LOCALISATION OF AUTOSOMAL GENES IN MAN USING CHROMOSOMAL ABERRATIONS.

by

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CHAPTER I.

INTRODUCTION

"So-called 'formal genetics' of the type carried on extensively in Drosophila and in maize in the early days -- the making of maps of the chromosomes -- would seem an almost Utopian objective for human material.. However, it should be beneath the dignity of man to be content to remain forever ignorant of such basic facts of his own structure". Muller, (1949).

General

In many chromosomal organisms which have been studied so far, genetic analysis has preceded cytological analysis. Although human cytogenetics is a comparatively recent field, cytological analysis has advanced more rapidly than genetic analysis in man. Worthwhile contributions to genetic analysis have been made in some organisms by combining it with cytological analysis. For example, Rhoades and McClintock (1935) made use of chromosome breaks connected with structural changes for the cytological localisation of genes in Zea mays. Slizynska (1938) located the white locus on the

3C1 band of the X chromosome of Drosophila melanogaster by correlating the loss of the normal allele at the white locus with the simultaneous loss of this band from the salivary gland X chromosome. In the mouse the linkage analysis of the joint segregation of a translocation chromosome and marker loci in the progeny of translocation heterozygotes has enabled localisation of genes on particular autosomes and at measurable distances from the break points producing the translocation. These studies were initiated by Snell (1946) and have been extended by Carter, Lyon and Phillips (1955) and by Slizynski (1957) with remarkable success.

Improved techniques in cytogenetics and painstaking microscopy have enabled the study of the morphology of the normal chromosomes in man (for historical review see Stern, 1959), and the identification of a wide range of chromosomal aberrations and variations. These aberrant chromosomes can be used in autosomal mapping. The problem has been treated in two different ways, in most cases as a side line to descriptive cytogenetics rather than as a deliberate attempt at localisation of genes on the autosomes. Some workers have attempted to find a direct association, whether qualitative or

quantitative, between the aberrant chromosome and a marker gene. Others have used familial chromosomal aberrations in linkage analysis. Most of these studies however, have been based on the examination of a few individuals only.

This thesis attempts to answer the question - how far can chromosomal aberrations that are known at present be useful in the localisation of autosomal genes in man?

Outline of the presentation

Chapters on the methods of analysis for linkage data in man and on the description of normal and aberrant human chromosomes precede a chapter where the experimental and analytical methods available for autosomal gene localisation using aberrant chromosomes are discussed. The body of the thesis describes the material and the laboratory techniques used by the author in seeking the linkage of a marker gene with an aberrant chromosome by following their joint segregation in a pedigree. Four pedigrees, each carrying one of three different chromosomal aberrations on three different autosomes have been studied. The data from the cytological investigation and marker gene investigation have been tabulated

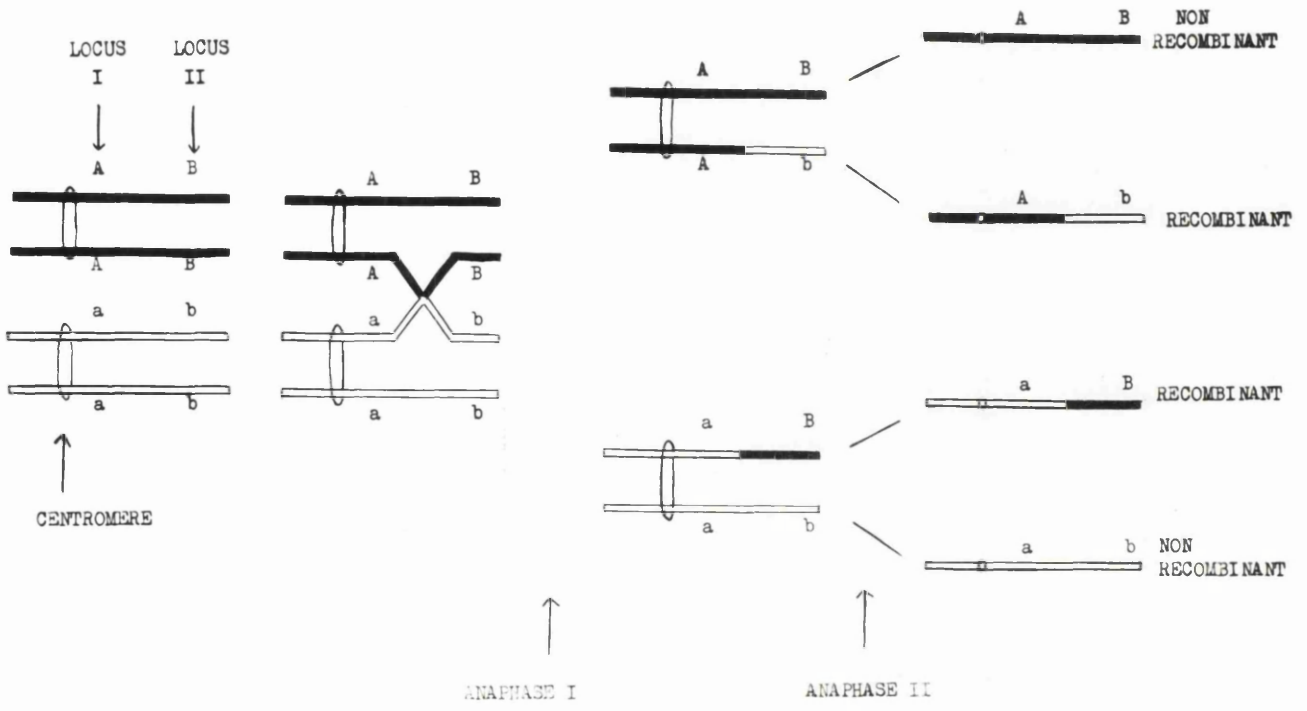
(Tables 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7 and 7.8). The data of one marker gene in one pedigree are used to illustrate variations of the likelihood method that are generally agreed to be the most suitable for the analysis of human linkage data (VIII A). The final probability that a marker locus is on any one of the marker chromosomes used in the study is given (VIII B). The results are discussed with reference to those of other workers in the field.

The phenomenon of genetical linkage

In the soma of a diploid organism the chromosomes are paired structures, one member of each pair being derived from each parent. The chromosomes of a pair have corresponding parts. The gene loci are linearly arranged on the chromosome and the two alleles at a particular locus may or may not be identical. Each parent contributes one of the two alleles at a locus to the offspring. In man there are 22 pairs of autosomes which behave in this way. The sex chromosomes, however, behave differently. The female has two X chromosomes and the male, one X and one Y. The gene loci on the unpaired part of the X chromosome have only one allele in the male.

In inheritance the alleles of loci which lie on different pairs of autosomes segregate and pass to haploid gametes independently. Alleles whose loci lie on the same chromosome may not show this independence of each other, i.e. they may show linkage with one another. Consider two homologous chromosomes each having two loci, I and II, with two alleles each, A, a and B, b. The alleles A and B, which are on the same member of the chromosome pair, are said to be in coupling, and the alleles A and b, which are on opposite members of the pair, are said to be in repulsion. At meiosis there is replication of the DNA of the chromosomes and pairing of the homologous parts. Fig. I.1 shows such a pair of chromosomes in the four strand stage. If sister-strand crossing over is assumed not to occur, a single crossing over event between two of the four strands in the interval between locus I and locus II, will yield one of four possible haploid chromosomal arrangements from the original diploid heterozygote AB/ab. These are AB and ab (non-recombinant or parental types) and Ab and aB (recombinant types). For linked loci, a 1:1:1:1 ratio for the four possible products of a meiotic cell division is not observed. The proportion of gametes that are recombinant will depend on the frequency of exchange of

FIG. 1.1 CROSSING OVER BETWEEN LINKED LOCI.



segments. If analysed in terms of coupling phase, recombination is a change from the coupling phase to the repulsion phase or vice versa during the formation of gametes. The probability of such a change, which is a measure of the degree of linkage between the two loci, is called the true recombination fraction, θ , and is estimated by $\hat{\theta}$.

$$\hat{\theta} = \frac{\text{number of recombinant gametes}}{\text{total number of gametes}}$$

The recombinant types, Ab and aB, in Fig. 1.1 are each expected to occur with a frequency $\theta/2$, and the parental types each with a frequency $(1-\theta)/2$.

Crossing over is the interchange of corresponding segments between chromatids of homologous chromosomes. Crossovers are the chromatids that have taken part in such interchanges. Crossing over between a pair of linked genes occurs at a stable frequency but the frequency will differ with the gene pairs involved. The map interval, w Morgans, is the average number of exchange events occurring per chromatid between two sites in a single meiosis. It is an approximate measure of the physical distance between the two sites, if the chance of a crossover is assumed to be roughly the same at all points on the chromosome. Map distance is thus based on the frequency of crossing over. Unfortunately, frequency of crossing over can only be measured indirectly - via recombination

frequency. Recombination frequency estimates are based on counting the proportion of gametes carrying 1, 3, 5, 7 ..... crossovers between locus I and locus II, since strands carrying an even number of cross-overs between locus I and locus II do not show recombination between alleles at those loci. Haldane (1919), by an application of the Poisson distribution, related θ to w in the absence of interference (i.e. if the exchange events are statistically independent of each other) as

$$\theta = \tfrac{1}{2}(1 - 1/e^{2w}).$$

Clearly, as w becomes larger, θ approaches 0.5. If w is small, i.e. for small map intervals, θ is approximately equal to w; as follows:-

Since, by definition, $\frac{1}{e^{2w}} \equiv 1 - 2w + \frac{(2w)^2}{2!} \cdots\cdots$

Haldane's formula can be expanded as

$\theta \simeq \tfrac{1}{2}(1 - (1 - 2w))$, if terms in w^2 and higher terms are negligibly small; i.e. $\theta = w$. For small intervals this is a valid approximation, even when there <u>is</u> interference, and a recombination fraction of 0.01 corresponds to the unit of map distance (1 map unit or 1 centimorgan, cM, or 0.01 Morgans, 0.01 M).

When there is complete interference, i.e. a maximum of one

crossover event per strand in the relevant interval, $\theta = w$ for all values of w (up to a maximum of 1 M), since every strand carrying an exchange is a recombinant strand.

In practice, the level of interference at meiosis in man is not accurately known. The observation of more than one chiasma on one chromosome pair (Ford and Hamerton, 1956) and the recovery of double recombinants (Graham, Tarleton, Race and Sanger, 1962) exclude both complete chiasma interference and complete chromatid interference respectively. For relating θ and w, the mapping function with intermediate levels of interference proposed by Kosambi (1944), $2w = \tan^{-1} 2\theta$, or Carter and Falconer (1951), $4w = \tan^{-1} 2\theta + \tanh^{-1} 2\theta$ can be used.

Estimation of recombination fraction.

The direct estimate, $\hat{\theta}$, of the recombination fraction, θ, is the proportion of recombinant types among the products of meiosis. This estimate is feasible when the phenotype defines the genotype precisely at each of the two loci. In a mating of a double heterozygote of known coupling phase with a double homozygote, any strand produced by the

heterozygote at meiosis and transmitted in a gamete later fertilised can then be classified in the zygote as recombinant or non-recombinant.

$$\hat{\theta} = \frac{\text{Number of recombinant progeny}}{\text{total number of progeny}}$$

Special statistical methods enable the extraction of recombinational data even from less directly informative matings (see Chapter II). The only known autosomal linkages in man have been identified by such analyses. The certain autosomal linkage pairs are given in Table 1.1. For the nail patella : ABO and Lutheran : secretor intervals the recombination fraction has been large enough and the data sufficient for the genetical comparison of the sexes. In the former, Renwick and Schulze (1965) calculated the recombination fraction in the female as 0.146 and that in the male as 0.084. In the latter, Cook (1965) quotes the figure of 0.177 for the female and 0.101 for the male.

At least one mimic locus for the elliptocytosis locus was detected by discrepancies in the linkage estimates in different pedigrees. Morton (1956) showed that in some pedigrees there was no linkage with the elliptocytosis locus. The phenotypes of individuals heterozygous at one or other of the mimic elliptocytosis loci have not yet been distinguished from each other.

Table 1.1 The certain autosomal linkage pairs in Man.

Pairs of loci	Recombination fraction	Authors	
Lutheran:Secretor	7.5%	Mohr	1951
Elliptocytosis:Rhesus	3.3%	Lawler	1954
Nail-patella:ABO	9.7%	Renwick & Lawler	1955
Haemoglobin, Hb_β:Hb_δ	0.2%	Ceppelini	1959
Cae cataract:Duffy	0%	Renwick & Lawler	1963
Transferrin:Cholinesterase E_1	16.0%	Robson, Sutherland & Harris	1966
Albumin:Gc	1.5%	Weitkamp, Rucknagel & Gershowitz	1966
Sclerotylosis:MNS	* 4%	Mennecier	1967
Adenylate kinase, AK:ABO	20%	Rapley, Robson, Harris and Maynard-Smith	1968

* By computer analysis (Renwick, personal communication).

In the congenital cataract : Duffy linkage there was no definite recombination between the loci, and the modal estimate of the recombination is 0 with a final probability of linkage of 0.977 (Renwick and Lawler, 1963).

The albumin : Gc linkage detected by Weitkamp, Rucknagel and Gershowitz, (1966), has been confirmed by Melatin and Blumberg (1967) using the variant albumin, albumin Naskapi.

With the identification of the AK : ABO linkage by Rapley, Robson and Harris (1968) the first linkage group consisting of the loci nail patella, ABO and AK was established. The order of these loci is probably ABO, nail patella and AK (Renwick, personal communication). With the allocation of the Duffy locus to chromosome no. 1 (Donahue, Renwick, de los Cobos, Borgoankar, Bias and McKusick, 1968), the congenital cataract : Duffy linkage can be assigned a definite chromosome.

Total map length

Each crossover event affects only two of the four strands. Therefore the map interval based on the events on a single strand relates to only half the total events. Similarly, the map interval in Morgans is equal to only half the number of visible chiasmata

between locus I and locus II, assuming that there is a 1:1 correspondence between crossover events and chiasmata. An idea of the total genetic map length can be obtained by counting the chiasmata. Ford and Hamerton (1956) studied testicular biopsies of 3 men of different ages. In 23 cells in late diplotene to mid-diakinesis they obtained an average number of chiasmata of 55.9. They counted the end to end association of the X and Y as a chiasma. No definite chiasma has been reported between an X and Y, however. When this association of the X and Y was not included the average number of chiasmata per cell was 54.9. McIlree, Tulloch and Newsam (1966) have confirmed these observations. The chiasma counts of 14 normal men studied by these authors had an average count per cell varying between 49.8 and 58.3. Taking one chiasma as being equal to 0.5 Morgans (see above), the mean value of the total autosomal map length in males would be 27.5 Morgans (2,750 centimorgans or map units). The number of chiasmata must be regarded as a minimal count, for the chiasmata that had undergone terminalisation at the time of observation would not have been included. Further, as already mentioned, there is a lower frequency of crossing over in males than in females.

Table 1.2 gives the mean values of arm measurements of the autosomes expressed as percentages of the haploid autosomal complement (Ferguson-Smith, 1964a). From these values, and assuming uniformity, the total map length can be apportioned between the individual autosomes.

Total number of loci

Vogel (1964) gives an upper limit to the number of human genes. The haemoglobin molecule consists of 2 alpha chains and 2 beta chains, each having about 150 amino acids. Many other proteins consist of sub-units of similar magnitude, each of which is probably the product of one structural gene. Since each amino acid is coded for by a triplet of nucleotide pairs, one structural gene might have the length of about 3 x 150 nucleotide pairs. The weight of one human spermatozoon, consisting almost entirely of the haploid chromosome complement, is approximately 3×10^{-12} g. The weights of the nucleotide pairs in DNA are

Adenylic acid + Thymidylic acid = 1.025×10^{-21} g

Cytidylic acid + Guanlyic acid = 1.027×10^{-21} g

Hence, the total haploid chromosome set contains about 3×10^{9} nucleotide pairs. Assuming that most of the DNA of the chromosome

Table 1.2 Centromere indices and relative arm lengths of the chromosomes.

Chromosome Number	Centromere Index	Relative Length (% Total Autosomal Length)		
		Short arm	Long arm	Total
1	46.9	4.184	4.743	8.93
2	38.5	3.152	5.027	8.18
3	46.6	3.221	3.690	6.91
4	29.0	1.873	4.587	6.46
5	28.4	1.671	4.214	5.89
6	38.3	2.282	3.676	5.96
X	40.2	2.177	3.244	5.42
7	39.1	2.060	3.213	5.27
8	31.9	1.604	3.418	5.02
9	34.7	1.727	3.254	4.98
10	32.5	1.487	3.093	4.58
11	39.9	1.827	2.754	4.58
12	32.0	1.404	2.977	4.38
13	14.6	0.554	3.241	3.80
14	14.7	0.508	2.957	3.46
15	15.7	0.513	2.764	3.28
16	39.9	1.310	1.975	3.28
17	32.8	1.047	2.140	3.19
18	27.8	0.802	2.084	2.89
19	45.1	1.230	1.499	2.73
20	43.5	1.063	1.380	2.44
21	26.2	0.526	1.485	2.01
22	26.9	0.480	1.306	1.79
Y	30.0	0.662	1.547	2.21

works as genetic material, and that an average of 450 nucleotide pairs are required for one structural gene, Vogel concludes that the total gene content is approximately 6.7×10^6. His estimate would be much too high if many copies of each structural gene are present, as postulated by Callan (1967).

The smallest difference in chromosomal length that can be detected is about 0.2% of the total chromatin of a cell at metaphase. This would represent a very large number of loci and direct analytical studies such as those on the banding patterns of the salivary gland chromosomes of Drosophila (Slizynska, 1938) may not be possible in man. However, a number of alternative methods for obtaining information about the position of gene loci on chromosomes are available, and the present study was undertaken with the hope that the systematic analysis of chromosomal aberrations by some of these methods would prove to be profitable.

CHAPTER II.

METHODS FOR ANALYSIS OF LINKAGE DATA

To demonstrate that two loci are linked a particular allele at one locus must be shown to have a tendency to travel together in inheritance with an allele at the other locus. If, in an individual, the alleles at a locus are the same on both members of the chromosome pair, e.g. if the genotype is AA or aa, A and a, being two of the possible alleles at this locus, the individual is said to be homozygous. If the alleles differ, e.g. if the genotype is Aa, he is said to be heterozygous. For detection of linkage between two loci at least one parent must be heterozygous at both loci studied. As described earlier, a double backcross mating, heterozygote x homozygote, gives the simplest information. Theoretically, any two loci can be tested for linkage between them. Linkage studies in man, however, are handicapped by the small size of the families, the long generation time and inability to plan matings. This last restriction, operating as it does in a natural population carrying a large number of alleles at each locus, makes the determination of genotype sometimes difficult, even when the phenotype can be tested. Difficulties brought in by illegitimacy, consanguinity,

refusal of some members to co-operate and non-availability of others due to emigration or death have also to be considered. The maximum amount of information is extracted from such restricted data by statistical analysis.

It is not practical to test two loci for linkage directly in a population which has a very low frequency of heterozygotes for both loci. In autosomal linkage investigation generally, the pedigree is chosen through the phenotypes at only one locus, the main locus. It is preferable to select a heterozygous condition which is infrequent in the population studied, so that mates will usually be homozygous normal. In the interest of large pedigree size, the presence of the rare allele should preferably not interfere with the viability of the zygote. Ideally, the various genotypes should be of easily distinguishable phenotype and scorable at all ages. If tests for homogeneity are satisfactory, information from different pedigrees can be added together.

The selected main locus is tested against a group of marker loci for which no allele frequency is too close to 100%. In the study presented, 23 such loci have been tested, allowing for 24 x 23/2 pairs of autosomal loci to be potentially available for linkage analysis, as

the main locus is also included. That a parent selected for study because of heterozygosity at the main locus is also heterozygous at the test locus depends on chance. The test loci used in this study were those for some of the erythrocytic antigens, erythrocytic enzymes, serum proteins and the ABH secretor status.

The amount of information that can be obtained from linkage analysis depends to a large extent on the size of the pedigree. Although even a sibship of two, with or without parents analysed, could give some information, the pedigree should preferably extend to as many generations as possible. In such large pedigrees the coupling phases of some of the parents might be known. Further, if a substantial amount of information can be gathered from a few large pedigrees, tests for heterogeneity are more readily interpreted.

Direct Count: If there are sufficient suitable data, linkage can be recognised by counting the proportion of the recombinant offspring with respect to the tested loci. This constitutes the simplest method of analysis. However, only certainties can be counted. This means that there must be a clear discrimination of recombinants from non-recombinants and a knowledge of the coupling phases in the parents. The uncertainties limiting the opportunity of performing such an

analysis are of two types:

i. the phenotypes of some members of each pedigree are unavailable or incomplete, and

ii. certain of the phenotypes represent more than one genotype.

Likelihood methods: i. General

Since at least some of the data cannot be analysed by the direct method, the detection and estimation of linkage from human pedigrees is best achieved by using the method devised by Bell and Haldane (1937) and developed further by Haldane and Smith (1947), Morton (1955) and Smith (1959). The basic principle employed in this method is the assumption that valid conclusions about the presence of linkage can be arrived at through the knowledge of the theoretically-based likelihood of actually finding, in the general population, families with the observed distribution of phenotypes. The likelihood of obtaining the observed family data, if the true recombination fraction is θ, is compared with the likelihood of obtaining the family (with its observed phenotypes), if there were truly no linkage at all; i.e. if $\theta = 0.5$.

Likelihood of finding family F, if the true recombination fraction is θ, is given by

$$L(F|\theta) \propto \theta^{n}(1-\theta)^{m}$$

where n = number of recombinants

m = number of non-recombinants.

Arithmetical manipulations of such expressions are easier if they are treated as logarithms, for then,

$$\log_{10} L(F|\theta) = k + n\log\theta + m\log(1-\theta)$$

where k is the proportionality constant.

The ratio $\frac{L(F|\theta)}{L(F|0.5)}$ is the standardised likelihood ratio.

Its logarithm

$$\log_{10}\frac{L(F|\theta)}{L(F|0.5)} = \log_{10} L(F|0) - \log_{10}(L(F|0.5)$$

is called the lod or z score.

By definition, a lod is the logarithm, to the base ten, of the standardised likelihood ratio.

ii. Lod Tables

Morton (1955) calculated the lod scores for families of up to 5 sibs, with values of θ from 0.1 to 0.4 and different mating types. He also set out corrections which have sometimes to be applied to the score when selection of matings depends on phenotypes at both

loci. The mating types as set out by Morton and the lod scores with the appropriate corrections are given in a condensed form in Maynard-Smith, Penrose and Smith (1961). These authors have included values of lod scores at $\theta = 0.05$ and 0. Lod scores from different matings of the same family or from different families which give information about the same loci can be added together to give a total lod score Z. The anti-logarithm of this score (antilod) is the standardised likelihood ratio.

iii. Hand likelihood

Instead of using the tables of Morton, the likelihood ratios for each pedigree treated as an integrated unit, can be calculated by hand. The analysis can be complete by this method, as all possible genotypes for the phenotype of any individual can be incorporated. Population genotype frequencies are used to weight these possible genotypes for each unrelated mate. The method, however, is tedious and time consuming.

iv. Computer analysis

A large computer, if appropriately programmed, can compute this full likelihood for almost any pedigree for various alternative

values of θ. It is possible to make the computer check the information regarding phenotypes, sort out all possible genotypes for each individual and reject pedigrees showing inconsistencies.

In Chapter VIII A, these different methods for the analysis of linkage data are illustrated and compared by the analysis of the pedigree JN1PW with respect to the possible linkage of the ABO locus to the locus controlling the presence of satellites on chromosome no. 17.

v. Sequential tests

Morton uses a sequential method for the detection of linkage. A particular value of θ say (θ_1), equal to 0.1 or 0.2 or 0.3, depending on the amount of data available, is chosen. The lod score values of families are added on to the total score as they are found. If at any time lod $(\theta_1) > 3$, it is concluded that linkage is probably present. If lod $(\theta_1) < -2$, linkage is thought to be unlikely. For values between these figures more data are necessary before a decision can be made. The sequential method is not used in this study.

vi. Probability that two loci lie on the same autosome

Smith (1959) uses $\bar{\Lambda}$ the average likelihood ratio, equivalent to

the height of a rectangle having an area equal to that under the likelihood/ θ curve, and lying on the same base. Λ is determined by planimetry, or by Simpson's rule or by integration of a polynomial specifying the curve.

a. Prior odds. Smith incorporates in the calculations the prior odds on linkage and the prior odds of a particular recombination fraction, if there is linkage. Two approximations have been made by him.

i. that all 22 autosomes have an equal probability of carrying a particular autosomal locus. Therefore, the prior odds that any two loci chosen at random may lie on the same chromosome are 1:21. This figure errs on the safe side, since the more accurate odds would be 1:18.4 if calculated on the assumption that the autosomes have individual probabilities of carrying a certain locus which are not equal but are proportional to the respective chromosome lengths in mitotic metaphase, as given in Table 1.2.

ii. that for linked loci the recombination fractions are equally distributed throughout all parts of the range $0 \leq \theta \leq 0.5$. This is justified theoretically and from observation for chromosomes of length

1 Morgan (Morton, 1955). For chromosomes larger than 1 Morgan, values of θ close to 0.5 are more likely than others and, since the average human autosomal map length even in males is 1.25 Morgan, the simple approximation errs on the unsafe side, i.e. it overestimates the odds on linkage. Allowance can be made for this and for the opposite bias from approximation in critical cases.

Acceptance of the simpler approximations allows us to multiply each point on the likelihood curve by a constant factor (r for convenience) to transform the curve into a probability curve without redrawing it. The constant r is such that the total probability is 1, but in practice it need not be evaluated.

b. Final probability that both loci are on the same chromosome

The odds on linkage derived from an average likelihood Λ will then be as follows:

Prior odds on linkage		1:21
Odds on linkage (from observations)	Λ	r:r
Final odds on linkage	Λ	r:21r
	or Λ	:21

Therefore the final probability that the two autosomal genes at locus I and at locus II are on the same chromosome is $\frac{\Lambda}{\Lambda + 21}$

vii. Estimating θ

The total lod scores from any one family or a group of homogeneous families can be used to obtain a maximum likelihood estimate of the recombination fraction θ. A curve is drawn plotting the total lod against θ. If linkage is present inspection of the curve will show the lod reaching its maximum value at a certain recombination fraction, and the value of θ for this maximum is the maximum likelihood estimate of the recombination fraction. The same estimate may also be obtained from the plot of likelihood ratio against θ.

viii. Limits of θ

After the estimate of θ in this sample has been calculated, it is necessary to give probability limits which express the confidence that the true value of θ in the population as a whole lies within a certain range of values. The 95% probability limits can be determined by planimetry by cutting off, at the upper and lower tails of the probability/θ curve, areas equivalent to 5% of the total area.

CHAPTER III.

THE HUMAN CHROMOSOMES - NORMAL, ABERRANT AND VARIANT

THE NORMAL CHROMOSOMES

The Karyotype

The chromosomal complement of man at somatic metaphase consists of 46 chromosomes (Tjio and Levan, 1956). The 22 pairs of autosomes and the pair of sex chromosomes are classified according to the standard system proposed by the Denver Human Chromosome Study Group (1960). This classification has been revised at the London Conference of the Ciba Foundation Guest Symposium on Human Chromosomes (1963) and at the Chicago Conference on the Standardisation in Human Cytogenetics Nomenclature (1966). The chromosomes are arranged in order of decreasing size, numbered from 1 to 22 and grouped into 7 groups, A to G. A standard arrangement of the chromosomes of a single somatic mitotic figure is termed a karyotype.

The Idiogram

The position of each centromere (area joining the 2 chromatids

of a metaphase chromosome) is constant. It helps in distinguishing between different groups as well as in identifying individual chromosomes. A relatively constant centromere index, $\frac{\text{length of short arm}}{\text{length of long arm}} \times 100$ can be calculated for each chromosome, as given in Table 1.2.

Some chromosomes show constrictions (secondary constrictions) other than the centromere (primary constriction). When the secondary constrictions are sub-terminal, the segment distal to the constriction appears as a satellite. Secondary constrictions at constant positions are useful in identifying individual members of a chromosome group (Ferguson-Smith, Ferguson-Smith, Ellis and Dickson, 1962).

An idiogram is a diagrammatic representation of the karyotype and is made by taking into consideration the relative lengths, the centromere indices, and the position of satellites or constant secondary constrictions of the chromosomes of a large number of normal karyotypes. In the present study the idiogram according to Ferguson-Smith et al (1962) shown in Fig. 3.1 was used. Fig. 3.2 is a normal male karyotype, arranged according to this idiogram.

Autoradiography as an aid to identifying individual chromosomes

The replication of DNA is asynchronous in the chromosomes of

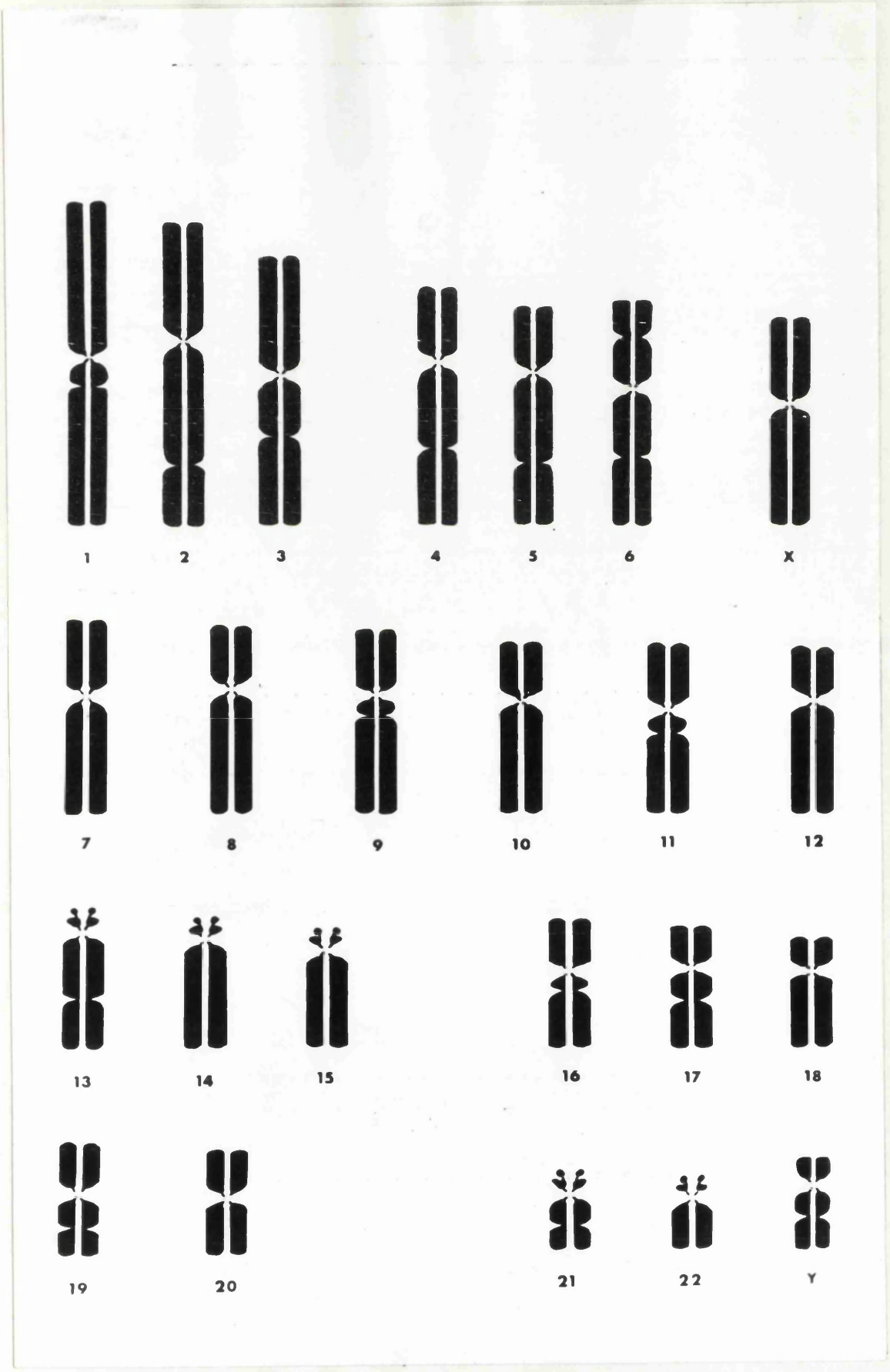


Fig. 3.1

Idiogram used in present study.
Autosomes numbered 1-22 Sex Chromosomes X Y

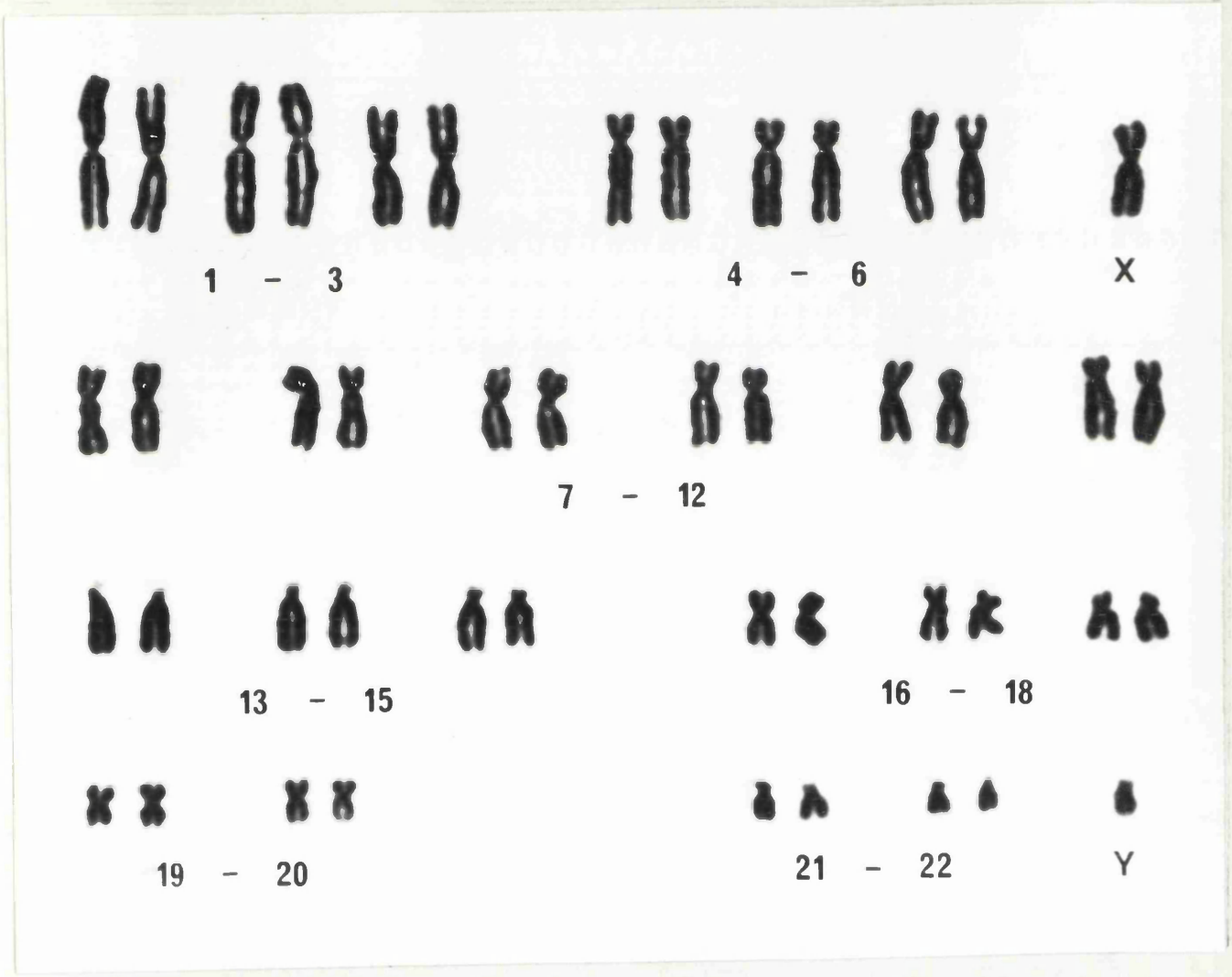


Fig. 3.2

Normal male karyotype, arranged according to idiogram of Fig. 3.1.

the human complement. The incorporation of tritiated thymidine during DNA replication enables the study of chromosome labelling behaviour by autoradiography. With the usual technique of terminal labelling the chromosomes that are labelled in the autoradiograph are the last to complete replication, while unlabelled chromosomes are earlier replicating. The sequence in which the chromosomes complete their replication enables the characterisation of specific chromosomal pairs or groups. The identification of the late replicating X (Gilbert, Muldal, Lajtha and Rowley, 1962) is now a standard procedure in sex chromosome investigations. Yunis, Hook and Mayer (1964a) used the same technique to distinguish between members of the D group in two cases of D trisomy and to distinguish between chromosome No. 17 and chromosome No. 18 in a case of E trisomy (Yunis, Hook and Mayer, 1964b). German, Lejeune, MacIntyre and De Grouchy (1964) and Patau, Therman and Inhorn (1964) identified the chromosome of the cri du chat syndrome as No. 5 and Yunis, Hook and Mayer (1965) the G chromosome of Down's syndrome as No. 22. Even autoradiographic studies, however, have not helped much in distinguishing between members of the C group.

Summary of groups

Group A. This group consists of chromosome pairs nos. 1, 2 and 3. They are the largest chromosomes and are easily distinguished from each other. Chromosome no. 1 is metacentric (centromere central in position). Chromosome no. 2 is sub-metacentric and chromosome no. 3 is metacentric.

Group B. This group consists of the next two pairs of chromosomes nos. 4 and 5. In the idiogram shown in Fig. 1.2 the largest sub-metacentric chromosome of the C group, C_6 is also included in this group. Chromosome no. 6 is commonly characterised by a conspicuous secondary constriction in the short arm which does not occur in a similar position in any other member of the C group. Both 4 and 5 are sub-metacentric chromosomes but no. 4 can be distinguished from no. 5 by the greater relative length of its short arm.

Autoradiography: Chromosome no. 4 replicates later than chromosome no. 5.

X chromosome. The X chromosome is about the same size as chromosomes nos. 4, 5 and 6, but is more metacentric.

Autoradiography: In the female one of the X chromosomes is late replicating.

Group C. This group consists of 7 pairs of medium-sized metacentric and sub-metacentric chromosomes, nos. 6 to 12. Chromosome C_6 has been described above. Chromosomes nos. 8, 10 and 12 are sub-metacentric and the smallest of these are designated no. 12. Chromosomes nos. 7, 9 and 11 are more metacentric. Chromosome no. 9 (C_1 of Patau (1965)) is easily distinguished by the secondary constriction in its long arm. Chromosome no. 11 is the most nearly metacentric in this group.

Group D. This group consists of 3 pairs of large satellited acrocentric chromosomes, nos. 13, 14 and 15.

Autoradiography: The lower half of the long arm of each of two D chromosomes is late replicating and this pair of chromosomes is designated D_1 (no. 13). The D_1 chromosome is found in triplicate in the trisomy D syndrome. Two D chromosomes are late replicating in the centromeric portions of the long arms and/or short arms, and are designated as D_2 (no. 14). The remaining D chromosomes, namely D_3 (no. 15), are earlier replicating (Yunis, et al, 1964).

Group E. This group consists of three pairs of chromosomes nos. 16, 17 and 18. No. 16 is metacentric; nos. 17 and 18 are

sub-metacentric, but no. 17 can usually be distinguished from no. 18 by its higher centromere index.

Group F. This group consists of two pairs of small metacentric chromosomes, nos. 19 and 20.

Group G. Group G consists of two pairs of small satellited acrocentric chromosomes, nos. 21 and 22 and the Y chromosome in the male.

Autoradiography: The two chromosomes with late replicating long arms are designated G_1. When morphologically distinguishable on the basis of size, the G_1 chromosomes are smaller than the other pair (G_2). In Down's syndrome the chromosome in triplicate is G_1, (Yunis et al, 1965).

Y chromosome. The Y chromosome is also acrocentric but is easily recognised by the fact that its chromatids lie close together and by the presence of a secondary constriction in the middle of its long arm. Further, the distal half of its long arm has a fuzzy appearance. The Y chromosome does not usually take part in satellite association with other acrocentric chromosomes.

THE ABERRANT CHROMOSOMES

Any deviation from the normal chromosomal complement that can be recognised cytologically is termed a chromosomal aberration. Chromosomal aberrations can be classified broadly into numerical aberrations and structural rearrangements. In numerical aberrations, the position of genes remains stable while the gene number is varied, either by gain or loss, of whole chromosomes or of whole complements of chromosomes. In structural rearrangements, the gene number is left unchanged, or varied slightly by small gains or losses, while new gene arrangements are created (Swanson, 1960).

Mechanism of origin

Numerical aberrations arise as a result of errors in cell division at meiosis or mitosis, or as a result of abnormal fertilisation. Structural rearrangements may arise spontaneously or be induced. Every organism has its own rate of spontaneous chromosome change, a rate governed by intrinsic factors such as age, or extrinsic ones like background radiation. Structural rearrangements are induced by ionising radiations, chemical mutagens or viral infection.

Conditions for survival of rearranged chromosomes

Any structural rearrangement involves at least two breaks at different points, either in the same chromosome or in different chromosomes. Only broken ends of chromosomes are capable of fusion and before such a rearrangement can survive and be perpetuated, several other conditions have to be satisfied. The rearrangement must still carry a linear order of the genes with one centromere and two telomeres per chromosome. It must not carry too great a deficiency or duplication. The change may involve a deficiency, duplication or both, or an inversion or translocation. With respect to arms, the change may be within the same or different arms, and with respect to chromosomes, it may be within a single chromosome, between members of a homologous pair or between non-homologous chromosomes. Individuals heterozygous for such rearrangements are termed structural heterozygotes.

Restrictions on identification of aberrant chromosomes

The identification of a chromosomal aberration will depend, to a large extent, on the refinement of the cytological techniques used.

In man no large polytene chromosomes similar to those in the salivary glands of insects occur, and, although good techniques for meiotic preparations are available, reports of studies using them are few. Human cytogenetics has, so far, been largely restricted to the examination of chromosomes in viable zygotes at somatic metaphase. Morphological analysis for differences in length and centromere position is restricted by the low power of resolution of the microscope. Only changes that amount to about 0.2% of the total chromatin content of a cell can be detected at the microscopic level. Considering a small chromosome like one in the G group this amounts to a doubling of the length of a satellite region before it is detectable as an increase in length. In a larger chromosome arm the same increase could be missed. If the heteromorphism is entirely in the length of one or the other arm of a chromosome, the normal variation in length between homologues is another hindrance to their detection. Patau (1965) claims that chromosome pairs identified as homologues have differences in length amounting to 5.3% of the length of one homologue. It is also probable that structural rearrangements that result in differences in length and in minor shifts in the position of the centromere but in the homozygous form, will not be detected.

In the account of the known human chromosomal aberrations that follows, each aberration will be defined and cytomorphological restrictions on its observation mentioned. Its effect, if any, on the phenotype will be mentioned, and its incidence and familial transmission, where established, will be given. Unless otherwise mentioned, all structural rearrangements described are in the heterozygous state.

a. Numerical aberrations

There is no restriction to identification of numerical aberrations in viable zygotes, as this depends only on a chromosome count.

i. Triploidy. In triploidy, a whole chromosome set is present in triplicate. Edwards, Yuncken, Ruston, Richards and Mittwoch (1967) described the first case of triploidy in a viable zygote. The effect on the phenotype is gross, although less striking than in for example the trisomy syndromes, and no single clinical syndrome has been described. Triploidy is a not uncommon cause of foetal loss. Carr (1963), in a study of abortus material, quotes a figure of 3% for its incidence.

ii. Trisomy. Trisomy is the presence in triplicate of a single chromosome. Lejeune, Gautier and Turpin (1959) found 47 chromosomes in patients with Down's syndrome and established the G_1 trisomy syndrome. (Down's syndrome cases with 47 chromosomes will be referred to as G_1 trisomies in this thesis.) The incidence of this syndrome is 1 in 600 live births (Carter, 1963). However, out of a possible array of 22 autosomal trisomies, only 3 others have so far been reported in viable zygotes. Patau, Smith, Therman, Inhorn and Wagner (1960) reported an autosomal disorder associated with an extra member of the D group chromosomes, trisomy D_1, and Edwards, Harnden, Cameron, Crosse and Wolff (1960) described the extra autosome of the E trisomy syndrome. Trisomy for chromosome no. 16 was described by Lewis, Hyman, MacTaggart and Poulding (1963). Studies in abortuses have shown trisomies of chromosomes 2, 3 and 16, in addition to the above common trisomies G, D and E (Geneva Conference, 1966).

iii. Centric fragments. Kodani (1958) described small supernumerary chromosomes in meiotic metaphases of human testes. This observation could not be confirmed by Hirschhorn and Cooper (1961).

Smith, Steinberger, Steinberger and Perloff (1965) report the karyotype of a male with a supernumerary deleted chromosome. His sex chromatin study was negative (i.e. deleted chromosome not part of an X chromosome). His mother was found to carry a similar fragment (i.e. deleted chromosome not a supernumerary Y chromosome).

iv. Monosomy. In monosomy one member of a pair of chromosomes is absent. It was generally believed that monosomy for an autosome was not compatible with life till Hall, Fredge and Svenningsen (1967) described a congenitally malformed male infant monosomic for a G group chromosome. Of 300 cells examined, only one cell had 46 chromosomes. The rest had 45 chromosomes.

v. Isochromosomes. These are formed when the centromere splits in a plane at right angles to the longitudinal axis of the chromatids instead of in a plane passing between them. An isochromosome of the short arm of an E group chromosome (no. 17 or 18) has been offered as an explanation of the extra chromosome in the second case of 2 trisomic children described by Gustavson, Atkins and Patricks (1964). Mukherjee and Burdette (1966a) report a familially transmitted

isochromosome of the G group, and Stevenson, Paterson, Goodman and Salem (1966) of one of the D group chromosomes.

A post-zygotically acquired isochromosome of the long arm of chromosome no. 2 has been implicated in Waldenstrom's macroglobulinaemia (Bottura, Ferrari and Veiga, 1961; Ferguson and McKay, 1963).

b. Structural rearrangements

Five forms of structural rearrangements are known.

i. Duplications. A duplication is the gain of a segment of a chromosome in one member of a normal complement. Although the part played by duplications in evolution has been stressed by Muller (1936) and evidence has been presented by Ingram (1961) supporting the evolution of the genes coding for the different polypeptide chains of haemoglobins α, β and γ, from one original ancestral gene through successive duplications followed by other chromosomal rearrangements, no cytological demonstration of a duplication has been reported in man. The long variants of the Y chromosome and of chromosome no. 16, which occur in phenotypically normal individuals, could possibly be duplications.

ii. Deletions. A deletion is a detachment and loss of a segment of a chromosome from a normal complement. A number of large easily identifiable deletions of autosomes associated with abnormal phenotypes have been described. Lejeune, Lafourcade, Berger, Vialatte, Borswillwald, Seringe and Turpin (1963) described 3 unrelated patients carrying a deletion of part of a short arm of a B group chromosome with similar clinical findings, later called the cri du chat syndrome. De Grouchy, Lamy, Thieffry, Arthuris and Salmon (1963) described the clinical findings in a case carrying a deletion of the short arm of chromosome no. 18. Subsequently Bühler, Bühler and Stalder (1964) and Van Dyke, Valdmanis and Mann (1964) have also described similar cases. Reisman, Kasahara, Chung, Darnell and Hall (1966) described a case with a large deletion of the long arm of the G group chromosome.

Small deletions which have no demonstrable effect on the phenotype and are transmitted in families occur. With the present powers of resolution, such deletions can be recognised only if they occur in the satellite regions of the acrocentric chromosomes or in small chromosomes. Small deletions in large chromosomes cannot

be recognised, while sizeable deletions of the same will not be compatible with a normal phenotype. Hall (1963), in a study of 38 patients with G_{21-22} trisomy, found in the karyotype of two male infants that the long arms of one of the five chromosomes of group G was deleted. The fathers of both infants had a similar chromosomal fragment replacing one of their four G group chromosomes. Migeon (1965) describes a father and daughter whose karyotype had a deletion of the short arm of one G group chromosome. Broyer, Chevrie, Aicardi, Le Tan Vinh and Thieffry (1966) and Neu, Leao and Gardner (1966) report other such families. Elmore, Nance, McGee, de Montmollin and Engel (1966) report a familially transmitted deletion of the whole of the short arm of a G group chromosome ascertained through a case of pycnodysostosis.

Familial transmission of a deletion of a short arm of a D group chromosome was established by Buchanan, Pearce and Wetherly-Mein (1964). Another such pedigree has been reported by Migeon (1965).

Post-Zygotic deletions. Nowell and Hungerford (1960) found in the haemopoietic cells of individuals with chronic myeloid leukaemia a chromosomal fragment (the Philadelphia chromosome, Ph^1) in

place of one G group chromosome.

iii. Ring chromosomes. A ring chromosome is formed by terminal deletions at both ends of a single chromosome and fusion of the broken ends. Gripenberg (1967) cites references to 29 reported cases of ring chromosomes. Rings have been observed in all but the F group chromosomes.

iv. Inversions. An inversion is a change in the linear sequence of genes such that the genes in the segment are in reverse order relative to those outside the segment. An inversion may be pericentric (breaks in both arms on either side of the centromere) or paracentric (both breaks on one arm). In organisms like Drosophila melanogaster, polymorphisms for paracentric inversions occur quite frequently. With cytology restricted to somatic metaphase however, paracentric inversions cannot be picked up in man. Even pericentric inversions, where the breaks occur at nearly equal distances from the centromere, will be missed.

Carr (1962) described a pericentric inversion of a chromosome no. 2 in a normal male. The inversions described by Gray, Mutton and Ashley (1962) and Chandra and Hungerford (1963) could be variant

acrocentric chromosomes. Familial transmission of a pericentric inversion of chromosome no. 1 was reported by Lele, Dent and Delhanty (1965). An inversion in a chromosome no. 2 has also been reported by De Grouchy, Emerit, Corone, Vernant, Lamy and Soulie (1963), and one in a B group chromosome by Morishima, Liu and Grumbach (1964). An inversion in a C group chromosome was reported by de Grouchy, Frezal, Bitan, Jammet and Lamy (1965) and 4 others showing familial transmission were reported by Ferguson-Smith (1967) and Jacobs, Cruickshank, Faed, Frackiewicz, Robson, Harris and Sutherland (1968). Cohen, Capparo and Takagi (1967) reported an inversion involving a D group chromosome.

v. Translocations. This is an exchange between two non-homologous or homologous chromosomes. A translocation may be reciprocal or insertional, the latter involving at least three breaks while the former can occur even with two breaks.

Reciprocal translocations involving arms of equal length cannot be recognised at somatic metaphase. Small translocations to the long arms of the large chromosomes are also missed. Reciprocal translocations that have been identified so far are of two types:

a. These are the common translocations involving the acrocentric chromosomes, viz. the D and G group chromosomes. These chromosomes are known to bear nucleolus organising areas (Ferguson-Smith, 1964b) and to associate by their satellites at metaphase (Ferguson-Smith and Handmaker, 1961). An extension of this association into interphase may predispose to the type of centric fusion found in these translocations (Swanson, 1960). Three forms occur: the D/D, D/G and G/G. All three are frequently transmitted in families. Lists of such translocations which have been shown to segregate in families are given by Hustinx (1966) and Hamerton (1966).

b. The second type include the infrequent translocations involving exchanges between larger segments of chromosome arms. Lists of such translocations have been made by Brogger (1967) and by Gripenberg (1967). At least one translocation involving each one of the autosomes occurs in these lists. Some chromosomes tend to be involved more frequently than others.

Translocations involving the X chromosome and an autosome are specially important for purposes of autosomal gene localisation. The first report of such a translocation was by Edwards (1961). Subsequently,

Lie, Coenegracht and Stalder (1964) described a large late replicating chromosome with a translocation of part of an autosome (probably a C group chromosome) on it. Mann, Valdmanis, Capps and Puite (1965) and Mukherjee and Burdette (1966b) described B/X translocations. Another C/X translocation was reported by Neuhauser and Back (1967).

Insertional translocations. The first claim for detecting an insertional translocation was by Patau, Therman, Inhorn, Smith and Ruess (1961). Studying the chromosomes of patients with oral-facial-digital syndrome, they found an abnormality of a chromosome no. 1 at or near the centromere in a mother and child with the syndrome. They concluded that this was an insertion into chromosome no. 1 of a segment of chromosome C_1 (no. 9 in the classification used in this study), and that the syndrome was caused by partial trisomy for that specific chromosomal segment. Ruess, Pruzansky, Lis, and Patau (1962) studied a further 6 cases with same syndrome and found a similar variant chromosome in 2 cases. Yunis (1965) studying 4 cases with the same syndrome could not confirm their findings.

Sex chromosomes

i. X chromosome. All the aberrations so far described have

also been observed in the X chromosome. In fact, many of them were first described as occurring in this chromosome. These aberrations have been reviewed by Ferguson-Smith (1965).

ii. Y chromosome. Aberrations in this chromosome have been reviewed by Jacobs and Ross (1966).

Chromosomal mosaicism.

Mosaicism is the existence in one individual of two or more cell lines with different karyotypes. Mosaicism has been demonstrated for most types of chromosomal aberrations. The presence of mosaicism for aberrant and normal cell lines seems generally to exert a diluting effect on the abnormal phenotype.

THE VARIANT CHROMOSOMES

Variations in chromosome morphology which cannot be classified as any one of the structural rearrangements already described occur in man. These variations occur in relation to heterochromatic regions of either the satellites of the acrocentric chromosomes or of the secondary constrictions of the other chromosomes. Their occurrence and incidence have been highlighted by a number of cytogenetic population surveys.

Sasaki, Makino and Kajii (1963) studied the chromosomes of 22 patients with congenital heart disease and correlated the presence of a heteromorphic chromosome no. 16 with this condition. More recently, Court-Brown, Buckton, Jacobs, Tough, Kuensberg and Knox (1966) studied a random sample of 438 subjects (207 males and 231 females) with respect to the frequency of individuals showing a structural abnormality or variant chromosome. They concluded that about 0.5% of subjects have a major structural rearrangement of the autosomes, and between 2 and 3% a variation confined to one autosome. The variant chromosomes described by them involved nos. 16 and 17 and the D and G groups. They observed that the variations are confined to autosomes which have a secondary constriction and are in the vicinity of such constrictions. They state "Morphologically, they are quite characteristic, they can be inherited and they probably are present in all cells. There are good reasons for believing that they occur through some medium other than through the production of chromosome breaks." German, Ehlers and Engle (1966) studied 35 index cases of families in each of which more than one member had cardiac anomalies. In 4 cases they found familially transmitted variant chromosomes of

nos. 16, 17 and the D group. Moores, Anders and Emanuel (1966) investigated the inheritance of marker chromosomes in 6 families from 250 cases of congenital heart disease. They found distinctive morphological variations in chromosomes nos. 9, 16, 17 and the D group. They concluded that the variation occurred at sites of secondary constriction and was due to inherited alteration in behaviour of these regions. Ferguson-Smith and Boyd (personal communication) report 10 cases with variant chromosomes in a total of 217 cases studied at the Human Cytogenetic Laboratory, Royal Hospital for Sick Children, Glasgow, in the year 1966-67.

Classification of variant chromosomes

The variant chromosomes described in the studies referred to above, and in other published isolated examples can be classified as:

a. prominent satellites on acrocentric chromosomes.

b. elongated secondary constrictions of non-acrocentric chromosomes.

a. Familially transmitted prominent satellites on one G group chromosome were reported by Cooper and Hirschorn (1962), Therkelsen (1964) and Court-Brown et al (1966).

Extra large satellites on one D group chromosome have been seen in the surveys of Court-Brown et al (1966), German et al (1966) and Moores et al (1966). Donahue, et al (1968) describe an inbred Amish community where this variant chromosome is present in 10% of the population. A mating between heterozygotes has been described, producing 2 presumptive homozygotes amongst eight offspring.

b. The karyotype (Fig. 3.3) illustrates the secondary constrictions of chromosomes nos. 1, 9 and 16, as also those in 3, 6 and 21. The extension of the heterochromatin at the secondary constriction of one member of a pair of the chromosomes mentioned will produce a structural heterozygosis for that pair.

Chromosome no. 1. The first description of a variant chromosome no. 1 was by Patau et al (1961), but the authors stated that the unusual segment was an insertion from chromosome no. 9 and postulated that a partial trisomy was the cause of the oral-facial-digital syndrome. Yunis and Gorlin (1963) described a similar chromosome in a family ascertained through a case of cysts of the jaw, basal cell carcinomata and bifid ribs. Several unaffected members also had the variant chromosome. They

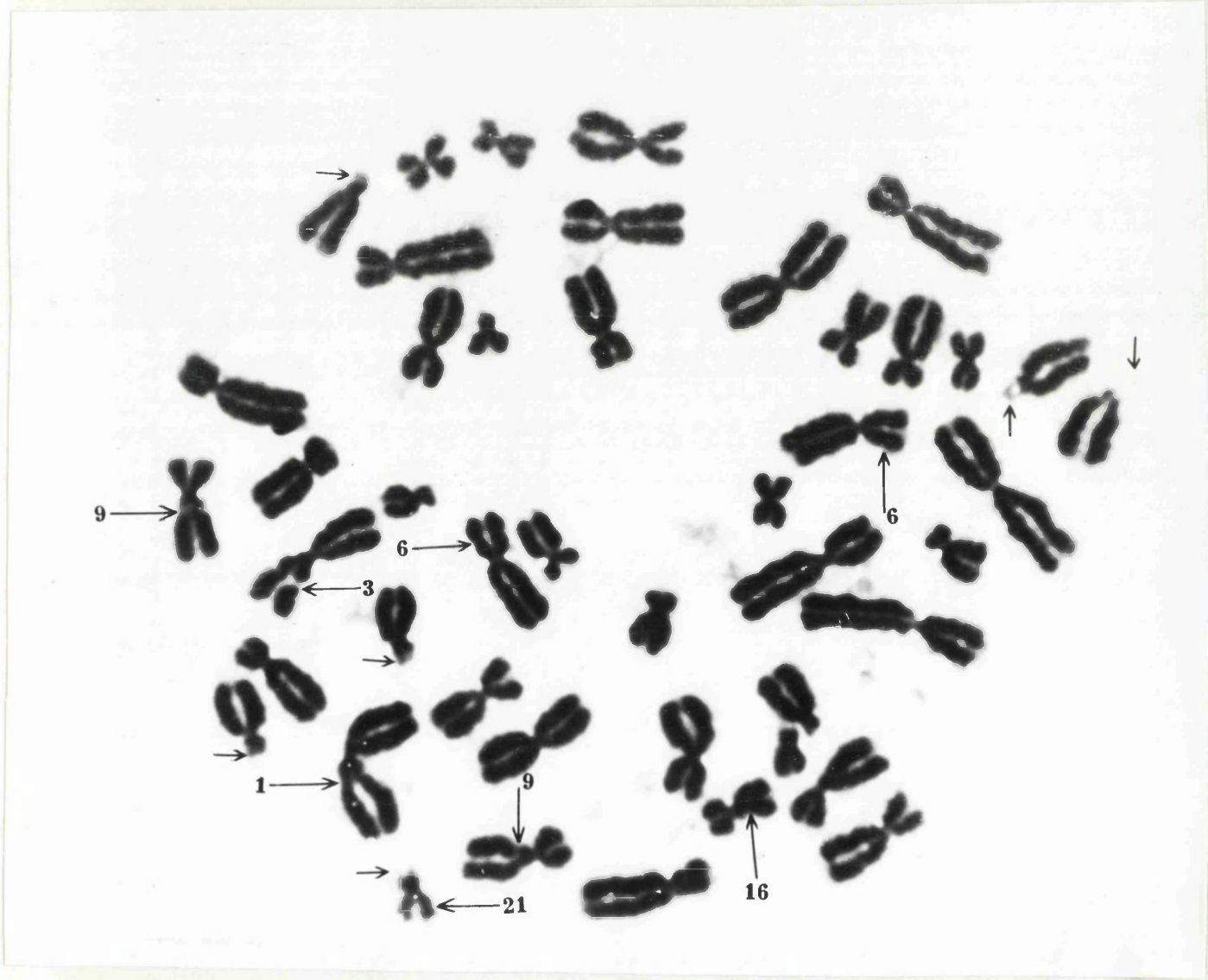


Fig. 3.3

Secondary constrictions of chromosomes Nos. 1, 3, 6, 9, 21 and of the acrocentric chromosomes.

also reported another family with the same clinical syndrome but no chromosomal anomaly and concluded that the variant chromosome was unrelated to the clinical syndrome. Cooper and Hernits (1963) described a family ascertained through a male pseudohermaphrodite in which a variant chromosome was segregating. Philip, Frydenberg and Sele (1965) ascertained a family through a case of primary amenorrhoea. They demonstrated the variant chromosome in cultured fibroblasts from the skin. Galperin (1966) has demonstrated that there is a significant difference between the brachial index of a variant no. 1 chromosome and that of its normal homologue. He has also observed a significantly greater number of secondary constrictions in chromosome no. 1 in carriers of the variant chromosome.

Chromosome no. 9. A heteromorphism for this chromosome was reported by Ferguson-Smith et al (1962). Moore et al (1966) described the segregation of such a variant chromosome in a family.

Chromosome no. 17. An extension of the heterochromatic region of the short arm of chromosome no. 17 produces a satellited chromosome of characteristic appearance. Ferguson-Smith et al (1962) first demonstrated such a chromosome. Court-Brown et al (1966), German et al

(1966) and Moores et al (1966) have reported familially transmitted satellited chromosomes no. 17. The satellite has not been demonstrated in cultured fibroblasts.

The long variant of chromosome no. 16. The earliest reference to such a chromosome is by Jennings and Turner (1961), who describe a mentally retarded child with other congenital anomalies in whose karyotype a chromosome with abnormal morphology replaced one normal chromosome no. 16. In it the paracentromeric region was elongated and the long arms had an inverted V appearance. He suggested that it was a reciprocal translocation. Sasaki et al (1963) found a similar heteromorphism in the chromosomes no. 16 in 12 out of 22 cases of congenital heart disease. They however, thought that the variation was in the smaller chromosome of the pair and that it represented a deletion of the long arm of that chromosome no. 16. Court-Brown (1964) describes a carrier with a similar variant as a translocation heterozygote. Patau (1964) refers to the stable polymorphic state of the same variant. Nuzzo, Caviezel and de Carli (1966) report the familial transmission of this variant chromosome. Subsequently, Court-Brown et al (1966), German et al (1966) and Moores et al (1966) describe other such pedigrees.

The mass of extra chromatin involved in the elongation of the chromosome arm is difficult to explain as an extension of existing heterochromatin. If the carriers of this heteromorphism are translocation heterozygotes, the unbalanced form of the translocation should also occur, but there is no evidence for this in the pedigrees described. The only other plausible explanation would be a duplication in the long arm.

CHAPTER IV.

EXPERIMENTAL AND ANALYTICAL METHODS AVAILABLE FOR AUTOSOMAL GENE LOCATION USING CHROMOSOMAL ABERRATIONS.

1. Methods using the phenotypic evidence of the number of alleles at a locus.
   - A. Qualitative determination of the minimum number of alleles at a locus.
     - i. Demonstration of the presence of three alleles at a locus.
     - ii. Demonstration in a deletion heterozygote of two alleles at a locus to place that locus on the normal disomic part of the complement.
   - B. Quantitative determination of the number of alleles at a locus.
     - i. Direct assay of allele product.
     - ii. Indirect assessment of allele number via differences in relative intensities of electrophoretic bands.

iii. Determination of allele number via population phenotype frequencies.

2. Methods using the concurrence of a rare event affecting a locus and a rare event affecting a whole chromosome or chromosome segment.

   A. Simultaneous loss of an allele and of a whole chromosome or chromosome segment.

   B. Transmission of both alleles at a locus and both chromosomes of a pair in a single gamete (following non-disjunction).

3. In vitro methods using chromosomal aberrations.

4. Methods based on the linkage analysis of the joint segregation of a heteromorphic pair of autosomes and polymorphic marker loci.

   Principle of methods.

   A. Variant chromosomes.

   B. Deletions.

   C. Pericentric inversions.

   D. Translocations.

CHAPTER IV.

EXPERIMENTAL AND ANALYTICAL METHODS AVAILABLE FOR AUTOSOMAL GENE LOCATION USING CHROMOSOMAL ABERRATIONS.

Introduction

The association of an abnormal phenotype with an abnormal karyotype is often used to associate particular clinical syndromes with particular autosomes. Thus it is usual to associate the abnormal phenotypes of the regular trisomy syndromes G, D and E with the particular autosomes 22, 13 and 18 respectively. These abnormal phenotypes however can be attributed in a greater or lesser degree to the relatively non-specific effect of excess or deficiency of chromosomal material leading to an unbalanced genotype at a critical stage in development. Even if a developmental effect on the phenotype is not involved, as in examples where abnormal phenotypes are correlated with abnormal karyotypes produced by somatic change in stem cells, no direct assignment of genes to a particular autosome is possible. For example, the abnormal granulopoiesis in chronic myeloid

leukaemia is associated with the appearance of the Ph^1 chromosome (page 38), but no assignment of gene loci to chromosome 21 can be made.

The methods that can be applied effectively to locate autosomal genes by studying aberrant or variant chromosomes are classified on page 50 and 51.

1.A.i.

Demonstration of the presence of three alleles at a locus.

If the presence of three different alleles at a locus could be demonstrated in a trisomic individual it would be conclusive evidence that the locus under study is carried on the chromosome present in triplicate. If for example a trisomic with acid phosphatase (AcP) phenotype ABC (AB, AC, mosaicism excluded) is found, this would mean that the chromosome in triplicate carries the AcP locus. The other loci that could be tested by this method are Rhesus (Rh) for triallelic phenotype $CC^W c$ and haptoglobin (Hpα) for phenotype 1F1S2. In populations where the abnormal haemoglobins occur at suitable levels, the haemoglobin phenotype ASC may also be looked for.

If p, q and r are the allele frequencies of the alleles A, B and C at any of these loci the frequency of the genotype ABC amongst trisomic individuals will be 6 pqr. Using British allele frequencies, the genotype frequencies at each of these loci would be:-

Locus	Genotype	Allele frequency			Genotype frequency of ABC. 6pqr
		p	q	r	
AcP	ABC	0.36	0.6	0.04	0.05
Rh	$CC^{W}c$	0.42	0.013	0.57	0.02
Hp	1F1S2	0.16	0.24	0.6	0.14

The use of this method is not restricted to trisomics. The presence of three alleles may be looked for in any individual carrying a chromosomal aberration involving a detectable duplication, i.e. a duplication per se, duplication carried by translocation heterozygotes or in heterozygotes for isochromosomes.

1.A.ii.

Demonstration in a deletion heterozygote of two alleles at a locus to place that locus on the normal disomic part of the complement.

The demonstration of a heterozygote at any locus will mean that there are at least two alleles present at that locus in the individual

concerned. If the individual is also heterozygous for a deletion, the locus studied cannot be on the deleted segment. The deleted segment may be a deletion per se or a deletion carried by heterozygotes for ring chromosomes, translocations or isochromosomes.

Salmon, Ropartz, De Grouchy, Lejeune, Salmon, Rivat, Liberge and Delarue (1966) first applied this method to analyse deletions and exclude certain markers from the deleted segments in cases studied by them. Bender, Ritter and Wolf (1967) have extended this analysis to deletions reported by other workers. Wolf, Reinwein, Gorman and Kunzer (1967) have listed all loci that have been excluded from the distal part of the long arm of chromosome no. 18, and Reinwein, Ritter and Wolf (1967) have presented a similar list for the short arm of chromosome no. 18.

The weakness of this method as a positive approach towards gene location is obvious. It is possible to exclude a small number of loci from, in most cases, a small segment of a chromosome arm. These loci may be on any one of the 43 normal autosome arms or on the non deleted segment of the autosome arm carrying the deletion. The weakness is intensified by the fact that it is generally not possible to define the deleted segment with any certainty. For example, a

deletion is recognised in the heterozygous condition by the presence of shortening of the short or long arm of a chromosome as compared with its homologue. The positions of two pairs of breaks producing an identical shortening may be variable.

However with deletions that can be defined, this method can prove useful, and in one instance has proved so already. Gerald, Warner, Singer, Corcoran and Umansky (1964 and 1967) postulated that the Hp$_{\alpha}$ locus is at the distal end of either the short arm or long arm of a D_1 chromosome (see page 64). Bias and Migeon (1967) found heterozygotes at the Hp$_{\alpha}$ locus in individuals also heterozygous for a nearly complete deletion of the short arm of a D_1 chromosome and narrowed the alternatives given by Gerald et al (1964) to the distal end of the long arm of the D_1 chromosome.

1.B.i.

Direct assay of allele product

By this method it is hoped to find a direct correlation between chromosome excess and excess of enzyme activity and between chromosome deficiency and deficiency of enzyme activity. Assuming that there are no feed back control mechanisms acting, an individual

trisomic for a particular chromosome would be expected to produce $1\frac{1}{2}$ times as much enzyme from alleles on the chromosome in triplicate as does a normal disomic individual, and an individual monosomic for a chromosome, half as much. The simplifying assumptions are that to a first approximation, alleles which we class together as 'normal' determine the production of enzyme in equal amount and with similar specific activities.

Investigations for excess enzyme activity have been done mainly with G_1 trisomics, and an excess of about 50% has been demonstrated for a number of enzymes. A few of these studies will be cited to illustrate the difficulties in interpreting the results simply as gene-dosage effect.

a. Studies on leucocyte alkaline phosphatase

The leucocyte alkaline phosphatase level of patients with chronic granulocytic leukaemia is low. The demonstration by Nowell and Hungerford (1960) that this disease was associated with a heterozygous deletion of the long arm of a G group chromosome prompted Alter, Lee, Pourfar and Dobkin (1962) to study the activity of this enzyme in 36 G_1 trisomics and 35 age-matched disomic controls. There was a significant excess of activity in the trisomic individuals and it was

postulated that the long arm of a G chromosome carried the locus for leucocyte alkaline phosphatase. However King, Gillis and Baikie (1962) who found a similar excess pointed out that the enzyme levels vary with a number of non-specific effects other than age, e.g. exertion, appearance of hormones at puberty, variation of hormone levels and the presence of infection. These factors could lead to a leucocytosis and to the production of immature cell stages in the peripheral blood which are known to have a high activity of the enzyme.

b. Studies on leucocyte acid phosphatase.

A high level of leucocyte acid phosphatase in G_1 trisomics was demonstrated by Mellman, Oski, Tedesco, Maceira-Coelho and Harris (1964). Stalder, Buhler, Buhler, Egli, Richterich and Colombo (1965) reported the elevation of this same enzyme in four patients, all in a partially trisomic state, but for a chromosome other than G_1. These included two cases partially trisomic for D in a D/G translocation, and one case partially trisomic for D in a D/E translocation, and another partially trisomic for a C group chromosome. If there is only one structural locus for leucocyte acid phosphatase these findings cannot be explained as gene- dose effect.

c. Enzyme activity in G_1 trisomics compared with translocation Down's syndrome.

Rosner, Ong, Paine and Mahanand (1965) compared the leucocyte, erythrocyte and whole blood galactose-1 phosphate uridyl transferase, leucocyte acid - and alkaline - phosphatase, erythrocyte glucose 6-phosphate dehydrogenase and leucocyte 5-nucleotidase in 12 cases of Down's syndrome with 46 chromosomes (translocation Down's syndrome) age-matched with 11 cases of Down's syndrome having 47 chromosomes (G_1 trisomy) and with 11 normal controls. They found an excess of enzyme activity in the trisomic cases as compared with the normal controls but not in the translocation Down's syndrome cases. If the excess of enzyme activity in the trisomics is due to a gene-dosage effect, a similar excess in enzyme activity is to be expected in the translocation Down's syndrome cases as they too, are almost totally trisomic for a G_1 chromosome. The deletion of a segment of the short arm of this chromosome which occurs when the translocation is formed could account for the absence of excess enzyme activity, if the loci investigated were on the deleted segment. It is however unlikely that all the loci studied could be located on the small segment that is deleted. It is also unlikely that a position effect caused by the inter change could inactivate all these loci.

1.B.ii.

Indirect assessment of allele number via differences in relative intensities of electrophoretic bands.

The presence of elevated amounts of enzymes in trisomics can also be demonstrated by comparing the intensities of electrophoretic bands of the enzymes in trisomics with that in disomics. For some enzymes the heterozygote shows one or more hybrid components not present in either homozygote. The hybrid molecule is formed by the random combination of the parental polypeptide sub-units. Consider two parental sub-units, α and α_1. If the units combine at random in a heterozygote, the distribution of the concentration of the enzymes should be in the proportion $(c+c_1)^2$, where c and c_1 are the concentrations of α and α_1 respectively, i.e. 1:2:1. Assuming a direct relation between dosage and rate of synthesis if one sub-unit is duplicated, the distribution would be $(2c+c_1)^2$, i.e. 4:4:1. If the genes of human placental alkaline phosphatase, a dimeric enzyme which shows three electrophoretic bands in the heterozygote, were located on the G_1 chromosome, the 4:4:1 distribution of the enzyme components should be demonstrable in the placenta of a heterozygous G_1 trisomic (Beckman, 1966).

1.B.iii.

Determination of allele number via population phenotype frequencies.

First suggested by Bateman (1960), these studies attempt to measure a shift of phenotype frequencies with respect to a marker locus in a population created by non-disjunction of the chromosome carrying the marker as against a normal population. The blood group locus studied most extensively has been the ABO. If p, q and r are the frequencies of the alleles A, B and O respectively in the normal diploid population mating at random, the genotype frequencies will be given by $(pA + qB + rO)^2$. For a triallelic locus they will be given by $(pA + qB + rO)^3$. There will be a shift in the frequencies of the phenotypes. The deficiency in the O group yielding r^3 instead of r^2, should be quite noticeable.

Shaw and Gershowitz (1962) studied a population of 793 G_1 trisomics and found a deficiency of the O phenotype and an excess of A and B phenotypes as compared with a control population of 1,000 individuals. They pointed out that the probability of detecting a shift from disomic phenotype frequencies would depend on the number of alleles at the given locus, the dominance relationships, dosage-effects, gene frequencies in the parental

population, time of non-disjunction, and recombination frequency between the locus and the centromere. They calculated on the basis of first division non-disjunction and no crossing-over between the locus and the centromere. The control population of Shaw and Gershowitz has been criticised by Kaplan, Li, Wald and Borges (1964) and Goodman and Thomas (1966), who repeated similar studies but were unable to demonstrate a significant shift of frequencies.

This method of analysis can be extended to D and E trisomics if sufficient cases can be found. By application of the same method to a monosomic population a shift of the frequency of the allele from the expected q^2 in the disomic population to q in a monosomic population might be demonstrable. In the same context, populations of deletion heterozygotes should also give rise to a similar shift of phenotype frequencies.

Three associations between deletion heterozygotes and rare recessive states have been published. Lele, Penrose and Stalland (1963) found a deletion heterozygote of the long arm of a D group chromosome manifesting a retinoblastoma. Uchida, McRae, Wang and Ray (1965) observed a case of arrhinencephaly and alopecia

congenita associated with a deletion of the short arm of chromosome no. 18, and Elmore et al (1966) a case of pycnodysostosis with a deletion of the short arm of a G group chromosome. These authors postulate that these are instances of mono-allelic expression of these rare recessive conditions when the allele is present in the hemizygous state. However the ascertainment bias involved in these observations is gross. If it were possible to distinguish the phenotype of the heterozygote from that of the normal homozygote and prove that one parent, and only one is heterozygous, these correlations could be treated as concurrences (see below). Otherwise the only way of confirming these observations is by demonstrating a shift of the phenotype frequency from q^2 to q in a population of deletion heterozygotes.

2.A.i.

Simultaneous loss of an allele and of a whole chromosome or chromosome segment.

Barring illegitimacy, the concurrence of two very rare events - the loss of an allele and of a whole chromosome or chromosome segment - would indicate that the locus concerned was on the deleted segment, e.g. an O offspring from an AB mother.

Gerald, Warner, Singer, Corcoran and Umansky (1964 and 1967) demonstrated anomalous inheritance of alleles at the haptoglobin locus. The phenotype at that locus was Hp-1 in one offspring of a mating of father Hp-2 and mother Hp-21. The karyotypes of the parents were normal but that of the child revealed one of the ~~ring~~ $\overset{D}{\chi}$ chromosomes in a ring form. The odds on non-paternity considering the results from the other markers was 1:100. Including a priori odds of, say, 1:30 the final odds on non-paternity would be 1:3,000. The probability of a silent Hp allele (say $q = \frac{1}{1,000}$) being present in the father Hp-2 and transmitted to the son is $\frac{1}{2}q = \frac{1}{2,000}$. The prior odds that the Hp_{α} locus is on the 1% of autosomal length deleted when the ring chromosome was formed in the child is 1:99. The final odds that the Hp_{α} locus is on the deleted part of the D chromosome is (probability that Hp is on deleted segment):(probability of non-paternity or silent allele) $(\frac{1}{100}) : (\frac{1}{3,000} + \frac{1}{2,000})$, i.e. 12:1.

2.B

Transmission of both alleles at a locus and both chromosomes of a pair in a single gamete (following non-disjunction).

The transmission of both alleles at a locus and both chromosomes

of a pair in a single gamete can be studied when non-disjunction is known to have occurred in one or other parent. For example, the demonstration of a trisomic offspring of O phenotype from a mating with a mother of phenotype AB and father of phenotype O would indicate that the ABO locus was on the chromosome present in triplicate. Such matings are, however, less than 2% of those in the general population. The parents of seven AB G_1 trisomics have been investigated. The mother was AB in only one instance, and in this case the father's phenotype in respect of ABO could not be studied (Shaw and Gershowitz, 1962).

3.

In vitro studies using chromosomal aberrations.

Simple techniques exist for establishing human somatic cell cultures. If differences of gene products present in a human cell population carrying a chromosomal aberration as compared to that of a normal cell population, are expressed in culture, either de novo or via techniques of enzyme induction, location of autosomal genes would be possible. With the exception of the population frequency studies, most of the methods described above could have a counterpart

in vitro. Such studies will not be restricted to viable zygotes as material for study can be obtained from abortuses or from aneuploid cell lines produced by age-effect, neoplasia or by virus transformation. As methods for obtaining clones from such cultures are also available, the study of cells from mosaic individuals could also prove useful. Since most mosaics are a mixture of normal and aberrant cell lines, cells carrying a normal diploid complement of chromosomes can be compared with cells from the same individual carrying a chromosomal aberration.

A. Quantitative determination in cell culture of the number of alleles.

Martin (1966) studied a mosaic G_1 trisomic having a triple cell stem line, a normal male diploid cell line, a cell line trisomic for G_1 and a cell line quadrisomic for G_1. He found heterogeneity in activity of alkaline phosphatase between the different clones but the differences could not be accounted for by the karyotypic variability alone.

Nadler, Inouye and Hsia (1967) present the data on the enzyme activities for alkaline phosphatase, acid phosphatase, glucose 6-phosphate dehydrogenase and galactose 1P uridyl transferase in cultured fibroblasts derived from eleven no. 18 trisomics, eleven D_1 trisomics and five G_1

trisomics compared with ten normal controls. No significant differences from normal could be demonstrated for any of the enzymes studied. They conclude that the elevated activity of certain leucocyte enzymes in trisomic individuals is probably due to a non-specific property of the leucocytes. They admit however, that the absence of the expected excess in enzyme activity in fibroblasts could be an effect of in vitro tissue culture.

B. In vitro concurrences

Effects of hemizygosity for loci can be studied in cell culture. If the new occurrence of a cytologically detectable chromosome deficiency or whole chromosome loss is accompanied by the change of a single marker from heterozygous to hemizygous, its location on the deleted segment or lost chromosome is established. If two or more heterozygous markers become hemizygous as a result of a single chromosome loss these markers are linked.

Reduction in hybrid tetraploid cells.

Barski, Soreiul and Cornefert (1960) first demonstrated that viable hybrids can be obtained by mixed culture of cells from different species. Evidence from studies on such cells suggested that these

hybrid cells with a 4n chromosomal complement undergo reduction to 2n. Weiss and Green (1967) demonstrated that stepwise reduction to near diploid from a hybrid mouse-human tetraploid cell line affects mainly the human genome, thus providing a suitable method for locating genes. In their study, hybrid cells between a normal human diploid cell strain and a thymidine kinase deficient mouse cell strain were isolated in a selective medium. The human gene for thymidine kinase permits the hybrid cells to grow in a medium containing aminopterin. Continued growth of the hybrid cells results in the slow elimination of the human chromosomes. The hybrid cells were karyotyped at regular intervals and it was possible to correlate the ability of the hybrid cells to survive in the selected medium with the presence of a particular pair of human C group chromosomes. It is postulated that the locus for human thymidine kinase is on this chromosome pair.

4.

Methods based on the linkage analysis of the joint segregation of a heteromorphic pair of autosomes and polymorphic marker loci.

Principle.

A pedigree in which a heteromorphic pair of autosomes segregates

provides an opportunity for testing for linkage between a specified locus on that particular autosome and a range of polymorphic loci. In such a study the presence of the heteromorphism is scored as the "main locus", the cytological phenotype being scored at somatic metaphase of viable zygotes. Theoretically, any chromosomal aberration or variation that produces a heteromorphic pair of autosomes at somatic metaphase and is transmitted from parent to offspring can be used.

Incidence of the heteromorphism.

As mentioned in Chapter 11, it is preferable to select as the main locus a condition which is infrequent in the population so that most matings will be heterozygous x homozygous normal. This applies even more strongly in studies using heteromorphic pairs of autosomes since it might be difficult to distinguish the abnormal homozygote from the normal homozygote even if it did occur. The variant chromosomes and the structural re-arrangements producing heteromorphic pairs of autosomes both occur in the population at frequencies which are suitable for use in linkage analysis.

Identification of the "site" of locus.

The "phenotype" scored is the presence or absence of a heteromorphic pair of autosomes at somatic metaphase, the structural heterozygote being scored as heterozygous at the main locus and the others homozygous normal. The exact site on the chromosome tested for linkage with the marker loci will depend on the aberration used. With the structural rearrangements, linkage is with a site on the chromosome close to the break-points producing the rearrangement. With the variant chromosomes it is assumed that there is a locus near the site of the anomaly controlling the anomalous appearance.

Harmlessness of the heteromorphism.

In the interest of large pedigree size and as the main locus is scored only at somatic metaphase of living zygotes, the effect of the heteromorphism on the rest of the phenotype should be minimal.

Ease of cytological scanning of phenotype.

To avoid errors of misclassification, the heteromorphism is preferable in an easily identifiable autosome that can be assigned a number. Ideally it should be expressed in all metaphases analysed.

Behaviour of the heteromorphism at meiosis.

Crossing-over between linked genes and segregation occur at meiosis and it would be helpful if the behaviour of the heteromorphic pair of autosomes at meiosis has been studied or is predictable through studies in other organisms. Some chromosomal aberrations affect the frequency of crossing-over, either in the region of the aberration itself or in regions adjacent to it. Crossing-over and segregation in some aberrant chromosomes can lead to unbalanced and therefore inviable gametes. Fortunately, such losses do not usually disturb the linkage analysis by likelihood methods. It is better not to score zygotes whose chromosome morphology or general phenotype suggest imbalance. Such zygotes probably carry duplications or deficiencies, so that a phenotype say Hp-2, may be genotypically interpreted as 2, 22, or 222 with ensuing loss of deductive power.

The chromosomal aberrations with established familial transmission that are useful for linkage analysis will now be discussed under the criteria outlined above. The nature of the linkage information obtained from an analysis of each aberration will be included.

A. The variant chromosomes

No breaks are postulated in the origin of the variant chromosomes, and it is assumed that there is a locus, on the same chromosome, controlling the presence of the variation. The position of this locus in a variant chromosome of one pedigree is assumed to be identical with that in another such variant in another pedigree. Linkage data can be added together without serious prior doubts about heterogeneity. The presence of the variant chromosomes does not produce a demonstrable effect on the phenotype of the individuals carrying them. The extended chromosome no. 1 and the long chromosome no. 16 (see pages 47 and 48) are easily scored because they occur in easily identifiable chromosomes and because the variation is expressed in a majority of cells. The enlarged satellites of the D and G group chromosomes are comparatively difficult to score. The scoring of the satellited chromosome no. 17 is even more difficult because of its erratic expression (see page 48).

No information regarding these variant chromosomes is available from meiotic studies. There may be reduced cross-over in the region carrying the heteromorphism, as established for such

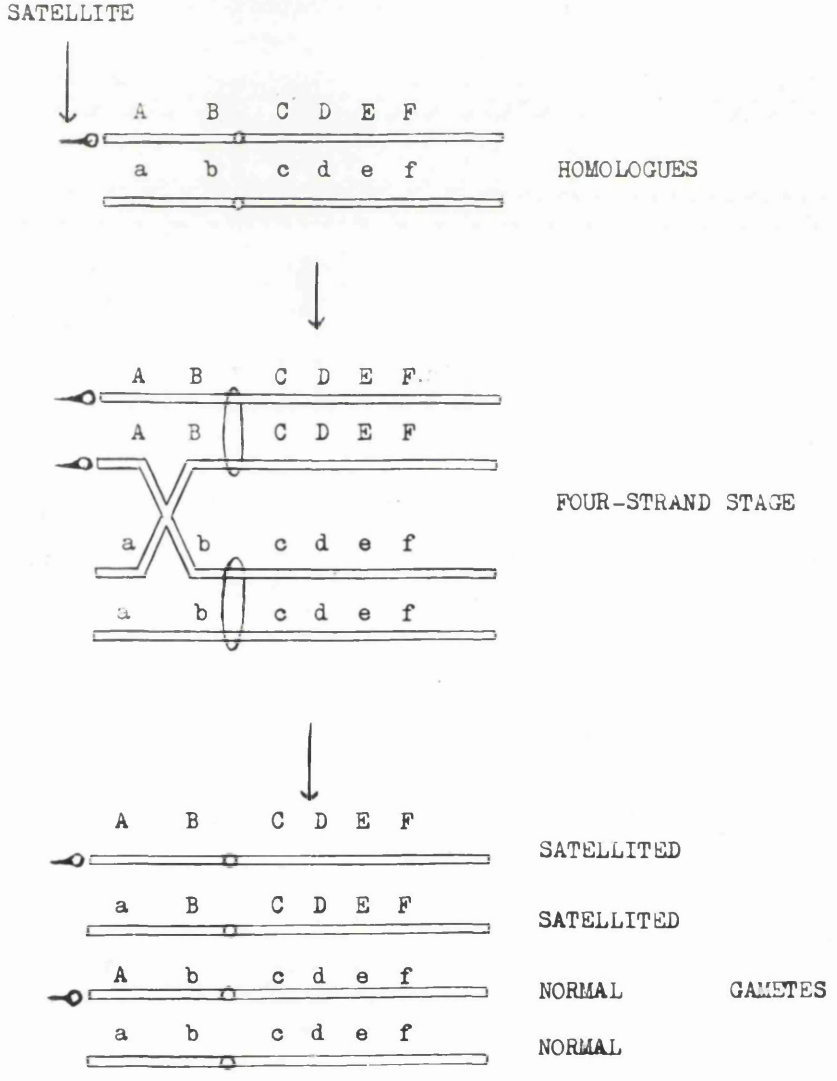
regions in Drosophila (Burnham, 1962). Fig. 4.1 illustrates crossing-over as it is presumed to occur in a heterozygote carrying a satellite on one homologue. The homologues are also heterozygous for the markers ABCDEF.

In the analysis, the phenotypes, variant or normal, are scored at somatic metaphase. Linkage of a marker gene A, with the variant chromosome locates the gene close to the locus controlling the anomalous appearance.

B. Deletions

A deletion is formed by at least two breaks and is identified at somatic metaphase by the decrease in length of one arm of a chromosome of a pair. It would be difficult to prove that two deletions producing a similar shortening in the same chromosome in two different pedigrees were identical. Deletions causing the same abnormal phenotype may be assumed to carry deficiencies for approximately the same segment of the chromosome. Large deletions produce abnormal phenotypic effects on the individuals carrying them, while small deletions may not cause a demonstrable effect on the phenotype. Deletions involving the small chromosomes

FIG. 4.1 CROSSING OVER AND SEGREGATION IN A HETEROZYGOTE FOR A SATELLITED CHROMOSOME.



or those of the satellite regions of acrocentric chromosomes are easy to score. No studies on the behaviour of such deletions at meiosis in man have been reported. Evidence from studies in Drosophila indicate that crossing-over is reduced on either side of the deletion (Burnham, 1962).

Fig. 4.2 illustrates crossing-over and segregation as it is presumed to occur in a heterozygote carrying a cytologically detectable deletion for a segment of the chromosome. The homologues are heterozygous for the loci ABDEF. The products of a cross-over between markers A and B are illustrated in Fig. 4.2.

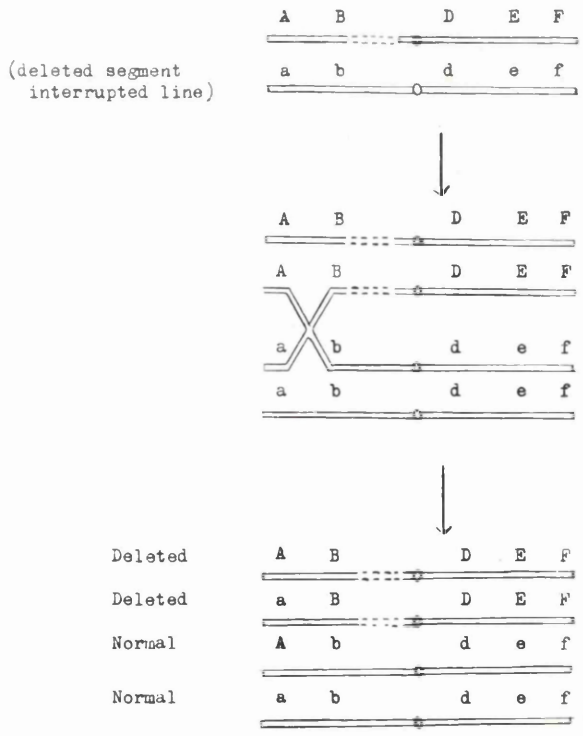
In the analysis, the phenotypes deleted or normal are scored at somatic metaphase. If the marker B is found to give a low recombination fraction with the "deletion" locus it must be placed close to one or other break point producing the deletion.

C. Pericentric inversions.

A pericentric inversion is identified at somatic metaphase by a disturbance in the arm ratio without a change in length of the chromosome. Other inversions can only be detected in meiotic preparations and are therefore not useful for linkage analysis. The position of the

FIG. 4.2

CROSSING OVER AND SEGREGATION IN A HETEROZYGOTE FOR A DELETION.



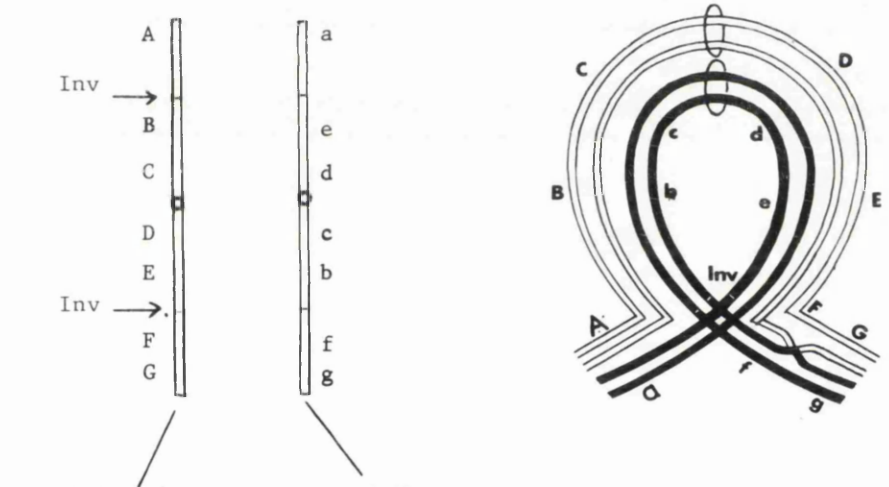
breaks producing the inverted segment may be variably placed, so that two inversions resulting in morphologically similar chromosomes may not have identical break points. If the centromere in either the inverted chromosome or its normal homologue is nearly terminal, the break points can be estimated with greater certainty.

Pericentric inversions in the autosomes which produce no demonstrable effect on the phenotype occur in man (see page 39). No meiotic studies on such inversions are available. Evidence from studies in Drosophila indicate intrachromosomal suppression of crossing-over in the portion of the chromosome in the inverted segment (Carson, 1953).

Fig. 4.3 illustrates crossing-over outside the loop in a pericentric inversion heterozygote, who is also heterozygous for the markers ABCDEFG. From each cross over four strands will be recovered - two parental or non-recombinant, (of these one will contain the inverted segment and the other will be normal) and two non-parental or recombinant strands, (one containing the inverted segment and the other being normal).

In the analysis the phenotypes "inverted" or "normal" are scored

Fig. 4.3 Crossing-over outside inversion loop in heterozygote for pericentric inversion.



Products of crossover between F and G

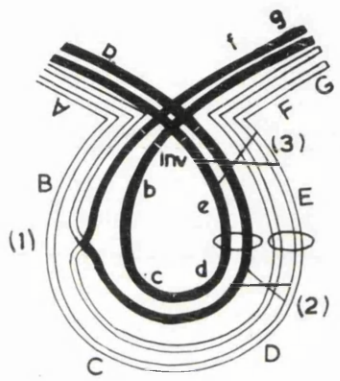
2 N strands	ABCDEFG	non-recombinant
	ABCDEFg	recombinant
2 Inv strands	abcdefg	non-recombinant
	abcdefG	recombinant

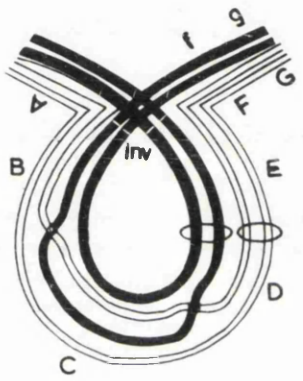
at somatic metaphase. If a low recombination fraction between any marker locus used in the study and the "inversion" locus is found it must be interpreted that this locus must be close to the break points producing the inversion but outside the inversion loop.

Fig. 4.4 illustrates crossing over within the inversion loop. The products recovered will depend on the type of cross-over. With a single cross-over at any point (say 1. in fig. 4.4) four strands will result. Of these, two will be parental or non-recombinant, (one inverted, one normal) and two will be recombinant. The recombinant strands however are seen to be duplication-deficient and most probably will not be recovered; even if they appear in viable zygotes they will give rise to a morphologically different chromosome to that of the original inverted chromosome. Therefore only parental or non-recombinant strands will be scored in the analysis. A similar outcome will result from a three-strand double cross-over (illustrated at points 2. and 3. in fig. 4.4). With a four-strand double cross-over as illustrated in Fig. 4.4 all the products will be duplication-deficient.

With a two-strand double cross-over within the loop however, four strands will be recovered - two parental or non-recombinant (one

Fig. 4.4 Crossing over within Inversion Loop.





Single Crossover at (1)

Products	
1 N	ABCDEFG
1 Inv.	aedcbfg
2 Duplication-deficient	ABcdea
	gfbCDEFG

Two-strand double crossover

Products	
2 N	ABCDEFG
	ABcDEFG
2 Inv.	aedcbfg
	aedCbfg

Three-strand double crossover

Crossovers at points (1) and (2)

Products	
1 N	ABcdEFG
1 Inv.	aeDCbfg
2 Duplication-deficient	aeDCBA
	gfbCDEFG

Four-strand double crossover

Crossovers at points (1) and (3)

Products	
4 duplication-deficient	ABCDea
	ABCDEa
	gfbCDEFG
	gfbcdeFG

inverted, one normal) and two non-parental or recombinant (one inverted, one normal).

As before, in the analysis when the normal and inverted phenotype is scored at somatic metaphase, if a low recombination fraction is found between any locus studied and the "inversion" locus, this locus must be placed on the inverted segment close to one or other break point. With loci thus placed within the inversion loop however a lower recombination fraction than expected may be obtained, as many of the recombinant strands are not recovered in the zygotes.

Considering the results together the interpretation of a low recombination fraction between a marker locus and the "inversion" locus will be that this locus is close to one other break point producing the inversion.

D. Reciprocal translocations

The two types of balanced translocation heterozygotes and the X-autosomal translocations will be considered separately. The use of reciprocal translocations in linkage analysis can be modelled on Snell's analysis of the mouse translocations (Snell, 1946). In his studies the phenotypes, balanced translocation heterozygote and

homozygous normal were scored at the main locus and tested for linkage with a group of marker loci. He did not depend on cytology for the scoring of these phenotypes, however, but used the litter size produced by each category, semi-sterile mice being scored as translocation heterozygotes and those with normal fertility as homozygous normal. It was found that only orthoploid gametes, i.e. either normal or balanced heterozygotes, were recovered in zygotes.

a. Translocations involving exchange of large segments of autosomes.

In the reciprocal translocations involving exchanges of large unequal segments between two chromosomes, the site of breakage in the chromosome can be roughly defined. The balanced translocation heterozygote is phenotypically normal, and the cytological scoring at somatic metaphase is easy. Meiotic studies have been reported in a few, and the presence of quadrivalents has been demonstrated (Hulten, Lindsten, PenMing, Fraccaro, Mannini, Tiepolo, Robson, Heikens and Tillinger, 1964; Lindsten, Fraccaro, Klinger and Zetterquist, 1965).

Crossing over and segregation in such a reciprocal translocation

is shown in Fig. 4.5. Crossing over can take place in the interchanged segments, non-interchanged segments or interstitial segments (region between centromere and point of break). Segregation of the chromatids can occur in 3 different ways:

i. Alternate. There is alternate segregation of the chromatids homologous centromeres passing to opposite poles, and giving rise to one normal gamete and one balanced gamete.

ii. Adjacent 1. In this type of segregation the homologous centromeres still pass to opposite poles but adjacent chromatids pass into the same gametes, giving rise to duplication-deficient gametes of different degrees, depending on the size of the exchange.

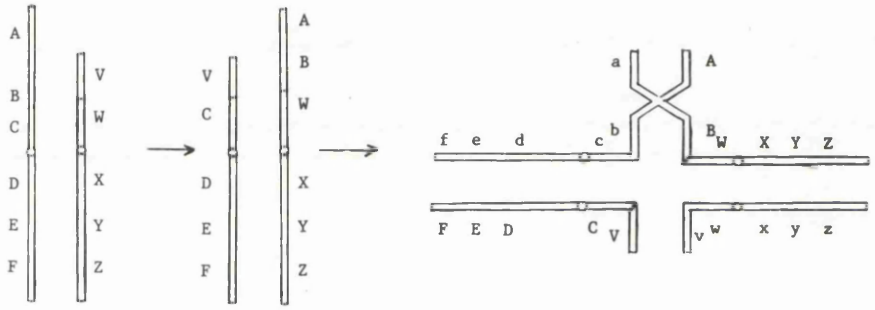
iii. Adjacent 2. In adjacent 2 segregation homologous centromeres pass to the same pole. It is unlikely that products of such segregation will be recovered.

If alternate segregation is the most likely, scoring of balanced translocation heterozygote or normal at somatic metaphase will give a 1:1 ratio of these products of meiosis.

Fig. 4.5 illustrates the products of a cross-over between markers A and B in the interchanged segments. Linkage of a

Fig. 4.5

CROSSING OVER AND SEGREGATION IN RECIPROCAL TRANSLOCATION HETEROZYGOTE



Original chromosomes

Translocated chromosomes

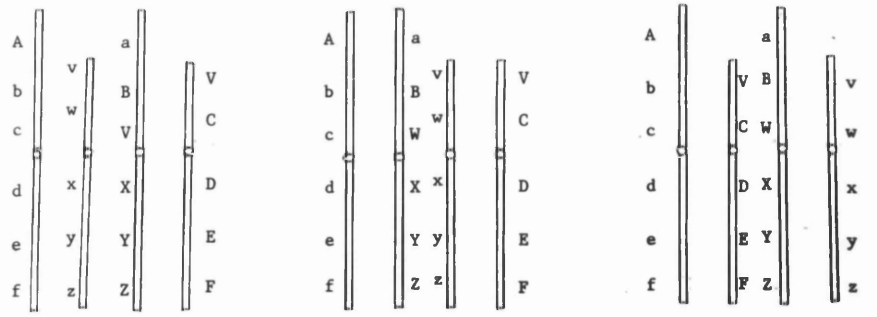
Four-strand stage (Only two of the four strands drawn for clarity)

GAMETES

Alternate segregation

Adjacent 1 segregation

Adjacent 2 segregation



1 Normal

1 Balanced heterozygote

Duplication-deficient gametes

marker gene with the "translocation" locus will mean that it is located close to the break point of one or other original chromosome which gave rise to the translocated chromosome.

Even if the products of an adjacent 1 segregation were recovered, they would give rise to chromosomes morphologically different from the original translocated chromosome, which can be identified at somatic metaphase and left out of the analysis.

b. Translocations between acrocentric chromosomes.

In the other type of reciprocal translocations, i.e. those involving the acrocentric chromosomes, there is a loss of one centromere with a portion of adjacent heterochromatin bearing the satellited regions. The break points are easy to define. The balanced structural heterozygote is phenotypically normal and is easily scored at somatic metaphase by a simple chromosome count for these individuals will have only 45.

Meiotic studies have been carried out on one male D/G translocation heterozygote (Hamerton, Cowie, Giannelli and Briggs, 1961; Mikkelsen, 1966), and one male D/D translocation heterozygote (Kjessler, 1964). A majority of cells showed a chain trivalent.

Crossing over can occur in the interchanged segments or non-interchanged segments. As breaks are close to centromeres no interstitial segments have to be considered. Segregation is by alternate, adjacent 1 or adjacent 2 orientation of the centromeres, but there may be a tendency for inco-ordination of centromeres resulting in the redistribution of chromatids to give other types of duplication-deficient combinations.(Brøgger, 1967).

Hamerton (1966) analyses the segregation in the translocations D/D, D/G and G_1/G_2. He tabulates the findings from 45 D/G translocation heterozygotes, 30 female and 15 male, 23 D/D translocation heterozygotes, 14 female and 9 male, and 7 G_1/G_2 translocation heterozygotes, 4 female and 3 male. He found a deficiency of progeny resulting from adjacent segregation, these being virtually absent in the D/D and G_1/G_2 translocations. In the D/G translocation, 19% of the progeny of female heterozygotes and 6.5% of the progeny of male heterozygotes result from adjacent segregation. For practical purposes, when translocations D/D and G_1/G_2 are used for linkage analysis, only alternate segregation need be considered. With D/G translocations, the presence of G_1 interchange trisomics through

adjacent segregation has to be kept in mind. These individuals can be easily identified by their trisomic phenotype and left out of the analysis.

c.

The X autosomal translocations are of special importance in a study designed to locate genes on autosomes. Such translocation carriers will show an X linked type of pedigree pattern for autosomal loci carried, on the X chromosome due to the translocation. A linkage of an X-linked gene to an autosomal marker gene in such a translocation would locate that marker on the autosome carrying the translocation and close to the break point producing the translocation.

CHAPTER V.

THE PEDIGREE MATERIAL

This study involves the linkage analysis of four pedigrees in each of which a heteromorphic pair of autosomes segregate. The code names of the pedigrees are V21AN, JM1MY, JN1AN and JN1PW.

Selection of pedigrees.

The probands in each pedigree were selected out of a number of cases found to have variant chromosomes from patients referred to Dr. M. A. Ferguson-Smith at the Human Cytogenetics Laboratory, Royal Hospital for Sick Children, Yorkhill, Glasgow. The initial chromosome analyses were done by the staff of that laboratory.

Pedigree V21AN.

The proband of this pedigree was a female child with Down's syndrome due to regular G_1 trisomy, investigated at the age of 6 months. Neither of the parents were translocation carriers; the mother's cells, however showed a heteromorphic pair of chromosomes no. 2. The chromosomes of this pair were equal in size but one was

nearly metacentric. Family studies were undertaken to investigate the transmission of this heteromorphism particularly with respect to the correlated transmission of alleles at the polymorphic loci.

Pedigree JM1MY.

In this pedigree the proband was a male child, aged 6 months referred to the clinic for the investigation of cryptorchidism. The karyotype analysis revealed a heteromorphic pair of chromosomes no. 16. The long arms of one of them was elongated to produce a chromosome which approximated in size and centromere position to a chromosome no. 12. The mother's karyotype too, showed the same heteromorphism. Linkage studies were undertaken.

Pedigree JN1AN.

In this pedigree the proband was a male, aged 33 years, under investigation for hypogonadism. His karyotype revealed a heteromorphism of the chromosomes no. 17: one was satellited. Linkage analysis was undertaken.

Pedigree JN1PW.

In this pedigree the proband was a mentally retarded female, manifesting the cri du chat syndrome, first investigated at the age

of 2 years, and reported on by McGavin, Ferguson-Smith and Ellis (1967). A karyotypic analysis failed to reveal the expected deletion of the short arm of a chromosome no. 5. The chromosomes no. 17 however showed the same heteromorphism as in pedigree JN1AN. Linkage analysis was undertaken.

Collection of material.

The probands and their close relatives were in and around Glasgow, with some scatter in other parts of Scotland and in England and Wales. Some individuals were lost to the study through emigration. The homes of the relatives were visited by the candidate. A complete pedigree was drawn up with the help of reliable members of the family. Full names, addresses and ages of all living members were recorded. The year and age at death of deceased members and the cause of death, if in infancy, was also noted. Special note was made of miscarriages, if any. To maintain the co-operation of the family by reducing discomfort to the volunteers, as well as to cut down unnecessary chromosomal studies, blood was taken only from members having a 50% probability of carrying the heteromorphism at the first visit. When collecting material outside the Glasgow area,

members carrying at 25% probability were also included at the first visit. A rapid clinical appraisal sufficient to observe gross clinical abnormalities was made. Congenital defects of the face and extremities were especially looked for.

From each subject tested, 20 ml of venous blood was drawn into a sterile disposable syringe and distributed as follows:

10 ml into sterile Evans' heparinised tube

5 ml into sterile bijoux for clot and serum

5 ml into sterile bijoux containing 1 ml of acid citrate dextrose ACD, (2 g. sodium citrate and 3 g. dextrose in 120 ml distilled water).

About 3 ml of saliva was also obtained from each subject. The saliva was collected in a universal container and a drop of 1/10,000 thiomersolate solution was added.

Each of these bottles was labelled with the full name and age of the subject and the date of collection.

CHAPTER VI.

LABORATORY TECHNIQUES

A. Cytological Investigation.

1. Leucocyte culture.

a. Plasma.

The 10 ml blood in the Evans' heparinised bottle was used for the culture of leucocytes. Chromosome preparations were made by a modification of the method used by Moorhead, Nowell, Mellman, Batipps and Hungerford (1960). The bottle was centrifuged gently for 10 min. at 200 r.p.m. and the plasma with the leucocytes transferred to a sterile container. A leucocyte count was made on the plasma and an aliquot of the plasma mixed with Waymouth's tissue culture medium (Paul, 1961) to give a mixture containing 1-2,000 cells/ml. Four to five ml. of this mixture were transferred to each of 6 two-ounce culture flasks, and 0.1 ml of phytohaemagglutinin (Burroughs Wellcome) added to each flask to initiate mitoses. The cultures were then refrigerated at 4^{o}C overnight before incubation at 37^{o}C. After incubation for 72 hr. 0.25 ml of a 80 µg. per ml solution of

colcemide (Ciba) was added to each flask to arrest divisions at metaphase. The incubation was continued for a further $2\frac{1}{2}$ hr. The cultures were transferred to centrifuge tubes and spun at 400 r.p.m. for 10 min. The supernatant was removed and 10 ml hypotonic 1.12% (w/v) solution of sodium citrate at 37°C was added. The tubes were incubated for a further 7 min. at 37°C and then centrifuged at 400 r.p.m. for 5 min. The supernatant was removed and the cells suspended in a few drops of the supernatant. 10 ml of a freshly prepared cold ethanol-glacial acetic acid (3:1) mixture were added to the cell suspension, mixed well and kept at 4°C for 30 min. The cells were then centrifuged at 600 r.p.m. for 6 min. and resuspended in a few drops of fresh fixative. One drop of this suspension was allowed to fall from a height of 6 ins. on to a clean, wet microscopic slide and air dried rapidly over a flame. The presence of mitoses and the quality of the preparation were checked under the phase-contrast microscope. Four slides from each culture were made, stained for $1\frac{1}{4}$ hr. in 2% natural orcein in 60% acetic acid, dehydrated and mounted in DEPEX (G. T. Gurr).

b. Whole blood cultures.

The blood left over in the heparinised bottle was stored at 4°C.

In some instances the plasma leucocyte cultures failed: further chromosome preparations were then made from the whole blood, using 1 ml of whole blood in 4 ml of Waymouth's tissue culture medium.

2. Chromosome analysis.

a. Microscopic.

The slides showing a reasonable number of well-spread metaphases of good chromosome morphology were chosen. These were scanned under the x10 objective to pick up a minimum of 20 metaphase figures for counting and analysis. The cells were analysed under oil, with a x100 objective. Cells with less than 45 chromosomes were rejected. The chromosomes were assigned to the groups from A to G and an attempt at identifying them by number was made, in group A nos. 1, 2 and 3, in group B nos. 4 and 5, in group C no. 6 and in group E nos. 16, 17 and 18. In preparations from males the Y chromosome was identified.

Special attention was paid to the chromosome group in which the marker chromosome was expected. Some cells selected by scanning were rejected because individual members of the group

under study could not be identified. Information from the chromosome analysis was tabulated, giving the position of the cell, the cell count and presence or absence of the marker. Any other noteworthy observation on each cell was recorded. In cells with 45 chromosomes the missing chromosome was indicated.

b. Photographic.

From the cells analysed microscopically a minimum of 3 cells was chosen for photographic analysis. These cells were photographed with a x100 objective and enlarged to give a final magnification of x4,000 of original object size. The chromosomes from the prints were then cut out and arranged on a card according to the idiogram (Fig. 3.1) to obtain the final karyotype. The arrangement was checked by at least 2 independent observers. The karyotypes were studied to confirm the presence or absence of the marker and also for other morphological differences between the chromosomes that might have been missed in the microscopic analysis.

The findings of the chromosome analyses, both microscopic and photographic, for the 4 pedigrees are summarised in Tables 7.1, 7.2, 7.3 and 7.4.

B. Marker Investigations.

a. Indexing, separation and storage of samples.

Each sample was given a serial index number after the full name, pedigree name, and age of the donor had been recorded.

About 0.5 ml of fresh serum was sent to Dr. J. Hirschfeld, State Institute of Blood Group Serology, Stockholm, Sweden, for investigation of Ag lipoprotein antigen phenotypes.

The plasma and serum, free of cells, were transferred to $3 \times \frac{3}{8}''$ tubes. The packed erythrocytes from the ACD bijoux were transferred to similar tubes and mixed with an equal volume of buffered citrate-glycerol for storage in the frozen state (Race and Sanger, 1958). The clot from the bijoux without ACD or cells from the ACD bijoux were left at $4^{o}C$ and used for testing for the phenotypes of the erythrocytic antigenic loci. These tests were carried out as soon as possible after collection of samples.

The universal containing the saliva was placed in a boiling water bath for 10 min. to inactivate the enzymes which would otherwise attack the ABH antigens and the saliva supernatant was transferred into labelled tubes.

The samples of plasma, serum, packed erythrocytes and saliva were stored at -20°C.

b. Tests.

The methods employed for testing the phenotypes at each marker locus were those in use in the laboratory of Prof. J. H. Renwick.

1 a. The erythrocytic antigenic loci.

The phenotypes of the individuals for the following erythrocytic antigenic loci were tested for: ABO, MNS, P, Rhesus, Lutheran, Kell, Lewis, Duffy and Kidd. Standard techniques, as described by Race and Sanger (1958) were used. The method used in testing for each of these antigenic loci is summarised in Tables 6.1 and 6.2. The phenotype-genotype relationships with respect to these loci are given in Table 6.3.

1 b. The ABH secretor locus.

The method for testing the phenotypes at the ABH secretor locus (Race and Sanger 1958) is included at the end of Table 6.2. A 1:2 dilution of the thawed saliva was used. If a deposit formed, the saliva was centrifuged and the supernatant used for the test.

Table 6.1 Summary of methods for red cell antigen testing (Race and Sanger, 1958).

System	Typing sera	temperature	Usual method	
ABO	anti-A	RT	Saline slide	15 mins.
		RT	Saline tube	1 hr.
	anti-B	RT	Saline slide	15 mins.
		RT	Saline tube	1 hr.
	anti-A_1	RT or 4°	Saline slide	
	anti-AB (O serum)	RT	Saline slide	15 mins.
			Saline tube	1 hr.
MNSs	anti-M	RT or 4°	Saline slide	5-10 mins.
	anti-N	RT or 4°	Saline slide	5-10 mins.
	anti-S	37°	Saline tube	1 hr.
	anti-s	37°	Saline and IAGT	1 hr.
P	anti-P_1	4°	Saline slide	10-15 mins.
Rhesus	anti-C^W+C Complete	37°	Saline tube	1 hr.
	anti-C^W	37°	Saline tube	1 hr.
	anti-c Incomplete	37°	Enzyme or Albumin	
	anti-D Incomplete	37°	Enzyme or Albumin	
	For D^u 4 anti-D	37°	IAGT	
	anti-E Incomplete	37°	Enzyme or Albumin	
	anti-e Complete	37°	Saline tube	1 hr.

RT - Room temperature 20°C

IAGT - Indirect Antiglobulin Test.

Table 6.2 Methods for red cell antigens (cont'd) and for ABH secretor status.

System	Typing sera	temperature	Usual Method
Lutheran	anti-Lu^a	4^oC or 11^oC	Saline tube 1 hr.
Kell	anti-K anti-k	37^oC 37^oC	Saline tube followed by IAGT 1 hr. Saline tube followed by IAGT 1 hr.
Lewis	anti-Le^a	$+4^oC$	Saline tube 1 hr.
Duffy	anti-Fy^a anti-Fy^b	37^oC 37^oC	Saline tube followed by IAGT 1 hr. Saline tube followed by IAGT 1 hr.
Kidd	anti-Jk^a	37^oC	Saline tube followed by IAGT 1 hr. Sometimes papain-treated cells followed by IAGT.
Secretor status (saliva)	Cells anti-A -A_2 anti-B -B anti-H -O	RT or 4^oC	Inhibition Tube test

Table 6.3 Erythrocytic Antigenic Loci.

Phenotype-genotype correlation

Loci	Tested phenotypes	Possible genotypes
ABO	A_1	A_1A_1, A_1A_2 or A_1O
	A_2	A_2A_2 or A_2O
	B	BB or BO
	A_1B	A_1B
	A_2B	A_2B
	O	OO
MNS	MMSS	MS/MS
	MMSs	MS/Ms
	MMss	Ms/Ms
	MNSS	MS/NS
	MNss	Ms/Ns
	NNSS	NS/NS
	NNSs	NS/Ns
	NNss	Ns/Ns
	MNSs	MS/Ns or Ms/NS
P	P+	P^1P^1, P^1P^{2*}
	P-	P^2P^2
Lu	Lu(a+)	Lu^aLu^a, Lu^aLu^b
	Lu(a-)	Lu^bLu^b

Loci	Tested phenotypes	Possibl
Kell	K(+)	K
	K(-)	
Lewis	L(+)	L
	L(-)	
Duffy	Fy (a+b+)	Fy^a
	Fy (a+b-)	Fy^a
	Fy (a-b+)	Fy^b
Kidd	Jk(a+)	Jk^aJk
	Jk(a-)	Jk

* P^2 includes p class of alleles.

Table 6.3 cont'd. Rhesus-Phenotype-Genotype correlation - Seven alleles tested for.

Reaction							Phenotype	Possible Genotypes
C	C^w	c	D	E	e	D^u		
+	-	+	+	-	+	.	$C\bar{c}Dee$	$CDe/\bar{c}de\ [R_1r]$ $Cde/\bar{c}De\ [R^1R_0]$ $CDe/\bar{c}De$
+	-	-	+	-	+	.	$CCDee$	$CDe/CDe\ [R_1R_1]$ $CDe/\bar{c}de\ [R_1R^1]$
-	-	+	+	+	+	.	$\bar{c}\bar{c}DEe$	$\bar{c}DE/\bar{c}de\ [R_2r]$ $\bar{c}dE/\bar{c}De\ [R^{11}R_0]$ $\bar{c}DE/\bar{c}De\ [R_2$
-	-	+	+	+	-	.	$\bar{c}\bar{c}DEE$	$\bar{c}DE/\bar{c}DE\ [R_2R_2]$ $\bar{c}DE/\bar{c}dE\ [R_2R^{11}]$
-	-	+	-	-	+	-	$\bar{c}\bar{c}ddee$	$\bar{c}de/\bar{c}de\ [r\ r]$
+	-	+	+	+	+	.	$C\bar{c}DEe$	$CDe/\bar{c}DE\ [R_1R_2]$ $Cde/\bar{c}DE\ [R^1R_2]$ $CDe/\bar{c}dE\ [R$
+	-	+	-	-	+	-	$C\bar{c}ddee$	$Cde/\bar{c}de\ [R^1r]$
+	-	-	-	-	+	-	$CCddee$	$Cde/Cde\ [R^1R^1]$
-	-	+	-	+	+	-	$\bar{c}\bar{c}ddEe$	$\bar{c}dE/\bar{c}de\ [R^{11}r]$
-	-	+	-	+	-	-	$\bar{c}\bar{c}ddEE$	$\bar{c}dE/\bar{c}dE\ [R^{11}R^{11}]$
+	+	-	+	-	+	.	$C^w\bar{c}Dee$	$C^wDe/\bar{c}de\ [R_1{}^wr]$ $C^wde/\bar{c}De\ [R^1{}^wR_0]$ C^wDe/c
+	+	+	+	-	+	.	C^wCDee	$C^wDe/CDe\ [R_1{}^wR_1]$ $C^wDe/C^wDe\ [R_1{}^wR_1{}^w]$ C^w $C^wDe/Cde\ [R_1{}^wR^1]$
+	-	+	-	-	+	+	$C\bar{c}D^uee$	$CD^ue/\bar{c}de\ [R_1{}^ur]$ $Cde/\bar{c}D^ue\ [R^1R^u]$ $CD^ue/\bar{c}D^ue$
+	-	-	-	-	+	+	CCD^uee	$CD^ue/CD^ue\ [R_1{}^uR_1{}^u]$ $CD^ue/Cde\ [R_1{}^uR^1]$
-	-	+	-	+	+	+	$\bar{c}\bar{c}D^uEe$	$\bar{c}D^uE/\bar{c}de\ [R_2{}^ur]$ $\bar{c}D^ue/\bar{c}dE\ [R^uR^{11}]$ $\bar{c}D^uE/\bar{c}$
-	-	+	-	+	-	+	$\bar{c}\bar{c}D^uEE$	$\bar{c}D^uE/\bar{c}dE\ [R_2^uR^{11}]$ $\bar{c}D^ue/\bar{c}D^uE\ [R_2{}^uR_2{}^u]$
-	-	+	-	-	+	+	$\bar{c}\bar{c}D^uee$	$\bar{c}D^ue/\bar{c}de\ [R_0{}^ur]$ $\bar{c}D^ue/\bar{c}D^ue\ [R_0{}^uR_0{}^u]$
+	-	+	-	+	+	-	$C\bar{c}ddEe$	$Cde/\bar{c}dE\ [R^1R^{11}]$ $CdE/\bar{c}de\ [R^yr]$

The phenotypes scored were Sec+ and Sec-.

Sec+ corresponds to genotypes SeSe and Sese, and Sec- corresponds to genotype sese.

2. The erythrocyte enzyme loci.

The phenotypes of the polymorphic erythrocyte enzymes acid phosphatase (AcP), adenylate kinase (AK), 6-phosphogluconate dehydrogenase (6PGD), and phosphoglucomutase (PGM) were demonstrated by differences in electrophoretic mobility.

a. The acid phosphatase (AcP) locus.

The polymorphism for the erythrocyte AcP was first demonstrated by Hopkinson, Spencer and Harris (1963) by differences in mobility during starch gel electrophoresis. A modification of their method was used.

Principle: Haemolysates of erythrocytes are subjected to electrophoresis in a starch gel at acid pH. The gel is incubated with substrate - a phenolphthalein salt - the enzyme liberates phenolphthalein from the substrate and the areas of enzyme activity are shown up as red zones when at an alkaline pH.

Solutions:

Gel buffer.	0.59 g. succinic acid 1.114 g. Tris dissolved in water adjusted to pH 6.0 and made up to 2,000 ml.
Tank buffer.	86.2 g. citric acid dissolved in 800 ml distilled water, adjusted to pH 6.0 with 1ON. NaOH and solution made up to 1 litre. A 1 in 5 dilution (0.08 M) of this solution was used in the electrode tanks.
Substrate solution.	0.1 g. phenolphthalein phosphate pyridine salt (Calbiochem) dissolved in 16.7 ml of 0.05 M citric - NaOH buffer (1 in 8 dilution of the concentrated tank buffer) and pH adjusted to 6.0, with 1 N NaOH.

Method:

The gel was prepared with 25 g. hydrolysed starch (Connaught) and 200 ml gel buffer following the method of Smithies (1955).

Haemolysates were made by adding 1 vol. distilled water to 1 vol. red cells which were then centrifuged for 30 min. Twelve samples were tested on each gel using thick filter paper inserts. Electrophoresis was carried out at 5°C for 17 hrs. with a potential gradient of 7 v. per cm. using double compartment electrode tanks containing 125 ml of tank buffer in each compartment. The gel was sliced and the anodic portion (about 8 cm. from origin) was stained for AcP by covering it with a freshly prepared solution of phenolphthalein phosphate pyridine salt, which is more stable than the sodium or calcium salt. After 5 hrs. incubation at 37°C the substrate was poured off and the surface of the gel exposed to ammonia. The phenotypes were read within a few minutes.

Reading of phenotypes.

The phenotypes A, AB, AC, B, BC and C, the different patterns for which are illustrated in Fig. 6.1, were looked for.

Phenotype - genotype relationship.

The above phenotypes correspond to the genotypes AA, AB, AC, BB, BC and CC, respectively.

Fig. 6.1 Acid phosphatase phenotypes

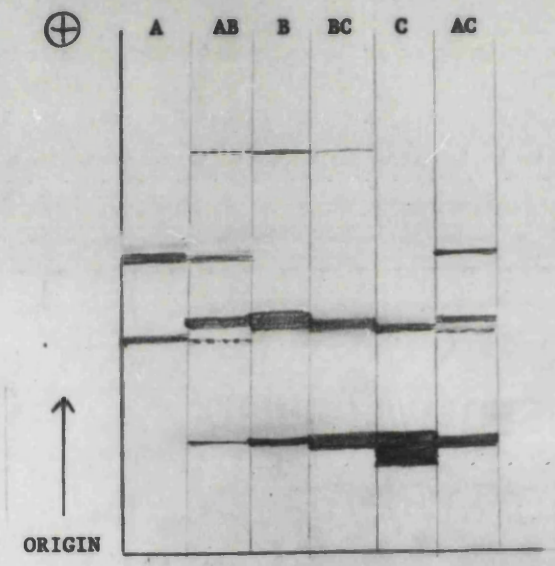
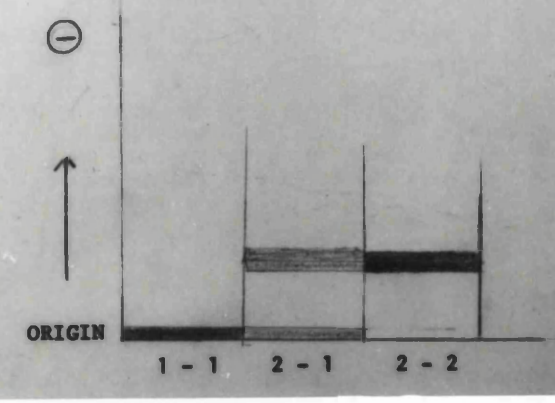


Fig. 6.2 Adenylate kinase phenotypes



b. The adenylate kinase (AK) locus.

Polymorphism for adenylate kinase was first demonstrated by Fildes and Harris (1966) using differences in mobility in starch gel electrophoresis. The AK phenotypes are distinguishable in the cathodic portion of the AcP gel described earlier.

Principle.

At the sites of AK activity, the substrate adenosine diphosphate (ADP) is converted to adenosine monophosphate (AMP) and adenosine triphosphate (ATP), which reacts with glucose in the presence of hexokinase to produce ADP and glucose-6-phosphate, which is then oxidised by glucose-6-phosphate dehydrogenase to 6-phosphogluconate with the concomitant reduction of nicotinamide adenine dinucleotide phosphate (NADP). The reduces NADP, in the presence of phenazine methosulphate (PMS), reduces 3- (4,5-dimethyl thiasolyl-2) - 2,5 diphenyl tetrazolium bromide (MTT) to a blue coloured insoluble formazan which is deposited at the sites of AK activity.

Solutions.

Tris Hcl buffer. 12.114 g. Tris (0.1 M) dissolved in 1 litre of distilled water and adjusted to pH 8.0 with N HCl.

0.94% agar. 4.7 g. IONAGAR no. 2 dissolved in 500 ml hot Tris buffer 0.05 g. merthiolate added and distributed in 8 oz. flasks.

Glucose-$MgCl_2$ in tris buffer. 0.45 g. glucose 1.5 g. $MgCl_2$, dissolved in 40 ml tris buffer.

MTT solution. 0.03 g. MTT (G. T. Gurr) dissolved in 5 ml 0.1 M tris buffer.

Substrate solution. 4.5 ml glucose - $MgCl_2$ solution
0.5 ml MTT (E. Gurr), solution,
1 drop hexokinase solution (Boehringer),
1 drop G6PD solution (Koch-Light),
0.01 g. ADP, 0.0075 g. NADP,
0.003 g. PMS, made up to 25 ml with melted 0.94% agar at 50°C.

Method.

The cathodic portion of the AcP gel (approximately 4 cm.) was overlaid with 25 ml of the freshly prepared substrate agar. The setting of the agar was speeded by cooling at 4°C for $\frac{1}{2}$ hr. and the

enzyme action was then encouraged by incubating for one hour at room temperature ($20^{o}C$).

Reading of phenotypes.

The phenotypes are 11, 21 and 22, the different patterns for which are illustrated in Fig. 6.2. The phenotypes 11 and 21 were readily scored on this gel. If there has been a hint of the rare phenotype 22 (a "2" band unaccompanied by any "1" band), the electrophoresis would have been repeated and the entire gel stained for AK phenotypes. No such requirement arose in this study.

Phenotype-genotype relationship.

The genotypes corresponding to the phenotypes mentioned above are also 11, 21 and 22.

c. 6-phospho-gluconate dehydrogenase (6PGD) locus.

The polymorphism for this enzyme was first demonstrated by Fildes and Parr (1963) using differences in mobility in starch gel electrophoresis.

Principle.

The activity of 6PGD is localised by using a substrate with

6-phosphogluconic acid which is oxidised by the enzyme with the simultaneous reduction of MTT in the presence of PMS to blue formazan.

Solutions.

Phosphate buffer (0.4 M, pH 7.0)

i. 170.4 g. Na_2HPO_4 in 3 litres distilled water.

ii. 108.9 g. KH_2PO_4 in 2 litres distilled water.

2,700 ml of (i) mixed with 1,800 ml of (ii) and adjusted to pH 7.0 with the appropriate solution.

Gel buffer.

Phosphate buffer 0.008M.

Phosphate buffer, 0.4 M, is diluted 1 in 50.

Tank buffer.

Phosphate buffer 0.2 M

Phosphate buffer, 0.4 M diluted 1 in 2.

Tris solution, 0.1 M.

1.21g.Tris dissolved in 100 ml distilled water and adjusted to pH 8 with 1N.HCl.

Substrate solution.

0.2 g. 6-phospho-gluconic acid, Na salt, 0.04 g. NADP, 0.04g. MTT, 0.008 g. PMS dissolved in 100 ml of 0.1 M tris.

Method.

A modification of the method of Fildes and Parr (1963) was used. The gel was prepared using 25 g. starch in 200 ml gel buffer. Haemolysates were prepared by the addition of 2 volumes of water to 1 volume of erythrocytes. 18 samples per gel were tested, using inserts of no. 1 filter paper. Electrophoresis was carried out for $5\frac{1}{2}$ hrs. at 5^{o}C at a potential gradient of 7 volts per cm., using double compartment tanks, with 125 ml tank buffer in each compartment, the gel tray being immersed in an ice-water mixture throughout. The sliced gel was stained with 5 ml of substrate solution and the colour allowed to develop at room temperature.

Reading of phenotypes.

Two phenotypes, A, which is shown up by a single band, and AB, shown up by a double or, ideally, a triple band pattern, were scored. They are illustrated in Fig. 6.3. The homozygote, BB, has only the slow moving band but is very rare and was not observed in this study.

Phenotype-genotype relationship.

Phenotype A represents the homozygote <u>AA</u>, and <u>AB</u> represents the heterozygote AB.

Fig. 6.3 6-phosphogluconate dehydrogenase phenotypes.

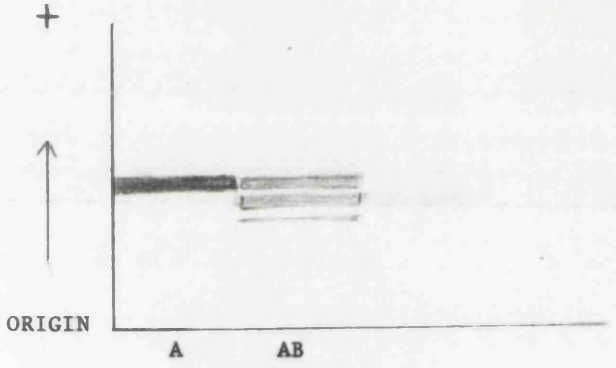
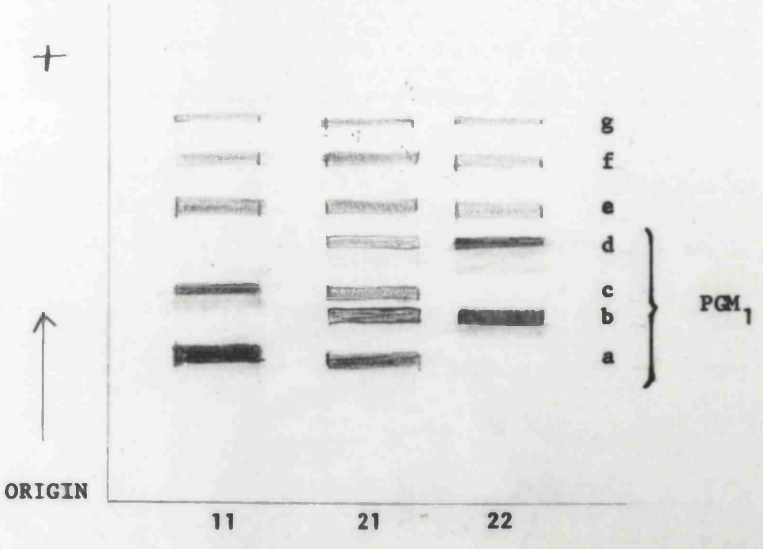


Fig. 6.4 Phosphoglucomutase PGM_1 phenotypes.



Phenotype-genotype relationship.

The phenotypes 11, 21 and 22 correspond to genotypes 11, 21 and 22 respectively.

3. The serum protein loci.

The polymorphisms in serum transferrins (Tf), haptoglobins (Hp), cholinesterases (E_1 and E_2), lipoproteins (Lp and Ag), alpha$_2$-glycoprotein or group specific component (Gc) and immunoglobulins (Gm and Inv) were also used as markers. The phenotypes at these loci can be demonstrated as follows:

a. Differences in electrophoretic mobility scored

i. directly by protein stain: Hp and Tf.

ii. by position of enzyme activity: E_2.

or iii. by position of antigenic activity: Gc.

b. Differences in sensitivity to specific enzyme inhibitors: E_1.

c. Differences in antigenic specificity detected by

i. Ouchterlony's double diffusion technique in agar or agarose gel: Lp and Ag.

or ii. inhibition of agglutination: Gm and Inv.

a.i[1] The haptoglobin (Hp) locus.

Smithies (1955) first demonstrated the genetic polymorphism in the serum haptoglobins.

Principle.

Haemoglobin is added to the serum which is electrophoresed in a starch gel with a discontinuous buffer system. The differences in mobility of the haemoglobin-haptoglobin complexes are demonstrated by treating the sliced gel with o-dianisidine. The haemoglobin part of the complex catalyses the oxidation of this compound in the presence of hydrogen peroxide, producing a green colour which localises the complexes.

Solutions.

Gel buffer.	504.25 g. Tris, 57.5 g. citric acid dissolved in 5 litres of water, adjusted to pH 8.6, diluted to 1 in 20 for use.
Tank buffer.	17.8 g. NaOH 166.1 g. boric acid dissolved in water, adjusted to pH 7.95 and made up to 5 litres, diluted 1 in 2 for use.

Haemoglobin solution. One ml erythrocytes in 20 ml water.

Ortho-dianisidine solution. 5 g. o-dianisidine shaken up in 95 ml glacial acetic acid.

H_2O_2 100 vols (= 30% w/v)

Method.

A modification of the method of Smithies (1959a) was used. The gel was prepared with 60 g. hydrolysed starch in 400 ml gel buffer. Serum samples were prepared by mixing 1 volume of haemoglobin solution with 3 volumes of serum. 20 samples were tested on each gel using thin Whatman no. 1 filter paper inserts. Electrophoresis was carried out using single compartment electrode tanks each containing 250 ml tank buffer for 5 hr. at room temperature at a potential gradient of 8 volts per cm.

A 10 cm. portion of the gel, measured from the origin to the anode, was sliced to obtain 4 slices. The 3rd or 4th slice was covered with o-dianisidine solution for 5 min., the stain then washed off and the gel covered with 50% H_2O_2 solution. The phenotypes were scored when the green colour developed.

Reading of phenotypes.

The phenotypes 11, 21 and 22 were scored, the patterns for which are illustrated in Fig. 6.5.

Phenotype-genotype relationship.

These phenotypes correspond to the genotypes 11, 21 and 22.

a.i[11] The sub-types at the haptoglobin locus.

Smithies, Connell and Dixon (1962) demonstrated that the phenotypes 11, and 21 at the haptoglobin locus could be sub-typed into 1F1F, 1F1S, 21F or 21S.

Principle.

Three distinct stages are involved.

(a) The serum haptoglobin is isolated and partially purified by adsorption to and elution from ion-exchange cellulose.

(b) The disulphide bonds involved in the combination of the polypeptide chains alpha and beta of the haptoglobin molecule are broken down by reductive cleavage, and the hydrogen bonds by concentrated urea.

(c) The constituent polypeptide chains of the haptoglobin

Fig. 6.5 Haptoglobin phenotypes

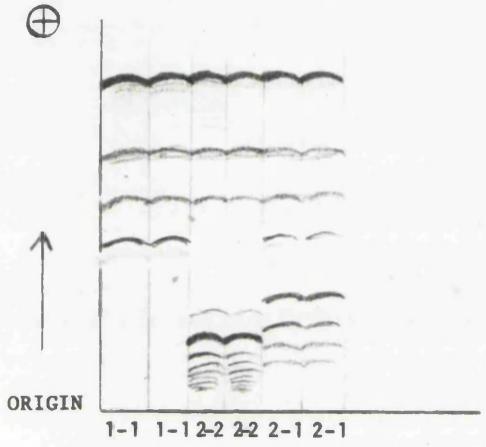
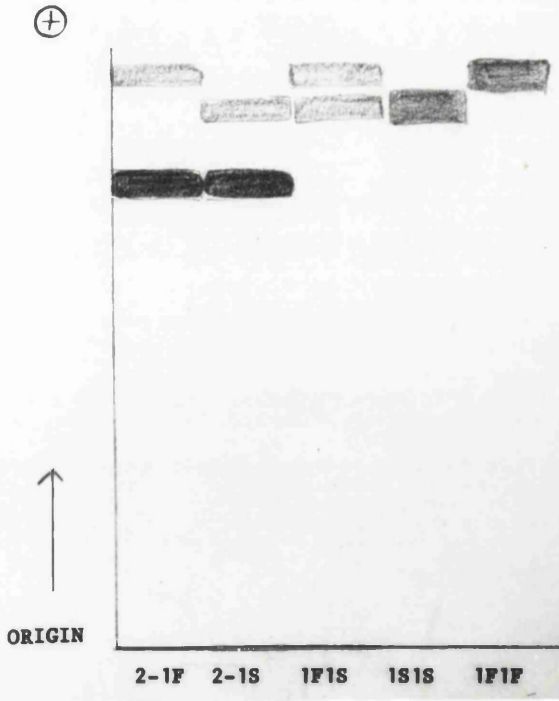


Fig. 6.6 Haptoglobin subtypes



molecules are separated by suitable starch gel electrophoresis, under continuance of cleavage conditions to prevent re-aggregation of polypeptides.

Method.

The method as described by Smithies et al (1962) was used.

Readings of phenotypes.

The phenotypes 1F1F, 1F1S, 21F and 21S were scored, the different patterns for which are illustrated in Fig. 6.6.

Phenotype-genotype relationship.

The above phenotypes represent the genotypes 1F1F, 1F1S, 21F and 21S respectively.

a.i[111] The transferrin (Tf) locus.

The polymorphism for the serum transferrins was first demonstrated by Smithies (1959b).

Principle.

Serum samples were subjected to electrophoresis in a discontinuous buffer system. The transferrins, which are iron-binding proteins, are localised by staining the gel with a protein stain dissolved in methanol-acetic acid.

Solutions.

The gel buffer and the tank buffer were those used in studying the haptoglobins.

Methanol-acetic acid-water mixture. 2.5 litres methanol
2.5 litres water
0.5 litres glacial acetic acid

Naphthalene Black solution. 6 g. Naphthalene Black 10B (Gurr) dissolved in 1 litre methanol-acetic acid mixture, agitated for 5 hr. and filtered.

Method.

A modification of the method of Smithies (1959a) was used. Electrophoresis was the same as for the haptoglobins. The first slice of the gel was covered for 5 to 10 min. with Naphthalene Black solution, washed with changes of methanol-acetic acid for 24 hrs. and the phenotypes scored.

Reading of phenotypes.

The transferrin C was identified in the band situated about 5.5 cm. from the origin. Although the fast variant B and the slow variant D were looked for, neither was found in the samples studied.

Phenotype-genotype relationship.

The phenotype C corresponds to the genotype CC.

a.ii. The cholinesterase (E_2) locus.

The polymorphism at the E_2 locus was first published by Harris, Hopkinson, Robson and Whittaker (1963) but their technique was not used in this study.

Principle.

Serum samples are electrophoresed using a discontinuous buffer system. The cholinesterase activity is localised by using β-naphthyl acetate as substrate, which is hydrolysed by the enzyme. The product of hydrolysis 2 -naphthol couples with Fast Blue "B" dye to produce a pink band at the sites of cholinesterase activity.

Solutions.

Gel and tank buffer as for haptoglobins

Phosphate buffer. 0.25 M 71 g. Na_2HPO_4 (anhydrous) in 2 litres water.

(b) 68 g. KH_2PO_4 in 2 litres water.

505 ml of (a) are added to 900 ml (b) and pH adjusted to 6.3 with appropriate solution.

Substrate solution. 50 mg. β-naphthyl acetate

25 mg. Fast Blue dye.

The naphthyl acetate was shaken up in 10 ml 50% aqueous acetone and poured slowly into 50 ml of phosphate buffer. The dye was added and mixed thoroughly.

Method.

The method of electrophoresis was the same as that of the haptoglobins. The second or third slice of the gel was pre-heated in a water bath at 37°C and the freshly prepared substrate solution was filtered on to it through Kleenex tissue paper. After a few minutes incubation at 37°C the gel was washed in tap water and the phenotypes recorded.

Reading of phenotypes.

The most common phenotype was C_5- which is a pink band situated about 2.5 cm. from the origin. The phenotype C_5+ is the presence of an additional slightly slower band as illustrated in Fig. 6.8.

Fig. 6.7 Gc phenotypes

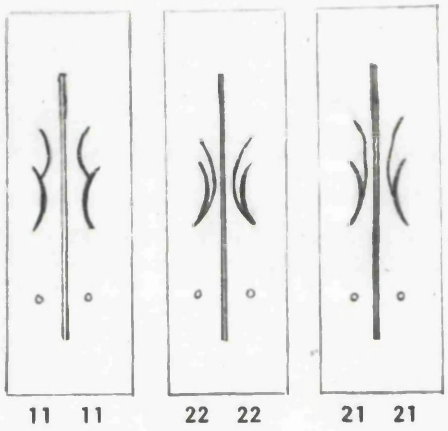
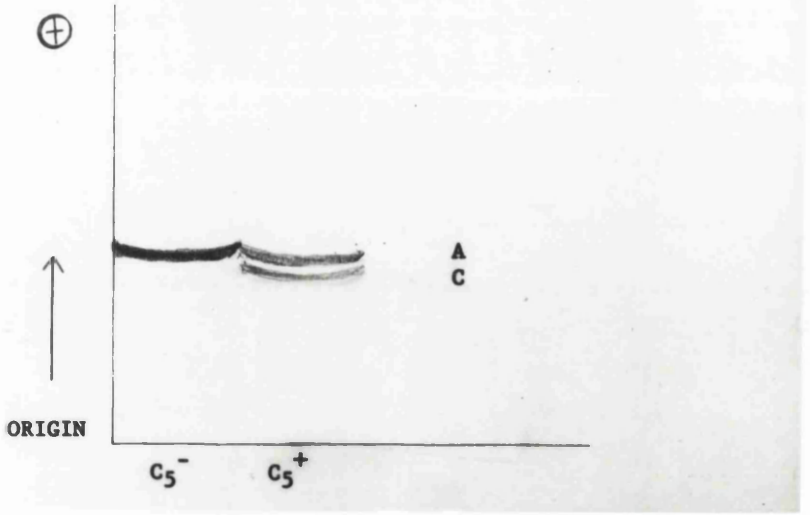


Fig. 6.8 Cholinesterase phenotypes. E_2.



Phenotype-genotype relationship.

The phenotype C_5- represents the common homozygote $\underline{E_2^- E_2^-}$ and C_5+ the heterozygote $\underline{E_2^+ E_2^-}$ or rarer homozygote $\underline{E_2^+ E_2^+}$.

a.2 The Group specific component (Gc) locus

The genetic polymorphism in the Gc proteins was first demonstrated by Hirschfeld (1960).

Principle.

Serum is subjected to electrophoresis in an agar gel. After separation of the proteins, anti-Gc serum is placed in a longitudinal slit parallel to the direction of electrophoresis and allowed to diffuse out. The antigen-antibody complexes are demonstrable as arc-shaped precipitates and the different phenotypes are distinguished by the position and the shape of the arcs.

Solutions.

Barbitone buffer (0.1 M), pH 8.5. 7.36 g. barbitone
41.2 g. sodium barbitone
0.2 g. merthiolate
dissolved in 2 litres of water.

Tank buffer. 1 in 2 dilution of barbitone buffer.

Agar solution, 1%. 10 g. Ionagar no. 2 dissolved completely in 500 ml hot water and mixed with 500 ml hot barbitone buffer, 50 mg. merthiolate added and distributed into 6 oz. flasks.

Methonal-acetic acid mixture and naphthalene black solutions as for transferrins.

Method.

The method of Hirschfeld (1960) was used. 2 ml of melted 1% agar was poured on each of 7 microscope slides placed on a level surface. After the agar had set, a narrow slit and two wells on each side of it and equidistant from it were cut with a scalpel on each gel, using a template. The agar from the wells was removed and serum samples (two per slide) were placed in the wells. The gels were electrophoresed in a closed tank containing barbitone buffer for $2\frac{1}{2}$ hr., a potential gradient of 90 volts being applied across the whole system. The agar from the central slit in each gel was then removed and the slit filled with a 30% dilution of horse

serum (Behringwerke) containing anti-Gc antibodies amongst antibodies to other human serum proteins. After 20 hr. diffusion the arc-shaped precipitates of the antigen-antibody complexes became visible. The gels were washed in 0.9% NaCl solution to remove the buffer and unprecipitated proteins, and finally in distilled water for 48 hrs. to remove the saline. The gels were dried at 37°C and covered with naphthalene black solution for a few min., washed in methanol-acetic acid mixture and the phenotype scored again.

Reading of phenotypes.

The patterns for the different phenotypes are illustrated in Fig. 6.7. Phenotypes 11, 21 and 22 were scored.

Phenotype-genotype relationship.

These phenotypes correspond to the genotypes 11, 21 and 22 respectively.

b. The cholinesterase (E_1) locus.

The existence of an atypical cholinesterase enzyme in patients sensitive to the muscle relaxant, succinyl choline was first demonstrated by Kalow (1956). The method for detecting it in serum by

determining the relative insensitivity to inhibition by dibucaine was described by Kalow and Genest (1957) and a screening test using an inhibitor RO 2 - 0683 was described by Kalow (1962). Harris and Robson (1963) have adapted this inhibition technique for a rapid agar diffusion test to score atypical and intermediate types of enzymes.

Principle.

The usual, and atypical forms of serum cholinesterase are differentially inhibited by RO 2 - 0683 (Roche). With appropriate dilutions of inhibitor and serum in an agar diffusion test it is possible to stain and distinguish between the usual, intermediate and atypical phenotypes by the amount of enzyme activity still present in an agar containing the inhibitor. The substrate is 1-naphthyl acetate which is hydrolysed by the enzyme: the 1-naphthol so formed is then coupled with a diazo reagent to give a brown colour.

Solutions.

Tris - HCl buffer, pH 7.4. 24.23 g. Tris dissolved in 2 litres distilled water and pH adjusted to 7.4 with 1 N HCl.

Agar solution, 1.5% 15 g. Oxoid agar no. 3 added to 1 litre hot Tris buffer and heating continued till all the agar dissolves. Merthiolate (0.1 g. per 10 ml) was added and the agar bottled in 100 ml volumes.

RO 2 - 0683 solution. 0.043 g. RO 2 - 0683 dissolved in 1 litre distilled water, diluted 1 in 10 (1×10^{-5}M).

Phosphate buffer, 0.2 M. (a) 42.59 g. Na_2HPO_4 in 1.5 litres water.

(b) 31.20 g. $NaH_2PO_4.2H_2O$ in 1 litre water.

1.5 litres of (a) mixed with 600 ml (b) and adjusted to pH 7.1.

Substrate solution. 0.1 g. 1-naphthyl acetate dissolved in 10 ml 50% aqueous acetone. 2 ml of this solution are added to 100 ml 0.2 M phosphate buffer and the mixture shaken up with 0.02 g. Fast red TR salt (Gurr).

Method.

The method of Harris and Robson (1963) was used. 100 ml of the 1.5% agar was melted and 50 ml poured into a levelled "sandwich box" marked 'control'. 0.5 ml inhibitor solution RO 2 - 0683 was added to the other 50 ml which was then poured into a second box marked 'inhibitor'. 24 equally spaced vertical wells were made in the agar using a cork borer. Dilutions were made of the unknown serum samples and one known intermediate sample (to be used as a control) in Tris buffer solution, 1 in 8 dilution for the 'inhibitor box and 1 in 32 dilution for the control box. Each well was filled with one of these dilutions. The boxes were covered and incubated overnight at 37°C. The cholinesterase zones were developed with substrate solution freshly prepared, using 50 ml for each box. The phenotypes were scored after 2 hr. at room temperature.

Reading of phenotypes.

The phenotypes usual (U) or intermediate (I) were scored. The rare 'atypical' phenotype (A) was not encountered. On the control plate, all sera give a brown zone of activity round the well. With the U sera, the corresponding well on the inhibitor plate is

lighter (enzyme inhibited by RO 2-0683). With I sera the zone of enzyme activity is more intense on the inhibitor plate than on the control plate.

Phenotype-genotype relationship.

The phenotype U corresponds to the genotypes $\underline{E_1^U E_1^U}$ or $\underline{E_1^U E_1^S}$ and I, to the heterozygote $\underline{E_1^U E_1^A}$.

c.i[1] The serum lipoprotein (Lp) locus.

The serum Lp system was detected by Berg (1963) and the genetic nature of the polymorphism was demonstrated by Berg and Mohr (1963).

Principle.

A rabbit antiserum against an Lp (a+) donor is prepared and is absorbed with serum from an Lp (a-) donor. The resulting antiserum distinguishes between Lp (a+) and Lp (a-) serum types. The unknown sera are tested by Ouchterlony's double diffusion test, against the absorbed antiserum.

Solutions.

2% agarose. 5 g. agarose (Behringwerke) were added

to 250 ml hot water and heated until all the agarose dissolved. 25 mg. merthiolate was then added and the solution distributed in 6 oz. flasks.

Buffer solution. 8.5 g. NaCl,
50 mg. merthiolate.
The above are added to 16.7 ml of 0.4 M phosphate buffer, pH 7.0, and diluted to 500 ml with distilled water.

Method.

The method of Berg (1963) was used. The agarose solution was melted and mixed with an equal quantity of buffer solution. 5.2 ml aliquots were poured into petri dishes, diameter 4.7 cm, placed on a level surface. After the gels set, a pattern of 7 wells, each of diameter 4 mm, was cut, one being central and the others equally spaced peripherally. Absorbed rabbit anti-Lp^a serum was placed in the central well and the sera to be tested in the peripheral wells. A known Lp (a+) serum was included in each dish and a known Lp (a-) serum in every 4th dish. The gels were incubated at $37^{o}C$ for 48 hr. and the phenotypes scored.

Reading of phenotypes.

The presence or absence of an arc shaped precipitate between the central well and the peripheral wells was scored, the presence of a precipitate indicating Lp (a+), and absence, Lp (a-), phenotypes.

Phenotype-genotype relationship.

Lp (a+) corresponds to genotypes $\underline{Lp^{a}Lp^{a}}$ or $\underline{Lp^{a}Lp}$ and Lp (a-) to genotype $\underline{LpLp}$.

c.i[11] The serum lipoprotein (Ag) locus.

The polymorphism at this locus was demonstrated by Allison and Blumberg (1961).

The phenotypes Ag(+) and Ag(-) are tested for by a method similar to that of the Lp factors.

Anti-Ag is not available in bulk as its only source is human. The Ag phenotyping given in this study was done by Dr. J. Hirschfeld, State Institute of Blood Group Serology, Stockholm, Sweden.

c.ii. The serum protein loci, Gm and Inv.

The Gm^{a} factor was detected by Grubb (1956) and the genetic nature of the polymorphism demonstrated by Grubb and Laurell (1956).

The Inv system, which is genetically independent of the Gm system, was demonstrated by Ropartz (1963). The Gm groups are determinants on the Heavy (H) polypeptide chains of the globulins of the γG only, while the Inv group are determinants on the Light (L) chains common to the γG, γM and γA globulins

Principle.

The antigenic phenotypes of the immunoglobulins are demonstrable by scoring competitive inhibition of the agglutination of erythrocytes coated with human immunoglobulins carrying the relevant antigenic specificity. In practice, this involves detecting whether or not test sera inhibit the agglutination by anti-Gm (or anti-Inv) sera or erythrocytes with the appropriate coating of Gm or Inv factor, the presence of Gm (or Inv) factor causing inhibition and giving a negative reading, whilst absence of such a factor causes agglutination, giving a positive reading on the test.

Method.

The method described by Harboe (1960) was used; Table 6.4 summarises its use in testing for Gm^a phenotypes. The best dilutions of the antisera and of the test sera were found for each combination of

Table 6.4 Method for testing Gm^a

Sensitisation of red cells				Dilutions		Technique		
Dilution of anti Rh	RBC / Anti Rh	Time	Temp.	Anti Gm^a	Test Sera	Method	Temp.	Time
$^1/_2$	$^V/_V$ 50% RBC dil. anti Rh	$1\frac{1}{2}$ hr.	37^oC	$^1/_8$	$^1/_4$	Slide Tube	RT RT	15 mins 1 hr.

Anti Gm^a + Unsensitised Cells + Saline	Gm a+ + Sensitised Cells + Saline	Gm a- + Sensitised Cells + Saline	Test Sera + Sensitised Cells + Saline
Anti Gm^a + Sensitised Cells + Saline	Gm a+ + Sensitised Cells + Anti Gm^a	Gm a- + Sensitised Cells + Anti Gm^a	Test Sera + Sensitised Cells + Anti gm^a

anti-Gm^a and test serum. It is essential to use the same reagents to test the whole pedigree.

Of the Gm factors only Gm^a, Gm^b and Gm^x (Gms 1, 2 and 3 of the WHO nomenclature) were tested for. Only Gm(a+) samples were tested for Gm^x since the allele $\underline{Gm^{a-,x+}}$ is extremely rare.

Of the Inv factors only Inv (1)(Inv 1 of WHO nomenclature) was tested for.

Reading of phenotypes.

The phenotypes scored were

Gm (a+) or Gm (a-)

Gm (b+) or Gm (b-)

Gm (x+) or Gm (x-)

Inv (1+) or Inv. (1-)

Phenotype-genotype relationships are given in Table 6.5. The Gm alleles are grouped into the 3 classes $\underline{Gm^{ax}}$, $\underline{Gm^a}$, $\underline{Gm^b}$ for this study, although each class contains many different alleles distinguishable by additional test reagents, not used here.

Relative usefulness of the marker loci.

As mentioned in Chapter II for useful linkage analysis it is

Table 6.5 Phenotype-genotype correlation. Gm and Inv. loci.

Locus	Phenotypes scored	Possible genotypes
Gm	a + b + x +	$Gm^{ax}\ Gm^{b}$
	a + b - x +	$Gm^{ax}\ Gm^{a}$, $Gm^{ax}\ Gm^{ax}$
	a + b - x -	$Gm^{a}\ Gm^{a}$
	a - b +	$Gm^{b}\ Gm^{b}$
Inv.	1+	$Inv^{1}\ Inv^{1}$, $Inv^{1}\ Inv$
	1-	Inv Inv

necessary that one of the loci tested should have a minimum of two distinguishable alleles occurring commonly in the population. The usefulness of a locus will depend on

i. The ease and reliability of recognising different phenotypes at the locus,

ii. the number of alleles at the locus,

iii. the dominance relationships between the alleles,

iv. and the allele frequency in the population studied.

The relative efficiency of a two allele marker showing dominance (only two phenotypes at locus) would be highest when the frequencies of p and q of the dominant and recessive alleles are in the ratio 1:3. When both genes are expressed in the heterozygote the highest efficiency of the marker would be when p and q have equal frequencies. The multiallelic loci of the blood groups are easily the most efficient. Rhesus, MNSs and A_1A_2BO have the added advantage in the reliability of the standard antisera.

The loci used in this study, the different alleles and their frequencies for the population studied are given in Tables 6.6 and 6.7.

Table 6.6 Erythrocyte antigen loci studied, their alleles and frequencies (Scottish).

Loci	Alleles and Allele Frequencies							
ABO	A_1	.14	A_2	.06	B	.07	O	.73
MNSs	MS	.25	MS	.28	NS	.08	Ns	.39
Rhesus	$CDe(R_1)$	.43	$cDE(R_2)$	.14	cde(r)	.40	$cDe(R_0)$	.03
P	P^1	.52	P^2	.48				
Lutheran	Lu^a	.02	Lu^6	.98				
Kell	K	.05	k	.95				
Lewis	L	.75	l	.25				
Duffy	Fy^a	.41	Fy^b	.59				
Kidd	Jk^a	.52	Jk^b	.48				
Sec	Se	.42	se	.58				

For Rhesus C^w is classed with C
and D^u with D

Table 6.7 Erythrocyte enzyme and serum protein loci studied, their alleles and allele frequencies (Scottish).

Loci	Alleles and Allele Frequencies					
Hp	Hp^{1F}	0.14	Hp^{1S}	0.23	Hp^{2}	0.63
AcP	AcP^{A}	0.36	AcP^{B}	0.6	AcP^{C}	0.04
Gm	Gm^{a}	0.22	Gm^{b}	0.66	Gm^{ax}	0.12
Inv	Inv^{r}	0.08	Inv^{-1}	0.92		
PGM_1	PGM_1^{1}	0.63	PGM_1^{2}	0.37		
6PGD	$6PGD^{A}$	0.98	$6PGD^{B}$	0.02		
AK	AK^{1}	0.96	AK^{2}	0.04		
$Che\ E_1$	$Che\ E_1^{U}$	0.98	$Che\ E_1^{I}$	0.02		
$Che\ E_2$	$Che\ E_2^{5}$	0.04	$Che\ E_2^{-5}$	0.96		
Tf	Tf^{C}	0.99	Tf^{B}	0.01		
Gc	Gc^{1}	0.72	Gc^{2}	0.28		
Lp	Lp^{a}	0.19	Lp	0.81		
Ag	Ag^{+}	0.4	Ag	0.6		

CHAPTER VII

RESULTS OF INVESTIGATIONS:

A. Cytological Investigations.

B. Marker Investigations.

C. Pedigree Data.

CHAPTER VII.

A. Results of cytological investigations

Pedigree V21AN.

The marker chromosome of pedigree V21AN was a heteromorphic chromosome no. 2 and is illustrated in Fig. 7.1. There was a shift of the position of the centromere, and the short arm was longer and the long arm shorter than in the homologue. Following the Chicago Conference nomenclature it is referred to as 2 p+ q-. Group A chromosomes are easy to identify and to number 1, 2 and 3. On microscopic analysis, in the heterozygous carriers of this heteromorphism there were five metacentric chromosomes and only one sub-metacentric in group A. In length the extra metacentric in most cells was equal in length to that of the normal chromosome no. 2. Rarely, i.e. in two cells of V.6 and in one cell of V10 and in three cells of III.33, all three heterozygotes, one chromosome no. 2 was very much larger than the other, with no shift of the position of the centromere (p+q+). However, a similar chromosome was seen in one cell of IV.13 in an individual not carrying the heteromorphism.

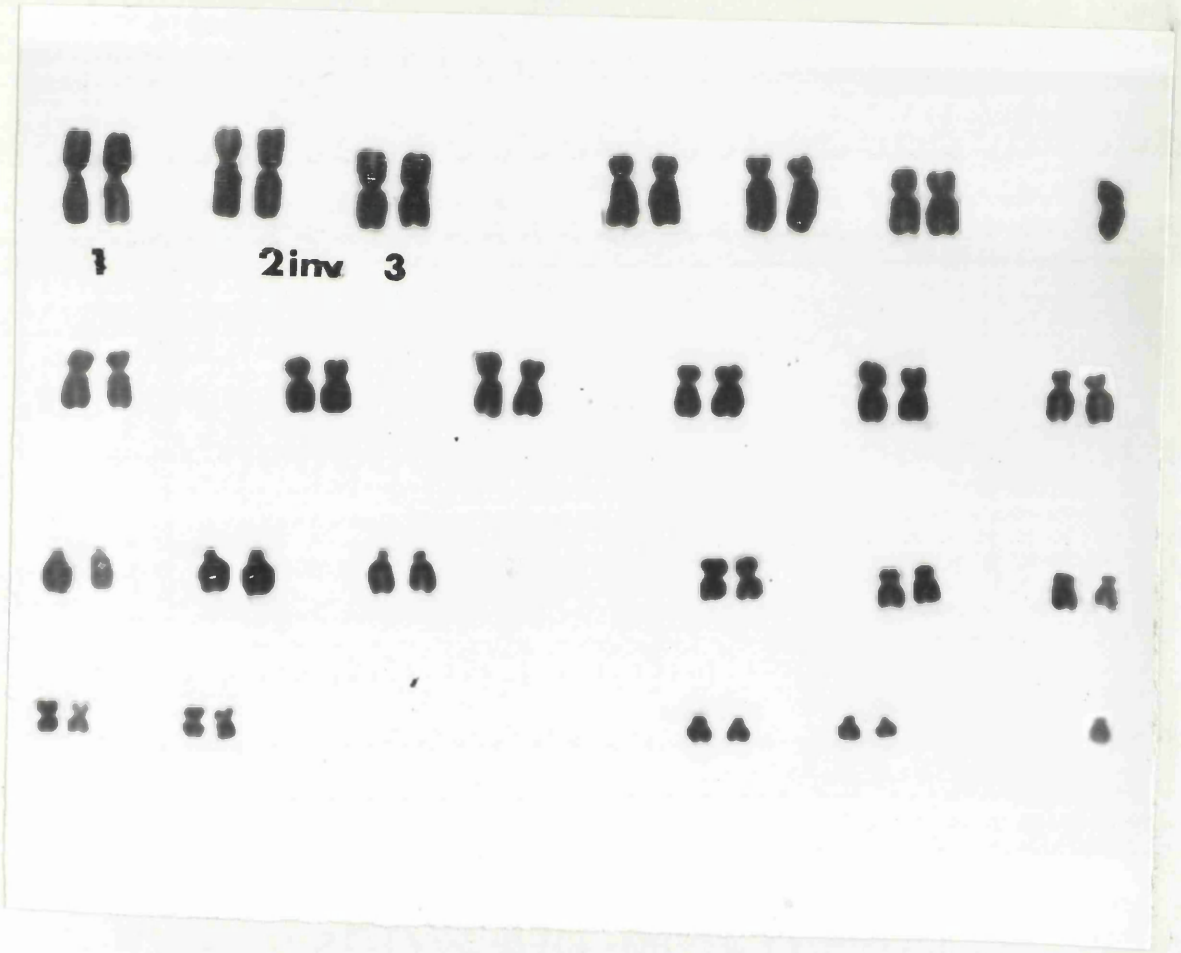


Fig. 7.1 The 'marker' chromosome of Pedigree V21AN

2 inv. = inversion in chromosome No. 2

i.e. 2p+q- (p = short arm)

(q = long arm)

Table 7.1 Chromosome analysis of Pedigree V21AN in number of cells.

Ped. No.	Count 45	46	47	total	microscopic analysis marker+	marker-	uncertain	photographic analysis marker+	marker-	uncertain	conclusion
III.13	1	18	1	20	18	0	2	0	3	0	normal
III.20	1	19	0	20	0	20	0	0	3	0	normal
III.21	0	20	0	20	0	18	2	0	3	0	normal
III.33	2	18	0	20	16	3	1	3	0	0	†heteromorphic
III.39	0	26	0	26	23	0	3	4	0	0	heteromorphic
III.40	0	21	1	22	0	22	0	0	3	0	normal
III.44	2	24	0	26	0	26	0	0	3	0	normal
IV.2	1	20	0	21	0	18	3	0	3	0	normal
IV.4	1	20	0	21	0	21	0	0	3	0	normal
IV.6	2	21	0	23	0	23	0	0	3	0	normal
IV.8	0	20	0	20	0	19	1	0	3	0	normal
IV.10	0	25	0	25	21	0	4	4	0	0	heteromorphic
IV.11	1	22	0	23	0	23	0	0	3	0	normal
IV.13	0	22	1	23	0	20	3	0	3	0	normal
*IV.14								0	4	0	normal
*IV.15								7	0	0	heteromorphic
IV.16	0	30	0	30	30	30	0	4	0	0	heteromorphic
IV.17	0	30	0	30	0	30	0	0	3	0	normal
V.5	1	20	0	21	21	0	0	3	0	0	heteromorphic
V.6	5	21	0	26	21	3	2	3	0	0	heteromorphic
* V.9											normal
V.10	1	20	21	21	17	3	1	3	0	0	heteromorphic

* Chromosome analysis done by staff of Human Cytogenetic Laboratory.

† heteromorphic = carrier of heteromorphic chromosome no. 2. 2 p+q-

One cell in V.6 had a pair of normal chromosomes no. 2 and two cells in V.10 had short' "long" arms (q-)2. Of a total of 169 cells analysed in the heterozygotes (Table 7.1) 150 cells have been scored as positive under direct microscopy, and ten cells as uncertain. Of the nine other cells, one had a normal pair of chromosomes no. 2, six had one chromosome no. 2 larger than the other and two had one chromosome no. 2 with shorter long arms.

On photographic analysis, one of the five metacentrics of group A was of intermediate size and could be distinguished from the chromosomes no. 1 and the chromosomes no. 3.

Pedigree JM1MY.

The marker chromosome of pedigree JM1MY is illustrated in Fig. 7.2. The length of the long arm of this chromosome was markedly increased as compared with its homologue. Following the Chicago Conference (1966) nomenclature it is referred to as 16 q+. On microscopic analysis it was identified by counting the smaller metacentrics as a group, i.e. 19, 20 and 16. In a heterozygous carrier of this heteromorphism there were only five chromosomes instead of six in this group. In some cases the heteromorphic

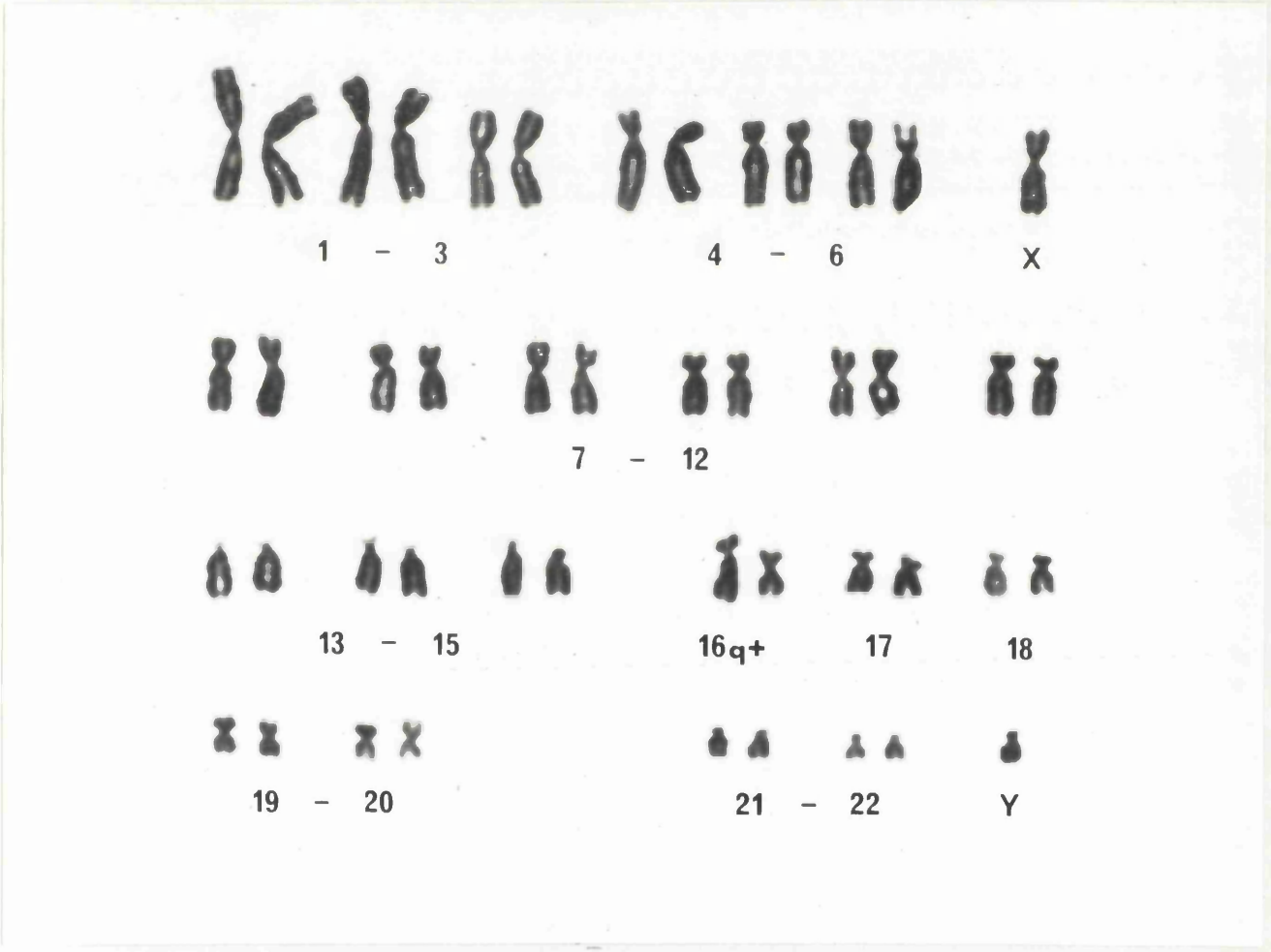


Fig. 7.2 The 'marker' chromosome of Pedigree JM1MY (16q+) q = long arm.

Table 7.2 Chromosome analysis of pedigree JM1MY in numbers of cells.

Ped. No.	Count				microscopic analysis			photographic analysis			conclusion
	45	46	47	total	marker+	marker-	uncertain	marker+	marker-	uncertain	
II.2	1	20	0	21	20	0	1	4	0	1	†heteromorphic
III.2	1	40	0	41	0	39	1	0	4	0	normal
III.3	2	58	0	60	58	0	2	6	0	0	heteromorphic
III.4	0	23	0	23	0	21	2	0	3	1	normal
III.6	0	20	0	20	18	0	1	4	0	0	heteromorphic
III.7	0	26	0	26	0	26	0	0	3	0	normal
III.8	1	22	0	23	21	0	2	3	0	0	heteromorphic
III.9	0	20	0	20	0	13	7	0	4	0	normal
III.11	0	20	0	20	0	18	2	0	4	0	normal
III.13	0	21	1	22	0	17	5	0	4	0	normal
*IV.2											heteromorphic
*IV.3											normal
IV.5	2	24	0	26	0	23	3	0	4	1	normal
IV.6	0	26	0	26	26	0	0	3	0	0	heteromorphic
IV.7	0	20	0	20	24	0	2	4	0	0	heteromorphic
IV.8	0	18	8	26	24	0	2	5	0	0	heteromorphic
IV.12	1	22	0	23	22	0	1	3	0	0	heteromorphic
IV.13	0	20	0	20	20	0	0	3	0	0	heteromorphic
IV.14	0	22	1	23	22	0	1	3	0	0	heteromorphic
V.2											heteromorphic
V.3	2	21	0	23	22	0	1	0	3	0	normal

* Chromosome analysis done by staff of Human Cytogenetic Laboratory.

† heteromorphic = Carrier of long variant of chromosome no. 16. 16q+.

chromosome could be picked out from the rest of the chromosomes by the inverted V shape of the distal portion of the elongated long arm. As shown in Table 7.2 out of 267 cells examined in ten heterozygotes, 255 cells showed the marker. The only difficulties in classifying were met when the chromosomes in the preparation were contracted. This chromosome provides an easily recognisable marker and the chances of misclassification are practically nil. With a good quality preparation it is possible to score carriers even without recourse to photography.

Pedigree JN1AN.

The heteromorphism studied in pedigree JN1AN was the presence of a satellite in one chromosome no. 17. It is illustrated in Fig. 7.3 and is referred to as 17s+. It was the most difficult of all the markers scored in this study. It is not expressed in all cells. As shown in Table 7.3 of the five heterozygotes scored, of a total of 116 cells, the satellite was present in 49 cells and absent in 51 cells. 16 cells were scored as uncertain.

Pedigree JN1PW.

The marker chromosome of this pedigree too was a satellited chromosome no. 17 which appeared identical to that in pedigree JN1AN.

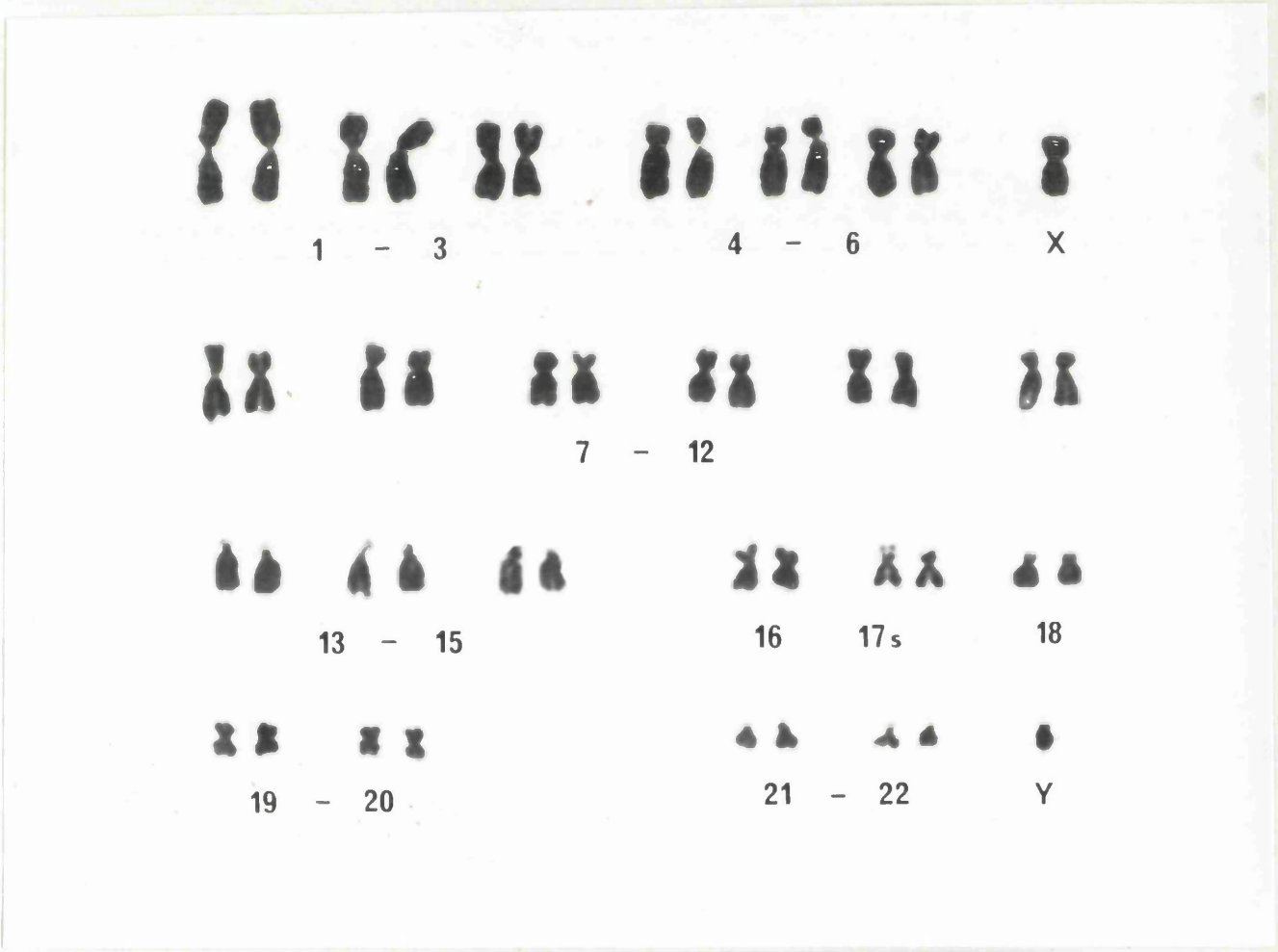


Fig. 7.3 The 'marker' chromosome of Pedigrees JN1AN and JN1PW
17s (s = satellite)

Table 7.3 Chromosome analysis of pedigree JN1AN in numbers of cells.

Ped. No.	Count 45	46	47	total	microscopic analysis marker+	marker-	uncertain	photographic analysis marker+	marker-	uncertain	conclusion
II.7	0	26	0	26	0	24	2	0	4	0	normal
II.9	1	20	0	21	9	9	3	4	0	0	†heteromorphic
II.10	1	26	0	27	0	27	0	0	4	0	normal
II.12	1	19	0	20	0	19	1	0	3	0	normal
II.13	1	22	0	23	0	19	4	0	3	0	normal
II.14	0	20	0	20	0	19	1	0	2	1	normal
III.2	3	18	0	21	0	21	0	0	3	0	normal
III.4	1	25	0	26	10	14	2	3	0	1	heteromorphic
III.6	0	19	1	20	0	20	0	0	3	0	normal
III.8	1	22	0	23	0	20	3	0	3	0	normal
III.13	1	24	0	25	6	11	8	3	0	2	heteromorphic
III.14	1	21	0	22	12	7	3	4	0	0	heteromorphic
III.15	0	21	0	21	0	21	0	0	3	0	normal
III.16	0	23	0	23	0	22	1	0	3	0	normal
*III.18											heteromorphic
III.19	1	2	0	22	12	10	0	3	0	0	heteromorphic
IV.8	0	21	0	21	0	21	0	0	3	0	normal
IV.9	1	19	0	20	0	20	0	0	3	0	normal
IV.12	2	18	0	20	0	19	1	0	3	0	normal
IV.13	1	19	0	20	0	20	0	0	3	0	normal
IV.14	2	18	0	20	0	20	0	0	3	0	normal
IV.15	2	18	0	20	0	17	3	0	3	0	normal
IV.16	0	20	0	20	0	17	3	0	3	0	normal

* Chromosome analysis done by staff of Human Cytogenetics Laboratory.

† heteromorphic = carrier of heteromorphic chromosome no. 17. S+ 17.

Table 7.4 Chromosome analysis of pedigree JN1PW in numbers of cells.

Ped. No.	Count 45	46	47	total	microscopic analysis marker+	marker-	uncertain	photographic analysis marker+	marker-	uncertain	conclusion
I.2	0	38	0	38	0	35	3	0	3	0	normal
I.3	0	33	0	33	0	33	0	0	3	0	normal
II.1	0	26	0	26	0	25	1	0	4	0	normal
II.2	1	28	0	29	13	13	3	4	0	1	†heteromorphic
II.5	0	37	0	34	0	31	3	0	4	0	normal
II.9	1	20	0	21	0	20	1	0	3	0	normal
*III.1											normal
*III.2											heteromorphic
III.3	2	49	0	51	0	49	2	0	3	1	normal
III.4	1	24	0	25	7	17	1	4	0	0	heteromorphic
IV.1	1	21	0	22	0	21	1				normal
*IV.2											heteromorphic
IV.3	1	19	0	20	0	20	0	0	3	0	normal

* Chromosome analysis done by staff of Human Cytogenetics Laboratory.

† heteromorphic = carrier of heteromorphic chromosome no. 17. S+ 17.

As shown in Table 7.4 of the heterozygotes scored, of 54 cells, in 20 cells the marker was present, in 30 cells the marker was absent, and 4 cells are recorded as uncertain.

Incidental findings.

JM1MY IV.8 was shown to be a mosaic Klinefelter, eight cells out of the 26 analysed had an extra group C chromosome. A buccal smear by the method of Moore and Barr (1955) showed 9% sex chromatin positive. The child two years of age was clinically normal and phenotypically male with fully descended testes.

JN1AN III.6 carried a variant chromosome, identical to that studied in JM1MY, in 13 of the 20 cells analysed. Her offspring IV.8 and IV.9 did not carry the variant chromosome.

B. Results of marker investigations.

The results from the marker investigations are set out in Tables 7.5, 7.6, 7.7 and 7.8.

Incidental findings.

V21AN IV.17 and JN1PW IV.1 were found to be extra-marital

Key to symbols used in Tables 7.5, 7.6, 7.7 and 7.8.

Column.

1. Pedigree number.
2. Pedigree number of parents.
3. Sex.
4. Y. of b. Year of birth.
5. L. or d. Living (1) or dead (d).

Phenotypes

6. Main locus
   + = heterozygous
   - = homozygous normal
7. ABO.
8. MNS.
9. P.
10. Rhesus.
11. Lutheran.
12. Kell.
13. Lewis.
14. Duffy.
15. Kidd.
16. ABH secretor.
17. Transferrin.
18. Haptoglobin.
19. Gm gammaglobulin.
20. Gc protein.
21. Cholinesterase E_2.
22. Cholinesterase E_1.
23. Acid phosphatase.
24. Phosphoglucomutase PGM_1.
25. 6-phosphogluconate dehydrogenase.
26. Inv gammaglobulin.
27. Adenylate kinase.
28. Lp lipo-protein.
29. Ag lipo-protein.

Table 7.5 Results of marker investigations of pedigree V21AN.

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
No.	Parents	Sex	Y. of b.	L. or d.	p+q-(2)	ABO	MNS	P	Rhesus	Lu (a)	K	Le (a)	Fy (a)(b)	Jk (a)	Sec	Tf	Hp	Gm (a)(b)(x)	Gc	E_2	E_1	AcP	PGM	6PGD	Inv.	AK	Lp (a)	Ag (x)(y)
I.1	.	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
I.2	.	F	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
I.3	.	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
I.4	.	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
I.5	.	F	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.1	.	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.2	I.1,2	F	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.3	I.1,2	F	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.4	I.1,2	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.5	.	M	1878	l.	.	A_2	MSMS	+	ccDEE	-	-	-	-	-	+	C	21	+ + -	11	-	U	A	11	A	-	11	+	-
II.6	I.1,2	F	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.7	.	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.8	I.1,2	F	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.9	.	F	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.10	I.1,2	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.11	I.2,3	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.12	I.4,5	F	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.16	I.4,5	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.17	.	F	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.18	I.4,5	F	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.19	.	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table 7.5, cont'd.

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
No.	Parents	Sex	Y. of b.	L. or d.	p+q-(2)	ABO	MNS	P	Rhesus	Lu (a)	K	Le (a)	Fy (a)(b)	Jk (a)	Sec	Tf	Hp	Gm (a)(b)(x)	Gc	E_2	E_1	AcP	PGM	6PGD	Inv.	AK	Lp (a)	Ag (x)(y)
III.1-11	.	.	.	.	.	.	.	.	.	.	.	.	. .	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III.12	.	F	1926	l.	.	O	NsNs	+	ccDEe	-	-	-	+ +	-	+	C	1F1S	+ + -	11	-	U	B	21	A	-	11	-	- +
III.13	II.3,4	M	1914	l.	-	A_1	MsNs	-	CcDee	-	-	-	+ -	+	+	C	22	- . .	11	-	U	BC	11	A	-	11	+	+ -
III.14-19			.			.	.	.	.	.	.	.	. .	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III.20	II.5,6	M	1909	l.	-	O	MNSs	+	CcDEe	-	-	+	+ +	+	-	C	21F	- + .	21	-	U	B	21	A	-	11	-	- .
III.21	II.5,6	F	1920	l.	-	O	MSMS	+	CcDEe	-	-	-	- .	+	+	C	22	- + .	11	-	U	B	11	A	-	11	-	- .
III.22-26			.			.	.	.	.	.	.	.	. .	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III.27-29			.			.	.	.	.	.	.	.	. .	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III.30	.	F	1896	l.	.	O	MNSs	-	CcDee	-	-	-	+ +	+	+	C	21	+ + -	11	-	U	AB	11	A	-	11	+	. .
III.31				d.	.	.	.	.	.	.	.	.	. .	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III.32				d.	.	.	.	.	.	.	.	.	. .	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III.33	II.11,12	F	1892	l.	+	O	MSMs	+	CcDee	-	-	.	+ +	+	+	C	11	- + .	11	±	U	B	21	A	-	11	+	. .
III.34	.	M	.	d.		.	.	.	.	.	.	.	. .	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III.35	II.11,12	M	.	d.		.	.	.	.	.	.	.	. .	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III.36	.	F	.	d.		.	.	.	.	.	.	.	. .	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III.37	II.11,12	M	.	d.		.	.	.	.	.	.	.	. .	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III.38	.	F	.	d.		.	.	.	.	.	.	.	. .	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III.39	II.11,12	M	1908	l.	+	O	MsNs	+	CCDee	-	-	-	- +	+	+	C	1F1S	+ + +	11	-	U	B	11	A	-	11	.	. .
III.40	.	F	1913	l.	-	O	MsNs	-	CcDee	-	-	+	+ -	+	-	C	21	+ + +	21	+	U	BC	21	A	-	21	.	. .
III.41	.	M	.	.	.	.	.	.	.	.	.	.	. .	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III.42,43	II.17,18		.	.	.	.	.	.	.	.	.	.	. .	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III.44	II.19,20	F	1921	l.	-	B	MsNs	+	CcDee	-	-	-	+ -	+	+	C	21S	- + .	21	-	U	BC	11	A	-	11	-	- .
III.45,46			.			.	.	.	.	.	.	.	. .	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .

Table 7.5, cont'd.

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
No.	Parents	Sex	Y.of b.	L.or d.	p+q-(2)	ABO	MNS	P	Rhesus	Lu (a)	K	Le (a)	Fy (a)	Fy (b)	Jk (a)	Sec	Tf	Hp	Gm (a)(b)(x)	Gc	E_2	E_1	AcP	PGM	6PGD	Inv.	AK	Lp (a)	Ag (x)(y)
IV.1	III.12,13	M	1948	l.	.	A_1	MsNs	-	CcDEe	-	-	-	-	+	+	+	C	21S	+ + -	11	-	U	AC	11	A	-	11	-	+ +
IV.2	III.28,29	F	1919	l.	-	A_1	NsNs	+	ccddee	-	-	-	+	+	+	+	C	22	+ + +	11	-	U	AB	11	A	+	11	-	. .
IV.3	.	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
IV.4	III.30,31	F	1922	l.	-	O	MSMs	-	CCDee	-	-	-	+	+	+	+	C	21	- + .	11	-	U	AB	11	A	-	11	+	. .
IV.5	III.30,31	F	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
IV.6	III.30,31	F	1925	l.	-	O	MsNs	-	CcDee	-	-	-	+	+	+	+	C	21	- + .	11	-	U	AB	11	A	-	11	-	. .
IV.7	III.30,31	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
IV.8	III.31,32	M	1919	l.	-	A_1	MNSs	+	CCDee	-	-	-	+	-	+	+	C	22	- + .	11	-	U	B	11	A	-	11	.	. .
IV.9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
IV.10	III.33,34	F	1930	l.	+	A_2	MsMs	+	CcDee	-	+	-	+	-	+	+	C	21	- + .	11	-	U	AB	21	A	-	11	-	- .
IV.11	.	M	1919	l.	-	A_1	MNSs	+	ccDEE	-	-	-	+	+	-	.	C	21	- + .	21	+	U	AB	11	A	-	11	-	. .
IV.12	.	M	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
IV.13	III.36,37	F	1936	l.	-	O	MsNs	+	CCDee	-	-	-	+	+	+	+	C	1S1S	- + .	11	-	U	B	21	A	-	11	-	- .
IV.14	.	M	1930	l.	-	O	MSMs	+	CcDee	-	-	-	+	-	-	+	C	22	- + .	11	-	U	B	11	A	-	11	.	. .
IV.15	III.39,40	F	1939	l.	+	O	MsNs	+	CcDee	-	-	-	+	-	+	+	C	11	- + .	21	-	U	B	21	A	-	11	.	. .
IV.16	III.39,40	F	1942	l.	+	O	MsMs	-	CCDee	-	-	-	+	-	+	+	C	1S1S	+ - +	11	+	U	B	11	A	-	21	+	. .
IV.17	III.40,41	M	1952	l.	-	O	NsNs	+	ccddee	-	-	-	+	-	-	+	C	21	+ - +	11	+	U	BC	11	A	-	11	.	. .
IV.18,19	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
IV.20	III.44,45	M	1953	l.	.	B	MSMs	+	CcDee	-	-	+	+	+	+	-	C	22	- + .	21	-	U	B	11	A	-	11	.	. .
V.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
V.2-4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
V.5	IV.10,11	M	1948	l.	+	A_1	MSMs	+	CcDEe	-	-	-	+	+	+	+	C	21	- + .	21	-	U	AB	11	A	-	11	-	. .
V.6	IV.10,11	M	1950	l.	+	A_1	MsNs	+	CcDEe	-	+	-	+	+	+	+	C	11	- + -	21	+	U	AB	11	A	-	11	-	. .
V.7,8	IV.12,13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
V.9	IV.14,15	F	1965	l.	-	O	MNSs	+	CcDee	-	-	-	+	-	+	.	C	21	- + .	11	+	U	B	11	A	-	11	-	. .
V.10	IV.14,15	M	1967	l.	+	O	MNSs	+	ccddee	-	-	-	+	-	+	.	C	21	- + .	21	-	U	B	11	A	-	11	+	- .

Table 7.6 Results of marker investigations of pedigree JM1MY

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
No.	Parents	Sex	Y.of b.	L.or d.	q+ (16)	ABO	MNS	P	Rhesus	Lu (a)	K	Le (a)	Fy (a)(b)	Jk (a)	Sec	Tf	Hp	Gm (a)(b)(x)	Gc	E_2	E_1	AcP	PGM	6PGD	Inv.	AK	Lp (a)
I.1	.	F	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.
I.2	.	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.
II.1	.	F	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.
II.2	I.1,2	M	1897	l.	+	O	NsNs	+	ccDEe	-	-	-	+	+	+	+	C	21	- + .	21	-	U	AB	11	A	-	21	.
II.3	I.1,2	M	.	d.	.	.	.		.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.
III.1	.	M	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.
III.2	.	F	1923	l.	-	A_2	MSMs	+	CcDee	-	-	-	+	+	+	+	C	11	+ + +	11	-	U	A	11	A	-	11	.
III.3	II.1,2	M	1921	l.	+	O	MNSs	+	CcDEe	-	-	-	-	+	+	+	C	11	- + .	11	+	U	A	11	A	-	11	.
III.4	II.1,2	F	1924	l.	-	O	MNSs	+	ccDEe	-	-	-	-	.	+	+	C	22	+ + +	11	-	U	A	21	A	-	21	.
III.5	.	M	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.
III.6	II.1,2	F	1928	l.	+	O	MsNs	+	ccDEe	-	-	+	-	.	+	-	C	11	- + .	21	+	U	AB	11	A	-	21	.
III.7	.	M	1920	l.	-	B	MsNs	+	ccDee	-	-	-	-	.	+	+	C	21	- + .	22	-	U	AB	11	A	-	11	.
III.8	II.1,2	M	1929	l.	+	O	MsNs	+	CcDEe	-	-	-	+	+	+	+	C	21	+ + +	22	-	U	A	11	A	-	11	+
III.9	II.1,2	M	1932	l.	-	O	MsNs	+	CcDee	-	-	+	-	.	+	-	C	21	+ + +	21	-	U	A	11	A	-	11	.
III.10	II.1,2	M	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.
III.11	II.1,2	F	1936	l.	-	O	MsNs	-	ccDee	-	-	-	+	+	+	+	C	11	- + .	21	-	U	AB	11	A	-	11	.
III.12	.	M	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.
III.13	II.1,2	M	1938	l.	-	O	MsNs	+	ccDEe	-	-	-	+	+	+	.	C	22	- + .	11	-	U	AB	21	A	-	11	.
III.14	.	F	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.

Table 7.6, cont'd.

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
No.	Parents	Sex	Y. of b.	L. or d.	q+ (16)	ABO	MNS	P	Rhesus	Lu (a)	K	Le (a)	Fy (a)(b)	Jk (a)	Sec	Tf	Hp	Gm (a)	(b)	(x)	Gc	E_2	E_1	AcP	PGM	6PGD	Inv.	AK	Lp (a)
IV.1	III.1,2	F	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
IV.2	III.2,3	F	1948	l.	+	A_2	MNSs	+	CCDee	-	-	-	+	+	+	.	C	11	+	+	+	11	-	U	.	11	A	-	.	.
IV.3	.	M	1943	l.	-	A_1	MsNs	-	ccddee	-	-	-	-	.	+	.	C	21	-	+	-	11	-	U	.	11	A	-	.	.
IV.4	III.2,3	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
IV.5	III.2,3	M	1950	l.	-	O	MSMs	+	CCDee	-	-	-	+	+	-	+	C	11	+	+	+	11	-	U	A	11	A	-	11	.
IV.6	III.2,3	M	1952	l.	+	O	MSMs	+	CcDee	-	-	-	-	+	+	+	C	11	-	+	.	11	-	U	A	11	A	-	11	.
IV.7	III.2,3	F	1958	l.	+	O	MSMs	+	CcDee	-	-	-	-	+	-	+	C	11	-	+	.	11	-	U	A	11	A	-	11	.
IV.8	III.2,3	M	1964	l.	+	O	MSMs	+	CcDee	-	-	-	-	+	+	+	C	11	+	+	+	11	-	U	A	11	A	-	11	+
IV.9	III.2,3	misc.	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
IV.10-11	III.4,5	.	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
IV.12	III.6,7	F	1951	l.	+	B	MsMs	+	ccDEe	-	-	-	-	.	+	+	C	11	-	+	.	21	-	U	B	11	A	-	21	-
IV.13	III.6,7	F	1954	l.	+	B	MsMs	+	ccDEe	-	-	+	-	.	+	-	C	11	-	+	.	21	-	U	AB	11	A	-	11	-
IV.14	III.6,7	M	1956	l.	+	B	MsNs	+	ccDEe	-	-	-	-	.	+	+	C	11	-	+	.	21	-	U	A	11	A	-	11	-
V.1	IV.2,3	misc.	.	d	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
V.2	IV.2,3	M	1965	l.	+	A_1	NsNs	-	CcDee	-	-	.	-	.	+	+	C	21	+	+	+	11	-	U	.	11	A	-	.	.
V.3	IV.2,3	M	1967	l.	-	A_1	MNSs	-	CcDee	-	-	-	+	+	+	.	C	21	+	+	+	11	-	U	AB	11	A	-	11	.

Table 7.7 Results of marker investigations of Pedigree JN1AN

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
No.	Parents	Sex	Y. of b.	L. or d.	s+ (17)	ABO	MNS	P	Rhesus	Lu (a)	K	Le (a)	Fy (a)(b)	Jk (a)	Sec	Tf	Hp	Gm (a)(b)(x)	Gc	E_2	E_1	AcP	PGM	6PGD	Inv.	AK	Lp (a)	Ag (x)(y)
I.1	.	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
I.2	.	F	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.1	I.1,2	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.2	I.1,2	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.3	.	F	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.4	I.1,2	F	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.5	I.1,2	M	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.6	.	F	.	d.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.7	I.1,2	M	1899	l.	-	O	NsNs	+	-CCDee	-	-	-	-	+	+	C	21F	+ + -	21	-	I	AB	21	A	-	11	+	+
II.8	.	F	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.9	I.1,2	M	1902	l.	+	O	NsNs	-	CCDee	-	-	-	-	+	+	C	21	- +	11	-	U	B	21	A	-	11	+	+
II.10	.	F	1907	l.	-	O	MSMS	+	ccDEE	-	-	-	+	+	+	C	22	- +	11	-	U	BC	11	A	-	11	-	-
II.11	.	M	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
II.12	I.1,2	F	1904	l.	-	O	MsNs	+	CCDee	-	-	-	-	+	+	C	22	+ + -	11	-	I	B	21	A	+	11	-	-
II.13	I.1,2	M	1907	l.	-	O	MsNs	-	CcDee	-	-	-	-	+	+	C	22	+ - -	22	-	I	B	11	A	+	11	+	+
II.14	I.1,2	M	1910	l.	-	O	MsNs	-	CcDee	-	-	-	-	+	+	C	21F	- +	22	-	U	B	11	A	-	11	+	+
II.15	.	F	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
III.1	.	M	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
III.2	II.2,3	F	1919	l.	-	O	MsNs	+	ccddee	-	-	-	+	+	+	C	22	+ + -	21	-	U	B	21	A	-	11	+	+
III.3	.	M	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
III.4	II.2,3	F	1920	l.	+	O	MNSs	+	CcDee	-	-	-	+	+	+	C	22	- +	21	-	I	B	21	A	-	11	-	+
III.5	.	M	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table 7.7, cont'd.

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
No.	Parents	Sex	Y. of b.	L. or d.	s+ (17)	ABO	MNS	P	Rhesus	Lu (a)	K	Le (a)	Fy (a)(b)	Jk (a)	Sec	Tf	Hp	Gm (a)(b)(x)	Gc	E_2	E_1	AcP	PGM	6PGD	Inv.	AK	Lp (a)	Ag (x)(y)
III. 6	II. 5, 6	F	1923	l.	-	O	MsMs	+	CcDee	-	-	-	-	+	+	C	22	+ . -	21	-	U	AB	11	A	-	11	+	- .
III. 7	.	M	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III. 8	II. 5, 6	F	1933	l.	-	A_1	MsMs	+	CcDee	-	-	-	+	+	+	C	22	- + .	21	-	U	AB	11	A	-	11	-	- .
III. 9-11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III. 12	.	M	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III. 13	II. 9, 10	F	1926	l.	+	O	MNSs	-	CcDEe	-	-	-	+	+	+	C	22	- + .	11	-	U	B	11	A	-	11	+	+ .
III. 14	II. 9, 10	M	1929	l.	+	O	MNSs	-	CcDEe	-	-	-	-	.	-	+	C	22	- + .	11	-	U	B	11	A	-	11	+	+ .
III. 15	.	F	1931	l.	-	O	MsNs	+	ccDEe	-	-	-	+	.	-	+	C	21S	+ - -	11	-	U	B	11	A	+	11	-	- .
III. 16	II. 9, 10	M	1930	l.	-	O	MNSs	-	CcDEe	-	-	-	-	.	+	+	C	21	- + .	11	-	U	BC	11	A	-	11	-	+ .
III. 17	.	F	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
III. 18	II. 9, 10	M	1934	l.	+	O	MNSs	-	CcDEe	-	-	-	-	.	-	+	C	21	- + .	11	-	U	BC	11	A	-	11	-	+ .
III. 19	II. 9, 10	M	1939	l.	+	O	MNSs	-	CcDEe	-	-	-	-	.	+	+	C	22	- + .	11	-	U	B	11	A	-	11	-	+ .
III. 20-22	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
IV. 1-11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .
IV. 12	III. 12, 13	F	1951	l.	-	O	MNSs	-	ccDEE	-	-	-	+	-	+	+	C	21	- + .	11	-	U	AB	21	A	-	11	+	+ .
IV. 13	III. 12, 13	F	1956	l.	-	A_2	MSMs	-	CcDEe	-	-	+	+	-	+	-	C	21	- + .	11	-	U	AB	11	A	-	11	-	+ .
IV. 14	III. 14, 15	F	1953	l.	-	O	MSMs	+	CcDEe	-	-	-	+	.	-	+	C	21F	+ + -	11	-	U	B	11	A	+	11	+	- .
IV. 15	III. 14, 15	F	1956	l.	-	O	MNSs	-	ccDEe	-	-	-	+	.	-	+	C	21S	+ + -	11	-	U	B	11	A	-	11	+	+ .
IV. 16	III. 14, 15	M	1959	l.	-	O	MNSs	+	ccDEe	-	-	-	-	.	-	+	C	21S	+ + -	11	-	U	B	11	A	-	11	+	+ .
IV. 17	III. 14, 15	M	.	l.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.	. .

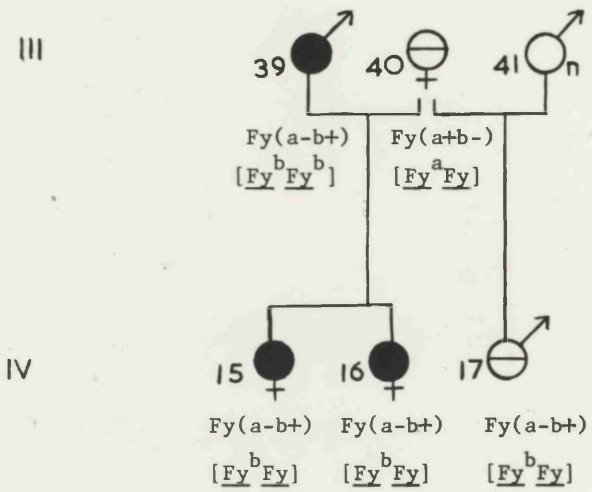
Table 7.8 Results of marker investigations of pedigree JN1PW

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
No.	Parents	Sex	Y. of b.	L. or d.	s+ (17)	ABO	MNS	P	Rhesus	Lu (a)	K	Le (a)	Fy (a)(b)	Jk (a)	Sec	Tf	Hp	Gm (a)(b)(x)	Gc	E_2	E_1	AcP	PGM	6PGD	Inv.	AK	Lp (a)
I.1	.	M	.	1.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.
I.2	.	F	1903	1.	-	O	NsNs	+	Ccddee	-	-	+	+	+	-	C	22	. + .	21	-	U	B	11	A	-	11	-
I.3	.	M	1901	1.	-	A_1	MsNs	+	CCDee	-	-	-	-	+	+	C	22	- + .	11	-	U	A	21	A	+	11	+
II.1	.	M	1915	1.	-	B	MNSs	+	CcDee	-	-	-	-	+	+	C	1S1S	+ + +	21	-	U	AC	11	A	-	11	.
II.2	I.1,2	F	1917	1.	+	A_2	MNSs	+	ccDEe	-	-	-	-	+	+	C	22	- + .	11	-	U	B	11	A	-	11	.
II.3-4	.	.	.	1.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.
II.5	I.2,3	M	1925	1.	-	A_1	MsNs	-	CcDee	-	-	-	+	+	+	C	22	- + .	21	-	U	AB	21	A	-	11	-
II.6-8	.	.	.	1.	.	.	.	.	.	.	.	.	.	.	.	.	.	. . .	.	.	.	.	.	.	.	.	.
II.9	I.2,3	F	1931	1.	-	A_1	NsNs	-	CCDee	-	-	-	+	+	+	C	22	- + .	11	-	U	AB	11	A	-	11	+
III.1	.	F	.	1.	-	A_1	MNSs	+	CCDee	-	-	-	+	+	+	C	22	+ + -	21	-	U	AB	11	A	+	.	.
III.2	II.1,2	M	.	1.	+	A_2	NsNs	+	CcDee	-	-	-	-	+	+	C	21	+ + +	11	-	U	B	11	A	-	.	.
III.3	II.1,2	M	1944	1.	-	A_2B	NsNs	+	ccDEe	-	-	-	-	+	+	C	21	+ + +	11	-	U	AB	11	A	-	11	.
III.4	II.1,2	M	1949	1.	+	B	MSMS	+	CcDee	-	-	-	-	+	+	C	21S	+ + +	21	-	U	BC	11	A	-	11	-
IV.1	III.1	F	1962	1.	-	A_1	MNSs	-	CCDee	+	-	+	+	+	+	C	21	- + .	11	+	U	B	11	A	-	11	.
IV.2	III.1,2	F	1964	1.	+	A_2	MNSs	-	CCDee	-	-	-	-	+	+	C	21S	+ - .	11	-	U	.	11	A	-	11	.
IV.3	III.1,2	F	1966	1.	-	A_2	NsNs	-	CCDee	-	-	.	-	+	+	C	21	+ + -	21	-	U	AB	11	A	+	11	.

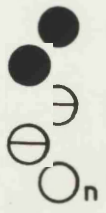
☌ age of child (6 days)

Fig. 7.4

Pedigree V21AN A rare silent allele, Fy, at the Duffy locus.
Duffy phenotypes are given followed by
postulated genotypes within brackets.



K E Y



Heterozygous for the inversion

Homozygous normal

Not tested

on the results from the marker investigations and were not used in the linkage analysis.

The presence of a Duffy Fy, silent allele was deduced in V21AN and is given in Fig. 7.4. The Duffy system has three common phenotypes, Fy(a+b-), Fy(a+b+) and Fy(a-b+), and one rare one Fy(a-b-), controlled by 3 alleles, Fy^a, Fy^b and a rare silent Fy, the last having an estimated frequency of 0.03 in the U.K. (Race and Sanger, 1962).

C. The Pedigree Data.

The pedigree data are set out in Fig. 7.6, 7.7, 7.8 and 7.9 and the key to the pedigrees in Fig. 7.5.

Table 7.9(a) gives the number of generations in each pedigree, the number of heterozygous matings at the main locus and the number of children tested. To be informative these heterozygotes for the main locus must be heterozygous at the marker loci also.

Segregation Ratios.

A simple count of the known offspring of known parents has been made and is set out in Table 7.9(b). The numbers involved

Fig. 7.5

KEY TO SYMBOLS USED IN PEDIGREES.

Structural heterozygote

Normal

Not examined cytologically

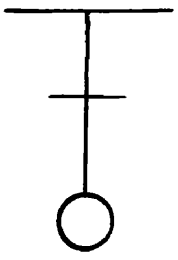
Male

Female

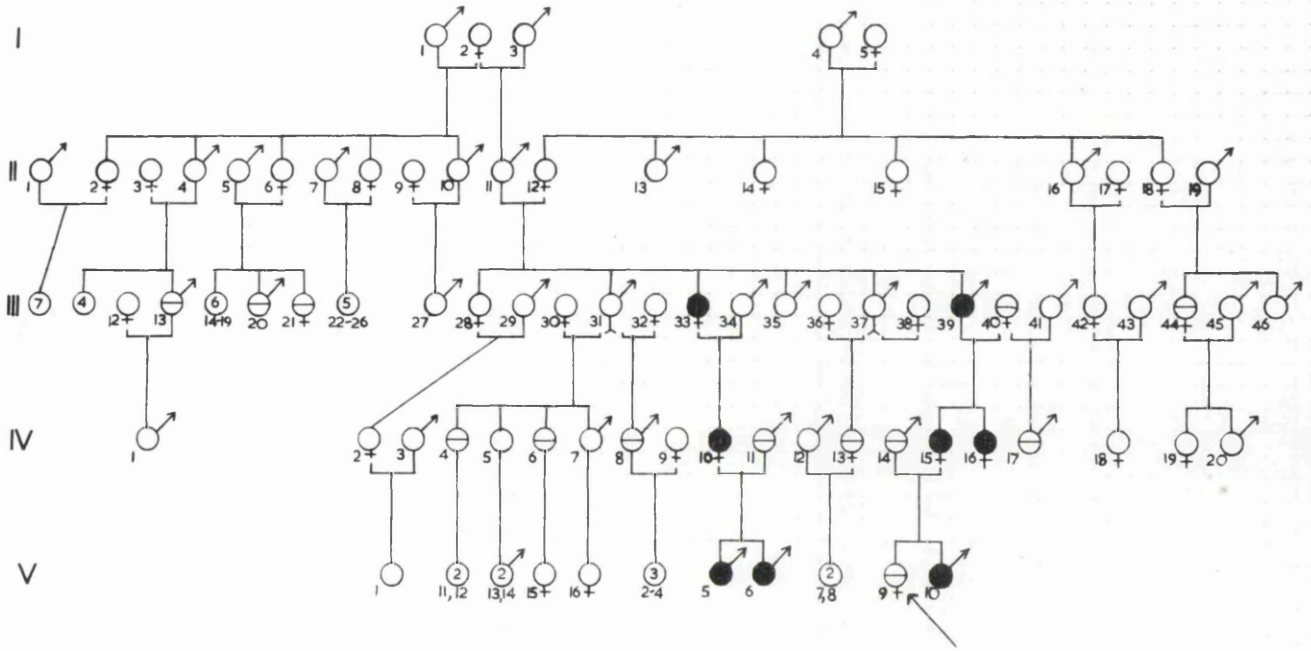
Propositus

Death in infancy

Miscarriage



'Marker' investigations indicate extra marital offspring



Fig, 7.6 Pedigree V21AN.

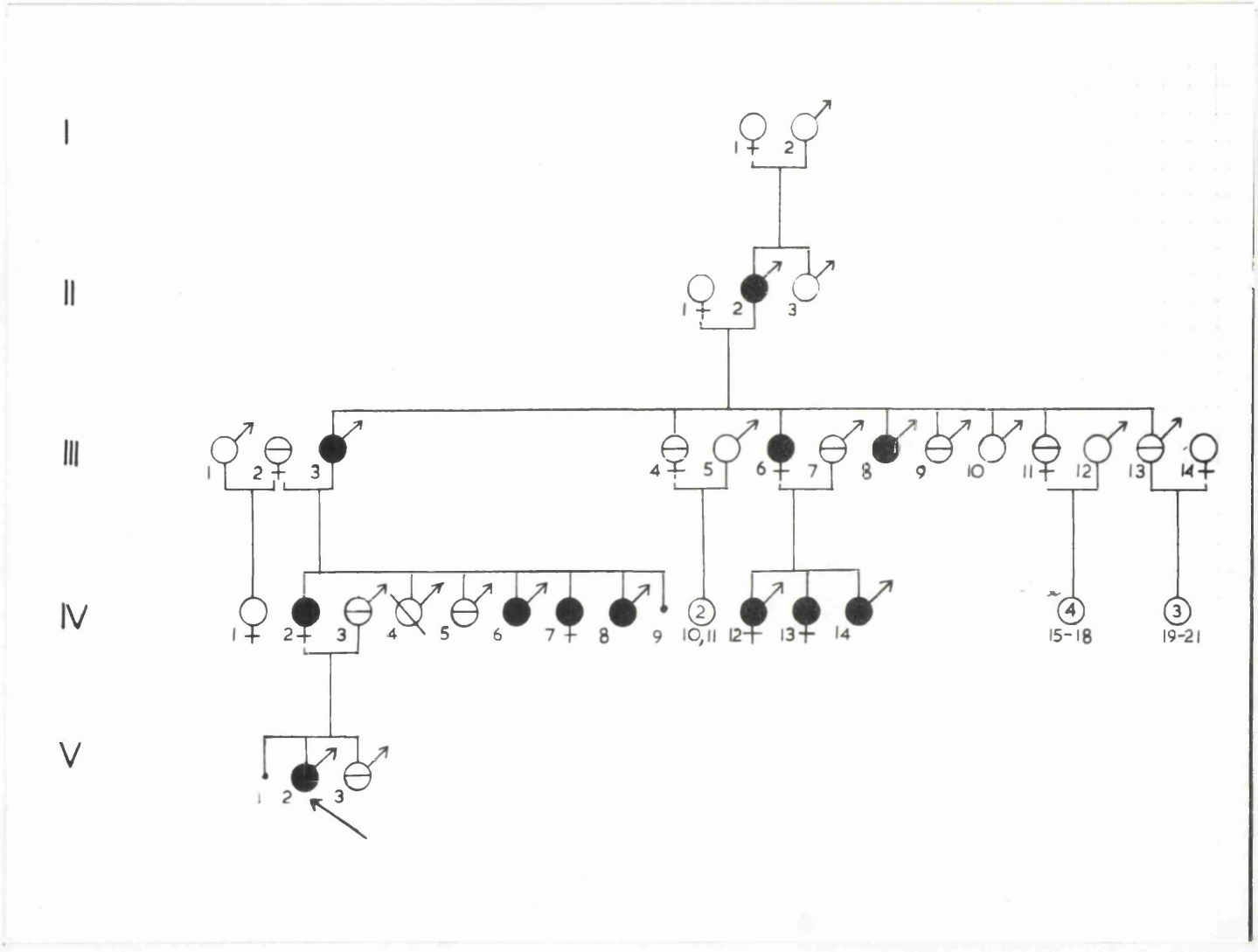


Fig. 7.7 Pedigree JM1MY.

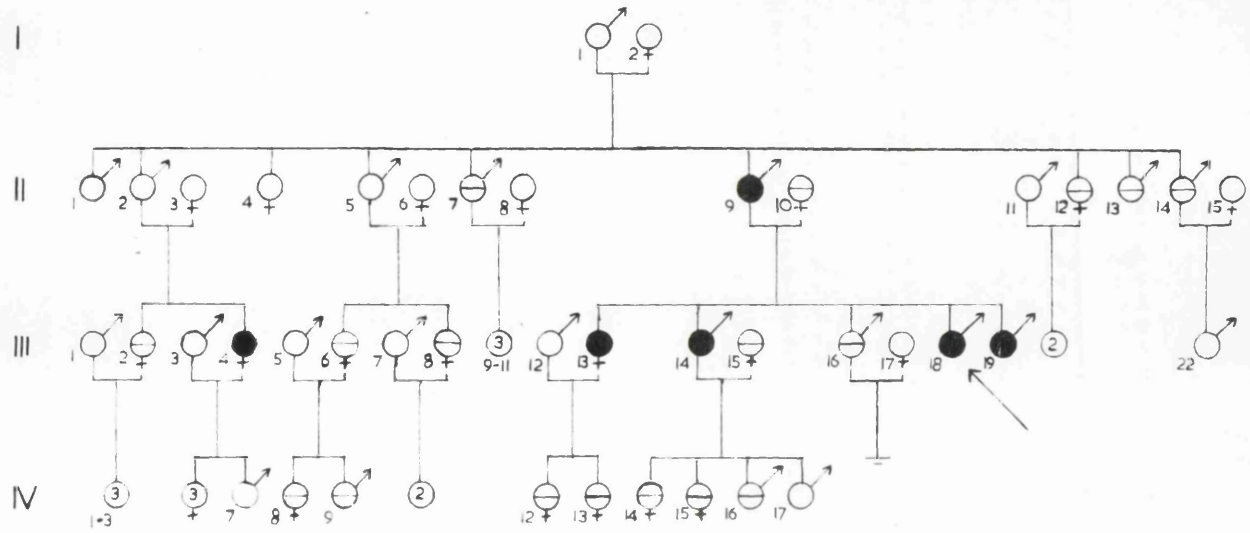


Fig. 7.8 Pedigree JN1AN.

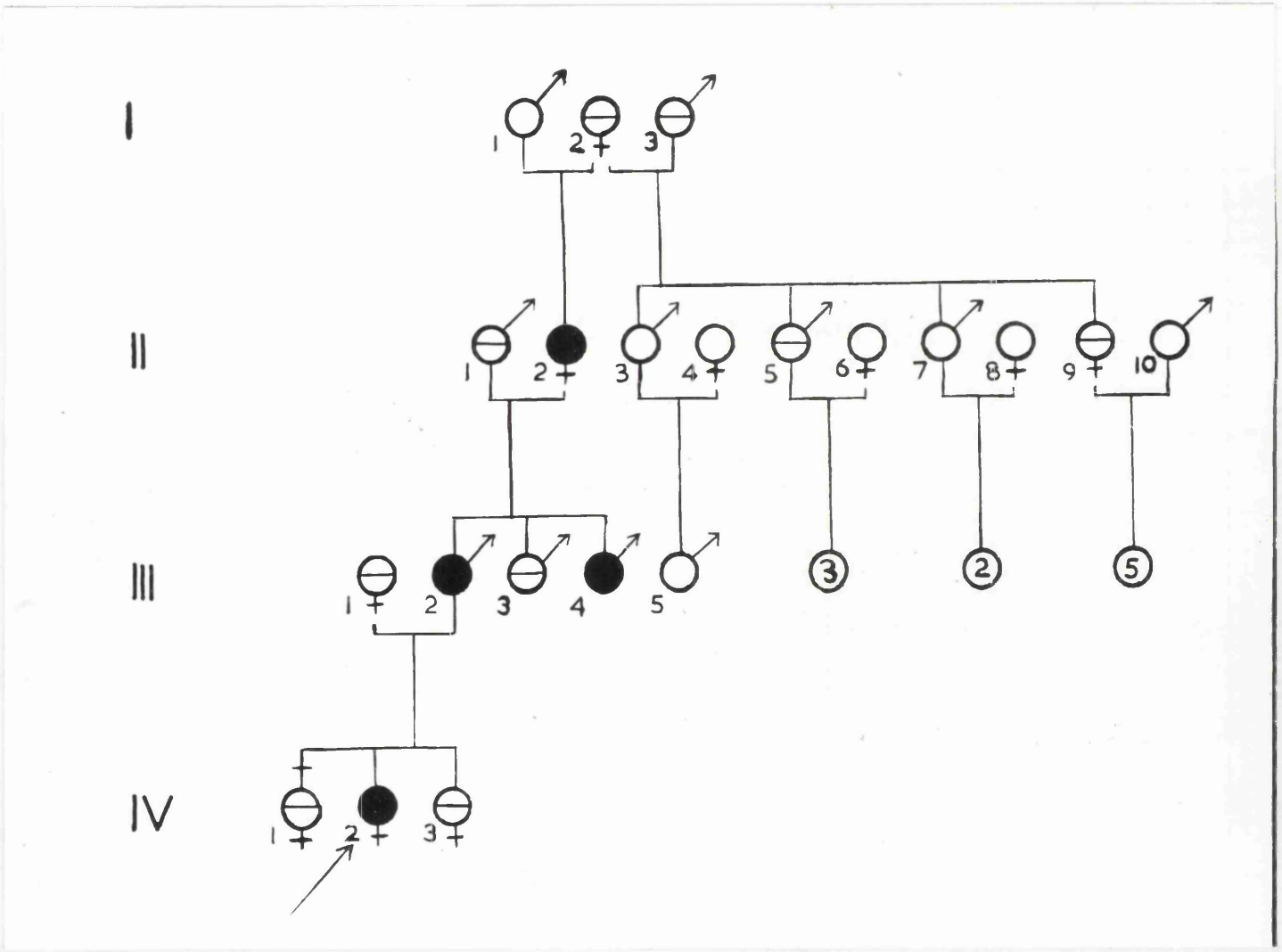


Fig. 7.9 Pedigree JN1PW.

Table 7.9(a) Pedigree data.

Pedigree	Number of generations	Number of single backcross matings	Number of tested children
V21AN	5	4	7
JM1MY	5	4	17
JN1AN	4	4	13
JN1PW	4	2	5

Table 7.9(b) Segregation ratios among tested children of matings of heterozygotes with normal homozygotes.

Pedigree	Marker chromosome	Number of heterozygotes	Number of normals	X^2 for 1:1 segregation
V21AN	2 p+ q-	6	1	
JM1MY	16 p+	11	6	$X^2=1.4$ P=0.3
JN1AN JN1PW	17 s+	8	10	$X^2=0.22$ P=0.7

in pedigree V21AN are few. The counts from pedigrees JN1AN and JN1PW have been added together. For the long variant of chromosome no. 16 (16q+) and the satellited chromosome no. 17 (17S+) a 1:1 segregation of heterozygotes to normals has been demonstrated.

CHAPTER VIII.

ANALYSIS OF RESULTS

A. Illustration of Computation of Linkage Probabilities.

B. Final Probability that a Locus is on a Marker Chromosome.

CHAPTER VIII.

A. Illustration of three methods for the computation of lods using the ABO locus and the heteromorphism of pedigree JN1PW.

The phenotypes at the ABO locus and the segregation of the satellited chromosome no. 17 are given in Fig. 8.1. The phenotype-genotype relationships at this locus are in Table 6.3.

I.1 is unavailable for study. Barring new mutation he can be assumed to be heterozygous for the satellited chromosome no. 17. The genotype of II.2 must be $\underline{A_2O}$, that of III.2 $\underline{A_2O}$ and that of III.4 $\underline{BO}$.

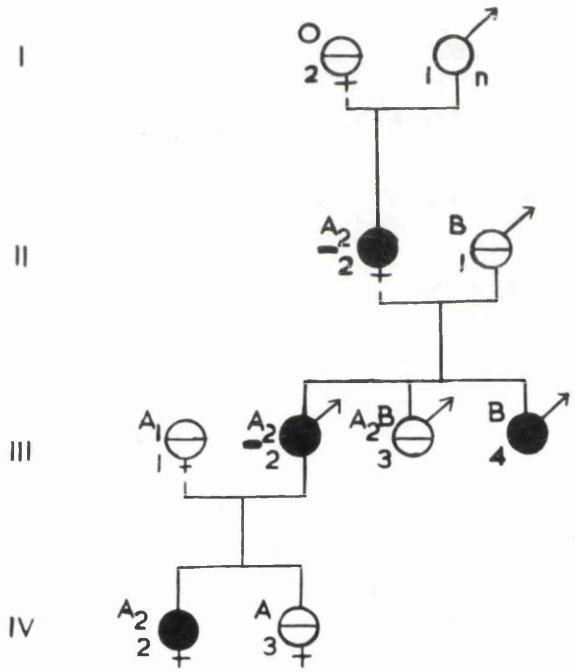
The coupling phases of II.2 and III.2 are $\underline{A_2}O$, where the underscoring indicates the allele in coupling with the locus controlling the heteromorphism.

Method a. Simple Count.

With the coupling phase known to be $\underline{A_2}O$, a count of the recombinants and non-recombinants in Gen. III can be made. There are 2 recombinants and 1 non-recombinant and the likelihood of such a family is proportional to $\left(\frac{\theta}{2}\right)^2 \cdot \left(\frac{1-\theta}{2}\right)$

Fig. 8.1

The ABO phenotypes of Pedigree JN1PW.



K E Y

Heterozygous at main (JN1) locus.

Homozygous at main (JN1) locus.

Not tested.

No information can be obtained from Gen. IV for, although the coupling phase of III.2 is known, there are alternative genotypes for III.1, IV.2 and IV.3. Only certainties can be counted.

The likelihood of the pedigree JN1PW at the ABO locus is therefore , $k\left(\frac{\theta}{2}\right)^2 \cdot \left(\frac{1-\theta}{2}\right)$ where k is a constant.

The lod scores for a family of such likelihood for different values of θ have been calculated using the formula given in Chapter II and are given in Table 8.1(a).

Method b. Use of Morton's lod score (z) Tables.

The tables given in Maynard Smith et al (1961) can be used. Considering the locus controlling the presence or absence of the satellite on chromosome no. 17 as G and the ABO locus as T, the information in pedigree JN1PW at the ABO locus in Gen. III could be scored as mating type $\left[22 \times 1111\right]$, i.e. $Gg\ T_1\ T_2 \times gg\ T_3\ T_4$ and could be scored by z tables if the coupling phase were unknown. But, as before, I.1 is assumed to be heterozygous at the main locus and the coupling phase of II.2 is therefore known to be $\underline{A_2}O$. Table 51(a) of Maynard Smith et al (1961) gives the scores for the offspring

Table 8.1(a) Lod scores by count

θ	$P(F\|\theta) \propto \theta^2.(1-\theta)$	$\text{Log}_{10} P\, F\|\theta$ $2 \log_{10}\theta + \log_{10}(1-\theta)$	Lod Score $\text{Log}_{10}P(F\|\theta) - \log_{10}P(F\|\theta = 0.5)$
0.5	$0.5^2 \times 0.5$	$\bar{1}.0970$	0
0.4	$0.4^2 \times 0.6$	$\bar{2}.9824$	-0.1146
0.3	$0.3^2 \times 0.7$	$\bar{2}.7993$	-0.2977
0.2	$0.2^2 \times 0.8$	$\bar{2}.5051$	-0.5919
0.1	$0.1^2 \times 0.9$	$\bar{3}.9542$	-1.1428
0.05	$0.05^2 \times 0.95$	$\bar{5}.3757$	-1.7213
0.	0×1	$-\infty$	$-\infty$

Table 8.1(b) Lod scores by Morton's Tables.

	θ	θ^2	$1-\theta$	Lod score $\theta\,(1-\theta)$
0.5	0	0	0	0
0.4	-0.097	-0.194	0.079	-0.115
0.3	-0.222	-0.444	0.146	-0.298
0.2	-0.398	-0.796	0.204	-0.592
0.1	-0.699	-1.398	0.255	-1.143
0.05	-1.000	-2.000	0.279	-1.721
0	$-\infty$	$-\infty$	0.301	$-\infty$

of II.1 and II.2 where the likelihood is k $\left(\frac{\theta}{2}\right)^2 \cdot \left(\frac{1-\theta}{2}\right)$ as in Table 8.1(b). Values for $\theta = 0$ have been included.

Morton's tables cannot readily be used for obtaining information from Gen. IV because two alternative genotypes $\underline{A_1A_2}$, $\underline{A_1O}$ have to be considered for III.1.

Method c. Hand and computer likelihood.

Information from Gen. IV can be included when the likelihoods are calculated in full, i.e. by hand or computer analysis. The two alternative genotypes $\underline{A_1O}$ and $\underline{A_1A_2}$ can be considered for III.1, weighted by the appropriate genotype frequencies. In IV.2, two alternative genotypes can be considered. IV.3, although typing as A_2 has been considered as being of phenotype A because of her age (Race and Sanger, 1958). The alternative genotypes for IV.3 are therefore, $\underline{A_1O}$, $\underline{A_1A_2}$, $\underline{A_2O}$ and $\underline{A_2A_2}$. Using Scottish genotype frequencies the total likelihood for pedigree JN1PW at the ABO locus can be set out as follows:

TOTAL LIKELIHOOD

$\underbrace{\text{III. 3.} \quad \text{IV. 4.} \quad \text{III. 2.}}_{k}$ III. 1

	Genotype frequency		
$\underline{A_1O}$	0.204	$\underline{A_2O}\left[\left(\frac{1-\theta}{2}\right) \cdot \frac{1}{2}\right]$	$\underline{A_2O}\left[\frac{\theta}{2} \cdot \frac{1}{2}\right]$
		$\underline{A_2O}\left[0\right]$	$\underline{A_1A_2}\left[\frac{\theta}{2} \cdot \frac{1}{2}\right]$
			$\underline{A_1O}\left[\left(\frac{1-\theta}{2}\right) \cdot \frac{1}{2}\right]$
$\underline{A_1A_2}$	0.0168	$\underline{A_2O}\left[\left(\frac{1-\theta}{2}\right) \cdot \frac{1}{2}\right]$	$\underline{A_2O}\left[\left(\frac{1-\theta}{2}\right) \cdot \frac{1}{2}\right]$
		$\underline{A_2O}\left[\frac{\theta}{2} \cdot \frac{1}{2}\right]$	$\underline{A_1A_2}\left[\frac{\theta}{2} \cdot \frac{1}{2}\right]$
			$\underline{A_1O}\left[\left(\frac{1-\theta}{2}\right) \cdot \frac{1}{2}\right]$
			$\underline{A_2A_2}\left[\frac{\theta}{2} \cdot \frac{1}{2}\right]$

i.e. TOTAL LIKELIHOOD

$$= \frac{k\,\theta^2.(1-\theta)}{8}\left[\left(0.204 \quad \frac{(1-\theta)}{4} \quad \frac{(\theta+1)}{4}\right) + \left(0.0168 \cdot \frac{1}{4} \cdot \frac{1}{2}\right)\right]$$

$$= k\,\theta^2.\frac{(1-\theta)}{2^3}\left[0.204 \quad \frac{(1-\theta^2)}{2^4} + \frac{0.0168}{2^3}\right]$$

$$= \frac{k\,\theta^2}{2^6}.(1-\theta)\left[0.102\,(1-\theta^2) + 0.0168\right]$$

The likelihoods and lod scores obtained with this formula are given in Table 8.2. The lod scores obtained from the computer analysis are also included for comparison.

The lod/θ curves for the lod scores obtained by the different methods are given in Fig. 8.2. Curve 1 represents the lod scores by computer or by hand and 2 the lod scores by count or by Morton's tables. The additional information obtained by the full likelihood is seen to be small in this case. It can be considerable.

The antilods (likelihood ratios) of the complete lod scores are given in Table 8.2. Fig. 8.3 is the likelihood/θ curve for the linkage between the ABO locus and the locus controlling the presence of the satellite. The area under the curve was measured by a planimeter. A rectangle with the same area was constructed on the same base as the curve. The height of this rectangle was 0.43 which is the average likelihood ratio and which agrees with the value of 0.46 for the average likelihood ratio obtained by the computer analysis.

B. The final probability that a marker locus is on a marker chromosome.

Table 8.3 gives the lod scores for each pair of loci for a series

Table 8.2 Lod scores by hand likelihood

θ	$P(F\ \theta)$	$\text{Log}_{10} P(F\ \theta)$	Lod score	Lod score (computer)	Antilod
0.5	0.0001822 k	$\bar{4}.2606$	0	0	1
0.4	0.0001537 k	$\bar{4}.1867$	-0.0739	-0.0739	0.8435
0.3	0.0001079 k	$\bar{4}.0330$	-0.2276	-0.2276	0.5921
0.2	0.00005736 k	$\bar{5}.7587$	-0.5019	-0.5020	0.3149
0.1	0.00001652 k	$\bar{5}.2180$	-1.0416	-1.0415	0.0909
0.05	0.000004399 k	$\bar{6}.6434$	-1.6172	-1.6172	0.0242
0	0	$-\infty$	$-\infty$	$-\infty$	

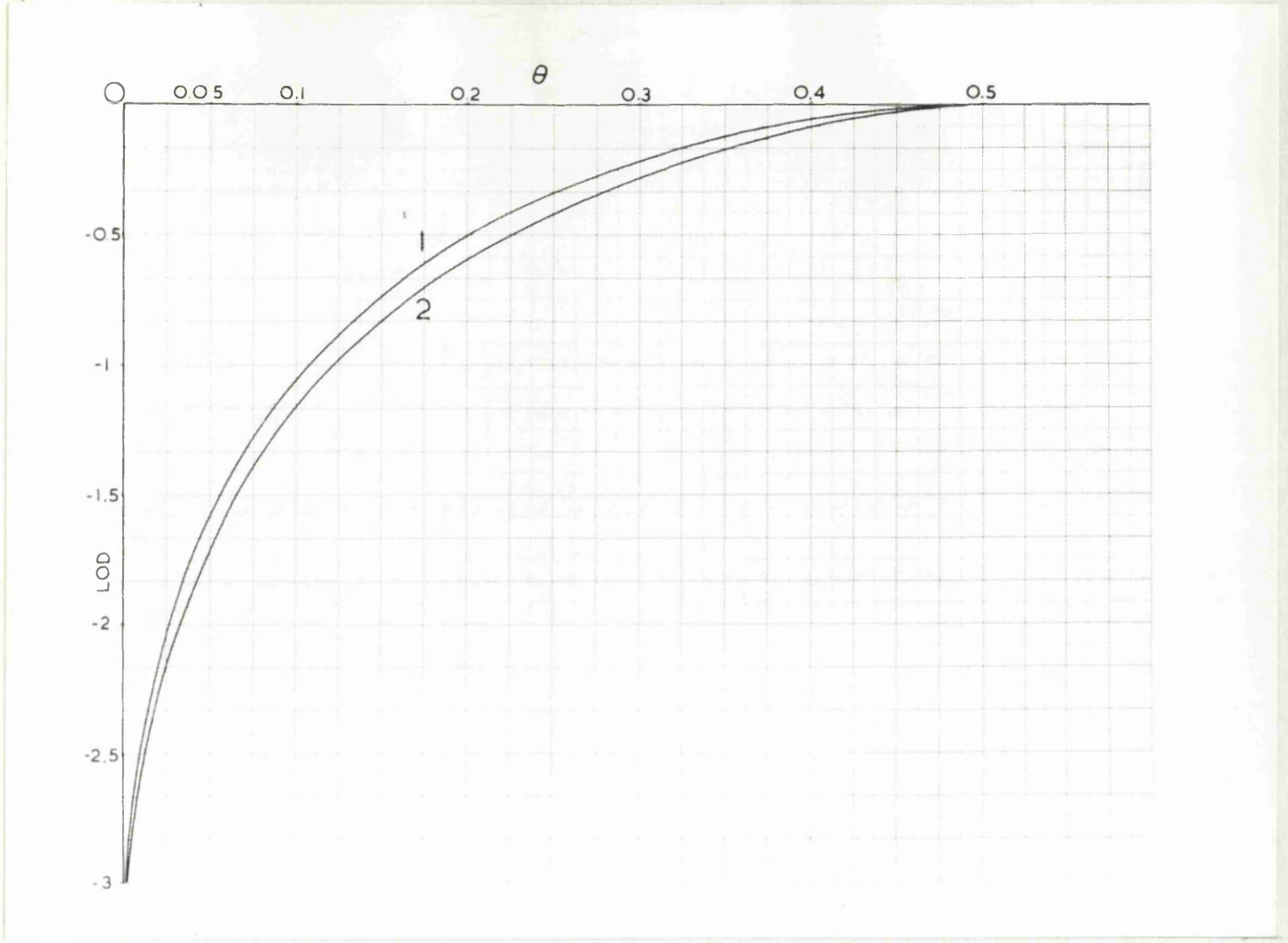


Fig. 8.2

LOD/θ Curve for linkage between the ABO locus and the locus controlling the satellite on chromosome No. 17 of pedigree JN1PW.

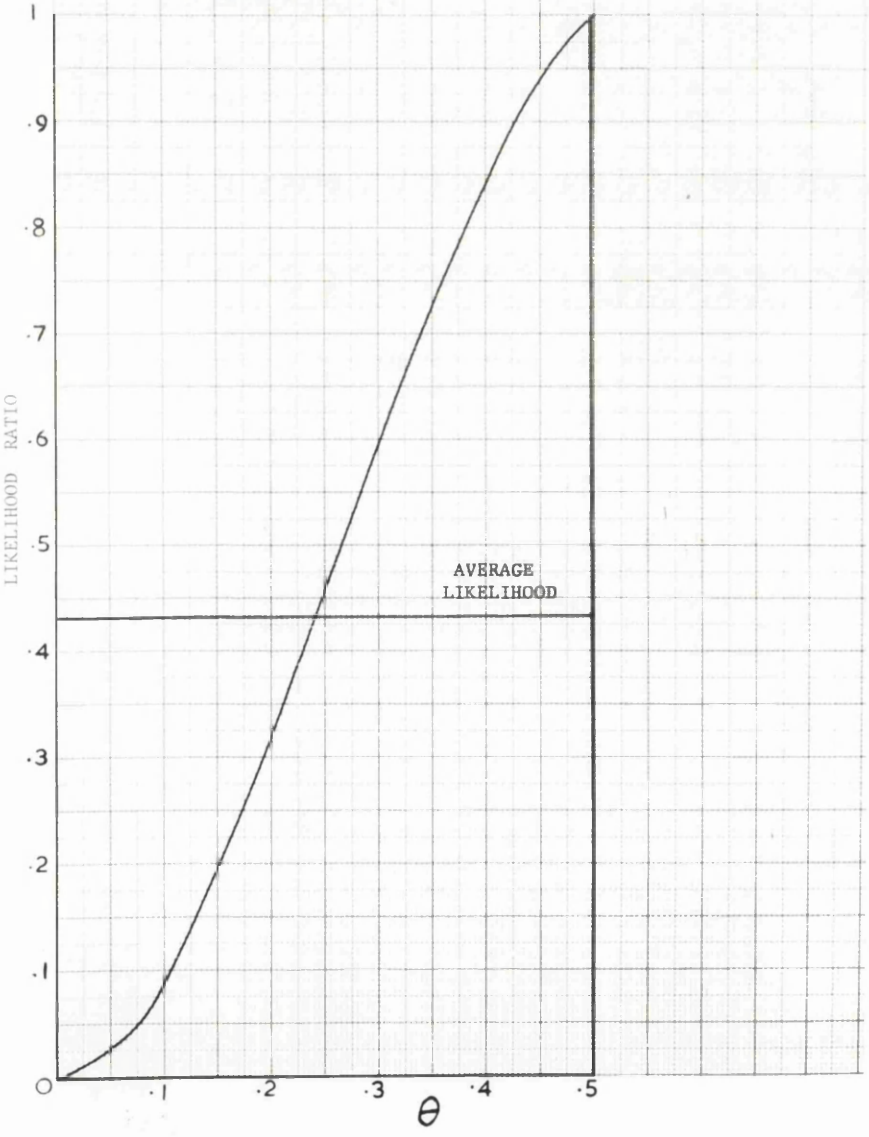
Curve 1. Lod score by computer or by hand.

2. Lod score by count or by Morton's Tables.

Fig. 8.3

Likelihood ratio/$_\theta$ curve for linkage between the ABO locus and the locus controlling the satellite on chromosome No. 17 of pedigree JN1PW.

The average likelihood = 0.432 has been obtained by planimetry.



of recombination fractions, θ from 0 to 0.5 as obtained by computer analysis of pedigree V21AN. These will be used to illustrate the extraction of the probability of a marker locus being on a marker chromosome.

Except for the linked pairs ABO:AK, Lutheran:Secretor, Transferrin:E_1, previous evidence for or against linkage between the marker loci has been ignored. The prior odds of a marker being on chromosome no. 2 are treated as if independent of the odds for the other markers. As discussed in the next Chapter, Fy is believed to be on chromosome no. 1 and Hp_α on chromosome no. 13, so these received a prior probability of 0 of being on chromosome no. 2. Of the other markers, 14 were informative.

For each marker Table 8.3 gives Λ, the average likelihood ratio (or average antilod), over the whole range of possible recombination values. Line (a) in Table 8.3 gives a priori odds that each marker is on chromosome no. 2, namely 4:43 based on the proportion of the complement represented by chromosome no. 2. The final odds that a particular marker locus is on chromosome no. 2 are (Λ:1) (4:43) are in line (b). Thus the final probability is given by the ratio $\frac{4\Lambda}{4\Lambda + 43}$.

Key to symbols used in Tables 8.3, 8.4 and 8.5.

Column.	Loci.
7.	ABO.
8.	MNS.
9.	P.
10.	Rhesus.
11.	Lutheran.
12.	Kell.
13.	Lewis.
14.	Duffy.
15.	Kidd.
16.	ABH secretor.
17.	Transferrin.
18.	Haptoglobin.
19.	Gm gammaglobulin.
20.	Gc protein.
21.	Cholinesterase E_2.
22.	Cholinesterase E_1.
23.	Acid phosphatase.
24.	Phosphoglucomutase PGM_1.
25.	6-phosphogluconate dehydrogenase.
26.	Inv gammaglobulin.
27.	Adenylate kinase.
28.	Lp lipo-protein.
29.	Ag lipo-protein.

Table 8.3 Lod scores for Pedigree V21AN with respect to possible linkage of one of the marker loci to a break-point of the inversion in chromosome 2.

Recombination fraction θ	7 ABO	8 MNS	9 P	10 Rh	11 Lu	12 K	14 Fy	15 Jk	16 Sec	18 Hpα	19 Gm	20 Gc	21 E_2	23 AcP	24 PGM_1	27 AK	28 Lp	29 Ag
0	0.043	-∞	-0.457	-∞	0	-0.870	-1.350	0.322	0.201	0.212	-∞	-∞	-0.153	0.078	0.685	0	-0.938	0.089
0.05	0.035	-0.705	-0.270	-1.130	0	-0.580	-0.069	0.272	0.170	0.306	-0.875	-0.729	-0.131	0.065	0.599	0	-0.644	0.072
0.1	0.028	-0.417	-0.141	-0.931	0	-0.444	0.099	0.227	0.143	0.350	-0.557	-0.450	-0.110	0.053	0.518	0	-0.434	0.057
0.2	0.015	-0.146	-0.084	-0.521	0	-0.194	0.155	0.149	0.098	0.314	-0.249	-0.198	-0.067	0.031	0.372	0	-0.185	0.036
0.3	0.007	-0.031	-0.006	-0.273	0	-0.076	0.104	0.086	0.054	0.213	-0.098	-0.077	-0.032	0.015	0.242	0	-0.069	0.022
0.4	0.002	-0.001	-0.016	-0.109	0	-0.018	0.038	0.036	0.022	0.099	-0.023	-0.018	-0.009	0.004	0.122	0	-0.017	0.010
0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Average likelihood ratio Λ:1	1.03:1	0.71:1	0.85:1	0.44:1	1:1	0.64:1	1.15:1	1.38:1	1.22:1	1.71:1	0.62:1	0.66:1	0.65:1	1.07:1	0.23:1	1:1	0.68:1	1.08:1
(a) Apriori odds on linkage = $l_2 : L - l_2$ = 4:43	4:43	4:43	4:43	4:43	see Sec	4:43	*0	4:43	4:43	†0	4:43	4:43	4:43	4:43	4:43	see ABO	4:43	4:43
(b) Probability that marker is on No. 2 = 4Λ/(4Λ+43)	.087	.062	.073	.039		.056	0	.110	.102	0	.055	.058	.057	.091	.021	.	.059	.091
(c) Probability of marker not being on No. 2	.913	.938	.927	.961		.944	1.0	.890	.898	1.0	.945	.942	.943	.909	.979		.941	.909

Probability that no marker is on No. 2 = 0.37

Probability that at least 1 marker is on No. 2 (initial prob. was $1-\left(\frac{39}{43}\right)^{14} = 0.71$) = 0.63

* Fy is now believed (P = 0.99) to be on chromosome No. 1

† Hp_{α} is believed (P = 0.99) to be on No. 13

[The Pedigree V21AN gave no information with respect to Lu, Le, E_1, 6PGD, Inv, AK or Tf loci.

Table 8.4 Lod scores for Pedigree JM1MY with respect to possible linkage between one of the marker loci and the locus controlling the anomalous appearance of chromosome no. 16.

Recombination fraction θ	7 ABO	8 MNS	9 P	10 Rh	11 Lu	13 Le	14 Fy	15 Jk	16 Sec	18 Hp_{α}	19 Gm	20 Gc	23 AcP	27 AK	28 Lp
0	0	-∞	-∞	-∞	0	-0.008	-∞	-∞	-∞	-0.865	-∞	-∞	-∞	-∞	-∞
0.05	0	-3.885	-0.832	-3.792	0	-0.007	-1.479	-0.454	-0.975	-0.186	-0.721	-0.906	-0.721	-3.871	-0.206
0.1	0	-2.474	-0.532	-2.197	0	-0.005	-0.763	-0.216	-0.639	0.022	-0.443	-0.418	-0.443	-2.467	-0.131
0.2	0	-1.117	-0.244	-0.845	0	-0.003	-0.154	-0.038	-0.291	0.124	-0.194	-0.055	-0.194	-1.171	-0.486
0.3	0	-0.525	-0.098	-0.284	0	-0.001	-0.072	0.016	-0.109	0.095	-0.076	0.042	-0.076	-0.524	-0.134
0.4	0	-0.168	-0.023	-0.057	0	-0.000	0.107	0.215	-0.020	0.031	-0.018	0.030	-0.018	-0.168	-0.002
0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Average likelihood ratio Λ:1	1	0.31:1	0.63:1	0.41:1	1	0.99:1	0.77:1	0.84:1	0.60:1	1.07:1	0.67:1	0.79:1	0.67:1	0.31:1	0.87:1
(a) A priori odds on linkage $= l_{16}:L-l_{16} = 1:30$	See AK	1:30	1:30	1:30	See Sec	1:30	*0	1:30	1:30	† 0	1:30	1:30	1:30	1:30	1:30
(b) Probability that marker is on No. 16 $= \Lambda / \Lambda + 30$	See AK	0.010	0.021	0.013	See Sec	0.032	0	0.026	0.020	0	0.022	0.025	0.022	0.010	0.027
(c) Probability that marker is not No. 16		0.990	0.979	0.987		0.968	1.0	0.974	0.980	1.0	0.978	0.975	0.978	0.990	0.973
Probability that no marker is on No. 16 = $\prod$ (previous line)									0.79						
Probability that at least one marker is on No. 16 = 1-(previous line) (initial prob. was $1 - (\frac{30}{31})^{11} = 0.30$)									0.21						

* Fy is now believed to be on No. 1 (P = 0.99)

† Hp_{α} is now believed to be on No. 13 (P = 0.99)

The pedigrees gave no linkage information with respect to K, E_2, E_1, PGM, 6PGD, Inv, Lu or Tf loci.

Table 8.5 Lod scores for Pedigree JN1AN followed by those for JN1PW. Subsequent calculations are based on the sums of the lods for JN1AN and JN1PW with respect to possible linkage of one of the marker loci with the locus controlling the satellite on chromosome no. 17.

Recombination fraction θ	7 ABO	8 MNS	9 P	10 Rh	11 Lu	13 Le	14 Fy	15 Jk	16 Sec	18 Hp_{α}	19 Gm	20 Gc	22 E_1	23 AcP	24 PGM_1	26 Inv	27 AK	28 Lp	29 Ag
0	-0.011	1.023	-0.428	0.602			0.585	0.427	-0.454	-∞	-∞	-∞	-0.488	-0.301	-∞	-0.352		-∞	-∞
0.05	-0.005	1.161	-0.385	0.561	0	0	0.549	0.346	-0.294	-0.237	-0.787	-0.299	-0.199	-0.275	-0.221	-0.337	0	-5.466	-2.062
0.1	-0.001	1.150	-0.317	0.530	0	0	0.509	0.271	-0.201	-0.022	-0.498	-0.088	-0.068	-0.229	-0.050	-0.300	0	-3.685	-1.441
0.2	0.002	0.968	-0.179	0.462	0	0	0.413	0.142	-0.094	0.091	-0.227	-0.032	-0.031	-0.132	0.107	-0.190	0	-1.892	-0.805
0.3	0.002	0.685	-0.078	0.360	0	0	0.296	0.055	-0.037	0.070	-0.092	0.035	-0.037	-0.058	0.082	-0.087	0	-0.928	-0.430
0.4	0.001	0.355	-0.019	0.211	0	0	0.160	0.011	-0.008	0.021	-0.022	0.012	-0.014	-0.014	0.025	-0.021	0	-0.344	-0.177
0.5	0	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0
0	-∞	-0.825	-∞	-∞		-0.010	0	-0.052	-0.048	-∞	-∞			-0.175				-∞	
0.05	-1.617	-0.442	-0.740	-3.721	0	-0.009	0	-0.033	-0.024	-1.000	-1.000	0	0	-0.155	0	0	0	-0.043	
0.1	-1.042	-0.188	-0.459	-2.540	0	-0.007	0	-0.028	-0.019	-0.699	-0.699	0	0	-0.135	0	0	0	-0.038	
0.2	-0.502	-0.010	-0.203	-1.139	0	-0.004	0	-0.019	-0.011	-0.398	-0.398	0	0	-0.097	0	0	0	-0.028	
0.3	-0.228	0.070	-0.081	-0.741	0	-0.002	0	-0.110	-0.005	-0.222	-0.222	0	0	-0.062	0	0	0	-0.019	
0.4	-0.074	0.061	-0.020	-0.308	0	-0.001	0	-0.005	-0.001	-0.097	-0.097	0	0	-0.030	0	0	0	-0.009	
0.5	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	
Average likelihood ratio Λ:1	0.47:1	0.57:1	0.54:1	0.37:1	1:1	0.99:1	0.23:1	1.37:1	0.78:1	0.54:1	0.41:1	0.92:1	0.95:1	0.67:1	1.03:1	0.73:1	1:1	0.20:1	0.34:1
(a) A priori odds on linkage = l_{17}:$(L-l_{17})$	1:31	1:31	1:31	1:31	see Sec	1:31	*0	1:31	1:31	†0	1:31	1:31	1:31	1:31	1:31	1:31	see ABO	1:31	1:31
(b) Probability that marker is on No. 17 = $\Lambda/(\Lambda + 31)$	0.015	0.018	0.017	0.012	see Sec	0.031	0	0.042	0.024	0	0.013	0.029	0.030	0.022	0.032	0.024	see ABO	0.006	0.011
(c) Probability that marker is not on No. 17	0.985	0.982	0.983	0.988		0.969	1.0	0.958	0.976	1.0	0.987	0.971	0.970	0.978	0.968	0.976		0.994	0.989
Probability that no marker is on No. 17 = $\prod$ (previous line) based on JN1AN, JN1PW										0.72									
Probability that at least one marker is on No. 17 (initial prob. was $1-(\frac{31}{32})^{15} = 0.38$)										0.28									

* Fy is believed to be on No. 1 (P = 0.99)

† Hp_{α} is believed to be on No. 13 (P = 0.99)

Neither JN1AN nor JN1PW gave linkage information with respect to the Lu, K, E_2, 6 PGD, AK or Tf loci.

The probability that none of the marker loci is on chromosome no. 2 can be calculated by multiplying the individual probabilities for each locus (line c). This probability is 0.37 leaving a probability of 0.63 that at least one marker is on chromosome no. 2, compared with an initial probability $1-(39/43)^{14} = 0.72$.

Table 8.4 similarly summarises the linkage data of pedigree JM1MY. Eleven markers (excluding Fy and Hp_{α} loci again) were informative and have been considered in this pedigree. As before the probability that at least one of these markers is on chromosome no. 16 is 0.21 compared with the initial probability of 0.31.

Table 8.5 gives the lod scores for the pedigrees JN1AN and JN1PW as obtained by computer analysis. As the marker chromosome appears identical in the two pedigrees the lod scores have been added together. It gives the average likelihood ratios for the combined scores. There were fifteen informative markers (excluding Fy and Hp_{α}) and the total probability that at least one of these is on chromosome no. 17 is 0.21 as compared with 0.38 initially.

CHAPTER IX.

DISCUSSION

An experimental study designed to seek a linkage between a locus or break point scored by cytology on a particular autosome and any one of 23 marker genes has been described. The nature of the information obtained from each analysis will now be discussed.

Pedigree V21AN autosome no. 2

After virus infection and after exposure to chemical mutagens breaks are common in chromosome no. 2. It is also one that is frequently eliminated after irradiation (Gripenberg, 1967). However, the aberrations of this autosome occurring in vivo are few. Table 9.1 gives all the reported cases of aberrations of autosome no. 2. To these must be added the suggestion that an isochromosome for the long arm of this autosome may be implicated in the abnormal phenotype of Waldenstrom's macroglobulinaemia (Page 34). In the aberrations tabulated, a ring chromosome, translocations and pericentric inversions appear. Of these only four translocations have been shown to be transmitted. Marker investigations have been carried out on all of

Table 9.1 Aberrations Involving Autosome no. 2.

Aberration	Familial Transmission	Marker Data	Authors
Ring chromosome	-	-	di Grado, Mendes and Schröder 1964
Pericentric Inversion	-	-	Carr 1962
			De Grouchy et al 1963
Translocations			
2/3	+	-	Lee, Rosenblum and Linsao 1964
2/B	+	+	Summitt 1966
2/C	+	-	Book, Santesson and Zetterquist 1961
			Hulten et al 1964
2/D	-	-	Mercer and Darakjian 1962
2/G	+	+	Lejeune, et al 1963
	-	-	Becker and Albert 1963
	-	-	Miller and Dill 1965

these (Lejeune, Lafourcade, Salmon and Turpin, 1963; Hulten et al, 1964; De Grouchy, 1965; and Summitt, 1966).

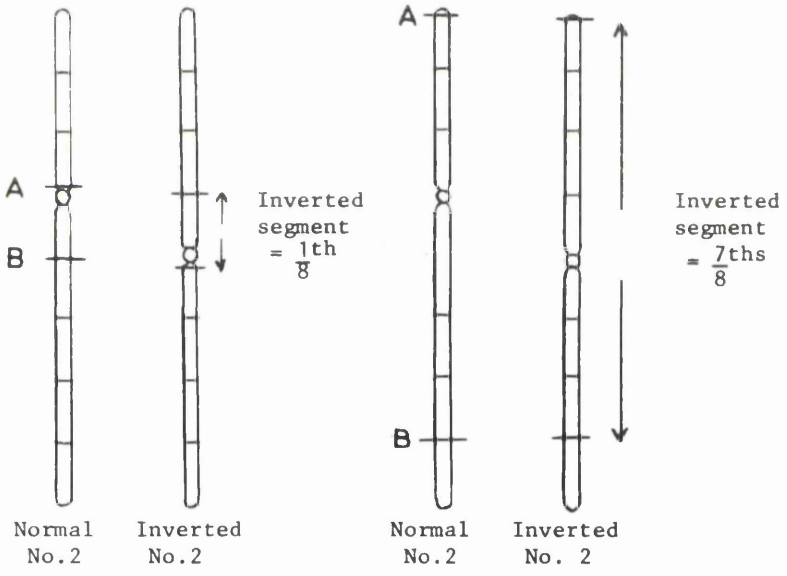
The heteromorphism for chromosome no. 2 in this study, where one chromosome of the pair is metacentric is presented as a pericentric inversion. Both chromosomes of the pair are of equal length (see Fig. 7.1) so that (a) trisomy for chromosome no. 1 or chromosome no. 3 and monosomy for chromosome no. 2, (b) a simple insertional translocation in a chromosome no. 2, and (c) an isochromosome of either arm of chromosome no. 2 need not be considered. The presence of a pericentric inversion has not been finally confirmed as meiotic studies have not yet been possible.

The inversion is carried in phenotypically normal males and females and transmitted to phenotypically normal male and female offspring. There is no history of abortions, nor was there concern about infertility among members of the pedigree. Whether the presence of the inversion in the mother IV.15 predisposed to non-disjunction and thus produced the G_1 trisomy in V.9 is uncertain. No other trisomic offspring from heterozygous parents occur in the pedigree.

Chromosome no. 2 is the second longest of the autosomes and represents 8.18% (3.15% + 5.03%) of the autosomal length (Table 1.1). The inversion chromosome is metacentric with a centromeric index of 50%. It is not possible to define the break points producing the inversion precisely. The size of the inverted segment will vary with the position of the breaks. The short arm constitutes $\frac{3}{8}$ths and the long arm $\frac{5}{8}$ths of the length of a normal chromosome no. 2. The centromeric shift in the inverted chromosome is $\frac{1}{8}$th of the length of the chromosome. Thus the shortest possible length of the inverted segment is $\frac{1}{8}$, and the greatest $(\frac{3}{8}^{th} + \frac{1}{8}^{th} + \frac{3}{8}^{th}) = \frac{7}{8}^{th}$ of the length of the chromosome (Fig. 9.2). Wherever the breaks occur, the proximal $\frac{1}{5}^{th}$ of the long arm will always be within the inverted segment and its distal $\frac{1}{5}^{th}$ outside.

The products of cross-overs in a heterozygote for a pericentric inversion have been analysed in detail on page

Fig. 9.2 Diagram to illustrate the possible extremes in size of the inverted segment of chromosome No. 2 in pedigree V21AN.



A = break on short arm

B = break on long arm

75. As seen, cross-overs outside the inversion loop lead to recombinant strands between loci placed outside the inversion loop and the 'inversion' locus, since the inverted or normal phenotype is scored in the analysis. With reference to loci placed on the inverted segment, a recovered strand can only be recombinant if it has experienced an odd number of cross-over events between the marker locus and one break point accompanied by an odd number of cross-over events between the same locus and the other break point.

If a low recombination fraction is found between a marker locus and the inversion locus eg. $\frac{\text{F.Inv.}}{\text{f.Nor.}}$ or $\frac{\text{B.Inv.}}{\text{b.Nor.}}$ (Fig. 4.4), it would mean that this locus is linked to one or other break point producing the inversion, and is either on the normal segment or on the inverted segment of the chromosome. If the locus is on the inverted segment the recombination fraction obtained would be smaller than if the same locus were tested with another marker at a similar distance away but on a normal chromosome segment.

In the linkage analysis using pedigree V21AN, linkage of one of the marker loci studied was sought with one or other break point producing the inversion.

The lod scores of pedigree V21AN are given in Table 8.3 and

give no strong evidence of linkage of a marker to a break point. There remains a probability of 0.63 that chromosome no. 2 carries at least one of the following: ABO, MNS, P, Rh, Jk, Se, K, Gm, Gc, E_2, AcP, PGM_1, Lp or Ag. The corresponding probability initially was 0.71, based on the length of chromosome no. 2.

Lejeune et al (1963) studied a family with a 2/22 translocation. No positive evidence of linkage with either break point producing the translocation, i.e. break point on chromosome no. 2 or on chromosome no. 22, was obtained by them. The marker investigations in a 2/B translocation reported by De Grouchy (1965) consist of only those for the propositus and the parents. No relevant information can be obtained. In the pedigree of a 2/3 translocation reported by Summitt (1966), in Gen. III out of a total of four offspring, only two, one normal and one a balanced translocation can be scored easily, the other two being products of duplication-deficient gametes. An informative mating at the Hp α locus does not favour linkage. This locus is believed to be on chromosome no. 13, anyway.

Hulten et al (1964) report the linkage analysis of a pedigree with a 2/C translocation. The presence of the translocation has been confirmed by the demonstration of a quadrivalent at meiosis.

No arm measurements are given but the break on chromosome no. 2 appears in the karyotype to be at the junction of the proximal $\frac{3}{4}$ with the distal $\frac{1}{4}$ of the short arm. In this family there was information at the Jk locus. There is a suggestion of linkage with the break points of the translocated chromosome, placing the Jk locus close to the break point on chromosome no. 2 or the break point of the C group chromosome involved in the translocation. The observational odds in favour of linkage are 7:1. The lod scores at the Jk locus of pedigree V21AN (Table 8.3) are also positive, observational odds in favour of linkage being 1.38:1. If the Jk locus was actually on chromosome no. 2 the combination of these two sets of odds 7:1 and 1.38:1 with the prior odds of 4:43 that the Jk locus is on chromosome no. 2,the final odds become 38.6:43; i.e. a probability of 0.47 compared with 0.09 initially.

Pedigree JM1MY, autosome no. 16.

In pedigree JM1MY, the heteromorphism is present in the autosome pair no. 16. This chromosome too, is frequently affected by breaks in its secondary constriction during virus infection and during chemical mutagenesis (Gripenberg, 1967). The variant

form of this chromosome has been described (page 48), and is the cause of the heteromorphism in this pedigree. Although the lengthening of the arm is in the region of the secondary constriction, the obvious increase in the amount of chromatin present is against the hypothesis that this represents an elongation of the secondary constriction. The only other explanation is a duplication or a balanced translocation. The secondary constriction is close to the centromere on the long arm. The centromere index is 37.7 (Table 1.1) and the lengths of the short arm 1.31% and of the long arm 1.98% of the total autosomal length. As described on page 73 linkage analysis will test for genes on either side of the anomalous appearance. The short arm represents 36 map units and the long arm 56 map units of a total autosomal length of 2,750 units (page 12).

Crawford, Punnett and Carpenter (1967) report a family in which a propositus had 46 chromosomes with a single heteromorphic pair. One chromosome of the pair (no. 16) was shorter than the other, and it was concluded that the long arm of chromosome no. 16 was deleted for a segment distal to the secondary constriction. From fifteen autosomal markers (not listed) they were able to

exclude MNS and Rh from the deleted segment. In the Duffy system the phenotype of the propositus was Fy (a+b-). From the Duffy alleles in the parents, her genotype should have been $\underline{Fy}^{a}\underline{Fy}^{b}$ or $\underline{Fy}^{b}\underline{Fy}^{b}$. There was no evidence from the other markers that the propositus was extra-marital and the authors concluded that the child carried a silent Duffy allele or was hemizygous at the Duffy locus.

The lod scores for the Duffy locus are negative in the pedigree JM1MY and give an average likelihood ratio of 0.77:1. Furthermore the Duffy locus has now been located with greater certainty (probability 0.99) on chromosome no. 1 (see page 147) (Donahue et al, 1968; Ives and Chown, unpublished) so the propositus of Crawford et al (1967) probably had a silent allele.

The family reported by Nuzzo et al (1966) shows segregation for a variant chromosome similar to that in pedigree JM1MY but the marker data from the mother and two sibs, are scanty and have not been subjected to linkage analysis.

The lod scores for pedigree JM1MY as obtained by computer analysis are given in Table 8.4. The evidence is scanty and does

not favour the localisation of MNS, P, Rh, Le, Fy, Jk, Se, Gm, Gc, AcP, AK, Lp on chromosome no. 16. There remains a probability of 0.21 that one or more of these is on this chromosome, compared with 0.30 initially.

Pedigrees JN1AN and JN1PW, autosome no. 17.

The heteromorphism present in these two pedigrees is that of a satellite on one of the chromosomes No. 17. As described earlier (page 47) several instances of the satellited chromosome no. 17 have been reported. Its expression is variable and extreme care has to be taken to avoid misclassification. It would be worthwhile in future investigations to try out special techniques such as those used for showing up secondary constrictions (Saksela and Moorhead, 1962; Sasaki and Makino, 1963), to make the heterozygote more conspicuous. The position of the satellite is well defined and linkage tests using its controlling locus could scan a considerable proportion of the chromosome which has an estimated map length of about 90 map units.

No marker investigations concerning chromosome no. 17 are available in the literature.

The site controlling the anomalous appearance of the chromosome no. 17 is assumed to be the same in both pedigrees JN1AN and JN1PW. Therefore their individual lod scores are added together to give a total lod score for both families, and further calculations have been made on the combined scores (Table 8.5). The combined data serve to reduce somewhat the probability that chromosome no. 17 carries ABO, MNS, P, Rh, Le, Jk, Se, Gm, Gc, E_1, AcP, PGM_1, Inv, Lp, Ag. There remains a probability of 0.28 that it carries one of these loci, compared with 0,38 initially.

Results from investigations on other autosomes.

Chromosome no. 1 and the Duffy locus.

Cooper and Hernits (1963) investigated a family in which a variant chromosome no. 1 was segregating. The matings were uninformative except at the Duffy locus, where a double backcross mating gave slightly positive lod scores. They suggested a hint that the Duffy locus was on chromosome no. 1, but the probability of this from their data was only 0.2. A family with an identical heteromorphic chromosome no. 1 investigated by Philip et al (1965) an intercross mating which also gave slightly positive lod scores at

this locus. The lod scores for the family with a pericentric inversion of chromosome no. 1 investigated by Lele et al (1965) are also positive.

The weak suggestion of linkage obtained from the pedigrees described above has been strengthened by the investigations on a large pedigree by Donahue, Bias, Renwick and McKusick (in preparation). The probability of linkage of the Duffy locus with the locus controlling the anomalous appearance of the variant chromosome no. 1 is now 0.9 and an unpublished large pedigree by Ives and Chown (personal communication) raises the probability to 0.99.

The C group chromosomes.

This group consists of seven pairs of autosomes which, by most workers, are classified together as pairs 6-12. If chromosomal aberrations or variations within this group are to be used as markers, it is imperative to attempt the morphological identification of these autosomes by number. The idiogram used in this study numbers these autosomes although admittedly in some cases the differentiation is difficult. The variant chromosomes that may be used from this group for future investigations could be chromosome no. 9 and chromosome no. 11.

Chromosome No. 6 and the Hageman factor.

De Grouchy, Veslot, Bonnette and Roidot (1968) describe a congenitally malformed child whose karyotype reveals a deletion of the short arm of a C group chromosome (most probably a chromosome no. 6). The karyotypes of the parents were normal. The assay of the coagulation factors expressed as % values gives a value of 126 and 160 for the Hageman factor (factor XII) for the parents, and only 40 for the child. They postulate that the gene for the Hageman factor is on the short arm of chromosome no. 6, the values obtained demonstrating the effect of a single dose in the child. If 99% reliability is assumed for the results of the test, P (error of classification in testing 3 persons) = 0.03., the prior odds in favour of the Hageman factor being on the deleted segment of chromosome no. 6 is 2.2:97.8, (based on the autosomal length represented by the deleted segment). The observational odds in favour are 0.97:0.03. Therefore the final odds that the Hageman factor is on the deleted segment of chromosome no. 6 are 2.12:2.93 i.e. a probability of 0.4 only.

Other investigations on the C group

The suggestion that the Jk locus could be on a member of the C

group (Hulten et al, 1964) has already been mentioned. If the Jk locus were actually on this chromosome the odds in favour of linkage are 7:1., combining this with the prior odds that the Jk locus is say, on chromosome no. 10, i.e. 5:95 the final odds in favour of linkage are 35:95., giving a probability of 0.27, compared with 0.05 initially.

Jacobs et al (1968) report the linkage analysis of three families carrying a pericentric inversion of a C group chromosome (most probably chromosome no. 10). The morphology of the chromosome carrying the inversion fixes one of the break points close to the centromere on one arm and the other at the terminal end of the other arm. Of the two possibilities either the short arm or the long arm is involved in the inversion and a large part of the other arm is outside the inverted segment. No evidence of linkage of any of the marker genes with the break points of the inversion was found even when the lod scores of all three families were combined. The long arm of chromosome no. 10 is about 85 map units and the short arm about 42 map units. Therefore the evidence from this analysis does not rule out the possibility that any of the markers used could be at the distal end of the long arm or short arm of the chromosome involved.

The D group chromosomes.

When aberrations of the D group chromosomes, which consist of three pairs 13, 14 and 15, are used in gene localisation, it is essential that an attempt be made to identify the chromosomes by number. Morphological identification by differences in size within the group is difficult. Use of autoradiography to study the labelling behaviour helps to distinguish between members of the group, see page 28.

The D group chromosomes are important in a discussion on autosomal gene localisation using chromosomal aberrations. Working with this group both qualitative and quantitative methods have been applied. Aberrations or variants in this group have also been used in linkage analysis. Most of the aberrations described in Chapter III have been used; viz. trisomy D_1, deletions of the short or long arms of D, ring D, enlarged satellites on D and translocations D/D and D/G.

Recognising the correlation in a propositus with retinoblastoma and a deletion in a D chromosome Lele et al (1963) postulated that the locus for retinoblastoma was at the distal end of the long arm of the D

chromosome. The significance to be attached to such correlations has been discussed (page 63).

Quantitative studies on D_1 trisomics.

Huehns, Hecht, Keil and Motulsky (1964) reported quantitative studies on seven D_1 trisomics. All these showed a persistence of a normal embryonic haemoglobin Hb Gower-2, and increased amounts of the haemoglobin γ_4. Hb Gower-2 was shown to be a tetramer with 2 normal α chains and 2 abnormal ϵ chains differing from the normal embryonic ϵ chains by more than one amino acid substitution. The presence of two prenatal haemoglobins in a new born trisomic could be due to immaturity. However no such increase has been observed in premature disomic infants. The variable increase of these haemoglobins could be due to a triplication of the structural loci for the γ and ϵ chains, but it is just as likely that the increase is an indirect effect of triplication of one or more other loci.

Linkage studies

Corcoran, Gerald, Diamond, Zergollern, Hoefnagel and Benirschke (1964) suggested linkage between the Gm locus and the break points in a D/D translocation analysed by them. The highest lod score was 0.418 at $\theta = 0.2$. No conclusions can be drawn without additional data.

The "D" chromosome and the haptoglobin locus.

Gerald et al (1964) reported the concurrence of the loss of an allele at the Hp_{α} locus and the loss of small segments of the terminal ends of a D chromosome resulting in the formation of a ring chromosome. More recently, Bloom, Gerald and Diamond (1966) studied the labelling pattern of the ring chromosome giving the anomalous inheritance at the Hp_{α} locus and defined it as D_1. They also reported a case of a B/D translocation apparently monosomic for a short segment of the short arm of D_1 but who was heterozygous at the Hp_{α} locus. De Grouchy, Salmon, Salmon and Maroteaux (1966) report a family with a short arm deletion of a D chromosome (which one not specified), in whom four carriers of the deletion typed Hp 21. Bias and Migeon (1967) report a pedigree in which a D_1 chromosome with a short arm deletion was segregating. Heterozygotes for the ABO, Rh, MNS, P, Kell, Kidd, Duffy, Lutheran, Lewis, Gm, Inv, Hp_{α}, Tf and Gc types were observed in the heterozygotes for the deletion, ruling out the possibility that the loci for these systems notably Hp_{α} are on the deleted segment of the D_1 chromosome which involves almost the whole of the short arm. Analysis for evidence of linkage of the Hp_{α}

locus with the break points of the deleted chromosome was also done, Renwick (personal communication). The average likelihood ratio was 0.20 and tends to make close linkage of the Hpα locus with the break points unlikely. The data do not rule out the possibility that the Hpα locus is at the distal end of the long arm of D_1 which is about 90 map units away.

As detailed on page 64 the odds in favour of the hypothesis that the Hpα locus is on the segment of chromosome no. 13 deleted with the formation of the ring chromosome, from the findings of Gerald et al (1967) are 12:1. Since the Hpα locus cannot be on the distal end of the short arm it must be on the distal end of the long arm. The findings of Cook, Gray, Robson and Brack (personal communication) who found a similar anomalous inheritance at the Hpα locus in a heterozygote for a deletion of the distal segment of a D chromosome strengthens the hypothesis.

The G group chromosomes.

The morphological identification of the two pairs of chromosomes of the G group nos. 21 and 22 is difficult. However, they can be distinguished by their labelling pattern on autoradiography. As with

the D group chromosomes, aberrations and variations of the G group trisomics, enlarged satellites, deletions and translocations have been studied both qualitatively and quantitatively.

Elmore et al (1966) described a family in which deletion of the short arm of a G group chromosome was segregating. The pedigree was ascertained through the propositus who presented with pycnodysostosis. It was postulated that the locus for pycnodysostosis is on the short arm of the deleted G chromosome (prior odds 1:250), the propositus carrying a single abnormal allele derived from a heterozygous mother. The father and the two other sibs carrying the deletion but not the abnormal phenotype are postulated to carry a normal allele on a normal G chromosome. As mentioned on page 63 this would be a concurrence only if it were possible to show that the mother alone was heterozygous for the pycnodysostosis allele. Otherwise these two observations may equally well be explained as the occurrence together of two unrelated rare events - a person being homozygous for a pycnodysostosis allele and carrying a deleted chromosome.

Quantitative studies

Studies using quantitative methods for the demonstration of the presence of a third allele in G_1 trisomics, whether in vivo or in vitro, are disappointing. The results from different workers are conflicting and difficult to interpret, and it seems unlikely that direct localisation of structural loci for the different enzymes would be possible by this method.

Shaw and Gershowitz (1962), comparing the ABO phenotypes of a population of G_1 trisomics with a control disomic population found a shortage of group O and an excess of group A and B. These results have not been confirmed.

Linkage studies

The linkage analysis of two pedigrees in which a G chromosome with enlarged satellites was segretating was reported by Cooper and Hirschorn (1962). No evidence of close linkage of the locus controlling the anomalous appearance with the marker loci used was found.

Shaw (1962), analysed two pedigrees, one with a D/21 and another with a 21/22 translocation and found a ratio of 5:14 of recombinants to non-recombinants between the ABO locus and the

break point of the G chromosome involved. This ratio does not differ significantly from that obtained with independent segregation. Hustinx (1966) analysed the ABO locus for linkage with the break points of the two translocations studied by him (families B and C), and those of Shaw (1962) and McIntyre, Staples, Steinberg and Hempel (1962) by the method of lod scores. He could find no evidence of linkage between the ABO locus and the break point on chromosome no. 21.

However, no claims that the ABO locus is on any other autosome have been made. It would therefore be worthwhile to continue the analysis of G_1 trisomic populations to demonstrate a phenotypic frequency shift, and also look for the critical mating between an AB mother and O father producing an AB G_1 trisomic offspring.

Conclusions.

The use of aberrant or variant chromosomes in linkage analysis is a reasonable approach to genetic analysis of the autosomes of man. However, as seen by the results of this study it is difficult to obtain conclusive evidence for or against linkage of a marker locus with a specified locus on a marker chromosome without a considerable amount of informative data. The success in the localisation of the

Duffy locus on chromosome no. 1 stresses the importance of studying large pedigrees and the presentation of linkage data in such a way that scores from different pedigrees can be added together. Even if no positive statement can be made about the presence of linkage, if the probability that each marker locus studied could be on the marker chromosome used is calculated the summated probabilities from different pedigrees can be used to strengthen any suggestion of linkage (or vice versa).

The first essential seems to be to mark each autosome by at least one reliable marker. At the present stage it is essential to use as many as possible of the markers that have been identified. It is likely that aberrations identified in somatic cells are on chromosomes that are less important genetically. Also the break-points producing the structurally rearranged aberrant chromosomes may vary from pedigree to pedigree. Although this will not interfere with the detection of linkage, such heterogeneity would render the estimation of recombination fractions more difficult. Variant chromosomes do not have this disadvantage. The identification of new variants will depend on the patient and detailed observations at the microscopic level on all individuals studied whether selected or in

random surveys. Techniques which would make variant chromosomes more conspicuous would help greatly.

Cytological analysis must in hopeful cases be extended to family studies and be accompanied by marker gene investigations. If 25% of the loci in man are polymorphic(Harris, 1966) the prospects for the identification of additional marker loci are good.

A uniform way of presenting linkage information should be adhered to. The method of analysis of the results used in this study seems adequate. It gives the markers used, the lod scores at the informative loci and the average likelihood ratios at each locus. Prior odds based on the lengths of the autosomes used can be incorporated. The final probability either for or against the hypothesis that any marker locus studied is on the marker chromosome can be deduced. If required each locus can be considered separately, or a final probability that at least one of the marker loci studied is on the marker chromosome can be given.

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