

THE NATURE AND SIGNIFICANCE OF

'BORDERLINE' DIABETES

A Thesis presented for the degree of

Doctor of Medicine

in the

University of London

by

Harry Keen, M.B., B.S., F.R.C.P.

Department of Medicine,
Guy's Hospital,
London S.E.1.

January, 1971.

"The transition from the normal to the pathological cannot be defined and nothing shows this intimate relationship between physiology and pathology better than diabetes".

Claude Bernard (1877). *Lecon sur le Diabete et la Glycogenose Animale*. Bailliere et Fils, Paris.

"We neglect unwisely the study of those differences between man and man of which, for the most part, physiology takes no cognisance, but which may yet prove of much importance in modifying the processes of disease".

Jonathan Hutchinson (1884). *The Pedigree of Disease*. London.

"Health and disease know no sharp boundary. They could only do so if it were possible for biology to adopt the dictionary definition of normality. But variability, both in time and in the species, is one of the most distinctive and necessary attributes of life, which thus admits no constant and no norm".

John Ryle (1947). *The Meaning of Normal*. *Lancet* 1, 1.

".... our chief difficulties in clinical medicine are encountered at the extremities of the range of natural variability. At what point, we may ask ourselves, does a pulse-rate (under standard conditions) cease to be a normal pulse-rate ? at what point is normality passed and should we account the organ, structure or function as diseased ? How is normality (local and constitutional) to be better assessed or measured in order to secure the answers to these and other questions bearing on the practical discrimination between the healthy, the unhealthy, and the borderline state ?"

John Ryle (1947). *The Meaning of Normal*. *Lancet* 1, 1.

CONTENTS

1.	Acknowledgements	-	-	-	-	-	-	1
2.	Synopsis	-	-	-	-	-	-	3
3.	Introduction and General Review	-	-	-	-	-	-	6
4.	Definitions and Methods	-	-	-	-	-	-	22
5.	Genetic Considerations	-	-	-	-	-	-	31
	Insert 1	-	-	-	-	-	-	33
	Insert 2	-	-	-	-	-	-	52
6.	The Bedford 'Borderline' Diabetic Study	-	-	-	-	-	-	55
7.	Findings in the 'Borderline' Group	-	-	-	-	-	-	63
	i General Biometric Observations	-	-	-	-	-	-	63
	ii The Kidney, the Eye and the Peripheral Nerves	-	-	-	-	-	-	71
	a) The Kidney	-	-	-	-	-	-	72
	Insert 3	-	-	-	-	-	-	73
	Insert 4	-	-	-	-	-	-	74
	Insert 5	-	-	-	-	-	-	75
	b) The Eye	-	-	-	-	-	-	83
	c) The Peripheral Nerves	-	-	-	-	-	-	96
	Insert 6	-	-	-	-	-	-	98
8.	Cardiovascular Disease: Prevalence, Incidence and the Effects of Treatment	-	-	-	-	-	-	101
	Insert 7	-	-	-	-	-	-	104
	Insert 8	-	-	-	-	-	-	105
	Insert 9	-	-	-	-	-	-	106
9.	Conclusion	-	-	-	-	-	-	115
10.	References	-	-	-	-	-	-	123
11.	Appendices 1, 2 and 3	-	-	-	-	-	-	In back pocket.

1.

ACKNOWLEDGEMENTS

It is clear from the scale of the work described in this Thesis that it represents the labours - physical and intellectual - of a group of individuals. Though the Bedford 'borderline' diabetic project was conceived and formulated initially by the author who has held and still holds responsibility for its direction and development, many valued associates have contributed to one or several phases of the study. Chief among these are Professor W. J. H. Butterfield, O.B.E., Director of the Department of Medicine, Guy's Hospital Medical School, and the late Dr. C. L. E. H. Sharp, Medical Officer of Health for Bedford, with both of whom I had the pleasure of sharing responsibility for the planning and execution of the initial diagnostic survey for diabetes in Bedford in 1962. To Professor Butterfield, I owe a deeply felt debt of gratitude for his continuing support and encouragement through the years. Much of the close planning and argument about the project have, over the last four years, been conducted with my valued colleague and friend, Dr. R. J. Jarrett. Over the years, Dr. D. A. Pyke of King's College Hospital, Dr. D. R. Boyns, Dr. C. Chlouverakis, Dr. J. H. Fuller, Dr. S. Mistry and Dr. J. D. Ward have, for varying periods, been members of the team which has maintained contact with and collected information from the population groups in Bedford. To Dr. G. A. Rose and his technical associates of the Department of Epidemiology and Medical Statistics, London School of Hygiene, acknowledgement is gladly made for their assistance with the initial cardiovascular evaluation of the population studied. Biochemical estimations have been made by our technical assistants Messrs. K. Kilborn, M. Wood, M. Shaw, M. Field, E. Quartay, C. Arden, Mrs. C. Ben-Dyke. Much of the secretarial burden

has been carried in London by Mrs. C. Boyns, Miss R. Lloyd and by Miss F. Phillips and in Bedford by Mrs. H. Cothill and Mr. F. Batson and the patient and painstaking labours in Minnesota coding of E.C.G.'s etc. by Mrs. N. Keen and Mrs. J. Gray are beyond praise. We have received assistance in the Bedford Clinic from Mrs. P. Gillam, S.R.N., Mrs. P. Jones, B.Sc., Mrs. L. MacRae, S.R.N. and Mrs. E. Inskip, S.R.N. and we gladly acknowledge the support of the Bedford municipal authority in making transport, communication and other facilities available. Substantial financial support for the project came initially from grants from the Research Fund of the British Diabetic Association. For the last four years the study has been financed by a special development grant from the Department of Health and Social Security. Gratefully acknowledged also, is assistance rendered from time to time by Messrs. Ames Ltd., Beechams Ltd., Hoechst Pharmaceuticals and other well wishing groups, firms and individuals. Not least is acknowledgement due to those several hundred, largely unprotesting, members of the public who, called upon to help only by reason of their blood sugar level, have responded by undergoing a variety of examinations and other inconveniences and minor indignities in the interest of the expansion of medical knowledge.

2.

SYNOPSIS

The application of ever more accurate methods to the diagnosis of diabetes, along with the increasing facility in automated chemical estimation, has made it possible to extend the diagnostic search for asymptomatic and oligosymptomatic cases of the disease to large, apparently healthy, population groups. This transition of diagnosis from the clinic to the community has brought with it many new problems. Among the most pressing is the question of the criteria to be employed when diagnosis is attempted on an epidemiological scale. Closely linked, is the question of the evaluation of various forms of treatment, for only the demonstration of some benefit to the individual concerned can ultimately justify the inconveniences and risks of earlier diagnosis and treatment (Wilson and Jungner, 1968; Keen and Jarrett, 1969).

The epidemiological approach to early diagnosis has raised to a level of high importance a number of problems which, in the hospital clinic, are only glimpsed. For example, when diagnosis is based upon the application of a fixed, critical level of blood sugar, the number of individuals qualifying for the diagnostic label of diabetes rises steeply with age. However, it is arguable whether a given level of blood sugar is of the same significance at all ages and there are strong arguments for a sliding diagnostic scale. Should the blood sugar differences between the sexes be regarded as of pathological significance, or do they represent physiological variation? What is the importance of the very considerable diurnal fluctuation in blood sugar levels and of the variation which accompanies pregnancy and the

menstrual cycle in women ? To what extent are elevations in blood sugar in the population related to agents such as corticosteroids, oral contraceptives and oral diuretics, now widely taken for a variety of conditions ?

When grossly elevated levels of blood sugar are found there is little difficulty in justifying the making of a diagnosis and the recommending of therapeutic action. It is in evaluating the significance and consideration of the management of the more frequently discovered, lesser elevations in blood sugar that the main difficulty lies. As with many biological variables (blood pressure, body weight, intelligence, serum uric acid concentrations, for example) a wide range of values are commonly accepted as normal. The decision as to the point in this range when the change in quantity becomes sufficiently extreme to merit the application of the diagnostic label of diabetes is still arguable and unclear.

It seemed fruitless to argue this important case on the basis of the blood sugar level itself. The question as to whether a given level of blood sugar (or response to a diagnostic test) represented diabetes or not was re-formulated in a way that was both of practical relevance and susceptible to answer. At what level of blood sugar (or degree of glucose intolerance) is there an enhanced risk, either present or in the future, of the development of those tissue disorders characteristic of the diabetic state ? Closely linked is the further question of whether, by therapeutic intervention, it is possible to alter the natural history of the untreated condition.

This Thesis embodies work related to these important questions. Its main concern is with the recognition and definition of intermediate degrees of glucose intolerance and the examination and fate of a defined group of 'borderline' diabetic subjects, brought to light by a population survey in Bedford, England, in 1962. In various of its sections, some of them already published, the Thesis considers certain biometric, biochemical and clinical findings in 248 members of this 'borderline' diabetic group, comparing them when possible with normoglycaemic controls and with the newly-found, unquestionably diabetic group, identified in the same population survey. Certain of the observations reported here were made during the special examination of a group of 735 diabetics and their first degree relatives. Others relate to studies made at the time of the Bedford survey and during the years of follow-up which succeeded it. A main preoccupation is the definition of the relationship between elevated blood sugar levels and the enhanced frequency of 'atherosclerotic' arterial disease in terms of prevalence at the outset of the study and incidence in the follow-up period. A practical clinical contribution is the evaluation of the effectiveness of long-term antidiabetic treatment, carried out in a double-blind, factorial trial of tolbutamide and carbohydrate restriction therapy, commenced in the 'borderline' diabetic group in 1962.

3. INTRODUCTION AND GENERAL REVIEW

The Region of Diagnostic Uncertainty

The nebulous area between the clearly normal and the clearly abnormal has been an increasing source of embarrassment in medicine, not only in terms of the 'neatness' of nosological classification but also in the practical question of diagnosis and treatment of disease in individual cases. Its growth in importance has depended largely upon technical advances making it possible to detect small deviations from the norm with greater reliability and on a quasi-industrial scale, coupled with an increasing drive towards the earlier recognition and treatment of disease. So, many physical and chemical investigations, used originally to support and explain the clinical picture of disease, have themselves become the main, and sometimes the only, indicator of the presence of 'disease'.

The doctor may thus find himself pressed to treat what is little more than a biochemical deviation from a statistical norm. Very reasonably he may question the utility of intervention, often prompted by the awareness of the harm his treatment may do. In addition to the physical risks of medication, there is a more subtle threat, the breaching of that poorly defined sense of wellbeing which is difficult to recognise until it is lost.

The general arguments against the overenthusiastic and uncritical acceptance of 'abnormal' laboratory results in initiating the mechanism of diagnosis and treatment have been well expressed by Cochrane and Elwood (1968). They, and others (Sharp, 1968), have also emphasized

the need for carefully controlled, unbiased studies in the grey region of the 'borderline' abnormality. It is in this phase that it is reasonable to expect disease to be in its earliest and most readily reversible condition. Here, too, not only is it ethically possible but even desirable that the influence of treatment should be evaluated in a scientifically controlled way. In a field of endeavour which, inevitably, is the scene of both evangelical enthusiasm and destructive cynicism, it is of obvious importance to encourage objective, critical appraisal of its advantages and its penalties.

'Borderline' Diabetes Mellitus

Diabetes mellitus provides an excellent example of the emergence of the diagnostic, therapeutic and ethical dilemmas described in general terms above, a situation stemming mainly from the widespread application of simple methods of blood sugar measurement to large sections of the general population. Two clear reasons for this diagnostic expansion are evident; the growth of the epidemiological approach to the non-infectious disorders (Morris, 1964) with improved understanding of the need for careful ascertainment; and the clinical recognition that a large proportion of diabetic patients coming to ordinary clinical diagnosis show evidence, in the form of well established diabetic complications, suggestive of many years of prior, unrecognised metabolic disorder. When populations are 'screened' for either or both of these reasons, many individuals fitting neither the normal nor the clearly diabetic category are found. It is this intermediate state that has been termed 'borderline' diabetes.

The use of the designation 'borderline diabetes' is certainly not meant to add yet another diagnostic label to the accumulation of terms which already encumber the diabetic scene (Keen and Jarrett, 1970). Indeed, it finds no place in the catechism officially 'approved' by the British Diabetic Association (Fitzgerald and Keen, 1964). The term is used in this study in a precisely defined way, its use being justified by convenience. It conveys its own basic meaning to those involved in its study, as well as to those individuals who are its subject.

In all population groups appropriately studied (Gordon, 1964; Butterfield, 1964; Welborn et al, 1968; Hayner et al, 1965; West and Kalbfleisch, 1966; College of General Practitioners, 1963; Prior et al, 1966; Mauratoff et al, 1969; Mimura et al, 1964), a continuum of blood sugar values is to be made out between the large number of clearly normal values on the one hand and the smaller numbers of equally clearly diabetic values on the other. In all but one population (Miller et al, 1968) described, the distribution is 'continuous' or unimodal, that is to say there is no obvious break in the frequencies, dividing the population into two (bimodal) or more (polymodal) separate segments. The form of the frequency distribution curve, however, is not strictly 'normal' in a statistical sense; it is positively skewed, as are many biological variates when their frequencies are plotted as a simple, untransformed numerical series (Gaddum, 1945; King and Wootton, 1959).

There is much controversy regarding the aetiological implications of the distribution curve of this form. What is in dispute is whether

it reflects the distribution of a variate in a single population, the members of which vary only quantitatively from each other in respect of the factors which govern the variate; or, whether the distribution curve in fact contains two or more distinct populations, differing qualitatively from each other in respect of a primary factor governing the variate in question, the distinction between them being blurred by secondary factors. The controversy proper opened over the question of the distribution of arterial blood pressure in the population (Pickering, 1961) and has extended to include the behaviour of blood sugar in population samples (Butterfield, 1964; Keen, 1966; Neel et al, 1965). Despite the importance of this general hypothesis, the issues that it raises will be considered here only in relation to the subject of this Thesis. Using a variety of mathematical manoeuvres (logarithmic transformation, use of square or cube root etc.) positively skewed distributions may be made, statistically, more 'normal', an important consideration if ordinary probability statistics are to be applied. Whatever transformation is employed, however, the intermediate or borderline region of values remains, and represents the chief concern of this Thesis.

The existence of intermediate or borderline responses to an oral glucose load has long been recognised, though for various reasons its frequency, its place in the spectrum of response and its long-term implications have received comparatively little attention. The concept of diabetes mellitus as a discrete entity, stemming from the historical origins of the diagnostic term (Keen, 1970), is partly responsible for this; decision has been directed to determining whether a given response was, or was not, diabetic in nature, an approach also imposed by practical

clinical and therapeutic need for determinants of action. The frequency of intermediate responses has also been obscured by prior selection of subjects for study by glycosuria; restriction of consideration only to the glucose tolerance of people initially selected for testing because of glycosuria markedly reduces the representation of borderline responses (Butterfield, Keen and Whichelow, 1967). In any case, the limits of the region of diagnostic uncertainty are of necessity vague and arbitrary and may therefore contain widely varying numbers, depending upon the criteria selected.

An early study of the genetics of diabetes by Pincus and White (1933; 1934) involved the measurement of glucose tolerance of close relatives of diabetics, regardless of the presence of glycosuria. A number of intermediate responses to oral glucose were noted and have been indicated by other workers subsequently (see Section 5). It was the growing realisation of their frequency in relatives of diabetics (Keen, 1957; Clarke, 1961), culminating in the specific study of Thompson (1965) which gave most substance to the now widely held views on the multifactorial nature of the inheritance of diabetes in man.

Walker and Kerridge (1961) described a special category of borderline response to oral glucose which they termed an Intermediate Group of Possible Pre-diabetics in their epidemiological study of the village community at Ibstock. Their use of glycosuria as an initial selective 'screen' obscured the true frequency of the borderline responses, but it was found in a quarter of the glycosuric subjects tested and outnumbered the newly found diabetics.

Grant and Moorhouse (1966) have directed special attention to the significance of the lesser grades of glucose intolerance, being concerned chiefly with the practical interpretation of results in population screening for diabetes. From this practical standpoint, they were disposed to reject the lesser grades of hyperglycaemia as clinically unimportant, especially those found in older people in whom they considered that they probably represented a 'normal' consequence of ageing rather than the advent of a pathological state. It is important, however, not to confuse the question of immediate clinical utility with that of more fundamental implications of hyperglycaemia, a distinction which they do not entirely grasp. The 'borderline' area is also recognised by the Committee of Experts of the World Health Organisation (1965) and by the European Diabetes Epidemiology Study Group (1970), each of whom gave its own criteria and special interpretation.

Is 'Borderline' Diabetes a Homogeneous Entity ?

Does a population composed of 'borderline' diabetic individuals consist of a single homogeneous class of people varying only quantitatively from each other in respect of blood sugar and the mechanisms which determine it, or is it composed of a mixture of two qualitatively distinct populations, one expressing the upper bounds of the normal and the other the lower limits of the abnormal, or diabetic ? The very mechanisms employed in the definition of the borderline group will ensure that its average blood sugar values will fall between mean values for the diabetic on the one hand and those for the normals on the other. It may be that, although the blood sugar level plays a

critical role in the clinical definition of diabetes in man (Keen, 1968; 1969), it only represents a peripheral manifestation of a central and specific fault responsible for the disease. Penrose (1951) has shown in phenylketonuria, for example, that the distinction between affected and unaffected individuals is unclear when such factors as head length or hair colour are compared, but that complete separation occurs when the concentration of phenylalanine in the blood plasma is compared. To give substance to the hypothesis of heterogeneity among the 'borderline' diabetic group, therefore, it would be necessary to apply some indicator of the 'diabetic state' other than the blood sugar itself. Such an indicator would clearly distinguish between undoubted diabetics and normals and, when applied to a borderline-by-blood-sugar population, it should effect a similar separation between 'true' and 'spurious' diabetic hyperglycaemics. Although a number of candidates for this role, in particular the synalbumin insulin antagonist of Vallance Owen (1964), have been proposed none can be considered a sufficiently reliable marker to resolve the question in this way (Keen, 1968; 1969; Keen and Jarrett, 1967).

It has been argued that certain of the so-called complications of diabetes, affecting the eyes, the kidneys, the peripheral nerves and, perhaps, the major arteries, are not the consequence of the diabetic metabolic abnormality, but concomitants or even the cause of it, distinct from, but linked in some way with, the factors responsible for the hyperglycaemia. The most recent protagonists of this view, Siperstein et al (1968) claim to demonstrate statistically significant thickening of muscle capillary basement membrane not only in diabetics compared

with normals, but also in "genetically prediabetic patients" (the offspring of two diabetic parents) with normal responses to the oral glucose tolerance test. One might choose these morphological changes as a marker and with it seek to distinguish affected and unaffected individuals among the 'borderline' group. However, even if some such distinction could be made out, it would not provide anything approaching proof of heterogeneity. For instance, the difference between affected and unaffected 'borderline' individuals might be explained by differences in the duration of the hyperglycaemic state, known to influence the appearance of the clinically evident tissue changes (Marble, 1967). The duration of the hyperglycaemia in the 'borderline' individuals is unknown. Degree as well as duration of hyperglycaemia might also be found to influence the rate of appearance of tissue changes but this could be interpreted as support for either hypothesis. It could be argued from such findings that the tissue changes are a direct consequence of the raised level of blood sugar and that those with the higher blood sugars will consequently manifest more tissue change; or alternatively it could be proposed that those people carrying the diabetic marker are both more likely to have the higher blood sugar and also to show the morphological manifestations of the diabetes. However, the case for using basement membrane changes as a marker for 'true' diabetes is much weakened by the contradictory evidence of Osterby Hansen (1965) who was unable to distinguish between the renal glomerular capillary basement membrane thickness in recent onset juvenile diabetics and normals. One is also deterred from the use of morphological markers by the demonstration that tissue changes, indistinguishable from those occurring in spontaneous diabetics, may be found in so-called secondary diabetes (Duncan et al, 1958)

and that they may also be produced in animals rendered diabetic by experimental means (Engerman and Bloodworth, 1965; Bloodworth, 1965).

A further approach to the resolution of this problem might lie in the collection of very much larger bodies of data from 'free-living' human populations in the hope that, with the elimination of statistical fluctuation in numbers, a true division of the population into 'normal' and diabetic components may become visible. In the large study of one-hour blood sugar values in an American population carried out by the U.S. Public Health Service in 1960 - 1962 (Gordon, 1964), however, simple inspection of the frequency distributions does not indicate the presence of any such natural division. The Pima Indian population (Miller et al, 1968) do show a convincingly bimodal distribution of two-hour post glucose blood sugar values becoming increasingly evident as age advances. This population is highly unusual in several respects, however, not least in the very great frequency of diabetes that it shows (diabetes is present in over 40% of men and 50% of women aged 40 years or more) and it would be unwise to argue from its behaviour to that of other groups.

An alternative approach might be to seek to reduce the 'blurring' due to variation in response introduced by environmental factors by the performance of repeated tests of glucose tolerance on a carefully defined, smaller group of people. Armitage et al (1966) in an analogous study with blood pressure measurements showed the improvement in precision achieved by repeated measurement. An approach of this sort might succeed in separating the variation 'between individuals' from the considerable variation 'within individuals' (MacDonald et al, 1965; 1969)

in respect of blood sugar.

The Long-Term Significance of Raised Blood Sugar

The long-term implications of grossly raised blood sugar levels are evident in the course of the ordinary clinical care of people with established diabetes, in many of whom persistent hyperglycaemia of considerable degree was the only demonstrable diagnostic abnormality. A proportion of newly found diabetics - of the order of 5% to 10% - will show characteristic retinal changes of the disease at the time of diagnosis (Pyke and Roberts, 1959; Soler et al, 1969), and in the rest, the rate of appearance of retinal changes is considerable (Caird et al, 1969, Section 7ii). The enhanced rate of development of clinical arterial disease in diabetics compared with non-diabetics is strongly suggested by the Framingham study (Kannel et al, 1967), supported by Epstein's (1967) follow-up studies in the Tecumseh community study and by our own findings in Bedford (Section 8). The emergence of renal and neurological changes is also a commonplace of clinical observation.

In contrast to this compelling evidence and experience relating to the consequences of gross hyperglycaemia, our understanding of the long-term significance of hyperglycaemia of lesser degree is much less sure. Intermediate or 'borderline' elevation of blood sugar, detected at a given point in time may be interpreted in several ways. It may represent a high-point in that individual's blood sugar behaviour, rarely if ever to be repeated. It may characterise an individual set at an intermediate position in the spectrum of glucose tolerance for the whole of his life. It may be a blood sugar value examined in a person in the

process of passing from clearly 'normal' to clearly 'diabetic' levels of blood sugar. Follow-up data from our Bedford study suggest that all of these interpretations may apply but that, in general, the higher the blood sugar at the time of first measurement, the more certainly and the more rapidly it may be expected to rise as time goes by. These observations are confirmed by those of O'Sullivan (1969) and also by the repeat study carried out in Birmingham by a Diabetes Working Party of the College of General Practitioners (1970) upon individuals found five years earlier (Diabetes Working Party, 1962) to have disturbances of glucose tolerance.

O'Sullivan restudied in 1963 the post-prandial blood sugars of 1181 people in the 34 to 55 year old age group among the population of Oxford, Mass., U.S.A., the seat in 1945 of one of the earliest epidemiologically valid population surveys for diabetes (Wilkerson and Krall, 1947). He was particularly concerned with two aspects. First, had there been any change in the prevalence of diabetes within the selected age group? Secondly, what information on the rate of development of diabetes and the factors influencing it could be obtained from a restudy of the 877 of the 1181 who had been examined and found non-diabetic in 1946? Criteria for the development of diabetes were either the finding of a single post-prandial blood sugar exceeding 199mg/100ml., or a post-prandial blood sugar exceeding 169mg/100ml. followed by another post-prandial level exceeding 139mg/100ml.

Using these criteria, the 1963 diabetes prevalence in the 34 - 55 year age group (2.4%) was lower than that in the 34 - 55 year age

group in 1946 (3.5%), though the difference was not statistically significant. Of the 887 people measured on both occasions, 1.8% qualified for the diagnosis of diabetes in 1963. The proportion becoming diabetic was much higher among those with a positive family history of the disease in 1946 (5.2%) than among those without diabetes in the family (0.9%). Of 706 people found to have blood sugars below 140mg/100ml. in 1946, 12 (1.7%) had become classifiable as diabetic in 1963, while of 121 exceeding this value in 1946, 4 (3.3%) had become diabetic in 1963. Among people with 1946 blood sugars below 140mg/100ml. only 0.8% of non-obese, compared with 6.0% of obese people had become diabetic by 1963. The presence in 1946 of both obesity and a positive family history of diabetes in this group determined the later emergence of diabetes in 15%. The numbers in this category are small and little weight can be put upon the precise value but a further indication of family history / overweight interaction is found in a study of diabetic families carried out by Baird (1969).

The Birmingham Diabetes Survey Working Party (1970) repeated oral glucose tolerance tests after a lapse of approximately five years in 527 of 630 (84%) persons tested in the Birmingham Diabetes Survey of 1960 - 1961. As in the earlier study (1962), they allocated individual test results to one of seven main categories and commented upon the transition from one class to another over the five years. As an index of the rate of development of diabetes, accession of individuals to the class showing the most marked abnormality of glucose tolerance - that of so-called 'florid' diabetes - can be used. This category of response was characterised by a one-hour capillary blood glucose exceeding

180mg/100ml., a two-hour figure exceeding 120mg/100ml. and a fasting blood sugar exceeding 130mg/100ml. Their classification of tests was based upon the various combinations of two characteristics of the glucose tolerance test response, the degree of hyperglycaemia (height of blood sugar attained) and the duration of hyperglycaemia. Each of these characteristics had predictive value in relation to the appearance of a 'florid'-type curve after five years; prolonged duration of hyperglycaemia was a better predictor than its degree; the presence of both was the most significant. The importance of sustained hyperglycaemia is reflected in an analysis which took as the predictive factor the blood sugar level measured two hours after the glucose load in the initial survey. When the same two hour classification that we used in Bedford (Sharp, 1964; Butterfield, 1964; Keen, 1964) was applied to the first test results (120mg/100ml; 120 - 199mg/100ml; 200mg/100ml. +), the follow-up placed 1%, 14% and 94% respectively into the 'florid' class, although 9%, 46% and 97% respectively fulfilled the British Diabetic Association criteria (Fitzgerald and Keen, 1964) for the diagnosis of diabetes. Changing the two-hour discriminating value from 120 to 135mg/100ml. also, as would be expected, increased the proportion of florid curves emerging from the intermediate group from 14% to 23%, scarcely affecting the other two, and changed the proportions using the British Diabetic Association criteria, to 11%, 61%, and 97% respectively. The cost in loss of selectivity for the increase in sensitivity of the higher two-hour cut-off point is small but clear.

One may conclude from the above studies that the higher the initial blood sugar the more likely is further deterioration in glucose

tolerance to occur; other factors, in particular the presence of a familial link with an established diabetic and of obesity, accentuate this trend. The data upon which these conclusions are based are comparatively limited in size and do not allow of a more critical analysis of the variation in the rate of blood sugar rise within initial blood sugar categories in order to determine whether distinct populations of 'fixed' hyperglycaemics and 'deteriorating' hyperglycaemics exist. They contain no information relating to the development of tissue changes which characterise the diabetic state, information which would enrich the conclusions as they relate to the natural history of diabetes as a whole. In this 'two-time point' type of study it is difficult to allow for the extent to which day to day individual variation is responsible for movement between classes of glucose intolerance.

Age and 'Borderline' Blood Sugars

The rising mean blood sugar levels with age noted in several studies in small groups (Deren, 1937; Horvath et al, 1947; Chesrow and Bleyer, 1954; Silverstone et al, 1957; Brandt, 1960) and larger population samples (Diabetes Working Party, 1963; Butterfield, 1964; Gordon, 1964; Hayner et al, 1965; Welborn et al, 1969) would lead one to suppose that the 'borderline' elevations of blood sugar would be found with ever increasing frequency as age advances. Although this, in fact, is true, close examination of the behaviour of glucose tolerance with age shows that, on average, the age effect is much more notably expressed in the height to which the blood sugar rises in the 60 minutes or so following absorption, than in fasting levels or the level achieved in the

post-absorptive phase, i.e. at the two-hour time point (Butterfield, 1964) which show little mean change with age. It is upon the latter level that the criterion of the 'borderline' diabetic has been rested, so that it is not purely an ageing effect which determines those selected. However, in addition to the increasing mean G.T.T. values at 30, 60, 90 minutes, an increasing variability of values at all time points, including 120 minutes, also characterises the successive age groups, leading to increasing numbers who exceed the cut-off level.

It is not correct, however, to assume that the borderline situation is limited to older people. In the Bedford group, the structure of the initial survey excluded people under the age of 20 years from the screening procedure so that it was not possible to make any comment about the situation in minors. Nevertheless, of the total of 248 individuals recruited to the 'borderline' group, 32 were under the age of 40, of whom 14 were under 30. Fajans et al. (1970) demonstrated that intermediate degrees of carbohydrate intolerance were also to be found among children and adolescents and were not necessarily associated with rapid and severe metabolic deterioration. In the great majority of the cases that they followed, no progressive worsening of the abnormality of glucose tolerance was found over periods of up to 16 years. They, too, draw attention to the considerable fluctuation of blood sugar responses within individuals which may be obscured when attention is limited to the average behaviour of groups. Although Fajans et al. establish the principle that 'borderline' diabetes may be found in the young, no estimates of prevalence are possible, for their examples are highly selected. It would seem unlikely that the phenomenon is a common

one in the very young, if only on the basis of cautious extrapolation of the Bedford data. However, the question can only be clarified by planned studies.

4.

DEFINITIONS AND METHODSGlucose Tolerance

The blood sugar response to an oral glucose or 'liquid glucose' load has been used as the index of glucose tolerance throughout the studies described, except in the few instances specially mentioned. In general, the recommendations of the Medical and Scientific Section of the British Diabetic Association (Fitzgerald and Keen, 1964) have been followed for the actual performance of the test. At the initial survey glucose tolerance evaluation in Bedford in 1962, the test was performed after an overnight fast and started at between 08.30 and 09.30 hours. In follow-up two-hour blood sugar measurements made in the 'borderline' group, the glucose load was consumed at home approximately one hour fifty minutes before arrival at the follow-up clinic, between 16.30 and 18.30 hours. Except when otherwise defined, the load was consumed after a fast lasting from approximately 08.00 hours on the day of the visit. As our further studies have shown (Jarrett and Keen, 1969), there is a systematic influence of time of day upon glucose tolerance and this has to be taken into account in comparisons involving the initial survey and the subsequent follow-up blood sugar estimates.

The Glucose Load

A standard dose of a lightly carbonated and flavoured liquid glucose preparation ('Lucozade') has been used as the 'challenge' throughout the work described here. Special studies showed it to be indistinguishable from an equivalent dextrose load, both in the blood sugar rise it

produced and in the curve of insulinaemia it evoked (Boyns and Keen, 1966). It was more palatable than simple dextrose solution and almost never provoked nausea. Its composition is as follows:-

Dextrose	3.58g/100ml.
Maltose	2.62g/100ml.
Tri- to Hepta - oses	7.82g/100ml.
Octa + - oses	4.44g/100ml.
Total (solids)	18.46g/100ml.

A standard dose of 235ml. Lucorade, equivalent to 50g. of anhydrous dextrose, was used throughout this study. Arguments have been advanced for other dosage schedules based upon body weight, surface area or other considerations but no convincing evidence or arguments have established the superiority of one dose level over another in adults. The 50g. load is widely used in Britain and elsewhere. It rarely provokes the nausea and consequent variation in gastric emptying encountered with larger loads. Use of a fixed load in this group of adults avoids the difficulties in interpretation involved when different 'stimuli' are applied to different people, or to the same person from time to time.

Blood Sugar Measurement

Blood sugar has been measured by the Autoanalyser, using the ferricyanide reduction method of Hoffman (1937) and employing the micromethod which requires 0.1ml. of whole blood diluted in a suitable volume (2.0 or 0.9ml. of 1% potassium fluoride in water). This method has been critically assessed by Whichelow et al (1967) and the reproducibility of the procedure, which has been evaluated by them, is

identical with our own, used by the same hands in the same laboratory. The accuracy of the ferricyanide method has been criticised but the strictures apply chiefly to the extremes of the blood sugar range, particularly in hypoglycaemic regions, and are not relevant to the range in which our values lie.

Except in very few instances, all of them clearly stated, capillary blood has been used in preference to venous samples. Whichelow et al, (1967) have outlined the arguments in favour of this source, an important one in epidemiological studies being that of economy of time, effort and materials.

Blood samples have been collected at half-hourly intervals during the course of the standard two, or two and one half hour glucose tolerance tests. In the case of the 'two-hour blood sugar', widely used in these studies as an abbreviated test of glucose tolerance, the procedure exactly parallels the full standard test but with the omission of all but the two-hour blood sample.

Other Chemical Methods

The various other estimations referred to in the Inserts or Appendices to this Thesis are described in the publications themselves and/or in the commentaries associated with them. They include immunoassays of urinary albumin (Inserts 3, 4 and 5), of plasma insulin and chemical estimation of plasma lipids (Appendix 1).

Physical and Biometric Measurements

Height and Weight - These measurements were made with the subject standing in stockinged feet and using standard clinical equipment which was checked from time to time. For certain of the analyses, these measurements have been combined as the 'Ponderal Index' (Tanner, 1959), calculated as height in inches divided by the cube root of the weight in pounds. This statistic gives a measure of body 'form' or shape, at one end of the scale being the sphere with a low value for ponderal index (i.e. minimising height for a given weight) and, at the other, the cylinder with a high value for the ponderal index (i.e. maximising height for a given weight). It may therefore be regarded, with some reservations, as a measure of rotundity/'cylindricity' and, hence, of obesity. Direct comparisons with alternative and independent estimates of total body fat validate it surprisingly well (Steinkamp et al, 1965).

Skin Fold Measurements - Skin folds were raised in the loose skin overlying the triceps muscle midway between the points of the acromion and olecranon processes in the left arm when hanging loosely by the side. The subscapular measurement was made over the inferior angle of the left scapular with the arm in the same position. In both cases, skin and subcutaneous tissue were lifted from the underlying deep fascial plane and the Harpenden skin fold callipers (British Indicators (Sales) Ltd., St. Albans, Herts.) applied so that the blades made contact with parallel skin surfaces. Readings were made after the initial rapid fall in thickness, lasting a second or two, had subsided. Reproducibility of repeated measurement depended considerably upon the experience of the measurer and improved to approximately $\pm 10\%$ in suitably experienced hands.

Threshold of Vibratory Perception - Measurement of the perception of a standard vibrating stylus of fixed frequency but variable amplitude was made using the Biothesiometer (Bio-Medical Instrument Co., Newbury, Ohio). When suitably loaded and mounted as described in Insert 6 this instrument gave surprisingly reproducible results for a measurement with a subjective endpoint.

Other neurological examination, in particular the eliciting of the tendon reflexes, was carried out by standard clinical methods. Reinforcement procedures were used when unassisted stimuli failed to evoke a response and reflexes were recorded as present when they were elicited by any of these manoeuvres.

Cardiovascular Evaluation - systematic examination included the application of the standard W.H.O. questionnaire (Rose and Blackburn, 1968) for the presence of the symptoms of angina pectoris, history of cardiac infarction and intermittent claudication. Standard electrocardiography was performed using the Cambridge Electrite machine; 12-lead records were made in men but chest leads were omitted in the case of women. A copy of the cardiovascular questionnaire is appended to this Thesis (Appendix 2). It was analysed by a standard coding procedure and individuals were classified as demonstrating 'likely', 'questionable' or 'absent' evidence of the three conditions (angina pectoris, history of myocardial infarction, intermittent claudication) in the enquiry. Electrocardiograms were analysed according to the Minnesota Coding procedure (Blackburn et al, 1960) and finally allocated to 'probable' (1:1, 1:2, 7:1), 'possible' (1:3, 4:1, 2, 3, 5:1, 2, 3) and 'absent' (and other abnormality or 1:0) categories in respect of evidence

of coronary disease. Each record was analysed by two independent observers and differences were reconciled by a third coder. Coding was carried out in ignorance of the clinical status of the individual concerned.

Arterial disease elsewhere, not included in this standardised form of data collection was evaluated on the basis of its clinical manifestations, employing hospital records and other clinical data where available. Such events as stroke or the advent of gangrene occurred comparatively rarely, do not figure as a very important element in the studies discussed, and were clinically unquestionable. This type of information was collected in ignorance of treatment which the individual might have been receiving.

Deaths were adjudged as due to arterial disease (coronary, cerebral etc.) or not on the basis of the causes stated upon the death certificate. No systematic autopsy studies were made.

Definitions

The definition of 'borderline' diabetes has been entirely arbitrarily made. It is meant to encompass that region of the blood sugar spectrum which, though not unequivocally diabetic is yet above the levels commonly regarded as normal. For the purpose of the study reported here, the designation was applied solely on the basis of the blood sugar, measured during the course of the 1962 survey in Bedford two hours after a standard glucose load as described above:-

Two-hour blood sugar less than 120mg/100ml. was classed as normal.

Two-hour blood sugar between 120 and 199mg/100ml. was classed as 'borderline'.

Two-hour blood sugar of 200mg/100ml. or more was classed as diabetic.

This definition of the 'borderline' category includes, at one extreme, a number of people whose blood sugars would be regarded by some as normal and, at the other, a number who would be regarded as diabetic.

Populations Studied

Two main population studies form the basis of this Thesis. The first was composed of a set of 470 established diabetic patients already attending the clinics at St. Mary's and King's College Hospitals, London, along with 1201 of their ostensibly normal first degree relatives; in addition, a group of 280 non-diabetic 'control' patients and 641 of their first degree relatives was also recruited for purposes of comparison. The age and sex composition is shown in Table 1A.

The second population was recruited from Bedford citizens cooperating in the 1962 Diabetes Survey in the town. It contains three subgroups. 248 subjects were found in the course of the survey to conform, by virtue of the two-hour blood sugar, to the criteria above for 'borderline' diabetes; an age and sex matched normoglycaemic control group was also selected; the comparisons were completed by studies of

the newly found diabetic group, numbering 116. Their age and sex composition is shown in Table 1B.

These two population groups are described here only in outline, for they are dealt with in suitable detail in the appropriate Inserts and Appendices to this Thesis.

TABLE 1A

Age and Sex Composition of Diabetic and Control
 Propositi and their Examined First Degree Relatives.

Age	Propositi*				Relatives**			
	Diabetic		Control		Diabetic		Control	
	Male	Female	Male	Female	Male	Female	Male	Female
0-					23	11	14	5
10-	35	38	11	8	54	65	38	46
20-	33	29	17	12	73	63	48	64
30-	29	21	22	13	90	138	50	61
40-	20	21	29	32	136	180	55	61
50-	19	50	31	30	77	116	39	58
60-	32	71	25	30	42	84	25	46
70-	12	55	5	11	17	26	11	18
80+	2	3	0	4	4	2	2	0
Totals	182	288	140	140	516	685	282	359

*Includes only propositi contributing one or more relatives
 to examination for study.

**Includes known diabetics among relatives.

TABLE 1B

Age and Sex Composition of Control, Borderline and
 Diabetic Groups, recruited from the 1962 Bedford
 Survey for further examination.

Age	Men			Women		
	Contl	B/line	Diabc	Contl	B/line	Diabc
20-	5	8	-	4	6	2
30-	8	10	5	8	8	7
40-	20	23	4	14	20	3
50-	30	38	14	21	22	20
60-	24	26	14	26	28	16
70-	17	20	11	12	30	17
80+	-	4	1	-	5	2
Total	104	129	49	85	119	67

5.

GENETIC CONSIDERATIONS

Insert 1 and Insert 2

INSERT 1

The problem of the 'borderline' diabetic, and the question of the influence of this state upon the genetic explanations of the familial nature of diabetes, became apparent during the course of the study of 735 diabetic patients and their relatives described in Insert 1 to this Thesis. This family study, which was started in 1953, was planned to examine the allegation that hypertension occurred with increased frequency in diabetics and their families (Balme and Cole, 1951). The study involved the examination of first degree relatives of diabetic patients attending St. Mary's and King's College Hospital Diabetic Clinics. Ostensibly non-diabetic relatives, who were found to be glycosuric after the ingestion of an oral 50g. glucose load, were submitted to standard oral glucose tolerance tests. Analysis of the blood sugar responses (and those of a comparable group of controls) disclosed many individuals with levels of glucose tolerance lying in the zone intermediate between the clearly normal on the one hand and the clearly diabetic on the other. Frequency distribution plots of the blood sugar data from the glucose tolerance tests of relatives of diabetics showed unimodal, positively skewed distributions at all time points of the G.T.T. It demonstrates the complexities that underlie the use of oral glucose tolerance tests in the genetic analysis of this type of material.

INSERT 2

A prima facie case has been made that the form of inheritance of diabetes of the so-called juvenile onset type (characterised, basically, by insulin dependence) differs from that of so-called maturity-onset diabetes (the non insulin dependent type). This case is based upon the relatively higher frequency of diabetes in the siblings of juvenile onset patients than in the siblings of maturity onset patients, using the frequency in the general population as base for both groups. Since the studies upon which these conclusions were based used verbal family history data and having regard to the frequency of undiagnosed cases of diabetes among older relatives of diabetics, the family data discussed in Insert 1 were further analysed in order to test the allegation of genetic heterogeneity. Other weaknesses in the argument were also explored. The conclusions reached suggested that the incorporation of those relatives with the lesser degrees of carbohydrate intolerance into the analysis cast considerable doubt on the simple conclusions of other workers.

INSERT 1

OBSERVATIONS ON THE INHERITANCE
OF DIABETES

by

Harry Keen, M.B., B.S., M.R.C.P.

Medical Unit, St. Mary's Hospital, W.2.

(Unpublished paper presented to the British
Diabetic Association 11th Banting Memorial
Meeting, Oxford).

Tables and Figures in this Insert are
numbered independently of those in the
body of the Thesis, and the references
are independently compiled.

An investigation into the blood pressures of diabetics and their families brought to light interesting and unexpected observations on the behaviour of oral glucose tolerance in the relatives. They are presented in the hope that they may cast fresh light on the mode of inheritance of diabetes mellitus itself.

Materials and Methods

In the course of an investigation into the blood pressure of diabetics, 735 diabetics over ten years of age were specially examined. 532 of these were consecutive proven cases of diabetes mellitus attending St. Mary's Hospital, and the other 203, selected only by age and sex, were younger diabetics attending King's College Hospital Diabetic Department. Each diabetic was questioned concerning the whereabouts or fate of all first degree relatives (parents, siblings, children) and further information was sought about all those alive at present. Relatives who lived far away from the Hospital were asked to report to their own doctor who examined them and sent us his findings by post. Those who lived near were invited to a special hospital appointment. It is only with this last class of relatives, 1,201 of them who came in response to the invitation and who were examined personally, that this report is concerned.

Table 1 shows the age and sex distribution of the 470 diabetic probands who contributed first degree relatives to the study. It is not representative of the general age distribution of diabetics as the size of the younger age groups has been boosted by special collection but it still shows the expected preponderance of elderly females.

Table 2 shows the frequency of the sizes of the examined kinships which each diabetic propositus contributed. Single representative and groups of 2 and 3 members constitute the greater part.

On arrival at the hospital, after explanation of the purpose of the study, each relative drank 50g. of glucose in 200ml. of water. This was followed by a simple standardised physical examination. At the end of one hour, the subject passed urine; 0.5ml. was added to 5ml. of qualitative Benedicts solution, and the result was recorded after boiling in a water bath for 5 minutes. The glucose drink was omitted when the relative was already known to be diabetic. Glycosuria was said to be present when there was at least green colouration of the Benedicts solution with a perceptible yellow precipitate.

The original purpose of the urine screening procedure was to identify an expected small proportion of undiagnosed diabetics among these ostensibly healthy relatives in relation to the study of blood pressure levels. However, the incidence of glycosuria was so much higher than that reported by other workers that a population composed of 'control' relatives was assembled. This group comprised 641 first degree relatives of patients attending St. Mary's Hospital for conditions, for the most part trivial, other than diabetes mellitus. The propositi themselves were not included in the group. The age and sex composition of these two sets of relatives are shown in Table 3.

The control relatives were invited to the hospital, given oral glucose and treated in the same manner as the relatives of diabetics. Each relative found to have glycosuria under these conditions was invited to the hospital for an oral glucose tolerance test (G.T.T.). This was done in the morning after an overnight fast, in almost all cases, within two weeks of the initial urine test. For the G.T.T. 50g. glucose was given by mouth and capillary blood sugar estimated at half hourly intervals for two hours by the copper reduction method of King and Wootton (1951). Of the 641 control relatives of non-diabetic controls, 110 had a positive family history of diabetes (F.H.+), and 5 were actually known to be suffering from diabetes. The group of (F.H.+) control relatives has been treated separately from those with no family history of the disease (F.H.-) in the analysis and so have known diabetics among both groups of relatives.

Results

1. Glycosuria

The incidence of glycosuria in the relatives of diabetics and in the two groups of control relatives is shown in Table 4. It was 24.6% and 30.4% in the diabetic relatives and the F.H.+ control relatives respectively, and these two values did not differ statistically significantly from each other. However, both significantly exceed the incidence in the F.H.- control relatives which is 14.9%, itself a surprisingly high figure.

2. Glucose Tolerance Tests

For the analysis of results, widely accepted but arbitrary criteria for the diagnosis of a diabetic curve were adopted. These criteria consisted of a blood sugar of 180mg/100ml. or more during the curve plus a value of 120mg/100ml. or more two hours after glucose. Many curves quite clearly satisfied these criteria for the diagnosis of diabetes mellitus and a large proportion were equally clearly non-diabetic. There were, however, a substantial number of intermediate curves which fulfilled the criteria neither of diabetes, nor of normality. Table 5 gives selected examples of curves with the diagnostic group to which they were allocated.

Table 6 shows how these classes of curves were distributed proportionately among the glucosuric relatives of each group, distinguishing diabetic, intermediate and normal responses and also showing those glycosuric relatives who failed to attend for the test. In addition to the greater frequency of glycosuria in the relatives of diabetics compared with controls, the proportion showing intermediate and abnormal responses was also considerably higher. In Table 7 the mean blood sugars and their standard errors at the various points of the G.T.T. are shown for relatives of diabetics and for relatives of the F.H.- controls. The means for the relatives of diabetics are all higher and the differences are statistically highly significant.

3. Frequency Distributions

Figure 1 shows the frequency distribution of blood sugars in the two groups of glycosuric relatives who had G.T.T.'s performed. There is a numerical excess of diabetic relatives at all levels of blood sugar, but, proportionately, they predominate increasingly as the level rises, so that, at the highest blood sugar levels, diabetic relatives only are represented. No natural break in the series of observations occurs at the commonly accepted upper limits of normality nor can one be observed elsewhere in this material. The distributions show leptocurtosis and positive skewing.

Discussion

It is clear that the conventional forms of genetic analysis in diabetes may be unsatisfactory in the light of the findings among the relatives in this study. Most of the earlier studies in man and the conclusions drawn from them have been derived from the analysis of verbal family histories. The hypothesis of Mendelian recessive inheritance, first seriously advanced by Pincus and White (1933; 1934), is much weakened by the deficiency of cases of diabetes among the offspring of the union of two diabetics. Theoretically, the incidence should be 100%, but that observed in several studies does not exceed 10%. This objection was partly met by Harris's hypothesis (1950) that the two forms of diabetes, distinguished clinically as the youthful onset, severe type and the older onset, mild type by Lawrence (1955), were differently inherited. Harris suggested that severe diabetics were homozygous, and mild diabetics heterozygous for an abnormal gene. However, one

would expect, on the basis of this suggestion, that all parents of severe young-onset diabetics should be or become, diabetic themselves. In fact, the incidence among such parents in this study, and in others, is only about 10%. His argument for the existence of the homozygous state in young-onset diabetics was supported by a demonstrated increase in consanguineous marriages among their parents. In assessing the significance of this apparent excess of consanguinity, he made no allowance for the racial composition of his diabetic group. Simple dominant inheritance was suggested by Cammidge (1928) and Wright (1931) from the study of selected family pedigrees. Most later workers have discarded this possibility because of the discrepancy between the theoretical 50% of parents who would be expected to develop diabetes and the much smaller observed incidence. Levitt and Pessikova (1934) also postulated inheritance by way of a single, dominant gene, explaining anomalies in the manifestation of the disease as evidence of 'variable expression' in the operation of the gene.

The absence of a clear separation of the relatives into a normal and an abnormal group, complicates the conventional genetical analysis. Other workers have encountered a similar problem. Sherrill, (1921) who performed G.T.T.'s on 38 ostensibly normal, first-degree relatives of diabetics, considered that 21 showed abnormalities varying from minimal to clearly diabetic in degree. In a 'control' group (made up of 28 patients, 12 of whom had hypertension) abnormal curves were found in 11 cases. Pincus and White (1933; 1934) performed glucose, or sucrose, tolerance tests on 95 diabetic relatives of unspecified degree. 25% of these showed values which they called 'abnormality high' while, of 48

controls, only one was so classed. The data recorded in their paper indicates that all grades of glucose intolerance were found and that there was no clear division of the blood sugars of these relatives into normal and abnormal groups. Steiner (1936) studied the glucose tolerance curves of 258 first degree relatives of diabetics. He found what he termed 'latent diabetes' in 3.5% and other, lesser abnormalities of carbohydrate metabolism in 7.8%. Lemser (1936) followed up some of these same relatives after 3½ years. Of the 6 latent diabetics, one had become symptomatic and of the 16 with 'lesser abnormalities', one was now classed as a latent diabetic.

The findings in the present study, and in those previously discussed, suggest that conclusions based only on verbal family histories may be misleading. Not only is material collected by questioning of patients inherently unreliable, particularly in the elderly, but it cannot identify the diabetics among the ostensibly normal relatives. Furthermore, by excluding from consideration individuals falling between the classes of normal and diabetic it artificially imposes a segregating (normal/abnormal) character upon the result with possibly incorrect genetic implications. An appreciable proportion of glycosuric relatives of diabetics do fall into this intermediate G.T.T. class and it is probable that more examples of lesser degrees of glucose intolerance would have been found in non-glycosuric relatives, had they also been subjected to G.T.T.

The failure of earlier studies to satisfy simple Mendelian ratios, and the existence of intermediate degrees of glucose intolerance among relatives makes it difficult to attribute the genetic factor in the aetiology of diabetes to the influence of a single gene. The results of this study suggest an alternative explanation, that the inheritance is polygenic, or multifactorial. This suggestion is not inherently unlikely when one considers the many factors which are known to influence the level of the blood sugar and glucose tolerance. These include insulin, growth hormone, glucagon, adrenal cortical steroids and autonomic factors. Dietary factors and obesity also contribute. The level of the blood sugar is the resultant of the actions of all these, and other influences. Variation in the inheritance of each, some, or all of these factors may be responsible for the inherited features of the syndrome of diabetes mellitus.

Summary

The incidence of glycosuria one hour after 50g. glucose by mouth in 1201 first degree relatives of diabetics was 24.6%. This was significantly higher than the incidence of 14.9% in 531 relatives of non-diabetics without a family history of the disease.

Glucose tolerance test results in those with glycosuria showed significantly higher blood sugar values among the relatives of diabetics than among the relatives of controls.

The distribution of the blood sugar values in the two groups showed a predominance of diabetic relatives at the higher levels, but, in this material, there was no natural division of response to glucose into discrete groups which could be regarded as normal and abnormal.

Current views on the mechanism of inheritance of diabetes are briefly reviewed, and a distinction drawn between conclusions based on verbal family histories, and those from actual examination of the relatives.

It is concluded that this study supports the hypothesis of multiple hereditary factors rather than a single one. Investigation of complete populations of relatives is required before this alternative can be established.

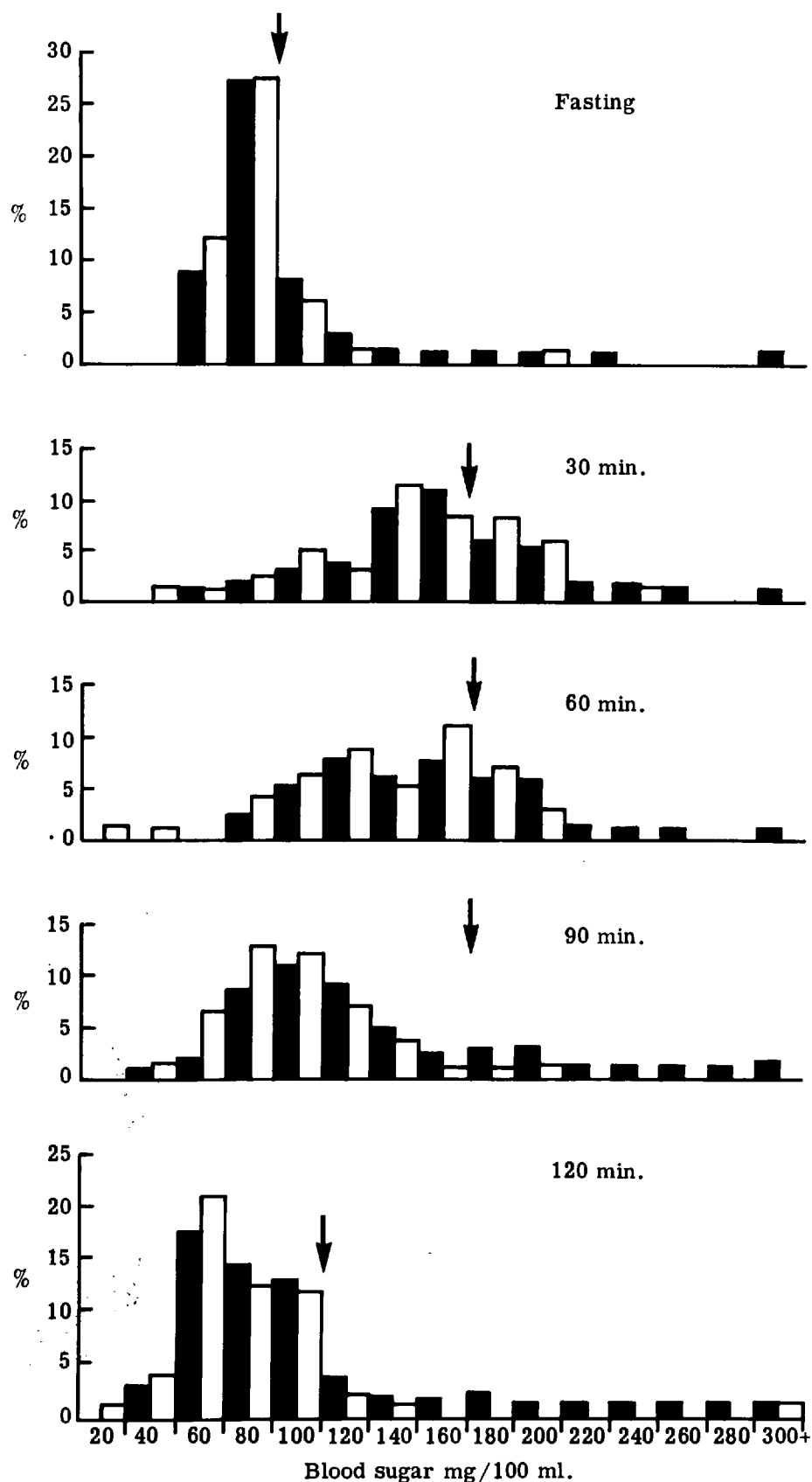


Fig. 1 Frequency distribution histograms of blood sugar values at the five time points of the glucose tolerance test in relatives of diabetics ■ and of controls □

TABLE 1

Age and Sex of Diabetic Propositi
contributing Relatives to Study

AGE	No. of Diabetic Propositi contributing Relatives		
	MALES	FEMALES	TOTAL
10-	35	38	73
20-	33	29	62
30-	29	21	50
40-	20	21	41
50-	19	50	69
60-	32	71	103
70-	12	55	67
80+	2	3	5
TOTAL	182	288	470

TABLE 2

Numbers of Kindships by Size
contributed to Study

139	propositi contributed	1 relative
130	propositi contributed	2 relatives
103	propositi contributed	3 relatives
53	propositi contributed	4 relatives
21	propositi contributed	5 relatives
12	propositi contributed	6 relatives
3	propositi contributed	7 relatives
3	propositi contributed	8 relatives
2	propositi contributed	9 relatives
3	propositi contributed	10 relatives
1	propositi contributed	11 relatives

TABLE 3

Age and Sex Composition of the 1,201 Relatives of Diabetics and the 641 Relatives of Non-Diabetic Controls (divided by absence and presence of diabetics in a blood relative) seen and examined in the Study. Numbers in brackets represent known Diabetics among them, excluded from later Analyses.

AGE	Relatives of:-								
	i Diabetics			ii Controls FH-ve			iii Controls FH+ve		
	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL
0-	23	11	34	13	5	18	1	0	1
10-	54 (1)	65 (2)	119 (3)	31	40	71	7	6	13
20-	73	63 (2)	136 (2)	39	53	92	9	11	20
30-	90	138 (3)	228 (3)	42	52	94	8	9	17
40-	136 (3)	180 (5)	316 (8)	42	54	96	13 (1)	7	20 (1)
50-	77 (1)	116 (5)	193 (6)	35	47	82	4	11 (1)	15 (1)
60-	42	84 (8)	126 (8)	23	34	57	2	12 (1)	14 (1)
70-	17	26	43	9	10	19	2 (1)	8 (1)	10 (2)
80+	4	2	6	2	0	2	0	0	0
TOTAL	516 (5)	685 (25)	1201 (30)	236	295	531	46 (2)	64 (3)	110 (5)

TABLE 4

Post-Glucose Glycosuria Rates in Relatives of Diabetics and of
Non-Diabetic Controls (divided by absence and presence of diabetes
in a blood relative). Known Diabetics Excluded.

Relatives of:-	Number Seen	Glycosuric	
		No.	Prop.
a) Diabetics	1,171	299	25.6%
b) Control FH+	105	32	20.4%
c) Control FH-	531	79	14.9%

		SE	P.
a-b	- 4.8	4.7	0.3
a-c	10.7	2.0	0.001
b-c	15.5	4.7	0.005

TABLE 5

**Examples of Types of Glucose Tolerance Response found among
Ostensibly Normal Relatives of Diabetics, selected to Illustrate
the Allocation Scheme.**

Code	Sex	Age	Mins. after Glucose					Allocation
			Fasting	30	60	90	120	
68/1	F	68	123	260	340	274	210	Diabetic
68/2	M	35	107	124	163	139	118	Normal
92/1	M	47	94	173	224	111	76	Inter.
199/2	M	42	177	238	279	327	341	Diabetic
269/1	F	65	105	251	266	196	82	Inter.
287/4	F	57	103	219	194	164	118	Inter.
419/4	F	47	116	162	174	152	140	Inter.
443/1	M	46	85	115	148	127	130	Inter.

TABLE 6

Classification of G.T.T. Results in Glycosuric Relatives of Diabetics and Controls (by family history). Known Diabetics Excluded. Norm G.T.T.:— No B.S. exceeding 180mg/100ml. or more; 2hr. B.S. 120mg/100ml. Abnormal G.T.T.:— Exceeds both these Limit Levels, Inter. G.T.T.:— Exceeds one or other Limit Level. Prop* expressed as %age of G.T.T.'s performed.

Relatives of:—	No. of glyco	GTT done		Norm GTT		Inter GTT		Abnorm GTT	
		No.	Prop	No.	Prop*	No	Prop*	No	Prop*
a) Diabetics	299	233	78.0%	119	51.1%	75	32.2%	39	16.7%
b) Control FH+	32	29	90.7%	15	51.7%	12	41.5%	2	6.9%
c) Control FH-	79	68	86.2%	43	63.4%	23	33.8%	2	2.9%

TABLE 7

**Mean Blood Sugar (mg/100ml.) in Relatives of Diabetics and Controls
(with absent family history). Differences are Highly Significant
Statistically at all Time Points. Known Diabetics are Excluded.**

	Relatives of Diabetics		Relatives of Controls (FH-)	
	Mean	SE	Mean	SE
Fasting	89.9	0.60	85.6	0.54
30 mins	165.8	1.04	151.7	1.44
60 mins	205.1	1.32	142.9	1.69
90 mins	131.0	1.26	99.5	1.22
120 mins	96.8	1.07	76.4	0.75

BIBLIOGRAPHY

- Cambridge, P.J. (1928). *British Medical Journal* 2, 738.
- Harris, H. (1950). *Annals of Eugenics* 15, 95.
- King, E.J. and Wootton, I.D.P. (1951). 'Microanalysis in Medical Biochemistry'. J. and A. Churchill Ltd., London.
- Lawrence, R.D. (1955). *Annals of Internal Medicine* 43, 1199.
- Lemser, F.H. (1938). *Munchen Medizinische Wochenschrift* 85, 1657.
- Levitt, S.G. and Pessikova, L.N. (1934). *Proceedings of the Maxim Gorky Research Institute for Medical Biology* 3, 132.
- Pincus, G. and White, P. (1933). *American Journal of Medical Science* 186, 1.
- Pincus, G. and White, P. (1934). *American Journal of Medical Science* 188, 159.
- Sherrill, J.W. (1921). *Journal of the American Medical Association* 77, 1779.
- Steiner, F. (1936). *Deutsche Archiv fur Klinische Medizin* 178, 497.
- Wright, L.S. (1931). *American Journal of Medical Science* 182, 484.

INSERT 2

AGE OF ONSET AND INHERITANCE OF DIABETES:
THE IMPORTANCE OF EXAMINING RELATIVES

by

Harry Keen, M.B., B.S., M.R.C.P.

and

Norman S. Track, B.Sc.

Diabetologia 4, 317-321, 1968.

ORIGINALS

Age of Onset and Inheritance of Diabetes: The Importance of Examining Relatives

H. KEEN and N.S. Track

Department of Medicine, Guy's Hospital Medical School, London University

Received: February 23, 1968

Summary. Suggestions that diabetes of younger- and older-onset are inherited differently have been examined in a family study which compares the first-degree relatives of 735 diabetic patients with those of 514 'control' patients. Verbally reported histories from the propositi indicated an excess prevalence of known diabetes among the siblings of younger-onset diabetics; however, when the ostensibly normal, first-degree relatives were examined a high frequency of unsuspected glucose tolerance test abnormality was found. When the diabetes prevalence in relatives of diabetics was compared with that in relatives of controls (*K* ratio), a method at present widely used to determine the mode of inheritance, a number of problems arose suggesting that this means of genetic analysis may be misleading in diseases such as diabetes. It is concluded, for reasons which are discussed, that differences in prevalence ratios cannot be accepted as good evidence for different modes of inheritance of younger- and older-onset diabetes in man.

Age de l'apparition du diabète et sa transmission héréditaire. Importance de l'examen des membres de la famille

Résumé. La suggestion a été faite que le diabète commençant dans la première moitié de la vie est transmis d'une façon différente du diabète diagnostiqué plus tard. — Nous avons examiné cette hypothèse dans une étude qui compare les membres du premier degré des familles de 735 malades souffrant du diabète avec ceux des 514 sujets de contrôle. Les histoires familiales des malades, rapportées oralement, ont indiqué une prévalence excessive de diabète connu parmi les frères et les sœurs des diabétiques juvéniles. Toutefois, lorsque les membres — supposés normaux — furent examinés, une haute fréquence d'intolérance au glucose non-soupçonnée fut trouvée. Quand la prévalence de la maladie dans les familles des diabétiques fut comparée avec celle des sujets de contrôle (rapport *K*). — méthode souvent employée actuellement afin de déterminer le mode de transmission du diabète — nous nous sommes trouvés en face de certains problèmes

qui sembleraient indiquer que cette méthode d'analyse génétique pourrait mener à certaines erreurs d'interprétation dans les maladies telles que le diabète. — Pour les raisons examinées, nous concluons que des différences dans les rapports de prévalence ne peuvent pas être acceptées comme preuves convaincantes d'un mode de transmission différent du diabète juvénile et du diabète d'apparition plus tardive.

Manifestationsalter und Vererbung des Diabetes: Die Wichtigkeit von Verwandten-Untersuchungen

Zusammenfassung. Die Hypothese eines unterschiedlichen Vererbungsmodus für den Diabetes mit frühzeitiger Krankheitsmanifestation und den Diabetes mit späterem Krankheitsausbruch wurde auf Grund von Familienuntersuchungen geprüft. Blutsverwandte 1. Grades von 735 Diabetikern wurden mit einer entsprechenden Kontrollgruppe von 514 Personen verglichen. Bei den Blutsverwandten der Patienten mit früher Diabetesmanifestation ist aus den Krankheitsanamnesen und mündlichen Berichten eine größere Diabeteshäufigkeit zu ermitteln. Wenn indessen die scheinbar normalen Verwandten ersten Grades untersucht wurden, fand man eine große Anzahl vorher unbekannter Fälle von Glucosetoleranzstörung. Bei einem Vergleich der Diabeteshäufigkeit unter den Verwandten der Zuckerkranken und den Verwandten der Kontrollfälle (*K* Vergleichszahl) einer Methode, die z. Zt. oft zur Bestimmung des Vererbungsmodus benutzt wird, ergaben sich einige Probleme, die andeuten, daß diese Art Vererbungsanalysen bei Erkrankungen, wie dem Diabetes, irreführend sein können. Aus den Ergebnissen der Untersuchungen wird gefolgert, daß die zahlenmäßigen Unterschiede, die sich bei dieser Methode des Vergleichs ergeben, keinen Beweis für einen unterschiedlichen Vererbungsmodus des früher oder später sich manifestierenden Diabetes darstellen.

Key-words: Genetics, glucose tolerance, family, heterogeneity, diabetes, juvenile, maturity.

Introduction

Are the clinical and biochemical differences between the two main types of diabetes in man (LAWRENCE, 1955) accompanied by differences in the mode of inheritance? Such differences have been asserted in the past from a consideration of diabetics' family histories alone (HARRIS, 1950). A more valid comparison may, however, be made using the prevalence in the general population (or a sample of it) as a base; thus there have been subsequent reports (SIMPSON, 1962; SIMPSON, 1964; Report of a working party appointed by the College of General Practitioners,

1965) that the excess prevalence of diabetes in the siblings of younger-onset patients over that in a like-aged general population sample is much greater than the excess prevalence in siblings of older-onset cases. There are, however, objections to drawing conclusions from such comparisons of diabetes prevalence, particularly when they are based upon verbal accounts of family history (STEINBERG, 1959; KLINT, MEINERT, HO and BRIESE, 1967). This paper re-examines the question of genetic heterogeneity in a group of diabetics, non-diabetics and the relatives of both with special reference to these and other objections.

Materials and Methods

The basis of the study was formed by 735 patients (diabetic propiiti) attending the diabetic clinics at St. Mary's and King's College Hospitals London, England. The age and sex distribution of this sample is shown in Fig. 1, which also shows the composition of a

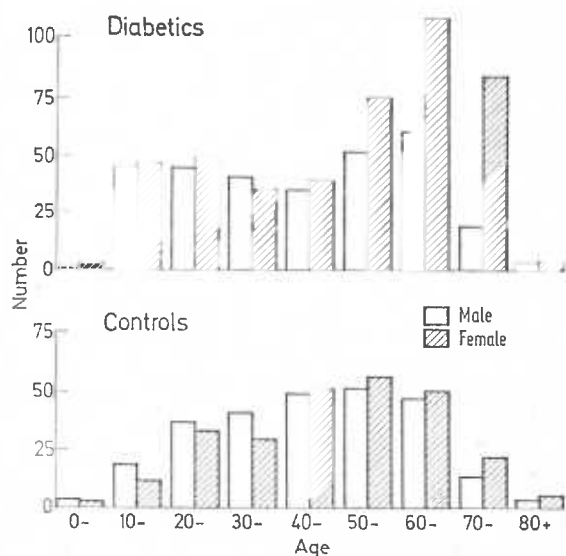


Fig. 1. Age and sex composition of 735 diabetic propiiti (above) and 514 control propiiti (below). The number of younger diabetics was specially augmented

control group of 514 randomly recruited outpatients attending St. Mary's Hospital. The only criterion of selection of the latter was that they were not known diabetics; in the main, they were attending for minor medical and surgical conditions. The age structure of the diabetic sample is not typical of the diabetic population in general for there was selective collection of younger patients to augment the numbers. The extra young patients were recruited without foreknowledge of their family history. A systematic enquiry for diabetes (the reported family history) in any member of the family was made of all diabetics and controls. Details were recorded of the status of all first-degree relatives (parents, siblings and offspring); and those who were reported to be free of diabetes and to live within the London area were invited to attend for examination. Significantly more relatives of diabetics responded than relatives of controls (Table 1). Each attender drank 50 g of glucose in 200 ml of water, answered a standardized verbal questionnaire and underwent a brief physical examination. The urine passed one hour after the glucose drink was tested for sugar with BENEDICT's solution. Of the 1149 relatives of diabetics tested, 26.4% had reducing activity in their post-glucose urine, significantly more than the 17.7% of the 609 relatives of controls similarly tested ($p < 0.002$). Relatives found to be glycosuric were asked to return for a glucose tolerance test (G.T.T.). Capillary blood

samples were taken following an overnight fast, and then 30, 60, 90 and 120 minutes after 50 g of glucose by mouth. Samples were estimated in duplicate, using KING's modification of the FOLIN-WU method (KING and WOOTON, 1951).

Table 1. The number of first-degree relatives of diabetic and control propiiti who were reported to be non-diabetic and who were invited for examination is shown, along with the percentage that attended

Relationship to Propiiti	Diabetic Propiiti		Control Propiiti	
	Invited	Attended	Invited	Attended
Parents	368	72.6%	236	41.5%
Siblings	704	65.6%	613	46.6%
Offspring	549	77.1%	421	53.2%
Overall	1621	70.9%	1270	47.9%

The analysis which follows is divided into two parts. The first part deals with the reported family history as collected from diabetic and control propiiti and is comparable, therefore, with most earlier studies. The second part re-assesses the pattern when the additional findings from the examination of the ostensibly normal, first-degree relatives are also embodied. In both cases, the ratios of frequency of diabetes in relatives of special classes of diabetics to relatives of corresponding controls (K ratios) have been calculated as suggested by PENROSE (1953).

Results

Analysis of Reported Family History

The proportions of diabetic and control propiiti knowing of diabetes in either non-first-degree or first-degree relatives are shown in Table 2. The sexes are

Table 2. The percentage of propiiti reporting diabetes in non-first-degree and first-degree relatives (in each case whether the types of history were single or combined) is shown for propiiti divided into twenty-year age groups. D/C , the ratio of percentage positive reporters in diabetics to controls, indicates the magnitude of excess family history given by diabetic propiiti over controls

Age of propiiti	Percentage of propiiti reporting:					
	Non-first degree ($\pm 1^\circ$) family history			First-degree ($\pm$ non- 1°) family history		
	Diabetic	Control	D/C	Diabetic	Control	D/C
0-19	40.7	17.6	2.3	8.2	—	—
20-39	27.5	12.9	2.1	13.8	2.2	6.3
40-59	17.6	7.2	2.4	31.2	6.3	4.9
60+	10.0	3.7	2.7	28.0	11.1	2.5

not separated as prior analysis had shown no clear differences between them. In successively rising age-groups of the diabetic propiiti, successively more patients report first-degree relatives with diabetes. Though the absolute frequencies are higher in the

diabetics, the rise with age is faster in the controls and so the ratio of relative frequency (D/C) falls with increasing age. This contrasts with the ratios for the non-first-degree family history, which are similar in all age groups.

K ratios comparable with those published by Simpson (1962, 1964) and the College of General Practitioners (1965) may be calculated from the frequency of reported diabetes in the relatives themselves. These frequencies for parents and siblings of diabetics and controls, and the derived *K* ratios are shown in Table 3 for *propositi* with onset diabetes under 35 years of age and for those aged 35 years or more at onset. The *K* ratios all have values clearly exceeding unity, indicating familial aggregation of known cases. Only among the siblings is there an indication that familial aggregation, judged in this way, is more conspicuous in the families of younger-onset than of older-onset patients. Due to the absence of reported diabetes among the offspring of controls, it was not possible to calculate *K* ratios. However, Table 3 shows that diabetes is reported three times as frequently in the offspring of younger-onset diabetics than among those of older-onset patients.

Reported family history analysis, therefore, supports the view that inheritance plays a larger role in determining the appearance of diabetes in the families of younger-onset cases than among relatives of older-onset patients.

upper end of the blood sugar scale by comparison with relatives of controls. At all time points, older relatives tend to higher values. Although a number of the blood sugar values shown are obviously abnormally high, there is no clear division of the distributions into normal and abnormal groups. In order to make a comparison of the prevalence of abnormality in relatives of diabetics and controls, it is, therefore, necessary to

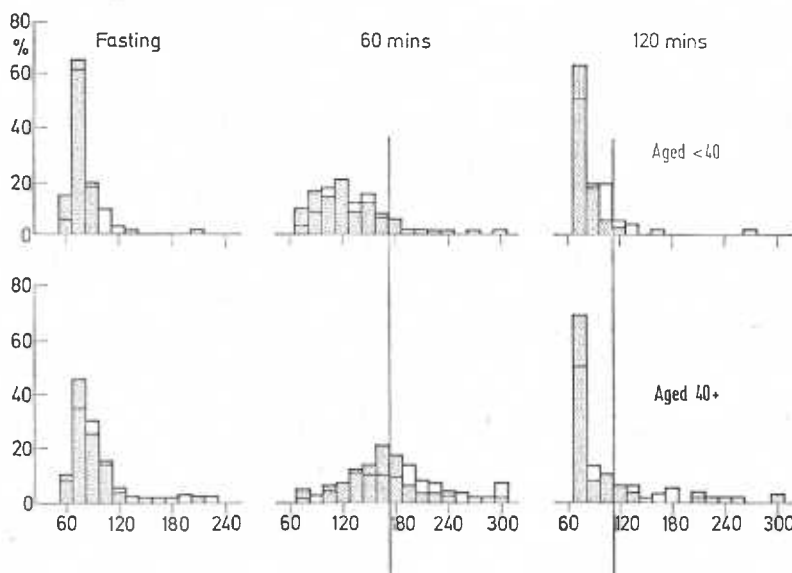


Fig. 2. Frequency distribution histograms of fasting, 60 min and 120 min blood sugar values (mg/100 ml on abscissa) from glucose tolerance tests on ostensibly normal, first-degree relatives of diabetics (white columns) and controls (dark columns) found to be glycosuric after oral glucose. Upper three blocks constructed from relatives aged less than 40 years; lower three represent relatives aged 40 years or more. Vertical lines indicate arbitrary dividing levels (see Table 4 and text)

Table 3. The percentage of total parents, siblings and offspring reported diabetic is shown for histories taken from diabetic *propositi* aged less than 35 years and those aged 35 years and more at time of clinical diagnosis. Control *propositi* are similarly separated at 35 years of age. Total numbers of relatives in each class are shown in brackets. Parents and siblings of four diabetics and two controls who knew nothing of their family origins have been excluded. *K* ratios indicate the degree of excess of affected relatives of diabetics over those of controls

	Diabetics Onset Age		Controls Age		<i>K</i> Ratio	
	< 35	35 +	< 35	35 +	< 35	35 +
Parents	4.0 (540)	9.7 (922)	1.1 (278)	2.9 (750)	3.6	3.3
Siblings	3.3 (596)	3.5 (2199)	0.5 (405)	0.8 (1797)	6.6	4.4
Offspring	2.7 (110)	0.9 (919)	0.0 (62)	0.0 (629)	—	—

Examination Findings in Relatives

G.T.T.'s were performed on 238 relatives of diabetics and 92 relatives of controls, all of whom were reported as normal by the *propositi* but found to be glycosuric at examination. The frequency distributions of their fasting, 1 h and 2 h blood sugar values are shown in Fig. 2, with results for younger and older relatives shown separately. In both age groups, the relatives of diabetics occupy positions towards the

make arbitrary definitions of abnormal responses. If a discriminating level is chosen high in the range, then the frequency of abnormality will be low and, due to the relative positions of the distributions, the calculated *K* ratio will be high. On the other hand, if the discriminating level is set low, although the frequency of abnormality will be higher, the *K* ratio will be lower. To compromise, we have made the division at the commonly accepted diagnostic levels shown as vertical lines on the frequency distributions (180 mg/100 ml at

60 min; 120 mg/100 ml at 120 min). In addition, a function of the area under the glucose tolerance curve has been calculated by summing the fasting and two hour blood sugars with twice the three intervening values. Division of areas into normal and abnormal groups was also made arbitrarily from a consideration of model curves. Four categories of abnormality so derived are defined in Table 4. Glucose tolerance curves which could not be assigned to these categories were classified as normal.

Table 4. *Criteria of abnormality of glucose tolerance test. Category 4 is a function of the area under the blood sugar curve and is calculated by adding to the sum of the fasting and two-hour blood sugar values (in mg/100 ml) twice the sum of the 30, 60 and 90 min values e.g. $a + 2b + 2c + 2d + e$*

1. Highest blood sugar 180 mg / 100 ml or more
2. Two hour blood sugar 120 mg / 100 ml or more
3. Abnormality 1 plus Abnormality 2
4. G.T.T. Area (2 h) 1200 or more

Table 5. *Rates of newly found G.T.T. abnormality (see Table 4) and of reported diabetes in the younger (under 40) and older (40 or more) relatives of younger (under 35) and older (35 or more) diabetic and control propositi; these rates are expressed as a percentage of all relatives seen in the particular group*

Relatives of Diabetics							Relatives of Controls							
Prop. Age of Onset	Relative Ago	Newly Found Abnormality				Previously Known D.M.	Prop. Age	Relative Age	Newly Found Abnormality				Previously Known D.M.	
		1	2	3	4				1	2	3	4		
<35	<40	2.7	0.7	0.7	1.3	3.3	<35	<40	3.0	0.0	0.0	0.0	0.4	
	40 +	12.8	4.5	3.8	6.4	4.0		40 +	11.7	1.5	0.0	4.4	1.1	
	35 +	<40	11.1	5.6	3.7	4.6	0.9	35 +	<40	1.9	0.5	0.5	1.0	0.0
		40 +	17.3	8.1	7.0	13.3	5.2		40 +	12.9	2.9	2.4	5.9	1.3

Table 5 shows the rates of these newly-found G. T. T. abnormalities calculated after making allowance for the small number of glycosurics defaulting at G.T.T. The Table also shows the rates of *reported* known diabetes in the first-degree relatives. The relatives are divided by the onset age of the propositus contributing them to the study (< 35 or 35+) and also by their own age at the time of examination (< 40 or 40+). Abnormality rates have been calculated separately for each group. It is possible to make several different estimates of the total frequency of carbohydrate intolerance in the relatives by adding the rates of *reported* diabetes into each of the other rates in turn, for it is reasonable to assume that *reported* diabetics qualify for all of the G. T. T. abnormality categories.

K ratios calculated from these different total rates are shown in Table 6. The degree to which they exceed unity indicates the systematically higher rates of abnormality in relatives of diabetics compared with relatives of controls. Although the absolute frequency of abnormality is higher in older relatives (Table 5), the

K ratios are higher for younger ones (Table 6). However, the values of *K* for relatives of younger- and older-onset diabetics do not show marked differences.

Analysis of *reported* family histories supplemented by examination of relatives, therefore, gives no clear indication of a larger inherited element in the families of younger-onset than older-onset diabetics.

Table 6. *Relative prevalence (K) ratios calculated for each class of relative after adding to each, in turn, of the rates of newly found G.T.T. abnormality, the rates of known diabetes. The resulting estimates of total prevalence of glucose intolerance in relatives of diabetics were divided by the corresponding control estimates to give the K ratios*

Prop. Age Group	Relative Age	Abnormality			
		1	2	3	4
< 35	< 40	1.8	10.0	10.0	11.5
	40 +	1.3	3.3	7.2	1.9
35 +	< 40	6.4	13.2	9.4	5.6
	40 +	1.6	3.2	3.3	2.6

Discussion

When estimates are based upon the analysis of verbal family histories, values of the *K* ratio for the relatives of diabetics of younger-onset are higher than those for the relatives of older-onset cases. However, when a fuller ascertainment of glucose tolerance status is made by submitting ostensibly normal relatives to examination and including abnormal responses to glucose in the calculation of the ratios, this difference between relative prevalence in the families of younger-onset and older-onset cases is found to be much less marked.

There are probably several reasons for the conflict between conclusions arising from family history study alone and from the history supplemented by examination of reported normal relatives. If diabetes breeds true symptomatically, younger-onset cases are more likely to know about affected relatives than the oligo or asymptomatic older-onset diabetics. This would lower the *K* ratio for relatives of older-onset cases.

A more serious objection to the use of the K ratio emerges when the prevalence of the condition in the general population shows a rise with age. To consider the extreme cases, if general population frequencies are extremely low or nil in youth, then a very few affected relatives of diabetic propoiti will give a very, perhaps infinitely, high value for the K ratio ($X\%/0 = \infty$) as with the offspring in Table 3; at the other age extreme, the general population frequency may be so high that it is not possible to exceed it significantly ($X\%/100 < 1$). Fig. 3 depicts a hypothetical model where diabetes

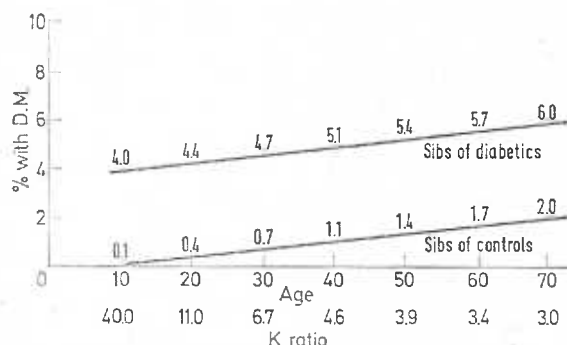


Fig. 3. A hypothetical model showing that, even assuming a constant genetic factor causing a fixed increase of 4% in the rate of affected siblings of diabetics at all ages, there is a considerable fall with age in calculated K ratios (see text for further explanation)

frequency in a population composed of siblings of diabetics is compared with that in a population composed of siblings of controls (or of the population at large). In its construction, two reasonable assumptions are made: (i) that the frequency of diabetes in the general population rises with age from zero to a total of 2%. (ii) that among siblings of diabetics a genetic factor, operating equally at all ages, causes diabetes in an extra 4% of them. The values of K ratios calculated on these assumptions are much higher at younger than at older ages. Thus, a result which has been proposed as evidence of different modes of inheritance can arise from a population which is, by definition, genetically homogeneous.

There are further difficulties in using the K ratio when the abnormality or disease in question represents an arbitrarily defined extreme of a continuously distributed variate, such as the blood sugar in this study. Not only will values for K depend upon the discriminating blood sugar level selected but, since it has been shown that there is a general rise in blood sugar with increasing age (Fig. 2), the K ratio will show a fall with age even though the blood sugar distributions, relative to each other, do not change!

Our findings suggest that the differences of K values published in support of the hypothesis that there are different modes of inheritance in the two clinical types of diabetes may arise in part from incomplete ascertainment of diabetes in the relatives, and that, in any case, the influence of age on diabetes prevalence

introduces objections to the simple comparisons of relative prevalence.

It is clear that lesser grades of glucose intolerance occur with unsuspectedly high frequency among the relatives of patients (and in the population at large). Further, it appears that any choice of diagnostic criteria will represent an arbitrary division imposed for extraneous reasons upon a continuously varying series. EDWARDS (1960) has shown that even when 'thresholds' of recognition are arbitrarily imposed, it is still possible to demonstrate the mode of operation of a hereditary element. It would seem unwise, however, to apply his general argument to the question of different modes of inheritance of the two clinical types of diabetes for their 'thresholds' of recognition are entirely dissimilar.

The purpose of this paper is not so much to deny the existence of genetic heterogeneity in diabetes as to point out some of the objections to the use of the K ratio as evidence supporting it.

Acknowledgements. This work was supported by a grant from the Research Fund of the British Diabetic Association. N.S.T. is aided by a grant from the Canadian Diabetic Association Foundation Fund. We would like to thank Messrs. AMES for their provision of printing facilities for coding purposes. Statistical analysis was computer-assisted and we wish to thank Professor BUCKINGHAM and Miss A. RUSSEL, B.Sc., of the University of London Institute of Computer Science for their assistance.

This paper formed the basis of a communication given to the British Diabetic Association, Medical and Scientific Section Meeting, Leicester, April, 1967.

References

- EDWARDS, J.H.: The Simulation of Mendelism. *Acta genet. (Basel)* **10**, 63—70 (1960).
- HARRIS, H.: The Familial Distribution of Diabetes Mellitus: A Study of the Relatives of 1241 Diabetic Propoiti. *Ann. Eugen.* **15**, 95—117 (1950).
- KING, E.J., and I.D.P. WOOTTON: *Micro-Analysis in Medical Biochemistry*, 2nd ed., London: J. & A. Churchill Ltd. 1951.
- KLIMT, C.R., C.L. MEINERT, I.P. Ho, and P.W. BRIESE: Study of Familial Patterns of Reported Diabetes: Evaluation of questionnaire Data. *Diabetes* **16**, 40—50 (1967).
- LAWRENCE, R.D.: Three Types of Human Diabetes. *Ann. Int. Med.* **43**, 1199—1208 (1955).
- PENROSE, L.S.: The Genetical Basis of Common Diseases. *Acta genet. (Basel)* **4**, 257—265, (1953).
- Report of a Working Party Appointed by the College of General Practitioners, The Family History of Diabetes. *Brit. med. J.* **1**, 960—962. (1965).
- SIMPSON, N.E.: The Genetics of Diabetes: A Study of 233 Families of Juvenile Diabetics. *Ann. hum. Genet. (Lond.)* **26**, 1—12 (1962).
- Multifactorial Inheritance: A Possible Hypothesis for Diabetes. *Diabetes* **13**, 462—471 (1964).
- STEINBERG, A.G.: The Genetics of Diabetes. *Ann. N.Y. Acad. Sci.* **82**, 197—207 (1959).

HARRY KEEN, M. R. C. P.
Department of Medicine
Guy's Hospital Medical School
London Bridge S.E. 1
England

Further Comments Upon Genetic Aspects

Diabetes has been termed the "geneticists nightmare" (Neel, 1969) and the existence of intermediate grades of glucose intolerance, discussed in Inserts 1 and 2, contribute no little part to this *cri de coeur*. The shortcomings of the traditional approach by the verbal questioning of patients are clear. It is not very accurate (Steinberg, 1959; Klimt et al, 1967), it is incomplete since many now normal may later become diabetic, and it imposes a 'segregating' character upon a disorder which has all grades of expression ranging without a break from the normal to the clearly diabetic. Edwards' (1960) case that even data collected in this way can indicate polygenic inheritance despite its inbuilt bias toward simple single gene Mendelian interpretation is weakened by the heterogeneity in age and type of expression of diabetes; that it still has some strength is indicated by the fact that Simpson (1964) did in fact come to this tentative conclusion from verbal-type family history data.

A more direct approach to mechanisms of inheritance by measurement of glucose tolerance in the offspring of various types of mating (between diabetics, between normals and between both) was made by Neel et al (1963) and more recently in the close relatives of established diabetics by Thompson (1965). Both demonstrated a general shift of glucose tolerance in relatives of diabetics towards higher blood sugar levels. Neither showed clear, or even suggestive segregation of the responses into normal and abnormal groups. It would therefore be reasonable to suppose that familial, perhaps genetic factors play a part in determining the state of borderline diabetes in man. The recent

report by Moorhouse (1970), suggesting a greater frequency of various grades of glucose intolerance with ageing in the relatives of diabetics compared with relatives of non-diabetics, supports and somewhat amplifies this general conclusion.

The contribution of genetic factors to the Bedford 'borderline' diabetic group presented later is difficult to evaluate. The frequency of a "positive family history" of diabetes in the 'borderline' group more closely resembled that in the controls than in the newly found diabetics. At face value this might seem to suggest a much greater importance of environmental factors in determining the lesser degrees of glucose intolerance. However, such a conclusion should be advanced with great reserve. Verbal family history, at best an imperfect genetic index, may be a particularly inappropriate datum in this situation for if, as seems likely, members of families tend to resemble each other not only in the presence of glucose intolerance but also in its degree, then there may be many unidentified examples of 'borderline' diabetes among ostensibly normal relatives of the 'borderline' probands. What is required is not so much an enquiry into the family pattern of diabetes mellitus, as a comprehensive study of the nature of inheritance across the whole range of blood sugar values (or glucose tolerance) preferably originating from a defined population group rather than a hospital or clinic population.

6. THE BEDFORD 'BORDERLINE' DIABETIC STUDY

(see Appendices 3, 4 and 5).

In order to explain the genesis of this study it is necessary briefly to describe the framework within which it originated.

The Diagnostic Survey

In May and June 1962 a diagnostic survey for undiagnosed diabetics was undertaken in the South Midlands town of Bedford (1961 Census population 63,000 approximately). The idea of a town-wide survey was originally conceived by Dr. C. L. E. H. Sharp, Medical Officer of Health for Bedford, who approached Professor W. J. H. Butterfield and members of the staff of the Department of Medicine, Guy's Hospital, in 1961. In concert, three main aims of the operation were defined.

1. To conduct an epidemiologically valid study of the prevalence of undiagnosed diabetes in a representative urban population.
2. To study the medical and social factors involved in the aetiology of diabetes found in this way.
3. To collect for the first time, a valid random sample of a normal population to define the variation in the normal glucose tolerance test, and to set acceptable criteria for the biochemical diagnosis of diabetes.

Prerequisites

Long before the survey commenced, the co-operation and participation of many individuals and bodies in the town were recruited. The study was largely financed from a Request for research into diabetes left by a Bedford citizen, Mr. A. Cullip. The Mayor and municipal authority gave their full support to the project and meetings were arranged with management and on the shop floor of local factories, with the Women's Institute, junior business men's Round Table Organisation, schools, the Civil Defense Organisation etc. Posters were widely displayed, interviews and articles published in the local Press and radio and television broadcasts arranged. Plans were discussed with the staff of Bedford General Hospital and the project as a whole presented to the profession through the Bedford Medical Society and personal letters written to each local family doctor.

Plan of Survey

In broad outline, it was planned to attempt to collect a sample of urine, passed an hour or so after Sunday breakfast, from every adult member of the population living within the statutory town boundary. The population approached was defined as that appearing on the 1961 Electoral Register for that part of the Bedford Parliamentary Constituency which lay within the above limits, numbering approximately 38,400. Urine testing stations were set up at the Public Health Clinic at Putnos and urine testing with the enzyme-tipped Clinistix (Ames Co. Ltd.) arranged on a conveyor belt system. Optimal rates and deployment had been worked out on a prisoner population in the local prison earlier. Information regarding age, sex, marital status, address, and the presence of known

diabetes was to be inscribed on the specially prepared lid of the plastic urine containers.

A further stage of the study concerned those people found to be glycosuric in this initial screening step. Modified, curtailed glucose tolerance tests, consisting of a single capillary blood sugar measurement exactly two hours after the ingestion of a liquid glucose solution, taken in the morning after an overnight fast, were arranged. Blood sugar was estimated automatically using Autoanalysers, at the rate of 40 per hour, programmed for the micro-ferricyanide method.

Superimposed upon these arrangements was the drawing, from the whole cooperating population (excluding only already known diabetics and people with other conditions likely to affect glucose tolerance) a random sample, stratified by decade of age and sex. Random sample subjects were to undergo a full 2½ hour oral glucose tolerance test with blood sugar measured fasting and at half hourly intervals. Glycosuric and random sample subjects (in some cases, these coincided) attended a special clinic for these tests at the rate of approximately 60 per day. During their stay at the clinic, a standard medical history was taken and examination carried out.

Results

25,701 of the Bedford citizens approached provided urine samples for analysis and 1,046 of these (4.1%) were positive to Clinistix testing. The modified glucose tolerance test was performed on 939 of these glycosurics. A further 25 glycosurics were drawn into the

random sample and, along with 517 non-glycosurics, were subjected to the full 2½ hour G.T.T.

Special Groups

Allocation to diagnostic groups was carried out, for glycosurics and random sample subjects alike, solely on the basis of the two-hour blood sugar value by the criteria described in the section on Materials and Methods. In this way, 228 'borderline' diabetic subjects were recruited, 74 from the random sample and 154 from the glycosuric group. (This total has been published, in error, as 229 due to the incorrect inclusion of one normoglycaemic glycosuric subject). In addition, a further 20 subjects with two-hour blood sugar levels conforming to the criteria laid down were recruited from a glaucoma screening clinic at which 50g. glucose loads were administered to fasting subjects submitting themselves voluntarily to eye examination. 210 people from the normoglycaemic segment of the random sample were selected on an age / sex - matching basis as a control group. The numbers fell short because of difficulty in finding suitable normoglycaemic individuals in the oldest age groups. 116 of the newly detected diabetics constituted the third group in certain of the comparisons which follow.

Examination and Follow-Up

All the people subjected to glucose tolerance tests, curtailed or full, had been questioned and examined at the time of the G.T.T. In addition, as many members as possible of each of the three special groups described above were re-examined during the 6 months following the survey using the standardised methods described in an earlier section. In the case of the diabetic group, in most cases treatment had already been started at the time of this re-examination.

'Borderline' diabetics were assigned randomly to their Treatment Group (see below) some time after the re-examination. The question of treatment did not arise for the control group and it was emphasised that they had been recalled for examination precisely because they were non-diabetic.

Since 1962, strenuous efforts have been made to re-examine all available members of the 'borderline' group at regular six-monthly intervals. The nature of these re-examinations has varied somewhat from 'cycle' to 'cycle' but within each examination cycle the same examination and questioning routine has been adopted for all. Certain of the enquiries and measurements have been made at all attendances (weight, blood sugar measurement, symptomatic enquiry etc); others have been made rather less frequently (blood pressure measurement, measurement of skin fold thickness and threshold of vibratory perception, timed urine collection, examination of lens and fundi, cardiovascular questionnaire, etc.). Some have been made on two or three occasions only (electrocardiography, venous blood sampling, quantitative urine culture etc.). A detailed schedule of examinations is shown in Table 2.

TABLE 2

		Open and Standardised Enquiries							Exam. Procedures								Investigs.				
		GHE	FHE	CVQ	UHQ	ATS	DMS	DDQ	Ht	Wt	LG	SF	BP	AP	TR	VS	OE	E	C	U	V
Survey	(1962)	+	+				+		+	+	+	+	+	+	+				+		
Visit	1 (1962)	+	+	+						+			+		+	+	+	+	+	+	
	2 (1963)	+				+			+	+	+	+		+	+	+			+	+	
	3 (1963)	+	+			+		+	+	+	+	+			+	+			+	+	+
	4 (1964)	+				+			+	+	+	+		+	+				+	+	
	5 (1964)	+	+	+		+	+		+	+	+	+	+	+	+	+	+		+	+	
	6 (1965)	+		+		+	+		+	+	+	+		+	+	+			+	+	
	7 (1965)	+				+	+		+	+	+	+	+	+	+	+	+		+	+	+
	8 (1966)	+	+	+		+				+		+			+	+		+	+	+	
	9 (1966)	+			+	+				+									+	+	
	10 (1967)	+	+	+		+	+	+		+	+	+	+	+	+	+			+	+	
	11 (1968)	+		+		+	+			+		+							+	+	
	12 (1968)	+	+	+		+	+			+	+	+	+	+	+	+			+	+	+
	13 (1969)	+				+				+		+							+	+	
	14 (1969)	+		+		+		+		+		+				+		+	+		
	15 (1970)	+	+	+		+	+			+	+	+	+	+	+	+			+	+	

TABLE

Summary of important component enquiry, examination and investigation procedures carried out to date in the Bedford 'borderline' diabetic group. Dates refer to the visits paid by subject 001 (Mr. E. N. A.). Periods in excess of the usual 6 months separated visits 9 and 10 and visits 14 and 15.

GHE = General Health Enquiry
 FHE = Family History Enquiry
 CVQ = Cardiovascular Questionnaire
 UHQ = Urinary History Questionnaire
 ATS = Adverse Treatment Symptoms
 DMS = Diabetic Symptoms
 DDQ = Dietary Diary Questionnaire

Ht. = Height
 Wt. = Weight
 LG = Limb Girth Measurement
 SF = Skin Fold Measurement
 BP = Blood Pressure Measurement
 AP = Arterial Pulse Palpation
 TR = Tendon Reflex Testing
 VS = Vibratory Perception Measurement
 OE = Ophthalmoscopic Examination

E = Electrocardiography
 C = 2hr. Capillary Blood Sugar
 U = Urinary Albumin Assay
 V = Venous Blood Taken
 * = Casual Blood Sugar
 F = Fasting Blood Sugar

TABLE 3

Allocation of 'Borderline' Diabetics by Age and Sex
to the Four Treatment Groups.

		TOLBUTAMIDE*					
		ACTIVE		PLACEBO			
		AGE	MEN	WOMEN		MEN	WOMEN
DIET**	20-		1	2		0	0
	30-		3	6		2	0
	40-		6	2		7	7
	50-		13	6		8	2
	60-		4	7		6	9
	70-		6	5		6	8
	80+		1	0	62	59	2
NO DIET	20-		3	1	61	66	4
	30-		1	1		4	1
	40-		7	6		3	5
	50-		6	10		11	4
	60-		8	8		8	4
	70-		2	7		6	10
	80+		0	1		1	2

*Tolbutamide Active consisted of 0.5g. twice daily.
 Placebo - identical tablet of 0.003g. twice daily.

**Diet Carbohydrate restricted by dietary prescription to 120g. daily.
 No diet involved only admonition to 'cut down on table sugar'.

At the first visit, 'borderline' subjects were allocated to Treatment Groups, approximately equal numbers receiving Tolbutamide 0.5g. twice daily without effective carbohydrate restriction, Tolbutamide with effective carbohydrate restriction, placebo tablets without carbohydrate restriction and placebo tablets with effective carbohydrate restriction. The factorial design of this controlled trial of treatment was developed to enable the effect of Tolbutamide and of dietary carbohydrate restriction to be assessed independently of each other and, when it appeared to be of value, in association with each other (Table 3).

7. FINDINGS IN THE 'BORDERLINE' GROUP

A variety of measurements and examinations were made on the occasion of the 1962 Survey. Some were repeated and others added over the course of the few months following the Survey in the individuals falling into the 'borderline' category and also in the control and diabetic groups. As previously described, the 'borderline' subjects were allocated to Treatment Groups after this first re-examination and then reviewed six-monthly. Control and diabetic subjects were seen for review approximately five years after the Survey, in 1967.

The material which follows was collected at one or other of these examinations or reviews; in each case, the source of the information is clarified. When appropriate, the findings in the 'borderline' group are set against corresponding data for the control and diabetic groups.

i. General Biometric Observations

a) Weight

The simplest presentation of data in relation to weight is shown in Table 4 which shows by age and sex groups the mean body weight in lbs. of the three blood sugar groups. Subjects were weighed without shoes and jackets to the nearest lb.

Except in the youngest age group (where numbers of newly found

diabetics were very small) in each of the others, and for both sexes, there is a strong trend for weight to increase with increasing blood sugar categories. So far as one is able to judge from these figures, the trends are steepest among the middle-aged (30 - 59) in both sexes suggesting that in the youngest and oldest age groups, the relationship between body weight and raised blood sugar is less notable. While this is a commonplace clinical observation among the young, in whom diabetes is usually characterised - at the time of clinical presentation at any rate - by wasting and weight loss, it is a comparatively novel observation among the elderly. Here, it must be emphasized, one is dealing with a survivor population, and it is possible that individuals with both obesity and hyperglycaemia have failed to survive to this stage of life, carried off perhaps by cardiovascular disease to which both factors would make them specially vulnerable. An alternative explanation might be that factors other than obesity might assume increasing importance in the elevation of blood sugar among the elderly. These explanations are not mutually exclusive and there is no way to distinguish which is the likelier.

The overall trends, shown in the last row of figures of Table 4 are significant ($p < 0.01$) using Armitage's modification of the χ^2 test for trends (1955), as are the individual differences between controls and diabetics using Student's t test (Snedecor, 1958). There is a suggestion that the trend is rather stronger among men than women. The increased variability in weight in the diabetics is indicated by the greater standard error, only partly explained by the smaller numbers in this group and an observation made by others (Pyke and Please, 1957).

TABLE 4

Body Weight in lbs. Mean and Standard Error
in the Three Blood Sugar Groups by Age and Sex.

Age		Men			Women		
		Cont1	B/line	Diabc	Cont1	B/line	Diabc
20-	Mean	153.6	140.2	127.0	143.7	139.8	113.5
	SE	5.0	9.4	-	20.0	14.1	2.5
30-	Mean	168.3	152.1	180.0	142.0	142.9	189.4
	SE	8.5	8.6	18.3	7.2	5.6	26.7
40-	Mean	158.3	157.9	186.0	136.7	140.8	170.0
	SE	3.8	4.4	20.7	4.9	5.5	25.5
50-	Mean	159.2	171.1	180.1	143.0	146.9	164.7
	SE	5.1	3.8	7.7	5.7	7.4	8.3
60-	Mean	152.2	166.2	165.4	137.6	150.6	150.0
	SE	4.0	7.6	7.1	3.3	5.5	8.5
70-	Mean	150.4	151.7	160.5	136.3	131.9	141.8
	SE	5.5	4.5	9.1	8.0	4.4	4.5
80-	Mean	-	160.5	132.0	-	130.4	130.5
	SE	-	9.0	-	-	4.9	7.5
All	Mean	150.5	160.8	169.3	139.0	141.7	155.5
	SE	2.2	2.5	7.1	2.4	2.5	8.1

b) Ponderal Index

The use of simple body weight as an expression of obesity is unsatisfactory, for it does not take height, or other indices of body build into consideration. This objection is partly met by the use of the 'ponderal index', an expression suggested by Tanner and derived from the height divided by the cube root of the weight, the former expressed in this case in inches and the latter in lbs. Since, for a given height, increasing weight will give a diminishing dividend, the actual value of the index will vary inversely with the degree of obesity. Since the divisor is a cube root function of weight, the index expresses the height per unit of volume and thus covers the range between the cylinder at one extreme (where for a given height, volume is minimised) to a sphere at the other (where, for a given volume, height is minimised) and the index may therefore be regarded as an expression of elongation / rotundity applied to man.

Table 5 shows the ponderal index by age and sex calculated from the standing height and the weights from which the preceding Table was derived. The trends shown in the previous Table are still present, indicating that they were not simply based upon systematic differences in height between the blood sugar groups. They are steepest in the 4th and 5th decades in both sexes. Owing to the complex nature of the ponderal index, it is not possible to contrast the relative strength of overall trend when compared with that for body weight.

TABLE 5

Ponderal Index (Height ins. /Weight lbs. ^{$\frac{1}{3}$})
Mean and Standard Error in the Three Blood Sugar
Groups by Age and Sex.

Age		Men			Women		
		Cont1	B/line	Diabc	Cont1	B/line	Diabc
20-	Mean	13.1	12.9	12.3	12.1	12.2	13.1
	SE	.12	.26	-	.49	.27	.46
30-	Mean	12.5	12.5	11.6	12.3	11.9	11.1
	SE	.11	.17	.15	.15	.18	.42
40-	Mean	12.3	12.3	11.2	12.2	12.0	11.2
	SE	.09	.11	.24	.14	.14	.44
50-	Mean	12.3	11.9	11.9	11.9	11.8	11.2
	SE	.10	.09	.13	.15	.19	.14
60-	Mean	12.4	12.3	11.9	11.9	11.6	11.6
	SE	.09	.18	.14	.11	.16	.22
70-	Mean	12.3	12.3	12.0	11.9	11.9	11.6
	SE	.13	.15	.13	.18	.12	.12
80-	Mean	-	11.8	12.4	-	11.8	11.8
	SE	-	.20	-	-	.22	.30
All	Mean	12.4	12.2	11.9	12.0	11.8	11.4
	SE	.05	.06	.08	.06	.07	.10

c) Subscapular Skin Fold

A more direct measurement of obesity is the thickness of a fold of skin and subcutaneous tissue, the mean absolute value of which is shown in mm., by age and sex for the three blood sugar groups in Table 6. The measurement of course, represents the thickness of a double fold, Unlike the body weight data in Table 4, the values for skin fold thickness are absolutely greater in women than men, suggesting either that a greater proportion of body fat in women is deposited in the subcutaneous tissue or that a higher proportion of the body weight is represented by adipose tissue in women than men. Apart from this, the findings support those expressed in the previous two Tables, indicating increasing obesity with progressively higher blood sugar groups, a gradient apparently greater in magnitude in the middle years than at the two extremes. In proportionate terms, the size of the difference between the groups is much larger in respect of skinfold thickness than in respect of body weight, a comparison summarised in Table 7.

These analyses support the generally accepted (though surprisingly poorly furnished) contention that obesity is an association of diabetes and further suggest that the association is stronger in the middle-aged than the young or the elderly.

TABLE 6

Subscapular Skin-Fold in mm. Mean and Standard
Error in the Three Blood Sugar Groups by Age and Sex.

Age		Men			Women		
		Cont1	B/line	Diabc	Cont1	B/line	Diabc
20-	Mean	11.4	13.3	15.0	20.6	19.8	14.2
	SE	0.9	3.5	-	7.8	4.4	5.8
30-	Mean	17.2	17.5	25.2	18.7	23.5	31.3
	SE	2.0	2.8	8.0	2.7	3.6	3.8
40-	Mean	16.3	17.2	28.6	17.1	21.9	36.1
	SE	1.5	1.3	5.8	1.7	2.1	13.9
50-	Mean	17.7	23.2	25.7	18.6	23.2	31.8
	SE	1.6	1.2	3.1	1.9	3.7	4.4
60-	Mean	16.8	18.9	22.0	22.3	26.0	27.6
	SE	1.2	2.0	1.5	1.7	3.8	4.7
70-	Mean	16.4	20.6	19.9	17.7	21.7	26.6
	SE	1.0	2.3	3.2	4.2	2.5	7.0
80-	Mean	-	19.3	17.0	-	20.3	28.0
	SE	-	0.1	-	-	10.7	-
All	Mean	16.6	19.8	22.7	19.6	23.1	28.5
	SE	0.7	0.8	1.4	1.0	1.4	2.5

TABLE 7

Comparison of Magnitude of Trends of Body Weight and Skin-Fold Thickness with Blood Sugar Grouping, by Sex. Mean Values for all Age Groups combined are standardised by expressing Mean Body Weight and Mean Skin-Fold Thickness for Male Controls as 100 and calculating the Proportionate Excess or Deficit in the other Groups with this as base.

	Men			Women		
	Contl	B/line	Diabc	Contl	B/line	Diabc
Body Weight	100	106.8	112.5	92.4	94.2	103.3
Skin-Fold	100	119.3	136.7	118.1	139.2	171.8

ii The Kidney, the Eye and the Peripheral Nerves.

The observations bearing upon the kidney, the eye and the peripheral nerves are considered together as representing a group of diabetic 'complications' which are relatively specific to the disease. Although differing widely in their clinical and morphological manifestations, abnormalities of these three systems are considered by some to share common underlying pathogenic mechanisms and have even earned the name of diabetic 'triopathy.

In the established, clinically apparent diabetic it is often many years before the clinical manifestations of these disorders become evident, and then commonly when structural and functional changes are so far advanced that their reversibility by treatment becomes doubtful. In making observations in the 'borderline' diabetic, the use of sensitive and quantitative indices of disorder or renal, ocular and neurological function seemed desirable. It was for this reason that urinary albumin immunoassay was developed and that the biothesiometer was used for numerical estimate of vibratory threshold. At the time in question, the method of fluorescein retinal angiography was insufficiently developed for application to the Bedford Study and clinical ophthalmoscopy was employed.

a) The Kidney (Inserts 3, 4 and 5).

Following upon the clinical observation that proteinuria is the first indication of renal involvement in diabetes, attention was focussed initially upon the development of a sensitive and accurate method for measuring urinary albumin. The method was then applied to urine samples collected as described and kept at -20°C in the deep freeze until assay.

The following three Inserts trace the development of the assay method (Insert 3), its preliminary application to samples of the three blood sugar groups (Insert 4) and a more comprehensive study of the urinary findings in them at the first visit, with an examination of the statistical associations of the microalbuminuria found (Insert 5). The long-term behaviour of urinary albumin excretion rates in the blood sugar groups remains to be determined, in particular the influence, if any, of the various forms of treatment employed in the 'borderline' group.

INSERT 3

AN IMMUNOASSAY METHOD FOR URINARY

ALBUMIN AT LOW CONCENTRATIONS

by

Harry Keen, M.B., B.S., M.R.C.P.

and

Costis Chlouverakis, M.D.

The Lancet 2, 913-914, 1963.

AN IMMUNOASSAY METHOD FOR URINARY ALBUMIN AT LOW CONCENTRATIONS

H. KEEN

M.B. Lond., M.R.C.P.

C. CHLOUVERAKIS

M.D. Athens

OF THE DEPARTMENT OF EXPERIMENTAL MEDICINE,
GUY'S HOSPITAL, LONDON, S.E.1

ALTERATIONS in the permeability of the renal glomerular membrane may be reflected by changes in the amount of albumin in the urine. The ordinary clinical tests for albuminuria, however, become positive only after a twenty to hundred-fold increase over the normal.

Most methods of measuring albumin at low concentrations are laborious because they depend upon the separation of protein by electrophoresis after the concentration of large volumes of urine. Immunoelectrophoresis or gel-diffusion techniques, although requiring smaller volumes and less preparation, give only semiquantitative estimates.

The method described here overcomes most of the difficulties outlined above: it is specific, it requires very small volumes of urine only, it is accurate and sensitive over a selected concentration range, and it is capable of dealing with batches of a hundred or so samples of urine at each "run".

Principle of Method

The method, based on isotope-saturation analysis, employs the same principle as the methods of Hales and Randle (1963) and Morgan and Lazarow (1963) for measuring insulin in human plasma. A known amount (W') of isotopically labelled human albumin (^{125}I -albumin) is added to a measured volume of the urine under investigation, containing the unknown quantity of albumin (W) to be measured. To this mixture is added antihuman-albumin serum (raised in the guineapig) in such a quantity that the ^{125}I -albumin is present in excess. After equilibration between the albumin in the urine and the added antibody, a part of the albumin is bound and some remains in the free form. An anti-guineapig-globulin serum (raised in the rabbit) serves to precipitate the bound albumin which is then separated from the free albumin by filtration. The radioactivity found in the precipitate is related to the amount of endogenous albumin (W). Theoretically, if a is the radioactivity of the precipitate in the absence of endogenous albumin (i.e., when $W=0$), and x is the radioactivity of the precipitate in the presence of endogenous albumin (i.e., when $W>0$), then

$$\frac{x}{a} = \frac{W'}{W'' : W'} \quad (\text{equation 1})$$

$\frac{x}{a}$ being measurable and W' being known, the value for the unknown quantity, W , is calculable:

$$W = \frac{W' (a-x)}{x} = W' \left(\frac{a}{x} - 1 \right). \quad (\text{equation 2})$$

This formulation would apply if the system were found to conform strictly to the dilution principle. In practice it departs significantly from it, and the value for the unknown is derived by interpolation in a standard curve.

Materials and Methods

Human albumin prepared at the Lister Institute was used for radioiodination, for immunisation of guineapigs, and for the standard albumin solutions.

The iodination of the albumin with ^{125}I was carried out by McFarlane's method (1958), free iodine being removed by passage through an ion-exchange resin ('De-acidite FF', Permutit Ltd.). The concentration of this albumin and its specific activity were adjusted to the desired level by mixing with buffered solutions of unlabelled albumin. The final specific activity of the ^{125}I -albumin solution varied between 100 and 200 μC per mg., and the concentration between 0.3 and 1 mg. per 100 ml. approximately.

Guineapig antibody against human albumin was obtained from animals injected with Lister albumin and Freund's adjuvant.

Anti-serum to guineapig globulin was produced by injecting a globulin preparation from normal guineapig serum (made by ammonium-sulphate precipitation) into rabbits. The dilutions of both anti-sera and of the labelled albumin were made in Michaelis' barbitone buffer ($\text{pH}=8.4$) containing 200 mg. per 100 ml. gelatin. Reactions were carried out in small disposable polystyrene precipitin tubes (Baird & Tatlock, $1\frac{1}{2} \times \frac{1}{4}$ in.), and reactants were adequately mixed with a homemade micro-vortex mixer. The urine (0.1 ml.), with admixed ^{125}I -albumin (0.1 ml.), was incubated for 18 hours at room temperature with 0.1 ml. of appropriately diluted guineapig antihuman-albumin serum. The precipitating antibody (0.1 ml. of appropriately diluted rabbit anti-guineapig-globulin serum) was added, and the reactants were mixed and incubated for a further hour at the same temperature. The precipitated complex was then separated from the free albumin in solution by filtering the contents of the tubes through cellulose-acetate-membrane filters (Oxoid).

The dilutions of the two antibodies, selected as a result of preliminary experiments, were such that the albumin antiserum complexed with 30-50% only of the added ^{125}I -albumin, and the precipitating serum brought down all of the complex.

During each experiment a standard albumin curve was constructed, substituting for the urine 0.1 ml. of suitable albumin solutions in buffer, in concentrations from 0 to 60 ng.

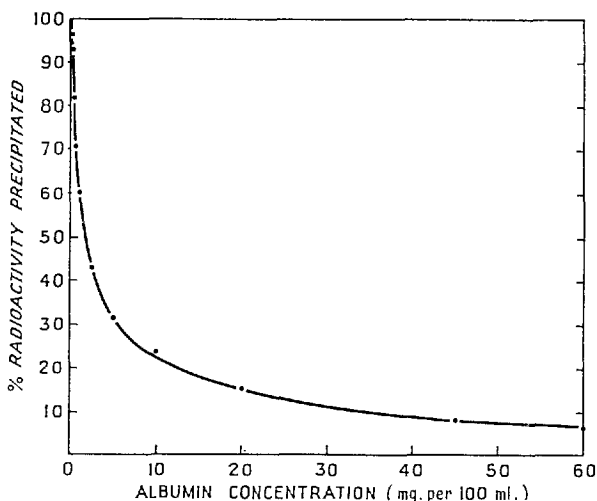


Fig. 1—Standard curve relating the proportion of radioactivity precipitated to the concentration of albumin.

per 100 ml. From this curve the amount of albumin in the urine under investigation was inferred.

Duplicate measurements were performed on all specimens of urine and standards, and the precipitates were counted in a well-type scintillation counter.

Results

Fig. 1 shows a typical standard curve with albumin concentrations which vary from 0 to 60 mg. per 100 ml. While the curve is extremely sensitive at low concentrations of albumin, large variations at higher concentrations result in only small changes in the radioactivity precipitated. For this reason urine specimens that gave a positive reaction with 'Albustix' (i.e., concentration > 30 mg. per 100 ml.) were diluted with distilled water before assay until the reaction with albustix became negative.

In fig. 2 the reciprocal of the proportion of the precipitated radioactivity shown in fig. 1 is plotted against the albumin content of the standards. The broken line represents the theoretical relationship calculated on the assumption that the dilution principle alone applies. The experimental line approximates to it over the very lowest range of albumin concentration only. At higher concentrations the manner in which the experimental line diverges from the theoretical reflects the fact that the radioactivity actually precipitated exceeds that predicted on the basis of simple dilution. This probably indicates that, as the albumin concentration increases, so does the combining

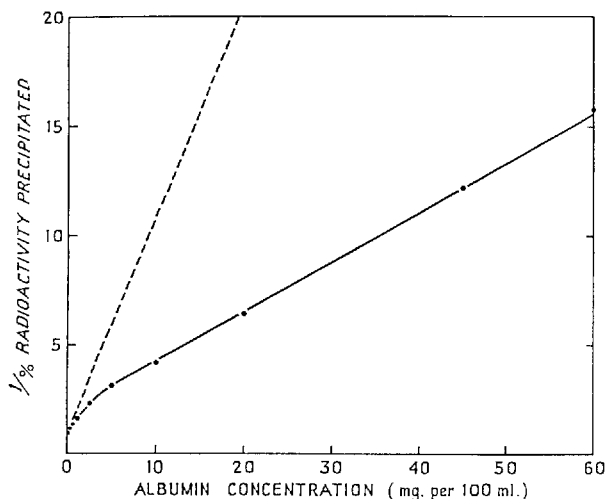


Fig. 2—Curve relating the reciprocal of the proportion of radioactivity precipitated to the albumin concentration.

The solid line indicates the experimental curve, and the broken line indicates the theoretical line based on the dilution principle.

power of the antibody. Unless this change in the "valency" of the antibody with albumin concentration can be defined, it is not possible to apply the dilution principle, and the unknown albumin concentration is found empirically by interpolation into the standard curve. The reproducibility which is obtained by the method is indicated by the following values of albumin concentration—1.08, 1.04, 0.99, and 1.07 mg. per 100 ml.—obtained for the same sample of urine assayed along with successive batches of urine on four different days and, on each occasion, with a new standard curve.

Summary

A simple immunological method for estimating the albumin concentration in human urine is described. It is sensitive and reproducible down to very low concentrations, and it requires less than 1 ml. of urine. Fifty to a hundred urine samples can be estimated in each experimental "run".

We should like to acknowledge the assistance of Mr. N. Veall, B.Sc., F.INST.P., who prepared the ^{125}I -albumin; and to thank Mr. C. Arden, Miss R. Emerson, and Mr. M. Shaw for technical help.

The work was supported by a grant from the United States Public Health Service and a research grant from the British Diabetic Association.

REFERENCES

- Hales, O. N., Randle, P. J. (1963) *Biochem. J.* **88**, 137.
 McParlane, A. S. (1958) *Nature, Lond.* **182**, 53.
 Morgan, C. R., Lazarow, A. (1963) *Diabetes*, **12**, 115.

INSERT 4

URINARY ALBUMIN EXCRETION AND
DIABETES MELLITUS

by

Harry Keen, M.B., B.S., M.R.C.P.

and

Costis Chlouverakis, M.D.

The Lancet 2, 1155-1156, 1964.

URINARY ALBUMIN EXCRETION AND DIABETES MELLITUS

HARRY KEEN
M.B. Lond., M.R.C.P.

C. CHLOUVERAKIS
M.D. Athens

Preliminary Communication

reprinted from THE LANCET, November 28, 1964, pp. 1155-1156

URINARY ALBUMIN EXCRETION AND DIABETES MELLITUS

OF the clinical features which characterise diabetic nephropathy, proteinuria has been recognised as the first clinical hint.¹ Light-microscopic examinations of renal tissue from longstanding diabetics shows gross alterations in the basement membrane of the glomerular capillaries.² More recent studies with the electron microscope have shown quite distinct changes of a similar nature at a very early stage of the diabetes, before any clinical evidence of renal disease is apparent, and even in so-called prediabetes in some cases.^{3 4}

Proteinuria was not found in these early cases using the ordinary clinical tests; these are, however, inadequate to measure small amounts of protein in the urine, and even a tenfold to twentyfold increase of urinary protein excretion over the normal would pass undetected. Thus it seemed possible that a counterpart of these very early pathological changes might be found in large but clinically undetectable increases in the rate of urinary protein excretion.

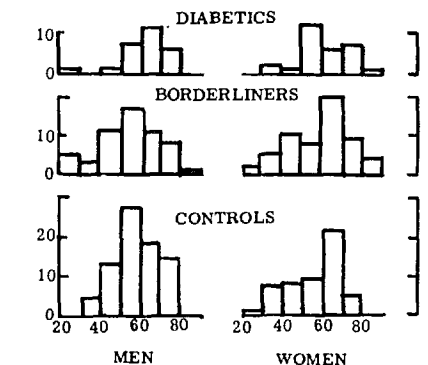
This possibility was examined in a sample of people studied in the Bedford diabetes survey.⁵⁻⁷

MATERIAL AND METHODS

Urine samples examined in the present study were collected from three groups of people who were identified in the survey by their blood-sugar 2 hours after 50 g. glucose by mouth, as follows:

1. Those with a 2-hour blood-sugar above 200 mg. per 100 ml. were designated "diabetics".

-
1. Gellman, D. D., Pirani, C. L., Soothill, J. F., Muchrache, R. C., Kark, R. M. *Medicine*, 1959, **38**, 321.
 2. Kimmelstiel, P., Wilson, C. *Amer. J. Path.* 1936, **12**, 83.
 3. Sabour, M. S., MacDonald, M. K., Robson, J. S. *Diabetes*, 1962, **11**, 291.
 4. Camerini-Davalos, R. A., Chaulfield, J. B., Rees, S. B., Lozano-Castaneda, O., Naldjian, S., Marble, A. *ibid.* 1963, **12**, 508.
 5. Sharp, C. L. *Proc. R. Soc. Med.* 1964, **57**, 193.
 6. Butterfield, W. J. H. *ibid.* p. 196.
 7. Keen, H. *ibid.* p. 200.



AGE-DISTRIBUTION OF GROUP EXAMINED

Fig. 1—Frequency-distribution histograms showing numbers (ordinate) of subjects in each 10-year age-group for the sexes separately, divided into diabetics, borderline diabetics, and normal controls.

2. Those with a 2-hour blood-sugar between 120 and 200 mg. per 100 ml. were designated "borderline diabetics".

3. Those with a 2-hour blood-sugar below 120 mg. per 100 ml. were designated "normal controls".

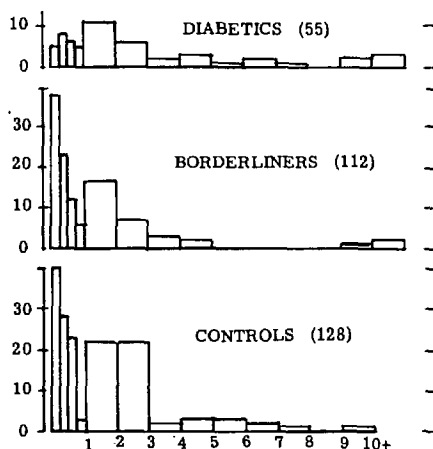
The reasons for and purpose of this grouping have been discussed elsewhere.⁵⁻⁷

Following the survey, people from the above three groups were subjected to a follow-up examination, including a further "2-hour blood-sugar", and the collection of a timed and measured 2-hour urine-output. The albumin content was estimated by a simple and sensitive radio-immunoassay method, specific for albumin, on frozen aliquots of a random sample of the three groups. A total of 295 urine samples were assayed: 55 from "diabetics", 112 from "borderline diabetics", and 128 from "normal controls". The age and sex distribution of these subjects is shown in fig. 1. The compositions of the borderline diabetic and control groups are very similar, though the diabetics contain an excess of older people.

OUTLINE OF ALBUMIN-ASSAY METHOD

The immunoassay method employed has been described elsewhere.⁸ In outline, a fixed quantity of human albumin labelled with ¹²⁵I is added to a measured volume of the test urine (0.1 ml.). To this mixture is added antihuman-albumin serum raised in the guineapig; and, after equilibration, albumin-antibody complexes are precipitated by an antigineapig-globulin serum raised in the rabbit. The radioactivity found in

8. Keen, H., Chlouverakis, C. *Lancet*, 1963, ii, 913.



the precipitate is related to the quantity of albumin in the test urine, which is derived by interpolation into a standard curve.

RESULTS

The distribution of the urinary-albumin concentrations in each of the three groups is shown in fig. 2. The distributions are very asymmetrical, and of the J type; more than half the urine samples had an albumin concentration of 1 mg. per 100 ml. or less.

The frequency-distribution of the 2-hour albumin excretion-rate values, calculated from the albumin concentration and the 2-hour volume, is shown in fig. 3. This distribution is also very asymmetrical, resembling that of the albumin concentration.

Differences of urinary albumin excretion-rates among the groups cannot be assessed by statistical tests based upon the assumption of normally distributed variates. To make the comparison, all the albumin excretion-rates were ranked by value and then divided into six equal segments. The percentage representation of each of the three groups—diabetics, border-

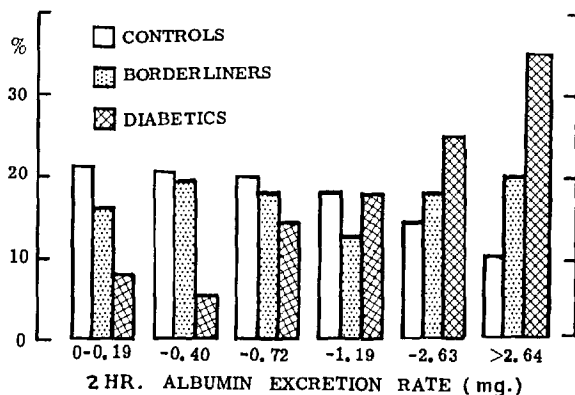


Fig. 4—Percentage representation of the three groups in each excretion-rate "hexile" (see text).

line diabetics, and normal controls—in each "hexile" is shown in fig. 4.

There is a clear tendency for diabetics to be better represented in the higher excretion-rate categories, with the reverse for the normal controls. Borderline diabetics occupy an intermediate position. On the basis of this classification a 6×3 contingency table was constructed and a χ^2 test showed that the different representation of the groups in each hexile was very unlikely to have arisen by chance ($P=0.0006$).

The table shows a further comparison of mean albumin excretion-rates when subjects were classified according to their 2-hour blood-sugar not only at the survey but also at their visit to the follow-up clinic. (D=200 mg. or more per 100 ml., B=120-199 mg. per 100 ml., N=119 mg. per 100 ml. or less.) It can be seen that the calculated albumin excretion-rate shows a stepwise decline from the "most" to the "least" hyperglycæmic group:

<i>Subgroup</i>	<i>No.</i>	<i>2-hr. albumin excretion (mg.)</i>
DD	29	5.65
DB	30	3.94
BB	58	2.32
ND	4	1.82
BN	52	0.87
NN	115	1.05
Total	288	2.05

DISCUSSION

The increased urinary albumin excretion demonstrated in the newly discovered and borderline diabetics in Bedford may well be due to an increased permeability of the glomerular basement membrane very early in the clinical course of the condition, and reflecting the morphological changes mentioned earlier.

Alternative explanations must be considered, however. Urinary-albumin excretion-rates will depend not only upon the rate of entry of albumin into the glomerular filtrate but also upon the rate of its reabsorption in the renal tubules. A diminution in tubular albumin reabsorption may occur as a result of some abnormality of the tubular epithelium or if reabsorption is competitively hindered by the presence of other small molecular weight proteins; there is no evidence at present supporting these possibilities in early diabetes.

Loss of plasma-proteins into the urinary tract at sites other than the renal glomerulus would also give rise to increased albuminuria. Such losses could arise from inflammation or hæmorrhage anywhere in the urinary tract and, in this present study, the intervention of such factors cannot be ruled out. But casts and cells per se in the urine do not contribute to the estimated albumin content measured as described (unpublished observations).

If we assume that the increased albuminuria associated with raised blood-sugar levels is a manifestation of a specific "diabetic" change in glomerular permeability, then the evidence of this study suggests that the grade of change may be quantitatively related to the degree of

hyperglycæmia. Such a relationship would be most simply explained by a direct effect of the circulating glucose level upon the structure or function of the glomerular basement membrane, although it is also clearly possible that blood-glucose levels and basement-membrane behaviour might both be influenced by a common factor. Too little information is at present available to make any more than speculative attempts to differentiate between these possibilities.

It remains to be seen whether these "subclinical" increases in albuminuria in the diabetic are correlated with morphological changes in the glomeruli or with subsequent clinical development of renal disease, and whether treatment will influence these events.

SUMMARY

Using a sensitive and specific method for measuring albumin in the urine of three groups consisting of early diabetics, "borderline diabetics", and normal people, a gradation in the rate of urinary-albumin excretion was found. Diabetics excreted the most and normals the least albumin of the three groups.

This gradation in the rate of albumin excretion occurred at concentrations undetectable by ordinary clinical tests. The increased albuminuria may be related to the glomerular basement-membrane changes reported by other workers, but further correlative studies are required.

This work was supported by a research grant from the British Diabetic Association and by a U.S. Public Health Service Grant. Acknowledgments are also due to Mr. N. Vcall for preparing the ^{125}I -labelled albumin, and to Mr. M. Field, Miss A. Southon, and Miss J. Williams for technical assistance.

Department of Medicine
Guy's Hospital,
London, S.E.1

HARRY KEEN
M.B. Lond., M.R.C.P.
C. CHLOUVERAKIS
M.D. Athens

INSERT 5

THE CONCOMITANTS OF RAISED BLOOD SUGAR:

STUDIES IN NEWLY-DETECTED

HYPERGLYCAEMICS

11. URINARY ALBUMIN EXCRETION, BLOOD PRESSURE

AND THEIR RELATION TO BLOOD SUGAR LEVELS

by

Harry Keen, M.B., B.S., M.R.C.P.

Costis Chlouverakis, M.D.

John Fuller

and

R. John Jarrett, M.A., M.D.

Guy's Hospital Reports 118, 247-254, 1969.

THE CONCOMITANTS OF RAISED BLOOD SUGAR: STUDIES IN NEWLY-DETECTED HYPERGLYCAEMICS

II. Urinary albumin excretion, blood pressure and their relation to blood sugar levels

By H. KEEN, C. CHLOUVERAKIS, J. FULLER and R. J. JARRETT
Department of Medicine, Guy's Hospital Medical School, S.E.1

RENAL disease is a complication of diabetes mellitus of growing importance and is credited as the primary cause of death in some 11–12 per cent of diabetics (Joslin 1959). The first clinical indication of diabetic nephropathy is proteinuria (Gellman, Pirani, Soothill, Muehrcke and Kark 1959) and much of this protein is albumin (Cameron, J. S., personal communication). By modern techniques, albumin can be detected and measured in very small quantities; we have used a radio-immunoassay procedure to measure micro-albuminuria, following an oral glucose load, in normal and hyperglycaemic individuals from the population of Bedford, co-operating in the Diabetes Detection Survey conducted in 1962 (Sharp, Butterfield and Keen 1964).

The reasons for the study were threefold: firstly, to determine whether abnormalities of albumin excretion were already present at the time of diagnosis; secondly, to see whether the degree of albuminuria could qualify the interpretation of doubtfully abnormal glucose tolerance tests; and thirdly, to form a 'baseline' for the evaluation of renal changes in a long-term double-blind trial of treatment (Keen, Jarrett, Boyns, Chlouverakis and Pyke 1968).

MATERIAL AND METHODS

The population, from which the subjects of this study were derived, and the composition of the three diagnostic groups—normal, borderline diabetic and diabetic—is described in the preceding paper (Jarrett, Keen, Boyns, Chlouverakis and Fuller 1969).

Following the Survey, the diabetics, borderline diabetics and controls were seen at a special clinic. Each individual was instructed to consume 50 g glucose (in the form of a flavoured, carbonated drink provided, dispensed to the exact quantity, by Beecham Laboratories, Ltd.), 1 hour and 50 minutes before the clinic

TABLE I
AGE AND SEX COMPOSITION OF THE THREE DIAGNOSTIC GROUPS

Age	Controls	Borderliners	Diabetics
Males:			
29-39	8	14	4
40-59	39	45	14
60+	35	32	24
Total	82	91	42
Females:			
29-39	11	12	6
40-59	24	32	17
60+	36	43	25
Total	71	87	48

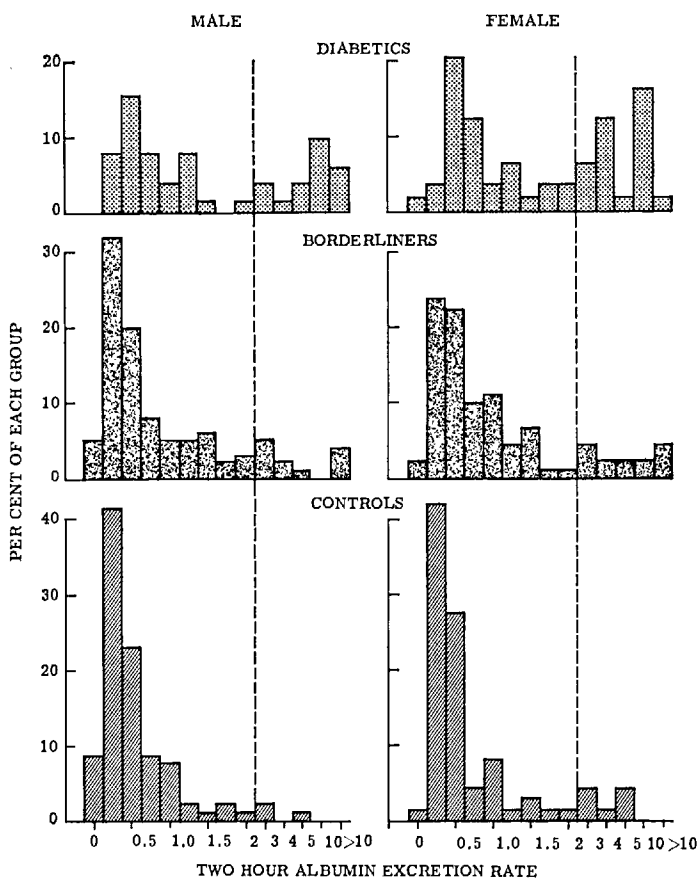


FIGURE 1.

Frequency-distribution histogram of the 2-hour albumin excretion rate in the three diagnostic groups.

appointment and to urinate immediately afterwards. At the clinic, a capillary blood sample, for estimation of the blood sugar, was taken 2 hours after the time of the glucose drink. The bladder was again emptied and the volume of the urine measured. An aliquot of this was taken and frozen for subsequent estimation of the albumin content by a sensitive specific radio-immunoassay method (Keen and Chlouverakis 1963).

The results can be expressed either as the albumin concentration or as the 2-hour albumin excretion rate in mg/2 hr. The latter has been used in this analysis.

Data analysis was performed using the London University Atlas Computer.

RESULTS

The age and sex distribution of the individuals in the three diagnostic groups considered in this paper is shown in Table I. The figures represent the total number of samples for which results are available.

The frequency distribution of the 2-hour albumin excretion rate is shown in Figure 1. Clearly, the distribution, particularly

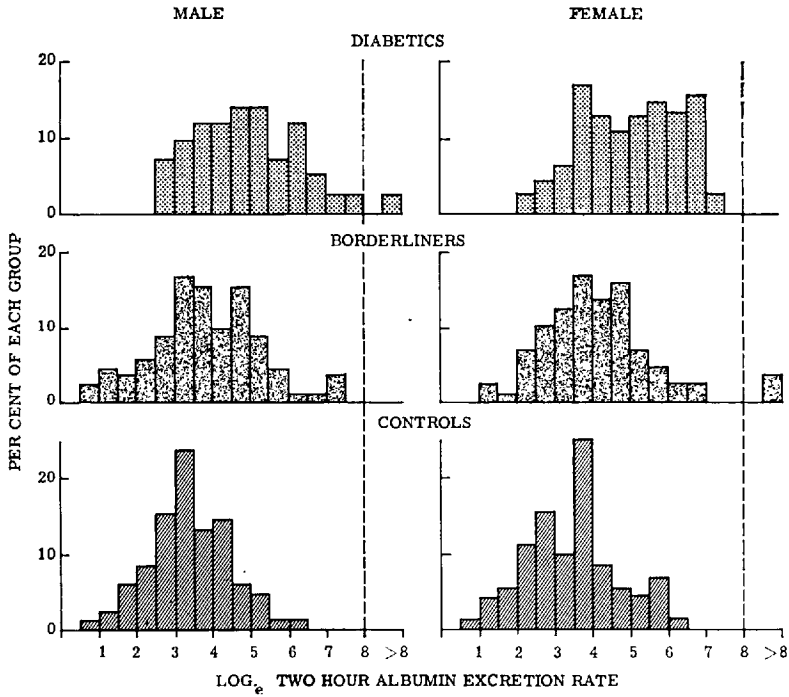


FIGURE 2.

Frequency-distribution histogram of the logarithm of the 2-hour albumin excretion rate ($\log_e (100 \times \text{albumin excretion rate})$) in the three diagnostic groups.

TABLE II
THE MEAN OF THE LOG_e ALBUMIN-EXCRETION-RATE
(Figures in parentheses are the standard errors of the means)

Age Group	Mean log albumin excretion rate			
	20-39	40-59	60 +	All
Male:				
Controls ..	3.986 (0.34)	3.123 (0.163)	3.571 (0.168)	3.399 (0.115)
Borderliners ..	4.472 (0.26)	3.392 (0.216)	4.249 (0.236)	3.859 (0.149)
Diabetics ..	4.515 (0.61)	4.741 (0.284)	5.051 (0.301)	4.897 (0.207)
Female:				
Controls ..	3.284 (0.35)	3.935 (0.226)	3.197 (0.215)	3.460 (0.149)
Borderliners ..	4.023 (0.479)	4.385 (0.242)	3.997 (0.219)	4.144 (0.156)
Diabetics ..	4.496 (0.58)	4.747 (0.259)	5.112 (0.249)	4.906 (0.177)

that of the control group, is positively skewed. In the borderline diabetic and the diabetic groups, there is a relative decrease in the proportion of lower values and an increase in that of the higher values. Following logarithmic transformation ($\log_e (100 \times \text{albumin excretion rate})$), the frequency distribution (Figure 2) was such that conventional statistical techniques could be applied. It is evident from Figure 2 that not only are there relatively more high values amongst the borderline diabetic and diabetic groups, but that the median values for these groups are higher. This is further demonstrated in Table II, which shows the mean log albumin-excretion-rate in the three diagnostic groups, further subdivided into three age groups and by sex. Within each age group, the same trend occurs, with mean values rising from the controls, through the borderline diabetics to the diabetics. On the other hand, there is no obvious effect of age upon the mean values within each diagnostic group.

Linear correlation coefficients were calculated between the log albumin-excretion-rate and a number of other variables (Table III and IV). There were no significant correlations with age or ponderal index ($= \text{height}/\sqrt[3]{\text{weight}}$) nor with weight, which was separately calculated. However, when observations in all the diagnostic groups were pooled, there was a statistically significant correlation between the log albumin-excretion-rate and the 2-hour blood sugar in both men and women. Within the individual

TABLE III
CORRELATIONS WITH LOG ALBUMIN-EXCRETION-RATE

				Age	Ponderal index
Males	- r	..	..	0.0753	-0.0235
Females	- r	..	..	0.0236	-0.1299

TABLE IV
CORRELATIONS WITH LOG ALBUMIN-EXCRETION-RATE

				Systolic B.P.	Diastolic B.P.	Blood sugar
All males	r	..	..	0.2087	0.1334	0.2993
	p	..	..	<0.01>0.001	—	<0.001
All females	r	..	..	0.2509	0.1447	0.3115
	p	..	..	<0.001	<0.05>0.01	<0.001
Controls:						
Males	r	..	..	0.0638	0.1171	-0.0851
	p	..	..	—	—	—
Females	r	..	..	0.1496	0.1861	-0.0626
	p	..	..	—	—	—
Borderliners:						
Males	r	..	..	0.0374	0.1665	0.1861
	p	..	..	—	—	—
Females	r	..	..	0.1436	0.0698	0.0940
	p	..	..	—	—	—
Diabetics:						
Males	r	..	..	0.4402	0.0573	-0.1091
	p	..	..	<0.01>0.001	—	—
Females	r	..	..	0.4433	0.2904	0.1124
	p	..	..	<0.001	<0.05>0.01	—

diagnostic groups, these correlations were not significant, possibly because of the smaller numbers and the artificially restricted range of blood sugars within each diagnostic group, inherent in the experimental design.

In the combined groups, significant correlations were also found between the log albumin-excretion-rate and the systolic blood pressure, both in men and women, and with the diastolic blood pressure in the women. When the groups were considered separately, the correlation coefficients were lower and non-significant in the control and borderline diabetic groups. In the diabetic group, however, in both sexes, the correlation coefficient for systolic blood pressure against albumin excretion rate was almost twice as high as that for the combined groups. In the diabetic women, only, the correlation coefficient for diastolic blood pressure against albumin excretion rate was also higher than that for the combined groups.

This significant correlation of systolic blood pressure and log albumin-excretion-rate in the diabetic group could be due to a greater incidence of hypertension, but this explanation seems unlikely in the light of the mean blood pressure results (Table V). Although the mean levels of the diabetics are higher than those of the controls, they are similar to those of the borderline diabetics, in whom there is no relation between blood pressure and albumin excretion.

TABLE V
MEANS OF BLOOD PRESSURES IN THE THREE GROUPS ACCORDING TO SEX

			Controls		Borderliners		Diabetics	
			Male	Female	Male	Female	Male	Female
Systolic	..	..	142	155	153	172	157	171
Diastolic	..	..	91	95	91	96	93	96

To examine the possibility that the demonstrated relationship between blood pressure and log albumin-excretion-rate might be due to a common effect of age upon both, an analysis of variance of these three factors was carried out. No significant effect of age was demonstrable.

DISCUSSION

This study clearly shows that at the time of diagnosis of 'hyperglycaemia', abnormalities of urinary albumin excretion may already be present. Our data do not suggest the cause of the increased albuminuria, but current opinion favours the view that the albumin concentration in the urine is the same as that in the glomerular filtrate, allowing for the changes brought about by water absorption (Hardwicke, Soothill and Cameron 1969). The albuminuria, if this view is correct, must therefore represent increased glomerular permeability. In long-term diabetics, gross albuminuria may be associated with histological changes, particularly in the glomerulus; in this study, we have found a significant correlation between the blood sugar measured 2 hours after 50 g glucose and the degree of albuminuria. It seems a not unreasonable assumption that the hyperglycaemia, or some undefined 'diabetic' process that it represents, is the cause of the increased albuminuria.

In advanced diabetic glomerulosclerosis, abnormality of the glomerular basement membrane is visible using light microscopy. There have been suggestions that thickening of the basement membrane is detectable, using electron microscopy, at the time of diagnosis (Sabour, MacDonald and Robson 1962; Camerini-Davalos, Caulfield, Rees, Lozano-Castaneda, Naldjian and Marble 1963), but more recent studies (Osterby-Hansen 1965; Kimmelstiel 1966) have not only contradicted the earlier findings, but have also cast doubt upon their validity. Nevertheless, histological normality of the basement membrane does not preclude a functional abnormality.

A possible alternative cause of increased albumin excretion is infection. At the time of this study, no observations upon quantitative bacteriuria or urine culture were made. However, we have

since estimated the incidence of significant bacteriuria, using quantitative methods, in the borderline diabetic group and found the incidence to be similar to that of control populations (Brumfitt, Harrison, Keen and Jarrett 1966). In addition, although histological pyelonephritis is more common in diabetics of long standing than in non-diabetics, the prevalence of urinary infection is now thought to be similar in both groups (O'Sullivan, Fitzgerald, Meynell and Malins 1961; Pometta, Rees, Younger and Kass 1967).

In the diabetic group, we found a significant correlation between the degree of albumin excretion and the systolic blood pressure and, to a lesser extent, with the diastolic blood pressure also. This correlation did not appear to be explicable by a common effect of age nor by a greater prevalence of hypertension among the diabetics. The simplest explanation may, therefore, be the correct one—that the results of hypertension and hyperglycaemia combine to increase the degree of albuminuria. The therapeutic implication of this is that treatment of hypertension associated with diabetes may ameliorate or delay the onset of renal disease.

SUMMARY

The albumin excretion rate following an oral glucose load was measured, using a sensitive radio-immunoassay method, in three groups drawn from the population of Bedford. The three groups—normal, borderline diabetic and diabetic—were classified by the level of the blood sugar 2 hours after the 50-g glucose load. The degree of albumin excretion was positively correlated with the 2-hour blood sugar level, suggesting that hyperglycaemia may cause renal functional abnormality, which may be present at or before the time of diagnosis. In the diabetic group, only, the degree of albumin excretion was also positively correlated with the height of the blood pressure.

ACKNOWLEDGEMENTS

We would like to acknowledge the unfailing assistance in the Bedford Study given by the late Dr. C. L. Sharp, Medical Officer of Health, and the members of the Bedford Public Health Department, in particular, Mr. F. Batson, Mrs. P. Gillam, Mrs. P. Jones, Mrs. L. Macrae, and Mr. J. Mundy. We also thank our own staff—Mrs. C. Boyns, Miss R. Lloyd, and Miss F. Phillips and the staff of the London University Institute of Computer Science, for their invaluable help. The financial support of the Ministry of Health and of the British Diabetic Association is gratefully acknowledged.

The continuing study owes most to the co-operation and friendliness of the Bedford population in general and our special groups in particular. At all times, the interest and support of Professor W. J. H. Butterfield have been greatly valued.

REFERENCES

- Brumfitt, W. F., Harrison, J., Keen, H. and Jarrett, R. J. (1966) Unpublished observations.
- Camerini-Davalos, R. A., Caulfield, J. B., Rees, S. B., Lozano-Castaneda, O., Naldjian, S. and Marble, A. (1963) *Diabetes*, **12**, 508.
- Gellman, D. D., Pirani, C. L., Soothill, J. F., Muehreke, R. C. and Kark, R. M. (1959) *Medicine*, **38**, 321.
- Hardwicke, J., Soothill, J. F. and Cameron, J. S. (1969) In 'Proteins in Normal and Pathological Urine'. Ed. Manuel, J., Revillard, J. P. and Betuel, H. Basel: Karger.
- Jarrett, R. J., Keen, H., Boyns, D. R., Chlouverakis, C. and Fuller, J. (1969) *Guy's Hosp. Rep.*, **118**, 237.
- Joslin, E. P. (1959) In *The Treatment of Diabetes Mellitus*. Ed. Joslin, E. P., Root, H. F., White, P. and Marble, A. Philadelphia: Lea and Febiger.
- Keen, H. and Chlouverakis, C. (1963) *Lancet*, **ii**, 913.
- Keen, H., Jarrett, R. J., Boyns, D. R., Chlouverakis, C. and Pyke, D. A. (1968) *Postgrad. med. J.*, **44**, 960.
- Kimmelstiel, P. (1966) *Diabetes*, **15**, 61.
- Osterby-Hansen, R. (1965) *Diabetologia*, **1**, 97.
- O'Sullivan, D. J., Fitzgerald, M. G., Meynell, M. J. and Malins, J. M. (1961) *Brit. med. J.*, **1**, 786.
- Pometta, D., Rees, S. B., Younger, D. and Kass, E. H. (1967) *New Engl. J. Med.*, **276**, 1118.
- Sabour, M. S., MacDonald, M. K. and Robson, J. S. (1962) *Diabetes*, **11**, 291.
- Sharp, C. L., Butterfield, W. J. H. and Keen, H. (1964) *Proc. Roy. Soc. Med.*, **57**, 193.

Further Comments on the Kidney

Support is dwindling for the earlier view (Sabour et al, 1962; Camerini-Davalos et al, 1963; Daysog et al, 1961) that in renal biopsy specimens examined with the electron microscope, thickening of the renal glomerular capillary basement membrane is demonstrable in 'prediabetic' subjects prior to the overt metabolic disturbance of diabetes. The strongest evidence against this important assertion is that of Osterby Hansen (1965), referred to earlier. She was unable to differentiate statistically between basement membrane thickness of the peripheral glomerular capillary loop in renal biopsy samples taken at onset from juvenile-type diabetics and that of a matched, non-diabetic, control group. Her findings are supported by those of Ireland (1970) who extended his basement membrane estimates to the mesangial 'stem' region of the glomerular capillary tuft.

The urinary albumin levels found in the Bedford study are not at variance with these morphological conclusions. The general conclusion to be drawn from the measurement of urinary albumin excretion rates suggests that, at any rate in the subjects of our study, they were positively associated with the degree of glucose intolerance, with an additional influence of blood pressure level superimposed. The effect of duration of hyperglycaemia prior to detection in the 1962 survey is, however, unknown. No histological material is available from the subjects in Bedford; we did not feel able to consider the possibility of carrying out renal biopsy procedures, though it would be highly desirable to conduct studies

along these lines to establish the relationship between basement membrane morphology and albumin excretion rates.

A Collaborative Study with "Prediabetics"

Some further light is cast upon renal glomerular basement membrane changes in "prediabetics" by a recently completed collaborative study with Dr. M. Balodimos and others at the Joslin Clinic, Boston, Mass., (Balodimos et al, 1970). Timed and measured overnight urine samples were collected from 196 subjects in Boston (Table 8). 59 of them were established diabetics attending the Joslin Clinic and 41 were non-diabetics, with a negative family history of the disease. In addition, urine collections were made from 96 offspring, both of whose parents were diabetic. 48 of these had normal standard oral glucose tolerance and 48 had minor impairment of glucose tolerance. Tables 9-11 show that the clinical diabetics differed significantly from the other groups in respect of the proportion showing higher urinary albumin concentrations and excretion rates and in the greatly increased proportion in the top quartile of the distribution of 8-hour excretion rates. There was no indication of systematically increased urinary albumin excretion in the genetically predisposed, even when a minor element of glucose intolerance was already manifest.

TABLE 8

Constitution of Boston Group

FHO = Offspring with no family history of diabetes
 FH⁺ = Unselected for or against family history of diabetes
 FH++ = Offspring both parents diabetic
 GTTN = Two or more normal G.T.T.'s
 GTD = Clinical Diabetes
 GTTC = Mild glucose intolerance
 Overnight urine collected, timed and measured.

	No				
	Male	Fem	Mean Age	FH	GTT
Controls	15	26	32.2	0	N
Diabetics	34	25	33.2	+	D
'Pre DM' (N)	33	15	28.5	++	N
'Pre DM' (C)	27	21	36.6	++	C

TABLE 9

Frequency distribution of albumin concentration in the Boston sub-groups.

	Alb. Conc. (mg/100ml.)				
	Total	% 0-0.5	% 0.5 - 1.0	% 1.0 - 2.0	% 2.0 +
Controls	41	65.8	22.0	9.8	2.4
Diabetics	59	45.8	25.4	18.6	10.2
'Pre DM' N	48	79.1	6.3	8.3	6.3
'Pre DM' C	48	81.2	12.5	2.1	4.2

TABLE 10

Frequency distribution of calculated 8-hour urinary albumin excretion rates in the Boston sub-groups.

		8-Hour Alb. Excrn. Rate (mg/8hr.)				
	Total	% 0 - 0.5	% 0.5 - 1.0	% 1.0 - 3.0	% 3.0 - 5.0	% 5.0 +
Controls	41	17.1	36.6	34.1	9.8	2.4
Diabetics	59	13.5	10.2	30.5	8.5	37.3
'Pre DM' N	48	23.0	25.0	35.4	6.2	10.4
'Pre DM' C	48	29.2	31.2	33.3	2.1	4.2

TABLE 11

Quartile distribution of 8-hour urinary albumin excretion rates in the Boston sub-groups.

		8-Hr. Alb. Excr. Rate Quartile			
	Total	I	II	III	IV
Controls	41	26.8	31.7	24.4	17.1
Diabetics	59	13.5	11.9	23.8	50.8
'Pre DM' N	48	27.1	27.1	29.2	16.6
'Pre DM' C	48	35.4	33.3	22.9	8.4
Total	196	25.0	25.0	25.0	25.0

Mechanism of 'Microalbuminuria'

Little can be said at this stage about the mechanisms responsible for the increased urinary albumin excretion of the diabetic group and the intermediate position of the 'borderline' group. Although it seems probable that the phenomenon indicates altered permeability characteristics of the renal glomerular basement membrane, there is no evidence that it is necessarily the functional counterpart of the thickening that has been demonstrated in electron microscopic or light microscopic studies. Indeed, it may seem difficult to reconcile an apparent thickening of the barrier between the blood and Bowman's space with the increased passage of plasma constituents. The increased glomerular filtration rate in early diabetics described by Ditzel (1966) may be related to increased proteinuria. Observation of the more advanced clinical situation, however, confirms the association of gross thickening of the glomerular basement membrane with heavy proteinuria (Gillman et al, 1959). One must suppose that, as well as the thickness, the permeability properties of the basement membrane are altered in addition. The role of the interlacing foot processes of the epithelial cells in regulating permeability properties of the glomerular capillary is uncertain. Part of the increased albumin 'leak' may be associated with the very considerable disorganisation of this microstructure.

b) The Eye

Attention has been directed to two vulnerable sites in the eye, to the retina for evidence of the microvascular disturbance so characteristic of diabetes, and to the lens, where opacities and cataract formation have been sought and recorded. At the first review, the eyes of as many subjects as possible in each of the three blood sugar groups were examined ophthalmoscopically in a darkened room after dilatation of the pupils with homatropin (1%). The retina was systematically examined and specified findings recorded. Directed search was made for capillary 'microaneurisms' (recognised as small, well-defined red dots), 'exudates', haemorrhages, abnormalities in existing vessels and the appearance of 'new' vessels. In the lens, opacity of any degree or form was sought with appropriate adjustment of the clinical ophthalmoscope. All abnormalities were recorded with the appropriate descriptors - number, colour, shape and size etc. Observed lenticular opacities were matched against a prepared set of drawings (Grades I, II and III), grading being made to the nearest drawing. Examinations were, in the main, carried out by a single observer (H.K.). In the case of questionable retinal lesions, a second opinion was obtained.

Eye examination was repeated in 1967 during the follow-up review. Of necessity, there were some losses of subjects by death, by change of residence or by default, from each of the original groups.

1. Retinopathy

In this presentation, only lesions considered to be 'microaneurisms' have been considered, whether or not they were associated with other fundal abnormalities. This selection is made because of the comparative specificity of this type of lesion in diabetics, though, uncommonly, microaneurisms may be associated with a variety of other ocular and general disorders (Ashton, 1951, 1963).

In considering the following results, three general points should be borne in mind. First, since microaneurisms were not seen in any member of the normoglycaemic control group this has not been considered in the analysis which follows. Second, not only was there a large difference between 'borderline' and diabetic individuals in the frequency of involvement but the degree of involvement differed markedly as well. In only one 'borderline' subject (no. 86) were more than 3 microaneurisms seen; of 20 diabetics showing the lesions, only 6 were recorded as having less than 6 microaneurisms. Further, 3 of the diabetics had advanced to the point of significant visual disability due to diabetic retinopathy by the end of the five years 1962 - 67, while none of the 'borderline' subjects were so affected. Finally, the decision that a given lesion was a capillary microaneurism was made on ophthalmoscopic examination alone. It is possible that in some cases the lesion seen actually represented a small, 'punctate' haemorrhage. This possible source of error has been discussed earlier (Keen and Smith, 1958).

Microaneurisms in 'Borderline' and Diabetic Groups

The frequency with which capillary microaneurisms were recorded as present in the 'borderline' and diabetic groups at the first examination and at the 5-year follow-up are shown in Tables 12 to 14. Among 'borderline' subjects, lesions were seen in only 3 people at the first examination (1.3% of those examined). Apart from a hypertensive woman who had already had one stroke and died of another before the 5-year re-examination, lesions were very few in number and seen only once. Hypertension and/or the more notable grades of glucose intolerance were present in all but 1 of the 5 subjects (3.3% of those examined) who were recorded as positive for microaneurisms at the 5-year re-examination. In none of them were more than 3 lesions detected.

A different picture is seen with the diabetic group. At the time of first examination, 7 of the 105 examined (6.7%) were recorded as having retinal capillary microaneurisms, in 5 cases showing more than 6 lesions. At the 5-year re-examination, 17 of the 73 examined (23.3%) showed capillary microaneurisms, 11 of them with more than 6 lesions. Of those positive at the first examination who were re-examined, all were again recorded as showing lesions 5 years later. The great majority of those unrecorded at 5 years had died in the interim.

Microaneurisms and Glucose Intolerance

An indication of a relationship between the degree of glucose intolerance and the presence of capillary microaneurisms is provided by Table 13. The survey (1962) 2-hour blood sugar distribution was divided into quartiles by setting out the blood sugar values in numerical series then dividing the blood sugar range at points which allocated approximately one fourth of the group to each successive section. The 'cutting points' so selected are recorded in the legend to Table 13. At the first examination, only 1 of the 54 subjects (1.85%) in the lower two blood sugar quartiles showed microaneurisms compared with 6 of 51 subjects (11.75%) in the upper two quartiles. This differs significantly from random distribution ($\chi^2 = 4.2$; $p < 0.05$) and supports the hypothesis of a positive association between higher levels of blood sugar and the increased frequency of retinal capillary microaneurisms. This association with the survey 2-hour blood sugar was no longer apparent at the 5-year re-examination ($\chi^2 = 1.33$; $p < 0.3 > 0.2$) but, during the interval, the diabetics had been subjected to a variety of treatments.

It is tempting to draw, from these data, the conclusion that the appearance of retinal microaneurisms is linked with the degree of glucose intolerance. At the time of initial eye examination, not only were the lesions conspicuously more frequent among diabetics than among 'borderline' subjects, but, within the diabetic group, there appeared to be a positive association between microaneurisms and 2-hour blood sugar level. In addition, the passage of 5 years witnessed a far

larger number of new lesions developing in diabetics than in 'borderline' subjects. In those developing lesions in the latter group, hypertension, or the more extreme degrees of hyperglycaemia were frequently to be found and in all cases lesions were very few in number. A tentative metabolic hypothesis explaining the link between hyperglycaemia and retinal microvascular changes has been proposed elsewhere (Keen and Chlouverakis, 1965).

Other Possible Explanations

Alternative explanations should be considered. There is no indication of the duration of the hyperglycaemic state prior to its discovery in 'borderline' and diabetic subjects in 1962. It is conceivable that those more hyperglycaemic in 1962 had been so longer, so that what appears to be an effect of blood sugar level on retinal abnormalities may in fact be the influence of duration. This hypothesis is not supported by the quite different behaviour of the two groups during the 5-year follow-up period although this period may have succeeded a longer prior period of hyperglycaemia in the diabetic than the 'borderline' group. In other words, the diabetics may already have been exposed to an 'incubation period' of retinopathy from which the 'borderline' subjects have yet to emerge. Only continued observation can decide the validity of this interpretation. Finally, both the infrequency of retinal changes and the lesser degrees of glucose intolerance in the 'borderline' group may be the expression of the lesser intensity of a common hypothetical 'diabetogenic factor', an unlikely explanation in the light of the earlier discussion on the

links between diabetes and its long-term complications in general.

It is concluded that the observations detailed here are compatible with, and offer some further support for, the hypothesis formulated elsewhere (Keen and Chlouverakis, 1965) that a close and causal link may exist between hyperglycaemia and retinal vascular abnormalities. The observations are also consistent with the view that it is not until certain critical, minimal levels of hyperglycaemia are attained, that the mechanism responsible for the blood sugar/retinopathy association comes into play.

TABLE 12

Retinal Capillary Microaneurysms (by 'grade') in the newly found Diabetics in 1962 and in 1967.

- Cap. m.a. 0:- Retina examined; no lesions seen.
- Cap. m.a. 1:- 1 to 6 microaneurysms.
- Cap. m.a. 2:- 6 to 12 microaneurysms.
- Cap. m.a. 3:- More than 12 microaneurysms.
- Cap. m.a. ?:- Not examined.

*Three with visual disability.

1962			1967					
			0	1	2	3	?	
Cap. m.a.	0	98	56	6	2	5	29	98
	1	2	0	0	0	2	0	2
	2	3	0	0	0	1	2	3
	3	2	0	0	0	1	1	2
	?	11	0	0	0	0	11	11
			56	6	2	9*	43	116

TABLE 13

Frequency of diabetic retinopathy (microaneurysms/haemorrhages) in newly found diabetics in 1962 and at follow-up examination in 1967. Relation with the Survey 2hr. blood sugar level is shown by quartile of distribution (division is made at the point where the population is divided into approximately equal fourths, or quartiles).

- Quartile 1 200 - 239mg/100ml.
- Quartile 2 240 - 284mg/100ml.
- Quartile 3 285 - 329mg/100ml.
- Quartile 4 330+ mg/100ml.

Quartile of B.S.	Total	Number (1962)		Number (1967)	
		Assessed	R/pathy+	Assessed	R/pathy+
4	26	25	2	15	5
3	29	26	4	19	5
2	31	29	1	22	5
1	30	25	0	17	2
	116	105	7 (6.7%)	73	17 (23.3%)

TABLE 14

Summary of characteristics of 'borderline' subjects in whom 1 or more capillary microaneurysm was recorded. Of those recorded at first exam., no. 86, a treated hypertensive, died of stroke before 5-year time point. Nos. 806 and 810 were recorded as showing two and one microaneurysm respectively and these could never be reidentified after the first exam. Of the five subjects recorded at the 5-year follow-up, nos. 36, 198 and 225 had clinically significant hypertension and nos. 20 and 36 (like no. 806) showed considerable 2hr. blood sugar elevation during the follow-up period.

	Serial No.	Sex	Age	Survey B.P.	Blood Pressure	Follow-Up B.S.'s	
First Exam. (1.3%)	86	F	51	158	200/100	-	
	806	F	75	139	150/ 75	2	200
	810	F	53	128	125/ 70	-	
Five Year F-Up (3.3%)	20	F	46	146	125/ 65	2	200
	36	F	64	171	200/110	1	200
	98	M	58	175	155/ 95	-	
	198	M	50	124	200/120	-	
	225	F	54	166	250/135	-	

11. Cataract

It has been asserted that lenticular opacities are more frequently to be found, age for age, in the diabetic than in the non-diabetic (Duke Elder, 1945; Heinsius and Arandt, 1950; Keen and Smith, 1960; Tulloch, 1962) though this assertion has not gone unchallenged (Waite and Beetham, 1935; McGuinness, 1967). In making comparisons of this sort, it is important to allow for such factors as the age and sex composition of the groups compared as well as the duration and degree of hyperglycaemia evidenced by the diabetics concerned. Poor 'control' of diabetes (as judged by the frequency and degree of glycosuria) was found to be associated with the increased appearance of lens opacities in younger patients by O'Brien and Allen (1942) and in middle-aged males by Barditt and Caird (1968). Keen and Smith (1960) found almost all of the excess of lenticular opacities in clinically evident diabetics to be in the 'worst controlled' part of the population they studied, judged on the basis of urine tests and blood sugar estimations. The failure of McGuinness (1967) to find a clear relation between blood sugar elevation and lenticular opacities might have been due to the minor degrees of hyperglycaemia which he considered in the course of his population study. Most of his subjects would have qualified for inclusion in the 'borderline' group described here.

A Glycaemic Threshold

The notion that a 'threshold' effect may be operating with lenticular opacity formation (that is to say, a minimal degree of hyperglycaemia which is necessary before the relationship between blood sugar and lenticular opacity becomes apparent) receives some support from the results of the lens examination in the Bedford subjects. Table 15 shows by age and sex the frequency and degree of lenticular opacities found in control, 'borderline' and diabetic subjects on the occasion of the first eye examination. In the Table, the figures in each category represent the percentage of individuals in that particular sex and blood sugar group. All groups show the expected rise of frequency of involvement with increasing age. Only among the diabetic women is there a suggestion of an excess of lenticular opacities to be seen. Frequencies in the 'borderline' group differ little, if at all, from those in the controls.

Experimental support that a glycaemic threshold effect may operate in the formation of cataract is offered by the work of Patterson (1952). In experimentally induced diabetes in rats, he found that lenticular opacities occurred only when the degree of hyperglycaemia induced exceeded about 225mg/100ml. Above this level, there appeared to be a rough, positive association between the degree of hyperglycaemia and the speed with which opacities developed. In vitro, Lou and Kinoshita (1967) suggested that no accumulation of glucose within the lens occurs until concentrations exceeding 200mg/100ml. are present in the suspending medium. It seems probable that excess intracellular glucose will be metabolised by way of the sorbitol pathway (Van Heyningen,

1959, 1962) and suggestive evidence has been adduced that the osmotic effects of this poorly diffusible sugar alcohol may contribute to the development of cataract in animals and in human diabetes (Pirie and Van Heyningen, 1964).

TABLE 15

Lenticular Opacities by Grade in the Three Bedford
Blood Sugar Groups by Age and Sex.

Figures represent percentage of individuals within
sex/blood sugar groups falling into described cataract
grades. 0 = no opacity. 1 = spokes. 2 = 'chunks'.
3 = vision limiting. All = all + grades.

Grade of Lenticular Opacity
(% of respective B.S. group by sex)

Age	B.S. Group	MEN					WOMEN				
		0	1	2	3	All +	0	1	2	3	All +
20	Contl.	4.8	-	-	-	0	2.4	-	-	-	0
	B/line	6.3	-	-	-	0	5.0	-	-	-	0
	Diabc.	2.3	-	-	-	0	3.4	-	-	-	0
30	Contl.	7.7	-	-	-	0	11.8	-	-	-	0
	B/line	8.1	-	-	-	0	7.9	-	-	-	0
	Diabc.	6.8	2.3	-	-	2.3	8.5	-	-	-	0
40	Contl.	18.3	-	-	-	0	15.3	1.2	-	-	1.2
	B/line	16.2	0.9	-	-	0.9	15.8	-	-	-	0
	Diabc.	6.8	-	-	-	0	3.4	1.7	-	-	1.7
50	Contl.	25.0	4.8	-	-	4.8	15.3	3.5	1.2	-	4.7
	B/line	27.9	0.9	0.9	-	1.8	17.8	-	1.0	-	1.0
	Diabc.	18.2	6.8	-	2.3	9.1	23.7	5.1	-	-	5.1
60	Contl.	9.6	8.7	4.8	-	13.5	20.0	12.9	-	-	12.9
	B/line	16.2	0.9	1.8	-	2.7	16.8	5.0	3.0	-	8.0
	Diabc.	25.0	9.1	-	-	9.1	8.5	6.8	6.8	-	13.6
70	Contl.	3.8	11.5	-	1.0	12.5	5.9	8.2	2.4	-	10.6
	B/line	7.2	7.2	2.7	-	10.9	7.9	11.9	5.0	-	16.9
	Diabc.	6.8	6.8	2.3	2.3	11.4	8.5	15.3	5.1	-	20.4
80	Contl.	-	-	-	-	-	-	-	-	-	-
	B/line	-	1.8	0.9	-	2.7	1.0	1.0	1.0	-	2.0
	Diabc.	-	2.3	-	-	2.3	-	3.4	-	-	3.4

c. The Peripheral Nerves (Insert 6)

The involvement of the nervous system in diabetes is probably confined to abnormalities of the peripheral nerves, though the possibility of changes in the spinal cord was suggested by Garland and Taverner (1953). Afferent nervous pathways are more often affected than efferent, the slower conducting, small diameter fibres being more vulnerable than the faster conducting large diameter fibres (Dolman, 1963). The peculiar vulnerability of these fibres, and of post-ganglionic autonomic fibres (Keen, 1962), may be due to their lesser investment by the myelin sheath, morphological abnormality of which has been clearly linked with clinical neuropathy (Thomas and Lascelles, 1966).

In the examination of the Bedford subjects presented here, assessment of neurological function has been restricted to consideration of vibratory perception and tendon reflexes in the legs. Although this limited examination was partly determined on the basis of practicability and convenience, it was also recommended by two further facts. Disorders of these neurological functions are characteristic clinical manifestations of diabetic neuropathy and a considerable body of data has built up concerning them (Williamson, 1897; Martin, 1953; Fry et al, 1962; Mayne, 1965; Collens et al, 1946; Rundles, 1945; Mirsky et al, 1953; Steiness, 1963). By the use of suitable apparatus it is easier to make quantitative assessments of the degree of disturbance of vibratory perception than of most of the other sensory modalities. It is also reasonably reliable to make a simple assessment of the presence or absence of a tendon reflex. No attempt was made to grade the response. As described in the examination and follow-up procedures, repeated

re-examination\$ of these two neurological functions have been made since 1962. This presentation, however, deals only with the findings at the time of identification of the three blood sugar groups.

INSERT 6

THE CONCOMITANTS OF RAISED BLOOD SUGAR:

STUDIES IN NEWLY-DETECTED

HYPERGLYCAEMICS

1. A COMPARATIVE ASSESSMENT OF NEUROLOGICAL

FUNCTIONS IN BLOOD SUGAR GROUPS

by

R. John Jarrett, M.A., M.D.

Harry Keen, M.B., B.S., M.R.C.P.

David R. Boyns, M.R.C.P.

Costis Chlouverakis, M.D.

and

John Fuller

Guy's Hospital Reports 118, 237-246, 1969.

THE CONCOMITANTS OF RAISED BLOOD SUGAR: STUDIES IN NEWLY-DETECTED HYPERGLYCAEMICS

I. A comparative assessment of neurological functions in blood sugar groups

By R. J. JARRETT, H. KEEN, D. R. BOYNS, C. CHLOUVERAKIS
and J. FULLER

Department of Medicine, Guy's Hospital Medical School, S.E.1

DEFECTIVE neurological function is a common feature of diabetes mellitus and is frequently localized to the legs. Williamson (1905) first reported that the perception of vibration was often lost or impaired in diabetics and he subsequently stated (1922) that vibration sense might be lost although other forms of sensation remained unimpaired. Although his results with tuning-fork tests have been confirmed by others using electro-magnetic instruments (Steiness 1963), the two major reported studies have given conflicting results regarding the relationship between duration of diabetes and impaired vibration perception (Mirsky, Futterman and Broh-Kahn 1953, Steiness 1963).

A large number of previously unsuspected hyperglycaemic individuals were discovered following the Bedford Diabetes Survey in 1962 (Sharp, Butterfield and Keen 1964). Together with a control group, the newly-found diabetics were recalled to a special clinic for examination and questioning. Among the examination procedures carried out on this occasion were a quantitative measurement of the threshold of vibratory perception and testing of patellar and Achilles tendon reflexes. The results are reported below.

MATERIAL AND METHODS

The initial screening procedure in the population survey consisted of a test for glycosuria in urine specimens passed after a carbohydrate-loaded breakfast. The capillary blood sugar level was measured in the glycosuric individuals 2 hours after 50 g glucose by mouth. In addition, full 2-hour oral-glucose tolerance tests were carried out upon a stratified random sample drawn from the whole co-operating population. Those people found to have a 2-hour blood sugar value of 200 mg/100 ml or more were called 'diabetic', those with values between 120-199 mg/100 ml were called 'borderline diabetic', and those with values below

120 mg/100 ml were regarded as normal. From the normal individuals in the random sample, a control group, matched for age and sex with the borderline diabetics, was recruited. The numbers shown in Table I represent those individuals examined at the follow-up clinic for whom complete neurological information is available.

TABLE I
AGE AND SEX COMPOSITION OF THE THREE DIAGNOSTIC GROUPS

								Total
<i>Controls</i>	Males	5	8	19	31	24	17	104
	Females	2	10	14	17	28	13	84
<i>Borderline diabetics</i>	Males	6	9	19	30	19	16	99
	Females	4	8	14	17	24	25	92
<i>Diabetics</i>	Males	1	4	3	12	16	10	46
	Females	2	5	3	16	13	17	56
<i>Age</i>		20-	30-	40-	50-	60-	70+	

The threshold of vibratory perception was measured with a Bio-Thesimeter (Bio-Medical Instrument Co., Newbury, Ohio). This instrument incorporates an electrically-driven stylus, with a plane surface, 13 mm in diameter, which vibrates at a constant frequency of twice the a.c. main electricity supply. The amplitude of the vibration which is in the horizontal plane, is proportional to the square of the voltage. The instrument was mounted on a pivoted, counterbalanced rod, so that the stylus was applied to the skin at a weight of 100 g. With the subjects in the sitting position measurements were made with the stylus resting on the pulp of the distal phalanx of the right index finger and the tip of the right great toe. For the latter measurement, the leg was elevated to the horizontal with the foot comfortably supported at a normal angle on a chair or stool. The instrument was first applied to the body away from the part to be tested and the stimulus increased to demonstrate the quality of the sensation. At the test site, the amplitude of the vibration was increased gradually until the subject announced that he or she was just able to feel it. The procedure was repeated several times until consistent results were obtained. In a number of individuals no vibration was perceived, even at the level of maximal stimulation (Table II). These cases have been included in the highest vibration perception threshold (VPT) category.

The measurements of VPT were expressed in volts and subsequently transformed to (volts²) for the statistical analysis. A

TABLE II
NUMBER OF INDIVIDUALS WITH MAXIMUM MEASURABLE VIBRATION PERCEPTION
THRESHOLD VALUES

		Male	Female	Male	Female	Male	Female
<i>Controls</i>	Finger	0	0	0	1	1	0
	Toe	0	0	0	0	9	4
<i>Borderline diabetics</i>	Finger	0	0	0	0	0	1
	Toe	0	0	0	2	8	9
<i>Diabetics</i>	Finger	0	0	0	1	0	1
	Toe	0	0	0	1	11	11
<i>Age</i>		20-39		40-59		60+	

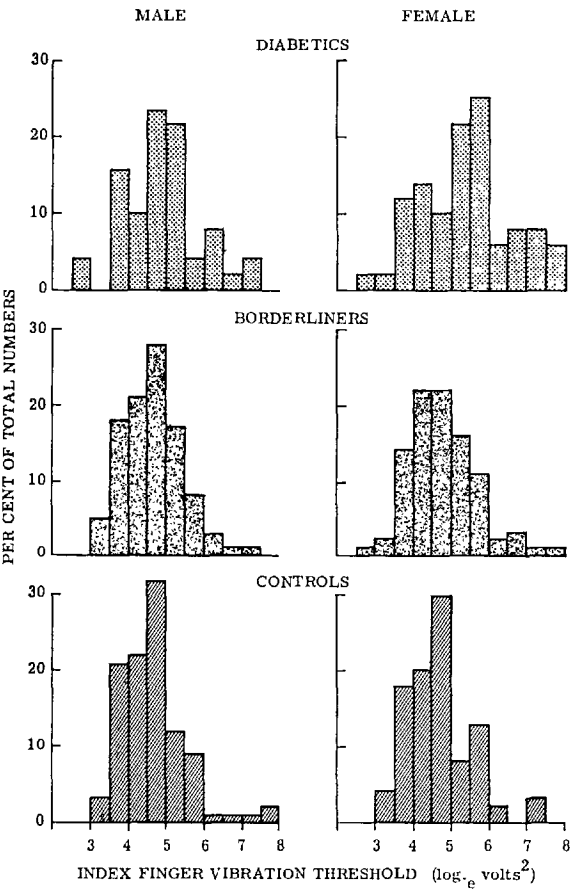


FIGURE 1.
The frequency distribution of the vibration threshold measured in the right
index finger. The values shown are the natural logarithms of the (volts²).

TABLE III
MEAN VIBRATION PERCEPTION THRESHOLD AS (VOLTS²) IN THE THREE DIAGNOSTIC GROUPS
(Figures in parentheses denote the standard error of the mean)

Finger VPT

<i>Controls</i>	Males	59.2 (8.2)	57.4 (9.8)	84.6 (11.4)	124.2 (14.3)	180.5 (49.3)	474.5 (179.4)
	Females	56.5 (5.3)	67.4 (9.7)	95.4 (19.9)	163.7 (11.4)	192.0 (59.1)	232.2 (38.8)
<i>Borderline diabetics</i>	Males	97.3 (48.4)	73.7 (12.6)	107.7 (22.9)	114.1 (15.2)	257.2 (60.0)	201.8 (50.0)
	Females	76.2 (27.4)	89.4 (32.4)	92.2 (18.5)	158.9 (29.3)	187.1 (46.1)	295.6 (94.2)
<i>Diabetics</i>	Males	81.0	47.0 (15.8)	204.7 (132.3)	165.6 (42.3)	309.0 (110.4)	232.0 (61.5)
	Females	464.0 (226.3)	127.0 (41.4)	103.0 (38.1)	377.1 (148.3)	465.2 (188.7)	592.9 (135.6)
Age		20-	30-	40-	50-	60-	70+

Toe VPT

<i>Controls</i>	Males	170.0 (47.9)	176.9 (48.5)	288.9 (54.0)	577.7 (99.7)	1408.6 (155.6)	1613.8 (180.8)
	Females	145.0 (16.9)	258.3 (63.4)	304.1 (44.4)	624.4 (130.0)	606.8 (68.8)	1440.5 (213.3)
<i>Borderline diabetics</i>	Males	84.5 (7.8)	266.7 (36.2)	312.1 (80.6)	384.5 (80.9)	1041.5 (168.8)	1405.5 (211.2)
	Females	112.5 (14.8)	168.6 (52.4)	405.1 (161.2)	614.6 (149.3)	754.6 (119.6)	1472.5 (158.7)
<i>Diabetics</i>	Males	144.0	103.2 (27.4)	416.3 (248.4)	683.1 (152.6)	1530.5 (222.5)	1411.9 (261.2)
	Females	404.0 (56.6)	167.8 (37.3)	383.0 (163.7)	506.9 (145.9)	1161.7 (231.1)	1980.4 (153.6)
Age		20-	30-	40-	50-	60-	70+

TABLE IV
MEAN VIBRATION PERCEPTION THRESHOLD AS LOG_e (VOLTS²) IN THE THREE DIAGNOSTIC GROUPS
(Figures in parentheses denote the standard error of the mean)

Finger VPT

<i>Controls</i>	Males	4.03 (0.14)	3.94 (0.16)	4.25 (0.14)	4.65 (0.10)	4.74 (0.17)	5.42 (0.26)
	Females	4.03 (0.95)	4.10 (0.15)	4.30 (0.19)	4.62 (0.18)	4.83 (0.14)	5.25 (0.18)
<i>Borderline diabetics</i>	Males	4.11 (0.35)	4.18 (0.51)	4.42 (0.15)	4.48 (0.13)	5.20 (0.18)	5.02 (0.17)
	Females	4.11 (0.32)	4.15 (0.27)	4.21 (0.23)	4.86 (0.15)	4.88 (0.15)	5.21 (0.17)
<i>Diabetics</i>	Males	4.39	3.64 (0.33)	4.58 (0.69)	4.72 (0.27)	5.20 (0.22)	5.09 (0.28)
	Females	5.82 (0.60)	4.36 (0.51)	4.44 (0.35)	5.13 (0.30)	5.48 (0.29)	5.94 (0.24)
Age		20-	30-	40-	50-	60-	70+

Toe VPT

<i>Controls</i>	Males	4.93 (0.30)	4.99 (0.19)	5.43 (0.15)	6.0 (0.16)	7.04 (0.15)	7.25 (0.13)
	Females	4.96 (0.12)	5.31 (0.22)	5.34 (0.23)	6.16 (0.17)	6.19 (0.14)	7.03 (0.22)
<i>Borderline diabetics</i>	Males	4.07 (0.40)	5.5 (0.31)	5.29 (0.21)	5.57 (0.15)	6.65 (0.20)	7.01 (0.19)
	Females	4.68 (0.15)	4.77 (0.30)	5.37 (0.29)	5.92 (0.26)	6.26 (0.19)	7.09 (0.14)
<i>Diabetics</i>	Males	4.97	4.46 (0.31)	5.43 (0.63)	6.25 (0.22)	7.1 (0.19)	7.03 (0.23)
	Females	5.98 (0.14)	5.01 (0.20)	5.69 (0.40)	5.78 (0.23)	6.75 (0.23)	7.53 (0.09)
Age		20-	30-	40-	50-	60-	70+

further transformation to the $\log_e$ of the squared voltage was also performed, because of the demonstration by Mirsky *et al.* (1953) that vibration perception is distributed log-normally both in groups of normal persons and of diabetics. Patellar and Achilles tendon reflexes were tested using the ordinary clinical techniques. If reflexes were absent, reinforcement techniques were employed and results recorded as positive or negative with no attempt at quantitation.

Statistical analysis was carried out on an Atlas Computer at the University of London Institute of Computer Science.

RESULTS

1. Vibratory Perception Threshold (VPT)

Plotting the frequency distribution of the arithmetic and logarithmic values of the VPT confirmed the finding of Mirsky *et al.* (1953) that they were log-normally distributed. The 'normalized' frequency-distribution of the log finger-VPT is shown for the three diagnostic groups in Figure 1, and is similar to the form of the distribution of the toe VPT.

The mean values for the finger and toe VPT are shown in Table III as (volts²) and Table IV as log (volts²) for the three diagnostic groups and according to sex and decade of age. It can be seen that, in all three groups and in both sexes, the mean values and the scatter about the mean rise with age. In Table V, the linear correlation coefficients of age on log VPT are given. In each sub-group, the correlation was highly significant.

Inspection of Table II reveals no systematic differences between groups except, perhaps, in the higher values for the older female diabetics. In five groups (three finger, two toe) the mean values for the diabetics suggestively exceeded those of the corresponding controls. Levels of statistical significance for the differences in

TABLE V
CORRELATION OF VIBRATION THRESHOLD (LOG VOLTS²) WITH AGE

		Fingers			Toes		
		r	n	p	r	n	p
<i>Controls</i>	Male	0.5004	104	<0.001	0.7323	104	<0.001
	Female	0.3717	84	<0.001	0.5702	84	<0.001
<i>Borderline diabetics</i>	Male	0.3975	102	<0.001	0.6600	102	<0.001
	Female	0.4697	95	<0.001	0.6854	95	<0.001
<i>Diabetics</i>	Male	0.4240	47	<0.001	0.7044	47	<0.001
	Female	0.3399	58	<0.001	0.6875	58	<0.001

TABLE VI

COMPARISON (USING STUDENT'S T TEST) OF THE MEAN VIBRATION THRESHOLD IN FEMALE DIABETICS COMPARED WITH THE FEMALE CONTROLS IN THE SAME TEN-YEAR AGE GROUP

	Age group	Significance of difference
Finger	50-59	0.10 > p > 0.05
Threshold	60-69	0.1 > p > 0.05
	70-	0.05 > p > 0.025
Toe	60-69	0.1 > p > 0.05
	70-	0.05 > p > 0.025

TABLE VII

PERCENTAGE OF INDIVIDUALS ACCORDING TO THE NUMBER OF THEIR ABSENT TENDON REFLEXES IN THE THREE DIAGNOSTIC GROUPS, BY SEX

Number of absent reflexes	Controls		Borderline diabetes		Diabetics	
	Male	Female	Male	Female	Male	Female
0	89.4	89.5	79.1	81.0	57.4	57.6
1	2.9	3.5	7.3	6.0	12.8	8.5
2	5.7	3.5	9.1	9.0	17.0	22.0
3	1.0	—	—	3.0	4.3	5.1
4	1.0	3.5	4.5	1.0	8.5	6.8

TABLE VIII

PERCENTAGE OF INDIVIDUALS ACCORDING TO THE NUMBER OF THEIR ABSENT TENDON REFLEXES IN THE THREE DIAGNOSTIC GROUPS, BY AGE GROUP

Number of absent reflexes	Controls			Borderline diabetes			Diabetics		
	20-39	40-59	60+	20-39	40-59	60+	20-39	40-59	60+
0	100	93.8	81.9	96.2	87.9	67.0	91.7	71.4	42.4
1	—	1.2	6.0	—	3.6	12.5	—	8.6	13.5
2	—	3.8	7.2	3.8	7.3	12.5	—	17.2	25.4
3	—	—	1.3	—	—	3.4	—	—	8.5
4	—	1.2	3.6	—	1.2	4.6	8.3	2.8	10.2

these groups are given in Table VI. If the borderline diabetes are pooled with the controls, all five differences become statistically significant ($p < 0.05$).

Tendon reflexes

Table VII shows the percentage of individuals in each of the three diagnostic groups set against the number of their absent tendon reflexes. In Table VIII the results are depicted in 20-year age groups; as there was no systematic difference, the sexes have been pooled.

A normal complement of tendon reflexes was found most frequently in the control subjects, least in the diabetics, with the borderline diabetics occupying an intermediate position.

Despite the apparent dissociation of the behaviour of the VPT and tendon reflexes in the three diagnostic groups, some relationship between them may be seen in Table IX, which shows the mean log toe-VPT by age and diagnostic group in individuals with one or no absent reflex and those with two or more absent reflexes. However, the association between VPT and absent reflexes is not strong and this will be discussed later.

TABLE IX
THE MEAN TOE VPT (LOG VOLTS²) IN THE THREE DIAGNOSTIC GROUPS
ACCORDING TO THE NUMBER OF ABSENT TENDON REFLEXES

	Number of absent reflexes	Mean toe VPT (log volts ²)					
Controls	0-1 2-4	4.94 —	5.16 —	5.39 —	6.04 6.3	6.49 7.26	7.11 7.46
Borderline diabetics ..	0-1 2-4	4.30 —	5.16 —	5.32 —	5.67 6.14	6.34 6.93	7.08 7.08
Diabetics	0-1 2-4	5.58 5.78	4.77 —	5.92 4.84	5.99 5.94	6.74 7.27	7.23 7.44
Age		20-	30-	40-	50-	60-	70-

DISCUSSION

The two chief quantitative studies in which vibratory perception in diabetics was compared with that in non-diabetics, are those of Mirsky *et al.* (1953) and Steiness (1963). The former authors measured the VPT in the index fingers and great toes of 102 established diabetics and 136 non-diabetics. They found that, in both groups, the VPT increased with age, that the increase was greater in the toes and that the patient with diabetes reacted 'as if he were approximately 20 years older, in this respect, than a person without diabetes'. However, they could find no relationship between the severity of VPT impairment and the known duration or 'severity' of the diabetes. In the non-diabetics, there were small, but significant, sex differences, the VPT measured in the great toe being higher in men than in women. The findings of Steiness (1963) were broadly similar to those of Mirsky *et al.* (1953). The major difference was that Steiness found a statistically significant correlation between the VPT and the known duration of the diabetes. With regard to the effects of age, our findings are in agreement with those of Mirsky *et al.*, and of Steiness.

The major difference between these studies and our own is in the nature of the group of diabetics studied. Their diabetic patients were established and under treatment, whereas ours were newly-found and, in over half the cases, were without symptoms. Because they were diagnosed in a Survey, they are as near to an 'at onset' group as one can get; even so, it is quite possible that many of them had been hyperglycaemic for months or even years.

The moderately hyperglycaemic or borderline diabetic group did not show any abnormality of vibration perception threshold, despite abnormalities of albumin excretion and an increased prevalence of arterial disease (Keen, Rose, Pyke, Boyns, Chlouverakis and Mistry 1965; Keen, Chlouverakis, Fuller and Jarrett 1969). What is perhaps more surprising is that the newly-found diabetics showed no more impressive abnormalities of vibration perception threshold compared with the other two groups. Only the older women had significantly higher thresholds, both in hands and feet. It is possible that, in women, hyperglycaemia may go unrecognized for longer periods than in men, who are, perhaps, more likely to have routine medical examinations or to have associated conditions, such as cardiac ischaemia, which brings their diabetes to light. The significantly lower renal threshold for glucose in men is also likely to lead to earlier recognition (Butterfield, Keen and Whichelow 1967).

The discrepancy between the results of the VPT levels and the findings of absent reflexes may be due to a greater vulnerability of the latter to a diabetic neuropathic process. Although the two functions share the same sensory pathway, additional abnormality on the motor side of the reflex arc (Gilliatt and Willison 1962) might account for the clearer association of reflex abnormalities with the presence of diabetes. An alternative and, perhaps, more likely explanation, is observer bias, in that knowledge of the status of the examinee (control, borderline diabetic or diabetic) is more likely to influence judgement of a reflex response than reading figures on a dial.

The demonstration by both Steiness and Mirsky *et al.*, and the experience of practising clinicians (Fry, Hardwick and Scott 1962) that established diabetics show clearly impaired vibration perception, stands in contrast to our finding that the newly-found diabetic, in general, has little or no impairment. This strongly supports the view that neurological impairment in diabetics is related to the duration of the metabolic disorder, a view that is further supported by the absence of impaired vibratory perception in our borderline diabetics. These individuals might be regarded as being at an even earlier stage of diabetes and, as a group, do show other abnormalities related to diabetes.

This contrast prompts the hypothesis that, in the ranking of tissue vulnerability to the diabetic process, the peripheral nerves do not stand very high. However, it cannot necessarily be assumed that the limited examinations we have carried out are representative of the whole of peripheral nervous function, despite the widely held clinical view that vibration sense suffers early in diabetes. There is, for instance, evidence of a special vulnerability of the autonomic nerve fibres (Keen 1962), which may show abnormalities of function in the absence of somatic nerve involvement. In deciding the important issue of the relation of duration and 'severity' of the diabetes to neurological damage, 'cross-sectional' surveys have inherent disadvantages, which can only be overcome by 'longitudinal' studies in defined groups. We hope to provide such data in subsequent reports.

SUMMARY

Neurological function was assessed in three groups drawn from the population of Bedford. The three groups—normal, borderline diabetic and diabetic—were classified by the level of the blood sugar 2 hours after a 50 g glucose load. The threshold of vibration perception was measured on the right great toe and right index finger with a Bio-Thesiometer and patellar and Achilles tendon reflexes were tested using ordinary clinical techniques.

The threshold of vibration perception was directly related to age, but was similar in each of the three diagnostic groups. Only among the older female diabetics were higher mean vibration thresholds observed.

REFERENCES

- Butterfield, W. J. H., Keen, H. and Whichelow, M. J. (1967) *Brit. med. J.*, **4**, 505.
 Fry, I. K., Hardwick, C. and Scott, G. W. (1962) *Guy's Hosp. Rep.*, **111**, 113.
 Gilliatt, R. W. and Willison, R. G. (1962) *J. Neurol. Neurosurg. Psychiat.*, **25**, 11.
 Keen, H. (1962) In 'Disorders of Carbohydrate Metabolism', ed. D. A. Pyke, p. 177. London.
 Keen, H., Chlouverakis, C., Fuller, J. and Jarrett, R. J. (1969) *Guy's Hosp. Rep.*, **118**, 247.
 Keen, H., Rose, G., Pyke, D. A., Boyns, D., Chlouverakis, C. and Mistry, S. (1965) *Lancet*, **ii**, 505.
 Mirsky, I. A., Futterman, P. and Broh-Kahn, R. H. (1953) *J. Lab. clin. Med.*, **41**, 221.
 Sharp, C. L., Butterfield, W. J. H. and Keen, H. (1964) *Proc. Roy. Soc. Med.*, **57**, 193.
 Steiness, I. (1963) *Acta med. scand.*, **173**, Suppl. 394.
 Williamson, R. T. (1905) *Lancet*, **i**, 855.
 Williamson, R. T. (1922) *Am. J. med. Sci.*, **164**, 715.

Further Comments on the Peripheral Nerves

The neurological disorder associated with diabetes has, until recently, been among the more baffling aspects of the disease. Difficult to define and defying explanation, much of the earlier investigation in this field was based upon demonstration, collection and classification. The studies reported here have, in themselves, added little to the understanding of the condition but acquire greater significance when considered along with the other findings in the three Bedford groups. The abnormalities demonstrated were largely confined to the diabetic group, the 'borderline' diabetics differing little, if at all, from the controls. This relationship recalls the similar situation which holds in respect of cataract, discussed in an earlier section, and equally with it, supports the suggestion that a glycaemic 'threshold' effect may be operating. The minimal degree of glucose intolerance necessary for nerve damage may be even higher than that for the development of lens opacities, for neurological deficit was only clearly displayed by a small number of the diabetic subjects studied.

It is of some interest that investigations of recent years have suggested that a common biochemical mechanism may be responsible for the damage to both the lens and the peripheral nerve in the diabetic. Sherman and Stewart (1966) reported the presence of the poorly diffusible glucose alcohol, sorbitol, in mammalian nerve. Gabbay et al (1966) demonstrated its accumulation in nerves in experimental diabetes in animals. Subsequent experiments by Gabbay and others (1968) and by Ward and Baker (1970) have confirmed and extended these findings, and shown that, in diabetic animals, the accumulation of sorbitol can be reversed

by the administration of insulin. Swelling of nerve trunks is demonstrable in the diabetic animal and Gabbay et al (1966) have produced evidence that this may be effected by the osmotic activity of the excess of sorbitol, a pathway activated by hyperglycaemia in the Schwann cell rather than the axon fibre. It is tempting to correlate this with the elegant demonstration in teased nerve fibre preparations (Thomas and Lascelles, 1966) of segmental demyelination in the nerves of human diabetics, also suggesting that the primary abnormality of neuropathy lies in the Schwann cell. However, more rigorous evidence is required before this hypothesis can be considered established.

8.

CARDIOVASCULAR DISEASEPrevalence, Incidence and the Effects of Treatment (Inserts 7, 8 and 9).

It is perhaps in aiding understanding of the metabolic background of atherosclerosis that the study of the lesser degrees of glucose intolerance has been most fruitful. The recognition of a spectrum of degrees of glucose tolerance and intolerance raised the question of the existence of a similar, parallel spectrum of enhanced liability to atherosclerosis. Hints that such a parallel relationship might exist had come mainly from those who had investigated carbohydrate metabolism in subjects with clinically evident atherosclerosis (Waddell and Field, 1960; Wahlberg, 1966; Kingsbury, 1966) and who had shown the increased frequency with which impaired glucose tolerance, short of overt diabetes, was found in atherosclerotic subjects.

However, objections can be raised to this approach to the study of the relationship between glucose intolerance and atherosclerosis. Both conditions are common, and since either or both may be present and unsuspected, the recognition of one may simply be the vehicle for the discovery of the other, creating the appearance of a special link. Dietary or drug treatment of one of the conditions may influence the manifestation of the other. Clinical interpretation of signs or symptoms may be biased by prior suppositions of a relationship.

It is possible to make some quantitative estimate of the degree of glucose intolerance but difficult to quantitate atherosclerosis in the same way, though a range of degrees of involvement of the arterial

wall must also be present. Atherosclerosis is itself an unsatisfactory term, allowing of both clinical and morbid pathological definitions and including processes affecting the arterial wall and the circulating blood (Bowyer and Gresham, 1970; Geer et al, 1970; Haust, 1970; Studer, 1970).

Epidemiological examination of the relationship has developed mainly over the last decade. It has sprung from two main approaches. On the one hand, interest in the epidemiology of atherosclerosis has, increasingly, included attempts to identify factors associated with enhanced risk of arterial disease (Ostrander et al, 1965; Kannel et al, 1961). On the other, it has grown from studies of the epidemiology of diabetes itself, and those disorders associated with it (Kaen et al, 1965; Welborn et al, 1969). The epidemiological approach meets most of the objections to clinical studies expressed above. Studies of population samples are, however, likely to identify principally the less well developed manifestations of the conditions sought, the more evident examples having declared themselves and come under care. In the case of glucose intolerance, for example, it is probable that the great majority of the juvenile onset type of insulin dependent diabetics will have been diagnosed and under dietary and insulin treatment. Similarly, those individuals most severely affected by atherosclerosis will have declared themselves clinically and have altered many of their habits of life accordingly. Nevertheless, despite these shortcomings, it seems likely that the examination of large population samples gives a truer picture of the relationship between the two conditions than clinic-based studies, the more so when augmented by long-term follow up examination.

The first of the papers which follows defines the link between blood sugar and the prevalence of 'arterial disease' as manifest in the control, 'borderline' and diabetic groups identified in the Bedford Survey. The second describes the progress and results at the five-year time point of the controlled trial of treatment with tolbutamide and dietary carbohydrate restriction in the 248 members of the 'borderline' group. The third paper extends these trial results to the seven-year time point, generally reviews the literature on the subject of the relationship between glycaemia and atherosclerosis and considers possible mechanisms which might account for the observed associations.

INSERT 7

BLOOD SUGAR AND ARTERIAL DISEASE

by

H. Keen, M.B., B.S., M.R.C.P.

G. Rose, M.A., D.M., M.R.C.P.

D. A. Pyke, M.D., F.R.C.P.

D. R. Boyns, M.B., B.Sc., M.R.C.P.

C. Chlouverakis, M.D.

and

S. Mistry, M.B.

The Lancet 2, 505-508, 1965.

BLOOD-SUGAR AND ARTERIAL DISEASE

H. KEEN

M.B. Lond., M.R.C.P.

SENIOR LECTURER IN MEDICINE, GUY'S HOSPITAL, LONDON, S.E.1

GEOFFREY ROSE

M.A., D.M. Oxon., M.R.C.P.

SENIOR LECTURER IN EPIDEMIOLOGY, LONDON SCHOOL OF HYGIENE AND
TROPICAL MEDICINE, LONDON, W.C.1, AND ST. MARY'S HOSPITAL
MEDICAL SCHOOL, LONDON, W.2

D. A. PYKE

M.D. Cantab., F.R.C.P.

PHYSICIAN, KING'S COLLEGE HOSPITAL, LONDON, S.E.5

D. BOYNS

M.B., B.Sc. Wales, M.R.C.P.

JUNIOR LECTURER

C. CHLOUVERAKIS

M.D. Athens

RESEARCH FELLOW

S. MISTRY

M.B. Bombay

RESEARCH FELLOW

DEPARTMENT OF MEDICINE, GUY'S HOSPITAL, LONDON, S.E.1

SURVEYS of glucose tolerance in general populations have revealed that raised levels of blood-sugar occur with unexpected frequency, especially among middle-aged and elderly people (College of General Practitioners 1963, Sharp, Butterfield, and Keen 1964, United States Department of Health, Education, and Welfare 1964). For every member of the population with known diabetes mellitus several others have unrecognised hyperglycaemia. Although, generally, this hyperglycaemia is symptomless and, in itself, of no immediate importance, even minor elevations of blood-sugar may possibly carry an increased risk of the development of cardiovascular disease.

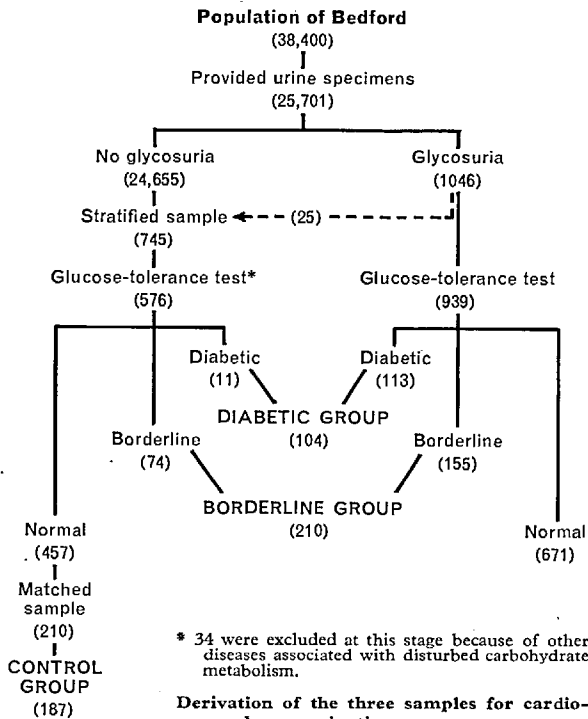
Clinicians have long believed that patients with diabetes mellitus have a special tendency to atherosclerosis and its complications (Liebow et al. 1955, Marble 1955, Bryfogle and Bradley 1957). Clinical impressions of an association

between two diseases can be misleading since a patient with both conditions may be more likely to enter hospital than a patient who has only one of them, and recognition of one disease increases the chance of discovery of the other. We have reduced these sources of error by examining the association between arterial disease and hyperglycæmia, defined by the blood-sugar response to oral glucose, measured in population samples, in a diagnostic survey for previously undiagnosed diabetics. This study was designed to answer the question whether an increased tendency to arterial disease was associated with hyperglycæmia both at "diabetic" levels and also in people with only "borderline" elevations of the blood-sugar.

Survey Population and Methods

Population Studied

The study forms part of a continuing follow-up of participants



in the Bedford diabetes survey (Sharp, Butterfield, and Keen 1964). Three sample groups were selected for cardiovascular examination during the period September, 1962, to April, 1963: a "control" group; a group with "borderline" hyperglycaemia; and a group with "diabetic" levels of blood-sugar (see accompanying figure).

Of 38,400 persons aged twenty and over on the electoral register of Bedford, 67% provided postprandial urine specimens. Persons already known to have diabetes mellitus were excluded; among the remainder, 1033 had glycosuria (by the 'Clinistix' test), and these were recalled for glucose-tolerance tests. Of the 939 who attended, 113 had blood-sugars of 200 mg. per 100 ml. or more two hours after 50 g. of glucose by mouth (group A), and 155 had two-hour values in the range 120-199 mg. per 100 ml. (group B).

A random sample of 745 people from the whole cooperating population (excluding only those already known to have diabetes) was stratified by age and sex; 77% of these attended for glucose-tolerance tests. 34 were excluded from further consideration because of conditions known to be associated with disturbed carbohydrate metabolism (e.g., steroid therapy, pregnancy, peptic ulcer, or hyperthyroidism). Of the remaining 542, 11 had two-hour blood-sugar values of 200 mg. per 100 ml. or more (group C), and 74 had blood-sugars in the range 120-199 mg. per 100 ml. (group D). Groups A and C were combined to provide the diabetic sample, and groups B and D were combined into a borderline sample. Each person in the borderline sample was matched for sex and age (± 2.5 years) with a person from the random sample whose two-hour blood-sugar was under 120 mg. per 100 ml. This provided the control sample.

When the members of the three groups were asked to attend for cardiovascular examination 187 (89%) of the control group, 210 (92%) of the borderline group, and 104 (84%) of the diabetic group attended.

Cardiovascular Examination

The examination was done during a further glucose-tolerance test (not the test used to classify the participants), so that all three groups had taken 50 g. of glucose two to two and a half hours previously. This may have slightly augmented the frequency of s-T changes and T changes in the electrocardiogram (E.C.G.) (Ostrand and Weinstein 1964), but there is no reason to suppose that comparisons between the different blood-sugar groups were affected.

Resting E.C.G.s were recorded and classified according to the Minnesota code (Blackburn et al. 1960); this system uses a purely descriptive approach. All reports were made by one observer, who was aware of the blood-sugar group to which the subject belonged, but "blind" re-reading of a sample revealed no evidence of bias. E.C.G.s were recorded on twelve

TABLE 1—AGE AND SEX DISTRIBUTIONS OF PERSONS EXAMINED IN THE THREE BLOOD-SUGAR GROUPS

Age (yr.)	No. of persons								
	Control			Borderline			Diabetic		
	Men	Women	Total	Men	Women	Total	Men	Women	Total
<50	33	25	58	33	29	62	6	11	17
50-59	29	17	46	34	15	49	13	17	30
60-69	24	28	52	24	28	52	15	13	28
≥70	17	14	31	20	27	47	10	19	29
All ages	103	84	187	111	99	210	44	60	104

leads on the men, but it was not practicable to record chest leads on the women. The resultant underestimation of the frequency of changes in women was taken into account in comparisons between the sexes.

The presence of angina of effort, a history of possible infarction, and intermittent claudication was assessed by a standard questionnaire, approved by the World Health Organisation (Rose 1962, 1965). This provided a simple means of identifying symptoms according to defined criteria, and lessened the risk of bias in comparisons between the three blood-sugar groups.

Casual blood-pressure (B.P.) were recorded in the sitting position with a standard sphygmomanometer. Diastolic pressures were recorded at the point of muffling.

Smoking-histories were also recorded, but the results bore no apparent relevance to blood-sugar level and were not considered further.

The findings presented here relate to the initial prevalence-study. Re-examinations are being undertaken and the results will be described elsewhere.

Results

Table 1 shows the age and sex distribution of the persons examined in each of the three blood-sugar groups. The composition of the control and borderline groups are similar, apart from difficulties in recruiting the older members of the control group. The diabetic group, especially the men, were a little older. The differences between the groups with regard to age and sex have been taken into account in the analysis by calculating "standardised prevalence-ratios" (S.P.R.) for manifestations of disease, obtained for symptoms and signs in the same way as standardised mortality-ratios would be calculated for mortality:

- (1) For each sex separately, age-specific rates have been obtained for all three blood-sugar groups combined.

TABLE II—STANDARDISED PREVALENCE-RATIO OF SYMPTOMS AND SIGNS OF ARTERIAL DISEASE ACCORDING TO BLOOD-SUGAR GROUP

Symptom or signs	Standardised prevalence-ratio (%)								
	Men			Women			Total		
	Control (103)	Borderline (111)	Diabetic (44)	Control (84)	Borderline (99)	Diabetic (60)	Control (187)	Borderline (210)	Diabetic (104)
<i>Arterial symptoms</i>	76 (7)	118 (12)	107 (5)	68 (8)†	85 (13)†	166 (15)†	72 (15)†	98 (25)†	146 (20)†
<i>Electrocardiographic changes:</i>									
<i>Q/QS waves</i>	121 (12)	74 (8)	117 (5)	98 (1)	77 (1)	147 (1)	119 (13)	75 (9)	121 (6)
<i>S-T depression</i>	71 (10)	116 (18)	123 (9)	89 (18)	106 (27)	104 (17)	81 (28)	110 (45)	110 (26)
<i>T-wave flattening or inversion</i> ..	64 (10)†	104 (18)†	161 (13)†	64 (10)*	108 (21)*	133 (16)*	64 (20)‡	106 (39)‡	144 (29)‡
<i>Arterial disease</i>	81 (23)	103 (32)	132 (18)	78 (27)*	103 (44)*	125 (33)*	79 (50)‡	103 (76)‡	127 (51)‡

* Trend significant at 10% level.

† Trend significant at 5% level.

‡ Trend significant at 2% level.

Numbers of participants are in parentheses.

(2) Applying these rates to the numbers examined in each group yields the number of cases that would be expected to occur in a group of that age structure if frequency of disease were unrelated to blood-sugar.

(3) The ratio of the observed to the expected number of cases, expressed as a percentage, is the S.P.R. It measures the relative excess or deficiency of the observed over the expected number of cases in the group, taking age into account.

(4) The S.P.R.s for both sexes combined are readily obtained. Since the expected numbers were obtained for each sex separately, the combined ratios take into account sex as well as age differences between the three blood-sugar groups.

Table II presents S.P.R.s in the three groups for various manifestations of atherosclerotic disease, together with the number of cases on which each ratio is based.

Arterial symptoms refer to angina of effort, pain of possible infarction, or intermittent claudication (as assessed by the standard questionnaire). Numbers were too small for study of each symptom separately, although each seemed to show a roughly similar pattern. A trend is apparent in both sexes towards increasing prevalence of these ischaemic symptoms with increasing blood-sugar. The significance of the trend was tested by a modification of the χ^2 test (Armitage 1955), and confirmed for both sexes combined and for women ($0.02 < P < 0.05$). The trend is present for men by themselves but did not reach the 5% level of significance.

In the E.C.G.s the presence of Q/Qs changes is the most specific index of myocardial ischaemia, but the low prevalence reduces the sensitivity of the analysis. No association with blood-sugar is apparent, but this might of course be only the result of the small total number of events. Depression of S-T segments and flattening or inversion of T waves, like the arterial symptoms, show clear gradations with blood-sugar; the gradients in the two sexes are roughly similar.

The final part of table II summarises the frequency of arterial disease, defined as any one or more of the signs or symptoms listed earlier in the table; this provides a measure of the association between blood-sugar and arterial disease. Disease in the diabetic group is approximately 50% more frequent than in the control group, with the borderline group occupying a half-way position ($0.02 < P < 0.05$). The gradient for women is

ERRATUM

TABLE III - PREVALENCE OF "ARTERIAL DISEASE" IN DIFFERENT AGE
AND BLOOD-SUGAR GROUPS (MEN AND WOMEN COMBINED)

Age (yr.)	Prevalence (%)		
	Control	Borderline	Diabetic
<50	17	23	41
50-59	20	29	37
60-69	33	40	39
≥70	45	56	76
Total	27	36	49

TABLE III—PREVALENCE OF "ARTERIAL DISEASE" IN DIFFERENT AGE AND BLOOD-SUGAR GROUPS (MEN AND WOMEN COMBINED)

Age (yr.)	Prevalence (%)		
	Control	Borderline	Diabetic
<50	17	29	41
50-59	24	40	37
60-69	49	68	39
≥70	45	56	76
Total	27	36	49

TABLE IV—RELATION BETWEEN ARTERIAL DISEASE AND BLOOD-SUGAR WITHIN DIFFERENT BLOOD-PRESSURE GROUPS (MEN AND WOMEN COMBINED)

Diastolic blood-pressure (mm. Hg)	Blood-sugar group					
	Control		Borderline		Diabetic	
	No.	No. with arterial disease	No.	No. with arterial disease	No.	No. with arterial disease
<90	95	18 (19%)*	84	19 (23%)*	41	18 (44%)*
90-109	76	23 (31%)*†	88	40 (45%)*†	48	20 (42%)*†
≥110	15	9 (60%)*	36	17 (47%)*	15	13 (87%)*
Not recorded	1	..	2	..	0	..

* Trend significant at 2% level.

† Trend significant at 1% level.

statistically more significant than that for men because of the larger numbers.

Results so far have summarised the findings at all ages. Table III presents the age-specific prevalence-ratios for arterial disease (defined as in table II). The numbers are small, especially in the diabetic group; but in general the association holds for both younger and older people.

The manifestations of arterial disease that have been described might be related to B.P. either (in the case of s-r and r items) directly, or (as for symptoms) because hypertension is associated with an increased risk of atherosclerotic disease (Kannel et al. 1961). Since there may be some correlation between blood-sugar and B.P., it is important to know to what extent the association between arterial disease and blood-sugar is independent of B.P. This is examined in table IV, which shows that within the group with normal B.P. there is a clear gradient of prevalence of arterial disease on blood-sugar ($0.02 < p < 0.05$). At higher levels of B.P. the association is less evident.

TABLE V—COMPARISON OF PREVALENCE FOR ARTERIAL DISEASE BETWEEN MEN AND WOMEN

Signs or symptoms	Sex	Mean age-specific prevalence (%)		
		Control	Borderline	Diabetic
<i>Arterial symptoms</i>	M	7.7	6.4	10.2
	F	7.8	11.6	23.4
<i>Electrocardiographic changes:</i>				
Q/QS waves	M	6.2	4.5	4.3
	F	1.7	1.0	2.2
S-T depression	M	6.8	14.5	10.8
	F	21.2	26.8	27.0
T-wave flattening or inversion	M	6.9	16.2	17.5
	F	12.7	21.2	26.0

Since chest leads were not available for women, male rates have been adjusted appropriately.

It is generally supposed that all forms of ischaemic heart-disease are commoner in men than in women and it was very surprising to find that this was true only for Q-wave evidence of infarction and not for arterial symptoms, S-T depressions, or T-wave changes (table V). For all these changes the prevalence, taken as the means of the individual age-specific rates, is consistently higher for women than for men; and sometimes the differences are large. In calculating the prevalence of changes in E.C.G. for men in table V a deduction has been made to allow for the proportion in whom the change in question appeared only in the chest leads; this was necessary because chest leads were not recorded in the women.

Discussion

All samples were taken from those members of the total adult population of Bedford who provided urine specimens; it is this group that represents the effective population for the study. There is no reason to suppose that the relation between arterial disease and blood-sugar would have been different among those who failed to provide a specimen, although the actual frequencies of abnormality may have been different.

The control group was drawn entirely from the random sample, while the borderline and diabetic groups were recruited in the main from glycosurics detected in the original study, with only a small contribution from the random sample (see accompanying figure). The relationship between glycosuria and blood-sugar in the Bedford survey had been described by Sharp, Butterfield, and Keen (1964); it suggests that in the present study glycosurics are over-represented in the borderline and

diabetic groups—i.e., these two groups contain an excess of persons with lower renal thresholds for sugar. There is, however, no reason to suppose that people with lower thresholds are more likely to have arterial disease; the reverse is more probable.

The cardiovascular examination was performed at the second follow-up attendance. Losses at these two attendances resulted in response-rates of 69% in the control group, 80% in the borderline group, and 76% in the diabetic group. The losses may have been biased with regard to the frequency of arterial disease, but the main purpose of the study would be frustrated only if losses occurred more often among persons with arterial disease and low blood-sugar than among persons with arterial disease and high blood-sugar. This seems very unlikely to have been the case, especially since the participants were not then aware that arterial disease was to be studied.

As a partial check on this inference we used the results of a limited cardiovascular examination (B.P. and unstandardised history) that was done at each examination. There was no indication that those participants who later defaulted were a notably biased group with regard to cardiovascular disease.

We conclude that the association between these various manifestations of arterial disease and the level of glucose tolerance is real, and that this conclusion holds even at those levels of blood-sugar at which a diagnosis of diabetes is considered doubtful. Both sexes, and probably all age-groups, are involved. Only part of the effect can be explained by differing levels of B.P. A similar conclusion was reached by Epstein et al. (1965) and Ostrander et al. (1965) from their study of the population of Tecumseh, Michigan; in their results an effect of hyperglycemia on arterial disease was found to be independent of both B.P. and serum-cholesterol. Participants in their survey received a glucose load without previous fasting—it is interesting that this does not seem to have interfered with the general pattern of the findings.

Bolt These studies in Tecumseh dealt with prevalence of disease, not its incidence, and therefore they present an incomplete and potentially misleading picture of the occurrence of disease. Prevalence depends upon the balance between the rate of occurrence of new cases (incidence) and the rate of their removal by death or

recovery. Thus high incidence by itself tends to produce high prevalence, but this can be counter-balanced by high death-rate. This may explain our anomalous findings in the comparison between men and women: the higher prevalence among women for symptoms and for ST-T changes may indicate not a higher incidence, but only that fewer of the affected women die of the disease. This possibility is consistent with the lower rate for Q/QS changes in women as compared with men, since this is an index of the frequency of the lethal form of the disease.

There was no indication that people with high blood-sugar had, proportionately, any less frequent Q/QS changes than those with low values. Probably, therefore, the association between blood-sugar and the prevalence of other manifestations of arterial disease does reflect a real increase in incidence. Nevertheless confirmation of this point is required, and evidence may be available in due course from prospective studies.

The design of the study and the limited numbers of participants made it necessary to group results in three blood-sugar categories only. The differences in prevalence of arterial disease seemed to be about as great between the two lower as between the two upper groups. It would be interesting to know whether there is a lower limit of blood-sugar below which further reductions are not associated with further falls in arterial-disease-rates. Alternatively there may be a continuous gradation of risk over the whole range of blood-sugars, analogous to that observed for B.P. and arterial disease. Such a study would require a large number of people with two-hour blood-sugars below 200 mg. per 100 ml.

To obtain a perspective on these problems one would like to know how big a contribution hyperglycæmia makes to arterial disease. From the knowledge of the prevalence of arterial disease at different blood-sugars, and of the prevalence of these levels in the whole Bedford population, we estimate that the number of persons affected by arterial disease (as defined earlier) would be reduced by approximately 10% if everyone experienced the rates which were observed in the control group. To put it differently, there may be (very approximately) 1000 adults in Bedford with arterial disease which might not have occurred if they had not had two-hour blood-sugar levels above 120 mg. per

100 ml.; about a half of this group will be over the age of 60.

This question requires an experimental answer, and it is hoped that in time this may emerge from the controlled trial of tolbutamide and diet which is now being conducted within the borderline group. The incidence for new manifestations of arterial disease in the ensuing years will be compared in the treated and control groups. Meanwhile judgment must be suspended as to whether or not a person with a minor degree of symptomless hyperglycæmia ought to have the level of blood-sugar reduced.

Summary

As a sequel to the Bedford diabetes survey a standardised cardiovascular examination has been done on three samples from the general population. The samples were defined by the levels of blood-sugar two hours after 50 g. of glucose by mouth; persons with levels of 200 mg. per 100 ml. or over formed a "diabetic" group, those with levels of 120 to 199 mg. per 100 ml. formed a "borderline" group, and a matched random sample of those under 120 mg. per 100 ml. formed a "control" group.

The age-adjusted prevalence of both symptoms and electrocardiographic changes of arterial disease was lowest in the control group, intermediate in the borderline group, and highest in the diabetics. It appears that symptomless impairment of glucose tolerance may be one of the important accompaniments of atherosclerotic disease in the general population, although confirmation of its causal role must await further evidence.

We are grateful for the mixture of criticism and support from Prof. W. J. H. Butterfield, for the generous provision of services by Dr. C. L. Sharp (medical officer of health) and the Bedford Borough Council, and for help in the collection and analysis of information by Miss V. Horrocks, S.R.N., Miss L. Colwell, Mrs. B. Hunt, Mrs. E. Killick (London School of Hygiene and Tropical Medicine), and Mrs. C. Boyns (Guy's Hospital).

The Bedford follow-up study is supported by a research grant from the British Diabetic Association and by a development grant from the Ministry of Health. Dr. S. Mystry participated in the study during tenure of a British Council research fellowship.

REFERENCES

- Armitage, P. (1955) *Biometrics*, **11**, 375.
 Blackburn, H., Keys, A., Simonson, E., Rautaharju, P., Punsar, S. (1960) *Circulation*, **21**, 1160.
 Bryfogle, J. W., Bradley, R. F. (1957) *Diabetes*, **6**, 159.
 College of General Practitioners (1963) *Br. med. J.* **ii**, 655.
 Epstein, F. H., Ostrander, L. D., Johnson, B. C., Payne, M. W., Hayner, N. S., Keller, J. B., Francis, T. (1965) *Ann. intern. Med.* **62**, 1170.
 Kannel, W. B., Dawber, T. R., Kagan, A., Revotskie, N., Stokes, J. (1961) *ibid.* **55**, 33.
 Liebow, I. M., Hellerstein, H. K., Miller, M. (1955) *Am. J. Med.* **18**, 438.
 Marble, A. (1955) *Diabetes*, **4**, 290.
 Ostrander, L. D., Francis, T., Hayner, N. S., Kjelsberg, M. O., Epstein, F. H. (1965) *Ann. intern. Med.* **62**, 1188.
 — Weinstein, B. J. (1964) *Circulation*, **30**, 67.
 Rose, G. A. (1962) *Bull. Wld Hlth Org.* **27**, 645.
 — (1965) *Milbank meml Fund q. Bull.* **43**, 32.
 Sharp, C. L., Butterfield, W. J. H., Keen, H. (1964) *Proc. R. Soc. Med.* **57**, 193.
 United States Department of Health, Education, and Welfare (1964) Glucose Tolerance of Adults. United States 1960-1962. National Center for Health Statistics, series 11, no. 2. Washington.

INSERT 8

THE EFFECT OF TREATMENT OF MODERATE HYPERGLYCAEMIA
ON THE
INCIDENCE OF ARTERIAL DISEASE

by

H. Keen, M.B., B.S., M.R.C.P.

R. J. Jarrett, M.A., M.D.

C. Chlouverakis, M.D.

D. R. Boyns, M.B., B.Sc., M.R.C.P.

Postgraduate Medical Journal 44, 960-965, 1968.

The effect of treatment of moderate hyperglycaemia on the incidence of arterial disease

H. KEEN M.B., M.R.C.P.

C. CHLOUVERAKIS M.D.

R. J. JARRETT M.A., M.D.

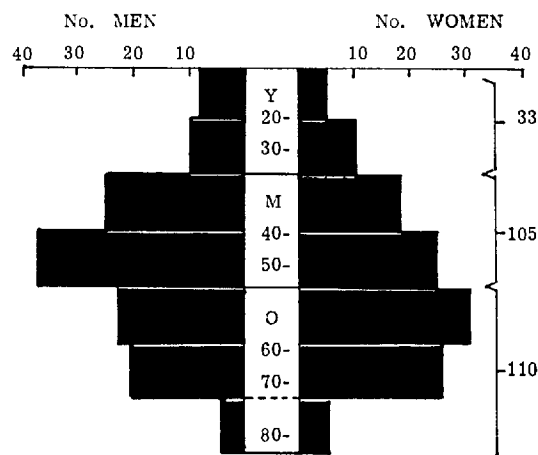
D. R. BOYNS M.B., M.R.C.P.

From the Department of Medicine, Guy's Hospital, London S.E.1

HYPERGLYCAEMIA is perhaps the newest addition to the list of 'risk factors' associated with major vascular disease even though, under its more familiar name of diabetes mellitus, it has long been supposed to enjoy a special clinical relationship with atherosclerosis (Joslin, 1927). There are two reasons for this minor paradox. First, the apparent clinical relationship between diabetes and atherosclerosis is still challenged in a way in which, for example, the relation between hypertension and arterial disease is not. Second, the new information about variation in glucose tolerance coming from population studies (Keen *et al.* 1965; Ostrander *et al.* 1965) is modifying our view of the nature of diabetes in man and putting the question of raised blood sugar and its associations in a rather different way.

A reasonably good case has been made over the last few years, however, for a statistically valid link between higher levels of blood sugar and clinically evident atherosclerosis (Keen *et al.* 1965; Ostrander *et al.* 1965; Epstein, 1967; Kingsbury, 1966). It is, of course, possible to formulate three standard hypotheses to explain the relationship—that A causes B, that B causes A or that both A and B are caused by C. We have chosen to examine what is perhaps the most likely and potentially the most useful of these explanations—that hyperglycaemia contributes causally to the development of the arterial lesions. It is a *useful* explanation because there is long experience and knowledge of methods aimed at lowering the blood sugar; the possibility of intervening in the progress of a disease process is one which stimulates both the interest of the doctor and the co-operation of the patient.

The Bedford diagnostic survey for diabetes of 1962 (Sharp *et al.* 1964) was carried out by the Department of Medicine, Guy's Hospital in association with the Bedford Public Health Department. It brought to light a large number of people with varying degrees of hyperglycaemia, when the capillary blood sugar was measured two hours after a 50 g-oral glucose load (Sharp *et al.* 1964). The frequency distribution of such 2-hour blood sugar values in a stratified random population sample, taken from the townfolk, showed no separation into normal and abnormal responses, so the tested population was arbitrarily divided into three



AGE DISTRIBUTION OF BORDERLINE GROUP BY AGE AND SEX

FIG. 1. Frequency distribution histogram showing numbers by sex and age in 248 recruits to the Borderline Group.

Y = 'young'
M = 'middle aged'
O = 'old'

groups on the basis of the blood sugar. Those below 120 mg./100 ml were classed as 'normal'; those above 200 mg./100 ml were called 'diabetics' and referred to the family doctor and the local diabetic clinic. It is with the intermediate group of 248 'moderate hyperglycaemics', or borderline diabetics, as we have chosen to call them, that this paper concerns itself.

Materials and Methods

When the Borderline Group was compared with an age/sex matched normoglycaemic control group on the one hand and with the newly found 'diabetics' on the other, the borderline diabetics lay between the other groups in the prevalence of several pathological changes. In particular, they showed an enhanced liability to arterial disease, assessed on the basis of a clinical history of angina pectoris, myocardial infarction and claudication and of pathological changes in the ECG (Keen *et al.*, 1965). We therefore felt justified in conducting a controlled clinical trial of treatment in these 'borderline' cases to answer the question "will treatment aimed at

Treatment of moderate hyperglycaemia

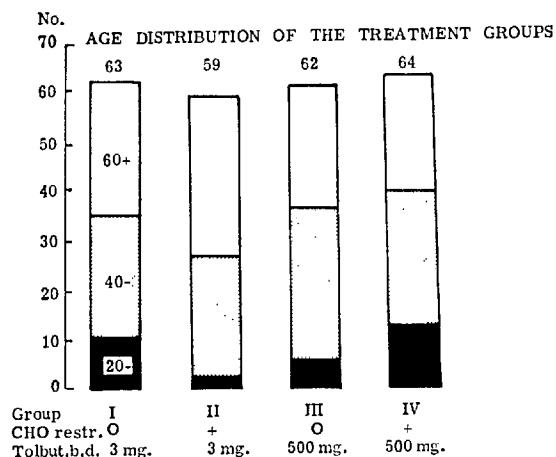


FIG. 2. Age distribution of the 4 treatment groups following random allocation of Borderline subjects. Sex representation approximately equal in age groups. For treatment abbreviations see text.

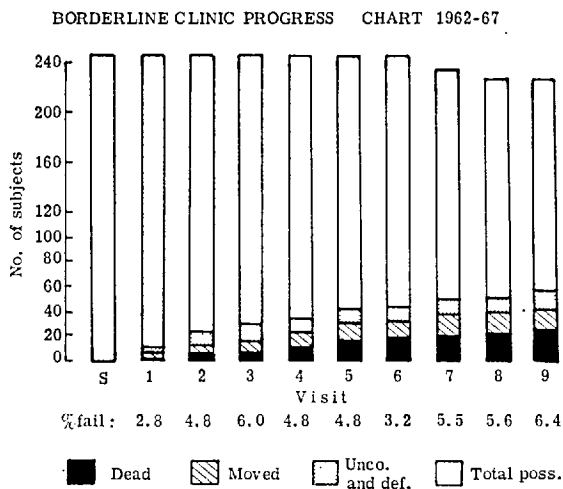


FIG. 4. Chart of attendance and default at successive Borderline Clinic visits. Reduced numbers at visits 7-9 represent a group entering the trial during the two years following the Survey and who have not yet reached these visit cycles.

Unco. = uncooperative.
 def. = default due to illness, etc.
 S = Survey visit.
 % fail = proportion of 'possibles' failing to attend.

RELEVANT SECTION OF BORDERLINE EXAM. PROCEDURES

	Q'aire	Trt. enq .	Weight	B. P.	Pulses	ECG	2-hr. B. S.
SURVEY	+		+	+	+		+
VISIT 1				+			+
2	+	+	+	+		+	+
3		+	+	+			+
4		+	+	+			FBS
5	+	+	+	+	+		+
6	+	+	+	+			+
7		+	+	+			+
8	+	+	+	+	+	+	+
9		+	+	+			+
10	+	+	+	+	+		+

FIG. 3. Relevant procedures carried out at Survey and successive visits to the Borderline Clinic.

Q'aire = questionnaire for arterial disease symptoms.
 Trt. enq = enquiry concerning adequacy of tablet taking or dietary restriction.

B.P. = blood pressure measurement.

Pulses = palpation of dorsalis pedis, posterior tibial and carotid pulses.

F.B.S. = fasting blood sugar (at 4th visit).

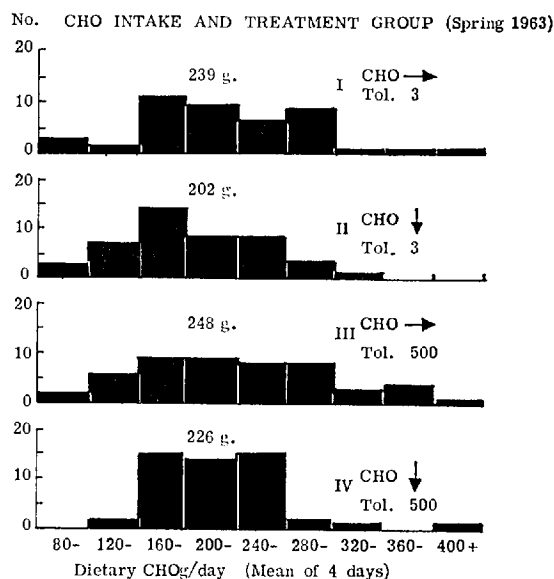


FIG. 5. Frequency distribution histogram (with means) of daily dietary carbohydrate intake in grams, estimated from four dietary questionnaires, completed by Borderline subjects.

CHO ↑ = recommended 120 g. carbohydrate diet.

CHO → = sugar limitation.

Tol. 3 = tolbutamide 3 mg. twice daily.

Tol. 500 = tolbutamide 500 mg. twice daily.

lowering the blood sugar affect the natural course of the condition?" An essential requirement of this project was to get the agreement of those who were going to take part in it, and this was achieved by interviewing the 'borderline' subjects in small groups, putting the case to them and then meeting each one privately to answer specific questions. 98% of those approached agreed to participate in the study and they were randomly allocated to one of four treatment groups. Fig. 1 shows the age and sex distribution of the 248 members of the Borderline Group; Fig. 2 shows the age distribution following random allocation to the four treatment groups.

Half the total were given tolbutamide tablets, 500 mg twice daily; the other half were given identical tablets containing 3 mg. tolbutamide to take twice daily. Half of each of these two groups were instructed by us in a dietary scheme which allowed them 120 g. of carbohydrate daily. The others were asked to reduce their intake of table sugar and sweets, but to make no other alterations in their diet.

The 'borderline' subjects have been seen at regular six-monthly visits, since 1962, at our special clinic in Bedford. At each visit, a number of standard procedures are carried out and those relevant to this communication are listed in Fig. 3. Although the procedures varied from visit to visit, measurement of blood sugar two hours after 50 g. glucose was made at every visit but one (when the fasting blood sugar was measured). Measurements were always made between 4 and 6.30 p.m.; the subjects consumed 50 g. glucose 1 hr. 50 mins. before arrival at the clinic, having taken nothing since breakfast time on

the day of the visit. Tablets were stopped for the ten days prior to the visit in all cases but one, when they were continued up to and including the day of the visit.

A 'life-table' for the group as a whole over the five years is shown in Fig. 4. Deaths and moves away from the district account for the major part of the losses. Excluding these, 94% of the possibles in the starting population continue to attend.

The 'success' of our dietary recommendations is shown in Fig. 5, a frequency distribution histogram of the mean daily carbohydrate intake of members of the four treatment groups. This is based upon four complete days' food intake assessed by questionnaire between six and nine months after the trial began. Differences between restricted and non restricted groups are disappointingly small.

Tablet-taking has been assessed in two ways, first by direct questioning at each visit and secondly by monitoring the rate of issue of bottles of tablets—500 in each—from the central store in Bedford. About half qualify as 'good' tablet takers—that is to say they miss less than 50 tablets in each six-month period.

2-HR. B.S. BEFORE AND AFTER 'TREATMENT'

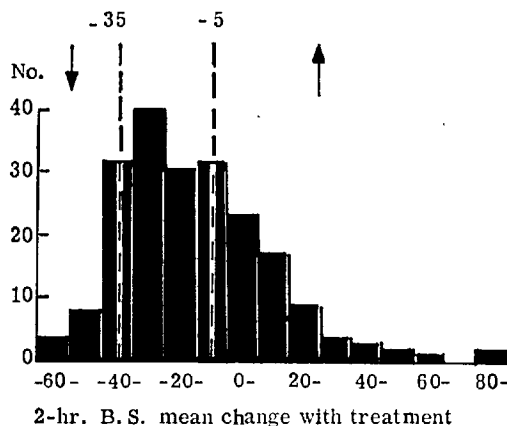


Fig. 6. This frequency distribution histogram shows the difference in values between the mean pre-treatment blood sugar (calculated from the Survey and first clinic visit) and the mean post-treatment blood sugar (calculated from all two-hour blood sugars on subsequent clinic visits) for each individual. All Borderline subjects, regardless of treatment group, are plotted here. The distribution has been divided into three parts. A blood sugar difference of -35 mg./100 ml. or more is classified as a 'relative fall'; a difference of -5 or less (i.e. a rise) is classified as a 'relative rise'. Intermediate differences are classified as 'modal changes'.

Results

Figure 6 is a histogram showing the distribution of the differences in mean blood sugar between the pre-treatment and the treatment periods for each subject. The figure suggests a mean blood sugar fall of about 20 mg./100 ml, but conceals a number of

Treatment of moderate hyperglycaemia

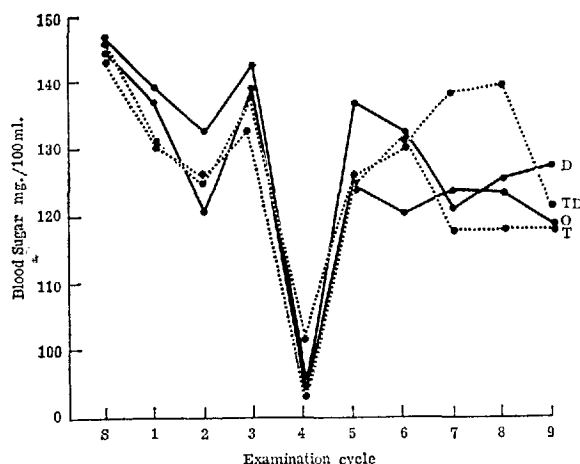
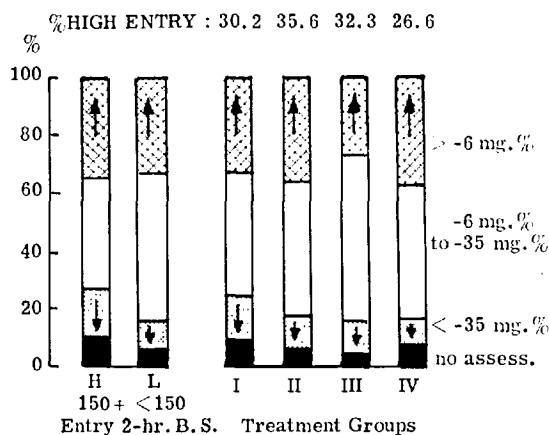


FIG. 7. The mean blood sugar for each of the treatment groups on each clinic visit. N.B. 4th visit fasting blood sugar; 9th visit tablets continued up to and including day of visit.
O = 'No treatment'.
T = therapeutic tolbutamide.
D = restricted carbohydrate intake (diet).

facts. First, as Fig. 7 shows, there was a large mean blood-sugar fall between the values recorded in the survey and the values measured at the first visit, before any treatment had been started! This apparent spontaneous 'improvement' is, we think, a statistical effect which we have called the 'fallacy of the missing middle'. It has wide general applications. Briefly, if a test group is recruited from people exceeding an arbitrary, fixed, 'cut-off' level when measured on a given occasion, then, if there is any spontaneous variation in that factor, on the next occasion upon which it is measured some of the higher observations may be expected to have fallen back towards the modal behaviour of the whole population. These falls will not be balanced by those, who in a complete population, would have risen from the lower values to 'take their place'. The overall effect will be a fall in the mean value for the test population. How readily might this fall be attributed to treatment!

The second observation from Fig. 7 shows that there is no systematic difference in the mean two hour blood-sugar behaviour among the four treatment groups. This is further emphasized by Fig. 8, which shows the relative proportions, within treatment groups, of 'relative rises', 'relative falls', and 'modal changes' in the blood sugar. This figure also shows that, as one might expect, those who came into the group with the higher initial screening levels showed rather more 'relative falls'.

Figure 9 tabulates the frequency of the various vascular events occurring during the five-year follow up period considered in this paper and their relative



B.S. RESPONSE OF TREATMENT GROUPS AND INFLUENCE OF 'ENTRY 2-HR. B.S.'

FIG. 8. Blood sugar response to treatment in each treatment group.

↓ = proportion showing relative fall.
↑ = proportion showing relative rise.
White area = proportion showing modal change.
The first two columns show these changes, respectively, in individuals in the whole follow-up group with entry mean blood sugars of 150 mg./100 ml. or more (H) or less than 150 mg./100 ml. (L). In a small proportion (black areas) no assessment was possible.

VASCULAR EVENTS

Worsened E. C. G. (0 → 1, 0 → 2, 1 → 2) *	18
Angina (1 episode allowed)	Poss. 13
	Prob. * 23
Infarct	Poss. 1
	Prob. * 16
Cerebro-vascular incident	Poss. 10
	Prob. * 4
Claudication	Poss. 15
	Prob. * 7
'Coronary' death	* 13
Cerebral arterial death	* 1
Pulse loss event (4 at onset, less x 2)	39

FIG. 9. Number of vascular incidents occurring during course of follow up of the Borderline Group.

Poss. = less certain events.
Prob. = more certain events.
Asterisked items classified as + in further analysis.
Other items classified as ? (query.)

number. Some of these events represent an arterial occlusion with greater certainty than others and these have been marked with an asterisk. In Fig. 10, the relative frequency of people experiencing 'certain' events (+) and 'questionable' events (?) is shown for each of the four treatment groups. On the left, the groups as a whole are shown, and on the right, those members of the groups who were

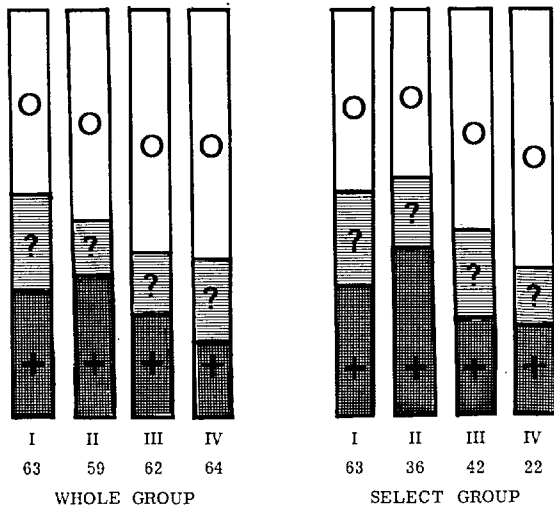


FIG. 10. Proportion of individuals in each treatment group experiencing more certain events (+), less certain events (?) and no events (O) during follow-up period. Left group of 4 columns represents all individuals. Right group of 4 columns represents those individuals adjudged to be adhering to recommended therapy.

classified as following their treatment reasonably closely. Satisfactory individuals in Group 4 are fewest in number since they had to satisfy both dietary and tablet criteria.

Inspection suggests that both groups 3 and 4 (who have in common that they are both taking tolbutamide 500 mg. b.d.) have fewer affected individuals than the other two groups. The frequency of 'certain' events in Group 4 is significantly less

than in Group 2 ($P < 0.05$) but just fails to achieve this significance level when compared with Groups 1 and 3. When Groups 3 and 4 (both tolbutamide-treated) are pooled, the frequency of 'certain' events in them is highly significantly less than in pooled Groups 1 and 2 (both placebo treated).

Another approach to the comparison is shown in Fig 11. Here, instead of the number of individuals, the frequency of events in each group is examined. This analysis therefore takes into consideration the fact that some individuals experienced more than one event, that there was some variation in the number of visits and that some subjects disappeared from the groups because of death, moves etc. The figure thus shows the frequency of events per six-month cycle computed from the total treatment time accumulated by each group. For all four groups, this exceeded 1000 'subject-years'. Once again group 4 fares better than the other three groups, with about two-thirds the event rate of the total. The table also indicates an absence of effect of blood sugar response on the event rate.

Discussion

Can the apparent advantage enjoyed by group 4 be explained by a different blood pressure composition? In Fig. 12, subjects were classified into three categories, low, middle and high, on the basis of their age and systolic blood pressures measured at the first visit. Treatment group 4 has, if anything, less than its share of low pressures and more of the middle and high pressures, a finding which adds to the significance of its apparently favoured position.

How is this apparently protective influence of tolbutamide to be explained, particularly in view

ANALYSIS OF ALL VASCULAR EVENTS BY TREATMENT GROUP AND BLOOD SUGAR RESPONSE (EVENTS/CYCLE)

B. S. Response	I	II	III	IV	All	Evs.	Subs.
Fall	.108	.066	.113	.050	.085	(22)	29
No change	.071	.088	.050	.049	.064	(69)	117
Rise	.076	.080	.100	.067	.080	(57)	83
No assess.	.081	.500	-	-	.114	(2)	19
Events/cycle	.07867	.08411	.07025	.05615	.07225	(150)	248
Aff. subs./cycle	.0559	.0590	.0488	.0482			

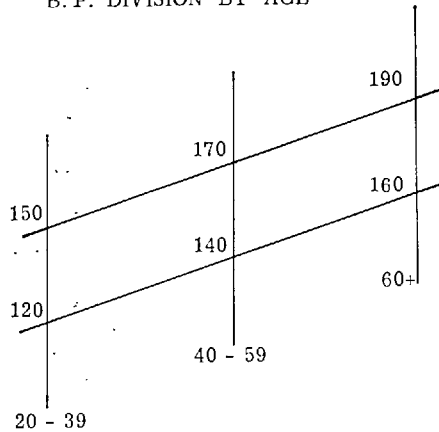
FIG. 11. Table showing calculated frequency of vascular events per six-month cycle in relation to the assessment of blood sugar change. Frequencies are shown for the individual treatment groups and for all subjects.

Evs. = number of events.

Subs. = number of affected subjects.

Treatment of moderate hyperglycaemia

B. P. DIVISION BY AGE



% DISTRIBUTION BY TREATMENT GROUP

	I		II		III		IV	
	m	f	m	f	m	f	m	f
'High'	14	30	32	54	12	33	26	41
'Middle'	33	41	40	29	31	28	37	41
'Low'	43	29	29	18	57	39	37	17

FIG. 12. The proportion of 'high', 'middle' and 'low' systolic blood pressure levels in each of the 4 treatment groups by sex. Classification of pressures (level of systolic at first follow-up visit) based on age of subjects as indicated in Fig. 1 above.

of our failure to demonstrate an effect on the blood sugar level? Perhaps it operates through alternative actions of tolbutamide upon fibrinolysis, lipid metabolism and platelet adhesiveness. It is possible that, like phenformin, tolbutamide may effect a 'redistribution' of glucose metabolism, without a net fall in the blood sugar; perhaps lower blood sugars ordinarily prevailed in the treated groups, but these were not reflected in our 'two-hour' post-glucose measurements.

Despite the suggestive nature of these findings we are aware of the dangers inherent in presenting

the results of a long-term study at a selected point in time. Relatively few events are necessary to wipe out the advantage of one group over another. In order to accept an effect with confidence, it is necessary to demonstrate a reasonably consistent trend with time and so exclude the possibility that a peculiarly favourable time point was selected for analysis. A more detailed analysis of the first 66 months of the study is in preparation and will be presented elsewhere; these results are therefore only to be regarded as an interim report on the Bedford Study, which continues.

Acknowledgments

The authors would like to take this opportunity of expressing their indebtedness to the very many people who have participated in this study. In particular, we would mention the late Dr. C. L. Sharp, Medical Officer of Health at Bedford, whose enterprise initiated the Survey and supported the Follow-Up. The Health Department and the Borough Council continue to provide generous facilities and assistance and financial support has been forthcoming from the Ministry of Health and the British Diabetic Association. We are grateful to Hoechst Pharmaceuticals for supplies of tolbutamide. The continuing study owes most to the co-operation and friendliness of the Bedford population in general and the 'Borderline' diabetics in particular.

References

- EPSTEIN, F. H. (1967). Hyperglycaemia. A risk factor in coronary heart disease. *Circulation* 36, 609.
- JOSLIN, E. P. (1927). Arteriosclerosis and diabetes. *Ann. clin. Med.* 5, 1061.
- KEEN, H., ROSE, G., PYKE, D. A., BOYNS, D., CHLOUVERAKIS, C., & MISTRY, S. (1965). Blood sugar and arterial disease. *Lancet* 2, 505.
- KINGSBURY, K. J. (1966). Relationship between glucose tolerance and atherosclerotic vascular disease. *Lancet* ii, 1374.
- OSTRANDER, L. D. JR., FRANCIS, T. JR., HAYNER, N. S., KJELSBURG, M. O., & EPSTEIN, F. H. (1965). Relationship of cardiovascular disease to hyperglycaemia. *Ann. intern. Med.* 62, 1188.
- SHARP, C. L., BUTTERFIELD, W. J. H., & KEEN, H. (1964). Diabetes Survey in Bedford, 1962. *Proc. roy. Soc. Med.* 57, 193.

INSERT 9

THE EFFECT OF CARBOHYDRATE TOLERANCE
ON PLASMA LIPIDS AND ATHEROSCLEROSIS
IN MAN

by

H. Keen, M.B., B.S., F.R.C.P.

and

R. J. Jarrett, M.A., M.D.

Atherosclerosis: Proceedings of the Second International
Symposium. Editor R. J. Jones. Springer-Verlag,
Berlin, Heidelberg, New York, pp. 435-444, 1970.

Reprint from

Atherosclerosis:
Proceedings of the Second International Symposium

Edited by
Richard J. Jones

(Springer-Verlag New York · Heidelberg · Berlin 1970.
Printed in Germany—Not for sale)

**THE EFFECT OF CARBOHYDRATE TOLERANCE
ON PLASMA LIPIDS AND ATHEROSCLEROSIS
IN MAN**

H. KEEN and R. J. JARRETT

References to the literature appearing in the text are available in a comprehensive bibliography which may be obtained at cost from the Chicago Heart Association, 22 West Madison Street, Chicago, Illinois 60603, U.S.A.

THE EFFECT OF CARBOHYDRATE TOLERANCE ON PLASMA LIPIDS AND ATHEROSCLEROSIS IN MAN*

H. KEEN and R. J. JARRETT

Although the clinical association of diabetes mellitus and arterial disease has been accepted for generations, it is only recently that a more general relationship

* The borderline diabetes follow-up study, originally supported by grants from the Research Fund of the British Diabetic Association, is now financed by the Department of Health and Social Security, London. Our many co-workers, noted in the bibliography, are thanked for their contribution of time and effort, as are our secretarial and technical assistants. We especially acknowledge the enduring support and interest of Prof. W. J. H. Butterfield and the subjects themselves.

between hyperglycemia and atherosclerosis has attracted attention. This development is largely due to a change in our concept of diabetes itself which, in the case of the non-insulin-requiring variety at any rate, is now increasingly regarded as the extreme of a spectrum of degrees of glucose intolerance, the resultant of many influences, and differing quantitatively rather than qualitatively from "normal" carbohydrate metabolism [785]. The analogy with high blood pressure is attractive and obvious.

The aim of this communication will be to review briefly the basis and strength of the association between raised blood sugar and atherosclerosis; to consider some of the possible mechanisms involved; and to report on the incidence of vascular disease in a seven-year follow-up of a group of moderately hyperglycemic people.

Though time-honored, the assumption of increased atherosclerosis in the clinical diabetic does not go unchallenged, as the following three statements of authoritative workers prove. "The mass of evidence demonstrating a markedly increased incidence and severity of atherosclerosis in diabetes is incontrovertible" [781]. "... the generally accepted association between diabetes mellitus and enhanced human 'atherogenesis' does not withstand critical examination" [1010]. "The relationship between diabetes and disease of arteries is confused. The subject is surrounded by conjecture and assumption. The facts are few" [1205]. Although bias, selection and artifact may confound interpretation of the seemingly clear association of the two diseases, it is difficult entirely to reject the great weight of evidence that coronary and peripheral artery disease, measured either by clinical manifestation or by post-mortem appearances, show a two to threefold excess in the clinical diabetic over the nondiabetic [236, 909]. The Framingham study [771] indicates a similar influence of diabetes on incidence. On one count there is no controversy—that there is a greatly enhanced risk of arterial disease in the diabetic woman compared with her nondiabetic counterpart [1205].

Approaching the association from the other limb, studies of both oral and intravenous glucose tolerance show that subjects with clinical atherosclerosis have hyperglycemic responses far more frequently than matched normals. This is most clearly shown by the studies of Wahlberg [1512] but many similar observations, well summarized by Epstein [436], further strengthen the association.

A special kind of support comes from epidemiological surveys which set some index of glucose tolerance against the manifestations of atherosclerosis, both assessed by the application of standard objective procedures in population samples. In comparatively few studies has the blood sugar/arterial disease relationship been given special attention. In the four of epidemiological proportions, the Tecumseh community study [1131], a similar survey in Busselton, Australia [1533], a survey restricted to male workers in Paris [520], and in our own Bedford Survey [787], the conclusions are essentially similar. After excluding known diabetics, about two to three times as much arterial disease is found among people in the top 10% to 20% of the total range of blood sugar values than in the rest of the population. This association is largely independent of age, of arterial blood pressure, of blood cholesterol level and of cigarette smoking. In common with other statistical "risk factors", the link between atherosclerosis and high blood sugar is least apparent in the oldest people.

Following our Bedford Survey in 1962 [1349], we looked for evidence of arterial disease in three groups of the townsfolk, characterized only by their levels of blood sugar measured two hours after 50 g oral glucose. The 124 people with two-hour blood sugars of 200 mg/100 ml or more were designated newly-found "diabetics"; 229 people had blood sugars between 120 and 200 mg/100 ml and these constituted a "borderline diabetic" group. They were matched as closely as possible by age and sex with a control group taken at random from

Table 1. *Prevalence of "arterial disease" by age in three groups of Bedford population characterized by level of blood sugar two hours after 50 g glucose*

Age (yr.)	Prevalence (percentage)		
	control	borderline diabetic	diabetic
< 50	17	23	41
50-59	20	29	37
60-69	33	40	39
70 +	45	56	76
Total	27	36	49

Table 2. *Incidence of individuals with cardiovascular events between 1962 and 1967 in three Bedford groups characterized by their blood sugar level^a*

Diagnostic group	Number of individuals		Coronary death		Angina or infarct		Claudication		Stroke		All events	
	M	F	M	F	M	F	M	F	M	F	M	F
			percentage		percentage		percentage		percentage		percentage	
Controls	63	56	4.8	—	4.8	5.4	1.6	1.8	—	—	11.2	7.2
Borderline diabetics	105	103	9.5	4.9	9.5	15.5	0.9	—	2.9	2.9	22.9	23.3
Diabetics	38	48	13.2	8.3	7.9	27.1	10.5	2.1	—	2.1	31.6	39.6

^a Each individual counts for only one event, coronary disease taking precedence; thus "claudication" and "stroke" refer to that symptom alone.

those people whose two-hour blood sugar values lay below 120 mg/100 ml. Estimates of arterial disease prevalence in these three blood sugar groups were formed by applying the World Health Organization questionnaire and performing, and Minnesota coding, electrocardiograms [1255]. Table 1 shows the relative prevalence by age groups when all these manifestations of arterial disease were counted. The overall gradient was statistically significant although the totals relied heavily on lesser ECG items such as T-wave and ST-segment abnormalities.

Cross-sectional prevalence studies such as this have their limitations in indicating the true force of an association. Those dead and disabled with the disease are not represented. It is the prospective *incidence* study which gives confidence in the reality of the association and here data are very sparse. Epstein [438] reports on the Tecumseh population, restudied after four years. There is a clear link

between prior hyperglycemia and subsequent coronary disease morbidity in women and mortality in men. In the Framingham follow-up [771], diabetes doubled coronary mortality in men and increased it by more than fivefold in women. We are just completing the analysis of a five-year follow-up of our three blood sugar groups in Bedford. Table 2 shows preliminary data indicating the profound effect of raised blood sugar on both morbidity and mortality from arterial disease, the former based upon a repeat questionnaire and the latter upon death information and certification.

A common opening move from an opponent in this discussion is to assert that hyperglycemia is not responsible for the vascular disease, but vascular

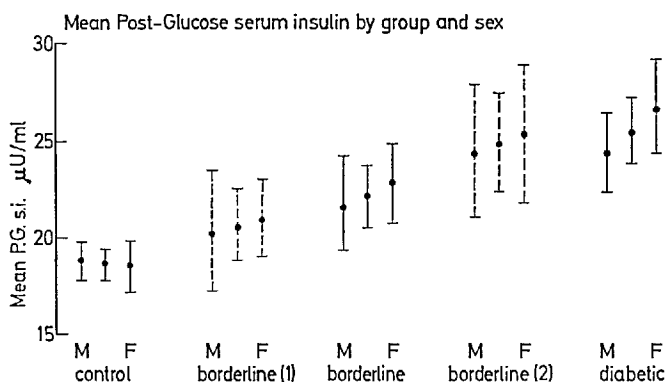


Fig. 1. The level of the serum immuno-reactive insulin (μ V/ml) two hours after 50 g glucose in the three Bedford blood sugar groups (see text). In the Borderline Diabetic group, the results are shown for the whole borderline group (two hour blood sugar 120–199 mg/100 ml; see text), and for the subdivisions 120–149 mg/100 ml (borderline 1) and 150–199 mg/100 ml (borderline 2). In each set of three points, these represent the mean for Males (M), all of the group and Females (F) in the group, with the bar indicating one standard deviation

disease for the hyperglycemia [901]. Against this reversed view, there is autopsy evidence that women with diabetes (but without cardiac infarction) have no more aortic atherosclerosis than an unselected group; furthermore, it is possible to demonstrate severe visceral atherosclerosis without evidence of sustained hyperglycemia [1010]. Also, diabetes in the young is not associated with atherosclerosis at onset, but appears to provide conditions for its accelerated development. However, it is possible that general arterial disease may have extra-pancreatic effects and restrict the tissue access of circulating glucose and insulin, an effect overcome, perhaps, by a compensatory increase in insulin production. The blood sugar may then be lowered only by consequent insulin overaction in the more accessible regions [305].

In fact, hyperinsulinemia is a commonly reported accompaniment of atherosclerosis [312, 1100, 1167, 1486, 1532]. The raised hormone concentration seems to occur independently of obesity which is also very closely associated with hyperinsulinemia [81, 779, 1165], but which, it must not be forgotten, may itself also be associated with atherosclerosis [771]. Hyperinsulinemia holds a

central role in many of the metabolic hypotheses of atherogenesis. It was operating in our three Bedford blood sugar groups in whom, when measured two hours after 50 g oral glucose, insulinemia showed a stepwise increase with rising blood sugar categories (see Fig. 1).

Insulin is no less a lipogenic than a carbohydrate hormone and it may be by way of effects upon lipid metabolism that an atherogenic role is played. Mahler [901] and others have shown *in vitro* that insulin favors triglyceride accumulation in aortic endothelium, probably both by stimulation of synthesis locally, and by inhibition of lipolysis. The role of *in situ* lipogenesis in atheroma induction is contested [12] but not disproved. Perhaps of greater importance is the association between insulin levels and circulating triglyceride concentrations. The studies of Farquhar et al. [449] in people with premature atherosclerotic disease would link this insulin-triglyceride relationship to carbohydrate intake, without regard to the type of carbohydrate ingested, itself the subject of controversy and discussion. They suggest that hyperlipidemia is a consequence of enhanced hepatic triglyceride production [1215], a conclusion that differs from that of Sandhofer et al. [1292] and Boberg et al. [199], who favor the view that hypertriglyceridemia results from diminished fractional removal rather than increased synthesis. It is suggested [1278] that hyperinsulinemia may be a consequence rather than a cause of the hyperlipidemia. Nevertheless, carbohydrate intolerance may relate to hypertriglyceridemia via the degree of hyperinsulinemia it evokes, though Bierman and Porte [160] consider the link to be much less direct, perhaps only operating through the common factor of obesity. By contrast, Ford et al. [478] provide statistical evidence, in a predominantly hypertriglyceridemic group, of an independent effect of blood sugar level on plasma triglycerides.

All of these relationships have been observed in highly selected groups—in people with arterial disease, with hyperlipidemias, with obesity or with diabetes. We examined the interaction of some of these metabolic variables in a normal population sample [7, 235]. The 220 members of the staff of a large British pharmaceutical firm were selected by an age/sex/ponderal-index stratifying, but otherwise random, procedure. Physical measurements were made and oral glucose tolerance tests performed after an overnight fast. Venous sugar and insulin levels were estimated on all blood samples and total glycerides and cholesterol on the fasting sample only. The sugar and insulin responses to the load were expressed as areas under the respective curves and their various interrelations are shown in Fig. 2. The very close link between "sugar area" and fasting glyceride in men is evident. Both also correlate with "insulin area", but only at low levels with the various indicators of obesity. The diminished level of correlation between "sugar area" and fasting glyceride level in women, compared with men, is explained in Table 3, which shows a complete absence of correlation in the premenopausal women, but an association as strong as that for men in the postmenopausal. It is tempting to link this finding with the comparative immunity of the nondiabetic, premenopausal woman to atherosclerotic disease, but at present one can only speculate upon its mechanism and significance.

Further evidence of a metabolic component involving carbohydrate metabolism in arterial disease comes from our long-term intervention study in Bedford. Since 1962, we have carried out a double-blind controlled therapeutic trial on

Insulin area	Log. fat fold	Arm circum.	Ponderal index	Glycerides	Cholesterol	Age	
0.322 ^c 0.315 ^c	0.078 -0.068	0.171 -0.016	-0.310 ^a -0.130	0.734 ^c 0.376 ^c	0.475 ^c 0.184	0.475 ^c 0.226 ^a	Blood sugar area
	0.353 ^c 0.163	0.319 ^b 0.200 ^a	-0.396 ^c -0.283 ^b	0.296 ^b 0.214 ^a	0.255 ^a 0.067	0.099 -0.078	Insulin area
		0.742 ^c 0.752 ^c	-0.603 ^c -0.683 ^c	0.046 0.111	0.160 0.227 ^a	-0.033 0.171	Log. fat fold
			-0.758 ^c -0.709 ^c	0.186 -0.106	0.222 ^a 0.186	0.093 0.339 ^c	Arm circum.
				-0.364 ^c -0.131	-0.341 ^c -0.245 ^a	-0.328 ^c -0.285 ^b	Ponderal index
					0.573 ^c 0.320 ^b	0.418 ^c 0.205 ^a	Glycerides
						0.555 ^c 0.527 ^c	Cholesterol

Fig. 2. Multiple correlation analysis of data derived from examination of a normal population sample. Areas calculated from glucose response curves. Ponderal index = height/ $\sqrt{\text{weight}}$. This figure is taken from the paper by M. E. Abrams, R. J. Jarrett, H. Keen, D. R. Boyns, and J. N. Crossley. ([7] by kind permission of the Editor)

^a $P < 0.05$.

^b $P < 0.01$.

^c $P < 0.001$.

Table 3. *Correlation^a of fasting plasma glycerides and the area under the blood sugar curve (50 g oral glucose tolerance test) in men and women separately*

Age	Men		Women	
	R	n	R	n
≤ 29	0.395	32	0.030	36
30 +	0.726	68	0.515	60
≤ 34	0.403	40	0.087	42
35 +	0.746	60	0.483	54
≤ 39	0.517	49	-0.002	53
40 +	0.750	51	0.697	43
≤ 44	0.465	58	0.088	59
45 +	0.815	42	0.712	37
≤ 49	0.497	65	0.256	67
50 +	0.802	35	0.655	29
All ^b	0.713	100	0.375	96

^a Correlations have been calculated for the groups above and below the ages 30, 35, 40, 45 and 50 years.

^b 24 people omitted from correlation because of incomplete data.

Table 4. *Trial of tolbutamide in "borderline hyperglycemic" group. Characteristics of the population studied*

	Men	Women	Total
Starting No.	129	119	248
Mean age	55.4	58.9	57.1
No. with arterial disease at entry	20	11	31
No. with significant hypertension at entry	15	38	53
No. "attacked" over 7 years	44	37	81
New "events" over 7 years	52	44	96

Table 5. *Age and sex composition of the 248 borderline diabetic subjects enrolled in the therapeutic trial of tolbutamide and diet*

Group No.	1		2		3		4	
TOL	0		0		+		+	
CHO	0		+		0		+	
Age group	M	F	M	F	M	F	M	F
20-39	8	4	2	0	4	2	4	8
40-59	14	9	15	7	13	16	19	10
60+	14	16	14	19	11	16	11	12

Group 1: 3 mg tolbutamide twice daily + sucrose "restraint".

Group 2: 3 mg tolbutamide twice daily + 120 g carbohydrate diet.

Group 3: 0.5 g tolbutamide twice daily + sucrose "restraint".

Group 4: 0.5 g tolbutamide twice daily + 120 g carbohydrate diet.

Table 6. *Trial of tolbutamide in "borderline hyperglycemic" group. Number and type of arterial events occurring in seven years of follow-up*

Event	Men	Women	Total
Cardiovascular death	13	8	21
Cardiac infarction	7	5	12
Onset of angina	10	13	23
E.C.G. worsening (M.C.) ^a	14	6	20
Onset of claudication	5	9	14
Stroke	3	3	6
Events in total group (n = 248)	52	44	96
Events in high-risk group (n = 75)	25	22	47

^a M.C. = worsening by 1 or 2 grades in Minnesota Code classification.

248¹ "borderline hyperglycemic" people found in the population survey. A randomly allocated half has been treated with tolbutamide, 0.5 g twice daily, and the other half with indistinguishable placebo tablets containing 3 mg of tolbutamide. In addition, one-half of each of these two groups was recommended to limit carbohydrate intake to 120 g daily and the other half simply to "cut down on table sugar". At regular six-month re-examinations, all have been subjected to repeated re-evaluation, the cardiovascular component of which includes the

¹ This total includes a further 19 subjects recruited shortly after the main survey.

Table 7. *Percentage of individuals by sex, who experienced an arterial event during the seven years 1962-1969^a*

Group No.		1		2		3		4	
		total	% +	total	% +	total	% +	total	% +
All Entrants	M	36	33.3	33	39.4	28	32.2	34	29.4
	F	29	41.5	25	36.0	33	27.3	30	20.0
	All	65	37.0	58	38.0	61	29.5	64	25.0
High Risk Entrants	M	4	100.0	10	60.0	8	75.0	6	83.3
	F	8	50.0	12	41.6	16	43.7	11	27.2
	All	12	66.6	22	50.0	24	54.2	17	47.0
Low Risk Entrants	M	32	25.0	23	30.4	20	15.0	28	17.8
	F	21	38.1	13	30.8	17	11.7	19	15.8
	All	53	30.2	36	30.6	37	13.5	47	17.0

Statistical treatment of combined placebo and tolbutamide groups

	All entrants		High risk		Low risk	
	total	% +	total	% +	total	% +
Groups 1 + 2 (Placebo)	123	37.4	34	55.9	89	30.3
Groups 3 + 4 (Tolbutamide)	125	27.2	41	51.2	84	15.5
Difference	10.2		4.7		14.8	
SE of diff.	5.91		11.55		6.27	
<i>t</i> =	1.73		0.41		2.36	
<i>p</i> =	<0.1 > 0.05		>0.1		=0.02	

^a Figures show total number in each group followed by percentage experiencing an event, in the whole group and the high- and low-risk entry subgroups (see text) successively. The effect of tolbutamide and its statistical significance is shown for the whole group and the two subgroups.

Table 8. *Trial of tolbutamide in "borderline hyperglycemic" group. Blood sugar measurements on "random" samples taken between 4:00 and 6:00 p.m. and without special preparation of the participants*

	Placebo (groups 1 and 2)	Tolbutamide (groups 3 and 4)
11th visit	diff. 4.56 90.77 mg/100 ml S.E. diff. 2.5	86.21 mg/100 ml " <i>t</i> " = 1.8
13th visit	diff. 6.26 86.09 mg/100 ml S.E. diff. 3.2	79.83 mg/100 ml " <i>t</i> " = 1.9
Both visits	diff. 5.35 88.23 mg/100 ml S.E. diff. 2.0	82.88 mg/100 ml " <i>t</i> " = 2.6 ^a

^a $P < 0.02 > 0.01$.

standard questionnaire and an electrocardiogram. A preliminary analysis of the results of treatment after five years [786] suggested a protective effect of tolbutamide against the mixed bag of cardiovascular incidents occurring since 1962, and this analysis has been brought up to the seven-year point for this communication. The composition and characteristics of the group are shown in Table 4 and Table 5 and the nature of the events in the follow-up period in Table 6. Subjects have been divided into low and high risk groups, the latter coming into the trial either with clear clinical or ECG evidence of cardiovascular disease or with clinically significant hypertension. As Table 7 shows, the frequency of cardio-

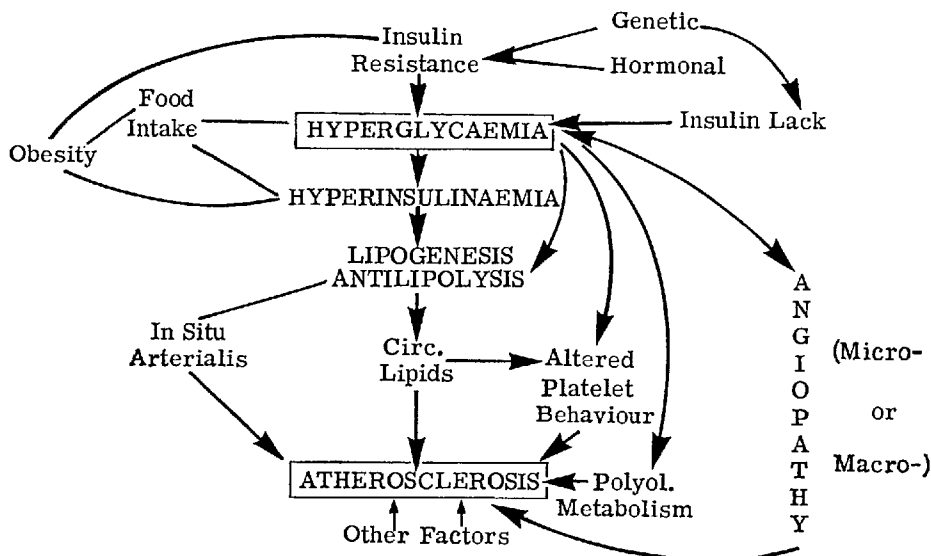


Fig. 3. A hypothetical scheme of metabolic interrelations in atherogenesis

vascular events during the follow-up period is considerably higher in the high, than in the low risk groups, and there is no indication of an effect of treatment. In the low risk individuals, however, the rate of events in the tolbutamide treated group is about half that in the placebo group, a difference significant at the 2% level.

When we tried to explain the protection at five years, we were unable to show a systematic difference between placebo and tolbutamide groups in the blood sugar measured two hours after glucose. However, Table 8 shows the blood sugar comparison when, on two occasions, it was measured without special preparation on a day when the subjects took meals and tablets as usual. On each occasion the mean "random blood sugar" was lower in the treated than the placebo group, the difference not quite attaining conventional levels of statistical significance. If the two sets are pooled, however, the statistical significance of the difference approaches the 1% level.

In conclusion, a statistical link between raised blood sugar levels and both the prevalence and incidence of atherosclerotic arterial disease appears to be established beyond reasonable doubt. The nature of the association is less clear.

The evidence suggests that the blood sugar/arterial disease link may be by way of changes in circulating insulin levels and lipid metabolism in blood and/or tissue, including the arterial wall itself. These associations may encompass and partly explain the dietary and obesity factors in atherogenesis and even provide some explanation for altered platelet behavior in the disease. A direct effect of raised blood sugar may involve localized osmotic damage to the arterial intima due to accumulation of nondiffusible sorbitol (Morrison, this volume). A tentative interlinking scheme is proposed in Fig. 3. Finally, the results of a long-term therapeutic trial are presented suggesting that a significant degree of primary protection against cardiovascular events can be conferred by tolbutamide in mildly and moderately hyperglycemic people.

Further Comments on Cardiovascular Disease

The chief additional commentary appropriate to this section relates to the evaluation of the influence of tolbutamide upon cardiovascular disease in the 'borderline' diabetic group. Few, if any, clinical studies of the use of this sulphonylurea preparation have addressed themselves to its long-term effects on the cardiovascular system though unconfirmed and disputed claims were made of an ameliorating short term effect of the drug on the symptoms of established angina pectoris and intermittent claudication (Fabrykant and Ashe, 1960; Singh et al, 1962; Clarke and Naylor, 1961). Such long-term controlled evaluations as have been made have concentrated upon the influence of tolbutamide on carbohydrate metabolism, as exemplified by glucose tolerance tests, at varying intervals after starting active or placebo treatment (Englehardt and Vecchio, 1965; Belknap et al, 1967; Feldman and Fitter, 1967; Camerini-Davalos, 1967).

Apart from the findings presented in this section, only two other controlled prospective trials have reported results on the long term influence of tolbutamide on cardiovascular disease. The study of Paasikivi (1970) was conducted upon 178 consecutive survivors of myocardial infarction. A large, multicentre, clinical trial in the U.S.A. (Universities Group Diabetes Program, 1970), was planned and carried out upon non-insulin dependent clinical diabetics, primarily to compare treatments in respect of the 'specific' vascular complications of diabetes and has issued its interim findings in respect of tolbutamide and cardiovascular death. The first of these trials presents data supporting the view that tolbutamide exerts a protective influence against

fatal and non fatal cardiac reinfarction; the second suggests that cardiovascular and sudden death occur significantly more frequently in diabetics treated with diet and tolbutamide than in those treated with diet and placebo.

Study on Survivors of First Myocardial Infarcts (Paasikivi, 1970)

On the basis of the frequently demonstrated evidence of impaired glucose tolerance in atherosclerotic patients (Wahlberg, 1966), Paasikivi administered up to 0.5g. tolbutamide thrice daily, or inert placebo, to 178 consecutive survivors of first myocardial infarcts. Glucose tolerance was monitored by intravenous glucose tolerance tests to determine if improvement in glucose tolerance was associated with improved cardiovascular prognosis. Overt diabetics were excluded from the group. Intravenous glucose tolerance in the remainder ranged between clearly normal and clearly diabetic responses. The treatment plan was 'single-blind', allocation to tolbutamide or placebo groups being by odd or even birth-dates.

In a mean follow-up period of 3 years (ranging from 12 to 66 months), tolbutamide did not significantly affect the overall mortality. Over the first 18 months, however, mortality was significantly lower in the tolbutamide group as was the mean survival time of those who died during the study period. This protective effect was most marked in subjects with 'borderline' and diabetic abnormality of intravenous glucose tolerance.

It has been argued that the rise in the cardiovascular mortality rate of the tolbutamide group in the latter part of the study period, as this group 'caught up' with the placebo group, was an indication of a late, adverse effect of the drug. However, isolated and selective presentation of rates in this way hardly constitutes a valid argument. Any measure which prolongs life must inevitably increase the later rate of deaths deferred.

Universities Group Diabetes Program (U.G.D.P.)

The information available to date (British Medical Journal, November, 1970) on the findings of this long-term multicentre trial is limited to verbal presentation of data at the 30th Annual Meeting of the American Diabetes Association, St. Louis, Mo., U.S.A., June, 1970, and the abstracts of that meeting (Goldner and Prout, 1970; Prout and Goldner, 1970a and b). Additional access to prepublication material has been permitted by Professor C. R. Klimt, M.D. (U.G.D.P.) and much of the commentary which follows is based upon detailed examination of this.

The U.G.D.P. study was constructed to make a controlled comparison of the relative success of a variety of antidiabetic treatments, including the use of oral hypoglycaemic agents and insulin injections, in delaying the long-term vascular complications of adult-onset, non insulin-dependent diabetes. 1,025 newly found diabetics, recruited at 12 Centres, were randomly allocated to one of 5 regimes of treatment, all of which included restriction of dietary carbohydrate and one of which

received, in addition, only placebo tablets. Another of the groups received 1.5g. tolbutamide daily. The comparison of the mortality experience of these two groups, in particular that part of it attributed to cardiovascular causes, has given rise to concern and is clearly relevant to this discussion.

In the period between the start of recruiting for the study in January, 1961 and September, 1969, when the use of tolbutamide was discontinued, 10.2% of the placebo group died compared with 14.7% of tolbutamide group patients, a difference that could readily have arisen by chance. However, deaths in the placebo group attributed to cardiovascular cause amounted to 4.9%, compared with 12.7% in the tolbutamide group. On the face of it, this demonstration of a $2\frac{1}{2}$ fold increase in cardiovascular mortality in the tolbutamide- compared with the placebo-treated patients would seem damning evidence against the drug. However, examination of the details of the study suggest features which considerably diminish the force of the *prima facie* case.

The population recruited to the trial was unrepresentative of both diabetic and general populations of the U.S.A. The slow rate of accession of patients to the trial (approximately one patient per centre per month) suggests that selective forces over and above those described as necessary criteria for recruitment must have operated. The female/male ratio of 3/1 and the racial composition (48% of the patients recruited were non-white) suggest that selective forces operated strongly against men and against whites.

The fact that overall mortality was not significantly higher in the tolbutamide group must raise the question of the degree of 'flexibility' in the allocation of cause of death within groups or differing manifestations or recognition differences between the groups. In fact, no death from myocardial infarction or atherosclerotic heart disease was recorded among the total of 21 deaths in the placebo group. This differs strikingly from the expected frequency in a like-aged non-diabetic population let alone among diabetics, in whom 40% - 50% of deaths could reasonably be expected to be attributed to this cause (Bradley and Partamian, 1965). The distribution of cardiovascular mortality in the study was far from uniform. Two of the 12 participating centres provided 22% of the cases but 47% of the total deaths and 50% of the cardiovascular deaths. Further, the apparent excess cardiovascular mortality in the tolbutamide group was confined to white females. In several respects, compared with the placebo group, the tolbutamide group was at an initial disadvantage in respect of several cardiovascular 'risk factors'. The average degree of glucose intolerance was greater, it contained substantially more hypercholesterolaemic subjects (300mg/100ml.), more of its patients were receiving digitalis, etc. While none of these differences achieved technical significance levels, their additive effects and their interactions may well have conferred a distinct disadvantage on the tolbutamide group over the years.

The degree of ascertainment of cause of death differed between the two groups. The proportion of deaths subjected to post mortem examination was twice as high for tolbutamide as for placebo fatalities; more of the former occurred in hospital. Precision in allocation of cause of death must, therefore, inevitably have been lower in the placebo group. In the ingenious statistical handling of the mortality results, no allowance was made for the option exercised in selecting cardiovascular mortality for comparison in preference to all other causes. Clearly, with a number of possible causes of death to select from, one in twenty comparisons could be expected to show a technically significant difference ($p = 0.05$), by chance.

Tolbutamide and Cardiovascular Disease

It is hazardous to draw firm conclusions about the role of tolbutamide in protecting against or predisposing to cardiovascular disease and death at present. The Bedford study, carried out by a single centre on 'borderline' diabetics, suggested some protective influence of tolbutamide, but this only achieved statistically satisfactory probability levels when many manifestations of arterial disease (including death) were pooled. Options could have been (but were not) exercised in deciding what to include or what to exclude to achieve the desired result. When component items included in the comparison are examined individually (Table 16), the comparison favours tolbutamide in most instances and, in the case of total mortality and deaths certified as due to cardiovascular causes, gives no hint of an adverse effect of the drug. The study of Paasikivi bases its

conclusions upon a single index of tolbutamide effect. At the least assessment it gives no indication of adverse effect after 5 years of tolbutamide treatment. At best, it suggests that reinfarction is postponed for a year or two by the drug. Other controlled clinical trials, involving the use of sulphonylureas and biguanides, are in progress (Carlstrom et al, 1970; Feldman and Crawford, 1970; Stowers, 1970; Kean et al, 1970 (a)) but in none are data yet available for analysis in respect of cardiovascular mortality. It will probably not be until these, and further, studies are available for consideration that a clear verdict on this important subject can be arrived at.

TABLE 16

Summary of cardiovascular events (and non-C.V. deaths)
in the 4 treatment groups in the Bedford 'borderline'
diabetic trial of tolbutamide vs. placebo, after 7 years.

Tolbutamide	0	0	+	+
CHO 120g./Day	0	+	0	+
Starting No.	65	57	62	64
C.V.	6	7	5	3
Deaths				
Other	3	4	6	4
Myoc. Infarct	3	2	3	4
Onset Angina	6	9	5	3
Onset Claud.	3	1	7	3
Stroke	2	2	2	0
E.C.G. Event	8	5	2	5
Total Events	28	26	24	18
Total Indiva	24	22	19	16
	37.7%		27.8%	
	p 0.1		0.05	

9.

CONCLUSION

Like many measurable human characteristics, glucose tolerance, whether evaluated by the blood sugar response to an oral or to an intravenous glucose 'challenge', shows considerable variation. There are clear differences between population groups, for example between the Pima people and the ethnically similar Athabaskan Indians and among the dissimilar geographical groupings tested by the standardised procedures of West and Kalbfleisch (1970). There is wide variation of response among the individuals going to make up each of these population groups, some of it attributable to age and sex composition. There is variation from time to time within individuals, even over the course of the day. To the uncertainties introduced by these many, ill-understood sources of variation must be added a further unknown, the truth with which the experimentally induced tests of glucose tolerance reflect the pattern of behaviour of the blood sugar under the ordinary conditions of life.

Bearing in mind these sources of uncertainty and lack of precision, it is all the more surprising that the blood sugar groups, defined in our Bedford study by a single measurement of blood sugar two hours after 50g. glucose by mouth, should have shown the associations as clearly as they did. The findings presented here support the view that the metabolic mechanisms displayed by the oral glucose tolerance are linked with processes involving the health or disease of the subjects studied.

The family studies which open this Thesis indicate the likelihood of the operation of genetic factors which, expressing themselves as overt, clinical diabetes in some family members, may also influence glucose tolerance to a lesser degree in ostensibly non-diabetic members of the family. It was the finding of the undue frequency of lesser degrees of glucose intolerance in the relatives that provoked the notion of the 'multifactorial' nature of the aetiology of diabetes mellitus and of its inheritance. It led directly to the design of the Bedford Survey in 1962 and, in particular, the planning of the structure of the random population sample.

Consideration of the frequency distributions of blood sugar levels in the Bedford random population sample and in all but one (Steinberg et al, 1970) of the other population groups published, is consistent with the view that glucose tolerance is a continuously distributed population characteristic and that the distinction between normal and diabetic responses is an 'operational' one, defining the action to be taken. Nevertheless the available data cannot be held to exclude the possibility that separate groups of responses do in fact exist, their boundaries blurred perhaps by secondary environmental and genetic factors. The positive skewing of many of the distribution curves is consistent with this alternative interpretation. Furthermore, the hypothesis of continuous distribution of glucose tolerance cannot comfortably accomodate the acute, insulin-sensitive juvenile-type of diabetes without some qualifications. Since the available data are consistent

with both of the alternative explanations, it would seem fruitless to continue to argue the matter without further information, preferably gathered with the need for critical distinction between the alternative hypotheses in mind.

Withdrawal from this argument in no way prejudices the examination of the clinically important questions which arise from the observations themselves. To what extent do the lesser degrees of glucose intolerance adversely affect the state of health and the length of life? Is there a critical degree of glucose intolerance which must be attained before any disadvantage is detectable? Are there any forms of intervention, by dietary adjustment or by medication, which will alter beneficially the adverse course of the condition? It is toward the answers to these questions that the material presented in this Thesis is directed.

Evidence has been brought to suggest that, in a number of respects, the 'borderline' diabetic differs from the normoglycaemic 'control'. These differences include an increased measure of obesity, evidence of disturbance of permeability of the basement membrane of the renal glomerular capillary and an increased prevalence and enhanced prospective risk of cardiovascular disease. In certain respects, however, the 'borderline' diabetic shows little or no disadvantage by comparison with the normoglycaemic, for example in the frequency of lenticular opacities, the development of retinal capillary microaneurisms and evidence of defective neurological function. The possible explanations of this dissociation are considered

below.

Only restricted answers can, as yet, be provided to the question of the efficacy of the intervention procedure of the Bedford trial on the natural course of 'borderline' diabetes. So far as its influence upon glycaemia was concerned, early analysis (Keen, 1966) suggested that neither tolbutamide nor diet had had a significant effect, either upon mean blood sugars of the treatment groups or upon the proportions in each group showing considerable rises (i.e. becoming 'diabetic') or falls (i.e. becoming 'normal') in blood sugar. A more recent review of treatment groups (Keen, 1970b) showed that 8, 6, 7, and 5 subjects had become 'diabetic' in the placebo group, the restricted carbohydrate group, the tolbutamide group and the combined therapy group respectively. Proportions so affected did not differ significantly between any pair of the four treatment groups, nor between the pooled tolbutamide versus placebo nor diet versus no diet groups. Though there was little evidence of an effect of treatment upon mean 2 hour post glucose blood sugar levels for the treatment groups, some influence of tolbutamide treatment upon 'everyday' blood sugar levels was suggested when non-glucose-loaded, random blood sugars were compared (Keen and Jarrett, 1970). So far as the effect of tolbutamide upon arterial disease in 'borderline' diabetics is concerned, the encouraging results of the Bedford study and the findings of others have been presented in Section 8. At present no clear general verdict can be pronounced. It is probable that differences between the clinical material, the forms of treatment and the characteristics

observed may account for the divergences in conclusions from the published studies. The data relating to ophthalmological, renal, neurological and other responses to therapy will be analysed and presented in due course.

'Threshold' and 'Non-Threshold' Consequences of Hyperglycaemia

The evidence assembled here suggests that the long-term sequels of hyperglycaemia may be separated into two groups which, for purposes of discussion, could be called threshold and non-threshold sequels. A threshold sequel is one for the development of which a certain minimal, or threshold, degree of hyperglycaemia is necessary and which is exemplified by the presence of lens opacities, by the appearance of retinal microaneurisms or by abnormalities of vibratory perception. In these respects, the 'borderline' diabetic group was almost indistinguishable from the normoglycaemic control but differed from the diabetic group, both in the frequency of abnormality at baseline and in the rate of appearance of newly affected individuals during the follow-up period where this was observed. A non-threshold sequel can be described as one which relates, in its frequency or intensity, to the degree of glucose tolerance across the whole range of the response to glucose. Arterial disease and proteinuria may be considered examples in this category, for in both cases, manifestations in the 'borderline' group were intermediate in degree between the normoglycaemic control on the one hand and the diabetic group on the other.

The general theory of threshold effects superimposed upon a continuously distributed variate is neither new, nor inherently improbable. One may readily accept, for example, a situation where, with rising arterial blood pressure there are increasing secondary changes in the arterial wall but upon which the threshold event of vascular rupture and, say, cerebral damage may be superimposed. Both classes of change are sequels to raised blood pressure, the one being continuously (though not necessarily simply), and the other discontinuously (though not necessarily predictably) related to a continuous variation in the main causal factor. Another special case of such a threshold effect is discussed by Carter (1969) in relation to the 'discontinuous' inheritance of hare lip and cleft palate against a background of multiple genetic components which predispose to the condition. His theoretical model presupposes that individuals beyond the threshold of genetic predisposition will actually manifest the abnormality by virtue of additional 'environmental triggers', the nature of which, in the main, are unknown. This hypothesis bears a close resemblance to that of Cerasi and Luft ("What is inherited - what is added?"), explaining the pattern of inheritance of diabetes mellitus itself (Cerasi and Luft, 1967a). Alternative models explaining an apparent threshold effect in the context of polygenic inheritance have also been developed by Edwards (1969) and Falconer (1965). Edwards related increase in the risk of manifestation to increase in the causal quantity in a direct but exponential fashion so that, in fact, no true absolute threshold exists. The model proposed by Falconer is probably more appropriate to the situation discussed here for it would take into

account all of the factors, genetic and environmental, influencing the likelihood of manifestation, relating its appearance to some total liability load made up of many components. Although these concepts were developed to explain the patterns of inheritance, the general arguments that they invoke have clear relevance to the question of hyperglycaemia and its associations considered here.

The separation of these two groupings of sequels may, however, simply indicate that quite different mechanisms are involved in their pathogenesis. Thus, the changes in the arterial wall which eventually predispose to atherosclerosis may be a consequence of the disturbance of circulating insulin levels and local lipid metabolism (Tzagournis et al, 1968), as suggested by the work of Mahler (1966) and Stout (1970), while the changes in the lens of the eye and the peripheral nerves may result from abnormality of sorbitol metabolism as discussed earlier, and the development of retinal microvascular abnormalities may involve the very specialised conditions in the retina of the diabetic, as postulated by Keen and Chlouverakis (1965).

It seems unlikely that the many sequels of long-standing diabetes can all be explained by the operation of a single mechanism. However, chronic hyperglycaemia is common to all of them, and since the changes discussed are so commonly associated with it and so rarely described in its absence, it would seem reasonable to suggest that the raised level of blood sugar itself plays a central, perhaps initiating, role. Reference has been made to the complex and varied chains of reactions which may result from this initiation. It is the further elucidation

of these which justifies the study of the nature and significance of 'borderline' diabetes.

10.

REFERENCES

- Abrams, M.E., Jarrett, R.J., Keen, H., Boyne, D.R. and Crossley, J.N. (1969)
British Medical Journal 1, 599.
- Armitage, P. (1955)
Biometrika 11, 375.
- Armitage, P., Fox, W., Rose, G.A. and Tinker, C.M. (1966)
Clinical Science 30, 331.
- Ashton, M. (1951)
British Journal of Ophthalmology 35, 189.
- Ashton, M. (1963)
British Journal of Ophthalmology 47, 521.
- Baird, J. (1969)
Personal Communication.
- Balme, H.W. and Cole, L. (1951)
Quarterly Journal of Medicine 20, 335.
- Balodimos, M.C., Keen, H., Gleason, R.E., Kahn, C.B., Soeldner, J.S., Chlouverakis, C. and Jarrett, R.J. (1970)
Diabetes 19, 377.
- Belknap, B.N., Bagdade, J.D., Amaral, J.A.P. and Bierman, E.L. (1967)
in 'Tolbutamide ... after ten years'.
Excerpta Medica, Amsterdam. P.171.
- Blackburn, H., Keys, A., Simonsen, E., Rautaharju, P. and Punsar, S. (1960)
Circulation 21, 1160.
- Bloodworth, J.M.B. Jr. (1965)
Archives of Pathology 79, 203.
- British Medical Journal (1970) 4, 444. (Leading Article).
- Bowen, A.J. and Reeves, R.L. (1967)
Archives of Internal Medicine 119, 261.
- Bowyer, D.E. and Gresham, G.A. (1970)
in 'Atherosclerosis: Proceedings of the Second International Symposium'. Editor R.J. Jones.
Springer-Verlag. P.3.
- Boyne, D.R., Crossley, J.N., Abrams, M.E., Jarrett, R.J. and Keen, H. (1969)
British Medical Journal 1, 595.

- Boyns, D.R. and Keen, H. (1967)
Unpublished Observations.
- Bradley, R.F. and Partamian, J.O. (1965)
The Medical Clinics of North America 49, 1093.
- Brandt, R.L. (1960)
Geriatrics 15, 315.
- Burditt, A.F. and Caird, F.I. (1968)
British Journal of Ophthalmology 52, 433.
- Butterfield, W.J.H. (1964)
Proceedings of the Royal Society of Medicine 57, 196.
- Butterfield, W.J.H., Keen, H. and Whichelow, M.J. (1967)
British Medical Journal 4, 505.
- Caird, F.I., Pirie, A. and Russell, T.G. (1969)
in 'Diabetes and the Eye'.
Blackwell Scientific Publications, Oxford & Edinburgh P.72 et seq.
- Camerini-Davalos, R.A. (1967)
in 'Tolbutamide ... after ten years'.
Excerpta Medica, Amsterdam. P.228.
- Camerini-Davalos, R.A., Chaulfield, J.B., Rees, S.B.,
Lozano-Castaneda, O., Maldjian, S. and Marble, A. (1963)
Diabetes 12, 508.
- Carlstrom, S., Lundquist, A., Lundquist, I., Norden, A.,
Schersten, B. and Wellmark, G. (1970)
Acta Medica Scandinavica (in press).
- Carter, C.O. (1969)
British Medical Bulletin 25, 52.
- Cerasi, E. and Luft, R. (1967)
Acta Endocrinologica 55, 278.
- Cerasi, E. and Luft, R. (1967a)
Diabetes 16, 615.
- Chesrow, E.J. and Bleyer, J.H. (1954)
Geriatrics 9, 276.
- Clarke, C.A. (1961)
Diabetes 10, 175.
- Clarke, S.D. and Maylor, F.D. (1961)
Lancet 2, 1327.

- Cochrane, A.L. and Elwood, P.C. (1968)
in 'Presymptomatic Detection and Early Diagnosis'.
Editors H. Keen and C.L.E.H. Sharp.
Pitman Medical Publishing Co. Ltd., London. P.359 et seq.
- Collens, W.S., Zilinsky, J.D. and Boas, L.C. (1946)
American Journal of Medicine 1, 638.
- Daysog, A., Dobson, H.L. and Brennan, J.C. (1961)
Annals of Internal Medicine 54, 672.
- Deren, M.D. (1937)
Journal of Laboratory and Clinical Medicine 22, 1138.
- Diabetes Survey Working Party (1962)
British Medical Journal 1, 1497.
- Diabetes Survey Working Party (1963)
British Medical Journal 2, 655.
- Diabetes Survey Working Party (1970)
British Medical Journal 3, 301
- Ditzel, J. (1966)
in '4th European Conference on the Microcirculation'.
Cambridge, S. Karger. P.509.
- Dolman, C.L. (1963)
Neurology 13, 134.
- Duke-Elder, W.S. (1945)
in 'Textbook of Ophthalmology'. Volume 3.
Kimpton, London. P.3204.
- Duncan, L.J.P., MacFarlane, A. and Robson, J.S. (1958)
Lancet 1, 822.
- Edwards, J.H. (1960)
Acta Genetica (Basel) 10, 63.
- Edwards, J.H. (1969)
British Medical Bulletin 25, 58.
- Engelhardt, H.T. and Vecchio, T.J. (1965)
Metabolism 14, 885.
- Engerman, R.L. and Bloodworth, J.M.B. Jr. (1965)
Archives of Ophthalmology 73, 205.

- Epstein, F.H. (1967)
Proceedings of the Royal Society of Medicine 60, 56.
- Eschwege, E. (1967)
in 'Enquête Épidémiologique sur les Facteurs de l'Atherosclérose'.
Bulletin de l'Institut National de la Santé et de la
Recherche Médicale 22, No. 2, P.264.
- Fabrykant, M. and Ashe, B.I. (1960)
New York State Journal of Medicine 60, 841.
- Fajans, S.S., Floyd, J.C. Jr., Conn, J.W. and Pek, S. (1970)
in 'Early Diabetes'.
Editor R.A. Camerini-Davalos.
Academic Press, New York and London. P.377.
- Falconer, D.S. (1965)
Annals of Human Genetics 29, 51.
- Feldman, R. and Crawford, D. (1970)
in 'Early Diabetes'.
Editor R. Camerini-Davalos.
Academic Press, New York and London. P.443.
- Feldman, R. and Fitterer, D. (1967)
in 'Tolbutamide ... after ten years'.
Excerpta Medica, Amsterdam. P.243.
- Fitzgerald, M.G. and Keen, H. (1964)
British Medical Journal 1, 1568.
- Fry, I., Hardwick, C. and Scott, G. (1962)
Guy's Hospital Reports 111, 113.
- Gabbay, K.H., Marola, L.O. and Field, R.A. (1966)
Science 151, 209.
- Gabbay, K.H. and O'Sullivan, J.B. (1968)
Diabetes 17, 239.
- Gaddum, J.H. (1945)
Nature 156, 463.
- Garland, H. and Taverner, D. (1953)
British Medical Journal 1, 1405.
- Geer, J.C., Panganamala, R.V., Newman, H.A.I. and Cornwell, D.G. (1970)
in 'Atherosclerosis: Proceedings of the Second International
Symposium'. Editor R.J. Jones.
Springer-Verlag. P.6.

- Gelin, J., Elgrishi, I., Ducimetière, P. and Richard, J.L. (1967)
in 'Enquête Epidémiologique sur les Facteurs de l'Atherosclerose'.
Bulletin de l'Institut National de la Santé et de la
Recherche Médicale 22, No. 2, P.279.
- Gellman, D.D., Pirani, C.L., Soothill, J.F., Muehrcke, R.C. and
Kark, R.M. (1959)
Medicine 38, 321.
- Goldner, M.G. and Prout, T.E. (1970)
Abstract. Diabetes 19, Supplement 1, 387.
- Gordon, T. (1964)
'Glucose Tolerance of Adults, United States, 1960-62'.
U.S. Public Health Service Publications, Series 11, No. 2.
U.S. Government Printing Office, Washington, D.C.
- Grant, D.R. and Moorhouse, J.A. (1966)
Canadian Medical Association Journal 94, 1213.
- Haust, M.D. (1970)
in 'Atherosclerosis: Proceedings of the Second International
Symposium'. Editor R.J. Jones.
Springer-Verlag. P.12.
- Hayner, N.S., Kjelsburg, M.O., Epstein, F.H. and Francis, T. (1965)
Diabetes 14, 413.
- Heinsius, E. and Arndt, G. (1950)
von Graefes' Archives fur Ophthalmologie 150, 555.
- Hoffman, W.S. (1937)
Journal of Biological Chemistry 120, 51.
- Horvath, S.M., Wisotsky, R. and Corwin, W. (1947)
Journal of Gerontology 2, 25.
- Ireland, J.T. (1970)
in 'Early Diabetes'.
Editors R. Camerini-Davalos and H.S. Cole.
Academic Press, New York and London. P.273.
- Jarrett, R.J. and Keen, H. (1969)
British Medical Journal 2, 341.

- Kannel, W.B., Dawber, T.R., Kagan, A., Revotskie, N. and Stokes, J. (1961)
Annals of Internal Medicine 55, 33.
- Kannel, W.B., Castelli, W.P. and McNamara, P.M. (1967)
Journal of Occupational Medicine 9, 611.
- Keen, H. (1957)
Abstract.
Lancet 2, 124.
- Keen, H. (1962)
in 'Disorders of Carbohydrate Metabolism'.
Editor D.A. Pyke.
Pitman, London. P.177.
- Keen, H. (1964)
Proceedings of the Royal Society of Medicine 57, 200.
- Keen, H. (1966)
Proceedings of the Royal Society of Medicine 59, 1169.
- Keen, H. (1968)
Hospital Medicine 5, 760.
- Keen, H. (1969)
Journal of Clinical Pathology 22, Supplement 2, 19.
- Keen, H. (1970)
Observations on the Epidemiology of Diabetes.
Proceedings of the 76th Congress of the German Association
of Internal Medicine, Wiesbaden, April, 1970. (in press).
- Keen, H. (1970)
The Natural History of Diabetes in the Population.
Paper given to the W.G. Oakley Valedictory Meeting,
King's College Hospital, June, 1970.
- Keen, H. and Chlouverakis, C. (1963)
Lancet 2, 913.
- Keen, H. and Chlouverakis, C. (1965)
in 'Biochemistry of the Retina'.
Editor C.N. Graymore.
Academic Press, London. P.123.
- Keen, H. and Jarrett, R.J. (1967)
Lancet 2, 214.
- Keen, H. and Jarrett, R.J. (1969)
in 'The New Management of Stable Adult Diabetes'.
Editor C. Weller.
Charles, C. Thomas, Springfield, Ill., U.S.A. P.3 et seq.
- Keen, H. and Jarrett, R.J. (1970)
Update 2, 889.

- Keen, H. and Jarrett, R.J. (1970a)
in 'Atherosclerosis: Proceedings of the Second International Symposium'.
Editor R.J. Jones.
Springer-Verlag, Berlin, Heidelberg, New York. P.435.
- Keen, H., Jarrett, R.J. and Ward, J.D. (1970)
A controlled trial of treatment of 'borderline' diabetics with phenformin and diet.
In preparation.
- Keen, H., Rose, G.A., Pyke, D.A., Boyns, D.R., Chlouverakis, C. and Mistry S. (1965)
Lancet 2, 505.
- Keen, H. and Smith, R.S. (1959)
Lancet 1, 849.
- Keen, H. and Smith, R.S. (1960)
Paper presented to the First Meeting of the Medical and Scientific Section of the British Diabetic Association, Guy's Hospital.
- King, E.J. & Wooton, I.D.P. (1959)
Microanalysis in Medical Biochemistry.
J. and A. Churchill Ltd., London.
- Kingsbury, K.J. (1966)
Lancet 2, 1374.
- Klimt, C.R., Meinert, C.L., Ho, I.P. and Briese, P.W. (1967)
Diabetes 16, 40.
- Lou, M.F. and Kinoshita, J.H. (1967)
Biochimica Biophysica Acta 141, 547.
- Mahler, R.F. (1966)
in 'Diabetes Mellitus'.
Editor L.J.P. Duncan.
Edinburgh University Press.
- Martin, M.M. (1953)
Brain 76, 594.
- Marble, A. (1967)
Diabetes 16, 825.
- Mayne, N. (1965)
Lancet 2, 1313.

- McDonald, G.W., Fisher, G.F. and Burnham, C.E. (1965)
Diabetes 14, 473.
- McDonald, G.W., Burnham, C.E. and Lewis, W.F. (1969)
Public Health Reports 84, 353.
- McGuinness, R. (1967)
British Medical Journal 1, 416.
- Miller, M., Bennet, P.H. and Burch, T.A. (1968)
in 'Biomedical Challenges presented by the American Indian'.
Scientific Publication No. 165, Pan American Health
Organisation, Washington, D.C.
- Mimura, G., Oshiro, S., Koganemaru, K., Haraguchi, Y.,
Jinnouchi, T. and Hashiguchi, J. (1964)
Kumamoto Medical Journal 17, 45 and 50.
- Mirsky, I.A., Futterman, P. and Broh-Kahn, R.H. (1953)
Journal of Laboratory and Clinical Medicine 41, 221.
- Moorhouse, J.A. (1970)
Diabetes 19, 362.
- Morgan, C.R. and Lazarow, A. (1963)
Diabetes 12, 115.
- Morris, J.N. (1964)
'Uses of Epidemiology'.
E. and S. Livingstone Ltd., Edinburgh.
- Mouratoff, G. J., Carroll, N.V. and Scott, E.M. (1967)
Journal of the American Medical Association 199, 961.
- Neel, J.V., Fajans, S.S., Conn, J.W. and Davidson, R.T. (1965)
in 'Genetics and the Epidemiology of Chronic Diseases'.
Editors J.V. Neel, M.W. Shaw and W.J. Schull.
U.S. Government Printing Office, Washington, D.C.
- Neel, J.V. (1969)
Proceedings of the 6th Congress of the International
Diabetes Federation.
Excerpta Medica. (in press).

- O'Brien, C.S. and Allen, J.H. (1942)
Journal of the American Medical Association 120, 190.
- Osterby Hansen, R. (1965)
Diabetologia 1, 97.
- Ostrander, L.D., Francis, T., Hayner, N., Kjelsberg, M.O. and Epstein, F.H. (1965)
Annals of Internal Medicine 62, 1188.
- O'Sullivan, J.B. (1969)
Diabetologia 5, 211.
- Paasikivi, J. (1970)
Supplement 507 to Acta Medica Scandinavica.
- Patterson, J.W. (1952)
American Journal of Ophthalmology 35, 68.
- Penrose, L.S. (1951)
Annals of Eugenics 16, 134.
- Pickering, G.W. (1961)
in 'The Nature of Essential Hypertension'.
Churchill, London. P.2.
- Pincus, G. and White, P. (1934)
American Journal of Medical Science 188, 782.
- Pincus, G. and White, P. (1933)
American Journal of Medical Science 186, 1.
- Pirie, A. and van Heyningen, R. (1964)
Experimental Eye Research 3, 124.
- Prior, I.A.M., Rose, B.S., Harvey, M.P.B. and Davidson, R. (1966)
Lancet 1, 333.
- Prout, T.E. and Goldner, M.G. (1970a)
Abstract.
Diabetes 19. Supplement 1, 374.
- Prout, T.E. and Goldner, M.G. (1970b)
Abstract.
Diabetes 19, Supplement 1, 375.
- Pyke, D.A. and Please, N.W. (1957)
Journal of Endocrinology 15, 216.
- Pyke, D.A. and Roberts, D.St.C. (1959)
Acta Medica Scandinavica 163, 489.

- Report of European Diabetes Epidemiology Study Group (1970)
Diabetologia 6, 453.
- Rose, G.A. and Blackburn, H. (1968)
'Cardiovascular Survey Methods'.
World Health Organisation Monograph Series No. 56.
World Health Organisation, Geneva.
- Rundles, R.W. (1945)
Medicine 24, 111.
- Ryle, J. (1947)
Lancet 1, 1.
- Sabour, M.S., MacDonald, M.K. and Robson, J.S. (1962)
Diabetes 11, 291.
- Sharp, C.L.E.H. (1964)
Proceedings of the Royal Society of Medicine 57, 193.
- Sharp, C.L.E.H. (1968)
in 'Presymptomatic Detection and Early Diagnosis'.
Editors H. Keen and C.L.E.H. Sharp.
Pitman Medical Publishing Co. Ltd., London. P.3. et seq.
- Sherman, W.R. and Stewart, M.A. (1966)
Biochemical and Biophysical Research Communications 22, 492.
- Silverstone, F.A., Brandfonbrener, M., Shock, N.W. and
Yiengst, M.J. (1957)
Journal of Clinical Investigation 36, 504.
- Simpson, N.E. (1964)
Diabetes 13, 462.
- Siperstein, M.D., Unger, R.M. and Madison, L.L. (1968)
Journal of Clinical Investigation 47, 1973.
- Snedecor, G.W. (1956)
'Statistical Methods'.
Iowa State University Press, Iowa, U.S.A.
- Singh, I., Chandel, I., Chatterjee, S.C., Brara, N.S. and
Joshi, M.J. (1962)
Journal of the Association of Physicians of India 10, 654.
- Soler, N.G., Fitzgerald, M.G., Malins, J.M. and
Summers, R.O.C. (1969)
British Medical Journal 2, 567.
- Steinberg, A.G. (1959)
Annals of the New York Academy of Science 82, 197.

- Steinberg, A.G., Rushforth, N.B., Bennett, P.H., Burch, T.A. and Miller, M. (1970)
in 'Early Diabetes'.
Editors R. Camerini-Davalos and H.S. Cole.
Academic Press, New York and London. P.11-21.
- Steiness, I. (1963)
Acta Medica Scandinavica 173, Supplement 394.
- Steinkamp, R.C., Cohen, N.L., Gaffey, W.R., McKey, T., Bron, G., Siri, W.E., Sargent, T.W. and Isaacs, E. (1965)
Journal of Chronic Diseases 18, 1291.
- Stout, R.W. (1970)
British Medical Journal 3, 685.
- Stowers, J.M. (1970)
in 'Early Diabetes'.
Editors R. Camerini-Davalos and H.S. Cole.
Academic Press, New York and London. P.449.
(and Personal Communication).
- Studer, A. (1970)
in 'Atherosclerosis: Proceedings of the Second International Symposium'. Editor R. J. Jones.
Springer-Verlag. P.20.
- Tanner, J.M. (1959)
British Nutritional Society 18, 148.
- Thomas, P.K. and Lascelles, R. (1966)
Quarterly Journal of Medicine 35, 489.
- Thomsen, A.C. (1965)
The Kidney in Diabetes Mellitus.
Kap 11, Munksgaard, Copenhagen.
- Thompson, G.S. (1965)
Journal of Medical Genetics 2, 221.
- Tulloch, J.A. (1962)
'Diabetes Mellitus in the Tropics'.
Livingstone, Edinburgh. Chapter 5.
- Tzagournis, M., Chiles, R., Ryan, J.M. and Skillman, T.G. (1968)
Circulation 38, 1156.

- University Group Diabetes Program (1970)
A Study of the Effects of Hypoglycaemic Agents on Vascular Complications in Patients with Adult-Onset Diabetes.
Supplement to Diabetes (in press).
- Vallance-Owen, J. (1964)
Diabetes 12, 502.
- Van Heyningen, R. (1959)
Nature 184, 194.
- Van Heyningen, R. (1962)
Experimental Eye Research 1, 396.
- Waddel, W.R. and Field, R.A. (1960)
Metabolism 9, 800.
- Wahlberg, F. (1966)
Acta Medica Scandinavica 180, Supplement 453.
- Waite, J.H. and Beetham, W.P. (1935)
New England Journal of Medicine 212, 367 and 429.
- Ward, J.D. and Baker, R. (1970)
Personal Communication.
- Wellborn, T.A., Cumpston, G.N., Cullen, K.J., Curnow, D.M., McCall, M.G. and Stenhouse, N.S. (1969)
American Journal of Epidemiology 89, 521.
- Wellborn, T.A., Curnow, D.M., Wearne, J.T., Cullen, K.J., McCall, M.G. and Stenhouse, N.S. (1969)
Medical Journal of Australia 2, 778.
- West, K.M. and Kalbfleisch, J.M. (1966)
Diabetes 15, 9.
- West, K.M. and Kalbfleisch, J.M. (1970)
Diabetes 19, 656.
- Whichelow, M.J., Wigglesworth, A., Cox, B.D., Butterfield, W.J.H. and Abrams, M.E. (1967)
Diabetes 16, 219.
- Wilkerson, H.L.C. and Krall, L.P. (1947)
Journal of the American Medical Association 135, 209.
- Wilson, J.M.G. and Jungner, G. (1968)
'Principles and Practice of Screening for Disease'.
World Health Organisation Public Health Paper No. 34, 83.

World Health Organisation Technical Report
Series No. 310 (1965)
Diabetes Mellitus.

Meeting October 24 1963

Diabetes Survey in Bedford 1962

Dr C L Sharp
(Medical Officer of Health, Bedford)

Introduction

Numerous surveys in the United States, of which mention might be made of that by Wilkerson & Krall (1947), and latterly in this country by Walker (1959) and Redhead (1960) have demonstrated that there is a large reservoir of undiagnosed diabetes in the population and have shown an incidence of diabetes mellitus of 1-2%, about half of whom were unknown and not receiving treatment. As diabetes in adults tends to be of insidious onset and the symptoms, when they do appear, do not immediately cause anxiety, there is a considerable delay before the patient seeks advice. Reid (1960) had shown in a survey in this country that 48% of patients had tolerated their symptoms daily for four months before seeking advice and 20% had tolerated symptoms for more than a year. Furthermore, about 20% of the patients coming up for diagnosis already had complications of the disease. Delay by the patient's doctor in making the appropriate investigation is probably also of significance.

It seemed that the failure to make progress in the United Kingdom had been caused by the lack of a large-scale demonstration of the value of a diabetes survey.

Large-scale demonstration, need of a multi-disciplinary team: The major difficulty is in setting up the initial large-scale demonstration. This probably results from a failure in communication between the various branches of medicine, confusion over responsibility and the lack of a team with the multiple skills to carry the project through. The experts and equipment lie in the teaching hospitals, but quite often the local community, particularly in large cities, is unsuited for a survey. What is required is a town of sufficient size, stability, means of communication (local press) and with local hospital and

clinic facilities. The major factor is in getting together those with an interest in preventive measures and those with the facilities and experience. Exploration of the field of screening methods for early, or in some cases presymptomatic, diagnosis can only be properly explored on a community basis. Bedford, a town of some 65,000 people, with good hospital and clinic facilities and within easy reach of London, offers an ideal opportunity for exploring these measures.

The Diabetes Survey

It was obvious that whilst the first step of discovering those with glycosuria could be carried out comparatively easily and cheaply with the glucose oxidase enzyme test, the follow-up with blood sugar estimations and clinical examinations would require facilities far beyond the capabilities of the local hospital and Health Department. The idea of the Bedford Diabetes Detection Survey was therefore conceived, and the aims were: (1) To find the expected 300-400 unknown and untreated diabetics in Bedford Borough. (2) To present an opportunity for further research into treatment. (3) To establish a screening technique for discovering diabetes. (4) To hasten the time when screening for diabetes becomes a routine part of preventive medicine by health departments.

Preliminary discussions with lay and professional people made two points clear: (1) Most lay people accepted the idea and became enthusiastic, though a few thought that public neurosis would be caused. (2) Doctors thought that (a) the idea had some merit and (b) it could not be done by reason of lack of funds and public co-operation.

It was decided to approach the Ministry of Health, and useful discussion took place with Dr J M G Wilson. The very great difficulties to be encountered in carrying out the survey made it imperative that powerful support should be found for the scheme. After careful consideration

I approached Professor W J H Butterfield and at a meeting at Guy's Hospital on December 23, 1960, the project was put forward, with some trepidation, for to screen the population of Bedford, then some 63,000 people, was a formidable undertaking. Professor Butterfield subsequently wrote confirming his interest and whole-hearted support for the plan, and after further discussions a meeting was called under his chairmanship. Those attending were: Dr Roy Acheson of the London School of Hygiene, now Professor at Yale; Mr Norman Allen of the British Diabetic Association; Mr Kennedy Brown and Mr John Laurie of Patrick Dolan Associates, public relations consultants; Mr Dennis Vobes of the Ames Company, makers of urine testing materials; Mr L J Evans, Managing Director of Technicon Limited, makers of auto-analysers for blood sugar testing; members of the Department of Experimental Medicine at Guy's Hospital and myself.

The idea was to utilize the glucose oxidase urine test for glucose to select those with glycosuria for further examination. This meant obtaining large numbers of specimens of urine, and the delivery and collection of the urine containers would require considerable numbers of voluntary helpers.

Public relations: A public relations master plan was prepared by Mr John Laurie. Its first aim was to get the goodwill of the medical profession, and its second to get community support and recruit organizers and voluntary helpers for the massive task of distribution and collection and also for testing of the urine samples.

Professor Butterfield wrote a personal letter to each of the doctors in the Borough, explaining the project and enclosing a memorandum setting out further details. He also came down to Bedford to discuss the project with the Consultant to the local Diabetic Clinic.

Before taking the matter to Council the Public Health Committee were approached and talks were given to the Association of Engineering Employers and to the Trades Council. A personal approach was made to many other people thought to be of importance in influencing organizations and public opinion generally: heads of schools, principals of colleges and, perhaps most important of all, the Editor of the *Bedfordshire Times*, the local newspaper.

Wanted 400 volunteers: Following Public Health Committee support of the project the Mayor made an appeal for support from the com-

munity and called a meeting of voluntary organizations. These were addressed by Professor Butterfield and by Mr Norman Allen of the British Diabetic Association. In the main those recruited were people who could organize the collection and delivery in various sections of the town, which for our purpose had been divided into twenty areas. However, a considerable number of helpers were needed and the British Diabetic Association gave a luncheon for heads of schools and principals of colleges, at which Professor Butterfield outlined the project and gained their support so that volunteers from schools and colleges helped in the delivery of a leaflet to each of the 20,000 households in the town, informing the householder of the proposed scheme; and subsequently in the delivery of a pack, consisting of a plastic pot and instruction leaflet in an envelope, to every house on Saturday, May 12, for collection on Sunday, May 13, 1962.

At the same time Mr John Laurie made the necessary approaches to the press, both local and national, radio and television and supervised the production of posters and other written material. Anglia Television and BBC radio and television programmes were arranged.

It was obvious that the overall control of the 400 volunteers would be an extremely difficult matter and an approach was made to Mr A S Hislop, Civil Defence Officer. He divided the town into four regions, each with an Area Organizer in charge, and subdivided these into groups of streets, each with a collection centre, making about 20 centres in all.

Nearly all the voluntary organizations in the town played a part in the work, but perhaps special mention should be made of the Bedford Round Tablers, who staffed most of the area centres.

An early decision had been to limit the number of tests by eliminating those under the age of 21 and using as our defined population those on the electoral roll. The volunteers were supplied with street lists, made up by cutting columns from the electoral roll, and were asked to deliver an envelope to each person named in each street. The whole delivery was accomplished without difficulty on the Saturday afternoon.

The same volunteers set about the more difficult task of collection on Sunday, and by four o'clock approximately 20,000 urine samples had been brought into the centres. These were collected by vans and conveyed to the urine testing centre, which had been set up in Putnoe

Clinic. A repeat collection was made the next week-end. Almost two-thirds of the citizens of Bedford submitted urine samples (25,700) out of the 38,400 persons on the electoral roll.

To cope with the very large number of urine tests to be made a programme had been worked out, using trays marked into thirty squares on which the urine samples were placed; the lids were removed and a Clinistix test carried out; a minute later the results were read and any which were positive were recapped before further testing to determine the amount of glucose present and to see whether there was any ketonuria. A work study engineer gave invaluable help during Sunday afternoon, to such good effect that on Monday, when the testing was resumed, some 13,000 urines were tested during the morning.

Results of urine testing: One thousand and forty-six urine samples were found to have glucose present, an overall incidence of 4%. The incidence of glycosuria was higher among men than women, average 5.9% and 2.5% respectively. In both sexes the incidence of glycosuria rose with age: whereas 4.6% of men in the 20s and 30s had glycosuria, 8% of men in the age group 70–80 were affected; amongst women the incidence rose from 2.9% in the 20–30-year-old group to 4.4% in the 70–80-year-olds. Of those with glycosuria 91% accepted the invitation to undergo the glucose tolerance test at another clinic.

Arrangements for blood sugar testing: A very large number of blood sugar estimations were needed every day, ranging up to 300 specimens for a single session; work which would have been impossible without the aid of auto-analysers. Of the glycosurics who attended for testing, just under a third were found to have diabetes by the accepted criterion, a blood sugar of 120 mg/100 ml two hours after taking 50 g of glucose by mouth. While many of the newly found diabetics had blood sugars between 120–200 mg/100 ml some were more severe, with blood sugar ranging as high as 440 mg/100 ml. Analysis showed that of these people who were previously unaware of suffering from any illness, about half had symptoms or signs on clinical examination. The percentage of diabetics among the glycosurics rose with age among men and women, indicating that with advancing years glycosuria tends to be more frequently associated with a high blood sugar level.

Of a random sample, called for glucose tolerance tests irrespective of glycosuria, 78% responded. The results were interesting. Of 543 persons who were without glycosuria in the

original survey, 76 were found to have blood sugar levels over 120 mg/100 ml. A high incidence of hyperglycaemia among people who had passed the first screening test showed that the loading was insufficient. The original instructions to the patients were that they were to produce a specimen of urine one to two hours after a main meal.

The total of diabetics found was 356. Those with blood sugars over 200 mg/100 ml were sent letters advising them to go to their general practitioners, who, in most cases, referred them to the Bedford Diabetic Clinic, which would have been overwhelmed but for the support of Guy's Hospital staff.

Borderline Diabetics

The remaining borderline group, with blood sugars of 120–200 mg/100 ml were called up in batches to one of the clinics, where they were addressed by either Professor Butterfield or Dr Harry Keen, who explained to them our interest in research and asked them to submit to a variety of tests and be kept under constant scrutiny, so that we could learn more of such cases. We hoped that they would benefit from the close control of their condition, but it was made abundantly clear to them that this was a research project in which we were asking them to take part. Questions were invited and lively discussions took place. Private interviews with patients were also offered.

Approximately 98% of those who attended these talks agreed to attend the 'borderline clinic', which is staffed by personnel from Guy's and King's College Hospitals and the London School of Hygiene.

All concerned with carrying out the survey in Bedford felt that the whole project had been eminently worth while: as an exercise in preventive medicine, in setting the stage for further research, and in producing a sufficiently large survey to excite the interest so badly needed to ensure that the work of diabetes detection is carried out without the long delays which have characterized other measures in preventive medicine. Many patients expressed their appreciation, both verbally and by letter, before and after receiving the benefits of treatment. Many appeared to be relieved of anxieties, for perhaps some had secretly feared that their ill health had a far more sinister origin.

REFERENCES

- Redhead I H (1960) *Brit. med. J.* i, 695
- Reid J J A (1960) *Med. Offr.* 103, 325
- Walker J B (1959) *Postgrad. med. J.* 35, 302
- Wilkerson H L C & Krall L P (1947) *J. Amer. med. Ass.* 135, 209

Professor W J H Butterfield

(On behalf of the Departments of Public Health, Bedford, and Experimental Medicine, Guy's Hospital Medical School, London)

Summary of Results of the Bedford Diabetes Survey

Before we went to Bedford, in addition to the detailed administrative planning we had to take three important medical decisions: First, we had to accept the differences in breakfast, alimentary digestion and timing in urine sampling as inherent variables in our screening procedure. Second, after much discussion and reading, we decided to accept the widely held criterion for the laboratory diagnosis of diabetes mellitus, namely, a capillary blood sugar exceeding 120 mg/100 ml, two hours after the administration of 50 grams of glucose by mouth. In the event, to diminish the incidence of nausea, which accompanies oral dextrose monohydrate, we used an equivalent dose of liquid glucose. Third, we decided to test the glucose tolerance, with conventional blood sugar curves, of an age/sex stratified random sample of about 500–600 people drawn from the co-operating population.

Glycosuria

Of a total resident population of 63,000 persons in Bedford, we took as our defined population for the study those persons over 21 years of age – the Electoral Roll – which comprised 38,400. Of these persons, 25,701 provided urine samples for testing, and 1,046 (4%) gave results which were positive to Clinistix. Such positive urines were also subjected to further investigation by Clinitest and Acetest methods, and the results will be published in due course: suffice it here to say that these more elaborate procedures did not discriminate diabetics more satisfactorily.

Table 1 shows the incidence of glycosuria by age and sex: it is evident that the incidence rises steadily among the men, but there is a plateau among the women. Since these 1,046 cases did not include the recognized diabetics in the town,

we wondered if the data for women in the middle age groups had been influenced by the cases of diabetes picked up at antenatal clinics.

Of the 1,046 glycosurics detected, 939 responded to our letter asking them to attend for blood sugar tests to determine if they had renal glycosuria or hyperglycaemia.

The procedure was to give them, on arrival at the testing centre established in a Public Health Clinic, 50 g liquid glucose, made up for drinking in a 250 ml aqueous solution. They then sat reading for half to three quarters of an hour before being asked to attend a brief clinical examination consisting of a history, including occupation and previous illnesses; obstetric history in the case of women; family history of diabetes, with specific questions on anginal pain, claudication, recent memory failure, cardiac infarction, gangrene or strokes, boils, thirst, pruritus, weight and eyesight. Examination included height, weight, skinfold thickness and other anthropological measurements, detection of the pulses at the ankle, and estimation of the blood pressure, recording double diastolic readings, i.e. change of sounds and disappearance of sounds (these often coincided). Two hours after the glucose drink, 0.1 ml capillary blood was taken from the earlobe and diluted in 3 ml of 1% potassium fluoride in water for glucose estimation (Hoffmann's method) using the micro-analytic method on auto-analysers.

Hyperglycaemia

Fig 1 is a histogram of the two-hour blood sugar values found. Three interesting points arise. Firstly, there is a smooth gradation of blood sugar values, plotted on an arithmetic scale, from low to high, without obvious segregation into two populations. This being so, the criterion for diagnosis of diabetes, marked in the figure, seems hard to justify. Secondly, we were surprised by the height of blood sugars to be found among a population which could be regarded as healthy. Until the present study these glycosurics were not recognized as diabetics. Thirdly, it is clear that in the natural history and pathology of diabetes,

Table 1

Incidence of glycosuria by age and sex

Age	Men			Women		
	Total	Glycosuric No.	%	Total	Glycosuric No.	%
20–29	1,898	83	4.37	2,007	57	2.84
30–39	2,745	141	5.13	2,801	61	2.18
40–49	2,780	143	5.13	3,012	58	1.92
50–59	2,100	157	7.48	2,577	51	1.98
60–69	1,375	110	8.00	1,959	55	2.80
70–79	616	48	7.80	1,056	49	4.65
80–89	134	12	8.95	272	12	4.42
90–99	9	1	12.1	12	–	–

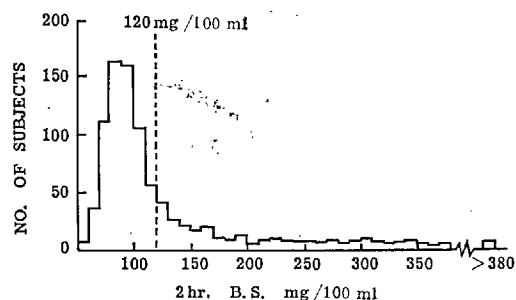


Fig 1 Two-hour blood sugars in glycosurics (see text)

hyperglycaemia of considerable extent may have occurred, at least from time to time and perhaps for years, before our present clinical practice, which hinges largely on self-detection by symptoms or submission to insurance examinations, will detect it.

Table 2

Incidence of diabetes (two-hour blood sugar over 120 mg/100 ml) amongst glycosurics subjected to glucose tolerance test

Age	Men			Women		
	Total	No.	%	Total	No.	%
20-29	74	4	5.4	52	3	5.8
30-39	127	14	11.0	54	11	20.4
40-49	132	19	14.4	53	13	24.5
50-59	146	47	32.2	47	32	68.0
60-69	101	30	29.7	47	34	72.2
70-79	37	22	59.4	27	22	81.5
80-89	7	4	57.1	7	6	85.7

Table 2 correlates hyperglycaemia with glycosuria by age and sex. It will be seen that the incidence of diabetes as indicated by raised blood sugars (two-hour B.S. of 120 mg/100 ml or more) among the glycosurics rises steadily with age so that, among the elderly, especially women, glycosuria is usually associated with hyperglycaemia. The proportion of glycosurics with raised blood sugar levels is lowest among the younger age groups and rises with age: one implication of this finding is that the renal threshold for glucose rises with age.

Table 3

Symptoms of diabetes and signs of arterial disease in 266 diabetics among 939 glycosurics

No. with arterial disease	No. with symptoms	No. with both arterial disease and symptoms	Total with arterial disease or symptoms
31	78	23	132
(11.0%)	(29.3%)	(8.6%)	(49.6%)

The criterion for diagnosis of diabetes was two-hour B.S. > 120 mg/100 ml

Table 3 shows the results of the clinical examinations in relation to hyperglycaemia. Of the 266 new cases who fulfilled the criterion for the diagnosis of diabetes, half had symptoms of diabetes or signs of vascular disease. The purpose of early diagnosis must be prevention of disability and, in the case of diabetes, to shield the ageing population from vascular complications. That quick and relatively crude clinical methods would detect so many cases with overt symptoms and signs suggests that there was in fact probably a much higher incidence of vascular disease. However, it is clear that education would be a major first step in detecting diabetes, for nearly a third of the new diabetics in this group had symptoms of arterial disease.

The Random Sample

The objects of studying an age/sex stratified random sample drawn from the Bedford population were to construct 'normal' glucose tolerance curves for comparison with the glycosuric cases, to find the incidence in the population of the symptoms and signs studied, and to evaluate the incidence of glycosuria after a standard glucose load for comparison with that found after variable English breakfasts.

Just over 600 persons drawn at random from the population providing urine samples were asked to come up for tests: it is a great tribute to the spirit abroad in Bedford, to Dr Sharp and to the excellent public relations established by Mr Laurie, that over 90% co-operated, except in the most elderly age group.

The results were somewhat surprising. The incidence of glycosuria after 50 g liquid glucose by mouth was 30%, indicating the importance of dietary habits in the detection of diabetes.

Of the 570 persons who co-operated, 90 had blood sugar values in the range accepted as diagnostic of diabetes. If these data are applied to the age/sex structure of the nation as a whole, we find an overall incidence of diabetes of 12-14%. A histogram constructed from the two-hour blood sugar values in the random sample is strikingly similar to Fig 1, again without obvious segregation into normal and diabetic populations. Closer scrutiny of the data indicated that glucose tolerance diminishes with age. The mean results for women are shown in Fig 2. Essentially similar trends were seen among the men and it is evident that we shall

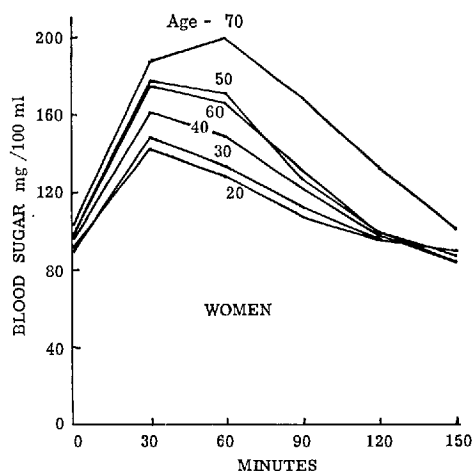


Fig 2 Mean glucose tolerance curves in 281 women of random sample, by age (see text)

soon be facing the questions: should we correct our glucose tolerance values for age, or is hyperglycaemia *per se* related to the diabetic complications? At this moment, I am opposed to correcting glucose tolerance for age, because of the high incidence of complications among elderly diabetics. All that such correction would do would be to give an excuse to avoid treating them. These views are supported by the data displayed in Table 4 summarizing the clinical findings in the random sample. It will be seen that there was a high incidence of symptoms and signs of vascular disease, using crude clinical methods of detection, among the hyperglycaemic group.

Another very important point, particularly germane to the question of the inheritance of diabetes, emerges from detailed analysis of the random sample. Fig 3 shows histograms of the glucose tolerance results in women, broken down by age groups. The vertical lines shown are widely accepted criteria for the laboratory diagnosis of diabetes, i.e. a fasting blood sugar above 100 mg/100 ml, one-hour values over 180 mg/100 ml and two-hour levels over 120 mg/100 ml. It will be seen that with increasing age, the proportion of diabetics increases. The findings among the men in the random sample were

Table 4

Glucose tolerance tests and vascular disease in age/sex stratified sample

	B.S. < 120 mg/100 ml	B.S. > 120 mg/100 ml	Total
Number in sample	480	90	570
With symptoms or signs of arterial disease	134 (27.9%)	39 (43.3%)	173 (30.35%)

$\chi^2 = 7$ $P < 0.01$

N.B. Criteria for vascular disease slightly different from Table 3

similar. Emphasis must be laid on the very high proportions of the randomly selected 'non-diabetic' elderly persons showing hyperglycaemia. This fact must undermine much of the current evidence for the inheritance of diabetes on a genetic basis, which rests on comparisons of the incidence of diabetes among the relatives of 'known' diabetics and controls. Our results indicate that if we subjected all the elderly grandmothers and grandfathers to glucose tolerance tests we would find that a very high proportion of the apparently normal relatives of the normal population fulfilled current criteria for the diagnosis of diabetes. Indeed it seems that the chances are loaded against anyone having four 'normoglycaemic' grandparents in the 70s today. These considerations are not of course complete. The smooth distribution curves contain an unknown proportion of persons with

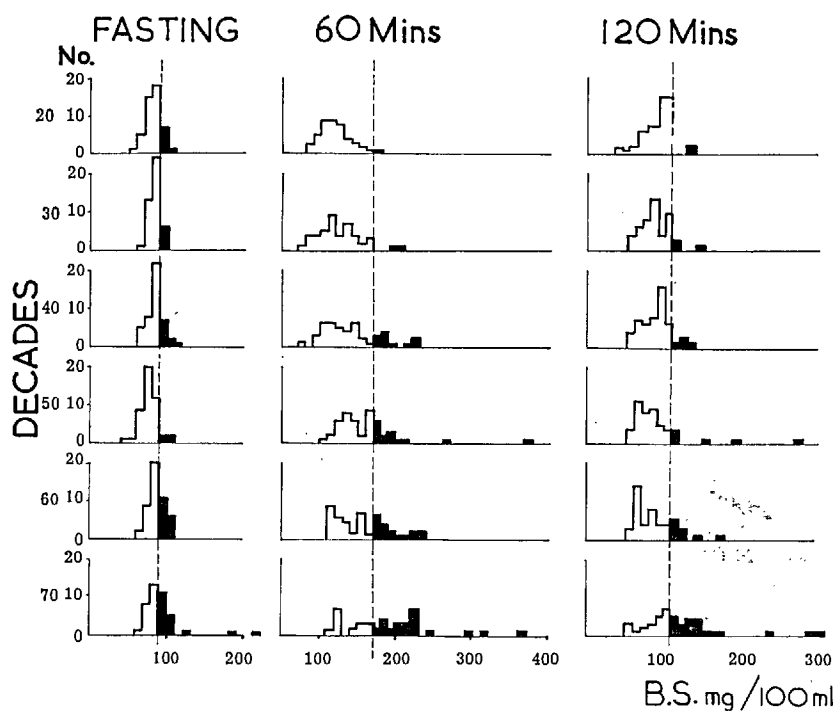


Fig 3 Blood sugars during glucose tolerance in women of random sample, by age (see text)

Table 5
The changing incidence of diabetes

Era	Detection	Treated cases			Untreated cases		Total incidence
		Symptoms: Severe	Mild	None	With symptoms	Without symptoms	
Before biochemistry	Clinical	0.1%	?	?	?	?	0.1%
1900-46	Clinical + biochemistry	0.1%	0.5%	0.1%	?	?	0.7%
1947-61	Clinical + biochemistry + urine surveys	0.1%	0.5%	0.1%	0.25%	0.45%	1.4%
1962-	Clinical + biochemistry + glucose tolerance	0.1%	0.5%	0.1%	up to 3%	up to 9%	up to 12%

decompensated carbohydrate tolerance on the day of their test due to social or physiological factors rather than disease. We should not discard the possibility of inheritance of some factors which cause hyperglycaemia. But the results as a whole do serve to show that all of us are glycaemic, but some are more glycaemic than others. It is possible to consider the diabetic as being older than his chronological age with respect to his blood sugar.

Conclusions

Table 5 indicates the changing incidence of diabetes, depending upon the method of case finding. It also divides the incidence of the disease among various sections of the community – those who have sought medical care, those who have symptoms but have not yet gone to the doctor and those who do not yet have recognizable symptoms, but in whom there may be biochemical abnormalities. Before the era of modern biochemistry diabetes was recognized as a clinical entity, a fatal wasting disease. We do not know the incidence accurately, but it is unlikely that it exceeded 0.1%. Between the turn of the century and 1946, with biochemical techniques applied to the persons in the community who turned to the medical profession for their health care (and that is not everyone) the incidence was taken as 0.7%, of whom approximately 0.1% were diabetics with severe symptoms, 0.5% were milder cases and approximately 0.1% had few, if any, symptoms. The latter were detected as diabetics at insurance examinations or in connexion with some other medical or surgical consultation. In 1947 and 1953, Wilkerson & Krall studied Oxford in Massachusetts and, using urine testing as the screening method, found a latent case for every known diabetic. Of these, we suspect that about a third had symptoms, and in Table 5 I have put the incidence of cases with symptoms as 0.25% and without symptoms as 0.45%. The total incidence of diabetes is given as 1.4%, a twofold increase on

previous values and fourteen times the earlier figure. With the introduction of glucose tolerance testing on a mass scale, another large increase in overall incidence has occurred, as shown in the bottom line, due largely to the detection of widespread intolerance to glucose. How much of this should be attributed to changing social patterns, such as lack of exercise and over-eating in affluent conditions, or to inherent metabolic factors remains to be decided. However, the important point is that we must cater for the appearance of very large numbers of hyperglycaemic patients in the immediate future.

At Bedford the 356 new diabetics represented a load far above that which could be referred to the Diabetic Clinic, and so we sent along only those with blood sugars over 200 mg/100 ml, or with clear evidence of clinical deterioration needing treatment. Rather over 200 persons with two-hour blood sugars between 120 and 200 mg/100 ml were called to special meetings and told that they represented borderline diabetic cases; it was explained that we were not yet sure what sort of therapy to recommend and they were asked to take part in a clinical trial to compare placebo, placebo plus diet, diet plus anti-diabetic therapy (tolbutamide) and antidiabetic therapy alone. This is now going on with additional support from the British Diabetic Association and the Ministry of Health, with Dr Keen and various members of the Department going to Bedford to follow the cases up each Thursday afternoon.

Finally, with all these cases emerging, what can the clinician do when they are found? Marked hyperglycaemia (we suggest two-hour blood sugar over 200 mg/100 ml) with acute symptoms (e.g. polyuria and polydipsia), weight loss and acetonuria indicates an urgent need for treatment and the institution of insulin therapy must be considered. If the two-hour blood sugar is over 200 mg/100 ml, but the symptoms are

milder, such as tolerable thirst, urinary, vascular, ocular or skin manifestations, the patient will need a diet and a trial of oral antidiabetic drugs. If these fail, insulin may be needed sooner or later. If the patient has a known diabetic relative or shows signs of renal or vascular disease or cataract, I think the blood sugar should be controlled to avoid further tissue changes and ischaemia. Obese patients usually respond well to diet, but oral antidiabetic drugs should be used if necessary to secure 'normoglycaemia' in them and in some of the leaner patients. The remainder with two-hour blood sugar over 120 mg/100 ml should be given advice about health, diet and exercise and kept under surveillance every year or two. A bout of over-eating or under-exercising may overtax their endogenous insulin resources and seriously aggravate their hyperglycaemia.

REFERENCES

- Wilkerson H L C & Krall L P
(1947) *J. Amer. med. Ass.* 135, 209
(1953) *J. Amer. med. Ass.* 152, 1322

Dr Harry Keen

(Guy's Hospital Medical School, London)

The Bedford Survey:

A Critique of Methods and Findings

The Bedford Survey provided an opportunity for a critical study of screening methods for identifying undiagnosed diabetics; it also furnished a picture of glucose tolerance in the population at large, and created new problems and ideas, some of which will be dealt with in this paper.

If the aim of the Survey was to identify as suspected diabetics all those people in Bedford whose capillary blood sugar two hours after 50 grams glucose by mouth (two-hour B.S.) would have been 120 mg/100 ml or more, then it failed quite remarkably. Accepting the random sample findings as representative of the population at large, one would have expected to find about 15% of the co-operating population, some 3,900 individuals in this two-hour B.S. class. In fact only 282, 1% of the population, were identified. This huge discrepancy points to inadequacy in the preliminary screening procedure, the 'post-prandial' urine test for sugar. The data of Kenny & Chute (1953) demonstrated the surprisingly small blood sugar rise after breakfast and lunch in a population group. The fact that about two-thirds of the Bedford Survey glycosurics were found to have two-hour B.S. levels below 120 mg/100 ml at glucose tolerance test (G.T.T.) suggests that the 'challenge' of the meal evoked glycosuria less successfully in those with chronic

hyperglycaemia than in those with low renal thresholds or transient hyperglycaemic peaks.

The relationship of glycosuria to the two-hour B.S. is further explored in Table 1 which shows the frequency distribution of two-hour B.S. levels in the 295 members of the random sample whose urines were retested between the sixty-minute period and the ninety-minute period when they came for G.T.T. The Table also shows the two-hour B.S. levels of the 12 survey glycosurics drawn by chance in this sample, and those of the 90 whose urines were positive when retested at G.T.T. It can be seen that a 4% incidence of glycosuria under survey conditions rose to 30% after the 50 gram glucose load at G.T.T. Many more of the people with the higher two-hour B.S. values were glycosuric at G.T.T. than at the survey; of the 50 with two-hour B.S. values of 120 mg/100 ml or more, only 10% were glycosuric at survey while 54% became glycosuric at G.T.T.; of the 10 with levels of 160 mg/100 ml or more, 9 became positive at G.T.T. The glucose load also provoked glycosuria in a large number of people with two-hour B.S. levels below 120 mg/100 ml (3% at survey against 26% at G.T.T.), but analysis of the glucose tolerance curves of these glycosurics showed that 57% of them achieved blood sugar levels of 180 mg/100 ml or more at some stage of the G.T.T. compared with only 16% of non-glycosurics.

Table 1

Two-hour blood sugar and glycosuria in 295 members of random sample

Two-hour blood sugar (mg/100 ml)	No. of persons in group	Positive urine test	
		At survey	At G.T.T.
40-59	5	0	3
60-79	81	2	22
80-99	90	3	23
100-119	69	2	15
40-119	245	7 (3%)	63 (26%)
120-139	31	1	15
140-159	9	0	3
160-179	5	2	5
180-	5	2	4
Over 120	50	5 (10%)	27 (54%)
Total	295	12 (4%)	90 (30%)

The distribution of raised two-hour B.S. levels among G.T.T. glycosurics and non-glycosurics by age and sex, is examined in Fig 1. This shows that a larger proportion of women than of men had a two-hour B.S. of 120 mg/100 ml or more; with increasing age, more glycosurics had raised two-hour B.S. levels (and so, to a lesser extent, did non-glycosurics). Among older people, more

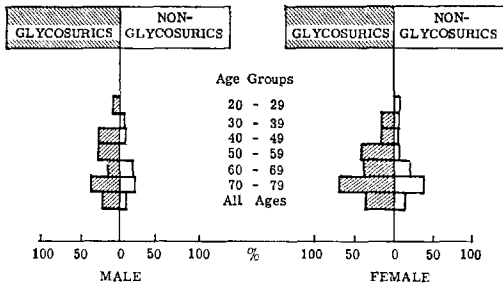


Fig 1 Elevated two-hour blood sugars in G.T.T. glycosurics and non-glycosurics. Horizontal bars denote percentage with two-hour B.S. of 120 mg/100 ml or more

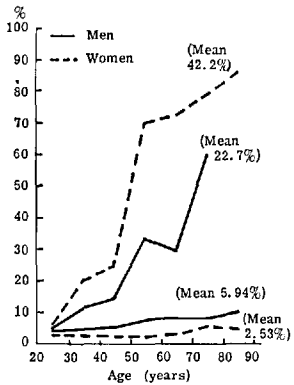


Fig 2 Incidence of glycosuria at survey and of raised two-hour B.S. in glycosurics, by age and sex. Lower two lines show percentage of population with glycosuria. Upper two lines show percentage of glycosurics with two-hour B.S. of 120 mg/100 ml and over

women than men had raised two-hour B.S. levels in the absence of glycosuria. The figure suggests that the chance that glycosuria represents a raised blood sugar level increases with age, and is higher in women than in men. Support for this conclusion is to be found in a similar analysis of the two-hour B.S. levels of all those people found to be glycosuric in the survey (Fig 2). The lower pair of lines show that the incidence of glycosuria was higher in men than women and rose a little with age. By contrast, the proportion of these survey glycosurics with two-hour B.S. values of 120 mg/100 ml or more (upper pair of lines) rose rapidly with age and was higher in women than in men. There are two explanations for these results. The first, and the less likely, is that non-diabetic glycosuria in youth presages diabetes in later life which develops more rapidly in women than in men. A more reasonable explanation is that the renal threshold for glucose rises with age, and that at all ages it is higher in women than men. Thus, as age advances, increasingly high levels of blood sugar are required to produce glycosuria;

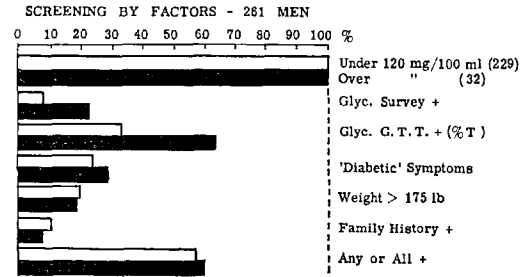


Fig 3A

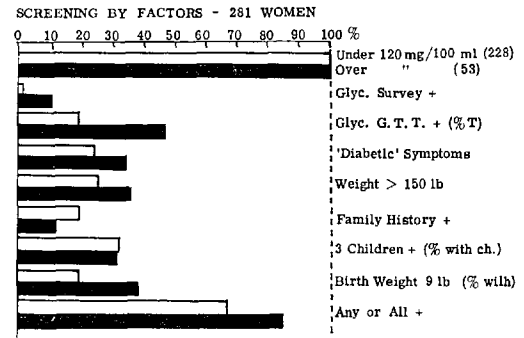


Fig 3B

Fig 3A, B Differentiating value of various factors between people with higher and lower two-hour B.S. levels. Black columns represent incidence of factor shown on right among those with two-hour B.S. of 120 mg/100 ml or more, and white columns among those with two-hour B.S. less than 120 mg/100 ml. Rates calculated from random sample data

Glyc. Survey+ = glycosuric at main survey
Glyc. G.T.T. + (%T) = glycosuric at G.T.T. as % of the 295 retested

'Diabetic' Symptoms = thirst, polyuria, recent visual change, skin sepsis

Family History+ = positive family history of diabetes mellitus. 3 Children + (% with ch.) = women with 3 live births or more, as % of all women with children

Birth Weight 9 lb + (% with) = women with baby of 9 lb or more at birth, as % of all women with babies

at any age, the level requires to be higher in women than in men. These relationships have importance in judging the significance of a positive urine test, for, while glycosuria in a young man carries only a 5% chance of representing the higher two-hour B.S. levels, in older women the chance is about 80%.

These limitations of urine testing led us to examine other factors which might have differentiated between people with higher and lower two-hour B.S. levels. Figs 3A and 3B show graphically, for men and women respectively, the differentiating value of a number of these factors taken from the random sample data. For

comparison, the efficiency of the urine test is also expressed in the same way. The length of the black bar represents the proportion of people with two-hour B.S. levels of 120 mg/100 ml or more who screened positive to the particular factor; ideally it should be 100%. The degree to which it falls short of this value represents the false negatives. The proportion of false positives is shown by the length of the white bar which, ideally, should be 0%. In men, none of the factors examined had discriminating value, and among the women only the history of bearing a baby with a birth weight of 9 lb or more pointed strongly to a high two-hour B.S. It remains to be seen whether different 'settings' of the dividing blood sugar level, or values of the screening factors would yield more useful discrimination. To identify the group with two-hour B.S. of 120 mg/100 ml and over, we conclude that there is little alternative to direct measurement of the blood sugar; for this, a simple blood sugar screening device, perhaps along the lines of the Clinistix, would be invaluable.

It must be emphasized that our choice of a two-hour B.S. of 120 mg/100 ml as a dividing level was quite arbitrary. Among people in the random sample there was no obvious two-hour B.S. level which recommended itself instead. While the apparently continuous distribution of two-hour B.S. values across the range regarded as demarcating normal from abnormal in this general population sample raises fascinating speculations about the nature of diabetes and its aetiology, the critical question for the clinician is: 'Who needs treatment?' An operational diagnosis has to be made on the basis of the blood sugar results. Harting & Glenn (1951) left '... the decision concerning the degree of hyperglycaemia and glycosuria necessary for a definite diagnosis of diabetes ... to the judgment of the person's physician'. We argued along the following lines: All clinicians would accept a two-hour B.S. of 200 mg/100 ml or more as falling into the diagnostic category 'diabetes mellitus', so we sent people with these levels to their family doctors with a definite diagnosis and a recommendation that they should be referred to the local diabetic clinic. A two-hour B.S. of less than 120 mg/100 ml would be accepted as excluding the diagnosis and we told people in this class that they were normal at present.

People with intermediate values (two-hour B.S. 120–199 mg/100 ml) were interviewed in small groups and their 'borderline' status explained to

them. With their agreement, we started for them a special 'borderline clinic' in Bedford, where they have been seen and examined at six-monthly intervals since the survey ended. They have been allocated at random to one of a number of treatment groups, and at each clinic visit special attention is being paid to the eyes, kidneys, peripheral nerves and blood vessels, along with repeated measurements of two-hour B.S., and other biochemical factors. Similar observations have been made on a matched control group composed of members of the random sample who were proved 'normal' in respect of the blood sugar at G.T.T.

We hope that our study of the 'borderline' group will, in time, answer the following questions: (1) In what ways, if any, do 'borderline' diabetics differ from normals? (2) Is there a critical level of blood sugar at which 'diabetic damage' starts? (3) If so, will treatment postpone or prevent the damage?

Conclusions

The Bedford Survey has emphasized the insensitivity of urine testing as a preliminary screening procedure in the identification of undiagnosed diabetics. Sensitivity may be greatly improved by testing the urine after a standard 50 g glucose load, but this has the disadvantage of yielding many false positive results. Most of these are among younger people, particularly men, and this probably reflects the fact that the renal threshold for glucose is lower in men than women and that it rises with age. As a screening procedure, blood sugar measurement after a glucose load is the method of choice.

Findings in the random sample of the population suggest that there is a large number of people with marginal elevation of blood sugar levels who are not readily assignable to either diabetic or normal categories. Careful evaluation of such people by means of controlled trials of treatment are of high importance, for not only may they be candidates for the development of symptomatic diabetes but it also seems possible to us that the widespread distribution of arterial disease and cataract in the population at large may in part be related to an unrecognized 'borderline' diabetic state.

REFERENCES

- Harting D & Glenn B (1951) *New Engl. J. Med.* 245, 48
 Kemy A J & Chute A L (1953) *Diabetes. N.Y.* 2, 187

Put X in appropriate box

(1)

APPENDIX 2

CARDIOVASCULAR QUESTIONNAIRE

Office use

IDENTIFICATION

Surname Country

1 ☐

Forenames Survey

2 ☐

Sample

3 ☐

Subject

4-7 ☐

Sex: Male ☐ 1

8 ☐

Female ☐ 2

Day Month Year

Date of birth Age at interview ☐

9-10 ☐

Nativity

11 ☐

Race.. .. .

12 ☐

Civil state

S M W Div Sep
1 2 3 4 5

13 ☐

Interviewer

14 ☐

Date of interview Day Month Year

15 ☐

16 ☐

17 ☐

18 ☐

19 ☐

20 ☐

SECTION A: EFFORT PAIN

Yes No

HAVE YOU EVER HAD ANY PAIN OR DISCOMFORT IN YOUR CHEST?

☐ ☐

21 ☐

If no, HAVE YOU EVER HAD ANY PRESSURE OR HEAVINESS IN YOUR CHEST?

☐ ☐

22 ☐

If no, proceed to Section B. If yes, ask next question.

If during the remainder of Section A an answer is recorded in a box marked *, proceed to Section A (optional) without asking any more Section A questions.

DO YOU GET IT WHEN YOU WALK UPHILL OR HURRY?

Yes ☐ 1

23 ☐

No ☐ *

Never hurries or walks uphill ☐ 2

Record yes if either walking uphill or hurrying causes pain or discomfort.

Yes No

DO YOU GET IT WHEN YOU WALK AT AN ORDINARY PACE ON THE LEVEL?

☐ ☐

24 ☐

If yes to either of last two questions,

WHAT DO YOU DO IF YOU GET IT WHILE YOU ARE WALKING?

Stop or slow down ☐ 1

Carry on ☐ 0

Record stop or slow down if subject carries
on after taking nitroglycerine (trinitrin).

IF YOU STAND STILL, WHAT HAPPENS TO IT? Relieved ☐ 1

Not relieved ☐ 0

HOW SOON? 10 minutes or less ☐ 1

More than 10 minutes ☐ 0

WILL YOU SHOW ME WHERE IT WAS?



Sternum (upper or middle)

Sternum (lower)

Left anterior chest

Left arm

Other

If other, mark on diagram.

DID YOU FEEL IT ANYWHERE ELSE?

If yes, record additional information above.

Yes No

--	--

25

☐

--	--

26

☐

--	--

27

☐

--	--

28

☐

--	--

29

☐

--	--

30

☐

--	--

31

☐

Yes No

--	--

32

☐

--	--

33

☐

SECTION A (OPTIONAL)

HAVE YOU HAD THIS PAIN OR DISCOMFORT MORE THAN THREE TIMES?

If no, proceed to Section B.

DOES ANY OTHER KIND OF EXERTION BRING IT ON? (Specify)

DO ANY OF THESE THINGS TEND TO BRING IT ON:

PLEASE SAY YES OR NO TO EACH -

- EXCITEMENT OR EMOTION?

- STOOPING?

- EATING?

- BREATHING?

- COLD WIND?

- COUGHING?

Yes No

--	--

34

☐

--	--

35

☐

--	--

36

☐

--	--

37

☐

--	--

38

☐

--	--

39

☐

--	--

40

☐

IS IT WORSE IF YOU HAVE A CHEST COLD OR BAD COUGH?

WOULD YOU DESCRIBE IT AS A PAIN OR A DISCOMFORT? Pain ☐ 1

Discomfort ☐ 2

41

☐

42

☐

43

☐

WOULD ANY OF THESE WORDS DESCRIBE THE SENSATION:

PLEASE SAY YES OR NO TO EACH -

- HEAVINESS?

Yes No

--	--

44

☐

- BURNING?

--	--

45

☐

- TIGHTNESS?

--	--

46

☐

- STABBING?

--	--

47

☐

- PRESSURE?

--	--

48

☐

SECTION B: PAIN OF POSSIBLE INFARCTION

HAVE YOU EVER HAD A SEVERE PAIN ACROSS THE FRONT OF
YOUR CHEST LASTING FOR HALF AN HOUR OR MORE?

Yes No

--	--

49

☐

If yes,

HOW MANY OF THESE ATTACKS HAVE YOU HAD?

50

☐

Date

Duration

Other information

1st attack _____

Last attack _____

Yes No

--	--

51

☐

HAVE YOU EVER HAD AN ELECTRICAL RECORDING OF YOUR HEART (ECG)?

If yes, WHERE? _____

WHEN? _____

Yes No

--	--

52

☐

DID YOU SEE A DOCTOR BECAUSE OF THIS PAIN?

If yes, WHAT DID HE SAY IT WAS? _____

SECTION C: INTERMITTENT CLAUDICATION

If an answer is recorded in a box marked *, no further
questions need be asked.

DO YOU GET PAIN IN EITHER LEG ON WALKING?

Yes No

	*
--	---

53

☐

DOES THIS PAIN EVER BEGIN WHEN YOU ARE STANDING STILL
OR SITTING?

*	
---	--

54

☐

IN WHAT PART OF YOUR LEG DO YOU FEEL IT?

Pain includes calf/calves

--

 1

55

☐

Pain does not include calf/calves

*

 0

If calves not mentioned, ask: ANYWHERE ELSE?

DO YOU GET IT IF YOU WALK UPHILL OR HURRY?

Yes

--

1

56

☐

No

*

0

Never hurries or walks uphill

--

2

(4)

DO YOU GET IT IF YOU WALK AT AN ORDINARY PACE ON THE LEVEL?

Yes	No
☐	☐

Office use

57 ☐

If yes to either of last two questions,

DOES THE PAIN EVER DISAPPEAR WHILE YOU ARE STILL WALKING?

☐	☒
--------------------------	-------------------------------------

58 ☐

WHAT DO YOU DO IF YOU GET IT WHEN YOU ARE WALKING?

stop or slacken pace

☐	1
--------------------------	---

Carry on

☒	0
-------------------------------------	---

59 ☐

WHAT HAPPENS TO IT IF YOU STAND STILL?

Relieved

☐	1
--------------------------	---

Not relieved

☒	0
-------------------------------------	---

60 ☐

HOW SOON?

10 minutes or less

☐	1
--------------------------	---

More than 10 minutes

☐	0
--------------------------	---

61 ☐

Conclusion

Effort pain If yes to (a)28 or (b)29 or (c)30 and 31:

YES

☐

62 ☐

If no to 24: GRADE 1

☐	1
--------------------------	---

If yes to 24: GRADE 2

☐	2
--------------------------	---

NO

☐	0
--------------------------	---

"Possible infarction"

If yes to 49: YES

☐	1
--------------------------	---

63 ☐

If no to 49: NO

☐	0
--------------------------	---

Intermittent claudication If 10 mins. or less to 61:

YES

☐

64 ☐

If no to 57: GRADE 1

☐	1
--------------------------	---

If yes to 57: GRADE 2

☐	2
--------------------------	---

If more than 10 mins. to 61: NO

☐	0
--------------------------	---

Oral Glucose Tolerance and Related Factors in a Normal Population Sample

I. Blood Sugar, Plasma Insulin, Glyceride, and Cholesterol Measurements and the Effects of Age and Sex

D. R. BOYNS,* M.B., M.R.C.P., D.C.H.; J. N. CROSSLEY,† M.B., B.S., B.Sc.; M. E. ABRAMS,‡ M.B., B.Sc., M.R.C.P.
R. J. JARRETT,* M.D.; H. KEEN,§ M.B., B.S., M.R.C.P.

Summary: Oral glucose tolerance tests were performed on 220 people, a representative sample of the employees of a large pharmaceutical company. Blood sugar and plasma immunoreactive insulin levels were measured on each sample of venous blood obtained before and at half-hourly intervals for two hours after 50 g. of glucose by mouth; plasma cholesterol and glycerides were measured on the fasting sample only.

Women had higher mean insulin levels throughout the test, though their mean blood sugar levels were higher only at 90 and 120 minutes. In both sexes there were positive correlations between age and the levels of blood sugar, plasma cholesterol, and plasma glycerides. Though the levels of glucose rose with age, those of insulin did not.

Introduction

During a diabetes screening survey in a large British pharmaceutical company the opportunity was taken to perform oral glucose tolerance tests on a representative sample of the employees in order to study the interrelationships of the circulating glucose and insulin responses to a glucose load with a number of other factors. In this paper the methodology of the study is described and the results in relation to age and sex are presented. In the accompanying paper an analysis of the interrelationships is presented.

Methods

Population.—The diabetes survey was carried out on the premises of Beecham Laboratories Ltd., where the population was mixed, containing people engaged in managerial, clerical, and both skilled and unskilled manufacturing occupations. Altogether 961 employees (84% of the total) took part in the screening survey, which also had a research aspect—namely, the study of various methods of preparation before tolerance testing. A total of 919 people were tested on two occasions, and this co-operating population was further asked to volunteer for the glucose tolerance tests of the present study. From the volunteers, 220 people were selected by a stratification procedure in such a way that there were about equal numbers in each age and sex group (Table I) and an even distribution of body build in the groups. The latter was achieved by calculating the "ponderal index" ($\text{height}^2 \div \text{weight}$) for all the

individuals in each age/sex group, then randomly selecting about equal numbers from the upper, middle, and lower tertiles of the distributions. Known diabetics and pregnant women were excluded before test, but no exclusions were made on the basis of the test results themselves.

TABLE I.—Age and Sex Composition of the Population Sample

Age	Men	Women
24 and below	24	22
25-34	22	22
35-44	20	20
45-54	22	23
55+	21	24
Mean	39.43	38.95
Range	16-74	15-63
Total No.	109	111

Testing Procedure.—After a clear explanation of the research nature of the examinations proposed, members of the subsample were requested to take extra carbohydrate in addition to their normal diet for three days, until the evening before the test, and to present themselves, fasting, at about 9 a.m. on the morning of the test. Venous blood samples were collected before and at 30, 60, 90, and 120 minutes after an oral load of liquid glucose (235 ml. of Lucozade, equivalent to 50 g. of anhydrous glucose). Blood was taken by repeated venepuncture through a tiny skin bleb of lignocaine (without adrenaline), put into chilled tubes containing heparin and Versenate, then carefully mixed and centrifuged at 2,000 r.p.m. for 15 minutes. The supernatant plasma was stored at -20°C . and not thawed until required for estimation.

Laboratory Methods

Blood Sugar.—This was measured on 0.1-ml. aliquots of the freshly drawn venous blood taken before centrifugation and diluted in 3 ml. of 1% aqueous solution of potassium fluoride. Estimations were carried out on the AutoAnalyzer (Technicon) by the microferricyanide reduction method (Whiclow *et al.*, 1967).

Plasma Immunoreactive Insulin.—Radioimmunoassay of the plasma samples against human insulin standard curves (human insulin was kindly supplied by Novo Industri A.S.) was carried out by a modification of the two-antibody method of Morgan and Lazarow (1963). The modification was minor, consisting chiefly of miniaturization, so that plasma samples of 0.1 ml. only were required. In addition, edetic acid was added to the buffer, as recommended by Sheldon and Taylor (1965). Estimates were carried out at least in duplicate. When plasma samples required dilution owing to high values, several dilution

* Lecturer, Department of Medicine.
† Lecturer, Department of Physiology.
‡ Senior Lecturer, Department of Medicine.
§ Reader in Medicine.
Guy's Hospital, London S.E.1.

levels were assayed to ensure parallel slope conditions. Assays were arranged so that complete "sets" of samples were measured in the same experimental run and so that, so far as was possible, all the stratification groups were equally represented in each run. The standard error of the assay was about 5% of the value right across the operative range of the assay.

Glycerides.—Plasma glycerides were estimated by a semi-automated procedure based on the method of Lofland (1964). Aliquots of the isopropanol extract of plasma were treated with 0.1 N ethanolic KOH at 70° C. for 20 minutes in order to hydrolyse the glycerides and the free glycerol was estimated on the AutoAnalyzer by the chromotropic acid method of

Lambert and Neish (1950). A measure of the reproducibility of the method was obtained from 10 estimations of the glyceride concentration on a pooled serum sample. These gave a mean serum glyceride of 73 mg./100 ml. (S.D. ± 4.8 mg./100 ml.), which did not differ significantly from the mean of 10 gravimetric serum glyceride estimations on the same sample (71 ± 4.4 mg./100 ml.).

Cholesterol.—The total plasma cholesterol was estimated by the automated method of Block *et al.* (1966). When applied to the pooled serum sample, 10 estimations gave a mean value 185 mg./100 ml. (S.D. ± 7.6 mg./100 ml.), which compared well with 10 gravimetric estimations on the same sample (182 ± 6.3 mg./100 ml.) (data of MacDonald and Jordan, (personal communication).

Data analysis was performed by means of an IBM 360/65 computer at University College, London. The necessary programmes were written in Fortran IV and use was also made of the BMD (Biomedical) computer programmes (Dixon, 1965).

Results

Blood Sugar and Plasma Insulin

The frequency distribution of blood sugar and plasma insulin values for the sexes separately are shown as histograms in Figs. 1 and 2, which also give the mean values, standard deviations, and range. The distribution of the plasma insulin values is positively skewed, but logarithmic transformation (Fig. 3) makes the distribution more nearly normal, a phenomenon previously reported by Welborn *et al.* (1966) and Chlouverakis *et al.* (1967). The mean blood sugar and plasma insulin values, by sex, are shown graphically in Figs. 4 and 5. The blood sugar values in men are greater at 0, 30, and 60 minutes but less at 90 and 120 minutes. These differences are statistically significant at 30 ($0.02 > P > 0.01$), 60 ($0.05 > P > 0.02$),

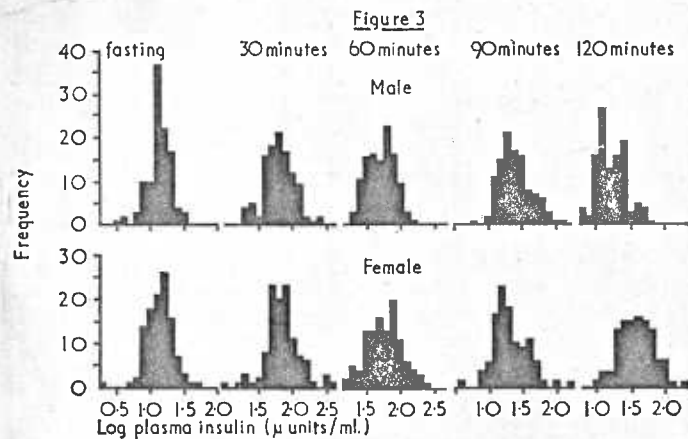
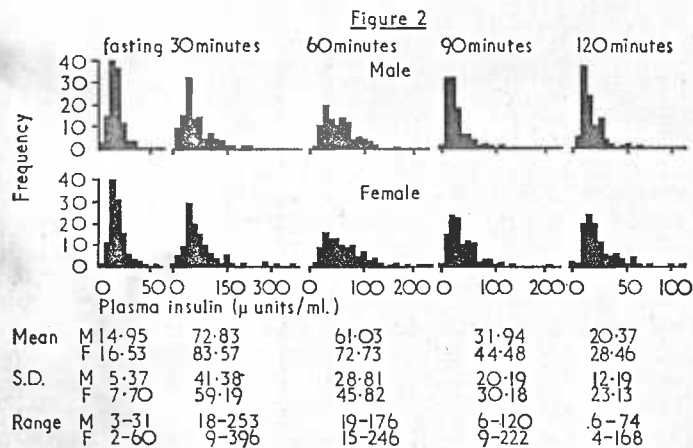
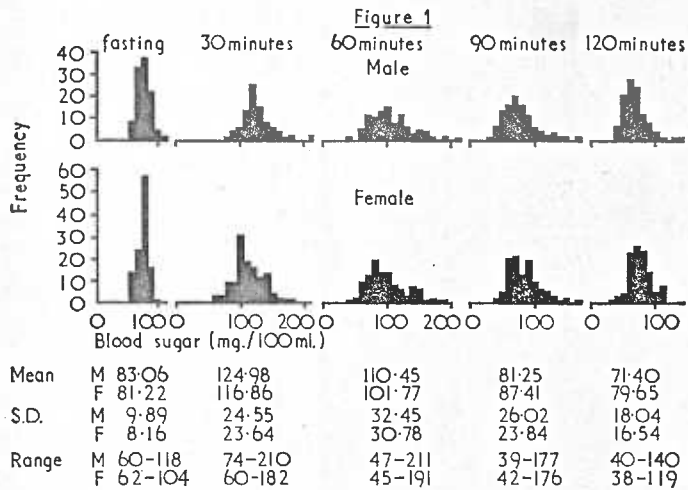


FIG. 1.—Histograms showing the frequency distribution by sex of blood sugar values at the five time points of the glucose tolerance test. FIG. 2.—Histograms showing the frequency distribution by sex of plasma insulin values at the five time points of the glucose tolerance test. FIG. 3.—Frequency distribution of plasma insulin values after logarithmic transformation.

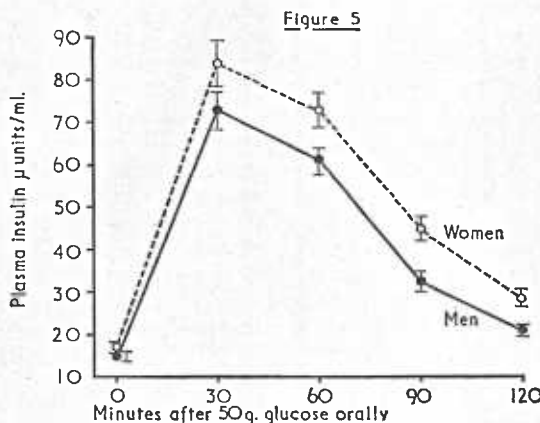
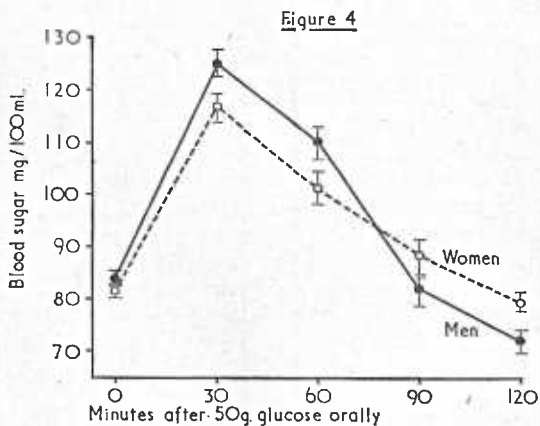


FIG. 4.—The mean ($\pm$ S.E.M.) levels of blood sugar by sex. FIG. 5.—The mean ($\pm$ S.E.M.) levels of plasma insulin by sex.

TABLE II.—Mean Blood Sugar Values (with Standard Deviation) at the Five Time Points of the Glucose Tolerance Test by Age and Sex

Age	Sex	n	Blood Sugar at									
			0 min.		30 min.		60 min.		90 min.		120 min.	
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
24	M	24	78.8	5.4	108.5	16.5	86.5	19.1	68.0	17.2	67.1	14.1
	F	22	80.1	7.3	113.5	21.4	91.5	22.2	86.0	17.6	81.7	13.8
25-34	M	22	81.3	10.4	120.5	25.9	97.5	23.8	73.2	20.7	68.3	13.4
	F	22	82.6	8.4	109.4	20.2	88.1	17.8	79.9	16.9	80.7	10.9
35-44	M	20	84.8	8.7	124.5	15.7	113.5	30.5	84.8	16.9	76.0	13.3
	F	20	78.7	7.7	112.9	22.8	101.2	39.1	89.4	28.9	77.0	18.0
45-54	M	22	85.4	11.0	133.9	27.5	122.5	31.0	86.8	28.0	71.9	20.2
	F	23	80.8	8.1	120.6	29.4	112.4	34.0	90.8	29.2	76.4	17.1
55+	M	21	85.8	11.8	139.7	23.4	135.9	32.5	95.6	35.1	74.6	26.1
	F	24	83.5	8.9	126.5	21.0	114.0	29.5	90.8	24.2	82.1	21.2

TABLE III.—Mean Plasma Insulin Levels (and Standard Deviation) at the Five Time Points of the Glucose Tolerance Test by Sex and Age Group

Age	Sex	n	Plasma Insulin at									
			0 min.		30 min.		60 min.		90 min.		120 min.	
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
24	M	24	15.2	4.4	72.5	35.9	47.3	17.1	25.2	10.3	17.9	7.0
	F	22	19.0	12.3	101.3	89.4	74.0	48.3	55.7	47.9	33.3	27.5
25-34	M	22	14.7	4.4	75.6	35.8	59.4	27.1	27.7	22.7	16.7	6.0
	F	22	17.6	5.7	75.7	44.1	60.9	43.7	35.4	16.1	31.2	33.2
35-44	M	20	15.7	4.7	78.8	54.2	65.4	30.2	36.4	19.1	20.8	11.2
	F	20	14.9	6.9	86.0	62.1	72.9	52.6	46.0	27.5	24.8	14.3
45-54	M	22	16.6	6.8	72.6	45.4	70.8	34.5	36.8	23.2	24.6	16.9
	F	23	14.6	5.2	76.1	36.7	74.1	38.6	43.3	24.5	23.7	12.5
55+	M	21	12.5	5.8	64.9	36.8	64.0	29.9	34.7	22.3	22.2	15.6
	F	24	16.5	6.1	79.6	52.6	81.0	47.5	42.3	24.7	29.2	21.9

TABLE IV.—Correlation of Age with Blood Sugar, Plasma Insulin, and Log Insulin

Time (min.)	Men (n=109)						Women (n=111)					
	Blood Sugar		Insulin		Log Insulin		Blood Sugar		Insulin		Log Insulin	
	R	P	R	P	R	P	R	P	R	P	R	P
0	0.241	<0.02	-0.1	—	-0.183	—	0.073	—	-0.182	—	-0.133	—
30	0.440	<0.001	-0.056	—	-0.091	—	0.213	<0.05	-0.134	—	-0.096	—
60	0.535	<0.001	0.202	<0.05	0.191	—	0.309	<0.01	0.064	—	-0.091	—
90	0.356	<0.001	0.161	—	0.144	—	0.118	—	-0.105	—	-0.051	—
120	0.124	—	0.166	—	0.066	—	-0.043	—	-0.110	—	-0.091	—

and 120 ($P<0.001$) minutes. By contrast, women have higher insulin values throughout the test, the differences reaching statistical significance at 60 ($P=0.02$), 90 ($P<0.001$), and 120 ($P<0.001$) minutes.

In Tables II and III the blood sugar and plasma insulin values, respectively, are given for each time point of the tolerance test and also in relation to age. In both men and women the mean blood sugar level (Table II) tends to rise with age at each time point except at 120 minutes. When linear correlation coefficients of age against blood sugar were calculated, statistically significant associations were found at 0, 30, 60, and 90 minutes for the men and at 30 and 60 minutes for the women. By contrast, plasma insulin levels, both arithmetic and logarithmic (Table III), did not show any significant trends with age. The correlation coefficients are given in Table IV.

Glycerides

The mean levels of fasting plasma glyceride by age and sex are given in Table V. The mean level in each age group is higher for the men. In both sexes there was a trend upwards with age, which was highly significant for the men ($r=0.414$; $P<0.001$) but only just significant for the woman ($r=0.204$; $P<0.05$). This rise in glycerides with age is similar to that found by Schaefer (1964) and Fredrickson *et al.* (1967), who also noted higher levels in men.

Cholesterol

The mean levels of total plasma cholesterol by age and sex group are shown in Table VI. The mean values, which are strikingly similar to those of Fredrickson *et al.* (1967), rise with age in both sexes.

TABLE V.—Mean Fasting Plasma Glyceride Levels (With Standard Deviation) by Age and Sex

Age	Plasma Glyceride (mg./100 ml.)					
	Men			Women		
	Glyceride	S.D.	n	Glyceride	S.D.	n
<24	77.7	16.9	23	75.8	30.6	21
25-34	83.3	26.4	17	73.0	22.9	21
35-44	96.6	21.2	18	88.6	27.0	17
45-54	101.0	36.0	22	84.7	26.0	18
55+	109.0	27.7	20	89.9	27.3	19
Overall mean	93.4	28.5	100	81.9	27.2	96
Range	47-210			36-185		

TABLE VI.—Mean Fasting Plasma Cholesterol Levels (With Standard Deviation) by Age and Sex

Age	Total Plasma Cholesterol (mg./100 ml.)					
	Men			Women		
	Cholesterol	S.D.	n	Cholesterol	S.D.	n
<24	190.3	41.3	22	192.3	30.7	22
25-34	212.4	37.7	17	191.8	27.0	20
35-44	235.8	31.7	17	219.9	32.4	17
45-54	249.1	35.7	21	238.8	48.7	18
55+	258.4	43.2	20	249.1	40.1	19
Mean	228.9	45.6	97	216.9	42.9	96
Range	133-328			152-363		

Discussion

Analysis of the blood sugar data confirms the different "shape" of the mean glucose tolerance curve in men and women previously described by Butterfield (1964), Keen (1964), and Sharp (1964) and the effect of age on oral glucose tolerance

(Butterfield, 1964 ; Gordon, 1964 ; Keen, 1964 ; Sharp, 1964). In both men and women the mean blood sugar two hours after the glucose load was unrelated to age, at least up to the seventh decade. This contrasts with the peak blood sugars, but confirms the results of the glucose tolerance tests performed on the Bedford random population sample, in which a mean two-hour blood sugar rise was observed in only the over-70 age group (Butterfield, 1964 ; Keen, 1964 ; and Sharp, 1964). This fact affects the choice of the one-hour or two-hour blood sugar sample for population screening for diabetes, a topic which is discussed in detail elsewhere (Keen and Jarrett, 1969).

There is a considerable literature concerning the levels of blood sugar and plasma insulin, both fasting and after a glucose load, but the insulin values reported in this paper are the first, to our knowledge, to be based on a normal population sample. Most reports are of small series (see Buchanan and McKiddie, 1967, for references), and direct comparison of reported levels is difficult because of differences in technique of measurement and the lack of universal insulin standards. In general, however, it has been found that the shape of the insulin curve parallels that of the blood sugar curve and that the distribution of the insulin levels is log normal.

The present study shows a sex difference in plasma insulin levels after a glucose load, not obviously attributable to differences in blood sugar levels. Thus women had higher mean insulin levels throughout the tolerance test, though their mean blood sugar levels were higher only at 90 and 120 minutes. The difference also could not be attributed to the effects of oral contraceptives, for only four women were using them at the time. In addition, one postmenopausal woman was receiving oestrogens.

In this study there was little evidence of a positive relationship between age and insulin levels, with the single exception of the peak levels in men. This is apparently at variance with an earlier study (Chlouverakis *et al.*, 1967) in which serum insulin was found to rise with age in a control group, but not in a group of borderline diabetics. This rise with age was statistically significant only when the fasting levels were considered, but a similar trend was apparent at one hour.

There are several differences between the two studies, however. Firstly, and probably most important, the control population in the first study was preselected by virtue of having a blood sugar less than 120 mg./100 ml. two hours after 50 g. of glucose on two occasions. Perhaps because of this selection, in this control population, blood sugar levels did *not* rise with age, a phenomenon which could be related, or even attributed, to the rise with age of circulating levels of insulin. This hypothesis is reinforced by the study of Hales *et al.* (1968), in which a positive correlation was found between age and insulin levels at 60 and 90 minutes after oral glucose. This was again

in a selected population, with normal glucose tolerance, in which blood glucose levels did not rise with age. The second major difference lies in the timing of the glucose tolerance tests. In the earlier study they were performed in the afternoon, and it has since become apparent that afternoon glucose tolerance and insulin responses may differ considerably from morning ones (Bowen and Reeves, 1967 ; Jarrett and Keen, 1968 ; Malherbe *et al.*, 1968).

At present little is known about the diurnal variation in insulin response to glucose. It is likely that it will be quantitatively rather than qualitatively different, and if that is true then the results of the present study and those of Chlouverakis *et al.* (1967) and of Hales *et al.* (1968) may be combined. The conclusion with respect to age is that, in the whole population, insulin levels do not rise with age, though glucose levels do. However, in selected subgroups, in which there is no positive correlation between age and blood glucose levels, there is discernible a positive correlation, albeit of low degree, between age and insulin levels. As we suggested earlier (Chlouverakis *et al.*, 1967), "older people, maintaining the same blood sugar levels as young ones, only do so under the influence of elevated circulating levels of insulin."

REFERENCES

- Block, W. D., Jarrett, K. J., jun., and Levine, J. B. (1966). *Clin. chim. Acta*, **12**, 681.
- Bowen, A. J., and Reeves, R. L. (1967). *Arch. intern. Med.*, **119**, 261.
- Buchanan, K. D., and McKiddie, M. T. (1967). *Diabetologia*, **3**, 460.
- Butterfield, W. J. H. (1964). *Proc. roy. Soc. Med.*, **57**, 196.
- Chlouverakis, C., Jarrett, R. J., and Keen, H. (1967). *Lancet*, **1**, 806.
- Dixon, W. J. (editor) (1965). *Biomedical Computer Programs*. University of California, Los Angeles.
- Fredrickson, D. S., Levy, R. I., and Lees, R. S. (1967). *New Engl. J. Med.*, **276**, 148.
- Gordon, T. (1964). *Vital and Health Statistics*, Series 11, No. 2. Washington.
- Hales, C. N., Greenwood, F. C., Mitchell, F. L., and Strauss, W. T. (1968). *Diabetologia*, **4**, 73.
- Jarrett, R. J., and Keen, H. (1968). Communication to the Medical and Scientific Section of the British Diabetic Association, Aberdeen, September 27-28.
- Keen, H. (1964). *Proc. roy. Soc. Med.*, **57**, 200.
- Keen, H., and Jarrett, R. J. (1969). "Modern Concepts in the Diagnosis of Diabetes" in *The New Management of Stable Adult Diabetes*, edited by S. Weller. Springfield, Illinois.
- Lambert, M., and Neish, A. C. (1950). *Canad. J. Res.*, **28**, Section B, p. 83.
- Lofland, H. B. (1964). *Analyt. Biochem.*, **9**, 393.
- Malherbe, C., de Gasparo, M., Heller, F., and Hoet, J. J. (1968). 4th Meeting of the European Association for the Study of Diabetes, Louvain, July 23-26.
- Morgan, C. R., and Lazarow, A. (1963). *Diabetes*, **12**, 115.
- Schaefer, L. E. (1964). *Amer. J. Med.*, **36**, 262.
- Sharp, C. L. (1964). *Proc. roy. Soc. Med.*, **57**, 193.
- Sheldon, J., and Taylor, K. W. (1965). *J. Endocr.*, **33**, 157.
- Welborn, T. A., Rubenstein, A. H., Haslam, R., and Fraser, R. (1966). *Lancet*, **1**, 280.
- Whiclow, M. J., Wigglesworth, A., Cox, B. D., Butterfield, W. J. H., and Abrams, M. E. (1967). *Diabetes*, **16**, 219.