DIABETES, THIOL SUBSTANCES AND DIABETOGENS*

JOHN K. MCKECHNIE, B.SC. (HONS.), M.D. (RAND), M.R.C.P. (EDIN.)

Medical Registrar, Diabetic Clinic, King Edward VIII Hospital, Durban

Diabetes is by no means a new clinical problem. I well remember visiting the tombs at Sakkara in Egypt in 1945, and being shown a mural which depicted Betahotep, the Egyptian physician, tasting a specimen of urine in order to test it for glycosuria! Times may have changed since then, and also our ideas on diabetes, but the concept that the pathogenesis of diabetes is related to some form of intoxication of normal body function is by no means new. In this paper I hope to review, very briefly, some present-day concepts.

INTRODUCTORY DEFINITIONS

Diabetes Mellitus

There is no completely satisfactory textbook definition of diabetes mellitus, and for the purposes of this paper my definition is based on that in Simpson's textbook.[20] Diabetes mellitus is a chronic metabolic disorder characterized by deficiency of insulin which may be absolute or relative, and manifested clinically by a syndrome of altered carbohydrate, protein and fat metabolism (i.e. hyperglycaemia, glycosuria, protein depletion and wasting of tissues in some cases, gluconeogenesis, and ketosis). It is also associated with alterations in mucopolysaccharide metabolism affecting the blood vessels, eyes, kidneys and nerves, and these changes are at present poorly understood.

'Thiols'

These are chemical substances which have an alcoholic configuration, but in which the oxygen of the alcoholic grouping is replaced by sulphur, e.g. R-OH = alcohol structure, R-SH = thiol structure; $CH_3.OH$ = methyl alcohol, R-SH = mono-thiol; and $CH_3.CH_2.OH$ = ethyl alcohol, R.SH.SH = di-thiol.

The example of a monothiol with which we are most familiar in medicine is methyl mercaptan. This is one of the substances which is present in the urine and the breath of a patient with hepatic failure ($CH_3.SH$), and which gives

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the characteristic asparagus-like smell. Other thiols are co-enzyme A (CoA-SH) and glutathione. An example of a dithiol is 2, 3-dimercapto-propanol, best known as 'BAL' (British Anti-lewisite), and this is a dithiol derivative of propionic acid.

Thiol substances are often referred to as mercaptans because of their ability to combine with mercury ('capture mercury'),[1] and this is the basis of the use of BAL in mercury and heavy-metal poisoning.[2]

The fact that thiol groups are oxidizable and will combine with heavy metals has been known for many years, and Ehrlich, in 1909, actually suggested that this was the reason for the biological action of arsenic, mercury and their compounds. Later work with trypanosomes and spirochaetes showed that thiol compounds, especially glutathione and the sulphur-containing amino acid, cysteine, protected these organisms against the action of bismuth, arsenic and mercury. It has been suggested that mercurial diuretics produce their action on the kidney by combining with the SH groups present in the enzymes of the tubules, thereby impairing their function and causing diuresis. In the same way, lewisite, which is an arsenical poison, acts by blocking the pyruvate-oxidase enzyme system, which depends on thiamine in a protein-magnesium-cocarboxylase complex, and which is one of the important enzyme systems in carbohydrate metabolism. BAL, in arsenical poisoning, provides sulphydryl groups which reactivate the 'poisoned' enzyme-SH groups.

SIMPLE CHEMISTRY

There are 8 essential amino acids (leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine),[62] and only one of these, methionine, contains sulphur. Two other amino acids which contain sulphur are cysteine, and its condensation product, cystine.

Proteins are formed by the chemical combination of amino acids by peptide linkages, and glutathione is a tripeptide formed from glutamic acid, cysteine and glycine. Gowland Hopkins of Cambridge first identified this substance in the blood and tissues in 1921, and its function was for many years unknown. Glutathione is a sulphur-containing compound, in which the SH group is free. It has been found that the presence of free SH groups is of great importance in enzyme reactions which catalyze oxidation and reduction reactions, and 2 SH groups may combine with loss of 2 hydrogen atoms to form an oxidized, or disulphide linkage:

$$\text{A-SH} + \text{B-SH} = \text{A-S-S-B} + 2\text{H}^+$$

The disulphide linkage is of great importance in the insulin molecule. Sanger and his co-workers at Cambridge (1945-55) established the structure of insulin, and the molecule, which has a molecular weight of 6,000, contains 51 amino acids, of which 12% (6 units) are cysteine. In spite of our having known insulin as a pure crystalline protein since 1926 (Abel), at present we still do not know whether or not it has a single point of action, whether it acts at different points of carbohydrate metabolism,[3] or whether it acts purely on the cell membrane by a physical process.[4] In 1935 and 1937, Freudenberg and Wegman,[5] and Stern and White,[6] respectively, established that the disulphide bridges between the cysteine components of the molecule are important, and that their integrity is essential for insulin activity.[7] In 1947, Barron[8] showed that BAL could inactivate insulin *in vitro* by breaching the disulphide bridges.

GLUTATHIONE

Glutathione is formed in the wall of the intestine and in the liver, and is transported within the erythrocytes to the various cells of the body. Very little is known about its transfer across the cell membranes or its place in normal physiology. Racker[9] summarized the position in the 'euphoristic theory'. This states that glutathione is present to 'keep the cell enzymes happy' by preventing their oxidation, and protecting them against toxic heavy metals. Calvin[10] stated that glutathione may be present in many different forms, and mentioned 7 different possible chemical configurations.

Glutathione is important in transpeptidation, oxidation-reduction and hydration-dehydration reactions. Hopkins established that it is the co-enzyme in the conversion of methylglyoxal to lactic acid. It is part of the co-enzyme in the oxidation of pyruvic acid (acetyl CoA-SH of the pyruvic-oxidase system), and is also essential in maintaining ascorbic acid in the reduced form in both animals and leafy vegetables, where its concentration parallels that of ascorbic acid. Living tissues maintain glutathione in its reduced form by means of triphosphopyridine-nucleotide (TPN) reducing systems and glutathione reductase.[11] Old erythrocytes which undergo haemolysis in certain forms of acquired haemolytic anaemia associated with glucose-6-phosphate dehydrogenase deficiency, e.g. favism; primaquine sensitivity; aspirin, sulphonamide, and phenacetin sensitivity, etc., have recently been shown to be deficient in glutathione. In sickle-cell anaemia, reducing substances, such as glutathione, cysteine, and bisulphite, as well as deoxygenation, cause sickling. Cortisone, which reduces the blood-glutathione level, has therefore been suggested as a form of therapy by Mazzia,[12] but it is not always successful.

Glutathione is formed in the presence of magnesium ions and adenosine triphosphate (ATP), firstly, by γ-peptide linkage of glutamic acid and cysteine to form glutamyl-cysteine; subsequently, glycine is added, also by peptide linkage. In the blood, small quantities of reduced glutathione are present (40 mg. per 100 ml.), as well as small amounts of glutamyl-cysteine and cysteinyl-glycine. Production of glutathione is limited by deficiency of the amino acid, serine (β-hydroxyl-glycine), cysteine and glutamic acid.[13]

Deficiency of vitamin B_{12}, folic acid, and pyridoxine also limit glutathione production. (Vitamin-B_{12} function is related to nucleic-acid synthesis, transmethylation, maintenance of SH forms of S-S compounds, and function of liver enzymes, and has a key rôle in protein synthesis;[14] pyridoxin is important in decarboxylation reactions.) In animals, vitamin-B_{12} deficiency has been shown to cause decreased carbohydrate and fat metabolism, manifest as hyperglycaemia and an impaired glucose-tolerance test,[15,61] but it has no effect on steroid-induced diabetes. As a point of interest, diabetic retinopathy and neuropathy have been treated with varying, but limited, success with vitamin B_{12}[16] and pyridoxine. More recently anabolic steroids have been used. All of these are important in glutathione

synthesis, and synthesis of glutathione may be the result of therapy and a possible cause of benefit.

Henneman *et al.*[17] have shown that blood-glutathione levels are (*a*) *decreased* in anaemia, ketosis, liver disease and obstructive jaundice, beri-beri, and sanguinarine intoxication; (*b*) *normal* in diabetes and after steroid administration; and (*c*) *increased* in febrile disorders (not a result of increased metabolic rate). Lazarow[18] has shown that the blood level of glutathione is *decreased* in sodium deficiency, cysteine and methionine deficiency, ketoacidosis caused by acetoacetate injections, and scurvy.

In beri-beri, which is due to thiamine deficiency, there is hyperpyruvicaemia. In India, a form of epidemic oedema has been found to occur as a result of ingestion of argemone oil, which contains an alkaloid, sanguinarine, and which depletes the blood glutathione. Here, too, hyperpyruvicaemia results. The 2 conditions are therefore similar, the major difference being that the one is due to a deficiency of the constituent of the enzyme (cocarboxylase), while the other is due to a deficiency of the co-enzyme (the glutathione component of the acetyl co-enzyme A).

SOME ASPECTS OF DIABETES

Hsia[19] classified diabetes as one of the diseases caused by an inborn error of metabolism. Approximately 60% of patients give a family history of diabetes,[20] and Pincus and White[21] have demonstrated that this is the result of an autosomal genetic transmission.[22] There are apparently both dominant and recessive genes, and penetrance may be related to age, parity, weight and ABO blood groups. A homozygous pattern is said to result in earlier emergence of the condition than a heterozygous pattern.

Dd×Dd=25% homozygous dd, Dd×dd=50% dd, and dd×dd=100% dd. The dd pattern is an autosomal recessive combination and produces diabetes with a variable age of onset.

Joslin[20] has expressed the opinion that all patients with true diabetes have a hereditary background, and that the latent or incubation period of the disease may vary greatly and extend up to 60 years. The incidence of diabetes increases with age,[23,24] owing to metabolic and environmental factors acting on inherited susceptibilities to these factors.[25] A possible acquired factor is multiparity in the female.[20,26]

It would appear that there are 4 factors which may be responsible for the emergence of diabetes:

1. Congenital aplasia or hypoplasia of islet cells.
2. Islet exhaustion following prolonged carbohydrate load.
3. Islet-cell intoxication (diabetogens).
4. Anti-insulin substances — (*i*) endocrine gland secretions, or (*ii*) antibodies, endocrine dependent.

The possibility of endocrine-dependent antibodies in the pathogenesis of diabetes has recently assumed importance as a result of the work of Vallance-Owen,[27,28] and may be associated with the β-lipoproteins, albumin, and α-globulin and γ-globulin fractions of the plasma. Rizzo *et al.*[29] have shown that obese non-insulin-dependent diabetic subjects have high levels of anti-insulin factors in their blood in association with high blood-sugar levels.

In diabetes, we cannot consider the rôle of the pancreas and insulin alone and, although it is customary, it is not necessarily correct to control diabetes according to demonstrable abnormalities in carbohydrate metabolism.[4] True complete pancreatic deficiency is mainly a result of surgical procedures, and it differs from other clinical forms of diabetes in that it usually requires only 30 - 40 units of insulin a day for 'control'. In diabetes, pancreatectomy has been shown to reduce the daily requirement of insulin, and this is thought to be the result of removal of glucagon-secreting α-cells.

CLASSIFICATION OF DIABETES

There are many different clinical classifications of diabetes and this has led to confusion. Recently, Gillman *et al.*[30] and Gilbert *et al.*[31] have studied experimental diabetes in baboons along the classical extirpation and substitution lines of von Bering and Minkowski, Houssay, and Young. Based on this work, and experience at the Diabetic Clinic of King Edward VIII Hospital, it is suggested that diabetes

TABLE I. COMPARISON OF KETOTIC AND NON-KETOTIC DIABETES

	Ketotic	*Non-ketotic*
Onset	Young	Maturity
Body habitus	Tall, thin	Short, obese
Insulin	Dependent	Independent
Insulin levels	Often deficient[4]	Normal[58]
Insulin resistance	++++	++
Insulin antibodies	α-globulin, albumin	β-lipoproteins
Hormones	STH (Ant. pituitary) +++	Adrenal (11, 17 oxysteroids) +++
Lipaemia	Absent	Present
Complications	Keto-acidosis	Vascular, especially retina
Oral therapy	Poor response	Good response
Glutathione	Decreased	Normal or slight decrease
Types	I (Hugh Jones) 'Insulin dependent' (Himsworth) 'Insulin dependent' (Lawrence)	II 'insulin independent' 'Lipoplethoric'

be classified as *ketotic* and *non-ketotic*, which corresponds roughly with *insulin dependent* and *non-insulin dependent*, as shown in Table I.

EXPERIMENTAL AND CLINICAL DIABETES

There is evidence of a possible functional defect of islet cells in all diabetics, possibly caused by anti-insulin factors.[32]

Experimentally, diabetes may be produced in many ways, among which are:

1. Pancreatectomy, removing at least 80% of the organ.
2. Partial pancreatectomy plus dietary modifications.
3. Diabetogens, especially alloxan.
4. Hormones and enzymes — glucagon, ACTH and steroids, STH (growth hormone), thyroxin, adrenaline, seminoma and interstitial-cell tumour of the testicle, and insulinase.

Lazarow[25] has summarized the above tabulation, by stating that anything which produces an increased rate of insulin removal, inactivation or destruction, would tend to lead to the development of diabetes.

Allen and Sherrill[33] demonstrated that high calorie diets produced β-cell changes in partially pancreatectomized

animals, and Dohan and Lukens[34] showed that hyperglycaemia in rats led to the development of a diabetic state. Houssay and Martinez[35] made the following interesting observations on the effect of diet on the development of diabetes in partially pancreatectomized animals:

A high-fat diet causes 100% diabetes;
A high-carbohydrate diet causes 78% diabetes (later 100%);
A high-protein diet causes 56% diabetes (later 82%).

In these experiments, the incidence of diabetes was independent of the calorie intake, but if the animals were given a high calorie diet as well, the onset of diabetes was more rapid. These observations accord with the clinical experience of 2 world wars in Europe, where with food rationing the incidence and mortality from diabetes fell. An interesting aspect of Houssay and Martinez' work is the fact that they have demonstrated experimentally that *single* daily feeds are more apt to produce hyperglycaemia than the same intake of food given in multiple feeds. This may be of importance clinically, and we should perhaps ensure that our obese patients on reducing diets should not starve themselves during the day and then insult their homeostatic mechanisms with only one big meal a day. Joslin[20] has shown that obesity tends to increase with age, and that even slight weight reduction by obese diabetics leads to amelioration of the diabetic state and increased longevity. He advises particularly against obesity in pregnancy in Jewish females.

Pancreatectomy of at least 80% of the pancreas leads to the development of diabetes unless supplementary insulin is given. This has given rise to the concept that exhaustion of the remaining β-cells may be an important factor in diabetogenesis. Jackson and Woolf[36] have shown that the infants of prediabetic or diabetic mothers are generally large babies, and in these infants the islets of Langerhans show gross proliferation, becoming 'continents'. This may be the result of a response to raised blood-sugar levels, and/or other endocrine stimuli, among which Gilbert[37] has suggested the anterior pituitary growth hormone. One of the actions of the sulphonylurea compounds which are used in oral diabetotherapy, is to cause stimulation and proliferation of the islet β-cells, and the fact that these compounds are only of use in the presence of an endogenous supply of insulin suggests some basis for the exhaustion theory (these compounds also inhibit insulinase[38]).

Another observation which supports the exhaustion theory is that phlorizin prevents hyperglycaemia from causing degranulation, hydropic degeneration, disappearance and fibrosis of the β-cells. The action of the biguanides is at present incompletely understood. Pomeranze *et al.*[39] have suggested that they cause transient tissue hypoxia, leading to anaerobic glycolysis and formation of lactic acid, but their action is possibly similar to that of synthalin A, which causes degeneration of α-cells and consequent decrease of glucagon production.[40] Another hypoglycaemic substance is hypoglycin, found in Jamaican ackee fruit. Hypoglycin B is the glutamic-acid amide of hypoglycin A, and has a simple amino-acid structure. In view of its glutamine content, it may have some action by way of glutathione metabolism and synthesis.

DIABETOGENS

The possibility of diabetogenic substances has been postulated for many years.[41] In 1889, Weiner[29] showed that alloxan could produce death from convulsions in rabbits, and Jacobs[42] demonstrated that these were caused by hypoglycaemia. Provided the initial hypoglycaemia is treated, a permanent diabetic state develops about 17 days after the injection of alloxan.[12]

Alloxan is a chemical substance with a very short life in the blood; it is a pyrimidine compound related to the nucleic-acid derivatives thymine and cytosine, to uracil, and also to the barbiturates. Evrett[43] stated that alloxan is an oxidation product of uric acid, and Lazarow[25] suggested that uric acid or one of its metabolites may be diabetogenic.

Uric acid has been shown to be capable of producing diabetes permanently under experimental conditions (1 G. per kg. body weight by intravenous injection), and Griffiths[44] was able to produce a diabetic state in protein-deficient rats by intraperitoneal injection of uric acid. Diets containing vegetable fats and protein protect against the diabetogenic action of alloxan. Methionine is also protective, whereas choline is not, and this is thought to be due to the SH groups in methionine.

Many enzymes contain SH groups which may be decreased by oxidants such as alloxan and dehydroascorbic acid, heavy metals, and protein deficiency. It has been shown that injection of alloxan results in the alloxan combining with erythrocyte glutathione to form dialluric acid and an unidentified substance with an absorption maximum in the spectrum at 305 Angstrom units. Heavy metals do not produce diabetes, because in combining with SH groups they act as enzyme poisons. Glutathione, like BAL, can reactivate disulphide linkages, and so protect them against the action of heavy metals such as mercury and arsenic. Both glutathione and BAL also protect against the diabetogenic action of alloxan provided they are given before the alloxan.[45] Because alloxan can combine with amino ($-NH_2$) groups as well as with sulphydryl groups, it is a more effective diabetogen, although it is also toxic in its action on enzymes. In the doses used, alloxan selectively affects the β-cells, which have a low glutathione content, and are therefore more susceptible to its action in low concentrations. When alloxan is injected intravenously it combines with the blood glutathione and is neutralized by it, causing a decrease in the blood-glutathione level as well as in the amount of effective circulating alloxan.

The action of alloxan is potentiated by factors which reduce the blood-glutathione level, e.g. protein deficiency and starvation;[44] increased dehydroascorbic acid in scurvy;[46] and raised blood-uric-acid levels — possible diabetogenic substances.[47]

Normally, insulin is destroyed by insulinase, and this process is decreased by hypophysectomy and thyroidectomy. *In vitro*, glutathione inactivates insulin (by opening the disulphide bridges), but Lazarow[18] mentioned that the use of antithyroid substances and thyroidectomy caused an increase in the tissue glutathione content, and protected against alloxan diabetes. Injection of anterior pituitary extracts causes a decrease in tissue glutathione and the

occurrence of diabetes, which may be permanent.[48,49] Insulin has been found to raise the tissue-glutathione level. In human diabetics, the blood level of glutathione may be normal in non-ketotic patients, or decreased if ketosis is present (acetoacetate increase and sodium depletion).

It has been suggested that reduction of the β-cell glutathione level may make the β-cell enzymes more susceptible to the action of diabetogens or toxins, especially when insulin demands are increased, as in hyperpituitarism, hyperthyroidism and hyperglycaemia. Conn *et al.*[50] have shown that ACTH causes an increase in uric-acid excretion, decreased tissue-glutathione levels, and decreased glucose tolerance. Herman[51] has reported decreased glucose tolerance in patients with gout. Uric acid may form dialluric acid in the liver, and this may be oxidized to alloxan, but it is not known if alloxan is an intermediate substance in the metabolism of uracil and cytosine in the formation of urea and ammonia.

CLINICAL DATA

Much of the work on glutathione is not conclusively established, mainly owing to poor techniques, and, as a result, the alterations in glutathione levels in various clinical conditions are not proved beyond doubt. Joiner[52] stated that blood-glutathione levels were unaltered by ACTH given to non-diabetic subjects, although 4 of his 11 patients developed lag-type glucose-tolerance curves. He concluded that the administration of ACTH did not cause diabetes by virtue of an alloxan-like action in the presence of decreased glutathione levels. Seltzer[53] estimated the blood-glutathione levels in diabetics, and found that they were lower than in controls both before and after administration of tolbutamide, but his results do not relate blood-glutathione levels to the erythrocyte levels. Other workers have not produced consistent results for blood-glutathione levels in diabetes, and this may be the result of poor selection of cases without due regard to the type of diabetes being investigated. Krahl[54] showed that, in diabetes, the liver production of glutathione is decreased, and that this is improved by adding both insulin and glucose, whereas insulin alone has no effect.

Administration of glutathione to normal persons has been found to cause an increased peripheral use of glucose that does not result from insulin potentiation or inhibition of adrenal steroid action.[17] *In vivo*, glutathione stimulates the secretion of adrenal steroids, whereas *in vitro* it decreases their secretion. In steroid diabetes, administration of glutathione produces inconsistent results. In rats, glutathione increases the hyperglycaemia of steroid diabetes, whereas it reduces the hyperglycaemia of Cushing's syndrome, in which glutathione levels are low. Steroid administration causes a decrease in the blood-glutathione level, and if the glucose-tolerance test is abnormal, the decrease in the blood-glutathione level coincides with the maximal rise in the blood-sugar level. Large doses of steroids and ACTH cause ketosis and insulin resistance, and may contribute to the lowered blood-glutathione levels by this mechanism. It is still not known whether or not diabetes mellitus and steroid diabetes are caused by similar metabolic changes.

In clinical practice, Bailey[55] has shown that about 10% of patients with severe burns exhibit signs of adrenal overactivity manifest as glycosuria, hyperglycaemia and eosinopenia. Conn[56] showed that intravenous injection of glutathione lowered the blood-sugar level in ACTH diabetes, and that by giving BAL to burn cases, the hyperglycaemia and hyperpyruvicaemia which were present were reduced. Butterfield[57] has given BAL to insulin-resistant diabetics (2 obese females), with subsequent decrease in their carbohydrate excretion. He has also shown that BAL administration benefits the control of brittle diabetics who have a hyperpyruvataemia, but this is of limited value because of the long-term dangers of BAL administration. β-Mercaptoethylamine has been given orally with inconsistent results (this is a component of co-enzyme A). The basis of these clinical experiments is the supply of sulphydryl groups to the body.

The whole position regarding alloxan has been summarized by Lazarow.[15] Glutathione protects against alloxan, and the level of glutathione in the blood and the β-cells determines sensitivity to alloxan. It is probable that the β-cell-glutathione level is an important factor in determining the age and speed of onset and the progression of diabetes in animals with decreased pancreatic reserve, but there is no direct evidence of this. It is not known whether or not tissue-glutathione levels are the result of hormone action or the metabolic disturbances caused by hormonal inbalance. The only established facts are that alloxan destroys β-cells, that alloxan reacts with glutathione, that glutathione protects against the effects of alloxan, and that factors which increase tissue-glutathione levels cause a decreased sensitivity to alloxan, and *vice versa*.

SUMMARY

The reasons for the emergence of diabetes in man are at present incompletely understood. It would appear that diabetes mellitus is an inborn error of metabolism that becomes manifest as the result of metabolic and environmental factors acting on inherited susceptibilities to these factors. The rôle of diabetogens in human diabetes is at present unsettled

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