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GENETIC AND EPIDEMIOLOGICAL ASPECTS OF DIABETES MELLITUS

BY

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THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANTHROPOLOGY

UNIVERSITY OF DURHAM

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Abstract

Using an interview-questionnaire technique,the frequency of insulin-taking diabetics in the families of non-insulin-taking diabetics,and vice-versa,is compared with the frequencies in control families and in the general population. The results suggest that these two forms of diabetes are not genetically distinct. Early-onset, insulin-dependent diabetics show indications of dominant inheritance in their families more often than do diabetics of other types. Cardiovascular complications,at least in late-onset,insulin-taking diabetics,are found to occur more rapidly,and to be more severe,in individuals with a strong family history.

Diabetics and controls are examined for a range of red and white cell polymorphisms,serum proteins and isoenzymes. In some of these systems,the control group exhibits significant heterogeneity with regard to age. Comparison of the diabetic population with matched controls shows no significant associations except in the case of the ABO system,where older diabetics,especially males,show a higher frequency of group A_1,and in the HLA system,where insulin-taking diabetics show higher frequencies of the B8 and Bw15 alleles. It is suggested that the apparent association with ABO is the result of differential mortality amongst non-diabetics rather than any increased liability to the disease of group A_1 individuals. Associations with the HLA system show a relationship with age at diagnosis which may explain certain inconsistencies in previously published reports.

The prevalence of clinically diagnosed diabetes in the Durham area in 1975 exceeds that reported previously for other British populations. The presentation of new cases of insulin-requiring diabetics has a marked peak in February.

Preface

Diabetes mellitus is a group of disorders whose aetiology and inter-relationship are not fully understood. The work of this thesis was commenced with two major objectives - to employ pedigree information to clarify the genetic inter-relationship between various sub-types of the disease and,secondly,to explore associations between these and certain genetic polymorphisms taking full account of the influence of factors such as age and place of birth.

As so often happens in studies of diabetes,the major difficulties encountered were those of definition. The diabetic group is divided mainly by current treatment and,to a certain extent, this method of subdivision has been superseded by developments in the immunological study of these diseases over the past two years. Apart from being unavailable in this case,these immunological techniques are, at present,unsuitable for the classification of an existing diabetic population,although they have done much to increase our understanding.

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Chapter 1

The role of heredity in diabetes mellitus - a review of the literature.

Chapter 1

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1.1 Introduction.

The realisation that diabetes runs in families may be almost as ancient as awareness of the disease itself. Hindu physicians of the first century A.D. recognised the condition of mahumeha (honey urine),which seems likely to have been diabetes mellitus,and mention that it could be transmitted from father to son (see Simpson,1976,for translation).

Cammidge (1928) considers Rondolet,a sixteenth century physician,to have been the first Western author to suggest the hereditary transmission of diabetes. In the next century, Richard Morton,an English physician,reported a family with four out of seven sibs affected by the disease (Morton,1696).

Other early writers on the subject do not seem to have been impressed by familial aggregation. Thomas Willis, for example,in his volume 'Pharmaceutice Rationalis or an Exercitation of the Operations of Medicines in Human Bodies', published in 1679,attributes the disease to 'good fellowship and gusling down chiefly of unallayed wine' (extract in Major, 1959).

Writers of the present century were convinced of the importance of heredity in diabetes from their own observations. Heredity was,to Naunyn (1906) a 'red thread which is intertwined in diabetes from youth to old age'. Joslin (1947) wondered whether 'heredity would not be demonstrable in every case if we had the facts'.

This first chapter is a review of the extensive body of literature concerned with the speculation that diabetes,or a predisposition to diabetes,is inherited.

1.2 Proposed hereditary mechanisms for diabetes mellitus.

Estimates of the percentages of diabetics giving a positive family history of the disease were published by many physicians in the first three decades of this century. Wright (1931) provides a useful list of these papers. The first demonstration,however,that the relatives of diabetics were significantly more often affected than the relatives of non-diabetic controls was made by Gregory Pincus and Priscilla White in 1933. Although their conclusion that the disease was inherited as an autosomal recessive was not new,Pincus and White were the first to consider the question of the variable age at onset of the condition by the comparison of age-matched individuals.

The concept of a recessive mode of inheritance for diabetes was supported by several subsequent papers,including an important review by Steinberg (1959). Other publications advocated alternatives,however,and soon every known mode of inheritance had been proposed. Table 1.1 lists the most important of these publications.

(a) Recessive inheritance.

Not all diabetics have affected parents or affected children,though they may have affected grandparents or affected grandchildren. This phenomenon of the disease 'skipping a generation' suggested that diabetes was not due to a simple autosomal dominant gene and that it might be due to a recessive.

Assuming that the disease is due to a single recessive allele (m) segregating at an autosomal locus with

Table 1.1 Pedigree analysis in diabetes mellitus.

(a) Autosomal recessive inheritance

Allan (1933)
Pincus and White (1933)
Pincus and White (1934)
Steinberg and Wilder (1952)
Thompson and Watson (1952)
Grunnet (1957)
Post (1962)
Nilsson (1962)
Nilsson (1964)
Barrai and Cann (1965)
Steinberg (1968)

(b) Autosomal dominant inheritance

Levit and Pessikova (1934)
Burnstein and Patterson (1949)
von Kries (1953)
Vallance-Owen (1964)
Pavel and Pieptea (1966)
Roy et al (1966)
O'Brien et al (1967)

(c) Sex-linked inheritance

Penrose and Watson (1954)

(d) Autosomal co-dominant

Cammidge (1934)
Harris (1950)
Stimmler and Elliott (1964)
Ehrlich and Martin (1966)

(e) One major gene and non-allelic modifiers

Lamy et al (1961)

(f) Polygenic

Keen (1957)
Simpson (1964)
Thompson (1965)
Neel et al (1965)
Falconer (1967)
Smith et al (1972)

(g) Effect of a single dose of a gene which, in the homozygous state, gives a recognised, rare abnormality

Swift et al (1972)
Fraser (1973)

(h) Early-onset diabetes the result of a major gene, late-onset diabetes either unifactorial or multifactorial

Goodman and Chung (1975)

its normal counterpart (M), then all diabetic individuals must be genetically mm, and, discounting any mutational events of M to m, all diabetics have parents who are either Mm or mm. Thus, diabetics result from the mating Mm x Mm, where neither parent is affected, from the mating Mm x mm, where one parent is affected and from the mating mm x mm, where both parents are affected.

These three informative matings will produce different frequencies of affected sibs of a diabetic propositus and it was the analysis of these frequencies which formed the corner-stone of the argument for the recessive gene hypothesis.

Pincus and White (1933) included data from 523 families of diabetic propositi and from 153 controls ('non-diabetic patients'). The numbers of affected and unaffected relatives were ascertained by interview-questionnaire. The age at onset of the affected relatives and details of the current age or age at death of affected and unaffected relatives were also recorded. They compared the incidence of the disease by decade of current age (or age at death) in parents and sibs of the diabetic and of the non-diabetic propositi, finding that in no decade did the incidence of the disease in the relatives of the non-diabetics exceed that in the relatives of diabetics.

In testing the data against the predictions of a recessive allele hypothesis they ignored the only two families they had of the mm x mm mating and considered 440 sibships from the hypothetical Mm x Mm mating and 81 from the Mm x mm type. The possibility of mm parents being misclassified as Mm was mentioned but ignored in the analysis.

Since ascertainment was through an mm child, the

expectation of mm among the sibs of the propositus is -

$$\frac{p}{1 - (q)^n}$$

where p is the probability of an mm individual appearing (0.25 in the Mm x Mm mating), q is the probability of an mm individual not appearing (1 - p) and n is the number of children per family.

In all the various sized families the expected numbers of mm individuals, assuming a recessive allele hypothesis, may thus be calculated and, ignoring the propositi, the expected numbers of mm sibs may be alloted to decades according to the observed numbers of sibs present. By summing across the families the expected numbers of mm sibs in each decade may be obtained. These mm sibs, are, genetically, potential diabetics, in order to calculate the expected number manifesting the condition in each successive decade, age incidence tables from diabetic clinics and percentage survival tables for the general population were used.

Two assumptions were necessary for this calculation - firstly, that potential diabetics, before the development of the disease, are subject to the same chances of death as other members of the population and, secondly, that all mm individuals will manifest the condition by the end of the ninth decade.

By means of these calculations the authors derived expected numbers of identifiably diabetic siblings in each decade. Summation of these expected numbers over all decades produced close agreement with the numbers observed (64.98 expected affected siblings in the Mm x Mm mating compared

with 64 observed,and 32.85 expected affected siblings in the *Mm* x *mm* mating compared with 32 observed).

The authors expressed some surprise at this close agreement and were critical of the interview-questionnaire approach they had used. In a later report of the use of glucose tolerance tests in close relatives of diabetics,however,(Pincus and White,1934),they reiterated their belief that diabetes was recessively inherited,although they conceded that the results were in accordance with a dominant mode of inheritance with 'limited expression' of the gene.

Thompson and Watson (1952) also compared the ratio of affected offspring from *Mm* x *Mm* matings and *Mm* x *mm* matings and found good agreement with the 1:2 values predicted by the recessive allele hypothesis.

The review of the subject published by Steinberg in 1959 supported the concept of recessive inheritance and re-analysed the data of advocates of other modes of inheritance to show that they were not incompatible with this view. There are many criticisms which can be levelled against the recessive allele hypothesis,however,and it is no longer tenable.

Firstly,it is difficult to explain the difference between early-onset,insulin-requiring diabetes and late-onset, insulin-independent diabetes on the basis of their both being due to the same genotype,without introducing additional modifying factors,environmental or genetic. With the introduction of these factors the hypothesis moves away from the simple recessive gene idea.

In addition,although the majority of families do

not show transmission of the disease through consecutive generations,there are some published pedigrees with affected persons in three and four generations (Pavel and Pieptea,1966) and even in five generations (Burnstein and Patterson,1949). If these reports are to be believed then,clearly,simple recessive inheritance is unlikely or diabetes is a genetically heterogeneous group of disorders.

Thirdly,according to the recessive allele hypothesis, all the children of conjugal diabetics will be of the genotype mm so that they should all develop diabetes. They do not,however, a phenomenon Joslin attributed to their dying 'too soon of other causes' (Joslin,1947). Other authors (e.g. Nilsson,1962) concede that it is difficult to explain this deficit without invoking additional modifying mechanisms.

Post (1962) considered this unnecessary and that the observed number of affected progeny from such marriages was not significantly different from that expected when appropriate corrections were made for the age-structure of the group of offspring and for the probability of death before diagnosis. It has been observed (Neel,1976) that the numbers of offspring from these unusual matings is so small that it is difficult to distinguish with any accuracy one genetic hypothesis from another.

In some cases (Nilsson,1962;Barrai and Cann,1965) analysis of segregation data was considered to be compatible with more than one genetic hypothesis. Nilsson stated that 'the values fit in best with an autosomal recessive hypothesis, though the results of the analysis are also to a certain degree

compatible with a dominant gene of low penetrance'. Further investigation with a larger sample (Nilsson,1964) was unable to produce a firmer conclusion. Barai and Cann were able to fit the data they analysed (taken from Simpson,1962) to recessive and polygenic models.

(b) Dominant inheritance.

The advocates of dominant inheritance have the problem of explaining the large numbers of parents and children of diabetics who are not affected themselves. The only way to do this is to postulate a greatly reduced penetrance for the allele in question. Levit and Pessikova (1934) suggested that only 10% of individuals carrying the gene actually develop diabetes.

Once one resorts to this 'escape clause' (Edwards, 1969) the hypothesis is no longer one of a simple dominant gene. There are only two possible reasons for the reduction of penetrance - the action of the environment or the influence of other genes. In the case of the first,the mechanism is then multifactorial,with the second,a major gene with modifiers. At the level of penetrance proposed by Levit and Pessikova the hypothesis is indistinguishable from multifactorial inheritance.

In their analysis of family data von Kries (1953) and Levit and Pessikova (1934) considered dominant inheritance more likely than recessive because the prevalence of the disease among parents of the probands in their sample was as great as that amongst sibs.It was pointed out,however,(Steinberg, 1959) that the ages of these individuals had not been taken into account and that the frequency of affected siblings would

increase with time and be greater than that amongst the parental group when these two groups were comparable in terms of age.

The most appealing suggestion for the dominant inheritance of diabetes came with the description of 'synalbumin antagonist' (Vallance Owen,1964). Here was a single factor, dominantly inherited,which interfered with the physiological action of insulin and explained the pathogenesis of carbohydrate intolerance and its inheritance in an elegant manner. Unfortunately the identification of this insulin antagonist in established diabetics was not universally reproducible (Keen,1963;Cameron et al,1964 and Levin and Recant,1967) and synalbumin-positive individuals without diabetes have been reported (Levin and Recant,1967).

(c) Sex-linked inheritance.

In their analysis of the families of 500 diabetics, Penrose and Watson (1945) found a significant deficit of brother sister diabetic pairs compared with pairs of affected,like-sexed siblings. This finding prompted them to suggest a sex-linked element in the inheritance of the condition.

If diabetes were due to a major gene and this were present on the X chromosome,there would be no inheritance of the condition by a son from his father. This was not the case,raising the possibility that the major genetic determinant was autosomal but that sex-linked modifying genes were present. If part of the action of these modifiers was in terms of age at onset,this age should be more highly correlated in like-sexed parent-offspring combinations than in unlike-sexed parent-offspring combinations.

Penrose and Watson's initial report suggested this.

A further report from the same centre (Thompson and Watson,1952) analysing data on 1,631 families of diabetics which included the original 500,failed to confirm a significant deficit of like-sexed affected sib pairs,and the authors considered the small discrepancy with respect to sex was due to differential prevalence of the condition in the two sexes. The hypothesis of sex-linked inheritance of diabetes has,therefore,been abandoned.

(d) Co-dominant inheritance.

Following earlier suggestions (Cammidge 1928 and 1934),Harris (1950) tackled the problem of clinical heterogeneity in the disease by considering that the early-onset form of the disease (diagnosed before the age of thirty)was due to the presence of an abnormal allele in the homozygous state. The late-onset form of the disease was considered to be the result of the inheritance of this same allele with its normal counterpart. Harris studied family histories from 1,241 diabetics and found that 7% of the sibs of early-onset propositi had developed the disease by the time they were forty,whereas only 1.3% of the sibs of late-onset propositi had become diabetic by this age.

This hypothesis was supported by the finding of greater consanguinity among the parents of early-onset propositi than among the parents of late-onset propositi. A similar finding was reported by Thompson and Watson (1952) who found that 0.88% of the parents of early-onset diabetics were first cousins and 0.43% of the parents of late-onset propositi. The number of first cousin marriages in the whole sample was,however,

only nine.

It has been pointed out (Steinberg,1959) that, according to Harris' hypothesis,the disease should be more frequent among the parents of early-onset cases than among the parents of late-onset patients. This prediction was not supported in the analysis of the data of Steinberg and Wilder (1952) nor in the analysis of Harris' data by these authors.

Steinberg also points out (Steinberg,1959) that the frequencies of the affected sibs of diabetics should be greater when the disease in the propositus is early-onset than when it is late and that this tendency should be independent of the affection status of the parent. In an analysis of three sets of data,however,(that of Harris,1950;Thompson and Watson,1952, and Steinberg and Wilder,1952),Steinberg demonstrates that, although frequency of affected sibs is,indeed,dependent upon the age at onset in the propositus,it is also dependent upon whether one or neither parent is diabetic. In this analysis Steinberg did not consider the current ages of siblings or parents and was considering clinically diagnosed diabetics rather than indivdiduals assessed by a glucose tolerance test.

Following the lead of Vallance-Owen,Ehrlich and Martin (1966) studied the plasma of non-diabetic sibs of diabetic children, a small sample of their parents and ten controls ('children with no obvious endocrine disorder selected at random from the wards of the Hospital for Sick Children'). The number of individuals positive for synalbumin antagonist in each group suggested that the phenomenon was inherited as an autosomal co-dominant.

(e) One major gene with non-allelic modifiers.

It was suggested by Lamy et al (1961) that,in the majority of cases,the diabetic phenotype is the result of the action of a major gene with non-allelic modifiers but that,in some instances,if enough modifiers are present,the major gene is unnecessary. In the latter instance the modifying genes are acting as components in a polygenic system. The distinction between this hypothesis and that of polygenic inheritance is largely a matter of semantics.

(f) Polygenic inheritance.

The terms 'polygenic' and 'multifactorial' are often used synonymously. It is useful,however,to differentiate between the two terms in the manner suggested by Carter (1969). He uses the term 'polygenic' to refer to a genetic component involving alleles at more than one locus and reserves 'multifactorial' to include environmental as well as genetic factors.

Advocates of single gene inheritance tended to view diabetes as a qualitative trait with the abnormal clearly differentiated from the normal. If glucose tolerance is taken to be the indicator of the diabetic state,however,diabetics are recognised as individuals in that portion of the population beyond an arbitrary threshold imposed upon a continuously varying character. Edwards (1960) has pointed out that such quasi-continuous traits,when common and familial,may simulate single factor inheritance so it is hardly surprising that family data are compatible with several simple genetic models.

This continuous distribution of glucose tolerance has been demonstrated by Neel et al (1965) and by Thompson (1965),who performed glucose tolerance tests on the first degree relatives of diabetics (Thompson) and on individuals without a positive family history of diabetes (both groups). It was suggested that this attribute was influenced by a large number of factors,genetic as well as environmental,in much the same way as height or I.Q.

This finding has been challenged by Steinberg et al (1970) and by Rushforth et al (1971) who considered that, even if the distribution were bi- or tri-modal,the number of abnormal individuals,even in a sample of first degree relatives of diabetics,would be so small that any discontinuity would be lost. Accordingly,they directed their attention to groups with a very high prevalence of glucose tolerance abnormalities - the Pima Indians of Arizona and Asiatic Indians resident in South Africa (the latter from unpublished data of Jackson and Campbell). In both populations,individuals over the age of 45 showed a bimodal distribution of the $\log_{10}$ values of blood sugar two hours after the loading dose.

This bimodality of response does not necessarily indicate the effect of a major gene,however,but merely signifies the presence of two clearly identifiable phenotypes. This could be accounted for by an environmental difference and it is only when environmental variables have been standardised over the whole population that a bimodal distribution is indicative of a major gene effect (Thompson,1965).

A multifactorial aetiology,with a polygenic hereditary

component has proved a useful working hypothesis for diabetes since the concept of heritability can be applied to estimate the magnitude of the genetic contribution (Falconer,1965 and 1967),to predict the risks of recurrence in relatives (Falconer, 1967;Darlow et al,1973;Smith,1971 and 1972) and for the investigation of genetic heterogeneity within the disease (Smith,1976). Some observations on the occurrence of diabetes within families can best be explained in terms of this type of inheritance. The first degree relatives of propositi who develop their diabetes before the age of 20 are more likely to be affected by the disease than the relatives of propositi with diabetes of later onset (Harris,1950,and Simpson,1964). This effect can be explained if it is assumed that early-onset cases have inherited a larger number of 'risk factors' so that first degree relatives will also tend to have a larger number of such factors and thus be at a greater risk of developing the disease.

(g) Effect of a single dose of a gene which,in the homozygous state,gives a recognised,rare abnormality.

There are many conditions,apparently determined by single genes,which give rise to syndromes with diabetes mellitus as a component part. A full list of these would be out of place in this account but may be found in the review by Rimoin and Schimke (1971),together with the references to the original descriptions. It is not an unreasonable hypothesis that the heterozygotes for these genes are at a greater risk with regard to developing diabetes.

Hallett et al (1965) investigated the diabetics

amongst 132 parents of children with cystic fibrosis. They compared the prevalence of the disease in this group with that in 118 parents of unaffected children (neighbours of the affected individuals). They found three diabetics amongst the cystic fibrosis gene carriers and none amongst the controls but concluded that there was no statistically significant excess of diabetics in the carrier group.

Swift et al (1974) pointed out that,assuming a prevalence of 1% for diabetes in the United States in individuals of the same age group as the control parents used by Hallett et al,about one affected individual would be expected in a group of 118. If the carriers of the cystic fibrosis gene were three times more susceptible to diabetes then the observed number of cases in the carrier group would be the result expected. That this result was not significant was only the result of the small sample considered. Restriction of sample size is inevitable if only obligatory heterozygotes are used and the group can be enlarged by the inclusion of more distant relatives who have a certain,calculable probability of being heterozygotes.

Swift had already done this (Swift,1971 and 1972) by considering the prevalence of neoplastic disease and of clinically diagnosed diabetes in all relatives whose probability of being heterozygote for the gene for Fanconi's anaemia was greater than 0.123. The result of this investigation was the claim that the females had a six-fold risk of developing diabetes compared with age-matched controls from the general population. Fanconi's anaemia is not one of the conditions known to be associated with diabetes in the homozygous state but Swift

suggested that the affected individuals died before diabetes became manifest.

Edwards (1973) felt that the increased incidence of diabetes in these female relatives was solely a consequence of the fact that the risk of becoming diabetic and of being included as the parent of a child with a rare recessive condition are both related to parity. Fraser (1973) felt that the point was valid though the magnitude of the bias exaggerated. He suggested that Swift's results be compared,not with the prevalence figures for the general population,but with data from the relatives of normal children. Swift (1973) pointed out that the sample of female relatives found to have an increased risk of diabetes included,not only mothers,grandmothers and great-grandmothers but also sisters,aunts,first cousins,grandparents' sisters and female first cousins once removed. The probability of ascertaining these relatives was independent of their own parity.

This line of investigation is intriguing and is of practical as well as theoretical importance,for such heterozygotes make up a significant proportion of the general population. The frequency of heterozygotes for cystic fibrosis amongst white residents of the United States,for example,is estimated at about 4% (Hallett et al,1965). The question is,as yet,unresolved.

(h) Early-onset diabetes the result of a major gene,late-onset diabetes either unifactorial or multifactorial.

Using the technique of complex segregation analysis, claimed to differentiate between inheritance by a single gene and

by a polygenic system (Rao et al,1975),Goodman and Chung (1975) studied questionnaire information on the first degree relatives of 6,559 diabetic propositi. The families were grouped,according to the propositus' age at diagnosis,into early-onset (0 - 19 yrs), middle-onset (20 - 39 yrs) and late-onset (over 40 yrs). The estimate of heritability in the early-onset group was so high (0.99) that,in accordance with the views of previous writers (Morton,1967,and Smith,1971(b)),they considered that the condition was due to the segregation of a major gene. The considerably lower heritability estimates for the two later-onset groups allowed them to fit either a single locus hypothesis,with penetrance qualifications,or a multifactorial hypothesis.

Despite considerable effort in the field of pedigree analysis a single mode of inheritance for diabetes has never been convincingly demonstrated. The main reasons for this failure are:-

(i) Lack of recognition of the genetic heterogeneity of the group of individuals labelled 'diabetic', or the use of sub-divisional criteria which are not valid genetically.

(ii) Ignorance of the fundamental lesion responsible for the various sub-types of the disease and the influence of environmental factors on the phenotype.

(iii) Failure to agree upon definitions of abnormality.

(iv) The analysis of pedigree data based on the assumption that dibetes is a qualitative trait.

(v) Failure to take into account the variable age at onset of the disease.

1.3 The definition of diabetes.

Although other features of the disease,especially cardiovascular abnormalities,are currently of prime importance with regard to the morbidity and mortality of diabetes,and other metabolic disturbances are as marked as that of the control of blood glucose,it is on the basis of abnormality of blood glucose control that diabetes is customarily defined.

This control is most conveniently measured by the glucose tolerance test but,despite wide use of this investigation, there is little conformity either in the definition of abnormality or in the standardisation of factors which may influence the result.

The standards of abnormality suggested on behalf of the British Diabetic Association by Fitzgerald and Keen (1964) and endorsed by the World Health Organisation (W.H.O.,1965) are that capillary blood glucose at two hours after the oral administration of 50 grams of glucose should not exceed 6.7mmol/l and that the peak of blood glucose should not be greater than 10.0mmol/l. The suggested upper limit of normality at two hours has been raised to 7.5mmol/l as a result of follow-up of borderline cases in the Birmingham diabetes survey (FitzGerald, 1976). Different upper limits of abnormality have been used by other workers (e.g.Köbberling et al,1969).

Several single figure indices which describe characteristics of the glucose tolerance curve have been

advocated. These have several advantages in that they eliminate the ambiguity of individuals abnormal with regard to one criterion blood value but normal with regard to the other,make graphical display of the distribution of response in a population considerably easier and simplify the consideration of change in the glucose tolerance of an individual with time.

The simplest of these - the two hour blood glucose value (Steinberg et al,1970,and Rushforth et al,1971) conveys nothing about the shape of the curve and has little,other than simplicity,to recommend it.

Jarrett and Graver (1968) measured the area under the curve by means of a formula:-

$$\text{area} = a + 2b + 2c + 2d + e$$

where a is the fasting blood sugar and b,c,d and e are subsequent half hour measurements. As pointed out by Billewicz et al (1973),this figure is disproportioately influenced by the absolute value of the fasting blood glucose.

The H index (Billewicz et al,1973) is an expression of the shape of the curve taking two of its most important features into consideration - the time taken to reach the peak and the degree to which fasting blood sugar level is regained within the two-hour test period. It is independent of the absolute value of blood glucose at the peak and the fasting blood glucose.

Comparison with assessments by other criteria (those of FitzGerald and Keen,1964) show that it gives clinically useful results. Its main advantage,however,is not greater accuracy of diagnosis but the fact that it is independent of the absolute level of fasting blood glucose,site of sampling,

method of measurement of blood glucose,loading dose and absolute value of peak blood glucose. The value of H correlates quite well ($r = 0.68$) with the mathematically more complex estimate of the efficiency of homeostatic control of blood glucose,ω_0, derived by Ackerman et al (1964).

Stringent rules for the conduct of the oral glucose tolerance test are laid down by FitzGerald and Keen (1964) but it is not often emphasised in family and epidemiological studies that these are adhered to. In addition,many other factors,age, time of day,current medication etc.,are known to influence the result (Jarret,1973) but are often paid scant attention.

1.4 Heterogeneity and lack of precise knowledge of pathogenesis.

Before the immunological and immunogenetic studies of the past few years,the major sub-division of diabetes was on the basis of age at onset and treatment. Two major forms were thus defined. Juvenile-onset diabetes (J.O.D.,juvenile diabetes mellitus (J.D.M.),early-onset diabetes,or classical diabetes) was recognised as that form of the disease which presented before a certain age (from 20 to 45,depending on the author concerned), was rapid in onset,with symptoms of polyuria,polydypsia and weight loss,a tendency to ketoacidosis and a requirement for treatment with insulin. The other major sub-type - maturity-onset diabetes (M.O.D.,adult- or late-onset diabetes) - was diabetes presenting after this age,with mild or absent symptoms, often associated with obesity without the requirement for insulin. The implication from this classification was that juvenile-onset and

maturity-onset diabetes were genetically and aetiologically different.

This sub-division was unsatisfactory in many ways. An arbitrary age limit,wherever it is set,is unlikely to distinguish effectively between two diseases and some individuals diagnosed after the critical age may require insulin therapy whereas other individuals diagnosed before this age may be treated perfectly well without it.

In addition,the treatment of diabetes is not immutable and may be the result of the preferences of the physician in charge. Individuals may commence their treatment with diet or oral hypoglycaemics and progress to insulin or vice versa and other patients,adequately controlled without insulin for the greater part of the time may require insulin during times of stress,such as infection.

Techniques for the detection of circulating antibodies directed against the insulin-producing cells and the study of the major histocompatibility locus antigens in diabetics have contributed to our understanding of the sub-division of diabetes although the situation is still fluid with many unresolved questions. The possible sub-types of diabetes will be discussed in the order in which they appear in table 1.2. This is a suggested working classification of diabetes based, with some modification,on that given by Cudworth (1976).

(a) Congenital diabetes.

It is uncertain whether truly congenital diabetes exists although Laurence and Dodge (1975) report an infant who

Table 1.2 Genetic and aetiological classification of diabetes.

PRIMARY DIABETES

(a) Congenital diabetes

(b) Diabetes associated with recognised genetic syndromes.

(c) Maturity-onset diabetes in young people (M.O.D.Y.)

(d) Type I diabetes mellitus

(e) Type II diabetes mellitus

SECONDARY DIABETES

Diabetes associated with obesity,pancreatitis or as a result of medication,acromegaly etc.

died shortly after birth in diabetic coma and in whom autopsy revealed a complete absence of the islets of Langerhans. One of the child's brothers had died in similar circumstances although no post-mortem evidence was available in this case.

MacDonald (1974) has written on the subject of diabetes in the first month of life giving references to 32 case reports. He concludes that in the affected cases 'small for dates' there was mention of neonatal diabetes amongst siblings or cousins in four out of eight cases for whom family information was available. Affected cases not 'small for dates' were found not to have a family history of the condition.

Diabetes was not permanent in every case and those of normal birth-weight or above tended to have transient diabetes. Whether this diabetes of early life is a distinct genetic entity or whether it is related to intra-uterine infection is unknown.

(b) Diabetes associated with recognised genetic syndromes.

Several syndromes,with recognised genetic or chromosomal aetiology,have been described as associated with abnormalities of glucose tolerance,with or without symptoms of diabetes. These comprise about 1% of clinical cases of diabetes (Neel,1976),and have been mentioned before (section 1.2(g)).

It is difficult to draw any firm conclusions as to the relationship between the basic genetic defect and the development of diabetes in these conditions. In some cases,ataxia telangiectasia,for example,there is insulin resistance similar to that found in some cases of type II diabetes (see below),in others insulin deficiency seems to be the result of secondary pancreatic

degeneration,cystic fibrosis,for example (Rimoin,1976). The mechanism of glucose intolerance in chromosomal aberations is not clear. (Schöffling and Schade,1976).

(c) Maturity-onset diabetes in young people.

In 1974 Tattersall described and named a rare form of diabetes characterised by clear autosomal inheritance and 'mildness' (in that insulin is rarely required and affected individuals seem to escape cardiovascular and neurological complications no matter how long the duration of their disease). The name given to this condition was 'maturity-onset diabetes of young people' (M.O.D.Y.) since it was defined as presenting before the age of twenty-five. A subsequent report (Tattersall and Fajans,1975) confirmed that the inheritance of the disease was clearly different from that of insulin-dependent diabetes presenting in the same age group. M.O.D.Y. is an unfortunate name since available information indicates that the condition is different,not only from the juvenile-onset,insulin-dependent form of the disease but also,in its dominant inheritance and lack of complications,from maturity-onset,insulin-independent diabetes.

Although the first to give the condition a name, Tattersall was not the first to describe the condition since it is mentioned in several early reports (Charles,1907,and Cammidge,1928) and may have been the disease segregating through several generations in the families described by Bernstein and Patterson (1949) and Pavel and Pieptea (1966).

The relationship of the condition to other forms

of diabetes is not wholly clear. A recent reviewer (Cudworth, 1976) included it in the same genetic category as type II diabetes,as did Irvine (1977) in his aetiological classification. There seems little justification for this. Barbosa (1977) suggests a tentative designation of 'type III' diabetes but, since an important component of the syndrome - the cardiovascular complications - is lacking,there is some justification for not regarding it as diabetes at all but using his alternative suggestion - 'maturity-onset type of hyperglycaemia in the young' (Barbosa,1977).

(d) Type I diabetes mellitus.

Early-onset,insulin-requiring diabetes has long been known to be accompanied in many cases by lymphocytic infiltration of the Islets of Langerhans (Warren and Root,1925,and Gepts,1965). These observations are derived from post-mortem examinations of individuals dying as a result of keto-acidotic episodes at the onset of their diabetes. A suggestion that the same process occurs in insulin-requiring diabetics with presentation after the age of 30 comes from the report of LeCompte and Legg (1972).

Significant associations between known auto-immune diseases and insulin-dependent diabetes (Ungar et al,1968,and Masi et al,1965) have strengthened the belief that cases of this type of diabetes are aetiologically related and that they involve some abnormality of the immune response.

The definition of type I diabetes and the exact pathological processes involved are still not totally clear

but the study of the human leucocyte antigen (H.L.A.) frequencies of diabetic subjects and the development of immunochemical techniques for the visualisation of pancreatic islet cell antibodies are contributing greatly.

The HLA system will be discussed in detail in the next chapter but,briefly,evidence has been presented (Cudworth and Woodrow,1976) that,in agreement with the evidence from post-mortem studies described above,insulin-requiring diabetics,at whatever age they are diagnosed,seem to have been subject to the same disease process and that they are different,genetically, from non-insulin-requiring diabetics.

Several groups have reported the detection of circulating IgG directed against pancreatic islet cells in insulin-requiring diabetics (Bottazzo et al,1974;MacCuish et al, 1974;Lendrum et al,1975,and Lendrum et al,1976). At first it was considered that these pancreatic islet-cell antibodies (P.I.C.A.) were only present in those diabetics with concomitant auto-immune disease of other organs (Bottazzo et al,1974,and MacCuish et al,1974) but it was later realised that the phenomenon is more generalised with detectable antibodies in all insulin-requiring diabetics immediately after the onset of the disease. With increasing duration of disease the titre declines in most individuals (Lendrum et al,1976). Type I diabetes was taken to be the result of auto-immune destruction of the insulin-producing cells in genetically predisposed individuals as a result of contact with an unknown environmental trigger factor or factors and corresponded,very largely,with the previous category of insulin-requiring diabetes (Cudworth,

1976).

Bottazzo and Doniach (1976) suggested a further sub-division of this group into a type IA and IB. The former, numerically the larger group,was considered to have transient circulating P.I.C.A. and to be the result of the environmentally triggered auto-immune reaction described above. Type IB had permanent P.I.C.A. and the propensity for the development of auto-immune disease of other endocrine organs.

Irvine (1977) has put forward evidence for the presence of P.I.C.A. in some individuals initially treated with oral hypoglycaemics who eventually progress to insulin therapy. These individuals are,therefore,in the same aetiological,and, presumably,genetic category as the type I diabetics who commence their treatment with insulin. Irvine (1977) further suggests that three sub-groups of the type I diabetes may be recognised - type Ia,a rare variety in which the disease is the result of auto-immune destruction of the beta cells as a result of a genetic diathesis,type Ic,also rare in which the diathesis is towards direct viral destruction of the beta cells with no auto-immune reaction and a commoner type Ib,in which both direct viral action and subsequent auto-immune destruction take place.

There has been no demonstration to date of anti-bodies directed specifically against the beta cell. The reports refered to above have been concerned with antibodies directed at all islet cells. The demonstration (Bottazzo and Lendrum, 1976) of antibodies specific to the glucagon- and somatostatin-producing cells (alpha and delta) suggests that anti-beta cell antibodies may soon be isolated.

P.I.C.A. has been demonstrated in 1.7% of the general population (Lendrum et al,1976),the significance of this is unknown.

For some time circumstantial evidence has been available suggestive that the environmental trigger for type I diabetes may be a virus or one of several viruses. New cases of insulin-dependent diabetes are more common in the autumn and winter months (Adams,1926;Gamble and Taylor,1969;Gamble et al,1973,and Bloom et al,1975). This pattern of incidence corresponds with the epidemiology of Coxsackie type B4 virus. Gamble et al (1973) reprted neutralising antibodies to Coxsackie types B1 to B5 in the sera of newly-diagnosed insulin-requiring diabetics.

A disease indistinguishable from human diabetes can be produced in genetically susceptible mice by the intra-peritoneal injection of Coxsackie B4 virus (Coleman et al,1973).

A prospective study (Dippe et al,1975) failed to support the hypothesis that infection with the Coxsackie B4 led to the development of diabetes although,in choosing an Eskimo community for this study,lack of genetically susceptible individuals may have accounted for the failure of any infected individuals to develop diabetes.

Other viruses,such as mumps (Khakpour and Nik-Akhtar, 1975) and rubella (Menser et al,1974),have been implicated in the pathogenesis of type I diabetes and the fact that the disease may be produced in mice by infection with encephalomyocarditis virus (Hayashi et al,1974,and Bloucher et al,1973) suggests that more than one virus may be involved.

In a study of 110 early-onset insulin-requiring newly-diagnosed diabetics (Cudworth et al,1977) antibody titres to Coxsackie types B1 to B5 were found to be higher in diabetics with certain HLA specificities (Bw15 and B8/Bw15) Bw15 positive individuals tended to present in the months of January,February and March. There was no correlation of HLA phenotypes or antibody titres with circulating P.I.C.A. levels.

The definition of type I diabetes rests with the demonstration of circulating P.I.C.A. but,since this phenomenon is transitory in most cases,it is not possible to classify all established diabetics in this manner. It seems that,in any population of diabetics,the overwhelming majority of insulin-taking diabetics and some of those treated with oral hypoglycaemics will belong to this category.

(e) Type II diabetes.

There is very little evidence concerning the fundamental lesion responsible for type II diabetes and there are some indications that it may be a repository for several sub-types.

Early investigations by Himsworth (1949) suggested that there was a group of diabetics who were insensitive to exogenous insulin and whose disease might be the result of the lack of effective action of their own insulin. The introduction of radio-immunoassay techniques for the measurement of endogenous insulin confirmed this view by demonstrating normal or increased amounts of circulating insulin in non-ketosis-prone diabetics (Yalow and Berson,1960;Berson and Yalow,1961,and Chiles and

Tzagournis,1970).

The finding of abnormally elevated serum insulin levels after a glucose load in chemically diabetic subjects (Jackson et al,1972,and Reaven et al,1972) prompted the suggestion that the initial lesion in maturity-onset diabetes might be peripheral insensitivity to circulating insulin (Jackson et al.1972) and that the cardiovascular complications seen in this disease might be the consequence of the resultant hyperinsulinaemia (Reaven et al,1972). Although further work (Reaven et al,1976) confirmed this feature of chemical diabetes and borderline clinical diabetes,insulin deficiency was found in some individuals with florid maturity-onset non-insulin-requiring diabetes with fasting hyperglycaemia. Whether this represents the normal progression in this type of diabetes - from insulin insensitivity to insulin deficiency by way of beta cell exhaustion - or whether the type II category includes at least two groups of individuals with regard to insulin effectiveness and insulin secretion is not clear.

In-vitro experiments (Rosenbloom et al,1976) have demonstrated that the number of insulin molecules required to activate cell receptor sites increases with age and is also greater in individuals with hereditary conditions characterised by precocious ageing (Rothmund's syndrome and progeira). The molecular basis for this change is unknown but the work does suggest that,in at least some of,the type II diabetics,hyper-glycaemia may be the result of accelerated cellular ageing processes resulting in peripheral insensitivity to circulating insulin.

There is considerable epidemiological evidence that diet contributes to the development of diabetes,especially type II diabetes. The prevalence of the disease differs in groups of the same ethnic background living under different dietary circumstances (West and Kalbfleisch,1971;West,1974;Prior, 1974,and West,1974(a)),and the incidence and mortality rates in this country declined during the dietary restrictions necessitated by the two world wars (Trowell,1974). It is uncertain whether increase in carbohydrate,fat or protein is the major contributor or whether total calorific intake irrespective of source is the most important factor (West,1975). Reduction in the vegetable fibre content of the diet with increase in the quantity of refined carbohydrate has also been implicated. It is likely that several of these factors are involved simultaneously. The rapid absorption of refined carbohydrate has been cited as antagonistic to circulating insulin (Kiehm et al,1976) whereas a diet in which 75% of the calories are in the form of carbohydrate but accompanied by a high fibre content leads to a lowering of fasting blood glucose in established diabetics and a reduction in insulin and oral-hypoglycaemic requirements (Kiehm et al,1976). It has been suggested (Jenkins et al,1976) that the addition of certain forms of dietary fibre to iso-caloric test meals reduces post-prandial hypoglycaemia in established diabetics.

The implication of dietary factors in the pathogenesis of diabetes does not diminish the role of heredity. Faced with a given dietary regime individuals react differently and it is overwhelmingly likely that some of these differences will have been inherited. Neel (1962) has introduced the concept of the 'thrify

genotype' to refer to those individuals genetically better adapted to some nutritional situations and has suggested that the genetically potential diabetic individuals may have been selectively favoured before the large-scale availability of refined carbohydrate foods and the increase of life-expectancy.

It is possible that trace elements in the diet, particularly chromium,may be related to insulin effectiveness. Impaired glucose tolerance can be produced in experimental animals by the reduction of trivalent chromium (Cr^{3+}) levels in the diet (Mertz and Schwarz,1959). Normal glucose tolerance can be restored in these animals by the administration of Cr^{3+} or of 'glucose tolerance factor' which contains chromium in this form (Schwarz and Mertz,1959). In addition to Cr^{3+} glucose tolerance factor (G.T.F.) contains nicotinic acid as its active component (Doisy et al,1976) and,certainly in vitro,seems to be necessary for maximum cellular response to insulin. (Mertz,1969). In humans the ingestion of Cr^{3+} improves glucose tolerance in late-onset diabetics (Glinsmann and Mertz,1966),elderly subjects (Levine et al,1968),and young adults (Doisy et al,1976). It is possible that low chromium levels,either due to dietary deficiency or inadequate absorption,may contribute to the insulin insensitivity of type II diabetes by reducing the amount of circulating G.T.F.

1.5 The contribution of twin studies.

Early twin studies in diabetes (Then Berg,1938;Harvald and Hauge,1963,and Gottlieb and Root,1968) tended to emphasise the fact that high concordance rates in monozygotic twin pairs

was evidence for an inherited component in diabetes. More recently, the study of twins has been less popular and the emphasis has shifted with the realisation that twins concordant for diabetes may be concordant because of environmental factors,whereas discordant pairs are discordant because the action of the environment has been different on the two individuals concerned. Modern studies of diabetes in twins tend to reveal what is not inherited rather than what is (Pyke and Nelson,1976).

In an analysis of 106 pairs of monozygotic twins from the United Kingdom,Pyke and Nelson (1976) found 71 pairs to be concordant (both individuals diabetic) and 35 discordant (one individual diabetic). A family history of diabetes was found in 45% of the concordant pairs but only 17% of the discordant pairs,suggesting that,in the discordant pairs the disease in the affected individual was less influenced by genotype than the disease in the concordant pairs. The discordance rates differed markedly when age at diagnosis of the index twin was considered. When this age was less than forty years the discordance rate was 50% (32 pairs discordant out of 64),when the age at diagnosis was greater than forty the discordance rate was 7.1% (3 discordant pairs out of 42). The age at diagnosis is strongly positively correlated in concordant twins (Pyke and Nelson,1976),with most pairs becoming concordant within three years of the diagnosis of the disease in the index twin so that this difference in concordance rates with age at diagnosis of the index case is unlikely to be the result of late-onset pairs having been followed up for longer than early-onset pairs.

This finding is in agreement with earlier studies

(Then Berg,1938;Harvald and Hauge,1963;Gottlieb and Root,1968) but the deduction which must be made,namely,that hereditary factors are more important in late-onset diabetes than in early-onset,is at variance with the estimates of higher heritability in early-onset disease than in late-onset disease (Goodman and Chung,1975,and Simpson,1964). There is no conflict,however, between this finding and the hypothesis that type I diabetes (most of the twins with onset before the age of forty must have been of this variety although there is no immunological confirmation of this) is the result of beta cell destruction in genetically predisposed individuals following an environmental stimulus. The non-diabetic twins of the pairs in which the index twin had early-onset diabetes presumably remained unaffected because,although they possessed the appropriate genotype,they did not encounter the relevant environmental stimulus. The present twin data suggest that,in early-onset diabetes,genetic factors are necessary for the precipitation of the disease but are probably not sufficient alone,whereas,in late-onset diabetes,genetic factors are necessary and,in most cases,are sufficient,or the environmental factors,whatever they are,are ubiquitous. If the non-diabetic twins of the pairs with early-onset index twins remain unaffected,as is proposed,it suggests that there is a critical period in the normal life-span,outside of which exposure to the environmental triggering mechanism is unlikely to lead to the development of diabetes.

1.6 Animal models.

Diabetes mellitus has been reported in many laboratory

and domesticated animals. The diversity of the pathological processes involved in animal diabetes and the several genetic mechanisms proposed reflect the heterogeneity and,to a certain extent,the confusion seen in human diabetes.

Diabetes in animals may be induced (by various substances toxic to the islets of Langerhans,such as alloxan) or may be spontaneous. The chemical induction of diabetes forms a convenient laboratory model for the study of the biochemistry of insulin lack,although it would seem more analagous to the diabetes induced in humans by pancreatectomy or pancreatitis rather than the primary diabetic syndromes where hereditary factors are involved.

Diabetes occurring spontaneously in animals has been reviewed (Dickie,1970,and Herberg and Coleman,1977). As in the human disease,the factors responsible are environmental and hereditary,and instances of simple and complex inheritance may be found. Changing the diet of some strains of sheep (Warren et al, 1966) and the Egyptian sand rat (Dickie,1970) leads to the precipitation of diabetes. In the Chinese hamster (Gerritsen and Dulin,1967) diabetes occurs spontaneously and inheritance of the condition seems to be polygenic,depending on the action of at least four loci (Butler,1967). In this animal the disease seems similar to that of type I diabetes in humans in that plasma insulin levels are below normal and plasma free fatty acid and ketone levels are elevated. In addition,small blood vessel changes develop that are indistinguishable from those found in human type I (and type II)diabetes.

In the mouse,several different conditions are known

in which animals become diabetic,with or without concurrent obesity (Herberg and Coleman,1977). The Japanese KK mouse,in addition to hyperphagia and obesity,has diabetes characterised by hyperinsulinaemia and,therefore,presumably,peripheral insulin resistance. The genetics of this condition has been explained as being polygenic (Iwatsuka and Shino,1970) although Butler and Gerritsen (1970),using different criteria,suggested a dominant gene with 25% penetrance,coupled with a recessive modifier which raised the penetrance to 75%. This conflict is rather reminiscent of human diabetes research.

Single gene models in mice include the 'diabetes mouse' where an autosomal recessive gene (db) is segregating at a chromosome 4 locus and in which the disease appears similar to human type II diabetes in that hyperglycaemia is the result of insulin resistance (Dickie,1970). Several other animal models exist and serve a useful purpose in the elucidation of biochemical aspects of diabetes although no clear understanding of the genetics of human diabetes has been gained from their study.

1.7 Complications and the concept of premature ageing.

The ability to maintain diabetics for many years on insulin and other supportive therapy has meant that cardiovascular complications contribute substantially to the mortality and morbidity of the disease. Type I and type II diabetics are equally likely to develop these changes given an equal duration of the disease (Tattersall,1974). Lamentably,there is little direct proof that good control of diabetes,in so far as this is judged by blood glucose estimates,can prevent or reverse

these complications (Fajans,1972;Keen and Jarrett,1975,and B.M.J., 1976) although some studies,both retrospective (Knowles,1964) and prospective (Pirart et al,1975) suggest that strict control of blood glucose from the time of diagnosis can delay their onset. The major problem in this field is lack of agreement on the definition and assessment of 'good control' in the diabetic.

The mechanisms responsible for cardiovascular complications in the diabetic are largely obscure. Their absence in the M.O.D.Y. sub-type (Tattersall,1974,and Tattersall and Fajans,1975) tends to argue against the view that they are due to elevated blood glucose alone and their presence in both type I and type II diabetes suggests that absolute blood insulin levels are not responsible,though they may be due to lack of effective insulin action. Plasma triglycerides are elevated in diabetics even when control is judged satisfactory in terms of blood glucose (Keiding et al,1952) and it is possible that elevated blood lipids are contributory. It would seem that the study of the M.O.D.Y. phenotype would be fruitful in the assessment of the contribution of various metabolic derangements to the pathogenesis of diabetic angiopathy.

Genetic factors have been said to be important in the production of cardiovascular complications. The study of identical twins concordant for diabetes (Pyke and Tattersall,1973,and Pyke and Nelson,1976) has shown that related individuals are similar in the rate of development and nature of these complications even when living apart and treated at different centres.

Superficially it would seem that the observation of retinopathy and glomerulosclerosis in the 'secondary diabetes'

associated with chronic pancreatitis (Duncan et al,1958,and Sevel et al,1971) was against this view. It has been shown,however, (Verdonk et al,1975) that the frequency of these complications in such patients is greater in those who also have a family history of diabetes. The 'potential diabetic',judged from family history may be more susceptible to pancreatitis and more susceptible to the complications of the resulting diabetes.

There can be little conflict as to whether cardiovascular changes are genetic or metabolic in origin. It is likely that, given a certain hereditary capability,one or several of the metabolic derangements seen in diabetes act as exacerbating factors in their development. Having come to terms with the possibility that both environmental and hereditary factors are acting to produce the disease state in the first place,it demands very little extrapolation to suggest that the same is true of other manifestations of the disease.

The physico-chemical properties of collagen in young, insulin-dependent diabetics have been shown to resemble those of collagen from older individuals.

Work discussed above (Rosenbloom et al,1976) has shown that the insulin resistance of maturity onset diabetes resembles that seen in older,non-diabetic individuals,at least in vitro.

The possibility that genetically determined acceleration of cellular ageing processes underlies the pathogenic process in all diabetics has been expounded by Vracko and Benditt (1972). These workers considered that the thickening of the capillary basement membrane characteristic of diabetic micro-angiopathy was due to abnormally rapid cell turnover and the subsequent

accumulation of cellular debris at these sites. That the same process might be responsible for the changes seen in macro-angiopathy was suggested by Martin and Sprague (1973).

Skin fibroblasts,when grown in culture,have a potential number of replications which is inversely proportional to the age of the donor (Martin et al,1970). Fibroblasts from diabetics exhibit about half the number of replications compared with age-matched controls (Vracko and Benditt,1975),a finding taken to indicate increased susceptibility of diabetics' cells to injury. Beta cells reach the limitation of replication in vitro after only a few generations (Logothetopoulos,1972).

Vracko and Benditt's hypothesis is appealing in that one genetic abnormality is responsible for all the manifestations of diabetes in its several forms. It has come under some criticism (Williamson and Kilo,1977) and there are several incongruities. The laminar nature of the thickened capillary basement membrane which Vracko and Benditt took to be due to the successive deposition of cellular material has not been reported in all anatomical sites. In addition,it is difficult to incorporate the suggestion that type I diabetes is due to an abnormal response to viral infection in individuals genetically distinct,at least in some respects,from the type II diabetic,and that diet and obesity seem to influence the development of the latter disease.

A hypothesis concerning the pathogenesis of the type I and type II diabetes suggesting a genetic relationship between them is developed in chapter three. The intervening chapter deals more specifically with the evidence for 'genetic markers' for diabetes.

Chapter 2

Genetic polymorphisms and diabetes mellitus.

Chapter 2

2.1 Introduction.

2.2 Red cell antigen systems.

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(b) The MNSs system.

(c) The P system.

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(b) Acid phosphatase.

(c) Phosphoglucomutase.

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2.4 The ability to taste phenylthiourea.

2.5 The major histocompatibility complex.

2.6 Methodological and statistical difficulties in the search for associations.

(a) Selection of patients.

(b) Selection of controls.

(c) Statistical significance.

2.7 The biological significance of associations with disease.

2.1 Introduction.

There are two major justifications for the investigation of associations between genetic polymorphisms and disease. Firstly,knowledge of such associations identifies genetic factors important in the aetiology of the condition. Secondly,the demonstration of differential susceptibility to disease,on the basis of genotype,may reveal mechanisms whereby balanced polymorphisms are maintained by selection.

There is considerable published material on this aspect of medical genetics. Before 1970 most investigators were concerned with the ABO polymorphism and several convincing associations were demonstrated such as that between carcinoma of the stomach and blood group A (Aird et al,1953) but this extensive investigation contributed little to our understanding of the pathology of individual diseases and less to the elucidation of the factors maintaining the ABO polymorphism and influencing the world-wide distribution of the A,B and O alleles.

Since 1970,however,the intensely polymorphic major histocompatibility complex (M.H.C.) has been studied and has increased our understanding of hereditary aspects of some disorders of obscure aetiology,among them diabetes.

This chapter reviews the studies concerned with associations between genetic polymorphisms and diabetes. A brief description of the polymorphism and some historical notes are included. The chromosome assignments for various loci are all taken from a series of articles in Cytogenetics and Cell Genetics, (1976),16,Nos. 1 - 5,which are reports from the 1975 International Congress on Cytogenetics.

2.2 Red cell antigen systems.

(a) The ABO system.

The ABO system was discovered by Landsteiner (1900). Four common alleles, designated $\underline{A}_1$, $\underline{A}_2$, $\underline{B}$ and $\underline{O}$ segregate at a locus on chromosome 9. The $\underline{A}_1$, $\underline{A}_2$ and B alleles are dominant to the $\underline{O}$ and co-dominant with respect to eachother, except in the case of $\underline{A}_1$ and $\underline{A}_2$, where $\underline{A}_1$ is dominant to $\underline{A}_2$. This system is the only one described so far with 'naturally occurring' antibodies - anti-A and anti-B in group O persons, anti-A in group B persons and anti-B in group A persons. Group AB persons have neither anti-A nor anti-B.

Available data on the distribution of the ABO blood group phenotypes in diabetic individuals are summarised in Table 2.1.

Several of the studies have revealed a significant excess of group A individuals amongst the diabetics taken as a whole, although in no case is this excess very great. Some have failed to find associations, others have found associations with phenotypes other than A.

A readily appreciated numerical representation of the result is the relative incidence (x), which, in the case of the A/O comparison is calculated as follows:-

$$x = {}^{n_1}/_{n_2} \quad x. \quad {}^{N_2}/_{N_1}$$

where n_1 and n_2 are the numbers of A and O individuals in the diabetic group and N_1 and N_2 the same categories for the control group. This ratio is an estimate of the relative risk, or the increased risk of the disease developing in group A persons

Table 2·1 Investigations dealing with diabetes and the ABO system

Reference	No.Diabetics	No.Controls	Source	Matching	Findings
(1) McConnell (1955)	815	N.G.	N.G.	N.G.	Increased group A amongst early onset diabetics These data included in ref.3.
(2) Craig and Wang (1955)	817	1) 7,418 2) 2,597	Donors Hospital patients	Area of residence	No significant difference group as a whole Deficiency group O in obese diabetics Deficiency group O in patients with family history of diabetes or obesity or both. Deficiency of group B in diabetics Increase in group O in non-obese males with no family history of diabetes or obesity.
(3) McConnell et al (1956)	1) 634 (S.W.Lancs) 2) 199 (W.Cheshire) 3) 500 (Oxford)	1) 6,510 (S.W.Lancs) 2) 1,247 (W.Cheshire) 3) 6,492 (Oxford)	Donors	Area of residence	Significant excess of group A in male diabetics, irrespective of age at onset. No difference in female diabetics. Excess in males is greater if sample is restricted to those with an affected first degree relative.
(4) Zeytinoglu (1956)	432	23,790	N.G.	N.G.	No significant difference with diabetic group taken as a whole. Significant excess of group A in diabetics with 'Kimmelsteil-Wilson syndrome'
(5) Speiser (1958)	239	1,000	-	-	Significant excess of B in females. Deficit of AB in males.

N.G. = not given

Table 2·1 (continued)

Reference	No.Diabetics	No.Controls	Source	Matching	Findings
(6) Otto-Servais et al (1958)	1,000	20,978	Donors & Soldiers	Country of origin	Higher B, lower AB in diabetics as a whole, more marked in females.
(7) Mähr (1959)	1,300	2,867	Donor	Country of origin	No significant association.
(8) Anderson and Lauritzen (1960)	992	1) 12,122 2) 27,332 3) 9,682	Medico legal series Donors Hospital patients	Country of residence	Excess of group O in diabetics of both sexes. Excess of group O more marked in early onset cases (before 30th birthday) Excess of group O more marked in males
(9) Henry and Poon-King (1961)	355	'over 5000'	recipients of blood transfusion	Country of residence	Increased group B and decreased group O in males
(10) Cornil and Pirart (1961)	564	300,000	Donors		Significant excess of A in diabetics
(11) Bibawi & Khatwa (1961)	951	10,045	'healthy soldiers'	Country of residence	Significant excess of group A (and AB) in diabetics, particularly in males

Table 2·1 (continued)

Reference	No. Diabetics	No. Controls	Source	Matching	Findings
(12) Doll, et al (1961)	102	1) 365	healthy persons	Not stated	Significant excess of A
(13) Simpson (1962)	101	1) 235	unaffected relatives	Residence	No significant difference
		2) 36,990	Donors	Area of residence	
		3) 1,000	Paediatric patients		
(14) Sauer, et al (1963)	1,709	1) 3,086	Donors	Country of origin	Excess of group A in series as a whole
(15) Macafee (1964)	865	11,027	Donors	Area of residence	No significant difference
(16) Serra, et al (1964)	900	1,000	Hospital patients	-	Excess of A and B male diabetics, of A and O in female diabetics.
(17) Buckwalter (1964)	1,459	49,979	Donors, Students, Physicians, Technicians, Spouses of diabetics, unaffected sibs	"	"Marginally significant" excess of B and deficieny of AB in diabetics.
(18) Berg, et al (1967)	176	3,089	Hartmann and Lundervall (1944)	Country of origin	No association

Table 2·1 (continued)

Reference	No. Diabetics	No. Controls	Source	Matching	Findings
(19) Révai and Konig	308	63,000	Donors	Area	Excess of A especially in male diabetics
(20) Keensfontor (1974)	100	400	Donors	Country of origin	Excess of group A in diabetics
(21) Scholz, et al (1975)	1,052	2,349	Donors	Area of residence	Non significant excess of group A in diabetics as a whole. Excess of group O in non obese diabetics. Slight deficiency of group C in late onset (after 40), diabetics.

compared with that in group O persons.

The relative incidence has the further advantage that data from numerous centres may be amalgamated by the method of Woolf (1955), resulting in a weighted mean relative incidence and a chi squared value which may be divided into two parts, the first (with one degree of freedom) gives the probability that the mean relative incidence deviates from unity by chance alone, the second gives the significance of heterogeneity between the pooled samples.

Table 2.2 shows the results of these investigations in the form of A/O ratios. The data of Tedeschi and Cavazzutti (1958) are taken from McConnell (1966).

In some cases it has been necessary to calculate the components of the A/O ratio from phenotype frequencies where only these and the total sample sizes are given. These are indicated (*). Series which form part of later reports (e.g. McConnell, 1955) are omitted, as are those in which the necessary data are not given (e.g. Ksenofontor, 1974). The raw data from which the ratios are calculated are given in table 1 of Appendix A.

Transforming the value of x into its logarithm avoids difficulties due to asymmetry (Woolf, 1955). If $y = \log_e x$, then the sampling variance of y is given as:-

$$V = 1/n_1 + 1/n_2 + 1/N_1 + 1/N_2$$

where n_1, n_2, N_1 and N_2 have the same designation as in the equation for x given above. The weight (w) of y is given by the reciprocal of V.

If x is unity, i.e. there is no association of blood groups with the diseased state, y is zero. The chi squared value (with one degree of freedom) for the significance of the

Table 2·2 ABO results in diabetics

Reference	x	chi squared	p
Craig & Wang (1955)	1·12	1·98	< 0·20 > 0·10
McConnell et al (1956) (1)	1·12	1·60	< 0·30 > 0·20
(2)	1·19	1·18	< 0·30 > 0·20
(3)	1·11	1·13	< 0·30 > 0·20
Zeytinoglu (1956	1·03	0·08	< 0·80 > 0·70
Tedeschi & Cavazzutti (1958)	1·28	4·53	< 0·05 > 0·025
Speiser (1958)	1·11	0·40	< 0·60 > 0·50
Otto-Servais et al (1958)	1·02	0·08	< 0·80 > 0·70
Mähr (1959) *	1·03	0·15	< 0·70 > 0·275
Anderson & Lauritzen (1960)*	0·83	7·31	< 0·01 > 0·005
Cornil & Pirart (1961) *	1·30	8.31	< 0·005 > 0·001
Bibawi & Khatwa (1961) *	1·34	12·93	< 0·0005
Doll et al (1961)	1·20	0·60	< 0·50 > 0·40
Simpson et al (1962) +	0·97	0·02	< 0·90 > 0·80
Sauer et al (1963) +	1·08	1·83	< 0·20 > 0·10
Macafee (1964) *	0·91	1·50	< 0·30 > 0·20
Serra et al (1964)	1·34	8·69	< 0·005 > 0·001
Buckwalter (1964) +	1·02	0·12	< 0·80 > 0·70
Berg et al (1967)	1·04	0·06	< 0·90 > 0·80
Scholz et al (1975) *	1·08	0·90	< 0·40 > 0·30

X (weighted mean relative incidence) = 1·08

Chi squared (significance) = 17·08 (d.f. = 1) p < 0·0005

Chi squared (heterogeneity) = 36·32 (d.f. = 19) p < 0·01

* calculated from frequencies

\+ control series combined

deviation of y from zero in each case is given by y^2/V or wy^2.

Combination of data from various centres yields a weighted mean, Y, given by $\Sigma wy/\Sigma w$. Its antilogarithm, X, is the weighted mean relative incidence. Chi squared (with one degree of freedom) for the significance of the deviation of Y from zero is given by $(\Sigma wy)^2/\Sigma w$ or $Y^2\Sigma w$ and heterogeneity between samples is tested for by chi squared given by $\Sigma wy^2 - (\Sigma wy)^2/\Sigma w$ or $\Sigma wy^2 - Y^2\Sigma w$. Degrees of freedom are given by $(N - 1)$ where N is the number of data sets.

From table 2.2 it is evident that the weighted mean relative incidence, X, (1.08) differs significantly from unity, but that the data sets are heterogeneous (chi squared, with 19 degrees of freedom = 36.32).

This heterogeneity is hardly surprising for a disease group such as diabetes. Several disease entities are included in the term and, presumably, these sub-groups are being included to differing degrees in the various populations studied.

Many of the published reports have tried to take this clinical heterogeneity into account, sub-dividing the sample on various criteria and examining them separately for associations (see table 2.3).

The sub-division of the diabetic population on the basis of presence or absence of a family history of the condition reflects the once-favoured distinction between 'hereditary' and 'acquired' diabetes and is of little utility. Of more potential merit is the distinction between early- and late-onset cases (the thirtieth birthday is usually taken as the division between them). Even more useful is a sub-division on the grounds of

Table 2·3 Criteria used for the sub-division of diabetic samples

Reference	Method of sub-division
McConnell (1955)	By mode of inheritance - 'recessive gene', 'dominant gene' and 'unclassifiable'. By age at diagnosis (division at 30 yrs.)
Craig and Wang (1955)	By sex. By presence of family history of diabetes or obesity. By presence or absence of obesity. By age at diagnosis (division at 30 years.)
McConnell et al (1956)	By sex. By age at diagnosis (division at 30.) By treatment*. By presence or absense of family history.
Zeytinoglu (1956)	With and without 'Kimmelsteil-Wilson syndrome'.
Tedeschi and Cavazzutti (1958)	By sex.
Speiser (1958)	By sex.
Otto-Servais et al (1958)	By sex.
Mähr (1959)	By sex.
Anderson and Lauritzen (1960)	By sex. By age at diagnosis (division at 30 years.) By presence or absence of family history. By current age.
Henry and Poon King (1961)	By sex. By race (East Indian and 'mixed').
Cornil and Pirart (1961)	By sex. Presence or absence of family history. Age at diagnosis (division at 30).
Bibawi and Khatwa (1961)	By sex.
Doll et al (1961)	By sex.
Simpson et al (1962)	All 'juvenile diabetics'.
Sauer et al (1963)	By sex.
Macafee (1964)	By sex (data for sexes separate not given). By age at onset (criteria not given). By presence or absence of family history.
Serra et al (1964)	By sex. By age at diagnosis (division at 20 years). By presence or absence of family history

* Classification 'by treatment' in all cases except Scholz et al (1975), involves division into insulin - taking and non - insulin - taking diabetics.

Table 2·3 continued.

Reference	Method of sub-division
Buckwalter (1964)	By sex.
Berg et al (1967)	None
Rĕvai and Konig (1968)	By sex (percentage for controls do not add up to 100 - no further analysis possible).
Ksenofontor (1974)	None
Scholz et al (1975)	By sex. By current age. By treatment. By age at diagnosis (division at 40). By body type (Rohrer index).

insulin-taking or non-insulin-taking since this approximates to the currently favoured type I and type II aetiological sub-divisions. These sub-divisions of the diabetic sample reveal no clear-cut differences with regard to ABO phenotype frequencies.

The data as published do not allow detailed consideration of current age or age at diagnosis. Anderson and Lauritzen (1960) found an excess of group O individuals amongst the early-onset diabetics and a more detailed analysis of this data (Mourant,1977, personal communication) shows a steady rise of the A/O ratio throughout life so that the adult diabetics show a significant excess of group A.

Most workers sub-divide their diabetics by sex. In general,the A/O ratio differs more markedly from unity in male diabetics than it does in females. Tables 2.4 and 2.5 show the results for males and females respectively in those reports where data are given for the sexes separately. The raw data used for these calculations are given in Appendix A,tables 3 and 4. In each case,male and female diabetics are compared with the total control group. The weighted mean relative incidence for male diabetics is 1.12 compared with 1.08 for females. The deviation of this incidence from unity is more significant in males than in females although the data sets for the male diabetics are heterogeneous. These values compare well with those computed,from an augmented set of reports,by Mourant (1977), although the heterogeneity found by him amongst the male data sets was non-significant.

This difference between the sexes does not imply a difference in the mechanism of hereditary transmission of

Table 2·4 ABO results for male diabetics only

Reference	x	chi squared	P
Craig & Wang (1955)	0·95	0·29	< 0·60 > 0·50
McConnell et al (1956) (1)	1·32	3·57	< 0·10 > 0·05
(2)	1·61	3·86	< 0·05 > 0·025
(3)	1·25	1·98	< 0·20 > 0·10
Tedeschi & Cavazzutti (1958)	1·23	1·60	< 0·30 > 0·20
Speiser (1958)	1·13	0·35	< 0·60 > 0·50
Otto-Servais et al (1958)	1·09	0·60	< 0·50 > 0·40
Mähr (1959) *	1·01	0·01	< 0·95 > 0·90
Anderson & Lauritzen * + (1960)	0·79	0·06	< 0·90 > 0·80
Cornil & Pirart (1961) *	1·43	5·68	< 0·025 > 0·01
Bibawi & Khatwa (1961) *	1·81	18·35	< 0·0005
Doll et al (1961)	1·56	1·56	< 0·30 > 0·20
Sauer et al (1963) +	1·09	1·17	< 0·30 > 0·20
Serra et al (1964)	1·60	12·47	< 0·0005
Buckwalter (1964) +	1·04	0·20	< 0·70 > 0·60
Scholz et al (1975) *	1.09	0.58	< 0.50 > 0.40

X (weighted mean relative incidence) = 1·12

Chi squared (significance = 14·52 (d.f. = 1) P < 0·0005

Chi squared (heterogeneity) = 37·81 (d.f. = 13) P < 0·0005

* calculated from frequencies

\+ control series combined

Table 2·5 ABO results for female diabetics only

Reference	x	chi squared	P
Craig & Wang (1955)	1·21	3·85	< 0·05 > 0·025
McConnell et al (1956) (1)	1·03	0·07	< 0·80 > 0·70
(2)	0·97	0·02	< 0·90 > 0·80
(3)	1·04	0·10	< 0·80 > 0·70
Tedeschi & Cavazzutti (1958)	1·32	3·34	< 0·10 > 0·05
Speiser (1958)	1·09	0·13	< 0·80 > 0·70
Otto-Servais et al (1958)	0·98	0·05	< 0·90 > 0·80
Mähr (1959) *	1·04	0·18	< 0·70 > 0·60
Anderson & Lauritzen * + (1960)	0·87	0·02	< 0·90 > 0·80
Cornil & Pirart (1961) *	1·24	3·53	< 0·10 > 0·05
Bibawi & Khatwa * (1961)	1·15	2·02	< 0·20 > 0·10
Doll et al (1961)	1·00	0·00	-
Sauer et al (1963) +	1·06	0·58	< 0·50 > 0·40
Serra et al (1964)	1·19	2·28	< 0·20 > 0·10
Buckwalter (1964) +	1·01	0·02	< 0·90 > 0·80
Scholz et al (1975) *	1·07	0·46	< 0·50 > 0·40

X (weighted mean relative incidence) = 1·08

Chi squared (significance = 7·99 (d.f. = 1) p < 0·005

Chi squared (heterogeneity) = 8·66 (d.f. =13) p<0·8 > 0·7

* calculated from frequencies

\+ control series combined

predisposition in males and females. If the A,B and O alleles influence the development of diabetes they do so in both sexes but their effect is less discernible in the female presumably because of the greater influence of non-genetic factors such as parity and obesity. Studies of heritability of liability (Falconer,1967) have shown that heritability is lower in the female.

Four series have presented results for the sub-types of A - Sauer et al (1963),Buckwalter (1964),Berg et al (1967) and Scholz et al (1975). In no case were the relative frequencies of A_1 and A_2,and A_1B and A_2B different in diabetics compared with controls.

Vogel and Krüger (1968),amalgamating twenty studies of ABO distribution in diabetics,found a significant excess of the B phenotype over the O phenotype,although,again,there was significant heterogeneity between samples. This tended to suggest that relative resistance to the disease of the O phenotype,rather than susceptibility of the A phenotype,was responsible for the associations. Analysis of the twenty sets of data in table 2.2 fails to confirm this with a weighted mean relative incidence of the B/O ratio of 1.01,which is insignificantly different from zero (chi squared,with one degree of freedom = 0.215).

The results of various comparisons using the data from these reports are shown in table 2.6.

(b) The MNSs system.

In 1927 the discovery of the M and N antigens was announced (Landsteiner and Levine,1927). These were

Table 2.6. The Results of various comparisons using data from reports quoted in table 2.2. (male and female diabetics combined).

Comparison	X	Chi square (sig)	p	Chi square (het)	p
A/O	1·08	17·08	< 0·0005	36·32	< 0·01 > 0·005
B/O	1·01	0·21	N.S.	77·76	< 0·0005
A/not A	1·07	12·30	< 0·0005	41·35	< 0·005
A+AB/B+O	1·07	11·86	< 0·001 > 0·0005	90·65	< 0·0005

N.S. = non-significant

considered to be determined by two co-dominant alleles M and N. The possibility that N is a precursor substance acted upon by one allele M with an allelic amorph m is,as yet,unresolved (Race and Sanger,1975). The discovery of another antigen,S (Walsh and Montgomery,1947),added interest since,although the corresponding gene,S,was not allelic to M and N,it was associated,being found more often with M than with N (Sanger and Race,1947,and Sanger et al,1948). The s antigen,product of the s gene,the allelic partner of S,was detected by Levine et al (1951). It is suggested that M and N,and S and s segregate at two closely linked loci on chromosome No.2.

Table 2.7 shows the results of investigations on diabetics using anti-M and anti-N antisera. No significant association has been found,except in the series reported by Ksenofontor (1974), where the diabetic group show a higher frequency of the N phenotype and a lower frequency of heterozygotes. Although not giving the data in full,Scholz et al (1975) state that the M phenotype is more common in diabetics diagnosed before the age of forty compared with those diagnosed after this age. This difference was not significant.

The only series to test for antigens of the Ss system is that of Scholz et al (1975) in which testing was only performed with anti-S. The data are not given but no association was found.

(c) The P system.

In the same report as they developed the MN system, Landsteiner and Levine described another individual difference in human blood (Landsteiner and Levine,1927(a)). Some individuals' red cells were agglutinated by the sera of immunised rabbits in

Table 2.7 MNSs Results in diabetics

Reference	Diabetics			Controls			Chi square (d.f. = 2)	P
	MM	MN	NN	MM	MN	NN		
McConnell et al (1955)	123 (24·60%)	112 (22·40%)	265 (53·00%)	N.G.	N.G.	N.G.	N.G.	Stated to be N.S.
Simpson et al (1962)	28 (27·72%)	44 (43·56%)	29 (28·71%)	363 (28·38%)	634 49·57%)	282 (22·05%)	2·46	N.S.
Buckwalter and Tweed (1964)	302 (26·19%)	614 (53·25%)	237 (20·56%)	587 (26·85%)	1208 (55·26%)	391 (17·89%)	3·54	N.S.
Macafee (1964)	135 (29·80%)	231 (50·99%)	87 (19·21%)	60 (29·70%)	102 (50·50%)	40 (19·80%)	0·05	N.S.
Berg et al (1967)	49 (27·22%)	90 (50·00%)	41 (22·78%)	668 (28·10%)	1565 (50·66%)	656 (21·24%)	0·248	N.S.
Ksenofontov (1974)*	29 (29·00%)	7 (7·00%)	64 (64·00%)	150 (40·00%)	55 (13 75%)	185 (46·25%)	10·56	< 0·005
Scholz et al (1975)	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	Stated to be N.S.

* calculated from frequencies N.G. = not given. N.S. = non-significant.

a way which cut across the previously described ABO and MN distinctions. Those individuals whose cells were agglutinated were designated P+,those whose cells were unaffected,P-. The antiserum was called anti-P.

The discovery that Tj(a-) individuals were also negative with anti-P (Sanger,1955) suggested that the Tj^a antigen belonged to the P system. The outlined scheme for the system (Race and Sanger,1975) suggests three alleles $\underline{P_1}$,$\underline{P_2}$ and $\underline{p}$,with $\underline{p}$ being a possible amorph. Individuals possessing at least one $\underline{P_1}$ allele correspond to those previously designated P+,in that they show agglutination with anti-P_1 (previously anti-P). Varying strengths of reaction to anti-P_1 have been shown to be inherited.

The exact chromosomal assignment of the P locus is in doubt,there is evidence for linkage with the HLA locus (chromosome 6) and with the ADA (adenosine deaminase) locus of chromosome 20.

Ksenofontor (1974) and Scholz et al (1975) performed P system grouping on their subjects. Scholz et al did not detect a significant association but do not give their data. Ksenofontor gives neither conclusions nor results for this system.

(d) The Rhesus system.

Soon after the discovery of the Rh system (Landsteiner and Weiner,1940) its complexity was realised. Fisher's synthesis (Fisher,1947) clarified the position considerably and his suggestions and nomenclature will be used here. Three loci,closely linked and now known to be situated on chromosome No.1,were postulated with the alleles $\underline{C}$ and $\underline{c}$ at one,$\underline{D}$ and $\underline{d}$ at the second and $\underline{E}$ and $\underline{e}$ at the third. Antisera corresponding to the gene products of five of these

six alleles are currently in use,anti-d alone has not been found. In view of the relative frequencies of rhesus haplotypes it was postulated (Fisher and Race,1946,and Fisher,1947) that the order of these loci on the chromosome was D,C,E. All populations so far studied show linkage disequilibrium between the alleles of this system,similar to that found in the MNSs and the HLA systems,in that the theoretically possible chromosome combinations do not occur with equal frequency. In English populations three orders of haplotype frequencies are found - CDe,cde and cDE which occur at frequencies of 12% or greater,cDe,cdE,Cde and CDE at less than 3% and the rare CdE (Race and Sanger,1975). The mechanisms responsible for this,presumably selective,have never been demonstrated.

There are often more than one Rh genotype which can,in theory,fit the results of an individual tested with anti-C,anti-c, anti-D,anti-E and anti-e. In practice the 'most likely genotype', based on previous knowledge of relative haplotype frequencies in the population to which the individual belongs,is suggested.

The results of investigations of diabetics with anti-D alone are given in table 2.8. No significant associations are demonstrated.

Testing for alleles at the other two loci and the assignment of probable genotypes has been performed by Buckwalter and Tweed (1962),Berg et al (1967) and Scholz et al (1975). D+ve and D-ve frequencies cannot be calculated from the data given by Berg et al (1967) and Scholz et al (1975) due to the inclusion of a category termed'other'.

Berg et al (1967) found a significant excess of the R_2 haplotype (cDE) amongst diabetics,the chi squared value,with five

Table 2·8 Investigations of rhesus frequencies (D & d alleles only) in diabetic population

Reference	Diabetics		Controls		Chi square (d.f. = 2)	P.
	D + ve	D - ve	D + ve	D - ve		
Craig and Wang (1955)	684 (83·72%)	133 (16·28%)	1255 (82·67%)	263 (17·33%)	1·08	N.S.
McConnell et al (1956)	401 (80·20%)	99 (19·80%)	N.G.	N.G.	-	stated to be N.S.
Zeytinoglu * (1956)	355 (82·18%)	77 (17·82%)	19,695 (82·79%)	4,095 (17·21%)	0·96	N.S.
Anderson and Lauritzen (1960)	823 (82·96%)	169 (17·04%)	3516 * (81·41%)	803 * (18·59%)	1·11	N.S.
Simpson et al (1962)	85 (85%)	15 (15·00%)	30,279 (84·01%)	5,763 (15·99%)	1·08	N.S.
Buckwalter & Tweed (1964)	943 (81·79%)	210 (18·21%)	1813 (83·36%)	363 (16·64%)	1·31	N.S.

* calculated from frequencies N.G. = not given

degrees of freedom being 18.53 ($p < 0.01 > 0.001$). Scholz et al (1975) found the opposite - an excess of $\underline{R}_1$ (CDe) and r (cde) amongst the diabetics with a deficiency of $\underline{R}_2$, although this was not significant. Buckwalter and Tweed (1962) did not find any significant differences between diabetics and controls.

(e) The Kell system.

The elucidation of the Kell system began in 1946 with the discovery of an antibody responsible for haemolytic disease of the newborn and which was not anti-D (Coombs et al,1946). This was named anti-Kell (anti-K) after the original family and it was not long before the antithetical antibody,anti-k,was found (Levine et al,1949). It seemed that the system consisted of two allele,K and k. Later came the detection of the Kp^a and Kp^b antigens and the realisation that the corresponding alleles (Kp^a and Kp^b) were associated with K and k but not allelic to them (Allen and Lewis, 1957). In 1965 the alleles of the Sutter system (Js^a and Js^b) were seen to be part of the same complex (Stroup et al,1965). It is now suggested that the loci involved are situated on chromosome 6.

Three groups from those listed in table 2.2 tested their subjects with anti-K. No significant differences were found between diabetics and controls. The data from Simpson et al (1962) and Berg et al (1967) is summarised in table 2.9. The data of Scholz et al are not given in their paper. There are no results available for diabetics tested with anti-Kp^a and anti-Kp^b,nor with anti-Js^a and anti-Js^b.

Table 2·9 Diabetics tested with anti-Kell

Reference	Diabetics		Controls		Chi squared (d.f. = 1)	P.
	Kell + ve	Kell - ve	Kell + ve	Kell - ve		
Simpson et al	9 (9·00%)	91 (91·00%)	59 (8·97%)	599 (91·03%)	0·0001	N.S.
Berg et al (1967)	8 (4·65%)	164 (95·35%)	90 (9·00%)	910 (91·00%)	3·639	N.S.

(f) The Lewis system and secretor status.

There are two features which distinguish the Lewis blood group system from those considered so far. Firstly, Le^a and Le^b substances are not produced by the red cells as integral parts of the membrane but are passively acquired from the surrounding plasma (Grubb,1951;Sneath and Sneath,1955 and 1959). Secondly,the Lewis system,ABO and sectretor systems are inter-related.

Anti-Le^a was found in 1946 (Mourant,1946) and anti-Le^b two years later (Andresen,1948). At first,family studies suggested that the Le^a antigen was recessively inherited (Andresen,1948) but it was soon realised that Le (a+b-) individuals were always non-secretors of ABH (Grubb,1948). In fact,individuals homozygous for the h allele at the H locus (the rare 'Bombay' phenotype) have Le^a substance on their red cells even when they are secretors but this does not affect the consideration of European populations.

At the Lewis locus (as yet not assigned to a particular chromosome) two alleles,Le and le,are segregating. The le gene is inactive but under the influence of the Le gene Le^a substance is produced. In the presence of the dominant secretor allele,Se,and at least one H allele at the H locus,most of this Le^a substance is converted to Le^b leaving weakly detectable Le^a in secretions but not on red cells. The secretor locus is also unassigned but is known not to be closely linked to the Lewis locus (Race and Sanger,1975).

Andersen and Lauritzen (1960) found a significant excess of Le^a +ve individuals amongst their diabetics (chi squared 17.15 for one degree of freedom,p less than 0.0005). They took their control data from Jordal (1975) and these subjects were grouped by a different technique and,presumably,by a different antibody,from

the diabetics. This finding suggested a link between diabetes and secretor status but the four series shown in table 2.10 failed to confirm this.

(g) The Duffy system.

The Duffy locus has been assigned to chromosome No.1. The two common European alleles, $\underline{Fy}^{a}$ and $\underline{Fy}^{b}$ are co-dominant. A third silent allele, $\underline{Fy}$, extremely rare in Caucasians, is very common in Negroes giving the phenotype Fy(a-b-) when in the homozygous state. Anti-Fy^{a} was initially reported in 1950 (Cutbush et al, 1950) and anti-Fy^{b} the following year (Ikin et al, 1951).

Only one of the studies referred to in table 2.2 tested for the Duffy system (Scholz et al, 1975) and only anti-Fy^{a} was used. Results are not given but no significant difference between diabetics and controls was found.

2.3 Serum proteins and iso-enzymes.

(a) Haptoglobin.

This alpha-2 glycoprotein present in the serum and capable of binding haemoglobin was first shown to have phenotypic variants in starch-gel electrophoresis by Smithies (1955, and Smithies and Walker, 1956). The two common alleles, $\underline{Hp}^{1}$ and $\underline{Hp}^{2}$, segregate at an, as yet, unassigned autosomal locus.

Several groups have studied this polymorphism in diabetics with conflicting results. Simpson et al (1962), in their study of 98 early-onset diabetics, found a reduction in the 2-1 phenotype and an increase in the 2-2 amongst diabetics. The results of Jörgensen and Hopfer (1967) showed a significant reduction in

Table 2·10 Secretor results in diabetics

Reference	Diabetics		Controls		Chi squared	P
	Secretors	Non-Secretors	Secretors	Non-Secretors		
Doll et al (1961)	85 (83·33%)	17 (16·67%)	303 (78·70%)	82 (21·30%)	1·07	N.S.
Macafee (1964)	387 (62·82%)	229 (37·18%)	306 (64·42%)	169 (35·58%)	0·30	N.S.
Buckwalter (1964	601 (78·25%)	167 (21·75%)	971 (77·00%)	290 (33·00%)	0·44	N.S.
McConnell (1966)	235 (73·90%)	83 (26·1%)	1845 (75·77%)	590 (24·23%)	0·54	N.S.

the frequency of the $\underline{Hp}^1$ allele in the diseased group. Berg et al (1969) found an increase of a similar nature though this was only marginally significant. No significant differences could be demonstrated by Scholz et al (1975).

The most significant demonstration of an association was by Ksenofontor (1974). His series showed a reduction of 2-2 individuals amongst the diabetics and an increase of the frequency of heterozygotes. This paper gives a combined analysis for the haptoglobin and MN loci and shows a significant excess of diabetic individuals heterozygote for the two systems (39% compared with 18.25% of controls). These studies are summarised in table 2.11.

(b) Acid phosphatase.

Hopkinson et al (1963) first reported electrophoretically distinct phenotypes of the phosphotransferase enzyme red cell acid phosphatase. The five phenotypes were designated A,BA,B,CA and CB, determined by three co-dominant alleles $\underline{P}^a$,$\underline{P}^b$, and $\underline{P}^c$. The predicted sixth phenotype,C,was soon reported (Lai et al,1964). The $\underline{P}^b$ allele is the most common in almost all groups studied to date. Populations of European origin tend to show higher $\underline{P}^a$ frequencies than those of non-Europeans and the $\underline{P}^c$ allele,though rare in Europeans is commoner than amongst Europeans.

There has been no study of red-cell acid phosphatase phenotype frequencies amongst diabetics.

(c) Phosphoglucomutase.

Phosphoglucomutase (PGM),which reversibly catalyses the transfer of phosphate from the first to the sixth position of the

Table 2.11 Haptoglobin results in diabetics

Reference	Diabetics			Controls			Chi squared	p
	1-1	2-1	2-2	1-1	2-1	2-2	(d.f. = 2)	
Simpson et al (1962)	13 (13.27%)	39 (39.79%)	46 (46.94%)	85 (13.49%)	318 (50.48%)	227 (36.03%)	4.68	0.10
Ksenofontor (1967)	11 (11.00%)	61 (61.00%)	28 (28.00%)	72 (18.00%)	172 (43.00%)	156 (39.00%)	10.55	0.01
Berg et al (1967)	39 (20.53%)	90 (47.37%)	61 (32.10%)	132 (13.20%)	462 (46.20%)	406 (40.60%)	8.97	0.01
Scholz et al (1975)	89 (15.08%)	289 (48.98%)	212 (35.94%)	328 (13.99%)	1173 (50.02%)	844 (35.99%)	0.50	N.S.

N.S. = non-significant

Data from Jörgensen and Hopfer (1967) not available.

glucose molecule,was found to show inherited,electrophoretically detectable variation by Spencer et al (1964). At least three loci code for this enzyme - PGM_1 (chromosome 1),PGM_2 (chromosome 4) and PGM_3 (chromosome 6). The placenta is the most satisfactory tissue for the study of the PGM_3 locus variants and it is the products of the PGM_1 and PGM_2 loci that may be seen in the electrophoresis of red-cell preparations. Starch-gel electrophoresis demonstrates seven bands,designated a,b,c,d,e,f and g. In the three common phenotypes,the e,f and g bands,representing the products of the PGM_2 locus do not vary. At the PGM_1 locus there are two common co-dominant alleles,$\underline{PGM_1^1}$ and $\underline{PGM_2^2}$ whose products are represented by the a and c,and the b and d bands respectively. The $\underline{PGM_1^1}$ allele is the commoner in most populations so far documented except for some Jewish groups and Finnish Lapps (Mourant et al,1976). There have been no reports of PGM_1 locus frequencies in diabetics.

(d) Adenylate kinase.

Adenylate kinase (AK) is a red cell enzyme catalysing the reversible conversion of two molecules of adenosine diphosphate to a molecule of adenosine triphosphate and a molecule of adenosine monophosphate. Electrophoretic variants of this enzyme were first demonstrated by Fildes and Harris (1966). Two co-dominant alleles, $\underline{Ak}^1$ and $\underline{Ak}^2$ segregate at a locus on chromosome 9. The former allele is the commoner in all populations so far studied. Nothing is known of the distribution of this polymorphism in diabetics.

(e) Adenosine deaminase.

The enzyme adenosine deaminase (ADA) is an aminohydrolase

catalysing the deamination of adenosine to inosine. Electrophoretic variants were discovered by Spencer et al (1968) and two co-dominant alleles $\underline{ADA}^1$ and $\underline{ADA}^2$, segregating at a locus on chromosome 20, give three recognised phenotypes. The ADA phenotype frequencies of diabetics have not been reported.

(f) Esterase D.

The first demonstration that the red-cell enzyme esterase D (EsD) was polymorphic was by Hopkinson et al (1973). Two co-dominant alleles, $\underline{EsD}^1$ and $\underline{EsD}^2$, segregate at a chromosome 13 locus and, of these, the $\underline{EsD}^1$ allele is the commoner in the populations reported on so far, although $\underline{EsD}^2$ is commoner in Asian populations than it is in European (Hopkinson et al, 1973; Welch and Lee, 1974, and Cartwright et al, 1976). There has been no previous report of EsD phenotype frequencies amongst diabetics.

(g) Other polymorphisms.

Diabetic patients have been investigated with regard to the Gm system (Berg et al, 1969; Scholz et al, 1975), the C3 component of complement (Farhud et al, 1972), Gc (Berg et al, 1969; Cleave, 1966; Jörgensen and Hopfer, 1976(a), and Scholz et al, 1975) and for the beta-lipoprotein systems Ag (Blumberg, 1964; Berg et al, 1969), Ld (Berg et al, 1969) and Lp (Berg et al, 1969). Significant associations were detected for Gm and Ag, although those for Gm were conflicting and that found by Blumberg (1964) for Ag was not confirmed by Berg et al (1969).

2.4 The ability to taste phenylthiourea.

The polymorphism for the ability to taste the substance phenylthiourea (also known as phenylthiocarbamide,or P.T.C.) was discovered in 1932 (Fox,1932). It is considered to be determined by the alleles T and t segregating at an,as yet,unassigned locus. The allele T,which confers the ability to taste the substance,is dominant to t.

Two studies,summarised in table 2.12,have shown a significant deficit of tasters in diabetics. This has been found in Caucasians (Terry and Segall,1947) and in Negroes (Terry,1950). The series of Harris et al (1949),drawn from a Caucasian population, failed to confirm this. An association between P.T.C. tasting and diabetes has never been satisfactorily explained and there has been no study on a sample of diabetics sub-divided on meaningful clinical grounds. The possibility exists that diabetics have an altered perception of taste leading to a misclassification of TT and Tt individuals as non-tasters.

2.5 The major histocompatibility complex.

Stimulated by the needs of transplant surgery and by the intensive study of the analogous system in the mouse,knowledge of the major histocompatibility complex (M.H.C.) in humans has increased enormously in the past two decades. The linkage relationships of the complex (situated on the short arm of chromosome 6) and the large number of alleles segregating at the human leucocyte antigen (HLA) loci,which are a part of the complex,are shown in figure 2.1.

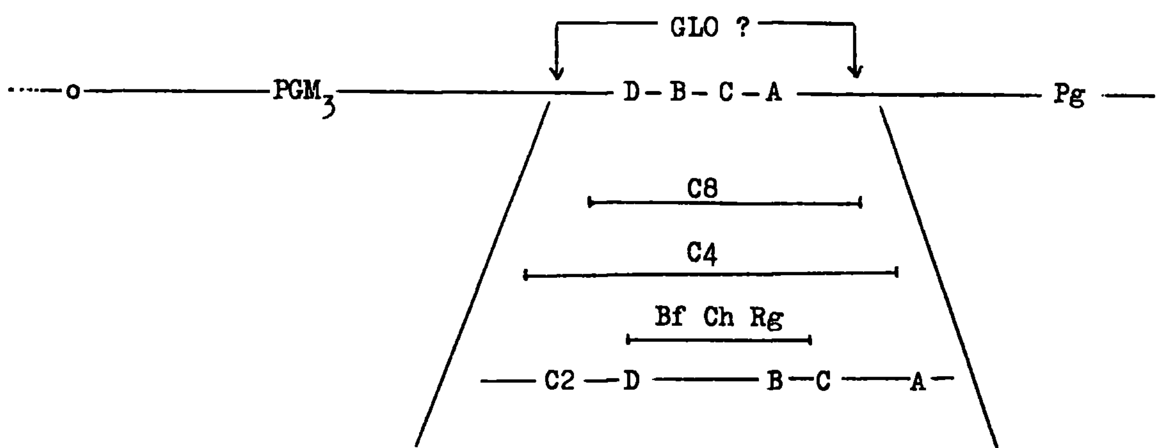
These alleles code for glycoprotein molecules which are integral parts of the cell surface of most cells (Colombani and Degos,

Table 2·12 P.T.C. tasting in diabetics

Reference	Diabetics		Non-diabetics		Chi squared (d.f. = 1)	P
	Taster	Non-Taster	Taster	Non-Taster		
Terry and Segal[+] (1947)	124 (58·77%)	87 (41·23%)	223 (74·09%)	78 (25·91%)	13·32	$<0·0005$
Harris et al (1949)	72 (69·91%)	31 (30·09%)	372 (68·76%)	169 (31·24%)	0·05	N.S.
Terry (1950)	99 (81·82%)	22 (18·18%)	583 (92·25%)	49 (7·75%)	12·93	$<0·0005$

+ Caucasian diabetics and caucasian controls.

Figure 2.1 Linkage relationships of HLA loci (adapted from Bodmer,1976).



Key: o = centromere, PGM_3 = phophoglucomutase locus 3, GLO = glyoxylase, D,B,C,A, = HLA loci, Pg = Pepsinogen, C2,C4,C8 = complement loci, Bf = Properdin factor B, Ch,Rg = Chido and Rodgers blood group loci.

Alleles at each locus:

A	B	C	D
A1	B5	Cw1	Dw1
A2	B7	Cw2	Dw2
A3	B8	Cw3	Dw3
A9	B12	Cw4	Dw4
A10	B13	Cw5	Dw5
A11	B14		Dw6
A28	B18		
A29	B27		
Aw30	Bw15		
Aw31	Bw17		
Aw32	Bw21		
Aw33	Bw22		
	Bw35		
	Bw37		
	Bw39		
	Bw38		
	Bw40		
	Bw41		

1972) and which seem to play a role in the recognition of self and the co-ordination of the immune response (Katz and Benacerraf,1975).

Several features of the system are worth noting. Firstly, the extent of the polymorphism at each locus is prodigious. The A and B loci have the greatest number of recognised alleles but this is probably a consequence of the fact that they have been most intensively studied to date. The number of possible haplotypes at these four loci is very large indeed. There has been considerable speculation on the reasons for this degree of polymorphism. It may be the result of frequency-dependent selection in that individuals possessing the rarer specificities may be favoured if pathogens and parasites are able to mimic the commoner specificities. Under such a system there would be continual pressure for the production of new alleles in higher animals and continual pressure for their copying in parasites and pathogens.

A further characteristic of the system is the linkage disequilibrium present between alleles at different loci. The alleles HLA-A1 and HLA-B8,for example,both occur commonly in European populations. The theoretical frequency of occurrence of the two alleles on the same chromosome (the '1 - 8' haplotype) is the product of their separate gene frequencies. This haplotype occurs much more commonly than this prediction,however,at least in European populations. The magnitude of this linkage disequilibrium is given by the difference between the observed haplotype frequency and the expected haplotype frequency and is known as delta. The value of delta may thus be positive or negative depending on whether the two alleles are associated more or less often than expected. Allele frequencies,haplotype frequencies and delta values exhibit

considerable geographic variation.

The reasons for this linkage disequilibrium and geographic variation are not clear. New alleles may have originated in a particular population and the linkage disequilibrium between these new alleles and other alleles characteristic of those populations may be the result of limited diffusion away from this centre. Alternatively,and more probably,selective forces,as yet undefined,make it advantageous,under certain conditions,to possess certain combinations of these alleles in the cis rather than the trans position (Thomson et al,1976).

Since the first tentative description of an association between a disease and alleles at the HLA loci (Amiel,1967) a large number of such associations have been suggested and subsequently confirmed. In general these associations are more striking than those with the ABO polymorphism.

Several studies have examined the distribution of HLA alleles in diabetics,complete agreement has not been achieved.

The first series (Finkelstein et al,1972) could detect no significant association. A group of 44 diabetic individuals were considered,all insulin-taking. With hindsight it is possible to see that the alleles implicated in the disease (B8 and Bw15) were both present at increased frequencies in the diabetic group. The small sample size probably accounts for the negative result.

The next report (Singal and Blajchman,1973) showed a significant excess of Bw15 individuals amongst the diabetic group although serological difficulties made this conclusion suspect. In 1974 Cudworth and Woodrow and Nerup et al using larger populations of diabetics were able to demonstrate convincing associations

between insulin-dependent diabetes and the alleles B8 and Bw15. The relative risks were 2.0,for B8,and 2.8,for Bw15 (Nerup et al, 1974). A sample of Austrian diabetics (Schernthaner et al,1975) confirmed these associations and suggested an excess of the C locus allele Cw3 in the diabetics.

Expansion of the Liverpool series (Cudworth and Woodrow, 1976) suggested further associations,a positive association between insulin-dependent diabetes and B18 and a negative association with B7. The latter has also been found by Ludwig et al (1976).

An investigation of 75 insulin-taking diabetics by Cathelineau et al (1975) found a significant excess of B8 positive individuals amongst the diseased group but was unable to confirm the association with Bw15.

Most of these insulin-taking diabetics had presented before the age of 30. The only series in which a substantial number of insulin-taking diabetics presenting after this age was studied is that of Cudworth and Woodrow (1976) they were able to report that,in these individuals,the frequency of the B8 allele was significantly increased and that of Bw15,though suggesting an association did not reach significance. Cathelineau et al (1975) found no increase in either antigen in their cases presenting after 35 though there were only 20 of them.

Insulin-taking diabetics in other populations have also been subject to scrutiny. Wakisaka et al (1976) showed a positive association between the disease and Bw22J,a specific Japanese variant of Bw22. The Japanese do not possess the B8 allele but they do possess the Bw15 allele. Interestingly,in the series of Japanese diabetics reported by these authors there were

fewer, though not statistically significantly fewer, Bw15 positive individuals amongst the diabetics than amongst the controls.

Patel et al (1977) report frequencies of the HLA-A and HLA-B locus alleles amongst Caucasian, Mexican-American and Negro diabetics in the United States. The frequencies of B8 and Bw15 were both raised amongst all three racial groups though the sizes of the samples were not sufficient to withstand correction for the number of alleles tested for (see below).

The HLA typing of 100 non-insulin-taking diabetics (Cudworth and Woodrow, 1976) showed no association with any of the HLA alleles.

On the basis of a small series (Nelson and Pyke, 1976) it has been claimed that M.O.D.Y. is not associated with the HLA system. This finding has been disputed in a preliminary report (Barbosa, 1977) which claims an association with the haplotype 3 - w15.

At the A locus significant associations have been demonstrated between early-onset insulin-dependent diabetes and the A1 and A11 alleles (Cudworth and Woodrow, 1976). The association with A1 was shown to be a consequence of the positive linkage disequilibrium with B8. The association with A11 was significant after correction for the number of specificities studied and was also present after pooling the Liverpool data with that of two other centres - Copenhagen (Nerup et al, 1975) and Montpelier (Seignalet et al, 1975). The weighted mean relative incidences of selected HLA alleles from these populations are shown in table 2.14 which has kindly been supplied by Dr.J.C.Woodrow.

Prospective reports on the HLA frequencies in diabetics

Table 2.14 Selected HLA allele results in diabetics (pooled data quoted by Cudworth and Woodrow, 1976)

Allele	X	Chi sq. (sig) d.f. = 1	P	Chi sq. (het) d.f. = z	P
A1	1·41	7·61	0·006	2·52	N.S.
A11	0·44	12·79	$3{\cdot}5 \times 10^{-4}$	1·54	N.S.
B7	0·44	26·39	$2{\cdot}8 \times 10^{-7}$	2·10	N.S.
B8	2·37	48·29	$3{\cdot}7 \times 10^{-12}$	3·01	N.S.
B18	2·02	12·87	$3{\cdot}3 \times 10^{-4}$	1·50	N.S.
BW15	2·04	21·63	$3{\cdot}3 \times 10^{-6}$	0·49	N.S.

are published by Cudworth et al (1977) and Garavoy et al (1977). They have the advantage over the above reports,which are all retrospective,in that they reflect differential susceptibility and are free of any bias possibly resulting from differential survival. The series reported by Garavoy et al was able to confirm the positive association with B8,though not that with Bw15,and demonstrated the seasonal variation in the presentation of new cases of juvenile diabetes. There was no difference in the frequency of new diabetics positive for B8 at any season of the year. The 110 cases reported by Cudworth et al showed an increase of both B8 and Bw15 and suggested clustering of the Bw15 positive individuals in the first three months of 1976. In addition,subjects with this specificity,especially if accompanied by B8,showed higher titres of antibodies to Coxsackie B1 - B4 viruses immediately after diagnosis. There was no correlation between HLA phenotype and the presence of P.I.C.A.

Jansen et al (1975) have suggested that the production of antibodies to exogenous insulin is related to HLA phenotype. They found that the frequencies of both B8 and Bw15 were increased in diabetics taking insulin but that the increase of Bw15 was confined to the group shown to have high levels of anti-insulin antibodies and that the increase of B8 was confined to those diabetics with low levels of antibodies.

HLA-D locus antigens show associations with insulin-dependent diabetes. Thomsen et al (1975) found a significant excess of insulin-taking diabetics positive for the D locus antigen LD-8a (now called HLA-Dw3). This allele is known to be in positive linkage disequilibrium with B8 and the relative risk for developing

the disease considering the D locus allele was considerably higher than that for the B locus allele. There was also an association with LD-w15a (now called Dw6),a D locus allele in positive linkage disequilibrium with Bw15,but the association with this D locus allele was considered to be secondary to that with Bw15.

Control populations in most of the above studies were composed of blood donors,often supplemented by laboratory staff and medical students. The controls were usually stated to be resident in the same geographical area as the patients. In some cases (e.g. Nerup et al,1974,and Wakisaka et al,1976) the exact source of the controls is not given.

2.6 Methodological and statistical difficulties in the search for associations.

The underlying rationale and methodology employed in the search for associations has been severely criticised by Wiener (1956, and 1970). Many of the points raised in this criticism are of great importance,they are concerned with the choice of patients,the selection of controls and the statistical and biological significance of the results.

(a) Selection of patients.

The series listed in table 2.2 and indeed most of the series concerned with other diseases report frequencies in patients collected retrospectively,after the diagnosis has been made. The practical advantages of this approach outweighed,in the minds of the investigators,the theoretical advantages of the prospective approach.

One criticism of the retrospective study is that it introduces unacceptable bias with regard to the selection of patients. More severe cases attending hospital may be included,omitting the less severe cases managed by the general practitioner. Peptic ulcers,for example,that bleed,perforate or give intractable pain will tend to be preferentially included in a retrospective sample of hospital patients.

Several workers have attempted to minimise this problem by including all the patients with a particular disease attending an out-patient department in a set period of time (for diabetics,Craig and Wang,1955;Serra et al,1964,for example) or by taking a 'random sample' of such a population (McConnell et al,1956). The inclusion of individuals in such samples,however,is heavily dependent upon the frequency of attendance. There is no series in the literature on diabetics which includes all the known cases,irrespective of hospital attendance,within a defined geographical area.

In addition,retrospective studies may give a completely false representation of the relationship between a genetic factor and a disease. An increased frequency in the living patients with a certain condition may give the impression that a particular genotype or phenotype is predisposed to the development of the disease when, in fact,it is associated with survival.

Uncertainty with regard to the definition of the disease also creates difficulties and this is especially true for type II diabetes where the definition of abnormality relies upon an arbitary point in a continuous distribution.

Unrecognised heterogeneity within a disease may mask a real association. Two equally common sub-types,for example,both

associated to an equal degree with the O phenotype,one positively and one negatively,would yield a normal ABO distribution if they were both taken together. An association between a polymorphism and a rare sub-type of a disease would be masked by amalgamation with a numerically superior category with no association. Subjectivity in the process of clinical subdivision of peptic ulcer patients has been suggested by Weiner (1956) as influencing the results of investigations concerning the ABO polymorphism.

(b) Selection of controls.

The blood donors commonly used as controls have many advantages. Data on a large number of reliably typed individuals are available for most countries of the world (Kopeć,1970,and Mourant et al,1976). Regional variation is defined to a very fine degree,particularly for the ABO system in the United Kingdom (Kopeć,1970). This obviates the need for typing large numbers of 'normal' people,a category difficult to define and even more difficult to locate.

Since the variance of a gene frequency estimate is inversely proportional to the sample size,large samples give better estimates than small samples although the gene frequencies being estimated by the use of donors are the frequencies in the donor population of the region. Whether these are the same as the frequencies in the general population is a matter for speculation at the present state of knowledge.

Buckwalter and Knowler (1958) have claimed that the presence of professional donors significantly increases the frequency of blood group O in this type of sample. Although this

does not apply to this country it may be influencing the frequencies quoted for other countries. It has also been suggested (Seeff et al, 1975) that blood donors,especially females,show a significant excess of rhesus negative individuals when compared with persons grouped for paternity cases. Kopeč (1970) found that blood donors,at least for ABO and Rh(D) frequencies,did not differ significantly from a large number of Royal Air Force recruits,though this does not mean that either group is representative of the general population.

There are several factors which might be considered important in the matching of controls to diseased persons. The factors which will be considered here are age,sex,area of birth and social class.

Age.

There are few data available on the question of whether gene frequencies change in a population as it ages. The question has been considered from a theoretical standpoint (Hiorns and Harrison,1970) though the model they considered was not directly applicable to blood group data. Most of the data available are concerned with the ABO system and some of them are conflicting. The ideal study - the collection of frequencies for a cohort of births and the prospective examination of these individuals for differential mortality on the basis of genotype - has not been carried out for reasons of practicality,although a study of this type on a limited scale is currently being attempted (Beardmore, personal communication). The nearest substitute for this ideal study is to sample different age groups of an existing population and see whether they differ with regard to phenotype frequencies.

If this difference cannot be attributed to other factors they are indicative of differential survival of individuals on the basis of phenotype.

Jörgensen and Schwartz (1968) have suggested that healthy individuals over the age of 75 years have a significantly higher frequency of O individuals than a population of donors from the same region. They suggest that the O phenotype is selectively favoured by way of resistance to disease,notably neoplasms,which select against the bearers of the A allele.

This finding has been confirmed by van Hoote and Kesteloot (1972) who,in a survey of 42,804 male soldiers between the ages of 16 and 60,found a significantly higher frequency of O and a significantly lower frequency of A in the 55 - 60 year age group than in all the younger age groups. They attributed this to differential mortality and showed that,in men aged 50 - 54, significantly more individuals of the A and AB blood groups had parents who had died before the age of 60,irrespective of cause, compared with the men of the O and B blood groups. They found that serum cholesterol levels tended to be higher in blood group A men and these individuals are known to be more often subject to cardiac infarction (Mourant et al,1971). However,van Houte and Kesteloot (1972) felt that differential mortality from coronary artery disease alone was unlikely to explain these differences. On the basis of these findings it was recommended (Kesteloot et al,1977) that age should be taken into account whenever blood group distributions are compared.

On the other hand,Roberts (1948),in an analysis of 30,000 war-time donors from the south-western counties of England,

found no significant differences in either sex between the mean ages for the four blood groups A,B,O and AB. He concluded that,if membership of any group did confer a selective advantage,as judged by longevity,it was either small or effective outside the age range covered by the data (18 - 60 years).

Buckwalter and Knowler (1958) found no change in the ABO phenotype frequencies with age and concluded that,although some diseases may seem to favour the O phenotype,others select against it making the net effect zero. Since their analysis was confined to individuals under 65 years of age they may have missed changes which occur later in life.

Bennett and Walker (1956) in an analysis of 3,028 East Anglian donors between the ages of 50 and 65 years could show no significant heterogeneity with regard to the A/(O+A) ratio with age although there was a trend for an increase of this ratio with increase in age of the sub-sample.

If blood group frequencies are related to age then the comparison of donor populations with diabetic populations is unsatisfactory. Few data are available for the age distribution of blood donors. The standard text for this country (Kopeć,1970) does not break down the data in terms of age since this would make an already complex analysis impossibly unwieldy. The age distribution of 1,517 unselected Isle of Wight donors (Smith,1977),shown in table 2.15,reveals that the commonest age group is 25 - 29 and that 50% of them are below the age of 35. This is very different indeed from the age structure of a diabetic population (to be discussed in chapter 4).

There are some data on this problem for the HLA system

Table 2·15 Age distribution in a sample of 1517 Isle of Wight donors

Age	No.	%	Cumulative %
under 20	91	6·00	6·00
20 - 25	209	13·78	19·78
25 - 30	258	17·01	36·78
30 - 35	222	14·63	51·42
35 - 40	191	12·59	64·01
40 - 45	149	9·82	73·83
45 - 50	123	8·11	81·94
50 - 55	107	7·05	88·99
55 - 60	93	6·13	95·12
60 - 65	74	4·88	100·00

although,again,there are contradictions. A preliminary report (Bender et al,1973) suggested that individuals heterozygous at both the A and the B loci were more frequent in older people than in controls. Gerkins et al (1974),in an analysis of healthy aged individuals,aged individuals with neoplasms,healthy young individuals and young individuals with neoplasms,found that the healthy aged individuals were more frequently heterozygous at the A and B loci than healthy young individuals and that,when matched for age,healthy individuals were more frequently doubly heterozygous than patients with neoplasms. This was disputed in a second report from the West German group (Bender et al,1976) who could find no significant differences with regard to heterozygosity in their enlarged samples. No convincing differences in the frequencies of individual alleles with age has been reported.

The remarks of Svejgaard et al (1974) on this point are worth quoting in full since,although they refer specifically to the HLA system,they could also be taken as relevant to other polymorphisms:-

> 'Very few studies have,as yet,tried to match patients and controls in respect of all possible variables which might influence the distribution of HLA antigens e.g. age and geographic origin. Race differences are usually taken into account,but considerable variation in HLA antigen frequencies have been found within races (Bodmer,1973). Little is known about age variation in the HLA antigen frequencies. In many studies the control series

included individuals suffering from diseases other than those under study. Often cadaveric kidney donors are included and it is as yet unknown whether brain tumours (in contrast to injuries) for example are associated with HLA antigens. Even healthy blood donors may not reflect the true distribution of HLA antigens in the population. In most European areas the effect of the above variables might be small (but needs investigating) and large differences between patients and controls would be real.'

Sex.

Suggested sex differences in the ABO frequencies (Fisher and Roberts,1943,and Allan,1953) may be related to the phenomenon of age specific mortality. Although donor records register sex,many studies do not include this in their analysis and the question of the relationship between sex,age and polymorphisms remains unanswered.

Area of birth.

For the ABO system,analysis has been carried out on a regional basis in the United Kingdom although the area of residence is considered rather than the area of birth of the donor or his antecedants (Kopeć,1970). As Mourant has pointed out in his foreword to this publication,this has the effect of describing a population a generation later than that described on the basis of birth place. The magnitude of the difference between the

distribution of a polymorphism on the basis of residence and that on the basis of birth place depends on the degree of migration into and out of the area and the localities from which the migrants are drawn. Whether this affects the study of associations with diseases depends on whether the diseased group and the control group are different enough with regard to their geographical and racial origins to show differences in their phenotype frequencies. This possibility seems unlikely at first sight but may apply if the diseased and control groups differ markedly in terms of age or social class both of which are undoubtedly related to social mobility. There are no data to suggest that blood donors form a representative sample of the population with regard to social class.

The variations in the HLA system on a regional basis within national boundaries is almost totally unknown,although,as stated above it is known that antigen frequencies in this system vary considerably within races. Until these variations have been quantified,it seems unwise to compare the frequencies of diseased individuals with the mean frequencies for their race.

(c) Statistical significance.

It has been pointed out (Weiner,1970) that an 'association' said to be significant at the 20% level will occur by chance once in 20 trials and that,with many groups working on polymorphisms and diseases,and positive findings being published in preference to negative,chance associations will be taken for real relationships. Vogel (1970) thought this objection unlikely. The acid test of a disease association is that it can be repeated in several centres

with different series of patients and controls.

The consideration of several genetic factors simultaneously may lead to the demonstration of a spurious association. This has been specifically mentioned in the case of the HLA system where a large number of alleles are studied at the same time (Svejgaard et al,1974) but it is none the less true of other investigations where a number of polymorphisms are examined in the same population. Several means of circumventing this difficulty,at least for HLA, have been suggested (Svejgaard et al,1974) although none take into account the fact that the factors studied are not totally independent (there being linkage disequlilbrium between some of the A and B locus alleles). The most convenient (and most widely employed) method for correcting for this is to multiply the p value for each allele by the number of alleles tested for. This means that only highly significant associations will be revealed and some real, though less marked associations will be lost. This difficulty is overcome by the finding of the same associations in other independent series.

2.7 The biological significance of associations with disease.

There has been no satisfactory explanation of the biological significance of the associations between the ABO system and disease. It would be satisfying if a convincing theory explaining the whole phenomenon were available rather than one explanation for every association.

The nearest thing to this is a theory expounded by Lennes (1961),which,although intended to explain the association with duodenal ulcer,is capable of explaining all ABO - disease

associations on the same principle. Lennes suggested that the development of duodenal ulcer had nothing to do with the ABO phenotype of the individual but that an interaction between the 'ulcer genotype' and the phenomenon of feto-maternal ABO incompatibility was responsible for the association. The presence of the 'ulcer genotype' in the fetus was suggested to make the abortion or failure of implantation of an embryo already incompatible with its mother more likely. Since ABO incompatibility affects O fetuses less often than it does A,B or AB fetuses,the result will be that,of those individuals born with the 'ulcer genotype',more will be O than A,B or AB. After birth the ABO locus has no effect. By extrapolation,the same phenomenon could account for associations with blood groups A or B by postulating a protective effect of the relevant disease genotype with regard to the effects of feto-maternal incompatibility.

It has been pointed out (McConnell,1966) that a prediction of this theory is that associations with ABO should be less marked in those areas where the frequency of the O gene is highest (and feto-maternal incompatibility lowest). In fact, this is not bourne out by the data on diabetes taken from the reports listed in table 2.2. When x,the value of the A/O ratio, is plotted against the value of O/N% for each corresponding control group there is no significant negative correlation demonstrated ($r = -0.072$). This is also true for the comparison of y ($\log_e x$) against O/N%.

The biological significance of the HLA associations is easier to comprehend. By analogy with the mouse,there is likely to be a region of chromosome 6,not far from the HLA loci,

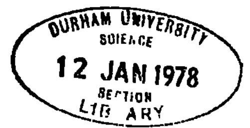
which contains the immune response (Ir) genes (McDevitt and Bodmer, 1974). If this is the case then the associations observed between the HLA alleles and,for example,diabetes,may be due to linkage disequilibrium between the B locus alleles and particular Ir genes which render the individual more likely to develop diabetes. It has been suggested (Nerup et al,1976) that the Ir gene associated with the B8 allele may code for an abnormal T lymphocyte response to certain infections and that this abnormal response results in the auto-immune destruction of insulin-producing cells. The finding of a greater association with the D locus allele Dw3 (Thomsen et al,1976) supports this general theory since the D locus is nearer the hypothetical Ir genes than the A,B or C loci.

Family studies (Cudworth and Woodrow,1975(a)) support the contention that it is not the HLA alleles themselves that are responsible since sib pairs,both of whom are diabetic,share HLA haplotypes more often than would be expected by chance even though these haplotypes may not include the B8,Bw15 or B18 alleles.

It is evident from twin studies (Nelson et al,1975) that different mechanisms are responsible for the association with Bw15. This is supported by the work with the D locus allele Dw6 and the fact that the relative risk for individuals possessing both B8 and Bw15 alleles is equal to the sum of the two individual relative risks (Svejgaard et al,1975) whereas the risks for being homozygous B8 or homozygous Bw15 are no different from having one B8 or Bw15 allele in combination with something else. It has been suggested (Nerup et al,1976) that the Bw15 positive unaffected sibs of diabetic individuals have lower levels of insulin response than do the Bw15 negative unaffected sibs of the same diabetics. It may

be that the Bw15 allele itself is connected with beta cell function.

A considerable amount of data has been presented concerning associations between diabetes and polymorphisms. It is evident, though, that, despite this volume of work, some systems have been examined inadequately, with conflicting results, or not at all. More important, however, is the failure of reported series to explore any effect of age, sex or area of origin in the distribution of phenotype frequencies in the general population which may be exaggerating or masking associations with diabetes.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Chapter 3

The genetic relationship between type I and type II diabetes.

Chapter 3

3.1 Introduction.

3.2 Family studies.

(a) Frequencies of affected relatives.

(b) Studies of heritability of liability.

3.3 A hypothesis for genetic predisposition to type I and type II diabetes.

(a) Propositions.

(b) Predictions.

3.1 Introduction.

The conclusions from the evidence reviewed in the previous chapter are that the major genetic determinants for type I diabetes are present somewhere in the major histocompatibility complex on chromosome 6 and that these are non-contributory in the genetic predisposition to type II diabetes. Although different genetic factors are,therfore,responsible for these two diseases it does not necessarily follow that they are genetically independent and, indeed,the evidence on this is contradictory.

Two things suggest that type I and type II diabetes may be inter-related genetically. Firstly,the finding of families in which both diseases are present,though data of this nature must be subject to rigorous analysis to determine whether such events are commoner than would occur by chance. Secondly,both types of diabetic suffer from cardiovascular complications of the same clinical and histological type,and,if there are genetic factors involved in these changes,it seems possible that type I and type II diabetics share some of these components.

If these sub-types of diabetes are genetically related then it follows that the chromosome 6 determinants involved in type I diabetes are not the only hereditary material contributing to the disease. This possibility has been suggested before (Cudworth and Woodrow,1975,and 1976),to explain the failure of sibs of diabetics to develop the disease when they were exposed to the environmental trigger at the same time as their HLA-identical affected brothers and sisters.

3.2 Family studies.

There have been no studies of the frequencies of type I and type II diabetes in families using an immunological criterion - the presence of P.I.C.A. The difficulties involved in such a study would be considerable,mainly because of the transient nature of these antibodies in the circulation. The closest approaches have been the consideration in families of disease subdivided on the basis of age at diagnosis or current treatment. Of the two the latter is the more acceptable since insulin-taking diabetics are largely type I, although the insulin-independent category may include some diabetics of this type (Irvine,1977).

Analysis of the family data from these studies has been of two types - the consideration of frequencies of affected relatives in families of diabetics and controls and the use of more complex techniques such as heritability of liability.

(a) Frequencies of affected relatives.

MacDonald (1974(a)) analysed family data,obtained by questionnaire,from the relatives of 118 insulin-taking diabetics diagnosed before the age of sixteen and of 108 non-diabetic children admitted to hospital for minor surgery. In the final analysis he used grandparental data only,since he considered this group to be the most likely to manifest the condition if they were genetically predisposed. There was information on 423 grand-parents of diabetics and 395 grandparents of non-diabetics. The groups were of comparable age.

Considering diabetics diagnosed over the age of 45 and irrespective of treatment,the prevalence in the grandparents of

diabetic children was 0.078,that in the granparents of non-diabetic children 0.071,an insignificant difference.

This late threshold of diagnosis was chosen to exclude cases which might have been ambiguous in the classification into early- and late-onset types. Curiously,MacDonald excluded from the control group any children with a relative of any type diagnosed as diabetic before the age of thirty - 'To reduce the incidence of genes which could lead to juvenile diabetes among the families of the controls'. It is unfortunate that the prevalence of non-insulin-taking diabetics amongst the grandparents was not considered rather that the prevalence of late-onset diabetes irrespective of treatment.

Lestradet et al (1972) considered insulin-independent and insulin-dependent subdivisions of diabetes. They studied the prevalences of these sub-types in selected relatives (fathers, mothers,grandparents and parents' sibs) of 1,000 insulin-dependent child diabetics and those of 1,000 controls (paediatric patients without diabetes). They found that 1.55% of the relatives of the diabetic propositi and 1.42% of the relatives of controls had insulin-independent diabetes,concluding that these two sub-types of the disease were genetically independent. In this study the relatives considered were not shown to be of comparable ages and sibs were eliminated from the analysis.

Köbberling (1969),using a different approach,concluded that early- and late-onset were genetically distinct because the prevalence of the former disease amongst the sibs of early-onset diabetics was twenty-five times that amongst siblings of late-onset diabetics. Similarly,Cudworth (1976) concluded that type I and type II diabetes

were 'genetically heterogeneous' because the prevalence of type II diabetics amongst the sibs of type II propositi was 4.5% compared with 0.7% for the prevalence of type I diabetes in these siblings, whereas the prevalence of type II diabetes in the sibs of type I propositi was 0.8% compared with 8.6% for type I siblings of type I propositi. Such statements are meaningless when,as Cudworth himself points out,unexpired risk in these relatives is not considered and the prevalence in the general population is not used for comparison.

(b) Studies of heritability of liability.

The application of techniques for the estimation of heritability of liability to early- and late-onset diabetes has produced ambiguous results. Analysing data from an Edinburgh population,Smith et al (1972) considered that these subdivisions of diabetes were genetically related,in that the 'genetic correlation' between them was 0.65 (Smith,1976). The limiting values for this correlation being zero for no relationship and unity for the same genetic disease.

Analysing a larger body of Canadian data by the same methods,Simpson (1969) reached the opposite conclusion,namely that early- and late-onset diabetes were distinct genetic diseases.

If the methodology is valid it would seem that the only way of explaining these conclusions are that the data sets were different (Simpson,1976). On the other hand,the classification of diabetes in this way is unsatisfactory,since,using diagnosis at 30 years as the division between early- and late-onset diabetes, as will be shown in chapter 8,nearly half of the insulin-taking diabetics in a population will be classified as late-onset cases

along with the non-insulin-taking diabetics. A spurious genetic relationship between the early- and late-onset categories could result from this alone. As suggested by Smith et al (1972) and MacDonald (1974(a)), the model of multifactorial inheritance with an arbitrary threshold may be inappropriate for early-onset diabetes.

3.3 A hypothesis for genetic predisposition to type I and type II diabetes.

The basis of this hypothesis is that the auto-immune reaction to the pancreatic beta cells triggered by the relevant environmental factor in an individual carrying the appropriate Ir genotype, varies in severity and only produces the insulinopaenia characteristic of the type I disease in the most severely affected individuals. The severity of this beta cell injury is determined by genes at other loci which are concerned with the response of all cells of the body to injury. Furthermore, these same genes comprise the whole, or the major part, of the genetic determinants of type II diabetes and of the cardiovascular changes characteristic of diabetic complications.

(a) Propositions.

It is proposed that a polygenic system determines the vulnerability of cells to local injurious factors - traumatic, immunological and metabolic - so that individuals at one end of the consequent normal distribution of genotypes have vulnerable cells with a rapid rate of cell death and a resultant rapid rate of cellular replacement. Individuals at the other end of the range have cells relatively resistant to injury, a slow rate of cell death and a slow

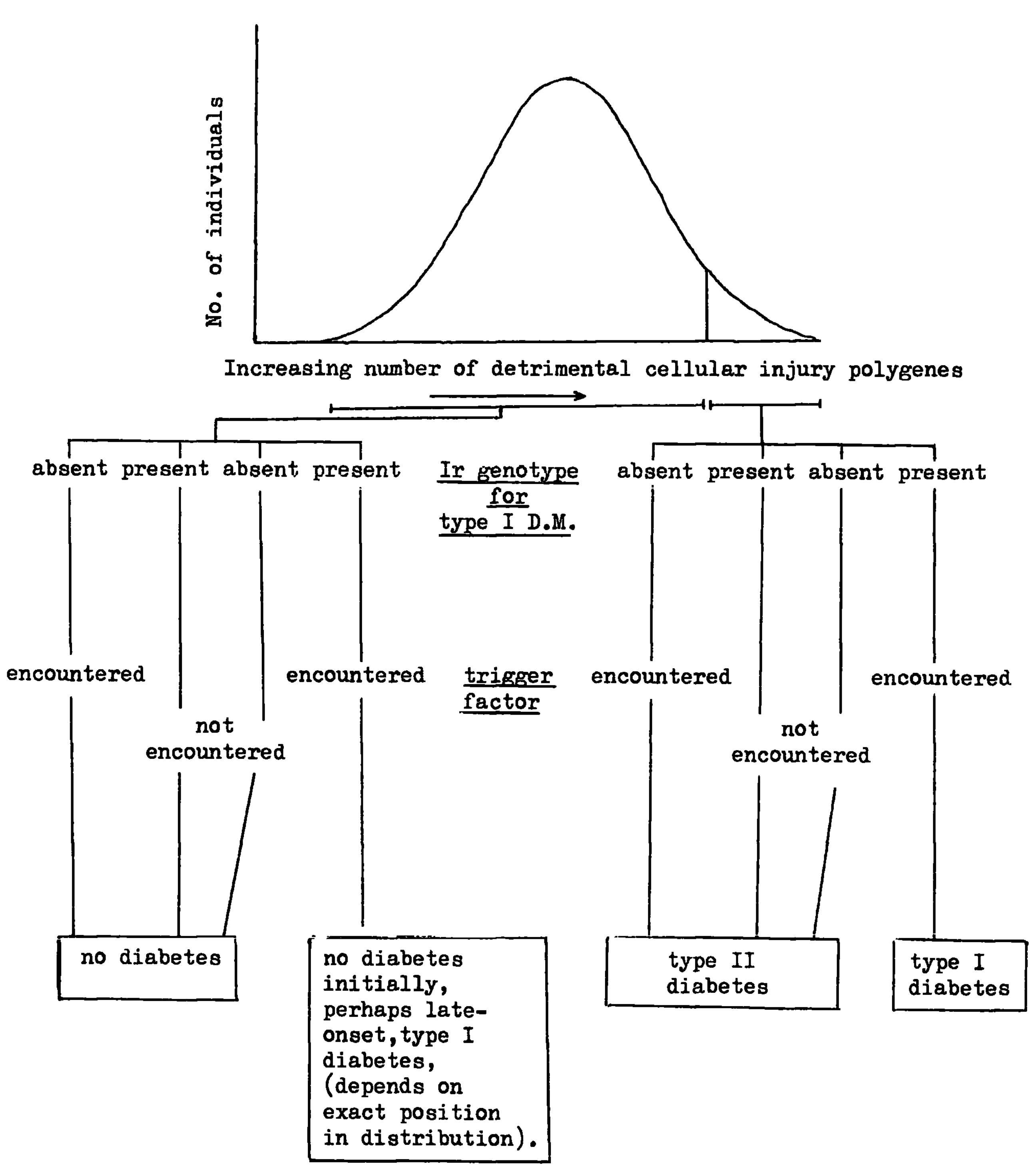
rate of cell replacement. Most people are intermediate (see figure 3.1).

There is evidence from tissue culture (Logethetopoulos, 1972,and Martin et al,1970) that cells have a limited number of regenerative cycles at their disposal,at least in vitro. If this also applies in vivo then individuals with rapid rates of cell death would approach this theoretical limit faster than those with slower rates of cell death.

In the individuals with a high turnover of cells the accumulation of debris around capillaries leads to basement membrane changes in the manner envisaged by Vracko and Benditt (1972). The development of insulin resistance in the type II diabetics is seen as another manifestation of this genetic make-up, either through the sensitivity of cell-surface receptor sites to injury or the presence of large numbers of 'aged' cells - in the sense that they are further removed from their stem cell in terms of mitotic divisions. Thus the major components of type II diabetes, micro-angiopathy and insulin resistance are considered the result of the possession of certain extreme combinations of genes in this polygenic system.

This proposition does not preclude the influence of environmental factors,both acceleratory and retardatory,in the pathogenesis of type II diabetes,given the required genetic make-up. Acceleratory changes may be a diet high in refined carbohydrate with a consequent metabolic insult to the cells in the form of rapid and extreme fluctuations in blood glucose. The development of obesity,frequent pregnancies or relative dietary chromium deficiency are also possible acceleratory environmental influences.

Figure 3.1 Schematic representation of hypothesis for genetic predisposition to type I and type II diabetes.



Deceleratory changes might include near starvation,diets low in refined carbohydrate and high in dietary fibre,low parity and diets adequate with regard chromium intake.

The proposition of such a multifactorial aetiology for type II diabetes fits well with the observed familial incidence of the disease. Groups with an unusually high prevalence of the disease,such as the Pima Indians of North America (Miller et al, 1965) show this prevalence because of their possession of a large number of the appropriate polygenes (perhaps,because,under certain circumstances,they are selectively favoured,or by chance) and because acceleratory environmental influences (in this case the recent adoption of a high carbohydrate,low fibre diet).

The pathogenesis of type I diabetes is more complex in that it involves the simultaneous influence of both the polygenic system outlined above,the relevant Ir genotype and the environmental components of each. When an infection of the appropriate type is experienced by an individual with the required Ir genotype and with 'vulnerable' cells an auto-immune reaction occurs which is sufficient to produce complete,or almost complete, insulin lack either immediately or after an initial,unsuccessful period of compensatory beta cell replication. The initial cellular insult is severe because the beta cells are particularly vulnerable to immunological injury and the recovery process is unsuccesful because the beta cells have already undergone a number of regenerative cell divisions and have a finite number available to them.

When an infection is encountered by an individual not at this extreme end of the polygenic distribution but who,nevertheless,

carries the relevant Ir genotype,there is a reaction against beta cells,with a transient appearance of P.I.C.A. and,perhaps,a short-lived deterioration of glucose tolerance. In these individuals, however,either because the cells are less vulnerable to injury, or because they have a greater regenerative capacity,or both,beta cell function is regained either permanently or for a considerable time,depending on where,precisely,the genotype of the person fits on the polygenic distribution. In the individuals whose recovery is only temporary,glucose tolerance will deteriorate with the insidious onset of insulin-dependent diabetes later in life.

The insulinopaenia and other metabolic derangements of type I diabetes are envisaged as acceleratory contributions to the development of cardiovascular changes but these changes are not possible without the appropriate polygenic background.

(b) Predictions.

The following predictions are generated by this hypothesis and some of them will be tested with family data:-

(i) There should be more individuals with type I diabetes in the families of type II diabetic propositi (and vice versa) than would be expected by chance. Because these two types of diabetes share certain genetic factors viz. the genes for 'vulnerable' cells.

(ii) The frequency of the occurrence of cardiovascular complications in diabetics should be directly related to the family history. The more affected relatives there are and the more closely they are related to the propositus,the more common and the more rapid should be the complications in the index case.

This is because both the density of family history and the development of complications are related to the number of deleterious cellular ageing polygenes present.

(iii) There should be a higher incidence of diabetic individuals in the families of type I and type II diabetics whose disease occurred early in life compared with those index cases whose disease was diagnosed later.

(iv) The hypothesis suggests that there are at least two types of late-onset insulin-dependent diabetic - those with sudden onset who are experiencing the environmental trigger agent for the first time and those with an insidious onset,perhaps with a period of initial insulin-independence,who have experienced the trigger at some time in the past but made a temporary recovery. Both of these types will show an increased frequency of certain HLA-B and HLA-D locus alleles but those of sudden onset should show the seasonal fluctuations characteristic of early-onset insulin-dependent diabetics,have a higher incidence of cardiovascular complications and a greater number of affected relatives. This follows from the fact that those with an insidious onset recovered initially because they possessed fewer cellular injury genes.

(v) Sibs of newly-diagnosed diabetics who share identical HLA haplotypes with their affected sibs will show a transient appearance of P.I.C.A. and a short-lived phase of glucose intolerance at the time of the onset of the disease in their affected sib. Recovery will follow if their component of polygenes is different from that of their sib with diabetes.

(vi) At any one time a certain number of the general population will show positive circulating P.I.C.A.,not all will

become insulin-requiring diabetics.

This hypothesis is similar to that expounded by Lamy et al (1961) in that predisposition to type I diabetes is considered to be the result of a major gene with non-allelic modifiers. It has the disadvantage that it proposes a whole new series of, as yet,unidentified polygenes but there is the advantage that these genes are the same in both types of diabetes.

Chapter 4

The populations studied.

<u>Chapter 4</u>

4.1 Introduction.

4.2 Diabetic subjects.

(a) Diabetics from the 1975 out-patient population.

(b) Diabetics from other sources.

(c) The total diabetic sample.

4.3 Control subjects.

(a) Neighbour controls.

(b) Controls from the orthopaedic wards.

(c) Student controls.

(d) The total control sample.

4.7 Characteristics of the diabetic and control populations taken as a whole.

(a) Place of residence.

(b) Place of birth.

4.4 Data analysis.

4.1 Introduction.

The collection of data for this study took place with the following major objectives in view:-

(i) To compare the distribution of some genetic polymorphisms in a sample of clinically diagnosed diabetics with that in an appropriately matched control group for the sake of clarification in systems already studied and to search for new associations in those hitherto unexplored.

(ii) To test some of the predictions of the hypothesis outlined in the previous chapter using pedigree data from diabetics and controls.

(iii) To examine the pattern of occurrence of new cases of the disease with regard to time of onset,area of residence at diagnosis and the genetic make-up of newly-diagnosed diabetics.

The information on diabetic individuals was collected from those attending the out-patient department of Dryburn hospital, Durham. A group of non-diabetic controls was assembled from several sources.

4.2 Diabetic subjects.

At Dryburn hospital,diabetic out-patients are seen at one of three clinics - during the course of a paediatric out-patient session and at one of two specific adult diabetic clinics, one for individuals up to the age of 65,the other for geriatric patients.

This particular hospital offers several advantages for the conduct of such a survey. Firstly,it is situated near the geographical centre of its catchment area which considerably

eases the logistics of visiting patients at their homes after a preliminary interview at the clinic.

Secondly, the diabetic patients are reviewed unselectively, i.e. there is no tendency to follow up only those patients on insulin or those with poor control, for example, and to discharge others to the care of the general practitioners.

Thirdly, diabetics are gathered together at one clinic and not seen at general medical sessions with other types of patient.

Fourthly, the co-operation of the consultant staff at Dryburn had been secured, both for the study of the diabetic patients and for the study of controls. This last consideration, of course, was the most important of all.

The only disadvantage of the diabetic clinics at this hospital was the lack of separate diabetic notes, which would have facilitated some aspects of the study and lack of accurate figures on the size of the current diabetic population. Using the estimate of 0.64% for the prevalence of clinically diagnosed diabetes (Malins, 1968) and estimating the population of the catchment area to be somewhere around 186,000 (Sutherland, 1974), it was calculated that just over a thousand diabetics should be resident in the area, a large number of these would be attending the local hospital.

It was originally intended that all clinically diagnosed diabetics resident in the area should be investigated. Time and resources did not allow for this. All clinically diagnosed diabetics in the area in 1975 were, in fact, located (see chapter 8) but they were not all contacted.

Diabetics were interviewed at the out-patient clinics

from November,1974,to June 1976. The definition of diabetes employed was that they should have been diagnosed as such by the physicians in charge and that they should be currently receiving treatment. Diagnosis had been made either on the basis of a glucose tolerance test or on the basis of a random blood sugar. Glucose tolerance tests had been judged on the basis of published criteria (FitzGerald and Keen,1962).

At the beginning of the interview the diabetics were told of the nature and purpose of the investigation. Venous blood was taken from those wishing to participate and name,address,date of birth and current treatment noted. Arrangement were made to see as large a number as possible of these individuals at their homes when,during a longer interview,details of family history,mode of onset of the disease,place of residence at the time of diagnosis, past treatment,place of birth of the patient,his parents,spouse and grandparents and details of occupation were ascertained. For each patient the date at which the diagnosis of diabetes was made was the date on the initial referral letter from the general practitioner. For patients whose date of diagnosis was unrecorded this date was ascertained by asking the individual when he had first been told he was diabetic. For individuals not visited at home,the place of residence at the time of diagnosis was noted from the referral letter.

During the home interview,each diabetic was asked to find an acquaintance suitable for study as a control. If necessary, a second sample of blood was taken at this time.

(a) Diabetics from the 1975 out-patient population.

During the course of 1975,936 clinically diagnosed diabetics were seen as out-patients at Dryburn hospital. Of these, 377 (40.3%) were male and 559 (59.7%) were female (sex ratio 0.67). By the first of January,1976,251 (26.8%) of them were being treated with insulin,336 (35.9%) with a combination of oral hypoglycaemic drugs and dietary therapy and 349 (37.3%) with dietary restriction alone. Their age distribution (in terms of age on the first of January,1976) is given in table 4.1.

Of these 936 individuals,693 (74.0%) participated in the study,32 (3.5%) were asked but refused and the remaining 211 (22.5%) were not contacted. These tended to be patients attending infrequently who happened to be missed on the days they were present at the clinic.

Table 4.1 also shows the age distribution of those individuals included in the sample,together with the percentage sampled of those available for each five-year group.

The percentage sampled of those available ranged from 100% (0 - 4 age group) to 52.9% (75 - 79 age group). This was partly a result of the frequency of attendance and partly of the total number available in each age group. Despite this,however, the 693 individuals represent a random sample of the total out-patient population in terms of current age in these categories. After amalgamation of the 0 - 4 and 5 - 9 age groups and the 90 - 94 and 85 - 89 age groups,the chi squared for the comparison of age group membership between diabetics available and diabetics sampled is 10.36 which,for 16 degrees of freedom,gives p between 0.9 and 0.8.

Table 4·1 Age distribution (on 1.1.76) of diabetics attending ; out-patients in 1975 and details of sampling of each age group.

Ages (yrs)	Number available	frequency (%)	number sampled	% sampled of those available	% of total sample
0-4	4	0·4	4	100·0	0·6
5-9	6	0·6	5	83·3	0·7
10-14	11	1·2	9	81·8	1·3
15-19	20	2·1	19	95·0	2·7
20-24	22	2·4	20	90·9	2·9
25-29	13	1·4	10	76·9	1·4
30-34	18	1·9	16	88·9	2·3
35-39	23	2·5	21	91·3	3·0
40-44	40	4·3	34	85·0	4·9
45-49	45	4·8	39	86·7	5·6
50-54	60	6·4	50	83·3	7·2
55-59	89	9·5	77	86·5	11·1
60-64	130	13·9	94	72·3	13·6
65-69	150	16·0	112	74·7	16·2
70-74	146	15·6	96	65·7	13·9
75-79	104	11·1	55	52·9	7·9
80-84	35	3·7	21	60·0	3·0
85-89	17	1·8	10	58·8	1·4
90-94	3	0·3	1	33·3	0·1
TOTALS	936	100	693	74·0	100

Mean age (total population) = 60.38 ($\pm$0.57) years (sample) = 58.08 ($\pm$0.67) years

Minimum, 3.07 Maximum, 92.23

Minimum, 3.07 Maximum, 91.59

Of the diabetics sampled,297 (42.9%) were male and 396 (57.1%) were female (sex ratio 0.75),so that the sample is slightly biased towards males,although this is insignificant when compared with the total out-patient group (chi squared,with one degree of freedom = 1.09).

The numbers in each treatment sub-group in the sample and in the total population are given in table 4.2. Although the sample is biased towards insulin-taking diabetics (who were the more frequent attenders),at the expense of the diet-controlled patients, this difference is non-significant (chi squared = 4.35 for 2 degrees of freedom).

(b) Diabetics from other sources.

In addition to these 693 individuals,28 other diabetics were included,bringing the total to 721. 27 of these were new-comers to the clinic in 1976 and one was a local medical practitioner interested in the venture.

(c) The total diabetic sample.

Table 4.3 shows the total diabetic sample sub-divided into 5 year age groups. Compared with the age distribution of the 1975 out-patient population,there are more young diabetics in the sample. The mean age of these 721 diabetics,on the first of January,1976,was 57.56 (± 0.68) years.

There were 314 male and 407 females (sex ratio 0.77 - insignificantly different from the complete 1975 diabetic clinic). Insulin-taking diabetics comprised 31.9% of the sample (230 individuals,129 male and 101 female),there were 252 (34.9%) treated

Table 4·2 Treatment of diabetics in sample and diabetics in the out - patient population

Treatment	Whole group	sample
Insulin	251 (26·8%)	215 (31·0%)
Oral hypoglycaemics + diet	336 (35·9%)	248 (35·8%)
Diet alone	349 (37·3%)	230 (33·2%)

Table 4·3 Age distribution (on 1.1.76) of all diabetics in the sample

(age)	Number	Frequency (%)	Cumulative frequency (%)
0-4	5	0·7	0·7
5-9	6	0·8	1·5
10-14	12	1·7	3·2
15-19	20	2·8	6·0
20-24	22	3·1	9·0
25-29	11	1·5	10·5
30-34	16	2·2	12·8
35-39	23	3·2	16·0
40-44	37	5·1	21·1
45-49	42	5·8	26·9
50-54	50	6·9	33·8
55-59	79	11·0	44·8
60-64	95	13·2	58·0
65-69	115	16·0	73·9
70-74	98	13·6	87·5
75-79	56	7·8	95·3
80-84	22	3·1	98·3
85-89	11	1·5	99·9
90·94	1	0·1	100·0

Total - 721

Mean age = 57·55 St.dev 18·13

Minimum 3·07 years Maximum 91·59 years

with oral hypoglycaemics (81 males and 171 females) and 239 patients (33.2%) treated with diet alone (104 males,135 females). This distribution with regard treatment is insignificantly different from that in the 1975 diabetic clinic (chi squared = 5.69 for 2 degrees of freedom).

Time and resources permitted 433 of these 721 individuals (60.0%) to be visited at home for further questioning. This represented between 40 and 50% of the current out-patient population in 1976.

4.3 Control subjects.

Control data for such studies as this are often difficult to obtain. Kopeč (1970) gives ABO and Rhesus (D) results for donors from this region but there are few such samples for other systems. Besides,theoretical disadvantages in using donors for comparison with diabetics have been raised in chapter 2. Accordingly,it was decided to collect blood samples from non-donor,non-diabetic individuals resident in the area for comparison with previously published material,investigation for the effect of sex,age and other factors and for comparison with the diabetics.

(a) Neighbour controls.

It was the original intention that the entire control sample should consist of individuals chosen by the diabetic subjects from amongst their acquaintances. By specifying that the diabetics should choose a non-diabetic friend of the same sex,within five years of their own age and living within the same parish,it was hoped that a control sample,matched with the diabetics in terms of age,sex,

social class and area of residence should be built up. The diabetic was to contact a suitable person and report the address of the individual,who would be contacted with a view to a home visit.

In practice this exercise was not a succes since only 74 (17.1%) of the 433 individuals asked were able to find a suitable individual who would co-operate. These 74 people were visited at home and information taken on their date of birth, occupation,family history of diabetes and place of birth of themselves and their antecedants. Of these 74,68 agreed to have blood drawn. A urine sample taken between one and two hours after the largest meal of the day was tested for glucose and,in fact,one individual was found to have glycosuria. Diabetes was confirmed with a glucose tolerance test and he was referred to the diabetic clinic leaving 73 neighbour controls.

There are several reasons for the failure of this part of the exercise. It may be that many diabetics did not appreciate the importance of studying normal individuals for comparison,or were unable,or unwilling,to persuade a suitable individual to take part. Alternatively,some individuals may not have known a suitable person sufficiently intimately.

(b) Controls from the orthopaedic wards.

As soon as it became obvious that the neighbours of the diabetics would be insufficient for a control population other sources of non-diabetics were considered. Many authors have rightly criticised the use of hospital in-patients as controls. They are a highly selected group and may include sufferers from diseases strongly associated with the polymorphisms under study. It was

considered justified,however,to use a sample of patients from the orthopaedic wards if the reasons for admission were traumatic conditions or minor orthopaedic surgery. The reasons for admission of these subjects were mostly head,injuries,fractures etc. (see table 4.4). Care was taken to exclude patients whose current admission was the result of a chronic illness. All patients with rheumatoid arthritis,for example,if this was the reason for their current admission,were excluded,similarly with pathological fractures. Cases of chronic back pain were also excluded.

From these patients details of name,address,date of birth, place of birth and the births of ancestors was taken,together with a sample of venous blood and,in some cases,a family history.

It is the policy of the orthopaedic wards to test all patients for glycosuria and all those included in this study were negative in this respect.

(c) Student controls.

It soon became clear that the controls showed some interesting trends with regard to age,but that insufficient numbers were available in the younger age groups for valid statistical comparison. As a result,students at the University were asked to volunteer as controls and data were collected from them in collaboration with Paul Converse,an M.Sc. student in the anthropology department. The only stipulation was that the volunteers should not be diabetic and that they should be permanently resident in the county of Durham. Forty six controls were collected in this way. Information on date of birth,place of birth of self,parents and grandparents and father's occupation was taken at the same time as 10 mls of venous blood.

Table 4·4 Reasons for admission of orthopaedic controls

Diagnosis	Number	Frequency (%)
Head injury	109	28·09
Fractured Femur (neck or shaft)	60	15·46
Keller's operation	44	11·34
Medical meniscectomy *	40	10·30
Lacerations	21	5·41
Back injuries	17	4·38
Amputation of digit *	14	3·61
Removal of ganglion	12	3·09
Fractured radius and/or ulna	12	3·09
Fractured tibia	10	2·57
Fractured pelvis	8	2·06
Repair of tendon *	7	1·80
Removal of toe nail	6	1·55
Fractured fibula	6	1·55
Patellectomy *	5	1·29
Fractured Ribs	5	1·29
Fractured nasal bones	3	0·77
Eye injury	2	0·52
Dislocated shoulder	2	0·52
Bruising to foot	2	0·52
Removal of bursa	2	0·52
Fractured humerus	1	0·26
Total	388	

* Following trauma

All these students had been screened for glycosuria at their entrance medical, though, in some cases, this was two and a half years prior to the interview.

(d) The total control sample.

The entire control sample from the three sources - neighbours, orthopaedic patients and students - amounted to 507 individuals. Of these, 262 (51.7%) were male and 245 (48.3%) female (sex ratio 1.06). Their age distribution (calculated as of the first of January, 1976) is shown in table 4.5. As can be seen, when compared with the diabetics (table 4.3) there is a deficiency of individuals in the older age groups. This was because the numbers of suitable orthopaedic in-patients in these groups were relatively few. The numbers of controls from each source are summarised in table 4.6.

4.7 Characteristics of the diabetic and control populations taken as a whole.

(a) Place of residence.

Throughout this study, 'old' County Durham is taken as the area of reference as are the 'old' counties of the rest of the United Kingdom. These areas refer to the counties before the reorganisation which took effect in April, 1974. The reason for using 'old' counties is simply that people still think in terms of these boundaries rather than in terms of 'new' county boundaries.

All individuals in the control group were resident within 'old' County Durham. All but four of the diabetics were.

Table 4·5 Ages (on 1.1.76) of all controls

Ages (yrs)	Number	Frequency (%)	Cumulative frequency (%)
0-4	3	0·6	0·6
5-9	2	0·4	1·0
10-14	1	0·2	1·2
15-19	69	13·6	14·8
20-24	57	11·2	26·0
25-29	32	6·3	32·3
30-34	24	4·7	37·1
35-39	25	4·9	42·0
40-44	26	5·1	47·1
45-49	28	5·5	52·7
50-54	31	6·1	58·8
53-59	40	7·9	66·7
60-64	45	8·9	75·5
65-69	33	6·5	82·1
70-74	31	6·1	88·2
75-79	29	5·7	93·9
80-84	22	4·3	98·9
85-89	6	1·2	99·4
90-94	2	0·4	99·8
95-99	0	0·0	99·8
100-104	1	0·2	100·0

Table 4·6 Source of controls

Source	No	% of total	Sex	No	% of total
Neighbours	73	14·40	Male	21	4·14
			Female	52	10·26
Orthopaedic patients	388	76·53	Male	217	42·80
			Female	171	33·73
Students	46	9·07	Male	24	4·73
			Female	22	4·34
Total	507				

(b) Place of birth.

The place of birth (by county) was only ascertained from 391 (54.2%)of the sample of diabetics,since the question was only asked of the 433 who were visited at home and 42 of those asked were unsure. There is no reason to think that this group is unrepresentative in terms of birth place. Of the 507 controls, 476 were asked their place of birth and information was obtained from 420 of them. The places of birth of diabetics and controls are summarised in table 4.7. The differences between the two samples are insignificant (chi squared = 0.188 for 2 degrees of freedom). This large proportion of native-born Durham inhabitants has been noted before (Teasdale,personal communication).

Tables 4.8,4.9 and 4.10 show information on the place of birth of parents,grandparents and the six immediate ancestors of controls and diabetics. There are no significant differences with regard to the parents or grandparents (chi squared values,with 2 degrees of freedom,are 4.2 and 4.7 respectively). With consideration of parents and grandparents simultaneously,there is a significant difference between the two samples with chi squared,for 3 degrees of freedom,equal to 14.03 (p between 0.005 and 0.001).

4.4 Data analysis.

Most of the analysis was performed with the assistance of the I.B.M. 370/167 computer available to researchers at the University of Durham. The raw data were stored in two files,one for clinical and polymorphism data on individuals,the other for pedigree information. Polymorphism data were analysed largely by means of the Statistical Package for the Social Sciences (S.P.S.S)

Table 4·7 Place of birth of diabetic and control samples

Place of birth	Diabetics	Controls
County Durham	326 (83·4%)	346 (82·4%)
Neighbouring counties (Northumberland, Westmorland, Cumberland and Yorkshire)	31 (7·9%)	34 (8·1%)
Other parts of the United Kingdom	34 (8·7%)	40 (9·5%)
Total	391	420

Table 4·8 Comparison of parental birth-place of diabetics and controls.

No.of parents born in Co.Durham	Diabetics	Controls
0	59 (16·5%)	73 (18·0%)
1	67 (18·7%)	54 (13·3%)
2	232 (64·8%)	279 (68·7%)
	358	406

Table 4·9 Comparison of grand-parental birth-places in diabetics and controls.

No. of g.parents born in Co.Durham	Diabetics	Controls
None or 1	51 (21·5%)	70 (21·2%)
2	52 (21·9%)	50 (15·2%)
3 or 4	134 (56·5%)	210 (63·6%)
	237	330

Table 4·10 Comparison of places of birth of six direct ancestors of diabetics and controls

No. of ancestors born in Co.Durham	Diabetics	Controls
None or 1	40 (17.02%)	54 (16·51%)
2 or 3	42 (17·87%)	46 (14·07%)
4 or 5	46 (19·57%)	34 (10·40%)
6	107 (45·53%)	193 (59·02%)
	235	327

with a small number of Fortran programmes for the calculation of gene frequencies etc. The family data were analysed with Fortran programmes written for the purpose. Programmes supplied by other workers for various aspects of the analysis are acknowledged in the appropriate sections.

Chapter 5

Pedigree analysis.

Chapter 5

5.1 Introduction.

5.2 The collection of data.

5.3 Methodological considerations.

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5.14 Subdivision of diabetes on the basis of current treatment.

5.15 Insulin-taking and non-insulin-taking diabetics.

5.16 The separate consideration of oral hypoglycaemic and diet-treated groups.

5.17 Comparison of the prevalence of diabetes in the relatives of diabetics and in the general population.

5.18 Correlations between pairs of first degree relatives.

(a) Age at diagnosis.

(b) Current treatment.

5.19 Correlation between family history and phenotype.

(a) The measurement of the severity of family history.

(b) The assessment of complications.

5.20 Summary and discussion.

5.1 Introduction.

Analysis of pedigree data, the mainstay of classical human genetic studies, has hitherto, revealed little of the mechanism of the inheritance of diabetes except in the case of M.O.D.Y. and the study of HLA haplotype frequencies amongst affected members of sibships. There is a continuing role for pedigree analysis, however, since such an approach may answer several relevant questions notably whether diabetes, subdivided on the basis of current treatment, for example, is a group of distinct genetic disorders or a group of genetically inter-related conditions.

Pedigree data were collected, by the technique of interview questionnaire, from a number of diabetic propositi and a number of non-diabetic controls. The prevalence of the various sub-types in the general population was also assessed. The analysis of the results and a critical discussion of the methodology form the substance of this chapter.

5.2 The collection of data.

Pedigree information was collected during an interview with the propositus or informant conducted by the same interviewer. Diabetic and non-diabetic propositi were seen, in most cases, once and once only between January, 1975, and January, 1977, using the same technique of questioning. Information was recorded for each relative working from the propositus and taking relatives in the same order each time - spouse, parents, children, grandchildren, grand-parents, sibs, sibs' children, sibs' grandchildren, parents' sibs, parents' children and grandparents' sibs. If information was available for more distantly related individuals this was also recorded.

For each relative the following data were noted - sex,relationship to propositus,whether alive or dead,current age or age at death (to within five years),whether affected by diabetes and,if affected,current treatment and age at diagnosis (again to within five years). For each living affected relative the centre at which their diabetes was managed was recorded and,if this proved to be Dryburn hospital,the name and address of the individual were noted.

In the case of children,the informant was always the parent,usually the mother. In these cases a second interview was often required if the necessary information on the relatives of the father could not be furnished.

At each interview the informant was asked to notify the investigator if any previously unsuspected diabetes came to light. Four pedigrees were later ammended because of this.

The information from each family was coded for computer analysis in the method detailed in Appendix B. Relatives were omitted if the current age or age at death were unknown or if there were any doubt about whether they were alive or dead,it was felt that,in these cases,information on the disease state of that particular individual was not reliable. Using a library of Fortran programmes written for the purpose,statistical analysis was performed on various aspects of the data.

5.3 Methodological considerations.

The accuracy of family data obtained on diabetes by the technique of interview questionnaire has been criticised elsewhere (see,for example,MacDonald,1974(a)). The presence of the

interviewer,however impartial he may believe himself to be,may influence the result of the interview by assisting the informant in a particular direction. In addition,it may well be,as MacDonald (1974a) suggests,that diabetics are very much more aware of the disease in their relatives than are non-diabetics and that this is sufficient to bias the results significantly.

The findings of the Edinburgh group (Smith et al,1973) are encouraging in this respect. They checked the information obtained on relatives by contacting a sub-sample directly and re-recording the information directly from the individual concerned. They found that diabetic propositi gave accurate information on the current age and disease status of relatives and that,for the diabetic relatives,age at diagnosis was also reliable. As far as treatment was concerned,all those said to be on insulin were,although two (out of 25) said to be on dietary therapy alone were also being treated with oral hypoglycaemics. There has been no assessment of the information given on diabetic relatives by non-diabetic propositi,however,and this might be expected to be less accurate.

Any mis-recording of affected relatives as unaffected will produce an underestimate of the frequency of the disease. The magnitude of this error will be greater in more distantly related individuals with whom contact is less intimate,and,since diabetics may be more aware of the disease in their relatives,of greater magnitude in the families of non-diabetics than in the families of diabetics. Different types of diabetes may suffer from this phenomenon to differing extents in that insulin-taking diabetic relatives,especially when diagnosed at an early age will be a greater

talking point in a family,with a consequently greater chance of being reported,than older diabetics with less dramatic presentation and treatment. In addition,diabetic propositi with a long history of the disease will have accumulated family information on the condition for a much greater period and may thus be expected to possess greater knowledge of this aspect of their families.

The omission of relatives for whom complete information has not been obtained will lead to the exclusion of proportionally greater numbers of distantly related individuals than close relatives. The effect of this may well be to overestimate the frequency of affected individuals in the more distantly related groups if there is a tendency to report information on affected individuals more assiduously than that on unaffected individuals. Many diabetics,after the diagnosis of their own disease will actively seek information about similarly affected individuals in the family and,since they are interested,not in the frequency of affected relatives,but in individual instances,they may not be given,or not retain,the information about unaffected relatives of a similar degree of relationship.

The very nature of diabetes leads to the possibility that affected relatives,with no symptoms,may be undetected. Several populations have been investigated to assess the extent of this phenomenon and,regardless of the methods used to detect undiagnosed diabetes,have consistently shown two things - that the prevalence of undiagnosed disease is approximately equal to that of diagnosed disease (Malins,1968) and that the prevalence of undiagnosed diabetes rises with age. It is reasonable to assume that,at any one time,the cases of undiagnosed diabetes

will be of type II and not type I.

MacDonald (1974a) has suggested that the immediate families of diabetic propositi will contain fewer undiagnosed diabetics than the immediate families of control propositi since greater awareness of the disease in the former group will have resulted in the screening of relatives for the presence of the disease. In his sample of diabetics and their families resident in the United States, twice the percentage of parents of diabetic children had been previously investigated for the disease than the parents of non-diabetic controls. This is less likely in this country where there is less emphasis on routine screening of relatives.

5.4 Families of individuals attending the diabetic clinic.

The number of individuals attending the diabetic clinic for whom pedigree information was obtained totalled 433. Of these, 180 were male and 253 female. Adult diabetics were all interviewed at home after a preliminary encounter in the clinic. Children were seen together with a parent in the paediatric clinic although, if a second interview were necessary, this usually took place at home. The total number of relatives of all types for whom sufficient information was obtained for inclusion in the analysis was 13,811. These individuals - propositi divided according to sex, current treatment and age at diagnosis, relatives divided according to sex - are shown in table 5.1.

Of these 433 propositi two were classified as latent diabetic. One of these was shown to have glycosuria during her first pregnancy with reversion to normal glucose tolerance after

Table 5.1 The families of diabetic propositi

Propositi			Relatives		
Type of propositus	Male	Female	Male	Female	Total
Treated with insulin,age at diagnosis under 30.	51	41	1664	1662	3326
Treated with insulin,age at diagnosis 30 or over.	36	33	1020	1130	2150
Treated with oral hypoglycaemics, age at diagnosis under 30.	1	0	14	15	29
Treated with oral hypoglycaemics, age at diagnosis 30 or over.	36	95	1885	1992	3877
Treated with diet,age at diagnosis under 30.	0	7	128	127	255
Treated with diet,age at diagnosis 30 or over.	55	76	1919	2174	4093
'Latent diabetic'	1	1	36	45	81
Totals	180	253	6666	7145	13811

delivery. The other was shown to have glycosuria at a routine medical examination but this could not be confirmed and his glucose tolerance test was normal. These two families constituted too small a group to be considered separately and,since they could not be amalgamated satisfactorily with any other group,were eliminated from further analysis.

The remaining 431 families yielded information on 13,730 relatives. First degree relatives (parents,offspring and sibs) comprised 3,114 individuals. Second degree relatives (grand-parents,grandchildren,parents' sibs and sibs' children) amounted to 5,847 and third degree relatives (great-grandparents,great-grand-children,parents' sibs' children and sibs' grandchildren) 3,148. There were 344 spouses,35 half-sibs and 1,242 individuals more distantly related than third degree. Tables 5.2,5.3 and 5.4 show the numbers of various types of relatives amongst the families of various categories of propositus.

The policy of omitting relatives for whom adequate information has not been obtained leads to the artificial reduction in the numbers of relatives more distantly related than second degree. This is least marked in the families of insulin-taking diabetics presenting before the age of thirty,since,in the majority of cases,the informant was the parent,to whom the propositus' third and fourth degree relatives were second and third degree relatives. In most other cases,however,the lack of information about these more distantly related individuals resulted in a small proportion only being suitable for inclusion. In the final analysis,the relatives in these more distant categories were discounted,as explained below.

The spouses of propositi,in theory an attractive source

Table 5.2 First degree relatives of diabetics

Propositi		First degree relatives			
Type	No.	Parents	Offspring	Sibs	Total
Treated with insulin,age at diagnosis under 30.	92	182	51	186	419
Treated with insulin,age at diagnosis 30 or over.	69	135	135	224	494
Treated with oral hypoglycaemics, age at diagnosis under 30.	1	2	0	2	4
Treated with oral hypoglycaemics, age at diagnosis 30 or over.	131	251	342	479	1072
Treated with diet,age at diagnosis under 30.	7	14	5	21	40
Treated with diet,age at diagnosis 30 or over.	131	246	303	536	1085
Totals	431	830	836	1448	3114

Table 5.3 Second degree relatives of diabetics

Propositi		Second degree relatives			
Type	No.	Grandparents	Grandchildren	Others	Total
Treated with insulin,age at diagnosis less than 30.	92	306	4	683	993
Treated with insulin,age at diagnosis 30 or over.	69	130	131	724	985
Treated with oral hypoglycaemics, age at diagnosis under 30.	1	4	0	4	8
Treated with oral hypoglycaemics, age at diagnosis 30 or over.	131	193	529	1131	1853
Treated with diet,age at diagnosis under 30.	7	24	0	77	101
Treated with diet,age at diagnosis 30 or over.	131	204	405	1298	1907
Totals	431	861	1069	3917	5847

Table 5.4 Other relatives of diabetics

Propositi		Relatives			
Type	No.	Spouses	Half-sibs	Third degree	Fourth degree etc.
Treated with insulin,age at diagnosis under 30.	92	34	2	966	915
Treated with insulin,age at diagnosis 30 or over.	69	65	2	518	86
Treated with oral hypoglycaemics, age at diagnosis under 30.	1	1	0	9	4
Treated with oral hypoglycaemics, age at diagnosis 30 or over.	131	119	18	721	94
Treated with diet,age at diagnosis under 30.	7	4	0	64	46
Treated with diet,age at diagnosis 30 or over.	131	121	13	870	97
Totals	431	344	35	3148	1242

of controls,had to be omitted from the analysis,since there was a tendency for conjugal diabetic pairs to be visited in preference to families where only one spouse was affected. Similarly,the group of 35 half-sibs were ignored,since,genetically speaking,these have to be considered in a separate category,between first and second degree relatives and there were too few for separate treatment.

5.5 The use of control data.

The occurrence of diabetes in the families of diabetic propositi may be compared either with the occurrence of the disease in the families of non-diabetic controls (as in the studies of Lestradet et al(1972)and MacDonald (1974(a)),or with estimates of the prevalence of the disease in the general population (see,for example,Simpson (1964),and Smith et al (1973)). The former permits the consideration of living and dead relatives,using the age at death as equivalent to the current age. In the comparison of the disease in affected relatives with the prevalence in the general population, living relatives only may be considered,since the general population figures deal only with living individuals. This method eliminates any inaccuracies due to changing attack and detection rates inherent in the former method,in which three or four successive generations are regarded as contemporaneous,but it is limited by the laboriousness and potential inaccuracies involved in a large-scale estimation of the numbers of diabetics in the community. In this account both of these methods are employed and the results compared.

A distinction will be drawn between the term 'frequency' of affected individuals in the sample of living and dead relatives and the term 'prevalence' which has the conventional meaning of the

proportion of the living population that are currently affected.

5.6 The families of non-diabetic propositi.

It was intended that all control data should be collected from the neighbours of the diabetic propositi. In practice,however,only 66 such families presented themselves and the group was supplemented by pedigrees of 35 orthopaedic patients giving a total of 101 control families. Of these,the propositi in 43 were male,in 58 females. The age distribution of the propositi is given in table 5.5 and the reasons for admission of the 35 orthopaedic patients in table 5.6.

These 101 propositi gave information on 3,520 relatives. The number of relatives in each category is shown in table 5.7. The fall-off in the numbers of relatives more distant than second degree is seen again in the case of these control families and is again the result of omission of distant relatives with insufficient information. In the case of controls,spouses were a useful addition to the group and both these and half-sibs were utilised when the relatives of all types were pooled.

5.7 'Doubtful diabetics' amongst relatives.

During the interviews a number of relatives were discussed whose diabetic status was uncertain. These included relatives shown to have glycosuria of pregnancy whose current disease status was unknown,or relatives thought to have glycosuria detected during a terminal illness but in whom details of the disease or treatment were unknown. These individuals (39 in the families of diabetic propositi and 3 in the families of controls) posed

Table 5.5 Age and sex distribution of propositi of control families

Age group	No. of propositi	
	Male	Female
0 - 19	5	5
20 - 39	14	18
40 - 59	18	17
60 and over	6	18
Totals	43	58

Table 5.6 Reasons for admission of orthopaedic patients

Diagnosis	Number
Medial meniscectomy	9
Head injury	7
Keller's operation	4
Amputation of digit*	4
Lacerations	3
Patellectomy*	2
Fracture of tibia	2
Removal of ganglion	1
Removal of toe nail	1
Repair of tendon*	1
Back injury	1
Total	35

* Following trauma

Table 5.7 Relatives of control propositi (101 families)

First degree				Second degree			
Parents	Offspring	Sibs	Total	Grandparents	Grandchildren	Others	Total
199	140	339	678	266	76	1075	1417

Spouses	Half-sibs	Third degree	Fourth degree etc.
80	8	959	378

something of a problem. In the end they were ignored completely in the analyses because their status was so ill-defined so that, if a number of them were indeed clinically diagnosed diabetics, the estimates of prevalence given are slightly under-estimates.

5.8 Individuals ascertained through two propositi.

The practice of recording the names and addresses of living, affected relatives being treated at Dryburn Hospital enabled the number of relationships between the 532 (431 diabetic and 101 non-diabetic) propositi to be determined. There were twelve pairs who were related. In ten of these cases diabetic propositi were related to other diabetic propositi. In two cases non-diabetic propositi were related to diabetic propositi. The number of pairs present for each degree of relationship is shown in table 5.8.

Examination of the pedigrees of these 24 individuals revealed that, for 188 relatives, information had been obtained twice, once from each propositus. This afforded the opportunity for assessing the measure of agreement between the information obtained for the same persons through two informants but also posed the problem of whether these individuals should be included once or twice in the analysis.

(a) Measure of agreement in information obtained.

In 142 instances (75.5%) the information derived from both informants on these common relatives was identical. When the disagreement regarded current age or age at death, in all but one case the estimates of age were within five years of eachother. In one instance (0.7%) there was disagreement on the disease state

Table 5.8 Numbers of pairs of related propositi for each degree of relationship

Degree of relationship	Number of pairs
First degree	4
Second degree	3
Third degree	4
Fourth degree	1
Total	12

of a relative and,in this case,a genuine diabetic had been reported as non-diabetic by one propositus. There were 23 cases in which common relatives were reported as affected by both propositi and, in all but two,there was agreement on the age at diagnosis,and,in all but three,agreement on the current treatment. In the three cases of disagreement regarding treatment the debate was between oral hypoglycaemics and diet not regarding insulin-taking or non-insulin-taking. This accords with the findings of Smith et al (1972) in that information gained from diabetic propositi on the disease status is reliable,especially if age and age at diagnosis are considered only to the nearest five years and treatment is considered as either insulin-taking or non-insulin-taking.

(b) The case for double inclusion of common relatives.

In cases where ascertainment of related propositi is independent,i.e. the ascertainment of the second case is not the result of their relationship with the first,Falconer (1967) has advocated that common relatives be included twice. Since,in this instance,ascertainment of the second propositus was by virtue of their attendance at Dryburn Hospital and not the result of the relationship with the other propositus,it was felt justifiable to include these 188 common relatives twice in the analyses.

5.9 The frequency of diabetes of all types in the families of diabetics and in the families of non-diabetics.

Ignoring the doubtful diabetics,the frequencies of diabetics of all types in the various relatives of diabetic and non-diabetic propositi are summarised in table 5.9(a) and table

Table 5.9(a) Diabetics in the families of diabetic propositi

Type of relative	No. affected	Total relatives	Frequency (%)
First degree	153	3098	4.94 (±.39)
Second degree	128	5837	2.19 (±.19)
Third degree	57	3141	1.81 (±.24)
Fourth degree etc.	42	1236	3.40 (±.51)
All relatives	380	13312	2.85 (±.14)

Table 5.9(b) Diabetics in the families of control propositi

Type of relative	No. affected	Total relatives	Frequency (%)
First degree	8	678	1.18 (±.41)
Second degree	16	1416	1.13 (±.28)
Third degree	6	958	0.63 (±.26)
Fourth degree etc.	3	377	0.80 (±.46)
All relatives	33	3429	0.94 (±.17)

5.9(b) respectively. Sex and unexpired risk are not taken into account. These frequencies are represented graphically in figures 5.1(a) and 5.1(b). The standard errors of these and all other frequencies quoted in this chapter are calculated from the formula:-

$$\text{standard error} = \sqrt{\frac{p(1 - p)}{N}}$$

where p is the frequency and N the number of individuals on which it is based.

The families of diabetic propositi have significantly higher frequencies of affected relatives in all four categories of relationship at this crude level of analysis. For first degree relatives, chi squared is 19.25 ($p < 0.0005$), for second degree relatives 6.63 ($p < 0.025$) and for third degree relatives 6.85 ($p < 0.01$) - all chi squared values are for one degree of freedom. The frequency of affected relatives of diabetic propositi in the fourth and subsequent degrees of relationship is also significantly greater than that in controls. The frequency of affected relatives in these more distant relatives of diabetic propositi, however, is significantly greater than that in second and third degree relatives. This increase in frequency in the distant relatives is inexplicable in genetic terms and may be the result either of the small number of such relatives (1,236) or the consequence of the inclusion of affected distant relatives in preference to unaffected because of lack of information on the latter. These more distantly related individuals in the families of diabetic propositi are excluded from further analysis.

In the families of non-diabetic propositi the degree of

Figure 5.1(a) Frequencies of diabetics in the families of diabetics

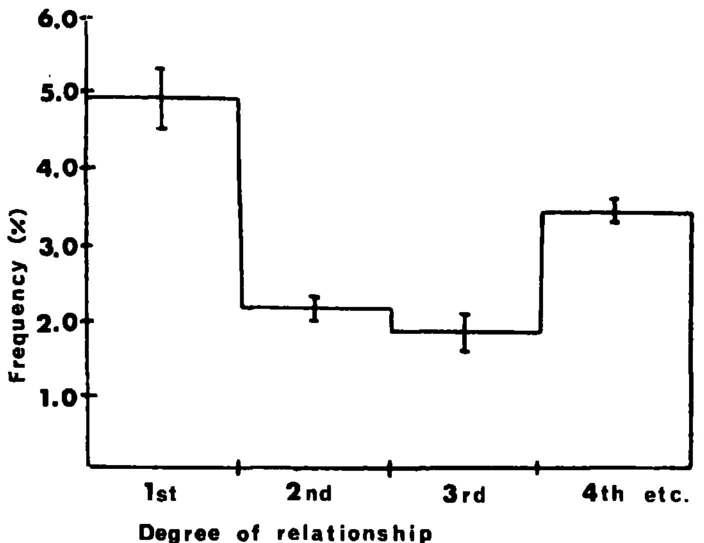
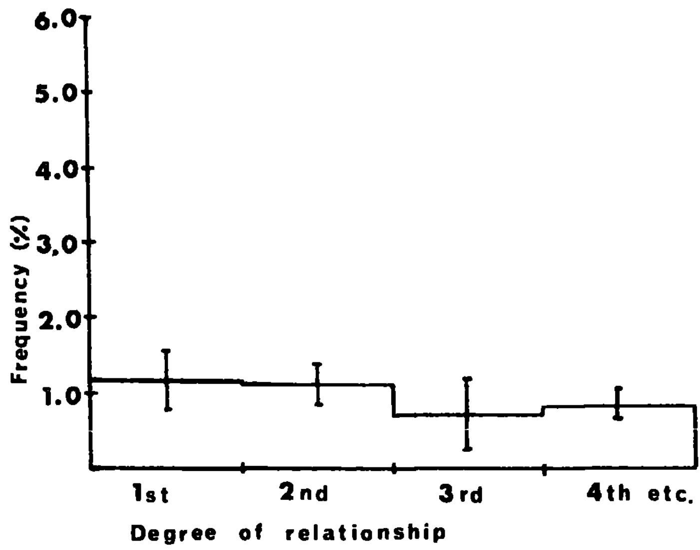


Figure 5.1(b) Frequencies of diabetics in the families of controls



Percentage frequencies are shown ± one standard error.

relationship to the propositus makes no detectable difference to the proportion of individuals that are affected. In figure 5.1(b), for example, the four categories of relatives, divided according to degree of relationship, are homogeneous with regard to their frequencies of affected individuals (chi squared = 2.00 for three degrees of freedom). The possibility that the close relatives of individuals chosen because they are non-diabetic should show a reduced frequency of affected individuals (Falconer, 1967) is not demonstrated in this sample. In the subsequent analysis the control population considered is the pooled sample of all living and dead relatives of the non-diabetic propositi including their spouses and half-sibs. This group consists of 3,517 individuals, 33 of whom were affected, a frequency of 0.94%. This figure is higher than the prevalence in the general population quoted by Malins (1968) and that found in this study (0.77%) because of the inclusion of dead relatives.

5.10 Diabetics diagnosed before the age of thirty and not taking insulin.

Out of the 936 diabetic individuals attending Dryburn out-patient departments during 1975, nine (0.96%) had been diagnosed before the age of thirty and were not being treated with insulin. This group represents 6.9% of the total number diagnosed before this age and 1.4% of non-insulin-taking diabetics in the sample. In view of the possibility that some of these individuals might correspond to the M.O.D.Y. type of diabetic, a particular interest was taken in this group.

Seven of these nine were contacted and a full family

history obtained,one refused to participate and one had left the area by the time she was contacted for a home visit. A further family was added to this group in 1976 making eight pedigrees which can be analysed in detail. In every case but one the proband through whom the family was ascertained was female. The one male index case had been diagnosed in 1973 and had been treated with oral hypoglycaemic agents since then. Six of the female index cases had been known diabetics for at least four years and had been treated with dietary restriction alone since diagnosis. In the seventh female,diagnosed in February,1976,there has been no need for insulin or oral hypoglycaemic treatment,at least until the time of writing (July,1977). It was considered that,although some of these families might correspond to the M.O.D.Y. type of diabetes,in others,the proband could be of the type II variety with a particularly early onset or a type I diabetic controlled on oral hypoglycaemics.

Figure 5.2 shows the pedigree of the only male propositus in this group. Presenting in June,1973,with thirst and polyuria,he has been treated so far with tolbutamide. There are no other recorded cases of diabetes of any type within the family. In view of the mode of presentation and the absence of family history,it is likely that this individual is of the type I variety initially controlled with oral hypoglycaemics. If this is correct he should progress to insulin treatment at some stage.

Two of the female cases did not have affected parents or grandparents,their pedigrees are shown in figures 5.3 and 5.4.

The proband of the family shown in figure 5.3 was found to be diabetic in August 1972 when she was being investigated for a rash by her general practitioner. She had no symptoms of diabetes

Legend for figures 5.2 to 5.9 and figure 5.19.

Symbol	Meaning
□ ○	Unaffected, male and female
■ ● (arrowed)	Proband
■ ●	Affected, male and female
◧ ◐	Glycosuria found on at least one occasion, not clinically diabetic at present
■ ● I	Diabetic on insulin
■ ● O	Diabetic on oral hypoglycaemic agents
■ ● D	Diabetic on dietary therapy alone
?	Details unknown

Current age or age at death for each individual included is known but not displayed in the figures. Details considered to be relevant to the discussion are given in the text.

For ease of drawing, the birth order of sibs has not been strictly adhered to.

Figure 5.2

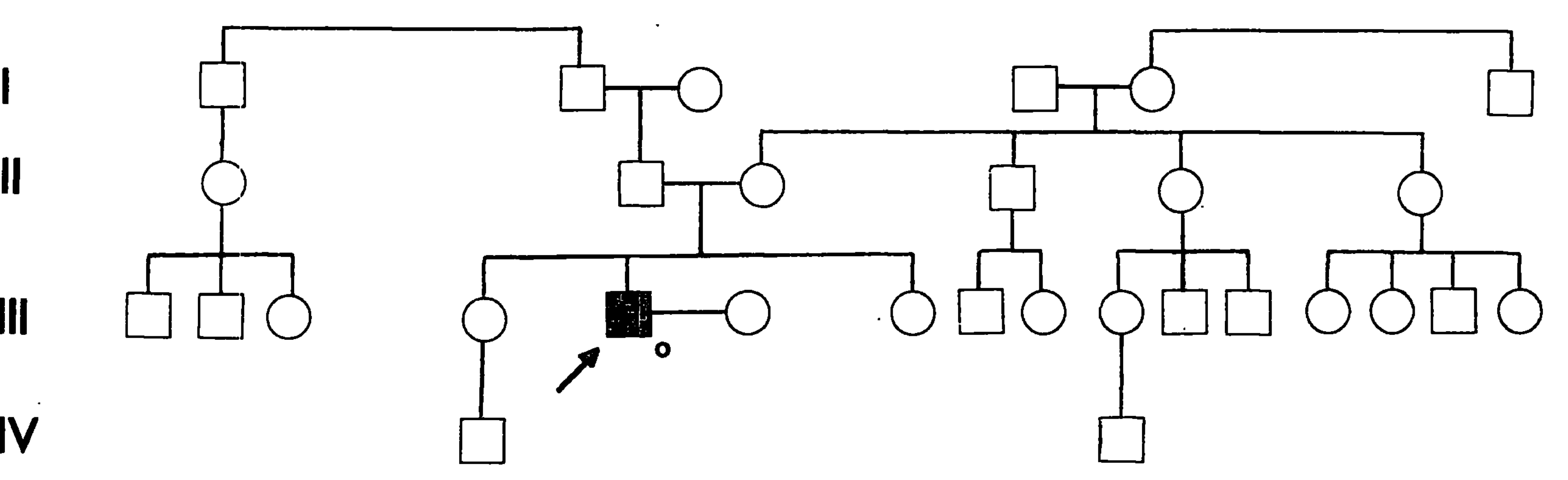


Figure 5.3

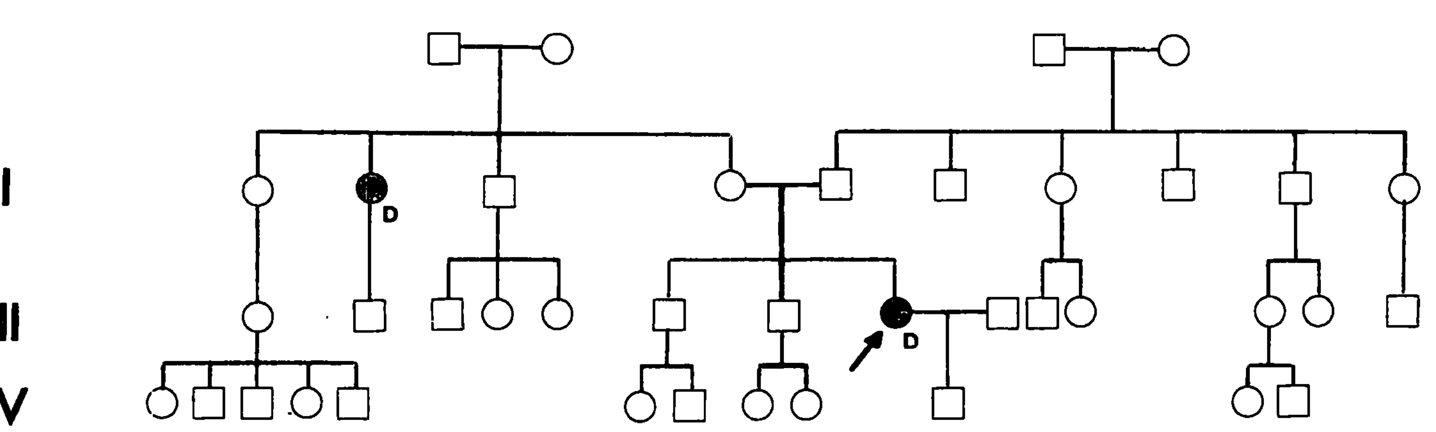
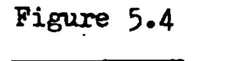
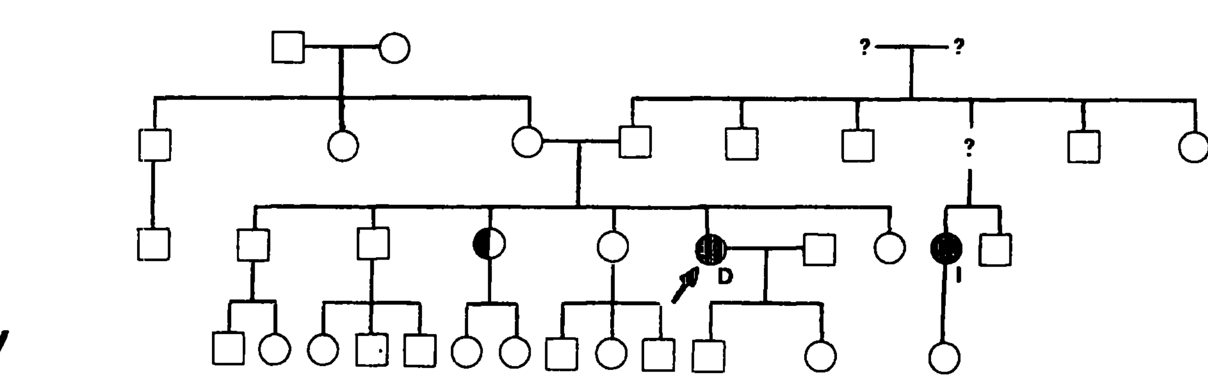


Figure 5.4





but showed a diabetic response in the glucose tolerance test performed on referal to hospital. She has no affected sibs and her one pregnancy was uneventful. The status of the father is unknown but the mother has been investigated for the disease (by urinalysis) by the general practitioner during the last year and found to be negative. There is no reliable evidence concerning the health of the grandparents when they were alive but neither is there any definite evidence of diabetes in them,or,for that matter, in any other relative except a maternal aunt. Discounting the possibility that the father and a paternal grandparent were affected but undetected,this pedigree does not fulfill the criteria for the M.O.D.Y. type of diabetes.

The proband of the family shown in figure 5.4 was found to have glycosuria during her first pregnancy in February 1970. The subsequent glucose tolerance test was abnormal and she has been treated with dietary restriction alone since diagnosis. This has provided sufficient control for two uneventful pregnancies. In this case,both the parents have been tested within the last two years and are known not to have glycosuria. One sib of the proposita has had glycosuria of pregnancy with a normal glucose tolerance test and a maternal first cousin has had diabetes since the age of four years,treated with insulin throughout. There is no evidence that this individual is of the M.O.D.Y. type excepting the possibilities of a mutation in her or of the non-manifestation of the condition in one of the parents,a possibility which runs counter to the published reports of the disease.

It is suggested that,in these two cases,the disease is not of the M.O.D.Y. variety but may be type II diabetes of particularly-

early onset.

In the other five families in this group there was evidence suggestive of M.O.D.Y. type inheritance. Their pedigrees are shown in figures 5.5 to 5.9.

On the paternal side of the family shown in figure 5.5 there is an affected relative in each of three successive generations. The proposita was diagnosed as diabetic following a routine medical examination for entry into Durham University when she was aged 18. There were no symptoms at that time and she has been adequately controlled on dietary restriction alone for the last three years. Her father and paternal grandfather are both diabetic and both are treated by dietary means. They were detected in a similar fashion with symptomless glycosuria though at rather greater ages than the proband (father 35 years,grandfather 67). There are no other members of the family affected though the only others specifically investigated (by urinalysis) have been the sibs of the proband.

In the family shown in figure 5.6 there is again an affected member in three cosecutive generations. The proband was found to have glycosuria of pregnancy with a diabetic glucose tolerance test. There were no symptoms and control has been satisfactory for two years without insulin or oral hypoglycaemics. Her father and paternal grandmother were both detected with symptomless glycosuria at the age of 47 and 60 respectively. They were both initially treated with diet alone but the father received oral hypoglycaemic treatment two years after diagnosis, He died at the age of 52 with coronary artery disease. The status of the paternal grandfather is unclear,he was found to have glycosuria during his terminal illness. There is no evidence that either of

Figure 5.5

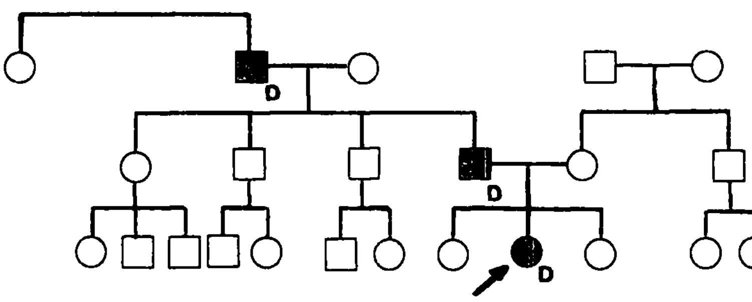


Figure 5.6

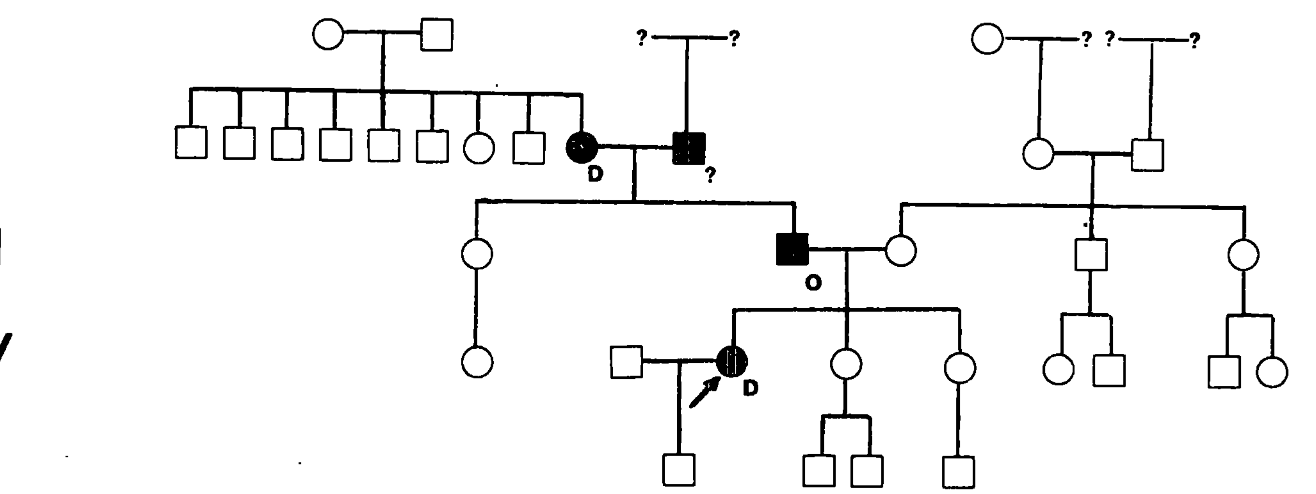


Figure 5.7

I
II
III
IV
V

?
D
?
D
D

Figure 5.8

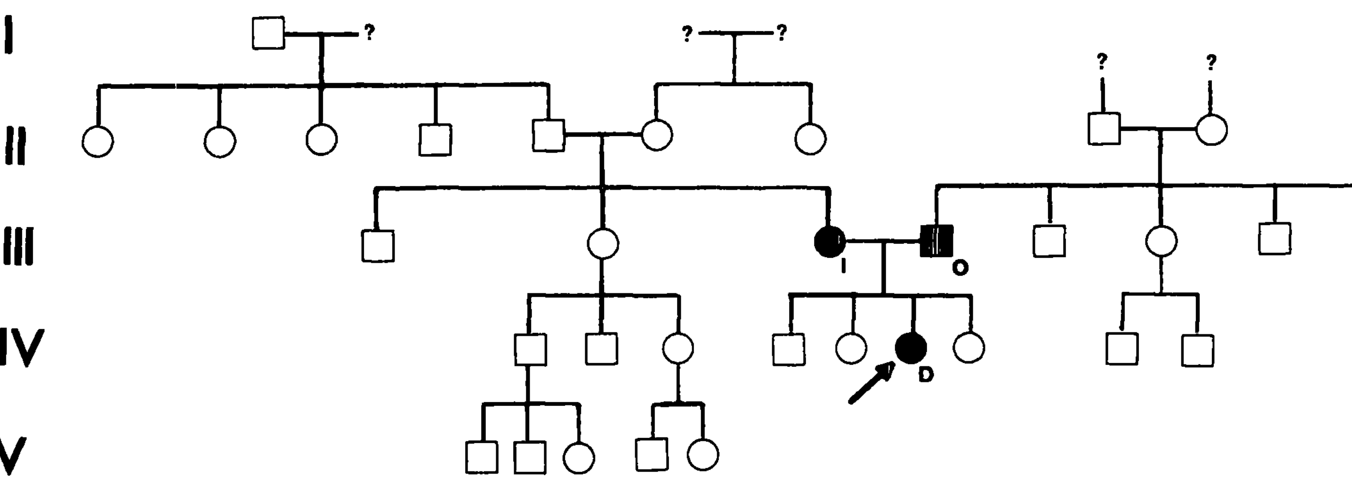
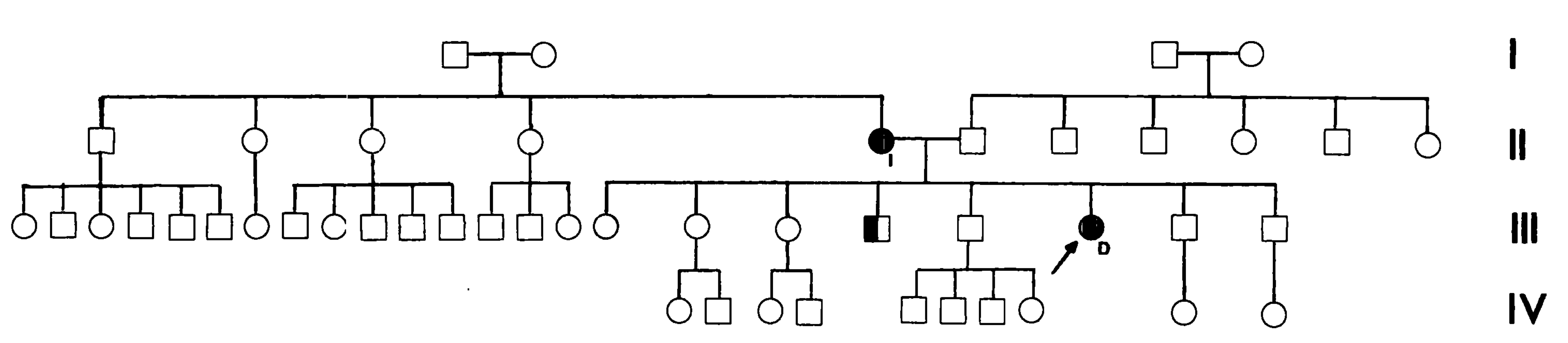


Figure 5.9



the paternal grandmother's parents had diabetes.

The proposita of the family shown in figure 5.7 was found to have glycosuria at a school medical examination at the age of twelve (in February,1976). She was refered to the hospital where diabetes was confirmed with a glucose tolerance test and has been treated with dietary therapy alone since that time. Her mother, also a patient at Dryburn,was diagnosed at the age of 31 and is managed with diet alone. Ther is no evidence of diabetes in either of the mother's parents,both of whom are deceased,though the mother's maternal grandfather was known to be diabetic,diagnosed at the age of 37. He preceeded the introduction of both insulin and oral hypoglycaemic agents and was managed for twenty years without them.

In these families there is suggestive evidence of the presence of the M.O.D.Y. gene though it is conceded that,as pointed out by Edwards (1960),the occurrence of affected relatives in three generations does not necessarily imply causation by a single dominant gene. In all three families the ratio of affected to unaffected sibs is too low to accord comfortably with the predictions of dominant inheritance. Assuming that individual II.8 of figure 5.7 was affected but undetected,there are 10 affected children of affected parents and 23 unaffected children (1:2.3). The published reports of M.O.D.Y.,where the affected:unaffected sib ratio has been close to the predicted 1:1 have been based upon families in which relatives were specifically investigated and it may be that,were the living and apparently unaffected sibs in these families to be investigated,they would also be shown to have the condition.

The status of the families shown in figure 5.8 and

5.9 are less clear. Both of the prbands were discovered to have symptomless glycosuria before the age of thirty and both have never received insulin or oral hypoglycaemics. In the family shown in figure 5.8,the picture is complicated by the proposita being the offspring of a conjugal diabetic pair. Her diabetes was detected at a routine medical in 1974 and she has been treated with dietary restriction since diagnosis. The mother's diabetes came on suddenly in 1965 when she was aged 44,with characteristic symptoms of thirst and weight loss. Insulin therapy was commenced at once and has continued since. The father was found to have symptomless glycosuria at the age of 40 and was treated with oral hypoglycaemics throughout. He died of a coronary at the age of 49. Both of the crucial paternal grandparents are dead though there is no positive evidence that either had the disease.

In the family shown in figure 5.9 the proband was found to have glycosuria in 1962 (she was then aged 28). There were no symptoms of diabetes and dietary restriction has been sufficient treatment for 12 years. The mother was affected though details of her disease are unclear. Diagnosis was at the age of 50 and she was treated with insulin for a time,the cause of death is unknown. The maternal grandparents died at a young age and there is nothing to suggest that they were affected. One sib of the proposita was found to have glycosuria at the age of 44,though a glucose tolerance test proved normal. It is impossible to be certain about these two families.

Tattersall (1974) has commented that M.O.D.Y. individuals are predominantly female and,in this population,the three index cases who are most likely to be of this type are all female,though there

are male affected relatives in every family. Five male relatives are known to be affected and five female,in fact.

All these families show the phenomenon of 'anticipation' in that the disease is diagnosed earlier in each generation. This has been reported before (Tattersall,1974). It may be simply a consequence of changing patterns of health care with more emphasis on routine urinalysis in medical examinations and during pregnancy. The affected parents and grandparents in these families were all diagnosed after the age of thirty. None of these individuals were attending the Dryburn clinic,except in the case of the mother of the family shown in figure 5.7,but,if they had been,and their offspring were not considered,they would have been classified as type II diabetics and not M.O.D.Y. This raises the possibility that several individuals,bearers of this gene,are being misclassified by virtue of the late detection of their glycosuria and that M.O.D.Y. may be much commoner than published reports imply.

The condition deserves considerable attention in population studies as a useful model for the investigation of the pathogenesis of cardiovascular complications. None of the propositi in the families suggested as examples of the condition had any recorded evidence of complications,though the duration of the disease was so short that this was hardly remarkable. It would be instructive to follow up such a group with appropriate controls.

It is interesting that the individual treated with insulin for the longest time amongst the diabetic patients at Dryburn has a family history suggestive of M.O.D.Y. This patient was found to be diabetic in 1928 when he was aged 24 and has been treated with insulin for the past 49 years with no recorded cardiovascular

complications. His sister is also diabetic treated with diet and his father and paternal grandfather were also affected. It is suggested that this individual is not a type I diabetic as would be assumed from his current treatment but a M.O.D.Y. individual commenced on insulin in the early days of that therapy and in whom endogenous insulin secretion has been almost totally suppressed.

The five families (figures 5.5 to 5.9) suggested as examples of M.O.D.Y. or of unclear designation were eliminated from further analysis. The propositus of the family illustrated in figure 5.2 was included with the insulin-taking diabetics and the families of figures 5.3 and 5.4 were included with the non-insulin-taking diabetics.

5.11 Unexpired risk.

In a disease with variable age of onset such as diabetes it is important to consider the unexpired risk to relatives. This may be accounted for by the matching of relatives for age using age at death or current age. Most conveniently these matched groups may be obtained by specifically matching affected and non-affected propositi for age,over a large sample it should follow that their relatives' ages will be comparable. Alternatively,relatives may be contrasted within specific age groups.

5.12 The consideration of unexpired risk with diabetes of all types.

The number and frequency of affected relatives (living and dead) of non-diabetic propositi,for each 20 year age group,are shown in table 5.10 together with the affected individuals in comparable age groups of the first,second and third degree relatives

Table 5.10 Diabetics in the families of diabetics and in the families of controls.

(Aff. = affected, Tot. = total)

Age	Relatives of controls			Relatives of diabetics								
(yrs)				First degree			Second degree			Third degree		
	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)
0 - 19	0	919	0	3	380	0.79 (±0.45)	6	1310	0.46 (±0.19)	6	1011	0.59 (±0.24)
20 - 39	1	812	0.12 (±0.12)	10	594	1.68 (±0.53)	11	1417	0.78 (±0.23)	7	687	1.02 (±0.38)
40 - 59	8	792	1.01 (±0.35)	36	855	4.21* (±0.69)	31	1091	2.84* (±0.50)	12	728	1.65+ (±0.47)
60 - 79	20	807	2.48 (±0.55)	81	1034	7.83* (±0.83)	65	1406	4.62* (±0.56)	30	589	5.09* (±0.91)
80 - 99	4	187	2.14 (±1.06)	17	210	8.09 (±1.88)	13	546	2.38 (±0.65)	2	73	2.74 (±1.91)
Totals	33	3517	0.94 (±0.16)	147	3073	4.78* (±0.38)	126	5770	2.18* (±0.19)	57	3088	1.85* (±0.24)

* chi squared test appropriate, significant difference between frequency in families of diabetics and frequency in families of controls, + test appropriate, no significant difference.

of diabetics. The control group is numerically insufficient to allow separate consideration of the sexes. In all 15 comparisons between the relatives of diabetics and the relatives of controls the frequency of affected individuals in the families of diabetics is greater than that in the relatives of controls. The probability of this occurring by chance is very low. This analysis is similar to that of Pincus and White (1933) but extended to second and third degree relatives. For some comparisons the numbers of affected individuals are too small for the chi squared test to be appropriate. In the cases where it is appropriate,and the frequency in relatives of diabetics is significantly greater than that in the relatives of controls,the frequency is marked (*). Where the chi squared test is appropriate but there is no significant difference is also indicated (+).

5.13 Subdivision of diabetes with regard to age at diagnosis.

When diabetes is subdivided according to age at diagnosis, two questions may be asked - firstly,do late-onset diabetics occur in the families of early-onset diabetic propositi more often than would be expected by chance and,conversely,do early-onset diabetics occur more often in the families of late-onset propositi? The previous negative answer to the first question (Lestradet et al, 1972,and MacDonald,1974(a)) are at odds with the findings of Smith et al (1972) and Smith (1976),who find that early- and late-onset diabetes are genetically related diseases.

Firstly,considering the comparison made by MacDonald (1974(a)), viz. that of grandparents of early-onset diabetics (age at diagnosis less than 30 years) with the grandparents of non-diabetic controls. MacDonald's propositi consisted of insulin-taking diabetics only,

the numbers of late-onset diabetic grandparents of these patients in MacDonald's study and in the present study, together with the frequencies for the control groups, are shown in table 5.11. The findings from County Durham confirm those of MacDonald in that the frequency of affected grandparents of diabetics is greater than that in the grandparents of controls but that this excess is non-significant (chi squared, with one degree of freedom, in the present sample = 2.13). The studies show widely differing frequencies of affected individuals which could be the result of a large number of factors - interview technique, greater awareness of diabetes in family members in the United States, the use of screening, regional variation in genetic and environmental factors or different definitions of the disease.

The frequencies of late-onset diabetics in relatives of early-onset diabetic propositi and in the relatives of controls in various age groups are seen in table 5.12. The numbers of affected individuals again limit the number of comparisons which can be tested with chi squared. It is appropriate in second degree relatives and shows that the frequency of late-onset diabetics in the younger (30 - 59) age group is significantly greater in the families of diabetics (chi squared = 9.48, with one degree of freedom $p < 0.0005$). the excess in the older age group of these relatives is non-significant (chi squared = 2.59, with one degree of freedom). Comparison of frequencies and their standard errors reveals that the excess frequency of affected individuals in first degree relatives and in the older third degree relatives is non-significant.

The frequency of early-onset diabetes in the relatives

Table 5.11 Late-onset diabetics amongst the grandparents of early-onset insulin-taking diabetics.

Status	Present study		MacDonald (1974)	
	Grandparents of diabetics	Grandparents of controls	Grandparents of diabetics	Grandparents of controls
Affected	17 (5.48%)	8 (3.01%)	39 (8.26%)	33 (7.64%)
Not Affected	293	258	433	399
Total	310	266	472	432

Table 5.12 Late-onset diabetics in the families of early-onset diabetics and in the families of controls.

Age (yrs)	Relatives of controls			Relatives of diabetics								
				First degree			Second degree			Third degree		
	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)
30 - 59	6	1208	0.50 ($\pm$0.20)	2	189	1.06 ($\pm$0.75)	8	357	2.24* ($\pm$0.78)	1	264	0.38 ($\pm$0.38)
60 and over	24	994	2.41 ($\pm$0.49)	3	81	3.70 ($\pm$2.10)	18	428	4.21+ ($\pm$0.97)	6	151	3.97 ($\pm$1.59)
Totals	30	2202	1.36 ($\pm$0.25)	5	270	1.85 ($\pm$0.82)	26	785	3.31* ($\pm$0.64)	7	415	1.69+ ($\pm$0.63)

* chi squared test appropriate, significant difference between frequency in families of diabetics and families of controls, + test appropriate, no significant difference.

of late-onset diabetic propositi has not,so far been reported. Table 5.13 shows these frequencies for this sample. Early-onset diabetes is not a common disease and only three affected relatives were encountered in the control population. The frequency of affected relatives in the families of diabetics exceeds that in the relatives of the controls in all cases though in no instance is this significant. Early-onset may,indeed,be more common in the relatives of late-onset diabetics than in the relatives of controls, though a much larger number of families are required to demonstrate this conclusively.

5.14 Subdivision of diabetes on the basis of current treatment.

Information was available on the treatment of most of the affected relatives in the families considered although,in some instances,particularly for the more distant relatives,this information was lacking. These individuals posed something of a problem - they could not be ignored since they were definitely affected and yet they could not be assigned unambiguously to current treatment groups. In all,there were 81 such relatives,61 in the families of controls and 28 in the families of diabetics.

It was decided to assign these individuals to treatment groups according to the distribution of affected relatives of known current treatment in the same age group of relatives of the same type of propositus. If,for example,the 30 - 59 year old affected relatives (whose treatment was known) of diabetic propositi treated with diet were distributed between the three treatment groups (insulin-taking:oral-hypoglycaemic-treated:diet-controlled) in the ratio of 1:3:2,then the affected relatives of this age,for whom

Table 5.13 Early-onset diabetics in the families of late-onset diabetics and in the families of controls.

Age (yrs)	Relatives of controls			Relatives of diabetics								
				First degree			Second degree			Third degree		
	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)
0 - 29	1	1315	0.08 ($\pm$0.08)	3	451	0.67 ($\pm$0.38)	8	1835	0.44 ($\pm$0.15)	7	823	0.85 ($\pm$0.32)
30 and over	2	2202	0.09 ($\pm$0.06)	6	2188	0.27 ($\pm$0.01)	4	2903	0.14 ($\pm$0.07)	6	1280	0.47 ($\pm$0.19)
Totals	3	3517	0.09 ($\pm$0.05)	9	2639	0.34 ($\pm$0.11)	12	4738	0.25 ($\pm$0.07)	13	2103	0.62 ($\pm$0.17)

* chi squared test appropriate,significant difference between frequency in families of diabetics and frequency in families of controls,+ test appropriate,no significant difference.

treatment was unknown would be distributed between these three treatment groups in the same ratio,with rounding off to the nearest whole number of individuals.

Oral-hypoglycaemic-treated and diet-controlled patients are initially amalgamated as non-insulin-taking diabetics.

5.15 Insulin-taking and non-insulin-taking diabetics.

Table 5.14 shows the frequencies of non-insulin-taking diabetics in the families of control propositi and in the families of insulin-taking diabetics. The numbers of affected individuals are small and so the relatives have been amalgamated into two age groups only - under 60 years and 60 and over. There are significantly more affected individuals in the total first and second degree relatives of diabetics but not in third degree relatives. Chi squared tests are only applicable in the older age group and the affected first degree relatives of diabetics are significantly more frequent than those of controls. The numbers are small in the other age sub-groups making the chi squared test invalid. The frequency of affected relatives in the diabetics' families is greater than those in controls' families for first and second degree relatives though comparison of these frequencies using their standard errors is unconvincing.

The results of the reciprocal comparison,the frequency of insulin-taking diabetics in the families of non-insulin-taking diabetics and in the families of controls,may be seen in table 5.15. In this case,the paucity of affected individuals in the families of controls has necessitated the subdivision of the relatives into two age groups only. The frequency of affected individuals in

Table 5.14 Non-insulin-taking diabetics in the families of insulin-taking diabetics and in the families of controls.

Age (yrs)	Relatives of controls			Relatives of diabetics								
				First degree			Second degree			Third degree		
	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)
Under 60	4	2523	0.16 (±0.08)	3	629	0.48 (±0.27)	10	1180	0.85 (±0.27)	0	1206	0
60 and over	19	994	1.91 (±0.43)	12	284	4.23* (±1.19)	23	802	2.87+ (±0.59)	11	283	3.89+ (±1.15)
Totals	23	3517	0.65 (±0.13)	15	913	1.64* (±0.42)	33	1982	1.66* (±0.29)	11	1489	0.74+ (±0.22)

* chi squared test appropriate,significant difference between frequency in families of diabetics and frequency in families of controls,+ test appropriate,no significant difference.

Table 5.15 Insulin-taking diabetics in the families of non-insulin-taking diabetics and in the families of controls.

Age (yrs)	Relatives of controls			Relatives of diabetics								
				First degree			Second degree			Third degree		
	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)
Under 60	5	2523	0.20 ($\pm$0.09)	11	1200	0.92* ($\pm$0.28)	13	2638	0.49+ ($\pm$0.14)	11	1220	0.90* ($\pm$0.27)
60 and over	5	994	0.50 ($\pm$0.22)	23	960	2.40* ($\pm$0.49)	14	1150	1.22+ ($\pm$0.32)	9	379	2.37 ($\pm$0.78)
Totals	10	3517	0.28 ($\pm$0.09)	34	2160	1.57* ($\pm$0.27)	27	3788	0.71* ($\pm$0.14)	20	1599	1.25* ($\pm$0.28)

* chi squared test appropriate,significant difference between frequency in families of diabetics and frequency in families of controls,+ test appropriate,no significant difference.

the first degree relatives of diabetics is significantly greater than that in controls in both age groups. Other significant comparisons are marked (*).

Within the numerical inadequacies of these data,the suggestion that these two forms of diabetes are genetically related is supported. This will be discussed further at the end of this chapter.

5.16 The separate consideration of oral-hypoglycaemic and diet-treated groups.

The finding by Irvine (1977) that some individuals initially treated with oral-hypoglycaemics are P.I.C.A. positive (and,therefore,type I diabetics) recommends that the diet and oral-hypoglycaemic treated groups be treated separately. There is also an implication from this work that insulin-taking diabetic relatives may be more common in the families of oral-hypoglycaemic-treated propositi than in the families of diabetics controlled with diet, although this is dependent upon the proportion of oral-hypoglycaemic-treated patients that are type I. Tables 5.16(a) and 5.16(b) show the frequencies of insulin-taking diabetic relatives in the families of oral-hypoglycaemic-treated propositi,diet-treated propositi and controls. There are no statistically significant excesses of insulin-taking relatives in the families of oral-hypoglycaemic-treated propositi compared with diet-managed propositi.

Table 5.16(a) Insulin-taking diabetics in the families of oral hypoglycaemic-taking diabetics.

Age (yrs)	Type of relative								
	First degree			Second degree			Third degree		
	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)
0 - 29	0	169	0	0	771	0	1	311	0.32 ($\pm$0.32)
30 - 59	5	414	1.21 ($\pm$0.54)	4	526	0.76 ($\pm$0.38)	0	224	0
60 and over	13	480	2.71 ($\pm$0.74)	10	554	1.81 ($\pm$0.57)	3	182	1.65 ($\pm$0.94)
Totals	18	1063	1.69 ($\pm$0.39)	14	1851	0.76 ($\pm$0.20)	4	717	0.56 ($\pm$0.28)

Table 5.16(b) Insulin-taking diabetics in the families of diet-managed diabetics and in the families of controls.

Age (yrs)	Relatives of diabetics									Relatives of controls		
	First degree			Second degree			Third degree					
	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)
0 - 29	1	182	0.55 (±0.55)	3	753	0.40 (±0.23)	6	355	1.69 (±0.68)	1	1315	0.08 (±0.08)
30 - 59	5	435	1.15 (±0.51)	6	588	1.02 (±0.41)	4	330	1.21 (0.60)	4	1208	0.33 (±0.17)
60 and over	10	480	2.08 (±0.65)	4	596	0.67 (±0.33)	6	197	3.05 (±1.23)	5	994	0.50 (±0.22)
Totals	16	1097	1.46 (±0.36)	13	1937	0.67 (±0.19)	16	882	1.81 (±0.45)	10	3517	0.28 (±0.09)

5.17 Comparison of the prevalence of diabetes in the relatives of diabetics and in the general population.

The method for the determination of the prevalence of diabetes in the general population using 1971 census data will be described in detail in chapter 8. Table 5.17 shows these figures for all types of diabetes in both sexes,considering the area immediately surrounding Durham city. The prevalences are expressed in percentages. Also present in this table are the prevalences of affected individuals in the families of diabetics (living relatives only are considered).

Thirty comparisons are possible between the relatives of diabetics and the general population. The prevalence in the diabetics' relatives exceeds that in the general population in 25 of these,in four cases the prevalence is zero in the families of diabetics and in one case the prevalence in the general population is the greater. The numbers of affected relatives in the families of diabetics are too small for the chi squared test to be valid except in the cases of individuals between 60 and 69 years old, those 70 years of age and over and the total samples irrespective of age. The comparisons for which the prevalence of affected relatives in the families of diabetics is significantly greater than that in the general population are marked.

Table 5.18 shows the prevalence of non-insulin-taking diabetics in the living relatives of insulin-taking diabetic propositi and the prevalence of this type of diabetes in the general population. Table 5.19 shows the prevalence of insulin-taking diabetics in the families of non-insulin-taking diabetic propositi and that in the general population.

Table 5.17 Prevalence of diabetes in the families of diabetics and in the general population.

Age (yrs)	General population			Relatives of diabetics								
				First degree			Second degree			Third degree		
	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)
0 - 9	5	13306	0.04 (±0.02)	0	67	0	1	464	0.22 (±0.22)	0	513	0
10 - 19	16	12882	0.12 (±0.03)	1	141	0.23 (±0.40)	3	717	0.42 (±0.24)	3	430	0.70 (±0.40)
20 - 29	25	13126	0.19 (±0.04)	2	174	1.15 (±0.81)	0	680	0	2	351	0.57 (±0.40)
30 - 39	36	10100	0.36 (±0.06)	6	310	1.94 (±0.78)	6	552	1.09 (±0.44)	2	275	0.73 (±0.51)
40 - 49	53	10305	0.51 (±0.07)	5	331	1.51 (±0.67)	3	469	0.64 (±0.37)	0	311	0
50 - 59	93	9242	1.01 (±0.10)	12	279	4.30 (±1.21)	13	326	3.99 (±1.08)	6	319	1.88 (±0.76)
60 - 69	201	8118	2.48 (±0.19)	14	324	4.32* (±1.22)	6	250	2.40+ (±0.97)	11	295	3.73+ (±1.10)
70 and over	208	5836	3.56 (±0.24)	23	234	9.83* (±1.95)	11	243	4.53+ (±1.33)	6	144	4.17+ (±1.67)
Totals	637	82915	0.77 (±0.03)	63	1860	3.39* (±0.42)	43	3701	1.16* (±0.18)	30	2638	1.14* (±0.21)

* chi squared test appropriate, significant difference between frequency in families of diabetics and frequency in families of controls, + test appropriate, no significant difference.

Table 5.18 Non-insulin-taking diabetics in the families of insulin-taking diabetics and in the general population.

Age	General population			Relatives of diabetics								
(yrs)				First degree			Second degree			Third degree		
	Aff.	Total	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)
Under 60	116	68961	0.17 (±0.02)	1	521	0.19 (±0.25)	6	960	0.63 (±0.25)	0	1110	0
60 and over	360	13954	2.58 (±0.13)	7	144	4.86 (±1.79)	8	291	2.75+ (±0.96)	4	129	3.10 (±1.53)
Totals	476	82915	0.57 (±0.03)	8	665	1.20 (±0.42)	14	1251	1.12* (±0.30)	4	1239	0.32 (±0.16)

* chi squared test appropriate,significant difference between frequency in families of diabetics and frequency in families of controls, + test appropriate,no significant difference.

Table 5.19 Insulin-taking diabetics in the families of non-insulin-taking diabetics and in the general population.

Age (yrs)	General population			Relatives of diabetics								
				First degree			Second degree			Third degree		
	Aff.	Total	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)	Aff.	Tot.	(%)
0 - 29	42	39314	0.11 (±0.02)	0	163	0	2	1376	0.15 (±0.10)	2	611	0.33 (±0.23)
30 - 59	70	29647	0.24 (±0.03)	4	618	0.65 (±0.32)	3	872	0.34 (±0.20)	2	478	0.42 (±0.30)
60 and over	49	13954	0.35 (±0.05)	5	414	1.21 (±0.54)	0	202	0	7	310	2.26 (±0.84)
Totals	161	82915	0.19 (±0.01)	9	1195	0.75 (±0.25)	5	2450	0.20 (±0.09)	11	1399	0.79 (±0.24)

Although, in most instances, the prevalence of both types of diabetes is more common in the relatives of diabetic propositi of the other type, it is impossible to be categorical with such small numbers of affected relatives.

5.18 Correlations between pairs of first degree relatives.

Information was available on 134 pairs of affected first degree relatives, one of whom being, in each case, a propositus. Of these pairs, 72 were sib-sib combinations, the remaining 62 were parent-offspring pairs. They were examined for the correlation with regard to age at diagnosis and current treatment. In the sib-sib pair group there were ten brother-brother pairs, 38 sister-sister pairs and 24 brother-sister pairs, twice as many like-sexed pairs as unlike-sexed pairs, an effect previously noticed by Penrose and Watson.(1945). There are significantly more females amongst these sib-sib pairs when they are compared with the sex ratio in the total 1975 out-patient population (44 males to 100 females compared with 377 males to 559 females, chi squared = 4.95 for one degree of freedom, $p < 0.05 > 0.025$). In the parent-offspring combinations there were seven father-son pairs, ten father-daughter pairs, 15 mother-son pairs and 30 mother-daughter pairs (like-sexed pairs/unlike-sexed pairs 37/25).

(a) Age at diagnosis.

Age at diagnosis was significantly correlated in the sib-sib pairs ($r = 0.68, p = 0.00001$). This is shown in figure 5.10 for 70 pairs, the age at diagnosis being unknown for one member of a pair. The age at diagnosis in the parent-offspring pairs was not significantly

Figure 5.10 Age at diagnosis in sib-sib pairs

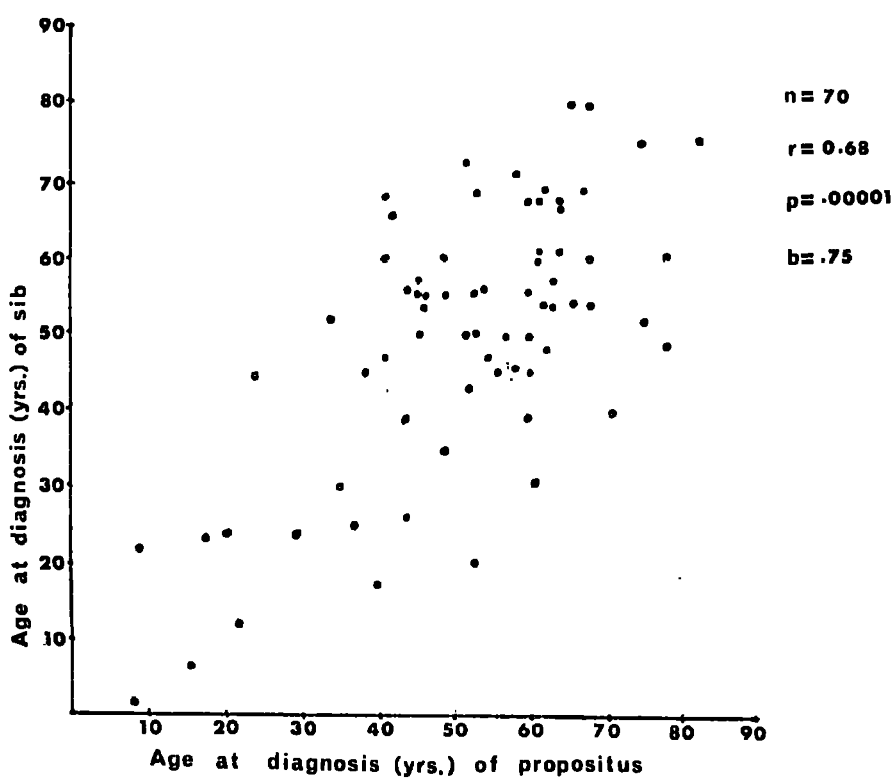
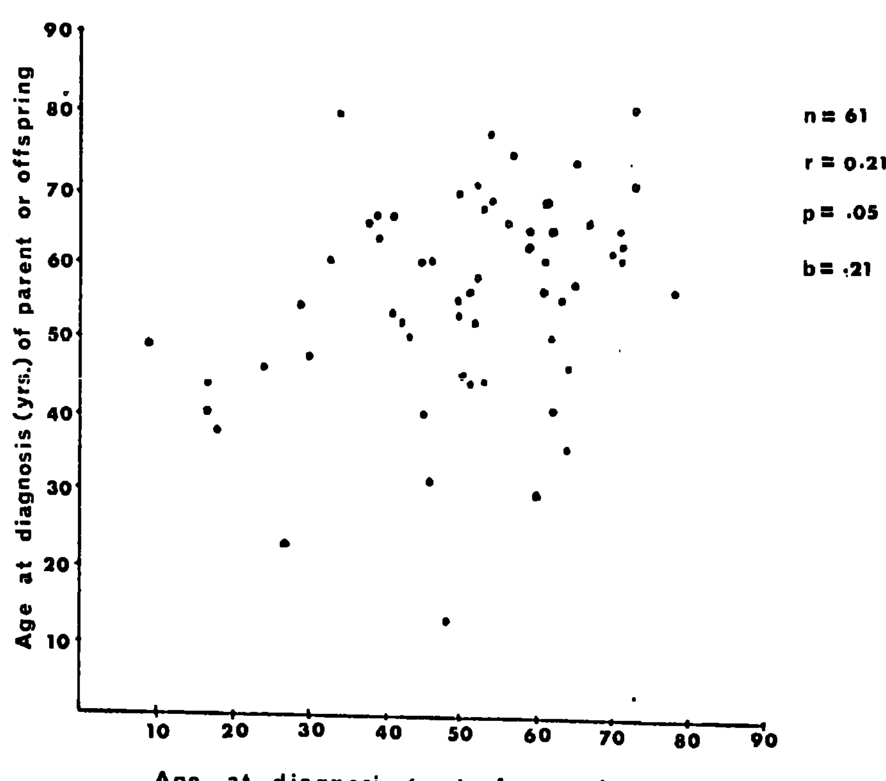


Figure 5.11 Age at diagnosis in parent-offspring pairs



correlated (r = 0.21,p = 0.05). The value for the 61 pairs in which age at diagnosis was known for both members are shown in figure 5.11. The higher correlation in the sib-sib pairs implies that common environmental effects,not present in the parent-offspring pairs,are contributing considerably to the determination of time of onset of the disease.

Age at diagnosis is more highly correlated (r = 0.78, p = 0.00001)if sib-sib pairs in which both members are in the same treatment group (insulin-taking or non-insulin-taking) are considered (figure 5.12). The lower cluster of points (with age at diagnosis less than about 40 years) consists,predominantly,of the pairs in which both members are insulin-taking diabetics. When these 13 pairs are considered alone (figure 5.13) age at diagnosis is significantly correlated (r = 0.67,p = 0.006). There is no significant correlation demonstrable in the 24 sib-sib pairs in which both members are non-insulin-taking diabetics (r = 0.34, p = 0.05). This distribution is shown in figure 5.14.

Like-treatment parent-offspring pairs (figure 5.15) show a significant correlation for age at diagnosis (r = 0.34, p = 0.02). There are too few parent-offspring pairs in which both members are insulin-taking but analysis of the parent-offspring pairs in which both are non-insulin-taking (figure 5.16) shows a significant correlation with regard to age at diagnosis (r = 0.40, p = 0.02).

(b) Current treatment.

Tables 5.20(a) and 5.20(b) show the correlation of current treatment in sib-sib and parent-offspring pairs respectively.

Figure 5.12 Age at diagnosis in like-treatment sib pairs.

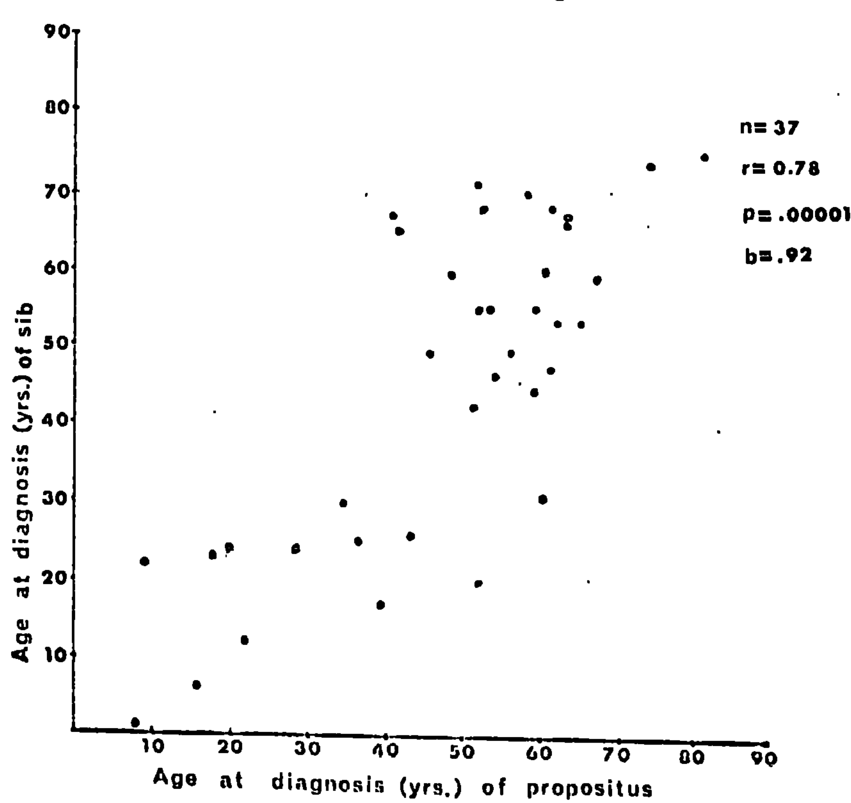


Figure 5.13 Age at diagnosis in insulin-taking sib pairs.

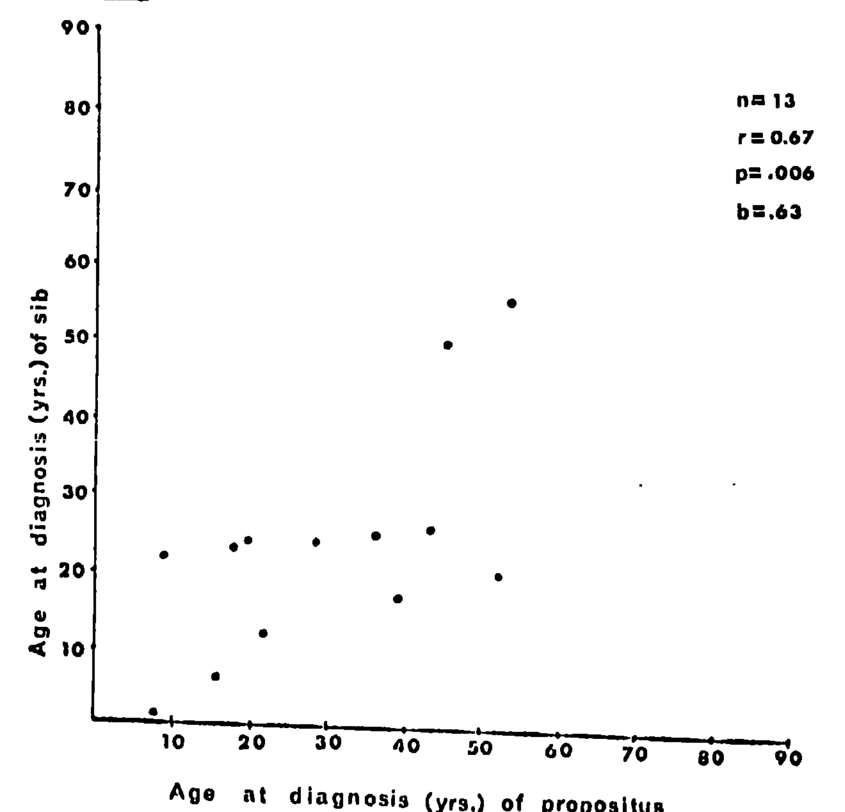


Figure 5.14 Age at diagnosis in non-insulin-taking sib pairs.

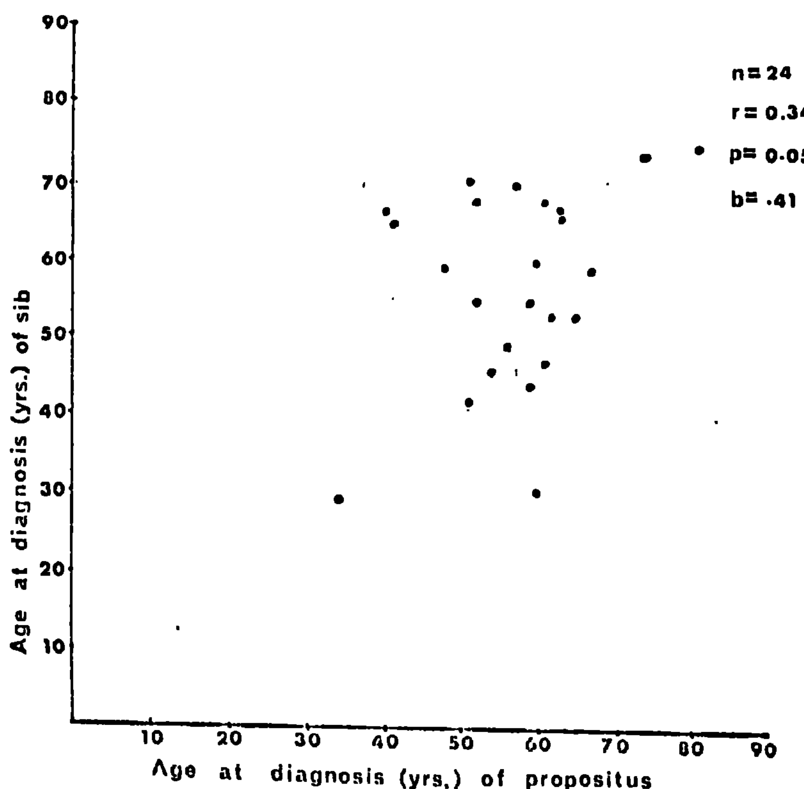


Figure 5.15 Age at diagnosis in like-treatment parent-offspring pairs.

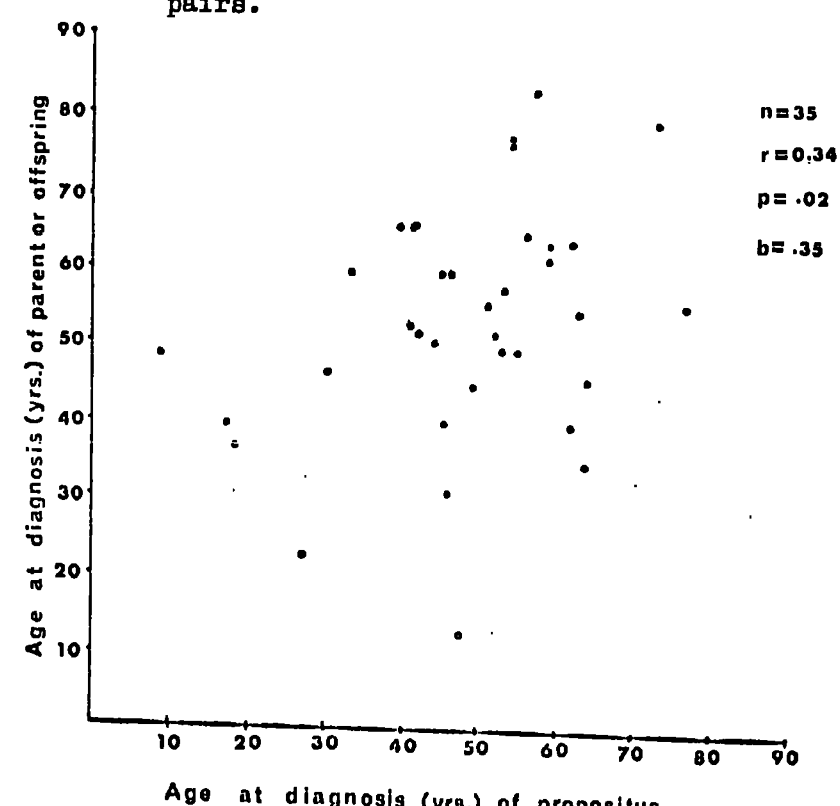


Figure 5.16 Age at diagnosis in non-insulin-taking parent-offspring pairs.

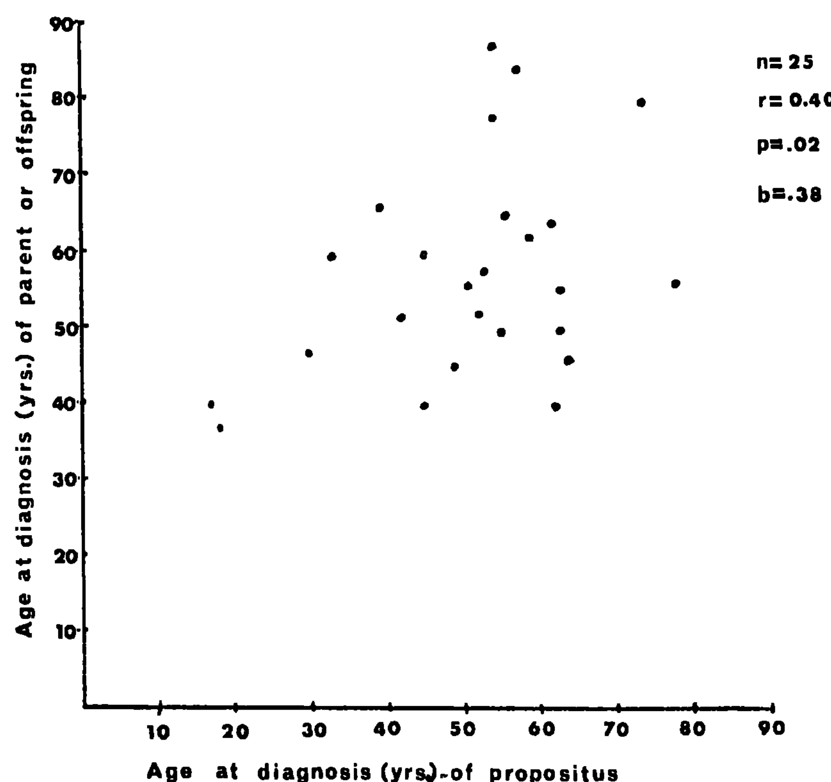


Table 5.20(a) Current treatment of sib-sib pairs.

(1)	Propositus insulin-taking, sib insulin-taking	- 13 (23.2%)
(2)	Propositus insulin-taking, sib non-insulin-taking	- 8 (14.3%)
(3)	Propositus non-insulin-taking, sib insulin-taking	- 10 (17.9%)
(4)	Propositus non-insulin-taking, sib non-insulin-taking	- 25 (44.6%)

Total number of pairs - 56

Concordant - 38 (67.9%)   Discordant - 18 (32.1%)

Table 5.20(b) Current treatment of parent-offspring pairs.

(1)	Propositus insulin-taking, parent or offspring insulin-taking	- 10 (19.6%)
(2)	Propositus insulin-taking, parent or offspring non-insulin-taking	- 3 (5.9%)
(3)	Propositus non-insulin-taking, parent or offspring insulin-taking	- 12 (23.5%)
(4)	Propositus non-insulin-taking, parent or offspring non-insulin-taking	- 26 (51.0%)

Total number of pairs - 51

Concordant - 36 (70.6%)   Discordant - 15 (29.4%)

As would be expected, there are more pairs concordant for current treatment than discordant, and this excess is significant.

5.19 Correlation between family history and phenotype.

It is a prediction of the hypothesis outlined in chapter 3 that, in affected individuals with a strong family history of the disease, cardiovascular complications should develop more rapidly than in diabetics with a less marked family history. This follows because the proportion of affected individuals in a pedigree is an indication of the number of deleterious 'rapid ageing' genes segregating in the family and affected individuals, on average, will tend to have a larger number of these genes, and will be subjected to more rapid complications than diabetics with fewer deleterious genes and fewer affected relatives.

Hereditary factors determining the rate of development of diabetic complications and their type in twins have been suggested before (Pyke and Tattersall, 1973) although there has been no investigation of other types of relatives. Such factors are one possible explanation for the differences in the nature and speed of development of these changes in racially distinct groups.

Two major difficulties are apparent - the measurement of the severity of family history so that diabetic propositi can be ranked for this attribute in a meaningful way, and, secondly, some means of assessing the rate of development of complications.

(a) The measurement of the severity of family history.

Smith (1972) has reported the use of a computer programme (RISKMF) for the calculation of recurrence risks of

multifactorial conditions in families. With some adaptation this programme may be used to give a probability for the likelihood of the propositus developing the disease in a particular age group, based on the consideration of his family history. This depends,not only upon the frequency of affected relatives but on their sex,the age at which they became affected,the heritability of the disease in both sexes,its prevalence in the general population and the current ages or ages at death of unaffected relatives. The RISKMF programme,by taking these factors into account,gives the probability of an unborn child developing the disease in each of four successive age groups (0 - 19,20 - 39,40 - 59 and 60 years and over),or the probability of an individual already born developing the disease in the present and subsequent age groups. The input requires the coded family history,the prevalence of the disease in the general population for each sex for the four age groups and the heritability of liability to the disease again for each sex and for each age class. The family history required are the sex of each relative,the relationship to the propositus and the disease status (i.e. affected or unaffected). If the relative is unaffected the programme uses the current age,if affected,the age at diagnosis. The programme assumes that the inheritance of the disease is polygenic and that diabetes diagnosed at all ages is genetically one disease,an assumption which is not wholly justified.

By inputing the coded family history of any diabetic propositus,but coding the propositus as unaffected and currently in the age group in which diabetes was diagnosed,the output gives the probability,based on the family history and on the above assumptions, that the propositus would develop the disease in the age group in

which it was diagnosed. The probability figure for the oldest age group is the probability,based on the present family history,that the propositus will develop the disease eventually. A specimen coded pedigree for the programme is shown in Appendix C.

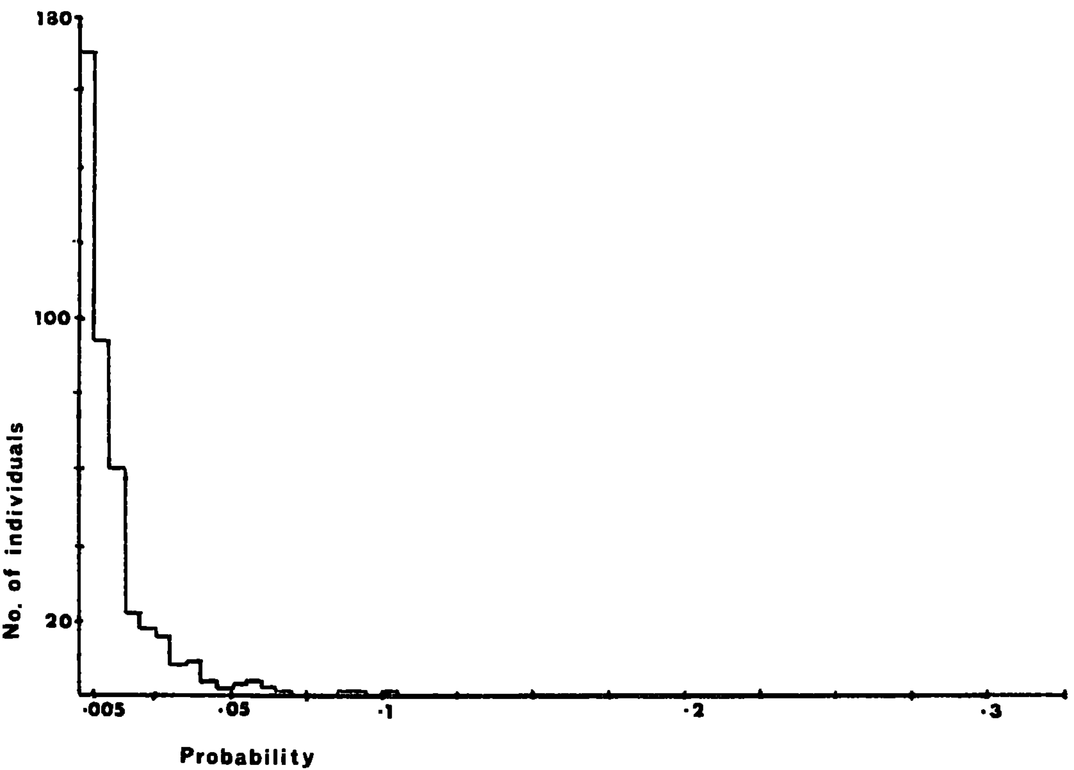
In order to analyse the pedigrees collected in this way it was necessary to write a Fortran programme which would recode the family data and present it in a form suitable for the RISKMF programme (supplied by Dr.C.Smith and Miss S.Holloway). This programme is not able to deal with information on relatives more distantly related from the propositus than those shown in Appendix C so that only the most immediate relatives for each family were included. The distribution of risks for the development of the disease in diabetic and non-diabetic propositi are shown in figures 5.17(a) and 5.17(b) respectively. For the diabetics,this is the risk of developing the disease in the age group in which they were diagnosed,for the controls it is the risk of developing the disease in their current age group. These figures are calculated using the population prevalence estimates and the heritability figures supplied with the RISKMF programme.

The risk of developing the disease is greatly influenced by age so that a more meaningful comparison is the risk of being diagnosed as diabetic in the final age group,i.e. the probability of becoming diabetic eventually,assessed from the present family history. This is shown,for diabetics,in figure 5.18(a) and,for controls,in figure 5.18(b).

The highest value for this figure is 0.3021 for the diabetic propositus in figure 5.19,shown for the purpose of illustration. This individual also had the highest risk of developing the disease

Figure 5.17 Probability of becoming diabetic, based on family history.

(a) Diabetic propositi (probability at age of diagnosis)



(b) Control propositi (probability at present age)

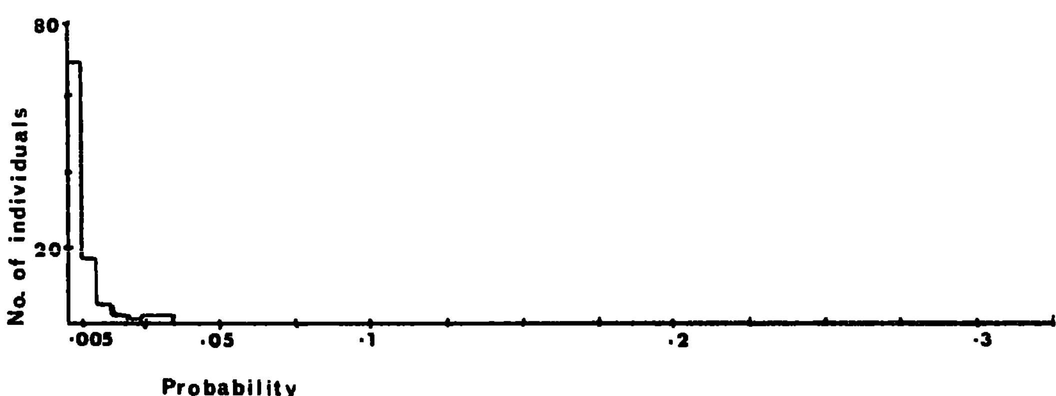
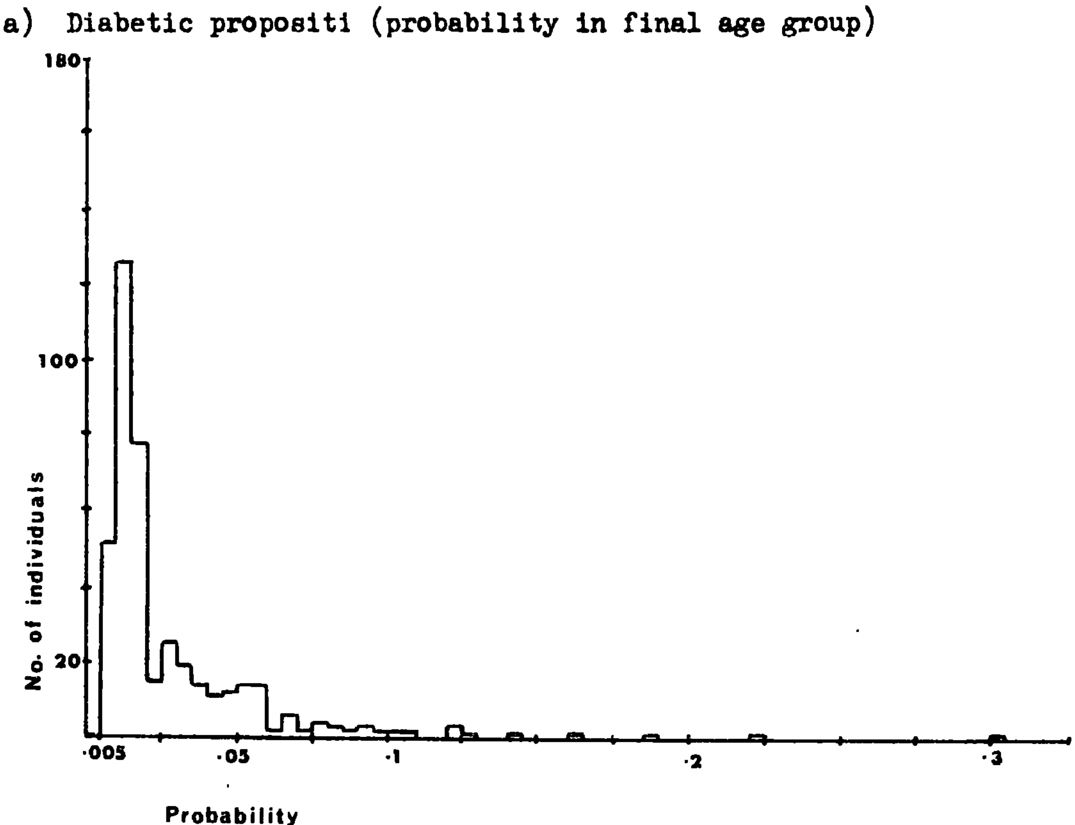


Figure 5.18 Probability of becoming diabetic, based on family history.

(a) Diabetic propositi (probability in final age group)



(b) Control propositi (probability in final age group)

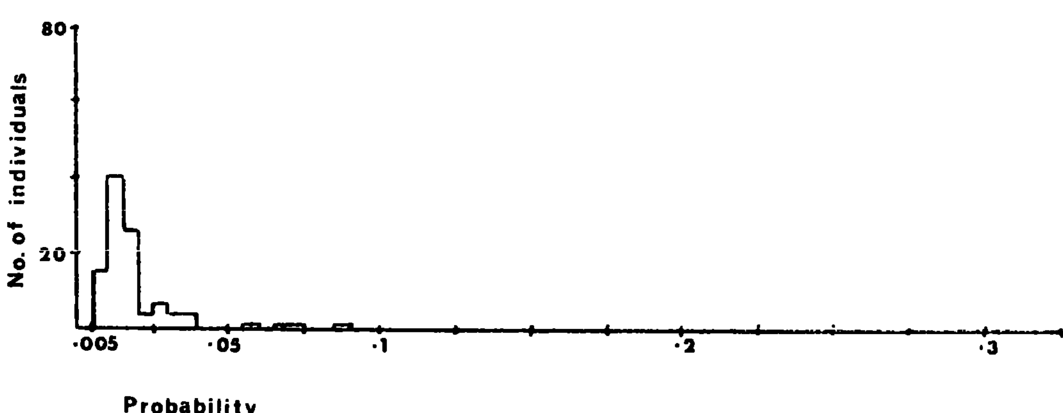


Figure 5.19

I
II
III
IV
V

in the age class of diagnosis (0.1727 in figure 5.17(a)).

(b) The assessment of complications.

The evaluation of the development of cardiovascular complications present in the whole diabetic group for whom family history was known and a value for the probability calculated (418 individuals,excluding the M.O.D.Y. propositi) was rejected as being too time consuming. Instead,the complications noted throughout the course of the disease in 20 insulin-taking individuals were noted from the hospital records. The individuals were chosen, 10 from those diagnosed before the age of 30, 10 from those diagnosed after this age,according to their position in the distribution of probabilities for becoming affected at the age at which they were diagnosed. For each age at onset category,the five with the highest value of this probability and five randomly chosen from the group with the lowest value of this probability.

For insulin-taking diabetics diagnosed before the age of 30,cardiovascular complications attributable to diabetes,together with the period after diagnosis of the disease at which they were first noted are shown in table 5.22(a) and 5.22(b). The complications developed to date are more severe in the diabetics with the greatest family history (table 5.22(a)) but the two groups are not comparable in that the duration of the disease in the former group is much longer,on average,than that in the latter.

Tables 5.23(a) and (b) show the same information for insulin-taking diabetics diagnosed after the age of 30. Although the patients with the high values for the probability of developing diabetes (table 5.23(a)) have been diagnosed as diabetic for less

Table 5.22(a) Cardiovascular complications in insulin-taking diabetics diagnosed before the age of 30 years, with a high risk of becoming diabetic as judged by current family history.

No.	Sex	Risk	Current age (yrs)	Duration of disease	Complications
52	F	0.0263	33.3	26 yrs.	Severe proliferative retinopathy first noted 5 yrs after diagnosis, now blind in both eyes (11 yrs after diagnosis).
941	M	0.0169	56.7	34½ yrs.	Proliferative retinopathy first noted 18 yrs after diagnosis, blind in both eyes 34 years after diagnosis.
73	M	0.0151	71.0	47 yrs.	None
10	M	0.0148	22.2	1½ yrs.	None
31	M	0.0119	28.7	19½ yrs.	None

Mean age - 42.4 years   Mean duration of disease - 25.7 years

Table 5.22(b) Cardiovascular complications in insulin-taking diabetics diagnosed before the age of 30 years, with a low risk of becoming diabetic as judged by current family history.

No.	Sex	Risk	Current age (yrs)	Duration of disease	Complications
105	F	0.0002	18.4	$4\frac{3}{4}$ years	None
429	M	0.0002	22.7	$4\frac{1}{4}$ years	None
486	F	0.0002	58.2	40 years	Vitreous haemorrhage in right eye 16 years after diagnosis, no visual impairment at present.
526	F	0.0002	21.7	$14\frac{1}{2}$ years	One microaneurysm noted 14 years after diagnosis.
562	F	0.0002	10.4	$5\frac{1}{4}$ years	None

Mean age - 26.3 years

Mean duration of disease - 13.75 years

Table 5.23(a) Cardiovascular complications in insulin-taking diabetics diagnosed after the age of 30 years, with a high risk of becoming diabetic as judged by current family history.

No.	Sex	Risk	Current age (yrs)	Duration of disease	Complications
709	M	0.1727	71.2	$25\frac{1}{4}$ yrs.	Diabetic retinopathy first noted 15 years after diagnosis. Myocardial infarction 15 years and 24 years after diagnosis. Below knee amputations of both legs for diabetic gangrene 21 years after diagnosis.
204	M	0.0694	66.1	$5\frac{1}{2}$ yrs.	Amputation of left leg 2 years after diagnosis (diabetic gangrene), amputation of right leg for the same reason 5 years after diagnosis.
267	F	0.0603	49.6	$1\frac{1}{2}$ yrs.	Myocardial infarction 1 year after diagnosis.
235	M	0.0394	62.8	9 yrs.	None
192	F	0.0319	64.6	$4\frac{1}{2}$ yrs.	Bilateral macular degeneration noted at diagnosis.

Mean age - 62.9 years

Mean duration of disease - 9.1 years

Table 5.23(b) Cardiovascular complications in insulin-taking diabetics diagnosed after the age of 30 years, with a low risk of becoming diabetic as judged by current family history.

No.	Sex	Risk	Current age (yrs)	Duration of disease	Complications
850	F	0.0009	49.0	$11\frac{1}{2}$ yrs.	None
651	M	0.0009	47.2	$13\frac{3}{4}$ yrs.	None
343	M	0.0008	56.8	$25\frac{1}{2}$ yrs.	None
129	F	0.0008	65.4	$28\frac{1}{4}$ yrs.	Early diabetic retinopathy noted 27 years after diagnosis, no progression since then.
136	F	0.0008	68.7	$37\frac{1}{2}$ yrs.	Some exudates noted in left eye 30 years after diagnosis.

Mean age - 57.4 years

Mean duration of disease - 23.3 years

time than those with a low probability of developing the disease, the recorded cardiovascular complications in the former group are more severe and developed more rapidly after the onset of diabetes in these individuals.

5.22 Summary and discussion.

Investigation of cases of insulin-independent diabetes diagnosed before the age of 30 supports the suggestion (Tattersall, 1974,and Tattersall and Fajans,1975) that many will show features suggestive of dominant inheritance. Out of eight such cases investigated in this diabetic population,three (37.5%) showed a family history with affected parents and offspring in successive generations. The affected sibs were too few in number to accord with the predictions of dominant inheritance but active investigation of their glucose tolerance might well have revealed further cases of mild,non-insulin-requiring diabetes. In the 425 other families of diabetics investigated only nine (2%) suggested dominant inheritance in the same fashion. It is suggested that some of these may have been examples of M.O.D.Y. missed as a result of the propositus being diagnosed after the arbitrary age of 30. Further investigation of this sub-type of diabetes is urged since it presents a potentially useful model for the investigation of the pathogenesis of cardiovascular complications.

The investigation of the occurrence of diabetes in families by the interview questionnaire technique becomes progressively less reliable with more distantly related individuals since lack of information leads to the exclusion of many of these relatives,especially those unaffected by the disease.

The data presented here on the occurrence of diabetes in the grandparents of insulin-taking early-onset diabetics agree with the findings of MacDonald (1974(a)) in that the frequency of affected individuals amongst the grandparents of diabetics is greater than that amongst the grandparents of controls but that this difference is non-significant. This strongly suggests that the difference may be a real one but that a larger sample of families than is presented either here or by MacDonald (1974(a)) is required to demonstrate this with confidence.

When the numbers of individuals are augmented by the inclusion of other relatives,the data strongly suggest that non-insulin-taking diabetics are found in the families of insulin-taking diabetics more often than in the families of non-diabetics and that insulin-taking diabetics are,similarly,more common in the families of non-insulin-taking diabetics than in those of controls. These findings are supported in the examination of prevalence figures for the general population although in no case does the number of affected individuals in all age groups for any type of relative reach a high enough value for this contention to be supported by statistical tests. A larger number of families than has been studied here is required to substantiate this suggestion. If it is true then the findings of Smith et al (1972) and Smith (1976) are supported rather than those of MacDonald (1974(a)),Lestradet et al (1972) and Simpson (1969).

Age at diagnosis is positively correlated in pairs of affected sibs and is most highly correlated when pairs currently on the same treatment are considered. The higher correlation in sibs than in parent-offspring pairs suggests the action of environmental agents which are more commonly shared in the case of sibs than in

parents and offspring.

The development of cardiovascular complications in insulin-taking diabetics has been investigated in relation to the family history of the disease. Although twins have been investigated for genetic factors causing the development of these complications (Pyke and Tattersall,1973),no investigation has,so far,been directed at the question of whether diabetics with a large number of affected relatives develop complications more rapidly and more severely than those with fewer affected relatives,a prediction which follows logically from the suggestions of the hypothesis in chapter 3. Within the limitations of the methods employed here there is suggestive evidence,especially in the group of late-onset insulin-taking diabetics,that there is, indeed,a correlation between family history of diabetes and the tendency to develop severe cardiovascular complications in the propositus.

This finding,which needs further investigation with a prospective type of study,gives some meaning to the question asked of each new diabetic - 'Is there any diabetes in the family?'.

Chapter 6

Polymorphism results. I Control subjects.

Chapter 6

6.1 Introduction.

6.2 Methods.

(a) Preparation of samples.

(b) Red cell grouping methods.

(c) Electrophoresis.

(d) HLA antigen determination.

6.3 The ABO system.

(a) With subdivision of group A.

(b) No subdivision of group A.

6.4 The MNSs system.

(a) The MN locus.

(b) The Ss locus.

(c) The MNSs complex.

6.5 The P system.

6.6 The Rhesus system.

(a) Phenotype frequencies considering the three loci, C,D and E.

(b) Separate consideration of the C,D and E loci.

6.7 The Kell system.

(a) Tests with anti-K and anti-k.

(b) Tests with anti-Kp^a and anti-Kp^b.

(c) Tests with anti-K,antik,anti-Kp^a and anti-Kp^b.

6.8 The Lewis system.

6.9 The Duffy system.

6.10 Haptoglobin.

6.11 Acid phosphatase.

6.12 Phosphoglucomutase.

6.13 Adenylate kinase.

6.14 Adenosine deaminase.

6.15 Esterase D.

6.16 The HLA system.

6.17 Summary and discussion.

6.1 Introduction.

The results of the study of genetic polymorphisms in diabetics and in non-diabetic controls are described in the present chapter and in the following chapter. A description of the materials and methods, together with a discussion of the results for controls are dealt with in this chapter, the comparisons between diabetics and controls are discussed in chapter 7. Specific descriptions of soultions and details of sources of antisera are relegated to Appendix D. The discussion of results in the control subjects takes the same form for each system.- firstly a description of the results for the total sample is presented, together with a comparison with previously published data. Comparative series have been selected for their geographical similarity to the present sample, and for as large a sample size as possible. Most are derived from data given in Kopeč (1970) or Mourant et al (1976).

Secondly, the control sample is examined for differences in the phenotype frequencies resulting from the sex, area of origin and source of the subjects. Regarding area of origin, individuals have been divided in two ways - according to the county of their own birth (either County Durham or elsewhere in the United Kingdom), and according to place of granparental birth (contrasting those subjects with three or four grandparents born within the County with those with one or none). The three sources of controls considered are neighbours of diabetics, trauma patients and students. Data are not given for these comparisons except where significant differences have been found.

Lastly, the control data are examined for effects related

to the current age of individuals in the sample. This has been carried out in several ways. Phenotype frequencies for different combinations of five-year age groups have been analysed for differences using the standard chi squared test. The mean ages of different categories of individuals (homozygotes vs. heterozygotes, for example) have been compared using the Student's t-test,and analysis for trends in proportions using various age groupings has been performed using the method suggested by Cox (1970). In general, where a significant trend in frequencies has been found,the data are given for the four sub-divisions 0 - 19 years,20 - 39 years,40 - 59 years and 60 years and over (ages at last birthday). Although these sub-divisions are arbitrary they have the advantage in that they give equal periods of time (there are few subjects over 80),which is required by the method for analysis of trends in proportions. The details of this method is given in the first section of analysis devoted to the ABO system.

Unless otherwise stated,the computation of gene frequencies is by the method of gene counting and populations have been tested for accordance with the predictions of the Hardy-Weinberg law in the standard way. The standard errors of the gene frequency estimates,again unless otherwise stated,have been computed according to the formula:-

$$\text{Standard error} = \sqrt{\frac{p(1 - p)}{N}}$$

where p is the gene frequency concerned and N is the total sample size.

Not all individuals have been tested for all systems. Beneath each table of gene frequencies the chi squared

for the deviation of each group from Hardy-Weinberg prediction and the chi squared value for the comparison of the present control sample with the previously published sample are given. P values are only given when they are less than,or close to,0.05.

6.2 Methods.

(a) Preparation of samples.

From each subject,10 mls of venous blood were taken into two 5 ml heparinised containers. The contents of one were used for the HLA antigen determination,the other 5 mls for the erythrocyte grouping and for electrophoresis.

Within twelve hours of the blood being taken,the red cells for grouping and electrophoresis were separated by centrifugation at 2,000 r.p.m. for 10 minutes,the serum was decanted and frozen immediately to -80°C. The remaining red cells were washed twice with saline (kept at 4°C) and a 4% suspension of the cells in saline prepared for red cell grouping. The remaining erythrocytes were immediately frozen and kept for the preparation of haemolysates in batches. In all cases,red cell grouping was completed within 24 hours of the blood samples being taken.

Haemolysates were prepared by thawing the lysed cell specimens and adding 6 mls of carbon tetrachloride solution. This mixture was spun at 1,500 r.p.m. for 1½ hours. The supernatant was decanted and kept at -80°C until required for electrophoresis.

(b) Red cell grouping methods.

Red cell grouping was performed,with antisera derived from a variety of sources,either by a tile technique,by tube technique or

indirect Coombs. Control cells were unavailable for the typing of the first 378 specimens owing to financial constraints but, after this,regular supplies of Ortho 'Identigen' were obtained and appropriate controls (homozygous positive,heterozygote and homozygous negative) were set up with each run. In the case of testing with anti-Kp^a,no suitable controls were ever available. Of these first 378 specimens tested without controls,260 were retested (when a second sample of blood was collected for tissue typing) and,for these individuals,the results of the second testing were used.

In the case of unusual phenotypes (for the rhesus and Kell systems) corroboration was obtained with the use of commercial antisera.

Tile technique.

Used for testing with:-

Antiserum	Conditions
anti-A, anti-B, anti A+B	10 mins. at room temp.
anti-A_1, anti-P_1	10 mins. at 4°C.
anti-N	30 mins. at 4°C.
anti-A(hel)	1 minute at room temp.

Aliquots of antiserum and cell suspension were placed on a scored glass tile and the result read after the appropriate time interval. The tile was intermittently rotated to mix the cells and antiserum. For investigations carried out at 4°C,the tile had previously been cooled to this temperature.

Tube technique.

Used for tests with:-

anti-M, anti-N - saline, room temp. 2 hours

anti-Le(a) - saline, 16°C 2 hours.

anti-e - Papain method, 37°C, 1 hour.

anti-C, anti-c, anti-D, anti-E, anti-C^{w} - Albumen displacement, 37°C, 2 hours.

One drop of cell suspension was incubated with one drop of the appropriate antiserum in a precipitin tube. In the case of anti-M, anti-N and anti-Le(a), a 1% saline suspension of the cells was used in the place of the usual 4% solution. The anti-Le(a) serum used contained anti-B, so that the results quoted for this system are for A and O individuals only.

For the papain method used with anti-e, the red cells were initially sensitized with 1% papain solution by aggitating one drop of the 4% saline suspension with 2 drops of the papain solution. The resulting suspension was spun so that the excess papain could be removed and the sensitized cells were re-suspended in two drops of saline. One drop of anti-e was added and the test read after incubation for 1 hour at 37°C.

For the antisera requiring an albumen displacement method, the albumen (30% in the case of anti-C, anti-c, anti-D and anti-E, 20% in the case of anti-C^{w}) was run gently down the inside of the precipitin tube after the cell suspension had been incubated with the antiserum for $1\frac{1}{2}$ hours. The test was read after a further incubation period of $\frac{1}{2}$ hour.

Coombs technique.

Used with:-

anti-Fy^a	anti-K	all 1 hour techniques.
anti-Fy^b	anti-k	
anti-S	anti-Kp^a	
anti-s	anti-Kp^b	

After one hour of incubation at 37°C the excess antiserum was removed by washing four times with saline. The resulting cells, resuspended in a drop of saline, were mixed with an equal volume of anti-human globulin for 10 minutes at room temperature on a scored tile. When available, Coombs control cells (Ortho) were used to corroborate negative results.

The sources from which the various antisera were obtained are shown in Appendix D.

(c) Electrophoresis.

All systems were investigated with starch-gel techniques. The haptoglobin and red cell acid phosphatase systems were run in 5 mm thick gels, the remaining systems (phosphoglucomutase, adenylate kinase, adenosine deaminase and esterase D) were run in 1 mm gels. The details of gel manufacture, buffers, staining solutions and the conditions for each run are described in detail in Appendix D.

(d) HLA antigen determination.

Tissue typing was performed by the method advocated by the National Institutes of Health (N.I.H.), which has now largely superseded the Trypan blue vital staining method of Kissmeyer-Nielsen. The N.I.H. method consists of separation of lymphocytes,

successive incubation with antiserum alone and then with a mixture of antiserum and complement and the reading of the test under phase-contrast with Eosin counter-staining. The antisera used were largely commercially produced, the prohibitive cost of these and the limited range available account for the reduced range of specificities tested for.

Separation of lymphocytes.

Where possible, the test was commenced within three hours of taking the blood samples. Where this was not possible it was found acceptable to store the heparinized blood samples for up to twelve hours at 4°C when, although the appearance of the lymphocytes was satisfactory, the reading of the test was made somewhat difficult by the presence of red cells in the test wells. It is suggested that stored red cells, by virtue of some change in their surface charge, possibly, are not removed as efficiently by centrifugation with Ficoll as are fresh cells.

About 10 μg of carbonyl iron powder was first added to the blood in the heparinized containers and this mixture was rotated gently at room temperature to enable the iron particles to adhere to the macrophages. Standing for five minutes on a magnet brought the iron particles and these cells to the bottom of the tube and the supernatant was carefully layered onto 5 mls of a Ficoll - Triosil mixture (composition given in Appendix D) in a plastic centrifuge tube. This was spun for 15 minutes at 2,000 r.p.m. and the result was that the erythrocytes, embedded in the Ficoll were contained in the lowest layer, and the white cells (lymphocytes and platelets) could be removed by careful pipetting

from a zone between a layer of Triosil and the uppermost layer of serum. These cells were placed in 5 mls of culture medium (R.P.M.I. 1640 - Gibco-Biocult) and centrifugation for 10 minutes at 1,000 r.p.m. produced a plug of lymphocytes and a suspension of platelets in culture medium which could be discarded. The lymphocytes were re-suspended in 10 drops of culture medium.

Incubation with antiserum.

The concentration of the lymphocytes was adjusted to about one million cells per ml.

Terasaki test plates had previously been prepared by introducing 1 µl of antiserum into each well. An equal quantity of the cell suspension was introduced into each antiserum aliquot with a Hamilton dispenser. The antiserum - lymphocyte mixture was then incubated at room temperature for one hour. Controls were set up with each plate - anti-lymphocyte serum (Biotest) as the positive control and a suspension of lymphocytes in the individual's own serum for a negative control. After this 1½ hours had elapsed 5 µl of rabbit complement were added to each well and incubation was continued for a further ½ hour,when,after counter-staining each well with 3 µl of Eosin solution,the tests were read under phase contrast.

Reading the test.

The N.I.H. method relies upon the cyto-toxic action of the antisera in the presence of complement so that a positive result is one in which a significant number of the lymphocytes have been killed. Dead lymphocytes are readily distinguishable

from live cells under phase contrast in that the latter are smaller and denser than the former with characteristic 'haloes' of bright light. Counting the number of dead cells in a representative field enabled the result to be scored according to the percentage of dead cells present:-

less than 20% dead cells	-	negative
21 - 40%	-	+
41 - 60%	-	++
61 - 80%	-	+++
81 - 100%	-	++++

Most antisera gave consistent 81 - 100% 'kills'. In the cases of weaker antisera, the presence of more than one for each specificity enabled a determination to be made with confidence in nearly every case.

The sources of antisera are listed in Appendix D.

6.3 The ABO system.

(a) With subdivision of group A.

Results for this system are shown in table 6.1 together with comparative data for English donors given by Sanger and Race (1951). No significant differences can be demonstrated between the two samples (groups A_1B and A_2B have been combined for the calculation of chi squared). The gene frequencies for the two populations are given in table 6.2. Formulae given by Mourant et al (1976) and attributable to Bernstein have been used to calculate these frequencies. The formulae are:-

$$p_1' = 1 - \sqrt{(\bar{O} + \bar{A}_2 + \bar{B} + \overline{A_2B})}$$

Table 6.1 Results for the ABO system, with subdivision of group A.

Phenotype	Number and frequency (%)	
	(1)Present study	(2)Sanger and Race (1951)
A_1	161 (31.3)	66 (28.8)
A_2	36 (7.0)	19 (8.3)
O	255 (49.5)	115 (50.2)
B	47 (9.1)	18 (7.9)
A_1B	12 (2.3)	9 (3.9)
A_2B	4 (0.8)	2 (0.9)
Totals	515	229

Chi squared ((1) vs. (2)) = 2.23 (d.f.=4), N.S.

Table 6.2 Gene frequencies for ABO system, with subdivision of group A.

Gene	Frequency	
	Present sample	Sanger and Race (1951)
A_1	0.1855	0.1793
A_2	0.0492	0.0573
B	0.0633	0.0650
O	0.7020 ($\pm$.02)	0.6984 ($\pm$.03)

$$p'_2 = \sqrt{(\bar{O} + \bar{A}_2 + \bar{B} + \overline{A_2B})} - \sqrt{(\bar{O} + \bar{B})}$$

$$q' = 1 - \sqrt{(\bar{O} + \bar{A})}$$

$$r' = \sqrt{\bar{O}}$$

where p'_1, p'_2, q' and r' are initial estimates of the $\underline{A}_1$, $\underline{A}_2$, $\underline{B}$ and $\underline{O}$ gene frequencies respectively.

From these initial estimates can be calculated a correction factor, D:-

$$D = 1 - (p'_1 + p'_2 + q' + r')$$

and final values are given by:-

$$p_1 = p'_1 (1 + \tfrac{1}{2}D)$$

$$p_2 = p'_2 (1 + \tfrac{1}{2}D)$$

$$q = q' (1 + \tfrac{1}{2}D)$$

$$r = (r + \tfrac{1}{2}D)(1 + \tfrac{1}{2}D)$$

These final values of p_1, p_2, q and r approximate to the maximum likelihood estimates for the gene frequencies. In practice the value of r was calculated from:-

$$r = 1 - (p_1 + p_2 + q)$$

as in Mourant et al (1976).

No significant differences with regard to sex, area of origin or source of controls could be demonstrated.

The results for the ABO system in controls subdivided by current age are shown in table 6.3. Although the suggested trend in the decrease of the A_1 frequency with increasing age is not significant. The data are given firstly to illustrate the method used to assess the significance of such trends and, secondly, because the results are pertinent to the arguments used in the next chapter.

Table 6.3 ABO phenotypes of controls subdivided by age.

Phenotype	Number and frequency (%) in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
A_1	29 (37.7)	42 (29.6)	42 (32.8)	48 (28.6)
A_2	9 (11.7)	8 (5.6)	7 (5.5)	12 (7.1)
0	33 (42.9)	73 (51.4)	61 (47.7)	88 (52.4)
B	3 (3.9)	14 (9.9)	14 (10.9)	16 (9.5)
A_1B	3 (3.9)	2 (1.4)	3 (2.3)	4 (2.4)
A_2B	0 (0)	3 (2.1)	1 (0.8)	0 (0)
Totals	77	142	128	168

Mean age of A_1 subjects = 44.05 ($\pm$1.7) years.

Mean age of 0 subjects = 47.76 ($\pm$1.8) years.

Z (A_1 vs. rest) = 1.022 (p=0.15)

The assessment of the significance of trends in proportions as advocated by Cox (1970) relies on the calculation of z in the following manner:-

age group	0 - 19	20 - 39	40 - 59	60 and over	Totals
coded age (x_i)	1	2	3	4	
A_1 (r_i)	29	42	42	48	161
not A_1 $(n_i - r_i)$	48	100	86	120	354
group total (n_i)	77	142	128	168	515

the figures in the above table denote the absolute number of individuals in each group, thence:-

$$r = \Sigma r_i = 161$$

$$n = \Sigma n_i = 515$$

$$t = \Sigma x_i r_i = 431$$

$$\bar{x} = \Sigma n_i x_i / n = 1417/515 = 2.75$$

$$nS_x = [(\Sigma n_i x_i^2 - (\Sigma n_i x_i)^2/n)] = (4485 - ((1417)^2/515))$$

$$= 586.19$$

$$E(T) = r\bar{x} = 442.75$$

$$\mathrm{var}(T) = \frac{r\,(n-r)\,S_x}{(n-1)} = 126.21$$

$$z = \frac{|t - E(T)| - \frac{1}{2}}{\sqrt{\mathrm{var}(T)}} = \frac{11.25}{126.21} = 1.00$$

The probability of the value of z exceeding 1.00 by chance alone is given by the table for the distribution of the normal probability integral (Fisher and Yates, 1974) and is equal to 0.16, i.e. it is not significant.

(b) No subdivision of group A.

The results for the ABO system without subdivision of group A are given in table 6.4,together with regional blood donor results derived from Kopeč (1970),which may now be used for comparison. The gene frequencies for the two samples are given in table 6.5. These have been calculated by the equations for three alleles given by Mourant et al (1976) and are similar to those used for the calculation of p_1,p_2,q and r above. The derivation of the blood donor data from Kopeč is shown in table 6.6. Frequencies for the twelve postal districts from which control subjects were derived have been amalgamated without weighting for the numbers coming from each. There are no significant differences between the present control sample and the donors. Standard errors for the frequencies are calculated by means of the formula given by Smith (1969):-

$$\text{standard error} = \sqrt{\frac{p(2 - p)}{4n}}$$

where p is the frequency of group A,for example, and n is the total sample size.

Sex,area of origin and source of controls reveal no significant differences and there is no significant trend in proportions with age although,as shown in table 6.7,the downward trend of group A with age just fails to achieve this.

It should be noted that the method used for examining the data for a trend in proportions with age will reveal only linear trends. More complex associations will not be demonstrated by this method.

Table 6.4 Results for the ABO system,no subdivision of group A.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Kopeč (1970)
A	197 (38.3)	1849 (38.3)
O	255 (49.5)	2367 (49.0)
B	47 (9.1)	443 (9.2)
AB	16 (3.1)	174 (3.6)
Totals	515	4833

Chi squared ((1) vs. (2)) = 0.13 (d.f.=3),N.S.

Table 6.5 ABO gene frequencies,no subdivision of group A.

Gene	Frequency (±S.E.)	
	Present sample	Kopeč (1970)
A	0.2347 (±.014)	0.2379 (±.005)
B	0.0633 (±.003)	0.0661 (±.003)
O	0.7020 (±.021)	0.7026 (±.007)

Table 6.6 Derivation of ABO phenotype data from blood donors resident in the Durham area, taken from Kopeč (1970).

Kopeč area						
No	Name	A	O	B	AB	N
32	Stanley	203	248	43	12	506
33	Birtley	130	152	30	17	329
34	Washington	46	75	14	6	141
35	Chester-le-Street	88	122	21	12	243
36	Houghton-le-Spring	136	169	31	13	349
37	Seaham	130	163	24	8	325
38	Easington	163	223	50	22	458
39	Horden	201	231	50	11	493
40	Durham	382	458	87	37	964
41	Spennymoor	72	86	17	11	186
43	Ferryhill	231	335	57	21	644
55	Crook	67	105	19	4	195
	Totals	1849	2367	443	174	4833

Table 6.7 ABO phenotype frequencies and age in controls.

Group	Number and frequency (%) in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
A	38 (49.3)	50 (35.2)	49 (38.3)	60 (35.7)
not A	39 (50.7)	92 (64.8)	79 (61.7)	108 (64.3)
Totals	77	142	128	168

Z = 1.404 (p=0.08)

6.3 The MNSs system.

(a) The MN locus.

Control subjects tested with anti-M and anti-N totalled 488 individuals. The results, together with comparative data for a Hertfordshire sample used as controls in a study of mental disorders (Thomas and Hewitt, 1939) is given in table 6.8. The differences between the two samples are not significant. The frequency of the heterozygote, MN, in the present sample is greater than the theoretical maximum of 50% predicted by the Hardy-Weinberg law. This is probably the result of the mis-typing of some M individuals as MN because of agglutination with anti-N as discussed by Mourant et al (1976). The deviation of the phenotype frequencies from that predicted has been considered unlikely to be due to heterozygote advantage in this system (Race and Sanger, 1975).

The gene frequencies for the present sample and that of Thomas and Hewitt (1939) are given in table 6.9. No significant differences with regard to sex, area of origin or source could be detected at this locus and there were no significant trends with age.

(b) The Ss locus.

400 controls were tested with anti-S alone, the results, together with those from 359 British and European subjects from London (Giles, 1964) are shown in table 6.10. There is no significant difference between these samples.

No significant sex differences are demonstrable within the present sample and, similarly, there are no differences with regard to area of origin or source.

Table 6.8 Results for the MN locus,control subjects.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Thomas and Hewitt (1939)
M	136 (26.5)	279 (31.0)
MN	266 (51.8)	436 (48.4)
N	86 (16.7)	185 (20.6)
Totals	488	900

Chi squared (H.W.) (1) = 5.05

Chi squared ((1) vs. (2)) = 4.72 (d.f.=2) N.S.

Table 6.9 Gene frequencies for the MN locus,control subjects.

Gene	Frequency (±S.E.)	
	Present sample	Thomas and Hewitt (1939)
M	0.5512 (±.023)	0.5522 (±.017)
N	0.4488	0.4478

Table 6.10 Results with anti-S, control subjects.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Giles (1964)
S+ve	209 (52.3)	201 (56.0)
S-ve	191 (47.7)	158 (44.0)
Totals	400	359

Chi squared ((1) vs. (2)) = 1.06 (d.f.=1) N.S.

Of the 400 controls tested with anti-S,273 were also tested with anti-s. These results are shown in table 6.11 together with comparative data from Germany (Wendt and Theile,1963),the only other published European population similarly tested. Table 6.12 gives the gene frequencies for these populations.

There were no significant differences at this locus with regard to sex,area of origin or source but table 6.13 demonstrates significant trends with age,the frequency of the heterozygote,Ss, increasing with age at the expense of the s homozygote. These trends are paralleled by changes in gene frequencies (see table 6.14).

(c) The MNSs complex.

Results from the 386 control subjects tested with anti-M,anti-N and anti-S are shown in table 6.15,together with comparative data from the largest published series of English subjects thus tested (Ikin et al,1952) and quoted in Mourant et al (1976). There are no significant differences between these two series. As in other reported samples,linkage disequilibrium can be demonstrated in this system in the present control sample with the S allele associated with the M allele significantly more often than it is with the N allele.

The results for the 271 controls tested with anti-M, anti-N,anti-S and anti-s are given in table 6.16,together with comparative data for 1,000 donors from the South East of England published by Cleghorn (1960). There are no significant differences

Table 6.11 Results for the Ss locus, control subjects.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Wendt and Theile (1963)
S	29 (10.6)	18 (16.7)
Ss	117 (42.9)	39 (36.1)
s	127 (46.5)	51 (47.2)
Totals	273	108

Chi squared (H.W.) (1) = 0.07

Chi squared ((1) vs. (2)) = 3.16 (d.f.=2) N.S.

Table 6.12 Gene frequencies for the Ss locus, control subjects.

Gene	Frequency (±S.E.)	
	Present sample	Wendt and Theile (1963)
S	0.3205 (±.028)	0.3472 (±.046)
s	0.6795	0.6528

Table 6.13 Ss phenotypes of controls subdivided according to age.

Phenotype	Number and frequency (%) in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
S	4 (8.9)	10 (12.5)	6 (9.4)	9 (10.7)
Ss	16 (35.6)	30 (37.5)	29 (45.3)	42 (50.0)
s	25 (55.6)	40 (50.0)	29 (45.3)	33 (39.3)
Totals	45	80	64	84

Z (heterozygotes vs. rest) = 1.853 p = 0.03

Z (s vs. rest) = 1.854 p = 0.03

Mean age S individuals = 43.19 (±4.0) years

Mean age Ss individuals = 48.04 (±2.0) years

Mean age s individuals = 43.47 (±1.9) years

Table 6.14 Gene frequencies Ss locus of controls according to age.

Gene	Frequency (±S.E.) for each age group			
	0 -19	20 - 39	40 - 59	60 and over
S	0.2667 (±.066)	0.3125 (±.052)	0.3203 (±.058)	0.3571 (±.052)
s	0.7333	0.6875	0.6797	0.6429

Table 6.15 Results for the MNSs system, controls tested with anti-M, anti-N and anti-S.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Ikin et al (1952)
MS	67 (17.4)	230 (19.7)
MNS	121 (31.3)	303 (26.0)
NS	17 (4.4)	56 (4.8)
Ms	37 (9.6)	113 (9.7)
MNs	97 (25.1)	264 (22.6)
Ns	47 (12.2)	200 (17.1)
Totals	386	1166

Chi squared ((1) vs. (2)) = 9.27 (d.f.= 5) N.S.

Table 6.16 Results for the MNSs system, controls tested with anti-M, anti-N, anti-S, and anti-s.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Cleghorn (1960)
MS	16 (5.9)	57 (5.7)
MNS	12 (4.4)	39 (3.9)
NS	1 (0.4)	3 (0.3)
MSs	30 (11.1)	140 (14.0)
MNSs	80 (29.5)	224 (22.4)
NSs	7 (2.6)	54 (5.4)
Ms	26 (9.6)	101 (10.1)
MNs	66 (24.4)	226 (22.6)
Ns	33 (12.2)	156 (15.6)
Totals	271	1000

Chi squared ((1) vs. (2)) = 11.61 (d.f. = 7) N.S.

between the two samples (categories MNS and NS have been amalgamated for the calculation of chi squared).

Figure 6.17 shows the relationship of phenotype requencies to age in these 271 subjects. Although none of these trends are significant the data are presented here as a preparation for the next chapter. Attention is drawn to the frequencies of the double heterozygote (MNSs) in the four age categories.

6.4 The P system.

A total of 509 controls were tested with anti-P_1. The results,shown in table 6.18,were recorded as positive or negative with the positive category subdivided according to strength of agglutination. All readings were performed by one of two individuals. The classification on the basis of strength of reaction is open to some criticism. Not only is there room for considerable observer error,but there is also a possibility that variations in the strength of the antiserum might lead to variation in the result. All the anti-P_1 used in this study was from the same source and the same batch but continual freezing and thawing of the antiserum over the time interval of the study might have affected the titre. Advantage was made of the fact that several blood samples were collected on two separate occasions,the second time specifically for HLA typing. One hundred and twenty-two specimens (diabetic and control) were collected twice, and were able to be tested,on the second occasion,by the individual who had assessed the reaction with anti-P_1 on the first occasion. The measure of agreement between the readings on these two occasions is shown in table 6.19. Only 63 (52%) of the samples gave the same result on the second occasion as they had on the first. Of the 59

Table 6.17 Results for the MNSs system, controls tested with anti-M, anti-N, anti-S and anti-s, subdivided according to age.

Phenotype	Number and frequency (%) in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
MS	2 (4.5)	5 (6.3)	3 (4.7)	6 (7.2)
MSs	4 (9.1)	6 (7.5)	13 (20.3)	7 (8.4)
Ms	3 (6.8)	11 (13.8)	5 (7.8)	7 (8.4)
MNS	2 (4.5)	5 (6.3)	2 (3.1)	3 (3.6)
MNSs	11 (25.0)	23 (28.8)	16 (25.0)	30 (36.1)
MNs	15 (34.1)	20 (25.0)	13 (20.3)	18 (21.7)
NS	0	0	1 (1.6%)	0
NSs	1 (2.3)	1 (1.3)	0	5 (6.0)
Ns	6 (13.6)	9 (11.3)	11 (17.2)	7 (8.4)
Totals	44	80	64	83

Table 6.18 Results for control subjects tested with anti-P_1.

Reaction with anti-P_1	Number and frequency (%)
-ve	128 (25.1)
1	185 (36.3)
2	130 (25.5)
3	65 (12.8)
4	1 (0.2)
Total	509

Table 6.19 Results for subjects tested with anti-P_1 on two occasions.

		Result on the second occasion			
		-ve	1	2	3
Result on the first occasion	-ve	9	1	1	0
	1	10	25	3	3
	2	5	14	15	4
	3	0	10	8	14

Table 6.20 Results for subjects tested with anti-P_1 on two occasions (classification into +ve and -ve only).

		Result on the second occasion	
		-ve	+ve
Result on the first occasion	-ve	9	2
	+ve	15	96

samples (48%) that were classified differently on the second occasion, 12 (20%) of them were recorded as reacting more strongly and 47 (80%) as reacting more weakly than they did on the first occasion.

If the results are classified as either positive or negative without grading of strength of reaction 86% of samples give the same result twice (table 6.20). Although this level of agreement is no reason for congratulation it was felt justifiable to consider positive and negative subdivisions of reaction but not, under these laboratory conditions,any finer subdivisions. The direction of change,predominantly from a stronger to a weaker category,of these samples with time suggests that the strength of the antiserum was being progressively reduced.

The present sample,classified as positive or negative,is shown in table 6.21 together with that of Ikin et al (1954) composed of 1,166 English blood donors. There are no significant differences between these samples. Table 6.22 shows the gene frequencies for the two populations.

There were no detectable differences between the sexes,individuals from different areas or source of controls. The P system showed no relationship with current age.

6.5 The Rhesus system.

The Rhesus phenotypes of 515 individuals from the control group were determined. These samples were all tested with anti-C,anti-c,anti-D and anti-E. Those positive with anti-E were also tested with anti-e. Unusual phenotypes were checked with the full range of commercial antisera. Results are given first for the complete phenotypes and,secondly,considering the C,D and E loci

Table 6.21 Results with anti-P_1, control subjects.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Ikin et al (1954)
+ve	381 (74.9)	893 (76.6)
-ve	128 (25.1)	273 (23.4)
Totals	509	1166

Table 6.22 Gene frequencies for the P system, control subjects.

Gene	Frequency (±S.E.)	
	Present sample	Ikin et al (1954)
P_1	0.4985 (±.022)	0.5161 (±.015)
P_2 + p	0.5015	0.4839

separately. Gene frequencies in the latter case are calculated by the method of gene counting, the haplotype frequencies are estimated from the phenotype data by means of a maximum likelihood computer programme.

(a) Phenotype frequencies considering the three loci C,D and E.

Table 6.23 shows the phenotype frequencies for the entire control sample compared with those given for 1,073 English subjects by Race et al (1948). Combining the categories R_or, $r'r$, R_zR_1, $r''r$ and R_zR_2 enables a chi squared to be calculated and this shows no differences between the two samples. Table 6.24 gives the haplotype frequencies for these two populations calculated by the same computer programme.

There are no significant differences attributable to sex, area of origin or source in these frequencies but table 6.25 shows that a significant relationship exists with age. The *rr* genotype increases in frequency with increasing age of the subjects. Table 6.26 shows the relationship of age and haplotype frequency. The value of z for this trend (considering *rr* vs not-*rr*) is 1.998, $p = 0.023$.

(b) Separate consideration of the C,D and E loci.

Tables 6.27, 6.28 and 6.29 show results for the C,D and E loci in the present series and in that of Race et al (1948). There are no significant differences between the populations. Tables 6.30 and 6.31 give the gene frequencies for the *C*, *c*, *E* and *e* alleles. The frequency of the *Cc* heterozygote is greater than the 50% theoretical maximum. If this is the result of typing errors, as is

Table 6.23 Results for the Rhesus system, control subjects.

Reaction with anti-					Probable	Number and frequency (%)	
C	c	D	E	e	genotype	(1) Present sample	(2) Race et al (1948)
-	+	-	-	+	r r	81 (15.7)	170 (15.8)
+	+	+	-	+	R_1r	194 (37.7)	363 (33.8)
+	-	+	-	+	R_1R_1	86 (16.7)	190 (17.7)
-	+	+	-	+	R_or	4 (0.8)	19 (1.8)
-	+	+	+	+	R_2r	53 (10.3)	137 (12.8)
-	+	+	+	-	R_2R_2	11 (2.1)	29 (2.7)
+	+	+	+	+	R_1R_2	77 (15.0)	144 (13.4)
+	-	-	-	+	r'r'	0	0
+	+	-	-	+	r'r	5 (1.0)	10 (0.9)
+	-	+	+	+	R_zR_1	0	4 (0.4)
+	-	-	+	+	$r'r^y$	0	0
+	+	-	+	+	r"r'	0	0
-	+	-	+	+	r"r	2 (0.4)	7 (0.7)
+	-	+	+	-	R_zR_z	0	0
+	+	+	+	-	R_zR_2	2 (0.4)	0
+	-	-	+	-	r^yr^y	0	0
+	+	-	+	-	r^yr''	0	0
-	+	-	+	-	r"r"	0	0
						515	1073

Chi squared ((1) vs. (2)) = 6.0 (d.f.=6) N.S.

Table 6.24 Rhesus haplotype frequencies, control subjects.

Haplotype	Present sample	Race et al (1948)
r	0.4018	0.3973
r'	0.0119	0.0114
r"	0.0044	0.0081
r^y	0.0019	0
R_o	0.0098	0.0262
R_1	0.4230	0.4066
R_2	0.1470	0.1513
R_z	0	0.0037

Table 6.25 Rhesus phenotype results, control subjects subdivided by age.

Reaction with anti-					Probable genotype	Number and frequency (%) in each age group								Totals	
C	c	D	E	e		0-19		20-39		40-59		60 and over			
-	+	-	-	+	r r	9	(11.7)	19	(13.4)	18	(14.1)	35	(20.8)	81	(15.7)
+	+	+	-	+	R_1r	29	(37.7)	50	(35.2)	54	(42.2)	61	(36.3)	194	(37.7)
+	-	+	-	+	R_1R_1	12	(15.6)	33	(23.2)	17	(13.3)	24	(14.3)	86	(16.7)
-	+	+	-	+	R_or	0		1	(0.7)	1	(0.8)	2	(1.2)	4	(0.8)
-	+	+	+	+	R_2r	2	(2.6)	18	(12.7)	18	(14.1)	15	(8.9)	53	(10.3)
-	+	+	+	-	R_2R_2	3	(3.9)	0		5	(3.9)	3	(1.8)	11	(2.1)
+	+	+	+	+	R_1R_2	20	(26.0)	20	(14.1)	13	(10.2)	24	(14.3)	77	(15.0)
+	-	-	-	+	r'r'	0		0		0		0		0	
+	+	-	-	+	r'r	1	(1.3)	0		2	(1.6)	2	(1.2)	5	(1.0)
+	-	+	+	+	R_zR_1	0		0		0		0		0	
+	-	-	+	+	$r'r^y$	0		0		0		0		0	
+	+	-	+	+	r"r'	0		0		0		0		0	
-	+	-	+	+	r"r'	0		0		0		2	(1.2)	2	(0.4)
+	-	+	+	-	R_zR_z	0		0		0		0		0	
+	+	+	+	-	R_zR_2	1	(1.3)	1	(0.7)	0		0		2	(0.4)
+	-	-	+	-	r^yr^y	0		0		0		0		0	
+	+	-	+	-	r^yr''	0		0		0		0		0	
-	+	-	+	-	r"r'	0		0		0		0		0	
Totals						77		142		128		168		515	

Table 6.26 Rhesus haplotype frequencies, control subjects subdivided by age.

Haplotype	Frequency in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
r	0.3247	0.3707	0.4257	0.4458
r'	0.0195	0	0.0180	0.0131
r"	0	0	0	0.0133
r^y	0.0065	0.0035	0	0
R_o	0	0.0096	0.0118	0.0125
R_1	0.4610	0.4789	0.3844	0.3887
R_2	0.1883	0.1373	0.1602	0.1266
R_z	0	0	0	0
No.	77	142	128	168

Table 6.27 Results for control subjects tested with anti-C and anti-c.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Race et al (1948)
C	86 (16.7)	194 (18.1)
Cc	278 (54.0)	517 (48.2)
c	151 (29.3)	362 (33.7)
Totals	515	1073

Chi squared ((1) vs. (2)) = 4.81 (d.f.=2) N.S.

Chi squared (H.W.) (1) = 4.85

Table 6.28 Results for control subjects tested with anti-D.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Race et al (1948)
d	88 (17.1)	187 (17.4)
D	427 (82.9)	886 (82.6)
Totals	515	1073

Table 6.29 Rhesus results for control subjects tested with anti-E and anti-e.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Race et al (1948)
E	13 (2.5)	29 (2.7)
Ee	132 (25.6)	292 (27.2)
e	370 (71.8)	752 (70.1)
Total	515	1073

Chi squared ((1) vs. (2)) = 0.52 (d.f.=2) N.S.

Chi squared (H.W.) (1) = 0.09

Table 6.30 Gene frequencies for the C locus, control subjects.

Gene	Frequency (±S.E.)	
	Present sample	Race et al (1948)
C	0.4369 (±.022)	0.4217 (±.015)
c	0.5631	0.5783

Table 6.31 Gene frequencies for the E locus, control subjects.

Gene	Frequency (±S.E.)	
	Present sample	Race et al (1948)
E	0.1534 (±.016)	0.1631 (±.011)
e	0.8466	0.8369

most likely, it does not conform to the most usual error (cited by Mourant et al, 1976) - false negative results with anti-c which give an excess of CC homozygotes and a deficit of heterozygotes.

Consideration of only the D and d alleles allows for comparison with regional blood donor data derived from Kopeč (1970). Table 6.32 shows the derivation of these data from the same twelve postal districts as were used for the ABO data. There is no significant difference between the D -ve frequency in the present sample and that in these donors.

There is a difference between the sexes in the frequency of D -ve individuals. Although this difference is not significant in this sample, the data are given (table 6.33) since they are pertinent to the argument concerning the relationship between the frequency of this phenotype and age. No significant differences between the sexes are detectable at the C and E loci, nor are there any differences at any of the three loci when area of origin and source are considered.

Table 6.34 and table 6.35 show the influence of age on the phenotype frequencies considering the C and D loci respectively. There is a significant upward trend in the frequency of the c phenotype with age and in the frequency of the D -ve phenotype. The C locus gene frequencies in the various age sub-classes are shown in table 6.36.

If, instead of categorisation into four age groups, the sample is divided into two, under 55 years and 55 years and over, the difference in the D -ve frequencies is significant by chi squared (table 6.37).

The mean age of the D -ve individuals is 51.67 years ($\pm$ 2.4) and the mean age of the D +ve individuals is 45.45 ($\pm$ 1.0)

Table 6.32 Derivation of comparative data for Rhesus (D) from Kopeč (1970).

Kopeč area		No. Rh -ve	N	$\frac{dd}{N}$ %
No.	Name			
32	Stanley	102	506	20.16
33	Birtley	58	329	17.63
34	Washington	29	141	20.57
35	Chester-le-Street	50	243	20.58
36	Houghton-le Spring	75	349	21.49
37	Seaham	69	325	21.23
38	Easington	83	458	18.12
39	Horden	91	493	18.46
40	Durham	182	964	18.88
41	Spennymoor	47	186	25.27
43	Ferryhill	108	644	16.77
55	Crook	33	195	16.92
	Totals	927	4833	19.18
	Present sample	88	515	17.1

Chi squared (comparison of Kopeč sample and present sample) = 1.32

(d.f. = 1) N.S.

Table 6.33 Rhesus phenotype results (D only), controls subdivided by sex.

Phenotype	Number and frequency (%)	
	Males	Females
d	38 (14.2)	50 (20.2)
D	230 (85.8)	197 (79.8)

Chi squared (comparison of males and females) = 3.34 (d.f.=1) N.S.

Table 6.34 Results with anti-C and anti-c, controls subdivided by age.

Phenotype	Number and frequency (%) in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
C	12 (15.6)	33 (23.3)	17 (13.3)	24 (14.3)
Cc	51 (66.2)	71 (50.0)	69 (53.9)	87 (51.8)
c	14 (18.2)	38 (26.8)	42 (32.8)	57 (33.9)
Totals	77	142	128	168

Z (c vs. rest) = 2.541 (p = 0.005)

Z (Cc vs. rest) = 1.358 (p = 0.09)

Table 6.35 Results with anti-D, controls subdivided by age.

Phenotype	Number and frequency (%) in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
d	10 (13.0)	19 (13.4)	20 (15.6)	39 (23.2)
D	67 (87.0)	123 (86.6)	108 (84.4)	129 (76.8)
Totals	77	142	128	168

Z = 2.343 (p = 0.0096)

Table 6.36 Gene frequencies, C locus, for control subjects subdivided by age.

Gene	Frequencies (±S.E.) in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
C	0.4870 (±.057)	0.4824 (±.042)	0.4023 (±.043)	0.4018 (±.038)
c	0.5130	0.5176	0.5977	0.5982

Table 6.37 Rhesus (D) frequencies in controls subdivided by age, division at 55 years.

Phenotype	Number and frequency (%) in each age group	
	Under 55	55 and over
d	43 (14.0)	45 (21.0)
D	264 (86.0)	163 (78.4)
Totals	307	208

years. This difference is significant, the 2-tailed pooled variance estimate of Student's t is 2.46 which, for 513 degrees of freedom, gives p = 0.014.

The four age categories considered do not contain equal proportions of males and females (table 6.38). Since there is a sex difference, albeit statistically insignificant, in the D -ve and D +ve frequencies (table 6.33) and there are more of the predominantly D -ve females in the oldest group, the question arises as to whether sex differences are producing a spurious relationship with age in this system. Tables 6.39 and 6.40 show that the upward trend of D -ve frequencies with age is present in both males and females although in neither of these small groups does it reach acceptable levels of significance.

6.6 The Kell system.

(a) Tests with anti-K and anti-k.

Five hundred and fourteen control subjects were tested with anti-K. Those found to be positive were also tested with anti-k. One individual of the genotype KK was found, this was confirmed with commercial anti-K and anti-k. Table 6.41 shows the phenotype results for the present control sample together with those published by Ikin et al (1954). There are no significant differences (K and Kk categories have been combined for the calculation of chi squared). Table 6.42 shows the gene frequencies for the two populations, neither show differences from Hardy-Weinberg expectation.

There are no significant differences with regard to sex, area of origin or source for this locus and, although the frequency of heterozygotes rises with age, this trend is not significant.

Table 6.38 Sex and age distribution of controls tested with anti-D.

Sex	Number and frequency (%) in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
Male	46 (59.7)	95 (66.9)	72 (56.3)	55 (32.7)
Female	31 (40.3)	47 (33.1)	56 (43.7)	113 (67.3)
	77	142	128	168

Table 6.39 Rhesus (D) results, male controls subdivided by age.

Phenotype	Number and frequency (%) in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
d	6 (13.0)	10 (10.5)	11 (15.3)	11 (20.0)
D	40 (87.0)	85 (89.5)	61 (84.7)	44 (80.0)
Totals	46	95	72	55

Z = 1.259 (p = 0.10)

Table 6.40 Rhesus (D) results, female controls subdivided by age.

Phenotype	Number and frequency (%) in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
d	4 (12.9)	9 (19.1)	9 (16.1)	28 (24.8)
D	27 (87.1)	38 (80.9)	47 (83.9)	85 (75.2)
Totals	31	47	56	113

Z = 1.514 (p = 0.07)

Table 6.41 Results with anti-K and anti-k,control subjects.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Ikin et al (1954)
K	1 (0.2)	1 (0.1)
Kk	34 (6.6)	89 (7.6)
k	479 (93.2)	1076 (92.3)
Totals	514	1166

Chi squared (H.W.) (1) = 0.23

Chi squared ((1) vs. (2)) = 0.42 (d.f.=2) N.S.

Table 6.42 Kell locus gene frequencies,control subjects.

Gene	Frequency (±S.E.)	
	Present sample	Ikin et al (1954)
K	0.0350 (±.008)	0.0390 (±.006)
k	0.9650	0.9610

(b) Tests with anti-Kp^a and anti-Kp^b.

A total of 423 controls were tested with anti-Kp^a and the subjects positive with this antiserum were also tested with anti-Kp^b. No examples of the genotype Kp^aKp^a were found. Table 6.43 shows the data for the present control sample and that of the only other published sample similarly tested and with the Kp^a allele present. This is the Canadian donor group of Dichupa et al (1969) which shows a higher frequency of Kp^a +ve individuals than the present sample, although the difference is not significant. The gene frequencies for the two samples are given in table 6.44.

There are no differences with regard to sex,area of origin or age which are statistically significant.

(c) Tests with anti-K,anti-k,anti-Kp^a and anti-Kp^b.

Table 6.45 shows the results for the 423 individuals tested with these antisera together with the frequencies in a group of Michigan donors of West European origin (Shreffler et al (1971). No significant differences can be demonstrated between these two populations.

There are no differences with regard sex,area of origin, source or age demonstrable in this sample.

6.7 The Lewis system.

The anti-Le (a) antiserum available contained anti-B and the results (table 6.46) are,consequently,for group A and group O controls only. There was only sufficient antiserum for the testing of 108 non-diabetic individuals. The comparative data are derived from Race and Sanger (1968),no significant differences were found

Table 6.43 Results with anti-Kp^a, control subjects.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Dichupa et al (1969)
Kp^a	0	5 (0.04)
Kp^aKp^b	6 (1.4)	269 (2.4)
Kp^b	417 (98.6)	10965 (97.6)
	423	11239

Chi squared (H.W.) (1) = 0.02

Chi squared ((1) vs. (2)) = 1.8 (d.f.=1) N.S.

Table 6.44 Gene frequencies for Kp^a locus, control subjects.

Gene	Frequency (±S.E.)	
	Present sample	Dichupa et al (1969)
Kp^a	0.0071 (±.004)	0.0124 (±.001)
Kp^b	0.9929	0.9876

Table 6.45 Results for the Kell-Kp^a complex, control subjects.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Shreffler et al (1971)
KKp^b	1 (0.2)	6 (0.1)
$KkKp^aKp^b$	0	5 (0.1)
$KkKp^b$	27 (6.4)	589 (7.0)
kKp^a	0	1 (0.01)
kKp^aKp^b	6 (1.4)	125 (1.5)
kKp^b	389 (92.0)	7717 (91.4)
Totals	423	8443

Chi squared ((1) vs. (2)) = 0.22 (d.f. = 2) N.S.

Table 6.46 Results for control subjects tested with anti-Lewis (a), group A and group O persons only.

Reaction with anti-Lewis (a)	Number and frequency (%)	
	(1)Present sample	(2)Race and Sanger (1968)*
+ve	30 (27.8)	203 (21.9)
-ve	78 (72.2)	725 (78.1)
Totals	108	928

Chi squared ((1) vs. (2) = 1.94 (d.f. = 1) N.S.

* All ABO blood groups included in this sample.

between the two samples.

There were insufficient individuals to allow subdivision on the basis of sex,area of origin or age.

6.9 The Duffy system.

Testing with anti-Fy^a alone was performed on 507 control specimens. Table 6.47 shows the results for these and the sample reported by Ikin et al (1954). There are no significant differences between the samples.

No significant trends were found with age neither were there any differences with regard to sex,area of origin nor source.

Of the 507 individuals tested with anti-Fy^a,289 were also tested with anti-Fy^b. Table 6.48 shows the results together with comparative data from Cleghorn (1965). There are no significant differences. Table 6.49 shows the gene frequencies for the two populations. In neither case do the phenotype categories differ from Hardy-Weinberg prediction.

There was no detectable difference when the sex,area of origin and source of controls were considered. There were no trends with age.

6.10 Haptoglobin.

Only 86 control subjects were typed for haptoglobin phenotype. The results are presented in table 6.50,together with the series published by Harris et al (1959). No significant differences can be demonstrated between the two samples. Table 6.51 shows the gene frequencies of these two groups.

There were insignificant numbers available for further

Table 6.47 Results for control subjects tested with anti-Fy^a.

Reaction with anti-Fy^a	Number and frequency (%)	
	(1)Present sample	(2)Ikin et al (1954)
+ve	326 (64.3)	764 (65.5)
-ve	181 (35.7)	402 (34.5)
Totals	507	1166

Chi squared ((1) vs. (2)) = 0.23 (d.f. = 1) N.S.

Table 6.48 Results for control subjects tested with anti-Fy^a and anti-Fy^b.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Cleghorn (1965)
Fy^a	55 (19.0)	130 (19.8)
Fy^a Fy^b	126 (43.6)	321 (48.9)
Fy^b	108 (37.4)	205 (31.3)
Totals	289	656

Chi squared (H.W.) (1) = 2.76

Chi squared ((1) vs. (2)) = 3.54 (d.f. = 2) N.S.

Table 6.49 Duffy system gene frequencies, control subjects.

Gene	Frequency (±S.E.)	
	Present sample	Cleghorn (1965)
Fy^a	0.4083 (±.029)	0.4428 (±.019)
Fy^b	0.5917	0.5572

Table 6.50 Haptoglobin results,control subjects.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Harris et al (1959)
1-1	16 (18.6)	33 (18.4)
2-1	44 (51.2)	88 (49.2)
2-2	26 (30.2)	58 (32.4)
Totals	86	179

Chi squared (H.W.) (1) = 0.12

Chi squared ((1) vs. (2)) = 0.13 (d.f. = 2) N.S.

Table 6.51 Haptoglobin gene frequencies,control subjects.

Gene	Frequency (±S.E.)	
	Present sample	Harris et al (1959)
Hp^1	0.4419 (±.336)	0.4302 (±.037)
Hp^2	0.5581	0.5698

subdivision by sex,area of origin,source or age.

6.11 Acid phosphatase.

The acid phosphatase results for 229 controls are given in table 6.52. These differ significantly from the series of Hopkinson and Harris (1969) in having a higher frequency of the $\underline{P}^b$ allele and a lower frequency of $\underline{P}^c$. There is no way of telling whether this is a regional effect (Hopkinson and Harris's sample was from the South of England) or the result of technical errors leading to an under-representation of the CA phenotype and an over-representation of the BA phenotype in the present sample. The gene frequencies for the system are given in table 6.53.

There are significant differences with age in that the A phenotype increases in frequency with increasing age (table 6.55). No significant differences are seen with regard to sex,area of origin or source.

6.12 Phosphoglucomutase.

The results for the 115 controls examined for the PGM_1 locus are shown in table 6.56. This sample differs significantly from that of Hopkinson and Harris (1966) in having a higher $\underline{PGM}_1^1$ gene frequency. The 2-1 and 2-2 categories were combined for the calculation of this chi squared. Table 6.57 shows the gene frequencies for the two series. Neither differs significantly from Hardy Weinberg expectation.

There are no significant differences with regard to sex,area of origin or source in this system. Table 6.58 shows that, when the sample is subdivided by age,the numbers in each group are

Table 6.52 Acid phosphatase results,control subjects.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Hopkinson and Harris (1969)
A	24 (10.5)	119 (13.5)
BA	111 (48.5)	379 (43.1)
B	80 (34.9)	282 (32.1)
CA	4 (1.7)	39 (6.9)
CB	10 (4.4)	39 (4.4)
Totals	229	880

Chi squared ((1) vs. (2)) = 7.86 (d.f. = 3) p 0.05

Table 6.53 Acid phosphatase gene frequencies,control subjects.

Gene	Frequency (±S.E.)	
	Present sample	Hopkinson and Harris (1969)
$\underline{P}^a$	0.3559	0.3727
$\underline{P}^b$	0.6135	0.5705
$\underline{P}^c$	0.0306	0.0568

Table 6.54 Acid phosphatase results, control subjects subdivided according to age.

Phenotype	Number and frequency (%) in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
A	1 (6.3)	4 (4.2)	6 (11.1)	13 (20.3)
BA	11 (68.8)	44 (46.3)	25 (46.3)	31 (48.4)
B	3 (18.8)	40 (42.1)	20 (37.0)	17 (26.6)
CA	0	6 (6.3)	3 (5.6)	1 (1.6)
CB	1 (6.3)	1 (1.1)	0	2 (3.1)
Totals	16	95	54	64

Z (A vs. rest) = 2.976 ($p = 0.001$)

Table 6.55 Acid phosphatase gene frequencies, control subjects subdivided according to age.

Gene	Frequency in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
$\underline{p}^a$	0.4063	0.3053	0.3704	0.4531
$\underline{p}^b$	0.5625	0.6579	0.6018	0.5234
$\underline{p}^c$	0.0312	0.0368	0.0278	0.0234

Table 6.56 Phosphoglucomutase results, control subjects.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Hopkinson and Harris (1966)
1-1	79 (68.7)	1238 (58.7)
2-1	32 (27.8)	754 (35.7)
2-2	4 (3.5)	118 (5.6)
Totals	115	2110

Chi squared (H.W.) (1) = 0.11 (d.f. = 3) N.S.

Chi squared ((1) vs. (2)) = 4.55 (d.f. = 1) p 0.05

Table 6.57 Phosphoglucomutase gene frequencies, control subjects.

Gene	Frequency (±S.E.)	
	Present sample	Hopkinson and Harris (1966)
$P.G.M._1^1$	0.8261 (±.035)	0.7641 (±.009)
$P.G.M._1^2$	0.1739	0.2348

Table 6.58 Phosphoglucomutase results, control subjects subdivided according to age.

Phenotype	Number and frequency (%) in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
1-1	2 (66.7)	29 (78.4)	23 (67.6)	25 (61.0)
2-1	0	7 (18.9)	11 (32.4)	14 (34.1)
2-2	1 (33.3)	1 (2.7)	0	2 (4.9)
Totals	3	37	34	41

Z (2-1 vs. rest) = 1.653 (p = 0.05)

Mean age of heterozygotes = 54 65 (±3.7) years

Mean age of homozygotes = 45.55 (±0.9) years

small but the trend for increasing frequency of heterozygotes with increasing age almost reaches significance at the 0.05 level.

6.13 Adenylate kinase.

Table 6.59 shows the results for 395 controls and a series reported by Rapley et al (1967). There are no significant differences between these samples and neither deviates significantly from Hardy-Weinberg expectation.

No differences with regard to sex,area of origin or source can be found and there is no significant trend of adenylate kinase phenotype frequencies with age.

6.14 Adenosine deaminase.

Phenotype frequencies for the controls are shown in table 6.61. The results do not differ significantly from those given for this system by Hopkinson et al (1969). Gene frequencies are given in table 6.62.

There are too few results for subdivision by sex,area of origin or age.

6.15 Esterase D.

Table 6.63 shows the results for 390 controls typed for esterase D. They show no significant differences from a sample reported by Hopkinson et al (1973). The gene frequencies for these samples are given in table 6.64. Phenotype frequencies accord with those predicted by the Hardy-Weinberg law.

Table 6.59 Adenylate kinase results, control subjects.

Phenotype	Number and frequency (%)	
	(1) Present sample	(2) Rapley et al (1967)
1-1	363 (91.9)	1720 (91.1)
2-1	30 (7.6)	165 (8.7)
2-2	2 (0.5)	2 (0.1)
Totals	395	1887

Chi squared (H.W.) (1) = 2.40

Chi squared ((1) vs. (2)) = 0.23 (d.f. = 1) N.S.

Table 6.60 Adenylate kinase gene frequencies, control subjects.

Gene	Frequency (±S.E.)	
	Present sample	Rapley et al (1967)
$\underline{A.K.}^1$	0.9570 (±.010)	0.9552 (±.005)
$\underline{A.K.}^2$	0.0430	0.0448

Table 6.61 Adenosine deaminase results, control subjects.

Phenotype	Number and frequency (%)	
	(1)Present sample	(2)Hopkinson et al (1969)
1-1	96 (94.1)	1223 (90.4)
2-1	6 (5.9)	127 (9.4)
2-2	0	3 (0.2)
Totals	102	1353

Chi squared (H.W.) (1) = 0.09

Chi squared ((1) vs. (2)) = 1.55 (d.f. = 1) N.S.

Table 6.62 Adenosine deaminase gene frequencies, control subjects.

Gene	Frequency (±S.E.)	
	Present sample	Hopkinson et al (1969)
ADA^1	0.9706 (±.017)	0.9508 (±.006)
ADA^2	0.0294	0.0492

Table 6.63 Esterase D results, control subjects.

Phenotype	Number and frequency (%)	
	(1) Present sample	(2) Hopkinson et al (1973)
1-1	304 (77.9)	705 (81.3)
2-1	82 (21.0)	154 (17.8)
2-2	4 (1.0)	8 (0.9)
Totals	390	867

Chi squared (H.W.) (1) = 0.35

Chi squared ((1) vs. (2)) = 1.92 (d.f. = 1) N.S.

Table 6.64 Esterase D gene frequencies, control subjects.

Gene	Frequency (±S.E.)	
	Present sample	Hopkinson et al (1973)
$\underline{ESD}^1$	0.8846 (±.016)	0.9020 (±.010)
$\underline{ESD}^2$	0.1154	0.0980

6.16 The HLA system.

The absolute phenotype frequencies and the percentage frequencies for the HLA-A,HLA-B and HLA-C locus specificities considered are given in table 6.65,together with comparative data (Murray et al,1976) of hospital staff resident in the Newcastle area. For each specificity,the chi squared value for the difference between the two samples is calculated from the contingency table of the numbers of individuals positive for the specificity and the numbers negative for the specificity in the two populations.

The only specificity for which the value of p is less than 0.05 is HLA-A9. When the large number of comparisons is taken into account by multiplying the value of p by 15,the result is non-significant so that this difference may be due to chance. The Newcastle sample was not tested with anti-HLA-Cw4.

Each HLA locus is analogous to the ABO system in that a series of co-dominant alleles and one recessive allele (for the HLA system,the 'blank',for the ABO,the O allele) segregate at each locus. A formula for the calculation of HLA gene frequencies,based on this analogy is given by Mam and Gart (1976).

The first stage is the calculation of an initial estimate (p_i) of the gene frequency of each allele,except 'blank', from the formula:-

$$p_i' = 1 - \sqrt{1 - (G_i/N)}$$

where G_i is the number of individuals positive for each specificity and N is the number of individuals tested. The initial estimate of the 'blank' frequency is given by:-

$$r' = \sqrt{f_o}$$

where f_o is the frequency of individuals who are

Table 6.65 HLA results,control subjects.

Locus	Allele	Number and frequency (%)		Chi squared (d.f.=1)	p
		Present sample n=427	Murray et al (1976) n=246		
A	HLA-A1	147 (34.4)	91 (37.0)	0.45	N.S.
	HLA-A2	207 (48.5)	133 (54.0)	1.94	N.S.
	HLA-A3	122 (28.6)	64 (26.0)	0.50	N.S.
	HLA-A9	57 (13.3)	47 (19.0)	3.96	0.05
	HLA-A10	48 (11.2)	30 (12.0)	0.14	N.S.
	HLA-A11	40 (9.4)	20 (8.0)	0.29	N.S.
B	HLA-B5	43 (10.1)	20 (8.0)	0.69	N.S.
	HLA-B7	119 (27.9)	74 (30.0)	0.38	N.S.
	HLA-B8	126 (29.5)	69 (28.0)	0.16	N.S.
	HLA-B12	145 (34.0)	81 (33.0)	0.08	N.S.
	HLA-B13	17 (4.0)	7 (3.0)	0.59	N.S.
	HLA-B14	31 (7.3)	20 (8.0)	0.17	N.S.
	HLA-B17	32 (7.5)	26* (12.0)	3.39	N.S.
	HLA-B27	35 (8.2)	20 (8.0)	0	N.S.
	HLA-Bw15	53 (12.4)	34* (14.0)	1.21	N.S.
C	HLA-Cw4	23+ (12.9)	Not tested		

* n = 219 + n = 373

homozygous 'blank'.

The following formula gives adjusted estimates of the gene frequencies which are close to the maximum likelihood estimates:-

$$p_i = \frac{G_i}{2N} + \frac{p_i}{p_i' + 2r' + D} x \left(\frac{n_i}{2N} \right)$$

where D is the correction factor calculated in exactly the same way for each HLA locus as it is for the ABO system and n_i is the number of individuals homozygous for the allele under consideration. The final value of r, the frequency of 'blank', is calculated from 1 - p .

Table 6.66 shows the gene frequencies for the present sample, those of the Newcastle population (Murray et al, 1976) cannot be computed because n_i is unknown.

From these gene frequencies, haplotype frequencies are calculated indirectly. The value of delta, the linkage disequilibrium, for each pair of A and B locus alleles is given by:-

$$\text{Delta} = \sqrt{\frac{d}{N}} - \sqrt{\frac{(b+d)}{N} \times \frac{(c+d)}{N}}$$

where b, c and d are, respectively, the numbers of individuals possessing the first only, the second only and both specificities under consideration. This formula is taken from Mattiuz et al (1970).

With the value of delta known for each combination, the haplotype frequency is calculated from the formula:-

haplotype frequency $= (p_A \times p_B) + \text{delta}$

where p_A and p_B are the gene frequencies of the A and B locus alleles under consideration.

The calculation of haplotype frequencies in this manner

Table 6.66 HLA gene frequencies,control subjects.

Locus	Allele	Frequency
A	HLA-A1	0.1882
	HLA-A2	0.2884
	HLA-A3	0.1547
	HLA-A9	0.0691
	HLA-A10	0.0585
	HLA-A11	0.0480
	Blank	0.1931
B	HLA-B5	0.0507
	HLA-B7	0.1498
	HLA-B8	0.1611
	HLA-B12	0.1892
	HLA-B13	0.0199
	HLA-B14	0.0367
	HLA-B17	0.0381
	HLA-B27	0.0421
	HLA-Bw15	0.0635
	Blank	0.2489

has been shown to agree well with direct assessment from the study of family data.(Svejgaard et al,1971).

The gene frequencies thus calculated,and,therefore,the haplotype frequencies,are influenced by the range of specificities tested for in that,the fewer the specificities considered,the greater will be the number of individuals regarded as homozygous for any one allele (n_i) above,and the greater will be the frequency of the 'blank' allele. Consequently these gene frequency estimates may not be compared with those of other samples which have been tested against a different range of antisera. They are,of course,valid for internal comparisons in this study between sub-samples tested with the same antisera. **For the results in the control group see table 6.67.**

The phenotype frequencies show two possible differences when the sexes are compared. Table 6.68 shows that HLA-A10 and HLA-B5 frequencies for males and females. These differences may just be the consequence of the number of specificities tested for since they are non-significant after multiplication of the p values by the number of comparisons made.

Haplotype frequencies do not differ significantly between the sexes or between the different groups with respect to sex,area of origin or source.

The relationship of HLA phenotype frequencies to age is shown in table 6.69. There are no obvious trends,except that of HLA-B12 which appears to increase in frequency with age,although this trend is non-significant ($z = 1.498, p = 0.07$).

Table 6.70,6.71 and 6.72 show the numbers and frequencies of individuals in the four age groups who are positive for two specificities at the A and B loci (tables 6.70 and 6.71) and for

Table 6.67 HLA haplotype frequencies and delta values, control subjects.

	B5	B7	B8	B12	B13	B14	B17	B27	Bw15	Blank
A1	0 -.0123*	.0195 -.0087	.1229 .0926	0 -.0367***	.0038 .0001	0 -.0071	.0175 .0103*	0 -.0125	.0129 .0009**	.0383 -.0085*
A2	.0205 .0059	.0247 -.0185*	.0105 -.0360***	.0969 .0423***	.0110 .0053	.0110 .0004	.0049 -.0061	.0057 -.0064	.0358 .0175**	.0735 .0017
A3	.0060 -.0018	.0600 .0368**	.0097 -.0152*	.0031 -.0262***	.0004 -.0027	.0119 .0062	.0075 .0016	.0093 .0028	.0038 -.0060	.0386 .0001
A9	.0066 .0031	.0121 .0018	0 -.0150**	.0156 .0025	.0023 .0009	.0021 -.0004	0 -.0033	0 -.0039	.0064 .0020	.0178 .0006
A10	.0034 .0004	.0099 .0011	.0030 -.0064	.0012 -.0099	.0047 .0035*	.0015 -.0006	.0036 .0014	.0063 .0038	.0021 -.0016	.0186 .0040
A11	.0065 .0041	.0090 .0017	.0022 -.0055	.0072 -.0019	.0006 -.0004	0 -.0021	.0007 -.0011	.0033 .0013	.0021 -.0009	.0647 .0528
Blank	.0123 .0025	.0264 -.0025	.0294 -.0017	.0414 .0049	.0038 .0009	.0085 .0014	.0076 .0003	.0093 .0012	.0101 -.0022	.0476 -.0005

* $p < .05$
** $p < .01$
*** $p < .005$

Table 6.68 HLA alleles differing in frequency between the sexes.

Allele	Males	Females	Chi sq. (d.f.=1)	p
HLA-A10	32 (15.7%)	16 (7.2%)	6.90	0.0086
HLA-B5	13 (6.4%)	30 (13.5%)	5.14	0.0234

Table 6.69 HLA phenotype frequencies of controls subdivided according to age.

Locus	Allele	Number and frequency (%) in each age group			
		0-19 n=69	20-39 n=90	40-59 n=115	60 and over n=153
A	HLA-A1	30 (43.5)	20 (22.2)	38 (33.0)	59 (38.6)
	HLA-A2	28 (40.6)	58 (64.4)	47 (40.9)	74 (48.4)
	HLA-A3	18 (26.1)	30 (33.3)	31 (27.0)	43 (28.1)
	HLA-A9	8 (11.6)	14 (15.6)	16 (13.6)	19 (12.4)
	HLA-A10	6 (8.7)	6 (6.7)	17 (14.8)	19 (12.4)
	HLA-A11	9 (13.0)	7 (7.8)	11 (9.6)	13 (8.5)
	Blank	2 (2.9)	1 (1.1)	7 (6.1)	4 (2.6)
B	HLA-B5	10 (14.5)	6 (6.7)	9 (7.8)	18 (11.8)
	HLA-B7	20 (29.0)	24 (26.7)	35 (30.4)	40 (26.1)
	HLA-B8	27 (39.1)	18 (20.0)	32 (27.8)	49 (32.0)
	HLA-B12	17 (24.6)	31 (34.4)	41 (35.7)	56 (36.6)
	HLA-B13	3 (4.3)	5 (5.6)	6 (5.2)	3 (2.0)
	HLA-B14	3 (4.3)	9 (10.0)	7 (6.1)	12 (7.8)
	HLA-B17	5 (7.2)	9 (10.0)	6 (5.2)	12 (7.8)
	HLA-B27	6 (8.7)	7 (7.8)	9 (7.8)	13 (8.5)
	HLA-Bw15	7 (10.1)	13 (14.4)	18 (15.7)	15 (9.8)
	Blank	3 (4.3)	5 (5.6)	5 (4.3)	12 (7.8)
C	HLA-Cw4	8 (17.4) (n=46)	10 (12.3) (n=81)	13 (12.0) (n=108)	17 (12.3) (n=138)

Table 6.70 Number of specificities detected at the HLA-A locus in controls subdivided by age.

No. of specificities detected at A locus	Number & frequency (%) in each group			
	0-19	20-39	40-59	60 and over
0 or 1	35 (50.7)	43 (47.8)	56 (48.7)	71 (46.4)
2	34 (49.3)	47 (52.2)	59 (51.3)	82 (53.6)
Totals	69	90	115	153

Z = 0.488 (p = 0.31)

Table 6.71 Number of specificities detected at the HLA-B locus in controls subdivided by age.

No. of specificities detected at B locus	Number & frequency (%) in each group			
	0-19	20-39	40-59	60 and over
0 or 1	34 (49.3)	42 (46.7)	52 (45.2)	58 (37.9)
2	35 (50.7)	48 (53.3)	63 (54.8)	95 (62.1)
Totals	69	90	115	153

Z = 1.687 (p = 0.05)

four specificities (table 6.72) at the two loci considered together. There is a significant trend for increase in the frequency of heterozygotes at the B locus with increasing age. This accords with the findings of Gerkins et al (1974) in suggesting that heterozygosity at this locus is associated with survival.

There are no significant trends of haplotype frequencies with age.

6.17 Summary and discussion.

The analysis presented in this chapter suggests a relationship with age in the distribution of phenotypes of the Rhesus,MNSs,acid phosphatase,phosphoglucomutase and HLA polymorphisms.

The data suggest that,in the Rhesus system,the d phenotype is at a selective advantage in that individuals of this type make up a greater proportion of the older age groups than they do of the younger age groups. The effects of sex ratio,area of birth etc. have been examined and found not to explain these results. Short of the polymorphism changing in its composition rapidly in these generations studied,the most likely conclusion is some differential mortality with regard to the phenotypes in this system.

Similarly,in the MNSs system,where the Ss heterozygote, and,consequently,the MNSs double heterozygote,seems selectively favoured,in the red cell acid phosphatase system,where the A phenotype is at an advantage,the heterozygote in the acid phosphatase system and the heterozygote at the HLA-B locus.

These findings have two corollaries - firstly that disease associations with the above systems must exist and should be sought. An association between the MNSs system and breast cancer

Table 6.72 Number of specificities detected at the HLA-A and HLA-B loci in controls subdivided by age.

No. of specificities detected at A & B loci	Number & frequency (%) in each group			
	0-19	20-39	40-59	60 and over
1,2 or 3	55 (79.7)	69 (76.7)	87 (75.7)	107 (69.9)
4	14 (20.3)	21 (23.3)	28 (24.3)	46 (30.1)
Totals	69	90	115	153

$Z = 1.594$ $(p = 0.06)$

has been suggested (Lancet,1971) and challenged (Morosini et al,1972) but this association alone seems unlikely to explain the present findings. For the other systems mentioned above it is only in the case of HLA that an association between longevity and heterozygosity has been suggested (Bender et al,1972,and Gerkins et al,1974).

The second corollary from these findings is that any investigation which sets out to identify the mechanisms involved in this differential mortality must take age into account by matching diseased and control populations.

Other systems suggest an association between heterozygosity and longevity though the numbers of individuals collected here are insufficient for statistical significance. In the Kell system,for example,the frequency of the heterozygote,Kk is greater amongst older individuals.

Table 6.73 shows the relationship between age and heterozygosity at six of the loci studied. These are MN,Ss,Kell,Kp^a,HLA-A and HLA-B. The number of individuals typed for all six of these loci is few but the results suggest that,in the older age groups,there are more individuals who are heterozygous at a greater number of these loci than in the younger groups.

The relationship between heterosis and longevity requires careful study with a large cohort of new-born infants studied for a large number of loci and followed up for as long as is practicable.

Table 6.73 Heterozygosity at six loci (MN, Ss, Kell, Kp^a, HLA-A and HLA-B) in controls subdivided by age.

No. of loci at which individual is heterozygous	Number & frequency (%) in each group			
	0-19	20-39	40-59	60 and over
1	3 (11.5)	7 (18.9)	7 (17.1)	4 (8.2)
2	15 (57.7)	12 (32.4)	19 (46.3)	18 (36.7)
3	6 (23.1)	15 (40.5)	12 (29.3)	18 (36.7)
4	2 (7.7)	3 (8.1)	3 (7.3)	7 (14.3)
5	0	0	0	2 (4.1)
Totals	26	37	41	49

Chapter 7

Polymorphism results. II Comparisons with diabetics.

Chapter 7

7.1 Introduction.

7.2 The ABO system.

(a) Group A subdivided.

(b) Without subdivision of group A.

7.3 The MNSs system.

(a) The MN locus.

(b) The Ss locus.

(c) The MNSs complex.

7.4 The P system.

7.5 The Rhesus system.

(a) Phenotype frequencies considering the three loci, C,D and E.

(b) Separate consideration of the C,D and E loci.

7.6 The Kell system.

(a) Tests with anti-K and anti-k.

(b) Tests with anti-Kp^a and anti-Kp^b.

(c) Tests with anti-K,anti-k,anti-Kp^a and anti-Kp^b.

7.7 The Lewis system.

7.8 The Duffy system.

7.9 Haptoglobin.

7.10 Acid phosphatase.

7.11 Phosphoglucomutase.

7.12 Adenylate kinase.

7.13 Adenosine deaminase.

7.14 Esterase D.

7.15 The HLA system.

7.16 Summary and discussion.

7.1 Introduction.

In the analysis of genetic polymorphism data from diabetic and non-diabetic populations many comparisons are possible depending on the ways in which the groups are subdivided. The data for all possible comparisons is not given here,to do so would have made this chapter prohibitively long,but,following the example of the previous chapter,all possible comparisons are mentioned and,in general,data are given where significant differences have been found.

The diabetic sample has been investigated for the effect of the following factors on each polymorphic system:- sex,area of origin (by county of the subject's birth and by county of grand-parental birth as in controls),current treatment,current age and age at diagnosis. The relationship between phenotype and month of diagnosis is explored in the following chapter. Regarding treatment,if no significant differences are found between oral-hypoglycaemic-treated diabetics and diet-managed diabetics,these are amalgamated as non-insulin-taking diabetics for comparison with insulin-taking diabetics and controls. For systems in which phenotype frequencies,in both controls and diabetics,show no relationship to age,comparisons are made without matching for age. For systems in which an age effect,either in diabetics or in controls, or both,has been demonstrated,diabetics and controls of comparable age are contrasted.

Statistical comparison of diseased and control groups is mostly by means of conventional chi squared tests,relative incidence and chi squared by Woolf's method have been calculated where appropriate. The test for significance of trends in proportions,described in chapter 6,is also used. In general,in this chapter,the values of chi squared are not given unless significance is

reached at $p = 0.05$.

7.2 The ABO system.

(a) Group A subdivided.

There are no significant differences in the A_1,A_2,B, O,A_1B and A_2B frequencies in diabetics with regard to sex or area of origin. Oral hypoglycaemic-treated diabetics and diet-treated diabetics are not significantly different,consequently,they have been amalgamated as non-insulin-taking diabetics. The phenotype frequencies for insulin-taking diabetics,non-insulin-taking diabetics, the entire diabetic sample and the entire control sample are given in table 7.1 with corresponding gene frequencies in table 7.2. The gene frequencies are calculated in the manner described in the previous chapter.

The frequency of group A_1 individuals is higher in both insulin-taking diabetics and non-insulin-taking diabetics than it is in controls. The relative incidences (A_1/O),together with the chi squared values (Woolf's method) are given in table 7.3.

The relative incidence is not significantly different from unity in this sample for the insulin-taking diabetics nor for the diabetic group as a whole,though it is for the non-insulin-taking diabetics.

ABO phenotype frequencies for diabetics subdivided into 20 year age groups is given in table 7.4. Although there are no significant differences between these age groups and there is no significant trend of these frequencies with age,the data are given in full since they are pertinent to the argument which follows.

Tables 7.5,7.6 and 7.7 show the comparisons between diabetics,irrespective of treatment,and controls for three age

Table 7.1 Results for the ABO system in diabetics and controls (group A subdivided).

Group	Diabetics subdivided by treatment		All diabetics	All controls
	Insulin-taking	Non-insulin-taking		
A_1	76 (33.0%)	186 (37.9%)	262 (36.3%)	161 (31.3%)
A_2	13 (5.7%)	34 (6.9%)	47 (6.5%)	36 (7.0%)
O	106 (46.1%)	215 (43.8%)	321 (44.5%)	255 (49.5%)
B	29 (12.6%)	44 (9.0%)	73 (10.1%)	47 (9.1%)
A_1B	4 (1.7%)	8 (1.6%)	12 (1.7%)	12 (2.3%)
A_2B	2 (0.9%)	4 (0.8%)	6 (0.8%)	4 (0.8%)
Totals	230	491	721	515

Table 7.2 Gene frequency results for ABO system (A subdivided).

Gene	Diabetics subdivided by treatment			
	Insulin-taking	Non-insulin-taking	All diabetics	All controls
A_1	0.1932	0.2221	0.2121	0.1855
A_2	0.0416	0.0514	0.0594	0.0492
B	0.0795	0.0587	0.0651	0.0633
O	0.6857 (±.031)	0.6678 (±.021)	0.6634 (±.018)	0.7020 (±.020)
N	230	491	721	515

Table 7.3 Relative incidence ($^{A1}/0$) in diabetics and controls.

Diabetics Group	Relative incidence (x)	Chi squared (wy^2)d.f=1	P.
Insulin-taking	1.13	0.4565	$<0.50>0.40$
Non-insulin taking	1.37	4.9159	$<0.05>0.025$
All	1.29	3.8000	$<0.10>0.05$

Table 7.4 ABO phenotype frequencies (with group A subdivided) of all diabetics, irrespective of treatment, subdivided by current age.

Group	Number and frequency (%) in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
A_1	17 (39.5)	20 (27.8)	71 (34.1)	154 (38.7)
A_2	4 (9.3)	2 (2.8)	15 (7.2)	26 (6.5)
O	18 (41.9)	37 (51.4)	99 (47.6)	167 (42.0)
B	3 (7.0)	11 (15.3)	20 (9.6)	39 (9.8)
A_1B	1 (2.3)	1 (1.4)	2 (1.0)	8 (2.0)
A_2B	0	1 (1.4)	1 (0.5)	4 (1.0)
Totals	43	72	208	398

Table 7.5 ABO frequencies in diabetics and controls, individuals aged 0-39.

Group	Diabetics	Control
A_1	37 (32.2%)	71 (32.4%)
A_2	6 (5.2%)	17 (7.8%)
O	55 (47.8%)	106 (48.4 %)
B	14 (12.2%)	17 (7.8%)
AB	3 (2,6%)	8 (3.7%)
Total	115	219

mean age diabetics = 23.97 (±0.9)
mean age controls = 24.52 (±0.5)

Table 7.6 ABO frequencies in diabetics and controls, individuals aged 40-59.

Group	Diabetics	Control
A_1	71 (34.1%)	42 (32.8%)
A_2	15 (7.2%)	7 (5.5%)
O	99 (47.6%)	61 (47.7%)
B	20 (9.6%)	14 (10.9%)
AB	3 (1.4%)	4 (3.1%)
Total	208	128

51.70 (±0.4)
50.80 (±0.5)

Table 7.7 ABO frequencies in diabetics and controls, individuals aged 60 and over.

Group	Diabetics	Control
A_1	154 (38.7%)	48 (28.6%)
A_2	26 (6.5%)	12 (7.1%)
O	167 (42.0%)	88 (52.4%)
B	39 (9.8%)	16 (9.5%)
AB	12 (3.0%)	4 (2.4%)
Total	398	168

70.32 (±0.32)
71.91 (±0.6)

groups, the two younger age groups in table 7.4 having been amalgamated. The relative incidences (A_1/O), together with the chi squared values for these age-specific comparisons are shown in table 7.8.

It is evident from this table that it is amongst the older subjects that the differences between diabetics and controls with regard to frequencies of A_1 lie, although it is not immediately apparent whether this is the result solely of current age (or age at diagnosis) or owing to the predominance of non-insulin-taking diabetics amongst this older group.

There is no relationship between age at diagnosis and ABO phenotype within the diabetic sample although it is amongst those diagnosed after the age of 60 that significant deviations from the control frequencies may be detected.

The biological significance of these apparent associations will be discussed at the end of the next section.

(b) Without subdivision of group A.

Consideration of the ABO system without subdivision of group A enables a large sample of donors from the same region to be used for comparison (Kopeć, 1970). The A, B, O and AB phenotype frequencies for this sample of donors, the present control sample and the diabetic samples are shown in table 7.9, with the corresponding gene frequencies in table 7.10.

The excess of group A is again confined to non-insulin-taking diabetics, the relative incidences, together with the chi squared values, are given in table 7.11(a) for the comparison with the present control sample, and 7.11(b) for the comparison with the

Table 7.8 Relative incidences ($^{A1}/O$) in diabetics and subdivided by current age.

Age group	Relative incidence $^{A1}/O$ (x)	Chi squared (d.f.=1)	P.
0-39	1.00	0	–
40-59	1.04	0.0239	<0.90>0.80
60 and over	1.69	6.1627	<0.025>0.01

Table 7.9 Results for the ABO system in diabetics and controls (group A not subdivided).

Group	Diabetics divided by treatment		All diabetics	All controls (this study)	Donor controls (Kopeć, 1970)
	Insulin-taking	Non-insulin taking			
A	89 (38.7%)	220 (44.8%)	309 (42.9%)	197 (38.3%)	1849 (38.3%)
O	106 (46.1%)	215 (43.8%)	321 (44.5%)	255 (49.5%)	2367 (49.0%)
B	29 (12.6%)	44 (9.0%)	73 (10.1%)	47 (9.1%)	443 (9.2%)
AB	6 (2.6%)	12 (2.4%)	18 (2.5%)	16 (3.1%)	174 (3.6%)
Totals	230	491	721	515	4833

Table 7.10 ABO gene frequencies in diabetics and controls (group A not subdivided).

Gene	Diabetics subdivided by treatment		All diabetics	All controls	Donor controls
	Insulin-taking	Non-insulin taking		(present study)	(Kopeć, 1970)
A	0.2348 (±.021)	0.2735 (±.015)	0.2715 (±.013)	0.2347 (±.014)	0.2379 (±.005)
B	0.0795 (±.013)	0.0587 (±.008)	0.0651 (±.007)	0.0633 (±.003)	0.0661 (±.003)
O	0.6857 (±.031)	0.6678 (±.021)	0.6634 (±.017)	0.7020 (±.021)	0.6960 (±.007)
N	230	491	721	515	4833

Table 7.11(a) Relative incidences (A/O) in diabetics and controls from present study.

Group of diabetics	Relative incidence (A/O) (x)	Chi squared (d.f.=1)	P.
Insulin-taking	1.09	0.25	<0.70>0.60
Non-insulin taking	1.33	4.47	<0.05>0.025
All	1.25	3.24	<0.10>0.05

Table 7.11(b) Relative incidence (A/O) in diabetics and donor controls (Kopeć, 1970) from the same region.

Diabetic group	Relative incidence (A/O) (x)	Chi squared (d.f.=1)	P
Insulin-taking	1.07	0.21	<0.70>0.60
Non-insulin taking	1.31	7.18	<0.01>0.005
All	1.23	5.86	<0.25>0.01

donor controls. For insulin-taking diabetes, the relative incidence does not differ significantly from unity either compared with the present control sample or the donor controls. In both comparisons non-insulin-taking diabetics have a significantly higher A/O ratio than controls. With consideration of the entire diabetic group, the ratio is significantly raised only when comparison is made with the numerically larger donor control group.

Consideration of current age when group A is not subdivided gives a similar result to that given for A_1 frequencies. The relative incidences (A/O) and their chi squared values are given in table 7.12. Data for the calculation of these incidences may be obtained by appropriate amalgamation of the categories given in tables 7.5, 7.6 and 7.7. Similar consideration using the donor controls is not possible since they are not subdivided with regard to current age.

The numbers of A_2 individuals in the samples is insufficient to reveal whether the apparent association is with the A_1 group or with A. The relative incidence (A_1/O) is greater than that for (A/O) and this may signify that the differences between diabetics and controls lies in the frequency of A_1 rather than A.

From the information collected in this study it is not possible to determine categorically whether the excess of group A_1 (or A) amongst the older diabetics is primarily the result of their age, age at diagnosis or of the fact that they are mostly non-insulin-taking diabetics. An indication that it may be age, rather than treatment, is given by the fact that the A_1/O relative incidence in insulin-taking diabetics currently aged 60 or above compared with

Table 7.12 Relative incidences (A/O) in diabetics and controls matched for age.

Age group	Relative incidence (A/O)	Chi squared (d.f.=1	P.
0-39	0.94	0.06	<0.80>0.70
40-59	1.08	0.10	<0.80>0.70
60 and over	1.58	5.29	<0.025>0.010

controls of the same age is 1.35. This is not demonstrably different from unity with this small sample,however,there are only 52 diabetics in this category,17 of group A_1 and 23 of group O. A similar indication is given by an A_1/O ratio of 1.06 for the non-insulin-taking diabetics between the ages of 40 and 59 compared with controls of the same age. This is statistically indistinguishable from unity,though again,the sample is small (104 diabetics,44 of group A_1,60 of group O).

On the basis of the results presented here it is apparent that diabetics,either non-insulin-taking or late-onset, have a higher frequency of A_1 than a comparable sample of controls. This is not necessarily evidence that individuals of this blood group are genetically more susceptible to this disease,on the contrary,it is suggested that this excess of A_1 individuals may be a spurious association which is the result of differential mortality amongst non-diabetics with respect to the ABO phenotype and lack of this differential mortality in diabetics.

The studies of Jörgensen and Schwartz (1968) and van Houte and Kesteloot (1972) discussed in chapter 2 have suggested that older individuals have lower frequencies of the A blood group (they did not sub-type for A_1) than do younger age groups. This tendency,which is best explained by greater mortality rates amongst A persons than amongst O persons in certain age groups is suggested by the control data presented here although the differences are not significant. The reduction in the A_1 frequency amongst older controls is not seen in the older diabetics and it is here that the significant differences between diabetics and non-diabetics occur. This is explained,either by suggesting that the disease processes

causing this differential mortality are non-operational in diabetics or,more plausibly,that that they are occurring and are such an integral part of the diabetic state that they are causing death independently of the ABO phenotype of the individual.

If,as van Houte and Kesteloot (1972) suggest,the differential mortality in non-diabetics is,at least partly,the result of coronary artery disease,this would explain the recurrent finding by previous authors of a greater A/O relative incidence in male diabetics than in female diabetics. The loss of A individuals in older non-diabetic males should be proportionally greater than that in females giving a greater difference between elderly male diabetics and male controls than between elderly female diabetics and female controls. This sex difference is bourne out in the present study with a higher value of the A_1/O relative incidence in males than in females (tables 7.13 and 7.14). The greatest difference of all lies between male diabetics and male controls when both groups are over 60 years of age.

7.3 The MNSs system.

(a) The MN locus.

Diabetics show no significant differences with regard to sex in the phenotype frequencies at the MN locus,neither do they show differences with regard to area of origin. Table 7.15 shows the phenotype frequencies for this locus for the entire diabetic sample,insulin-taking diabetics,non-insulin-taking diabetics (there being no differences between oral-hypoglycaemic-treated and diet-managed diabetics) and the entire control sample. Since no differences with regard to age could be demonstrated in the diabetic

Table 7.13 A_1 and 0 phenotype frequencies in diabetics and controls sub-divided by sex.

Group	Diabetics		Controls	
	Males	Females	Males	Females
A_1	117 (37.3%)	145 (35.6%)	80 (29.9%)	81 (32.8%)
0	141 (44.9%)	180 (44.2%)	135 (50.4%)	120 (48.6%)
Totals	314	407	268	247

Table 7.14 Relative incidence $(^{A1}/0)$ in male and female diabetics compared with male and female controls.

Sex	Relative incidence	Chi squared (d.f.=1)	P.
Male	1.40	3.18	$<0.10>0.05$
Female	1.19	0.22	$<0.70>0.60$

Table 7.15

Results for the MN locus,diabetics and controls.

Phenotype	Number and frequency (%)			
	Diabetics		(3) All Diabetics	(4) All Controls
	(1) Ins.-taking	(2) Non-ins.-taking		
M	63 (27.9)	128 (27.1)	191 (27.3)	136 (26.5)
MN	117 (51.8)	257 (54.3)	374 (53.5)	266 (51.8)
N	46 (20.4)	88 (18.6)	134 (19.2)	86 (16.7)
Totals	226	473	699	488

Table 7.16 MN gene frequencies,diabetics and controls.

Gene	Frequencies (±S.E.)			
	Diabetics		All Diabetics	All Controls
	Ins.-taking	Non-ins.-taking		
M	0.5376 (±.033)	0.5423 (±.023)	0.5408 (±.019)	0.5512 (±.023)
N	0.4624	0.4577	0.4592	0.4488

sample, age matching has not been taken into account. Gene frequencies for these samples are given in table 7.16.

There are no significant differences between diabetics and controls and there are no differences between insulin-taking diabetics and non-insulin-taking diabetics.

(b) The Ss locus.

Tests with anti-S were performed on samples from 684 diabetic individuals. No differences were found with regard to sex, area of origin, current age or age at diagnosis. There are no significant differences amongst the diabetics with regard to treatment and the phenotype frequencies of diabetics do not differ from those in controls. These frequencies are set out in table 7.17.

Of the 684 individuals tested with anti-S, 232 were also tested with anti-s. There were no detectable differences with regard to sex or area of origin but the same trend with regard to current age was found in diabetics as in controls. Table 7.18 shows how the frequencies of heterozygotes increases with increasing age at the expense of the frequency of ss homozygotes. These trends have been tested for significance by the same method as was used in chapter 6. The mean age of ss homozygotes is significantly less than that of the heterozygotes.

Tables 7.19 and 7.20 show Ss locus phenotype frequencies for insulin-taking and non-insulin-taking diabetics, together with age-matched controls. There are no significant differences between diabetic and control groups, nor between the insulin-taking diabetics and the non-insulin-taking diabetics. Tables 7.21 and 7.22 show the

Table 7.17 Results for diabetics and controls tested with anti-S.

Phenotype	Number and frequency (%)			
	Diabetics		(3) All Diabetics	(4) All Controls
	(1) Ins.-taking	(2) Non-ins.-taking		
S+ve	105 (48.2)	256 (54.9)	361 (52.8)	209 (52.3)
S-ve	113 (51.8)	210 (45.1)	323 (47.2)	191 (47.7)
Totals	218	466	684	400

Table 7.18 Ss phenotype results for diabetics subdivided according to age and irrespective of treatment.

Phenotype	Number and frequency (%) in each age group			
	0 - 19	20 - 39	40 - 59	60 and over
S	5 (18.5)	4 (8.9)	23 (19.0)	24 (10.3)
Ss	6 (22.2)	12 (26.7)	47 (38.8)	104 (44.8)
s	16 (59.3)	29 (64.4)	51 (42.1)	104 (44.8)
Totals	27	45	121	232

Z (heterozygotes vs. rest) = 2.931 ($p = .0017$)

Z (s homozygotes vs. rest) = 2.061 ($p = .0197$)

Mean age of S individuals = 55.82 (± 2.5) years

Mean age of Ss individuals = 60.01 (± 1.2) years

Mean age of s individuals = 54.59 (± 1.4) years

Table 7.19 Results for the Ss locus, diabetics and controls aged 0-45.

Phenotype	Number and frequency (%)			
	Diabetics		(3)	(4)
	(1) Ins.-taking	(2) Non-ins.-taking	Diabetics	Controls
S	8 (10.4)	3 (15.8)	11 (11.5)	14 (10.6)
Ss	23 (29.9)	6 (31.6)	29 (30.2)	51 (38.6)
s	46 (59.7)	10 (52.6)	56 (58.3)	67 (50.8)
Totals	77	19	96	132

Table 7.20 Results for the Ss locus, diabetics and controls aged 45 and over.

Phenotype	Number and frequency (%)			
	Diabetics		(3)	(4)
	(1) Ins.-taking	(2) Non-ins.-taking	Diabetics	Controls
S	10 (15.1)	35 (13.3)	45 (13.7)	15 (10.6)
Ss	23 (34.9)	117 (44.5)	140 (42.5)	66 (46.8)
s	33 (50.0)	111 (42.2)	144 (43.8)	60 (42.5)
Totals	66	263	329	141

Table 7.21 Ss locus gene frequencies,diabetics and controls aged 0 - 45.

Gene	Frequencies (±S.E.)			
	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	Diabetics	
S	0.2532 (±.049)	0.3158 (±.229)	0.2656 (±.045)	0.2992 (±.040)
s	0.7468	0.6842	0.7344	0.7008

Table 7.22 Ss locus gene frequencies,diabetics and controls aged 45 and over.

Gene	Frequencies (±S.E.)			
	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	Diabetics	
S	0.3258 (±.058)	0.3555 (±.030)	0.3495 (±.026)	0.3404 (±.040)
s	0.6742	0.6445	0.6505	0.6596

corresponding gene frequencies.

(c) The MNSs complex.

Table 7.23 shows the phenotype frequencies for the 662 diabetic individuals tested with anti-M,anti-N and anti-S,together with the data for controls. There are no significant differences revealed.

When the results from the 454 diabetics tested with anti-M,anti-N,anti-S and anti-s are examined for age trends (table 7.24(a)),a significant upward trend of the frequency of the double heterozygote is seen. This was suggested in the control sample but was non-significant. This result is hardly surprising considering the situation already described in the Ss locus alone. Table 7.24 (b) and 7.24(c) show the phenotype frequencies for diabetics and age-matched controls. No significant differences can be demonstrated.

7.4 The P system.

For reasons given in the previous chapter,results for the P system are given as positive or negative reactions with anti-P_1 antiserum without grading of the strength of agglutination of the positives. Amongst the 718 diabetics tested with this anti-serum,there were no significant differences with regard to sex or area of origin and oral-hypoglycaemic-treated diabetics were indistinguishable from diet-treated diabetics. The phenotype frequencies for the entire diabetic group and for this group sub-divided by current treatment are given in table 7.25 together with the results for the entire control sample. Gene frequencies are given in table 7.26. Since there are no significant age differences

Table 7.23 Results for diabetics and controls tested with anti-M, anti-N and anti-S.

Phenotype	No. & frequency (%)	
	Diabetics	Controls
MS	120 (18.1)	67 (17.4)
MNS	197 (29.8)	121 (31.3)
NS	32 (4.8)	17 (4.4)
Ms	63 (9.5)	37 (9.6)
MNs	155 (23.4)	97 (25.1)
Ns	95 (14.4)	47 (12.2)
Totals	662	386

Table 7.24 (a) MNSs phenotype results in diabetics subdivided by age irrespective of treatment

Phenotype	Number and frequency (%) in each age group			
	0 - 19	20 - 39	40 - 59	60 & over
MS	2 (7.7)	2 (4.4)	9 (7.4)	11 (4.7)
MSs	3 (11.5)	4 (8.8)	15 (12.4)	29 (12.5)
Ms	3 (11.5)	7 (15.5)	11 (9.1)	23 (9.9)
MNS	3 (11.5)	2 (4.4)	12 (9.9)	13 (5.6)
MNSs	3 (11.5)	7 (15.5)	28 (23.1)	64 (27.6)
MNs	9 (34.6)	14 (31.1)	24 (19.8)	49 (21.1)
NS	0	0	2 (1.7)	0
NSs	0	1 (2.2)	4 (3.3)	11 (4.7)
Ns	3 (11.5)	8 (17.8)	16 (13.2)	32 (13.8)
Totals	26	45	121	232

Z (MNSs individuals vs. rest) = 2.296 ($p = 0.02$)

Table 7.24(b) Phenotype frequencies for diabetics (irrespective of treatment) and controls tested with anti-M, anti-N, anti-S and anti-s. Individuals aged under 40.

Phenotype	Number and frequency (%)	
	Diabetics	Controls
MS	4 (5.6)	7 (5.7)
MSs	7 (9.9)	10 (8.1)
Ms	10 (14.1)	14 (11.3)
MNS	5 (7.0)	7 (5.6)
MNSs	10 (14.1)	34 (27.4)
MNs	23 (32.4)	35 (28.2)
NS	0	0
NSs	1 (1.4)	2 (1.6)
Ns	11 (15.5)	15 (12.1)
Totals	71	124

Table 7.24(c) Phenotype frequencies for diabetics (irrespective of treatment) and controls tested with anti-M,anti-N, anti-S and anti-s. Individuals aged 40 and over.

Phenotype	Number and frequency (%)	
	Diabetics	Controls
MS	20 (5.7)	9 (6.1)
MSs	44 (12.5)	20 (13.6)
Ms	34 (9.6)	12 (8.2)
MNS	25 (7.1)	5 (3.4)
MNSs	92 (26.1)	46 (31.3)
MNs	73 (20.7)	31 (21.1)
NS	2 (0.5)	1 (0.7)
NSs	15 (4.2)	5 (3.4)
Ns	48 (13.6)	18 (12.2)
Totals	353	147

Table 7.25 Results for diabetics and controls tested with anti-P_1.

Phenotype	Number and frequency (%)			
	Diabetics		(3) All	(4) All
	(1) Ins.-taking	(2) Non-ins.-taking	Diabetics	Controls
-ve	22 (9.6)	61 (12.4)	83 (11.6)	128 (25.1)
+ve	206 (90.4)	429 (87.6)	635 (88.4)	381 (74.9)
Totals	228	490	718	509

Chi squared ((1) vs. (4)) = 23.33 (d.f. = 1) $p < 0.001$

Chi squared ((2) vs. (4)) = 26.23 (d.f. = 1) $p < 0.001$

Chi squared ((3) vs. (4)) = 38.61 (d.f. = 1) $p < 0.001$

Chi squared ((1) vs. (2)) = 1.19 (d.f. = 1) N.S.

Table 7.26 Gene frequency results for the P system,diabetics and controls.

Gene	Frequencies (±S.E.)			
	Diabetics		All	All
	Ins.-taking	Non-ins.-taking	Diabetics	Controls
P_1	0.6894 (±.031)	0.6472 (±.022)	0.6600 (±.018)	0.4985 (±.022)
P_2+p	0.3106	0.3528	0.3400	0.5015

amongst diabetics,comparisons are made without matching for age.

There is no significant difference between the two diabetic sub-groups but both of these groups are significantly different from the controls,as is the diabetic group as a whole,with a lower frequency of anti-P_1 negative individuals amongst the diabetics. The relative incidences (anti-P_1 +ve/anti-P_1 -ve) in insulin-taking and non-insulin-taking diabetics compared with controls are 3.15 (chi squared = 21.67,$p < 0.0005$) and 2.36 (chi squared = 25.28,$p < 0.0005$) respectively with a relative incidence of 2.57 (chi squared = 37.03,$p < 0.0005$) in the diabetic group taken as a whole.

This apparent association with the P system is entirely spurious and is the result of deterioration in the strength of the anti-P_1 antiserum with time. The diabetic samples were obtained towards the beginning of the project and no control samples at all were obtained until about 250 diabetics had been studied. From this point,until about the eight hundredth sample,diabetics and controls were obtained in approximately equal numbers but,from there on,the specimens were mostly from non-diabetics. As a consequence,diabetics were tested with predominantly fresher antiserum than controls and the excess of positive reactions in the former group can be attributed entirely to this. Table 7.27 shows the relationship between the P phenotype and the series sample number in diabetics.

7.5 The Rhesus system.

(a) Phenotype frequencies considering the three loci,C,D and E.

No sex differences can be detected in the phenotype

Table 7.27 Relationship of reaction with anti-P_1 to number of specimen,diabetics and controls.

Reaction with ant-P_1	Number and frequency (%)			
	Nos. 1-249*	Nos. 250-499	Nos. 500-649	Nos. 750 & over
+ve	223 (97.8)	150 (88.8)	137 (87.3)	125 (76.2)
-ve	5 (2.2)	19 (11.2)	20 (12.7)	39 (23.8)
Totals	228	169	157	164

* Number refers to identification number of specimen in series, collected chronologically from number 1.

frequencies for this system in diabetics. Similarly,there are no significant differences with regard to area of origin. The age trends found in non-diabetics were discussed in chapter 6 and cannot be demonstrated in the diabetic population. Since these age differences were present in controls,however,comparisons are made between age-matched groups of individuals.

Tables 7.28,7.29 and 7.30 show the phenotype frequencies in diabetics and controls for the three age groups 0 - 39 years, 40 - 59 years and 60 years and over. Tables 7.31,7.32 and 7.33 give,for each of these age groups,the haplotype frequencies calculated by means of the maximum likelihood computer programme.

Oral-hypoglycaemic-treated diabetics do not differ significantly from diet-managed diabetics and the two groups have been amalgamated. There are too few of these non-insulin-taking diabetics in the youngest age group to treat as a separate sample.

Several of the less common specificities (probable genotypes $\underline{R_zR_2}$,$\underline{r''r}$,$\underline{r'r}$,$\underline{R_2R_2}$,$\underline{R_2r}$ and $\underline{R_0r}$) have been amalgamated for the calculation of chi squared. No significant differences are demonstrated in any age group between insulin-taking diabetics and non-insulin-taking diabetics nor between either of these sub-groups and the control group. There is no significant difference in any age-specific comparison between the total diabetic group and the control group.

As discussed in chapter 2,there have been conflicting results in the comparison of rhesus results from diabetic and control populations. The studies of Berg et al (1967),Scholz et al (1975) and Buckwalter and Tweed (1964) finding associations with different haplotypes (Berg et al and Scholz et al) or no

Table 7.28 Rhesus phenotype results, diabetics and controls under 40 years of age.

Reaction with anti-					Probable genotype	Number and frequency (%)		
						Diabetics		Controls
C	c	D	E	e		(1) Ins.-taking	(2) All diabetics	
-	+	-	-	+	r r	16 (16.3)	19 (16.5)	28 (12.8)
+	+	+	-	+	R_1r	32 (32.7)	37 (32.2)	79 (36.1)
+	-	+	-	+	R_1R_1	24 (24.5)	27 (23.5)	45 (20.6)
-	+	+	-	+	R_or	0	0	1 (0.5)
-	+	+	+	+	R_2r	8 (8.2)	9 (7.8)	20 (9.1)
-	+	+	+	-	R_2R_2	2 (2.0)	2 (1.7)	3 (1.4)
+	+	+	+	+	R_1R_2	14 (14.3)	19 (16.5)	40 (18.3)
+	-	-	-	+	r'r'	0	0	0
+	+	-	-	+	r'r	0	0	1 (0.5)
+	-	+	+	+	R_zR_1	0	0	0
+	-	-	+	+	$r'r^y$	0	0	0
+	+	-	+	+	r"r'	0	0	0
-	+	-	+	+	r"r	1 (1.0)	1 (0.9)	0
+	-	+	+	-	R_zR_z	0	0	0
+	+	+	+	-	R_zR_2	1 (1.0)	1 (0.9)	2 (0.9)
+	-	-	+	-	r^yr^y	0	0	0
+	+	-	+	-	r^yr''	0	0	0
-	+	-	+	-	r"r"	0	0	0
Totals						98	115	219

Table 7.29 Rhesus phenotype results, diabetics and controls aged 40 to 59.

Reaction with anti-					Probable genotype	Number and frequency (%)			
						Diabetics			(4) Controls
C	c	D	E	e		(1) Ins.-taking	(2) Non-ins.taking	(3) All	
-	+	-	-	+	r r	12 (15.2)	19 (14.8)	31 (15.0)	18 (14.1)
+	+	+	-	+	R_1r	22 (27.8)	47 (36.7)	69 (33.3)	53 (41.4)
+	-	+	-	+	R_1R_1	19 (24.1)	26 (20.3)	45 (21.7)	17 (13.3)
-	+	+	-	+	R_or	2 (2.5)	1 (0.8)	3 (1.5)	1 (0.8)
-	+	+	+	+	R_2r	8 (10.1)	13 (10.2)	21 (10.1)	18 (14.1)
-	+	+	+	-	R_2R_2	1 (1.3)	0	1 (0.5)	5 (3.9)
+	+	+	+	+	R_1R_2	12 (15.2)	20 (15.6)	32 (15.5)	13 (10.2)
+	-	-	-	+	r'r'	0	0	0	0
+	+	-	-	+	r'r	1 (1.3)	0	1 (0.5)	2 (1.6)
+	-	+	+	+	R_zR_1	0	0	0	0
+	-	-	+	+	$r'r^y$	0	0	0	0
+	+	-	+	+	r"r'	0	1 (0.8)	1 (0.5)	1 (0.8)
-	+	-	+	+	r"r	0	0	0	0
+	-	+	+	-	R_zR_z	0	0	0	0
+	+	+	+	-	R_zR_2	2 (2.5)	1 (0.8)	3 (1.5)	0
+	-	-	+	-	r^yr^y	0	0	0	0
+	+	-	+	-	r^yr''	0	0	0	0
-	+	-	+	-	r"r"	0	0	0	0
Totals						79	128	207	128

Table 7.30 Rhesus phenotype results, diabetics and controls aged 60 and over.

Reaction with anti-					Probable genotype	Number and frequency (%)			
						Diabetics			(4) Controls
C	c	D	E	e		(1) Ins.-taking	(2) Non-ins.-taking	(3) All	
-	+	-	-	+	r r	9 (17.3)	60 (17.3)	69 (17.3)	35 (20.8)
+	+	+	-	+	R_1r	11 (21.1)	127 (36.7)	138 (34.7)	62 (36.9)
+	-	+	-	+	R_1R_1	11 (21.1)	56 (16.2)	67 (16.8)	23 (13.7)
-	+	+	-	+	R_or	1 (1.9)	1 (0.3)	2 (0.5)	2 (1.2)
-	+	+	+	+	R_2r	4 (7.7)	30 (8.7)	34 (8.5)	15 (8.9)
-	+	+	+	-	R_2R_2	3 (5.8)	5 (1.5)	8 (2.0)	3 (1.8)
+	+	+	+	+	R_1R_2	13 (25.0)	62 (17.9)	75 (18.8)	24 (14.3)
+	-	-	-	+	r'r'	0	0	0	0
+	+	-	-	+	r'r	0	3 (0.9)	3 (0.8)	2 (1.2)
+	-	+	+	+	R_zR_1	0	0	0	0
+	-	-	+	+	$r'r^y$	0	0	0	0
+	+	-	+	+	r"r'	0	1 (0.3)	1 (0.3)	0
-	+	-	+	+	r"r	0	0	0	2 (1.2)
+	-	+	+	-	R_zR_z	0	0	0	0
+	+	+	+	-	R_zR_2	0	1 (0.3)	1 (0.3)	0
+	-	-	+	-	r^yr^y	0	0	0	0
+	+	-	+	-	r^yr''	0	0	0	0
-	+	-	+	-	r"r"	0	0	0	0
Totals						52	346	398	168

Table 7.31 Rhesus haplotype frequencies in diabetics and controls, aged under 40 years.

Haplotype	Diabetics		Controls
	Ins.-taking	All	
r	0.3725	0.3696	0.3545
r'	0	0	0.0064
r"	0.0161	0.0139	0
r^y	0.0051	0.0043	0.0046
R_o	0	0	0.0063
R_1	0.4796	0.4783	0.4730
R_2	0.1267	0.1339	0.1552
R_z	0	0	0

Table 7.32 Rhesus haplotype frequencies in diabetics and controls aged 40 - 59.

Haplotype	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	All	
r	0.3458	0.3846	0.3691	0.4258
r'	0.0167	0	0.0064	0.0180
r"	0	0	0	0
r^y	0.0127	0.0078	0.0097	0.0039
R_o	0.0275	0.0099	0.0173	0.0117
R_1	0.4453	0.4648	0.4574	0.3805
R_2	0.1519	0.1328	0.1401	0.1602
R_z	0	0	0	0

Table 7.33 Rhesus haplotype frequencies in diabetics and controls aged 60 and over.

Haplotype	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	All	
r	0.3193	0.4056	0.3939	0.4487
r'	0	0.0107	0.0095	0.0130
r''	0	0	0	0.0129
r^y	0	0.0029	0.0025	0
R_o	0.0173	0.0034	0.0057	0.0126
R_1	0.4423	0.4286	0.4302	0.3858
R_2	0.2211	0.1488	0.1583	0.1270
R_z	0	0	0	0

association at all (Buckwalter and Tweed). It is suggested that these conflicting results have arisen by comparisons with control populations of different age structures with consequent variations in haplotype frequencies.

(b) Separate consideration of the C,D and E loci.

The results for the C,D and E loci considered separately are given for the same age subdivisions in tables 7.34 to 7.48. No significant differences can be detected either between the treatment sub-groups of the diabetics,between these sub-groups and controls nor between the diabetic groups as a whole and the controls.

7.6 The Kell system.

(a) Tests with anti-K and anti-k.

There are no significant differences between the sexes for Kell locus phenotypes. Similarly,area of origin,current age and age at diagnosis have no significant effect. The results for insulin-taking and non-insulin-taking diabetics are given in table 7.49 together with those for the entire diabetic population and the controls. The K and Kk phenotypes have been amalgamated for the calculation of chi squared and the tests show no significant differences either between the diabetics and the control group, within the diabetic group with regard to current treatment or between these treatment sub-groups and the controls. Gene frequency results for this locus are given in table 7.50.

Table 7.34 C locus phenotype frequencies for diabetics and controls aged under 40.

Phenotype	Number and frequency (%)		
	Diabetics		(3) Controls
	(1) Ins.-taking	(2) All	
C	24 (24.5)	27 (23.5)	45 (20.5)
Cc	47 (48.0)	57 (49.6)	122 (55.7)
c	27 (27.5)	31 (26.9)	52 (23.7)
Totals	98	115	219

Table 7.35 C locus gene frequencies for diabetics and controls aged under 40.

Gene	Frequency (±S.E.)		
	Diabetics		Controls
	Ins.-taking	All	
C	0.4847 (±.051)	0.4826 (±.047)	0.4840 (±.034)
c	0.5153	0.5174	0.5159

Table 7.36 D locus phenotype frequencies for diabetics and controls aged under 40.

Phenotype	Number and frequency (%)		
	Diabetics		(3) Controls
	(1) Ins.-taking	(2) All	
d	17 (17.3)	20 (17.4)	29 (13.2)
D	81 (82.7)	95 (82.6)	190 (86.8)
Totals	98	115	219

Table 7.37 E locus phenotype frequencies for diabetics and controls aged under 40.

Phenotype	Number and frequency (%)		
	Diabetics		(3) Controls
	(1) Ins.-taking	(2) All	
E	3 (3.0)	3 (2.6)	5 (2.3)
Ee	23 (23.5)	29 (25.2)	60 (27.4)
e	72 (73.5)	83 (72.2)	154 (70.3)
Totals	98	115	219

Table 7.38 E locus gene frequencies for diabetics and control aged under 40.

Gene	Frequency (±S.E.)		
	Diabetics		Controls
	Ins.-taking	All	
E	0.1479 (±.036)	0.1522 (±.033)	0.1598 (±.025)
e	0.8521	0.8478	0.8402

Table 7.39 C locus phenotype frequencies for diabetics and controls aged 40-59.

Phenotype	Number and frequency (%)			
	Diabetics		(3)	(4)
	(1) Ins-taking	(2) Non-ins.-taking	Diabetics	Controls
C	19 (24.1)	26 (20.3)	45 (21.7)	17 (13.3)
Cc	37 (46.8)	69 (53.9)	106 (51.2)	69 (53.9)
c	23 (29.1)	33 (27.8)	56 (27.1)	42 (32.8)
Totals	79	128	207	128

Table 7.40 C locus gene frequencies, diabetics and controls aged 40-59.

Gene	Frequencies (±S.E.)			Controls
	Diabetics			
	Ins.-taking	Non-ins.-taking	Diabetics	
C	0.4747 (±.056)	0.4727 (±.044)	0.4734 (±.035)	0.4023 (±.043)
c	0.5253	0.5273	0.5266	0.5977

Table 7.41 D locus phenotype frequencies for diabetics and controls aged 40-59.

Phenotype	Number and frequency (%)			
	Diabetics		(3)	(4)
	(1) Ins.-taking	(2) Non-ins.-taking	Diabetics	Controls
d	13 (16.5)	20 (15.6)	33 (15.9)	21 (16.4)
D	66 (83.5)	108 (84.4)	174 (84.1)	107 (83.6)
Totals	79	128	207	128

Table 7.42 E locus phenotype frequencies for diabetics and controls aged 40-59.

Phenotype	Number and frequency (%)			
	Diabetics		(3)	(4)
	(1) Ins.-taking	(2) Non-ins.-taking	Diabetics	Controls
E	3 (3.8)	1 (0.8)	4 (1.9)	5 (3.9)
Ee	20 (25.3)	34 (26.6)	54 (26.1)	32 (25.0)
e	56 (70.9)	93 (72.7)	149 (72.0)	91 (71.1)
Totals	79	128	207	128

Table 7.43 E locus gene frequencies for diabetics and controls aged 40-59.

Gene	Frequency (±S.E.)			
	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	All	
E	0.1645 (±.042)	0.1406 (±.031)	0.1498 (±.025	0.1641 (±.033)
e	0.8355	0.8594	0.8502	0.8359

Table 7.44 C locus phenotype frequencies for diabetics and controls aged 60 and over.

Phenotype	Number and frequency (%)			
	Diabetics		(3)	(4)
	(1) Ins.-taking	(2) Non-ins.-taking	Diabetics	Controls
C	11 (21.1)	56 (16.2)	67 (16.8)	23 (13.7)
Cc	24 (46.2)	194 (56.1)	218 (54.8)	88 (52.4)
c	17 (32.7)	96 (27.7)	113 (28.4)	57 (33.9)
Totals	52	346	398	168

Table 7.45 C locus gene frequencies for diabetics and controls aged 60 and over.

Gene	Frequency (±S.E.)			
	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	All	
C	0.4423 (±.069)	0.4422 (±.027)	0.4422 (±.025)	0.3988 (±.038)
c	0.5577	0.5578	0.5578	0.6012

Table 7.46 D locus phenotype frequencies for diabetics and controls aged 60 and over.

Phenotype	Number and frequency (%)			
	Diabetics		(3)	(4)
	(1) Ins.-taking	(2) Non-ins.-taking	Diabetics	Controls
d	9 (17.3)	64 (18.5)	73 (18.3)	39 (23.2)
D	43 (82.7)	282 (81.5)	325 (81.7)	129 (76.8)
Totals	52	346	398	168

Table 7.47 E locus phenotype frequencies for diabetics and controls aged 60 and over.

Phenotype	Number and frequency (%)			
	Diabetics		(3)	(4)
	(1) Ins.-taking	(2) Non-ins.-taking	Diabetics	Controls
E	3 (5.8)	6 (1.7)	9 (2.3)	3 (1.8)
Ee	17 (32.7)	93 (26.9)	110 (27.6)	41 (24.4)
e	32 (61.5)	247 (71.4)	279 (70.1)	124 (73.8)
Totals	52	346	398	168

Table 7.48 E locus gene frequencies for diabetics and controls aged 60 and over.

Gene	Frequency (±S.E.)			
	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	All	
E	0.2211 (±.057)	0.1517 (±.019)	0.1608 (±.018)	0.1399 (±.027)
e	0.7789	0.8483	0.8392	0.8601

Table 7.49 Results for diabetics and controls tested with anti-K and anti-k.

Phenotype	Number and frequency (%)			
	Diabetics		(3) All Diabetics	(4) All Controls
	(1) Ins.-taking	(2) Non-ins.-taking		
K	0	3 (0.6)	3 (0.4)	1 (0.2)
Kk	23 (10.0)	44 (9.1)	67 (9.4)	34 (6.6)
k	206 (90.0)	438 (90.3)	644 (90.2)	479 (93.2)
Totals	229	485	714	514

Table 7.50 Gene frequency results for diabetics tested with anti-K and anti-k.

Gene	Frequency (±S.E.)			
	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	All	
K	0.0502 (±.014)	0.0515 (±.010)	0.0511 (±.008)	0.0350 (±.008)
k	0.9498	0.9485	0.9489	0.9650

(b) Tests with anti-Kp^a and anti-Kp^b.

Results for 699 diabetic individuals are given in table 7.51. There are no significant differences amongst the diabetics with regard sex or area of origin. There is a tendency for heterozygotes to be more common amongst the older diabetics than amongst the younger although,with this sample,the difference is not significant. Possibly as a result of this,there is a significant difference between non-insulin-taking diabetics and the entire control sample. This difference is not seen in the comparison between the entire diabetic group and the controls. There are insufficient insulin-taking diabetics for separate treatment.

Gene frequencies are given in table 7.52.

(c) Tests with anti-K,anti-k,anti-Kp^a and anti-Kp^b.

Table 7.53 shows the results for the individuals tested with these four antisera. There are no significant differences either amongst diabetics or between diabetics and controls.

7.7 The Lewis system.

Table 7.54 shows the phenotype frequencies for diabetics and controls tested with anti-Le(a). These results are for A and O individuals only. The diabetic groups show a higher frequency of Le(a) positive individuals and this is in agreement with Anderson and Lauritzen (1960),although the excess is not significant with such small samples. There are no significant differences in the diabetic group with regard to sex,area of origin,age or treatment.

Table 7.51 Results for diabetics and controls tested with anti-Kp^a and anti-Kp^b.

Phenotype	Number and frequency (%)			
	Diabetics		(3) All Diabetics	(4) All Controls
	(1) Ins.-taking	(2) Non-ins.-taking		
Kp^aKp^b	2 (0.9)	17 (3.5)	19 (2.7)	6 (1.4)
Kp^b	218 (99.1)	462 (96.5)	680 (97.3)	417 (98.6)
Totals	220	479	699	423

Chi squared ((2) vs. (4)) = 4.12 (d.f. = 1) $p < 0.05 > 0.02$

Chi squared ((3) vs. (4)) = 2.06 (d.f. = 1) N.S.

Table 7.52 Gene frequencies for diabetics and controls tested with anti-Kp^a and anti-Kp^b.

Gene	Frequency (±S.E.)			
	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	All	
Kp^a	0.0045 (±.005)	0.0177 (±.006)	0.0136 (±.004)	0.0071 (±.004)
Kp^b	0.9955	0.9823	0.9864	0.9929

Table 7.53 Phenotype frequencies for diabetics and controls tested with anti-K, anti-k, anti-Kp^a and anti-Kp^b.

Phenotype	Number and frequency (%)			
	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	All	
$KKKp^aKp^b$	0	3 (0.6)	3 (0.4)	1 (0.2)
$KkKp^bKp^b$	21 (9.5)	44 (9.2)	65 (9.3)	27 (6.4)
$kkKp^aKp^b$	2 (0.9)	17 (3.5)	19 (2.7)	6 (1.4)
$kkKp^bKp^b$	197 (89.5)	415 (86.6)	612 (87.5)	389 (92.0)
Totals	220	479	699	423

Table 7.54 Results for diabetics and controls tested with anti-Le(a), (A and O persons only).

Reaction with anti-Le (a)	Number and frequency (%)			
	Diabetics		(3) All Diabetics	(4) All Controls
	(1) Ins.-taking	(2) Non-ins.-taking		
+ve	41 (34.2)	78 (34.4)	119 (34.3)	30 (27.8)
-ve	79 (65.8)	149 (65.6)	228 (65.7)	78 (72.2)
Totals	120	227	347	108

Chi squared ((3) vs. (4)) = 1.59 (d.f. = 1) N.S.

7.8 The Duffy system.

Table 7.55 shows the phenotype frequencies for the 621 diabetics and 507 controls tested with anti-Fy^a. No significant differences were found in the diabetic sample with regard sex, area of origin, current age or age at diagnosis. There were no differences between oral-hypoglycaemic-treated diabetics and diet-controlled diabetics. There are no significant differences between the treatment sub-samples and controls and no differences between the diabetics as a whole and controls.

Of these 621 diabetics tested with anti-Fy^a, 270 were also tested with anti-Fy^b. Their results are given in table 7.56, with gene frequencies in table 7.57. Again, the diabetics are internally consistent with regard to sex, area of origin, age, age at diagnosis and treatment and show no significant differences when compared with controls.

7.9 Haptoglobin.

There were no significant differences between male and female diabetics with regard to the haptoglobin phenotype frequencies, nor were there any differences with regard to area of origin. There were insufficient data for analysis in terms of current age or age at diagnosis.

Phenotype frequencies for the system are given in table 7.58 with gene frequencies in table 7.59. There are no significant differences between insulin-taking and non-insulin-taking diabetics nor between these sub-groups and controls. The entire diabetic group does not differ significantly from the control group. The numbers available were small, especially for the comparison between

Table 7.55 Results for diabetics and controls tested with anti-Fy^a.

Phenotype	Number and frequency (%)			
	Diabetics		(3) All Diabetics	(4) All Controls
	(1) Ins.-taking	(2) Non-ins.-taking		
+ve	136 (64.5)	263 (64.1)	399 (64.3)	326 (64.3)
-ve	75 (35.5)	147 (35.9)	222 (35.7)	181 (35.7)
Totals	211	410	621	507

Table 7.56 Phenotype frequencies for diabetics and controls tested with anti-Fy^a and anti-Fy^b.

Phenotype	Number and frequency (%)			
	Diabetics		(3) All	(4) All
	(1) Ins.-taking	(2) Non-ins.-taking	Diabetics	Controls
Fy^a	21 (24.1)	50 (27.3)	71 (26.3)	55 (19.0)
Fy^aFy^b	38 (43.7)	71 (38.8)	109 (40.4)	126 (43.6)
Fy^b	28 (32.2)	62 (33.9)	90 (33.3)	108 (37.4)
Totals	87	183	270	289

Table 7.57 Gene frequencies for diabetics and controls tested with anti-Fy^a and anti-Fy^b.

Gene	Frequency (+S.E.)			
	Diabetics			
	Ins.-taking	Non-ins.-taking	All	Controls
$\underline{Fy}^a$	0.4598 (±.053)	0.4672 (±.037)	0.4648 (±.030)	0.4083 (±.029)
$\underline{Fy}^b$	0.5402	0.5328	0.5352	0.5917

Table 7.58 Haptoglobin results,diabetics and controls.

Phenotype	Number and frequency (%)			
	Diabetics		(3) All Diabetics	(4) All Controls
	(1) Ins.-taking	(2) Non-ins.-taking		
1-1	11 (16.4)	24 (15.5)	35 (15.8)	16 (18.6)
2-1	26 (38.8)	65 (41.9)	91 (41.0)	44 (51.2)
2-2	30 (44.8)	66 42.6)	96 (43.2)	26 (30.2)
Totals	67	155	222	86

Table 7.59 Gene frequency results for the haptoglubin locus,diabetics and control.

Gene	Frequency (±S.E.)			
	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	All	
Hp^1	0.3582 (±.059)	0.3645 (±.039)	0.3626 (±.032)	0.4419 (±.054)
Hp^2	0.6418	0.6355	0.6374	0.5581

insulin-taking diabetics and controls.

The finding of Ksenofontor (1974), that twice as many diabetics as controls were heterozygous at both the MN and haptoglobin loci, could not be confirmed in this study. Of the 222 diabetics investigated for haptoglobin 54 (24.3%) were heterozygous at both loci. In the control sample 24 (27.9%) were of this type.

7.10 Acid phosphatase.

Acid phosphatase phenotype frequencies do not differ significantly in male and female diabetics. There is no relationship with area of origin and the trend found with age in controls is not present in the diabetics. There is no relation with age at diagnosis. Because of the heterogeneity of the control sample with respect to age, comparisons are made between diabetics and non-diabetics matched for age. Tables 7.59 and 7.60 show the acid phosphatase phenotype frequencies for diabetics and controls less than 40 years old and greater than 40 years old respectively. The corresponding gene frequencies are given in tables 7.61 and 7.62. There are insufficient non-insulin-taking diabetics for separate analysis in the under 40 group.

The differences between the groups are not significant.

7.11 Phosphoglucomutase.

No significant differences in phosphoglucomutase phenotype frequencies could be detected amongst the diabetics with regard to sex, area of origin or age at diagnosis. The trend of increasing heterozygote frequency with age, seen in the controls,

Table 7.59 Acid phosphatase phenotype results, diabetics and controls under 40 years of age.

Phenotype	Number and frequency (%)		
	Ins.-taking Diabetics	All Diabetics	Controls
A	12 (14.8)	12 (12.4)	5 (4.5)
BA	39 (48.1)	44 (45.4)	55 (49.5)
B	24 (29.6)	33 (34.0)	43 (38.7)
CA	1 (1.2)	2 (2.1)	2 (1.8)
CB	5 (6.2)	6 (6.2)	6 (5.4)
Totals	81	97	111

Mean age of diabetics = 25.28 (±.97) years

Mean age of controls = 26.312 (±.62) years

Table 7.60 Acid phosphatase results, diabetics and controls aged 40 and over.

Phenotype	Number and frequency (%)			
	Diabetics		(3) All Diabetics	(4) All Controls
	(1) Ins.-taking	(2) Non-ins.-taking		
A	18 (14.1)	52 (11.5)	70 (12.1)	19 (16.1)
BA	47 (36.7)	192 (42.5)	239 (41.2)	56 (47.5)
B	51 (39.8)	172 (38.1)	223 (38.5)	37 (31.4)
CA	4 (3.1)	11 (2.4)	15 (2.6)	2 (1.7)
CB	7 (5.5)	25 (5.5)	32 (5.5)	4 (3.4)
C	1 (0.8)	0	1 (0.2)	0
Totals	128	452	580	118

Mean age of diabetics = 63.95 (±.4) years

Mean age of controls = 62.68 (±1.2) years

Table 7.61 Acid phosphatase gene frequencies for diabetics and controls aged under 40 years of age.

Gene	Frequency (±S.E.)		
	Ins.-taking Diabetics	All Diabetics	Controls
A	0.3951	0.3608	0.3018
B	0.5679 (±.06)	0.5979 (±.05)	0.6622 (±.05)
C	0.0370	0.0413	0.0360

Table 7.62 Acid phosphatase gene frequencies for diabetics and controls aged 40 and over

Gene	Frequency (±S.E.)			
	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	All	
A	0.3398	0.3396	0.3397	0.4068
B	0.6094 (±.04)	0.6206 (±.02)	0.6181 (±.02)	0.5678 (±.05)
C	0.0508	0.0398	0.0422	0.0254

was not present in the diabetic sample.

Ideally, because of this trend, the diabetics should have been compared with age-matched controls but the size of the control sample was insufficient to allow for this. Table 7.63 shows the phenotype frequencies for this system and table 7.64 shows the gene frequencies. There are no significant differences in the diabetics with regard to current treatment and the treatment sub-groups are not significantly different from the control group. There is no difference between the diabetic group as a whole and the control group.

7.13 Adenylate kinase.

The results for 708 diabetics investigated for this system are given in table 7.65. There were no detectable differences between the sexes and none with regard to area of origin, current age or age at diagnosis. Oral-hypoglycaemic-treated diabetics and diet-managed diabetics were statistically indistinguishable from each other and have been combined as non-insulin-taking diabetics. The gene frequencies for this system are given in table 7.66.

There are no significant differences between insulin-taking diabetics and non-insulin-taking diabetics and neither sub-group differs significantly from the controls. The entire diabetic group is statistically indistinguishable from the controls. For the calculation of chi squared the 2-2 and 2-1 categories have been amalgamated.

7.13 Adenosine deaminase.

The numbers of diabetics and controls investigated for

Table 7.63 Phosphoglucomutase results, diabetics and controls.

Phenotype	Number and frequency (%)			
	Diabetics		(3) All Diabetics	(4) All Controls
	(1) Ins.-taking	(2) Non-ins.-taking		
1-1	64 (66.0)	151 (71.9)	215 (70.0)	79 (68.7)
2-1	31 (32.0)	44 (20.9)	75 (24.4)	32 (27.8)
2-2	2 (2.1)	15 (7.1)	17 (5.5)	4 (3.5)
Totals	97	210	307	115

Table 7.64 Phosphoglucomutase gene frequency results, diabetics and controls.

Gene	Frequency (±S.E.)			
	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	All	
PGM^1	0.8196 (±.039)	0.8238 (±.026)	0.8225 (±.022)	0.8261 (±.035)
PGM^2	0.1804	0.1762	0.1775	0.1739

Table 7.65 Adenylate kinase results, controls and diabetics.

Phenotype	Number and frequency (%)			
	Diabetics		(3) All Diabetics	(4) All Controls
	(1) Ins.-taking	(2) Non-ins.-taking		
1-1	210 (93.8)	454 (93.8)	664 (93.8)	363 (91.9)
2-1	14 (6.3)	29 (6.0)	43 (6.1)	30 (7.6)
2-2	0	1 (0.2)	1 (0.1)	2 (0.5)
Totals	224	484	708	395

Table 7.66 Adenylate kinase gene frequencies, controls and diabetics.

Gene	Frequency (+S.E.)			
	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	All	
$\underline{AK}^1$	0.9687 (±.017)	0.9680 (±.008)	0.9682 (±.007)	0.9570 (±.010)
$\underline{AK}^2$	0.0313	0.0320	0.0318	0.0430

this system are insufficient for investigation of sex differences or those related to area of origin,current age,age at diagnosis or treatment. The phenotype frequencies for the entire diabetic group and the entire control group are given in table 7.67,with gene frequencies in table 7.68.

There is a greater frequency of heterozygotes amongst the diabetics but this is not significant.

7.14 Esterase D.

The diabetic sample investigated for the esterase D system showed no significant differences when sub-divided by sex, area of origin,current age or age at diagnosis. The non-insulin-taking diabetics were homogeneous in their phenotype frequencies and these,together with the frequencies for insulin-taking diabetics and controls are given in table 7.69. There are no significant differences between insulin-taking and non-insulin-taking diabetics and neither these,nor the diabetic group as a whole,differ significantly from the control sample. Gene frequencies for this system are given in table 7.70.

7.15 The HLA system.

The diabetics showed no significant differences when HLA phenotype frequencies were compared in males and females and there were no differences with regard area of origin. The comparison between oral-hypoglycaemic-treated diabetics and diet-managed diabetics shows no convincing differences (table 7.71). The apparent excess of HLA-A3 positive individuals amongst the former group does not stand up to correction for the number of

Table 7.67 Adenosine deaminase phenotype results for diabetics and controls.

Phenotype	No. & frequency (%)	
	All Diabetics	All Controls
1-1	60 (85.7)	96 (94.1)
2-1	10 (14.3)	6 (5.9)
Totals	70	102

Table 7.68 Gene frequencies for diabetics and control, adenosine deaminase locus.

Gene	Frequency (±S.E.)	
	All Diabetics	All Controls
$\underline{ADA}^1$	0.9286 (±.031)	0.9706 (±.017)
$\underline{ADA}^2$	0.0714	0.0294

Table 7.69 Esterase D results,diabetics and controls.

Phenotype	Number and frequency (%)			
	Diabetics		(3) All Diabetics	(4) All Controls
	(1) Ins.-taking	(2) Non-ins.-taking		
1-1	185 (81.9)	398 (81.4)	583 (81.5)	304 (77.9)
2-1	39 (17.3)	86 (17.6)	125 (17.5)	82 (21.0)
2-2	2 (0.9)	5 (1.0)	7 (1.0)	4 (1.0)
Totals	226	489	715	390

Table 7.70 Esterase D gene frequency results,diabetics and controls.

Gene	Frequency (±S.E.)			
	Diabetics			Controls
	Ins.-taking	Non-ins.-taking	All	
ESD^1	0.9049 (±.019)	0.9018 (±.013)	0.9028 (±.011)	0.8846 (±.016)
ESD^2	0.0951	0.0982	0.0972	0.1154

Table 7.71 HLA Phenotype frequencies in oral hypoglycaemic treated diabetics and in diet treated diabetics.

Locus	Allele	Number & frequency (%)		Chi sq. (d.f.=1)	P	P x 18
		Oral hypo glycaemic n=150	Diet n=153			
A	HLA A1	51 (34.0)	62 (40.5)	1.11	0.2914	
	HLA A2	60 (40.0)	68 (44.4)	0.44	0.5049	
	HLA A3	45 (30.0)	30 (19.6)	3.85	0.0494*	0.8892
	HLA A9	18 (12.0)	28 (18.3)	1.87	0.1713	
	HLA A10	27 (18.0)	25 (16.3)	0.05	0.8175	
	HLA A11	12 (8.0)	8 (5.2)	0.55	0.4593	
	Blank	4 (2.7)	6 (3.9)	0.08	0.7720	
B	HLA B5	17 (11.3)	20 (13.1)	0.08	0.7744	
	HLA B7	48 (32.0)	40 (26.1)	0.99	0.3192	
	HLA B8	43 (28.7)	43 (28.1)	0.00	0.9849	
	HLA B12	42 (28.0)	48 (31.4)	0.27	0.6054	
	HLA B13	10 (6.7)	8 (5.2)	0.08	0.7746	
	HLA B14	10 (6.7)	6 (3.9)	0.66	0.4172	
	HLA B17	9 (6.0)	8 (5.2)	0.002	0.9665	
	HLA B27	12 (8.0)	20 (13.1)	1.56	0.2116	
	HLA BW15	30 (20.0)	24 (15.7)	0.69	0.4060	
	Blank	7 (4.7)	8 (5.2)	0.002	0.9686	
C	HLA CW4	20 + (17.7)	16 Δ (13.4)	0.51	0.4758	

+n = 113 Δn = 119

comparisons made (18),so that this difference may well be due to chance. The slight excess of HLA-Bw15 positive individuals in the oral-hypoglycaemic-treated group is worthy of note. This could, again,be a chance finding,alternatively,it could be the result of a small number of type I diabetics amongst this group (Irvine,1977).

Tables 7.72 and 7.73 show separate comparisons between controls and oral-hypoglycaemic-treated and diet-controlled diabetics respectively. In the former category there is an apparent excess of HLA-A10 and HLA-Bw15 positive individuals,whilst in the latter group there appears to be a deficit of HLA-A3 individuals. The relative incidences and chi squared values (Woolf's method) for these specificities are given in tables 7.74(a) and 7.74(b). None of these have a sufficiently low p value to withstand the correction for the number of comparisons and,may,therefore be the result of chance. The possible association between HLA-Bw15 and oral-hypoglycaemic-treated diabetes would be worth investigating in another sample of diabetics.

In the absence of any clear-cut differences between these two treatment sub-groups,they have been amalgamated as non-insulin-taking diabetics. This group is compared with controls in table 7.75. No significant differences are demonstrated.

Insulin-taking diabetics show several differences when compared with non-insulin-taking diabetics (table 7.76) and with controls (table 7.77). Relative incidences (insulin-taking diabetics vs. controls)of some specificities and various combinations are given in table 7.78. The starred p values are less than 0.05 even after correction for the number of specificities compared. The positive associations with HLA-B8 and HLA-Bw15 agree with most

Table 7.72 HLA Phenotype frequencies in oral hypoglycaemic treated diabetics and controls.

Locus	Allele	Number & frequency (%)		Chi sq. (d.f.=1)	P	P x18
		Diabetics n=150	Controls n=427			
A	HLA A1	51 (34.0)	147 (34.4)	0.00003	0.9957	
	HLA A2	60 (40.0)	207 (48.5)	2.88	0.0898	
	HLA A3	45 (30.0)	122 (28.6)	0.05	0.8202	
	HLA A9	18 (12.0)	57 (13.3)	0.08	0.7783	
	HLA A10	27 (18.0)	48 (11.2)	3.91	0.0481*	.8658
	HLA A11	12 (8.0)	40 (9.4)	0.11	0.7358	
	Blank	4 (2.7)	14 (3.3)	N.A.		
B	HLA B5	17 (11.3)	43 (10.1)	0.08	0.7791	
	HLA B7	48 (32.0)	119 (27.9)	0.73	0.3925	
	HLA B8	43 (28.7)	126 (29.5)	0.01	0.9279	
	HLA B12	42 (28.0)	145 (34.0)	1.54	0.2151	
	HLA B13	10 (6.7)	17 (4.0)	1.24	0.2649	
	HLA B14	10 (6.7)	31 (7.3)	0.003	0.9533	
	HLA B17	9 (6.0)	32 (7.5)	0.18	0.6686	
	HLA B27	12 (8.0)	35 (8.2)	0.01	0.9222	
	HLA BW15	30 (20.0)	53 (12.4)	4.59	0.0321*	.5778
	Blank	7 (4.7)	25 (5.9)	0.11	0.7342	
C	HLA CW4	20+ (17.7)	48Δ (12.9)	1.30	0.2535	

+n = 113 Δn = 373

Table 7.73 HLA Phenotype frequencies in diet managed diabetics and controls.

Locus	Allele	Number & frequency (%)		Chi sq. (d.f.=1)	P	P x 18
		Diabetics n=153	Controls n=427			
A	HLA A1	62 (40.5)	147 (34.4)	1.56	0.2114	
	HLA A2	68 (44.4)	207 (48.5)	0.58	0.4455	
	HLA A7	30 (19.6)	122 (28.6)	4.23	0.0398*	.7164
	HLA A9	28 (18.3)	57 (13.3)	1.83	0.1761	
	HLA A10	25 (16.3)	48 (11.2)	2.21	0.1364	
	HLA A11	8 (5.2)	40 (9.4)	2.02	0.1546	
	Blank	6 (3.9)	14 (3.3)	0.01	0.9079	
B	HLA B5	20 (13.1)	43 (10.1)	0.76	0.3830	
	HLA B7	40 (26.1)	119 (27.9)	0.09	0.7605	
	HLA B8	43 (28.1)	126 (29.5)	0.05	0.8226	
	HLA B12	48 (31.4)	145 (34.0)	0.23	0.6296	
	HLA B13	8 (5.2)	17 (4.0)	0.18	0.6745	
	HLA B14	6 (3.9)	31 (7.3)	1.58	0.2087	
	HLA B17	8 (5.2	32 (7.5)	0.58	0.4455	
	HLA B27	20 (13.1)	35 (8.2)	2.58	0.1084	
	HLA BW15	24 (15.7)	53 (12.4)	0.78	0.3760	
	Blank	8 (5.2)	25 (5.9)	0.01	0.9335	
C	HLA CW4	16 (13.4)+	48 (12.9)Δ	0.00004	0.9949	

+n = 119 Δn = 373

Table 7.74(a) Relative incidences in oral-hypoglycaemic treated diabetics and controls.

Allele	Relative incidence	Chi squared (d.f.=1)	P.
HLA A10	1.73	4.38	<0.05>0.025
HLA BW15	1.76	5.06	<0.025>0.01

Table 7.74(b) Relative incidences in diet-managed diabetics and controls.

Allele	Relative incidence	Chi squared (d.f.=1)	P.
HLA-A3	0.61	4.61	<0.05>0.025

Table 7.75 HLA Phenotype frequencies in non-insulin-taking diabetics and controls.

Locus	Allele	Number & frequency (%)		Chi sq. (d.f.=1)	P
		Diabetics n=303	Controls n=427		
A	HLA-A1	113 (37.3)	147 (34.4)	0.52	0.4723
	HLA-A2	128 (42.2)	207 (48.5)	2.53	0.1118
	HLA-A3	75 (24.8)	122 (28.6)	1.13	0.2888
	HLA-A9	46 (15.2)	57 (13.3)	0.35	0.5532
	HLA-A10	52 (17.2)	48 (11.2)	4.77	0.0290
	HLA-A11	20 (6.6)	40 (9.4)	1.45	0.2284
	Blank	10 (3.3)	14 (3.3)	0.04	0.8458
B	HLA-B5	37 (12.2)	43 (10.1)	0.63	0.4282
	HLA-B7	88 (29.0)	119 (27.9)	0.07	0.7922
	HLA-B8	86 (28.4)	126 (29.5)	0.06	0.8047
	HLA-B12	90 (29.7)	145 (34.0)	1.28	0.2576
	HLA-B13	18 (5.9)	17 (4.0)	1.09	0.2960
	HLA-B14	16 (5.3)	31 (7.3)	0.85	0.3572
	HLA-B17	17 (5.6)	32 (7.5)	0.73	0.3942
	HLA-B27	32 (10.6)	35 (8.2)	0.92	0.3370
	HLA-BW15	54 (17.8)	53 (12.4)	3.72	0.0536
	Blank	15 (5.0)	25 (5.9)	0.13	0.7159
C	HLA-CW4	36+ (15.7)	48Δ (12.9)	0.63	0.4265

+n = 229 Δn = 373

Table 7.76 HLA Phenotype frequencies in insulin-taking and non-insulin-taking diabetics.

Locus	Allele	Number & frequency (%) ins.-taking diabetics n=179	non-ins. taking diabetics n=303	Chi squared	P	P x 18
A	HLA-A1	66 (36.9)	113 (37.3)	.000	0.9961	
	HLA-A2	110 (61.5)	128 (42.2)	15.85	0.0001*	.0018**
	HLA-A3	30 (16.8)	75 (24.8)	3.76	0.0524	
	HLA-A9	30 (16.8)	46 (15.2)	0.11	0.7414	
	HLA-A10	23 (12.8)	52 (17.2)	1.28	0.2576	
	HLA-A11	13 (7.3)	20 (6.6)	0.008	0.9272	
	Blank	3 (1.7)	10 (3.3)	N.A.		
B	HLA-B5	13 (7.3)	37 (12.2)	2.46	0.1171	
	HLA-B7	26 (14.5)	88 (29.0)	12.34	0.0004*	.0072**
	HLA-B8	81 (45.3)	86 (28.4)	13.40	0.0003*	.0054**
	HLA-B12	45 (25.1)	90 (29.7)	0.95	0.3305	
	HLA-B13	9 (5.0)	18 (5.9)	0.05	0.8290	
	HLA-B14	10 (5.6)	16 (5.3)	0.004	0.9482	
	HLA-B17	3 (1.7)	17 (5.6)	3.45	0.0634	
	HLA-B27	15 (8.4)	32 (10.6)	0.39	0.5346	
	HLA-BW15	48 (26.8)	54 (17.8)	4.93	0.0264*	.4752
	Blank	6 (3.4)	15 (5.0)	0.36	0.5487	
C	HLA-CW4	15+ (10.9)	36Δ (15.7)	1.15	0.2835	

+n = 137 Δn = 229

Table 7.77 HLA Phenotype frequencies in insulin-taking diabetics and controls.

Locus	Allele	Number & frequency (%)		Chi sq. (d.f.=1)	P	P ×18
		Diabetics n=179	Controls n=427			
A	HLA-A1	66 (36.9)	147 (34.4)	0.23	0.6298	
	HLA-A2	110 (61.5)	207 (48.5)	8.00	0.0047*	.0846
	HLA-A3	30 (16.8)	122 (28.6)	8.75	0.0031*	.0558
	HLA-A9	30 (16.8)	57 (13.3)	0.93	0.3343	
	HLA-A10	23 (12.8)	48 (11.2)	0.18	0.6723	
	HLA-A11	13 (7.3)	40 (9.4)	0.46	0.4970	
	.	3 (1.7)	14 (3.3)	N.A.		
B	HLA-B5	13 (7.3)	43 (10.1)	0.87	0.3498	
	HLA-B7	26 (14.5)	119 (27.9)	11.62	0.0007*	.0126**
	HLA-B8	81 (45.3)	126 (29.5)	13.21	0.0003*	.0054**
	HLA-B12	45 (25.1)	145 (34.0)	4.16	0.0415*	.7470
	HLA-B13	9 (5.0)	17 (4.0)	0.13	0.7186	
	HLA-B14	10 (5.6)	31 (7.3)	0.33	0.5680	
	HLA-B17	3 (1.7)	32 (7.5)	6.81	0.0091*	.1638
	HLA-B27	15 (8.4)	35 (8.2)	0.01	0.9306	
	HLA-BW15	48 (26.8)	53 (12.4)	17.82	0.0000*	0.0000**
		6 (3.4)	25 (5.9)	1.15	0.2829	
C	HLA-CW4	15+ (10.9)	48Δ (12.9)	0.19	0.6656	

+n = 137 Δn = 373

Table 7.78 Relative incidences in insulin-taking diabetics and controls.

Specificity	Relative incidence	Chi squared (d.f.=1)	P.
HLA-A2	1.69	8.35	<0.005>0.001
HLA-A3	0.50	9.33	<0.005>0.001
HLA-B7	0.44	11.90	<0.001>0.0005*
HLA-B8	1.97	13.60	<0.0005*
HLA-B12	0.65	4.62	<0.05>0.025
HLA-B17	0.21	6.53	<0.025>0.01
HLA-BW15	2.59	18.11	<0.0005*
Homozygons HLA-B7	0.51	2.81	<0.10>0.05
Homozygons B8	2.39	12.55	<0.0005*
Homozygons BW15	2.96	6.68	<0.01 0.005
Heterozygons B7/B8	0.63	1.13	<0.30>0.20
Heterozygons B7/BW15	1.20	0.09	<0.80>0.70
Heterozygons B8/BW15	5.95	12.90	<0.0005*

previous authors. The negative association with HLA-B7 reported by some is also evident. There is a large relative incidence of the B8/Bw15 combination. The negative association with HLA-A3 is probably due to the positive linkage disequilibrium that allele shows with HLA-B7,whilst the positive association with HLA-A2 may be the result of linkage disequilibrium with HLA-Bw15. The negative association with HLA-A11 reported by Cudworth and Woodrow (1976) cannot be demonstrated. The lack of HLA-B17 positive individuals amongst the diabetics may be due to chance.

The 179 insulin-taking diabetics show significant differences with regard to age at diagnosis. Subdivided at the customary age of 30,the phenotype frequencies in the two groups are shown in table 7.79. They differ in their frequency of HLA-B8 and comparison of each sub-group in turn with controls (tables 7.80 and 7.81) shows that the frequency of this specificity in the later onset diabetics is indistinguishable from that in the general population whereas the excess of HLA-Bw15 positive individuals is highly significant. In the insulin-taking diabetics diagnosed before this age,the reverse is the case with a highly significant excess of the HLA-B8 allele and only a marginally significant excess of HLA-Bw15 positive individuals. A deficit of the HLA-B7 allele is suggested in both groups. The relative incidences are shown in table 7.82.

These data are taken to imply that insulin-taking diabetics are heterogeneous with regard to associations with alleles at the HLA-B locus. The positive association is largely confined to diabetes diagnosed before about the age of 40 (see figure 7.1(a)) but this does not apply to HLA-Bw15,shown in figure 7.1(b).

Table 7.79 HLA Phenotype frequencies in insulin-taking diabetics subdivided by age at diagnosis.

Locus	Allele	Number & frequency (%) Diag. <30 n=101	Diag. 30+ n=77	Chi sq. (d.f.=1)	P	P x18
A	HLA-A1	40 (39.2)	26 (33.8)	0.35	0.5540	
	HLA-A2	59 (57.8)	51 (66.2)	0.97	0.3237	
	HLA-A3	19 (18.6)	11 (14.3)	0.32	0.5701	
	HLA-A9	16 (15.7)	14 (18.2)	0.06	0.8100	
	HLA-A10	14 (13.7)	9 (11.7)	0.03	0.8590	
	HLA-A11	6 (5.9)	7 (9.1)	0.28	0.5974	
	Blank	2 (2.0)	1 (1.3)	0.06	0.8054	
B	HLA-B5	5 (4.9)	8 (10.4)	1.23	0.2671	
	HLA-B7	16 (15.7)	10 (13.0)	0.09	0.7694	
	HLA-B8	54 (52.9)	27 (35.1)	4.96	0.0259*	.4662
	HLA-B12	28 (27.5)	17 (22.1)	0.42	0.5180	
	HLA-B13	7 (6.9)	2 (2.6)	0.90	0.3434	
	HLA-B14	4 (3.9)	6 (7.8)	0.62	0.4309	
	HLA-B17	2 (2.0)	1 (1.3)	0.06	0.8054	
	HLA-B27	9 (8.8)	6 (7.8)	0.00067	0.9794	
	HLA-BW15	22 (21.6)	26 (33.8)	2.73	0.0982	
	Blank	2 (2.0)	4 (5.2)	0.59	0.4408	
C	HLA-CW4	5* (6.3)	10+ (17.2)	3.04	0.0811	

*n = 78 +n = 58

Table 7.80 HLA Phenotype frequencies in insulin-taking diabetics diagnosed before age of 30 and in controls.

Locus	Allele	Number & frequency (%)		Chi sq. (d.f.=1)	P	P x18
		Diabetics n=102	Controls n=427			
A	HLA-A1	40 (39.2)	147 (34.4)	0.63	0.4273	
	HLA-A2	59 (57.8)	207 (48.5)	2.52	0.1120	
	HLA-A3	19 (18.6)	122 (28.6)	3.67	0.0554	
	HLA-A9	16 (15.7)	57 (13.3)	0.21	0.6490	
	HLA-A10	14 (13.7)	48 (11.2)	0.28	0.5965	
	HLA-A11	6 (5.9)	40 (9.4)	0.86	0.3540	
	Blank	2 (2.0)	14 (3.3)	N.A.		
B	HLA-B5	5 (4.9)	43 (10.1)	2.08	0.1496	
	HLA-B7	16 (15.7)	119 (27.9)	5.80	0.0160*	.2898
	HLA-B8	54 (52.9)	126 (29.5)	19.11	0.0000*	0.0000
	HLA-B12	28 (27.5)	145 (34.0)	1.30	0.2538	
	HLA-B13	7 (6.9)	17 (4.0)	0.98	0.3214	
	HLA-B14	4 (3.9)	31 (7.3)	0.99	0.3188	
	HLA-B17	2 (2.0)	32 (7.5)	3.32	0.0684	
	HLA-B27	9 (8.8)	35 (8.2)	0.00004	0.9949	
	HLA-BW15	22 (21.6)	53 (12.4)	4.95	0.0262*	.4716
	Blank	2 (2.0)	25 (5.9)	1.84	0.1754	
C	HLA-CW4	5+ (6.3)	48Δ (12.9)	2.10	0.1474	

+n = 78   Δn = 373

Table 7.81 HLA Phenotype frequencies in insulin-taking diabetics diagnosed aged 30 or over and in controls.

Locus	Allele	Number & frequency (%)		Chi sq. (d.f.=1)	P	P x 18
		Diabetics n=77	Controls n=427			
	HLA-A1	26 (33.8)	147 (34.4)	0.0003	0.9856	
	HLA-A2	51 (66.2)	207 (48.5)	7.54	0.0060*	0.1080
	HLA-A3	11 (14.3)	122 (28.6)	6.14	0.0132*	0.2376
A	HLA-A9	14 (18.2)	57 (13.3)	0.89	0.3451	
	HLA-A10	9 (11.7)	48 (11.2)	0.01	0.9351	
	HLA-A11	7 (9.1)	40 (9.4)	0.02	0.8918	
	Blank	1 (1.3)	14 (3.3)	0.33	0.5641	
	HLA-B5	8 (10.4)	43 (10.1)	0.01	0.9047	
	HLA-B7	10 (13.0)	119 (27.9)	6.82	0.0090*	0.1620
	HLA-B8	27 (35.1)	126 (29.5)	0.71	0.4001	
	HLA-B12	17 (22.1)	145 (34.0)	3.69	0.0546	
B	HLA-B13	2 (2.6)	17 (4.0)	N.A.		
	HLA-B14	6 (7.8)	31 (7.3)	0.01	0.9422	
	HLA-B17	1 (1.3)	32 (7.5)	3.14	0.0763	
	HLA-B27	6 (7.8)	35 (8.2)	0.01	0.9148	
	HLA-BW15	26 (33.8)	53 (12.4)	20.92	0.0000*	0.0000**
	Blank	4 (5.2)	25 (5.9)	N.A.		
C	HLA-CW4	10^{+} (17.2)	48^{Δ} (12.9)	0.49	0.4333	

$^{+}n = 58$ $^{\Delta}n = 373$ N.A. = not applicable.

Table 7.82 Relative incidences in insulin-taking diabetics and controls.

Specificity	Insulin-taking diabetics diagnosed before 30 vs. controls			Insulin-taking diabetics diagnosis 30 or older vs. controls		
	Relative incidence	Chi sq. (d.f.=1)	P	Relative incidence	Chi sq. (d.f.=1)	P
HLA-B7	0.48	6.28	<0.025 >0.01	0.39	7.00	<0.01 >0.005
HLA-B8	2.69	19.35	<0.0005	1.29	0.95	<0.40 >0.30
HLA-BW15	1.94	5.52	<0.025 >0.01	3.60	20.61	<0.0005
HLA-B8/BW15	6.79	12.61	<0.0005	4.87	6.55	<0.025 >0.01

Figure 7.1(a) Frequency of HLA-B8 in insulin-taking diabetics according to age at diagnosis.

Figure 7.1(b) Frequency of HLA-Bw15 in insulin-taking diabetics according to age at diagnosis.

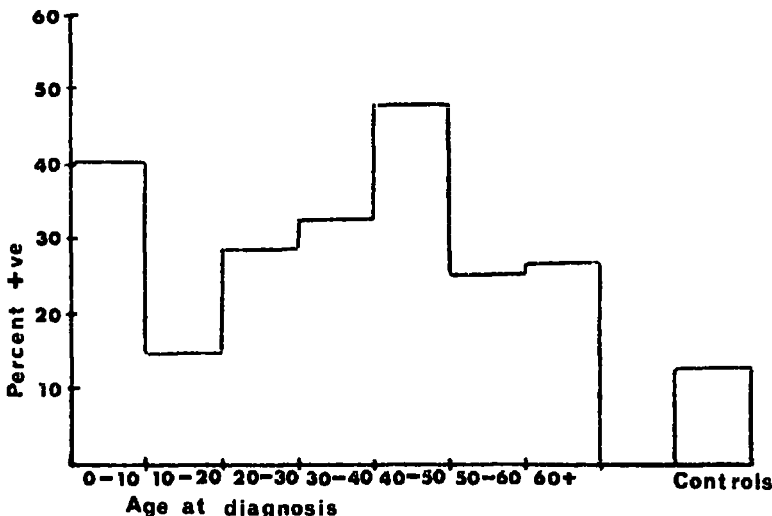
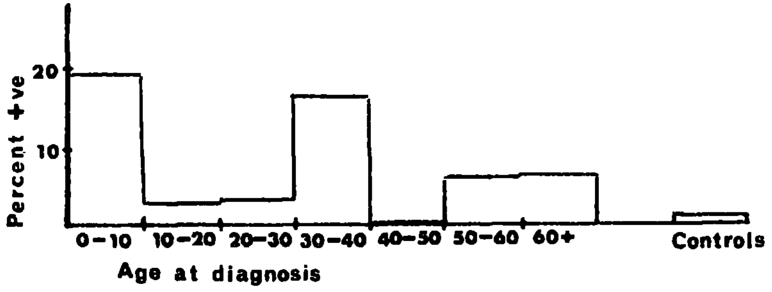


Figure 7.1(c) Frequency of B8/Bw15 heterozygotes in insulin-taking diabetics.



For the first four decades of life the frequency of HLA-B8 positive diabetics is significantly above the mean for the population of 29.5% whereas in the next three groups it is indistinguishable from the control value. The frequency of the HLA-Bw15 antigen is far more variable but in all four decades of onset except the second the frequency in diabetics is above that in the general population. Figure 7.1(c) shows that the number of diabetics presenting in the first decade of life and heterozygous B8/Bw15 is fourteen times that in the general population and in no other decade does the excess frequency approach this.

These results are at variance with those of Dausset (1977) which are given in figures 7.2(a) and 7.2(b) for the purposes of comparison. The numbers of individuals on which these conclusions are based are considerably smaller than those of the present sample,especially in the case of diabetics diagnosed over the age of 40,(Dausset et al (1977) - 21 cases,present study - 50). It may be that an increased sample of French diabetics would support the present finding of a raised incidence of HLA-Bw15 positive individuals prsenting after this age. For HLA-B8,given the expected lower frequency of the specificity in the French control sample,the pattern of the results is similar.

The heterogeneity of insulin-dependent diabetics with regard to HLA-B8 and HLA-Bw15 frequencies may explain some of the discrepancies in published results. Singal and Blajchman (1973) found a significant excess of HLA-Bw15 positive individuals amongst their sample of insulin-taking diabetics but not an excess of HLA-B8. They used a comparatively old population of diabetics (mean 59.3 years,range 18 - 84 years) and,although they do not give the

Figure 7.2(a) Frequency of HLA-B8 in insulin-taking diabetics according to age at diagnosis (from Dausset,1977).

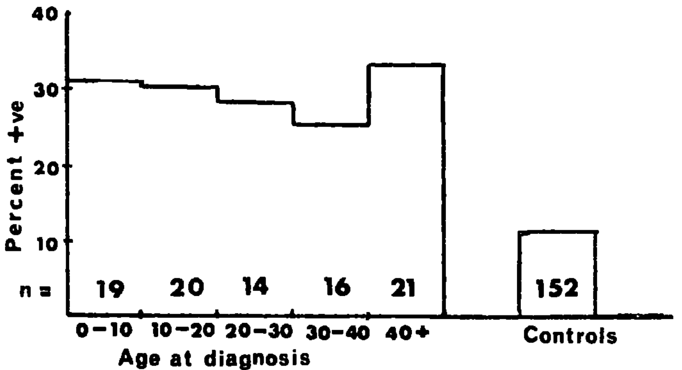
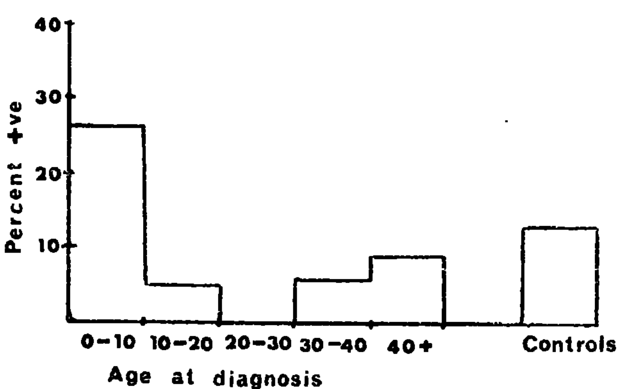


Figure 7.2(b) Frequency of HLA-Bw15 in insulin-taking diabetics according to age at diagnosis (from Dausset,1977).



ages at diagnosis of these patients,because of the phenomenon of 'frequency reduction' (see chapter 8),it is most likely that they were also of comparatively late age at diagnosis. This may account for their result. Both Cathelineau et al (1975) and Garavoy et al (1977) found the reverse - a significant excess of HLA-B8 positive individuals amongst insulin-taking diabetics but no excess of HLA-Bw15. They both studied a comparatively young population. Cathelineau et al (1975) had only 20 individuals who had presented after the age of 35 years. All the patients studied by Garavoy et al (1977) were under 20 years of age at diagnosis.

The findings of Cudworth and Woodrow (1976) differ from all these reports and the findings of the present study in suggesting associations with both HLA-B8 and HLA-Bw15 in insulin-taking diabetics diagnosed before 30 and an association only with HLA-B8 in diabetics diagnosed after this age. Their data show a suggested increase in HLA-Bw15 for insulin-taking diabetics diagnosed after the age of 30 and this may have reached significance with increase of the sample size. The effect on the relative incidence of a certain allele in a disease of the frequency in the general population will be discussed at the end of this chapter.

The HLA gene frequencies for diabetics of various types and for controls are given in table 7.83. Linkage disequilibrium (delta) values and haplotype frequencies were computed for every allelic combination and for each group. The results are not given in full but table 7.84 shows the haplotype frequencies and delta values for some important haplotypes.

Table 7.83 HLA gene frequencies in diabetics and in controls

Locus	Allele	Ins.-taking diabetics diagnosis <30	Ins.-taking diabetics diagnosis 30+	All ins.-taking diabetics	All non-insulin-taking diabetics	All controls
A	HLA-A1	0.2213	0.1866	0.2062	0.2118	0.1882
	HLA-A2	0.3521	0.4199	0.3805	0.2443	0.2884
	HLA-A3	0.983	0.0743	0.0879	0.1349	0.1547
	HLA-A9	0.0821	0.0957	0.0879	0.0804	0.0691
	HLA-A10	0.0775	0.0604	0.0667	0.0914	0.0585
	HLA-A11	0.0300	0.0464	0.0371	0.0342	0.0480
	Blank	0.1447	0.1167	0.1337	0.2030	0.1931
B	HLA-B5	0.0256	0.0548	0.0381	0.0633	0.0507
	HLA-B7	0.0845	0.0690	0.0777	0.1583	0.1498
	HLA-B8	0.3245	0.1995	0.2679	0.1544	0.1611
	HLA-B12	0.1532	0.1205	0.1388	0.1622	0.1892
	HLA-B13	0.0361	0.0134	0.0262	0.0303	0.0199
	HLA-B14	0.0205	0.0408	0.0292	0.0269	0.0367
	HLA-B17	0.0102	0.0067	0.0087	0.0286	0.0381
	HLA-B27	0.0466	0.0408	0.0441	0.0545	0.0421
	HLA-BW15	0.1182	0.1913	0.1489	0.0939	0.0635
	Blank	0.1806	0.2632	0.2204	0.2276	0.2489

Table 7.84 HLA haplotype frequencies (upper figure) and delta values for selected haplotypes in diabetics and controls.

Haplotype	Diabetics		Controls
	Insulin-taking	Non-insulin-taking	
1-8	0.1645 +0.1093	0.1116 +0.0789	0.1229 +0.0926
2-12	0.0848 +0.0320	0.0473 +0.0077	0.0969 +0.0423
3-7	0.0068 +0.0185	0.0527 +0.0314	0.0600 +0.0368
2-w15	0.0567 +0.0181	0.0257 +0.0028	0.0358 +0.0175
1-5	0 -0.0140	0.0071 -0.0063	0 -0.0123
1-12	0.0072 -0.0358	0.0279 -0.0064	0 -0.0367
2-8	0 -0.0569	0.0239 -0.0138	0.0105 -0.0360
3-8	0.0043 -0.0278	0.0065 -0.0143	0.0097 -0.0153

7.16 Summary and discussion.

The positive findings in the analysis of polymorphism results in diabetics and controls are that associations exist in the ABO and HLA systems but not in any of the others examined.

The association with the ABO system confirms the findings of a large number of previous authors (Craig and Wang,1955;McConnell et al, 1956;Zeytinoglu,1956;Cornil and Pirart,1961;Bibawi and Khatwa, 1961;Doll et al,1961;Sauer et al,1963;Serra et al,1964;Revai and Konig,1968,and Ksenofontor,1974) in finding an excess of group A individuals amongst the diabetics. The present findings that this excess is greater amongst males than amongst females and greater amongst the older diabetics than amongst the younger,are also in agreement with some of these authors.

This association is suggested to be the result,not of any greater predisposition of group A individuals to the disease but of an absence of differential mortality with regard to the ABO system in diabetics,whereas this differential mortality is present in non-diabetics. The result is that the non-diabetic population sees a reduction in its frequency of group A individuals with age, whereas the diabetic population does not,giving an apparent excess of group A individuals amongst older diabetics.

Cardiovascular disorders are a likely candidate for this. Diabetic individuals suffer from these diseases more frequently and more severely than non-diabetics and it may be that,once diabetic,these diseases progress swiftly irrespective of ABO blood group whereas,in the non-diabetic the blood group A person is more severely affected as suggested by Mourant et al.(1971).

The associations between insulin-requiring diabetes and

the B8 and Bw15 alleles of the HLA-B locus found by previous workers (for example,Nerup et al,1974,Cudworth and Woodrow,1974) have been confirmed. A larger number of non-insulin-taking diabetics than has been previously reported is considered here for the HLA system. No significant associations have been demonstrated though oral hypoglycaemic-taking diabetics show an interesting excess of B8 positive individuals. Investigation of a larger number of such diabetics might reveal that this is a true association and,if this were the case, would be most likely to be the result of the inclusion of some type I diabetics under this heading.

Heterogeneity with regard to age at diagnosis and HLA genotype is suggested with B8/w15 heterozygotes presenting predominantly in the first decade of life,B8 positive individuals presenting in the first four decades predominantly and the Bw15 positive individuals presenting throughout this time but making the greatest contribution to diabetics diagnosed after the age of 40. This heterogeneity has been noted before (Cudworth,personal communication) and it is suggested that this phenomenon can explain at least part of the disagreement between authors on associations between diabetes and this sytem.

The necessity of age matching when conducting an investigation of this kind is emphasised. Comparison of the Rhesus phenotype frequencies without age matching,for example,shows a significant difference between insulin-taking and non-insulin-taking diabetics with an excess of $\underline{R}_1\underline{R}_1$ individuals amongst the latter group (chi squared = 12.36,for five degrees of freedom,$p < 0.05 > 0.025$). These differences disappear when age is taken into account.

There seems to be little justification for strict age matching when phenotype frequencies only are considered in the HLA system. When gene frequencies or haplotype frequencies are considered, however,a case may be made out for matching the populations considered since the calculation of these frequencies takes into account the numbers of individuals homozygous for each specificity and this has been shown,at least for the HLA-B locus to be related to age.

Little attention has been paid to the possible relationship between the frequency of an allele in the general population and the magnitude for the relative risk of that allele in a disease where association exists. The relative risk for the HLA-B8 allele in insulin-dependent diabetics may be deduced from the data of Dausset(1977),shown in figure 7.2(a) to be 4.6. In the present series this relative risk is 2.69 (considering early-onset insulin-dependent diabetes in both cases). This difference may be accounted for by the difference in the frequency of this allele in the general population in France (about 10%) and that in County Durham (29.5 %). Where the population frequency is zero (e.g. Japan) the relative risk is either zero or infinity (if HLA-B8 positive individuals are found amongst diabetics but not amongst the non-diabetic controls).

Chapter 8

Clinical diabetes in the Durham area.

Chapter 8

8.1 Introduction.

8.2 Description of the 1975 Dryburn diabetic outpatient population.

(a) Age of the patients attending.

(b) Age at diagnosis.

(c) Duration of the disease.

(e) Appointments.

8.3 Newly-diagnosed diabetics in 1975 and 1976.

8.4 Retrospective analysis of month of diagnosis.

8.5 Assessing the prevalence in the general population.

(a) Determining the number of clinically diagnosed diabetics in the population.

(b) Census statistics.

(c) The prevalence of diabetes.

8.6 Summary.

8.1 Introduction.

The epidemiological aspects of this study arose as a natural consequence of the genetic investigations. The diabetic outpatient clinics at Dryburn had to be sampled and,to put the sample into perspective,it was necessary to determine the characteristics of the complete outpatient population from which it was drawn. Since the date of onset of diabetes in each patient had to be ascertained it was a natural extension to investigate retrospectively the pattern of occurrence of new cases of diabetes with time. For the analysis of family data,an estimate of the prevalence of various types of diabetes in the general population was required and this necessitated identifying all known cases of diabetes in the area and the use of Census statistics.

These three aspects - the characteristics of the outpatient population,the pattern of occurrence of new cases and the estimation of age-specific prevalence rates for the area - constitute the subject matter of this chapter.

8.2 Description of the 1975 Dryburn diabetic outpatient population.

The advantages of using this particular clinic for this investigation have been discussed in chapter 4 and some of the data were also given in that chapter.

Although diabetics were studied from November,1974 to June,1976,the year of 1975 has been chosen as a convenient temporal unit for discussion.

(a) Age of the patients attending.

Confirmed diabetics being treated as out-patients during this period totalled 936. Of these, 377 (40.3%) were male and 559 (59.7%) were female. The ages of all patients were calculated as of 1.1.76 and are given in tables 8.1(a) (males) and 8.1(b) (females). The mean age of the male patients was 56.6 years (standard deviation ±17.9), with the youngest patient attending being 4.1 years old and the oldest 87.9. The mean age of the females was 62.9 years (standard deviation ±16.4) with a range of 3.1 to 92.2 years. The figures in tables 8.1(a) and (b) are given separately for treatment sub-groups. Diagramatic representation may be seen in figures 8.1(a) and 8.1(b).

(b) Age at diagnosis.

The date of diagnosis (in most cases the date on the initial referal letter) of 23 individuals was unknown. For the remaining 913 the age at diagnosis in five year age groups is shown in tables 8.2(a) (males) and 8.2(b) (females). The mean age for diagnosis in males is 48.3 years (standard deviation 19.4), range from 0.2 years to 84.9years. The mean age in females was 54.7 years (standard deviation 17.8) with a range of 1.8 years to 90.6 years.

Figure 8.2 shows the distribution of age at diagnosis for both sexes combined, the population being divided into diabetics currently receiving insulin and diabetics being treated without it. Insulin therapy is by no means the exclusive prerogative of diabetics diagnosed early in life. 129 (51.4%) of those on insulin were diagnosed after the age of 30 although there may be a tendency in a diabetic clinic to review patients on insulin preferentially. The nine individuals not receiving insulin and diagnosed before the age of 30 were discussed in chapter 5.

Table 8.1 (a) Breakdown by age (on 1.1.76) and current treatment of male diabetics attending Dryburn out-patients in 1975.

Age (yrs)	Current treatment			All males	Cumulative frequency
	Insulin	Tablets	Diet		
0-4	2 (1.5%)	0	0	2 (0.5%)	0.5%
5-9	3 (2.3%)	0	0	3 (0.8%)	1.3%
10-14	5 (3.8%)	0	0	5 (1.3%)	2.7%
15-19	10 (7.6%)	0	0	10 (2.7%)	5.3%
20-24	10 (7.6%)	1 (1.0%)	0	11 (2.9%)	8.2%
25-29	10 (7.6%)	0	0	10 (2.7%)	10.9%
30-34	9 (6.9%)	0	1 (0.7%)	10 (2.7%)	13.5%
35-39	7 (5.3%)	1 (1.0%)	3 (2.1%)	11 (2.9%)	16.4%
40-44	10 (7.6%)	4 (3.9%)	6 (4.2%)	20 (5.3%)	21.8%
45-49	13 (9.9%)	9 (8.7%)	6 (4.2%)	28 (7.4%)	29.2%
50-54	9 (6.9%)	8 (7.8%)	20 (14.0%)	37 (9.8%)	39.0%
55-59	18 (13.7%)	8 (7.8%)	14 (9.8%)	40 (10.6%)	49.6%
60-64	7 (5.3%)	17 (16.5%)	22 (15.4%)	46 (12.2%)	61.8%
65-69	8 (6.1%)	21 (20.4%)	26 (18.2%)	55 (14.6%)	76.4%
70-74	7 (5.3%)	15 (14.6%)	21 (14.7%)	43 (11.4%)	87.8%
75-79	1 (0.8%)	14 (13.6%)	15 (10.5%)	30 (8.0%)	95.8%
80-84	1 (0.8%)	4 (3.9%)	6 (4.2%)	11 (2.9%)	98.7%
85-89	1 (0.8%)	1 (1.0%)	3 (2.1%)	5 (1.3%)	100.0%
Total	131	103	143	377	

Table 8.1 (b) Breakdown by age (on 1.1.76) and current treatment of female diabetics attending Dryburn out-patients in 1975.

Age (yrs)	Current treatment			All females	Cumulative frequency
	Insulin	Tablets	Diet		
0-4	2 (1.7%)	0	0	2 (0.4%)	0.4%
5-9	3 (2.5%)	0	0	3 (0.5%)	0.9%
10-14	6 (5.0%)	0	0	6 (1.1%)	2.0%
15-19	8 (6.7%)	0	2 (1.0%)	10 (1.8%)	3.8%
20-24	11 (9.2%)	0	0	11 (2.0%)	5.7%
25-29	2 (1.7%)	0	1 (0.5%)	3 (0.5%)	6.3%
30-34	6 (5.0%)	0	2 (1.0%)	8 (1.4%)	7.7%
35-39	6 (5.0%)	2 (0.9%)	4 (1.9%)	12 (2.1%)	9.8%
40-44	10 (8.3%)	5 (2.1%)	5 (2.4%)	20 (3.6%)	13.4%
45-49	6 (5.0%)	6 (2.6%)	5 (2.4%)	17 (3.0%)	16.5%
50-54	6 (5.0%)	9 (3.9%)	8 (3.9%)	23 (4.1%)	20.6%
55-59	10 (8.3%)	23 (9.9%)	16 (7.8%)	49 (8.8%)	29.3%
60-64	11 (9.2%)	36 (15.5%)	37 (18.0%)	84 (15.0%)	44.4%
65-69	13 (10.8%)	42 (18.0%)	40 (19.4%)	95 (17.0%)	61.4%
70-74	7 (5.8%)	54 (23.2%)	42 (20.4%)	103 (18.4%)	79.8%
75-79	7 (5.8%)	43 (18.5%)	24 (11.7%)	74 (13.2%)	93.0%
80-84	3 (2.5%)	9 (3.9%)	12 (5.8%)	24 (4.3%)	97.3%
85-89	3 (2.5%)	4 (1.7%)	5 (2.4%)	12 (2.1%)	99.5%
90-94	0	0	3 (1.5%)	3 (0.5%)	100.0%
Total	120	233	206	559	

Figure 8.1(a) Breakdown by age of male diabetics attending Dryburn in 1975.

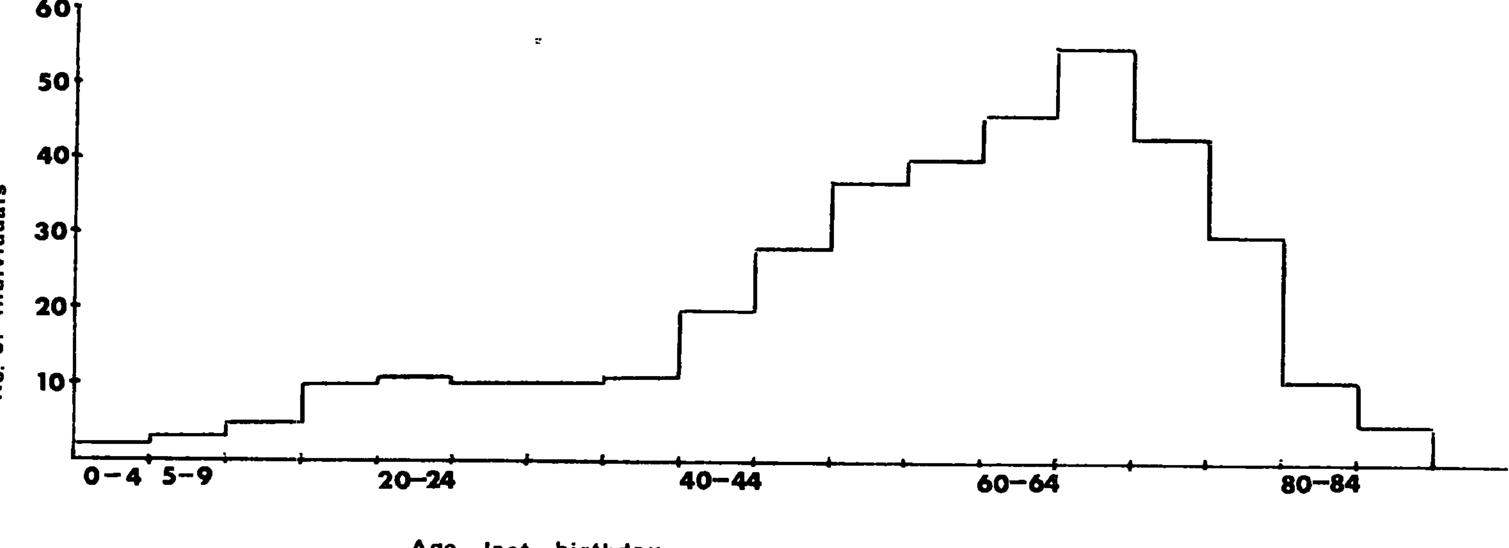


Figure 8.1(b) Breakdown by age of female diabetics attending Dryburn in 1975

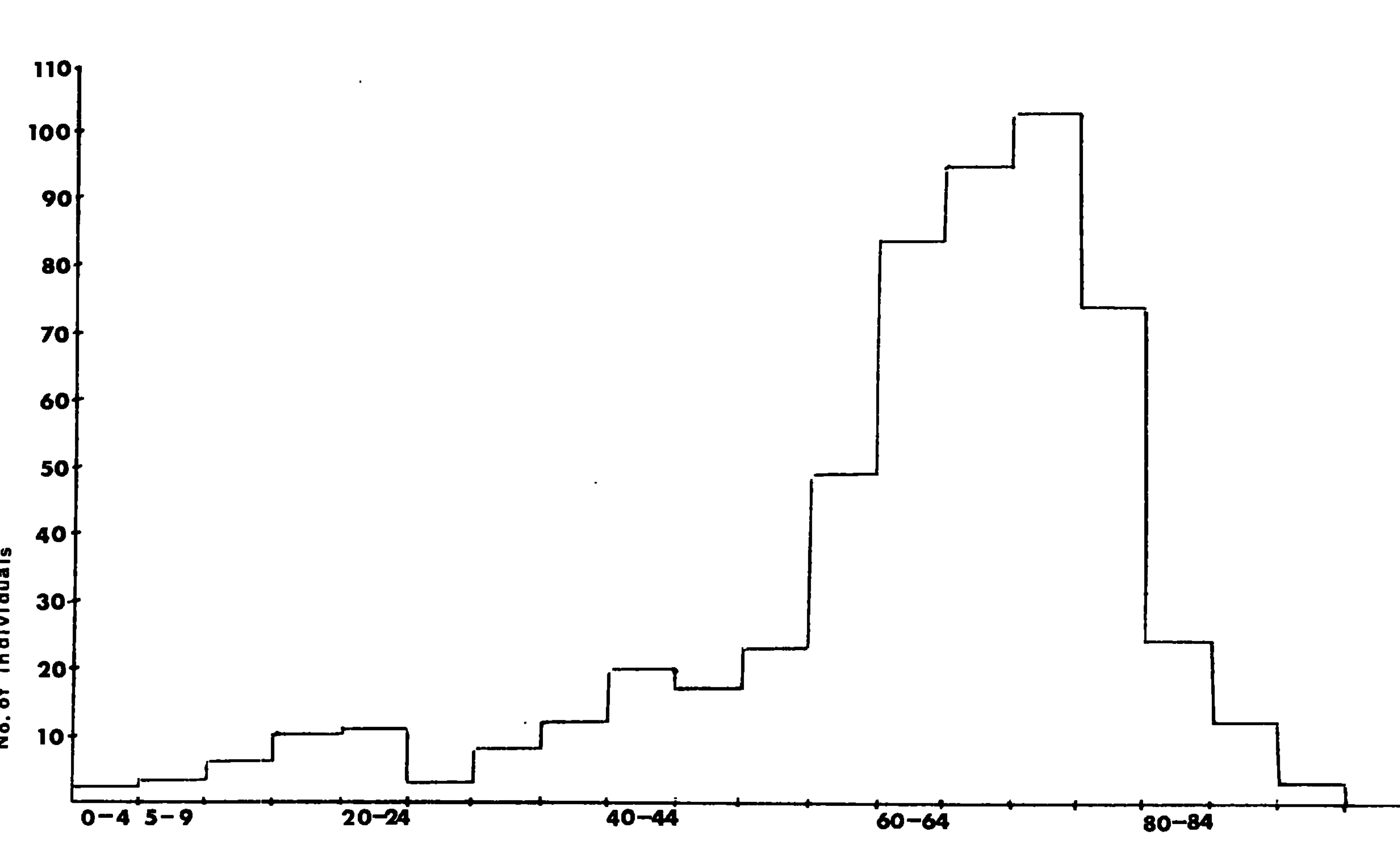


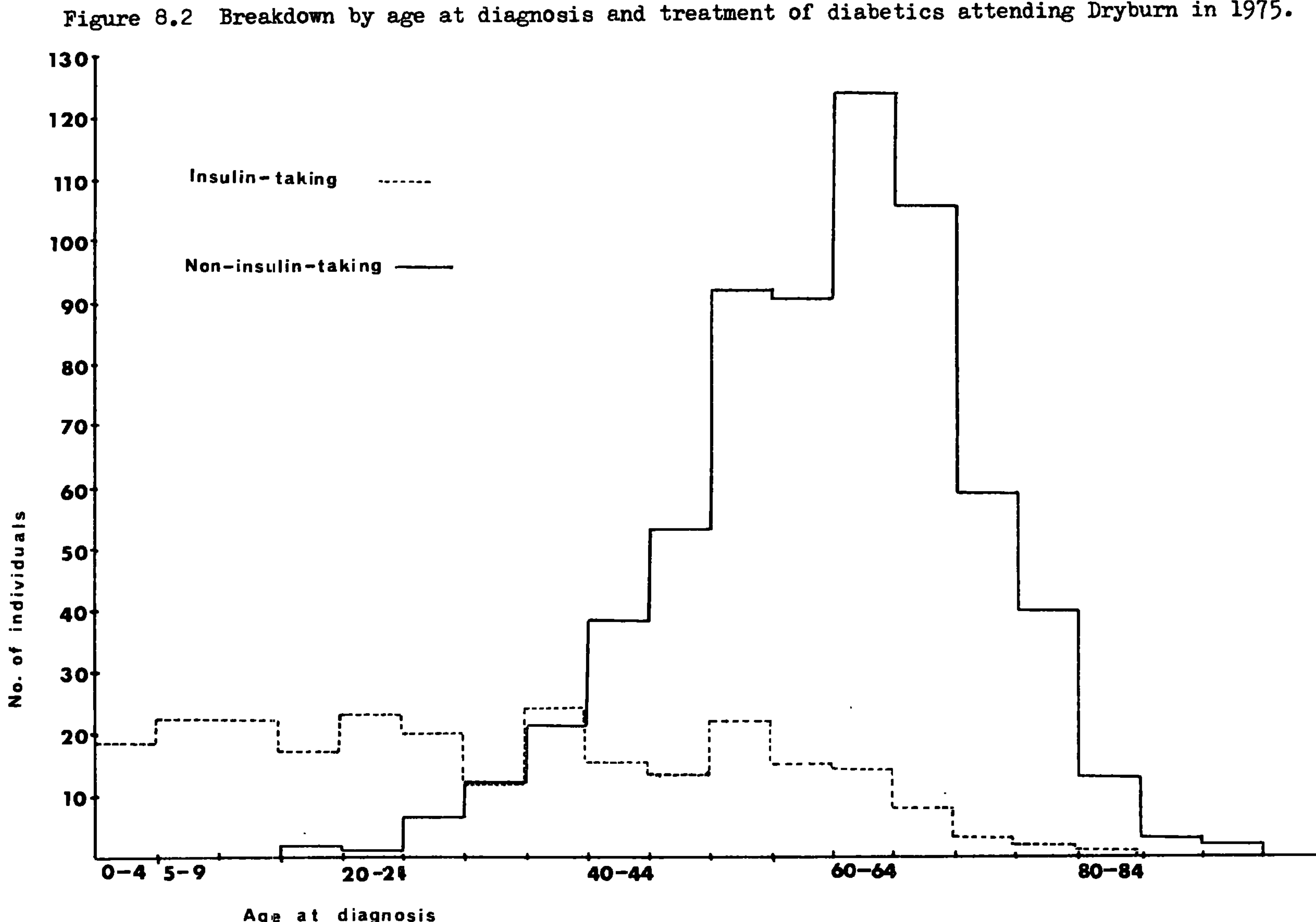
Table 8.2 (a) Breakdown by age at diagnosis and current treatment of male diabetics attending Dryburn out-patients in 1975.

Age at diag. (yrs.)	Current treatment			All males	Cumulative frequency
	Insulin	Tablets	Diet		
0-4	11 (8.4%)	0	0	11 (3.0%)	3.0%
5-9	9 (6.9%)	0	0	9 (2.5%)	5.5%
10-14	12 (9.2%)	0	0	12 (3.3%)	8.7%
15-19	10 (7.6%)	0	0	10 (2.7%)	11.5%
20-24	13 (9.9%)	1 (1.1%)	0	14 (3.8%)	15.3%
25-29	15 (11.5%)	0	0	15 (4.1%)	19.4%
30-34	7 (5.3%)	1 (1.1%)	4 (2.9%)	12 (3.3%)	22.7%
35-39	14 (10.7%)	5 (5.3%)	4 (2.9%)	23 (6.3%)	29.0%
40-44	8 (6.1%)	8 (8.4%)	11 (7.9%)	27 (7.4%)	36.3%
45-49	7 (5.3%)	11 (11.6%)	14 (10.0%)	32 (8.7%)	45.1%
50-54	7 (5.3%)	11 (11.6%)	29 (20.7%)	47 (12.8%)	57.9%
55-59	7 (5.3%)	10 (10.5%)	11 (7.9%)	28 (7.7%)	65.6%
60-64	7 (5.3%)	16 (16.8%)	27 (19.3%)	50 (13.7%)	79.2%
65-69	3 (2.3%)	14 (14.7%)	23 (16.4%)	40 (10.9%)	90.2%
70-74	0	12 (12.6%)	10 (7.1%)	22 (6.0%)	96.2%
75-79	0	6 (6.3%)	4 (2.9%)	10 (2.7%)	98.9%
80-84	1 (0.8%)	0	3 (2.1%)	4 (1.1%)	100.0%
Total	131	96	140	366	

Table 8.2 (b) Breakdown by age at diagnosis and current treatment of female diabetics attending Dryburn out-patients in 1975.

Age at diag. (yrs.)	Current treatment			All females	Cumulative frequency
	Insulin	Tablets	Diet		
0-4	7 (5.8%)	0	0	7 (1.3%)	1.3%
5-9	13 (10.8%)	0	0	13 (2.4%)	3.7%
10-14	10 (8.3%)	0	0	10 (1.8%)	5.5%
15-19	7 (5.8%)	0	2 (1.0%)	9 (1.6%)	7.1%
20-24	10 (8.3%)	0	0	10 (1.8%)	9.0%
25-29	5 (4.2%)	1 (0.4%)	5 (2.5%)	11 (2.0%)	11.0%
30-34	5 (4.2%)	4 (1.8%)	3 (1.5%)	12 (2.2%)	13.2%
35-39	10 (8.3%)	5 (2.2%)	7 (3.5%)	22 (4.0%)	17.2%
40-44	7 (5.8%)	11 (4.9%)	8 (4.0%)	26 (4.8%)	21.9%
45-49	6 (5.0%)	21 (9.3%)	7 (3.5%)	34 (6.2%)	28.2%
50-54	15 (12.5%)	32 (14.2%)	20 (9.9%)	67 (12.2%)	40.4%
55-59	8 (6.7%)	31 (13.8%)	39 (19.3%)	78 (14.3%)	54.7%
60-64	7 (5.8%)	43 (19.1%)	38 (18.8%)	88 (16.1%)	70.7%
65-69	5 (4.2%)	33 (14.7%)	36 (17.8%)	74 (13.5%)	84.3%
70-74	3 (2.5%)	20 (8.9%)	17 (8.4%)	40 (7.3%)	91.6%
75-79	2 (1.7%)	20 (8.9%)	10 (5.0%)	32 (5.9%)	97.4%
80-84	0	3 (1.3%)	7 (3.5%)	10 (1.8%)	99.3%
85-89	0	1 (0.4%)	2 (1.0%)	3 (0.5%)	99.8%
90-94	0	0	1 (0.5%)	1 (0.2%)	100.0%
Total	120	225	202	547	

Figure 8.2 Breakdown by age at diagnosis and treatment of diabetics attending Dryburn in 1975.



(c) Duration of the disease.

The duration of the disease (measured up until 1.1.76) for those individuals whose date of diagnosis was known is given in tables 8.3(a) and 8.3 (b). The mean duration of diabetes in males was 7.9 years (standard deviation 7.3) with a range of 0.05 years to 47.0. The mean duration for females was 8.2 years (standard deviation 7.4), range 0.05 years to 40.0 years. The distribution of duration of disease is skewed to the right in both sexes with the majority having been diagnosed as diabetics for less than seven years. This is related to the phenomenon of 'frequency reduction' noted by Darlow et al (1973) to be enlarged upon later in this chapter.

The exceptionally long-established diabetics with diagnosis of their disease more than 40 years ago (one male,one female) are noteworthy for their lack of complications. They have both been maintained on insulin since diagnosis. The male patient,diagnosed at the age of 24 (in 1928) is a member of the family discussed as being a possible example of the M.O.D.Y. type of diabetes in chapter 5. His endogenous insulin secretion may well have been suppressed by the administration of insulin for over 40 years. The female in this group is one of the insulin-taking diabetics with the lowest value for the probability of becoming diabetic assessed on family history and it is tempting to cite this as supportive of the conclusions made about this matter in chapter 5.

The phenomenon of frequency reduction is illustrated in table 8.4. This shows a crosstabulation of current age (in decades) against decade of age at diagnosis. The percentage figures in brackets denote the proportion that number in any decade of age at diagnosis represents of the total in the current age decade.

Table 8.3 (a) Breakdown by duration of disease (on 1.1.76) and current treatment of male diabetics attending Dryburn out-patients in 1975.

Durn. (yrs)	Current treatment			All males	Cumulative frequency
	Insulin	Tablets	Diet		
0-4	38 (29.0%)	53 (55.8%)	78 (55.7%)	169 (46.2%)	46.2%
5-9	30 (22.9%)	24 (25.3%)	31 (22.1%)	85 (23.2%)	69.4%
10-14	18 (13.7%)	12 (12.6%)	24 (17.1%)	54 (14.8%)	84.2%
15-19	23 (17.6%)	3 (3.2%)	6 (4.3%)	32 (8.7%)	92.9%
20-24	7 (5.3%)	3 (3.2%)	1 (0.7%)	11 (3.0%)	95.9%
25-29	8 (6.1%)	0	0	8 (2.2%)	98.1%
30-34	6 (4.6%)	0	0	6 (1.6%)	99.7%
35-39	0	0	0	0	99.7%
40-45	0	0	0	0	99.7%
45-49	1 (0.8%)	0	0	1 (0.3%)	100.0%
Total	131	95	140	366	

Table 8.3 (b) Breakdown by duration of disease (on 1.1.76) and current treatment of female diabetics attending Dryburn out-patients in 1975.

Durn. (yrs)	Current treatment			All females	Cumulative frequency
	Insulin	Tablets	Diet		
0-4	31 (25.8%)	94 (41.8%)	114 (56.4%)	239 (43.7%)	43.7%
5-9	27 (22.5%)	58 (25.8%)	42 (20.8%)	127 (23.2%)	66.9%
10-14	20 (16.7%)	38 (16.9%)	29 (14.4%)	87 (15.9%)	82.8%
15-19	19 (15.8%)	18 (8.0%)	8 (4.0%)	45 (8.2%)	91.0%
20-24	9 (7.5%)	14 (6.2%)	5 (2.5%)	28 (5.1%)	96.2%
25-29	7 (5.8%)	3 (1.3%)	3 (1.5%)	13 (2.4%)	98.5%
30-34	3 (2.5%)	0	1 (0.5%)	4 (0.7%)	99.3%
35-39	3 (2.5%)	0	0	3 (0.5%)	99.8%
40-44	1 (0.8%)	0	0	1 (0.2%)	100.0%
Total	120	255	202	547	

Table 8.4 Distribution of diabetics attending Dryburn out-patients in 1975 according to decade of age and decade of age at diagnosis (age at diagnosis unknown for 22 individuals).

Decade of current age	Decade of age at diagnosis: 0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99	Total
	10 (100%)										10
10-19	17 (54.8)	14 (45.2)									31
20-29	10 (28.6)	13 (37.1)	12 (34.3)								35
30-39	2 (5.0)	9 (22.5)	18 (45.0)	11 (27.5)							40
40-49	1 (1.2)	4 (4.8)	11 (13.3)	28 (33.7)	39 (47.0)						83
50-59		1 (0.7)	7 (4.7)	23 (15.5)	48 (32.4)	69 (46.6)					148
60-69			1 (0.4)	7 (2.6)	23 (8.5)	110 (40.7)	129 (47.8)				270
70-79			1 (0.4)		9 (3.7)	39 (16.0)	109 (44.7)	86 (35.2)			244
80-89						2 (4.1)	14 (28.0)	18 (36.7)	15 (30.6)		49
90-99									2 (66.7)	1 (33.3)	3
	40	41	50	69	119	220	252	104	17	1	

For any decade of current age a greater number of patients were diagnosed within that decade than would be expected by chance and the frequency,in most cases,becomes reduced as one moves backwards in terms of decade of age at diagnosis.

As suggested by Darlow et al (1973) this could be the result higher detection rates for diabetes in the present decade compared with previous decades. Alternatively,diabetics could be moving out of the population once diagnosed. This is more of a possibility in this case than in the case of the Edinburgh population since the figures given in table 8.5 are for an outpatient population. Another possible explanation,and perhaps the most likely,is that frequency reduction is the result of heavy mortality amongst the diabetic population.

(e) Appointments.

Of the 936 confirmed diabetics attending Dryburn hospital in 1975,17 (nine males and eight females) were attending the paediatric clinic,the remainder attending the general medical and geriatric departments. In addition to these 919 adult diabetics these latter clinics saw 19 new referals who were found to have glycosuria by their general practitioners or at a routine medical examination but who were not confirmed as diabetic on investigation at the hospital.

These 938 adult patients had 3,259 booked appointments during the course of the year. It is difficult to estimate how many of them were actually kept since not all patients attending the clinics are recorded as doing so by the medical records department. There is a record of 2,228 definite attendances in 1975 so that the true number of attendances lies somewhere between these figures.

The distribution of the number of booked appointments per individual is shown, for the adults only, in table 8.6. The number of such appointments ranges from 16 for one individual to one, with a modal value of two, or one appointment every six months. The consultants' work-to-rule in December of that year make these figures somewhat atypical of the work-load of these clinics.

8.3 Newly-diagnosed diabetics in 1975 and 1976.

During the course of 1975, 120 individuals were refered to the various out-patient clinics at Dryburn with a presumptive diagnosis of diabetes. Of these, 101 were confirmed as diabetic after a glucose tolerance test, the remaining 19 were labeled as 'renal glycosuria' or 'low renal threshold'. In the following year 101 individuals were refered to the clinics, 96 being confirmed as diabetic and 15 regarded as having a normal glucose tolerance test with a 'low renal threshold'. It is noteworthy that the 197 confirmed diabetics refered in these two years show an even distribution of the sexes with 97 males and 100 females whereas, in contrast, the 'low renal threshold' group show a preponderance of males (26 males to 8 females). Comparison of the new diabetics and the 'low renal threshold' group reveals that this difference is significant with a chi squared value of 8.65 (d.f. = 1) and $p < 0.005$.

This interesting group with 'low renal threshold' would be worthy of a follow-up study in Durham to determine how many subsequently became confirmed as diabetic. Certainly many of the current diabetics in this population were confirmed as diabetic after a finding of glycosuria which was not their first.

Table 8.6 Number of booked appointments at Dryburn adult diabetic clinic during 1975.

No. appointments	No. of individuals
1	139
2	250
3	140
4	168
5	88
6	48
7	40
8	15
9	11
10	7
11	5
12	4
13	2
14	1
15	0
16	1
Total	919

The mean age of diabetics diagnosed in 1975 was 56.8 years, the youngest being 2.5 years old and the oldest 84.0. Of those diagnosed in 1976 the mean age was 54.3 years at diagnosis, range being 4.5 years to 86.1 years.

The distribution of the ages at diagnosis of these groups are shown in table 8.7.

8.4 Retrospective analysis of month of diagnosis.

The month of diagnosis of 800 diabetics attending at Dryburn is shown in table 8.8. In that these data include those diagnosed after the survey was initiated, the data are partly prospective but the majority of the information is retrospectively derived and suffers from the defects of such a study.

The onset of the disease in non-insulin-taking diabetics is randomly distributed throughout the year with 288 (49.4%) presenting in the winter half of the year and 295 (50.6%) in the summer half.

The 217 cases of insulin-dependent diabetes, irrespective of age at diagnosis, show a slight prediliction for diagnosis during the winter months (112 or 51.6% diagnosed between October and March), although this is not statistically significant.

Tables 8.9 and 8.10 show the months of diagnosis of insulin-dependent diabetes diagnosed before the age of 30 years (8.9) and after this age (8.10). The only striking feature is the peak for presentation of the under 30 group in February, a peak which is not seen in the insulin-taking patients with a later age of onset.

Table 8.7 Age at diagnosis of diabetics diagnosed in 1975 and 1976.

Age (yrs)	No.& frequency (%) diagnosed in 1975	1976	1975 & 1976
0-4	2 (2.0)	1 (1.0)	3 (1.5)
5-9	2 (2.0)	1 (1.0)	3 (1.5)
10-14	1 (1.0)	4 (4.2)	5 (2.5)
15-19	1 (1.0)	2 (2.1)	3 (1.5)
20-24	0	6 (6.3)	6 (3.0)
25-29	3 (3.0)	2 (2.1)	5 (2.5)
30-34	0	2 (2.1)	2 (1.0)
35-39	7 (6.9)	3 (3.1)	10 (5.1)
40-44	8 (7.9)	4 (4.2)	12 (6.1)
45-49	5 (5.0)	6 (6.3)	11 (5.6)
50-54	9 (8.9)	9 (9.4)	18 (9.1)
55-59	7 (6.9)	10 (10.4)	17 (8.6)
60-64	20 (19.8)	11 (15.5)	31 (15.7)
65-69	12 (11.9)	15 (15.6)	27 (13.7)
70-74	9 (8.9)	7 (7.3)	16 (8.1)
75-79	12 (11.9)	8 (8.3)	20 (10.1)
80-84	3 (3.0)	3 (3.1)	6 (3.0)
85-89	0	2 (2.1)	2 (1.0)
Totals	101	96	197

Table 8.8 Month of diagnosis of diabetics by current treatment.

Month of diagnosis	Number and frequency (%)			
	Ins.-taking	Non-ins.-taking		
		Tablets	Diet	Total
January	18 (8.3)	28 (10.6)	26 (8.2)	54 (9.3)
February	25 (11.5)	21 (7.9)	26 (8.2)	47 (8.1)
March	20 (9.2)	13 (4.9)	30 (9.4)	43 (7.4)
April	23 (10.6)	18 (6.8)	26 (8.2)	44 (7.5)
May	16 (7.4)	23 (8.7)	31 (9.7)	54 (9.3)
June	20 (9.2)	26 (9.8)	27 (8.5)	53 (9.1)
July	16 (7.4)	24 (9.1)	26 (8.2)	50 (8.6)
August	18 (8.3)	25 (9.4)	20 (6.3)	45 (7.7)
September	12 (5.5)	20 (7.5)	29 (9.1)	49 (8.4)
October	17 (7.8)	21 (7.9)	40 (12.6)	61 (10.5)
November	19 (8.8)	22 (8.3)	28 (8.8)	50 (8.6)
December	13 (6.0)	24 (9.1)	9 (2.8)	33 (5.7)
Totals	217	265	318	583
Winter	112 (51.6)	129 (48.7)	159 (50.0)	288 (49.4)
Summer	105 (48.4)	136 (51.3)	159 (50.0)	295 (50.6)

Table 8.9 Month of diagnosis of insulin-taking diabetics diagnosed before the age of 30.

Month of diagnosis	No. & frequency (%)
January	7 (6.4)
February	19 (17.4)
March	6 (5.5)
April	11 (10.1)
May	11 (10.1)
June	9 (8.3)
July	8 (7.3)
August	12 (11.0)
September	5 (4.6)
October	7 (6.4)
November	8 (7.3)
December	6 (5.5)
Total:	109
Winter	53 (48.6)
Summer	56 (51.4)

Table 8.10 Month of diagnosis of insulin-taking diabetics diagnosed after the age of 30.

Month of diagnosis	No. & frequency (%)
January	11 (10.2)
February	6 (5.5)
March	14 (13.0)
April	12 (11.1)
May	5 (4.6)
June	11 (10.2)
July	8 (7.4)
August	6 (5.5)
September	7 (6.5)
October	10 (9.3)
November	11 (10.2)
December	7 (6.5)
Total	108
Winter	59 (50.0)
Summer	59 (50.0)

The genetic polymorphisms studied were examined for patterns in the genotypes of individuals presenting as diabetics at various times of the year (in the three-monthly groups, January - March, April - June, July - September and October - December). No significant heterogeneity was detected. The data are not given here except for the HLA system (tables 8.11 and 8.12) for insulin-taking diabetics diagnosed before and after the age of 30. The distribution of HLA-B8 individuals agrees with the prospective study of Garavoy et al (1977) in that no significant clustering of HLA-B8 positive individuals is seen.

There is a suggestion of the clustering of Bw15 positive individuals during the second six months of the year (for the early-onset patients) and in the first three months of the year (for late-onset patients) but these are not significant. Cudworth et al (1977), in their prospective study of diabetes in Merseyside, found a significant clustering of Bw15 positive individuals in the first three months of 1976.

8.5 Assessing the prevalence in the general population.

For comparative purposes it was necessary to obtain an estimate for the prevalence of various types of diabetes in the general population living in the Durham area.

This was performed in three stages - firstly a detection survey for the enumeration of all clinically diagnosed diabetics in the area at a given time, secondly, the extraction of population

Table 8.11 Month of diagnosis and HLA phenotype in insulin-taking diabetics diagnosed before the age of 30.

	Month of diagnosis			
	Jan to March	April to June	July to September	October to December
HLA-B8+ve	10 (45.5%)	12 (48.0%)	10 (58.8%)	7 (43.7%)
HLA-B8-ve	12	13	7	9
HLA-Bw15+ve	4 (18.2%)	2 (8.0%)	5 (29.4%)	5 (31.3%)
HLA-Bw15-ve	18	23	12	11

Table 8.12 Month of diagnosis and HLA phenotype in insulin-taking diabetics diagnosed after the age of 30.

	Month of diagnosis			
	Jan to March	April to June	July to September	October to December
HLA-B8+ve	5 (45.5%)	4 (40.0%)	5 (83.3%)	7 (63.6%)
HLA-B8-ve	11	10	6	11
HLA-Bw15+ve	4 (66.7%)	6 (42.9%)	4 (36.4%)	4 (22.2%)
HLA-Bw15-ve	2	8	7	14

figures from the Census data and, thirdly, the calculation of age and sex specific prevalence rates for all diabetics and for diabetes subdivided by treatment.

(a) Determining the number of clinically diagnosed diabetics in the population.

A method for identifying these individuals was suggested by the local Family Practitioner Committee. All diabetics are entitled to claim exemption from prescription charges under category (b) of leaflet E.C.91 (Rev. 4/71) as sufferers from a chronic disease. The Durham Family Practitioner Committee was able to supply lists of the names, addresses, dates of birth and N.H.S. numbers of all such patients. These lists included, not only diabetics but others who can also claim exemption (such as those with myxoedema, hypothyroidism and Addison's disease) and many diabetics, either those who have not claimed exemption or those who are over 60 or over 65 and who are exempt anyway, are not included on the lists.

To correct for these unwanted inclusions and exclusions the lists were sent to the appropriate G.P. with a request to delete the names of patients who were not diabetic and to insert the names and relevant information for known diabetics who were not on the lists already.

As a pilot project, patients living in the area surrounding Durham city were ascertained. This area consists of the Durham Rural District and the parishes of Brandon and Byshottles and New Brancepeth. The parishes making up this area are shown in the map in figure 8.3.

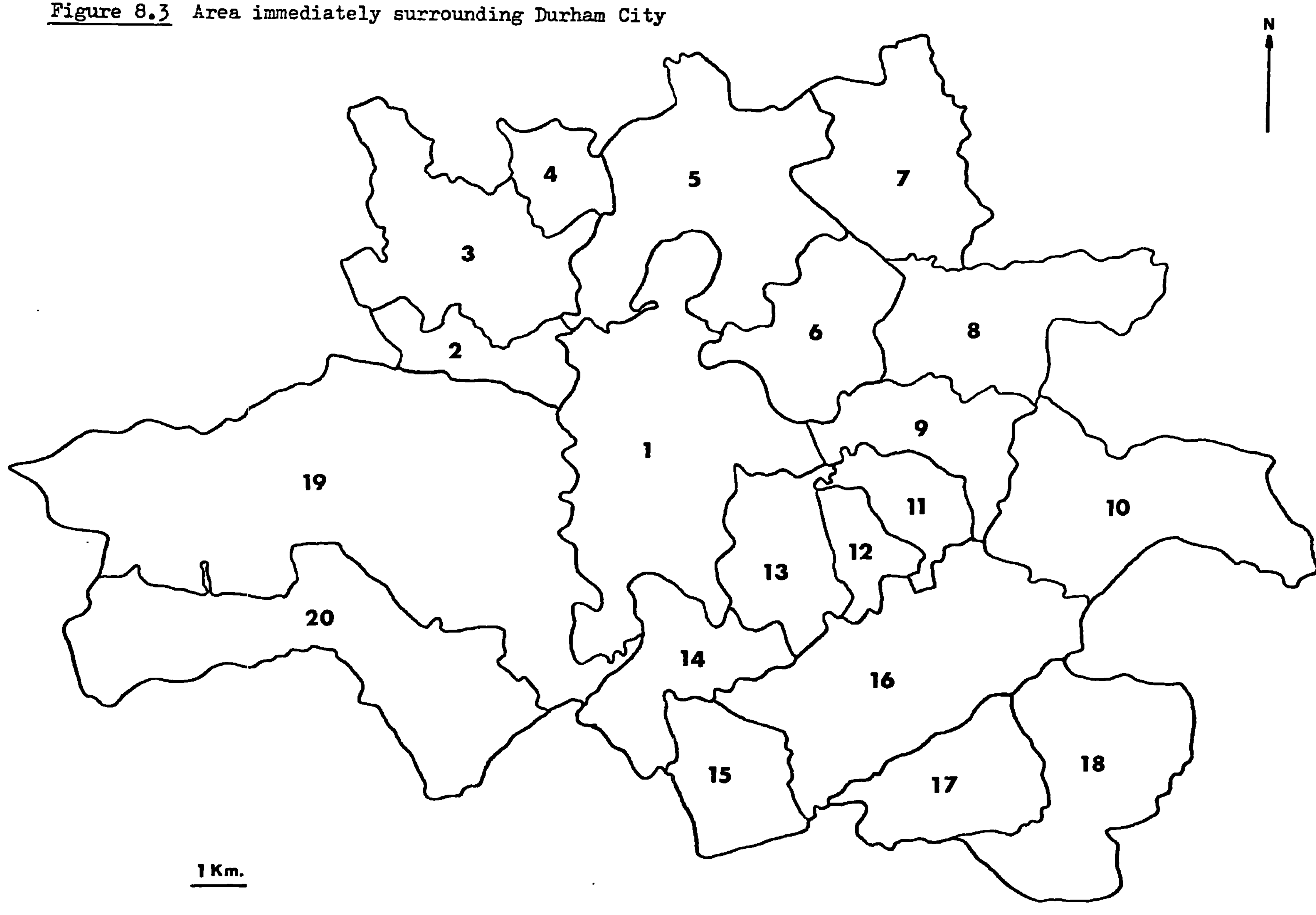
These patients were derived from nine general practices

Key to figure 8.3 Parishes comprising area under study.

Population (1971) - 82,915 Area - 194 sq kilometers

Code	Parish
1	Durham
2	Bearpark
3	Witton Gilbert
4	Kimblesworth
5	Framwellgate Moor
6	Belmont
7	West Rainton
8	Pittington
9	Sherburn
10	Shadforth
11	Sherburn House
12	Whitwell House
13	Shincliffe
14	Sunderland Bridge
15	Hett
16	Cassop-cum-Quarrington
17	Coxhoe
18	Kelloe
19	Brandon and Byshottles
20	Brancepeth

Figure 8.3 Area immediately surrounding Durham City



and returned lists were available for all but one of them. It was possible to confirm the diagnoses of most of the patients on the one outstanding list from the records of Dryburn Hospital but it was not,of course possible to add diabetics known to the practice but not on the list and not attending at Dryburn.

The original lists for these nine practices contained 278 individuals living in the designated area in 1975. Of these, 37 were removed by the practitioners as not being diabetic and 20 were added,making 261 known diabetics. Of these,145 (55.5%) had attended Dryburn at some time during 1975,the remaining 116 were either being reviewed by their general practitioner or being seen at another hospital. The records of the diabetic outpatient clinics at Dryburn revealed a further 376 diabetics resident in this area who had not been included on the original exemption lists and had not been added by the general practitioners showing that this method is unacceptable for the ascertainment of diabetics without the support of another method,since it only revealed information about 41% of the known diabetics in the area.

These diabetics,subdivided by current age and treatment are shown in table 8.13 (males) and 8.14 (females). Those with unknown treatment come from the general practitioners.

This method was extended to include the whole of County Durham which necessitated circulating 92 general practices out of which less than half returned the ammended lists. In consequence, the prevalence figures presented here are for the area involved in the pilot study only.

The response from General Practitioners was best from those particularly interested in the disease,especially if they

Table 8.13 Numbers of known diabetics in each treatment group (males).

Age	Treatment				Total
	Insulin	Tablets	Diet	Unknown	
0-9	1	0	0	0	1
10-19	8	0	0	0	8
20-29	10	1	0	3	14
30-39	7	1	3	8	19
40-49	11	9	8	2	30
50-59	17	10	21	4	52
60-69	9	23	30	12	74
70 +	8	26	29	1	64
Totals	71	70	91	30	262

Table 8.14 Numbers of known diabetics in each treatment group (females).

Age	Treatment				Total
	Insulin	Tablets	Diet	Unknown	
0-9	3	0	0	1	4
10-19	6	0	2	0	8
20-29	9	0	1	1	11
30-39	7	0	6	4	17
40-49	11	3	7	2	23
50-59	6	15	12	8	41
60-69	15	48	55	9	127
70 +	14	71	56	3	144
Totals	71	137	139	28	375

possessed a diagnostic index of their patients. From small single-handed practices with no administrative assistance the response was understandably poor with,at best,a list of patients derived from the doctors' memories. The numbers of diabetics included in tables 8.13 and 8.14 are underestimates of the total diabetic population and there is no way of assessing from the data the magnitude of the underestimation.

(b) Census statistics.

The Geography Department of the University of Durham has census data which are available to researchers who have obtained clearance from the O.P.C.S. These data are stored on the basis of 1 km grid squares so that,for ease of comparison,the statistics for diabetes were computerised in this form also. The area shown in figure 8.3 is made up of a series of 194 of these squares both for the general population figures and for the details of the places of residence of the diabetics. It was hoped to compare disease prevalence figures for 1975 with those for the partial census planned for 1976 but this census was abandoned at the last minute. Population projections on the basis of 1 km squares do not exist so that the disease figures for 1975 are compared with population figures for 1971. The estimated home population projection for the entire Northern area for 1976 (Registrar General's Statistical Review for 1971,part II,table A6) shows an increase over the 1971 estimate of home population of .09% (from 3,301,000 to 3,304,000) so that the error produced by this imperfect comparison is,hopefully,not large,but must be influencing the estimates of prevalence.

The figures for table 8.15 are built up from data for each

Table 8.15 1971 Census data for the general population.

Age	Sex		Both
	Males	Females	Sexes
0-9	6812	6494	13306
10-19	7039	5843	12882
20-29	7049	6077	13126
30-39	5349	4851	10100
40-49	5176	5129	10305
50-59	4553	4689	9242
60-69	3705	4413	8118
70 +	2244	3592	5836
Totals	41827	41088	82915

grid square and do not necessarily correspond exactly with the O.P.C.S. statistics for the whole area because of the 'adjustment procedures' employed in the interests of confidentiality.

(c) The prevalence of diabetes.

The prevalence of clinically diagnosed diabetes in this area is given, for males only, in table 8.16(a), for females only in table 8.16(b) and, for both sexes together, in table 8.16(c). Comparative data from the Edinburgh survey are also given.

The prevalence of diabetes of all types irrespective of age and sex in the Durham sample is 7.68 per thousand (±0.3) compared with 6.26 (±0.1) in Edinburgh in 1969. The difference is significant though there is no way of telling whether this is the result of differences in ascertainment, a difference in attack rate or detection rate in Durham in 1975 compared with Edinburgh in 1969, or a difference in the disease characteristics of an urban (Edinburgh) and semi-rural (Durham) population. The prevalence rates for all diabetics in the older age groups are considerably higher in the present sample. The Edinburgh workers admit the possibility of under-estimation in these groups, although this is also true of the Durham sample.

For the calculation of prevalence rates for treatment sub-groups. it was necessary to assign individuals of unknown treatment to particular sub-groups. This was done on the same basis as for affected relatives of unknown treatment (chapter 5). This is open to criticism since those attending general practitioners and not the local hospital might be expected to be predominantly non-insulin-taking.

Table 8.16(a) Prevalence (per thousand) in males of diabetes. Data from Durham area (1975) with comparative data from Edinburgh (1969).

Age	Durham				Edinburgh
	Insulin	Tablets	Diet	Total	(Total)
0-9	0.15	0	0	0.15	0.10
10-19	1.14	0	0	1.14	0.92
20-29	1.84	0.14	0	1.99	2.09
30-39	2.29	0.38	0.95	3.62	3.06
40-49	2.32	1.93	1.55	5.80	6.41
50-59	3.95	2.42	5.05	11.42	9.95
60-69	2.97	7.29	9.72	19.97	17.50
70 +	3.57	12.03	12.92	28.52	19.45
All ages	1.98	1.86	2.46	6.26	5.74

Table 8.16(b) Prevalence (per thousand) in females of diabetes. Data from Durham area (1975) with comparative data from Edinburgh.

Age	Durham				Edinburgh
	Insulin	Tablets	Diet	Total	(total)
0-9	0.62	0	0	0.62	0.16
10-19	1.03	0	0.34	1.37	0.94
20-29	1.65	0	0.16	1.81	1.95
30-39	1.85	0	1.65	3.50	2.59
40-49	2.34	0.58	1.56	4.48	4.34
50-59	1.49	4.05	3.20	8.74	8.17
60-69	3.63	11.78	13.37	28.78	18.80
70 +	3.90	20.32	15.87	40.09	20.75
All ages	1.90	3.58	3.65	9.13	6.72

Table 8.16(c) Prevalence (per thousand) of diabetes in both sexes considered together, Durham data and Edinburgh data.

Age	Durham			Edinburgh
	Insulin	Tablets & Diet*	Total	
0-9	0.37	0	0.37	0.13
10-19	1.09	0.15	1.24	0.93
20-29	1.75	0.15	1.90	2.02
30-39	2.08	1.48	3.56	2.82
40-49	2.33	2.81	5.14	5.31
50-59	2.81	7.25	10.06	8.98
60-69	3.33	21.43	24.76	18.30
70 +	3.77	31.87	35.64	19.82
All ages	1.95	5.73	7.68	6.26

* i.e. non-insulin-taking diabetes.

8.6 Summary.

This chapter, in many ways a by-product of the genetic studies carried out for this thesis, does not pretend to give a definitive account of the epidemiology of diabetes mellitus in the Durham area. There are too many methodological imperfections for it to merit that description. Rather, an attempt has been made to describe in detail a diabetic out-patient population in a, presumably, typical general hospital (as opposed to a teaching hospital where the selection of patients may be different), in the hope that it will serve as a base-line for the study of future trends in the constitution of the out-patient diabetic population of future years. From this changes in the sex-ratio of newly-diagnosed diabetics and of currently known diabetic patients may be studied, for example.

The study of the time of diagnosis of new diabetics is predominantly retrospective in nature and the results should be seen with this in mind. Despite this, it confirms the suggestions of previous authors that February is the commonest single month for the diagnosis of early-onset insulin-dependent diabetes.

There seems to be little information available on the number of diabetics in the non-insulin-taking category who are being treated with oral hypoglycaemic agents or diet alone. This study will help to fill that gap.

Chapter 9

Discussion

The opening chapters of this thesis are concerned with a review of previous findings on diabetes which are worthy of re-iteration before proceding to a fuller discussion of the results of the present investigation.

Segregation analysis,although widely employed in the study of diabetes has failed to demonstrate a convincing mode of inheritance when diabetes is considered as one disease. The reasons for this failure have been discussed at length,the most important being that such a consideration of diabetes as one disease is unjustified in the first place. When diabetes is subdivided such analysis has still failed to produce meaningful results except in the case of M.O.D.Y.,where the influence of environmental factors seems to be minimal in contrast to the important role of such non-genetic events in the precipitation of other forms of the disease in genetically susceptible individuals.

The study of genetic polymorphisms in established diabetics, summarised in chapter 2,has revealed reproducible associations only with the B8 allele of the HLA system and with the A allele of the ABO system although some workers have failed to confirm the latter. Many studies have found a positive association between insulin-dependent diabetes and the HLA-Bw15 allele and a negative association with HLA-B7 although others have failed to do so.

The general conclusions from the reports discussed in the first two chapters are that diabetes may be divided into two,or possibly three categories - type I (roughly equivalent to insulin-dependent diabetes),type II (roughly equivalent to insulin-independent diabetes) and a possible type III or M.O.D.Y. variety. The aetiologies of these sub-types have been shown to be different and it is tacitly,

and sometimes explicitly,stated that the genetic predisposition to each is independent. The evidence discussed in the preliminary sections of chapter 3 by no means convincingly demonstrates this for type I and type II diabetes,however.

The hypothesis expounded in chapter 3 incorporates the notion that genetic factors are involved in the evolution of the cardiovascular changes seen in the established diabetic. Two predictions of this hypothesis are tested by the data - firstly,that insulin-taking diabetics should occur in the families of non-insulin-taking diabetics (and vice versa) more often than expected by chance and that established diabetics should show the more rapid onset of cardiovascular complications if they have a strong family history of the disease,irrespective of the type of diabetes their affected relatives have.

Chapter 5 deals with the analysis of family data and this suggests,but does not categorically demonstrate,that both of these predictions may be true. The interview-questionnaire method employed for the examination of families of diabetics and controls is a poor one,it is time-consuming and its potential inaccuracies are discussed in detail. Current information on first degree relatives may be reliable,however,and the analysis of these individuals does suggest that insulin-dependent diabetes occurs in the relatives of insulin-independent diabetics more often than in the families of controls and more often than in the general population and that the converse is also true viz. that insulin-dependent propositi have more insulin-independent relatives than expected. This suggests that the two forms of diabetes have familial factors in common (although some of these may be non-genetic). Extrapolation of this to type I

and type II diabetes is unwise in the present state of knowledge since the correspondence between the two modes of classification may not be absolute. The study of diabetes subdivided according to the recently advocated immunological criteria is advocated.

Examination of existing and previous cardiovascular complications in insulin-taking diabetics suggests a relationship between the strength of family history of the disease and the development of these complications,at least in older individuals. A prospective study is required to evaluate the predictive value of the family history of a new diabetic at presentation with regard to the cardio-vascular pathology he may be expected to develop during the course of his disease.

Detailed examination of the current family history of early-onset (defined as under 30 years of age at diagnosis) non-insulin-taking diabetics confirms previous findings that patterns of dominant inheritance are more common in such individuals than in diabetes of other types.

The analysis of polymorphism data from non-diabetics focuses on two main aspects - comparisons with previously published samples of 'normal' individuals and the analysis of the present sample for heterogeneity,especially regarding age (chapter 6).

Data derived from the same region are only available for the ABO system (Kopeć,1970),rhesus (D) (Kopeć,1970) and the HLA system (Murray et al,1976). The quantitation of regional variation in this country for most polymorphisms is sparse in the extreme. In most systems,the phenotype and gene frequencies seem to accord well with those found in the reports considered but where they do not accord, it is not clear whether the differences are the result of geographic

clines,of sampling difficulties or of technical errors.

Heterogeneity with regard to age is certain to be demonstrated by chance alone in a certain number of systems when several are considered simultaneously. Of those considered here, the most convincing demonstration of a trend with age is that found in the MNSs system where the heterozygote Ss seems at an advantage over the homozygote s in terms of longevity. This finding becomes convincing when it is seen in the independent analysis of the diabetic group (chapter 7). Both male and female controls show an increase in the frequency of the rhesus -ve phenotype with age. This is not seen in the diabetic group so that it may be a chance finding or,alternatively that the selective forces acting on this locus are relevant in non-diabetics but not in diabetics. In the HLA system,heterozygosity at the B locus seems a genetic marker for longevity and combined heterozygosity at several loci may be associated with survival,although a much larger population study is required to confirm this.

Comparison of controls and diabetic subjects,with matching for age where necessary (chapter 7) reveals no convincing differences in phenotype frequencies except in the cases of the ABO and HLA systems. In the former,a significantly greater number of older male diabetics are of the phenotype A_1 compared with male controls. Examination of the data suggests that this is primarily the result of a deficiency in older male non-diabetics of this blood group rather than a pure excess of group A_1 diabetics. That this difference is the result of differential mortality in non-diabetics (with regard to the ABO system) which is not operational in diabetics is advocated as an explanation of this phenomenon.

The association with the HLA system confirms the results

of those authors who found that both HLA-B8 and HLA-Bw15 are positively associated with insulin-dependent diabetes and that HLA-B7 shows a negative association with this disease. When age at diagnosis is considered,however,HLA-B8 positive individuals and those possessing both the B8 and the Bw15 antigens develop their disease earlier than Bw15 positive subjects who are not also B8 positive. This heterogeneity is likely to have produced the disparity in published reports where the composition of the diabetic groups,with regard to age at diagnosis,has differed from author to author.

Suggested associations with other systems put forward by previous workers are considered to be the result of lack of attention to age effects in the control population since strict matching for age (in the rhesus system,for example) shows that these associations are not reproducible.

The penultimate chapter of the thesis is not intended to be a definitive account of the epidemiology of diabetes in the Durham area,rather it contains a detailed account of the structure of the diabetic out-patient clinic at Dryburn Hospital with details of the months of diagnosis over two successive years of new diabetics and a retrospective analysis of the months of diagnosis of existing diabetics at the clinic. The estimate of the prevalence of clinically diagnosed diabetes in the surrounding area has been undertaken because no such estimate considering different categories of diabetes subdivided by treatment is available,and this was required for comparison with the family data. As an estimate of prevalence it suffers from a number of deficiencies.

Examination of the clinic population reveals that over half of those currently being treated with insulin were diagnosed

after the age of 30. Early-onset insulin-taking diabetics were diagnosed in February more often than in any other single month. No convincing relationship between genotype and month of onset can be demonstrated in the retrospective analysis of this diabetic population. The prevalence estimates of the disease are higher than those presented by previous workers for Edinburgh in 1969 (see chapter 8 for references). No conclusions about the disease in the community and changes in prevalence may be made from this comparison.

Acknowledgements.

Assistance from the following individuals and organisations is gratefully acknowledged:-

At the Department of Anthropology, University of Durham: Dr.R.A. Cartwright and Professor E.Sunderland for advice and constructive criticism. Mr.M.A.Carr and Miss L.Bailey for their technical expertise.

At Dryburn Hospital, Durham: Dr.R.Mowbray and Dr.T.A.Grimson (Consultant Physicians), Dr.J.Yell (Consultant Geriatrician), Dr. J.M.Stansfeld (Consultant Paediatrician), Mr.A.J.M.Birnie, Mr.B.B. Porter and Mr.J.J.Williams (Consultant Orthopaedic Surgeons). Mr.J.Mulhall (Medical Records).

At the Department of Health, County Hall, Durham: Dr.M.O'Brien.

At the R.V.I.Hospital, Newcastle upon Tyne: Dr.J.Anderson.

At Chester-le-Street General Hospital: Mrs.A.Layfield.

At Bishop Auckland General Hospital: Dr.G.Ismay and Dr.F.Robertson.

At the Department of Geography, University of Durham: Dr.M. Visvalingam.

1971 Census of Population Small Area Statistics for kilometer squares supplied by the Office of Population Census and Surveys.

Financial Support: Addison Wheeler Research Fund, University of Durham; Peel Medical Research Trust; The Catherine and Lady Grace James Foundation.

For gifts of antisera: Newcastle Blood Transfusion Service, Miss L. Kennedy (Guy's Hospital), Dr.C.C.Entwhistle (National Tissue Typing Reference Laboratory, Bristol), Dr.D.Tills (British Museum of Natural History), Blood Group Reference Laboratory (Gatliff Road).

For computer programmes: Dr.C.Smith and Miss S.Holloway (Edinburgh), Professor E.G.Knox (Birmingham).

For typing assistance: Mrs.K.Dunn and Mrs.H.A.Coppen.

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Appendix A

Tables giving previously published blood group data for the calculation of x (relative incidence) and giving y ($\log_e x$), w (weight) and wy^2, necessary for the calculation of X (weighted mean relative incidence) and chi squared values for the significance of the deviation of X from unity and for the significance of heterogeneity amongst the data (Woolf's method).

Appendix A. Table 1. ABO investigations - summary of results (all diabetics together)

Reference	Diabetics				Controls				A/O
	O	A	B	AB	O	A	B	AB	
(1) Craig & Wang (1955)	411	296	80	30	1) 3853	2,485	817	263	1·12
(2) McConnell, et al (1956)	1) 280	265	61	28	1) 3146	2,648	546	170	1·12
	2) 86	98	11	4	2) 546	521	135	45	1·19
	3) 213	233	31	23	3) 2888	2,839	557	208	1·11
(3) Zeytinoglu (1956)	167	201	48	14	9,686	11,303	1,951	850	1·03
(4) Tedeschi & Carazzutti (1958)	169	193	55	19	831	742	165	61	1·28
(5) Speiser (1958)	79	103	43	14	367	430	130	73	1·11
(6) Otto-Servais et al (1958)	443	438	103	16	9,508	9,189	1,672	609	1·02
(7) Mähr (1959)	443	576	202	79	1,003*	1,270*	393*	201*	1·03
(8) Anderson and Lauritzen (1960)	463	401	94	34	20,342+*	21,276+*	5,209+*	2,309+*	0·83+*
(9) Cornil & Pirart (1961)	218	272	52	22	136,500*	130,500*	24,000*	9,000*	1·30

* calculated from frequencies + combined control series

Appendix A. Table 1. (continued)

Reference	Diabetics				Controls				
	O	A	B	AB	O	A	B	AB	A/O
(10) Bibawi and Khatra (1961)	279	408	132	132	3,275*	3,566*	2,451*	753	1·34
(11) Doll et al (1961)	39	45	12	6	272	262	50	16	1·20
(12) Simpson, et al (1962)	49	41	9	2	17,611+	15,125+	4,053+	1,436+	0·97
(13) Sauer, et al (1963)	641	768	210	90	5,111+	5,691+	1,622+	662+	1·08
(14) Macafee (1964)	446	307	91	21	5,522*	4,192*	1,229*	384*	0·91
(15) Serra et al (1964)	351	434	89	26	444	409	95	52	1·34
(16) Buckwalter (1964)	556	634	164	35	22,392+	21,144+	4,695+	1,748+	1·02
(17) Berg et al (1967)	69	91	13	3	1,199	1,521	259	110	1·04
(18) Scholz et al (1975)	406*	473*	118*	35*	951*	1,027*	254*	117*	1·08

* calculated from frequencies + combined control series

Appendix A. Table 2. (all diabetics combined)

Ref.	x	y	w	wy^2
1.	1·12	0·11333	154·8	1·98
2.(1)	1·12	0·11333	124·4	1·60
(2)	1·19	0·17395	39·1	1·18
(3)	1·11	0·10436	103·3	1·13
3.	1·03	0·02956	89·6	0·08
4.	1·28	0.24686	73·3	4·53
5.	1·11	0·10436	36·5	0·40
6.	1·02	0·01980	210·3	0·08
7.	1·03	0·02956	173·1	0·15
8.	0·83	-0·18633	210·5	7·31
9.	1·30	0·26236	120·8	8·31
10.	1·34	0·29267	151·0	12·93
11.	1·20	0·18232	18·1	0·6
12.	0·97	-0·03046	22·3	0·02
13.	1·08	0·07696	309·3	1·83
14.	0·91	-0·09431	168·9	1·50
15.	1·34	0·29267	101·5	8·69
16.	1·02	0·01980	313·1	0·12
17.	1·04	0·03922	37·1	0·06
18.	1·08	0·07696	151·5	0·90

References are as those for Table 1.

Appendix A. Table 3. ABO investigations - summary of results (male diabetics only).

Reference	Diabetics				Controls				A/O
	O	A	B	AB	O	A	B	AB	
(1) Craig & Wang (1955)	151	92	23	9	3,853	2,485	817	263	0·95
(2) McConnell, et al (1956)	(1) 91	101	19	10	3,146	2,648	546	170	1·32
	(2) 30	46	5	1	546	521	135	45	1·61
	(3) 74	91	10	6	2,888	2,839	557	208	1·25
(3) Tedeschi and Cavazzutti (1958)	79	87	23	12	831	742	165	61	1·23
(4) Speiser (1958)	46	61	16	3	367	430	130	73	1·13
(5) Otto-Servais et al (1958)	159	168	34	6	9,508	9,189	1,672	609	1·09
(6) Mähr (1959)	175	224	76	25	1,003*	1,270*	393*	201*	1·01
(7) Anderson and Lauritzen (1960)	246	203	44	16	+ 20,342*	+ 21,276*	+ 5,209*	+ 2,309*	0·79
(8) Cornil & Pirart (1961)	77	105	24	3	136,500*	130.500*	24,000*	9,000*	1·43

* calculated from frequencies + combined control series

Appendix A. Table 3. (continued)

Reference	Diabetics				Controls				A/O
	O	A	B	AB	O	A	B	AB	
(9) Bibawi & Khatwa (1961)	81	160	40	42	3,275*	3,566*	2,451*	753*	1·81
(10) Doll, et al (1961)	14	21	3	2	272	262	60	16	1·56
(11) Sauer, et al (1963)	304	370	91	43	+ 5,111	+ 5,691	+ 1,622	+ 662	1·09
(12) Serra, et al (1964)	129	190	41	12	444	409	95	52	1·60
(13) Buckwalter (1964)	265	261	72	14	+ 22,392	+ 21,144	+ 4,695	+ 1,748	1·04
(14) Scholz et al (1975)	172*	202*	51*	16*	951*	1,027*	254*	117*	1·09

* calculated from frequencies + combined control series

Appendix A. Table 4. ABO investigations - summary of results. (female diabetics only)

Reference	Diabetics				Controls				A/O
	O	A	B	AB	O	A	B	AB	
(1) Craig & Wang (1955)	260	203	57	21	3,853	2,485	817	263	1·21
(2) McConnell, et al (1956)	189	164	42	18	3,146	2,648	546	170	1·03
	56	52	6	3	546	521	135	45	0·97
	139	142	21	17	2,888	2,839	557	208	1·04
(3) Tedeschi and Cavazzutti (1958)	90	106	32	7	831	742	165	61	1·32
(4) Speiser (1958)	33	42	27	11	367	430	130	73	1·09
(5) Otto-Servais et al (1958)	284	270	69	10	9,508	9,189	1,672	609	0·98
(6) Mähr (1959)	268	352	126	54	1,003*	1,270*	393*	201*	1·04
(7) Anderson and Lauritzen (1960)	217	198	50	18	+ 20,342*	+ 21,276*	+ 5,209*	+ 2,309*	0·87

* calculated from frequencies   + combined control series

Appendix A. Table 4. (continued)

Reference	Diabetics				Controls				A/O
	O	A	B	AB	O	A	B	AB	
(8) Cornil & Pirart (1961)	141	167	28	19	136,500*	130,500*	24,000*	9,000*	1·24
(9) Bibawi & Khatwa (1961)	198	248	92	90	3,275*	3,566*	2,451*	753*	1·15
(10) Doll, et al (1961)	25	24	9	4	272	262	60	16	1·00
(11) Sauer, et al (1963)	337	398	119	47	5,111+	5,691+	1,622+	662+	1·06
(12) Serra, et al (1964)	222	244	48	14	444	409	95	52	1·19
(13) Buckwalter (1964)	391	373	92	21	22,392+	21,144+	4,695+	1,748+	1·01
(14) Scholz et al (1975)	234*	271*	67*	19*	951*	1,027*	254*	117*	1·07

* calculated from frequencies   + combined control series

Appendix A. Table 5. (male diabetics only)

Ref.	x	y	w	wy^2
1.	0·95	-0·05129	55·08	0·29
2.(1)	1·32	0·27763	46·33	3·57
(2)	1·61	0·47623	17·00	3·86
(3)	1·25	0·22314	39·68	1·98
3.	1·23	0·20701	37·45	1·60
4.	1·13	0·12222	23·16	0·35
5.	1·09	0·08618	80·28	0·60
6.	1·01	0·00995	83·59	0·01
7.	0·79	-0·23572	110·04	0·06
8.	1·43	0·35767	44·39	5·68
9.	1·81	0·59333	52·13	18·35
10.	1·56	0·44469	7·90	1·56
11.	1·09	0·08618	157·14	1·17
12.	1·60	0·47000	56·46	12·47
13.	1·04	0·03922	129·92	0·20
14.	1·09	0·08618	78·19	0·58

References are as those for Tables 3 and 4.

Appendix A. Table 6. (female diabetics only)

Ref	x	y	w	wy^2
1.	1·21	0·19062	106·00	3·85
2.(1)	1·03	0·02956	82·75	0·07
(2)	0·97	-0·03046	24·49	0·02
(3)	1·04	0·03922	66·96	0·10
3.	1·32	0·27763	43·30	3·34
4.	1·09	0·08618	16·90	0·13
5.	0·98	-0·02020	134·43	0·05
6.	1·04	0·3922	119·66	0·18
7.	0·87	-0·013926	102·51	0·02
8.	1·24	0·21511	76·36	3·53
9.	1·15	0·13976	103·43	2·02
10.	1·00	0·0000	11·22	0·00
11.	1·06	0·05827	170·90	0·58
12.	1·19	0·17395	75·19	2·28
13.	1·01	0·00995	187·60	0·02
14.	1·07	0·06766	100·11	0·46

References are as those for Tables 3 and 4.

Appendix A. Table 7. (considering $^B/_O$ ratio in all diabetics)

Ref	x ($^B/_O$)	y	w	wy^2
1.	0•92	-0•08338	60•91	0•42
2.(1)	1•25	0•22314	45•22	2•25
(2)	0•52	-0•65393	8•95	3•83
(3)	0•75	-0•28768	25•58	2•12
3.	1•43	0•35767	36•45	4•66
4.	1•64	0•49470	31•88	7•8
5.	1•54	0•43178	21•58	4•02
6.	1•32	0•27763	78•93	6•08
7.	1•16	0•14842	93•03	2•05
8.	0•79	-0•23572	76•69	4•26
9.	0•55	-0•59784	41•90	14•98
10.	0•63	-0•46204	84•22	17•98
11.	1•39	0•32930	7•73	0•84
12.	0•79	-0•23572	7•59	0•42
13.	1•03	0•02956	140•17	0•12
14.	0•92	-0•08338	70•29	0•49
15.	1•19	0•17395	37•23	1•13
16.	1•19	0•17395	126•91	3•84
17.	0•87	-0•13926	10•40	0•20
18.	1•09	0•08618	62•79	•47

References are as those for Table 1.

Appendix A. Table 8. (considering A/not A ratio in all diabetics)

Ref	x $(\frac{A}{not\ A})$	y	w	wy^2
1.	1·13	0·12222	169·41	2·53
2.(1)	1·05	0·04879	140·45	0·33
(2)	1·35	0·30010	42·73	3·85
(3)	1·12	0·11333	115·43	1·48
3.	0·97	-0·03046	105·15	0·10
4.	1·13	0·12222	86·28	1·29
5.	1·00	0·0000	47·3	0·00
6.	1·00	0·0000	234·96	0·00
7.	1·00	0·0000	220·71	0·00
8.	0·89	-0·11653	234·27	3·18
9.	1·21	0·19062	140·56	5·11
10.	1·37	0·31481	211·54	20·96
11.	1·05	0·04879	21·53	0·05
12.	1·04	0·03922	24·29	0·95
13.	1·05	0·04879	373·64	0·89
14.	0·94	-0·06188	184·23	0·71
15.	1·35	0·30010	116·45	10·49
16.	1·01	0·00995	353·51	0·03
17.	1·10	0·09531	41·58	0·38
18.	1·09	0·08618	177·52	1·32

References are as those for Table 1.

Appendix A. Table 9. (considering $(A+AB)/(B+O)$ ratio in all diabetics)

Ref.	x $\frac{A+AB}{B+O}$	y	w	wy^2
1.	1·13	.12222	175·99	2·63
2.(1)	1·13	·12222	143·45	2·14
(2)	1·27	·23902	42·83	2·45
(3)	1·19	·17395	115·97	3·51
3.	0·96	-0·04082	105·59	0·18
4.	1·17	0·15700	87·49	2·16
5.	0·95	-0·05129	48·21	0·13
6.	0·95	-0·05129	236·65	0·62
7.	0·96	-0·04082	223·55	0·37
8.	0·85	-0·16252	239·48	6·33
9.	1·25	0·22314	140·48	6·99
10.	1·74	0·055389	213·17	65·40
11.	1·19	0·17395	21·82	0·66
12.	0·97	-0·03046	24·63	0·02
13.	1·07	0·06766	377·86	1·73
14.	0·90	-0·10536	189·48	2·10
15.	1·22	0·19885	118·05	4·67
16.	0·97	-0·03046	357·81	0·33
17.	1·02	0·01980	41·44	0·02
18.	1·02	0·01980	179·18	0·07

References are as those for Table 1.

Appendix B

Giving details of the transformation of pedigree data into a form acceptable for computer analysis.

The following method was devised to store in computer files the relevant information collected for pedigree analysis so that the original pedigree could be reconstructed from the computer input and the required analysis could be performed.

A standard computer punched card has 80 columns in each of which a single character may be punched. These 80 columns were used in the following way:-

Columns 1 - 4

A four digit numerical label unique to each family, right justified within its field.

Column 5

The number of the card within the sequence of cards used for each family,using the digits 1 to 9 and then repeating.

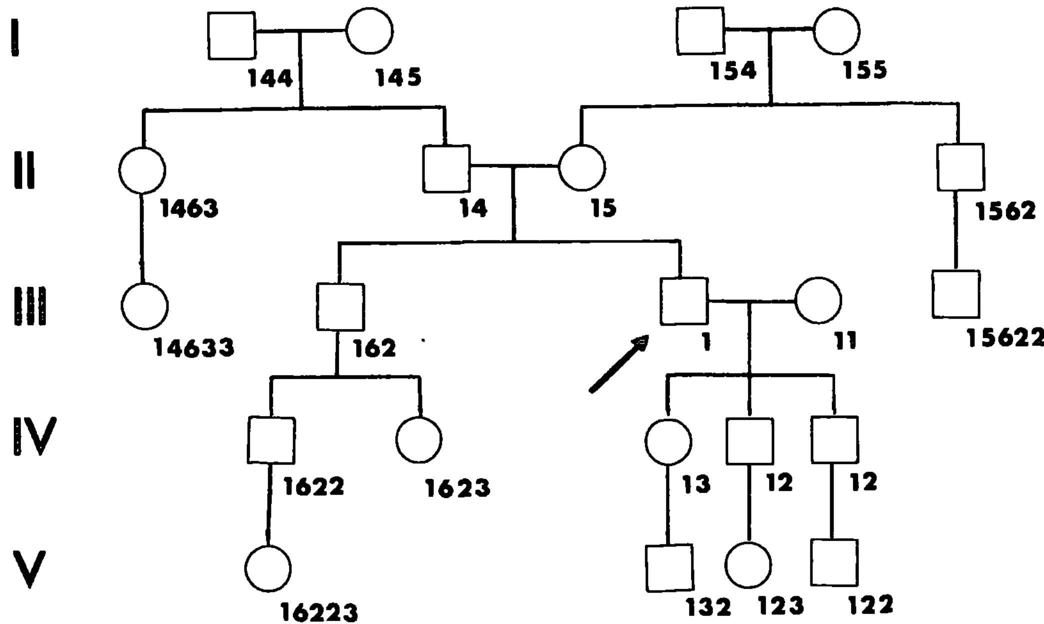
Columns 6 - 80

The required information on five members of the family per card using 15 columns for each person. The following items of information were coded in these 15 columns:-

In the first seven columns for each person (i.e. columns nos. 6 to 12 for the first person on each card) a numerical label encapsulating the sex of the individual,the relationship with the propositus and the sex of the propositus.

The codes for relatives of a male propositus are illustrated in figure B.1. They are built up logically from the

Figure B.1 The use of codes in an illustrative family



propositus,who is coded as '1'. Sons of the propositus have a code of '12',daughters '13',father '14' and mother '15'. A grandson, related to the propositus via a son is coded as '122',a granddaughter related in a similar fashion has a code of '123',whilst grandsons and granddaughters related to the propositus through a daughter are coded as '132' and '133' respectively. Paternal grandmothers and grandfathers are coded as '145' and '144',maternal grandmothers and grandfathers as '155' and '154'. The code for wives is '11'.

A complication occurs with sibs because of the necessity to distinguish full sibs from half-sibs. The full brother of a male propositus is coded as '162',the full sister of a male propositus is '163'. A half-brother,related to a male propositus through their father is '142',when related through the mother,'152'. Half-sisters,related through father and mother respectively are coded '143' and '153'. By extension of this method,the full brothers of the father of a male propositus are all coded '1462'. The daughters of such individuals (first cousins of the propositus) are coded '14623' and so on.

A female proband is coded as '2' and all her relatives are coded in exactly the same way as those of male propositi except that the code commences with a '2' in each case. The seven columns devoted to this coding will allow consideration of relatives up to and including the sixth degree.

Thus,although each relative in a pedigree may not have a unique code,every path relationship from the propositus is designated in a unique fashion. The number of steps from the propositus to an individual is equal to one less than the number of digits in the appropriate code for that relative and,with the

exception of propositi,all male relatives have an even code,all females an odd coding. These fixed rules greatly facilitate the writing of computer programmes for the analysis of such data.

The next column (column no. 13 for the first person on each card) is used to signify whether the relative is alive or dead, using the codes '1' and '2' respectively.

The next two columns (nos. 14 and 15 for the first person on each card) give the current age or ages at death. The codes 1 to 20 are given for successive five-year age groupings up to 100 years.

Birth order appears in the next two columns (nos. 16 and 17 for the first person on each card).

The last three columns (nos. 18 to 20 for the first person on each card) are devoted to details of the affection status of the individual. The first of these three columns uses the following codes for one of nine possible categories:-

Code	Category
1	Diabetic treated with insulin,diagnosis before the age of 30.
2	Diabetic treated with oral-hypoglycaemic agents,diagnosis after the age of 30.
3	Diabetic treated with diet alone,diagnosis after the age of 30.
4	Unaffected.
5	Diabetic treated with diet alone,diagnosis before the age of 30.
6	Diabetic,treatment unknown,diagnosis before the age of 30.

Code	Category
7	Diabetic, treatment unknown, diagnosis after the age of 30.
8	Diabetic treated with insulin, diagnosis after the age of 30.
9	Possible diabetic or 'latent diabetic!.

The last of these categories covers a wide range of individuals from thos diagnosed as diabetic during pregnancy,and now declared not to be diabetic,to those the informant thought might have had the disease as part of a terminal illness,for example. In practice this category was of little use and the small number of individuals in it were ignired in all calculations.

The second and third of these last three columns contain the age (the appropriate code for the five-year age group) at which diabetes was diagnosed in an affected individual.

A missing value for current age or age at death was not tolerated,the individual was ignored. The reasoning behind this was that if the informant were unsure of a person's age to within five years then they were unlikely to be sufficiently in contact with him to know about his medical status. Similarly for the 'alive or dead' category.

The missing value for birth order was '99' and also for the age at diagnosis of diabetes in an affected individual. The same code was also used for 'does not apply' for age at diagnosis in an unaffected individual.

When a pedigree was being coded,each individual is

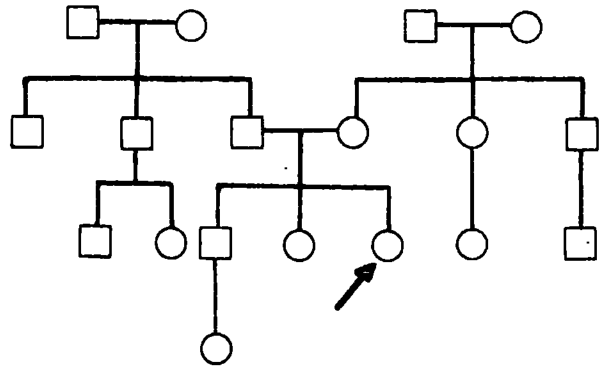
taken in turn with children following their parents (except in the case of the propositus and his parents). Successively numbered cards are used until each relative has been dealt with and a blank series of columns is left after the last relative of each family.

Information on the families of the 101 control propositi was stored in a line file of 783 lines. The 433 families of diabetic propositi were contained in a file of 2,466 lines. Files of this size allowed for the storage of information on 3,621 individuals in the case of control families and 14,244 individuals in the case of the families of diabetics. The latter file was sequentially structured so that,for example,the first 745 lines contained the families of propositi treated with insulin and diagnosed before the age of 30. Lines 746 to 1219 contained the families of insulin-taking diabetic propositi diagnosed after this age,so that,reading the first 1,219 lines of the file enabled the relatives of all insulin-treated diabetics to be analysed.

Fortran programmes,written specifically for the purpose, were used to analyse these data.

Appendix C

Relatives included in the calculation of probability of becoming diabetic (taken from Smith,1972).



Appendix D.

Electrophoresis methods.

Haptoglobins and acid phosphatase were examined in standard thickness starch gels. The remaining systems (phosphoglucomutase, adenylate kinase,adenosine deaminase,esterase D) were conducted in 1mm thick gels. This appendix lists the constituents of tank and gel buffers for each system,the staining procedures,conditions of the run and any special precedures required.

Haptoglobin.

Tank buffer:	0.06M	NaOH	2.4 g/l
	0.3M	Boric acid	18.55 g/l
	Adjusted to pH 8.5		
Gel buffer:	0.076M	Tris	9.2 g/l
	0.005M	Citric acid	1.05 g/l
	Adjusted to pH 8.65		

The gels are prepared by the addition of 150 mls of gel buffer to 100 mls of distilled water and 25 grms of starch per gel. The mixture is heated and pressure boiled. Inserts are filter paper impregnated with 4% lysate mixed 1:3 with serum. Electrophoresis run carried out cathode to anode,constant current 50 mA.. Duration of run about 3 hrs. Staining mixture consists of 250 mls of 1% acetic acid mixed with a small amount of benzidine with 3-5 drops of hydrogen peroxide. This mixture is poured on each gel and the bands appear rapidly.

Acid phosphatase.

Tank buffer:	0.41 M	Citric acid	86.16	g/l
		NaOH	45	g/l

Adjusted to pH 6.0

Gel buffer:	0.0025 M	Succinic acid	0.2952 g/l
	0.0046 M	Tris.	0.5572 g/l

Adjusted to pH 6.0

For each gel required, 250 mls of gel buffer (to which have been added 0.931 grms of E.D.T.A.) are mixed with 25 grms of starch. After heating 1 ml of 2-mercaptoethanol is added and the mixture is pressure boiled. Filter paper inserts are used and the run is conducted cathode to anode at constant voltage of 115 volts, current about 40mA. 16 or 17 hour run.

Incubation buffer: 0.05m Citric acid 10.50 g/l

Adjusted to pH 6.0

0.005M Phenolphthalein diphosphate (0.2944 grms/100 ml)

After slicing the gel 25 mls of the above incubation buffer are added to filter paper placed over each half. The gels are incubated for 3 hrs at 37°C. After incubation 4-5 mls of ammonia soln. are added per gel. Bands appear immediately.

Phosphoglucomutase.

Tank buffer:	Distilled water	1 litre
	Tris	12.11 grms
	Maleic acid	11.62 grms
	E.D.T.A.(acid)	2.92 grms
	$MgCl_2$	2.03 grms
	Adjust to pH 7.4 with 40% NaOH	
Gel buffer:	Tank buffer diluted 1:5	

Electrophoresis is carried out cathode to anode at constant voltage (110v) and at a current of 5-8mA. for about 17 hours.

For this and other thin layer methods, 40 mls of the gel

buffer are mixed with 3.5 grms of 10% starch, for each gel required.

Incubation buffer:	Tris	7.28 grms
	Distilled water	1 litre

Adjusted to pH 8.0 with conc. HCl, after which 20 mgs of $MgCl_2$ are added per 10 ml of buffer. The following ingredients are added to the incubation buffer and the resultant staining mixture placed in the oven at 37°C:-

for each 10 mls of incubation buffer	
Glucose 1 phosphate	30 mg
(containing G 1:6 diphosphate)	
NADP	1.5 mg
MTT	2.5 mg
PMS	2.5 mg
G 6 PDH	20 micro litre
Agar (2% soln.)	10 mls

The incubation buffer containing the first five ingredients in the above list is mixed into the agar after boiling and cooling to about 55-60°C. The mixture is applied to the gels by an overlay method and, after incubation at 37°C for 30 minutes the bands should appear.

<u>Adenylate kinase</u>.

Tank buffer:	Citric acid	28.72 grms
	NaOH	10.6866 grms
	Distilled water	1 litre

Adjusted to pH 4.9.

Gel buffer	Succinic acid	1.89 grms
	Tris	2.226 grms
	Distilled water	1 litre

Adjusted to pH 5.0

Electrophoresis run is carried out with specimens running from anode to cathode, constant voltage (250v) for 4 hrs.

Staining is again by the agar overlay method. The following

ingredients are mixed in 10 mls of the incubation buffer (see below) and the resultant solution is mixed into 10 mls of 2% agar which has been boiled and cooled to 55-60°C as for phosphoglucomutase above.

Glucose	18 mg
$Mg\ Cl_2$	40 mg
A.D.P.	4.9 mg
N.A.D.P.	3.1 mg
M.T.T.	2.5 mg
P.M.S.	2.5 mg
G6PDH	20 microlitre
Hexokinase	20 microlitre

Incubation buffer:	Tris	12.12 grms
	Distilled water	1 litre

Adjusted to pH 8.0 using conc HCl.

Bands appear after 30 minutes incubation at 37°C.

Adenosine deaminase.

Tank buffer:

Soln. A	NaH_2PO_4	46.802 grms
	Distilled water	3 litres
Soln B	Anhydrous $Na_2H\ PO_4$	19.874 grms
	Distilled water	1.4 litres

Add solution A to solution B and adjust pH to 6.5.

Gel buffer: Tank buffer diluted 1 in 10.

The electrophoresis is carried out cathode to anode at constant voltage (loo v) for 4 hours. Staining is by agar overlay method with following incubation buffer and staining mixture:-

Incubation buffer:

Solution A	NaH_2PO_4	0.195 grms

Distilled water		50 mls
Solution B	Anhydrous Na_2HPO_4	0.7098 grms
	Distilled water	200 mls

Solution A is added to solution B and the pH adjusted to 7.5. The solution is diluted 1 in 4 with distilled water.

Staining mixture:

2% agar solution in water	10 mls
Incubation buffer	10 mls
Adenosine	15 mg
M.T.T.	2 mg
P.M.S.	2 mg
Xanthine oxidase	10 microlitre
Nucleoside phosphorylase	10 microlitre

The agar solution is prepared as for the above two methods and incubation is for 30 minutes at 37°C. Clellands reagent is added to specimens before insertion into gels (see below).

Esterase D.

Gel buffer:	Tris	1.636 grms
	Citric acid	0.756 grms
	Boric acid	0.272 grms
	Lithium hydroxide	0.016 grms
	Distilled water	1 litre

Adjusted to pH 7.4 (stored at 4°C). For 10 gels 240 ml gel buffer are mixed with 240 mls water. 42 grms of starch are mixed with this solution for 10 gels.

Tank buffer:	Boric acid	108.84 grms
	Lithium hydroxide	6.72 grms
	Distilled water	4 litres

Adjusted to pH 7.2 and stored at 4°C.

Before insertion into the gel lysates are diluted with one drop of Clelland's reagent (see below) solution in the gel buffer.

Electrophoresis carried out cathode to anode for 2 hrs at constant voltage (300 volts), about 5 mA.

Incubation buffer: 41 mg. anhydrous sodium acetate (Soln.A)
10 mls distilled water

4 mg. 4-methylumbelliferyl acetate (Soln.B)
a few drops of acetone

Prior to incubation, solutions A and B are mixed and the gels incubated under filter paper strips impregnated with this mixture for 5 minutes at 37°C. Results are read under u.v. light.

Clelland's reagent, for use in adenosine deaminase and esterase D methods.

Clelland's reagent (dithiothreitol) is diluted with the appropriate gel buffer for that system by adding 37.5 mg to 5 mls of gel buffer. This may be stored at 4°C. Addition of this solution to the lysate before insertion into the gel is appropriate for specimens stored for any length of time and reduces the confusing storage bands seen with these specimens.

All methods using thin layer gels have specimens inserted by means of linen thread wicks.

Ficoll® Triosil® solution (for use in the separation of lymphocytes for HLA determination).

Soln. A 45 grms of Ficoll® disolved in 500 mls of distilled water. (This solutions requires over-night incubation to disolve).

Soln.B 100 ml Triosil® made up to 225 mls with distilled water, well mixed. (Protect from u.v. light).

Final solution is made up from 480 mls of soln.A and 200 mls of solution B.

Antisera

Antisera for red cell grouping and for HLA typing were derived from a variety of sources,commercial and private. All HLA antisera were commercially prepared (Biotest,Searle and Hoescht Pharmaceuticals) except for HLA-A9,B12 and Bw15 specificities which were kindly donated by other laboratories (see acknowledgements).

Red cell antisera were derived from the Newcastle Blood Transfusion Service (anti-A,anti-B,anti AB,anti-M,all rhesus antisera anti-K and some anti-Fy^a),from Lancaster Blood Transfusion Service (anti-P_1 and anti-Le (a)),Blood Group Reference Laboratory (anti-N,anti-M) and the British Museum of Natural History (anti-Fy^a and anti-Kp^a). Other antisera were commercially derived (Ortho and Biotest). Anti-human globulin was from Ortho.

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