The aetiology of juvenile-onset diabetes mellitus

Thesis submitted for the degree of Doctor of Medicine

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"The proper science and subject for man's contemplation is man himself."

Charron. Of Wisdom. Book 1, Ch. 1.

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Preface

My interest in the aetiology of juvenile-onset diabetes mellitus was stimulated by the sudden increase in the number of children who presented with diabetes in Sunderland during 1976.

During the ten year period from 1967 - 1976, the incidence, seasonal onset and clustering of diabetic children in the Sunderland area were analysed to detect possible environmental factors which could be associated with the onset of the disease. Other factors, such as the family history, human leucocyte antigens (H.L.A.) and the height of the diabetic child at the onset of the illness were studied in relationship to the aetiology of juvenile-onset diabetes mellitus.

Abstract

The aetiology of juvenile-onset diabetes mellitus

In Britain the incidence of juvenile-onset diabetes in children appears to be increasing. The incidence in Northamptonshire in 1949 was 1 : 7000 children, aged 0 - 15 years and a survey of diabetic children of similar age in Sunderland during a ten year period, 1967-76, revealed an incidence of 1 : 400.

The Sunderland survey comprised ninety-five diabetic children who were divided into three age groups 0 - 4 years, 5 - 9 years and 10 - 14 years. Overall more boys than girls develop diabetes, particularly in the younger 0 - 4 year group but girls increase in number and almost equal boys in the two older groups 5 - 9 years and 10 - 14 years.

The peak age of onset is eight and eleven years, consisting mainly of girls; however, boys predominate at twelve years of age. These peak age ranges related to sex could indicate that the underlying provoking factor in the aetiology of diabetes at these ages may be adrenarche or puberty.

Environmental factors also appear to play a role in the aetiology of juvenile-onset diabetes as more diabetic children present with their illness in the winter six months, October to March. This winter onset tendency was more significant in the older children (10 - 15 years), and spatial clustering of the disease in Sunderland children occurred, tending to confirm environmental influences such as infection in the community. Clustering of cases was also related to the highter social class areas of the town, consequently 80% of the diabetic children came from social groups I, II and III.

Only 7% of the diabetic children had a first degree insulin dependent diabetic relative compared to the national figure of 11%. A study of the extended family history revealed that almost half the Sunderland diabetic girls and one-third of the boys had a relative with insulin dependent diabetes. The diabetic child with the phenotypes H.L.A. - B8, BW15 and BW18 had a very

significant tendency to an extended family history.

An analysis of the H.L.A. phenotype showed that 75% of the diabetic children had H.L.A. - B8, BW15 or BW18 phenotype. 53% had H.L.A. - B8 compared to 24% controls. This B8 phenotype accounted for half the children in each of the three age groups. The majority of boys with H.L.A. - B8 presented in the winter six months, whilst girls with this phenotype presented evenly throughout the year. This could suggest that the provoking factor in the aetiology of diabetes in boys with H.L.A. - B8 could be an infection. The H.L.A. - B40 phenotype, not previously associated with a diabetogenic tendency, appears to fall into a similar category to boys with H.L.A. - B8.

The height of the diabetic children at the onset of the illness was compared to the height of local children and also to the national centile values. The mean height of the local children (25th centile) was found to be significantly below the national 50th centile. The Sunderland diabetic children were equally distributed about the national 50th centile value and consequently were significantly taller than the local children.

Although 80% of the diabetic children were above the local mean height, the diabetogenic phenotypes H.L.A. - B8, BW15 and BW18 were not associated with the very tall diabetic child. Most of the diabetic boys and girls above the 75th height centile had H.L.A. - B7 or B12 phenotypes. If H.L.A. - B8 was combined with either of these two phenotypes the children were shorter in stature.

It is possible that the provoking factor in the development of diabetes in boys with H.L.A. - B8 is an infection, whilst diabetes in girls with H.L.A. - B8, BW15 or BW18 appears to be related to puberty or adrenarche. Children with H.L.A. - B7 or B12 may be associated with a different endocrine imbalance producing excessive tallness prior to diabetes becoming evident.

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Review of the Literature

Chapter 1

THE INCIDENCE, SEASONAL ONSET, AGE AND SEX OF CHILDREN WITH DIABETES MELLITUS

One of the first studies done in the Western World on the incidence of diabetes in children was reported by Joslin (1946), who in 1935 - 36 was involved in a U.S.A. National Health Survey of 2,500,00 people. In the age range 0 to 14 years, one diabetic child was detected in 2,500 children.

In 1949, Henderson, Medical Officer to the Ministry of Education, arranged for a survey of diabetic children in England. With the assistance of school medical officers, and hospital paediatric departments in sixteen cities and fifteen county areas a total child population of 1,307,00 was sampled.

A total of 183 diabetic children were found in the age range 0 - 15 years, an incidence of 1 in 7,000. Most of the diabetic children (148) lived in the city urban areas (1 : 5000) whereas only 35 came from the rural areas (1 : 3000).

Henderson also reported that the incidence of diabetes increased as the children became older e.g. under the age 5 the incidence was 1 : 180,000, age 5 - 9, 1 : 8000 and 10 - 15 years, 1 : 3000.

No further surveys on the incidence of diabetes in children were done in Britain until a pilot study was carried out in London and Middlesex by Robertson in 1960, who reported his findings to the Banting Memorial Meeting of the British Diabetic Association. This study suggested a minimum incidence of one diabetic child per 3,300 school children aged 5 to 17.

In the same year, July, 1960, Nurse Beardsmore, a Health Visitor working with Reid, County Medical Officer for Northamptonshire surveyed a population of 45,000 school children (aged 5 to 15 years) and found fourteen

diabetic children, an incidence of 1 : 3250. Later in 1964 the same workers extended the age range of their survey from 2 years to 16 years, and from this increased children's population forty-three diabetic children were detected. Thirty-nine of the forty-three children were of school age (5 to 16 years), an incidence of 1 : 1,200. The results suggested that diabetes in Northamptonshire school children had more than doubled in four years.

The increase in the incidence of children's diabetes was also noted in Israel where Cohen (1971) reported an increase in successive cohorts of children born between 1947 - 51, 1952 - 56, and 1957 - 61. Cohen also noted a higher prevalence of diabetes among children of parents born in Europe and North America compared to children of parents born in Asia or Africa.

Further studies in Britain were done by Wandsworth and Jarrett working on the National Survey of Health and Development. This National Survey was started in 1946 as an investigation into social and economic aspects of 13,687 births occurring during March 3rd to 9th 1946. A sub-group of 5,362 children have been studied more closely, and in 1974 when the children had grown up and were 26 years old, sixteen young adults from the sub-group had developed diabetes, an incidence of 3 per 1,000. Ten of the sixteen patients (4.9 per 1,000) were from non-manual homes whilst six (1.8 per 1,000) were from a manual worker's background. Interestingly only one of the sixteen diabetics had developed their illness whilst at school.

A similar type of survey was carried out by the National Child Development Study Group (Calnan and Peckham 1977) which is concerned with the follow-up of 15,500 children born during one week in March 1958. By the time the children were 11 years old, eight had developed diabetes, and at 16 years of age, twenty-two were diabetics.

Table 1

Reported incidence rates of juvenile-onset diabetes mellitus

U.S.A.		
Joslin (1946)	0 - 14 years	1 : 2,500
U.K.		
Henderson (1949)	0 - 15 years	1 : 7,000
Robertson (1960)	5 - 17 years	1 : 3,300
Beardsmore & Reid (1960)	5 - 15 years	1 : 3,250
Beardsmore & Reid (1964)	5 - 15 years	1 : 1,200
Calnan & Peckham (1977)	0 - 16 years	1 : 700

In this study, the prevalence of diabetes was 0.2 per 1,000 by 7 years of age; 0.5 per 1,000 by 11 years of age; and 1.42 at 16 years.

In East Germany where diabetic screening is encouraged and diabetics are registered, Schliack (1974) reported the incidence in the age group 0 to 9 years at 0.2 per 1,000 at risk; 10 to 19 years, 0.8; and 20 to 29 years, 1.6. These figures are very similar to the results from the National Child Development Study, and are comparable to the National Survey of Health and Development.

In November, 1972, the British Diabetic Association sponsored the "Register of Newly Diagnosed Diabetic Children" and doctors were invited to report children up to the age of fifteen who had developed diabetes.

Results were published in 1975 by Bloom, Hayes and Gamble, who reported a minimum yearly incidence in Great Britain and Eire of 7.6 cases per 100,000 children age 0 - 15 years for the combined years of 1973 and 1974. Regional variations were striking, ranging from 7.5 cases per year per 100,000 children in North East England, to 5.6 in Greater London and 9.1 in South Western England, and 10.1 cases per 100,000 in South Eastern England. These variations in incidence between regions suggested the influence of environmental factors in the development of diabetes in children.

(ii) <u>Environmental factors and seasonal variation</u>

In 1969 Gamble and his colleagues found raised antibody titres to Coxsackie virus B4 in diabetics of recent onset and this gave impetus to the theory that "infection" may play a part in provoking diabetes. Later in 1973 Gamble and another group of workers reported on virology tests of 162 insulin dependent diabetics of recent onset, and in several found high titres to Coxsackie virus B1 - 5 compared to controls. They also noted more cases were diagnosed in the winter months than the summer, tending to confirm their impression that juvenile diabetes may be caused by a virus

infection(s) occurring in the colder months.

The Register of Newly Diagnosed Diabetic Children (1973) reported a difference in the number of diabetic children in 1973 (1216) compared to 1974 (1058). The decrease in 1974 was thought to be significant and was most noticeable in the South of England. It was thought unlikely that doctors had failed to notify new cases, and assuming the difference between 1973 and 1974 was genuine, it could be accounted for by environmental factors such as infection.

Gamble's finding of a seasonal variation in the onset of juvenile diabetes was confirmed by the "Register", more children presenting during the autumn, winter and spring months compared to the summer.

Interestingly, children presenting with diabetes under five years of age did not have a seasonal variation in onset.

Two years later Cudworth and his colleagues (1977) reported twice as many new diabetics in the autumn and winter months than the spring and summer. 110 Merseyside diabetics under 30 years were studied and their tissue typing was carried out. It was found that patients with the phenotype HLA - BW15 developed their diabetes more frequently during the winter and this group of patients also had high neutralising antibody titres to Coxsackie virus types B1 - 4.

These reports suggested that virus infections occurring during the winter months could provoke juvenile onset diabetes in certain young people.

However, in 1975, Dippe and his colleagues in U.S.A. reported an outbreak of Coxsackie virus B4 infection in two inhabited islands in the Bering Straits with a population of 350 and 110 respectively, all of Russian descent. Although the peak of the virus illness occurred in September 1969, which was confirmed by virology tests; five years later in 1973, none of the population had developed diabetes. It could be argued

that the numbers in the study were small and that Coxsackie virus B4 infection may not be the provoking factor in that particular racial group.

Further British support for an infective aetiology came from Birmingham where Rolles and Rayner (1975) reported the seasonal incidence of fifty-six diabetic children and found that 81% of the children who had the tissue type HLA - B8 developed their diabetes within a five month period, from October to the end of February.

(iii) Age incidence

Nancy Simpson (1962) studying diabetic families in Toronto found the commonest age range at onset to be between 10 to 14 years for both boys and girls.

The 1973 - 74 British Register showed that the incidence increased with age to a peak at 11 years for both sexes. After the age of 12, the incidence fell sharply. Simpson's results showed a similar dramatic fall after 14 years of age.

Cudworth and colleagues (1977) also confirmed a peak at 11, 12 and 13 years of age, with a decline in new cases in the mid and late teens.

(iv) Sex ratio

Simpson (1962) reported more boys among the 0 to 4 year olds, but more girls in the 5 to 9 year range, and equal numbers of boys and girls aged 10 to 14. Similar results were found in the British Register (1973/74) the only variation being more boys than girls in the older 10 to 14 year group. Cudworth and his colleagues (1977) found a slight preponderance of male patients (65 : 45), and the British Register (1973/74) recorded 1279 boys and 1145 girls.

SUMMARY

It would appear from the literature that the incidence of juvenile-onset diabetes is steadily increasing. However, in Britain, there appears to be a marked regional variation of the illness in children. This may be

due to social and/or environmental factors. In certain diabetic children with specific human leucocyte antigens, a seasonal onset is suggested. The peak age at onset from various studies is 11 and 12 years of age and more boys tend to develop diabetes than girls.

Chapter 2

Family History and Genetic Factors in Juvenile Diabetes

(i) Family History Studies

Many papers have been written about the genetic and hereditary predisposition to diabetes.

Apparently as early as the 7th century the family tendency to diabetes was noted in India (Joslin and Root).

In recent years workers have tried to throw further light on the hereditary tendency.

In 1955 White reported from the Joslin Clinic 16 diabetics out of a total of 175 offspring of young diabetic patients.

Following on from this study Nancy Simpson (University of Toronto) in 1962 made a detailed survey of the families of 233 juvenile diabetics who had developed their illness before they were 20 years old.

There were 466 parents, 566 siblings and 114 offspring in the study.

Of the 466 parents, 11 mothers and 7 fathers were found to be diabetics, all insulin dependent, an incidence of 7%. There was only one family where mother and father were both diabetics and only one of their four children had diabetes.

None of the 566 siblings had developed diabetes at the time of the survey.

Simpson also found that 102 of the 233 juvenile diabetics had become parents, having 114 offspring and only one child had developed diabetes. However, most of the offspring were still very young at the time of the study.

Dr. Simpson suggested that her findings indicated there was some genetic influence in the development of juvenile diabetes but the pattern did not fit the usual features of recessive, intermediate or dominant inheritance.

In 1968, Oakley, Pyke and Taylor found 13% of diabetic children gave a history of diabetes in a first degree relative, whilst the Register of Newly Diagnosed Diabetic Children 1973 - 1974, found that from 1400 young diabetics, 154 (11%) had a first degree relative.

Oakley also found that in a control group of non-diabetic children only 2% gave a positive family history in a first degree relative.

This difference would support the suggestion that diabetes does have a genetic factor but obviously not a strong one and that other factors such as environment and immunological factors seem to play a part.

(ii) Diabetes in Twins

Further studies on the genetic origin of diabetes was carried out by Tattersall & Pyke (Kings College, London) in 1972.

They analysed data from 96 pairs of identical (monozygotic) twins. 65 pairs were concordant (both diabetic) and 31 pairs were disconcordant (one twin diabetic).

In 75% of concordant twins the interval between diagnosis in the other twin was less than three years.

When diabetes developed in the index twin before the age of 40, half the pairs were disconcordant.

It is suggested by Tattersall and Pyke that the unaffected twin in the disconcordant pairs will probably remain non-diabetic, since half the pairs had been disconcordant for more than twelve years.

A family history was more common in concordant twins than in discon-cordant twins.

From this study these authors later suggested in an article in the Lancet in 1976 that diabetes is not a single entity, that inheritance is variable and in some cases especially when diagnosed in early life, there is good reason to suppose that non genetic factors are important.

Rosenthal and his colleagues in San Francisco (1976) argued against Tattersall and Pyke's evaluation of their study on diabetes in monozygotic twins.

Rosenthal felt that it takes longer to develop diabetes in the other twin if the diabetes developed in the index twin before they reached 40 years of age, but if the twins are followed long enough concordance in the younger diabetic eventually reaches 82%. They further stated that regardless of age of onset diabetes is a disease of genetic origin in most cases.

However, Cudworth and Woodrow (1976) disagreed with Rosenthal and his colleagues stating that the study of the Human Leucocyte Antigen system (HL-A) in diabetes has produced strong evidence that the major susceptibility to juvenile onset diabetes is determined by a gene(s). They quote their work on HL-A typing and twins in association with Wilson, Pyke and Batchelor, 1975, when they found an increased incidence of HLA-B8 and BW15 in diabetic identical twins with an onset before 40 years of age, and this suggested a genetic factor irrespective of concordance or disconcordance. Whether the second child became diabetic they suggested was purely a matter of a chance environmental effect.

They further argued that the genetic susceptibility to a maturity onset, non-insulin dependent diabetes is different as there was no evidence of an HLA linked gene, either in the diabetic population studies or in maturity onset diabetic identical twins.

<u>Summary</u>

The inheritance of diabetes is obviously complex. It would appear that in Britain, the percentage of diabetic children with a diabetic first degree relative is 11% - 13% compared to 2% in controls.

It is possible that the recently developed H.L.A. assessment of juvenile onset diabetics will assist in the better understanding of the inheritance of the illness.

Chapter 3

The Human Leucocyte Antigen System (H.L.A.)

(i) Introduction

The most sophisticated defence mechanism to find expression in vertebrate organisms is the immune response; that is, the capacity, after foreign macro molecular or allogenic cells are introduced, to produce specifically sensitized lymphocytes and to synthesize and secrete specific antibodies capable of reacting with these foreign substances (antigens) (Benaceraf and McDevitt 1972).

This immune response mechanism appears to be one of the important functions attributed to a major histocompatibility complex (M.H.C.) of cell surface antigens.

The first systematic studies on the antigens carried by cells which determine whether a graft will be rejected or not were performed on inbred strains of mice of known genetic make-up. It was found that just as in the red cell antigenic system, some antigens were very strong and others weak. The transplantation or histocompatibility antigens were found to be strong antigens and experiments suggested that these antigens in the mouse were controlled by the H - 2 genetic locus (Weir).

In man, these strong tissue antigens are found in most tissues throughout the body but are absent from the red blood cells which contain only the ABH antigens. Fortunately blood leucocytes carry all the known major histocompatibility (M.H.C.) antigens and it is possible to test white blood cells for the presence or absence of the M.H.C. antigens and therefore they were referred to in man as the human leucocyte antigens or the H.L.A. system.

Techniques to demonstrate the antibody to human lymphocytes have been developed. Walford, 1964, evolved the cytotoxic or complement dependent cell killing method and later in the same year Terasaki and McClelland developed the presently used microlymphocytotoxicity test which economised

Table 2

The nomenclature of the H.L.A. system

New Locus A	Previous first, LA locus	New Locus B	Previous second, 'four'
HLA-A1	HL-A1	HLA-B5	HL-A5
HLA-A2	HL-A2	HLA-B7	HL-A7
HLA-A3	HL-A3	HLA-B8	HL-A8
HLA-A9	HL-A9	HLA-B12	HL-A12
HLA-A10	HL-A10	HLA-B13	HLA-A13
HLA-A11	HL-A11	HLA-B14	W14
HLA-A28	W28	HLA-B18	W18
HLA-A29	W29	HLA-B27	W27
HLA-AW23	W23	HLA-BW15	W15
HLA-AW24	W24	HLA-BW16	W16
HLA-AW25	W25	HLA-BW17	W17
HLA-AW26	W26	HLA-BW21	W21
HLA-AW30	W30	HLA-BW22	W22
HLA-AW31	W31	HLA-BW35	W35
HLA-AW32	W32	HLA-BW37	TY
HLA-AW33	W19.6 Fe55	HLA-BW38	W16.1
HLA-AW34	Malay 2	HLA-BW39	W16.2
HLA-AW36	Mo*	HLA-BW40	W10
HLA-AW43	BK	HLA-BW41	Sabell
		HLA-BW42	MWA

New Locus C	Previous third	New Locus D	Previous LD, MLC
HLA-CW1	T1	HLA-DW1	LD 101
HLA-CW2	T2	HLA-DW2	LD 102
HLA-CW3	T3	HLA-DW3	LD 103
HLA-CW4	T4	HLA-DW4	LD 104
HLA-CW5	T5	HLA-DW5	LD 105
		HLA-DW6	LD 106

From D. Voak and A.H. Goldstone (1976)
H.L.A. Antigens and their significance
Hospital Update, Vol. 2 484

on the use of rare antisera and the numbers of lymphocytes needed for each test.

Following extensive research by many workers and international collaborative workshops begun in 1964 the present classification of the H.L.A. system has evolved.

Four major gene loci are now represented and are designated.

1. H.L.A. - A formerly HL - A
2. H.L.A. - B formerly HL - 4
3. H.L.A. - C formerly HL - AJ or Third
4. H.L.A. - D formerly M.L.C. or L.D.

These four gene loci are thought to be located on chromosome 6.

The present nomenclature of the H.L.A. system is shown in Table 2.

(ii) <u>Genetics of the H.L.A. System</u>

A. Experiments in animals

In 1968 Lilley demonstrated that there was an association between genetic linkage in the murine major transplantation antigen (H-2) complex and a relative resistance to virus induced leukaemogenesis.

This was followed in 1971 by reports of the H-2 linkage of genetic factors predisposing to murine autoimmune thyroiditis (Vladutiu and Rose) and to susceptibility of mice to acute lymphocytic - choriomeningitis virus infection (Oldstone et al 1973).

The work of Vladutiu and Rose in Buffalo, U.S.A., involved injections of mouse thyroid extract and inducing an autoimmune disease with measurable circulating thyroid autoantibodies and damage to the target organ by infiltration of the thyroid by mononuclear cells. They found a striking correlation between the response to thyroid antigen and H-2 type, e.g. the murine strain with the genetic make-up of H - 2^S and H - 2^K were consistently excellent responders, producing good quantities of thyroid antibodies and thyroid damage whilst other strains of mice H - 2^a,

H - 2^b, H - 2^d and H - 2^v gave a poor reaction.

This study on autoimmune thyroiditis is of particular interest because this is the first disease model in which an association has been established between a particular transplantation antigen type, a specific (antithyroglobulin) antibody response and the pathological severity of disease. The demonstration of all three factors go far in showing that histocompatibility linked specific immune response genes may play a part in disease pathogenesis.

The susceptibility of mice to acute lymphocytic-choriomeningitis virus infection (L.C.M.) was studied in 1973 by Oldstone, Dixon, Mitchell and McDevitt. These workers were interested in the natural host variation in the ability to resist infectious diseases. Various strains of mice were mated and the L.C.M. virus introduced intracranially and the mice were H - 2 typed, and virus absorption tests and histology was done. They found that different strains had different immune responses to this virus infection and linked the immune response to the genetic H - 2 linkage.

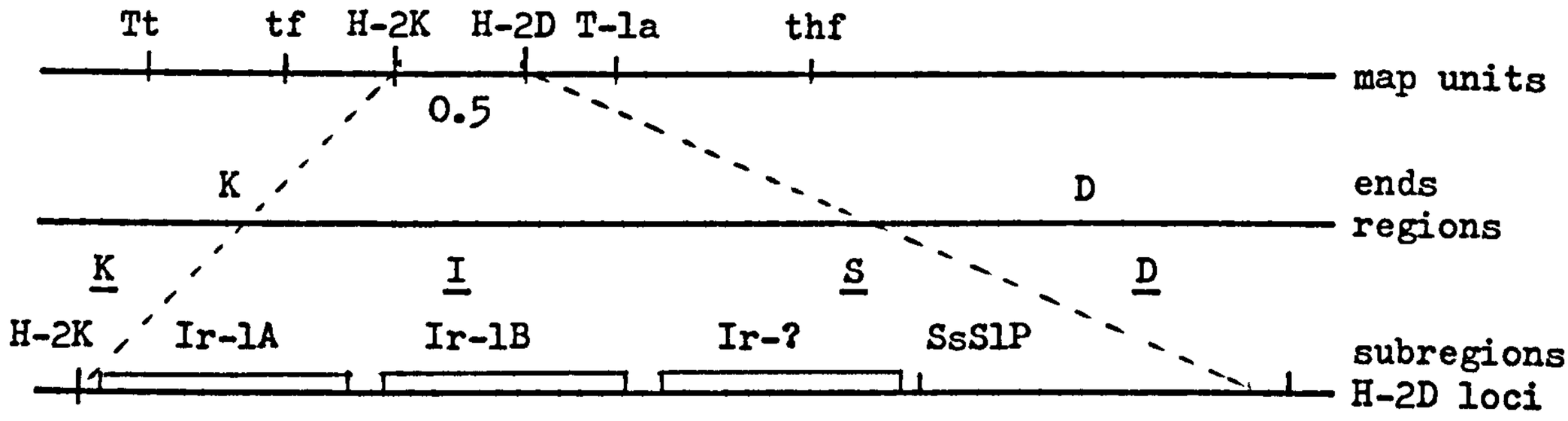
They suggested that their results:-

(a) ruled out the theory that M.H.C. antigens may represent specific receptor sites for attachment of L.C.M. virus since H - 2^q mice which were more susceptible to L.C.M. than H - 2^k mice, absorbs the same amount of L.C.M. virus in each case.

(b) They also refuted the theory that M.H.C. antigens and L.C.M. virus may share antigenic determinates, and fail to recognise the invading virus. This would suggest that the mouse host would then fail to respond immunologically to the virus. However, they found the H - 2^k resistant strain capable of mounting both humoral and cellular responses against L.C.M. infection.

Fig 1

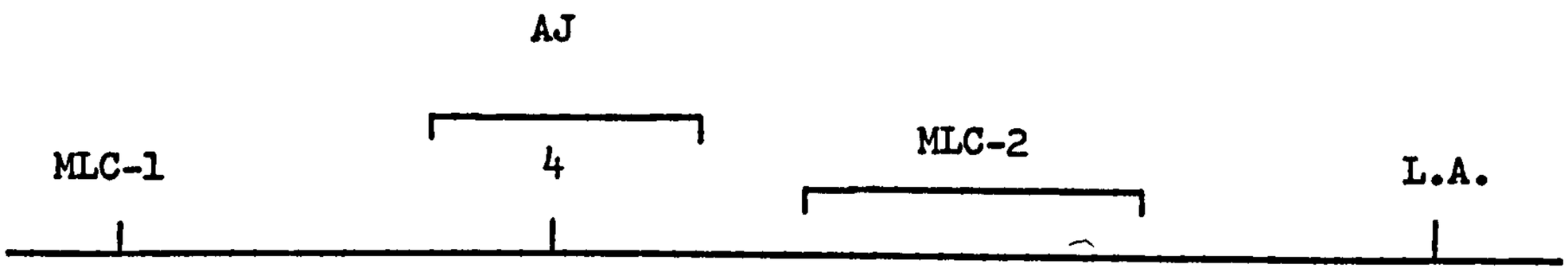
Schematic representation of the XVII mouse chromosome
from McDevitt and Bodmer (1974)



The first line is an approximate linkage map of the entire chromosome. The second line is a subdivision of the H-2 complex into its four major regions, K. I. S. D. The third line is a subdivision of the I region into its major subregions.

Fig 2

Schematic diagram of the
human major histocompatibility complex



The LA and four loci appear to be analagous to the D and K loci of H-2. AJ is a third serologically detected histocompatibility difference whose map position is not clearly established. The major MLC region is designated MLC-1 and lies at the outer limits of the human major histocompatibility complex. The position of human Ir genes have not yet been clearly mapped.

(c) It was thought that an immune response gene analagous to the Ir genes, controls the ability of the host to respond to L.C.M. virus antigens and this appears to be the answer in the work on H - 2 mice and L.C.M. infection.

B. Genetic mapping

The genetic mapping in mouse has been carried out in the past few years (Fig. 1). Most of the H - 2 linked specific immune response genes controlling immune responsiveness to a variety of polypeptide and protein antigens and iso antigens map in or near to the centre of the H - 2 complex lying between the H - 2^K locus (which is the gene for one of the two major serological transplantation antigens) and the SS - S1P locus, a marker near the centre of the H - 2 complex controlling serum α globulin. This region also controls a series of lymphocytic allo-antigens, now designated as Ia (Ir-associated) antigens, found primarily on B. lymphocytes.

Studies by Festenstein and Demant (1973) have shown that the major antigens eliciting the mixed lymphocytic culture (M.L.C.) reaction in vitro also map in the same region as the Ir genes. It is not yet clear whether M.L.C. genes, Ir genes and Ia antigens are different effects of the same genes or separate genes mapping in the same part of the major H - 2 complex.

At present, it seems that the M.L.C. reaction reflects the effect of Ia antigens and that both are separate from specific Ir genes. The situation in man is analagous, although not yet as precisely defined as in the mouse. The currently accepted version of the major histocompatibility complex is shown in Fig. 2.

No H.L.A. linked immune response genes have been mapped in man as yet. However, there is clear evidence that genes controlling antigens eliciting the M.L.C. reaction (H.L.A. - D) in vitro are clearly linked with the genes for the major serologically detected transplantation

antigens H.L.A. - A, H.L.A. - B and H.L.A. - C. The H.L.A. - D (M.L.C.) locus in man seems to be close to the H.L.A. - B locus. This point is of considerable interest because of the fact that most of the disease associations with the H.L.A. system that have been reported are with antigens of the H.L.A. - B locus. This is in striking analogy with the situation in the mouse when the Ir and M.L.C. group map close to the H - 2^K locus.

(iii) <u>H.L.A. and disease in man</u>

The search for associations between H.L.A. and specific diseases in man has been much more extensive than that in the mouse, but unfortunately because of the inability in man to carry out planned breeding experiments as can be done in mice, less clear cut results are obtained in the work done so far.

Most of the disease association found so far in man are with alleles of the B series. The suggested reason is that the B and D loci lie close together on chromosome 6 and hence these particular alleles are likely to be in strong linkage disequilibrium, i.e. given pairs of antigens of the B and D series occur more frequently in the population than would be the case if the alleles of the two loci had segregated independently at random (Leading article, Lancet 1975). Linkage disequilibrium is already recognised between alleles of the more widely separated A and B loci. In Whites, for example, the combination A1 and B8 are particularly common.

In addition, progress has been made in the serological identification of determinants of restricted tissue distribution (the Ia (immune-associated) series). These antigens appear to be more closely allied to the H.L.A. - D antigens which if they parallel experiments so far done im mice may eventually be shown to be more closely associated with disease in man than the H.L.A. - A and B series so far reported.

One of the first reports in man that the HLA system was

associated with disease was by Forbes and Morris (1970) who studied two groups of patients with Hodgkin's disease in Melbourne. Out of 35 patients in the first group, 56% were HL - W5 (now re-classified HLA - B5) and out of 75 in the second group, 45% were in the same HLA group compared to 25% of the normal Australian population. Later, following an international collaborative study of 450 Hodgkin's patients, HLA - A1, B5 and B18 were found to be commonly associated with this illness (McDevitt and Bodmer 1974).

However, probably the most important of the early studies was done by Levine and Stember (1972) who were the first to demonstrate the association in man between H.L.A. phenotype; a specific immune response and disease severity.

This work done in New York was on Ragweed hayfever which is an example of an allergic disease mediated by IgE antibodies (Reagins) to airborne allergens (pollen particles). Genetic studies on reagin (IgE) production in inbred strains of mice have demonstrated two kinds of genetic control of reagin production.

One genetic factor permits the production of high serum levels of reagins to many antigens. The other control system is by genes at a locus (or loci) closely linked to the H - 2 system; the Ir (immune response) genes. The Ir genes control immune responsiveness to synthetic polypeptide antigens and to minute doses of protein antigens. This system shows antibody specificity. In most mouse strains reagin production is a prominent part of the immune response. Ragweed hayfever in man shows many features similar to these immune response systems in the mouse.

Levine and Stember studied seven kindreds in which Ragweed hayfever occurred in more than one member. In one family, 67% of the members were HLA - B8 and they had an intense immediate wheal and flare reaction to

antigen E and developed severe hayfever. The rest of the family were HLA - A10 and B12 and did not have an immediate skin reaction or clinical illness.

This work suggested that the association with a specific H.L.A. haplotype was specific for antibodies to ragweed allergen, and not for reaginic antibodies to timothy-grass allergen and also that the genetic factor associated with H.L.A. affected the level of IgE antibodies to ragweed allergens in these patients.

Perhaps the closest association between histocompatibility genes and disease so far discovered in man is that between Ankylosing spondylitis and HLA - B27 where more than 90% of patients were found to carry the B27 allele compared to less than 8% controls (Brewerton et al 1973). However, it has been estimated that fewer than 5% of individuals with HLA - B27 will develop ankylosing spondylitis and even within affected families the disease does not show 100% linkage with the B27 antigen (Leading article, Lancet 1975).

Stokes and his colleagues (1972) reported the increased incidence of HL - A1 and HL - A8 in adult Coeliac disease. 78% of the patients studied had HL - A1 (now re-classified HLA - A1) compared to 33% controls, and 88% had HL - A8 (now HLA - B8) as against 29% controls. The frequency of the combination HL - A1 and HL - A8 in the coeliac patients was 75% compared to 20% in controls.

Dermatitis Herpetiformis is thought to have an association with coeliac disease and in a joint study published from Copenhagen and Newcastle upon Tyne, Janet Marks (1976) and her colleagues reported an increased incidence of HLA - DW3 and HLA - B8 in the Dermatitis Herpetiformis patients.

An interesting study was done by Thompson and colleague (1976) on the HLA antigens and atopic symptoms in children with steroid-responsive nephrotic syndrome. They found hayfever, positive prick test to grass

pollen antigens and a higher mean serum IgE antibody to be more common in nephrotic children who had HLA - B12 antigens. Whilst in the non-atopic patients there was an increased incidence of the haplotype HLA - B8. Their data suggested that in children who have both HLA - B12 and a history of atopy the risk of steroid-responsive nephrotic syndrome developing is thirteen times greater than in those with neither factor.

Another interesting study on adults with intrinsic asthma who did not have a history of atopy and negative prick skin test with 18 common allergens, - 80% of the intrinsic asthmatic patients were found to be HLA - W6. In contrast, a group of extrinsic asthmatics were found to have tissue antigens similar in distribution to the normal population.

It has also been established that multiple-sclerosis is associated with an increase in HLA - A3, HLA- B7 and HLA - DW2 (Jersild et al 1975).

(iv) <u>H.L.A. and juvenile-onset diabetes mellitus</u>

In October 1974, Nerup and his colleages reported a study on 146 diabetic patients in Denmark.

Eighty-five patients developed their diabetes before age forty (juvenile diabetes) and sixty-one had maturity-onset diabetes.

H.L.A. studies revealed an increase in HL - A8 (now HLA - B8) in juvenile diabetics and the W15 antigen (now HLA - BW15) was increased in both the juvenile and maturity-onset diabetes, but was not significantly increased in the maturity-onset group.

Nerup suggested that HL - A8 and W15 seem to be genetic markers for insulin dependent diabetics.

This work was followed by a Liverpool study, which Cudworth and Woodrow published one month after Nerup's article. One hundred diabetic patients were HLA typed, fifty patients with an onset before age 30 and fifty with an onset after that age. 54% of the juvenile group were

HL - A8 compared to 32% controls and W15 was found in 18% of juvenile onset patients compared to 12% controls. No such increase in HL - A8 and W15 was found in the fifty maturity-onset patients.

A further report by Cudworth and Woodrow (1975) on one hundred and fifty patients who developed diabetes before age 30 confirmed the above findings. Risk factors were suggested for the various H.L.A. antigens and diabetes mellitus. Those who had HL - A8 the relative risk was 2.17, and for W15 the risk was 2.35. If the patient had both HL - A8 and W15 antigens then the relative risk was 4.67.

Interestingly, a Japanese study by Wakisaka and colleagues (1976) from Sapporo where HLA - B8 is rare but HLA - BW15 is common, found 44% of the juvenile-onset group of thirty-two patients were HLA - BW22J compared to 13% controls. The HLA - B15 antigen was found to be present in only 6% of patients compared to 24% controls. This would suggest that if there is an HLA preponderance to diabetes mellitus it may vary from race to race.

(v) <u>H.L.A. phenotype and seasonal variation in juvenile-onset diabetes</u>

Gamble (1973) and his colleagues have been interested in the possibility that juvenile-onset diabetes mellitus (J.O.D.M.) may be provoked and precipitated by a virus infection. They tested 162 Insulin dependent diabetics for neutralising antibodies to Coxsackie virus and compared the results to a control group. They found an increase in B4 antibody especially in the 10 - 19 year old group.

If Coxsackie virus infection could be implicated in provoking diabetes in young people then the onset of symptoms would presumably follow the infection by some weeks or months. Gamble's group therefore plotted the month of onset of diabetes and found a definite increase in cases during the autumn and winter months and a low incidence from April to August.

In 1975, Rolles and his colleagues at Birmingham Children's Hospital analysed the months of onset of fifty-six Birmingham diabetic children who had also been HLA typed. They found that 46% of the diabetic children were HLA - B8 and of these 81% presented in the five months October to February. No seasonal variation was found in the children who did not have HLA - B8.

This work reinforced the suggestion that certain juvenile-onset diabetics were more likely to develop diabetes in the colder months, probably provoked by a Coxsackie virus infection.

Cudworth and his colleagues (1977) reported one hundred and ten new diabetics in the Merseyside area, under age 30, and found twice as many cases presenting in the autumn and winter months compared to the spring and summer. They found that HLA - BW15 patients clustered in a winter peak and also they were found to have an increased neutralising antibody titre to Coxsackie virus types B1 - 4.

It would therefore appear from the work reported to date that there is a seasonal incidence in juvenile-onset diabetes possibly related to the HLA phenotype and virus infection.

Chaper 4

The height of diabetic children at the onset of their illness

(i) The earliest report

In 1928 Harvey Spencer of Boston was one of the first physicians to report the height of children at the onset of their diabetes. He measured twenty-three diabetic children under 13 years of age at the onset of their illness who were attending the Infants and Childrens Hospital, Boston. In order to increase the number of children in his survey he added results from a study done by Allen and Sherriff, making a total of forty-five patients.

In order to compare his diabetic children, Spencer used standards in height and weight from Griffith's and Mitchell's Textbook of Diseases of Infants and Children. Thirty-six (80%) diabetic children were normal or above normal in height for their age and twenty-six of these (59%) were 1" or more above the normal average. Only nine children were below normal. In his report he did not differentiate between boys and girls but grouped them together.

It seemed apparent from this original work that very few diabetics were smaller than the average for their age at the onset of the illness.

(ii) Further studies in U.S.A.

In 1946 Jackson and Kelly, Iowa, studied eighty-six diabetic children, 92% of whom had developed their diabetes before their 13th birthday. Most of the children were third generation European-Americans and the researchers used the Iowa City childrens' growth charts to compare the heights of the children.

They found that thirty-six children (42%) deviated less than $\pm$ 1S.D. from the average height and almost a similar number, namely thirty-five (41%) were 1 S.D. or more below the average, whilst fifteen children

(17%) were 1 S.D. or more above the average in height for their age.

Unfortunately, as in Spencer's report, no sex difference was recorded, but at least the two studies did reveal conflicting results and gave an indication that further research was required.

(iii) Studies in Europe

Several European studies have been carried out but these have also varied in their results. Sterky (1967) in Stockholm examined sixty-six diabetic children as part of an assessment of the growth pattern in juvenile diabetes. Referring to the height of the children at the onset of their illness, he states "most diabetic boys have a slight increase in height in comparison with normals". No mention was made about the height of diabetic girls.

In Zurich, Bihrer (1970) did not find any difference in height between seventy-six diabetic and control children.

(iv) British studies

In Britain the interest in the height of diabetic children has only been apparent for the past few years. Helen Pond (1970), Kings College Hospital, London, studied the height of one hundred and one diabetic children all born before 1952. There were fifty boys and fifty-one girls. The onset of diabetes in these children was from 1 year of age to 14 years.

Pond used Tanner and Whitehouse growth charts, taking the percentile values and the space between the centile lines as "channels" and grouped the children irrespective of age into these channels according to their height.

The mean percentile value (M.P.V.) of boys of all ages at the onset of the illness was 57.1 with a standard error 3.5. These results suggested a shift to tallness in diabetic boys but was regarded as being borderline at the 5% level of significance. The diabetic girls followed a similar pattern but the shift in girls was highly significant ($P < 0.001$).

Table 3

Surveys of the height of diabetic children at the onset of their diabetes

Location of survey	Investigator	No. of diabetic children studied	Compared to controls the diabetic children are:-
U.S.A.			
Boston	Spencer (1928)	45	Taller
Iowa City	Jackson & Kelly (1946)	86	Shorter
EUROPE			
Stockholm	Sterky (1967)	66	"Boys slight increase in height"
Zurich	Bihrer (1970)	76	Same height
Holland	Drayer (1974)	62	Boys taller Girls shorter
U.K.			
London	Pond (1970)	101	Boys and girls taller
Glasgow	Craig (1970)	80	Boys and girls taller than local controls
Birmingham	Jivani & Raynor (1973)	104	Boys shorter Girls same height

In another group of seventy-four diabetic children born after 1952, Pond suggested that there was a definite significant increase in the height of boys at the onset of their diabetes. The increased height was more marked in boys than girls in the 5 - 9 age group, but there was a fall in girls age 10 - 14 years.

In the same year, 1970, Craig reported his study on eighty diabetic children attending the Hospital for Sick Children, Glasgow. These children presented with diabetes over a fifteen year period between 1954 to 1969.

Referring to the height of the children at the onset of their illness he said, "The boys at onset were on the average on the 50th centile and the girls on the 49th centile. Pond (1970) quoted the 69th centile for her diabetic children in London. This is in fact in keeping with the present figures as the average height of the Glasgow children falls about the 33rd centile on the Tanner - Whitehouse charts. (Craig 1963). This does stress the need for appropriate controls."

In 1973, Jivani and Rayner analysed the height of forty-eight diabetic boys and fifty-six girls in the Birmingham area. These authors used the Tanner - Whitehouse growth charts in order to compare the heights of their diabetic children, as they did not have standard measurements for Birmingham children.

Jivani and Rayner found the boys to be shorter than the normal boy at the onset of diabetes whilst the girls were near the 50th centile. The method of assessing the height was similar to that of Helen Pond, using the mean percentile values.

The results of these surveys are set out in Table 3.

(v) <u>The Latest Report</u>

The most recent published work on the height of diabetic children at the onset of their illness was done in 1974 by Drayer who measured the

height at the onset of diabetes in thirty-one boys and thirty-one girls, living in the three northern provinces of Holland.

The ages of the diabetic children ranged from 4 to 14 years. Their heights were recorded as standard deviational scores and these were compared with the heights of Dutch children taken from a National Survey.

The diabetic children and controls were divided into sexes and then compared at the 4 - 14 age group and also in the sub groups (a) 4 to 9 years, and (b) 9 to 14 years.

The diabetic boys as a whole (4 to 14 years) were found to be significantly taller than the Dutch boys ($P < 0.001$) but the diabetic girls were not taller than the control girls.

The sub groups showed that the diabetic boys aged 4 to 9 years were taller ($P < 0.01$) but the boys aged 9 to 14 years were not significantly taller than the control group.

The heights of the diabetic children were then compared and the boys (4 to 14 years) were found to be significantly taller than the girls ($P < 0.01$). However, the difference in the sub groups 4 to 9 years and 9 to 14 years was not significant.

Summary

The results of the height studies in Europe (including Britain) and U.S.A. are conflicting. However, five of the eight reports suggest that diabetic children, especially boys, are taller at the onset of their illness.

Part B

The results of the Sunderland study into the aetiology of juvenile-onset diabetes

Chapter 5

The incidence of juvenile-onset diabetes in the Sunderland area

(i) The geographical area and population of the study

This study into the aetiology of juvenile-onset insulin dependent diabetes mellitus was carried out in the Sunderland area, in the county of Tyne and Wear. The Sunderland Area Health Authority serves a population of 350,000. The catchment area includes the Sunderland County Borough, as well as parts of the Sunderland Metropolitan District; that is, Houghton-le-Spring, Hetton-le-Hole, Shiney Row and part of Washington. The catchment area also includes the Boldon and Whitburn areas of the South Tyneside Metropolitan District and the Seaham, Easington and Peterlee areas of County Durham.

The number of children under fifteen years in the Sunderland Metropolitan District is 76,300 (1971 census). Certain adjustments to this figure had to be made as the District boundaries do not coincide with the Sunderland hospitals catchment area.

It is known from Regional Health Authority figures that eighty per cent of the Washington population attend hospitals in Gateshead and Chester-le-Street; whilst the population of East Durham from towns such as Seaham, Murton, Easington and most of Peterlee attend hospitals in Sunderland. Hence the population of children under fifteen years of age for this diabetic survey comprised:-

Metropolitan District of Sunderland		76,300
Less 80% Washington children		10,000
		66,300
Plus children from Murton, Seaham and Peterlee		13,500
	Total	79,800

(ii) Selection of patients for survey

A ten year period was selected, from 1st January, 1967, to 31st December, 1976. Any child under the age of 15 years at the time of diagnosis of their diabetes mellitus, who presented at a Sunderland hospital was included in the survey. The records of diabetic children admitted to all the Sunderland hospitals during this ten year period were obtained. 95 diabetic children were traced and the numbers were confirmed by the Regional Health Authority Statistics Department. All but one of the diabetic children were diagnosed at the Children's Hospital, Sunderland. The exception was a 14 year old who was referred to a physician at the Royal Infirmary, Sunderland. Apart from this 14 year old, all the diabetic children were patients of myself and my colleague, Dr. John Heycock.

The individual hospital records were examined for the age and sex of child; date of onset of the illness; place of residence and the school attended and a note was made of any family history of diabetes mellitus.

From these facts, the age of onset, sex ratio, month of onset of diabetes and possible clustering of cases and family history was evaluated. The results are as follows:-

(iii) Incidence of juvenile-onset diabetes

The yearly incidence of juvenile-onset diabetes in the Sunderland area is recorded in table 4. The incidence during the first five years of the survey (1967 - 1971) varied between 3 to 6 cases per year, with a total of 23 diabetic children in the first five years.

However, during the second five years 1972 - 1976, there was a two to three fold increase in the yearly incidence during the first four years, but a sudden 'explosion' occurred of 23 cases in the fifth and final year.

Thus out of 95 children in the ten year survey, 23 developed

Table 5

Year of birth of diabetic children X Age at onset of illness (1967-76)

Age at onset	1953	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	Total	M	F	Incidence rate per 1,000
0													0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1												0	0	0	0	0	0	1	1	0	0	0	0		2	2	0	0.055
2											0	0	0	0	0	0	0	0	0	0	1	1			2	2	0	0.055
3										0	1	1	0	0	0	0	1	2	0	0	11	0			7	6	1	0.190
4									0	1	0	0	0	0	0	1	1	0	1	1					5	2	3	0.134
5								0	0	0	3	0	0	0	1	1	0	1	1						7	5	2	0.187
6							0	0	1	0	0	0	1	1	12	0	1	1							8	3	5	0.213
7						0	0	0	0	0	0	1	1	1	0	1	0								4	3	1	0.106
8					0	0	1	1	1	1	1	11	1	1	22	1									14	5	9	0.345
9				0	0	1	0	0	0	0	1	12	3	1	0										9	6	3	0.265
10					0	0	1	0	0	0	0	1	0	11	11	11									8	3	5	0.184
11			0	0	0	1	0	2	1	11	0	0	21	3											12	4	8	0.342
12		0	0	1	0	0	1	1	2	0	2	3	0												10	8	2	0.211
13		0	0	1	0	1		1	0	0	2	0													5	3	2	0.159
14		0	0	0	0	0	0	1	0	0	1														2	1	1	0.053
Total		0	0	2	0	3	2	6	4	4	8	9	12	11	6	8	4	3	5	3	3	1	1	0	0	95	53	42	2.499

diabetes in the first five years and 72 in the second five years.

For each group of children born in a particular year their rate of presentation with diabetes per year studied has been derived (table 5).

Considering the year of birth for the nine year period from 1960 to 1968 there has been no significant variation in the incidence between groups. There is more extreme variation in the incidence rates for those children born at either end of the period studied, but this data is more susceptible to variation because there are fewer children included. Within age groups, however, there has been an increase in the number presenting with diabetes in the final years of the study, which cannot entirely be explained by variation in the base population, since the number of births have remained relatively constant over the period considered. Examination of the ten years' data available for each age group of children presenting with diabetes shows that the proportion of those that presented within the last five years, 1972 - 1976 (76%), was significantly higher than the number presenting in the first five years 1967 - 1971 ($P < 0.001$).

The incidence rates per thousand were calculated for each age group (table 5). The base population was calculated as the mean of the number of births occurring in Sunderland County Borough plus the mean of half the births at Thorpe Maternity Hospital, Easington.

Over the ten year study period, 1967 to 1976, the incidence rate per thousand children up to age 15 years was 2.5 (1 in 400) and during the last five years of the survey, 1972 to 1976, the rate had increased to 3.8 per 1,000 (1 in 263).

(iv) <u>Sex ratio throughout the ten year survey</u>

The ninety-five diabetic children comprised fifty-three (56%) boys and forty-two (44%) girls. This difference between the sexes is not significant when the sex ratio of the children born in Sunderland after

Table 6

Yearly incidence of juvenile-onset diabetes in Sunderland children under 15 years of age from 1967 to 1976

First Quinquennium

	1967	1968	1969	1970	1971	Total
	M F 4 2	M F 2 3	M F 1 2	M F 1 3	M F 2 3	M F 10 13
Total	6	5	3	4	5	23

Second Quinquennium

	1972	1973	1974	1975	1976	Total
	M F 5 4	M F 6 8	M F 10 2	M F 10 4	M F 12 11	M F 43 29
Total	9	14	12	14	23	72

Total in 10 years 1967 - 1976 = 95 diabetic children
First Quinquennium 1967 - 1971 = 23 children
Second Quinquennium 1972 - 1974 = 72 children

Table 7

Age at onset of diabetes in Sunderland children (1967-76)

Age	0-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Male	0	2	2	6	2	5	3	3	5	6	3	4	8	3	1	53
Female	0	0	0	1	3	2	5	1	9	3	5	8	2	2	1	42
Total	0	2	2	7	5	7	8	4	14	9	8	12	10	5	2	95

1951 is compared. The ratio at that time was 1.03 males to every one female.

In the first quiquennium of the survey, 1967 - 1971 (table 6), there were ten boys and thirteen girls with diabetes but in the second five years, 1972 - 1976, forty-three boys and twenty-nine girls developed the illness. Although the number of boys developing diabetes in the second quinquennium appears to have increased at the expense of girls, the difference was not found to be statistically significant.

There is very little difference in numbers between the sexes throughout the ten years, apart from 1974 to 1975, when in each year ten boys developed diabetes whilst there were only two girls in 1974 and four in 1975. However, the total number of diabetic children in each of these two years was near the yearly average for that phase of the survey.

(v) Age and sex of the children at the onset of diabetes

The age of the children at the onset of their illness is shown in Table 7.

Throughout the ten years no child under one year of age developed diabetes. In fact, before the third birthday, only four cases of diabetes were recorded.

After three years, the children are randomly distributed, although there is a non-significant numerical trend towards a slightly increased incidence between the ages of eight and eleven years. After twelve years of age, there is a sudden decline in the number of diabetic children.

Two peaks are clearly evident at eight and eleven years of age. Fourteen children developed diabetes when eight years old, followed by twelve children who were eleven years and ten diabetic children who were twelve years of age.

At each year of age, apart from the first year when no diabetic children presented, boys predominated in nine of the remaining fourteen

Table 8

Diabetic children in age - sub groups

0 - 4 years			5 - 9 years			10 - 14 years		
12M	4F		22M	20F		19M	18F	
Total	16	(17%)	42		(44%)	37		(39%)

Table 9

The month of onset of diabetes in Sunderland children 1967-76

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Male	5	4	3	2	6	2	3	2	5	10	6	5	53
Female	5	4	3	4	5	2	3	2	3	3	6	2	42
Total	10	8	6	6	11	4	6	4	8	13	12	7	95

years. The difference in the sexes was most marked in boys aged three and twelve. Girls were in excess at age four, six, eight, ten and eleven years. Here the difference was most evident at the age of eight and eleven.

The ninety-five diabetic children were subdivided into three age groups, each of five year periods.

(i) From birth to four years eleven months (0 - 4 years);

(ii) Fifth birthday to nine years eleven months (5 - 9 years);

(iii) Tenth birthday to fourteen years eleven months (10 - 14 years).

There are sixteen children in the 0 - 4 year group, compared to forty-two children aged 5 - 9 years and thirty-seven in the 10 - 14 year range (table 8).

In the younger 0 - 4 age group, boys outnumbered girls in a ratio of 3:1. In the first three years, there were ten boys and only one girl. After this age, the number of diabetic girls dramatically increased, so that in the 5 - 9 years and 10 - 14 year groups the number of girls are almost equal to boys.

(vi) Seasonal variation

In assessing the time of onset of diabetes in epidemiological studies, the date of diagnosis of the illness is considered suitable. (Rolles, Rayner and Mackintosh 1975).

The 95 diabetic children were grouped according to the month in which their illness was diagnosed (table 9).

The number of cases occurring in any month varied from four patients in June and August to thirteen children presenting in October.

The months were grouped into three, e.g. January to March, and then into two six month periods, October to March, and April to September.

The results (table 10) show a definite trend to fewer cases during the warmer summer months, eighteen patients in July, August and

Table 10

Number of patients in each quarter of the year

January - March		April - June		July - September		October - December		
12M	12F	10M	11F	10M	8F	21M	11F	
Total	24		21		18		32	Total

Number presenting in six month periods

October - March		April - September	
33M	23F	20M	19F
56 (59%)		39 (41%)	

Table 11

Age groups and seasonal incidence

Age	October - March	April - September
0 - 4 years	5	11
5 - 9 years	25	17
10 - 14 years	26	11
Total	56	39

$P < 0.05$ in 10 - 14 year group,
other age groups not significant

September, compared to an increase in the colder latter quarter of the year with thirty-two patients in October, November and December. In the six months October to March, 56 (59%) children developed their illness and 39 (41%) from April to September.

The children who presented with their diabetes in the six month periods October to March and April to September were divided into three sub groups (table 11) and were then tested for significance using the binomial distribution of equal probabilities. No significant difference in seasonal variation was found in the children as a whole (0 - 14 years) but the older children, 10 - 14 years, had significantly more juvenile diabetics presenting during October to March ($P < 0.05$). The other two age groups, 0 to 4 years and 5 to 9 years, did not show this significant difference.

A 3 x 2 chi squared test was used to compare the individual age groups; to determine if seasonal onset depended significantly on the age of the child. A definite difference was noted between the very young groups (0 - 4 years) and the older groups (10 - 14 years) ($P < 0.05$), suggesting that the older children are more likely to present with their illness during the October to March period whilst the younger children tend to have their onset in the summer. A similar trend was noted between the 0 to 4 years and 5 to 9 year groups but the results were not mathematically significant.

Although more boys than girls were diagnosed during the October to March six months, this difference in sex-onset was not significant.

(vii) <u>Age at onset, sex ratio and seasonal distribution</u>

As mentioned in the previous section, out of 95 diabetic children, 56 developed their diabetes in the six month period October to March and only 39 in the months April to September.

The children were sub-divided into three age groups 0 - 4 years,

Table 12

Number of Sunderland diabetic children in age sub-groups who presented with diabetes between October to March and April to September

Age	October - March	April - September
0 - 4 years	M 4, F 1, 5	M 8, F 3, 11
5 - 9 years	M 14, F 11, 25	M 8, F 9, 17
10 - 14 years	M 15, F 11, 26	M 4, F 7, 11
Total 0 - 14 years	M 33, F 23, 56	M 20, F 19, 39

10 - 14 years $P < 0.05$

Other age groups not significant

5 - 9 years and 10 - 14 years (see table 12).

In the 0 to 4 group, five children (4 boys and 1 girl) developed diabetes in the winter period compared to eleven children (8 boys and 3 girls) during the warmer period April to September. Of the twelve young boys throughout the year, four presented in May and three in September.

The largest group, 5 - 9 year olds, comprised twenty-five children (60%) in the October to March period and seventeen (40%) in the summer-time. Very little difference in sex ratio was noted.

The 10 to 14 year group had twenty-six (67%) children in October to March period and only eleven (33%) in the summer six months.

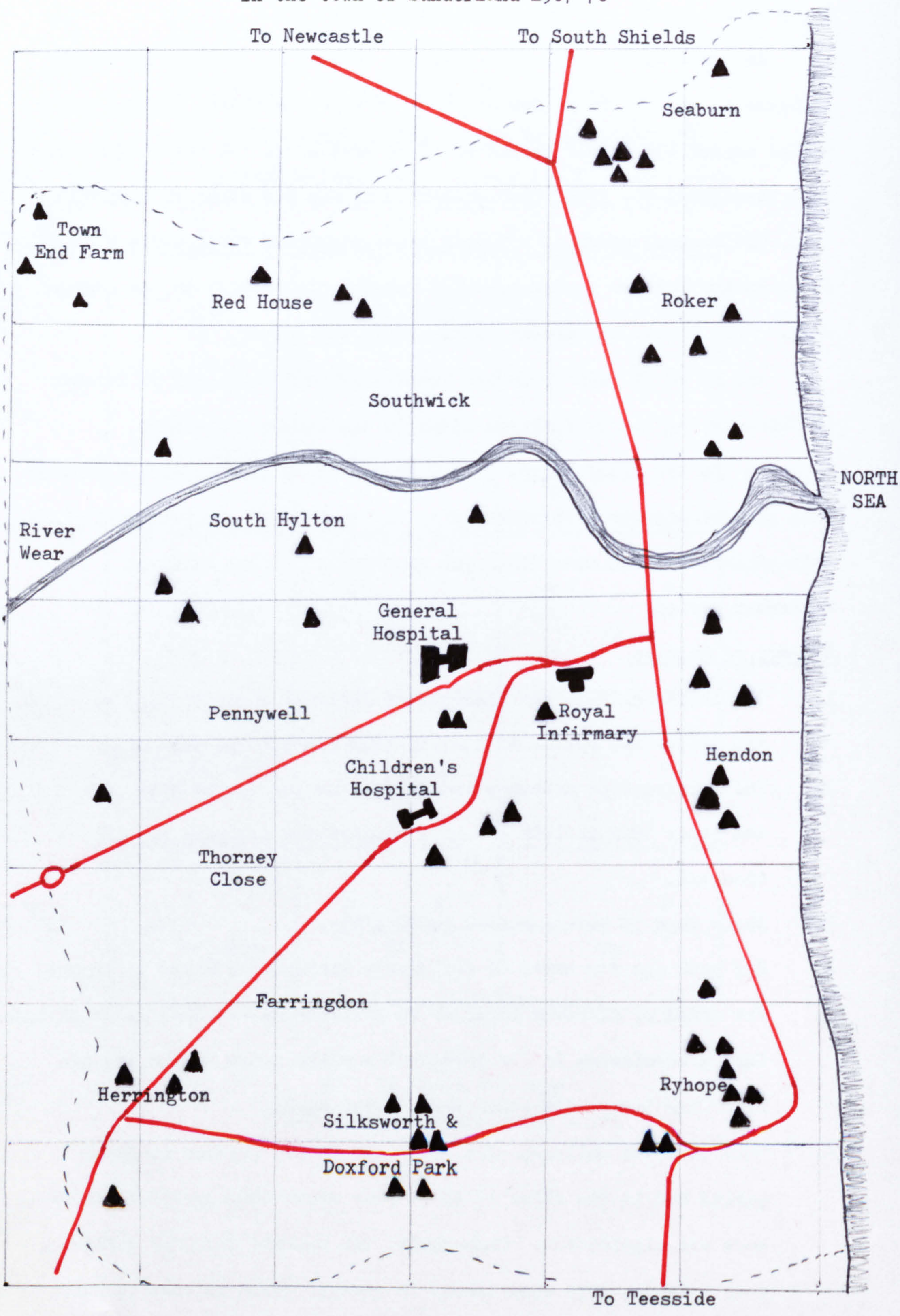
In the two older groups 5 to 9 years and 10 to 14 years, more boys than girls presented with diabetes in the winter six months and slightly more girls than boys were diagnosed as diabetic in the April to September period.

Summary of Results

1. The incidence rate for diabetes in children under fifteen years in the Sunderland area from 1967 to 1976 was 2.5 per thousand.
2. The incidence of diabetes in children in the second five years of the study 1972 to 1976 showed a significant increase to 3.8 per thousand.
3. There were 53 boys compared to 42 girls.
4. The peak age for onset of children's diabetes is 8 and 11 years.
5. Few cases of diabetes occurred in children under three years of age. Boys predominated in the younger 0 - 4 age group and in the two older age groups the sexes were almost equal.
6. More diabetic children were diagnosed in the October to March period and in the older 10 to 14 year group this seasonal difference was significant. Conversely, the younger diabetic children, 0 to 4 years, were more likely to develop their illness in the summer months.

Fig 3

Map showing diabetic children's place of residence
in the town of Sunderland 1967-76



Squares = National grid 1 km.

Chapter 6

An investigation into possible clustering of cases of juvenile diabetes

(i) Theory of "clustering" in relation to disease

One provoking factor thought to be associated with the onset of juvenile diabetes is virus infection(s), especially Coxsackie B-4 virus (Gamble 1969 and 1973). If this theory is correct then infection would be expected to occur in community areas such as streets or housing estates; places of work, or in the case of children, their school or nursery environment, including local authority swimming pools and playgrounds. It would therefore be reasonable to assume that new diabetic patients may present in "clusters" related to housing areas or school, rather than being sporadically spread throughout the community.

In order to examine this theory a list of the 95 diabetic children with their home address and if possible the name of the school which they attended at the time of the onset of the illness was recorded. The children were classified according to the day and month and year of onset during the ten year period of the survey and on a Geographia street map and on Ordnance Survey Map of the area, the patient was represented by a triangle ▲ . Clustering in groups of cases could therefore be easily identified by this technique (fig. 3).

(ii) Apparent clustering in the Sunderland Diabetic Survey

There appear to be three distinct clusters of diabetic children in Sunderland; one in the north-eastern part of the town, Seaburn Dene; another in the southern part of Sunderland, Ryhope. The third area is in the south-west part of the town, Silksworth and Doxford Park.

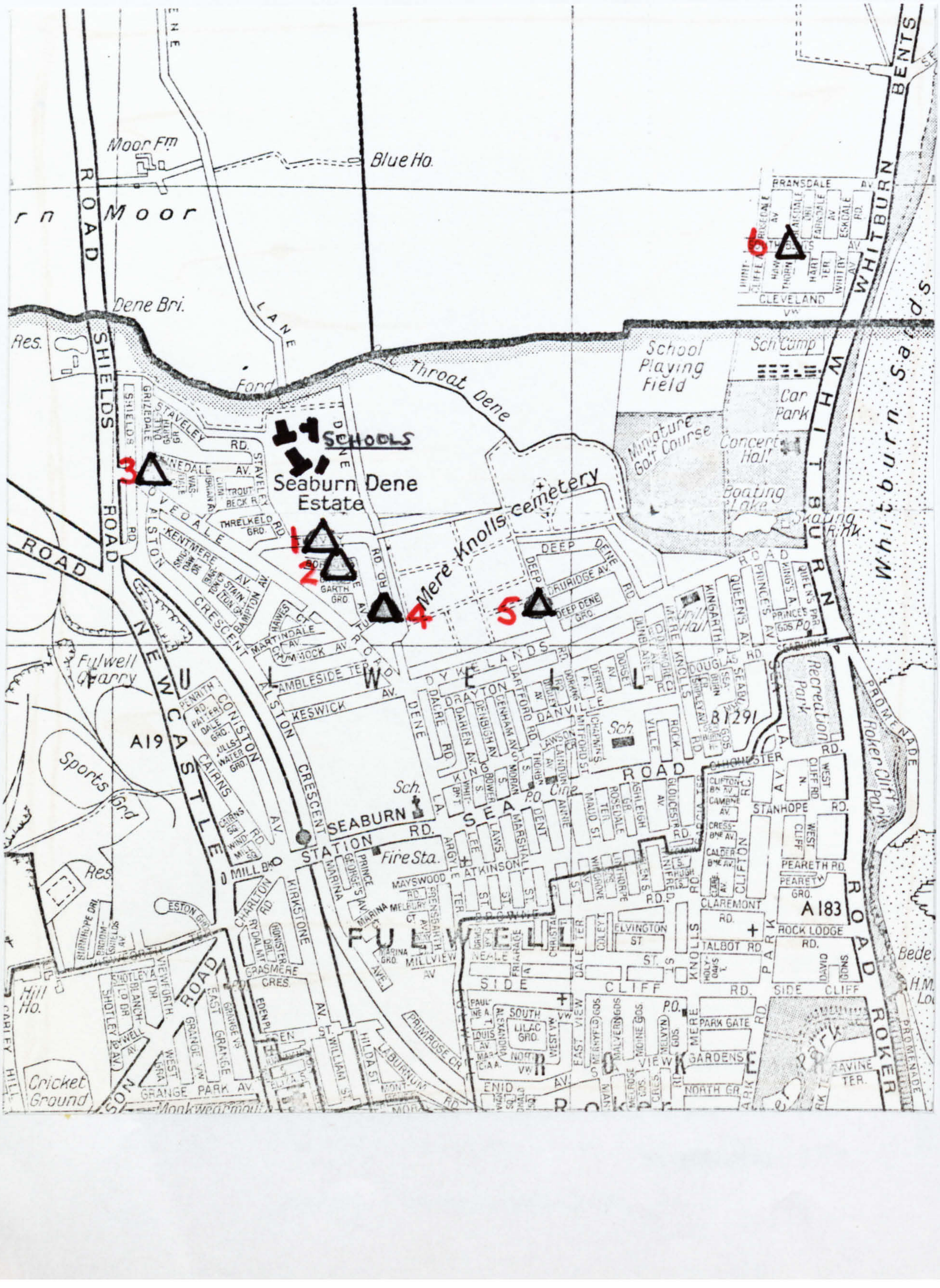
(a) Seaburn Dene

This area consists of a private housing estate comprising

Fig 4

Map of Seaburn showing the residence of diabetic children

National grid square = 1 km.



approximately 1,000 semi-detached houses. The children attend Seaburn Dene Primary School (infants and juniors) comprising 350 children. During the ten years of the study, six children developed diabetes when under age 15.

	Initials of patients	Age	Sex	Onset	Address	
1.	I.A.	3.7	M	March 1967	Torver Crescent	
2.	T.H.	9.2	M	April 1967	Borrowdale Avenue	Junior School
3.	L.S.	10.2	F	October 1967	Grisedale Court	" "
4.	C.S.	9.1	M	November 1973	Torver Crescent	" "
5.	A.C.	7.9	M	July 1974	Deepdene Road	" "
6.	C.H.	6.4	F	December 1976	South Bents	Infant School

Apart from the first patient (I.A.) who was not yet school age, all the other children were at the same local primary school when their diabetes started. Apart from this focal point, the children also lived very close to each other on the same estate (fig. 4) and presumably played together.

Just 1.0 kilometre away in the Roker area of Sunderland two older boys, both at the same Monkwearmouth Comprehensive School, developed diabetes in February, 1976. The children from Seaburn Dene also progress to Monkwearmouth Comprehensive School and consequently in this school at December 31st, 1976, there were four diabetic children under 15 years of age in this large comprehensive school who have 900 children in the 11 to 15 age group.

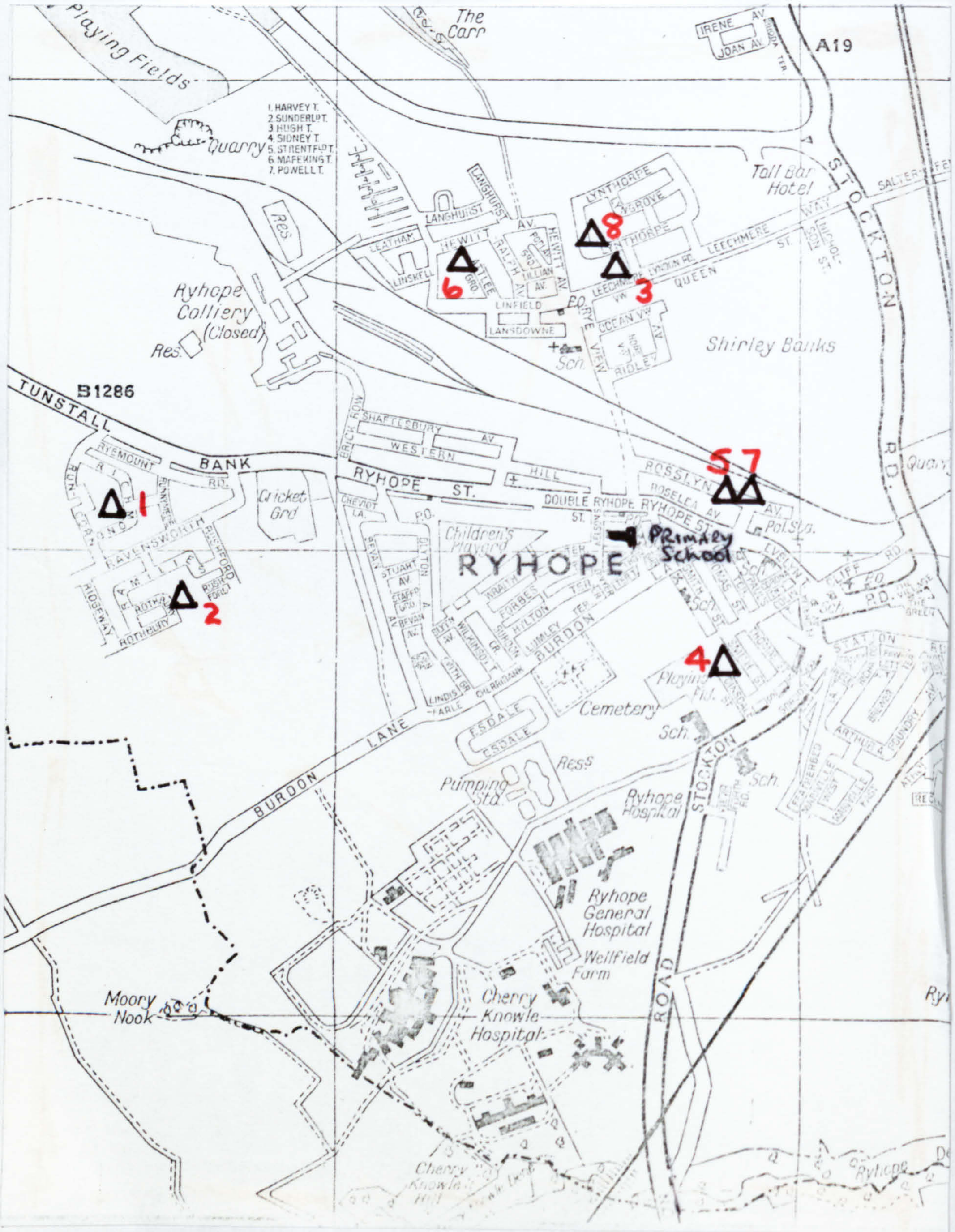
(b) Ryhope

A similar situation occurred in the village setting of Ryhope in the southern part of Sunderland. This area is divided by the busy Sunderland - Teesside trunk road. Approximately two thirds of Ryhope lies to the north of the trunk road and 650 children attend Ryhope Junior and

Fig 5

Map of Ryhope area showing the
residence of children with diabetes

National grid squares = 1 km.



Infant School. During the ten year survey, eight children under 15 years of age developed diabetes, all living on the north side of the trunk road.

No cases occurred in the southern part of Ryhope which lies to the south of the trunk road and has its own small primary school. However, the children do mix at Senior School level.

All the diabetic children in the Ryhope area presented in the last five years from 1972 (fig. 5).

Ryhope diabetic children 1967 - 1976

	Initials of patients	Age	Sex	Onset	Address	
1.	J.F.	1.6	M	May 1972	Rothbury	Not at school
2.	M.P.	4.5	M	May 1974	Richmond	Not at school
3.	A.P.	3.5	M	June 1974	Leechmere View	Not at school
4.	P.W.	10.9	F	January 1975	Smith Street	Ryhope Junior
5.	C.G.	2.0	M	September 1975	Rosslyn Avenue	Not at school
6.	G.H.	12.9	M	June 1976	Hewitt Avenue	Ryhope Comprehensive
7.	S.B.	8.2	M	November 1976	Rosslyn Avenue	Ryhope Junior
8.	D.A.	10.2	F	December 1976	Lynthorpe	Ryhope Junior

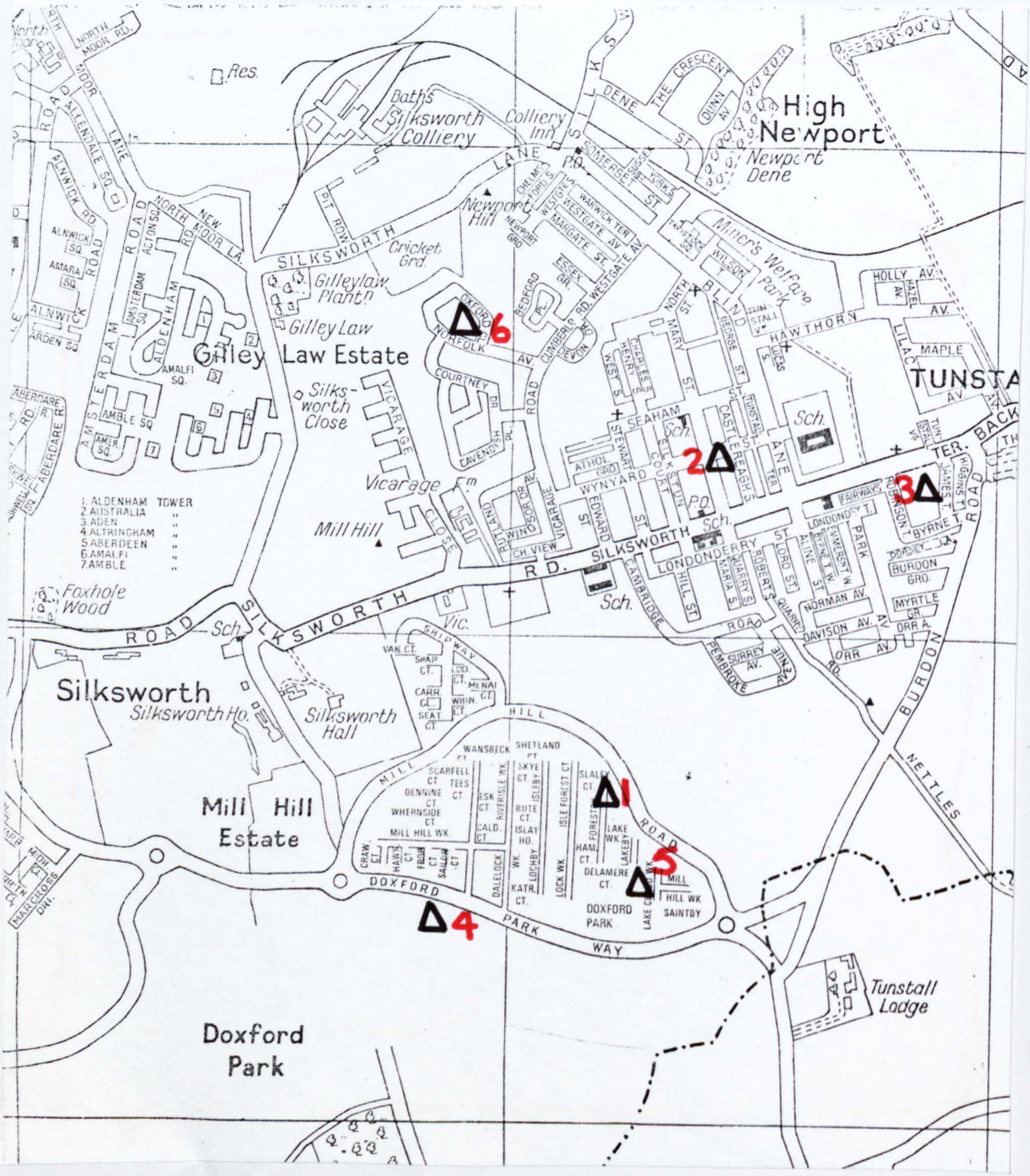
Two toddlers M.P. and A.P. lived within 1.0 km. of each other and developed their diabetes within forty days of each other, while two children at the same junior school, S.B. and D.A., developed their diabetes within forty days of each other and lived just less than 0.5 km. apart. There are also two diabetics in the same street, C.G. and G.H., with fourteen months between their illness.

(c) Silksworth and Doxford Park

In this south-western area of Sunderland, which was previously a local rural council district, six young children developed diabetes between August 1973 and December 1976. All the children at the time of diagnosis attended two local primary schools less than 0.5 km. apart.

Fig 6

Map of Silksworth showing the residence
of the diabetic children in the survey
National grid squares = 1 km.



One school is in the older Silksworth area (800 pupils) and the other school on a new small council estate, Doxford Park (550 pupils).

	Initials of patients	Age	Sex	Onset	Address	
1.	N.H.	8.9	M	August 1973	Doxford Park	Mill Hill Juniors
2.	C.H.	6.2	F	November 1973	Silksworth	Silksworth Juniors
3.	T.B.	8.9	F	April 1975	Silksworth	Silksworth Juniors
4.	S.T.	8.1	M	September 1975	Doxford Park	Mill Hill Juniors
5.	S.C.	10.2	F	September 1975	Doxford Park	Mill Hill Juniors
6.	N.B.	4.1	M	December 1975	Silksworth	Not at school

Two children, S.T. and S.C., were in the same Junior School and developed diabetes in the same month. Four children developed the illness between April and December, 1975,all within a radius of 0.5 km. (Fig. 6).

(d) Areas outside the Sunderland County Borough Boundary

Whitburn

This small village is situated on the coast, just north of the Sunderland town boundary. In 1969, two girls at the local senior school presented in the same month. They lived less than 0.5 km. of each other.

S.G. 11.8 years F March 1969

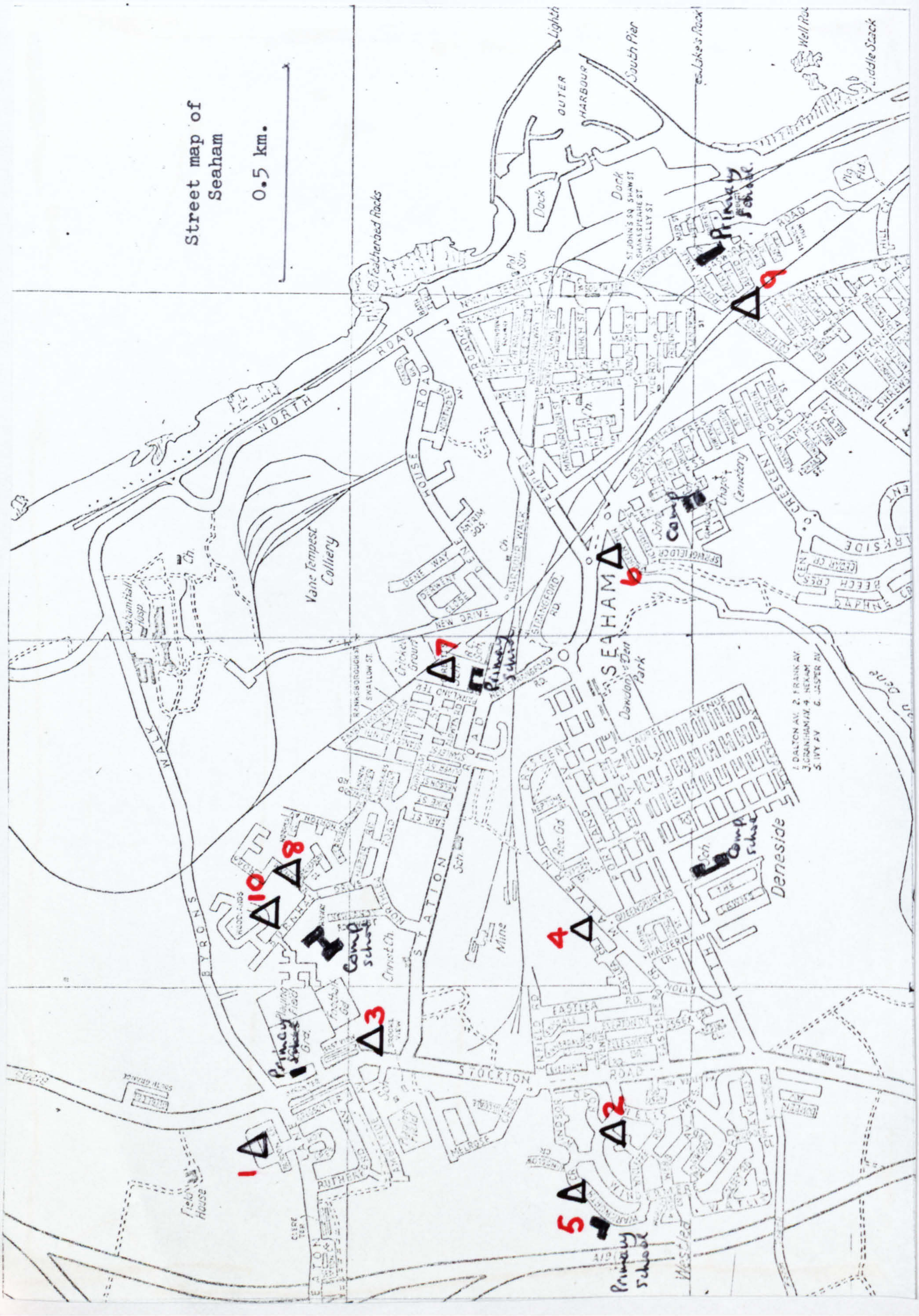
J.P. 13.8 years F March 1969

Seaham

This town lies four miles south of Sunderland on the coast and has 5,800 school children under 15 years (1971 Census). During the ten year survey, ten children developed diabetes (fig. 7).

Fig 7

Map of Seaham showing the residence of diabetic children in the survey



	Initials of patients	Age	Sex	Onset	Address
1.	S.L.	4.9	F	July 1967	Burdon Crescent
2.	P.L.	11.3	M	May 1972	Westlea
3.	S.F.	8.7	F	June 1972	East View
4.	C.G.	13.5	M	October 1972	Malvern Crescent
5.	C.C.	8.3	F	September 1973	Warkworth Crescent
6.	M.T.	9.3	M	December 1974	Maureen Terrace
7.	J.D.	11.0	F	May 1975	Corbett Street
8.	W.L.	13.6	M	March 1976	Neasham Road
9.	D.G.	13.9	M	April 1976	Albert Street
10.	K.N.	7.5	M	July 1976	Woodlands

Three children in 1972, P.L., S.F. and C.G. all lived within 0.6 km. and developed their diabetes between May and October and in 1976, W.L. and K.N. presented in May and July, living 100 metres from each other.

(iii) Areas in the survey without "clustering"

In contrast to the areas where groups or clusters of diabetic patients occurred, there are several areas in and around Sunderland where few, if any, cases of juvenile diabetes were recorded.

Such an area is Thorney Close, a large council estate, just west of the Children's Hospital. This estate has three primary schools and one senior school, comprising in total 3,280 pupils. No diabetic children were recorded from the estate or schools on the estate in the ten year survey. Similarly, an adjacent council estate, Pennywell, with a total school population of 2,500 children, produced only one diabetic child in the ten years.

On the north side of the River Wear, the main road to Newcastle and South Shields divides the seafront housing areas of Seaburn and Roker from the older housing area of Southwick and Red House council estate. This

lower socio-economic area has several primary schools and two comprehensive schools comprising 6,600 pupils. Only three children developed diabetes in this area of Sunderland in the ten year survey. This is in marked contrast to the large number of diabetic children in the Seaburn - Roker area.

A few miles south of Sunderland lies the mining area of Murton, between Seaham and Hetton-le-Hole. Although several children developed diabetes in Seaham and Hetton, no diabetic child was recorded from Murton between 1967 - 1976. The school population for the Murton area is 2,100.

(iv) <u>The investigation of clustering using the Knox Space - T formula</u>

The above results suggested the possibility of clustering of cases in juvenile diabetes. In order to test the hypothesis of clustering of cases both in time and space, the method suggested by Knox (1962, 1964) was used.

The Knox SPACE - T. Analysis (1967) was kindly carried out by Professor E. G. Knox at the University of Birmingham Health Services Research Centre.

The general purpose of the programme is to display and detect space - time interactions among events (e.g. disease events) specified jointly according to the date of occurrence and their location (i.e. map reference). The programme is written in FORTRAN - IV and consists of a main programme with two sub programmes (see Appendix 1).

93 patients in the diabetic study were classified according to their street address using a Geographia street map which incorporates National grid lines. The mid-point of the street was taken as the patient's home and this point was given a six figure grid reference to the nearest 0.1 kilometre. This grid reference combines the Eastings and Northings.

The day, month and year of onset was recorded for each case and combined with the grid reference and the information was written on a

Fortran computer sheet and the data analysed.

(v) Results of Space - T. analysis

93 patients gave 4278 possible pairs (93 x 92/2) and the time of onset and geographical distances between them was classified in the first table - "Analysis of all possible pairs". The number of days is expressed down the left hand column, i.e. 30, 60, 100 ..... and the distance in kilometres in the other column, e.g. 3.0, 6.0, 10.0.

There were 15 pairs within 3.0 km. of each other and 30 days onset. 9 pairs occurred within 60 days and 3.0 km. of each other. 25 pairs presented within 30 days and 6.0 km. of each other.

This data was then added to give cumulative values, i.e. the number 70 below 6.0 and alongside 60 days is the sum of the four top left hand numbers in the previous table, (15 + 25 + 21 + 9).

The expected cumulative values were then computed. These values are those which would be expected if there were no interaction between distances and times. Most of the expected values correlated closely to the cumulative values.

The final table is the standard error deviations of the cumulative values in terms of their deviations from the expected cumulative value in units of standard value in units of standard error. To be significant a deviation of $\pm$ 2 standard error should be produced and no such values were recorded.

SUMMARY

1. The distribution of diabetic children on a map of the Sunderland district revealed certain areas where diabetes is more prevalent. The cases seemed to group or cluster in these areas in contrast to other areas where diabetic children were few in number.
2. Although there is little doubt that many cases in this diabetic study were very close in time of onset and in the distance

separating the patients, the results from the Knox Space - T. analysis were not statistically significant.

Table 13

Diabetes in the extended family of diabetic and control children

	Diabetic boys	Control boys	Diabetic girls	Control girls
With family history	21 (37%)	21 (20%)	24 (45%)	18 (18%)
Without family history	36 (63%)	84 (80%)	25 (55%)	82 (82%)
Total	57	105	53	100

Chapter 7

A family history study of juvenile diabetes and the association with Human Leucocyte Antigens

(i) The family history of juvenile-onset diabetes in Sunderland

Any epidemiological study into juvenile-onset diabetes must take into account the family history of the disease.

Most family studies have reported only the incidence of diabetes in first degree relatives, and therefore I thought an inquiry into the extended family would be of interest.

To the 95 Sunderland diabetic children were added 15 who are currently attending the paediatric unit at South Shields General Hospital.

The 110 diabetic children were all under 15 years of age at the onset of their illness. There were 57 boys and 53 girls.

Apart from inquiring about diabetes in a first degree relative (the child's siblings and parents), I also inquired about other relatives, e.g.

(1) The parents' siblings and their offspring, i.e. the diabetic child's uncles, aunts and full cousins;

(2) The grandparents and their siblings.

The details of the inquiry are in Table 13.

21 boys (37%) had a diabetic relative compared to 36 (63%) without.

24 (45%) diabetic girls gave a similar positive history and 29 (55%) did not have a diabetic relative (Table).

When first degree relatives are assessed the numbers are very low. From the 57 diabetic boys, three fathers had diabetes. No mothers had the illness but two boys each had a brother with diabetes (one of the fathers and brother are in the same family).

None of the 53 diabetic girls had a diabetic parent. Interestingly, as the boys had brothers with diabetes, two girls each had one sister

with diabetes. In fact, all four girls are in the survey.

Hence, from the survey of 110 children, eight had a first degree relative with diabetes, an incidence of 7%.

In order to compare the diabetic children with a control group, I asked the same questions of 205 children (age 0 - 15 years) who were attending the Children's Hospital Out-patients, Sunderland, for reasons other than diabetes.

Out of 105 boys, only twenty-one (20%) had a relative with diabetes and eighteen (18%) of the 100 control girls had a positive family history, (Table 13).

Boys in the control group with a diabetic first degree relative were few, only one mother had the illness. No fathers or siblings were recorded.

From 100 control girls, two fathers were diabetics and two brothers but no mothers had the illness. Therefore, only five children from 205 controls had a first degree relative with diabetes, an incidence of 2%.

(ii) Human Leucocyte Antigens (H.L.A.) and family history

When studying the family history of juvenile-onset diabetes, examination of the H.L.A. phenotype certainly seems appropriate. These antigens appear to be derived from loci on chromosome 6 which may be closely linked to a locus which governs susceptibility to juvenile diabetes.

The family history used in this part of the study was similar to that used in the first section, i.e. parents, siblings, uncles and aunts, full cousins and grandparents.

Fifty-two diabetic children from the 110 had H.L.A. typing performed. The H.L.A. antigens which appear to be associated with juvenile diabetes are HLA-B8, BW15 and BW18, and therefore these three antigens were analysed in this family study.

Table 14

The association of family history with the H.L.A. phenotypes H.L.A. - B8, BW15 and BW18

Males	+ve F.H.	-ve F.H.	Total
With specific antigens	7	13	20
Without specific antigens	2	5	7
Total	9	18	27

P = 0.57

Females	+ve F.H.	-ve F.H.	Total
With specific antigens	13	5	18
Without specific antigens	0	7	7
Total	13	12	25

P = 0.00165

Both sexes combined	+ve F.H.	-ve F.H.	Total
With specific antigens	20	18	38
Without specific antigens	2	12	14
Total	22	30	52

$X^2 = 6.36$ Exact Probability $P = 0.0129$

$(0.01 < P < 0.05)$

From a total of 27 boys, nine had a positive family history, and of those, seven had the specified antigens. Thirteen of the remaining eighteen boys without a family history had these antigens (Table 14).

There were 25 girls who were H.L.A. phenotyped. Thirteen had a family history of diabetes, and all thirteen girls had the specified antigens. Whereas only five of the twelve girls without a family history had the specified antigens.

Tests of significance were carried out to determine the association of family history with the three H.L.A. antigens.

It was found that when both sexes are combined, there is a significant association between the H.L.A. phenotypes, HLA- B8, BW15 and BW18 and a positive family history of diabetes ($.01 < P < .05$).

This significance is even greater when female diabetic children are analysed, where the exact probability is $P = 0.0017$.

Although the boys show a trend in the same direction, the P value of 0.57 was not statistically significant.

(iii) <u>H.L.A. phenotypes in two diabetic families</u>

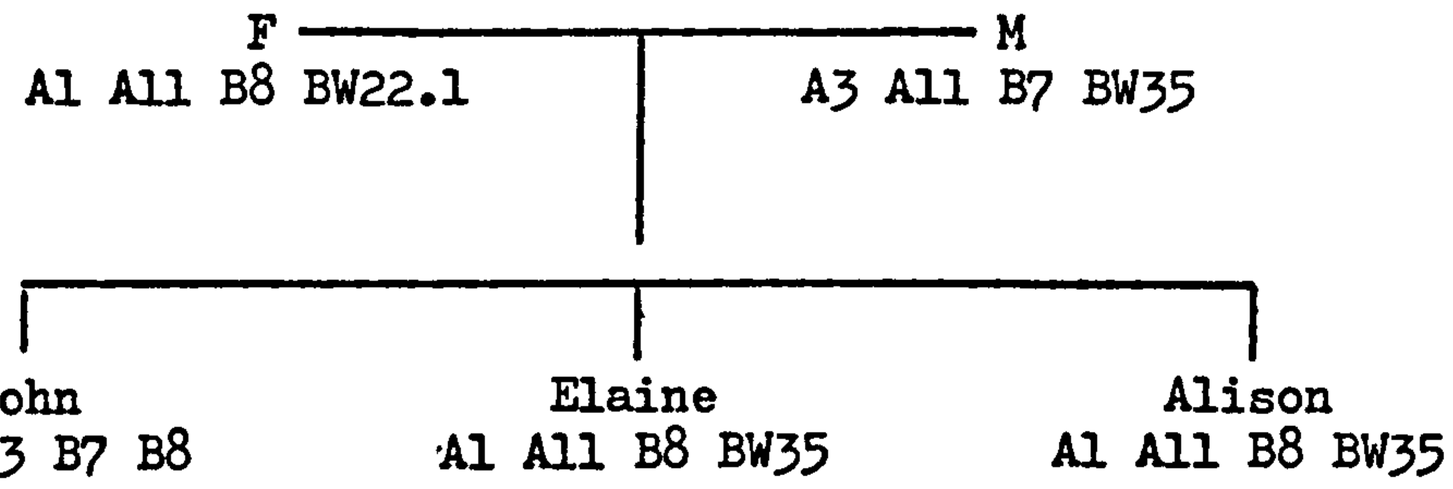
In order to determine how the H.L.A. haplotypes are passed from non-diabetic parents to affected diabetic children, I took blood samples for H.L.A. testing from two families, both of whom had two young diabetic sisters in the survey group.

Family 'A' consister of Father (38 years) and Mother (35 years), both well parents without diabetes. They have three children, John (13 years) a non-diabetic, Elaine who was eleven years old when she developed diabetes and Alison who was four when her illness started.

Family 'B' had a similar structure, an elder non-diabetic son (14 years) and two younger diabetic daughters. Father (40 years) and Mother (37 years) are both well. Judith was aged twelve when she developed diabetes and Maureen was ten at the start of her illness.

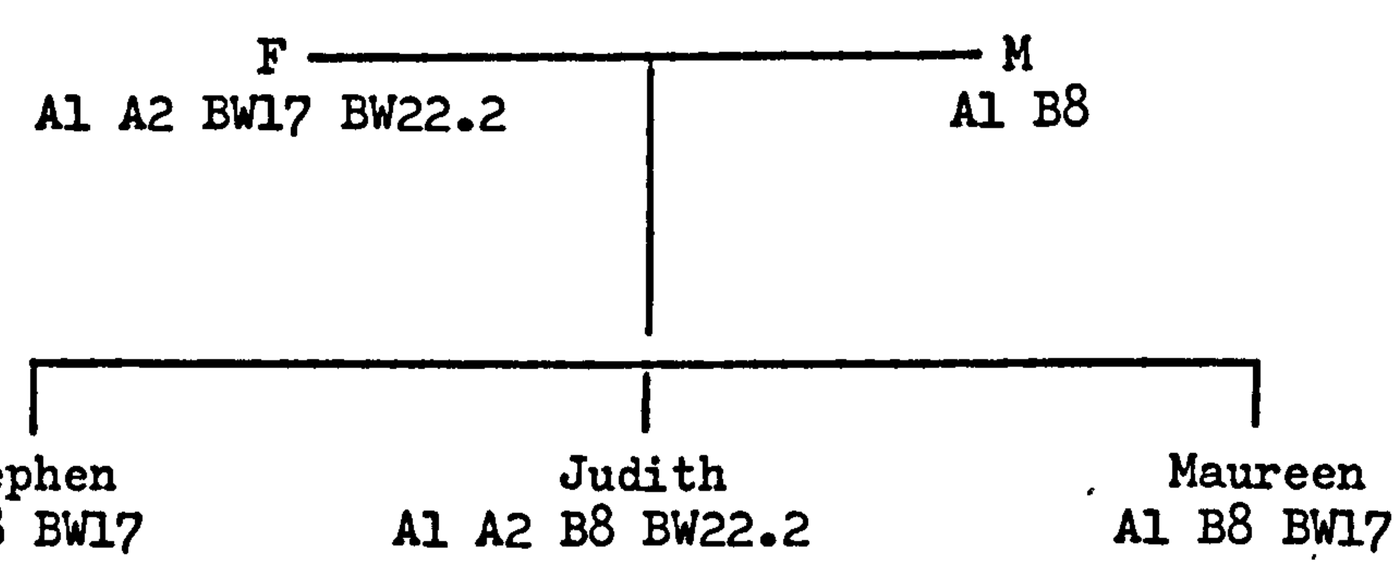
Apart from the sisters in each family with diabetes, no-one else in the extended families were known to have diabetes.

Family 'A'



All three children have inherited the paternal HLA-A1B8 haplotypes. The two affected daughters have also inherited the maternal A11, BW35 haplotypes.

Family 'B'



All three children have inherited the maternal HLA-B8 haplotypes. Stephen and Maureen have also inherited BW17 from Father, but Stephen has so far not developed diabetes.

Summary of results

1. 37% male and 45% female diabetic children had an 'extended' family history compared to 20% control boys and 18% girls.
2. Only eight (7%) diabetic children from a total of 110 had a first degree relative with diabetes compared to 2% controls.
3. There is a significant association between diabetic children

with HLA-B8, BW15 and BW18 and a family history of diabetes. This association is highly significant in girls.

Table 15b

H.L.A. Phenotype of Female Diabetic Children in the Sunderland survey (1967-76)

	Initials	Age/Years	Onset	H.L.A. Phenotype	
1.	E.S.	12.5	January 1968		B8: BW15
2.	L.M.	8.2	February 1968	A1: A11:	B8: B27
3.	T.B.	2.6	March 1969	A1: A2:	B5: B12
4.	C.E.	3.5	July 1969	A2: A11:	B8: B8
5.	E.A.	11.1	April 1970	A1: A11:	B8: BW35
6.	G.L.	8.2	May 1970	A3: A11:	BW35: BW35
7.	D.K.	9.3	January 1971	A2: A2:	BW15: BW40
8.	J.B.	12.1	May 1971	A1: A2:	B8: B22.2
9.	C.G.	11.8	June 1971	A1: AW24:	B7: B8
10.	J.M.	12.0	February 1972	AW24: AW32:	B12: BW15
11.	S.P.	11.6	March 1972	A2: A2:	B7: B12
12.	S.F.	8.8	June 1972	A1: A2:	B8: B12
13.	J.C.	9.2	September 1972	A2: A2:	B12: BW39
14.	M.B.	10.6	October 1972	A1: A1:	B8: BW17
15.	F.C.	8.2	October 1972	A3: A11:	BW40: BW40
16.	L.K.	5.3	April 1973	A3: A3:	BW15: BW18
17.	M.T.	9.6	April 1973	A2: AW30:	BW18: BW40
18.	L.C.	11.7	May 1973	A1: A2:	B8: BW15
19.	D.H.	11.1	May 1973	A2: A3:	B7: BW15
20.	A.A.	4.9	August 1973	A1: A11:	B8: BW15
21.	R.G.	4.7	December 1974	A2: A2:	B13: BW21
22.	K.M.	8.7	February 1976	A2: AW28:	B8: B27
23.	P.S.	12.5	March 1976	A1: AW24:	B7: B8
24.	L.S.	11.4	November 1976	A2: A2:	B7: B27
25.	J.H.	4.1	November 1976	A3: AW24:	B7: BW18
26.	D.L.	11.7	November 1976	A3: A3:	B8: BW15
27.	C.H.	6.4	December 1976	A2: AW28:	B8: BW40

Table 15a

H.L.A. Phenotype of Male Diabetic Children in the Sunderland survey (1967-76)

	Initials	Age/Years	Onset	H.L.A. Phenotype	
1.	B.D.	6.8	November 1967	A1: A2:	B5: B8
2.	A.W.	5.4	October 1968	A2: AW24:	BW35: BW40
3.	M.K.	3.1	October 1968	A1: AW24:	B8: BW39
4.	D.B.	9.0	October 1968	A1: AW24:	B8: BW39
5.	A.C.	4.9	January 1972	A1: A1:	B8: B8
6.	P.L.	11.3	January 1972	A1: AW24:	B8: B8
7.	J.F.	1.6	May 1972	A3: A3:	B7: BW15
8.	G.D.	11.1	December 1972	A2: A3:	B7: BW40
9.	A.H.	12.5	May 1973	A1: A2:	B5: BW17
10.	M.C.	8.1	July 1973	A2: A2:	B7: BW15
11.	C.S.	9.1	November 1973	A1: AW24:	B8: BW40
12.	H.T.	3.2	January 1974	A1: A2:	BW17: BW40
13.	C.H.	6.4	February 1974	A2: A3:	B8: BW21
14.	A.C.	7.9	July 1974	A2: AW24:	B12: BW15
15.	T.W.	12.2	October 1974	A1: A2:	B8: BW38
16.	S.T.	8.1	September 1975	A2: A2:	B8: BW15
17.	G.E.	3.0	October 1975	A1: A2:	B8: BW18
18.	P.M.	5.0	October 1975	A1: AW24:	B7: B8
19.	G.W.	11.5	January 1976	A2: AW28:	BW18: BW35
20.	M.V.	12.6	February 1976	AW23: AW29:	B12: BW18
21.	C.C.	11.6	February 1976	A1: A2:	B7: B8
22.	W.L.	13.6	March 1976	A2: A2:	B8: BW18
23.	G.H.	12.9	June 1976	AW32: AW32:	BW18: BW40
24.	K.M.	7.5	July 1976	A2: A2:	B5: B8
25.	R.B.	2.5	September 1976	A2: A2:	B12: B12
26.	C.C.	13.9	October 1976	A2: A2:	B12: B12
27.	D.B.	10.1	October 1976	A2: A11:	B7: B8
28.	S.B.	8.2	November 1976	AW24: AW28:	B7: B12

Chapter 8

Human Leucocyte Antigens (H.L.A.) and juvenile-onset diabetes in Sunderland children

(i) Introduction

Certain human leucocyte antigens (H.L.A.) have been found to be increased in patients with juvenile diabetes (Nerup 1974, Cudworth & Woodrow 1974, Rolles, Rayner & Mackintosh 1975).

In order to determine if their findings are applicable to diabetic children in the Sunderland area, H.L.A. testing was carried out on fifty-five children who developed their diabetes during the ten year period from January 1st 1967, to December 31st, 1976, and who were under fifteen years of age at the time of diagnosis.

(ii) Laboratory method of H.L.A. testing

The H.L.A. tests were carried out at the Regional Blood Transfusion Centre, Newcastle, by P. J. Dewar, Senior Chief Technician.

The author took blood samples comprising fifteen millilitres of heparinised blood from all fifty-five diabetic children in the H.L.A. study. The blood samples were transported at room temperature to the Regional Transfusion Centre within twenty-four hours and the H.L.A. testing was carried out using the micro droplet lymphocyte cytotoxicity test. The principle of this test is as follows. The blood leucocytes are known to carry all the known H. L. Antigens and it is therefore possible to test for the presence or absence of antigens of the H.L.A. system using leucocyte cytotoxicity tests. A purified suspension of blood lymphocytes is mixed with antiserum to H. L. Antigens in the presence of complement. If the lymphocytes carry the appropriate antigens the antiserum will combine and in the presence of complement will damage the cell membrane. The resulting increase in cell membrane permeability is usually

Table 16

Age and sex distribution of fifty-five Sunderland diabetic children who had H.L.A. phenotype assessed

Age	Male	Female	Total	%	Ten year study
0 - 4 years	6	5	11	20%	17%
5 - 9 years	11	10	21	38%	44%
9 - 14 years	11	12	23	42%	39%
Total	28	27	55	100%	100%

Table 17

H.L.A. - B8 Phenotype in Sunderland diabetic children

Age	Male	Female	Total - B8	Total no. in H.L.A. study
0 - 4 years	3	2	5 (9%)	11
5 - 9 years	7	4	11 (20%)	21
10 - 14 years	5	8	13 (24%)	23
Total	15	14	29 (53%)	55

detected by dye uptake into the cells and consequently the particular antigen can be identified.

The individual patient's H.L.A. phenotype is listed in Table 15a and 15b.

(iii) General analysis of the H.L.A. results

The fifty-five diabetic children comprised twenty-eight boys and twenty-seven girls. They were divided into three age sub-groups and as Table 16 reveals there was little difference between the sexes in each age group.

There were eleven children in the younger group (0 - 4 years); twenty-one in the 5 - 9 year group and twenty-three children in the 10 - 14 year group. Despite the fact that the children had been randomly selected from the original ninety-five diabetic children, the percentages in the various age groups for the H.L.A. tested children were very similar to the 10 year incidence study.

The diabetic children's phenotype was compared with a control group of two hundred and forty healthy individuals, who have been used as H.L.A. controls in other research studies carried out by the Regional Blood Transfusion Centre (Appendix 2).

The most commonly found HLA - B phenotype in the Sunderland diabetic children was HLA - B8 where twenty-nine of the fifty-five children had this phenotype (Table 17). This meant that 53% of the Sunderland H.L.A. tested children had HLA - B8 compared to 24% in the control group ($P<0.01$).

The twenty-nine children with HLA - B8 comprised fifteen boys and fourteen girls and in each of the three age sub-groups approximately half the children had the HLA - B8 phenotype.

Slightly more boys than girls in the two younger age groups had HLA - B8, but in the older 10 - 14 year group this was reversed. The difference between the sexes and the HLA - B8 phenotype was not statistically significant.

Table 18

H.L.A. - B8, BW15 and BW18 phenotype in Sunderland diabetic children

H.L.A. Phenotype	0 - 4 years		5 - 9 years		10 - 14 years		Total
	M	F	M	F	M	F	
B 8	2	2	6	4	4	5	23
BW 15	1	0	2	1	0	2	6
BW 18	0	1	0	1	3	0	5
B8 BW 15	0	0	1	0	0	3	4
B8 BW 18	1	0	0	0	1	0	2
BW 15 / BW 18	0	0	0	1	0	0	1
Total	4	3	9	7	8	10	41
	7		16		18		

41 (75%) of 55 diabetic children
21 boys, 20 girls

Apart from an increase in the number of diabetic children with H.L.A. - B8, other B antigens were increased above the control values. These were H.L.A. - BW15, eleven cases (20% compared to 14% controls); H.L.A. - BW18, eight patients (15% compared to 10% controls) and H.L.A. - BW40, nine cases (16% compared to 13% controls).

None of the diabetic children had the phenotypes H.L.A. - B13, B14, BW21.1 or B37. The control group, however, had small percentages of all these B antigens.

(iv) <u>H.L.A. - B8 and associated B phenotypes in juvenile diabetes</u>

Apart from H.L.A. - B8, other phenotypes have been implicated in association with juvenile-onset diabetes. These other H.L.A. - B phenotypes are BW15 and BW18 (Cudworth & Woodrow 1977).

In the Sunderland H.L.A. study, forty-one (75%) of the fifty-five diabetic children had H.L.A. - B8, BW15 or BW18 phenotypes, combined with each other or with other B phenotypes (Table 18).

Twenty-nine (53%) of the children had H.L.A. - B8 of which twenty-three (42%) had H.L.A. - B8 in combination with B - phenotypes other than BW15 or BW18. Only three children,two boys and one girl were homozygous H.L.A. - B8, B8.

There were six children who had H.L.A. - B8 phenotype combined with BW15 or BW18. Four had H.L.A. - B8 BW15 and two children were H.L.A. - B8 BW18.

Eleven children had H.L.A. - BW15 phenotype, of whom four were combined with H.L.A. - B8 and one child had the combination H.L.A. - BW15 BW18. The remaining six children had the H.L.A. - BW15 phenotype combined with B - antigens other than B8 or BW18.

The H.L.A. - BW18 phenotype was present in eight diabetic children. Two had H.L.A. - B8 BW18 and one child was H.L.A. - BW15 BW18. Five children had H.L.A. - BW18 in combination with other B - antigens.

Table 19(a)

Seasonal incidence of fifty-five diabetic children irrespective of H.L.A. phenotype

Age	October - March	April - September
0 - 4 years	Males 4 Females 3 = 7	Males 2 Females 2 = 4
5 - 9 years	Males 7 Females 5 = 12	Males 4 Females 5 = 9
10 - 14 years	Males 9 Females 7 = 16	Males 2 Females 5 = 7
Total	20M 15F = 35 (64%)	8M 12F = 20 (36%)

Table 19(b)

Seasonal incidence of H.L.A. - B8 BW15 and BW18 patients

Age	October - March		April - September	
0 - 4 years	Male 2 B8, 1 B8 BW18 Female 1 BW18	4	Male 1 BW15 Female 2 B8	3
5 - 9 years	Male 5 B8 Female 3 B8, 1 BW15	9	Male 1 B8, 2 BW15 1 B8 BW15 Female 1 B8, 1 BW18, 1 BW15 BW18	7
10 - 14 years	Male 4 B8, 2 BW18 1 B8 BW18 Female 2 B8, 1 BW15 2 BW8 BW15	12	Male 1 BW18 Female 3 B8, 1 BW15 1 BW8 BW15	6
Total	Males 15 Females 10 = 25 (61%)		Males 6 Females 10 = 16 (39%)	

Most of the children in the various age sub-groups had the three specific B - antigens B8, BW15 or BW18 (Table 19b).

In the younger 0 - 4 year group, seven (63%) of the eleven children had these antigens and in the 5 - 9 year age group sixteen (76%) of the children had H.L.A. - B8, BW15 or BW18. An almost similar percentage, 78% eighteen children from twenty-three in the older 10 - 14 year group had three antigens.

(v) <u>H.L.A. phenotype and the seasonal incidence of juvenile diabetes</u>

The fifty-five H.L.A. tested diabetic children were grouped according to the month of onset of their illness and the year was divided into two six-month periods - October to March and April to September (Table 19a). Thirty-five (64%) of the children presented with diabetes in the "colder" October to March period. In each sub-group, more children presented in the winter than summer time and slightly more boys than girls had a winter tendency.

The diabetic children who had H.L.A. - B8, BW15 and BW18 phenotypes were then grouped according to their seasonal onset (Table 19b). Twenty-five (61%) of the forty-one children with these three antigens had their onset between October and March. There were fifteen boys and ten girls in the winter group, whilst in the April to September period, there were sixteen children, six boys and ten girls.

Fourteen children from the fifty-five did not have H.L.A. - B8, BW15 or BW18. This group of fourteen were equally divided into seven boys and seven girls. Although these diabetic children did not have the diabetogenic phenotypes H.L.A. - B8, BW15 or BW18, ten of the fourteen children presented with diabetes in the winter six months; five boys and five girls.

One particular phenotype appeared to be more frequent in this group, that was H.L.A. - BW40, where all four children with this B - antigen

Table 20

Seasonal onset of diabetes in twenty-nine children with H.L.A. - B8

	October - March	April - September	Total
Males	13	2	15
Females	7	7	14
Total	20 (69%)	9 (31%)	29

presented between October and March (three boys and one girl).

The children with H.L.A. - B8 phenotype were next assessed according to seasonal onset. Twenty-nine children, fifteen boys and fourteen girls, had this phenotype (Table 20). Thirteen of the fifteen boys presented between October and March, whilst the girls were equally divided with seven girls in each six month period.

Eleven diabetic children had H.L.A. - BW15 combined with H.L.A. - B8, BW18 or other B - antigens. There were four boys and seven girls, of whom all four boys presented with diabetes in the summer six months. Only four of the seven girls presented between October and March.

H.L.A. - BW18 was present in eight children, five boys and three girls. Four of the five boys and one of the three girls presented in the winter six months.

Summary of results

1. Fifty-five diabetic children (28 boys and 27 girls) were included in the H.L.A. phenotype study. The percentages in each age sub-group were similar to the percentages in the 10 year incidence study.
2. The commonest H.L.A. phenotype in the Sunderland diabetic children was H.L.A. - B8, 53%, compared to 24% in the control group. The sexes were almost equal, fifteen boys and fourteen girls. Almost half the children in each age sub-group had H.L.A. - B8 phenotype.
3. Other B - antigens which were increased above the control values were H.L.A. - BW15, BW18 and BW40.
4. 75% of the children had H.L.A. - B8, BW15 or BW18.
5. 64% of the diabetic children irrespective of H.L.A. phenotype presented with diabetes between October to March. There were forty-one children with the diabetogenic phenotypes H.L.A. - B8, BW15 and BW18 and twenty-five (61%) presented in the winter six

months.

6. Thirteen of the fifteen boys with H.L.A. - B8 presented between October to March, whilst the girls with H.L.A. - B8 were equally divided between a winter and summer onset.

7. All four children with H.L.A. - B40 presented in the winter six months.

Chapter 9

The height of Sunderland diabetic children at the onset of their illness

(i) Selection of diabetic and control children

As there were several conflicting reports on the height of diabetic children at the onset of their illness, a study of the Sunderland diabetic children appeared to be worthwhile, comparing them to the height of local children as well as the standard height values. If the results confirmed a tendency to tallness in diabetic children, it begs the question "Why are they tall?" I intend to see if there is any association between tallness in diabetic children and certain human leucocyte antigens.

There were forty-five diabetic children, twenty-one boys and twenty-four girls, aged 4 to 14 years, who presented during the 10 years of the study (January 1st 1967 to December 31st 1976) and were found suitable for inclusion in the height survey.

The children's height had been recorded at the time of diagnosis. No child was included if the height had been measured later than the date of diagnosis.

The age range 4 years to 14 years excluded the toddler group whose height measurement may not be precise. Also this age grouping could allow two sub-groups, each of five years to be analysed separately if the number of cases permitted it.

For comparison I used the height standards of Tanner and Whitehouse 1966. These standards are thought to be suitable for British children. "In many European countries, children growing up in villages in the countryside are smaller than those in big towns, but in England such a difference is very slight if present at all. Children in the north of England may be a trifle smaller than those in the south. These standards

Fig 8

THE
HARPENDEN
STADIOMETER

The "Harpenden" Stadiometer is a counter recording height instrument, with an effortless counter balanced movement. It will give an accurate and direct reading, to the nearest millimetre, over a range of 600 mm to 2100 mm.

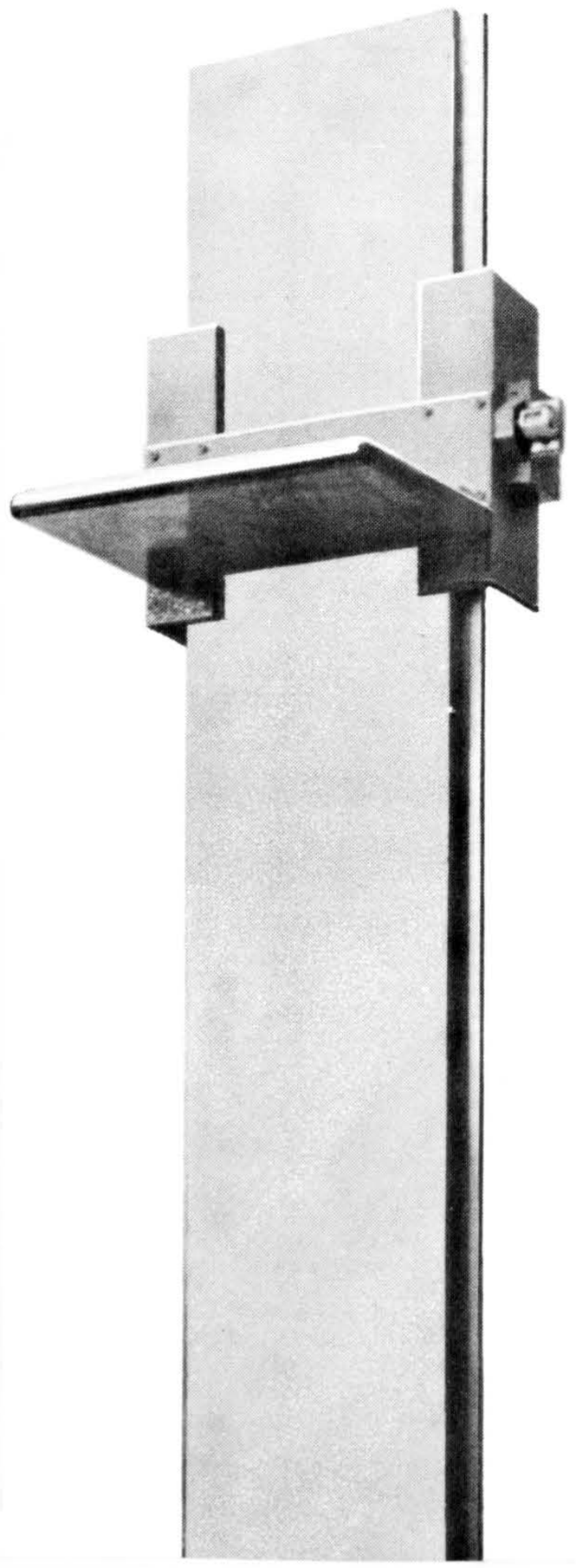


Table 21

TABLE OF DECIMALS OF YEAR

	1 JAN.	2 FEB.	3 MAR.	4 APR.	5 MAY	6 JUNE	7 JULY	8 AUG.	9 SEPT.	10 OCT.	11 NOV.	12 DEC.
1	000	085	162	247	329	414	496	581	666	748	833	915
2	003	088	164	249	332	416	499	584	668	751	836	918
3	005	090	167	252	334	419	501	586	671	753	838	921
4	008	093	170	255	337	422	504	589	674	756	841	923
5	011	096	173	258	340	425	507	592	677	759	844	926
6	014	099	175	260	342	427	510	595	679	762	847	929
7	016	101	178	263	345	430	512	597	682	764	849	932
8	019	104	181	266	348	433	515	600	685	767	852	934
9	022	107	184	268	351	436	518	603	688	770	855	937
10	025	110	186	271	353	438	521	605	690	773	858	940
11	027	112	189	274	356	441	523	608	693	775	860	942
12	030	115	192	277	359	444	526	611	696	778	863	945
13	033	118	195	279	362	447	529	614	699	781	866	948
14	036	121	197	282	364	449	532	616	701	784	868	951
15	038	123	200	285	367	452	534	619	704	786	871	953
16	041	126	203	288	370	455	537	622	707	789	874	956
17	044	129	205	290	373	458	540	625	710	792	877	959
18	047	132	208	293	375	460	542	627	712	795	879	962
19	049	134	211	296	378	463	545	630	715	797	882	964
20	052	137	214	299	381	466	548	633	718	800	885	967
21	055	140	216	301	384	468	551	636	721	803	888	970
22	058	142	219	304	386	471	553	638	723	805	890	973
23	060	145	222	307	389	474	556	641	726	808	893	975
24	063	148	225	310	392	477	559	644	729	811	896	978
25	066	151	227	312	395	479	562	647	731	814	899	981
26	068	153	230	315	397	482	564	649	734	816	901	984
27	071	156	233	318	400	485	567	652	737	819	904	986
28	074	159	236	321	403	488	570	655	740	822	907	989
29	077		238	323	405	490	573	658	742	825	910	992
30	079		241	326	408	493	575	660	745	827	912	995
31	082		244		411		578	663		830		997
	JAN. 1	FEB. 2	MAR. 3	APR. 4	MAY 5	JUNE 6	JULY 7	AUG. 8	SEPT. 9	OCT. 10	NOV. 11	DEC. 12

Chart prepared by J. M. Tanner and R. H. Whitehouse University of London, Institute of Child Health, for The Hospital for Sick Children Great Ormond Street. London, W.C.1.

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can be regarded as quite suitable for all English children." (Tanner, Whitehouse and Takaishi 1966). However, I thought it would also be more accurate to compare diabetic children in the Sunderland area with children from the same area. Therefore, during 1976 and 1977 I was able to measure 416 children, aged 4 to 14 years, attending the out-patient clinic of the Sunderland Children's Hospital. The 416 control children all attend local primary and secondary schools and none have any serious illness or handicap. They came from an almost similar social background as the diabetic children, namely social group II and III with smaller numbers from social groups I, IV and V.

(ii) Method of measuring height

Prior to January 1976, all the children, diabetic or otherwise, attending the out-patients were measured by using a wooden vertical rod graduated in inches. This rod was fixed to a wall and it had a sliding horizontal wooden bar which was gently lowered on to the child's head which was held in the erect position by gentle pressure under the mastoid processes. The child's height was therefore recorded in inches but for this study, the height was converted into centimetres.

From January 1976, all children were measured in centimetres using the Harpenden Stadiometer (Fig. 8).

(iii) Decimal age

In order to assess the children's height in relation to their age, all children were given a decimal age. The method used was that described in the Tanner and Whitehouse height and weight standard charts (1966). "Thus the year is divided in 10 not 12. Each date is marked (see Table 21) in terms of thousands of the year. Thus January 7th 1962 is 62.016. The child's birthday is similarly recorded, e.g. a child born on 23rd June 1959 has a birthday 59.474. The age of the child at the height examination is then obtained by simple subtraction:-

Table 22

Table showing the height of the control sample of 416 non-diabetic Sunderland children

Age Range (Years)	Number	Mean Height (cm.)	Standard Deviation (cm.)	Mean S.D.S.	Standard Deviation
Boys					
4.0 - 4.9	26	103.04	3.21	- 0.43	0.71
5.0 - 5.9	25	109.44	4.83	- 0.42	0.98
6.0 - 6.9	24	115.42	5.06	- 0.41	0.95
7.0 - 7.9	23	120.35	6.29	- 0.55	1.12
8.0 - 8.9	20	129.65	5.88	+ 0.13	1.01
9.0 - 9.9	25	130.80	6.86	- 0.57	1.12
10.0 - 10.9	21	134.90	7.64	- 0.68	1.19
11.0 - 11.9	24	141.46	7.96	- 0.47	1.15
12.0 - 12.9	17	145.35	5.77	- 0.66	0.77
13.0 - 13.9	10	149.10	6.33	- 0.95	0.78
Girls					
4.0 - 4.9	24	100.79	5.40	- 0.53	1.20
5.0 - 5.9	23	107.13	5.17	- 0.88	1.05
6.0 - 6.9	23	113.13	5.64	- 0.84	1.06
7.0 - 7.9	13	120.77	3.92	- 0.47	0.70
8.0 - 8.9	17	123.59	7.16	- 0.62	1.22
9.0 - 9.9	19	130.79	5.80	- 0.58	0.96
10.0 - 10.9	12	134.08	2.94	- 0.76	0.46
11.0 - 11.9	23	142.17	6.23	- 0.36	0.90
12.0 - 12.9	12	147.17	5.67	- 0.42	0.76
13.0 - 13.9	19	152.95	5.60	- 0.48	0.69

62.016 - 59.474 = 2.542. The figures can be rounded off for practical use."

(iv) Method of estimating control groups height values

The height and decimal age of 416 control children were entered on a Fortran computer sheet, boys and girls being entered separately.

The mean age for children in each year group, i.e. 4.0 to 4.9 years, 5.0 to 5.9 years and so on, was calculated and the standard deviation in years was also assessed (Table 22).

The mean height and standard deviation in centimetres of each year group was then determined.

In the majority of the year groups, the mean age was within 0.1 years of the 0.5 point and therefore the standard deviation scores (S.D. score) could be calculated using the Tanner tables of national values at the 50th centile level and the 0.5 point for each year group (Tanner, Whitehouse and Takaishi).

The standard deviation score (S.D.S.) is calculated from the following:-

Mean height of each year group minus the mean national height at relevant age divided by the S.D. at the relevant age group.

The degrees of significance between the Sunderland control children and the national values were then determined.

The Sunderland boys were found to be significantly shorter ($P < 0.05$) in nine of the ten age groups assessed and the girls were also significantly shorter ($P < 0.05$) in seven of the ten groups.

The age and height of the Sunderland boys and girls were also graphically computed and the national 50th centile for each sex was drawn on the computer graphs (Appx. 3a & b). Each asterisk represents one control child. A represents two children with an identical height and age, B three children, C four or more children. As can be seen from the

Table 23

The number of diabetic and control children within each height centile range using Growth & Development records (Tanner & Whitehouse 1966)

Males (4 - 14 years)

Centile Range	> 3	3-10	10-25	25-50	50-75	75-90	90 <	Total
non-diabetic Sunderland children	14 (6%)	33 (15%)	41 (18%)	55 (25%)	50 (22%)	21 (9%)	12 (5%)	226
diabetic Sunderland children	0	0	3 (14%)	6 (29%)	7 (33%)	3 (14%)	2 (10%)	21

Females (4 - 14 years)

Centile Range	> 3	3-10	10-25	25-50	50-75	75-90	90 <	Total
non-diabetic Sunderland children	15 (8%)	24 (13%)	44 (23%)	53 (28%)	37 (19%)	9 (5%)	8 (4%)	190
diabetic Sunderland children	0	2 (8%)	4 (17%)	7 (29%)	4 (17%)	7 (29%)	0	24

Total

Centile Range	> 3	3-10	10-25	25-50	50-75	75-90	90 <	Total
non-diabetic Sunderland children	29 (7%)	57 (14%)	85 (20%)	108 (25%)	87 (21%)	30 (7%)	20 (5%)	416
diabetic Sunderland children	0	2 (4%)	8 (18%)	12 (27%)	10 (23%)	11 (24%)	2 (4%)	45

graph, most of the Sunderland control children of both sexes fall below the national 50th centile line.

In order to confirm this finding that Sunderland children were shorter than the national average, I obtained the height of 100 children who had been examined by local authority clinical medical officers at school entry.

Fifty children (25 boys, 25 girls) came from one school in a high socio-economic area of Sunderland and the other fifty children were at school in a lower socio-economic area (social class IV and V).

The mean age of the children was 5.1 years at school examination and the mean height for boys at this age was 105.5 cm. and for girls 104.5 cm. These results are just above Tanner's 25th national centile value for each sex and confirms the above findings that Sunderland children are shorter than the national average.

(v) <u>The height of Sunderland diabetic children in relation to local control children and national values</u>

The individual diabetic child's height and decimal age are given in Appendix 4a & b. The children were then grouped into their centile height for their age and sex using the Growth and Development Record Chart (Tanner and Whitehouse 1966). The Sunderland control children were grouped in a similar manner and the diabetic and control children were then contrasted in relation to the national centile values for height.

64% of the control boys were below the national 50th centile value and 36% were above it. 43% of the diabetic boys were below the 50th centile whilst 57% were above this value (Table 23).

72% of the control girls and 54% of the diabetic girls were below the 50th centile whilst 28% of the control girls and 46% of the diabetic girls were above this level.

As a group the diabetic boys and girls were evenly spread above and

Table 24

The mean height (m.h.) and mean centile value (m.c.v.) of diabetic and control children

	Diabetic m.h.	m.c.v.	Control m.h.	m.c.v.
Males				
4 - 9 years	120 cm.	55th	115.5 cm.	28th
9 - 14 years	146 cm.	60th	140 cm.	25th
4 - 14 years	133 cm.	58th	128 cm.	25th
Females				
4 - 9 years	116 cm.	48th	113 cm.	25th
9 - 14 years	145 cm.	50th	141 cm.	25th
4 - 14 years	130 cm.	49th	127 cm.	25th

Table 25

Diabetic children in relation to national 25th centile

	Below 25th centile		Above 25th centile		Total
Boys	3	(14%)	18	(86%)	21
Girls	6	(25%)	18	(75%)	24
Total	9	(20%)	36	(80%)	45

below the national 50th centile for height with twenty-three diabetic children (51%) above the 50th centile and twenty-two children (49%) below it (Appendix 5a & 5b).

The diabetic and control children were then divided into two age groups; 4 to 9 years, and 9 to 14 years. The mean height and mean centile values were calculated for each group and overall, i.e. 4 to 14 years (Table 24). The mean height of the diabetic children in the two age groups and overall was considerably taller for both diabetic boys and girls when compared to the control children.

The mean centile value for the control boys and girls aged 4 to 14 was 25th centile. The diabetic boys mean centile value was 58 and the diabetic girls were in the 49th centile level.

Unfortunately, the number of diabetic boys and girls were too few to obtain a reliable test of significance on the height in the individual sex groups. However, when the diabetic boys and girls were grouped together the number of children became large enough to compare them with the control children using the chi squared test of proportions. The diabetic children were found to be significantly taller than the control children ($P < 0.01$).

As the Sunderland control children's mean centile value was the 25th centile, the diabetic children were compared with the local mean centile value (Table 25). Eighteen of the twenty-one diabetic boys were above this level whilst three were on or below the 25th centile (one boy, 4 - 9 years; and two boys, 9 - 14 years). Eighteen of the twenty-four diabetic girls were above the 25th centile and six girls were on or below this value (four girls, 4 - 9 years; and two girls, 9 - 14 years).

When the diabetic boys and girls were grouped together, thirty-six (80%) were above the 25th centile in height and only nine (20%) were on or below this value.

Table 26

Standard deviation score of Sunderland diabetic children age 4 to 9 years

$$\text{S.D.S.} = \frac{\text{mean height of diabetic children} - \text{mean height of controls}}{\text{standard deviation}}$$

Diabetic boys

4 - 9 years $\quad \dfrac{119.8 \text{ cm.} - 115.5 \text{ cm.}}{5.02} = +0.86$

Diabetic girls $\quad \dfrac{115.7 \text{ cm.} - 113.0 \text{ cm.}}{5.40} = +0.50$

4 - 9 years

(vi) The standard deviation score (S.D.S.) of the Sunderland diabetic and control children

The standard deviation score (S.D.S.) for each year of age for Sunderland control children was calculated using the mean height of the local children minus the mean national value at that age divided by the standard deviation at the relevant age (Table 22).

The mean S.D.S. for the Sunderland control children varied from -0.36 to -0.95 except for the eight year old boys (+0.13). The S.D. score for the control children also had a standard deviation approximating to 1.0, which indicates that the population from which they come is normally distributed (Tanner, Whitehouse, Hughes and Vince).

The standard deviation score for the diabetic children in the height study was then calculated. The children were grouped by sex into the age range 4 to 9 years and compared to the mean height and standard deviation of the Sunderland control boys and girls using the above formula (Table 26).

The diabetic boys (4 to 9 years) S.D.S. was +0.86 and the girls (4 to 9 years) S.D.S. was +0.50. These scores confirm the tallness of diabetic boys and girls at the onset of their illness, compared to local children.

The standard deviation score for the older diabetic children age nine to fourteen was not calculated and the reason for this will be discussed later.

(vii) The height of diabetic children in association with Human Leucocyte Antigens (H.L.A.)

From the H.L.A. study in Chapter 8 it was found that 75% of the diabetic children had the diabetogenic H.L.A. phenotypes, H.L.A. - B8, BW15 or BW18.

As the present height study found that the diabetic children in Sunderland were significantly taller than local healthy children, I was

Table 27

Sunderland diabetic children's H.L.A. phenotype compared to the local mean height (25th national centile)

	Diabetic boys		Diabetic girls	
	with HLA B8, BW15 or BW18	without specific phenotypes	with HLA B8, BW15 or BW18	without specific phenotypes
Above local mean height	14	4	12	5
Below local mean height	2	1	3	1
Total	16	5	15	6

Table 28

Diabetic children's average height above the local mean height (25th national centile)

	with HLA-B8 BW15 or BW18	without specific phenotypes
Boys 4 - 9 years	+ 4.2 cm	+ 3.5 cm
9 - 14 years	+ 5.1 cm	+ 7.0 cm
Girls 4 - 9 years	+ 2.4 cm	+ 4.3 cm
9 - 14 years	+ 1.3 cm	+ 11.0 cm

interested in finding out if tallness in diabetic children could be associated with a particular H.L.A. phenotype.

Forty-two of the forty-five children in this height survey were available for H.L.A. testing. There were twenty-one boys and a similar number of girls.

The children were grouped into those with H.L.A. - B8, BW15 and BW18 phenotype and those without these antigens.

Sixteen of the twenty-one boys had H.L.A. - B8, BW15 or BW18. Fourteen of them were above the local mean height. Similarly, four of the five boys without these specific antigens were also above the local mean height (Table 27).

Twelve of the fifteen girls with H.L.A. - B8, BW15 or BW18 were above the mean height for Sunderland girls and in similar manner to the boys, five of the six girls without these antigens were also above the local mean height.

I then estimated the mean height of the diabetic boys and girls with H.L.A. - B8, BW15 and BW18 and those without these antigens and grouped them into age groups, 4 - 9 years and 9 - 14 years. The groups were then compared to the local mean height (25th national centile). The diabetic children without the specific antigens H.L.A. - B8, BW15 and BW18 in the age groups boys 9 - 14 years and girls 4 - 9 years and 9 - 14 years were taller than the children who had the diabetogenic phenotypes (Table 28).

When the diabetic children with their individual phenotypes are grouped into the centile height channels, a more detailed analysis of the height of the diabetic child in relation to H.L.A. - B phenotype is possible (Table 29).

There are eleven girls with H.L.A. - B8, eight are below the 50th centile in height, whilst most of the boys are evenly spread around the 50th centile. Only two boys are above the 75th centile.

The nine children with H.L.A. - B12 (4 boys and 5 girls) are all tall

Table 29

Diabetic children's centile height and HLA phenotype

Boys

	3 - 10	10 - 25	25 - 50	50 - 75	75 - 90	90
4 - 9 years		B35 BW40	B8 B8 B5 B8 B8 BW15	B7 B8 B8 BW21 B5 B8 B7 BW15	B7 B12	B12 BW15
9 - 14 years		B8 BW40 B18 BW40	B8 B8 B5 BW17	BW18 BW35 B7 B8 B12 B12	B8 BW39	B7 BW40 B8 BW35 B12 BW18
Total	0	3	5	7	2	4

Girls

	3 - 10	10 - 25	25 - 50	50 - 75	75 - 90	90
4 - 9 years			BW15 BW18 B8 BW27 BW35-35 B8 B12 B8 B27	B12 B21 B8 BW40	B7 BW18 B8 BW15 BW40-40	
9 - 14 years		B7 B8 B8 BW15	BW18 B40 B8 BW35 B8 BW15 B7 B8		B12 BW39 B7 B27 B7 B12 B8 BW18 B12 BW15	
Total	0	2	9	2	8	0

except for one young girl, age 4 - 9 years, who is below the 50th centile and has H.L.A. - B8 phenotype in association with B12.

There are ten children (5 boys and 5 girls) with H.L.A. - B7 phenotype. The two girls with H.L.A. - B7 B8 are below the 50th centile, whilst the remaining three girls with H.L.A. - B7 BW18, B7 B27 and B7 B12 were all in the 75 - 90th centile height range. Two B7 B8 boys and one B7 BW15 are in the 50 - 75th centile channel and another two boys, B7 B12 and B7 BW40 are above the 75th centile.

There are eight girls in the 75 - 90th centile range and only two have H.L.A. - B8 phenotype. The other six girls are H.L.A. - B7 or B12. Similarly, from the six diabetic boys in the 75 - 90th and 90th channels, four are H.L.A. - B7 or B12 whilst two are H.L.A. - B8.

In the 'short' height channels, three boys are in the 10 - 25th centile and all three are H.L.A. - BW40. However, there is a fourth and last boy with H.L.A. - BW40 whose height is on the > 90th centile but his phenotype is B7 BW40.

Summary of results

1. The Sunderland children age 4 to 14 years, as determined by the control height study, revealed that both boys and girls were significantly shorter than the national 50th centile value.
2. The diabetic children at the onset of their illness were significantly taller than the control children. However, when compared to the national 50th centile, the diabetic boys and girls were grouped symmetrically about the 50th centile and therefore were not significantly taller than the national average.
3. The diabetogenic H.L.A. phenotypes H.L.A. - B8, BW15 and BW18 do not appear to be associated with the tendency to tallness found in Sunderland diabetic children; however, diabetic children with other B phenotypes were taller than children with H.L.A. - B8, BW15 or BW18.

PART C

The analysis of the Sunderland diabetic study and a comparison with published work

Chapter 10

The analysis of the Sunderland diabetic study and a comparison with published work

(i) Analysis of the incidence, age, sex and seasonal onset of diabetic children

One of the major problems when assessing the incidence of a particular illness in a local area, is the ability to estimate with reasonable accuracy, the base population. The difficulty lies in the fact that hospital records of a particular illness may not correspond to local borough or district boundaries, as hospital catchment areas vary enormously.

The base population for the Sunderland diabetic study did require a certain amount of readjustment. Apart from using the mean of the number of births occurring in the Sunderland County Borough during the ten year period, I also estimated that half the births at a smaller hospital, south of Sunderland (Thorpe Maternity Hospital, Peterlee) should be incorporated into the base population, as these children are in the Sunderland hospital catchment area, covering towns such as Seaham, Murton, Easington and part of Peterlee. This counterbalances the births in the Washington area which are included in the Sunderland birth rate figures but whose children tend to be centred on Gateshead for their hospital services.

The population of children under fifteen years of age in the survey also had to be readjusted to take into account the local catchment area in relation to the number of children in the Sunderland Metropolitan District.

When the yearly incidence of juvenile-onset diabetes is studied in the Sunderland area, there seems little doubt that the incidence has increased in the second quinquennium (Table 30).

Table 30

Yearly incidence of juvenile-onset diabetes in Sunderland children under 15 years of age from 1967 to 1976

First Quinquennium

	1967	1968	1969	1970	1971	Total
	M. F.	M. F.	M. F.	M. F.	M. F.	M. F.
	4 2	2 3	1 2	1 3	2 3	10 13
Total	6	5	3	4	5	23

Second Quinquennium

	1972	1973	1974	1975	1976	Total
	M. F.	M. F.	M. F.	M. F.	M. F.	M. F.
	5 4	6 8	10 2	10 4	12 11	43 29
Total	9	14	12	14	23	72

Total in 10 years 1967 - 1976 = 95 diabetic children.
First Quinquennium 1967 - 1971 = 23 children.
Second Quinquennium 1972 - 1976 = 72 children.

During the first five years, the number of children and the sexes are very similar. In the sixth year of the survey, there is a slight increase to nine cases per year, followed by an increase to between twelve and fourteen patients during the next three years. In the tenth and final year, twenty-three children developed diabetes in the Sunderland area.

A similar type of increase in the incidence of children's diabetes was reported from Northampton in 1960 and 1964; and in Israel, Cohen (1971) found an increase in successive quinquennia between 1947 and 1961.

The incidence rate in Sunderland of 2.5 per 1000 children under 15 years compares favourably with the National Survey of Health and Development incidence level of 3 per 1000 at 16 years of age (Wandsworth and Jarrett 1974). In contrast, the National Child Study group (Calnan and Peckham 1977) reported an incidence of 1.42 per 1000 at sixteen years from a group of 15,500 children born in 1958. These two studies were national surveys whilst the Sunderland and Northampton studies were local projects, reflecting the local incidence of diabetes.

There is little doubt that although national studies can give an overall picture of an illness, regional variations do exist and this was shown in the Register of Newly Diagnosed Diabetics (1975) where there were clear variations in the incidence rates for children under fifteen years, in various parts of Britain. The regional variation in juvenile diabetes may be due to a combination of factors, such as different genetic backgrounds, varying standards of living and the exposure and response to infections in local areas.

The Sunderland study revealed slightly more boys (56%) than girls (44%) developed diabetes in the ten years 1967-76. These results are very similar to the British "Register" (53% boys and 47% girls) and Cudworth's Liverpool study (59% boys and 41% girls).

During the first quinquennium in Sunderland, slightly more girls than boys presented with diabetes but in the second five years far more boys than girls developed the illness, 43 boys to 29 girls. The base population and the sex ratio remained fairly constant in the Sunderland area during the ten years, hence the increase in young male diabetics in the second quinquennium is not due to more boys being born during that time and is obviously quite significant.

When the sexes are analysed on a yearly basis in Sunderland, the difference between the number of boys and girls presenting each year with diabetes varied very little, except for 1974 and 1975, when there were ten diabetic boys in each year compared to two and four girls respectively. Three-quarters of the twenty boys developed their diabetes in the October to March period whereas the six girls were equally divided throughout the year. It could therefore be argued that the boys during these two years were more affected than girls by environmental factors.

One of the theories put forward by Gamble and his colleagues (1969) is that juvenile diabetes may be provoked by virus infection(s). It may be that boys who only have one X chromosome may have a greater susceptibility to infection, both bacterial and viral. Although male patients with dysgamma-globulinaemia appear to respond to virus infections with a normal antibody response in contrast to bacterial infection.

I also think that the 'infection' theory in susceptible children is further borne out by the results of the age of the child at the onset of their diabetes. Very few cases of diabetes occurred in the Sunderland area before the third birthday. After this age, many toddlers start play group or nursery school and mix outside in play; consequently there is a higher risk of exposure to infection.

In the Sunderland study there was an increase in the number of children with diabetes in their fifth and sixth year, corresponding with the

first two years of infant school and there is a further increase in the number of diabetic children at the age of eight when the children enter junior school and a further rise in the eleventh and twelfth years when children start senior school. It is feasible that on each of these occasions, certain children are put at greater risk by a changing environment and exposure to infections.

These results vary slightly from the Register of Newly Diagnosed Diabetics (1975) where the peak age range was between ten and twelve years. The Sunderland results were more similar to Cudworth and his Liverpool colleagues who reported a peak age of eleven years in their study (1977) with a smaller increase at seven years of age.

After the age of eleven or twelve, the Liverpool and Sunderland studies both revealed a sharp decline in the number of cases at thirteen, fourteen and fifteen years of age. One criticism of diabetic surveys is the difficulty in ensuring that correct figures are given for teenage diabetics, as they may be admitted either to a paediatric or an adult ward. However, both the Sunderland and Liverpool studies included teenage children seen in all Units. Nancy Simpson's (Toronto) study in 1962 revealed a similar decline in young teenage diabetic patients. There now seems little doubt that the fall in new diabetic patients from the age of thirteen onwards is a genuine decline in the illness and is not due to lack of information and co-operation between various departments.

When the children were divided into age subgroups, I was able to compare the Sunderland results with figures from Simpson's Toronto Study and the Register of Newly Diagnosed Diabetics. In the younger 0 - 4 year age range, all three studies revealed more boys than girls. However, in the 5 - 9 year group, Simpson and the "Register" found more girls than boys, whereas in the Sunderland study slightly more boys than girls had diabetes. The older 10 - 14 year group showed very similar results

between Simpson's study and the Sunderland survey, where the sexes were almost equal. The "Register" on the other hand found more boys than girls.

Most studies therefore tend to agree that in the younger 0 - 4 year group diabetic boys predominate (in Sunderland twice as many boys as girls) and this could be due to the increased susceptibility of young males to infection. All three studies revealed a marked increase in the number of girls with diabetes in the two older groups, where girls are either in excess or almost equal the boys in number.

The Liverpool survey (Cudworth and colleagues 1977) studied the number of children and their sex at each year of age and I was able to compare these figures with the Sunderland results and found a similar increase in the number of cases at seven years (Liverpool), eight years (Sunderland) and eleven (Liverpool and Sunderland). In all these peak age ranges, girls predominate and it is probable that one of the provoking factors could be the adrenarche in the younger girls and puberty in the older girls. In both studies, boys showed an increase in numbers compared to girls, at twelve (Sunderland)and thirteen years of age (Liverpool). The increase at this time could be due to male puberty which occurs later than in females.

Since Gamble (1973) reported that a higher percentage of new young diabetics presented with their illness during the winter months, October to March, further studies by Cudworth (1977) and Rolles, Rayner and Mackintosh (1975) have confirmed this winter-onset tendency. The Sunderland study certainly found more children (59%) presenting with their diabetes in the October to March six months.

Throughout the year from the January quarter with twenty-four cases, there was a steady decline into the spring with the least number of patients in the late summer quarter, July, August and September with

Table 31

The month of onset of diabetes in Sunderland children 1967-76

MONTH	JAN.	FEB.	MAR.	APR.	MAY.	JUN.	JLY.	AUG.	SEP.	OCT.	NOV.	DEC.	TOTAL
MALE	5	4	3	2	6	2	3	2	5	10	6	5	53
FEMALE	5	4	3	4	5	2	3	2	3	3	6	2	42
TOTAL	10	8	6	6	11	4	6	4	8	13	12	7	95

Number of patients in each quarter of the year

	Jan. - March	April - June	July - Sept.	Oct. - Dec.
	12 M. 12 F.	10 M. 11 F.	10 M. 8 F.	21 M. 11 F.
Total	24	21	18	32

Number presenting in six month periods

Oct. - March	April - Sept.
33 M. 23 F.	20 M 19 F.
56 (59%)	39 (41%)

eighteen cases. There is then a marked increase in the last quarter of the year with thirty-two cases. Throughout the earlier three quarters of the year the sexes were about equal but in the latter quarter twice as many boys as girls developed diabetes. This increase in the number of cases in the winter period, October to December, would support the "infection" theory particularly for boys rather than girls.

During most months of the year, the sexes are almost equal in number and only in October and December is there much variation, when boys exceeded girls (Table 31). This increase in boys in the October to December period accounts for the fact that twice as many boys as girls developed diabetes at that time of the year.

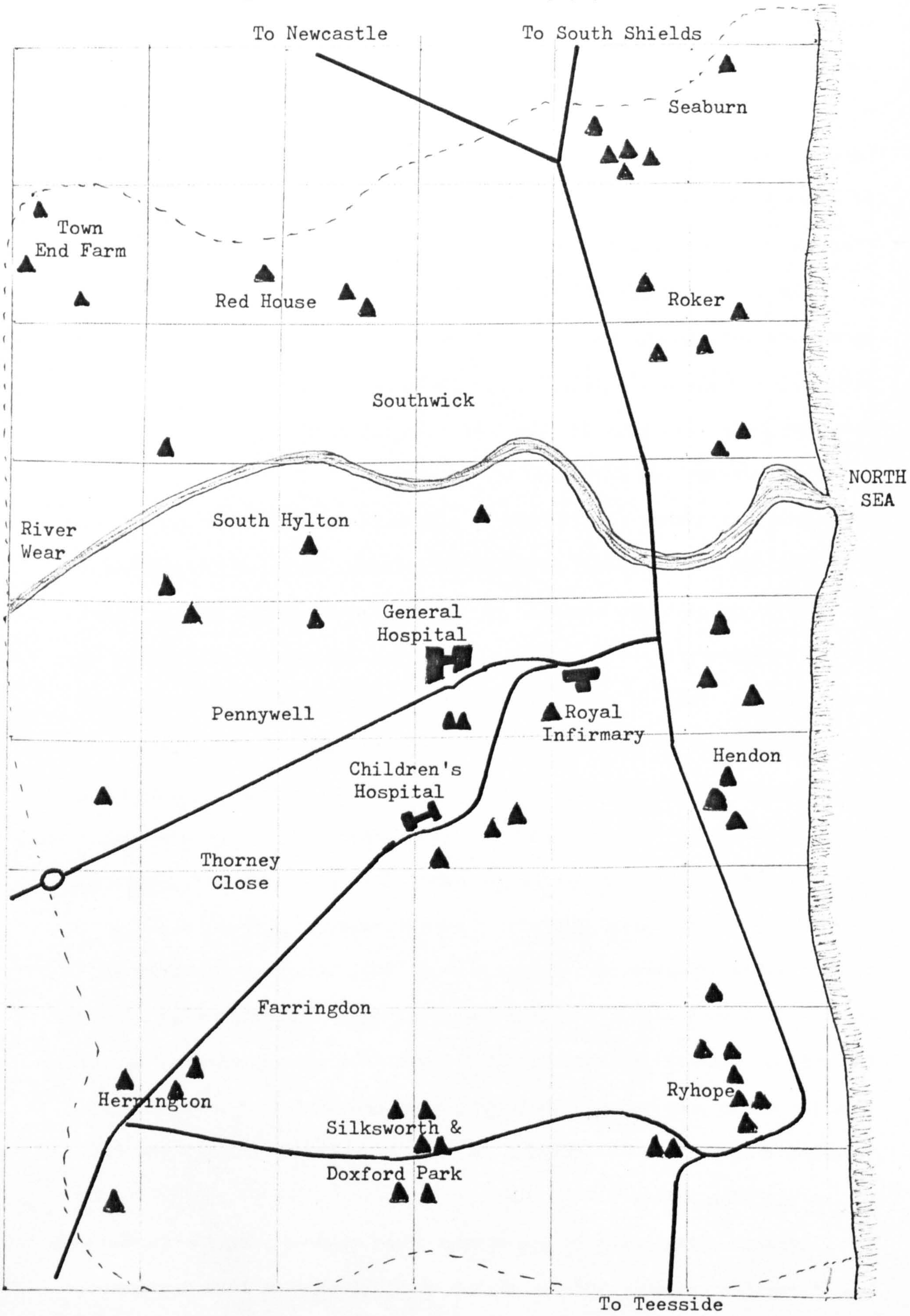
One interesting result that is difficult to explain in the monthly onset figures is the sudden increase in diabetic children who presented in May. There were six boys and five girls. Four of the six boys were in the very young 0 - 4 year group, and there was one in each of the two older groups. None of the five girls were in the 0 - 4 age range, two were 5 - 9 years and the remainder were in the 10 - 14 year group.

When the children were divided into three age groups, the Sunderland study revealed that the younger 0 - 4 year group had a greater tendency to a summer onset (April to September) whilst the older children, especially 10 - 14 years had a very significant winter tendency. These results closely followed those of the Register of Newly Diagnosed Diabetic Children (1975) when a winter tendency was associated with increasing age of the child. None of the surveys published so far have mentioned the difference in sex in connection with onset. The Sunderland study certainly revealed a more variable seasonal onset in relation to the age and sex of the diabetic child.

Diabetes developing in the winter time would be more in keeping with the "infection" theory, but the summer onset in younger boys is more

Fig 9

Map showing diabetic children's place of residence
in the town of Sunderland 1967-76



Squares = National grid 1 km.

difficult to ascribe to this theory. However, it could well be that diabetes in both the younger and older boys is triggered off by a virus infection but the immune response mechanism in the younger child may produce a varying time reaction in the onset of diabetes.

This variation in seasonal onset is not as marked in girls. This could suggest that the provoking factor(s) in the two sexes is different. The lack of seasonal onset in girls and the striking association with the adrenarche and menarchy could indicate an endocrine stress factor as the basis of the illness.

(ii) Analysis of clustering in juvenile-onset diabetes

The Sunderland study demonstrates that the diabetic children 'clustered' in certain areas of the town during the ten year period of the survey. This is particularly evident when each recorded case is marked on a map of Sunderland. The map (Fig 9) shows areas where patients with juvenile diabetes are concentrated and other areas which are lacking in cases of diabetes in childhood. This visual impression of clustering in certain parts of the town would suggest that environmental factors such as infection could be one of the provoking factors in juvenile-onset diabetes. In retrospect, it would have been very helpful to have arranged virology studies, particularly for Coxsackie viruses, when the children first develop their diabetes. However, as the study covered a ten year period, the major problem is whether results taken from these blood tests in 1977 would have enabled firm conclusions to be drawn from the results.

Apart from clustering of diabetes suggesting an environmental-infective basis, it was interesting to note that the clustering of diabetic children was mainly limited to the higher socio-economic housing areas.

I am sure that the town of Sunderland is similar to other large towns where housing areas can be divided into social class districts. These vary from the exclusive executive estates or certain streets of large older

houses where social class I and II families reside. The next category of housing appears to be the less expensive private housing estate or older house for social group III as well as the new high rental, rather exclusive council estates for the highly skilled craftsman. The last housing group is the older council estates or very old rented property, often in flats, for social group IV and V.

Relating these socio-economic housing groups to the diabetic children we find a large cluster of children in the Seaburn-Roker area of Sunderland residing on a private housing estate or the older large private houses near the seafront. On the Seaburn estate three children living within 200 metres of each other developed diabetes between March and October 1967. This could be the result of an infective illness on the estate or the local school. The second wave of children to develop diabetes on this estate occurred between 1973 and 1976 when three more children developed the illness. It is possible that these three children or even all six children developed their diabetes from the same infection in the community but the time taken for the diabetes to develop varied in each child. The three children in the Roker area, just south of the Seaburn estate, all developed their diabetes in the five month period between October 1975 and February 1976. These children live in large, older private houses within 0.5 km. of each other. Two of the three children were at the same school.

This north-east part of Sunderland bordering the seafront from Seaburn to Roker near the mouth of the River Wear produced twelve diabetic children in the ten year study. The three primary schools in the area supply children for one larger comprehensive school, 'Monkwearmouth', and the total school population forthese four schools is 4100 (4 - 18 years).

In contrast to the high social class area in the north-eastern part of Sunderland, there is an area of lower social class housing in the

northern part of the town, comprising Southwick and the Red House council estate. In this area of old rented property and older council houses there are 6600 school children and only three diabetic children were diagnosed in the ten year survey.

Another clustering situation occurred in Ryhope in the south-eastern part of Sunderland. Eight children developed diabetes between 1972 and 1976. Although the time factor is spread over four years, the children all lived very near each other on one of the better council estates. In Ryhope there are 3170 school children. None of the diabetic children came from the older terraced property in Ryhope.

Areas such as Seaburn and Ryhope stood out on the map in marked contrast to other areas in Sunderland where few cases of juvenile diabetes occurred such as in Southwick and Red House. Other examples of areas with a low incidence of juvenile diabetes is the older Thorney Close council estate, near the Children's Hospital. No diabetic child was recorded from this estate in the ten year study, whilst in an adjoining estate, Pennywell, only one child developed diabetes in the ten years. The total number of school children on these two estates if 5940. Conversely, on a new high-rental council estate, Doxford Park, which is very popular for families of skilled workers, three diabetic children presented in 1973 and 1975 from a school population of 550.

Variations in the number of cases of children with diabetes also occurred in smaller towns outside Sunderland. A marked contrast was noted between two adjacent populations of Seaham and Murton. Seaham produced ten diabetic children between 1967-76, whilst no diabetic child was recorded from Murton during the ten year survey.

It would certainly appear that specific areas of the town of Sunderland have a higher incidence of children's diabetes and more patients originate from the "better housing areas". I was able to confirm this impression by

Table 32 a

Social class distribution (percentage)

Social class	I	II	IIIn	IIIm	IV	V
National 1971 census	3[illegible].6	17.8	21.1	28.5	20.9	8.2
Sunderland ward census 1976	2.6	13.1	18.0	37.1	19.0	10.2

Table 32 b

Social class % - Sunderland wards

Social class	I	II	IIIn	IIIm	IV	V
Seaburn	4.5	27.6	30.9	27.2	7.7	2.1
Silksworth/Doxford	1.5	12.0	20.7	41.0	15.6	9.2
Ryhope	2.3	10.5	18.3	38.0	18.5	12.3
Thorney Close	0.7	8.2	16.3	38.0	20.8	15.7
Southwick	0.0	3.4	14.1	44.0	22.6	16.0

Table 32 c

Social class of Sunderland diabetic children

Social class	I	II	IIIn	IIIm	IV	V
%	10.9	21.7	7.6	39.7	11.9	8.7

comparing the social class structure nationally (1971 census) with the socio-economic status in Sunderland, taking the statistics from a 1976 ward census conducted by the Programme Planning Department of the Sunderland Borough Council.

The local socio-economic status does show some variation from the national figures (Table 32a). Sunderland have fewer families in social class I, II and non-manual III compared to the national figures but we do have a higher proportion of skilled manual III. The semi-skilled (IV) and unskilled (V) are, however, closer to the national level.

When individual council wards are examined in Sunderland, the areas with larger numbers of diabetic children; Seaburn, Silksworth and Ryhope, all have a higher percentage of families in social class I, II and non-manual III, compared to areas such as Thorney Close and Southwick, where few diabetic children occur (Table 32b).

An analysis of the social class of ninety-two of the ninety-five Sunderland diabetic children in shown in Table32c. Social class I and II account for 33% of the Sunderland diabetic children; 47% are from class III (manual and non-manual), whilst 20% are from classes IV and V. These results would confirm the impression that diabetic children originate from houses with a higher standard of living and could also account in part for the impression of "clustering".

The concentration of diabetic patients in certain areas compared to areas with few cases would appear to confirm the theory of clustering. However, the Knox-Space-T programme is trying to relate one case with another in relation to a certain short period of time, e.g. 30 or 60 days and also considering the distance in kilometres between them. The results of this analysis did not prove that clustering of diabetic cases existed during the ten year period of the survey but the Space-T programme is unable to assess the overall visual impression of "grouping" which is

easily detected when cases are marked out on a map of the area.

Mann, Thorogood and Smith (1978) when analysing 106 diabetic children under 16 years also concluded that the Knox-Space-T programme did not suggest clustering in the Oxfordshire area. However, when they divided Oxfordshire into its five administrative districts and compared the number of cases in each district, a significant difference was found. The districts were then separated into parishes (total 344) and pairs of cases in each parish were compared, taking into account the expected number of pairs. The results showed a significant clustering of pairs in the parishes of one of the five districts. Mann, Thorogood and Smith concluded that their finding is certainly compatible with some local environmental cause producing the disease in that area.

The Oxford work would help to confirm the Sunderland study which found clusters of diabetic children in certain parts of the town. Further prospective studies would be interesting and combined with blood virology tests would assist in clarifying this problem.

(iii) <u>Analysis of the family history in juvenile diabetes</u>

Most family history studies in diabetes have only analysed information relating to first degree relatives. This survey deals with the extended family history as well as first degree relatives with insulin dependent diabetes.

Inquiries into an extended family history are naturally fraught with the problem of personal knowledge about members of the family and doubt still exists in the mind of the investigator that the information imparted to him is correct. Fortunately, diabetes is an illness of which most laymen have some knowledge and they appear to know if a relation is having insulin injections or not.

As far as possible every effort was made to determine that the individual mentioned in the extended family history and first degree relative

study was an insulin dependent diabetic.

The Sunderland study revealed that 41% of the diabetic children had an extended family history of the illness. This figure is very similar to Hutchinson's statement that 40% of diabetic children may have a family history of diabetes (1975).

An extended family history was found in almost half the Sunderland diabetic girls (45%) and one third of the boys (37%). These figures are well in excess of the control group of 20% boys and 18% girls with a family history of diabetes. These results in the diabetic and control children would suggest that diabetes is a fairly common illness in the general population but they would also indicate that diabetes is more common in certain families.

When first degree relatives are considered, the Sunderland study of 7% compare exactly with Nancy Simpson's result in Toronto. However, Simpson's work was done sixteen years ago and as diabetes is increasing in western civilised countries the Toronto figure could now be out of date. It is also probable that there are regional variations in the "family history" studies just as there are in the "incidence" studies. For example, the Sunderland level of 7% is below other British reports. Oakley and his colleagues (1968) found that 13% diabetic children had a first degree relative, whilst the Register of Newly Diagnosed Diabetics (1975) gave a figure of 11%.

The low incidence of first degree relatives in children with diabetes is very interesting. If the illness has a genetic-family relationship then I would expect a higher incidence than 7% in first degree relatives, as presumably their genetic factors are spread throughout the immediate family of parents and siblings.

Several reasons could be advanced for other members of the family not developing diabetes. All members of the family may not have been exposed to the same environmental factors which may have "triggered off" diabetes

in the young patient in their family. Although the genetic make-up may be present in the non-diabetic members of the immediate family, it may be present in a slightly altered form and/or the stimulus required to provoke diabetes may not be of the required level. It is also possible that if families are studied for a longer period of time, then other members may eventually develop diabetes.

These theories could well be borne out by the fact that out of fifty-seven diabetic boys, only three fathers but no mothers have the illness. Two diabetic boys in the survey each had a brother with diabetes. None of the boys had a sister with the illness.

In a similar fashion, from the fifty-three diabetic girls, two pairs of sisters were recorded. None of the girls had a diabetic parent or brother.

It would appear that despite the fact that a family may have the same genetic make-up, certain families, for various reasons, environmental or genetic, may have a diabetic tendency in favour of one sex rather than the other.

There is little doubt that since H.L.A. antigens have been studied in juvenile insulin dependent diabetics, the genetic pattern of the disease is better understood. The Sunderland study showed a definite tendency for children with the H.L. Antigen B8, BW15, BW18 to have an extended family history of diabetes compared to children without these antigens. This tendency was very much greater in girls (P.00165) than boys (P0.57).

Cudworth and Woodrow (1975) analysed the genotypes of seventeen families with two or more siblings with diabetes. Ten families had inherited identical haplotypes, four had inherited one identical haplotype and in one family the affected siblings had not inherited a common haplotype.

This study suggested that the pattern of zygotic association of H.L.A. haplotype in siblings with juvenile diabetes differed significantly from that expected, had the zygotic assortment been randomly distributed.

There was a striking increase in the number of siblings in which both haplotypes were identical and a marked reduction in those members of the family with neither haplotypes identical.

The two Sunderland families studied, both had two affected siblings and the H.L.A. - phenotype results follow a similar pattern to the Liverpool study. The four diabetic children (all girls) in the two families had inherited the haplotype H.L.A. - B8; in one family from father and in the other from mother. It was very interesting to note that in both families there was an unaffected brother. In family A, brother was H.L.A. - B7 B8, whilst his diabetic sisters were both H.L.A. - B8 BW35. However, in family B, brother was H.L.A. - B8 BW17 and one diabetic sister had the same phenotype, whilst the other sister was H.L.A. - B8 BW22.2.

It would certainly appear appropriate that a locus in the H.L.A. - chromosome region constitutes a major component of the genotype of juvenile-onset diabetes. However, the fact that siblings of affected parents do not develop diabetes indicates two other sources of variability. It may be that other genes may be needed to interact with H.L.A. - linked genes in order to acquire the necessary threshold to develop diabetes and secondly environmental factors such as infection most probably initiate the illness in susceptible individuals. It is also possible that non-diabetic siblings may develop the illness when they are older.

(iv) <u>An analysis of H.L.A. phenotypes in juvenile-onset diabetes</u>

Several diseases have been described where the incidence of H.L.A. - B8 phenotype has been increased above normal control levels. Examples of such diseases are Ragweed hay fever, adult coeliac disease, dermatitis herpetiformis, Grave's and Addison's disease. Juvenile-onset diabetes mellitus also appears to fall into this category.

In order to determine if H.L.A. - B8 phenotype was increased in

Sunderland diabetic children, fifty-five diabetic children were selected from the original ninety-five children in the ten year incidence study.

In both the H.L.A. group of children and the ten year study group, boys were slightyin excess of girls.

Ten year study	56% boys	44% girls
H.L.A. study	51% boys	49% girls

The proportion of children in the three age subgroups were also similar in the H.L.A. and ten year study.

In 1974, Nerup reported from Denmark an increase in H.L.A. - B8 phenotype in juvenile diabetes. Later in the same year, Cudworth and his colleagues from Liverpool found 54% of their juvenile diabetics under 30 years of age had H.L.A. - B8 phenotype compared to 32% controls. The Sunderland study detected H.L.A. - B8 in 53% of diabetic children compared to 24% controls. The percentages of diabetics with H.L.A. - B8 in Liverpool and Sunderland are very similar but the control populations interestingly shows a difference of 8% in the two areas which could indicate a regional variation in the hereditary background between the West and East coasts of England.

In the Midlands, Rolles, Rayner and Mackintosh in 1975 found 46% of diabetic children had H.L.A. - B8 phenotype but unfortunately no control values were given.

The Sunderland H.L.A. - B8 results certainly confirm a significant increase compared to the local control population and would strongly support the Danish, Liverpool and Birmingham studies which have shown an increase in H.L.A. - B8 phenotype in juvenile-onset diabetes.

It is reasonable to assume from all these studies that at least half juvenile-onset diabetics will have H.L.A. - B8 phenotype.

There were twenty-nine Sunderland diabetic children with H.L.A. - B8 phenotype; fifteen boys and fourteen girls. This would suggest that the

B-8 phenotype affects diabetic children equally irrespective of the sex of the child.

The H.L.A. - B8 phenotype was also evenly spread throughout each of the three age subgroups (0-4 years, 5-9 years and 10-14 years) where almost half the children in each group had H.L.A. - B8. These results suggest that irrespective of age, the commonest phenotype in children with diabetes is H.L.A. - B8.

As the ten year survey had shown a tendency to a summer onset in younger children and a winter onset in older children, I had anticipated that the seasonal variation would reveal some difference in the H.L.A. phenotypes between these two age groups. However, no striking difference was found in the phenotypes of these age groups.

I think it is important to carry out a larger "phenotype seasonal onset study" in order to determine if the younger diabetic child definitely has a summer onset tendency and if so whether there is a more variable phenotype. If this proves to be the case, then it could indicate a differing aetiology between the younger and older diabetic child.

The Liverpool research also suggested that H.L.A. - BW15 and BW18 are increased compared to controls and that these two antigens plus H.L.A. - B8 are the three H.L.A. - B antigens most likely to be associated with a tendency to juvenile-onset diabetes.

The Sunderland results were quite striking in that from the nineteen H.L.A. - B antigens tested, three-quarters of the diabetic children had three specific antigens, H.L.A. - B8, BW15 and BW18.

The percentage of H.L.A. - BW15 patients in Liverpool was 18% compared to 12% controls. The Sunderland results were very similar where 20% diabetic children had BW15 compared to 14% controls.

Nerup (Denmark) did combine his H.L.A. - B8 and BW15 patients and found that 59% had these two antigens. This result compared favourably

Table 33

Percentage comparisons of H.L.A. phenotypes

H.L.A. - B8, BW15 and BW18 in diabetic children

from Liverpool (Liv) and Sunderland (Sun)

Age	0 - 4 years		5 - 9 years		10 - 14 years	
	Liv	Sun	Liv	Sun	Liv	Sun
H.L.A. - B8	29%	45%	51%	53%	55%	56%
H.L.A. - B8 BW15 & BW18	59%	63%	78%	76%	77%	78%

to 62% in the Sunderland study.

In Sunderland 75% of the diabetic children had H.L.A. - B8, BW15 or BW18, either alone or in combination with each other. Cudworth (1976) produced figures for juvenile diabetes with these three antigens, and I have calculated the percentages and compared them with the Sunderland study. Cudworth found 159 children under fifteen years with these antigens out of 214 diabetic children which gave a percentage of 74%, very similar to the Sunderland result of 75%. The percentage of children with these antigens in the three age sub-groups, 0 - 4 years, 5 - 9 years, 10 - 14 years are also similar apart from a slight variation in the younger 0 - 4 year group (Table33).

Liverpool found 29% H.L.A. - B8 young diabetic children compared to 45% in Sunderland. This discrepancy may well be due to the smaller numbers in the Sunderland group and could explain why the young Sunderland H.L.A. tested children had a winter-onset compared to the ten year survey which showed a trend to a summer onset. It could also explain why I did not find a greater variation in the H.L.A. phenotypes in the younger Sunderland diabetic children.

From the Liverpool and Sunderland studies it would appear that three-quarters of juvenile diabetics have H.L.A. - B8, BW15 or BW18, either alone or in combination with each other.

Cudworth (1977) suggested that if a person had H.L.A. - B8 BW15 phenotype, then the risk for juvenile diabetes was greater than having H.L.A. - B8, BW18 or H.L.A. - BW15 BW18.

In the Sunderland study only one combination (B8 BW18) of these antigens was found in the 0 - 4 year age group. Two children, one boy (H.L.A. - B8 BW15) and a girl (BW15 BW18) were in the 5 - 9 year range. In the older 10 - 14 year group, three girls had H.L.A. - B8 BW15 and one boy had H.L.A.- B8 BW18.

From all age incidence studies there are more older than young children with diabetes and more diabetic children with combinations of the three specific antigens could be expected in the older children as the number of patients is larger. However, if the H.L.A. - B8 BW15 phenotype places an individual at greater risk in developing diabetes, then the risk is presumably present from birth and therefore it is reasonable to assume that a child with H.L.A. - B8 BW15 phenotype should develop diabetes more frequently and at a younger age than children with other combinations of B antigens, but this does not appear to be so in the Sunderland study.

The Liverpool workers also suggested that individuals who are homozygous for H.L.A. - B8 were at greater risk than hetrozygous individuals. In their study, 12.9% of juvenile diabetics under thirty years of age were H.L.A. - B8 homozygous, compared to 7.2% controls. Only three of the fifty-five (5%) Sunderland diabetic children were H.L.A. - B8 homozygous which is approximately equal to the control population figure. There were two boys, four years and eleven years old, both presenting in January and one girl, age three, who presented in May. There is certainly no indication of an increase in H.L.A. - B8 homozygous patients amongst the Sunderland diabetic children.

No diabetic children in this study were homozygous for H.L.A. - BW15 or H.L.A. - BW18. The only other homozygous patients were two boys with H.L.A. - B12 B12 age two years and thirteen years, presenting in September and October respectively, and one girl age eight, H.L.A. B35 B35, with an onset in May.

Apart from the specific B antigens, H.L.A. - B8, BW15 and BW18 which certainly seem to be associated with juvenile-onset diabetes, I was impressed by the results of the other B antigens H.L.A. - BW40 and B12. H.L.A. - BW40 in the Sunderland study was increased above the control group with

nine diabetic children (16% compared to 13% controls) and certainly has a seasonal tendency. Five children had H.L.A. - BW40 in combination with either H.L.A. - B8, BW15 or BW18. The remaining four children had BW40 combined with other H.L.A. - B antigens.

H.L.A. - B12 also proved interesting, as Cudworth and Woodrow (1976) suggested that this phenotype was a low risk factor for juvenile diabetes. There were twelve Sunderland children with this phenotype, two were homozygous H.L.A. - B12, and five had H.L.A. - B12 combined with H.L.A. - B8 or BW15 or BW18. Although H.L.A. - B12 children comprised 22% of the Sunderland study compared to 28% controls, this still represented a large percentage of the study group (almost one-quarter). I therefore think it is unlikely that H.L.A. - B12 phenotype has a low risk association with juvenile diabetes; certainly in Sunderland and it could well be that H.L.A. - B12 and B40 may well act in a similar way to the H.L.A. - B8, BW15 combination which is thought to greatly increase the diabetic tendency. The H.L.A. - B12 and B40 phenotypes when combined with the H.L.A. - B8, BW15 or BW18 may well potentiate rather than retard their diabetic tendency.

There certainly seems little doubt from the British and Continental studies that H.L.A. - B8, BW15 and BW18 phenotypes are increased in Caucasians with juvenile diabetes. However, there may be racial or even regional variations, as Wakisaka in Japan (1976) found an increase in H.L.A. - B22J in juvenile diabetes, whilst H.L.A. - B8 which is uncommon in Japan was certainly not increased, nor was H.L.A. - BW15.

Apart from particular phenotypes which may be associated with an increased tendency to diabetes, it would appear that some B phenotype have an infrequent association with juvenile diabetes. Cudworth and Woodrow (1976) found H.L.A. - B5, B7, B12 and B35 to be in this category. In the Sunderland study no diabetic child had H.L.A. - B13, B14, BW22.1 or B37. H.L.A. - B5 was in fact slightly increased occurring in 7% of patients

compared to 5% controls, but patients with H.L.A. - B7, B12 and B35 were certainly less than the control group. It is therefore probable that several H.L.A. - B phenotypes have a low association with juvenile diabetes and may afford some genetic protection against developing the illness.

Since Gamble (1973) found a seasonal onset in older diabetic children this has been confirmed by Rolles, Rayner and Mackintosh (1975) in Birmingham who linked seasonal onset with H.L.A. - B8 phenotype. They found that 81% of their B8 diabetic children presented in the five months October to February. The percentage in Liverpool for the six months October to March was 66% whilst the Sunderland study was very similar with 69% H.L.A. - B8 diabetic children in the winter six months.

The Birmingham and Liverpool studies did not mention a sex difference in association with seasonal onset. In Sunderland, it was quite striking that thirteen of the fifteen boys with H.L.A. - B8 presented between October and March compared to the fourteen girls who were equally divided with seven in each six month period. These results would suggest that male diabetic children with H.L.A. - B8 have a strong tendency to present in the winter six months, which is not evident in girls.

Twelve children in the Sunderland H.L.A. study had the two other "diabetic-associated antigens" H.L.A. - BW15 and BW18 in combination with B- antigens other than B8.

Cudworth and his colleagues (1977) suggested that juvenile diabetics in Liverpool with H.L.A. - BW15 had a greater tendency to present with diabetes between December and April. In the Sunderland study, eleven children had H.L.A. - BW15, four boys and seven girls. All four boys presented in the summer six months, but four of the seven girls did present in the winter time, but only two girls presented between December and April. Hence, out of eleven children, only two H.L.A. - BW15 children presented between

December and April; certainly the Liverpool finding is not applicable to the Sunderland study.

In all, forty-one of the fifty-five Sunderland H.L.A. tested children had one or more of the 'specific' antigens (H.L.A. - B8, BW15 or BW18), and 61% of them presented in the winter six months. However, the winter tendency for H.L.A. - BW15 and BW18 is not as specific as for the male diabetic child who has the H.L.A. - B8 phenotype.

Two other B- antigens in the Sunderland study appeared to be associated with a winter seasonal onset. There were twelve children with H.L.A. - B12 phenotype and ten of these patients presented between October to March whilst seven of the nine children with H.L.A. - BW40 presented in the winter months. These two B- phenotypes have so far not been incriminated in a seasonal onset with juvenile diabetes.

(v) <u>An analysis of the height of the diabetic child at the onset of the illness</u>

One of the problems when dealing with the height at onset of a particular disease is that in many hospitals it is not a standard procedure to measure the height of a patient when they are admitted to hospital. Routine admission measurements tend to be limited to weighing the patient. However, in many paediatric out-patients it is a common practice to measure the height as well as the weight of the child.

This difference in procedure between out and in-patients became evident in this study on the height of diabetic children at the onset of their illness.

Only forty-five diabetic children between four and fourteen years of age were considered suitable for inclusion in the study. The remaining children were either outside the height study age range or had not been measured at the onset of their illness before insulin treatment had been started.

It seemed obvious that apart from comparing the Sunderland diabetic

Fig 10a & b

Fig. 10b

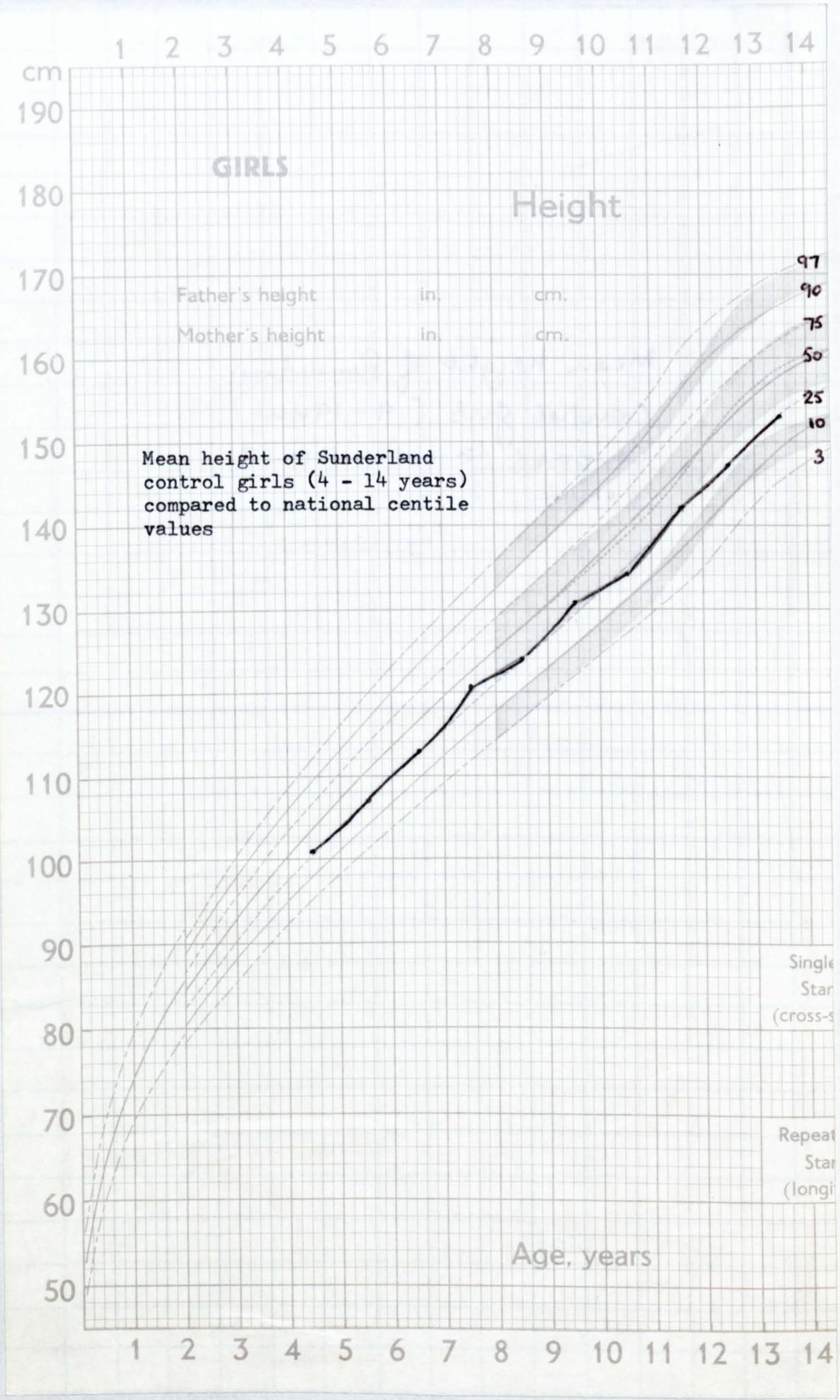
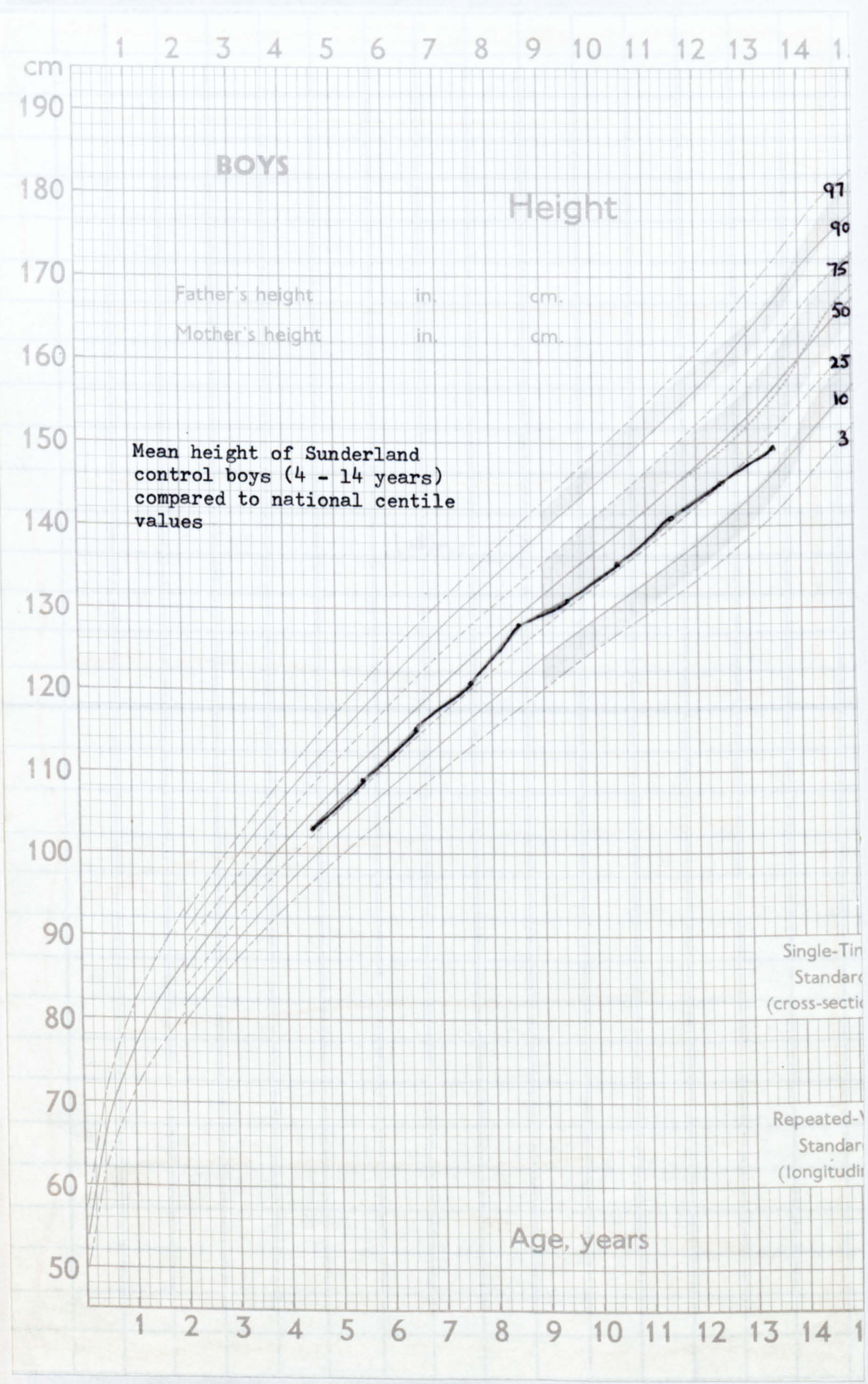


Fig. 10a



children with national height values, a comparison should also be drawn between local healthy children and diabetic children. I therefore estimated the height of four hundred and sixteen children attending the outpatient department of Sunderland Children's Hospital. It became evident from the measurements that there was a significant difference in height between local children and the national values as supplied by Tanner and Whitehouse.

It was a surprise to find such a large difference between the mean national height values for boys and girls and the Sunderland control children. Tanner and Whitehouse (1966) stated that their values could be "regarded as quite suitable for all English children. Children in the North may be a trifle smaller than those in the South". However, the mean height of the Sunderland boys and girls between the ages of four and fourteen years when charted on the Tanner and Whitehouse Growth and Development charts ran along the 25th centile (Fig10a&b).This interesting variation has since made me more cautious in the assessment of Sunderland children who are being investigated because of small stature.

Although there is a difference between the mean national height and Sunderland children, the local boys and girls did follow the national pattern in growth. The control boys were taller than girls until they became eleven years old then girls became taller until they were fourteen years old. This presumably is due to girls having an earlier onset in puberty than boys.

The Sunderland diabetic children were equally grouped above and below the national 50th centile for height, but for true comparisons the mean height of healthy local children should be used and when this is done, the diabetic children are significantly taller than local control children ($P<0.01$). The 25th national centile value was found to equate to the mean value for local children and using this standard, eighteen (86%) of the

twenty-one diabetic boys were above this value and eighteen (75%) of the twenty-four girls were above the 25th centile. These results tend to confirm the statistical tests of significance showing that the diabetic boys and girls tend to be taller than the average Sunderland child at the onset of their illness.

Craig (Glasgow) in 1970 reported similar results to the Sunderland height study. He found that the Glasgow diabetic children tended to be grouped around the national 50th centile value in height but also found that the Glasgow school children had a mean height corresponding to the 33rd national centile. The Sunderland diabetic children were equally grouped around the 50th centile whilst the control children's height corresponded to the 25th centile.

Helen Pond (1970) produced almost similar results from the Kings College, diabetic London clinic. She found that diabetic boys and more particularly diabetic girls were above the national 50th centile. Pond used the Tanner and Whitehouse growth charts as the control values, which would be quite appropriate as the national values are bases on the height of London school children and children attending the Harpenden Growth clinic.

Pond in an addendum to the publication in 1970 reported a further series of diabetic children she had studied. She confirmed the increased height of the diabetic child at the onset of the illness but also found that when she divided them into two groups, 5 to 10 years and 10 to 15 years, the younger boys and girls were very much taller in comparison to the control group. The older boys (10 - 15 years) were also taller than the controls but the difference was not as marked as in the younger boys. The diabetic girls (10 - 15 years) were also taller than the control girls but they tended to 'fall away' in height as they became older. In comparison, the Sunderland diabetic children showed a greater increase in height in the

older 9 - 14 year group. The diabetic boys increased from the 55th centile (4 - 9 years) to the 60th centile (9 - 14 years). However, the older girls increase in height was very small, from the 48th centile (4 - 9 years) to the 50th centile (9 - 14 years). The Sunderland diabetic girls would therefore appear to follow the height pattern reported by Helen Pond.

I had intended to calculate the standard deviation scores in two age groups - 4.0 to 8.9 years and 9.0 to 13.9 years. It was intended to compare the Sunderland diabetic and control children in these two age groups and also contrast the results with a similar survey done by Drayer in Holland (1974). However, Tanner, Whitehouse, Hughes and Vince (1971) stated that the S.D. score "is meaningful up to the age of ten in girls and twelve in boys, but about then the adolescent growth spurt of the standard makes their application to prepubescent children erroneous". I therefore restricted the comparison of S.D. scores to the 4 to 9 year old diabetic and control children.

Drayer (1974) found Dutch diabetic boys (4 - 14 years) to be taller than a control group but the diabetic girls were the same height as the control girls. In the age range 4 to 9 years,the diabetic boys were significantly taller than the control group but the older boys 9 - 14 years although taller than the control group were not significantly so.

These results are rather interesting when compared to the mean heights of Sunderland diabetic children. The Sunderland boys, 4 - 14 years, were 1 cm. shorter than the Dutch diabetic boys. The Dutch diabetic girls were taller than the Sunderland girls in all age groups by 3 to 6 cm. As the Dutch girls were equal in height to the control girls, this would indicate that the Dutch girls in general are much taller than Sunderland girls, whether diabetic or not.

As I had estimated the height at onset and obtained the H.L.A. phenotype of forty-two diabetic children, it seemed obvious to determine if

there was a link between the tallness of the diabetic child and the diabetogenic phenotypes H.L.A. - B8, BW15 and BW18.

The results of this study demonstrate that although children with the diabetogenic haplotypes H.L.A. - B8, BW15 and BW18 were taller than the local school children, the diabetic children with other B- haplotypes, especially B-12 and B7 were even taller, particularly girls in the 9 - 14 age group.

Although H.L.A. - B8 phenotype is associated with diabetic children who are above the local mean height, this B antigen is not associated with very tall children, i.e. those above the 75th centile height level. From eleven H.L.A. - B8 girls, eight are below the 50th centile and only two of the eleven boys are above the 75th height centile.

Another B- phenotype with a similar tendency to be associated with a shorter diabetic child is H.L.A. - BW40. Three of the four boys with BW40 are in the 10 - 25th centile, whilst the fourth boy B7 BW40 is in the 90th centile range.

Conversely, H.L.A. - B7 and H.L.A. - B12 phenotypes appear to be associated with taller diabetic children. Six of the eight girls in the 75 - 90th centile range are H.L.A. - B7 or B12 and four of the six boys in the 75th plus centile range are H.L.A. - B7 or B12. However, when H.L.A. - B8 is combined with B7 or B12, the diabetic child tends to be shorter in stature. It would appear that H.L.A. - B8 may in some way affect the growth of the pre-diabetic child and could indicate that there may be different mechanisms provoking diabetes in certain children. Boys with H.L.A. - B8 certainly have a tendency to a winter onset, suggesting that an infection may trigger diabetes in boys. Girls do not have such a definite seasonal onset and more girls present with diabetes when they are eight and eleven years old, which could indicate an endocrine provoking mechanism. However, even the eight and eleven year old girls show some variation in that five of the six girls from the

height study who were on or below the 25th height centile were in these two age groups, and four of the short girls had the H.L.A. - B8 phenotype. These results suggest that there are two types of diabetic girl at the age of eight and eleven. One type is short in stature and has the H.L.A. - B8 haplotype, whilst the other type of diabetic girl is tall and does not have H.L.A. - B8.

It is possible that this difference in the diabetic girls may be accounted for by a different aetiology. The short H.L.A. - B8 girls may have an "infection" basis for their diabetes whilst the taller, non H.L.A. - B8 girl may have puberty or adrenarche as the provoking factor.

The majority of reports now seem to suggest that diabetic children are taller at the onset of their illness. It is interesting to speculate why these children are taller. From the Sunderland study, children's diabetes is primarily a disease of the upper and middle social classes. It is known that these children are better nourished and taller compared to socially deprived children. However, in the Sunderland height study, four of the six children in social groups IV and V were above the local mean height. This would suggest that social class alone does not appear to be the answer to tallness in diabetic children.

The most likely explanation is that the metabolic abnormalities inherent in juvenile diabetes causes an inbalance in the neuro-endocrine regulation of growth or produces a disturbance in the tissues responsible for growth. Hypoglycaemia is known to stimulate growth hormone release (Roth, Glick, Yalow and Berson 1963) and hypoglycaemic episodes are known to occur before the classical symptoms of diabetes appear (Allen 1953). However, the mechanism of such hypoglycaemic episodes is not clear. Could it be due to release of extra quantities of insulin or some factor potentiating the action of insulin?

Svejgaard and Ryder (1976) have suggested that the H.L.A. system may play a part in interfering with the interaction between hormones and their

receptors on cell surfaces. If an H.L.A. antigen has some incidental resemblance to the binding site of a cell surface receptor molecule for a given hormone, there could be competition between the receptors and the H.L.A. antigen molecule. H.L.A. molecules could also react with the "carrier" part of the ligand or facilitate their reaction with ligand or the production of intracellular transmitters, resulting in either inhibition or enhancement.

Svejgaard and Ryder gave examples to explain this hypothesis. They state that the mouse H - 2 system has recently been shown to control the sensitivity of target organs to testosterone. A case has been recorded of a tetragenetic male, carrying twice the number of H.L.A. specificities (compared to normal individuals) who had several endocrine disorders. They also mentioned a large kindred where several members have juvenile diabetes. Healthy relations carrying the disease associated haplotypes had a tendency to have a lower insulin response to glucose than the remaining healthy individuals without these haplotypes.

If this theory by Svejgaard and Ryder is correct regarding the association of H.L.A. and disease, then it could explain why certain diabetic children are taller at the onset of their illness. The children with H.L.A. - B7 and B12 are taller than those with B8, BW15 and BW18 and this difference could indicate a different aetiology. It is possible that children with H.L.A. - B7 and B12 phenotypes alter the effect of insulin on the tissues and cause an increase or potentiate the action of growth hormone to a greater extent than children with H.L.A. - B8, BW15 or BW18. Perhaps a larger study may help to clarify this interesting problem.

Chapter 11

Recent thoughts on the aetiology of the disease

The results of the Sunderland study show that the aetiology of juvenile diabetes mellitus is a complex problem, because many different genetic, environmental, immunological, metabolic and hormonal influences interact to produce the clinical picture.

However, the studies on the H.L.A. system in diabetes have provided convincing evidence for the existence of genetic heterogeneity. It is now suggested that certain diseases associated with H.L.A. - B locus alleles show stronger associations with the H.L.A. - D locus and in some instances with the H.L.A. - DR antigens or Ia types. Recent studies (Jeannet et al Tissue Antigens 1977: 10, 196) indicate an association between insulin dependent diabetes and H.L.A. - DRw3 and DRw4. It is known that H.L.A. -Dw3 is in linkage disequilibrium with B8 and B18, whilst H.L.A. - Dw4 is linked to B15 and B40.

The Sunderland results suggested that B40 may well be a high risk diabetogenic phenotype and this now seems to be possible as Ludvigsson et al in 1977 (Diabetologia 13: p13-17) reported from Uppsala an increase in B40 in Swedish insulin dependent diabetic patients and placed H.L.A. - B40 second to H.L.A. - B8 in relative risk. The east coast of Britain with its Viking Scandinavian background may well account for the association between the Sunderland B40 results and those from Uppsala. When the Sunderland children with B40 phenotype are included with those children with H.L.A. - B8, BW15 and BW18, forty-five (82%) of the fifty-five H.L.A. tested children have these high risk genes.

The large number of new diabetic children in Sunderland during 1976 (23 cases) was not repeated in 1977. The number reverted to fourteen, similar to 1973, 74 and 75. This could reinforce the theory that environmental factors such as infection may play an essential role in the

development of diabetes in certain children.

Identification of such environmental factors or the precise nature of the immune mechanisms involved could lead to a new approach in the treatment of insulin dependent diabetes. This approach could be aimed at reducing the β-cell destruction and/or the enhancement of regeneration of the β-cells.

Appendix 1

The Knox Space - T analysis

7

STANDARD ERROR DEVIATIONS OF CUMULATIVE VALUES

	3.00	6.00	10.00	20.00	40.00	40.00	40.00	40.00	40.00	40.00	TOTAL
30.000	-0.033	-0.057	-0.632	-0.104	0.000	0.000	0.000	0.000	0.000	0.000	0.000
60.000	0.523	0.139	-0.387	-0.059	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100.000	0.530	0.091	-0.728	-0.126	0.000	0.000	0.000	0.000	0.000	0.000	0.000
182.000	0.325	-0.151	-0.728	-0.120	0.000	0.000	0.000	0.000	0.000	0.000	0.000
365.000	0.244	-1.371	-1.432	-0.159	0.000	0.000	0.000	0.000	0.000	0.000	0.000
547.000	-0.171	-1.000	-0.981	-0.086	0.000	0.000	0.000	0.000	0.000	0.000	0.000
730.000	-0.414	-0.953	-0.743	-0.051	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1095.000	-1.097	-1.193	-0.516	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9999.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*********	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

STOP

Appendix 2

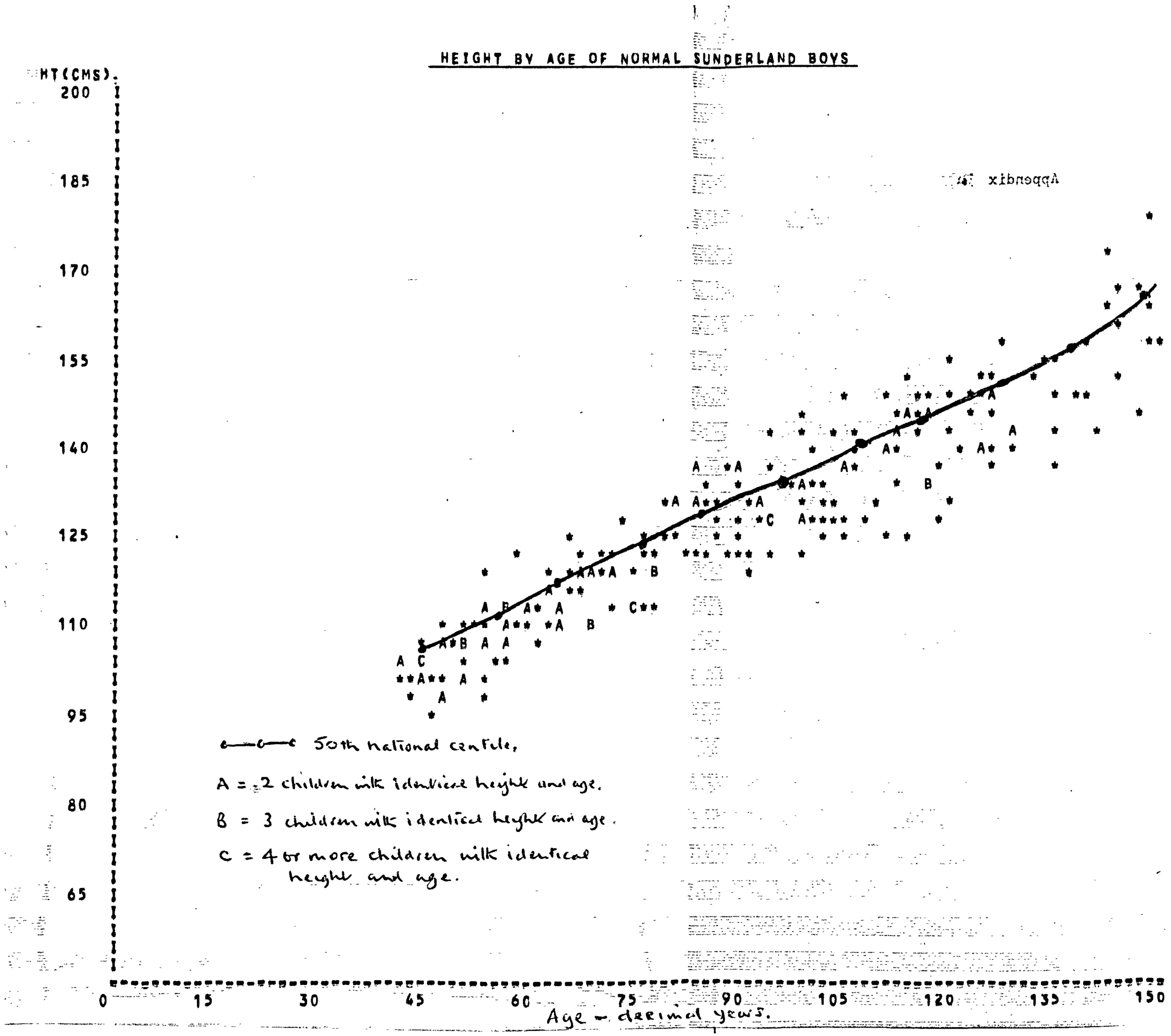
Control values of HLA-B phenotypes as supplied by the Regional Blood Transfusion Centre, Newcastle-upon-Tyne

HLA-B phenotypes

B5	5%
B7	30%
B8	24%
B12	28%
B13	5%
B14	7%
B15	14%
B17	11%
B18	10%
ET	1%
SL-ET	4%
BW22.1	4%
BW22.2	2%
B27	5%
B35	13%
B37	3%
B38	1%
B39	4%
B40	13%

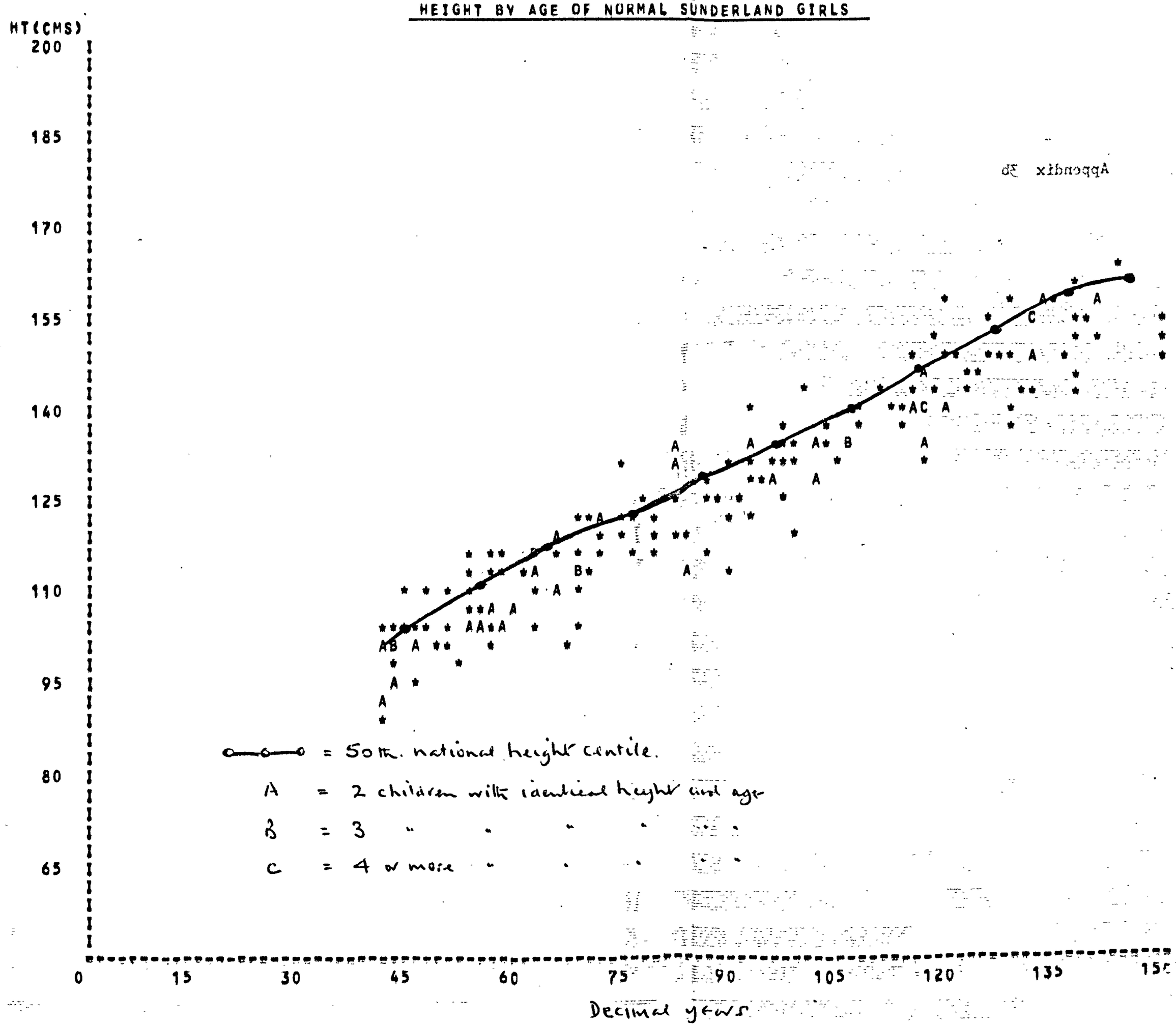
Appendix 3a

Computer print-out of height and age of Sunderland control boys



Appendix 3b

Computer print-out of height and age of Sunderland control girls



Appendix 4 (a)

Sunderland diabetic boys' HLA phenotype height and centile value

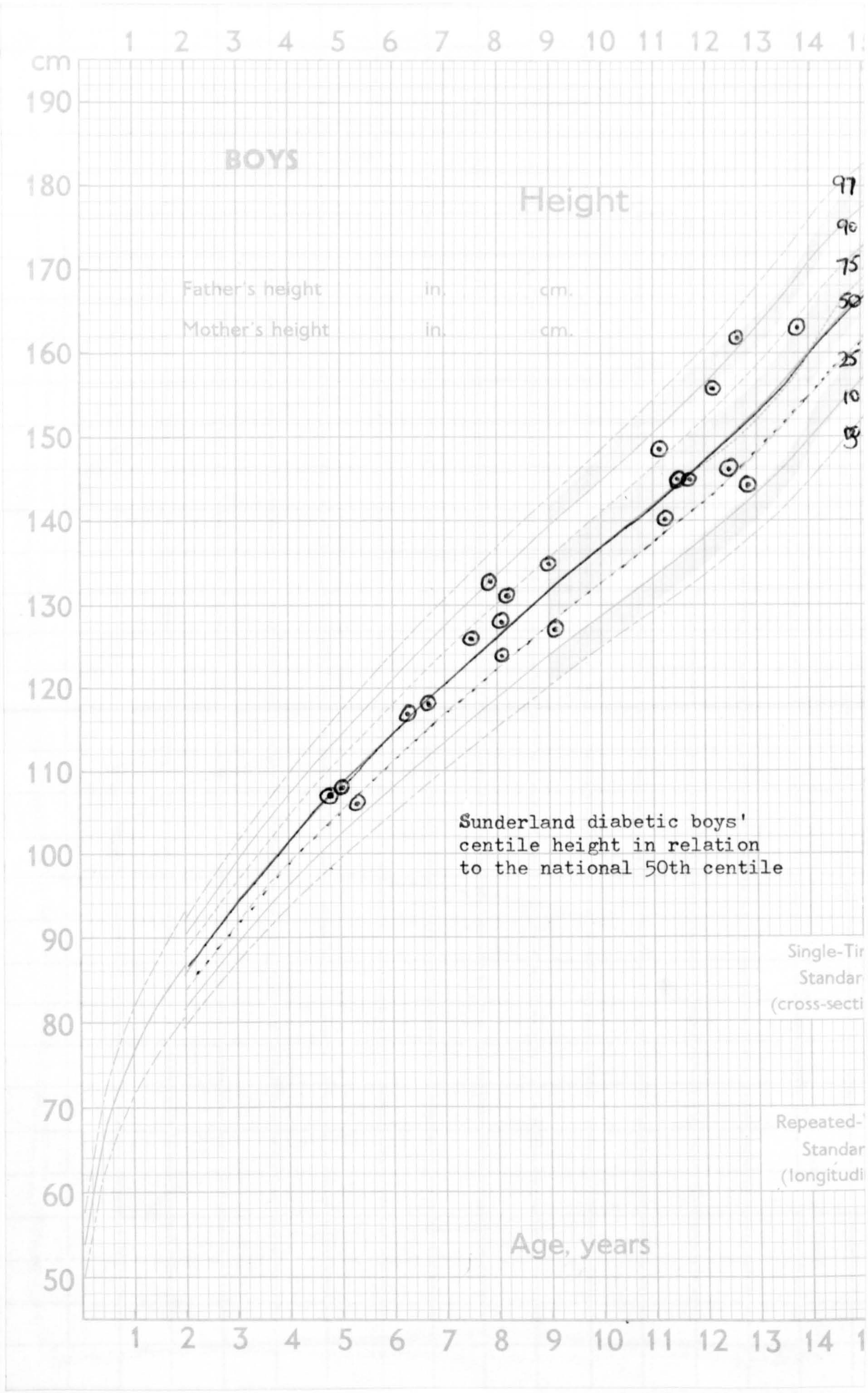
	Initials	Age/Years	HLA phenotype	Height/cm.	Centile value
1.	A.C.	4.9	B8 B8	107 cm	45th
2.	P.M.	5.0	B7 B8	108 cm	50th
3.	A.W.	5.4	B35 BW40	106 cm	15th
4.	C.H.	6.4	B8 BW21	117.5 cm	55th
5.	B.D.	6.8	B5 B8	118 cm	40th
6.	K.N.	7.5	B5 B8	126 cm	70th
7.	A.C.	7.9	B12 BW15	133 cm	90th
8.	S.T.	8.1	B8 BW15	124 cm	40th
9.	M.C.	8.1	B7 BW15	128 cm	65th
10.	S.B.	8.2	B7 B12	131 cm	80th
11.	D.B.	9.0	B8 BW39	135 cm	75th
12.	C.S.	9.1	B8 BW40	126.5 cm	20th
13.	G.D.	11.1	B7 BW40	149 cm	90th
14.	P.L.	11.3	B8 B8	140 cm	40th
15.	G.W.	11.5	B18 BW35	145 cm	60th
16.	C.C.	11.6	B7 B8	145 cm	55th
17.	T.W.	12.2	B8 BW38	156 cm	90th
18.	A.H.	12.5	B5 BW17	146 cm	40th
19.	M.V.	12.6	B12 BW18	162 cm	95th
20.	G.H.	12.9	B18 BW40	144 cm	20th
21.	C.C.	13.9	B12 B12	162.5 cm	70th

Appendix 4 (b)

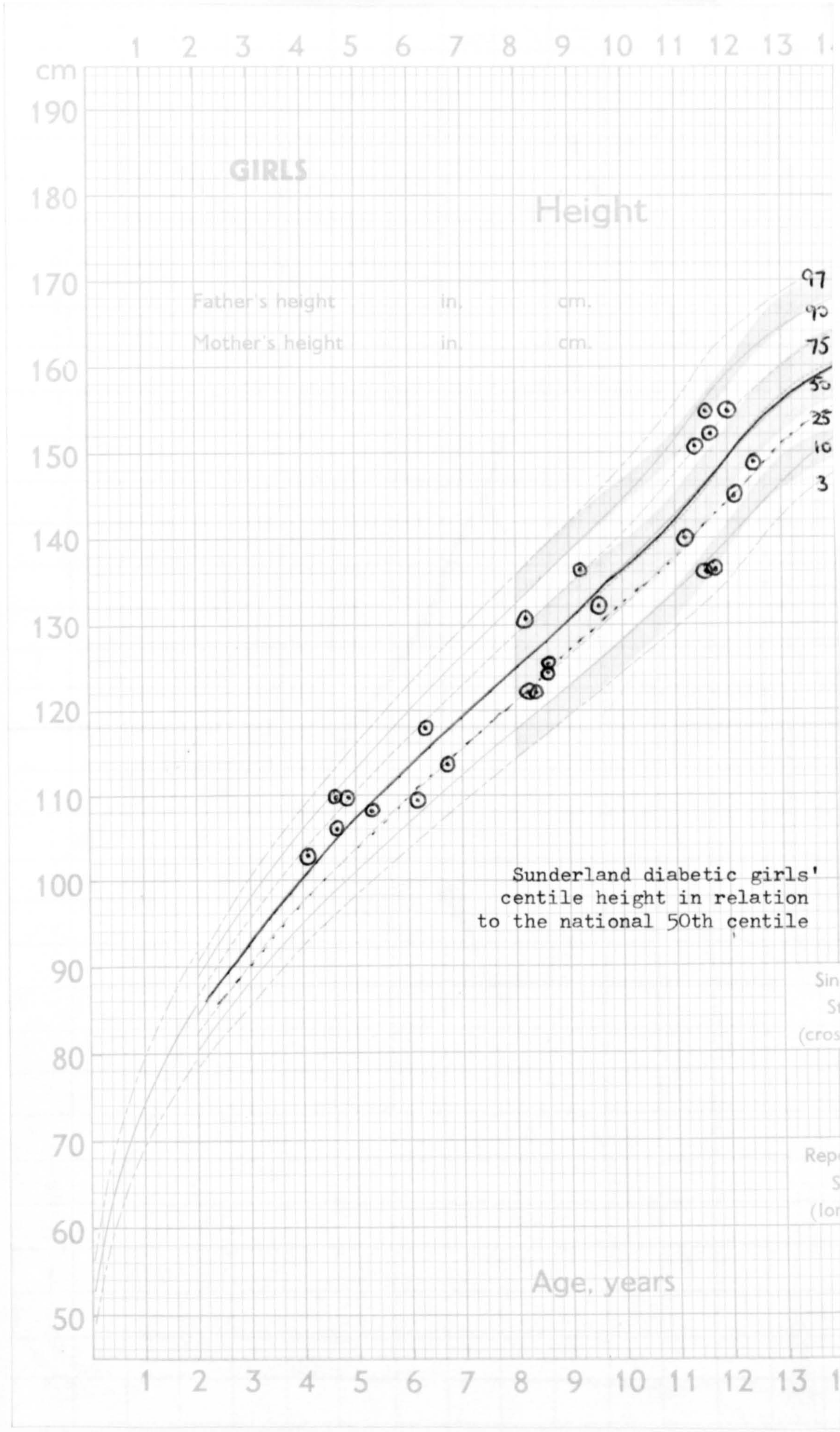
Sunderland diabetic girls' HLA phenotype height and centile value

	Initials	Age/Years	HLA phenotype	Height/cm.	Centile value
1.	J.H.	4.1	B7 BW18	103 cm	75th
2.	R.G.	4.7	B12 B21	106 cm	60th
3.	A.A.	4.9	B8 BW15	110 cm	75th
4.	L.K.	5.3	BW15 BW18	108 cm	40th
5.	C.H.	6.4	B8 BW40	118 cm	60th
6.	F.C.	8.2	BW40 BW40	131 cm	80th
7.	L.M.	8.3	B8 B27	122 cm	25th
8.	G.L.	8.3	BW35 BW35	122 cm	25th
9.	S.F.	8.7	B8 B12	124.5 cm	30th
10.	K.M.	8.7	B8 B27	125.5 cm	35th
11.	J.C.	9.2	B12 BW39	136.5 cm	80th
12.	M.T.	9.6	BW18 BW40	132 cm	40th
13.	E.A.	11.2	B8 BW35	140 cm	30th
14.	L.S.	11.4	B7 B27	151.5 cm	80th
15.	S.P.	11.6	B7 B12	155 cm	85th
16.	L.C.	11.7	B8 BW15	152.5 cm	80th
17.	C.G.	11.8	B7 B8	136 cm	10th
18.	D.L.	11.8	B8 BW15	136 cm	10th
19.	J.M.	12.0	B12 BW15	155 cm	80th
20.	E.S.	12.1	B8 BW15	145 cm	30th
21.	P.S.	12.5	B7 B8	149 cm	40th

Appendix 5a


BOYS
Height
Father's height in. cm.
Mother's height in. cm.
cm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
97
90
75
50
25
10
3
Sunderland diabetic boys' centile height in relation to the national 50th centile
Single-Tir
Standar
(cross-secti
Repeated-
Standar
(longitudi
Age, years
1 2 3 4 5 6 7 8 9 10 11 12 13 14 1

Appendix 5b


cm
GIRLS
Height
Father's height in. cm.
Mother's height in. cm.
97
90
75
50
25
10
3
Sunderland diabetic girls' centile height in relation to the national 50th centile
Age, years

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Dr. M. Parkin, Child Health Department, University of Newcastle read the completed manuscript and gave helpful advice.

Finally, I am deeply indebted to my secretary Miss Sandra Scott who undertook the monumental task of typing the script and tables.


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LIST OF CASES

13	3	67	39.40	60.20	0.
4	4	67	39.50	60.20	0.
8	5	67	34.10	50.00	0.
4	7	67	40.60	50.20	0.
6	10	67	39.10	60.40	0.
20	11	67	38.20	53.80	0.
16	1	68	36.80	57.00	0.
18	1	68	39.50	58.40	0.
24	9	68	32.30	50.10	0.
20	10	68	40.40	55.40	0.
25	11	68	40.40	58.20	0.
6	3	69	40.60	62.60	0.
29	3	69	40.60	61.90	0.
28	5	69	37.10	61.20	0.
7	2	70	36.80	59.50	0.
16	4	70	38.30	56.10	0.
13	5	70	33.40	50.30	0.
15	7	70	40.10	59.20	0.
4	3	71	43.50	40.50	0.
19	5	71	43.80	41.90	0.
22	6	71	36.60	61.60	0.
4	11	71	40.20	55.60	0.
11	11	71	40.10	58.00	0.
25	1	72	40.70	49.20	0.
2	5	72	43.30	43.50	0.
27	5	72	39.60	52.90	0.
14	6	72	40.90	49.90	0.
8	9	72	36.90	53.60	0.
5	10	72	43.80	41.90	0.
5	10	72	41.40	49.50	0.
8	12	72	38.50	57.40	0.
20	12	72	38.10	45.10	0.
2	1	73	32.40	50.00	0.
25	1	73	40.60	56.10	0.
8	3	73	42.70	43.60	0.
9	4	73	36.10	58.30	0.
18	5	73	40.90	50.20	0.
25	5	73	40.20	56.50	0.
29	5	73	37.60	57.50	0.
7	8	73	38.10	52.70	0.
6	9	73	35.80	53.50	0.
21	8	73	38.30	56.10	0.
12	9	73	40.40	49.20	0.
23	10	73	34.60	61.60	0.
19	11	73	38.40	53.40	0.
23	11	73	39.40	60.20	0.
12	1	74	38.40	55.30	0.
5	2	74	37.70	59.40	0.
8	2	74	36.60	53.50	0.
8	2	74	35.00	59.10	0.
21	2	74	40.40	55.40	0.
6	5	74	39.50	53.10	0.
13	6	74	40.50	53.50	0.
3	7	74	39.90	60.20	0.

2	10	74	40.00	56.40	0.
6	10	74	40.50	61.70	0.
12	10	74	34.70	59.80	0.
14	12	74	42.20	49.20	0.
10	1	75	36.80	61.60	0.
21	1	75	40.90	52.30	0.
11	4	75	38.90	53.30	0.
5	5	75	41.80	49.60	0.
30	8	75	34.60	59.40	0.
4	9	75	38.20	52.20	0.
7	9	75	37.20	52.30	0.
18	9	75	40.70	53.20	0.
10	10	75	32.20	52.10	0.
24	10	75	35.30	47.50	0.
22	10	75	39.80	59.00	0.
27	11	75	38.00	45.20	0.
22	12	75	37.90	53.60	0.
30	12	75	44.10	41.20	0.
6	1	76	37.50	56.80	0.
6	1	76	39.00	55.50	0.
1	2	76	33.00	53.50	0.
3	2	76	40.30	58.90	0.
10	2	76	40.60	59.10	0.
12	3	76	41.20	50.30	0.
2	4	76	43.10	48.90	0.
5	4	76	35.60	55.50	0.
21	6	76	40.50	53.60	0.
1	7	76	35.70	46.50	0.
12	7	76	41.30	50.20	0.
15	7	76	30.40	56.50	0.
31	8	76	40.50	54.40	0.
14	9	76	39.00	55.50	0.
8	10	76	37.90	59.10	0.
12	11	76	40.60	53.20	0.
16	11	76	34.40	52.90	0.
18	11	76	35.90	52.70	0.
23	11	76	36.60	56.90	0.
12	12	76	40.50	60.90	0.
21	12	76	40.60	53.70	0.

TOTAL CASES IN THIS SET = 93

'S OF ALL POSSIBLE PAIRS

DISTANCES IN KILOMETRES

TIMES	3.00	6.00	10.00	20.00	40.00	0.00	0.00	0.00	0.00	0.00	OVER	TOTALS
TO 30.	15	25	33	15	0	0	0	0	0	0	0	88
TO 60.	9	21	23	16	1	0	0	0	0	0	0	70
TO 100.	20	36	46	22	0	0	0	0	0	0	0	124
TO 182.	41	70	77	49	2	0	0	0	0	0	0	239
TO 365.	81	162	152	82	3	0	0	0	0	0	0	480
TO 547.	70	97	118	89	6	0	0	0	0	0	0	380
TO 730.	65	98	108	82	5	0	0	0	0	0	0	358
TO 1095.	130	189	200	151	10	0	0	0	0	0	0	680
TO 9999.	292	506	612	429	20	0	0	0	0	0	0	1859
TO 0.	0	0	0	0	0	0	0	0	0	0	0	0
TO 0.	0	0	0	0	0	0	0	0	0	0	0	0
TO 0.	0	0	0	0	0	0	0	0	0	0	0	0
TO 0.	0	0	0	0	0	0	0	0	0	0	0	0
TO 0.	0	0	0	0	0	0	0	0	0	0	0	0
TO 0.	0	0	0	0	0	0	0	0	0	0	0	0
TO 0.	0	0	0	0	0	0	0	0	0	0	0	0
OVER	0	0	0	0	0	0	0	0	0	0	0	0
TOTALS	723	1204	1369	935	47	0	0	0	0	0	0	4278

CUMULATIVE VALUES

	3.00	6.00	10.00	20.00
30.	15	40	73	88
60.	24	70	126	157
100.	44	126	228	281
182.	85	237	416	518
365.	166	480	811	995
547.	236	647	1096	1369
730.	301	810	1367	1722
1095.	431	1129	1886	2392
9999.	723	1927	3296	4231
100000.	723	1927	3296	4231

40.00	40.00	40.00	40.00	40.00	40.00	TOTAL
88	88	88	88	88	88	88
158	158	158	158	158	158	158
282	282	282	282	282	282	282
521	521	521	521	521	521	521
1001	1001	1001	1001	1001	1001	1001
1381	1381	1381	1381	1381	1381	1381
1739	1739	1739	1739	1739	1739	1739
2419	2419	2419	2419	2419	2419	2419
4278	4278	4278	4278	4278	4278	4278
4278	4278	4278	4278	4278	4278	4278

EXPECTED CUMULATIVE VALUES

	3.00	6.00	10.00	20.00
30.0	14.87	39.64	67.80	87.03
60.0	26.70	71.17	121.73	156.26
100.0	47.66	127.03	217.27	278.90
182.0	88.05	234.68	401.41	515.28
365.0	169.17	450.89	771.22	990.00
547.0	233.39	622.06	1064.00	1365.83
730.0	293.90	783.32	1339.82	1719.89
1095.0	408.82	1089.62	1863.73	2392.42
9999.0	723.00	1927.00	3296.00	4231.00
100000.0	723.00	1927.00	3296.00	4231.00

40.00	40.00	40.00	40.00	40.00	40.00	TOTAL
88.00	88.00	88.00	88.00	88.00	88.00	88.00
158.00	158.00	158.00	158.00	158.00	158.00	158.00
282.00	282.00	282.00	282.00	282.00	282.00	282.00
521.00	521.00	521.00	521.00	521.00	521.00	521.00
1001.00	1001.00	1001.00	1001.00	1001.00	1001.00	1001.00
1381.00	1381.00	1381.00	1381.00	1381.00	1381.00	1381.00
1739.00	1739.00	1739.00	1739.00	1739.00	1739.00	1739.00
2419.00	2419.00	2419.00	2419.00	2419.00	2419.00	2419.00
4278.00	4278.00	4278.00	4278.00	4278.00	4278.00	4278.00
4278.00	4278.00	4278.00	4278.00	4278.00	4278.00	4278.00