METHODS OF STUDYING ANTIDIABETIC SUBSTANCES
A REVIEW

By

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INTRODUCTION

Diabetes mellitus is a condition of an impaired metabolic status caused by dietetic factors or deficiency of insulin—relative or absolute—other endocrinal imbalances causing hyperglycemia being excluded from such a definition unless they diminish the effective insulin action in the body.

The disease was first recognised and described in Ayurveda by Susruta who called it Madhumeha, the glycosuria having been recognised by the taste and attraction to ants of the urine. The ancient concepts of this disease were based on polyuria and glycosuria as the main smyptoms and the older remedies were therefore directed towards alleviation of these and improvement in general health and well-being. These remedies were non-specific as shown by the multiple and unrelated indications of each. The enormous number of such remedies described in Ayurvedic treatises have been systematically arranged so as to facilitate identification and further study by Kirtikar and Basu (1933), Nadkarni (1954) and Chopra (1933). Modern studies carried out on some of these remedies have been reviewed by Lewis (1949) and Mukerji (1957).

Some of the notable studies on indigenous drugs are the works on Gymnema sylvestre (Chopra *et al.*, 1928 ; Mhaskar and Caius, 1930) ; Pterocarpus marsupium (Ojha *et al.*, 1949; Bose *et al.*, 1955; Sepaha and Bose, 1956) Eugenia jambolana (Mukerji, 1953 ; Vaish and Kehar, 1954; Gujral *et al.*, 1954; Sepaha and Bose, 1956); Coccinia indica (De and Mukerji,1953); Vinca rosea (Jones 1955) ;Cabbage (McDonald and Wischicki, 1938, cited by Lewis, 1949) ; and Ficus bengalensis and glomerata (Gujral *et al.*, 1954 ; Shrotri and Aiman, 1960). With increasing knowledge of metabolism and newer methods of study, better criteria for evaluation of antidiabetic substances have evolved and hopeful reports in the lay press can be more thoroughly assessed.

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In contrast to the older remedies, some of the new synthetic substances are notorious for their toxic effects. Search has therefore continued for better substances (Goldner, 1957) which can be safely and easily taken orally. The pioneer works on synthetic substances are of Frank *et al.* (1926) on synthalin ; Janbon (1942) and Loubatieres (1944 ; 1946a and b) on IPTD ; Franke and Fuchs (1955) and Achelis and Hardebeck (1955) on carbutamide ; Erhardt (1956) and Crentzfeldt *et al.* (1956) on tolbutamide ; Ungar *et al.* (1957) on DEBG or DBI and West and McCampbell (1958) on Chlorpropamide.

The methods used for studying antidiabetic substances can be broadly classified as (a) those directed towards demonstrating an antidiabetic action and (b) those which aim at elucidation of the mechanism of action, once it has been demonstrated.

Thus far no substance known can permanently restore to normal a gross diabetic status; nor even temporarily restore to the normal homoeostasis the carbohydrate metabolism of such an individual. Antidiabetic action would therefore imply merely correction to as near normal as possible the impaired metabolic status. Both the metabolic derangement and the antidiabetic action are judged by the nature and changes respectively of the blood-sugar level and the glucose tolerence. It is possible that there may be substance which can lower an elevated blood sugar but have no action on the normal blood sugar, just as we have antipyretic substances having no hypothermic activity.

Methods of estimating Blood-Sugar

The methods of estimation of blood-sugar have been continuously improving during the last fifty years and up to date newer methods are being evolved, which can specifically determine the amount of glucose in the blood. Bang (1913) first introduced a method needing only a small amount of blood. His method and that of Lewis and Benedict (1913-1914) were based on the reduction of yellow picric acid to orange picramic acid by the aldehyde group of the glucose molecule. These methods, together with many of the methods that followed had the disadvantage of being non-specific, the discrepancies being more in pathological bloods containing higher amounts of creatinine, acetone or diacetic acid.

Another group of methods based on the reduction of yellow Potassium ferricyanide to colourless ferrocyanide was introduced by Hagedorn and Jensen (1923). This was a more faithful reducing procedure, requiring less time and producing stabler results. For the quantitative estimation of the reduction various procedures have been adopted. These include : (1) Iodometric method of Hegedorn and Jensen (1923), where ferricyanide is allowed

to react with iodide and the liberated iodine is allowed to react with sodium thiousulfate. (2) Gasometric method of Van Slyke and Hawkins (1928-29), in which the unreduced ferricyanide is allowed to react with hydrazine to liberate nitrogen and (3) Colorimetric methods, which measure either the disappearance of the yellow colour of the ferricyanide (Schales & Schales; Hoffman, cited by Sunderman, 1951) or of the colour developed on adding ceric sulfate (McFadyen and Van Slyke, 1943., Miller and Van Slyke, 1936) or of the secondary development of the prussian blue colour of ferric ferrocyanide on adding a solution of ferric iron to the reduced ferrocyanide (Folin and Malmros, 1929. This is a micromethod).

The reduction of cupric salts to cuprous by glucose has been utilised in many methods, first of which was introduced by Shaffer and Hartman in 1921. In their procedure they used a tungstic acid filtrate of blood, which did not remove all the non-sugar reducing substances. Somogyi (1931) modified this method by using zinc hydroxide filtrate giving lower values. In both these the cupric salt is reduced to cuprous by iodine and the excess of iodine is titrated with sodium thiosulfate. Nelson (1944) appreciated the reliability of the Somogyi-Shaffer-Hartmann reagents and adopted this method to photometry, using an arsenous-molybdate reagent which was changed to a coloured compound by the cuprous salt.

In all these reduction procedures, the proteins have to be removed, and for this various agents e. g. tungstic acid (Folin and Wu, 1919), trichloracetic acid (Greenwald, 1915), salts of zinc, copper and iron (Somogyi, 1930, 1931), salts of mercury (West *et al.*, 1929), molybdic acid and tungstomolybdic acid (Benedict and Newton, 1929a & b; Benedict, 1931 a &b) are used. Folin (1930) had also recommended the use of unlaked blood as a basis of blood analysis, using hypertonic sodium sulfate to prevent hemolysis. The various methods of blood glucose analysis have been briefly summarised and reviewed by Sunderman *et al.* (1951).

More specific methods of glucose estimation include the anthrone method of Roe (1955), a condensation method of Athanail and Cabaud (1958) and an enzymatic method by Saifer and Gerstenfeld (1958). It is claimed that the values for non-glucose reducing substances—"Saccharoids" a term introduced by Benedict (1931a)— are lowest with this method. The older method of Benedict (1931) has also received renewed attention and Sunderman *et al.* (1951) have devised special procedures to increase the accuracy of that method.

Glucose Tolerance Tests

Besides studying the effect on the fasting blood-sugar, the effect on glucose tolerance has been very widely studied during recent years, after the introduction of the sulfonylureas. The oral glucose tolerance test was first

introduced by Liebman and Stern in 1906 and Baudouin in 1908. Later, after the establishment of micromethods for blood glucose estimation, more thorough studies were carried out by Bang and Jacobsen. Himsworth in 1933 investigated the blood glucose changes in detail and pointed out the difference between the capillary and venous sugar curves. Peters and Van Slyke (1946) have reviewed the factors taking part in the tolerance. In normal subjects, after the administrating of 30-50 gms. of glucose, the blood-sugar reaches a certain height which is not altered by increasing the dose. If more glucose is given, the absorption from the intestine, which has reached its maximum, is prolonged and the peak of blood-glucose is maintained for a longer time, but its height is not raised. In diabetic subjects the height of the curve increases with the dose up to a certain limit (Hansen 1923, cited by Van Slyke, 1943). Other factors which must be considered in performing glucose-tolerance-test (GTT) are the state of previous carbohydrate depletion which reduces the tolerance and exercise which can modify the peak hyperglycemia either way depending on whether it is performed before or after the administration of glucose. These alterations are due to utilisation of glucose by muscle and depletion of glycogen from the liver, and can be obviated by giving plenty of carbohydrate the previous night, and by complete rest. The reactions to previous starvation and exercise are more marked in infants, who also require more glucose per kilo body weight, to produce the same degree of hyperglycemia. In old age, the height and duratiou of the hyperglycemia are increased. Huidobro *et al.* (1948) have tried to study the role of the autonomic nervous system in the absorption of glucose from the intestine, but have not reached any definite conclusion.

For the detection of mild diabetes Exton and Rose in 1934 proposed a two-dose tolerance test and this test has been found to be better than the single-dose test as compared by Mathews *et al.* in 1939. A more prolonged study up to five hours after the administration of glucose has been advocated by Ralli and Shannon in 1931 but has not been widely accepted. Goldberg and Luft (1948) compared the oral single and two-dose one-hour tests and intravenous test in 32 healthy individuals and concluded that the two-dose test was the least variable as far as the reproducibility in the same individuals was concerned. Capillary blood was as reliable as venous, only three samples were required and the duration of the test was short, making this test a very convenient clinical procedure.

So far no drug has been found to correct the impaired pattern of glucose tolerance curve in Diabetes mellitus. The administration of sulfonylureas lowers the fasting blood-sugar (FBS) and the tolerance curve also runs at a lower level but the pattern is unchanged (Duncan, 1956; Kulkarni and Aiman, 1958a).

The reactions to intravenous glucose were studied in detail in the early days of blood-sugar estimation by Bang in 1913 (cited by Peters and Van Slyke, 1946) using varying doses of glucose. Later, Jorgensen in 1923 introduced the intravenous glucose tolerance test to obviate variations in the oral test resulting from impaired absorption from the intestine. Tisdell in 1925 used this test in infants. This test has the advantage of using small amounts of glucose and taking only the fasting and the 90 min. or 2 hours sample (Lozner *et al.*, 1941). The excretion of a large amount of glucose in urine is one of the objections but Amutzio *et al.* (1953) have showed that this is not more than 10 percent of the administered glucose at the ordinary dose level of 20-25 gms. Pyrexia and local venous thrombosis are often noticed after the test, but the latter can be prevented by concurrent administration of heparin (Duncan, 1956)

Amutzio *et al.* (1953) have studied the rate of removal of glucose, in terms of percentage of given glucose, from blood after intravenous glucose and have found it to be constant in the same subject on different occasions, independent of dosage. On the basis of this "assimilation index", a sharp separation of normal, mild diabetic and severe diabetic subjects was possible. Duncan (1956) has put forward the concept of "total index" and "increment index" depending on whether the rate of disappearance of glucose is calculated in terms of the total blood-sugar or of the increase above the FBS. He has found these indices constant in the same individual and has recommended the "increment index" as the superior one, as it does not vary with the dose of glucose.

Glycosuria.

Reduction of glycosuria has been a criterion for an antidiabetic effect, widely employed by many and lead to fallacious reports of a true antidiabetic action. Normally, the human urine contains about 0.02 to 0.1 percent glucose or other reducing substances (Peters and Van Slyke, 1946) which do not give positive tests with any of the commonly used reagents. Above this value, their presence can be detected by methods based on principles similar to those of blood glucose estimation. The Benedict's test (Benedict, 1911) and Fehling's test (Dukes, 1939) have been the most commonly used procedures over a long period. These tests, if appropriately carried out (Lawrence, 1956) can give fairly reliable values for all purposes. Care must be taken to remove albumin if present in large amount by boiling and filtering (Dukes, 1939) and in interpreting results with concentrated urines in which creatinine, glucoronates and uric acid may give positive results. With the Fehling's procedure, a greenish precipitate of phosphates may be formed (Dukes, 1939) and this is more true with the urine of experimental animals like rabbits which excrete a large amount of phosphates. False positive tests may also be obtained with patients taking penicillin in large doses (Whipple and

Bloom, 1950), Streptomycin (Neuberg, 1954), the broad spectrum antibiotics (Lippman, 1952) thiamine (Hart and Wise, 1939) and para-amino-benzoic-acid (Zarafonetis and Chandler, 1951). Other normal constituents of urine, like salicylate derivatives, galactose, fructose, and the pentoses may also give false evidence of glycosuria. Most of these false glycosurias can be distinguished by yeast fermentation, but fructose and glucuronic acid may present difficulty (Dukes, 1939; Zarafonetis and Chandler, 1951). Simultaneous dextrose tolerance test is also helpful in detecting renal glycosuria due to low renal threshold.

In recent years, more specific tests have been introduced, based on the enzymatic degradation of glucose. Essentially, the test consists of the breakdown of glucose by glucose oxidase to glucoronic acid and hydrogen peroxide and reaction of peroxide with an indicator to give a colour reaction (Moran *et al.*, 1957). Since the introduction of this method in 1956, glucose testing papers are commercially available and have been found to be qualitatively good (Leonards, 1957). Moran *et al.* (1957) have compared this test with the older copper reduction procedures and have found them more sensitive, but useful only for a rough quantitative estimation for clinical use (Jablokow *et al.*, 1957). Occasionally, a false positive test due to contamination with Hydrogen peroxide has been recorded (Phillips, 1958).

As there is a raising of renal threshold for glucose in diabetes, desugarisation of urine does not necessarily mean a lowering of the blood-glucose (Dukes, 1939).

Design of experiment

General Considerations

In order to get a correlation in the results of metabolic studies which are carried out on different animals under different conditions, factors modifying the results of such studies are found out and eliminated as far as possible. Ingle (1951, 1953) has concisely reviewed this subject and has introduced the term "Heteropoietic factors" to cover all such factors.

(a) *Genetic* : Dogs, rats, cats, mice and rabbits are the commonly used animals in studies on diabetes. Houssay *et al.* (1957b) have recently used toads. The alpha and beta cell content of the islets of animals vary to a great extent as shown by results of alloxan injection (Lazarow, 1954). Gourley (1952) has reviewed the effects of insulin in various animals and has concluded that mammals are most sentitive to insulin injections, birds are least sensitive and the poikilotherms intermediate. Even among the mammals wide differences in the hypoglycemic response exist. The steepness of the fall in blood-sugar is maximum in rabbits, less steep in cats, dogs and rats and least so in man. In the same species, strains differ in their responsive-

ness to various agents, especially when studies on isolated tissues such as rat diaphragm are considered (Randle, 1957; Vallance-Owen, 1957).

(b) *Sex*: The behaviour of the two sexes in different animals has been found to vary in different procedures. Foglia *et al.* (1947) found that the onset of pancreatectomy diabetes was earlier in males and that the female hormones afforded a protection in both sexes. Rodriguez (1954) reported recovery from alloxan diabetes in rats after treatment with estrogens, whereas Beach *et al.* (1951) have found that in the female rats the development of alloxan diabetes was more rapid. Besides these, if female animals are not isolated for a sufficient length of time before taking them up for experimental studies, pregnancy may prove to be a complicating factor.

c) *Age*: The sensitivity of experimental animals as well as of man to hormons and drugs changes with age (Ingle, 1951). Procedures like hypophysectomy and growth hormone administration may fail to be effective if carried out at too early an age (Walker, 1950 and Young, 1941 cited by Ingle, 1951.)

(d) *Environment*: Pioneer workers in metabolic problems have been impressed from time to time by the importance of temperature control, while working under constant conditions (Ingle, 1951). Chen (1943) has demonstrated that a striking increase in the sensitivity of mice to convulsive doses of insulin occurs on raising the temperature from 20°C to 40°C. This makes temperature control an essential though elaborate procedure in the assay of insulin by the mouse convulsion method (Burn *et al.*, 1950). Ingle and Nezamis (1949c) have shown that in eviscerated rats an increase in temperature raises the glucose tolerance. In pancreatectomised rats Ingle *et al.*, (1953a) have demonstrated a gradual loss of weight and diminution in glycosuria with increased urinary non-protein-nitrogen (NPN) when the temperature was lowered from 26°C to 3°C. In comparing results one has to bear in mind that modern studies in western countries are carried out in air-conditioned laboratories.

(e) *Diet and Fasting*: Houssay and Martinez (1947) have summarised the effect of protein and fat variation in the diet on the effect of pancreatectomy and shown that a high protein and low fat diet can afford much protection against subtotal pancreatectomy. Rodriguez and Krehl (1952) have put forth similar evidence for alloxan diabetes and have further pointed out the difference between long and short chain fatty acids. Various standard diets that can be fed to different animals are available (Reinecke 1939; Ingle, 1952). The effects of force feeding and feeding ad libidum on the responses to drugs are also important (Ingle *et al.*, 1947).

In our own laboratories, recently we have found that a change in the period of fasting of rabbits fed ad libidum with natural diets can alter the

response to administration of water, insulin and sulfonylureas(Kulkarni *et al.*, 1958) and to indigenous plant extracts (Shrotri and Aiman, 1960).

Specific Considerations

Experimental Diabetes

Various methods are employed to produce in animals a state of hyperglycemia. Surgical removal of the pancreas, selective chemical damage to beta cells, growth hormone and steroid administration etc. are all useful procedures for testing the antidiabetic action of druges, but none of them completely resembles the clinical state, thus making clinical trials indispensable.

I. *Pancreatectomy* : This has been attempted in various animals and the reactions of the animal differ from species to speciss (Peters and Van Slyke, 1946). The commonly used animal is the dog, in which an intense hyperglycemia, glycosuria and ketonuria with an accelerated nitrogen catabolism follows the operation. Owing to the anatomical peculiarities (Bradley, 1948) the procedure is lengthy, but can be mastered with practice and adequate equipment (Aiman, unpublished observations). Cat exhibits a reaction similar to that of dog, whereas the goat and the pig show least intense reactions, ketonuria being marked in the latter (Peters and Van Slyke, 1946; Ingle, 1953). The duck shows no changes in metabolism and the rabbit can survive for long periods without insulin. In rat, partial pancreatectomy is an easy procedure, taking a few minutes (Ingle and Griffith, 1942). In macacus rhesus monkey and in man, a mild diabetes is produced. Peters and Van Slyke (1946) suggest that the species differences result from differences in the inherent metabolic processes, upon which the action of insulin is superimposed. The removal of the alpha cells and the exocrine tissue of the pancreas leads to great changes in the metabolism, requiring an elaborate regime of maintenance, making the animal less suitable for the study of antidiabetic substances. Acute pancreatectomy has also been used in the studies on the mechanism.

II. *Alloxan Diabetes* : Since the observation of Dunn *et al.* (1943) that the intravenous injection of alloxan in rabbits leads to hyperglycemia and glycosuria, this subject has received wide attention. A similar state was soon shown to be produced in dogs, (Goldner and Gomori, 1943) rats and monkeys (Bannerjee, 1944). The histological changes in the islets of Langerhans were described by Duff (1945, cited by Lukens, 1948) and damage to beta cells by this agent was found to be the lesion. Lukens (1948) has reviewed the subject and Lazarow (1954) has studied in detail the biochemical lesions involved. The diabetes can be prevented by clamping the blood-supply to the pancreas for a few minutes after injection (Gomori and Goldner, 1945) thus showing that alloxan is rapidly destroyed in the body, and rapid injection of an adequate dosage is essential. The diabetogenic action can be altered either way

by different substances, a protection being afforded by thio-compounds(Lazarow, 1949., Martinez, 1951),antithyroid substances(Lazarow, 1949)and Chlorpromazine (Simoe *et al.*, 1955) and a potentiation obtained by methylene blue (Lazarow, 1950). Loubatieres (1954) has suggested a probable role of alloxan in human diabetes. Chemical damage to beta cells has also been attempted using 8-hydroxy quinoline (Kadota, 1950., Root and Chen, 1951, 1952) oxine and dithizone (Kadota, 1950) and various other substances (Kadota and Midorikawa, 1951). Alloxan has however remained the most commonly employed substance. The diabetes produced by alloxan is marked by an intense hyperglycemia and glycosuria, but ketosis is not so common as in pancreatectomy. The animals often require large doses of insulin, but survive for a long time.

III. *Miscellaneous Methods* : Besides these two commonly used types of experimental diabetes, many other diabetogenic procedures are used in the more detailed analysis of antidiabetic activity. Repeated glucose administration (Dohan and Lukens, 1946); injection of anterior pituitary growth hormone (Campbell *et al* , 1950., Houssay and Anderson, 1949) to normal or partially depancreatised animals so as to induce a temporary (Cotes *et al.*, 1949) or permanent (Campbell *et al.*, 1954) diabetes; steroid injection (Conn 1954;Abelove and Paschkis,1954; Ingle 1955; Buse *et al.*,1957) thyroid(Houssay, cited by Ingle, 1953) and estrogens (Ingle cited by Ingle, 1953) have all been used from time to time to produce a hyperglycemic glycosuric condition. Prolonged administration of insulin has also been shown to results into such a condition (Mirsky *et al.*, 1942 cited be Ingle, 1953).

Apart from these laboratory procedures, clinically occurring conditions such as acromegaly diabetes (Bergenstal *et al.*, 1957)and steroid administration diabetes (Fajans *et al.*, 1957) are often used to assess the role of the pituitary-adrenal axis in the antidibetic action.

Studies on the Mechanism of Action

As noted above, the establishment of the antidiabetic activity is the first step in the scrutiny to be followed by a detailed analysis of the mechanism of action.

Screening for antidiabetic activity has to be very thorough in order to detect the slightest activity. Studies on many species, using multiple experimental procedures and with varying doses of the drug are carried out. In some species parenteral administration may be necessary in case non-absorption by oral route is suspected. The material is extracted with different solvents and artefacts due to the chemical nature of the substance are avoided by direct testing (Loubatieres, 1957). Spontaneous cure, as sometimes occurs in alloxan diabetic rabbits must be borne in mind. The severity of diabetes by alloxan injection or pancreatectomy differs with the purpose

of the experiment, those for the mechanism of action requiring more drastic procedures, so as to eliminate all other mechanisms.

The possible mechanisms of action of reputed antidiabetic substances can be divided into false and true antidiabetic actions.

By false antidiabetic activity is meant the improvement in the clinical picture by an indirect action, which is not focussed on the impaired metabolic status. Examples of such an action are :

(i) Masking of the taste for sugar (Sollman, 1956) by the leaves of Gymnema Sylvestre, by action on the taste-buds, thereby diminishing the sugar intake. (ii) High tannin content of plant materials interfering with the absorption of glucose from the gut (Joglekar *et al*, 1958). Diminished absorption has also been claimed in the case of sulfonylureas (Friedlich *et al.*, 1956). (iii) High potassium content of plants, affecting an improvement in the symptoms of potassium depletion in severe diabetes. (iv) Antidiuretic action, which relieves the polyurea. (v) Improvement in general health by a tonic action (De and Mukerji, 1953; Gujral, 1954). (vi) Diminution in appetite, leading to reduction in glycosuria. Sturtevent and Fuller (1954) have studied the effects of such an anorexigenic substance in diabetic rats and have suggested an index relating glucose intake to glycosuria, which can distinguish between false and true antidiabetic action. (vii) Besides these, the various dietetic regimes which are followed during drug therapy, are themselves effective in relief of symptoms.

True antidiabetic action may be exerted through one or more of the following modes :

(i) *Pancreas* : Stimulation of the beta cells of the islets to secret more insulin, is suggested by Loubatieres (1955) as mode of action of sulfonylureas. Intrapancreatic perfusion of the drug (Colwell *et al.*, 1956, 1957), estimation of the total insulin content of the pancreas (Root, 1956) and histological study of the changes in islets Volk *et al.* (1957) are the methods employed to investigate this mechanism. Damage to the alpha cells, has also been suggested (Creutzfeldt, 1956).

(ii) Inhibition of insulinase, so as to prolong the action of insulin has been suggested by Mirsky *et al.* (1957) as the mode of action of the sulfonylureas. Campbell *et al.* (1953) and William (1956) have carried out *in vitro* studies to assess the effect on insulinase activity of the plasma. However, the *in vitro* activity may not have any relation with the hypoglycemic action, if any, in the case of many compounds (William, 1957).

(iii) In the liver, the substance may act at various enzyme levels to inhibit glycogenolysis and thus keep the blood-sugar low. Various studies

are therefore carried out to measure the glucose output by the liver (Clarke *et al.*, 1956; Tyberghein *et al.*,1956.,Ashmore *et al.*, 1956), to measure the glucose-6-phosphatase activity (Hawkins *et al.*, 1956), to study the serum potassium and phosphorous levels as reflecting the enzyme activities in the liver (Mohnike and Bibergeil, 1956), and to estimate the glycogen depletion (Lang and Sherry, 1956). Selective action at a particular metabolic level is also often studied by noting changes in blood glucose in response to fructose, galactose and glucose administration (Renolds *et al.*, 1956) and by the effect on alcohol metabolism (Forney and Hulpien, 1957). The intermediate metabolic processes have also been studied using C 14 labelled fructose and pyruvates (Ashmore *et al.*, 1956). Overall effects on the hepatic function are indicated by tests like the BSP clearace (Purnell *et al.*, 1956). Very often the hyperglycemic effect of glucagon has been shown to be modified by the sulfonylureas (Vaughan, 1956, 1957) suggesting an inhibition of the glucagon action in the liver.

(iv) *Peripheral action* The ultilisation of glucose by the tissues may be improved, resulting in a hypoglycemia and this mode of action has been studied either with the use of the rat-diaphram method (Cahill *et al.*, 1957) or by noting changes in the A-V glucose difference (Goldner *et al.*, 1956). The former method has been reviewed by Kulkarni and Aiman (1958 b) and can be used to see whether the drug itself has any effect on glucose utilisation or whether it can potentiate the insulin action of the plasma of a subject to whom the drug is administered (Aiman and Kulkarni, 1957). Perfusion of depancreatised and eviscerated animals with insulin and the drug has been carried out by Houssay *et al.* (1957a) for the same purpose. Recently Butterfield and Hardwick (1959) have studied the glucose uptake of the forearm tissue of human volunteers, and have observed a lowering of the glucose utilisation threshold produced by metahexamide. Liberation of the bound insulin at the periphery has also been postulated as one of the possible mechanisms of action of the sulfonylureas by Chaudhary and Aiman (1959).

SUMMARY

The various experimental procedures employed in the study of antidiabetic substances have been reviewed. The methods employed in the study of the mechanism of action have been only briefly dealt with, as they form a separate subject.

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