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Review Article

A Survey of Diabetes Mellitus

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REFERENCE to the disease known to us as diabetes mellitus goes back almost as far as recorded history itself. The Ebers Papyrus, an Egyptian document dating from about 1500 B.C., has outlined the symptoms of diabetes as well as a number of "cures" for the disease. Diabetes was described in some detail by a physician known as Aretaeus the Cappadocian as early as the first century A.D., but it was not until the seventeenth century that Thomas Willis, an English physician, observed that the urine of diabetics had an extremely sweet taste. This appears to have been the first evidence to connect diabetes mellitus with the metabolism of sugars.

A recent study on the prevalence of diabetes (1) indicates that there are somewhat over 2,000,000 known diabetics in the United States. Other evidence cited by the same workers (1) shows that the frequency rate for undiagnosed cases of diabetes in this country is about 8 per 1000 persons. Taking these two figures into account leads to an estimate that there are close to 3,500,000 diabetics in the U. S. alone. This represents nearly 20 diabetics per 1000 population and qualifies the disease as a major medical and public health problem.

THE METABOLIC EFFECTS OF DIABETES

Although more is known about the biochemical basis of diabetes mellitus than for most other diseases, too little is known to give an accurate definition of the disease. Some of the characteristic symptoms of diabetes are hyperglycemia, glycosuria, polyuria, polydipsia, and possible loss of weight and strength. Ketone bodies (acetoacetic acid, acetone, and β-hydroxybutyric acid) may be present in the blood and urine of diabetic subjects.

Diabetes may be classified into at least 2 types (2): the growth-onset (juvenile) type and the maturity-onset (adult) type of diabetes. The juvenile type of diabetes which is contracted early in life is characterized by a greatly diminished capacity to produce insulin (3) which approaches zero within a few years. Maturity-onset diabetics are frequently obese and develop symptoms quite gradually, usually after age 40. These individuals often have substantial quantities of pancreatic insulin (4) and normal or near normal levels of circulating insulin (3).

THE DEVELOPMENT OF INSULIN

The first evidence that the pancreas is concerned with diabetes came as early as 1889 when von Mering and Minkowski showed that pancreatectomy in laboratory animals produced the same symptoms as diabetes. This led workers to propose that the pancreas secretes a substance which regulates blood sugar as well as other factors concerned with diabetes. In 1909 de Meyer coined the name *insuline* to refer to the then hypothetical substance secreted by the islets of Langerhans of the pancreas.

It was not until 1922, however, that the biological activity of insulin was finally demon-

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strated by Banting and Best (5), who showed that a pancreatic extract could be used to alleviate the symptoms of diabetes. For this monumental discovery, Dr. Banting received a Nobel Prize. Four years later Abel and his associates (6) obtained crystalline insulin by the extraction of animal pancreases, and proposed that insulin was a protein.

Sanger was awarded the Nobel Prize in chemistry in 1958 in recognition of his work on the elucidation of the chemical structure of the insulin molecule. His work involved the cleavage of insulin into 2 peptide chains by performic acid oxidation (7), followed by degradation studies on the 2 individual chains to determine the amino acid sequence of each (8, 9). This work has been summarized by Sanger (10). As a result of the work of Sanger and others, it is known that insulin has a molecular weight of approximately 6,000 and is composed of 2 peptide chains. The A, or glycyl chain, is composed of 21 amino acid residues while the B, or phenylalanyl chain, is composed of 30. The 2 chains are connected by disulfide bridges between A 7 and B 7 and between A 20 and B 19, with a third disulfide bond found in the A chain from A 6 to A 11.

The absolute proof of the structure of insulin must come from the preparation of a synthetic insulin which exhibits a significant amount of insulin activity in a variety of biological assays. Because of this, a great deal of recent research has been directed toward this goal. Katsoyannis and his associates (11–13) prepared the A and B chains of sheep insulin by synthetic procedures requiring over 200 steps. Zahn, Meienhofer, and their co-workers have also reported the synthesis of A and B chains of insulin (14–16).

It can be readily seen that the combination of synthetic A chain with synthetic B chain to produce insulin is no mean task. The number of ways in which the two chains can combine *via* disulfide bonds is extremely high, so it is no surprise that the yields of synthetic insulin prepared in this manner have been extremely low. It has been reported (12) that Dr. G. H. Dixon, working in cooperation with Katsoyannis' group, was able to combine the synthetic A chain with the synthetic B chain to produce a product which showed insulin activity, but the details of the combination experiments have not yet been reported. Zahn and his associates (17) have combined synthetic A and B chains to produce a preparation containing 0.5% of the activity of crystalline insulin. Their calculations are based on a natural insulin preparation containing 26.7 I.U./mg.

A recent publication by a group of Chinese workers (18) describes the cleavage of insulin with sodium sulfite and sodium tetrathionate to produce the *S*-sulfonates of A and B chains. The chains were separated and then recombined by reduction and subsequent reoxidation. The insulin produced in these experiments showed an activity of 12 I.U./mg. when measured by the mouse convulsion test. This represents nearly 50% of the activity of natural insulin. Zahn and Brinkhoff have recently combined natural A chain with natural B chain to produce a product exhibiting 40% of the activity of natural insulin (19). These results make it seem quite likely that the yields for the combination of synthetic A and B chains can be improved significantly in the future.

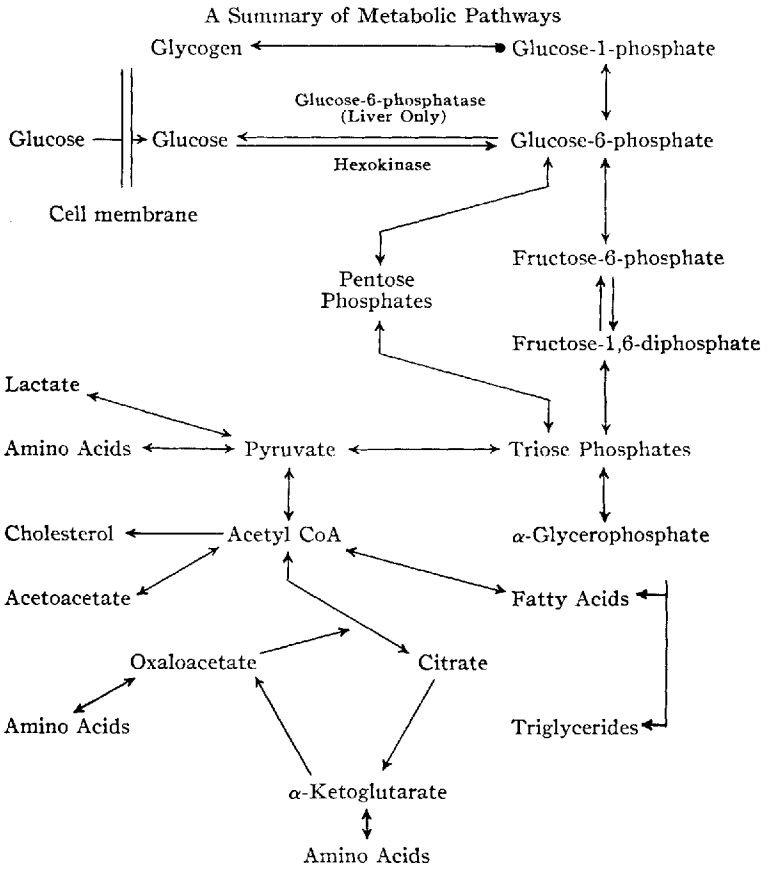
THE BIOLOGICAL EFFECTS OF INSULIN

It is well known that insulin exerts a profound effect not only on carbohydrate metabolism but also on the metabolism of fats and proteins. The biological effects of insulin have been extensively reviewed by numerous authors (20–25) and will not be treated in great detail here. The following discussion will simply summarize the effects of insulin on the metabolism of carbohydrates, fats, and proteins. Scheme I is included to allow the reader to follow the general metabolic pathways of these materials and to emphasize the interrelationship between the metabolism of all 3 classes of compounds.

Effects on Carbohydrate Metabolism

The important sites of carbohydrate metabolism which are sensitive to insulin are the liver, where glycogen is formed, stored, and broken down, the skeletal muscles, where glucose is oxidized to produce energy, and adipose tissue, where glucose may be converted to fatty acids, glycerol phosphate, and triglycerides. An appreciable quantity of glucose is also metabolized in the brain, but this tissue does not respond to insulin stimulation. The effects of insulin on carbohydrate metabolism are therefore different in the various tissues.

Effects in Skeletal Muscle.—Insulin is known to increase the uptake of glucose by skeletal muscle. Gemmil (26, 27) was the first to show that insulin stimulates glucose consumption in the isolated rat diaphragm. This system has been studied thoroughly by Vallance-Owen and has been developed as an assay for insulin in blood and other fluids (3). The increased glycogen synthesis promoted under these conditions is accounted for by Larner and Villar-Palasi (28, 29) by an insulin stimulation of uridine diphosphoglucose glycogen transglucosylase, an enzyme which catalyzes the rate-determining step in glycogen synthesis.



Scheme I

The glucose uptake of most peripheral tissues in humans has been shown to be stimulated by insulin. Evidence for this has been obtained by measuring the difference in glucose concentrations of venous and arterial blood in the limbs of humans (30).

The actual mechanism or mechanisms by which insulin promotes glucose utilization by skeletal muscle are not understood in biochemical terms. It does, however, seem reasonable to suppose that since insulin appears to promote all known pathways of glucose disposal and utilization (31), it probably exerts its primary action early in the metabolic sequence. This observation led to a number of investigations concerning the effect of insulin on the hexokinase reaction, a reaction which must occur before glucose can be metabolized by any route (32–35). Workers have been unable to show that insulin stimulates this reaction, nor have they been able to show consistently that it counteracts inhibitors of the reaction (36). It is, therefore, difficult to connect this reaction with the biochemical mechanism of action of insulin.

More recent studies have been concerned with the effect of insulin on the transfer of glucose and other sugars across cell membranes. Much of the research in this area has been carried out by Levine and his co-workers and has recently been summarized (31). These workers studied the effect of insulin on the transport of galactose, a sugar which is transported in the same manner as glucose but which is not metabolized in skeletal muscle. They observed that insulin definitely stimulates galactose transfer in most tissues. Narahara and Ozand (37) made similar observations in connection with their studies on the transport of 3-*O*-methyl-D-glucose, another non-utilizable sugar. Park and his associates have found that insulin also stimulates glucose transport in isolated muscle (38).

This evidence has led Levine (31) to propose that the primary effect of insulin on carbohydrate metabolism is at the level of the cell membrane. According to this theory, insulin increases the permeability of the cell membrane to glucose, thus allowing glucose to pass freely in either direction. Since the glucose inside of the cell is

rapidly phosphorylated, the process is essentially unidirectional. This idea appears to be in agreement with the available facts, but insulin might also have a specific action on other steps involved with the utilization of glucose.

Effects in Adipose Tissue.—In adipose tissue, carbohydrate metabolism is somewhat different than in muscle. In adipose tissue a large portion of the glucose-6-phosphate is oxidized through the pentose phosphate pathway and much is converted to glycerol phosphate, fatty acids, and triglycerides, whereas in the muscle the major synthetic product is glycogen.

Insulin has been shown to increase glucose oxidation by rat epididymal adipose tissue *in vitro* (39) and also increases the synthesis of fatty acids from glucose in this tissue (40, 41). The work of Winegrad and Renold (42), who worked with the oxidation of glucose-U-^{14}C, glucose-1-^{14}C, and glucose-6-^{14}C in isolated rat epididymal adipose tissue, indicates that in this tissue insulin stimulates catabolism by the pentose phosphate pathway to the same extent as by the Embden-Meyerhof pathway. This further suggests that the major effect of insulin is at a point early in the metabolic pathway. It is believed that the increased oxidation of glucose by adipose tissue in the presence of insulin is a result of an increased transport of the sugar across cell membranes (43).

Effects in the Liver.—Less is known about the effects of insulin on carbohydrate metabolism in the liver than about its effects in skeletal muscle and adipose tissue. It is exceedingly difficult to ascertain which of these effects are direct and which are secondary, thus making it difficult to present a detailed discussion of the exact effects of insulin on carbohydrate metabolism in the liver.

The liver is an important organ in the regulation of blood sugar levels. When blood glucose levels are high the liver takes up glucose and when they are below normal it produces glucose from glycogen. It has been shown (44) that the rate of removal or production of glucose by the liver is directly proportional to the degree of hyper- or hypoglycemia. Unlike skeletal muscle and adipose tissue, the cell membrane in the liver is freely permeable to glucose and other sugars (45) which indicates that transport is not rate limiting and does not account for the effect of insulin on net glucose uptake by the liver.

The fate of glucose in the liver must be considered in searching further for a possible effect of insulin on carbohydrate metabolism in the liver. Glucose is first phosphorylated by ATP to form glucose-6-phosphate; the enzyme catalyzing this reaction in the liver is quite specific and is referred to as "glucokinase." Once it is formed, glucose-6-phosphate may be reconverted to glucose under the influence of glucose-6-phosphatase, an enzyme present in appreciable quantities only in the liver, or it may be converted to glycogen and stored. The glucose-6-phosphate may also be oxidized in the liver, either by Embden-Meyerhof glycolysis or *via* the pentose phosphate pathway (45). The glucokinase reaction limits glucose utilization by the liver (46), and the glucose-6-phosphatase reaction regulates hepatic glucose output (47).

In severe diabetes hepatic glucokinase activity falls to one-fifth of normal, while glucose-6-phosphatase activity doubles (45). Both the decreased glucokinase activity (45) and the increased glucose-6-phosphatase activity of diabetics can be corrected by the administration of insulin over a period of time (45). The effect of insulin on carbohydrate metabolism in the liver then appears to be to decrease net hepatic glucose output.

Initially, it was found to be quite difficult to demonstrate an immediate decrease in hepatic glucose output after the administration of insulin to intact animals, and many workers were unable to produce a prompt effect (48–50). On the other hand, Madison and his associates (51) showed that insulin has a prompt effect on hepatic glucose output in unanesthetized dogs, providing the insulin is administered by slow intravenous infusion. More recently, other workers have shown that insulin promptly depresses net hepatic glucose output in intact animals quite rapidly (52–54). These workers have found that animals on a high carbohydrate diet respond better than those on a high protein diet, presumably because of the higher plasma insulin levels produced by the high carbohydrate diet. Their studies also indicate that significant hypoglycemia must be avoided in order to produce an insulin effect. This may be accomplished by the slow intravenous infusion of insulin and by administering small quantities of glucose to the animals.

Bishop and his co-workers have recently studied the effect of insulin on glucose output in intact dogs quite extensively (55) and have found that the first effect of insulin on the liver is to decrease glycogen breakdown, thereby decreasing the release of free glucose into the bloodstream. A later effect (after 2 hr.) is an increased glucose utilization for glycogen synthesis. The over-all result is then an immediate and sustained depression of net hepatic glucose output.

Effects on Fat Metabolism

The presence of ketone bodies in the blood and urine of uncontrolled diabetics indicates that

these individuals exhibit abnormal fat metabolism as well as abnormal carbohydrate metabolism. Stadie and his group (56) observed that liver slices from pancreatectomized cats produced 5 times more ketone bodies than liver slices from normal cats. This increased production of ketone bodies by diabetic animals results from an increased oxidation of fatty acids in these animals.

The work of Brady and Gurin (57) has shown that liver slices from alloxan-diabetic rats have a reduced ability to synthesize long chain fatty acids from acetate. It has also been demonstrated that insulin is able to stimulate the incorporation of acetate into longer chain fatty acids *in vitro* (58). Insulin has been shown to inhibit the release of free fatty acids (FFA) from glyceride stores in adipose tissue (59). Since insulin is able to stimulate fat synthesis and inhibit fatty acid mobilization in adipose tissue, it comes as no surprise that it is able to lower the elevated FFA levels observed in diabetic subjects (60).

The impaired carbohydrate metabolism of diabetics could be partially responsible for the decreased rate of fatty acid synthesis. This is indicated by the fact that fatty acid synthesis in liver slices is promoted by the presence of glucose in the medium. The problem is, however, probably much more complex than this and insulin undoubtedly has some more direct effect on fat synthesis. Chernick and Chaikoff (61) have demonstrated that the diabetic liver can oxidize lactate, pyruvate, and acetate but cannot incorporate these substrates into fatty acids. This malfunction in fatty acid synthesis can be corrected by treatment of the animal with insulin for several days (24), which is indicative of a direct effect on fatty acid synthesis.

Effects on Protein Metabolism

It is well established that diabetes results in a loss of body weight, a depletion of body protein, and a negative nitrogen balance (22). This nitrogen loss can be prevented by the administration of insulin (62), which suggests that insulin has some effect on the synthesis and/or degradation of proteins.

It is difficult to study the effects of insulin on protein metabolism in intact animals, although certain pertinent observations have been made in this manner. It has been demonstrated (63) that insulin lowers plasma amino acid levels, and it has also been shown (64) that it promotes the uptake of amino acids by skeletal muscle.

The best evidence that insulin affects protein metabolism comes from studies carried out in a number of *in vitro* systems. Numerous workers have used the isolated rat diaphragm to show that insulin stimulates the incorporation of labeled amino acids into protein (65–68). These studies show that insulin concentrations as low as 50 microunits/ml. will stimulate the incorporation of amino acids into proteins in this system. Since it is effective at this low concentration, the response is probably a physiological one.

It was first thought that the effect of insulin on protein synthesis was secondary to its effect on carbohydrate metabolism. It was felt that insulin stimulated glucose metabolism which in turn provided energy that could then be utilized for protein synthesis. More recent research, however, has made it appear highly unlikely that this is the case. Experiments carried out by Wool and Krahl (69) show that the effect of insulin on protein synthesis in the rat diaphragm *in vitro* is independent of the concentration of glucose in the medium for glucose concentrations between zero and 600 mg. %. In the same study it was shown that insulin stimulates the incorporation of labeled amino acids into proteins in diaphragms isolated from fed rats even when there is no extracellular glucose present in the diaphragm. This evidence strongly suggests a more direct effect of insulin on protein synthesis in this system.

It was found (70) that insulin stimulates the uptake of α-aminoisobutyric acid, a nonutilizable amino acid, by the isolated rat diaphragm. This led to the speculation that the effect of insulin on protein synthesis could be a result of its facilitating the transport of amino acids into cells. Further studies by Wool and Krahl (71) have essentially ruled out the possibility that this is the sole effect of insulin on protein synthesis. In these studies, the labeled amino acid was injected into intact rats, thus allowing the amino acid to accumulate in the diaphragm of the intact animals. The animals were then sacrificed, the diaphragms removed and incubated with or without insulin, and the rate of protein synthesis determined. The results showed that insulin stimulates the synthesis of proteins from labeled amino acids even when the amino acids are already present in the diaphragm, thus indicating a direct effect of insulin on protein synthesis.

The results of subsequent studies have led Wool to propose that the effect of insulin on protein synthesis is mediated by its effect on messenger RNA synthesis (72). He believes that the effect results from insulin stimulating the transfer of messenger RNA from the nucleus to the cytoplasm, a proposal which seems to agree with the existing facts.

POSSIBLE CAUSES OF DIABETES

The actual etiology of diabetes is not yet understood, although there are a number of factors which could account for the abnormal glucose tolerance exhibited by diabetics. The 3 most obvious possibilities are (*a*) a decreased production of insulin, (*b*) an increased rate of destruction of the hormone, and (*c*) an inability of tissue to respond to stimulation by insulin.

Juvenile diabetes is quite likely the result of the first. The pancreas loses the ability to produce and secrete insulin and the plasma insulin levels become very low, possibly even reaching zero (73).

As early as the 1940's, Mirsky (74) proposed that diabetes could be due to an increased destruction of insulin by an enzyme which he then referred to as "insulinase." Since that time numerous studies have been carried out to determine the nature of the enzyme responsible for the degradation of insulin. This led to the isolation of a purified preparation from acetone powders of livers which is capable of reducing insulin to its A and B chains (75–78). The reaction utilizes hydrogen from reduced glutathione, and the enzyme is therefore referred to as glutathione insulin transhydrogenase. There is no direct evidence for any increased activity of this enzyme nor any increased destruction of insulin in diabetic subjects, so its connection with the etiology of diabetes has not been established.

Neither of the first 2 factors seems to explain the impairment of glucose tolerance in cases of maturity-onset diabetes since individuals of this type may be characterized by normal or above normal insulin levels (79, 80). According to Lacy (81), the ultrastructure of the β granules in maturity-onset diabetics is similar to that of the normal human β cell. This suggests that the pancreas has not lost its ability to produce insulin, and another possible cause for the disease must be sought.

Maturity-onset diabetes could result from an inability of the peripheral tissue to respond to insulin. This decreased tissue response could very well be the result of the presence of substances which antagonize insulin. Numerous insulin antagonists have been described in some detail, and many of these are themselves hormones. It has been demonstrated (82) that growth hormone and the adrenal corticosteroids can inhibit the effect of insulin on glucose transport. Epinephrine, another insulin antagonist, increases blood sugar levels by increasing hepatic glucose output (83), and a similar effect on the liver is also produced by glucagon (83).

Insulin is a protein of sufficient molecular weight to produce antibodies (84) which are capable of neutralizing its action. This phenomenon may also be referred to as "insulin antagonism."

Bornstein and Hyde (85) have described a pituitary peptide with a molecular weight of about 4,500 which is able to inhibit the uptake of glucose by the isolated rat diaphragm. This substance is also capable of inhibiting the incorporation of acetate into fats by liver slices and the incorporation of amino acids into proteins in rat muscle (86). Its actual physiological significance has not been determined.

The studies of Samaan (87) with "typical" and "atypical" insulin and of Antoniades (88) with "bound" and "free" insulin indicate that binding of insulin to plasma proteins antagonizes its action in certain *in vitro* systems. Antoniades (88) has reported that bound insulin is biologically active in rat epididymal adipose tissue *in vitro* but is inactive in the isolated rat diaphragm, while free insulin is active in both systems. Recent studies (89) indicate that bound insulin increases the incorporation of labeled glucose into glycogen in muscle and adipose tissue and into fat in adipose tissue in intact rats. This suggests that the binding of insulin to plasma proteins does not antagonize its biological activity *in vivo*.

The insulin antagonists which are currently receiving the most attention are the synalbumin antagonist and plasma FFA, either or both of which could contribute significantly to the cause of diabetes.

The Synalbumin Insulin Antagonist.—It has been mentioned previously that insulin promotes the uptake of glucose by the rat diaphragm *in vitro*. This system has been used as a biological assay for insulin and under the proper conditions is an accurate method of determining the amount of insulin present in a given sample (3). The assay has also been used by Vallance-Owen (90) for the detection of insulin antagonism in the plasma of normal and diabetic subjects. His work on insulin antagonism has recently been summarized (91).

Vallance-Owen and Hurlock (92) found that the assay can be carried out using plasma from humans as the incubation medium. If insulin is added *in vitro* to the plasma of normal, fed subjects or obese, nonketotic diabetics, the insulin activity can be recovered almost quantitatively. In contrast to this, it was found that when plasma from uncontrolled or insulin-requiring diabetics is used as the incubation medium, the action of insulin is definitely antagonized.

It was subsequently shown by Vallance-Owen and his co-workers (93, 94) that the antagonist actually resides in the albumin fraction. The

albumin used in these studies was prepared by T.C.A.–ethanol fractionation, a procedure which has been shown by Schwert (95) to produce electrophoretically pure albumin.

Similar antagonism can be demonstrated in plasma-albumin from normal subjects, although this albumin is not nearly so active as that obtained from the plasma of diabetics. Vallance-Owen, Dennes, and Campbell (94) found that concentrations of 3.5–5% of diabetic plasma-albumin will completely antagonize the effect of 1,000 microunits of insulin in the rat hemidiaphragm assay and will also antagonize insulin *in vitro* at concentrations as low as 1.25%. Plasma-albumin from normal subjects will completely antagonize 1,000 microunits of insulin when used at concentrations of 3.5–5%, will show some antagonism at 2.5%, but exhibits no insulin antagonism at a concentration of 1.25%. These observations have recently been confirmed by Alp and Recant (96), who performed similar studies using a wide variety of albumin preparations. These workers studied albumin preparations isolated by the Cohn procedure (97) as well as a series of preparations isolated by the Debro procedure (98). Insulin antagonism was observed in all of these preparations, although the effect was not so great as that demonstrated by Vallance-Owen and his group. All of this evidence suggests that excessive insulin antagonism could have some connection with the etiology of diabetes.

Subsequent work by Vallance-Owen and his group (99) indicates that the antagonist is dependent on the presence of a functioning pituitary gland. This research involved a study of the plasma-albumin from 3 hypophysectomized patients, and the albumin obtained from these patients was found to be nonantagonistic in concentrations as high as 4%. One of the patients had been studied prior to hypophysectomy and his albumin had been shown to be antagonistic at that time.

It has also been shown (100) that the antagonist is dependent on the adrenal steroids. In 2 patients with a bilateral adrenalectomy and with cortisone therapy discontinued for at least 50 hr. prior to testing, the plasma-albumin was found not to antagonize insulin *in vitro*. After 1 of these patients subsequently was sustained on cortisone therapy, his plasma-albumin was retested and was found to be antagonistic.

It has been demonstrated (99) that when antagonistic albumin is passed through a partially acetylated cellulose column, it is nonantagonistic when eluted. This was the first indication that it is not the albumin itself but rather something associated with the albumin which is responsible for the insulin antagonism. Because of this, the term *synalbumin* antagonist was coined.

The synalbumin antagonist can be dialyzed away from the albumin after heat coagulation (100), but this, however, results in some loss in activity of the antagonist. The antagonist cannot be extracted from albumin with ethanol, chloroform, or a mixture of *n*-octane and acetic acid, which indicates that the antagonist is not a lipid, fatty acid, or steroid. This suggests that it is not a simple case of albumin-bound fatty acids inhibiting the action of insulin as shown in the studies of Randle, Garland, Hales, and Newsholme (101). The evidence shows that the antagonist could be a relatively low molecular weight polypeptide which led to the postulation (91) that the synalbumin antagonist might be the B chain of insulin.

Ensinck and Vallance-Owen (102) have shown that when ^{131}I-labeled insulin is administered to humans, the molecule is cleaved enzymatically and some of the reduced ^{131}I B chain becomes bound to the plasma-albumin. The A chain, on the other hand, appears to associate with the α_2 globulin fraction. These workers (102) have pointed out several similarities between B chain and the synalbumin antagonist; for example, both have a molecular weight of less than 4,000, both are capable of binding to albumin, and both dissociate from the albumin at extremes in pH.

Ensinck, Mahler, and Vallance-Owen (103) have recently presented further evidence to support the theory that the synalbumin antagonist could be B chain. They performed a series of experiments using reduced B chain, *S*-sulpho-B chain, and oxidized B chain. Their work showed that *S*-sulpho-B chain and reduced B chain antagonize the action of insulin in the hemidiaphragm assay, while oxidized B chain does not. They found that their *S*-sulpho-B chain preparation contained 0.5–0.9 sulfhydryl groups per mole of the chain. This, they feel, is responsible for the antagonistic activity of the *S*-sulpho-B chain and for the lack of antagonism shown by the oxidized B chain which contains no free sulfhydryl groups. It is indicated that the free sulfhydryl groups are essential for antagonistic activity since the reduced B chain preparations were rendered nonantagonistic by prior incubation with alkylating agents such as iodoacetamide or *N*-ethylmaleimide. The antagonistic activity of the reduced and *S*-sulpho-B chains is enhanced greatly by prior incubation with albumin which is probably because B chain itself is not very soluble unless complexed with albumin. These in-

vestigators (103) further demonstrated that B chain could be dissociated from albumin by passing the complex through an acetylated cellulose column or a Dowex 50 column in the sodium phase. The synalbumin antagonist had previously been shown to behave in a similar fashion.

It is proposed (103) that the B chain could result from the cleavage of insulin by glutathione-insulin transhydrogenase as shown by Tomizawa (104) and by Katzen and Stetten (78). It could antagonize the action of insulin by competing for the insulin binding sites at the cell membrane. It should be emphasized that insulin B chain has never been isolated from human serum or any of its components. If this could be accomplished, it would certainly add credence to the hypothesis that the synalbumin antagonist could be B chain.

Vallance-Owen (105) has shown that the plasma-albumin from prediabetics is antagonistic *in vitro* at a concentration of 1.25%. The term "prediabetes" may be defined as the metabolic state of a person at a period before he or she exhibits definite symptoms of diabetes. The presence of excessive synalbumin antagonism could therefore develop into a method of identifying prediabetics before they become overtly diabetic.

It is generally agreed that diabetes mellitus is hereditary in some way, although the mode of inheritance seems to be open to debate. Vallance-Owen (105) has studied a number of families with regard to the inheritance of excessive synalbumin antagonism, which he feels may indicate "essential diabetes." His evidence indicates that the transmission of excessive synalbumin antagonism could be by a dominant mode of inheritance, although the evidence is far from conclusive.

Lowry, Blanchard, and Phear (106) have performed experiments to show that the synalbumin antagonist does not inhibit the effect of insulin on adipose tissue. This could point to a fallacy in the whole line of reasoning or it could simply mean that synalbumin is capable of antagonizing some, but not all, of the actions of insulin. Also, no one has yet been able to demonstrate antagonistic activity for synalbumin or for B chain *in vivo*. A number of technical problems must be overcome before this can be accomplished; *i.e.*, B chain, if it is the antagonist, is quite insoluble and probably could not be administered alone, which would necessitate complexing it with a rather large quantity of albumin before infusing it into animals.

There are a number of questions yet to be answered about the synalbumin antagonist. For example, if it actually is B chain, does it arise from the incomplete synthesis of insulin or from increased destruction of insulin by glutathione insulin transhydrogenase? Also, what factors regulate the level of antagonist present in the plasma of normal and diabetic subjects? Nevertheless, these proposals merit serious thought and could provide answers to several important questions. It would certainly be advantageous to have a biochemical marker for the identification of prediabetics. This could also conceivably lead to fundamental knowledge about the etiology of diabetes mellitus and to possible ways of treating the disease.

The Glucose Fatty Acid Cycle.—It has been mentioned previously that the diabetic exhibits abnormal lipid metabolism as well as abnormal carbohydrate metabolism. Until quite recently, it had been generally agreed that the disturbance in lipid metabolism was secondary to the abnormalities in carbohydrate metabolism. Recent studies (107) suggest that the over-all disturbance in metabolism could result from high levels of FFA which result from an increased metabolism of fats by the diabetic. The implication is that the relationship between carbohydrate and lipid metabolism is reciprocal, and good evidence has been put forth to help verify this (107).

Randle and his associates (107) propose that the metabolism of glucose by the tissues inhibits the release of FFA from glyceride stores and that conversely the release of FFA inhibits the metabolism of glucose. According to this line of thought, the diabetic is unable to carry on normal carbohydrate metabolism because of elevated FFA levels.

Newsholme and Randle (108) demonstrated that anoxia, salicylate, and 2,4-dinitrophenol increase the rate of phosphorylation of fructose-6-phosphate to form fructose-1,6-diphosphate, a reaction which is catalyzed by phosphofructokinase. The reaction is inhibited in hearts from diabetic or starved rats or in hearts from normal rats which are perfused with media containing fatty acids or ketone bodies.

The stimulation of the phosphofructokinase reaction caused by anoxia is attributed (108) to increased concentrations of AMP and inorganic phosphate, which stimulate the reaction, and decreased concentrations of ATP, which inhibits the reaction. Alloxan-diabetes, starvation, fatty acids, and ketone bodies have no consistent effect on cencentrations of AMP, ATP, and inorganic phosphate and must, therefore, inhibit the reaction by a different mechanism. It is proposed (108) that the inhibition is caused by the increased concentration of citrate which these factors have been shown (109) to produce.

Randle's work (110) shows that the membrane transport of D-arabinose and D-glucose is greater in fed rats than in starved rats, a phenomenon which might be due to the presence of higher insulin concentrations in the fed animals. Fatty acids and ketone bodies will inhibit transport in fed rats but not in starved rats (110). Inhibition of transport by fatty acids and ketone bodies was demonstrated in starved rats when low concentrations (0.5 milliunit/ml.) of insulin but not when high concentrations (0.1 unit/ml.) of insulin were added. These workers feel that the above facts are explained by the hypothesis that fatty acids and ketone bodies impair the sensitivity of the transport system to stimulation by insulin.

Randle and his co-workers (110) also found that the phosphorylation of glucose by the perfused rat heart is inhibited by fatty acids and ketone bodies. A similar inhibition of phosphorylation of glucose was demonstrated in hearts from diabetic or starved rats.

It was found (110) that the rate of glycolysis was decreased in isolated rat hemidiaphragms and perfused rat hearts by fatty acids, ketone bodies, or pyruvate. These workers feel that the decreased rate of glycolysis is a result of a decreased uptake of glucose. The fact that more $^{14}CO_2$ is produced from 1-^{14}C-glucose than from 6-^{14}C-glucose under these conditions is an indication that some glucose is being oxidized through the pentose phosphate pathway.

Garland and Randle (109) showed that fatty acids, ketone bodies, and alloxan-diabetes inhibit the oxidation of pyruvate in the perfused rat heart and isolated hemidiaphragm. It is proposed (109) that this inhibition of pyruvate oxidation is the result of an increased acetyl CoA/CoA ratio which is produced by these conditions.

Garland and Randle (111) have reported that the concentration of fatty acyl CoA was increased in the rat heart *in vitro* by alloxan-diabetes, starvation, and perfusion with fatty acids. This is presumably caused by the increase in concentration of FFA which is due to an increased lipolysis and a decreased rate of fat synthesis. It was further shown (111) that the increased production of fatty acids and fatty acyl CoA could result in an increase in the concentration of citrate which then can inhibit the phosphofructokinase reaction. The concentration of citrate in diabetic rat hearts is lowered by insulin which might account for some of the increased carbohydrate metabolism brought about by insulin.

Garland *et al.* (112) illustrated that the oxidation of glucose in perfused rat hearts is inhibited when the perfusion media contains fatty acids or ketone bodies. They also found, however, that there is no decrease in oxygen consumption under these conditions. This was taken to mean that in the presence of fatty acids and ketone bodies, there is an increased oxidation of fatty acids to compensate for the decrease in glucose oxidation. This is further evidence for the reciprocal relationship between carbohydrate and fat metabolism.

From this evidence it is postulated (111) that the increased release of fatty acids from glyceride stores in diabetic rats results in increased levels of FFA, fatty acyl CoA, and ketone bodies. This in some way causes an increase in citrate concentration, and the over-all result is an inhibition of glucose transport, glucose phosphorylation, the phosphofructokinase reaction, and the oxidation of pyruvate.

INSULIN THERAPY

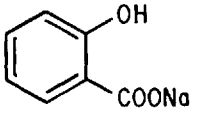
Insulin has been the mainstay of diabetes therapy ever since its discovery by Banting and Best. There are at least 7 different insulin preparations currently available in this country, the principal difference between these being in their duration of action. Crystalline insulin is effective but has an extremely short duration of action (6–12 hr.) (113). Longer acting preparations have been obtained by combining insulin with higher molecular weight proteins such as protamines and globins. Further work in this area resulted in the discovery of the lente insulins (114) in which duration of action can be controlled by a selective precipitation scheme. All of these preparations are insulin and exhibit the biological effects of insulin; they differ only in duration of action.

Although insulin offers many advantages in the treatment of diabetes, it also has a number of disavantages. It is a protein and is capable of producing allergic reactions. This can be further complicated when insulin is combined with a higher molecular weight, more highly antigenic protein, to increase its duration of action. Insulin is not effective orally and must therefore be administered parenterally which is an inconvenience at best. Insulin is a potent hormone and can produce dangerous hypoglycemia when doses are not regulated to correspond with conditions of diet and exercise. Good control of diabetes with insulin does not always prevent the occurrence of complications of diabetes such as diabetic neuropathy, glomerulosclerosis, diabetic retinopathy, and vascular disorders (115). Also, some patients are resistant to insulin (116) and require well over 200 units of insulin a day. This indicates a significant degree of resistance since it has been estimated (117) that a normal daily secretion of

insulin is about 55 units. Because of these numerous disadvantages, a great deal of research has been carried out to produce an insulin substitute which does not have these drawbacks.

ORAL HYPOGLYCEMICS

The Salicylates.—Long before the discovery of insulin it was known that sodium salicylate (I) is capable of lowering blood sugar in diabetics. Ebstein in 1876 (118) and Muller in 1877 (119)

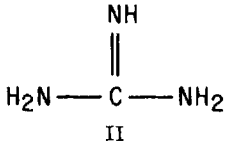


I

published reports on the ability of sodium salicylate to lower blood sugar. Not all salicylates exert this property. Acetylsalicylic acid, for example, has hypoglycemic acitivity when given in suitably high doses, while sodium gentisate which is chemically similar has no hypoglycemic acitivity.

The hypoglycemic salicylates cause an increased oxygen consumption in experimental animals (120) by the uncoupling of oxidative phosphorylation as shown by Brody (121). This uncoupling action increases aerobic glycolysis and increases glucose utilization, but the energy derived from the process is relatively little. The dosage of salicylates necessary to bring about a hypoglycemic effect is very high. It has been reported (122) that a daily dose of 4.8 Gm. is required to produce an adequate hypoglycemic effect. At this dosage level numerous side effects such as nausea, vomiting, hearing difficulties, and tinnitus appear quite frequently and thus the salicylates are not used clinically as hypoglycemic agents.

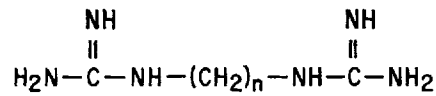
Guanidine.—The first reports on the ability of guanidine (II) to lower blood sugar were in 1918



II

(123). Hollunger (124) has shown that it inhibits respiration which leads to an increased glucose uptake by muscle, an increased glycolysis, and a decrease in blood glucose levels. Because of the relatively high toxicity of guanidine it was never widely used, although it did serve as a pattern for newer compounds which have been used as oral hypoglycemics.

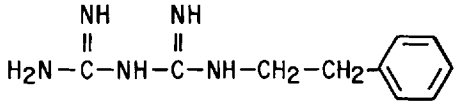
The Diguanidines.—These compounds (III), may be visualized as 2 guanidine molecules joined by a series of methylene groups. For the



III

most part, these compounds are more potent and less toxic than guanidine. Hypoglycemic activity increases with an increase in the number of methylene groups and apparently reaches a maximum in the decamethylene derivative which is 150 times more active than guanidine (125). This substance, known as synthalin A, was introduced into diabetes therapy in the 1920's The dodecamethylene diguanidine (synthalin B) is somewhat less active than synthalin A. Neither of these compounds compares favorably with insulin for effectiveness, and both also have a rather high tendency to cause kidney and liver damage (126), so their use as hypoglycemics was discontinued.

The Biguanides.—Further attempts to produce useful compounds resulted in 1929 in a series of biguanides which exhibited hypoglycemic activity (127). This discovery received little attention at the time, and little work was done in the area until 1957 when Unger and his associates (128) reported on the hypoglycemic activity of phenformin (IV) a newly synthesized biguanide.



IV

As a result of these studies, hundreds of new biguanides were prepared and tested for hypoglycemic activity, but none has been found to be superior to phenformin.

A great deal of information on the effects of phenformin and other biguanides on various biological systems is available, but the exact mechanism by which these compounds lower blood sugar is not understood. Phenformin was shown to increase the glucose uptake of the isolated rat diaphragm (129), but this effect was produced by a higher dose of the drug than is normally found in a physiological system. It has been found that glucose uptake by the diaphragm is accompanied by a decreased oxygen consumption and a decreased glycogen content of the diaphragm (130) which is opposite from the effect of insulin in this system. The increased glucose uptake of the diaphragm in the presence of phenformin appears to be a result of increased anaerobic glycolysis in the muscle (130). This is consistent with the findings of Steiner and Williams (131), who demonstrated that phen-

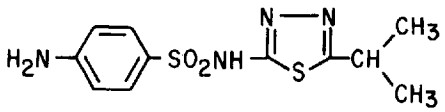
formin produces elevated levels of lactic acid, the end product of anaerobic glycolysis in muscle.

It has been shown (132) that phenformin increases the uptake of glucose by the rat epididymal fat pad *in vitro*. Daweke and Bach (133) used Buformin (N^1-*n*-butylbiguanide) to show an increased oxidation of glucose-1-^{14}C by rat adipose tissue at relatively low concentrations, but on the other hand, Wick and his co-workers (134) found that higher doses of phenformin inhibit glucose oxidation by adipose tissue.

Steiner and Williams (135) have proposed that the biguanides might act by inhibiting certain enzyme systems of the mitochondria which are concerned with Krebs cycle oxidations. This would lead to an indirect stimulation of the anaerobic glycolysis of glucose and in turn would cause an increased uptake of glucose. This theory accounts for the increased lactic acid production associated with biguanide treatment and is also consistent with the observations of Randle and Smith (136), who demonstrated that anaerobiosis can increase the glucose uptake of the isolated rat diaphragm.

The biguanides serve a useful function in diabetes therapy in that they are useful in treating certain cases which are unresponsive to other oralhypoglycemics. They affect carbohydrate metabolism directly but are not used in the absence of some supply of insulin since acidosis develops in such cases. Side effects such as anorexia, nausea, vomiting, weight loss, and muscular weakness occur quite frequently in phenformin treated subjects (137), although this compound does not exhibit the liver toxicity of the parent synthalins (138).

The Sulfonamides.—Savagone (139) was the first to publish information indicating that certain sulfonamides lower blood sugar in man. This led to the investigation of a number of sulfonamides as potential oral hypoglycemic agents. The work of Loubatieres (140) showed that one of the sulfonamides, 2-(*p*-aminobenzenesulfonamide)-5-isopropylthiodiazole (IPTD) (V), lowers



V

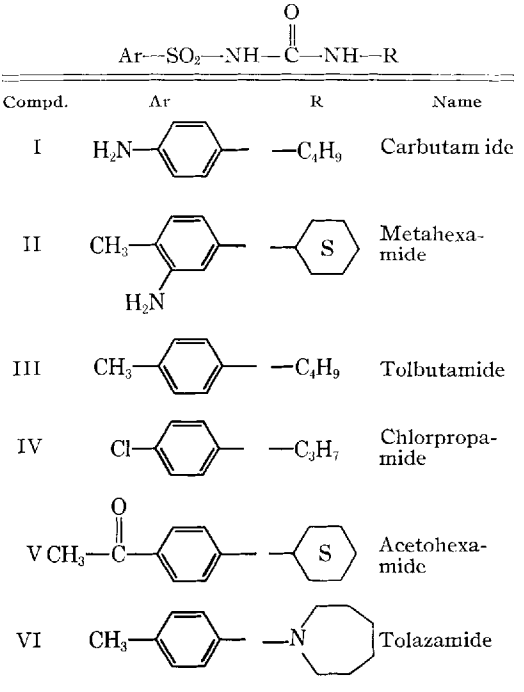
blood sugar levels, apparently by stimulating the β cells to secrete insulin.

Relatively little research was done on the sulfonamides as hypoglycemics until the advent of the sulfonylureas in the 1950's. At that time IPTD was studied clinically in human diabetics (141) and was found to lower blood sugar in some patients. It was found effective in the same general type of cases as the sulfonylureas; but since it is somewhat less potent than most sulfonylureas and has no obvious advantages over the sulfonylureas it never has been marketed.

The Sulfonylureas.—From a practical standpoint the sulfonylureas are currently the most important oral hypoglycemics. Their ability to lower blood sugar was first discovered by a group of German workers in 1951. Carbutamide (BZ 55) was the first of the sulfonylureas to be studied extensively from a clinical standpoint (142–144). The discovery that this compound is capable of controlling the effects of diabetes in some patients led to the synthesis and testing of literally hundreds of analogs and derivatives of carbutamide. It is beyond the scope of this review to consider the chemistry and pharmacology of large numbers of individual sulfonylureas, particularly since there are already numerous excellent reviews on the subject (145–148). This discussion will be concerned primarily with the pharmacological action of the sulfonylureas as a group.

Some of the more familiar sulfonylurea hypoglycemics are listed in Table I. Carbutamide, the first of the series to be studied thoroughly, was found to be an effective hypoglycemic but also exhibits liver toxicity which in some cases proved to be fatal (149). Because of this, the compound was never marketed in the U. S., although it is still in use in some European countries. Metahexamide, a somewhat newer compound, is an

TABLE I. – SULFONYLUREA HYPOGLYCEMICS



effective hypoglycemic (150) but also exhibits liver toxicity and has produced cases of obstructive jaundice (151) so is no longer being used clinically. Tolbutamide, chlorpropamide, and acetohexamide are all effective oral hypoglycemics which are currently in use in this country. Tolazamide, which was first synthesized in 1962 (152), is one of the newer sulfonylureas. It has been tested in animals and humans and shows promise of being a useful oral hypoglycemic (153).

All of the sulfonylurea hypoglycemics appear to act by the same mechanism, produce the same effects, and are effective in the same general types of cases. They differ mainly in potency, duration of action, and the frequency with which side effects occur. Of the many hundreds of sulfonylureas which have been synthesized, only a relatively few have a substantial amount of hypoglycemic activity and many of those are too toxic for clinical use.

The relationship between chemical structure and pharmacological activity for this class of compounds has been summarized by Mahler (147). The benzene ring should contain at least one substituent; best results are obtained when it is in the 4-position. Active compounds have been prepared where this substituent is methyl, amino, acetyl, chlorine, methylthio as well as several other groups or atoms. If a carboxyl, hydroxyl, nitro, or hydroxymethyl group is placed in the 4-position the resulting compound is inactive.

The group attached to the terminal nitrogen of the urea nucleus should be a certain size and should impart lipophilic properties to the molecule. Simple aliphatic groups are quite frequently placed in this position. The methyl derivatives are usually inactive, the ethyl derivatives exhibit some activity, and maximum activity is reached when the substituent contains 3–6 carbons. Activity is lost altogether if the substituent contains as many as 12 carbons. A number of active compounds have been prepared where this substituent is an alicyclic ring (154) with optimal activity usually being attained when this is a 5, 6, or 7 membered ring.

Most of the sulfonylureas are poorly soluble in water at a neutral pH but because of their weakly acidic character are more soluble at a slightly alkaline pH. This facilitates dissolution in the intestinal juices and absorption from the intestine. The absorption of tolbutamide from this site is nearly complete in most species (155). Most of the sulfonylureas are absorbed quite rapidly; Ridolfo and Kirtley (156) showed that carbutamide can be detected in the blood within 30 min. after oral administration.

The mode of metabolism of the sulfonylureas is quite variable and is dependent on the structure of the compound as well as the species of the animal. The mode and rate of metabolism of these drugs is important since many are converted to inactive metabolites, thus governing their potency and duration of action.

Carbutamide, which is structurally similar to the sulfa drugs, is metabolized by acetylation of the 4-nitrogen to produce an inactive metabolite (157). The 4-methyl group of tolbutamide is oxidized in the body to a carboxyl group (158) and the metabolite is without hypoglycemic activity. Chlorpropamide, which has a chlorine in the 4-position, is not metabolized in humans (159) and is excreted in the urine as the free drug. Acetohexamide is metabolized by the reduction of the ketone to a secondary alcohol (160) but the metabolite has hypoglycemic activity (161).

The biological half-life of these drugs is one of the best measures of their duration of action. The term "biological half-life" has been defined as (161) "the time required for the blood levels of the drug to decrease by 50 per cent during the postabsorptive, concentration-dependent disappearance phase." The biological half-life of a drug such as chlorpropamide, which is not metabolized, is dependent only on its rate of excretion. Johnson and his associates (159) determined the biological half-life of this drug to be 35 hr. The biological half-life of a drug which is metabolized in the body is dependent on the rate of metabolism as well as the rate of excretion of the unchanged drug. Baird and Duncan (162) estimated the biological half-life of carbutamide to be between 30 and 60 hr. The half-life of tolbutamide has been reported by a number of authors (163–165), and the average value is in the neighborhood of 5 hr. Recent studies by Smith, Vecchio, and Forist (161) indicate that the half-life of acetohexamide is 1.3 hr., indicating that the drug is metabolized very rapidly. Since the metabolite of this drug is an active hypoglycemic, the half-life of the metabolite should also be taken into consideration in estimating the duration of action of acetohexamide. The half-life for the metabolite is 4.6 hr. (161), which indicates that acetohexamide has a duration of action comparable to that of tolbutamide.

It should be emphasized that, although the duration of action of these compounds is proportional to their biological half-life, the 2 values are not equal to each other. Tolbutamide, for example, has a biological half-life of about 5 hr., but effective blood levels of the active drug are maintained for 12 to 18 hr. after oral administration (161).

Since all of these compounds are metabolized by different routes and at different rates it would be expected that the blood levels of drug would vary from compound to compound. This would quite logically have an effect on the relative hypoglycemic potencies of the compounds. McMahon and his co-workers (148) have determined the relative clinical potencies of a number of the common sulfonylurea hypoglycemics. The clinical potency was arrived at from considering the acute potency as well as the biological half-life of the various compounds. The clinical potencies of some of the more common sulfonylureas are: tolbutamide, 1; carbutamide, 1–3; chlorpropamide, 4–7; metahexamide, 10; acetohexamide, 1–4; and tolazamide, 5–15.

Numerous investigations have been carried out in recent years to determine the exact mechanism of action of the sulfonylurea hypoglycemics. A great deal of information has been accumulated and a number of facts have been well established. Some of the more pertinent observations are mentioned here.

Mirsky and Gitelson (166) have reported results which indicate that these compounds might decrease the activity of the insulin degrading enzyme in the rat liver. The decreased enzymatic activity which was observed was, however, very slight and it required an extremely high concentration of the sulfonylurea to bring it about. On the other hand, it has been found (167) that tolbutamide does not prolong the half-life of injected ^{131}I insulin. Because of this, it is rather doubtful that this effect is very important to the action of these compounds as hypoglycemics.

Some workers (168) have shown that sulfonylureas produce a slight increase in the glucose uptake of the rat diaphragm *in vitro*. This effect was not accompanied by an increased glycogen synthesis by the diaphragm as is the case when glucose uptake is stimulated by insulin. Most workers, however, have been unable to show an increased glucose uptake by the rat diaphragm in the presence of sulfonylurea hypoglycemics (169–171) which is in accord with the theory that sulfonylureas have no direct effect on carbohydrate metabolism.

The effect of sulfonylureas on the oxidation of labeled glucose by the rat epididymal fat pad *in vitro* appears to be somewhat confusing. Renold and his associates have reported that tolbutamide and chlorpropamide have a slight stimulatory effect on the oxidation of glucose by this tissue (172). Marques and Correa (173), on the other hand, found that acetohexamide, carbutamide, and tolbutamide did not increase glucose oxidation by isolated rat epididymal adipose tissue. They found that high concentrations (50 mcg./ml.) of chlorpropamide or tolazamide increased glucose oxidation slightly, but the effect was not nearly so great as that produced by insulin at a concentration of 20 microunits/ml. It is interesting to note that even in Renold's studies there was found to be no stimulation of fatty acid synthesis as is the case when insulin is added to the medium. It should also be pointed out that a number of substances other than hypoglycemics are able to stimulate the oxidation of glucose by adipose tissue *in vitro*. Some of these agents are epinephrine (174), ACTH (175), glucagon (176), serotonin (177), growth hormone (178), and prolactin (179). These observations suggest that even if the sulfonylureas do stimulate glucose oxidation in adipose tissue the effect is not necessarily important to their action as hypoglycemics.

It has been shown (180) that sulfonylureas are able to decrease the glucose output of the liver. This might contribute to their action as hypoglycemics, but it is unlikely to be their major action since Dulin and Johnston (181) have shown that sulfonylureas act as hypoglycemics in hepatectomized animals. It has been shown (182) that sulfonylureas are capable of inhibiting glucose-6-phosphatase activity in liver slices, but the concentration of drug required to bring about this response is well above the concentration normally present in blood. This makes it seem highly improbable that inhibition of glucose-6-phosphatase activity is important to the action of these compounds. It is also interesting that a similar concentration of sulfanilamide can inhibit glucose-6-phosphatase activity (182), but this compound does not lower blood sugar in intact animals.

It is generally agreed that the sulfonylureas are incapable of lowering blood sugar in totally pancreatectomized animals (183, 184, 157). They also have no hypoglycemic effect in alloxan-diabetic animals providing there is complete β cell destruction (181). This indicates that a functioning pancreas is essential to the action of these compounds.

It has been observed that pretreatment of animals with growth hormone or prolonged fasting of the animals decreases the response of these animals to sulfonylureas (185). Both of these conditions are known to decrease the insulin content of the pancreas. This further suggests that the action of the sulfonylureas is dependent on the presence of some insulin.

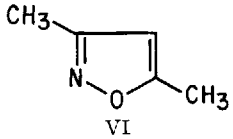
The storage and secretion of insulin by the pancreas has recently been reviewed by Lacy (81), who points out that insulin is stored in the β cells

in the form of β granules which are visible with the electron microscope. The process by which insulin is secreted is referred to as emiocytosis and involves the migration of the β granules to the cell membrane where they are liberated into the intercellular spaces. Williamson, Lacy, and Grisham (186) have demonstrated that tolbutamide stimulates emiocytosis in the β cells of the rat. Other workers (187) have shown a similar effect in the guinea pig. Vallance-Owen and his associates (188) have shown that a single oral dose of tolbutamide will increase plasma insulin-like activity by a factor of 2 within 2.5 hr. Yalow and Berson (189) made similar observations by using an immunoassay to measure plasma insulin levels.

The above observations indicate that the sulfonylureas have no direct effect on the metabolism of carbohydrates. The current feeling is that the primary action of the sulfonylureas is a stimulation of the pancreas to secrete insulin, but it should be emphasized that the mechanism by which they promote this release of insulin is not understood. This theory points out why it is essential to have a functioning pancreas for these compounds to be effective and illustrates why they cannot be used successfully to treat juvenile diabetics.

The sulfonylureas are an important class of therapeutic agents and can be used effectively in a great number of cases, but proper patient selection is an important factor in effective sulfonylurea therapy. In a recent report involving 9,214 diabetics studied over a 6-year period (190) it was concluded that the subject most likely to be controlled on these compounds is one who contracted diabetes after age 40, is overweight, has a daily insulin requirement of less than 30 units, and has had the disease for less than 5 years.

The Pyrazoles and Isoxazoles.—Dulin and Gerritsen were the first to report that 3,5-dimethylisoxazole (VI) is a potent oral hypo-



VI

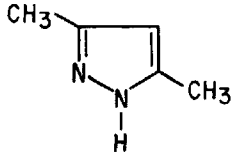
glycemic agent (191) in fasted, intact rats which are injected with 100 mg. of glucose at the time of treatment. The compound is effective at a dose as low as 10 mcg./150 Gm. rat which indicates that it is about 188 times more potent than tolbutamide and is the most potent oral hypoglycemic reported. These observations have opened a whole new area of research in oral hypoglycemics and have led to a great deal of information concerning this compound as well as a number of similar compounds.

This study (191) demonstrated that 3,5-dimethylisoxazole increases the oxidation of labeled glucose by fasted, intact rats, an effect which is shared by insulin but not the biguanides (129). The action of 3,5-dimethylisoxazole is different from that of the sulfonylureas as it is effective in alloxan-diabetic rats and the sulfonylureas are not. The compound was found to be ineffective in lowering blood sugar in spinal transectomized, eviscerated rats, which suggests that the action of the compound is dependent on the liver and/or the intestine.

In a subsequent study by Dulin, Lund, and Gerritsen (192) it was demonstrated that this isoxazole derivative also has a profound effect on fat metabolism as it is capable of lowering plasma FFA levels within 30 min. after oral administration to fasted, glucose-primed, intact rats. The compound had no effect on the rate of oxidation of labeled palmitate which was continuously infused into the animals, but there was an increase in the specific activity of plasma FFA during the infusion of the labeled palmitate. This led the authors to suggest that the depression in plasma FFA was probably due to an inhibition of lipolysis rather than to a stimulation of fatty acid oxidation.

It is interesting to note that the FFA levels are depressed within 30 min., while it requires about 2 hr. for blood sugar to be depressed significantly. This could mean that the effect on blood sugar is secondary to the effect on plasma FFA levels, although there is no proof that the two effects are directly related.

The work with 3,5-dimethylisoxazole has led to subsequent publications dealing with related compounds derived from pyrazole (193, 194). 3,5-Dimethylpyrazole (VII) was found to pro-



VII

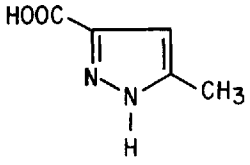
duce biological effects similar to those reported earlier for the analagous isoxazole and was shown to be 50 times as potent as tolbutamide in lowering blood sugar in glucose-primed, fasted, intact rats. It was also found to have a significant effect on fat metabolism (193).

It was shown (193) that 3,5-dimethylpyrazole increases the rate of oxidation of labeled glucose in fasted, intact rats and was also found to be effective in alloxan-diabetic rats. It has no effect

on the rate of oxidation of labeled glucose by rat epididymal adipose tissue *in vitro* and also has no effect on the liberation of FFA from this tissue *in vitro*.

As was found to be the case with 3,5-dimethylisoxazole, the pyrazole was found to lower plasma FFA levels in glucose-primed, fasted, intact rats. The administration of the pyrazole to rats continuously infused with labeled palmitate again resulted in no increased oxidation of the palmitate but did produce an increase in the specific activity of the plasma FFA. This again is suggestive that the FFA depression is probably a result of a decreased mobilization of fatty acids from glycerides in fat depots. The depression of plasma FFA occurred within 15 min., while it required 2 hr. to depress glucose levels significantly, which further suggests that the effect of these compounds on blood glucose levels could be secondary to their effect on fat metabolism.

Since 3,5-dimethylpyrazole depresses fat mobilization *in vivo* but not *in vitro*, since it depresses blood glucose levels in fasted, glucose-primed, intact rats but not in eviscerated animals, and since its action on blood sugar is antagonized by SKF 525-A, an inhibitor of drug metabolism, it was suggested that the action of the compound could be due to a metabolite rather than to the compound itself. A study carried out by Smith, Forist, and Dulin (195) has shown that 3,5-dimethylpyrazole is metabolized to 5-methylpyrazole-3-carboxylic acid (VIII) in the rat. Further work by Gerritsen and Dulin (194) shows that this metabolite is a potent hypoglycemic and is probably responsible for many of the effects which are produced when 3,5-dimethylpyrazole is administered to animals.



VIII

5-Methylpyrazole-3-carboxylic acid is 116 times more potent than tolbutamide in intact, fasted, glucose-primed rats (194). Like the dimethyl derivative, it also increases the rate of oxidation of labeled glucose in fasted, intact rats, is effective in alloxan-diabetic rats, and rapidly depresses plasma FFA. It is readily apparent from Fig. 1 that the depression in plasma FFA occurs much more rapidly than the depression in blood sugar.

This study also points out a number of differences between 3,5-dimethylpyrazole and 5-methylpyrazole-3-carboxylic acid (194). The carboxy compound has hypoglycemic activity in spinal transectomized, eviscerated rats, a property which is not shared by the dimethyl derivative. This indicates that the dimethyl derivative must be oxidized to the acid before it is able to lower blood sugar. The evidence indicates that this oxidation probably occurs in the liver and apparently occurs quite rapidly since blood sugar is depressed within 2 hr. in animals treated with the dimethyl compound.

Another difference between the two pyrazole derivatives is that 5-methylpyrazole-3-carboxylic acid increases glucose oxidation by rat epididymal adipose tissue *in vitro* and also inhibits the release of FFA by the same tissue *in vitro* (194). Neither of these effects is exhibited by 3,5-dimethylpyrazole which further suggests that it is the metabolite which is the active form of the drug. Like other pyrazoles and isoxazoles, but unlike insulin, the carboxy pyrazole derivative does not increase glucose uptake by the isolated rat diaphragm (194).

There is not sufficient information available to understand completely the mechanism of action of these compounds, although a number of points are quite clear. They affect both carbohydrate and fat metabolism in a variety of biological systems; but whether these two effects are related or independent of each other has not been established. If the two effects are related, the effect on fat metabolism is almost certainly the primary effect since this effect is manifested so much more rapidly than the blood sugar depression, a point which is made abundantly clear by Fig. 1. Further evidence for the primary effect on fat metabolism is that there have been no instances reported where these compounds lowered blood sugar without first having lowered plasma FFA levels.

There is good evidence to support the theory

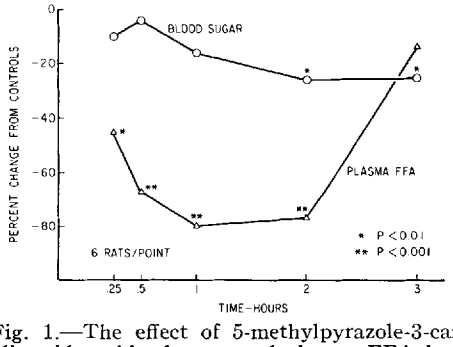


Fig. 1.—The effect of 5-methylpyrazole-3-carboxylic acid on blood sugar and plasma FFA levels in fasted, intact, glucose-primed rats (194). Reprinted with the permission of the *Journal of Pharmacology and Experimental Therapeutics*.

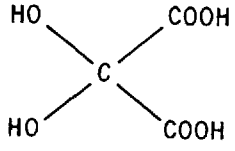
that a depression of plasma FFA levels could produce a depression in blood sugar. At low FFA levels the oxidation of fatty acids is minimized thus allowing for the oxidation of relatively large quantities of glucose for energy production which would result in a lowering of blood sugar levels. This idea is also in agreement with the work of Randle and his associates (107) which shows that high levels of FFA inhibit the utilization of glucose. The exact mechanism of action of these compounds is, however, probably much more complex than this.

One wonders why certain barbiturate anesthetics are able to inhibit the effect of these compounds on carbohydrate metabolism, but not on fat metabolism as shown by Gerritsen and Dulin (193). One possibility is that the barbiturate antagonizes the secondary action of the compounds on carbohydrate metabolism, but not the primary action on fat metabolism. Another question which must be answered is why the effect of 3,5-dimethylpyrazole on carbohydrate metabolism is blocked by evisceration, but the effect on FFA depression is not (193). There appears to be no ready answer to this question at this time.

A recent publication by Wright, Dulin, and Markillie (196) describes the preparation of 41 pyrazole derivatives which were tested for hypoglycemic activity. A number of the compounds having methyl groups in the 3 and 5 positions were found to be active oral hypoglycemics, 1 of them being about 100 times more potent than tolbutamide. It seems likely that these compounds act by the same mechanism as the original isoxazoles and pyrazoles, so little if any difference would be expected.

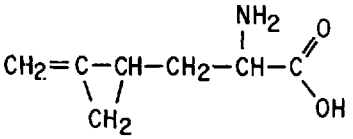
In summary, the pyrazoles and isoxazoles are among the most interesting hypoglycemics to be studied. Further study of this group of compounds could result in the discovery of important information concerning the causes, effects, and treatment of diabetes. It is also conceivable that an effective and useful antidiabetic drug could evolve from the series.

Miscellaneous Hypoglycemics.—Mesoxalic acid (IX) has been reported to decrease blood sugar levels in laboratory animals (197). Relatively little information has been published on the compound, but it appears to have an action similar to the sulfonylureas. It is effective in the presence of some endogenous insulin, but exerts no hypoglycemic effect in totally pancreatectomized animals (197). This led to the speculation (198) that the compound exerts its action by stimulating the β cells to secrete insulin. The calcium salt of this acid is on the Japanese market but has not been studied extensively in this country.



IX

Two compounds, hypoglycin A (X) and hypoglycin B, that have been isolated from the unripe fruit of a tropical tree (*Blighia sapida*) have been found to possess hypoglycemic activity (199). Chen and his associates (200) found hypoglycin A to be more effective than hypoglycin B, but found both compounds to be highly toxic.



X

Patrick (201) has studied the effects of hypoglycin A on various biological systems. He found that it is effective in alloxan-diabetic rats, but its action is different from that of insulin. This compound does not increase oxygen consumption in intact rats nor does it increase muscle glycogen. It does not increase the glucose uptake of the isolated rat diaphragm and does not stimulate the oxidation of glucose by rat epididymal adipose tissue *in vitro*. It does not appear to increase carbohydrate utilization greatly by any biological system. Patrick (201) has proposed that the liver is the primary site of action of the compound.

L-Leucine has been reported to lower blood sugar in some children with idiopathic hypoglycemia (202) and in some patients with islet cell tumors (203). Yalow and Berson (204) have shown that L-leucine produces elevated plasma insulin levels in these patients which probably accounts for its hypoglycemic effect. Fajans and his associates (205) have reported that L-leucine can also reduce blood sugar in normal healthy subjects who have been treated with insulin or sulfonylureas to increase plasma insulin levels. They have also shown that a modest but significant hypoglycemic effect is produced when L-leucine is administered to healthy subjects without sulfonylurea or insulin pretreatment. Fajans (206) has recently summarized his findings which indicate that L-leucine-induced hypoglycemia results from a decreased hepatic glucose output and an increased peripheral utilization of glucose. The stimulation of insulin release is proposed as the primary mechanism producing these effects.

TABLE II.—THE BIOLOGICAL EFFECTS OF VARIOUS HYPOGLYCEMIC AGENTS ON VARIOUS ANIMAL SYSTEMS

Effect	Insulin	Sulfonyl-ureas	Biguanides	3,5-Dimethyl-pyrazole	5-Methyl-pyrazole-3-carboxylic Acid
Effective in alloxan-diabetic animals	+	−	+	+	+
Effective in eviscerate animals	+	−	+	−	+
Increase glucose oxidation in intact animals	+	+	−	+	+
Increase glucose uptake by rat diaphragm *in vitro*	+	−	+	−	−
Increase glycogen content of isolated rat diaphragm	+	−	−	−	−
Increase glucose oxidation by rat epididymal adipose tissue *in vitro*	+	−	−	−	+
Lower plasma FFA levels	+	+	−	+	+

A large number of other substances have been found to exhibit hypoglycemic activity, but most of these show no real advantages over compounds which are in current use. 4-Dimethylamino-*N*-methyl-2,2-diphenylvaleramide was shown to be 4 times more potent than tolbutamide in fasted intact rats (207); it is proposed that it acts by decreasing the glucose output of the liver. Goldner and Jauregui (208) have reported that certain antihistaminic drugs possess hypoglycemic activity. Hultquist (209) has shown that iodoacetic acid and iodoacetamide exhibit hypoglycemic activity in rats, seemingly by the stimulation of the β cells to secrete insulin. Tris-(hydroxymethyl) aminomethane has also been reported to possess hypoglycemic properties (210).

This is only a partial list of substances which have been reported to have significant hypoglycemic activity. A complete list would be far too extensive for the purposes of this review.

Summary.—It may be seen from Table II that, although many different classes of compounds are capable of lowering blood sugar and counteracting certain other effects of diabetes, they do not all act by the same mechanism. It points out that the sulfonylureas, which are dependent on a functioning pancreas for their action, are not effective in alloxan-diabetic or eviscerate animals (181, 184). The biguanides, on the other hand, have been found to lower blood sugar in alloxan-diabetic (129) and eviscerate (138) animals. The biguanides do not increase glucose oxidation in intact animals, whereas insulin and the pyrazoles do (193). The biguanides are the only compounds in the table other than insulin which are generally agreed to stimulate glucose uptake by the isolated rat diaphragm (129), but even these compounds do not increase the glycogen content of the diaphragms as does insulin (211). The pyrazoles (193) and the sulfonylureas (212) as well as insulin are capable of lowering plasma FFA levels in intact animals. The table also illustrates that 5-methylpyrazole-3-carboxylic acid can be differentiated from 3,5-dimethylpyrazole by virtue of the effectiveness of the acid in eviscerate rats as well as by its effect on glucose oxidation in rat epididymal adipose tissue *in vitro* (194). It is obvious by now that none of these compounds qualifies as an oral insulin since none exhibits all of the biological effects of insulin. They are properly classified as "oral hypoglycemics" or "oral insulin substitutes."

CONCLUSIONS

The above observations lead to the realization that although a substantial amount of information about the metabolic effects of diabetes has been accumulated, a great deal more must be learned before certain important conclusions can be reached. The etiology of diabetes is not well understood, nor are its hereditary aspects. The effects of insulin on various biological systems are quite well documented, but the mechanisms by which it produces these effects are not clearly understood. Treatment of diabetes with oral hypoglycemics must be termed only partially successful at this time, and more research in the area is required to produce new compounds which are more similar to insulin in their biological action and which are effective in treating a greater number of diabetic patients. Long-range goals should be aimed at learning more about the causes of diabetes so that steps can be taken to prevent the disease rather than simply treating the symptoms. The cause is a worthy one and merits the vast amount of research being carried out in the area.

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