution of chromosome variant frequencies (intense and brilliant levels of fluorescence combined) subdivided according to paternal age, and the results of statistical analysis of these date.

The only effect of paternal age detected is again the increase of homozygosity of the short arm of chromosome 13 with paternal age.

Tables 6.54 and 6.55 give the distributions of the grouped chromosome variants subdivided according to paternal age, and results of analysis by means of the X^2 test.

TABLE 6.48: Chromosome variant phenotype frequencies subdivided according to age of father.

Age(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. Chromosome 3												
BB	0		0		0		1	(1.6)	0		1	(0.5)
BI	0		3	(9.4)	5	(6.4)	5	(8.1)	2	(9.1)	15	(7.5)
II	0		7	(21.9)	7	(9.0)	9	(14.5)	2	(9.1)	25	(12.5)
IN	3	(50.0)	14	(43.8)	42	(53.8)	17	(27.4)	11	(50.0)	87	(43.5)
BN	1	(6.7)	2	(6.3)	10	(12.8)	13	(21.0)	3	(13.6)	29	(14.5)
NN	2	(33.3)	6	(18.8)	14	(17.9)	17	(27.4)	4	(18.2)	43	(21.5)
Total	6		32		78		62.		22		200	
2. Chromosome 13p												
BB	0		0		0		1	(1.6)	0		1	(0.5)
BI	0		2	(6.3)	1	(1.3)	3	(4.8)	1	(4.5)	7	(3.5)
II	3	(50.0)	5	(15.6)	18	(23.1)	21	(33.9)	3	(13.6)	50	(25.0)
IN	2	(33.3)	23	(71.9)	48	(61.5)	24	(38.7)	14	(63.6)	111	(55.5)
BN	0		1	(3.1)	2	(2.6)	1	(1.6)	0		4	(2.0)
NN	1	(16.7)	1	(3.1)	9	(11.5)	12	(19.4)	4	(18.2)	27	(13.5)
Total	6		32		78		62		22		200	
3. Chromosome 13s												
IN	1	(16.7)	7	(21.9)	13	(16.7)	9	(14.5)	3	(13.6)	33	(16.5)
BN	0		1	(3.1)	0		3	(4.8)	1	(4.5)	5	(2.5)
NN	5	(83.3)	24	(75.0)	65	(83.3)	50	(80.6)	18	(81.8)	162	(81.0)
Total	6		32		78		62		22		200	
4. Chromosome 14s												
BI	0		0		1	(1.3)	1	(1.6)	0		2	(1.0)
II	0		0		1	(1.3)	0		1	(4.5)	2	(1.0)
IN	2	(33.3)	13	(40.6)	29	(37.2)	16	(25.8)	8	(36.4)	68	(34.0)
BN	1	(16.7)	0		1	(1.3)	4	(6.5)	0		6	(3.0)
NN	3	(50.0)	19	(59.4)	46	(59.0)	41	(66.1)	13	(59.1)	122	(61.0)
Total	6		32		78		62		22		200	
5. Chromosome 15s												
BI	0		1	(3.1)	0		0		0		1	(0.5)
II	0		0		0		2	(3.2)	0		2	(1.0)
IN	5	(83.3)	12	(37.5)	30	(38.5)	19	(30.6)	11	(50.0)	77	(38.5)
BN	0		0		0		3	(4.8)	0		3	(1.5)
NN	1	(16.7)	19	(59.4)	48	(61.5)	38	(61.3)	11	(50.0)	117	(58.5)
Total	6		32		78		62		22		200	
6. Chromosome 21s												
II	1	(16.7)	1	(3.1)	0		0		0		2	(1.0)
IN	1	(16.7)	4	(12.5)	19	(24.4)	20	(32.3)	4	(18.2)	48	(24.0)
BN	0		1	(3.1)	0		1	(1.6)	0		2	(1.0)
NN	4	(66.7)	26	(81.3)	59	(75.6)	41	(66.1)	18	(81.8)	148	(74.0)
Total	6		32		78		62		22		200	
7. Chromosome 22s												
BI	0		1	(3.1)	3	(3.8)	2	(3.2)	1	(4.5)	7	(3.5)
II	1	(16.7)	1	(3.1)	3	(3.8)	3	(4.8)	0		8	(4.0)
IN	3	(50.0)	12	(37.5)	31	(39.7)	24	(38.7)	9	(40.9)	79	(39.5)
BN	0		0		2	(2.6)	4	(6.5)	2	(9.1)	8	(4.0)
NN	2	(33.3)	18	(56.3)	39	(50.0)	29	(46.8)	10	(45.5)	98	(49.0)
Total	6		32		78		62		22		200	

TABLE 6.49: Statistical data for Table 6.48.

	Method of subdivision	X^2	d.f.	P
1. Chromosome 3				
	E	9.72	8	.285
	F	7.68	10	.660
2. Chromosome 13p				
	E	12.74	6	.047
	F	7.94	4	.094
3. Chromosome 13s				
	E	0.83	3	.842
	F	1.23	3	.745
4. Chromosome 14s				
	E	1.01	3	.799
	F	0.31	3	.957
5. Chromosome 15s				
	E	1.69	3	.639
	F	5.19	3	.158
6. Chromosome 21s				
	E	3.29	4	.511
	F	0.37	3	.946
7. Chromosome 22s				
	E	1.37	6	.968
	F	2.31	6	.889

TABLE 6.50: A comparison of the mean paternal ages of homozygotes and heterozygotes of the chromosome variants.

	mean paternal age: heterozygote	mean paternal age: homozygote	t	P	Mann - Whitney U	z	P	Analysis for trends: z	Analysis for trends: P
Chromosome 3	28.8320	28.4783	0.46	.643	4485.0	-0.089	.929	0.577	.281
Chromosome 13p	28.1803	29.5385	-1.84	.067	3777.0	-2.463	.014	1.741	.041
Chromosome 13s	28.5263	28.7531	-0.25	.806	2937.5	-0.439	.661	0.377	.352
Chromosome 14s	28.3947	28.9032	-0.68	.497	4451.0	-0.659	.510	0.517	.302
Chromosome 15s	28.5555	28.8151	-0.35	.726	4672.5	-0.367	.714	0.167	.433
Chromosome 21s	29.2000	28.5467	0.78	.436	3445.5	-0.861	.389	0.103	.460
Chromosome 22s	29.1383	28.3302	1.12	.266	4477.0	-1.239	.215	1.145	.125

TABLE 6.51: Chromosome variant phenotype frequencies (intense and brilliant levels of fluorescence combined) subdivided according to age of father.

Age(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. Chromosome 3												
II	0		10	(31.3)	12	(15.4)	15	(24.2)	4	(18.2)	41	(20.5)
IN	4	(66.7)	16	(50.0)	52	(66.7)	30	(48.4)	14	(63.6)	116	(58.0)
NN	2	(33.3)	6	(18.8)	14	(17.9)	17	(27.4)	4	(18.2)	43	(21.5)
Total	6		32		78		62		22		200	
2. Chromosome 4												
IN	0		3	(9.4)	6	(7.7)	3	(4.8)	1	(4.5)	13	(6.5)
NN	6	(100.0)	29	(90.6)	72	(92.3)	59	(95.2)	21	(95.5)	187	(93.5)
Total	6		32		78		62		22		200	
3. Chromosome 13p												
II	3	(50.0)	7	(21.9)	19	(24.4)	25	(40.3)	4	(18.2)	58	(29.0)
IN	2	(33.3)	24	(75.0)	50	(64.1)	25	(40.3)	14	(63.6)	115	(57.5)
NN	1	(16.7)	1	(3.1)	9	(11.5)	12	(19.4)	4	(18.2)	27	(13.5)
Total	6		32		78		62		22		200	
4. Chromosome 13s												
IN	1	(16.7)	8	(25.0)	13	(16.7)	12	(19.4)	4	(18.2)	38	(19.0)
NN	5	(83.3)	24	(75.0)	65	(83.3)	50	(80.6)	18	(81.8)	162	(81.0)
Total	6		32		78		62		22		200	
5. Chromosome 14p												
IN	0		0		1	(1.3)	1	(1.6)	0		2	(1.0)
NN	6	(100.0)	32	(100.0)	77	(98.7)	61	(98.4)	22	(100.0)	198	(99.0)
Total	6		32		78		62		22		200	
6. Chromosome 14s												
II	0		0		2	(2.6)	1	(1.6)	1	(4.5)	4	(2.0)
IN	3	(50.0)	13	(40.6)	30	(38.5)	20	(32.3)	8	(36.4)	74	(37.0)
NN	3	(50.0)	19	(59.4)	46	(59.0)	41	(66.1)	13	(59.1)	122	(61.0)
Total	6		32		78		62		22		200	
7. Chromosome 15p												
IN	0		0		1	(1.3)	2	(3.2)	0		3	(1.5)
NN	6	(100.0)	32	(100.0)	77	(98.7)	60	(96.8)	22	(100.0)	197	(98.5)
Total	6		32		78		62		22		200	
8. Chromosome 15s												
II	0		1	(3.1)	0		2	(3.2)	0		3	(1.5)
IN	5	(83.3)	12	(37.5)	30	(38.5)	22	(35.5)	11	(50.0)	80	(40.0)
NN	1	(16.7)	19	(59.4)	48	(61.5)	38	(61.3)	11	(50.0)	117	(58.5)
Total	6		32		78		62		22		200	
9. Chromosome 21p												
IN	0		1	(3.1)	3	(3.8)	0		1	(4.5)	5	(2.5)
NN	6	(100.0)	31	(96.9)	75	(96.2)	62	(100.0)	21	(95.5)	195	(97.5)
Total	6		32		78		62		22		200	

TABLE 6.51 contd.:

Age(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
10. Chromosome 21s												
II	1	(16.7)	1	(3.1)	0		0		0		2	(1.0)
IN	1	(16.7)	5	(15.6)	19	(24.4)	21	(33.9)	4	(18.2)	50	(25.0)
NN	4	(66.7)	26	(81.3)	59	(75.6)	41	(66.1)	18	(81.8)	148	(74.0)
Total	6		32		78		62		22		200	
11. Chromosome 22p												
II	0		0		1	(1.3)	0		0		1	(0.5)
IN	1	(16.7)	8	(25.0)	14	(17.9)	12	(19.4)	5	(22.7)	40	(20.0)
NN	5	(83.3)	24	(75.0)	63	(80.8)	50	(80.6)	17	(17.3)	159	(79.5)
Total	6		32		78		62		22		200	
12. Chromosome 22s												
II	1	(16.7)	2	(6.3)	6	(7.7)	5	(8.1)	1	(4.5)	15	(7.5)
IN	3	(50.0)	12	(37.5)	33	(42.3)	28	(45.2)	11	(50.0)	87	(43.5)
NN	2	(33.3)	18	(56.3)	39	(50.0)	29	(46.8)	10	(45.5)	98	(49.0)
Total	6		32		78		62		22		200	

TABLE 6.52: Statistical data for Table 6.51.

Method of subdivision	X^2	d.f.	P	Spearman's rho	P
1. Chromosome 3					
E	5.98	6	.425	-.0235	.742
F	1.91	6	.928	.0093	.896
2. Chromosome 4					
E	0.72	2	.697	-.0524	.461
F	0.23	1	.630	.0183	.796
3. Chromosome 13p					
E	13.07	6	.042	-.0284	.689
F	7.56	4	.109	-.0021	.976
4. Chromosome 13s					
E	0.83	3	.842	-.0232	.744
F	1.23	3	.745	-.0433	.543
5. Chromosome 14p					
E	0.24	1	.624	.0220	.758
F	0.06	1	.812	-.0459	.518
6. Chromosome 14s					
E	1.19	4	.897	-.0395	.579
F	0.31	3	.957	-.0333	.640
7. Chromosome 15p					
E	0.08	1	.777	.0532	.455
F	0.37	1	.544	.0311	.662
8. Chromosome 15s					
E	5.49	4	.241	-.0136	.848
F	5.19	3	.158	.0133	.852
9. Chromosome 21p					
E	0.23	1	.634	-.0338	.635
F	0.02	1	.885	-.0105	.883
10. Chromosome 21s					
E	3.41	3	.333	.0406	.568
F	0.37	3	.946	-.0358	.614
11. Chromosome 22p					
E	0.65	4	.958	-.0138	.846
F	1.10	3	.776	-.0220	.757
12. Chromosome 22s					
E	0.95	6	.987	.0334	.638
F	2.75	4	.601	.0180	.800

TABLE 6.53: A comparison of the mean paternal ages of homozygotes ard heterozygotes of chromosome variants (intense and brilliant levels of fluorescence combined).

	mean paternal age: homozygote	mean paternal age: heterozygote	t	P	Mann - Whitney U	z	P	Analysis for trends: z	Analysis for trends: P
Chromosome 3	28.7738	28.6638	0.15	.881	4735.0	-0.340	.734	0.069	.472
Chromosome 4	28.7380	28.3077	0.29	.770	1195.0	-0.102	.919	0.058	.476
Chromosome 13p	29.4823	28.1391	1.85	.066	3864.5	-2.535	.011	1.765	.038
Chromosome 13s	28.7531	28.5263	0.25	.806	2937.5	-0.439	.661	-.377	.352
Chromosome 14p	28.7121	28.5000	0.06	.954	198.0	0.0	1.000	0.125	.448
Chromosome 14s	28.9127	28.3649	0.73	.466	4384.0	-0.705	.481	0.586	.279
Chromosome 15p	28.6904	30.0000	-0.44	.661	216.0	-0.801	.423	0.029	.488
Chromosome 15s	28.7667	28.6250	0.19	.848	4727.0	-0.183	.855	0.018	.492
Chromosome 21p	28.7282	28.0000	0.31	.754	405.0	-0.647	.518	-0.228	.409
Chromosome 21s	28.5467	29.2000	-0.78	.436	3445.5	-0.861	.389	0.103	.460
Chromosome 22p	28.7875	28.4000	0.43	.669	3131.5	-0.210	.834	0.289	.386
Chromosome 22s	28.3894	29.1264	-1.01	.314	4456.0	-1.136	.256	1.106	.134

TABLE 6.54: Frequencies of grouped chromosome variants subdivided according to age of father.

Age(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. D group satellites (intense)												
0	0		9	(28.1)	23	(29.5)	22	(35.5)	6	(27.3)	60	(30.0)
1	4	(66.7)	15	(46.9)	36	(46.2)	31	(50.0)	8	(36.4)	94	(47.0)
2	2	(33.3)	6	(18.8)	18	(23.1)	9	(14.5)	8	(36.4)	43	(21.5)
⩾3	0		2	(6.3)	1	(1.3)	0		0		3	(1.5)
Total	6		32		78		62		22		200	
2. D group satellites (brilliant)												
0	5	(83.3)	30	(93.8)	76	(97.4)	52	(83.9)	21	(95.5)	184	(92.0)
⩾1	1	(16.7)	2	(6.3)	2	(2.6)	10	(16.1)	1	(4.5)	16	(8.0)
Total	6		32		78		62		22		200	
3. D group satellites (all)												
0	0		9	(28.1)	22	(28.2)	19	(30.6)	6	(27.3)	56	(28.0)
1	4	(66.7)	14	(43.8)	36	(46.2)	28	(45.2)	8	(36.4)	90	(45.0)
2	1	(16.7)	6	(18.8)	19	(24.4)	13	(21.0)	7	(31.8)	46	(23.0)
⩾3	1	(16.7)	3	(9.4)	1	(1.3)	2	(3.2)	1	(4.5)	8	(4.0)
Total	6		32		78		62		22		200	
4. G group satellites (intense)												
0	1	(16.7)	15	(46.9)	30	(38.5)	20	(32.3)	10	(45.5)	76	(38.0)
1	3	(50.0)	15	(46.9)	38	(48.7)	32	(51.6)	10	(45.5)	98	(49.0)
2	1	(16.7)	0		9	(11.5)	10	(16.1)	2	(9.1)	22	(11.0)
⩾3	1	(16.7)	2	(6.3)	1	(1.3)	0		0		4	(2.0)
Total	6		32		78		62		22		200	
5. G group satellites (brilliant)												
0	6	(100.0)	30	(93.8)	73	(93.6)	55	(88.7)	19	(86.4)	183	(91.5)
⩾1	0		2	(6.3)	5	(6.4)	7	(11.3)	3	(13.6)	17	(8.5)
Total	6		32		78		62		22		200	
6. G group satellites (all)												
0	1	(16.7)	14	(43.8)	28	(35.9)	16	(25.8)	8	(36.4)	67	(33.5)
1	3	(50.0)	15	(46.9)	37	(47.4)	34	(54.8)	11	(50.0)	100	(50.0)
2	1	(16.7)	1	(3.1)	12	(15.4)	11	(17.7)	3	(13.6)	28	(14.0)
⩾3	1	(16.7)	2	(6.3)	1	(1.3)	1	(1.6)	0		5	(2.5)
Total	6		32		78		62		22		200	
7. Total intense satellites												
0	0		7	(21.9)	8	(10.3)	6	(9.7)	4	(18.2)	25	(12.5)
1	0		7	(21.9)	23	(29.5)	26	(41.9)	7	(31.8)	63	(31.5)
2	3	(50.0)	11	(34.4)	31	(39.7)	17	(27.4)	4	(18.2)	66	(33.0)
3	2	(33.3)	3	(9.4)	15	(19.2)	11	(17.7)	5	(22.7)	36	(18.0)
⩾4	1	(16.7)	4	(12.5)	1	(1.3)	2	(3.2)	2	(9.1)	10	(5.0)
Total	6		32		78		62		22		200	
8. Total brilliant satellites												
0	5	(83.3)	28	(87.5)	72	(92.3)	47	(75.8)	18	(81.8)	170	(85.0)
⩾1	1	(16.7)	4	(12.5)	6	(7.7)	15	(24.2)	4	(13.3)	30	(15.0)
Total	6		32		78		62		22		200	

TABLE 6.54 contd.:

Age(yrs)	15 - 19		20 - 24		25 - 29		30 - 34		35+		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
9. Total variant satellites												
0	0		7	(21.9)	7	(9.0)	5	(8.1)	3	(13.6)	22	(11.0)
1	0		6	(18.8)	21	(26.9)	17	(27.4)	6	(27.3)	50	(25.0)
2	3	(50.0)	11	(34.4)	33	(42.3)	25	(40.3)	6	(27.3)	78	(39.0)
3	1	(16.7)	3	(9.4)	14	(17.9)	11	(17.7)	4	(18.2)	33	(16.5)
⩾4	2	(33.3)	5	(15.6)	3	(3.8)	4	(6.5)	3	(13.6)	17	(8.5)
Total	6		32		78		62		22		200	
10. Total intense bands												
⩽2	0		3	(9.4)	8	(10.3)	11	(17.7)	5	(22.7)	27	(13.5)
3	1	(16.7)	7	(21.9)	22	(28.2)	19	(30.6)	5	(22.7)	54	(27.0)
4	2	(33.3)	9	(28.1)	26	(33.3)	16	(25.8)	6	(27.3)	59	(29.5)
5	1	(16.7)	10	(31.3)	13	(16.7)	9	(14.5)	2	(9.1)	35	(17.5)
6	2	(33.3)	1	(3.1)	5	(6.4)	6	(9.7)	4	(18.2)	18	(9.0)
⩾7	0		2	(6.3)	4	(5.1)	1	(1.6)	0		7	(3.5)
Total	6		32		78		62		22		200	
11. Total brilliant bands												
0	4	(66.7)	23	(71.9)	57	(73.1)	34	(54.8)	13	(59.1)	131	(65.5)
1	2	(33.3)	6	(18.8)	18	(23.1)	15	(24.2)	8	(36.4)	49	(24.5)
⩾2	0		3	(9.4)	3	(3.8)	13	(21.0)	1	(4.5)	20	(10.0)
Total	6		32		78		62		22		200	
12. Total variant bands												
⩽2	0		2	(6.3)	4	(5.1)	4	(6.5)	2	(9.1)	12	(6.0)
3	1	(16.7)	6	(18.8)	19	(24.4)	12	(19.4)	7	(31.8)	45	(22.5)
4	1	(16.7)	9	(28.1)	22	(28.2)	19	(30.6)	3	(13.6)	54	(27.0)
5	1	(16.7)	7	(21.9)	24	(30.8)	15	(24.2)	4	(18.2)	51	(25.5)
6	3	(50.0)	5	(15.6)	5	(6.4)	9	(14.5)	6	(27.3)	28	(14.0)
⩾7	0		3	(9.4)	4	(5.1)	3	(4.8)	0		10	(5.0)
Total	6		32		78		62		22		200	

TABLE 6.55: Statistical data for Table 6.54.

Method of subdivision	X^2	d.f.	P	Spearman's rho	P
1. D group satellites (intense)					
E	5.81	6	.444	-.0651	.360
F	4.68	4	.321	-.0539	.448
2. D group satellites (brilliant)					
E	6.10	2	.047	.0880	.215
F	0.34	2	.845	.0736	.301
3. D group satellites (all)					
E	1.85	6	.933	-.0277	.697
F	2.30	6	.890	-.0243	.732
4. G group satellites (intense)					
E	2.10	6	.910	.0200	.778
F	0.44	4	.979	.0307	.666
5. G group satellites (brilliant)					
E	2.20	2	.333	.1061	.135
F	2.52	2	.284	.0677	.341
6. G group satellites (all)					
E	2.81	6	.833	.0581	.414
F	1.09	4	.895	-.0049	.946
7. Total intense satellites					
E	11.19	9	.263	-.0687	.334
F	10.91	8	.207	-.0961	.176
8. Total brilliant satellites					
E	7.65	3	.054	.1327	.061
F	1.98	2	.372	.0982	.166
9. Total variant satellites					
E	6.46	9	.693	-.0111	.876
F	7.72	8	.461	-.0472	.507
10. Total intense bands					
E	10.21	12	.598	-.1401	.048
F	6.68	8	.571	-.1143	.107
11. Total brilliant bands					
E	14.83	6	.022	.1513	.032
F	6.13	4	.190	.1398	.048
12. Total variant bands					
E	10.20	9	.335	-.0495	.487
F	7.02	10	.724	-.0478	.502

It appears that the incidence of brilliant satellites of the D group chromosomes is associated in some way with paternal age. The association is not simply linear, or not significantly so, as shown by the low value of the rank correlation coefficients. However, the total brilliant satellites, and the total brilliant bands both show associations with paternal age which lead to significant X^2 values amd significant rank correlation coefficients. These results are similar to those found in the examination of fluorescence variant frequemcies and maternal age, but are more obvious. In each case the associations are found particularly when paternal age is divided into five-year intervals, and less so when six-year intervals are used.

C. Birth order.

The data concerning the birth orders of the newborn infants are subject to a few sources of inaccuracy. No information was collected regarding miscarriages and still-births. It is also possible that other children who have died went unrecorded. The mothers were not asked to state whether or not the infant's siblings were all alive, and there is some possibility that only living siblings were recorded, and the birth order was calculated from this information. Another limitation of the data is that as the subjects were newborn infants they were always the last-born members of the sibship and therefore, in many cases, only the incomplete family size is known; this restricts the analytical techniques available. The present data have been analysed using the X^2 test. Although the inapplicability of this test has been shown by Halperin (1953), his suggested alternative requires knowledge of completed family sizes; as do other methods suggested by Penrose (1934) and Haldane and Smith (1948). The P values obtained by Halperin using the X^2 test and the modified method were very close, because although the actual values for X^2 were very different, so too were the degrees of freedom.

Walzer et al. (1969) gives some information about the effect of birth order on the incidence of major abnormalities and minor variants (including enlarged satellites, for example), of chromosomes in a series of newborn infants. By analysing their data with the X^2 test it appears that there is a significant difference (P=.004) in the incidence of minor variants with birth order. Only one infant with a birth order of three or more had a minor variant, however, and if the data for second and third or more birth orders are combined, no significant difference (P=.895) is found between first-born and other infants with respect to the incidence of minor variants.

(i) Blood groups and isoenzymes.

Table 6.56 shows the phenotype frequencies of the blood groups and isoenzymes subdivided according to order of birth. Table 6.57 gives the results of statistical analysis of these data. Table 6.58 gives the mean birth orders of the homozygotes and heterozygotes of these markers, the results of the t-test and the Mann Whitney U test, and the results of the analysis for trends across birth orders. The only marker to show any effect of birth order is the isoenzyme adenylate kinase. A significant result is found with each statistical test. It appears that the frequency of the 2-1 phenotype increases with increasing birth order. Unfortunately the numbers of infants in the birth order categories 3, 4 and 5 are very low. However, when these are combined, as was done for the X^2 test, the frequency of the 2-1 phenotype is still much higher than in the first or second birth order ranks.

(ii) Chromosome variants.

Table 6.59 shows the chromosome variant frequencies subdivided according to birth order. Tables 6.60 and 6.61 show the results of statistical analysis of these data. The data for chromosome 3 give an almost significant X^2 value.

TABLE 6.56: Blood group and isoenzyme phenotype frequencies subdivided according to order of birth.

Birth order	1		2		3		4		5		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. ABO												
A	43	(39.4)	40	(47.1)	6	(37.5)	4	(50.0)	1	(25.0)	94	(42.3)
O	55	(50.5)	33	(38.8)	8	(50.0)	4	(50.0)	3	(75.0)	103	(46.4)
B	9	(8.3)	10	(11.8)	2	(12.5)	0		0		21	(9.5)
AB	2	(1.8)	2	(2.4)	0		0		0		4	(1.8)
Total	109		85		16		8		4		222	
2. Rhesus												
r r	21	(19.6)	15	(17.9)	1	(6.7)	1	(12.5)	2	(50.0)	40	(18.3)
R_1r	39	(36.4)	29	(34.5)	6	(40.0)	3	(37.5)	1	(25.0)	78	(35.8)
R_1R_1	15	(14.0)	16	(19.0)	2	(13.3)	1	(12.5)	0		34	(15.6)
R_2r	4	(3.7)	3	(3.6)	4	(26.7)	1	(12.5)	0		12	(5.5)
R_2R_2	2	(1.9)	1	(1.2)	0		0		0		3	(1.4)
R_1R_2	22	(20.6)	18	(21.4)	2	(13.3)	2	(25.0)	1	(25.0)	45	(20.6)
r"r'	1	(0.9)	0		0		0		0		1	(0.5)
r"r	2	(1.9)	2	(2.4)	0		0		0		4	(1.8)
R_zR_2	1	(0.9)	0		0		0		0		1	(0.5)
Total	107		84		15		8		4		218	
3. Rhesus(D)												
D+ve	85	(78.0)	68	(80.0)	15	(93.8)	7	(87.5)	2	(50.0)	177	(79.8)
D-ve	24	(22.0)	17	(20.0)	1	(6.3)	1	(12.5)	2	(50.0)	45	(20.2)
Total	109		85		16		8		4		222	
4. MN												
M	35	(33.7)	23	(28.4)	2	(13.3)	3	(37.5)	2	(50.0)	65	(30.7)
MN	49	(47.1)	49	(60.5)	9	(60.0)	4	(50.0)	2	(50.0)	113	(53.3)
N	20	(19.2)	9	(11.1)	4	(26.7)	1	(12.5)	0		34	(16.0)
Total	104		81		15		8		4		212	
5. S												
S	17	(16.0)	9	(11.1)	0		3	(37.5)	0		29	(13.6)
Ss	49	(46.2)	39	(48.1)	6	(37.5)	4	(50.0)	0		98	(45.8)
s	40	(37.7)	33	(40.7)	10	(62.5)	1	(12.5)	3	(100.0)	87	(40.7)
Total	160		81		16		8		3		214	
6. MNS												
MS	7	(6.9)	5	(6.5)	0		1	(12.5)	0		13	(6.4)
MSs	19	(18.8)	11	(14.3)	0		2	(25.0)	0		32	(15.7)
Ms	6	(5.9)	5	(6.5)	2	(13.3)	0		1	(33.3)	14	(6.9)
MNS	9	(8.9)	4	(5.2)	0		1	(12.5)	0		14	(6.9)
MNSs	23	(22.8)	21	(27.3)	3	(20.0)	2	(25.0)	0		49	(24.0)
MNs	17	(16.8)	22	(28.6)	6	(40.0)	1	(12.5)	2	(66.7)	48	(23.5)
NS	1	(1.0)	0		0		1	(12.5)	0		2	(1.0)
NSs	5	(5.0)	5	(6.5)	2	(13.3)	0		0		12	(5.9)
Ns	14	(13.9)	4	(5.2)	2	(13.3)	0		0		20	(9.8)
Total	101		77		15		8		3		204	

TABLE 6.56 contd.:

Birth order	1 No.	%	2 No.	%	3 No.	%	4 No.	%	5 No.	%	Total No.	%
7. Duffy												
Fy^a	18	(17.0)	13	(15.5)	2	(13.3)	0		1	(25.0)	34	(15.7)
Fy^aFy^b	47	(44.3)	37	(44.0)	9	(60.0)	6	(75.0)	1	(25.0)	100	(46.1)
Fy^b	41	(38.7)	34	(40.5)	4	(26.7)	2	(25.0)	2	(50.0)	83	(38.2)
Total	106		84		15		8		4		217	
8. Kell												
Kk	11	(10.1)	5	(5.9)	1	(6.3)	0		0		17	(7.7)
k	98	(89.9)	80	(94.1)	15	(93.8)	8	(100.0)	4	(100.0)	205	(92.3)
Total	109		85		16		8		4		222	
9. Phosphoglucomutase												
1 - 1	53	(53.0)	55	(66.3)	10	(62.5)	4	(50.0)	4	(100.0)	126	(59.7)
2 - 1	37	(37.0)	23	(27.7)	5	(31.3)	3	(37.5)	0		68	(32.2)
2 - 2	10	(10.0)	5	(6.0)	1	(6.3)	1	(12.5)	0		17	(8.1)
Total	100		83		16		8		4		211	
10. Esterase-D												
1 - 1	74	(74.7)	63	(76.8)	12	(75.0)	4	(50.0)	4	(100.0)	157	(75.1)
2 - 1	23	(23.2)	18	(22.0)	3	(18.8)	4	(50.0)	0		48	(23.0)
2 - 2	2	(2.0)	1	(1.2)	1	(6.3)	0		0		4	(1.9)
Total	99		82		16		8		4		209	
11. Adenylate kinase												
1 - 1	98	(97.0)	79	(95.2)	12	(75.0)	8	(100.0)	3	(75.0)	200	(94.3)
2 - 1	3	(3.0)	4	(4.8)	4	(25.0)	0		1	(25.0)	12	(5.7)
Total	101		83		16		8		4		212	
12. Acid phosphatase												
A	13	(13.0)	12	(14.6)	1	(6.3)	3	(37.5)	1	(25.0)	30	(14.3)
BA	41	(41.0)	38	(46.3)	8	(50.0)	2	(25.0)	2	(50.0)	91	(43.3)
B	37	(37.0)	30	(36.6)	6	(37.5)	2	(25.0)	1	(25.0)	76	(36.2)
BC	4	(4.0)	2	(2.4)	1	(6.3)	1	(12.5)	0		8	(3.8)
CA	5	(5.0)	0		0		0		0		5	(2.4)
Total	100		82		16		8		4		210	

TABLE 6.57: Statistical data for Table 6.56

	X^2	d.f.	P	Spearman's rho	P
1. ABO	3.64	4	.457		
2. Rhesus	4.36	8	.823		
3. Rhesus(D)	0.83	2	.660	-.0656	.104
4. MN	4.17	4	.381	.0124	.858
5. S	4.44	4	.349	.0847	.217
6. MNS	10.27	7	.174		
7. Duffy	2.28	4	.685	-.0020	.977
8. Kell	1.95	2	.377	.0954	.156
9. PGM	3.72	4	.445	-.1223	.076
10. ESD	0.34	2	.844	.0051	.941
11. AK	9.28	2	.010	.1564	.023
12. AP	4.00	6	.697		

TABLE 6.58: A comparison of the mean birth orders of homozygotes and heterozygotes of the blood groups and isoenzymes.

	mean birth order: heterozygote	homozygote	t	P	Mann - Whitney U	z	P	Analysis for trends: z	P
MN	1.77	1.65	1.00	.318	4974.5	-1.529	.126	1.013	.156
S	1.64	1.74	-0.84	.403	5506.5	-0.433	.665	0.461	.323
MNS	1.72	1.65	0.44	.663	3505.0	-0.894	.371	0.420	.337
Rhesus	1.72	1.68	0.38	.703	5284.0	-0.358	.721	0.425	.334
Duffy	1.77	1.66	0.92	.357	5486.5	-0.868	.385	1.048	.147
Kell	1.41	1.73	-1.43	.154	1414.5	-1.419	.156	1.285	.099
PGM	1.62	1.79	-1.31	.192	4356.5	-1.337	.181	1.085	.138
ESD	1.75	1.73	0.12	.908	3850.5	-0.040	.968	0.206	.417
AK	2.33	1.70	2.42	.016	772.5	-2.272	.023	2.116	.017
AP	1.67	1.75	-0.66	.510	5927.0	-0.627	.530	0.611	.271

TABLE 6.59: Chromosome variant phenotype frequencies subdivided according to order of birth.

Birth order	1		2		3		4		5		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. Chromosome 3												
BB	0		1	(1.2)	0		0		0		1	(0.5)
BI	5	(5.2)	4	(4.9)	2	(12.5)	3	(37.5)	1	(25.0)	15	(7.3)
II	11	(11.3)	12	(14.8)	0		1	(12.5)	1	(25.0)	25	(12.1)
IN	44	(45.4)	36	(44.4)	7	(43.8)	1	(12.5)	1	(25.0)	89	(43.2)
BN	20	(20.6)	8	(9.9)	2	(12.5)	1	(12.5)	0		31	(15.0)
NN	17	(17.5)	20	(24.7)	5	(31.3)	2	(25.0)	1	(25.0)	45	(21.8)
Total	97		81		16		8		4		206	
2. Chromosome 13p												
BB	0		0		0		1	(12.5)	0		1	(0.5)
BI	5	(5.2)	2	(2.5)	1	(6.3)	0		0		8	(3.9)
II	22	(22.7)	22	(27.2)	0		4	(50.0)	3	(75.0)	51	(24.8)
IN	59	(60.8)	42	(51.9)	10	(62.5)	3	(37.5)	1	(37.5)	115	(55.8)
BN	1	(1.0)	3	(3.7)	0		0		0		4	(1.9)
NN	10	(10.3)	12	(14.8)	5	(31.3)	0		0		27	(13.1)
Total	97		81		16		8		4		206	
3. Chromosome 13s												
IN	18	(18.6)	10	(12.3)	4	(25.0)	2	(25.0)	1	(25.0)	35	(17.0)
BN	3	(3.1)	1	(1.2)	0		0		1	(25.0)	5	(2.4)
NN	76	(78.4)	70	(86.4)	12	(75.0)	6	(75.0)	2	(50.0)	166	(80.6)
Total	97		81		16		8		4		206	
4. Chromosome 14s												
BI	1	(1.0)	0		0		1	(12.5)	0		2	(1.0)
II	0		1	(1.2)	1	(6.3)	0		0		2	(1.0)
IN	32	(33.0)	27	(33.3)	6	(37.5)	4	(50.0)	1	(25.0)	70	(34.0)
BN	1	(1.0)	3	(3.7)	0		1	(12.5)	1	(25.0)	6	(2.9)
NN	63	(64.9)	50	(61.7)	9	(56.3)	2	(25.0)	2	(50.0)	126	(61.2)
Total	97		81		16		8		4		206	
5. Chromosome 15s												
BI	0		1	(1.2)	0		0		0		1	(0.5)
II	0		2	(2.5)	0		0		0		2	(1.0)
IN	40	(41.2)	33	(40.7)	6	(37.5)	1	(12.5)	2	(50.0)	82	(39.8)
BN	1	(1.0)	2	(2.5)	0		0		0		3	(1.5)
NN	56	(57.7)	43	(53.1)	10	(62.5)	7	(87.5)	2	(50.0)	118	(57.3)
Total	97		81		16		8		4		206	
6. Chromosome 21s												
II	2	(2.1)	0		0		0		0		2	(1.0)
IN	22	(22.7)	22	(27.2)	1	(6.3)	2	(25.0)	2	(50.0)	49	(23.8)
BN	3	(3.1)	0		0		0		0		3	(1.5)
NN	70	(72.2)	59	(72.8)	15	(93.8)	6	(75.0)	2	(50.0)	152	(73.8)
Total	97		81		16		8		4		206	
7. Chromosome 22s												
BI	3	(3.1)	3	(3.7)	1	(6.3)	0		0		7	(3.4)
II	5	(5.2)	3	(3.7)	0		0		0		8	(3.9)
IN	40	(41.2)	31	(38.3)	7	(43.8)	1	(12.5)	2	(50.0)	81	(39.3)
BN	4	(4.1)	3	(3.7)	2	(12.5)	0		0		9	(4.4)
NN	45	(46.4)	41	(50.6)	6	(37.5)	7	(87.5)	2	(50.0)	101	(49.0)
Total	97		81		16		8		4		206	

TABLE 6.60: Statistical data for Table 6.59.

	χ^2	d.f.	P
1. Chromosome 3	15.37	8	.052
2. Chromosome 13p	2.47	6	.872
3. Chromosome 13s	3.88	3	.275
4. Chromosome 14s	4.67	3	.198
5. Chromosome 15s	2.31	3	.511
6. Chromosome 21s	1.18	2	.554
7. Chromosome 22s	0.59	4	.964

TABLE 6.61: A comparison of the mean birth orders of homozygotes and heterozygotes of the chromosome variants.

	mean birth order: heterozygote	homozygote	t	P	Mann - Whitney U	z	P	Analysis for trends: z	P
Chromosome 3	1.69	1.85	-1.18	.239	4278.0	-1.385	.166	1.070	.142
Chromosome 13p	1.65	1.90	-1.86	.065	4394.5	-1.637	.102	1.764	.039
Chromosome 13s	1.83	1.72	0.64	.522	3279.0	-0.133	.894	0.328	.371
Chromosome 14s	1.87	1.66	1.61	.110	4540.0	-1.192	.233	1.550	.061
Chromosome 15s	1.69	1.78	-0.78	.447	4962.5	-0.512	.608	0.790	.215
Chromosome 21s	1.73	1.75	-0.11	.912	3875.5	-0.379	.705	0.212	.417
Chromosome 22s	1.70	1.78	-0.62	.533	5128.5	-0.405	.685	0.607	.271

The frequency of the NN type appears to rise with increasing birth order, and the frequencies of the IN and BN types to decline. The frequency of the BI type appears to rise with increasing birth order but the numbers involved are very small. There is no significant difference between the mean birth orders of the homozygotes and heterozygotes of chromosome 3. The difference between the mean birth orders of homozygotes and heterozygotes of chromosome 13p is nearly significant in the t-test, but not in the Mann Whitney U test. However, the trend for increasing homozygosity with increasing birth order for this variant was significant.

Tables 6.62, 6.63 and 6.64 give the frequencies of chromosome variants (intense and brilliant levels of fluorescence combined) subdivided according to birth order, and the results of statistical analysis of these data. No effects of birth order on the frequencies of any of the variants were detected by means of the X^2 test or by calculation of the rank correlation coefficient. A significant difference was found, however, between the mean birth orders of the homozygotes and heterozygotes of chromosome 3. Increasing birth order was associated with a rise in homozygosity.

Tables 6.65 and 6.66 give the distributions of the grouped chromosome variants subdivided according to birth order and the results of statistical analysis of these data. A significant X^2 value was obtained in the case of the total number of intense variant bands per individual. However, there was no simple linear trend for increasing or decreasing number of bands being associated with increasing birth order, as shown by the non-significant rank correlation coefficient. This finding can probably be discounted as being the significant (P=.05) finding expected when a large number of comparisons of this type are made.

TABLE 6.62: Chromosome variant phenotype frequencies (intense and brilliant levels of fluorescence combined) subdivided according to order of birth.

Birth order	1		2		3		4		5		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. Chromosome 3												
II	16	(16.5)	17	(21.0)	2	(12.5)	4	(50.0)	2	(50.0)	41	(19.9)
IN	64	(66.0)	44	(54.3)	9	(56.3)	2	(25.0)	1	(25.0)	120	(58.3)
NN	17	(17.5)	20	(24.7)	5	(31.3)	2	(25.0)	1	(25.0)	45	(25.0)
Total	97		81		16		8		4		206	
2. Chromosome 4												
IN	6	(6.2)	4	(4.9)	2	(12.5)	1	(12.5)	0		13	(6.3)
NN	91	(93.8)	77	(95.1)	14	(87.5)	7	(87.5)	4	(100.0)	193	(93.7)
Total	97		81		16		8		4		206	
3. Chromosome 13p												
II	27	(27.8)	24	(29.6)	1	(6.3)	5	(62.5)	3	(75.0)	60	(29.1)
IN	60	(61.9)	45	(55.6)	10	(62.5)	3	(37.5)	1	(25.0)	119	(57.8)
NN	10	(10.3)	12	(14.8)	5	(31.3)	0		0		27	(13.1)
Total	97		81		16		8		4		206	
4. Chromosome 13s												
IN	21	(21.6)	11	(13.6)	4	(25.0)	2	(25.0)	2	(50.0)	40	(19.4)
NN	76	(78.4)	70	(86.4)	12	(75.0)	6	(75.0)	2	(50.0)	166	(80.6)
Total	97		81		16		8		4		206	
5. Chromosome 14p												
IN	2	(2.1)	1	(1.2)	0		0		0		3	(1.5)
NN	95	(97.9)	80	(98.8)	16	(100.0)	8	(100.0)	4	(100.0)	203	(98.5)
Total	97		81		16		8		4		206	
6. Chromosome 14s												
II	1	(1.0)	1	(1.2)	1	(6.3)	1	(12.5)	0		4	(1.9)
IN	33	(34.0)	30	(37.0)	6	(37.5)	5	(62.5)	2	(50.0)	76	(36.9)
NN	63	(64.9)	50	(61.7)	9	(56.3)	2	(25.0)	2	(50.0)	126	(61.2)
Total	97		81		16		8		4		206	
7. Chromosome 15p												
IN	1	(1.0)	2	(2.5)	0		0		0		3	(1.5)
NN	96	(99.0)	79	(97.5)	16	(100.0)	8	(100.0)	4	(100.0)	203	(98.5)
Total	97		81		16		8		4		206	
8. Chromosome 15s												
II	0		3	(3.7)	0		0		0		3	(1.5)
IN	41	(42.3)	35	(43.2)	6	(37.5)	1	(12.5)	2	(50.0)	85	(41.3)
NN	56	(57.7)	43	(53.1)	10	(62.5)	7	(87.5)	2	(50.0)	118	(57.3)
9. Chromosome 21p												
IN	2	(2.1)	2	(2.5)	0		1	(12.5)	0		5	(2.4)
NN	95	(97.9)	79	(97.5)	16	(100.0)	7	(87.5)	4	(100.0)	201	(97.6)
Total	97		81		16		8		4		206	

TABLE 6.62 contd.:

Birth order	1 No.	%	2 No.	%	3 No.	%	4 No.	%	5 No.	%	Total No.	%
10. Chromosome 21p												
II	2	(2.1)	0		0		0		0		2	(1.0)
IN	25	(25.8)	22	(27.2)	1	(6.3)	2	(25.0)	2	(50.0)	52	(25.2)
NN	70	(72.2)	59	(72.8)	15	(93.8)	6	(75.0)	2	(50.0)	152	(73.8)
Total	97		81		16		8		4		206	
11. Chromosome 22p												
II	1	(1.0)	0		0		0		0		1	(0.5)
IN	18	(18.6)	17	(21.0)	3	(18.8)	2	(25.0)	0		40	(19.4)
NN	78	(80.4)	64	(79.0)	13	(81.3)	6	(75.0)	4	(100.0)	165	(80.1)
Total	97		81		16		8		4		206	
12. Chromosome 22s												
II	8	(8.2)	6	(7.4)	1	(6.3)	0		0		15	(7.3)
IN	44	(45.4)	34	(42.0)	9	(56.3)	1	(12.5)	2	(50.0)	90	(43.7)
NN	45	(46.4)	41	(50.6)	6	(37.5)	7	(87.5)	2	(50.0)	101	(49.0)
Total	97		81		16		8		4		206	

TABLE 6.63: Statistical data for Table 6.62.

	X^2	d.f.	P	Spearman's rho	P
1. Chromosome 3	5.73	4	.220	-.0025	.971
2. Chromosome 4	1.18	2	.555	.0265	.706
3. Chromosome 13p	2.03	4	.730	.0022	.976
4. Chromosome 13s	3.88	3	.275	-.0093	.895
5. Chromosome 14p	0.01	1	.919	-.0556	.427
6. Chromosome 14s	4.67	3	.198	.1130	.106
7. Chromosome 15p	0.01	1	.919	.0108	.877
8. Chromosome 15s	2.31	3	.511	-.0148	.833
9. Chromosome 21p	0.02	1	.895	.0319	.649
10. Chromosome 21s	1.18	2	.554	-.0535	.445
11. Chromosome 22p	0.14	2	.933	-.0047	.947
12. Chromosome 22s	1.07	4	.898	-.0654	.350

TABLE 6.64: A comparison of the mean birth orders of homozygotes and heterozygotes of the chromosome variants (intense and brilliant levels of fluorescence combined).

	mean birth order							Analysis for trends:	
	heterozygote	homozygote	t	P	Mann - Whitney U	z	P	z	P
Chromosome 3	1.60	1.94	-2.59	.010	4228.0	-2.418	.016	2.568	.005
Chromosome 4	1.85	1.74	0.43	.671	1182.5	-0.379	.705	0.373	.356
Chromosome 13p	1.66	1.86	-1.57	.118	4675.0	-1.299	.194	1.437	.075
Chromosome 13s	1.83	1.72	0.54	.594	3279.0	-0.133	.894	0.328	.371
Chromosome 14p	1.33	1.75	-0.79	.431	230.0	-0.796	.426	0.463	.323
Chromosome 14s	1.86	1.68	1.37	.172	4528.5	-1.091	.275	1.292	.099
Chromosome 15p	1.67	1.74	-0.15	.884	290.0	-0.155	.877	-0.228	.409
Chromosome 15s	1.68	1.79	-0.80	.423	4910.5	-0.603	.546	0.837	.201
Chromosome 21p	2.00	1.74	0.64	.521	447.5	-0.457	.647	0.475	.316
Chromosome 21s	1.73	1.75	-0.11	.912	3875.5	-0.379	.705	0.212	.417
Chromosome 22p	1.73	1.75	-0.14	.891	3275.5	-0.144	.886	-0.090	.464
Chromosome 22s	1.70	1.78	-0.60	.551	5039.0	-0.467	.641	0.600	.274

TABLE 6.65: Frequencies of grouped chromosome variants subdivided according to order of birth.

Birth order	1 No.	%	2 No.	%	3 No.	%	4 No.	%	5 No.	%	Total No.	%
1. D group satellites (intense)												
0	26	(26.8)	27	(33.3)	4	(25.0)	2	(25.0)	2	(50.0)	61	(29.6)
1	53	(54.6)	33	(40.7)	7	(43.8)	4	(50.0)	0		97	(47.1)
2	16	(16.5)	19	(23.5)	4	(25.0)	2	(25.0)	2	(50.0)	43	(20.9)
⩾3	2	(2.1)	2	(2.5)	1	(6.3)	0		0		5	(2.4)
Total	97		81		16		8		4		206	
2. D group satellites (brilliant)												
0	91	(93.8)	75	(92.6)	16	(100.0)	6	(75.0)	2	(50.0)	190	(92.0)
⩾1	6	(6.2)	6	(7.4)	0		2	(25.0)	2	(50.0)	16	(7.8)
Total	97		81		16		8		4		206	
3. D group satellites (all)												
0	25	(25.8)	24	(29.6)	4	(25.0)	2	(25.0)	2	(50.0)	57	(27.7)
1	49	(50.5)	35	(43.2)	7	(43.8)	2	(25.0)	0		93	(45.1)
2	21	(21.6)	17	(21.0)	4	(25.0)	4	(50.0)	0		46	(22.3)
⩾3	2	(2.1)	5	(6.2)	1	(6.3)	0		2	(50.0)	10	(4.9)
Total	97		81		16		8		4		206	
4. G group satellites (intense)												
0	38	(39.2)	30	(37.0)	7	(43.8)	5	(62.5)	0		80	(38.8)
1	42	(43.3)	41	(50.6)	9	(56.3)	3	(37.5)	4	(100.0)	99	(88.1)
2	14	(14.4)	9	(11.1)	0		0		0		23	(11.2)
⩾3	3	(3.1)	1	(1.2)	0		0		0		4	(1.9)
Total	97		81		16		8		4		206	
5. G group satellites (brilliant)												
0	88	(90.7)	75	(92.6)	13	(81.3)	8	(100.0)	4	(100.0)	188	(91.3)
⩾1	9	(9.3)	6	(7.4)	3	(18.8)	0		0		18	(8.7)
Total	97		81		16		8		4		206	
6. G group satellites (all)												
0	32	(33.0)	27	(33.3)	6	(37.5)	5	(62.5)	0		70	(34.0)
1	45	(46.4)	41	(50.6)	8	(50.0)	3	(37.5)	4	(100.0)	101	(49.0)
2	16	(16.5)	12	(14.8)	2	(12.5)	0		0		30	(14.6)
⩾3	4	(4.1)	1	(1.2)	0		0		0		5	(2.4)
Total	97		81		16		8		4		206	
7. Total intense satellites												
0	11	(11.3)	11	(13.6)	2	(12.5)	2	(25.0)	0		26	(12.6)
1	29	(29.9)	26	(32.1)	5	(31.3)	3	(37.5)	2	(50.0)	65	(31.6)
2	36	(37.1)	24	(29.6)	5	(31.3)	1	(12.5)	0		66	(32.0)
3	15	(15.5)	15	(18.5)	4	(25.0)	2	(25.0)	2	(50.0)	38	(18.4)
⩾4	6	(6.2)	5	(6.2)	0		0		0		11	(5.3)
Total	97		81		16		8		4		206	
8. Total brilliant satellites												
0	84	(86.6)	70	(86.4)	13	(81.3)	6	(75.0)	2	(50.0)	175	(85.0)
⩾1	13	(13.4)	11	(13.6)	3	(18.8)	2	(25.0)	2	(50.0)	31	(15.0)
Total	97		81		16		8		4		206	

TABLE 6.65 contd.:

Birth order	1 No.	%	2 No.	%	3 No.	%	4 No.	%	5 No.	%	Total No.	%
9. Total variant satellites												
0	10	(10.3)	8	(9.9)	2	(12.5)	2	(25.0)	0		22	(10.7)
1	22	(22.7)	24	(29.6)	3	(18.8)	1	(12.5)	2	(50.0)	52	(25.2)
2	41	(42.3)	29	(35.8)	6	(37.5)	3	(37.5)	0		79	(38.3)
3	16	(16.5)	12	(14.8)	5	(31.3)	2	(25.0)	0		35	(17.0)
≥4	8	(8.2)	8	(9.9)	0		0		2	(50.0)	18	(8.7)
Total	97		81		16		8		4		206	
10. Total intense bands												
≤2	11	(11.3)	11	(13.6)	5	(31.3)	1	(12.5)	0		28	(13.6)
3	29	(29.9)	22	(27.2)	3	(18.8)	2	(25.0)	0		56	(27.2)
4	22	(22.7)	28	(34.6)	7	(43.8)	2	(25.0)	1	(25.0)	60	(29.1)
5	26	(26.8)	7	(8.6)	1	(6.3)	0		3	(75.0)	37	(18.0)
6	7	(7.2)	8	(9.9)	0		3	(37.5)	0		18	(8.7)
≥7	2	(2.1)	5	(6.2)	0		0		0		7	(3.4)
Total	97		81		16		8		4		206	
11. Total brilliant bands												
0	63	(64.9)	58	(71.6)	9	(56.3)	3	(37.5)	1	(25.0)	134	(65.0)
1	23	(23.7)	16	(19.8)	6	(37.5)	2	(25.0)	3	(75.0)	50	(24.3)
≥2	11	(11.3)	7	(8.6)	1	(6.3)	3	(37.5)	0		22	(10.7)
Total	97		81		16		8		4		206	
12. Total variant bands												
≤2	4	(4.1)	6	(7.4)	2	(12.5)	1	(12.5)	0		13	(6.3)
3	25	(25.8)	16	(19.8)	5	(31.3)	0		0		46	(22.3)
4	19	(19.6)	30	(37.0)	5	(31.3)	1	(12.5)	0		55	(26.7)
5	31	(32.0)	14	(17.3)	3	(18.8)	2	(25.0)	2	(50.0)	52	(25.2)
6	13	(13.4)	9	(11.1)	1	(6.3)	4	(50.0)	2	(50.0)	29	(14.1)
≥7	5	(5.2)	6	(7.4)	0		0		0		11	(5.3)
Total	97		81		16		8		4		206	

TABLE 6.66: Statistical data for Table 6.65.

	X^2	d.f.	P	Spearman's rho	P
1. D gp. sat.(intense)	4.99	4	.288	.0336	.632
2. D gp. sat. (brilliant)	2.01	2	.365	.0913	.192
3. D gp. sat. (all)	3.92	4	.417	.0489	.485
4. G gp. sat. (intense)	0.32	3	.957	-.0644	.358
5. G gp. sat. (brilliant)	0.35	2	.838	-.0124	.860
6. G gp. sat. (all)	2.96	4	.565	-.0778	.260
7. Total intense sat.	2.89	6	.823	-.0299	.669
8. Total brilliant sat.	2.51	2	.285	.0724	.301
9. Total variant sat.	2.76	6	.838	-.0140	.842
10. Total intense bands	15.48	8	.050	-.0379	.588
11. Total brilliant bands	6.02	4	.198	.0490	.485
12. Total variant bands	12.41	8	.134	-.0184	.793

4. Occupational class.

There is a high level of assortative mating occurring in present day modern societies for factors such as social class background, I.Q. and education (Garrison et al. 1968). The most obvious personal characteristics which appear to be associated with social class, such as height, weight and growth rates are those very much influenced by environmental conditions such as nutrition and overcrowding.

There are relatively few studies concerning differences in frequency between social classes of characteristics with an almost entirely genetic determination.

Dawson (1964) found no evidence for any differences by social class in the distribution of ABO phenotypes in Ireland.

Wheatcroft (1973) found no significant association with social class of PTC tasting ability when a group of school-children were divided into tasters and non-tasters. Significant differences were found between the professional (grades I and II) and the manual groups in males, However, when the whole distributions of tasting ability were considered, and in the females differences were found between the manual group and group III and between the former and groups I, II and III combined.

Beardmore (pers. comm.) has found in a series of newborn infants in the Cardiff area a "suggestion" of an association between ABO phenotypes and social class. Blood group O was relatively more common in the manual classes, and blood group B more common in the non-manual. An association was also found between MN phenotypes and social class.

Patil et al. (1977) found that there was no strong correlation between the incidence of chromosome abnormalities and social class (using 5 grades of the Socioeconomic Index)

in a series of 7 and 8 year old American children. No correlation with social class was found either in the X and Y body survey of newborn infants in Denver, U.S.A. (Goad et al. 1976) or in the chromosome survey of newborn infants in Boston, U.S.A. (Walzer and Gerald 1977).

In the present study the occupations of the fathers of the newborn infants and the students, and the adults, with the exception of the geriatric patients, were classified according to the system devised by the Office of Population Censuses and Surveys (1970). Whether or not these categories represent any meaningful social divisions of the population is open to question. It seems likely that a classification system based solely on occupation would not be a very good indication of possible genetic stratifications of society. However, this system has been used in other studies, as indicated above.

Table 6.67 shows that there are highly significant differences between newborn infants and the adults with regard to the distributions of occupational class. This is a result of the fact that many of the adults were technicians at the University of Durham, and according to the classification system used, technicians of all grades, from the most junior upwards, are classed as grade II. This means that there could be persons in lower grades employed in positions which require the same or higher degree of educational qualification and technical skill, and which pay the same or higher levels of income. Therefore, the difference observed in this table is not thought to be important, and is not considered to be evidence of a difference in social classes between the two generations sampled.

Table 6.68 shows the distributions of blood group phenotypes subdivided according to occupational class and the results of statistical analysis of these data. No significant differences were found when the grades I, II,

TABLE 6.67: Series comprising the total sample (excluding geriatric patients) subdivided according to occupational class.

Series	I No.	%	II No.	%	III NM + M No.	%	IV + V No.	%	Total No.
Newborn infants	29	(13.5)	47	(21.9)	98	(45.6)	41	(19.1)	215
Students	13	(33.3)	10	(25.6)	8	(20.5)	8	(20.5)	39
Adults resident in Co. Durham	22	(12.0)	106	(57.9)	27	(14.8)	28	(15.3)	183
Orthopaedic patients	0		4	(13.8)	20	(69.0)	5	(17.2)	29
Total	64	(13.7)	167	(35.8)	153	(32.8)	82	(17.6)	466

$X^2 = 100.18$ d.f. = 9 P = .000

Infants v. Adults:

$X^2 = 47.59$ d.f. = 3 P = .000

TABLE 6.68a: Blood group phenotype frequencies subdivided according to occupational class.

Class	I		II		III NM + M		IV + V		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. ABO										
A	26	(40.6)	57	(34.1)	67	(44.1)	37	(45.1)	187	(40.2)
O	31	(48.4)	94	(56.3)	62	(40.8)	35	(42.7)	222	(47.7)
B	6	(9.4)	12	(7.2)	20	(13.2)	8	(9.8)	46	(9.9)
AB	1	(1.6)	4	(2.4)	3	(2.0)	2	(2.4)	10	(2.2)
Total	64		167		152		82		465	
2. Rhesus(D)										
D+ve	57	(89.1)	135	(80.8)	117	(77.0)	71	(86.6)	380	(81.7)
D-ve	7	(10.9)	32	(19.2)	35	(23.0)	11	(13.4)	85	(18.3)
Total	64		167		152		82		465	
3. MN										
M	19	(31.1)	54	(32.7)	45	(30.6)	19	(27.9)	137	(31.1)
MN	29	(47.5)	74	(44.8)	76	(51.7)	39	(57.4)	218	(49.4)
N	13	(21.3)	37	(22.4)	26	(17.7)	10	(14.7)	86	(19.5)
Total	61		165		147		68		441	
4. S										
S	3	(4.9)	20	(12.2)	16	(11.1)	8	(10.5)	47	(10.6)
Ss	25	(41.0)	78	(47.6)	66	(45.8)	34	(44.7)	203	(45.6)
s	33	(54.1)	66	(40.2)	62	(43.1)	34	(44.7)	195	(43.8)
Total	61		164		144		76		445	
5. MNS										
MS	1	(1.7)	14	(8.6)	8	(5.8)	3	(4.8)	26	(6.2)
MSs	9	(15.5)	32	(19.8)	19	(13.7)	8	(12.9)	68	(16.2)
Ms	8	(13.8)	7	(4.3)	14	(10.1)	5	(8.1)	34	(8.1)
MNS	1	(1.7)	6	(3.7)	7	(5.0)	4	(6.5)	18	(4.3)
MNSs	11	(19.0)	31	(19.1)	39	(28.1)	18	(29.0)	99	(23.5)
MNs	15	(25.9)	36	(22.2)	28	(20.1)	14	(22.6)	93	(22.1)
NS	1	(1.7)	0		1	(0.7)	0		2	(0.5)
NSs	4	(6.9)	14	(8.6)	6	(4.3)	2	(3.2)	26	(6.2)
Ns	8	(13.8)	22	(13.6)	17	(12.2)	8	(12.9)	55	(13.1)
Total	58		162		139		62		421	
6. Duffy										
Fy^a	14	(22.2)	25	(15.2)	34	(22.8)	13	(16.0)	86	(18.8)
Fy^aFy^b	31	(49.2)	73	(44.5)	67	(45.0)	42	(51.9)	213	(46.6)
Fy^b	18	(28.6)	66	(40.2)	48	(32.2)	26	(32.1)	158	(34.6)
Total	63		164		149		81		457	
7. Kell										
K	0		2	(1.2)	0		0		2	(0.4)
Kk	7	(10.9)	16	(9.6)	9	(5.9)	6	(7.3)	38	(8.2)
k	57	(89.1)	149	(89.2)	143	(94.1)	76	(92.7)	425	(91.4)
Total	64		167		152		79		465	
8. Penney										
Kp^aKp^b	0		4	(2.4)	0		2	(2.5)	6	(1.3)
Kp^b	64	(100.0)	163	(97.6)	145	(100.0)	79	(97.5)	451	(98.7)
Total	64		167		145		81		457	

TABLE 6.68a contd.:

Class	I		II		III MM + M		IV + V		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
9. P_1										
P_1+ve	26	(74.3)	95	(79.8)	35	(63.6)	30	(73.2)	186	(74.4)
P_1-ve	9	(25.7)	24	(20.2)	20	(36.4)	11	(26.8)	64	(25.6)
Total	35		119		55		41		250	

TABLE 6.68b: Statistical data for Table 6.68a.

	All classes					Manual v. non-manual		
	X^2	d.f.	P	Spearman's rho	P	X^2	d.f.	P
1. ABO	9.25	6	.160			6.46	3	.091
2. Rhesus(D)	5.99	3	.112			0.23	1	.634
3. MN	4.01	6	.676	.1326	.050	2.13	2	.345
4. S	4.72	6	.581	.0100	.883	0.35	2	.839
5. MNS	20.94	21	.462			5.99	8	.649
6. Duffy	6.47	6	.372	.0651	.338	1.95	2	.378
7. Kell	3.01	3	.390	-.0814	.229	3.76	1	.053
8. Fenney	0.15	1	.701			0.01	1	.935
9. P_1	5.22	3	.156	-.0802	.238	0.80	1	.370

III manual + non-manual and IV + V were compared, but evidence of a difference between the manual and non-manual groups was found for the phenotypes of the Kell system. There is apparently a higher frequency of the K allele in the non-manual classes.

Table 6.69 shows the distributions of phenotypes of the serum proteins and isoenzymes subdivided according to occupational class, and the results of statistical analysis. A significant difference was found in the case of haptoglobin, but, as the non-significant value of the rank correlation coefficient shows, there was not a consistent trend for any phenotype across social class. There is an apparent excess of the 2-2 and, to a lesser extent, 2-1 phenotypes of the ESD system in the manual groups compared with the non-manual.

Table 6.70 shows the chromosome variant frequencies subdivided according to occupational class, together with the results of statistical analysis for these data. Significant differences were found for variants of chromosome 3 and 21s. In the case of chromosome 3, the frequency of the NN type appears to increase with increasing (that is I to V) occupational class. In the case of chromosome 21s no consistent trend was found for any phenotype.

Table 6.71 shows the distribution of chromosome variant frequencies (intense and brilliant levels of fluorescence combined) subdivided according to occupational class. Again the above results for chromosome 3 were noted, but the rank correlation coefficient was not significant. Chromosome 4 showed a significant increase in frequency of the NN type with increasing occupational class. The difference between the manual and non-manual groups was also significant for this variant.

Table 6.72 shows the distributions of the grouped chromosome variants subdivided according to occupational

TABLE 6.69a: Serum protein and isoenzyme phenotype frequencies subdivided according to occupational class.

Class	I		II		III NM + M		IV + V		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. Haptoglobin										
1 - 1	8	(24.2)	21	(17.9)	5	(11.9)	6	(16.7)	40	(17.5)
2 - 1	9	(27.3)	53	(45.3)	28	(66.7)	15	(41.7)	105	(46.1)
2 - 2	16	(48.5)	43	(36.8)	9	(21.4)	15	(41.7)	83	(36.4)
Total	33		117		42		36		228	
2. Phosphoglucomutase										
1 - 1	43	(68.3)	93	(58.9)	80	(59.3)	43	(54.4)	259	(59.5)
2 - 1	17	(27.0)	52	(32.9)	43	(31.9)	29	(36.7)	141	(32.4)
2 - 2	3	(4.8)	13	(8.2)	12	(8.9)	7	(8.9)	35	(8.0)
Total	63		158		135		79		435	
3. Esterase-D										
1 - 1	50	(82.0)	125	(79.6)	105	(76.6)	52	(68.4)	332	(77.0)
2 - 1	11	(18.0)	30	(19.1)	30	(21.9)	20	(26.3)	91	(21.1)
2 - 1	0		2	(1.3)	2	(1.5)	4	(5.3)	8	(1.9)
Total	61		157		137		76		431	
4. Adenylate kinase										
1 - 1	58	(92.1)	152	(93.8)	131	(94.2)	72	(91.1)	413	(93.2)
2 - 1	5	(7.9)	10	(6.2)	8	(5.8)	7	(8.9)	30	(6.8)
Total	63		162		139		79		443	
5. Acid phosphatase										
A	12	(20.3)	21	(13.1)	17	(12.4)	7	(9.1)	57	(13.2)
BA	20	(33.9)	60	(37.5)	50	(36.5)	29	(37.7)	159	(36.7)
B	22	(37.3)	64	(40.0)	58	(52.3)	30	(39.0)	174	(40.2)
BC	3	(5.1)	10	(6.3)	7	(5.1)	7	(9.1)	27	(6.2)
CA	1	(1.7)	5	(3.1)	5	(3.6)	4	(5.2)	15	(3.5)
C	1	(1.7)	0		0		0		1	(0.2)
Total	59		160		137		77		433	

TABLE 6.69b: Statistical data for Table 6.69a.

	All classes					Manual v. non-manual		
	X^2	d.f.	P	Spearman's rho	P	X^2	d.f.	P
1. Hp	12.39	6	.054	-.0330	.620	5.07	2	.079
2. PGM	3.25	6	.777	.0604	.209	1.28	2	.528
3. ESD	4.63	3	.201	.1039	.031	6.23	2	.044
4. AK	1.00	3	.801	.0058	.903	0.01	1	.913
5. AP	5.86	12	.923			2.84	4	.586

TABLE 6.70a: Chromosome variant phenotype frequencies subdivided according to occupational class.

Class	I No.	%	II No.	%	III M + NM No.	%	IV + V No.	%	Total No.	%
1. Chromosome 3										
BB	0		0		1	(0.7)	1	(1.3)	2	(0.4)
BI	1	(1.7)	14	(8.4)	17	(12.0)	4	(5.1)	36	(8.1)
II	2	(3.3)	26	(15.7)	23	(16.2)	10	(12.8)	61	(13.7)
IN	36	(60.0)	57	(34.3)	55	(38.7)	29	(37.2)	177	(39.7)
BN	9	(15.0)	25	(15.1)	13	(9.2)	9	(11.5)	56	(12.6)
NN	12	(20.0)	44	(26.5)	33	(23.2)	25	(32.1)	114	(25.6)
Total	60		166		142		78		446	
2. Chromosome 13p										
BB	1	(1.7)	0		1	(0.7)	0		2	(0.4)
BI	2	(3.3)	4	(2.4)	7	(4.9)	4	(5.1)	17	(3.8)
II	12	(20.0)	50	(30.1)	30	(21.1)	16	(20.5)	108	(24.2)
IN	30	(50.0)	88	(53.0)	80	(56.3)	48	(61.5)	246	(55.2)
BN	1	(1.7)	1	(0.6)	2	(1.4)	1	(1.3)	5	(1.1)
NN	14	(23.3)	23	(13.9)	22	(15.5)	9	(11.5)	68	(15.2)
Total	60		166		142		78		446	
3. Chromosome 13s										
BI	0		1	(0.6)	0		0		1	(0.2)
IN	13	(21.7)	28	(16.9)	26	(18.3)	15	(19.2)	82	(18.4)
BN	3	(5.0)	5	(3.0)	6	(4.2)	0		14	(3.1)
NN	44	(73.3)	132	(79.5)	110	(77.5)	63	(80.8)	349	(78.3)
Total	60		166		142		78		446	
4. Chromosome 14s										
BI	0		2	(1.2)	2	(1.4)	0		4	(1.9)
II	1	(1.7)	3	(1.8)	2	(1.4)	3	(3.8)	9	(2.0)
IN	19	(31.7)	60	(36.1)	51	(35.9)	24	(30.8)	154	(34.5)
BN	4	(6.7)	6	(3.6)	5	(3.5)	3	(3.8)	18	(4.0)
NN	36	(60.0)	95	(57.2)	82	(57.7)	48	(61.5)	261	(58.5)
Total	60		166		142		78		446	
5. Chromosome 15s										
BI	4	(6.7)	1	(0.6)	0		2	(2.6)	7	(1.6)
II	1	(1.7)	6	(3.6)	0		1	(1.3)	8	(1.8)
IN	19	(31.7)	64	(38.6)	54	(38.0)	37	(47.4)	174	(39.0)
BN	1	(1.7)	6	(3.6)	6	(4.2)	1	(1.3)	14	(3.1)
NN	35	(58.3)	89	(53.6)	82	(57.7)	37	(47.4)	243	(54.5)
Total	60		166		142		78		446	
6. Chromosome 21s										
BI	1	(1.7)	0		0		3	(3.8)	4	(0.9)
II	1	(1.7)	2	(1.2)	1	(0.7)	4	(5.1)	8	(1.8)
IN	19	(31.7)	57	(34.3)	51	(35.9)	12	(15.4)	139	(31.2)
BN	0		3	(1.8)	3	(2.1)	1	(1.3)	7	(1.6)
NN	39	(65.0)	104	(62.7)	87	(61.3)	58	(74.4)	288	(64.6)
Total	60		166		142		78		446	
7. Chromosome 22s										
BB	0		1	(0.6)	0		0		1	(0.2)
BI	2	(3.3)	2	(1.2)	4	(2.8)	2	(2.6)	10	(2.2)
II	3	(5.0)	8	(4.8)	3	(2.1)	4	(5.1)	18	(4.0)
IN	26	(43.3)	68	(41.0)	56	(39.4)	26	(33.3)	176	(39.5)
BN	1	(1.7)	11	(6.6)	5	(3.5)	1	(1.3)	18	(4.0)
NN	28	(46.7)	76	(45.8)	74	(52.1)	45	(57.7)	223	(50.0)
Total	60		166		142		78		446	

TABLE 6.70b: Statistical data for Table 6.70a.

	All classes			Manual v. non-manus		
	χ^2	d.f.	P	χ^2	d.f.	P
Chromosome						
1. 3	24.92	12	.015	3.74	4	.442
2. 13p	11.16	9	.265	1.11	4	.892
3. 13s	4.33	6	.632	1.10	2	.577
4. 14s	1.20	6	.977	0.62	4	.961
5. 15s	6.41	6	.379	7.03	4	.134
6. 21s	17.30	6	.008	5.99	3	.112
7. 22s	6.77	9	.661	5.95	4	.203

TABLE 6.71a: Chromosome variant phenotype frequencies (intense and brilliant levels of fluorescence combined) subdivided according to occupational class.

Class	I		II		III NM + M		IV + V		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. Chromosome 3										
II	3	(5.0)	40	(24.1)	41	(28.9)	15	(19.2)	99	(22.2)
IN	45	(75.0)	82	(49.4)	68	(47.9)	38	(48.7)	233	(52.2)
NN	12	(20.0)	44	(26.5)	33	(23.2)	25	(32.1)	114	(25.6)
Total	60		166		142		78		446	
2. Chromosome 4										
IN	4	(6.7)	24	(14.5)	6	(4.2)	4	(5.1)	38	(8.5)
NN	56	(93.3)	142	(85.5)	136	(95.8)	74	(94.9)	408	(91.5)
Total	60		166		142		78		446	
3. Chromosome 13p										
II	15	(25.0)	54	(32.5)	38	(26.8)	20	(25.6)	127	(28.5)
IN	31	(51.7)	89	(53.6)	82	(57.7)	49	(62.8)	251	(56.3)
NN	14	(23.3)	23	(13.9)	22	(15.5)	9	(11.5)	68	(15.2)
Total	60		166		142		78		446	
4. Chromosome 13s										
II	0		1	(0.6)	0		0		1	(0.2)
IN	16	(26.7)	33	(19.9)	32	(22.5)	15	(19.2)	96	(21.5)
NN	44	(73.3)	132	(79.5)	110	(77.5)	63	(80.8)	349	(78.3)
Total	60		166		142		78		446	
5. Chromosome 14p										
IN	1	(1.7)	3	(1.8)	2	(1.4)	1	(1.3)	7	(1.6)
NN	59	(98.3)	163	(98.2)	140	(98.6)	77	(98.7)	439	(98.4)
Total	60		166		142		78		446	
6. Chromosome 14s										
II	1	(1.7)	5	(3.0)	4	(2.8)	3	(3.8)	13	(2.9)
IN	23	(38.3)	66	(39.8)	56	(39.4)	27	(34.6)	172	(38.6)
NN	36	(60.0)	95	(57.2)	82	(57.7)	48	(61.5)	261	(58.5)
Total	60		166		142		78		446	
7. Chromosome 15p										
II	0		1	(0.6)	0		0		1	(0.2)
IN	2	(3.3)	7	(4.2)	1	(0.7)	2	(2.6)	12	(2.7)
NN	58	(96.7)	158	(95.2)	141	(99.3)	76	(97.4)	433	(97.1)
Total	60		166		142		78		446	
8. Chromosome 15s										
II	5	(8.3)	7	(4.2)	0		3	(3.8)	15	(3.4)
IN	20	(33.3)	70	(42.2)	60	(42.3)	38	(48.7)	188	(42.2)
NN	35	(58.3)	89	(53.6)	82	(57.7)	37	(47.4)	243	(54.5)
Total	60		166		142		78		446	

TABLE 6.71a contd.:

	I		II		III NM + M		IV + V		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
9. Chromosome 21p										
MM	0		6	(3.6)	3	(2.1)	2	(2.6)	11	(2.5)
MN	60	(100.0)	160	(96.4)	139	(97.9)	76	(97.4)	435	(97.5)
Total	60		166		142		78		446	
10. Chromosome 21s										
II	2	(3.3)	2	(1.2)	1	(0.7)	7	(9.0)	12	(2.7)
IN	19	(31.7)	60	(36.1)	54	(38.0)	13	(16.7)	146	(32.7)
NN	39	(65.0)	104	(62.7)	87	(61.3)	58	(74.4)	288	(64.6)
Total	60		166		142		78		446	
11. Chromosome 22p										
II	1	(1.7)	3	(1.8)	1	(0.7)	0		5	(1.1)
IN	10	(16.7)	41	(24.7)	32	(22.5)	13	(16.7)	96	(21.5)
NN	49	(81.7)	122	(73.5)	109	(76.8)	65	(83.3)	345	(77.4)
Total	60		166		142		78		446	
12. Chromosome 22s										
II	5	(8.3)	11	(6.6)	7	(4.9)	6	(7.7)	29	(6.5)
IN	27	(45.0)	79	(47.6)	61	(43.0)	27	(34.6)	194	(43.5)
NN	28	(46.7)	76	(45.8)	74	(52.1)	45	(57.7)	223	(50.0)
Total	60		166		142		78		446	

TABLE 6.71b: Statistical data for Table 6.71a.

	All classes					Manual v. non-manual		
	X^2	d.f.	P	Spearman's rho	P	X^2	d.f.	P
Chromosome								
1. 3	20.70	6	.002	.0117	.854	2.70	2	.260
2. 4	12.29	3	.007	-.1421	.037	8.85	1	.003
3. 13p	6.16	6	.406	.0110	.872	0.13	2	.937
4. 13s	1.35	3	.717	-.0264	.700	0.05	1	.815
5. 14p	0.00	1	.971	-.0498	.466	0.05	1	.829
6. 14s	0.50	3	.920	-.0217	.752	0.51	2	.775
7. 15p	4.65	3	.199	-.0019	.978	0.91	1	.660
8. 15s	2.58	3	.461	-.0332	.627	2.66	2	.265
9. 21p	0.00	1	.964	-.0708	.300	0.01	1	.933
10. 21s	4.22	3	.239	-.0117	.865	4.02	2	.134
11. 22p	3.67	3	.299	-.0076	.912	1.12	2	.572
12. 22s	4.89	6	.558	-.0423	.537	3.47	2	.177

TABLE 6.72a: Frequencies of grouped chromosome variants subdivided according to occupational class.

Class	I		II		III NM + M		IV + V		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. D group satellites (intense)										
0	17	(28.3)	42	(25.3)	42	(29.6)	18	(23.1)	119	(26.7)
1	27	(45.0)	82	(49.4)	66	(46.5)	36	(46.2)	211	(47.3)
2	16	(26.7)	34	(20.5)	31	(21.8)	22	(28.2)	103	(23.1)
⩾3	0		8	(4.8)	3	(2.1)	2	(2.6)	13	(2.9)
Total	60		166		142		78		446	
2. D group satellites (brilliant)										
0	48	(80.0)	145	(87.3)	125	(88.0)	72	(92.3)	390	(87.4)
⩾1	12	(20.0)	21	(12.7)	17	(12.0)	6	(7.7)	56	(12.6)
Total	60		166		142		78		446	
3. D group satellites (all)										
0	13	(21.7)	35	(21.1)	37	(26.1)	16	(20.5)	101	(22.6)
1	27	(45.0)	79	(47.6)	62	(43.7)	35	(44.9)	203	(45.5)
2	16	(26.7)	40	(24.1)	35	(24.6)	24	(30.8)	115	(25.8)
⩾3	4	(6.7)	12	(7.2)	8	(5.6)	3	(3.8)	27	(6.1)
Total	60		166		142		78		446	
4. G group satellites (intense)										
0	17	(28.3)	58	(34.9)	50	(35.2)	34	(43.6)	159	(35.7)
1	30	(50.0)	75	(45.2)	68	(47.9)	34	(43.6)	207	(46.4)
2	13	(21.7)	27	(16.3)	21	(14.8)	5	(6.4)	66	(14.8)
⩾3	0		6	(3.6)	3	(2.1)	5	(6.4)	14	(3.1)
Total	60		166		142		78		446	
5. G group satellites (brilliant)										
0	56	(93.3)	150	(90.4)	130	(91.5)	71	(91.0)	407	(91.3)
⩾1	4	(6.7)	16	(9.6)	12	(8.5)	7	(9.0)	39	(8.7)
Total	60		166		142		78		446	
6. G group satellites (all)										
0	16	(26.7)	49	(29.5)	44	(31.0)	33	(42.3)	142	(31.8)
1	29	(48.3)	76	(45.8)	69	(48.6)	30	(38.5)	204	(45.7)
2	14	(23.3)	34	(20.5)	25	(17.6)	9	(11.5)	82	(18.4)
⩾3	1	(1.7)	7	(4.2)	4	(2.8)	6	(7.7)	18	(4.0)
Total	60		166		142		78		446	
7. Total intense satellites										
0	7	(11.7)	11	(6.6)	18	(12.7)	9	(11.5)	45	(10.1)
1	14	(23.3)	53	(31.9)	45	(31.7)	19	(24.4)	131	(29.4)
2	21	(35.0)	57	(34.3)	39	(27.5)	32	(41.0)	149	(33.4)
3	13	(21.7)	32	(19.3)	27	(19.0)	11	(14.1)	83	(18.6)
⩾4	5	(8.3)	13	(7.8)	13	(9.2)	7	(9.0)	38	(8.5)
Total	60		166		142		78		446	
8. Total brilliant satellites										
0	45	(75.0)	131	(78.9)	115	(81.0)	67	(85.9)	358	(80.3)
⩾1	15	(25.0)	35	(21.1)	27	(19.0)	11	(14.1)	88	(19.7)
Total	60		166		142		78		446	

TABLE 6.72a contd.:

Class	I		II		III NM + M		IV + V		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
9. Total variant satellites										
0	4	(6.7)	8	(4.8)	15	(10.6)	9	(11.5)	36	(8.1)
1	13	(21.7)	44	(26.5)	35	(24.6)	15	(19.2)	107	(24.0)
2	22	(36.7)	54	(32.5)	47	(33.1)	32	(41.0)	155	(34.8)
3	13	(21.7)	41	(24.7)	25	(17.6)	11	(14.1)	90	(20.2)
⩾4	8	(13.3)	19	(11.4)	20	(14.1)	11	(14.1)	58	(13.0)
Total	60		166		142		78		446	
10. Total intense bands										
⩽1	3	(5.0)	5	(3.0)	5	(3.5)	3	(3.8)	16	(3.6)
2	5	(8.3)	18	(10.8)	17	(12.0)	9	(11.5)	49	(11.0)
3	20	(33.3)	35	(21.1)	34	(23.9)	19	(24.4)	108	(24.2)
4	9	(15.0)	33	(19.9)	36	(25.4)	24	(30.8)	102	(22.9)
5	16	(26.7)	35	(21.1)	27	(19.0)	13	(16.7)	91	(20.4)
6	6	(10.0)	24	(14.5)	17	(12.0)	7	(9.0)	54	(12.1)
⩾7	1	(1.7)	16	(9.6)	6	(4.2)	3	(3.8)	26	(5.8)
Total	60		166		142		78		446	
11. Total brilliant bands										
0	38	(63.3)	98	(59.0)	86	(60.6)	53	(67.9)	275	(61.7)
1	15	(25.0)	53	(31.9)	40	(28.2)	18	(23.1)	126	(28.3)
⩾2	7	(11.7)	15	(9.0)	16	(11.3)	7	(9.0)	45	(10.1)
Total	60		166		142		78		446	
12. Total variant bands										
⩽2	5	(8.3)	11	(6.6)	13	(9.2)	6	(7.7)	35	(7.8)
3	14	(23.3)	30	(18.1)	26	(18.3)	18	(23.1)	88	(19.7)
4	12	(20.0)	34	(20.5)	32	(22.5)	21	(26.9)	99	(22.2)
5	16	(26.7)	36	(21.7)	30	(21.1)	19	(24.4)	101	(22.6)
6	11	(18.3)	25	(15.1)	28	(19.7)	8	(10.3)	72	(16.1)
⩾7	2	(3.3)	30	(18.1)	13	(9.2)	6	(7.7)	51	(11.4)
Total	60		166		142		78		446	

TABLE 6.72b: Statistical data for Table 6.72a.

	All classes					Manual v. non-manual		
	X^2	d.f.	P	Spearman's rho	P	X^2	d.f.	P
D group satellites								
1. intense	2.22	6	.898	-.0014	.984	0.87	3	.832
2. brilliant	4.75	3	.191	-.1601	.019	3.86	1	.050
3. i + b	3.47	9	.923	-.0529	.439	3.43	3	.330
G group satellites								
4. intense	4.77	6	.574	-.0317	.643	5.95	3	.114
5. brilliant	0.51	3	.916	.0306	.655	0.48	1	.488
6. i + b	11.93	9	.217	-.0284	.679	4.73	3	.193
All satellites								
7. intense	9.60	12	.651	-.0199	.772	4.35	4	.361
8. brilliant	2.85	3	.415	-.1242	.068	1.74	1	.187
9. i + b	11.36	12	.498	-.0456	.505	13.77	4	.008
All variant bands								
10. intense	19.10	18	.386	-.0526	.442	8.78	6	.187
11. brilliant	3.12	6	.794	-.0380	.579	1.87	2	.393
12. i + b	18.28	15	.248	-.0624	.361	5.92	5	.314

class, and the results of statistical analysis of the data. A significant difference was found between the manual and non-manual groups with regard to the frequency of brilliant satellites of the D group chromosomes. A greater number of these satellites per individual was found in the non-manual classes. A highly significant difference was found between the manual and non-manual groups with regard to the total number of satellites of all types per individual; however, there was no consistent trend for a higher or lower number up or down the occupational grades.

5. Geographical origin.

Information was collected from most individuals participating in this study concerning their place of birth and the places of birth of both their parents and all their grandparents. From Table 4.8, it can be seen that by far the majority of individuals came from within 'old' County Durham (Durham prior to the local government re-organisation.) Thus there seemed little point in analysing the chromosomal and other genetic data with respect to this geographical information. The data were analysed in order to investigate differences between sub-groups of the sample based on smaller geographical units within the county, but as no consistent differences were found, the many space-consuming tables showing this analysis are not given here.

Chapter 7: A COMPARISON BETWEEN CHROMOSOME VARIANT FREQUENCIES OF THE PRESENT STUDY AND THOSE OF OTHER, PUBLISHED, REPORTS.

Surveys of the extent of chromosomal variability in the human species have taken a variety of forms. The karyotypes of many different groups of individuals have been examined with respect to several different aspects of variability. The methodology and findings of some of these studies have been discussed in an earlier section (see above, page 67). In this section studies which sampled populations similar to those of the present study (that is, unselected newborn infants, 'normal' healthy adults and elderly (over 65 years) adults), and investigated the same type of chromosome variants (that is, the response to quinacrine staining of the centromeric regions of chromosomes 3, 4 and 13, and the short arms and satellites of all the acrocentric chromosomes) are considered. Certain of these have been mentioned earlier with regard to aspects of chromosomal variability not examined in the present study (see above, page 65).

Table 7.1 lists those studies which best fit this description and gives the sizes and compositions of the samples involved.

Unfortunately, for the purposes of comparison with the present study, most published reports have not been of random samples of the general population, or if they have, the samples used have often been too small to attach a reasonably small standard of error to the frequencies observed; that is, too small to draw any precise conclusions about the distribution of chromosomal variability in that population.

Often the samples included close relatives, persons

TABLE 7.1: Key to reports quoted in Tables 7.2 to 7.17

Series	Reference	Population	No.
A	Pearson et al. (1973)	Oxford	60
B	"	Leiden	170
C	Geraedts and Pearson (1974)	Dutch	221
D	McKenzie and Lubs (1975)	Newborn infants, Colorado, U.S.A.	77
E	Mikelsaar et al. (1975)	Normal adults, Estonian	208
F	Müller et al. (1975)	Newborn infants, New York, U.S.A.	357
G	Buckton et al. (1976)	Newborn infants, Edinburgh	482
H	"	14-year old children, Edinburgh	109
I	"	65+ year old adults, Barra (Outer Hebrides)	212
J	"	As I, only one person per sibship included	151
K	"	G + H + J	742
L	Lin et al. (1976)	Newborn infants, Ontario, Canada	930
M	Barker et al. (1977)	Amniotic fluid cultures, California, U.S.A.	108
N	Lubs et al. (1977b)	7- and 8-year old Black Americans	210
O	"	7- and 8-year old White Americans	205
P	van Dyke et al. (1977)	Like-sexed caucasian twins, preadolescents and young adults	80
Q	Verma et al. (1977)	Blood donors and volunteers, Denver and New York, U.S.A.	100
R	Yamada and Hasegawa (1978)	Japanese hospital patients and healthy volunteers	400
1	Present study	Total sample	670
2	"	Newborn infants	237
3	"	'Normal' adults	224
4	"	Geriatric patients	174
5	"	Total adults	433

with previously known chromosomal abnormalities, or other abnormalities and diseases, at higher frequencies than occur in the total population of the area. Sometimes it is possible to eliminate from the sample such sources of bias, for example, in the study of Buckton et al. (1976) the sample of elderly persons from Barra is given in a modified form, including only one individual per sibship. In other instances, however, mention is made of the fact that the sample is not recruited at random but no modification of the sample is made so that the variant frequencies of a random sample may be known. For example, in the same study (Buckton et al. 1976), 74 of the newborn infants were included because they were considered in the first three days of life to have a major or minor congenital abnormality. No figures are given to show the variant frequencies of the newborn infants without these 74, and none is given to indicate whether or not inclusion of them distorts the final frequencies.

As will be seen in the following section, several reports include in their samples a number of first-degree relatives.

The 'racial' composition of the sample is often mentioned but as the terms used necessarily embrace rather ill-defined human groups, no real significance can be attached to this information. The samples reported come from geographically widely separated places, but often these locations are hospitals in large cities, and therefore it is unlikely that the samples (being usually fairly small) could possibly represent the population of that place, therefore, it is impossible to say whether or not frequency differences between the samples indicate geographically-related differences.

The sexual composition of the sample is usually noted in published reports, but only rarely are variant frequencies given separately for the two sexes, even when a significant

difference in frequency between the sexes is reported.

In all cases an indication of the broad age-range of the sample is given; (for example, by the use of such terms as 'adult' or 'pre-adolescent'), but the age structure of the sample, or even the average age, is rarely quoted.

Methods of chromosome preparation and staining are not mentioned in the following comments on published reports where they are the same as, or modified versions of those used in the present study. Variations in technique become important with regard to the number of cells analysed per individual to determine the presence or absence of variants, the standards used to classify these variants, and whether the variant regions are analysed directly (by eye) from a microscopic preparation or from a photograph of the stained cell. After 1971, most researchers referred to the recommendations of the Paris Conference (1971) when defining the levels of fluoresecnce scored. Theoretically, this should lead to direct comparability between studies, but actual comparisions lead one to believe that observer-related differences occur in the application of these standards.

A failing of the present study is that there was no consistent attempt to check the repeatability of the classification of the variants throughout the period of analysis, by means of 'blind' analyses on repeat blood specimens. This is a common criticism of similar studies, but there are instances of attempts to check the maintenance of standards of scoring, and to quantify the "objectiveness" of the method of scoring (for example, by Van Dyke et al. 1977). Mckenzie and Lubs (1975) and Lubs et al. (1977). both investigated the relationship between the quality of chromosome preparation and the frequency of chromosome variants. Such a relationship was found in both studies (see above, page 115).

Many researchers have compared their results with those obtained by others, but statistical evidence for the often-reported conclusions that their frequencies coincide with those from other series, or are "substantially different" is almost never given. Nor is any indication given that these comparisons have even been subjected to a statistical analysis.

In some cases variant frequencies only are given, with no indication as to whether they occurred in the homzygous or heterozgous state. A certain amount of information is lost by this manner of presentation, and therefore only an incomplete comparison with other studies can be made.

Many authors compared their chromosome variant frequencies with those expected under a Hardy-Weinberg equilibrium. Agreement with such expectations was noted more commonly than lack of agreement.

Published Reports Giving Results Comparable With the Present Study.

A brief summary is given here of the findings of other studies which are comparable with present one. The reports are dealt with in chronological order of their publication. The series code, given alongside the reference to the report, is that given in table 7.1 and is used to refer to the series in the tables of this chapter.

The results of a statistical comparison of some of these studies with the present study is to be found in the following section.

1. Wahlstrom, J. (1971)

The results if this study were published before the general agreement on standards of scoring was reached by

the Paris conference in 1971. The frequency of the apparently brilliant regions of chromosome 3 was reported in a very small sample (N=46) which included 5 members of one family. The simple Mendelian inheritance of this variant region was shown in this family.

2. Pearson, P.L. et al. (1973). Series A and B.

This paper gives the results of chromosome analysis of a Dutch group from Leiden, and an English group from Oxford. Three categories of fluorescence are recognised; intense, normal and negative. The intense category is equivalent to level 5 of the Paris conference (brilliant). It is unclear what 'normal' refers to. It seems to be equivalent to the brightness of "some" satellites. The authors mention that the variant on the short arm of chromosome 13 is the most common but do not make a distinction in the table of results between the short arm and the satellites of the acrocentric chromosomes, and so comparisons with frequencies in other reports is only possible for chromosomes 3 and 4. It is stated that the frequencies of all the polymorphisms fit Hardy-Weinberg espectations, and that there are no overall differences between the Dutch and English samples.

An average of 2 variants per individual were found, but this figure includes C-band variants in chromosomes 1, 9 and 16 (not examined in the present study).

3. Geraedts, J.P.M. and Pearson, P.L.(1974) Series C.

This paper reported the chromosome variant frequencies from a Dutch sample (N=221) of both sexes, which included 14 patients "referred for diagnostic purposes". Most individuals were sampled in connection with family studies. 19 families were involved, and therefore each person in the sample was related on average to about 9 others in the sample.

The variants were classified into the same three categories as in Pearson et al. (1973).

An average of 4 variants per individual was found, with approximately half of these on chromosomes 3 and 13. No distinction was made between the short arms and the satellites of the acrocentric chromosomes. No significant sex differences were found.

4. Schnedl, W. (1974)

The results of chromosome analysis of 58 Viennese persons by this author were quoted in Schwarzacher (1976). The term 'strong' fluorescence was used to define the variants; it is not clear to which level of fluorescence (Paris conference) this refers.

5. Hauge, M. et al. (1975).

This paper reports the frequency of chromosome variants in a sample consisting of 50 mother/foetus pairs. A group of 40 unrelated Icelandic persons was also examined, but all these individuals were available for analysis because of their having qualified to participate in another study for reasons which definitely indicate that they do not form a random sample of the Icelandic population.

The same markers as in the present study were examined. Fluorescence scores of 0 to 3 were given to the variant regions but it is not clear to what these scores refer. Regions having a score of 1 or more classified as "variants".

Perhaps the most useful result of this study is the confirmation of the genetic determination of the fluorescent markers, and the demonstration that foetal cells can be distinguished from maternal cells using these variables.

6. McKenzie, W.H. and Lubs, H.A. (1975). Series D.

This paper reports the chromosome variability found in a sample of 77 newborn infants (both sexes) from Grand Junction, Colorado, U.S.A. C-band variability was also examined. The Q-bands were scored usually from photographs, with each presumed variant being verified densitometrically. (This is the only instance found of such verification.) All 5 levels of fluorescence (Paris conference 1971) were identified, but an intermediate category of "borderline Q-intensity variants" had to be designated. These borderline variants were included in comparisons with the present study according to whether they were classified as borderline-intense or borderline-medium by the authors.

A relationsip between the technical quality of the cell preparation and the frequency of fluorescent variants was demonstrated by the authors. There were more variants in excellent as opposed to good cultures (3.26 ± 0.18 Q-band variants in excellent cultures, 2.40 ± 0.22 in good; a difference which is significant at the 1% level). It appears that the sample contains results from both good and excellent cultures as it was stated that the average number of Q-band variants per subject is 2.92 ± 0.15 (4.44 per subject if borderline-intense variants are included). No sex difference is reported for the separate Q-band variant regions. It is noted that the mean number of all variants whether Q or C per subject is slightly higher in males than in females, but not significantly so.

The authors also reported that there was no significant difference between the frequencies of chromosome variants in newborn infants born to mothers who had been subjected to higher than usual levels of radiation and those exposed to lower levels.

7. Mikelsaar, A.-V.N. et al. (1975). Series E.

In the last of a series of 3 papers on human karyotype

polymorphisms, these authors report Q-band variant frequencies in a group of 208 normal adult Estonians. Two other groups are also examined:

(i) 80 children with mental retardation of unknown aetiology, and

(ii) 61 children with Down's syndrome,

but neither of these two groups will be compared with the present study.

These authors use only the term 'brilliant' to describe fluorescent variants, but in an earlier paper in the series (Mikelsaar et al. 1973) certain satellites are marked with an asterisk, others with two. This apparently reflects differences in the intensity of fluorescence, but whether or not the distinction is between intense and brilliant, or brilliant and very brilliant is not made clear.

A size factor is introduced into the assessment of the fluorescence of the satellites on the acrocentric chromosomes, viz.:

"Only brilliant satellites equal or greater in size to the proximal part of the acrocentric chromosomes in the overwhelming majority of cells (practically 100% of optimal metaphases) were taken into consideration."

In the second paper in the series (Mikelsaar et al. 1974) the authors stated that there was no deviation from Hardy-Weinberg expectations for any chromosome variable when the two sexes were combined. It is nowhere explicitly stated that the sample analysed on the third paper is the same as that reported in the second, but the two sample sizes are very similar (N=208 and N=207) and the variant frequencies are very similar for both. Therefore it would seem that the adult series of the third paper in the series (Mikelsaar et al. 1975) was the same as the series reported in the second paper. However, in the third paper of the series it is reported that chromosomes 14s, 21s, 22p and 22s

show an excess of homozygotes when compared with Hardy-Weinberg expectations. For chromosome 14s, this excess occurs in both sexes, but in 21s and 22p is confined to the males and in 22s to the females.

A significant difference in the distribution of homozygotes and heterozygotes for chromosome 3 between the sexes is also reported. (Excess heterozygotes were found in the females, compared with the males.)

8. Müller, H.J. et al. (1975) Series F.

This paper reported the chromosome polymorphism frequencies found in a sample of 357 newborn infants from New York, U.S.A.. Q- and C-band variants were both analysed and each infant was found to have a unique karyotype, when either Q- (including length variations of these regions) or C-bands were considered separately. The karyotype of each infant was first examined using G-banding methods, and any found to have an abnormality was excluded from the Q-band study.

Only "bright" variants were distinguished in this study. This term includes regions fluorescing at both level 4 and level 5 (Paris Conference).

All variant frequencies were found to agree with Hardy-Weinberg expectations, with the exception of chromosomes 3 and 22; in both cases there was a significant excess of heterozygotes.

The authors also investigated relationships between the different acrocentric chromosome variants. They found that satellites were present randomly on any particular D group chromosome, and that there was no relationship between the fluorescence intensity of the short arm of a chromosome and that of its satellite. They found that both 22p and 22s

variants were slightly more common, respectively, than 21p and 21s variants.

It is stated the "there is good general agreement between this study and others regarding the general magnitude of variants on specific chromosomes" but there is no mention of whether or not statistical tests have been used to show this.

9. Buckton, K.E. et al. (1976). Series G,H,I,J and K.

Q- and C-band polymorphism frequencies of three Scottish populations are reported in this paper. The three series are (i) 482 newborn infants and (ii) 109 14 year-old children, both from the Edinburgh area, and (iii) that part of the population of Barra (Outer Hebrides) aged 65 years or older. Individuals with a "constitutional" chromosomal abnormality were excluded from all series. As mentioned earlier (see above, page 334), the newborn infant series included quite a large proportion (74/482) with a major or minor congenital abnormality. The children were sampled over a period of two years and therefore some may belong to the same sibship, but this proportion is unknown.

Fluorescence levels 4 and 5 were distinguished in this study. The average number of variant bands per individual for each series is given as 4.17 for the newborn infants, 3.9 for the children and 2.9 for the elderly group from Barra.

The authors report that there is no obvious inconsistency with Hardy-Weinberg expectations, and no sex difference in the frequencies for chromosome 3.

10. Lin C.C. et al. (1976). Series L.

This paper gives the results of chromosomal analysis of a series of 930 consecutive newborn infants from Ontario, Canada. Two sets of multiple births were included (one pair of twins and one set of triplets). 87% of the parents are described as being Caucasian, the rest being Negro and Oriental. There is no subdivision of the sample into these categories with respect to chromosome variant frequencies. Five infants were found with major chromosomal abnormalities (2 47, XYY; 1 47, XXY; and two autosomal abnormalities).

Fluorescence levels 4 and 5 (Paris Conference) were distinguished, and an average of 2.1 variants found per individual. Sex differences were examined for chromosomes 3, 4 and 13p, and none found.

Comparisons were made between these results and those of other studies and indications of the significance of any difference given.

11. Schwinger, E. and Wehner, H. (1976).

This paper reports the chromosome variant frequencies of a group of 336 persons. However, as 247 of these individuals were included because of being suspected of having various chromosomal aberrations, the results are of little value for comparison with studies such as the present one. The 247 persons were placed in 13 diagnostic categories (with a maximum of 75 in one category, and five categories having less than 10), and therefore, not surprisingly, no useful conclusions may be drawn regarding the chromosome variant frequencies in the different categories.

Results from a group of 58 'normal' persons, whose chromosome preparations showed type B fluorescence (see above, page 115) characteristics, are given. These normal individuals were said to be a random sample of the population,

but as they were either participants in family examinations, or students, they clearly are not. There is no indication of how many of these individuals were related to each other.

The variant regions examined were the same as those of the present study, but as their fluorescence was classified as medium (level 3, Paris Conference) or 'strong' (level 5, Paris Conference) the results obtained are not comparable with those of the present study, nor with those of most other published reports.

12. Barker, P.E. et al. (1977). Series M.

This paper reports the fluorescent chromosome polymorphism results of a series of 108 mid-trimester diagnostic amniotic cell cultures. Unfortunately this series does not form a random sample of foetuses in the mid-trimester stage of development, as amniocentesis is only performed if there is some indication for it. Chromosome aberrations were found at a higher frequency than in studies on newborn infants; this may indicate a real difference found at different development stages or may be a reflection of, for instance, the raised mean maternal age of the sample.

The variants were scored as either present or absent, the category 'present' meaning present with at least an intense level (level 4) of fluorescence. The most frequent variants were on chromosomes 13p and 3. The modal number of variants per individual was 4, with a range of 0 to 11. The results were compared with those of other series, and found to be similar to one (Geraedts and Pearson (1974)) but not to the other (Hauge et al. (1975)).

13. Lubs, H.J. et al. (1977). Series N and O.

This study examined a group of 7 and 8 year old American children categorised into two racial groups; Black and White.

A further subdivision was made in the analysis on the basis of IQ test scores. The chromosome variants of a total of 415 children were investigated, and scored according to the Paris Conference standards (negative, intense and brilliant).

Following the discovery that almost twice as many Q-band variants were found in good quality cell preparations as in poor, a fairly thorough analysis of the results was carried out to eliminate technical variations as a source of error in interpretation of the results. For example, the quality of the preparations from different centres (where the children might have been predominantly black or predominantly white) was compared. No differences in banding quality were found in the different racial or IQ score groups.

Significant differences were found between the racial groups regarding variant frequencies. In most cases, the polymorphism was more common in the Black children than in the White. In other instances where the difference was not significant, the variant frequency was often higher in the Black than in the White children. Significant racial differences were found in the following variant regions: Chromosomes 3 (intense), 4 (brilliant and intense combined together), 13p (intense), 13p (brilliant), 13s (intense and brilliant combined), and 22s (intense and brilliant combined).

Only in the case of chromosome 4 were the brighter variants more common in the White children.

An average of 3.3 variants was found per child.

14. Van Dyke, D.L. et al. (1977). Series P.

The purpose of this study was not so much to report the variant frequencies of a sample of a particular population as to evaluate the heritability and stability of the variants, and the reliability of the method of scoring

them, and to demonstrate the application of chromosome analysis for the purposes of zygosity determination of twins. With regard to this latter aim, the chromosome polymorphism data obtained from a series of 40 pairs of twins was in complete agreement with similar blood group antigen data for the twins.

Repeated Q-band analysis showed that out of 216 opportunities for errors of scoring to occur, 10 (two 2-step and eight 1-step) were found. These errors mostly involved the chromosome regions 4c and 22p.

An average of 3.4 variants was found per individual. The variants were classified according to the usual standards (Paris Conference), intense and brilliant levels of fluorescence being combined.

The authors report that the frequencies found were similar to those of Caucasian populations for chromosomes 3c, 4c and 13c, with the frequency of chromosome 22c variants being "somewhat higher". No statistical evidence for this statement is given.

15. Verma, R.S. et al. (1977). Series Q.

This paper reports the Q-band and acridine-orange (RFA)-band variant frequencies of a sample of adults (aged 25 to 65 years) consisting of 70 blood donors from Denver, 20 blood donors from New York and 10 volunteers from New York. The individuals were all healthy and unrelated.

Only variant regions on the acrocentric chromosomes were examined. The Q-band variants were classed as either intense or brilliant and were usually scored from photographic prints. No sex differences were found in the variant frequencies. The results were compared with those of other studies and the authors found "some coincidental frequencies,

and some substantial differences."

16. McCracken, A.A. et al. (1978).

This study involved the examination of the fluorescent chromosome variants of 24 pairs of twins. The zygosity of the twins determined by the chromosomal polymorphisms agreed with that found from an examination of certain blood group antigens. The authors reported that there was only a random association between the polymorphisms, and that "the variant regions were distributed randomly according to their frequency values."

It was also reported that the variant frequencies corresponded well with those of other studies, but no results of a statistical comparison were given.

17. Yamada, K. and Hasegawa, T. (1978). Series R.

This paper reports the chromosome polymorphism frequencies of a series of 400 Japanese individuals of whom 350 were hospital patients with a variety of diseases (cancer, leukaemia and others) and 50 healthy volunteers. Patients with hereditary diseases were excluded, but not those with a disease which had a possible hereditary component in its aetiology.

The same chromosome regions as in the present study were examined, according to the same standards of scoring. An average of 3.83 (S.D. 1.86) variant bands was found per individual, with a range of 0 to 8. There was no significant sex difference in any variant frequency.

The authors compared these results with those of other studies but unfortunately some of the reports they chose were not reporting comparable frequencies.

Results of a Statistical Comparison Between the Chromosome Variant Frequencies of the Present Study and Published Reports.

Studies using samples consisting of less than sixty persons have not been included in the various comparisons made, although mention has been made of their findings in the previous section. The study of Van Dyke et al. (1977) (Series P) has been included, however, although the adjusted sample size is only 47.67 (allowance having been made for the presence of monozygotic and dizygotic twins in the sample).

'Phenotype' frequencies of chromosome variants of two published reports, Buckton et al. (1976) and Lin et al. (1976) are given in table 7.2. The results of comparisons between these series and the various series comprising the present study by means of the X^2 test are given in table 7.3. In many cases the P value of the X^2 is not recorded. This is because of its very small size in these cases. An idea of the magnitude of P can be gained by reference to table 7.4, which gives P values for some very high X^2 values. As is usual in the X^2 test it was often necessary to amalgamate certain phenotype classes when making the comparisons. At times, the phenotype frequencies were so different from each other that amalgamation of rare classes in one series led to amalgamation of fairly common phenotype classes in the other series. This seemed to make a nonsense of the procedure and in these cases the comparison was made between only those classes that did not require amalgamation in either series. This obviously gives a much lower X^2 value than would otherwise have been obtained, and in fact the need to do this shows the two series to be very different with regard to phenotype frequencies without any statistical test being performed on the data.

Table 7.3 shows that only in the case of chromosome 21p were the phenotype frequencies found in the total sample of the present study to be not very significantly

TABLE 7.2: Phenotype frequencies of chromosome variants in published reports.

		chromosome 3		4		13p		13s		14p		14s	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Series													
	BB	59	(12.2)	3	(0.6)	48	(9.9)	2	(0.4)			0	
	BI	98	(20.3)	21	(4.4)	19	(3.9)	1	(0.2)			1	(0.2)
G	II	48	(9.9)	98	(20.3)	15	(3.1)	2	(0.4)			2	(0.4)
—	IN	96	(19.9)	196	(40.7)	63	(13.0)	23	(4.8)			46	(9.5)
	BN	120	(24.9)	26	(5.4)	139	(28.8)	52	(10.7)			47	(9.7)
	NN	61	(12.6)	138	(28.6)	198	(41.0)	402	(83.4)			386	(80.1)
X^2(HW)		0.47		2.18		10.11		1.21				1.89	
	BB	5	(4.6)	0		2	(1.8)	0				0	
	BI	23	(21.1)	5	(4.6)	4	(3.7)	3	(2.7)			0	
H	II	20	(21.1)	8	(7.3)	8	(7.3)	0				2	(1.8)
—	IN	39	(35.7)	42	(38.5)	23	(21.1)	5	(4.6)			11	(10.0)
	BN	14	(12.8)	5	(4.6)	14	(12.8)	6	(5.5)			14	(12.8)
	NN	8	(7.3)	49	(44.9)	58	(53.2)	95	(87.2)			82	(75.2)
X^2(HW)		1.47		0.75		2.63		2.34				0.30	
	BB	3	(1.4)	0		4	(1.8)	1	(0.5)			0	
	BI	44	(19.3)	1	(0.5)	2	(0.9)	1	(0.5)			0	
I	II	41	(19.3)	3	(1.4)	5	(2.6)	0				0	
—	IN	72	(34.0)	76	(35.8)	35	(16.5)	12	(5.6)			18	(8.5)
	BN	24	(11.3)	3	(1.4)	25	(11.8)	28	(13.2)			7	(3.3)
	NN	31	(14.6)	129	(60.8)	141	(66.5)	170	(80.2)			187	(88.2)
X^2(HW)		2.25		2.68		2.91		0.02				0.06	
	BB	1	(0.7)	0		3	(1.9)	1	(0.7)			0	
	BI	24	(15.8)	1	(0.7)	2	(1.3)	1	(0.7)			0	
J	II	27	(17.8)	0		4	(2.6)	0				0	
—	IN	53	(35.5)	52	(34.4)	28	(18.5)	10	(6.6)			12	(7.9)
	BN	19	(12.6)	1	(0.7)	18	(11.9)	20	(13.2)			6	(3.9)
	NN	27	(17.9)	97	(64.2)	96	(63.6)	119	(78.8)			133	(88.0)
X^2(HW)		1.80		3.69		1.82		0.01				0.03	
	BB	65	(8.8)	3	(0.4)	53	(7.1)	3	(0.4)			0	
	BI	145	(19.5)	27	(3.6)	25	(3.4)	5	(0.7)			1	(0.1)
K	II	95	(12.8)	106	(14.3)	27	(3.6)	2	(0.3)			4	(0.5)
—	IN	188	(25.3)	290	(39.1)	114	(15.4)	38	(5.1)			69	(9.3)
	BN	153	(20.6)	32	(4.3)	171	(23.0)	78	(10.5)			67	(9.0)
	NN	96	(12.9)	284	(38.3)	352	(47.4)	616	(83.0)			601	(81.0)
X^2(HW)		0.24		3.26		18.46*		1.54				3.83	
	BB	83	(8.9)	0		2	(0.2)	0		0		0	
	BI	124	(13.3)	0		6	(0.6)	0		0		0	
L	II	112	(12.0)	47	(5.1)	114	(12.3)	0		5	(0.5)	0	
—	IN	275	(29.6)	169	(18.2)	321	(34.5)	29	(3.1)	5	(0.5)	0	
	BN	119	(12.8)	0		18	(1.9)	6	(0.6)	0		3	(0.3)
	NN	217	(23.3)	714	(76.8)	469	(50.4)	895	(96.2)	920	(99.0)	927	(99.7)
X^2(HW)		28.11*		21.06*		11.17*		0.01		0.65		0.16	

* significant at the 5% level

TABLE 7.2 contd.:

		chromosome											
		15p		15s		21p		21s		22p		22s	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Series													
	BB			3	(0.6)	0		1	(0.2)	0		0	
	BI			1	(0.2)	0		2	(0.4)	0		5	(1.0)
G	II			1	(0.2)	0		1	(0.2)	2	(0.4)	4	(0.8)
—	IN			63	(13.1)	5	(1.0)	50	(10.3)	19	(3.9)	67	(13.9)
	BN			48	(10.0)	2	(0.4)	35	(7.3)	6	(1.2)	60	(12.4)
	NN			366	(75.9)	475	(98.5)	393	(81.5)	455	(94.4)	346	(71.8)
X^2(HW)				0.91		0.00		0.12		1.66		0.80	
	BB			0		0		0		0		2	(1.8)
	BI			0		0		0		0		1	(0.9)
H	II			0		0		1	(0.9)	0		0	
—	IN			17	(15.6)	1	(0.9)	22	(20.2)	2	(1.8)	13	(11.9)
	BN			6	(5.5)	0		11	(10.1)	2	(1.8)	10	(9.2)
	NN			86	(78.9)	108	(99.1)	75	(68.8)	105	(96.3)	83	(76.1)
X^2(HW)				0.13		0.50		1.11		0.12		0.66	
	BB			0		0		0		0		0	
	BI			0		0		0		0		0	
I	II			0		0		0		1	(0.5)	1	(0.5)
—	IN			26	(12.3)	1	(0.5)	20	(9.4)	4	(1.8)	22	(10.4)
	BN			17	(8.0)	0		7	(3.3)	0		23	(10.8)
	NN			169	(79.7)	211	(99.5)	185	(87.3)	207	(97.6)	166	(78.3)
X^2(HW)				0.16		0.48		0.05		0.00		0.94	
	BB			0		0		0		0		0	
	BI			0		0		0		0		0	
J	II			0		0		0		1	(0.7)	1	(0.7)
—	IN			21	(13.9)	1	(0.7)	10	(6.6)	1	(0.7)	18	(11.9)
	BN			11	(7.3)	0		5	(3.3)	0		15	(9.9)
	NN			119	(78.8)	150	(99.3)	136	(90.1)	149	(98.7)	117	(77.5)
X^2(HW)				0.15		0.50		0.02		0.00		0.46	
	BB			3	(0.4)	0		1	(0.1)	0		2	(0.3)
	BI			1	(0.1)	0		2	(0.3)	0		6	(0.8)
K	II			1	(0.1)	0		2	(0.3)	3	(0.4)	5	(0.7)
—	IN			101	(13.6)	7	(0.9)	82	(11.1)	22	(3.0)	98	(13.2)
	BN			65	(8.8)	2	(0.3)	51	(6.9)	8	(1.1)	85	(11.5)
	NN			571	(77.0)	733	(98.8)	604	(81.4)	709	(95.5)	546	(73.6)
X^2(HW)				2.73		0.00		0.36		2.50		0.45	
	BB	0		2	(0.2)	0		0		0		0	
	BI	0		1	(0.1)	0		0		0		0	
L	II	0		0		0		1	(0.1)	0		0	
—	IN	2	(0.2)	2	(0.2)	2	(0.2)	18	(1.9)	5	(0.5)	2	(0.2)
	BN	1	(0.1)	1	(0.1)	0		0		0		4	(0.4)
	NN	927	(99.7)	925	(99.5)	928	(99.8)	911	(98.0)	925	(99.5)	924	(99.4)
X^2(HW)		0.16		0.31		0.23		0.00		0.10		0.06	

TABLE 7.3: A comparison of the phenotype frequencies of chromosome variants in the present study with those in published reports.

Series		chromosome 3	4	13p	13s	14p	14s	15p	15s	21p	21s	22p	22s
G v. 1	X^2	197.60	336.24*	503.42	70.55*		107.25		115.40	1.20	93.98	66.68	104.33
	d.f.	5	2	5	3		4		4	2	5	3	4
	P	**								.55			
H v. 1	X^2	50.65	123.94*	144.03	19.91		38.64		24.28*	0.20	27.31	25.02	33.03
	d.f.	5	2	5	3		3		2	1	3	2	3
	P				$.18 \times 10^{-3}$					.35			
J v. 1	X^2	16.51	74.56	228.17	39.77		41.26*		37.72*	0.72	39.79*	32.28	50.86
	d.f.	5	1	5	3		2		2	1	2	1	3
	P	$.55 \times 10^{-2}$								.40			
K v. 1	X^2	157.79	303.20	550.98	86.47		143.32		147.55	1.92	113.75	107.54	146.54
	d.f.	5	2	5	3		4		4	2	4	3	5
	P									.38			
L v. 1	X^2	82.81	57.22	222.91	121.71	5.83	419.07	23.01	489.21	11.99	301.19*	196.69	510.96*
	d.f.	5	1	5	2	1	1	1	2	1	2	1	2
	P					.02				$.05 \times 10^{-2}$			

* some classes have been ignored in the calculation of X^2

** P values are not given where they are very small. For an indication of their magnitude, see Table 7.4.

TABLE 7.3 contd.:

Series		chromosome 3	4	13p	13s	14p	14s	15p	15s	21p	21s	22p	22s
	X^2	95.40	263.45	305.03	27.99		68.58		78.77	0.11	30.80	32.80	77.46
G v. 2	d.f.	5	2	5	2		3		4	1	3	2	4
	P									.74			
	X^2	34.93	81.94	102.83	8.81*		28.99		67.25*	0.12	15.08	13.18	31.45
H v. 2	d.f.	5	1	5	2		3		2	1	3	1	3
	P				.01		$.22 \times 10^{-5}$			.73	.0018	.0003	
	X^2	38.43	34.82	127.00	56.98	0.88	21.20*	3.84	416.44	8.42	162.39	152.30	427.89
L v. 2	d.f.	5	1	5	2	1	1	1	3	1	1	1	2
	P					.35		.05		.004			
	X^2	10.69	32.21	101.14	25.93		34.21		33.38	0.65	39.93	29.20	31.36
J v. 4	d.f.	4	1	4	3		2		2	1	2	1	3
	P	.0303			$.98 \times 10^{-5}$					.42			
	X^2	42.81	90.58*	115.66	18.26		34.49		23.43*	0.17	25.05	23.65*	30.01
H v. 5	d.f.	5	2	5	3		3		2	1	3	2	3
	P				.00039					.68	$.15 \times 10^{-4}$		
	X^2	13.58	53.48	180.31	33.33		40.20*		34.54*	0.63	47.37*	33.85	44.94
J v. 5	d.f.	5	1	5	3		2		2	1	2	1	3
	P	.0185								.43			

* some classes have been ignored in the calculation of X^2

TABLE 7.4: Probabilities that high X^2 values are obtained by chance alone.

X^2	degrees of freedom				
	1	2	3	4	5
15	.00011	.00055	.0018	.0047	.0104
20	$.77 \times 10^{-5}$	$.45 \times 10^{-4}$	.00017	.00050	.0012
25	$.57 \times 10^{-6}$	$.37 \times 10^{-5}$	$.15 \times 10^{-4}$	$.50 \times 10^{-4}$	.00014
30	$.44 \times 10^{-7}$	$.31 \times 10^{-6}$	$.14 \times 10^{-5}$	$.49 \times 10^{-5}$	$.15 \times 10^{-4}$
35		$.26 \times 10^{-7}$	$.12 \times 10^{-6}$	$.47 \times 10^{-6}$	$.15 \times 10^{-5}$
40			$.11 \times 10^{-7}$	$.45 \times 10^{-7}$	$.15 \times 10^{-6}$
45					$.15 \times 10^{-7}$

different from those in the published reports. Comparisons were made with the newborn infants, adults and adults over the age of 65 years where appropriate, and again, the only similarity noted was in the case of chromosome 21p.

The frequencies of the individual variant classes found in published reports are recorded in table 7.5. These have been calculated from the published 'phenotype' frequencies. They have been compared with those of the present study by finding the ratio of the difference between the frequencies over the standard error of that difference, which is determined using the following formula:

$$SE_{diff} = \sqrt{SE_1^2 + SE_2^2}$$ (Sturtevant and Beadle, 1962).

If this ratio exceeds 1.96 then the two frequencies being compared are significantly different at the 5% level. The results of such a comparison (the ratios) are given in table 7.6, with those values that are equal to or below 1.96 marked with an asterisk. It appeared from a glance at the table that it was the more common variants that seemed to differ significantly from each other, whilst the more rare variants tended to be similar in published reports and in the present study.

This idea is represented graphically in figure 7.1. It can be seen that for the very frequent variants (3 and 13p) and the very rare variants (21p and 14p) this relationship holds. The other variants (particularly 15p, 22p and 13s), with the exception of chromosome 4, occupy a position which might indicate that the relationship mentioned above exists but is not obviously linear.

An explanation for this supposed relationship between the frequency of chromosome variants and the coincidence of these frequencies in different reports does not come readily to mind. It is possibly due to an increased frequency of

TABLE 7.5: Frequencies of chromosome variants ±1.96 S.E. in published reports.

Series	chromosome 3 B	3 I	3 N	4 B	4 I	4 N	13p B	13p I	13p N	13s B	13s I	13s N
D	.4091 ±.0901	.3506 ±.0849	.2403 ±.0726	.0130 ±.0179	.3961 ±.0890	.5909 ±.1019	.0325 ±.0282	.4091 ±.0901	.5584 ±.1002	.0065 ±.0127	.0197 ±.0219	.9740 ±.1116
G	.3485 ±.0347	.3008 ±.0319	.3506 ±.0339	.0550 ±.0146	.4284 ±.0366	.5166 ±.0391	.2635 ±.0302	.1162 ±.0209	.6203 ±.0413	.0591 ±.0151	.0291 ±.0107	.9118 ±.0445
H	.2156 ±.0582	.4679 ±.0795	.3165 ±.0685	.0459 ±.0281	.2890 ±.0660	.6651 ±.0884	.1009 ±.0411	.1972 ±.0560	.7018 ±.0896	.0413 ±.0267	.0367 ±.0252	.9220 ±.0936
I	.1675 ±.0373	.4599 ±.0566	.3726 ±.0524	.0094 ±.0092	.1958 ±.1400	.7948 ±.0659	.0825 ±.0268	.1108 ±.0308	.8066 ±.0660	.0731 ±.0253	.0307 ±.0165	.8962 ±.0669
J	.1490 ±.0419	.4338 ±.0657	.4172 ±.0648	.0066 ±.0091	.1755 ±.0451	.8179 ±.0784	.0861 ±.0324	.1258 ±.0387	.7881 ±.0779	.0762 ±.0305	.0364 ±.0213	.8874 ±.0792
K	.2884 ±.0253	.3524 ±.0274	.3592 ±.0276	.0438 ±.0105	.3565 ±.0275	.5997 ±.0330	.2035 ±.0218	.1301 ±.0177	.6664 ±.0339	.0600 ±.0123	.0317 ±.0090	.9083 ±.0358
L	.2199 ±.0201	.3349 ±.0240	.4452 ±.0267	.0000	.1414 ±.0224	.8586 ±.0224	.0151 ±.0056	.2984 ±.0229	.6866 ±.0305	.0032 ±.0026	.0156 ±.0057	.9812 ±.0321
N	.3964 ±.0539	.2794 ±.0469	.2986 ±.0482				.1961 ±.0402	.3424 ±.0509	.4567 ±.0568			
O	.3737 ±.0534	.1594 ±.0371	.4632 ±.0577				.0557 ±.0225	.2436 ±.0448	.7006 ±.0653			
Q							.0405 ±.0291	.2400 ±.0637	.7150 ±.0939	.0200 ±.0195	.0200 ±.0195	.9600 ±.0979
R							.4275 ±.0402	.0200 ±.0098	.5525 ±.0438	.0600 ±.0167	.0613 ±.0169	.8788 ±.0486

TABLE 7.5 contd.:

Series	chromosome 14p B	14p I	14p N	14s B	14s I	14s N	15p B	15p I	15p N	15s B	15s I	15s N
D	.0000	.0000	1.0000	.0000	.0455 ±.0465	.9545 ±.0465	.0000	.0000	1.0000	.0000	.0130 ±.0253	.9870 ±.0253
G				.0498 ±.0139	.0529 ±.0143	.8973 ±.0444				.0570 ±.0151	.0685 ±.0162	.8745 ±.0443
H				.0642 ±.0281	.0688 ±.0342	.8670 ±.0930				.0275 ±.0219	.0780 ±.0363	.8945 ±.0933
I				.0165 ±.0122	.0425 ±.0194	.9410 ±.0672				.0401 ±.0189	.0613 ±.0232	.8986 ±.0670
J				.0199 ±.0158	.0397 ±.0222	.9404 ±.0796				.0364 ±.0213	.0695 ±.0292	.8940 ±.0763
K				.0485 ±.0075	.0526 ±.0115	.9016 ±.0358				.0485 ±.0111	.0701 ±.0132	.8814 ±.0357
L	.0000	.0081 ±.0058	.9919 ±.0058	.0016 ±.0026	.0000	.9984 ±.0026	.0005 ±.0010	.0011 ±.0015	.9984 ±.0321	.0032 ±.0026	.0011 ±.0015	.9957 ±.0321
N												
O												
Q	.0000	.0000	1.0000	.0100 ±.0138	.0150 ±.0169	.9750 ±.0980	.0000	.0050 ±.0138	.9950 ±.0138	.0000	.0150 ±.0238	.9850 ±.0238
R	.0225 ±.1030	.0163 ±.0088	.9613 ±.0490	.1000 ±.0214	.0825 ±.0195	.8175 ±.0482	.0375 ±.0133	.0375 ±.0131	.9263 ±.0489	.1313 ±.0243	.0875 ±.0200	.7813 ±.0478

TABLE 7.5 contd.:

Series	chromosome 21p B	21p I	21p N	21s B	21s I	21s N	22p B	22p I	22p N	22s B	22s I	22s N
D	.0000	.0000	1.0000	.0000	.2060 ±.0355	.9740 ±.0355	.0000	.0064 ±.0178	.9936 ±.0178	.0065 ±.0127	.0130 ±.0179	.9805 ±.1117
G	.0021 ±.0029	.0052 ±.0045	.9927 ±.0446	.0405 ±.0126	.0560 ±.0147	.9035 ±.0444	.0062 ±.0050	.0239 ±.0097	.9699 ±.0446	.0674 ±.0161	.0830 ±.0178	.8496 ±.0441
H	.0000	.0046 ±.0127	.9954 ±.0127	.0505 ±.0295	.1101 ±.0428	.8394 ±.0926	.0092 ±.0127	.0092 ±.0127	.9816 ±.0939	.0688 ±.0342	.0642 ±.0331	.8670 ±.0930
I	.0000	.0024 ±.0066	.9976 ±.0066	.0165 ±.0122	.0472 ±.0204	.9363 ±.0672	.0000	.0142 ±.0159	.9858 ±.0159	.0542 ±.0219	.0566 ±.0223	.8892 ±.0669
J	.0000	.0033 ±.0091	.9967 ±.0091	.0166 ±.0145	.0331 ±.0203	.9503 ±.0797	.0000	.0099 ±.0158	.9901 ±.0158	.0497 ±.0253	.0662 ±.0285	.8841 ±.0792
K	.0013 ±.0018	.0047 ±.0035	.9939 ±.0360	.0371 ±.0097	.0593 ±.0122	.9036 ±.0358	.0054 ±.0037	.0189 ±.0070	.9757 ±.0360	.0640 ±.0127	.0768 ±.0138	.8592 ±.0356
L	.0000	.0011 ±.0021	.9989 ±.0021	.0000	.0108 ±.0066	.9892 ±.0066	.0000	.0027 ±.0033	.9973 ±.0033	.0022 ±.0021	.0011 ±.0015	.9968 ±.0321
N												
O												
Q	.0050 ±.0098	.0100 ±.0138	.9850 ±.0980	.0150 ±.0109	.0350 ±.0257	.9500 ±.0979	.0050 ±.0098	.0100 ±.0138	.9850 ±.0980	.0200 ±.0195	.0050 ±.0098	.9750 ±.0980
R	.0138 ±.0081	.0113 ±.0073	.9750 ±.0490	.1988 ±.0293	.0486 ±.0151	.7525 ±.0475	.1225 ±.0235	.0200 ±.0098	.8575 ±.0485	.0850 ±.0198	.0230 ±.0106	.8913 ±.0487

TABLE 7.6: Chromosome variant frequencies of the present study compared with those of published reports, by means of the ratio = Difference in variant frequency/S.E.diff.

Series		chromosome 3	4	13p	13s	14p	14s	15p	15s	21p	21s	22p	22s
	B	6.51	1.34*	0.08*	1.35*								2.93
D v. 1	I	0.52*	7.73	2.48	5.32		5.32		11.95		7.21	8.21	14.27
	N	6.89	6.60	2.25	1.38*		5.26		10.58		6.38	4.97	4.22
	B	12.64	7.26	14.39	5.05		3.68		3.34	0.33*	3.66	1.71*	2.83
G v. 1	I	3.33	22.57	20.61	6.89		9.89		10.63	1.09*	9.22	8.55	9.75
	N	7.03	15.86	6.89	0.70*		3.47		4.35	0.12*	3.28	2.75	4.18
	B	3.59	3.15	3.24	1.77*		2.58		0.00*		2.37	1.17*	1.60*
H v. 1	I	2.16	7.22	10.02	3.67		5.61		6.64	0.63*	2.82	9.24	8.05
	N	3.57	5.94	5.45	0.60*		1.41*		2.88	0.29*	0.64*	1.81*	2.75
	B	1.93*	1.25*	3.19	3.75		0.01*		0.75*		0.30*		0.73*
J v. 1	I	1.61*	5.61	15.51	4.13		9.12		8.09	1.07*	9.64	8.41	8.72
	N	2.79	3.31	8.17	0.08*		3.25		3.30	0.36*	3.20	4.90	3.52
	B	5.91	7.95	14.24	6.08		3.83		2.89	0.14*	3.87	1.79*	2.90
K v. 1	I	1.08*	20.78	20.74	6.52		10.48		11.13	1.37*	9.48	9.62	10.95
	N	7.33	13.95	9.69	0.65*		4.06		5.15	0.17*	3.68	3.30	5.06
	B	8.62		2.91	3.55		4.67		5.20				6.81
L v. 1	I	2.04	7.78	11.23	8.93	1.12*	15.54	3.33	18.32	2.82	14.59	11.99	18.67
	N	3.48	4.37	11.04	3.57	1.12*	8.14	0.99*	10.00	0.49*	9.48	5.67	10.86
	B	10.13		7.82									
N v. 1	I	3.37		6.05									
	N	7.44		0.56*									
	B	9.43		1.96*									
O v. 1	I	8.90		10.07									
	N	1.70*		7.10									

* ≤ 1.96, that is, the difference between the two frequencies is not significant at the 5% level.

TABLE 7.6 contd.:

Series		chromosome 3	4	13p	13s	14p	14s	15p	15s	21p	21s	22p	22s
	B			0.88*	0.34*		1.26*			0.69*	0.09*	0.69*	1.73*
G v. 1	I			7.92	5.65		12.03	1.56*	12.21	0.25*	8.41	8.89	17.05
	N			5.48	1.29*		3.37	1.56*	10.66	0.08*	2.68	1.01*	7.64
	B			18.82	4.73		6.91		7.87	2.88	12.06	10.05	3.97
R v. 1	I			28.74	2.64	0.33*	6.85	2.08	8.66	0.51*	9.70	8.93	15.45
	N			4.16	0.39*	0.96*	0.73*	2.16	1.12*	0.46*	1.76*	0.98*	5.27
	B	6.10		0.20*	0.75*								1.96*
D v. 2	I	0.25*	19.59	2.57	3.27		4.38		8.46		4.18	4.25	9.94
	N	6.05	18.63	2.48	0.92*		3.79		6.22		2.89		7.18
	B	10.24		12.89	5.00		3.25		5.22		4.64		2.43
G v. 2	I	2.08	17.88	14.00	3.34		6.26		6.74	0.76*	4.03	3.68	6.92
	N	5.07	19.43	6.07	0.04*		2.39		2.58	0.14*	0.93*	2.25	3.89
	B	3.05		2.87	1.96*		2.51		1.41*		2.86		1.57*
H v. 2	I	2.20	7.16	8.64	2.09		4.27		5.01	0.64*	0.61*	4.33	6.58
	N	4.59	6.46	5.42	0.15*		1.11*		2.10		0.48*	1.52*	2.60
	B	5.82		2.29	1.79*				1.50*				4.01
L v. 2	I	1.01*	6.57	8.00	4.63	0.33*	7.53	1.22*	10.63	1.40*	7.16	4.89	11.38
	N	2.47		8.85	1.87*			0.39*	6.21		3.77		7.88
	B	9.01		7.20									
N v. 2	I	2.41		5.29									
	N	5.96		1.05*									
	B	8.35		1.39*									
O v. 2	I	6.49		8.26									
	N	1.46*		6.71									

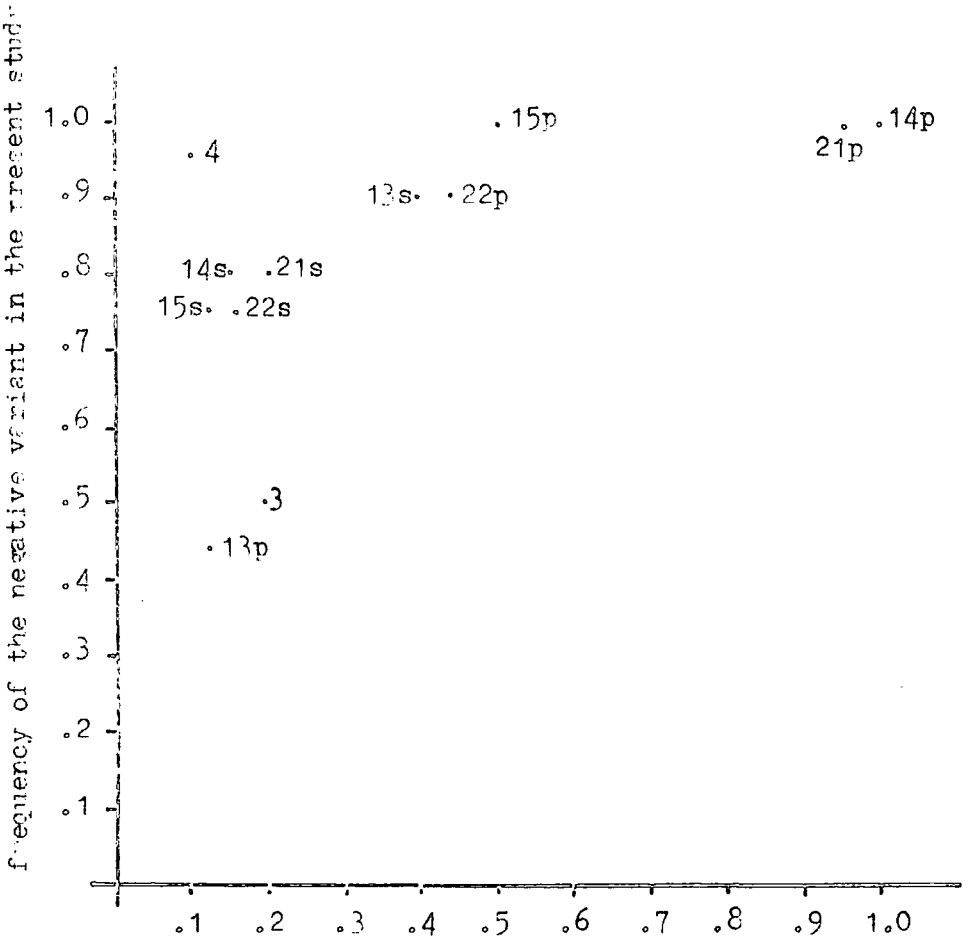
* ≤ 1.96

TABLE 7.6 contd.:

Series		chromosome 3	4	13p	13s	14p	14s	15p	15s	21p	21s	22p	22s
	B			1.09*	0.19*		1.96*			0.51*	0.27*	0.51*	0.87*
Q v. 3	I			6.82	4.83		8.64	1.29*	8.63	0.13*	7.19	6.76	10.52
	N			5.10	2.55		3.55	1.29*	7.28	0.06*	2.98	1.98	4.12
	B	2.15		2.86			0.53*		0.25*		0.55*		0.05*
J v. 4	I	0.82*	4.63	9.77	3.57		5.59		6.26	0.63*	6.71	3.90	5.24
	N	2.02	3.16	6.26	0.25*		2.36		3.44	0.63*	2.96	3.90	2.62
	B	3.69	3.11	3.31	1.59*		2.46		0.74*		2.04	1.03*	1.52*
H v. 5	I	1.94*	7.01	9.12	3.97		5.38		6.35	0.49*	3.72	2.04	7.18
	N	4.98	5.60	5.04	0.81*		1.46*		3.07	0.23*	1.25*	1.90*	2.55
	B	2.09	1.12*	3.27	3.55		0.10*		0.05*		0.22*		0.65*
J v. 5	I	1.37*	5.31	13.67	4.37		8.28		7.54	0.86*	9.73	7.77	7.65
	N	2.61	2.86	7.58	0.18*		3.21		3.47	0.29*	3.77	4.38	3.26
	B	10.10		7.85									
N v. 5	I	3.34		5.33									
	N	6.82		0.18*									
	B	9.41		2.09									
O v. 5	I	8.30		8.92									
	N	1.56*		6.45									
	B			1.01*	0.14*		1.26*			0.51*	0.36*	0.51*	1.70*
Q v. 5	I			7.25	5.69		10.57	1.88*	11.02	0.25*	8.79	8.09	13.86
	N			5.09	1.47*		3.35	1.88*	9.67	0.08*	3.20	1.90*	4.41
	B			18.73	4.28		6.63		14.56	2.59	11.52	9.93	3.67
R v. 5	I			23.11	3.04	0.01*	6.20	1.22*	7.77	0.66*	9.62	7.91	12.58
	N			3.40	0.00*	0.88*	0.81*	1.90*	1.46*	0.42*	0.63*	0.65*	4.69

* ≤ 1.96

Figure 7.1: The relationship between the frequency of variants and their similarity in the present and other studies.



misclassification of variants in the case of fairly frequent variants. Obviously, the more common a variant is, the more often one is going to come across examples of regions which lie on the borderline between levels of fluorescence. If the intensely fluorescent type of chromosome region is not common, then presumably slightly less than intense types of the same region will not be very common either (as all variant regions seem to show a continuous range of fluorescence), and opportunities for misclassification of the region will be few.

That chromosome 4 is an exception to this generalisation is not altogether surprising. It is this variant which seems to have the widest range of possible values (see below, page 389). That is, in some reports it is one of the more common variants but in others it is one of the rarest (table 7.11).

A similar comparison was made, where appropriate, between published frequencies and those found separately in the newborn infants, adults and adults aged over 65 years of the present study. Generally, it seems that when these comparisons are made fewer instances of significantly different frequencies are found than when the published reports are compared with the total sample of the present study. This, of course, is only to be expected if factors such as the sex and age composition of the sample have an influence on the amount of genetic variability detected in that sample.

As noted in the previous section, many published studies did not distinguish between the intense and brilliant levels of fluorescence of variant regions of the chromosomes. These levels were therefore combined for the series comprising the present study, and for those published reports that did make the distinction, and comparisons similar to those described above were made again. Table 7. 7 gives the phenotype frequencies of chromosome variants (intense

and brilliant levels of fluorescence combined) and the X^2 values which result from comparing them with those expected under a Hardy-Weinberg equilibrium. Table 7.8 gives the results of a comparison by means of the X^2 test between these frequencies and those of the present study. Again, the series were compared separately with the newborn infants, adults and elderly adults of the present study where appropriate, as well as with the total sample. Also again, P values are not given where these are very small and reference should be made again to table 7.4 for an indication of their magnitude.

Chromosome 21p has phenotype frequencies similar to those of the present study more often than the other variant regions. Also in this comparison, the frequencies of the chromosome 13s variants often do not differ significantly from those of the present study. As noted earlier (see above, page 362), fewer significant differences in phenotype frequencies are found when the series are matched, broadly, for age. However, it is still true that the similarities between series are always less noticeable than the enormous differences usually observed.

Table 7.9 gives the frequencies of chromosome variants (intense and brilliant levels of fluorescence combined) of published series. In some cases, they are as listed in the published paper (series A, B, and P), in other cases they were calculated from reported phenotype frequencies (series C, E, F, G, H, I, J, K, L and M) and from the actual numbers of variant chromosomes found (disregarding the homozygous or heterozygous condition) (series D, Q and R). For series N and O the results were published in the form of numbers of carriers of the variant (homozygous and heterozygous combined). Variant frequences were calculated from these as if the intense variants had been dominant to the negative and the populations sampled were in Hardy-Weinberg equilibrium.

TABLE 7.7: Phenotype frequencies of chromosome variants (intense and brilliant levels of fluorescence combined) in published reports.

		chromosome											
		3		4		13p		13s		14p		14s	
Series		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
C**	II	48	(21.7)	0		39	(17.6)			1	(0.5)		
	IN	118	(53.4)	12	(5.4)	143	(64.7)			61	(27.6)		
	NN	55	(24.9)	209	(94.6)	39	(17.6)			159	(17.9)		
X^2(HW)		1.05				19.12*				3.69			
E	II	87	(42.0)	8	(7.8)	148	(72.2)	1	(0.5)			7	(3.4)
	IN	95	(45.9)	41	(39.8)	50	(24.4)	15	(7.4)			27	(13.3)
	NN	25	(12.1)	54	(52.4)	7	(3.4)	187	(92.1)			169	(83.3)
X^2(HW)		0.01		0.00		1.13		1.27				14.52*	
F	II	100	(28.4)	9	(2.5)	188	(52.7)	3	(0.8)	1	(0.3)	5	(1.4)
	IN	196	(54.6)	85	(23.8)	150	(42.0)	48	(13.5)	14	(3.9)	75	(21.0)
	NN	61	(17.1)	263	(73.7)	19	(5.3)	306	(85.7)	342	(85.7)	277	(77.6)
X^2(HW)		4.42*		0.45		2.46		0.53		0.02		0.00	
G	II	205	(42.5)	123	(25.5)	82	(17.0)	5	(1.0)			3	(0.6)
	IN	216	(44.8)	222	(46.1)	202	(41.9)	75	(15.6)			93	(19.3)
	NN	61	(12.7)	138	(28.6)	198	(41.1)	402	(83.4)			386	(80.1)
X^2(HW)		0.12		3.08		5.86*		0.50				1.06	
H	II	48	(44.1)	13	(11.9)	14	(12.9)	3	(2.7)			2	(1.9)
	IN	53	(48.6)	47	(43.1)	37	(33.9)	11	(10.1)			25	(22.9)
	NN	8	(7.3)	49	(45.0)	58	(53.2)	95	(87.2)			82	(75.2)
X^2(HW)		1.67		0.11		3.89*		9.69*				0.00	
I	II	85	(40.1)	4	(1.9)	11	(5.2)	2	(0.9)			0	
	IN	96	(45.3)	79	(37.3)	60	(28.3)	40	(18.9)			25	(11.8)
	NN	31	(14.6)	129	(60.8)	141	(66.5)	170	(80.2)			187	(88.2)
X^2(HW)		0.21		4.30*		1.83		0.04					
J	II	52	(34.4)	1	(0.7)	9	(6.0)	2	(1.3)			0	
	IN	72	(47.7)	53	(35.1)	46	(30.5)	30	(19.9)			18	(11.9)
	NN	27	(17.9)	97	(64.2)	96	(63.6)	119	(78.8)			133	(88.1)
X^2(HW)		0.06		4.80*		1.17		0.00					
K	II	305	(41.1)	137	(18.4)	105	(14.2)	10	(1.4)			5	(0.7)
	IN	341	(46.0)	322	(43.4)	285	(38.4)	116	(15.6)			136	(18.3)
	NN	96	(12.9)	284	(38.3)	352	(47.4)	616	(83.0)			601	(81.0)
X^2(HW)		0.00		7.13*		13.74*		2.76				0.82	
L	II	319	(34.3)	47	(5.0)	122	(13.1)	0		5	(0.5)	0	
	IN	394	(42.4)	169	(18.2)	339	(36.5)	35	(3.8)	5	(0.5)	3	(0.3)
	NN	217	(23.3)	714	(76.8)	469	(50.4)	895	(96.2)	920	(98.9)	927	(99.7)
X^2(HW)		18.85*		58.87*		21.79*				409.98*			
M	II	17	(15.7)	0		25	(23.1)	2	(1.8)	0		0	
	IN	58	(53.7)	24	(22.2)	49	(45.4)	30	(27.8)	11	(10.2)	21	(19.4)
	NN	33	(30.6)	84	(77.8)	34	(31.5)	76	(70.4)	97	(89.8)	87	(80.6)
X^2(HW)		1.04				0.80							

* significant at the 5% level

** p and s variants are given in a combined frequency for

TABLE 7.7 contd.:

Series		chromosome 15p No.	%	15s No.	%	21p No.	%	21s No.	%	22p No.	%	22s No.	%
C	II	1	0.5			10	4.5			6	2.7		
	IN	93	42.1			88	39.8			85	38.5		
	NN	127	57.5			123	55.7			130	58.8		
X^2(HW)		13.48*				1.35				3.33			
E	II			1	0.5			3	1.5	16	19.5	4	2.0
	IN			24	11.8			27	13.3	27	32.9	25	12.3
	NN			178	87.7			173	85.2	39	47.5	174	85.7
X^2(HW)				0.04				2.34		6.67*		6.25*	
F	II	4	1.1	2	0.6	0		12	3.4	40	11.2	34	9.5
	IN	11	3.1	67	18.7	16	4.5	90	25.2	163	45.7	126	35.3
	NN	342	95.8	288	80.7	341	95.5	255	71.4	154	43.1	197	55.2
X^2(HW)		3.36		0.82		0.01		1.30		0.10		4.16*	
G	II			5	1.0	0		4	0.8	2	0.4	9	1.9
	IN			111	23.0	7	1.5	85	17.6	25	5.2	127	26.4
	NN			366	75.9	475	98.5	393	81.5	455	94.4	346	71.8
X^2(HW)				1.16				0.06		5.96*		0.46	
H	II			0		0		1	0.9	0		3	2.7
	IN			23	21.1	1	0.9	33	30.3	4	3.7	23	21.1
	NN			86	78.9	108	99.1	75	68.8	105	96.3	83	76.2
X^2(HW)												0.79	
I	II			0		0		0		1	0.5	1	0.5
	IN			43	20.3	1	0.5	27	12.7	4	1.9	45	21.2
	NN			169	79.7	211	99.5	185	87.3	207	97.6	166	78.3
X^2(HW)										22.22*		1.25	
J	II			0		0		0		1	0.7	1	0.7
	IN			32	21.2	1	0.7	15	9.9	1	0.7	33	21.9
	NN			119	78.8	150	99.3	136	90.1	149	98.7	117	77.5
X^2(HW)												0.67	
K	II			5	0.7	0		5	0.7	3	0.4	13	1.7
	IN			166	22.4	9	1.2	133	17.9	30	4.0	183	24.7
	NN			571	77.0	733	98.8	604	81.4	709	95.6	546	73.6
X^2(HW)				3.65				0.63		15.81*		0.27	
L	II	0		3	0.3	0		1	0.1	0		0	
	IN	3	0.3	10	1.1	2	0.2	18	1.9	5	0.5	6	0.6
	NN	927	99.7	917	98.6	928	99.8	911	98.0	925	99.5	924	99.4
X^2(HW)				127.04*				7.57*					
M	II	0		4	3.7	0		8	7.4	3	2.8	5	4.6
	IN	15	13.9	26	24.1	7	6.5	68	63.0	21	19.4	36	33.3
	NN	93	86.1	78	72.2	101	93.5	32	29.6	84	77.8	67	62.0
X^2(HW)				0.92				11.38*		1.33		0.00	

* significant at the 5% level

TABLE 7.8: A comparison of the phenotype frequencies of chromosome variants (intense and brilliant levels of fluorescence combined) in the present study with those in published reports.

Series		chromosome 3	4	13p	13s	14p	14s	15p	15s	21p	21s	22p	22s
	X^2	0.15	1.97										
C v. 1	d.f.	2	1										
	P	.93	.16										
	X^2	42.32	110.13	130.74	19.47		40.36		76.82		31.95	91.98	78.80
E v. 1	d.f.	2	1	2	2		2		2		1	2	2
	P	*			$.59 \times 10^{-4}$								
	X^2	11.76	56.30	68.38	9.11	0.96	28.82	0.10	75.32	3.89	6.19	47.58	11.62
F v. 1	d.f.	2	1	2	2	1	2	1	2	1	2	2	2
	P	.003			.01	.33		.75		.05	.05		.003
	X^2	71.54	483.67	94.90	6.46		48.14		62.16	0.33	41.92	54.52	53.03
I v. 1	d.f.	2	1	2	2		2		2	1	2	2	2
	P				.04					.57			
	X^2	34.96	153.37	80.30	12.96		8.23		23.51	0.20	0.64	17.77	24.39
H v. 1	d.f.	2	1	2	2		2		1	1	1	1	2
	P				.002		.02			.35	.42	$.25 \times 10^{-4}$	

* P values are not given where they are very small.
For an indication of their magnitude, see Table 7.4.

TABLE 7.8 contd.:

		chromosome											
		3	4	13p	13s	14p	14s	15p	15s	21r	21s	22r	22s
Series													
	X^2	13.81	74.56	156.51	1.62		38.95		31.21	0.72	37.01	32.28	96.84
J v. 1	d.f.	1	1	2	2		1		1	1	1	1	2
	P	.001			.45					.40			
	X^2	79.08	426.18	166.56	9.30		69.44		91.41	1.19	55.40	91.27	80.76
K v. 1	d.f.	2	1	2	2		2		2	1	2	2	2
	P				.01					.55			
	X^2	38.27	57.22	210.20	119.96	5.83	119.07	23.01	189.21	11.99	325.60	106.69	551.72
L v. 1	d.f.	2	1	1	1	1	1	1	2	1	2	1	1
	P					.02				.0005			
	X^2	2.33	16.74	15.85	3.20	11.64	14.49	18.64	13.53	5.26	46.98	1.68	7.03
M v. 1	d.f.	2	1	2	1	1	1	1	2	1	2	2	2
	P	.31	$.43 \times 10^{-4}$	.0004	.07	.0007	.0001	$.12 \times 10^{-4}$	.0012	.02		.43	.03
	X^2	8.59	34.90	35.46	0.77	1.33	16.32	2.15	39.48	1.71	3.99	89.07	2.95
F v. 2	d.f.	2	1	2	1	1	2	1	2	1	2	2	2
	P	.01			.38	.25	.0003	.14		.19	.13		.23

TABLE 7.8 contd.:

		chromosome											
		3	4	13p	13s	14p	14s	15p	15s	21p	21s	22p	22s
Series													
	X^2	44.11	263.45	63.98	0.02		26.13		27.12	0.11	5.24	22.80	24.90
G v. 2	d.f.	2	2	2	1		2		2	1	2	2	2
	P				.90					.74	.07		
	X^2	30.43	81.94	68.86	0.80		5.77		14.63	0.12	1.12	13.18	17.03
H v. 2	d.f.	2	1	2	1		2		1	1	2	1	2
	P				.37		.06		.0001	.73	.57	.0003	.0002
	X^2	27.44	34.82	118.65	54.64	0.88	355.38	3.84	372.29	8.42	162.39	152.30	716.52
L v. 2	d.f.	2	1	2	1	1	1	1	2	1	1	1	1
	P					.35		.05		.004			
	X^2	2.40	15.85	19.50	6.04	9.19	10.38	18.94	9.43	3.02	64.77	0.30	3.74
M v. 2	d.f.	2	1	2	1	1	1	1	2	1	2	1	2
	P	.30	$.70 \times 10^{-4}$		.01	.002	.0013	$.13 \times 10^{-4}$	.009	.08		.58	.15
	X^2	8.48	32.21	67.90	0.29		26.18		31.95	0.13	34.60	24.42	23.99
J v. 4	d.f.	2	1	2	2		1		1	1	1	1	1
	P	.01			.86					.72			

TABLE 7.8 contd.:

		chromosome											
		3	4	13p	13s	14p	14s	15p	15s	21r	21s	22p	22s
Series													
	X^2	0.10	2.98	8.73									
C v. 5	d.f.	2	1	2									
	P	.95	.08	.01									
	X^2	33.42	82.01	115.49	22.72		38.50		77.34		43.54	62.45	72.71
E v. 5	d.f.	2	1	2	2		2		2		2	2	2
	P												
	X^2	29.83	90.97	58.98	11.61		8.46		25.15	0.17	5.10	18.93	23.25
H v. 5	d.f.	2	1	2	2		2		1	1	2	1	2
	P				.003		.01			.68	.08	$.14 \times 10^{-4}$	
	X^2	10.39	53.48	116.64	1.06		34.75		72.76	0.63	47.78	33.85	33.94
J v. 5	d.f.	2	1	2	2		1		1	1	1	1	2
	P	.006			.59					.43			

TABLE 7.9: Frequencies of chromosome variants (intense and brilliant levels of fluorescence combined) ±1.96 S.E. in published reports.

Series		chromosome 3	4	13p	13s	14p	14s	15p	15s	21p	21s	22p	22s
A*	I	.56	.11	.36		.04		.24		.06		.24	
	N	.44	.89	.64		.96		.76		.94		.76	
		±.13	±.08	±.12		±.05		±.11		±.06		±.11	
B*	I	.42	.15	.26		.09		.17		.05		.13	
	N	.58	.85	.74		.91		.83		.95		.87	
		±.07	±.05	±.07		±.04		±.06		±.03		±.05	
C*	I	.4842	.0271	.5000		.1425		.2149		.2443		.2195	
	N	.5158	.9729	.5000		.8575		.7851		.7557		.7805	
		±.0659	±.0214	±.0659		±.0461		±.0452		±.0566		±.0546	
D	I	.7597	.4091	.4416	.0260	.0000	.0455	.0000	.0130	.0000	.0260	.0065	.0195
	N	.2403	.5909	.5584	.9740	1.0000	.9545	1.0000	.9870	1.0000	.9740	.9935	.9805
		±.0954	±.1098	±.1109	±.0355		±.0465		±.0253		±.0355	±.0179	±.0309
E	I	.6497	.2767	.8439	.0419		.1010		.0640		.0813	.3598	.0813
	N	.3501	.7233	.1561	.9581		.8990		.9360		.9187	.6402	.9187
		±.0650	±.0864	±.0497	±.0276		±.0415		±.0337		±.0376	±.1039	±.0374
F	I	.5546	.1443	.7367	.0756	.0224	.1190	.0266	.0994	.0224	.1597	.3403	.2717
	N	.4454	.8557	.2633	.9244	.9776	.8810	.9334	.9006	.9776	.8403	.6597	.7283
		±.0516	±.0365	±.0457	±.0274	±.0154	±.0336	±.0163	±.0310	±.0154	±.0380	±.0492	±.0461

* in these reports p and s variants were given in a combined frequency for chromosomes 13, 14, 15, 21 and 22

TABLE 7.9 contd.:

Series		chromosome 3	4	13p	13s	14p	14s	15p	15s	21p	21s	22p	22s
	I	.6494	.4845	.3797	.0882		.1027		.1255	.0073	.0965	.0301	.1504
G	N	.3506	.5155	.6203	.9118		.8973		.8745	.9927	.9035	.9699	.8496
		±.0426	±.0446	±.0433	±.0253		±.0271		±.0296	±.0076	±.0264	±.0153	±.0319
	I	.6835	.3349	.2982	.0780		.1330		.1055	.0046	.1606	.0183	.1330
H	N	.3165	.6651	.7018	.9220		.8670		.8945	.9954	.8394	.9817	.8670
		±.0873	±.0886	±.0859	±.0503		±.0637		±.0577	±.0127	±.0689	±.0252	±.0637
	I	.6274	.2052	.1934	.1038		.0590		.1014	.0024	.0637	.0142	.1108
I	N	.3726	.7948	.8066	.8962		.9410		.8986	.9976	.9363	.9858	.8892
		±.0651	±.0544	±.0532	±.0411		±.0317		±.0406	±.0066	±.0329	±.0159	±.0423
	I	.5828	.1821	.2119	.1126		.0596		.1060	.0033	.0497	.0099	.1159
J	N	.4172	.8179	.7881	.8874		.9404		.8940	.9967	.9503	.9901	.8841
		±.0787	±.0616	±.0652	±.0504		±.0378		±.0491	±.0091	±.0347	±.0158	±.0511
	I	.6408	.4011	.3336	.0916		.0984		.1186	.0061	.0964	.0243	.1408
K	N	.3592	.5989	.6664	.9084		.9016		.8814	.9939	.9036	.9757	.8592
		±.0345	±.0353	±.0339	±.0209		±.0214		±.0233	±.0056	±.0212	±.0111	±.0250
	I	.5548	.1414	.3134	.0188	.0081	.0016	.0016	.0086	.0011	.0108	.0027	.0032
L	N	.4452	.8586	.6866	.9812	.9919	.9984	.9984	.9914	.9989	.9892	.9973	.9968
		±.0319	±.0224	±.0298	±.0087	±.0058	±.0026	±.0026	±.0059	±.0021	±.0066	±.0033	±.0036

TABLE 7.9 contd.:

Series		chromosome 3	4	13p	13s	14p	14s	15p	15s	21p	21s	22p	22s
	I	.4259	.1111	.4583	.1574	.0509	.0972	.0694	.1574	.0324	.3889	.1250	.2130
M	N	.5741	.8889	.5417	.8426	.9491	.9028	.9306	.8426	.9676	.6111	.8750	.7870
		±.0933	±.0593	±.0940	±.0687	±.0415	±.0559	±.0479	±.0687	±.0334	±.0919	±.0624	±.0772
	I	.9024	.0488	.6414	.0488		.0665	.0144	.0513	.0096	.0691	.0192	.0716
N	N	.0976	.9512	.3586	.9512		.9335	.9856	.9487	.9904	.9309	.9808	.9284
		±.0401	±.0291	±.0649	±.0291		±.0337	±.0161	±.0298	±.0132	±.0343	±.0186	±.0349
	I	.6289	.1083	.3157	.0222		.0578	.0049	.0526	.0024	.0398	.0098	.0297
O	N	.3761	.8917	.6843	.9778		.9422	.9951	.9474	.9976	.9602	.9902	.9703
		±.0663	±.0425	±.0636	±.0202		±.0319	±.0096	±.0306	±.0067	±.0268	±.0135	±.0232
	I	.68	.27	.88	.03		.05		.08		.08	.49	.07
P	N	.32	.73	.12	.97		.95		.92		.92	.51	.93
		±.14	±.12	±.10	±.04		±.06		±.08		±.08	±.14	±.08
	I			.2850	.0400	.0000	.0250	.0050	.0150	.0150	.0500	.0150	.0250
Q	N			.7150	.9600	1.0000	.9750	.9950	.9850	.9850	.9500	.9850	.9750
				±.0885	±.0384		±.0306	±.0138	±.0238	±.0238	±.0427	±.0238	±.0306
	I	.2662	.0413	.4475	.1212	.0387	.1825	.0737	.2187	.0250	.2475	.1425	.1087
R	N	.7338	.9587	.5525	.8788	.9613	.8175	.9263	.7813	.9750	.7525	.8575	.8913
		±.0433	±.0195	±.0487	±.0320	±.0189	±.0379	±.0256	±.0405	±.0153	±.0423	±.0433	±.0305

The variant frequencies were compared with those of the present study by the method described earlier (see above, page 354). The results of this comparison are given in table 7.10. Again, it seemed as if the rare variants occurred with similar frequencies to the present study's more often than the more common ones, and again it appeared that more similarity was seen when series similar in composition with respect to broad age-groups were compared.

Figure 7.2 shows the frequencies of the intense variants $\hat{=}$ 1.96S.E. of published reports in relation to the frequencies found in the present study. This demonstrates visually the conclusion that must be drawn from the statistical results given in tables 7.6 and 7.10; that the frequencies of the chromosome variants differ greatly between various published reports and the present study. It is natural to speculate, as many previous authors have done, that this lack of similarity is due to a lack of objectivity in the method of scoring the chromosome variants, especially in view of the difficulties which arise in trying to maintain consistency of standards within a single laboratory. Geographically-related differences in frequency may, of course, occur, but it would be unwise to postulate these as an explanation of the large differences between laboratories encountered here until variations in methodology can be eliminated.

In table 7.11 the variant chromosome regions have been listed in order of frequency of the positive variant for each series examined in this chapter. It can be seen quite clearly that certain variants are the commonest in many studies (for example, chromosome 3 and 13p) and others are the rarest in nearly every study (for example 21p, 14p and 15p). This leaves a group of variants of 'medium' frequency (21s, 14s, 13s, 15s, 22s). The order of variants within each of these groupings alters between series but usually the variants tend to occur in these broad frequency groups. If,

TABLE 7.10: Chromosome variant frequencies (intense and brilliant levels of fluorescence combined) of the present study compared with those of published reports, by means of the ratio-

Difference in variant frequency/S.E.diff.

	chromosome											
	3	4	13p	13s	14p	14s	15p	15s	21p	21s	22p	22s
Series												
A v. 1	1.39*	3.75										
B v. 1	1.21*	1.62*										
C v. 1	0.13*	1.20*										
D v. 1	5.36	6.47	2.01	3.81		5.62		11.33		7.06	6.92	10.84
E v. 1	4.45	5.21	10.63	3.63		3.97		7.87		4.57	4.56	7.42
F v. 1	3.21	5.00	5.79	1.81*	0.90*	3.72	0.79*	6.65	1.38*	1.36*	3.19	0.02*
G v. 1	5.86	18.32	6.23	1.18*		4.92		5.65	0.58*	4.76	5.66	5.15
H v. 1	4.21	6.36	5.51	1.09*		2.00		4.35	0.78*	0.85*	5.29	3.79
J v. 1	2.33	4.29	9.12	0.13*		5.87		4.87	1.18*	6.15	6.99	5.01
K v. 1	6.19	18.22	8.84	1.09*		5.61		6.54	0.90*	5.18	6.52	6.15
L v. 1	3.00	7.07	10.16	7.03	1.12*	13.00	3.17	14.32	2.30	11.68	8.88	15.56
M v. 1	1.04*	2.17	2.01	1.31*	1.69*	3.32	2.03	2.46	1.25*	3.97	0.38*	1.38*
N v. 1	5.04	0.33*	2.08	3.15		5.97	0.44*	8.90	0.12*	0.53*	6.01	8.11
O v. 1	3.85	2.82	6.53	5.48		6.53	1.93*	8.75	1.55*	7.50	7.30	11.62
Q v. 1			5.65	3.00		8.16	1.56*	11.38	0.35*	5.39	5.63	10.65
R v. 1	7.26	0.16*	3.64	0.60*	2.30	0.91*	3.91	1.19*	1.66*	2.05	1.44	7.06

* ≤ 1.96, that is, the difference between the two frequencies is <u>not</u> significant at the 5% level.

TABLE 7.10 contd.:

Series	chromosome 3	4	13p	13s	14p	14s	15p	15s	21p	21s	22p	22s
D v. 2	4.84	6.56	2.20	2.35		4.30		7.07		3.74	4.25	7.87
F v. 2	1.86*	5.02	3.85	0.47*	1.16*	2.49	1.75*	4.02	1.16*	0.91*	7.70	0.27*
G v. 2	4.42	17.60	5.26	0.08*		3.20		3.23	0.42	2.10	3.23	3.90
H v. 2	3.75	6.45	5.27	0.27*		1.52		3.00	0.64	0.67	3.40	3.38
L v. 2	2.15	6.57	7.65	3.60	0.33*	7.53	1.12*	7.95	1.40*	5.47	7.89	9.49
M v. 2	0.88*	2.38	2.19	1.79*	1.82*	2.58	2.42	1.54*	1.20*	4.94	0.75*	1.38*
N v. 2	11.09	0.79*	1.24*	1.60*		4.18	0.59*	5.60	0.10*	2.27	3.63	7.98
O v. 2	3.25	3.02	5.89	3.07		4.54	0.46*	5.53	1.09*	3.59	4.27	7.97
Q v. 3			5.04	3.00		6.53	1.29*	7.85	0.27*	5.01	4.61	7.31
J v. 4	1.71*	3.15	6.35	0.38*		3.67		4.39	0.64*	4.56	3.90	3.38
A v. 5	1.35*	3.47										
B v. 5	1.16*	1.48*										
C v. 5	0.09*	1.42*										
E v. 5	4.14	5.04	8.44	3.77		3.79		7.56		5.22	4.34	6.51
H v. 5	4.01	6.18	5.04	1.44*		2.03		4.47	0.72*	1.62*	5.03	3.47
J v. 5	2.19	4.04	8.25	0.29		5.48		4.93	1.05*	6.60	6.27	4.52
K v. 5	5.39	17.00	7.32	1.56*		4.99		6.13	0.76*	5.69	5.77	5.12
N v. 5	13.37	0.02*	2.25	3.35		5.52	0.89*	8.28	0.10*	5.90	5.52	7.08
O v. 5	3.59	2.49	5.80	5.28		5.98	2.18	8.16	1.34	7.67	6.46	9.78
Q v. 5			5.18	3.24		7.40	1.88*	11.11	0.35*	5.95	5.31	9.20
R v. 5	6.57	0.50*	2.96	0.00*	1.97	1.01*	3.30	1.67*	1.57*	0.72*	0.96*	6.04

* ≤ 1.96

Figure 7.2: Diagrams showing the chromosome variant frequencies given in published reports in relation to those found in the present study.

A. Key to the symbols used.

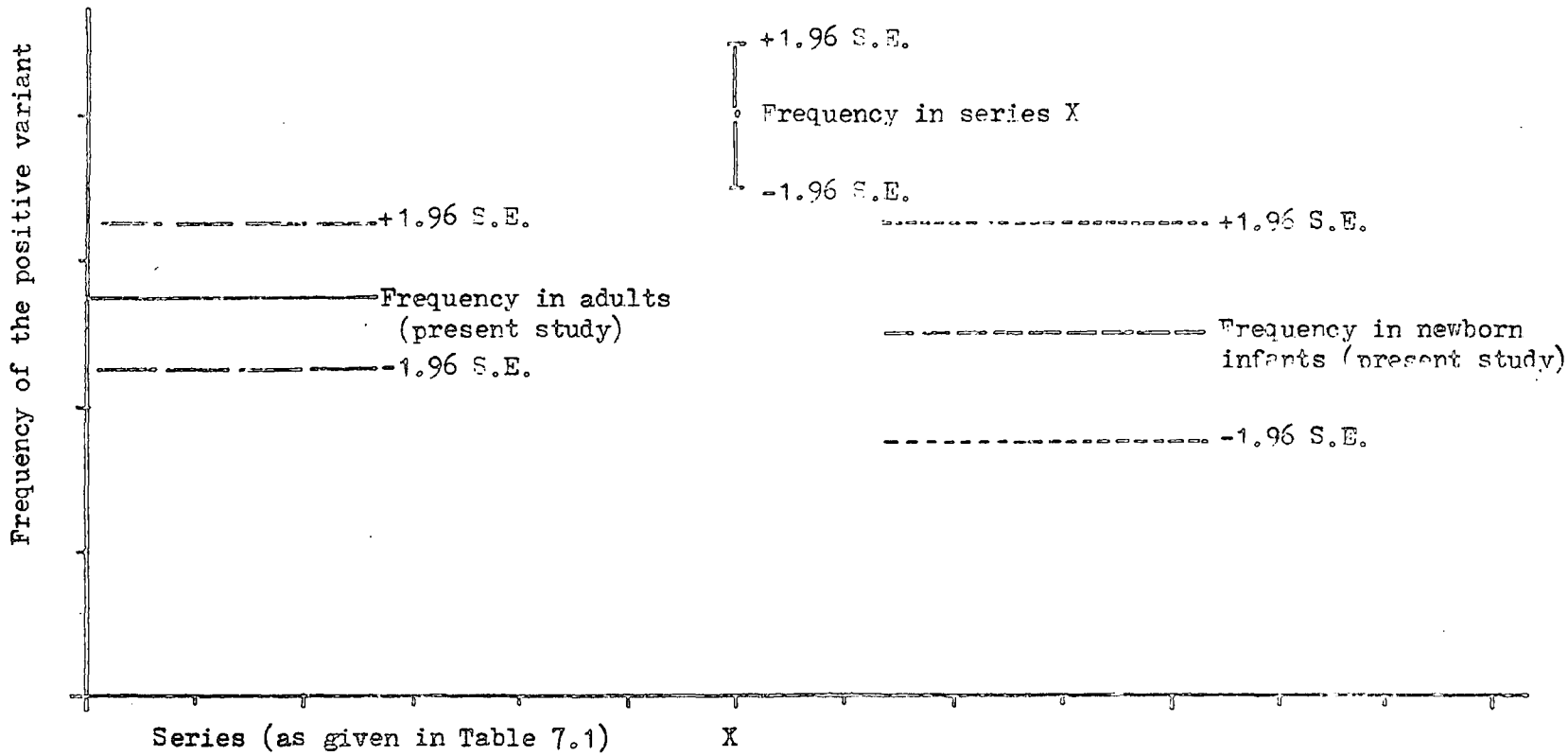


Figure 7.2 contd.:

B. Chromosome 3

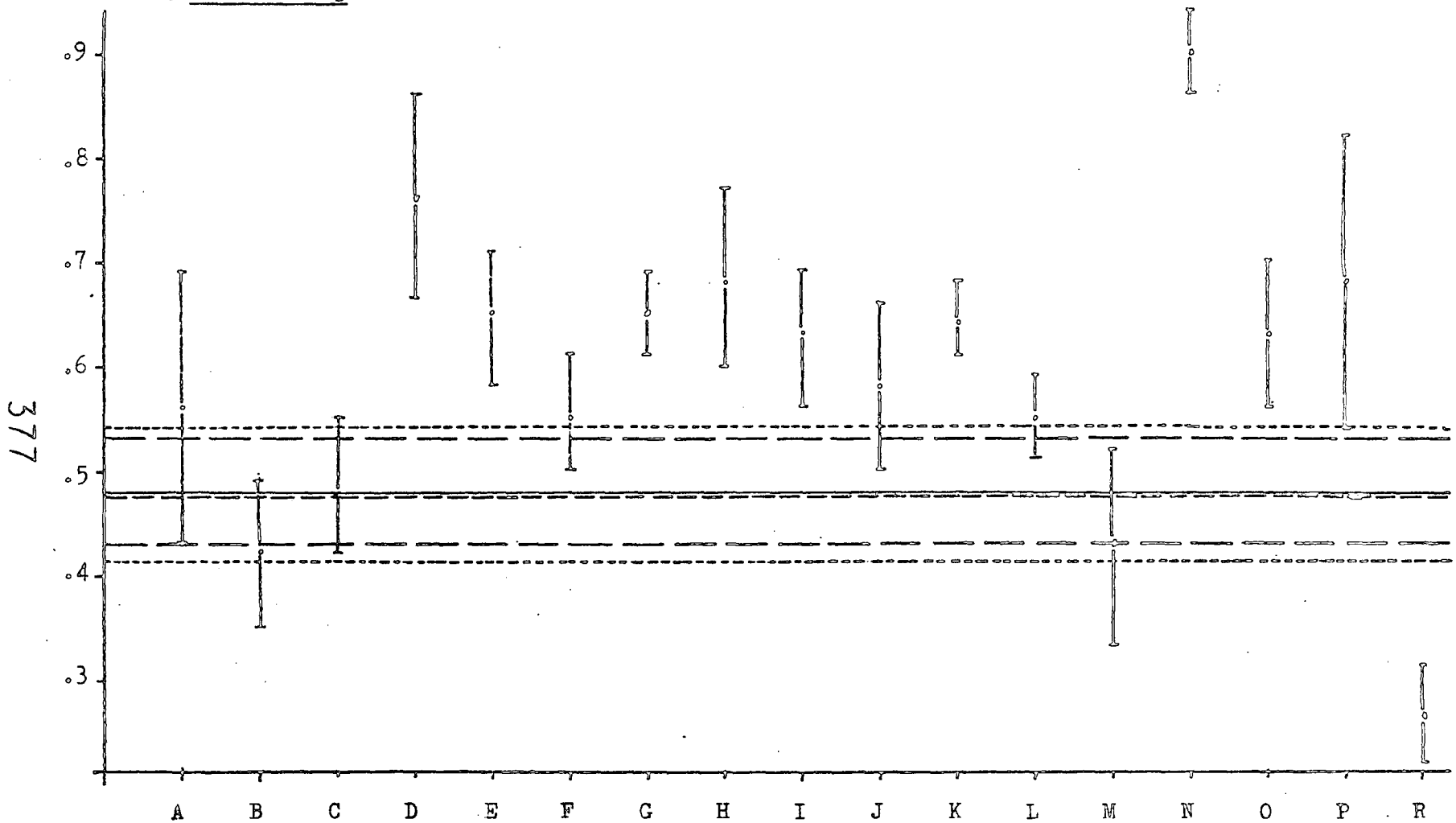


Figure 7.2 contd.:

C. Chromosome 4

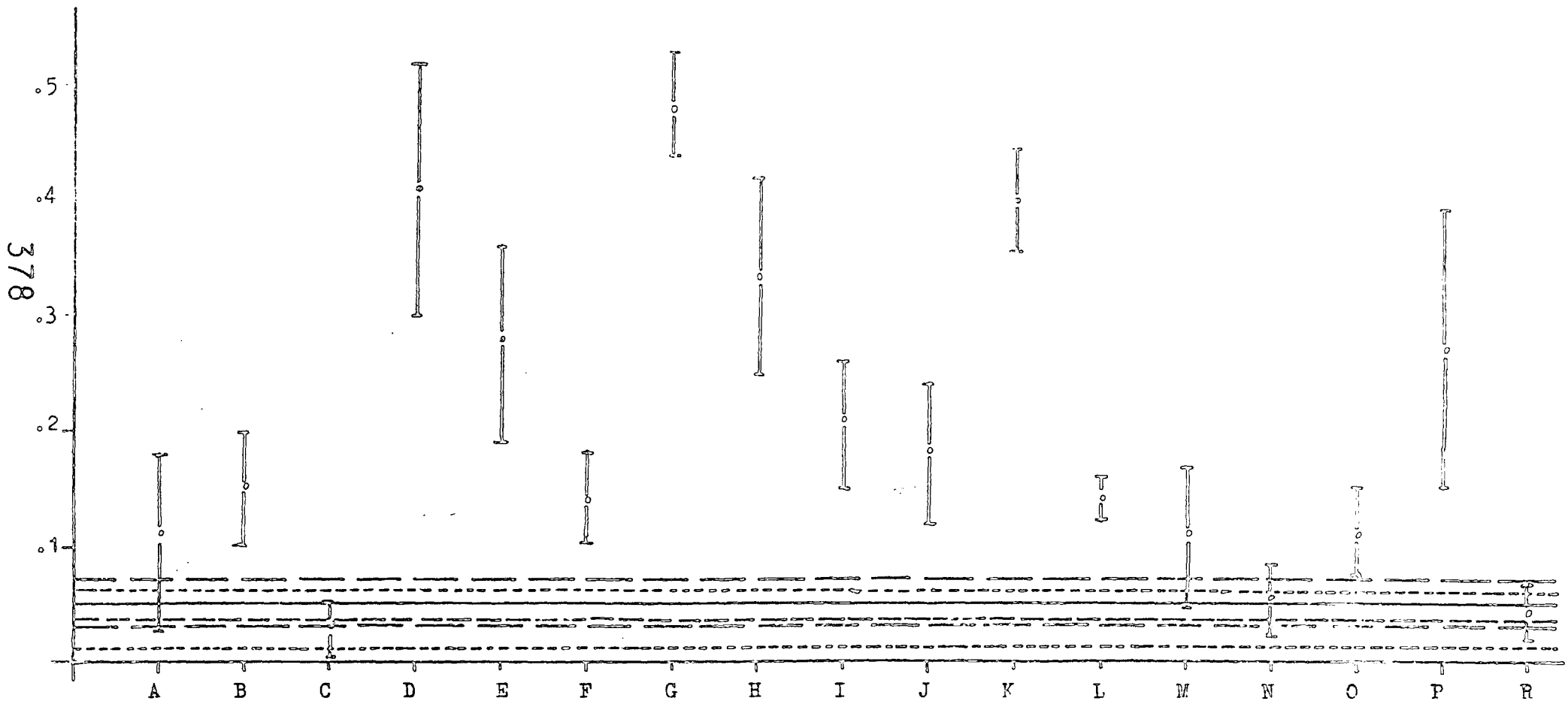


Figure 7.2 contd.:

D. Chromosome 13p

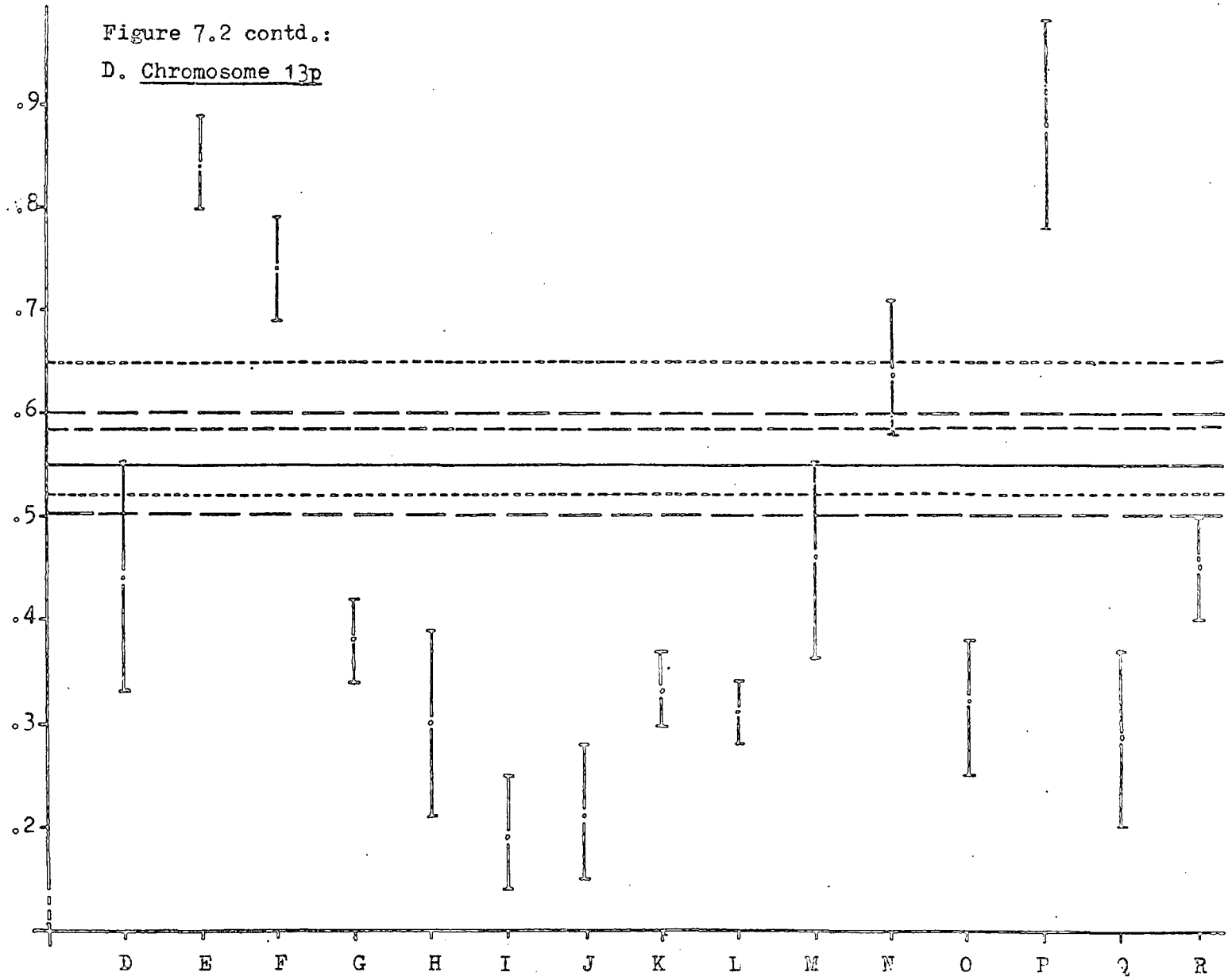


Figure 7.2 contd.:

E. Chromosome 13s

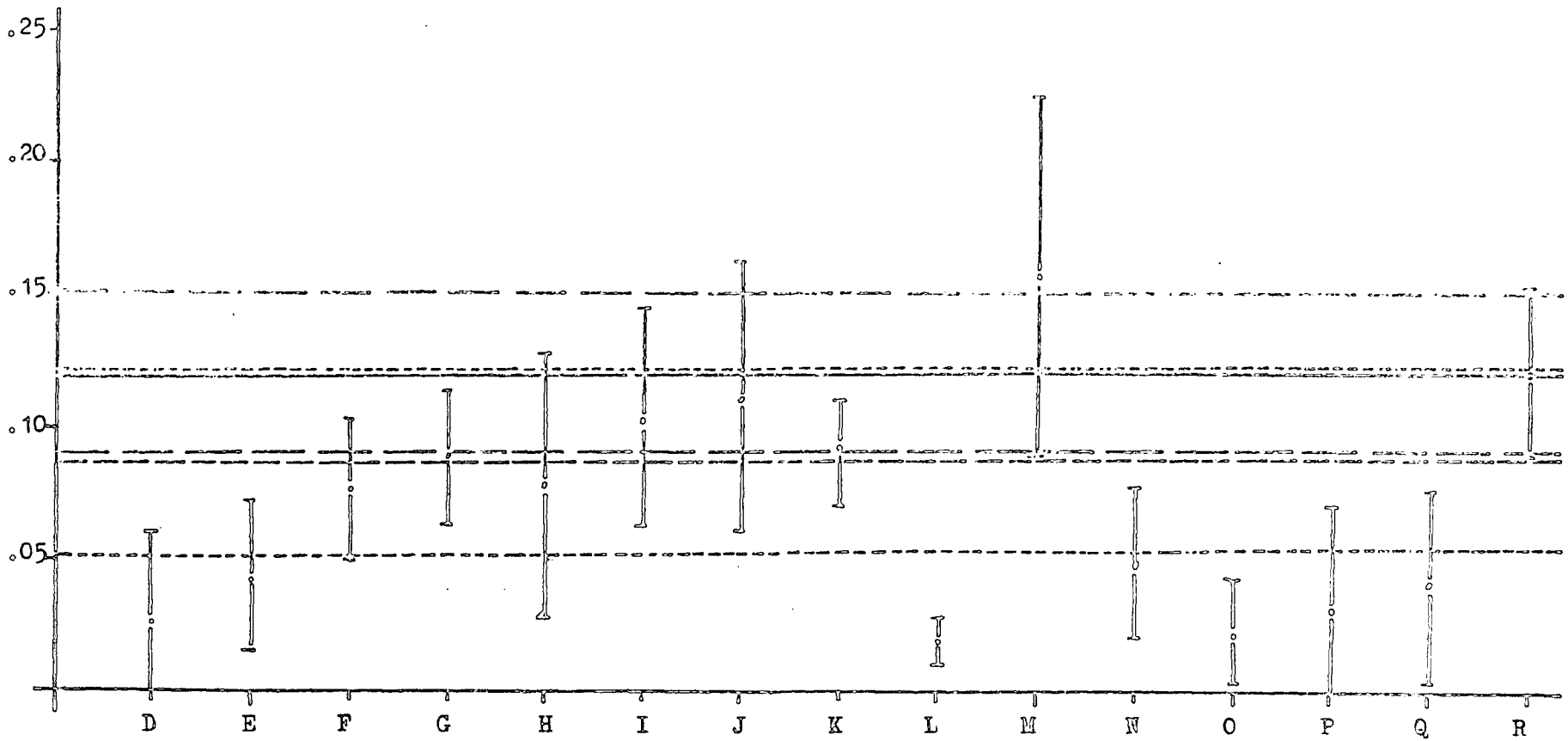
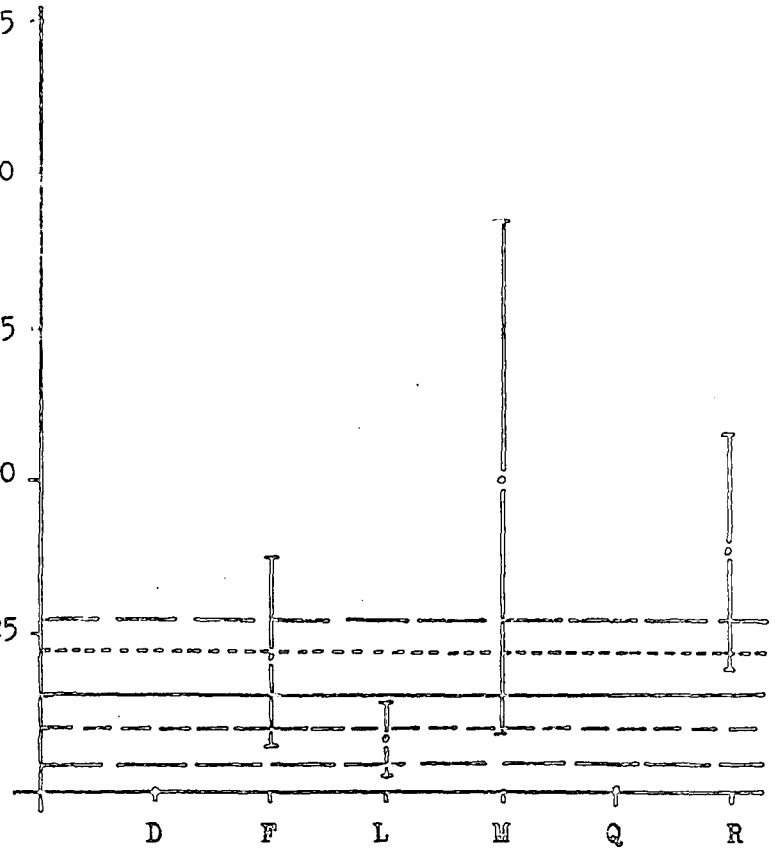


Figure 7.2 contd.:

F. Chromosome 14p



G. Chromosome 15p

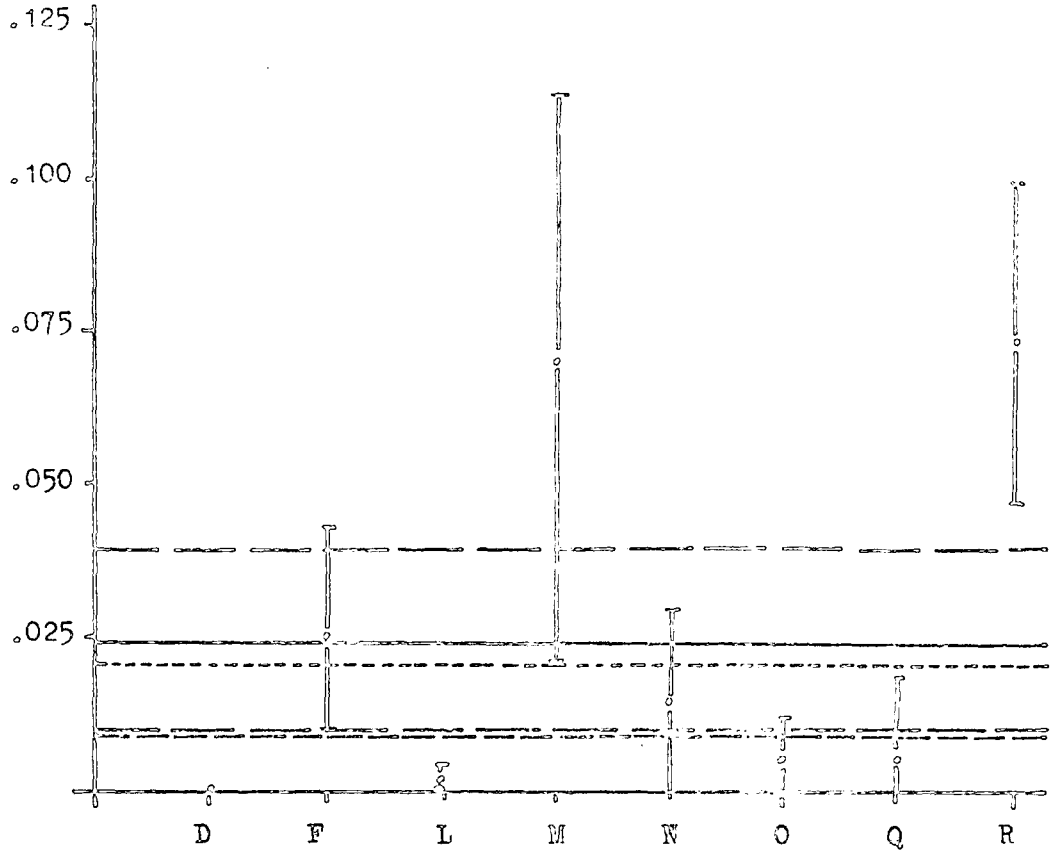


Figure 7.2 contd.:
H. Chromosome 14s

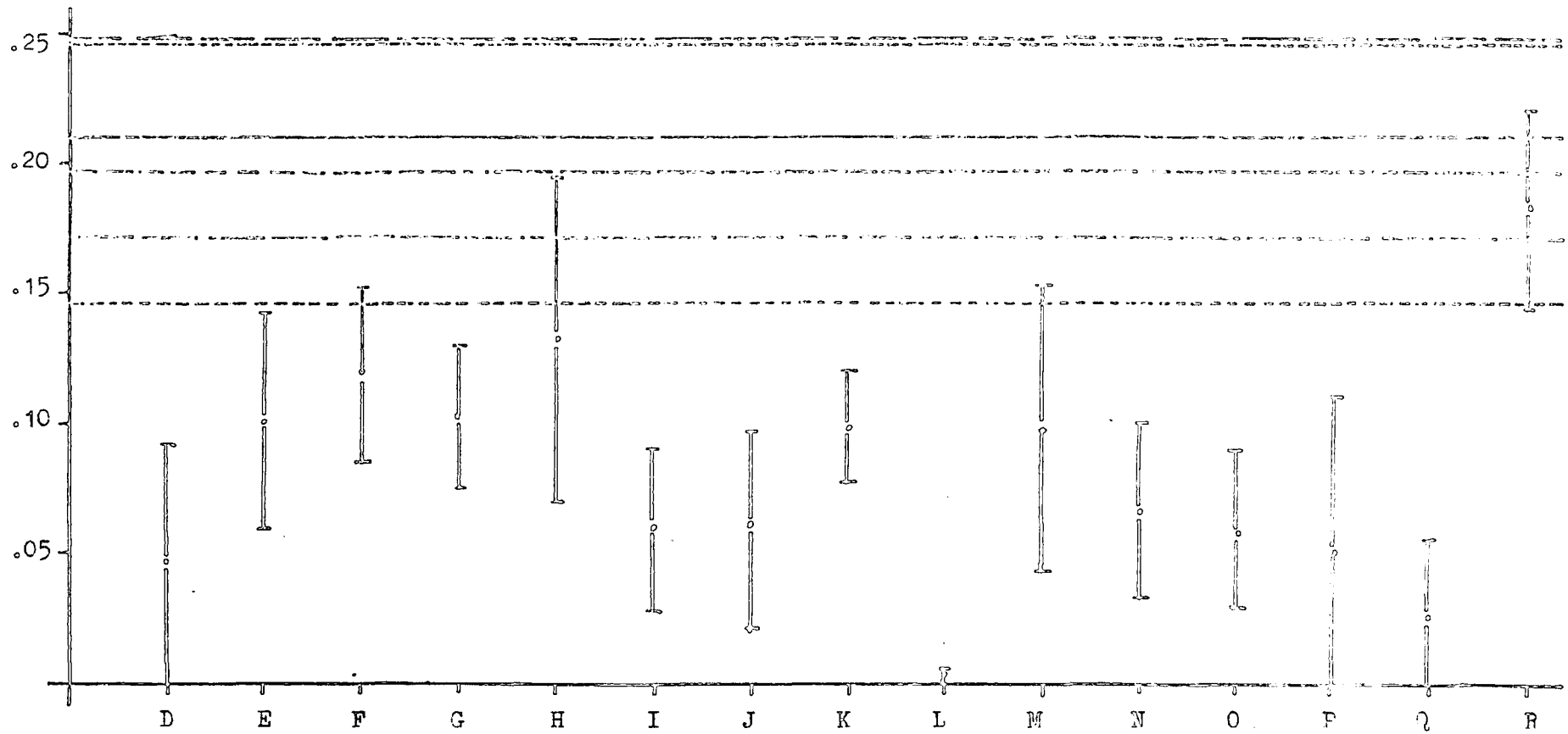


Figure 7.2 contd.:

I. Chromosome 15s

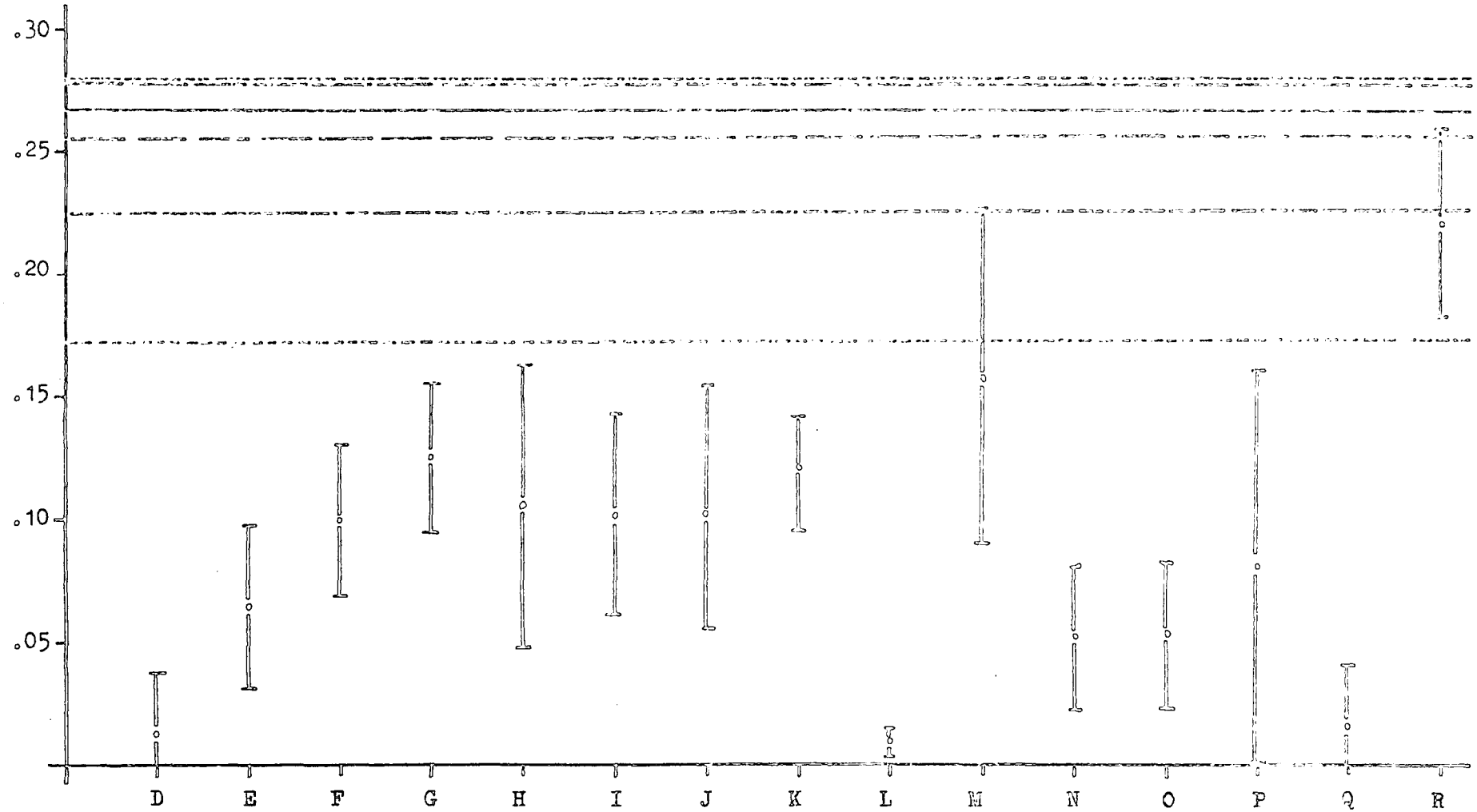


Figure 7.2 contd.:

J. Chromosome 21p

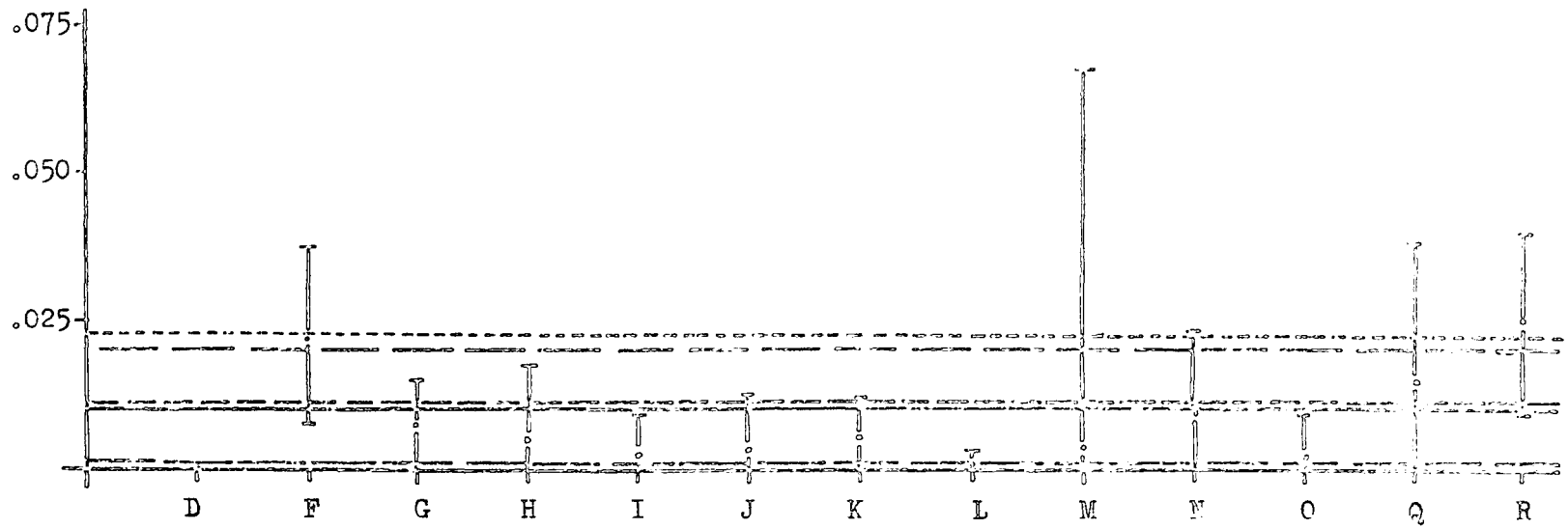


Figure 7.2 contd.:

K. Chromosome 21s

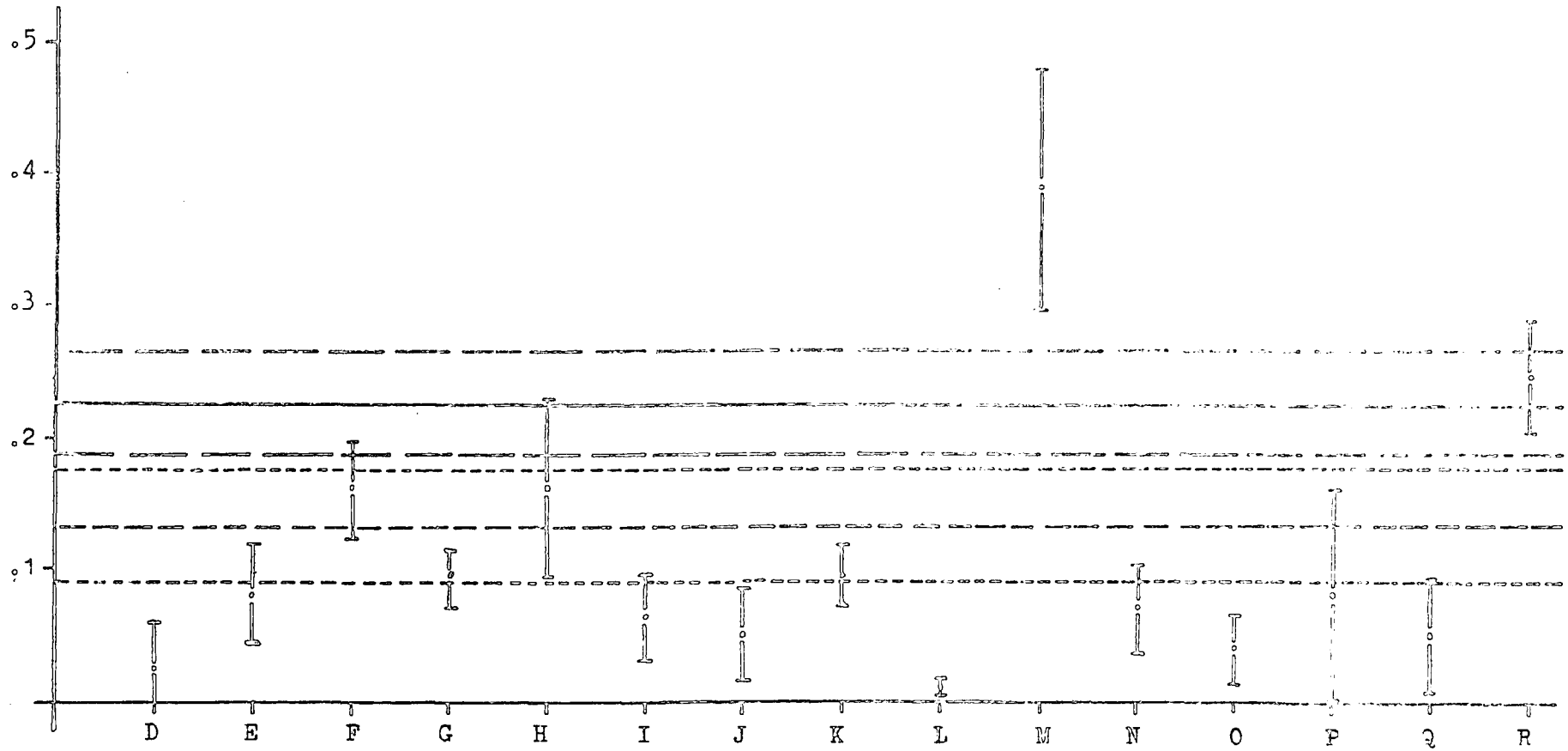


Figure 7.2 contd.:

L. Chromosome 22p

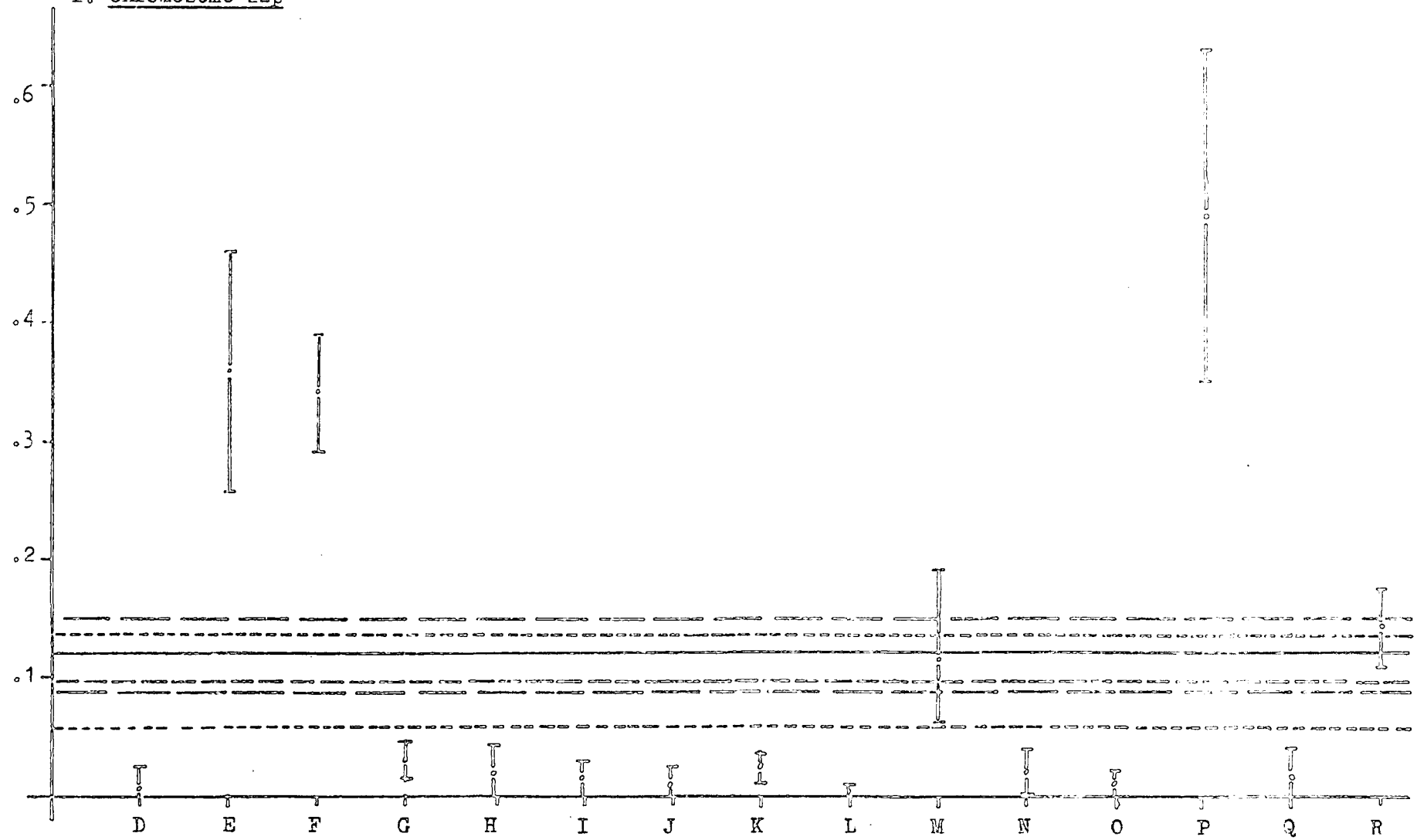


Figure 7.2 contd.:

M. Chromosome 22s

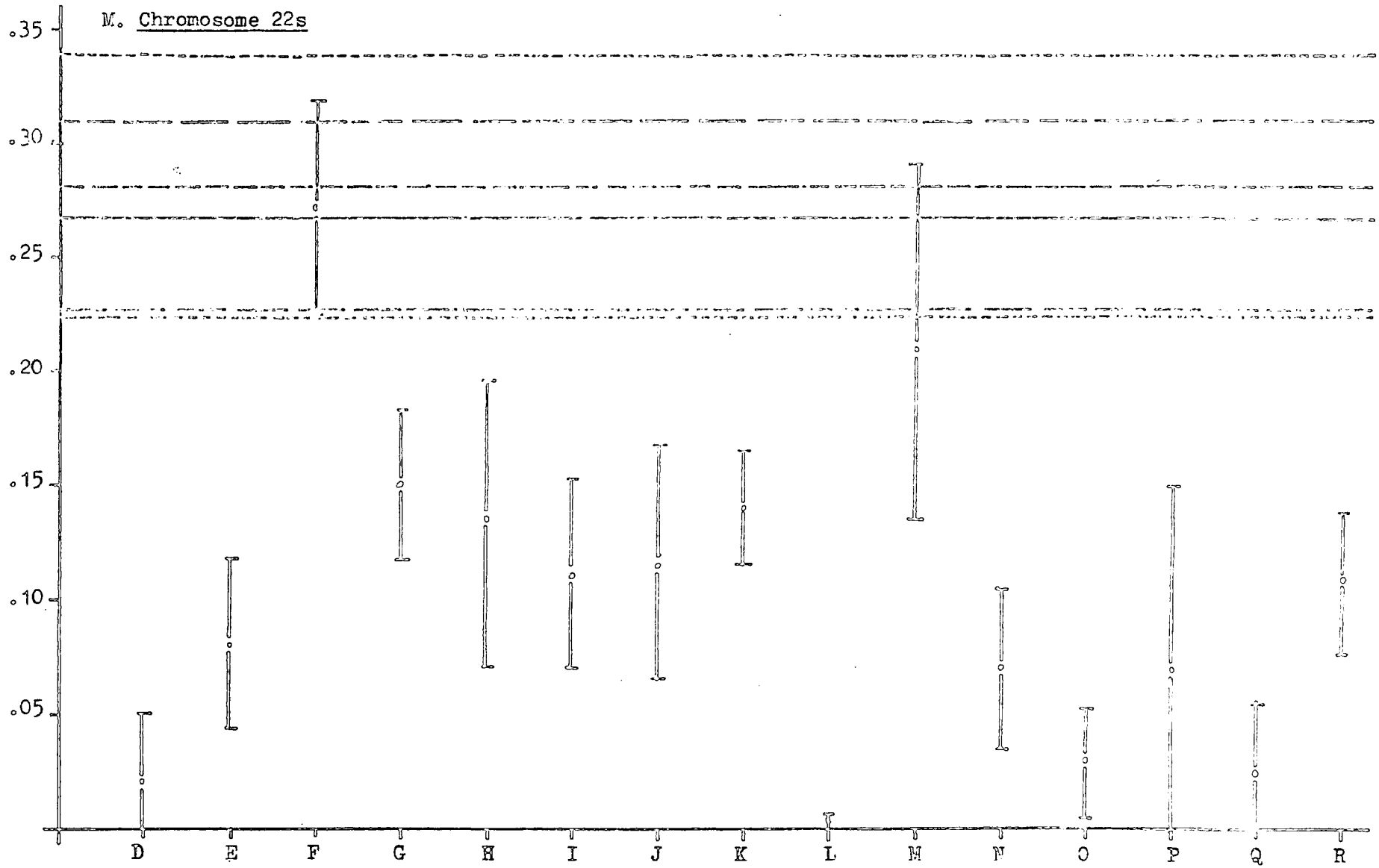


TABLE 7.11: Order of frequency of the chromosome variants (intense and brilliant levels of fluorescence combined) in the present study and in published reports.

Series												
1	13p	3	22s	15s	14s	21s	22p	13s	4	15p	14p	21p
2	13p	3	22s	15s	14s	21s	22p	13s	4	21p	14p	15p
3	13p	3	22s	15s	14s	21s	22p	13s	4	15p	21p	14p
4	13p	3	15s	22s	21s	14s	13s	22p	4	14p	15p	21p
5	13p	3	22s	15s	21s	14s	13s	22p	4	15p	14p	21p
A	3	13	15	4	22	14	21					
B	3	13	15	22	4	21	14					
C	13	3	21	21	15	14	4					
D	3	13p	4	14s	13s	21s	22s	15s	22p	15p	21p	14p
E	13p	3	22p	4	14s	21s	22s	15s	13s			
F	13p	3	22p	22s	21s	4	14s	15s	13s	15p	21p	14p
G	3	4	13p	22s	15s	14s	21s	13s	22p	21p		
H	3	4	13p	21s	14s	22s	15s	13s	22p	21p		
I	3	4	13p	22s	13s	15s	21s	14s	22p	21p		
J	3	13p	4	22s	13s	15s	14s	21s	22p	21p		
K	3	4	13p	22s	15s	14s	21s	13s	22p	21p		
L	3	13p	4	13s	21s	15s	14p	22s	22p	14s	15p	21p
M	13p	3	21s	22s	13s	15s	22p	4	14s	15p	14p	21p
N	3	13p	4	14s	15s	21s	22s	13s	22p	15p	21p	
O	3	13p	22s	21s	14s	15s	13s	4	22p	15p	21p	
P	13p	3	22p	4	15s	21s	22s	14s	13s			
Q	13p	21s	13s	14s	22s	15s	21p	22p	15p	14p		
R	13p	3	21s	15s	14s	22p	13s	22s	15p	4	14p	21p
'X'	3	13p	22s	15s	14s	21s	13s	4	22p	15p	14p	21p

for example, the frequencies of the 'medium' frequency group of variants in any one study are compared they often are found to be not significantly different from each other, and therefore within the frequency grouping the order of frequency of the variants is not important. Chromosome 4, and to a lesser extent, 22p, seems to have a 'floating' frequency, as mentioned earlier. This may be because these variants are fairly difficult to score visually, as they are often of very small size. Therefore more inconsistency between laboratories might be expected to occur for these variants. Table 7.11 also attempts a 'most typical' order of frequency of the variants (X).

Table 7.12 gives the average number of variant regions per individual for all series. These figures have been calculated by summation of the individual variant frequencies. This is not an accurate method of arriving at this figure, as can be seen by comparing the figures for the series of the present study calculated by finding the mean number of variants per individual with figures obtained by this method, and by comparing the figures with those found in some of the published reports. However, some reports do not give this information and so it was the only method available.

Allowance must be made when comparing the values given for different series for the different numbers of variable regions examined in some studies. Only 5 studies were concerned with exactly the same regions as the present study. With the exception of series L (Lin et al. 1976), the mean number of variants per individual appears to be similar in these studies. Unfortunately, it is not possible to compare these means more accurately, for example by means of the t-test, as it was impossible to know the standard deviations of the means.

TABLE 7.12: The mean numbers of variant bands (intense and brilliant levels of fluorescence combined) per individual found in the present study and in published reports.

Series	mean	S.E.	S.D.
Present study:			
Infants	4.287	0.090	1.385
Adults	4.686	0.087	1.816
Total	4.545	0.065	1.686
A	3.22		
B	2.54		
C	3.665		
D*	3.4938		
E	4.9992		
F*	5.1454		
G	4.2286		
H	3.8992		
I	2.9626		
J	2.8676		
K	3.9034		
L*	2.116		
M*	4.6058		
N	3.8862		
O	2.5442		
P	5.26		
Q	0.95		
R*	3.82		1.86

* these studies examined the same chromosomal regions as the present study.

Many published reports state that no sex difference was found in the variant frequencies, and occasionally a sex difference is noted. Only rarely, however, are the frequencies in the separate sexes recorded. Therefore it is possible to make only a limited comparison between frequencies found in the separate sexes in published reports and those found in the present study.

Tables 7.13 and 7.14 give the phenotype and variant frequencies in separate sexes in three published studies. These are compared with the frequencies found in the present study in tables 7.15, 7.16 and 7.17. As in comparisons between samples containing both sexes, few similarities in frequency were noted.

TABLE 7.13: Phenotype frequencies of chromosome variants (intense and brilliant levels of fluorescence combined) in published reports, subdivided according to sex.

		chromosome 3		4		13p		13s		14s		15s		21s		21c		22s	
Series		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
C** male	II	22	(19.4)	0				23	(20.4)	0		1	(0.9)	7	(6.2)			3	(2.7)
	IN	52	(48.7)	4	(3.5)			65	(57.5)	30	(26.5)	45	(39.8)	45	(39.8)			44	(38.9)
	NN	36	(31.9)	109	(96.5)			25	(22.1)	83	(73.5)	67	(59.3)	61	(54.0)			66	(58.4)
X^2(HW)		0.01						2.57				0.02		0.12				1.91	
C** female	II	26	(24.1)	0				16	(14.8)	1	(0.9)	0		3	(2.7)			3	(2.7)
	IN	63	(58.3)	8	(7.4)			78	(72.2)	31	(28.7)	48	(44.4)	43	(39.8)			41	(38.0)
	NN	19	(17.6)	100	(92.6)			14	(13.0)	76	(70.4)	60	(55.6)	62	(57.4)			64	(59.3)
X^2(HW)		3.18						21.38*		1.28				1.97				1.43	
E male	II	51	(51.0)	4	(8.5)	75	(75.0)	1	(1.0)	2	(2.0)	1	(1.0)	3	(3.1)	9	(23.7)	1	(1.0)
	IN	36	(36.0)	16	(34.0)	23	(23.0)	5	(5.1)	11	(11.2)	10	(10.2)	13	(13.2)	11	(29.0)	10	(10.2)
	NN	13	(13.0)	27	(57.5)	2	(2.0)	92	(93.9)	85	(86.8)	87	(88.8)	82	(83.7)	18	(47.3)	87	(88.8)
X^2(HW)		2.51		0.52		0.02		6.59*		4.15*		1.24		5.76*		5.68*		1.24	
E female	II	36	(33.6)	14	(7.1)	73	(69.5)	0		5	(4.8)	0		0		7	(15.9)	3	(2.9)
	IN	59	(55.1)	25	(44.6)	27	(25.7)	10	(9.5)	16	(15.2)	14	(13.3)	14	(13.3)	16	(36.3)	15	(14.3)
	NN	12	(11.3)	27	(48.2)	5	(4.8)	95	(90.5)	84	(80.0)	91	(86.7)	91	(86.7)	21	(47.8)	87	(82.8)
X^2(HW)		2.78		0.31		1.37				9.30*		0.54				1.60		4.47*	
L male	II	170	(34.5)	24	(4.8)	66	(13.4)												
	IN	202	(41.0)	88	(20.0)	173	(35.1)												
	NN	121	(24.5)	381	(77.2)	254	(51.5)												
X^2(HW)		14.64*		30.67*		15.75*													
L female	II	149	(34.0)	21	(4.7)	56	(12.8)												
	IN	192	(44.0)	83	(19.1)	166	(38.0)												
	NN	96	(22.0)	333	(76.2)	215	(49.2)												
X^2(HW)		5.11*		22.16*		6.76*													

** in this study p and s variants are given in a combined frequency for chromosomes 13, 14, 15, 21 and 22

TABLE 7.14: Frequencies of chromosome variants (intense and brilliant levels of fluorescence combined) ±1.96 S.E. in published reports - males and females separartely.

Series		chromosome 3 3	4	13p	13s	14s	15s	21s	22p	22s
C* male	I	.4381	.0177		.4912	.1327	.2079	.2611		.2212
	N	.5619	.9823		.5088	.8673	.7521	.7389		.7788
		±.0915	±.0243		±.0922	±.0626	±.0729	±.0810		±.0765
C* female	I	.5324	.0370		.5093	.1527	.2222	.2269		.2176
	N	.4676	.9630		.4907	.8473	.7778	.7731		.7824
		±.0941	±.0356		±.0943	±.0678	±.0784	±.0790		±.0778
E male	I	.6900	.2553	.8650	.0357	.0765	.0612	.0969	.3816	.0612
	N	.3100	.7447	.1350	.9643	.9235	.9388	.9031	.6184	.9388
		±.0906	±.1247	±.0670	±.0367	±.0526	±.0475	±.0586	±.1545	±.0475
E female	I	.6121	.2946	.8238	.0476	.1238	.0667	.0667	.3409	.1000
	N	.3878	.7054	.1762	.9542	.8762	.9333	.9333	.6591	.9000
		±.0923	±.1194	±.0729	±.0407	±.0630	±.0477	±.0477	±.1401	±.0574
L male	I	.5497	.1379	.3093						
	N	.4503	.8621	.6907						
		±.0439	±.0304	±.0408						
L female	I	.5606	.1430	.3181						
	N	.4394	.8570	.6819						
		±.0465	±.0328	±.0437						

* in this report, p and s variants were given in a combined frequency for chromosomes 13, 14, 15, 21 and 22

TABLE 7.15: A comparison of phenotype frequencies of chromosome variants (intense and brilliant levels of fluorescence combined) in the present study with those in published reports - MALES only.

Series		chromosome 3	4	13p	13s	14s	15s	21s	22p	22s
C v. 1	X^2	2.14	2.42							
	d.f.	2	1							
	P	.34	.12							
C v. 5	X^2	0.69	2.36							
	d.f.	2	1							
	P	.71	.12							
E v. 1	X^2	41.08	38.21	69.82	8.76	19.46	34.34	14.78	50.50	39.86
	d.f.	2	1	2	1	2	2	2	2	2
	P	*			.003	$.59 \times 10^{-4}$		.0006		
E v. 5	X^2	26.76	30.25	66.97	12.25	18.32	34.56	20.49	30.76	30.36
	d.f.	2	1	2	1	2	2	2	2	2
	P				.0005	.0001		$.36 \times 10^{-4}$		
L v. 1	X^2	26.17	25.19	94.50						
	d.f.	2	1	2						
	P									
L v. 2	X^2	26.78	11.18	61.98						
	d.f.	2	1	2						
	P		.0008							

* see note to Table 7.8.

TABLE 7.16: A comparison of chromosome variant phenotype frequencies (intense and brilliant levels of fluorescence combined) in the present study with those in published reports - FEMALES only.

Series		chromosome 3	4	13p	13s	14s	15s	21s	22p	22s
C v. 1	X^2	2.11	0.10							
	d.f.	2	1							
	P	.35	.76							
C v. 5	X^2	2.04	0.46							
	d.f.	2	1							
	P	.36	.50							
E v. 1	X^2	10.90	69.57	61.11	8.32	22.87	40.40	17.01	41.41	38.73
	d.f.	2	1	2	1	2	1	1	2	2
	P	.0042	*		.0040			$.37 \times 10^{-4}$		
E v. 5	X^2	10.36	49.64	49.79	8.47	20.92	40.26	22.48	31.75	41.80
	d.f.	2	1	2	1	2	1	1	2	2
	P	.0056			.0040					
L v. 1	X^2	13.75	31.21	113.50						
	d.f.	2	1	2						
	P	.0010								
L v. 2	X^2	5.76	19.43	56.93						
	d.f.	2	1	2						
	P	.06	$.10 \times 10^{-4}$							

* see note to Table 7.8.

TABLE 7.17: Chromosome variant frequencies (intense and brilliant levels of fluorescence combined) of each sex of the present study compared with those of published reports, by means of the ratio - Difference in variant frequency/S.E.diff.

Series	chromosome								
	3	4	13p	13s	14s	15s	21s	22p	22s
C v. 1 (males)	0.49*	1.48*							
C v. 1 (females)	0.77*	0.31*							
C v. 5 (males)	0.56*	1.37*							
C v. 5 (females)	0.77*	0.93*							
E v. 1 (males)	4.13	3.28	6.97	2.49	3.40	5.13	2.77	3.37	5.58
E v. 1 (females)	2.26	4.39	5.65	2.61	2.30	5.93	3.78	3.09	4.95
E v. 5 (males)	3.73	3.23	6.83	2.80	3.09	4.78	3.33	3.08	4.52
E v. 5 (females)	2.18	3.75	5.21	2.53	2.30	5.69	4.14	3.05	4.74
L v. 1 (males)	2.32	4.89	6.94						
L v. 1 (females)	1.99	5.01	7.32						
L v. 2 (males)	1.81*	4.01	5.78						
L v. 2 (females)	1.28*	3.12	5.04						

* ≤ 1.96, that is, the difference between the two frequencies is not significant at the 5% level.

Chapter 6: THE DISTRIBUTION OF QFQ-BAND CHROMOSOME VARIANTS WITHIN A HUMAN POPULATION, AND THE EVIDENCE FOR THEIR ADAPTIVE SIGNIFICANCE.

Certain regions of human chromosomes show a variable response to illuminations by ultra-violet light after staining with quinacrine dihydrochloride. The purpose of this study was to record the frequency of the variants of these regions within a population, and to discover from their distributions any evidence of the mechanism by which they are maintained within the population. Several other genetic polymorphisms (blood groups and isoenzymes) were examined within the same population. Their distributions were compared with those of the chromosome variants so that similarities and dissimilarities in the factors responsible for their maintenance might be inferred.

With only a few exceptions (for example, the polymorphisms associated with malaria) attempts to demonstrate the action of natural selection on the maintenance and distribution of genetic markers within human populations have, for several reasons, been unsuccessful. At various times alternative approaches to the detection of such processes have been proposed. These include the measurement of average heterozygosity, and the total genetic variance within a population. Heterozygosity has been examined in the analysis of the data of the present study. However, even this approach is limited by the fact that weak selection pressures, or those which are only periodic or are transient in time may not be detected by the means available (Friedlander 1975). In the present case it seems justifiable to use techniques which can be sure of detecting only relatively strong and consistent selection, simply because a new class of polymorphisms is being studied and there is no *a priori* knowledge of what degree of selection they may be subjected to. In this study the evidence for the adaptive significance of all genetic

markers derives from the statistical significance of various tests of association between the genetic and demographic data. Whilst this is not the most comprehensive method of demonstrating a polymorphism's evolutionary significance it provides the best preliminary analysis of data of the type collected. It is felt that even if this aspect of the data analysis is limited by the coarseness of the resolution permitted by the numbers available, the descriptive aspect of the results of chromosome variability within the population is valuably informative for comparison with other polymorphisms and populations.

A review of information about the structure of chromosomes and the arrangement of the different classes of DNA base sequences within them, together with the evidence which suggests a relationship between the different classes and the patterns of differential staining observed with several cytological techniques, leads one to suppose that in observing the differences in staining behaviour between homologous chromosomes and between the chromosomes of separate individuals, one is in fact, making visible differences in the composition of the DNA in the variable regions, or at least something which closely reflects such differences.

Among the higher Primates, the brilliantly fluorescing material occurs in the karyotypes of Man, chimpanzee and gorilla only (Pearson 1972). Because this type of material does occur in other groups of organisms one must presume that it either has a very ancient origin, and has subsequently been lost completely from several species and higher taxa or alternatively, it has had an independent origin in different evolutionary lineages. If the latter explanation is correct then one would not expect brilliantly fluorescing material from different groups of organisms to have the same biochemical composition; this would indicate that identical reactions to the stain need not imply identity of the base

compositions. If this can be so between groups of organisms, then one wonders whether or not the brilliantly fluorescent material within a species (either at the same chromosomal location or not) could have a multiple origin. That is, it may be that all brilliantly fluorescing material within the human karyotype has the same biochemical composition, or that this applies only to material found in homologous regions, or that not even this can be assumed. Thus, it may be relevant to consider either all the brilliant material together in the analysis of this data, or only that which is allelic, or it may be that even that which is apparently allelic is not, and that the different examples of brilliantly fluorescing material at any given chromosomal location form a class of alleles. An analogous situation seems to be revealed in the case of other genetic markers. For example, in some isoenzymes, variants previously thought of as single alleles in fact comprise of groups of related alleles (Selander 1976). Such findings have implications for the acceptability of the Neutral hypothesis of genetic variability; the existence of large allelic classes rather than a few alleles being consistent with the expectations of this theory.

In the present analysis, it seemed that the best way to handle the data was to treat chromosome variants of the same fluorescence intensity as if they were truly allelic, and also variants of different chromosome regions which had the same fluorescence intensity were grouped together, although it is recognised that these treatments (particularly the latter) may be based on false assumptions.

The acceptance of QFQ-band intensity variants as genetic polymorphisms which are susceptible to involvement in adaptation and evolutionary change of the organism necessitates making several assumptions about their nature. These are:

1. The non-genetic DNA which probably comprises the variants has some function, even though it does not code for any enzyme or protein structure.

2. Different types of this non-genetic DNA have different capacities for fulfilling this (these) function (s).
3. Those different capabilities have a result on the metabolism in the cell, and ultimately on the phenotype and genetic fitness of the organism.
4. These functional differences in the non-genetic DNA are reflected in their reaction to being stained by quinacrine dihydrochloride.

The truth of these assumptions is unproven. The alternative view would be that the DNA of the variant regions is non-functional, or that all types of non-genetic DNA are equally capable of fulfilling the function. Then, variations with respect to this DNA might be observed within human populations simply because of random accumulation of base differences, but no adaptive significance would be found to be attached to any particular kind of variant. If this were true then the study of chromosome variants within the population would be less useful if the objective were to elucidate the genetic structure of the population and the adaptive processes affecting this.

Several sources of evidence have been approached to discover whether the non-genetic DNA is as much susceptible to evolutionary processes as genetic DNA. These are:

1. Correlations between the frequency and distributions of chromosome variants and demographic parameters, and comparisons with the distributions of other genetic markers in a human population.
2. Comparisons between the results of chromosome variant analysis of different studies.
3. The existence of chromosome variants in related species.
4. The incidence and effects on the phenotype of other types of chromosome variability.
5. The facts of chromosome structure and the molecular mechanisms by which patterns of differential staining are produced.

1. The Analysis of Chromosome and Other Genetic Markers in the Present Study.

The first thing to be noticed in the analysis of these results is that the intense, brilliant and negative (normal) variants of each chromosome region examined are distributed randomly in the population. That is, most occur at frequencies not unlike those expected in a Hardy-Weinberg equilibrium. Technical reason have been suggested to account for deviations from such expectations where these have been found. No sex differences were found with respect to the distributions of any individual variants, but the total number of intense variants per individual, and consequently the total number of positive variants per individual, was found to be higher for the female adults than for the male adults. No such sex difference was found in the newborn infants.

Examination of the data with the aim of detecting an age-related disturbance in the frequencies of the chromosome variants constituted the most exhaustive aspect of the analysis. The variants of several chromosome regions appeared to show some change in frequency with age, but not in a manner which could be easily interpreted. In the case of variants of the satellites of chromosome 21, all variant phenotypes were less common amongst the newborn infants than amongst the adults, but none showed a clear relationship with age within the group of adults (age range 15 to 95 years). There was no tendency for similar phenotypes at different chromosome regions to show the same type of relationship with age. That is, in some cases the NN type increased in frequency with increasing age, in others the IN type did. Such increases were rarely consistent over all age groups, nor with the different methods used for subdividing the sample into age groups. The trends that could be detected were rarely significant statistically.

When the variants were grouped together by intensity of fluorescence, it was found that D and G group satellites, and consequently all satellites of both intense and brilliant fluorescence occurred at higher frequencies in the adults compared with the infants, but again no clear correlation with age could be detected within the adults. This increase in frequency did not occur in the case of variants of chromosomes 3 and 4, nor of those of the short arms of the acrocentric chromosomes, despite a general increase in the adults seen when all the variant regions were considered together.

There was no marked, or even slight but consistent, association between heterozygosity of any of the variants, or of total heterozygosity of the chromosome variants, with age, except in the case of chromosome regions of which positive variants were rare (for example, the short arm of chromosome 14). In this case the significantly older mean age of the heterozygotes compared with that of the homozygotes can probably be dismissed, as the heterozygotes were so infrequent (there were only 14 in the adult series).

The main conclusion to be drawn from this analysis is that no effect of the general fitness of the carrier (as indicated by longevity) has been detected for any of the chromosome variants examined in this study, either considered singly or together. It is not possible to show from this data, of course, whether or not these variants have an effect on the reproductive fitness of the carriers, except that they seem to occur in similar frequencies in newborn infants as they do in the general adult population.

In the case of the blood group and isoenzyme polymorphisms examined in the same sample, again with only a few exceptions (notably the decline in frequency of the rhesus negative phenotype with age) there is also no clear correlation of frequencies with age.
In recent years much evidence has accumulated for possible

selectiveadvantages and disadvantages of certain of the blood groups, from disease studies and primate studies for example (Reed 1975). Polymorphism of red cell antigens seems so widespread in human populations that it is difficult to believe that they are not involved in some important physiological reaction. It has been suggested that this would be immunological (Mourant et al. 1976). However, the analysis of the present data has not revealed any indication of a selective advantage or disadvantage of any of the blood group phenotypes, except, as mentioned above, in the case of the rhesus negative phenotype. This latter finding directly contradicts the findings of Williams (1977), who also examined a series of adults from this area (County Durham). Therefore, even this result must be held in some doubt.

It could be that the selection pressures which influence or have imfluenced blood group frequencies are of the type that are now relaxed and are not effective in the 1970s in County Durham, and therefore one would not expect to be able to detect their influence in a sample of the present-day population, even if selection pressures have influenced the frequencies of blood group phenotypes in earlier times.

As, at the moment, there is no indication about the nature of possible selection pressures favouring or disfavouring certain chromosome variants, it is not possible to say whether or not they are likely to be more or less relaxed nowadays as compared with other periods of evolution, or with those affecting the frequencies of other genetic polymorphisms. However, it is a general truth that the decline in infant mortality combined with the reduction in family size and in variance of family size which has occurred in modern Western societies, has greatly reduced the opportunity for selection at whatever genetic locus is considered (Kirk 1968).

The incidence of several chromosome abnormalities has been shown to be correlated with parental (particularly maternal) ages. Frequencies of the chromosome variants were also examined with respect to these parameters, but no similar linear (or other simple) associations were clearly demonstrated for any variant, nor for any group of variants. No interpretable associations were found between the incidence of any chromosome variants and the birth order of the infants.

With regard to the blood groups and isoenzymes, the mean maternal age for the heterozygotes of the MNS system was higher than that of the homozygotes. This effect was not related to any particular phenotype. A similar association was found between this marker and paternal age, but the trend was not consistent and probably merely reflects the association with maternal age. No other remarkable correlations were found. The 2-1 phenotype of adenylate kinase increased with increasing birth order, but this enzyme phenotype has a very low frequency, and consequently might be expected to show spurious associations.

The data were analysied with regard to the occupational class of the subjects, as this parameter has often been cited as a factor in the genetic stratification of human populations. In the case of two chromosomes, 3 and 4, the NN phenotype appears to increase in frequency from class I to class V, and in the case of the latter chromosome, the comparison between manual and non-manual classes also showed that significant differences existed.

The positive satellite variants of all acrocentric chromosomes considered together gave a highly significant result when the manual and non-manual classes were compared using the X^2 test, but this did not indicate any general trend for either higher or lower numbers of satellite variants per individual in the manual or non-manual classes.

For the blood groups, only the Kell system showed any stratification by occupational class. The frequency of the KK phenotype was higher in the non-manual classes than in the manual. The 2-2, and to a lesser extent the 2-1, phenotype of the Esterase D had an increased frequency in the manual classes compared with the non-manual. Thus there is little evidence of an influence of social class on the distribution of any of the genetic variants studied in this population sample.

In conclusion, this study has provided no convincing evidence that any of the demographic variables considered are associated with an equivalent stratification in the genetic structure of the population. The alternative explanations for this are that, firstly, this reflects the real situation; secondly, the population has not been sampled in a way necessary to reveal any such stratification. An obvious factor to turn to when considering this explanation is sample size. It seems obvious that the required size of sample depends very much on the degree to which the frequencies of these genetic and chromosomal markers are affected by the various demographic parameters. The problem in the present thesis is not so much that such effects are not detected, and are not significant, but that biologically explicable relationships are not detected, whether statistically significant or not. While it is expected that many disturbances of gene frequencies might indicate evolutionary or adaptive significance without having statistical significance, it is difficult to explain, say, the importance of a phenotype which declines in frequency with age (indicating a disadvantageous effect on survival or longevity) but which shows a sudden increased frequency in the oldest age-groups.

The three major ascertainment groups of the subjects of this sample also group the subjects according to age, and

to some extent, according to occupational class (although this is not known for the geriatric patients). While any differences in phenotype frequencies that may occur have not been attributed to the ascertainment of the subjects, this factor may aggravate or neutralise any effects of age or other demographic parameter.

2. A comparison between the results of different studies.

The major result of the comparison between this study and other published series is that while the actual frequencies of the different variants show enormous variation from study to study, the orders of frequency of the variants show quite marked similarities in almost all cases. There seem to be chromosome regions with quite common positive variants, others with rare variants and (naturally) a group of 'medium' frequency variants. In most cases the common and rare variants are the same in the different studies, although the absolute frequency varies. This probably indicates that something quite real has been measured in these researches (despite an apparent arbitrariness felt during the scoring of the variants) but that either quite marked geographic variation occurs with respect to the frequencies of the variants, or that, much more reasonably, technical difficulties and methodological variations have led to the lack of similarity between absolute variant frequencies in the different studies. Of course, until this latter factor is eliminated, there is no way of measuring the extent of the former, but there is no reason to think that geographical variation has not contributed at all to the observed inter-study differences.

3. Chromosome variants in the Primates.

Amongst the higher primates the intense and brilliant fluorescence variants are found only in the karyotypes of

the chimpanzee and gorilla. The satellites of the acrocentric chromosomes of these organisms have been shown to vary in their intensity of staining. No large surveys of the extent of this variation have been conducted but it is thought that the positive variants of the satellites are more common in the chimpanzee chromosome complement than in that of the human.

Other chromosome regions which have quite common positive variants in the human karyotype (such as the centromeres of chromosomes 3 and 13) have not been recorded as being variable in the karyotypes of related organisms. This contrasts with the situation regarding other genetic polymorphisms, such as the red cell antigens, which seem to be widespread not only amongst the higher primates, but amongst other mammalian species also (Mourant et al. 1976).

These considerations allow speculation about the time of origin of the variant material, but such speculations are hindered by the lack of knowledge about the composition of the variant regions. It is not known whether or not this material disperses in the genome after it has originated, or whether it has had a separate origin in each region where it is found in the present-day human karyotype.

Great similarity between variants of the higher primates and those of human chromosomes might indicate a selective value in either the retention or loss of the variants but there is insufficient primate data to make an adequate comparison; population studies would be required.

4. Other aspects of chromosome variability.

Disturbances in the amounts of large sections of the chromosome material (including structural genes) obviously have a fairly drastic effect on the phenotype. It is less

obvious what the effects of balanced rearrangements are. Quantitative differences in the heterochromatic material have been implicated in various deleterious phenotypic conditions, for example, an increased likelihood of congenital abnormalities of chromosomal nondisjunction and of development of malignancies. None of these aspects of chromosome variability (with the possible exception of the last) is directly comparable with the chromosomal variations which have been investigated in this study. They all involve larger amounts of chromatin, probably qualitatively different chromatin. But studies of their incidence does indicate that substantial variations from the norm, as far as the genomic material is concerned, usually have an effect on the phenotype which is not good. However, some of the chromosome variants studied here are sufficiently frequent to be considered 'normal' although almost without exception they occur at a frequency of less than 50%.

5. The facts of chromosome structure and the mechanisms of banding.

These point to no clear functional role of the material of these variant regions. They are perhaps involved in the regulation of genes but this seems unlikely as the material is so localised. It is perhaps involved in some aspect of chromosome maintenance, but this may be doubted as it does not occur on all chromosomes. Jones (1977) suggests that satellite DNAs are important during periods of speciation only, and if the DNA of the variant regions is of this type, perhaps this suggestion is true of the variant regions also. Perhaps they are merely the consequence of past unique events which had no effect ever and even what they are a consequence of is not important now.

In conclusion.

The comparability of the relative frequencies of the variants in different studies from many regions of the world suggests that these are real genetic polymorphisms. But whether or not they have, or have had adaptive significance is very difficult to demonstrate. Correlations with demographic parameters as in the present study, considerations of their existence in related animals, and of their biochemical nature and function have been as unfruitful for the elucidation of this question as have equivalent studies on most other human populations.

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Appendix 1

Forms used for the collection of demographic information.

1.1 Newborn infants.

1.2 Students and University staff.

1.3 University staff.

1.4 Hospital staff and orthopaedic patients.

University of Durham Department of Anthropology

South End House, South Road,
Durham, DH1 3TG, England
Telephone: Durham 64971 (STD code 0385)

Dear Madam,

I am at present carrying out a research project, financed by the Medical Research Council, which involves looking at the variability of human chromosomes. This work involves collecting small samples of cord blood from newly-born infants of the Durham area. I have approached the medical staff of this hospital and they are agreed that, given your permission, they are willing to co-operate inthe collection of the sample.

The blood will be collected from the afterbirth after delivery and neither you nor your child will be involved.

If you are willing to give your consent, please sign below, and also fill in the attached questionnaire.

Yours faithfully,

Barbara L. Hudson

Barbara L. Hudson

Signed ..... Date .....

All answers will be treated as strictly confidential

1. Surname of Child ..... 2. Sex .....
4. Hospital Case No. ..... 3. Date of Birth .....
5. Address .....
6. Your birthplace ..... 7. Your husband's birthplace .....
8. Birthplace of your mother .....
9. Birthplace of your father .....
10. Birthplace of your husband's mother .....
11. Birthplace of your husband's father .....
12. Husband's occupation .....
13. Your Date of Birth ..... 14. Your husband's Date of Birth .....
15. Ages and sexes of child's brothers and sisters:

	Age	Sex
1.	.....	.....
2.	.....	.....
3.	.....	.....
4.	.....	.....

Name..... No. P.C.....

Address

Date of birth / /

Places of birth self
spouse
father
mother
ff
fm
mf
mm

Occupation self
father

I hereby consent to the taking of a sample of blood for research the purpose of which has been explained to me.

signed.....

date.....

University of Durham Department of Anthropology

South End House, South Road,
Durham, DH1 3TG, England
Telephone: Durham 64971 (STD code 0385)

Dear Sir/Madam,

I am at present carrying out a research project, financed by the Medical Research Council, which is concerned with the variability of human chromosomes. The work involves the collection of small samples of venous blood from normal healthy adults resident in the Durham area. I am writing to ask you if you would be willing to participate in this survey. If so, would you please sign the consent statement below, fill in the questionnaire, and go to the University Student Health Centre, Old Elvet, between the hours of 9.30 a.m. and 12.30 p.m. any day from Tuesday 30th August until Friday 30th September. (Monday to Friday only) The medical staff there have kindly agreed to help me by taking the blood sample. The whole procedure only lasts a few minutes!

Thank you,

Yours faithfully,

Barbara L. Hudson

PLEASE BRING THIS WITH YOU TO THE HEALTH CENTRE

I hereby give voluntary consent for the withdrawal of a small sample (approximately 5 - 6 ml) of venous blood for the purposes of scientific research.

Signed ..... Date .....

NAME .....

DATE OF BIRTH .....

PLACE OF BIRTH .....

FATHER'S BIRTHPLACE .....

MOTHER'S BIRTHPLACE .....

FATHER'S FATHER'S BIRTHPLACE.....

FATHER'S MOTHER'S BIRTHPLACE .....

MOTHER'S FATHER'S BIRTHPLACE .....

MOTHER'S MOTHER'S BIRTHPLACE .....

OCCUPATION .....

SPOUSES OCCUPATION .....

University of Durham Department of Anthropology

South End House, South Road,
Durham, DH1 3TG, England
Telephone: Durham 64971 (STD code 0385)

ear Sir/Madam,

I am at present carrying out a research project, financed by the edical Research Council, which involves looking at the variability of uman chromosomes. This work involves the collection of small samples of enous blood from adults of the Durham area. I have approached the medical taff of this hospital and they have agreed that, given your permission, hey are willing to cooperate in the collection of the sample.

If you are willing to take part in this survey, please give your onsent by signing this form and also fill in the questionaire below. All nformation given will be treated in the strictest confidence.

Yours faithfully,

Barbara L Hudson

Barbara L. Hudson

Signed . . . . . Date . . . . . .

NAME . . . . . DATE OF BIRTH . . . . . .

ADDRESS . . . . . .

. . . . . .

YOUR BIRTHPLACE . . . . . .

MOTHER'S BIRTHPLACE . . . . . .

FATHER'S BIRTHPLACE . . . . . .

MOTHER'S MOTHER'S BIRTHPLACE . . . . . .

MOTHER'S FATHER'S BIRTHPLACE . . . . . .

FATHER'S MOTHER'S BIRTHPLACE . . . . . .

FATHER'S FATHER'S BIRTHPLACE . . . . . .

OCCUPATION . . . . . .

SPOUSE'S OCCUPATION . . . . . .

Appendix 2

Forms used for the recording of laboratory results

2.1 Demographic data and chromosome results.

2.2 Blood groups and isoenzymes.

DEPARTMENT OF ANTHROPOLOGY

Srs	Subject No.				Card	Sex	Date of Birth					Asct	Cty of Birth			Father C.C.B.			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Mth CofB		FF C of B			FM C of B			MF C of B			MM C of B			Occ	Focc	Diagnosis			
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
																Chrom 3		Chrom 4	
41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Chromosome 13					Chromosome 14			Chromosome 15				Chromosome 21				Chromosome 22			
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80

Srs	Subject No.				Card	Sex	Date of Birth					Asct	Cty of Birth			Father C.C.B.			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Mth CofB		FF C of B			FM C of B			MF C of B			MM C of B			Occ	Focc	Diagnosis			
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
																Chrom 3		Chrom 4	
41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Chromosome 13					Chromosome 14			Chromosome 15				Chromosome 21				Chromosome 22			
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80

Srs	Subject No.				Card	Sex	Date of Birth					Asct	Cty of Birth			Father C.C.B.			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Mth CofB		FF C of B			FM C of B			MF C of B			MM C of B			Occ	Focc	Diagnosis			
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
																Chrom 3		Chrom 4	
41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Chromosome 13					Chromosome 14			Chromosome 15				Chromosome 21				Chromosome 22			
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80

Srs	Subject No.				Card	Sex	Date of Birth					Asct	Cty of Birth			Father C.C.B.			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Mth CofB		FF C of B			FM C of B			MF C of B			MM C of B			Occ	Focc	Diagnosis			
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
																Chrom 3		Chrom 4	
41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Chromosome 13					Chromosome 14			Chromosome 15				Chromosome 21				Chromosome 22			
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80

DEPARTMENT OF ANTHROPOLOGY — BLOOD GROUP RESULTS — DATE:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
	NUMBER						A	B	AB	A_1	A_2	M	N	S	$\bar{s}$	C	$\bar{c}$	D	E	e	c^w	Jk^a	Jk^b	Fy^a	Fy^b	K	$\bar{k}$	Kp^a	Kp^b	P_1									
							1	Φ	1	1	9	A_1																											
							1	1	1	1	Φ	A_1B																											
							Φ	Φ	Φ	Φ	Φ	O																											
							Φ	1	1	Φ	Φ	B																											
							1	Φ	1	Φ	1	A_2																											

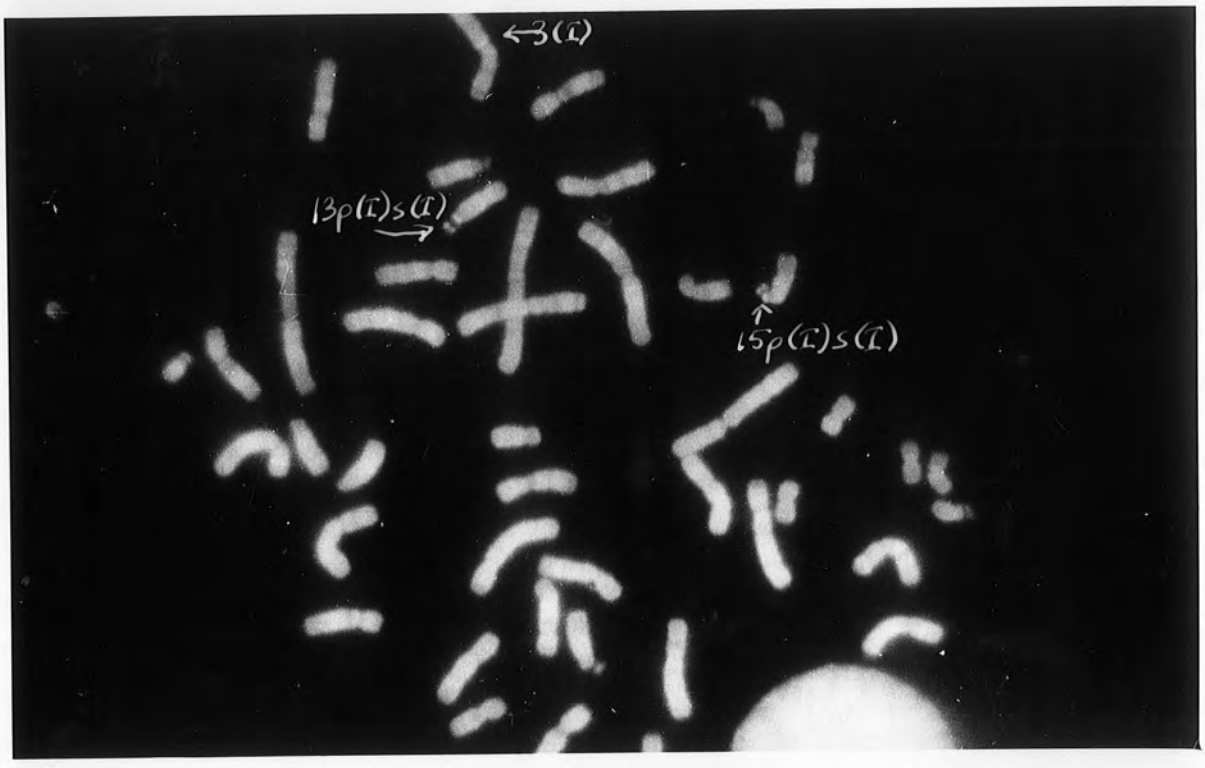
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
-							A	B	AB	A_1	A_2	M	N	S	$\bar{s}$	C	$\bar{c}$	D	E	e	c^w	Jk^a	Jk^b	Fy^a	Fy^b	K	$\bar{k}$	Kp^a	Kp^b	P_1									
												1	Φ	M																											
												1	1	M	N																										
												Φ	1	N		etc.																									

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
							A	B	AB	A_1	A_2	M	N	S	$\bar{s}$	C	$\bar{c}$	D	E	e	C^w	JK^a	JK^b	Fy^a	Fy^b	K	$\bar{k}$	Kp^a	Kp^b	P_1									

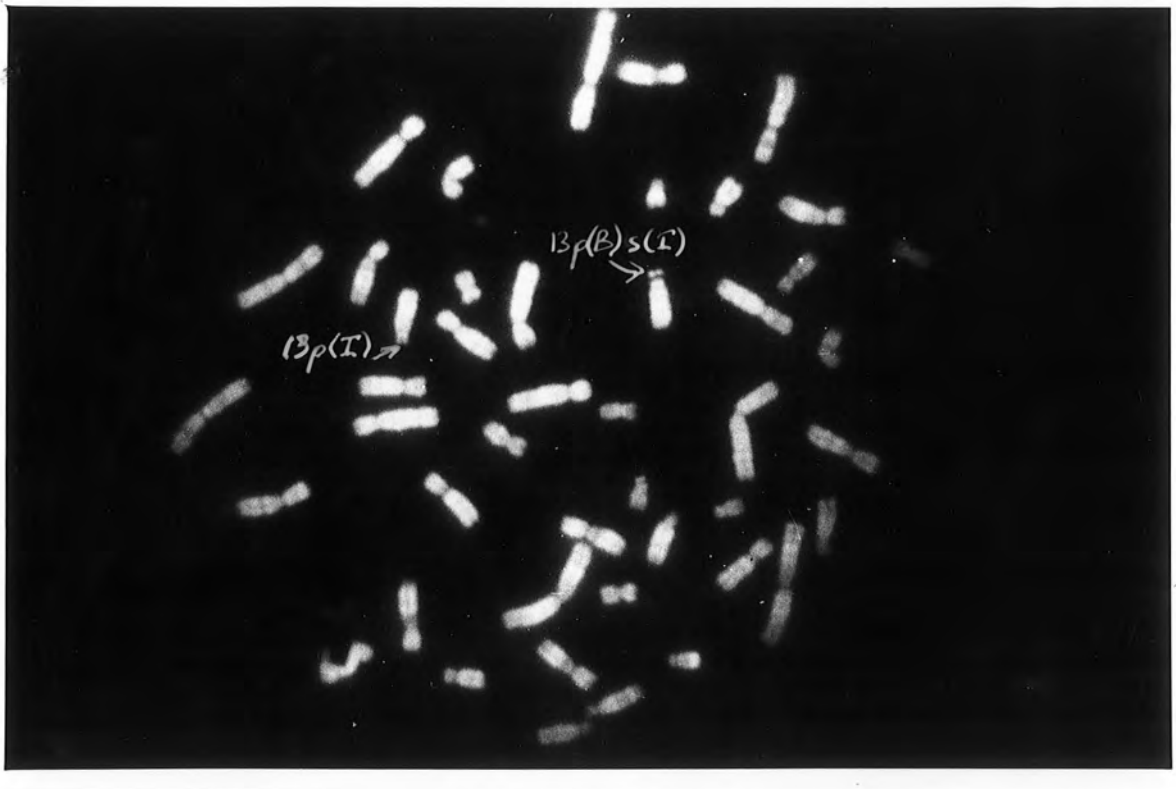
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							A	B	AB	A_1	A_2	M	N	S	$\bar{s}$	C	$\bar{c}$	D	E	e	C^w	JK^a	JK^b	Fy^a	Fy^b	K	$\bar{k}$	Kp^a	Kp^b	P_1									

Appendix 3

Photographs of quinacrine-stained human metaphase chromosomes showing variant regions.



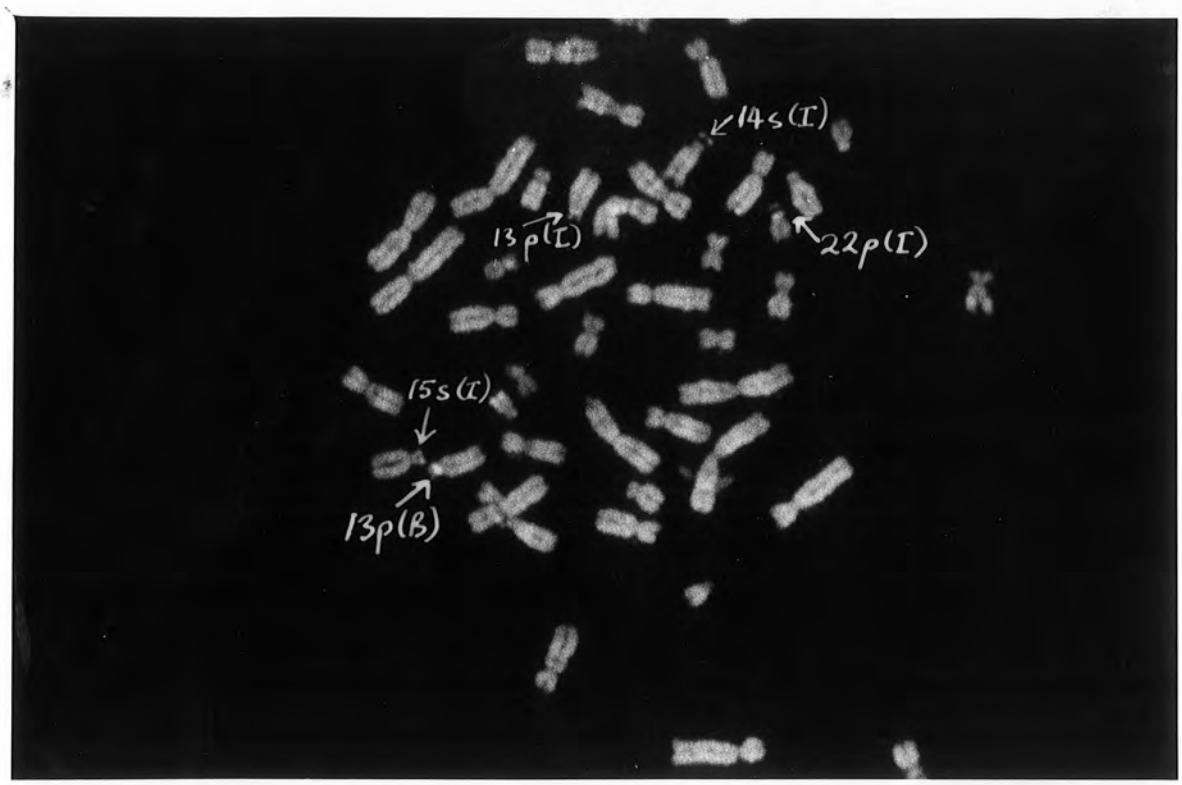
Variants: 3(I), 13p(I)s(I), 15p(I)s(I)



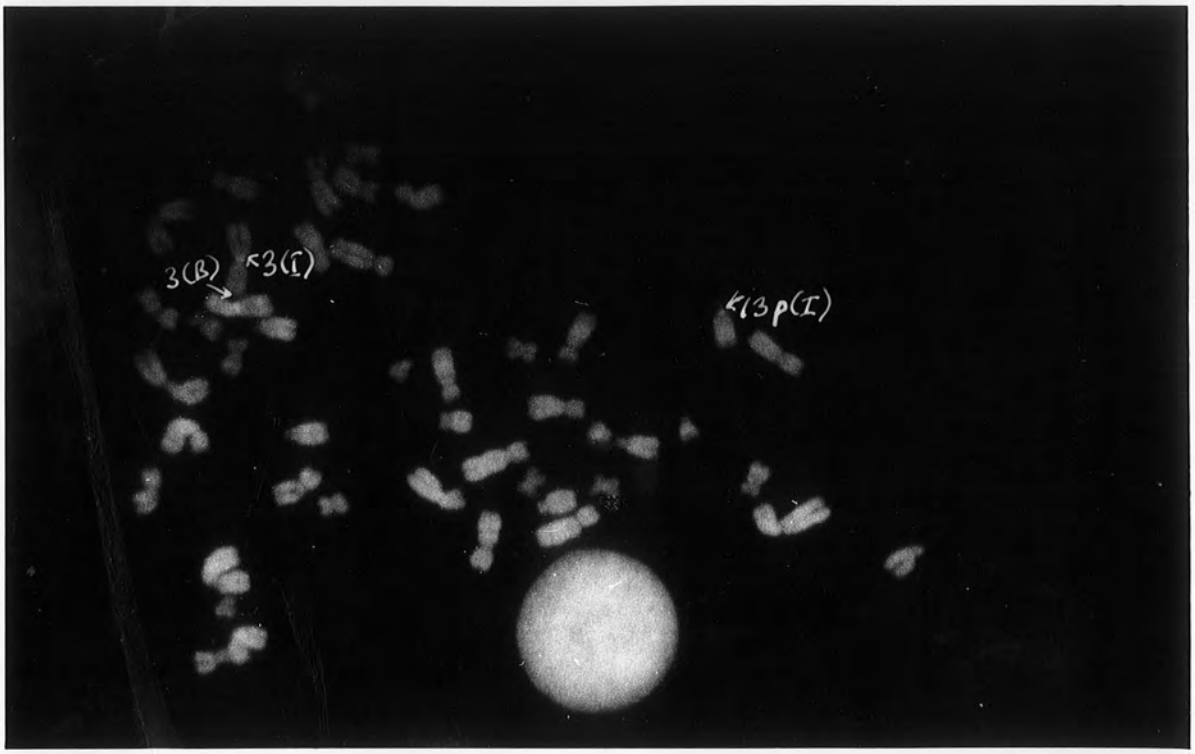
Variants: 13p(B)s(I),13p(I)



Variants: 13p(B), 14s(I), 15s(I)



Variants: 13p(B), 13p(I), 14s(I), 15s(I), 22p(I)



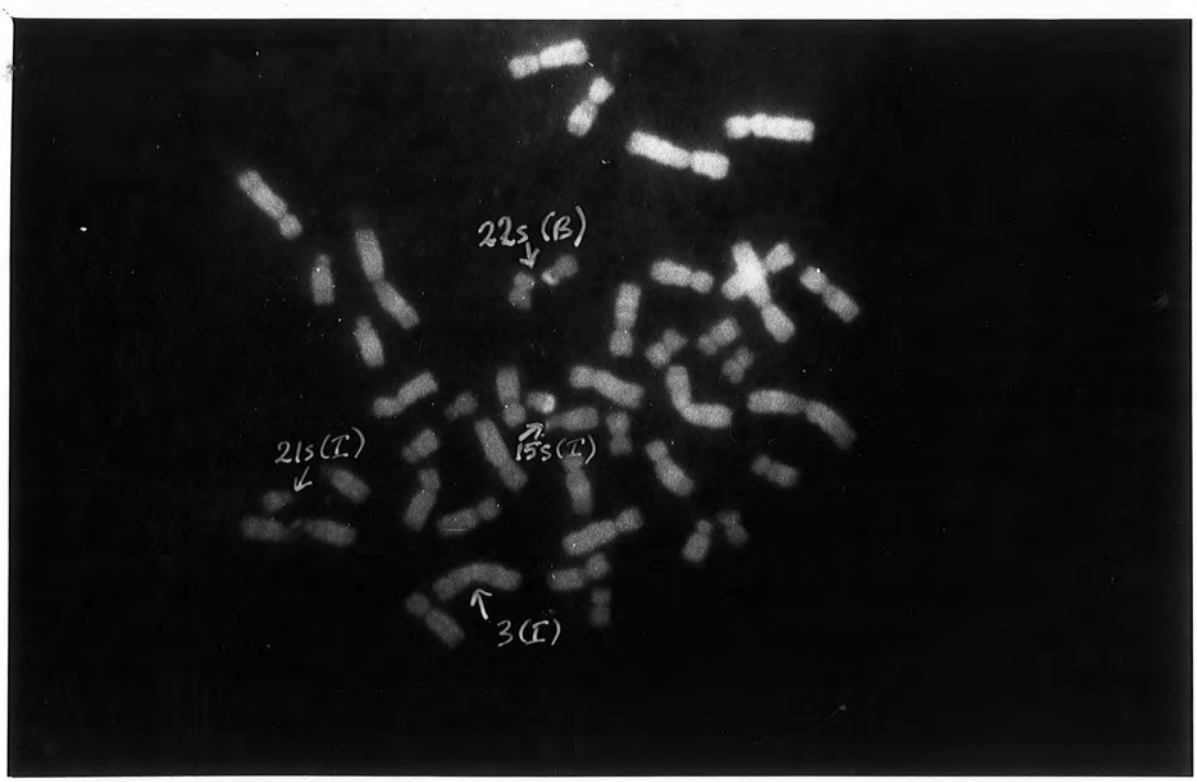
Variants: 3(I), 3(B), 13p(I)



Variants: 3(B), 4(B)



Variants: 3(I), 13p(I), 15s(B), 22s(B)



Variants: 3(I), 15s(I), 21s(I), 22s(B)