PHARMACOLOGICAL REVIEWS
Vol. 21, No. 3

Printed in U.S.A.

REGULATION OF INSULIN SECRETION

DALE A. MAYHEW[1], PETER H. WRIGHT[2], AND JAMES ASHMORE[2]

Medical Research Laboratories, Chas. Pfizer & Co., Inc., Groton, Connecticut, and Department of Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana.

TABLE OF CONTENTS

AGENTS

diazoxide	[Hyperstat, Mutabase; 7-chloro-3-methyl-2H-1,2,4-benzothiadiazine 1,1-dioxide]
carbachol	[choline chloride carbamate; carbamylcholine chloride; Carcholin; Moryl; Doryl; Coletyl; Lentin; (2-hydroxyethyl)-trimethyl ammonium chloride carbamate]
tolbutamide	[Orinase; Rastinon; 1-butyl-3-(*p*-tolylsulfonyl)urea]
phenoxybenzamine	[Dibenzyline; N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine]
phentolamine	[Regitine; 2-[N-(m-hydroxyphenyl)-*p*-toluidinomethyl]-imidazoline]
Isoproterenol	[isoprenaline; isopropylarterenol; 3,4-dihydroxy-α-[(isopropylamino)methyl]-benzyl alcohol]

[1] Address: Medical Research Laboratories, Chas. Pfizer & Co., Inc., Groton, Conn. 06340.
[2] Address: Department of Pharmacology, Indiana University School of Medicine, Indianapolis, Ind. 46202.

propanolol	[propranolol; Inderal; 1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol]
chlorpropamide	[Diabinese; 1-(*p*-chlorophenylsulfonyl)-3-propylurea]
acetohexamide	[Dymelor; 1-[*p*-acetylphenyl)sulfonyl]-3-cyclohexylurea]
carbutamide	[1-butyl-3-sulfanilylurea]
phenformin	[1-phenethylbiguanide]
chlorothiazide	[Diuril; 6-chloro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide]

I. INTRODUCTION

Glucose is the major stimulus for insulin release from the pancreas, but the response of the islet to this agent may be markedly altered by physiological, pharmacological, and pathological interactions. Thus it would appear that intestinal factors released during glucose absorption enhance insulin release due to alimentary hyperglycemia. A variety of drugs (sulfonylureas, adrenergic agents, chlorothiazides) and hormones can either inhibit or stimulate insulin release in the presence of glucose. The normal response of the pancreas to glucose may also be modified in obesity and pregnancy and in certain "genetic strains of mice." Accumulation of this information has resulted from the development of techniques for the isolation of mammalian islets and assays sufficiently sensitive and specific to measure *in vitro* the secretion of insulin by pieces of pancreas (27, 127), isolated islets (78), and the perfused pancreas (63).

The first studies on the regulation of insulin secretion were undertaken with fish islets. In mammals, the islets comprise about 1% of the total pancreatic mass, and separation of these cells from the acinar tissue seemed a formidable task. In the fish, however, the islet tissue exists as a distinct body within the mesentery and is easily separated from the more diffuse acinar tissue. A systematic analysis of the metabolic and enzymatic pathways in the fish islet was undertaken by Lazarow *et al.* (101, 102) in order to provide a useful frame of reference for analyzing the insulin secretory mechanism. These studies indicated that the pentose phosphate pathway of glucose metabolism is operative in the islet and provided data on the existence of various glycolytic and oxidative enzyme systems within islet tissue.

Fish islets also provided a good system for the study of incorporation of labeled amino acids into insulin. The glycogen content of islets was examined under various conditions, particularly after the effects of toxic concentrations of alloxan. Attempts to study the regulation of insulin secretion from the isolated fish islet were not quite as successful. In initial experiments the "fat pad" assay was used to estimate insulin release, and neither glucose nor tolbutamide appeared to influence insulin secretion in this system. Studies in other species with the fat pad assay indicated that glucose and other agents could modify insulin release.

The first extensive experiments with mammalian pancreas were performed by Coore and Randle (27). They used pieces of rabbit pancreas and incubated the tissues for only short periods of time. These experiments proved successful for

several reasons. First of all, insulin is destroyed more slowly by enzymes of the acinar tissue of the rabbit than by those of many other species. The relatively short period of incubation further minimized insulin degradation by proteolytic enzymes elaborated by the acinar tissue. Equally important was the fact that insulin was measured by a radioimmunoassay of sufficient specificity and sensitivity to detect the quantities of insulin released during the incubation period.

Evaluation of insulin secretion *in vivo* is more difficult since the hormone is released into the portal vein and must traverse the liver before entering the systemic circulation. The extent to which the liver may remove insulin from the portal blood has yet to be fully investigated. Thus, direct measurement of insulin secretion *in vivo* still presents practical problems when, as in man, samples of peripheral blood are the only ones readily available for study. Yet, much useful information on the regulation of insulin secretion has been derived from measurements of the hormone in plasma. These studies on regulation of insulin release *in vitro* and *in vivo* are reviewed in some detail and various limitations in evaluating such results are discussed.

II. MEASUREMENT OF INSULIN SECRETION

A. Assay of insulin

Initial attempts to assay insulin were based upon the biological activity of the hormone. Quantities of insulin required to produce hypoglycemic convulsions, even in hypophysectomized mice, were several orders of magnitude greater than quantities readily recovered from blood. Since these assays *in vivo* lacked sufficient sensitivity, various tissue methods *in vitro* were developed. These included glucose uptake or glycogen deposition by the rat diaphragm (213) and the oxidation of ^{14}C-labeled glucose by rat epididymal fat pads (132). The former method proved to be lacking in sensitivity and the latter proved to be too nonspecific for the assay of insulin in biological systems (55, 213). The solution to the insulin assay problem was found in the various modifications of the radioimmunoassay as developed by Yalow and Berson (228).

Insulin induces production of antibodies in guinea pigs after repeated injection in a water-in-oil emulsion (151, 225) or as a suspension (12). Anti-insulin serum from such animals will react with insulin *in vitro* (12) and will induce insulin deficiency in a variety of experimental animals *in vivo* (150, 222). For this reason, amongst others, antibodies to insulin produced by the guinea pig are thought to be capable of reacting with both endogenous and extracted insulin (222), but insulin antibodies produced by other species do not appear to react very strongly, if at all, with this hormone (149, 223). Guinea pig anti-insulin serum is therefore the only one now used for the immunoassay of insulin.

Various techniques are used to assay insulin and all are based on the same fundamental reaction. A constant amount of anti-insulin serum is allowed to react with an excess of insulin, a trace amount of which is radioactively labeled with ^{131}I or ^{125}I. At the end of the period of incubation, commonly carried out over 1 to 4 days at 4°C, two forms of insulin can be discerned: part of the hormone is bound by antibodies in the anti-insulin serum, and the excess remains free in

solution. The two forms will be termed "bound" and "free." To separate and measure the amounts of bound and free insulin in all such incubated mixtures, several techniques have been used. Yalow and Berson (228), who developed the first practical method for immunoassay of insulin in plasma, use chromato-electrophoresis on filter paper; free insulin then adheres to the filter paper at the point of application and the bound hormone migrates with the β-γ globulins. Morgan and Lazarow (156) and Hales and Randle (68) precipitate bound insulin with an antibody to guinea pig globulins produced in the rabbit and goat; free insulin remains in solution. Bound insulin can also be precipitated with sodium sulfite under suitable experimental conditions (67). Free insulin in the incubated mixture can also be adsorbed from solution with dextran-coated charcoal (71) or cellulose (224, 232) and can be destroyed selectively with insulinase (8) or a similar proteolytic enzyme (148). The specific activities of bound and free insulins will be identical if the labeled and unlabeled hormones behave identically in their reactions with the antibodies during incubation. Thus the greater the amount of unlabeled insulin in the original mixture, the greater will be the amount of radioactivity associated with the free insulin separated after incubation.

From the relative amounts of radioactivity associated with the free and bound fractions of insulin obtained with samples and with standard solutions of insulin, the insulin contents of the samples can be determined. These various techniques, which are extensively used to assay insulin in plasma, should be used with care if meaningful results are to be obtained. For example, insulin from the species of animal under investigation should always be used for reference, since guinea pig anti-insulin serum exhibits some degree of species-specificity in its reactions with the hormone. These and other theoretical considerations have been discussed in detail and need not be considered further here (12).

B. Techniques for the study of insulin secretion

Insulin secretion has been examined *in vivo* and *in vitro*, but these studies are as yet in their infancy. Interpretations of experimental observations have therefore to be viewed with caution and will be dependent upon characteristics of the techniques employed. Of the two basic procedures carried out *in vitro*, one, introduced many years ago by Anderson and Long (2), involves perfusion of the rat's isolated pancreas with diluted blood or a synthetic medium. The second, more recent in origin, involves media of known composition in which pieces of pancreatic tissue, isolated islets, or explants of cultured pancreatic tissue are incubated.

Two groups of investigators have been mainly responsible for recent observations obtained with the perfused pancreas. Sussman *et al.* (203) used pancreatic tissue completely separated from surrounding organs while Grodsky *et al.* (62) left the stomach, spleen, and duodenum attached. Diluted blood or bicarbonate-buffered medium is infused through the aorta or celiac axis, and effluent fluid is collected from the portal vein. These media are either recycled or allowed to collect after a single passage, substances under study being incorporated in recycled media or injected as "pulses" for a single passage through the tissue.

Insulin output from the perfused pancreas is stated either in absolute units or, more commonly, in terms of its concentration in the effluent medium.

Pieces of pancreatic tissue from a number of species have been incubated for periods up to about 2 hrs in bicarbonate-buffered media. Under these conditions, with tissue from the rabbit, Coore and Randle (27) found that some secreted hormone is destroyed during the period of incubation. The lytic substance released by pancreatic tissue of the rat appears to be more abundant (127). The lytic substance must be derived from acinar tissue since isolated islets of the rat do not destroy added or secreted insulin during incubation for 60 min (122). The lytic agent appears to be a proteolytic enzyme but does not resemble either trypsin or chymotrypsin (27). It has no detectable effect on proteins of high molecular weight but destroys small polypeptides such as glucagon (123). Two approaches have been used in attempts to prevent or allow for destruction of secreted insulin. Coore and Randle (27) found that meaningful but variable results could be obtained by incubating pieces of tissue in successive media for short periods of 15 to 30 min. Malaisse *et al.* (127) added known amounts of guinea pig anti-insulin serum to all media and used it for two purposes: the antibodies prevented destruction of insulin when added in excess (123, 127) and could be used to assay induced secretion (127). To protect glucagon and other polypeptides that might exert some action on the secretory process *in vitro*, kallikrein inhibitor (Trasylol, Bayer; FBA Research, New York) has proved useful. In high concentrations this agent reduces but does not abolish the destruction of insulin (123) and has proved essential to demonstrate an effect with glucagon *in vitro* (123). These techniques have been extensively used in recent years and have proved valuable in screening the actions of potential stimulants and inhibitors of insulin secretion. However, secretion rates under identical conditions do show wide variation between pieces of tissue from the same animal or groups of pieces from animals of the same or different species.

Individual islets have been isolated by several methods. By manual dissection at moderate magnification (10 to 20 x), Hellerstrom (69) has been able to obtain them from pancreatic tissue of the mouse, rat, and guinea pig. The relatively large islets of obese hyperglycemic mice have been isolated by this method in several centers; they are largely (90%) composed of *beta* cells. Alternatively, the techniques first used to destroy acinar tissue and extract insulin from the dog's pancreas have been applied to isolation of islets. One or more of the pancreatic ducts of the rat are ligated and islets are dissected out 4 to 6 weeks later, when they stand out as distinct ovoid bodies against a background of translucent fatty or fibrous tissue (16, 91, 144). These techniques are likely to be superceded by a method based on observations by Moskalewski (158) and described in more detail by Lacy and Kostianovsky (98), Vance *et al.* (214), and Howell and Taylor (73). In this procedure, pancreatic tissue is disrupted by injection of saline into the main pancreatic duct or directly into the pancreatic tissue. The resultant gelatinous mass is cut into small pieces and incubated with collagenase. The digested tissue is then suspended and washed in buffered or unbuffered medium, and the islets are allowed to separate out. Under gravity or light centrifugation,

islets are deposited more rapidly than the acinar elements and can therefore be concentrated. Eventually they have to be picked out individually under a dissecting microscope with either small glass loops (98) or a micropipette (214). By this means 50 to 100 islets can be obtained from the pancreas of a single adult rat, and 120 to 450 have been isolated from that of the rabbit. During 60 min, a single islet incubated in the presence of a high concentration of glucose (300 mg/100 ml) will secrete about 150 μU of insulin (120). With suitable experimental conditions and a method sensitive enough for assay of insulin, it should therefore now be possible to obtain much useful information by using very small numbers of islets for individual incubations.

Finally, mention must be made of the technique described by Vecchio and Gonet (215) for the culture of fetal pancreatic tissue. They removed pancreatic tissue from fetal rats after 18 days of gestation, when granulation is first observed in the β cells. This tissue was incubated for 4 days in a medium containing an extract of fetal rat tissue, normal rat serum, and a high concentration of glucose (300 mg/100 ml). Explants removed after this time contained well granulated *beta* cells, no identifiable *alpha* cells, and few acinar elements. When these were incubated in simple bicarbonate-buffered medium, they secreted insulin in response to a number of stimulants.

Since blood leaving the pancreas drains into the portal circulation, direct measurement of insulin secretion *in vivo* requires surgical intervention. To circumvent this difficulty, various devices are used to get an approximate estimate. In early studies in experimental animals, glucose was infused in high concentration but in small absolute amounts into the pancreatic artery. It was then found that glucose (18) and other sugars (172) administered in this manner produced systemic hypoglycemia in dogs. More direct evidence of the stimulant effect of glucose was obtained by Metz (143) and Seltzer (188), who collected blood from the pancreaticoduodenal veins of anesthetized dogs and measured its insulin content (insulin-like activity) with the rat diaphragm. Similar techniques with indwelling plastic catheters placed in a pancreatic vein have been described (83, 131). In many recent studies, no attempt has been made to measure pancreatic blood flow, and changes in rate of insulin secretion have been inferred from corresponding changes in the concentration of insulin in pancreatic venous blood. Most of the studies have been carried out in anesthetized animals, but in some cases chronic surgical preparations have been used.

In an attempt to estimate total pancreatic insulin secretion in conscious rats, Wright *et al.* (227) injected guinea pig anti-insulin serum intravenously. This serum induces transient hyperglycemia and, as the blood sugar concentration approaches its maximum, the concentration of reactive insulin antibodies in the injected animal's plasma falls. ^{125}I-labeled human albumin was injected to measure the volume of distribution of unneutralized insulin antibodies. In alloxan-diabetic rats, the concentrations of the antibodies and of labeled albumin fall at the same rate. From the concentrations of reactive insulin antibodies and their volumes of distribution at intervals (15 to 45 min) up to about 2 hrs after injection, rates of insulin secretion in the intervening periods could be calcu-

lated. It should be emphasized that during the period of study the injected animals were hyperglycemic to varying degrees according to circumstances.

Studies in man are based largely upon estimates of the insulin content of peripheral blood measured under varying experimental conditions. Ever since the first methods for assay of insulin in plasma became available, it has been assumed that any rapid increase in plasma-insulin concentration in peripheral venous blood is due to a corresponding increase in pancreatic insulin secretion. In order to confer some numerical value upon changes induced by some procedures, Kalkhoff *et al.* (82) estimated the area subtending the insulin response curve; the bigger the area, they argued, the larger the secretion evoked. Many investigators have since adopted the same method and some have actually done statistical comparisons of responses evoked in different subjects or under different experimental conditions. Metz (143) has shown in dogs that output of insulin from the pancreas is proportional to a function of the blood sugar concentration. Seltzer and Harris (190) related observed concentrations of insulin in peripheral blood to those of glucose, and the ratio of these values has been termed the "insulinogenic index." A low insulinogenic index, they reasoned, implies reduced secretory activity of the pancreatic islets. In diabetic Chinese hamsters, a good correlation between "insulinogenic index" and insulin release *in vitro* has been obtained (119). Neither of these devices can be used to get accurate quantitative estimates of rates of pancreatic insulin secretion, but both have proved useful in the study of pancreatic function in man.

C. Factors that may influence estimates of insulin secretion

Each of the techniques outlined above has its value, but it should be remembered that each also has its limitations. These should be recognized if meaningful interpretations are to be placed on experimental observations. There seems little doubt that any rapid increase in plasma-insulin concentration in peripheral blood is usually due to increased endogenous secretion of insulin. However, it is also true to say that changes in concentration of insulin in plasma reflect alterations in the equilibrium between pancreatic secretion on the one hand and uptake of the hormone by tissues on the other. It is known that exogenous ^{131}I-labeled (13) or unlabeled (133, 160) insulin and endogenously secreted insulin (181) are rapidly taken up by the tissues; half-lives of less than 20 min have been reported. A large proportion of this insulin is removed by the liver (111, 157, 185), and the rate of hepatic uptake of insulin may be markedly and rapidly affected by acute physiological processes. Thus it should not be assumed that increased pancreatic insulin secretion is necessarily the only cause for an increase in the level of circulating insulin.

Failure to demonstrate increased secretion as judged by absence of any rise in peripheral venous insulin concentration could be factitious. This is well illustrated by some observations of Kaneto *et al.* (85). Within minutes of stimulating the thoracic dorsal vagus trunk of the dog, they found that insulin concentration in portal venous plasma rose from 200 to 300 μU/ml but observed no significant change in the femoral vein (14 to 17 μU/ml). Thus, extensive dilu-

tion of insulin upon entering the systemic circulation and removal of insulin by the liver could mask actual increases in pancreatic secretion. For similar reasons, inhibition of secretion might not be detectable. It is difficult or impossible to show any decrease in plasma-insulin concentration when the resting level is at the lower limit of sensitivity for methods of plasma-insulin assay now in use. Schalch (187), for example, detected no change in plasma-insulin concentration in venous blood after exercise, and Cochran *et al.* (23) were able to do so only in subjects with high resting levels. To demonstrate inhibition *in vivo*, it may be necessary to apply some known stimulant first. By inducing hyperglycemia with anti-insulin serum, Wright and Malaisse (226) were able to show that epinephrine, exercise, and stress will inhibit insulin secretion in rats. Porte *et al.* (171) used a similar device in man when studying the effects of epinephrine. This drug, like glucose, induces hyperglycemia but, unlike glucose, it does not raise plasma-insulin concentration until the infusion of epinephrine is stopped. When peripheral blood is studied, it may not therefore be possible to demonstrate stimulation of insulin secretion, and to show inhibition, it may first be necessary to induce secretion with some stimulant.

Whether effects upon secretion are observed *in vivo* or *in vitro*, they could be the result of indirect rather than direct actions of the agents concerned. It is now well established, for example, that 2-deoxyglucose can inhibit the stimulant effect of glucose upon secretion of insulin *in vitro* (93, 127). It has a similar effect *in vivo* but, in this case, part of its action may be mediated through the adrenal medulla by stimulating the secretion of catecholamines (89, 93, 94, 100). The inhibitory effects of urethan (87), glucosamine (134), and diazoxide (193) may also be mediated, at least in part, through the adrenal medulla. As a corollary to this phenomenon, it might also be mentioned that surgical operations, coronary infarction, and acute infection (1, 191) produce effects consistent with inhibition of insulin secretion. Failure to observe a stimulant effect upon insulin secretion in acute experiments with animals under anesthesia may be due to increased sympathetic stimulation under these conditions. Even *in vitro*, an observed effect could be the result of indirect action. Glucagon, for example, has a striking stimulant effect upon insulin secretion *in vivo* (21, 29, 89, 92, 184) and *in vitro* (38, 66, 123, 208) and is produced by the *alpha* cells in the islets. It is possible, therefore, that increased insulin secretion observed *in vitro* could be due to stimulation of glucagon release. Vecchio *et al.* (216, 217) have postulated this thesis on the basis of their observations with explants of fetal rat pancreas. This tissue, which does not appear to contain any *alpha* cells, does not secrete much insulin when incubated in the presence of high concentrations of glucose unless glucagon is also present. Until more is known of factors controlling glucagon secretion and maturation of the function of *beta* cells, it will not be possible to tell whether this hypothesis is tenable.

It is perhaps too early to assess the physiological significance of many of the observations now being reported. In many cases, the concentrations of agents that have been shown to have effects are extremely high. In addition, the tissues upon which they are being tried may be in an unphysiological condition. In rats

injected with anti-insulin serum (227), for example, estimated rates of secretion (6 mU/100 g body weight/min) for normal animals are far in excess of the doses of injected insulin (0.55 to 0.88 mU/100 g/min) needed to control alloxan-diabetes in this species (197). For reasons that are not yet clear, it seems that for a few hours after an injection of anti-insulin serum, the islets are abnormally hyperresponsive to glucose, an effect observed *in vitro* that could well explain the findings *in vivo* (128). As another example, it has been shown in the perfused rat's pancreas (66, 202) and *in vivo* in the dog (83) that glucose and some other stimuli evoke very prompt increases in insulin secretion. Yet in pieces of pancreas (127) and in rats injected with anti-insulin serum (227) no secretion is normally detected for about 15 min. This discrepancy could be factitious since small changes in secretion rate cannot be detected when anti-insulin serum is used *in vivo* or *in vitro*, and there is some evidence that the pancreatic response, at least to glucose, occurs in two phases. The first is rapid and transient and the second is prolonged and generally seems to involve greater absolute rates. In addition to apparently incompatible results obtained in different systems, different species *in vivo* and their tissues *in vitro* show varying responses to the same agent. In the rat, carbamylcholine and acetylcholine do not appear to have any consistent effect *in vitro* when glucose is present in almost physiological concentrations (100 to 150 mg/100 ml) but inhibit secretion at high levels of glucose (136). In the dog *in vivo* (56, 85) and in pancreatic tissue from the dog, the rabbit, and man *in vitro* (136), on the other hand, vagal stimulation or the addition of carbamylcholine result in stimulation. In the sheep, unlike the dog or rat, glucose has a much weaker stimulant effect than propionate or butyrate (130, 131). These and other examples, some of which are considered in more detail later, will suffice to show that it is unwise at this stage to be dogmatic in assessing differences in effects observed in different systems or with different species. As further evidence accumulates and methods, especially *in vitro*, are made more specific and precise, the true values of existing observations will become clearer.

III. PHYSIOLOGICAL AND PHARMACOLOGICAL STIMULATION

A. Glucose and oxidative metabolism

Glucose is clearly one of the major physiological stimuli for insulin secretion. Tables 1 and 2 summarize the effects of glucose and numerous other agents that cause its secretion.

Many investigations have clearly shown that when the glucose concentration is altered *in vivo* or *in vitro* there is a parallel change in insulin release from pancreatic tissue (27, 63, 127, 143, 203). In studies with the perfused pancreas, "pulse" administration of glucose leads to an almost immediate transient release of insulin (31, 32, 62, 203). Recent work with the perfused rat pancreas indicates that when glucose is perfused for periods up to 1 hr there is a second and more sustained phase of insulin release. Glucose appears to act directly on the secretory mechanism, for quantities of dinitrophenol sufficient to block insulin synthesis do not block the initial secretory response (63). However, the secondary rise in insulin secretion is partially inhibited (30%) by addition of puromycin (31, 32),

and the concentration of puromycin used was enough to block insulin biosynthesis. Under these conditions the initial rise in insulin secretion not was blocked by puromycin, and it may be concluded that the secondary rise in secretion is, in part, due to release of newly synthesized hormone. Quantitative relation-

TABLE 1

Effects of various compounds on insulin release

Agent	Conditions	Effect on Insulin Secretion	Species	References
Glucose	*In vivo*	Stimulates	Man, dog, rat	82, 83, 131, 143, 190
	In vitro	Stimulates	Rat, rabbit, hamster	27, 119, 127, 153
Mannose	*In vitro*	Stimulates	Rat, rabbit	27, 127, 153
Galactose	*In vitro*	No effect	Rat	127
Xylitol	*In vitro*	Stimulates	Rat	153
Ribitol	*In vitro*	Stimulates	Rat	153
Ribose	*In vitro*	Stimulates	Rat	153
Xylose	*In vitro*	No effect	Rat	62, 127
Arabinose	*In vitro*	No effect	Rat	62, 127
10 amino acid mixture	*In vivo*	Stimulates	Man	48, 49
Arginine	*In vivo*	Stimulates	Man	48, 49
	In vitro	Stimulates	Rat	117
Leucine	*In vivo*	Stimulates	Man	48, 49
	In vitro	Stimulates	Rat	117
Phenylalanine	*In vivo*	Stimulates	Man	48, 49
	In vitro	Stimulates	Rat	117
Lysine	*In vivo*	Stimulates	Man	48, 49
	In vitro	Stimulates	Rat	117
Propionate	*In vivo*	Stimulates	Sheep, cow	72, 130, 131
	In vivo	No effect	Rat, rabbit, pig	72
Butyrate	*In vivo*	Stimulates	Sheep, cow	72, 130, 131
	In vitro	Stimulates	Rat	154
Octanoate	*In vitro*	Doubtful	Rat	154, 186
Acetoacetate	*In vivo*	Stimulates	Dog	140
	In vivo	No effect	Sheep	72
	In vitro	Doubtful	Rat, rabbit	27, 117, 174, 177
β-Hydroxybutyrate	*In vivo*	Stimulates	Dog	140
	In vivo	No effect	Sheep	72
	In vitro	Doubtful	Rat, rabbit	27, 117, 174,177

TABLE 2

Agents that inhibit insulin release induced by glucose in vitro

Agent	Species	References
Mannoheptulose	Rat, rabbit	27, 115
2-Deoxyglucose	Rat	94, 127
2,4-Dinitrophenol	Rat, rabbit	27, 127
Cyanide	Rat, rabbit	27, 127
Anoxia	Rat, rabbit	27, 127

ships between insulin secretion and glucose concentration have been reported for incubated pancreatic tissue from the rabbit (27, 54, 144) and rat (127). In each species, small increases in secretion were observed when the glucose concentration was increased from 0 to 100 mg/100 ml. A large and rapid increase in insulin release occurs as the concentration is raised to 300 mg glucose/100 ml. In the rat, secretion reaches a maximum at glucose concentrations of 300 to 500 mg/100 ml. A similar relationship between glucose and insulin secretion is also found with isolated islets of rats (122, 214) and mice (4).

On direct infusion into the pancreatic artery, glucose stimulates insulin release *in vivo* in dogs (143, 188). In man, in whom direct measurement of secretion is not possible, the serum level rises in peripheral blood after an intravenous or oral glucose load (82, 190). Reports on the histology of *beta* cells indicate there is a marked degranulation after prolonged hyperglycemia (60, 103). Under these conditions there is also a decrease in extractable insulin in the pancreas (103).

The mechanism of a glucose stimulation of insulin secretion is not known. Islets appear to be freely permeable to glucose, since agents (*e.g.*, phlorizin and 3-O-methyl glucose) that inhibit active glucose transport in other tissues have no effect on the stimulation of insulin secretion by glucose (27, 102, 115). It was initially believed that glucose stimulates secretion by virtue of its structural specificity, but it was subsequently shown that sugars with structures identical to glucose in the first three carbons (galactose, xylose, and *l*-arabinose) do not stimulate secretion *in vitro* (27, 54, 62). Current investigations center on the hypothesis that some direct or indirect product of glucose metabolism provides the stimulus. Many of the enzymes involved in glycolysis, the hexose monophosphate shunt, the tricarboxylic acid cycle (Krebs cycle), and glycogen synthesis have been found in islet tissue (5, 17, 101, 204–206). Some of the enzymes have been studied quantitatively, and others have been demonstrated histochemically. It therefore appears that pancreatic islets contain a complement of enzymes similar to those in other tissues.

Several observations indicate that insulin secretion involves oxidative metabolism of glucose and energy production. Thus, glucose-stimulated insulin secretion is blocked by anoxia (27, 127); an oxidative process is therefore necessary. This observation would also suggest that glycolysis may play a minor role in triggering secretion since glycolysis in other tissues is stimulated under anaerobic conditions. Glucose-stimulated secretion is also inhibited by potassium cyanide or 2,4-dinitrophenol (27, 127), which are known to interfere with oxidative metabolism in other tissues.

Sugars that are metabolized by pathways similar to that of glucose exert lesser effects on insulin release. Mannose is utilized by pancreatic islets to a lesser extent than glucose (78) and exerts a variable stimulant effect on secretion (27, 62, 127). Fructose, which is not metabolized as well as glucose by islets (74, 78), is considerably less potent in stimulating secretion (62, 127). Galactose does not stimulate insulin secretion (27, 62, 154) and is not metabolized by pancreatic islets (78). The use of three agents (mannoheptulose, glucosamine, and 2-deoxyglucose) that block glucose metabolism in other tissues and islets lends additional support to the idea that glucose needs to be metabolized to stimulate secretion. In a recent

review, several effects of mannoheptulose on carbohydrate metabolism and insulin secretion were discussed (192). Mannoheptulose, a seven-carbon sugar, inhibits insulin secretion both *in vivo* (162, 227) and *in vitro* (28, 115). In liver (26) and in isolated islets of the rat (115), it competitively inhibits the phosphorylation of glucose. In addition, mannoheptulose completely blocks glucose oxidation by isolated islets of the mouse (4). Glucosamine, which also inhibits glucose phosphorylation in other tissues, also blocks insulin secretion (115, 134). These studies indicate that glucose must first be phosphorylated before it can stimulate insulin secretion. Insulin secretion is also inhibited (94, 123) by 2-deoxyglucose, a substance that competitively inhibits phosphohexose isomerase in other tissues. If 2-deoxyglucose is acting in islets as it does in other tissues, it appears that glucose not only must be phosphorylated but also must be further metabolized before it can trigger insulin secretion.

Many recent studies on the isolated pancreatic islets indicate that metabolism of glucose *via* the pentose phosphate pathway may be important in the release of insulin. Xylitol, ribitol, and ribose stimulate insulin secretion from rat pancreatic islets (153). The reduction of NAD^+ in islet homogenate and the stimulation of insulin release in the presence of mannoheptulose and 2-deoxyglucose by xylitol and ribitol suggest that glucose and mannose as well as xylitol and ribitol stimulate insulin release by their metabolism *via* the pentose pathway (153). Addition of butyrate, octanoate, and citrate to glucose-containing media causes a parallel increase in insulin release and glucose-6-phosphate levels in rat islets. Since citrate inhibits phosphofructokinase and free fatty acids inhibit glycolysis in other tissues, it was suggested that the glucose-6-phosphate formed in the islets must be metabolized by a pathway other than glycolysis (154). Measurement of 6-phosphogluconate levels in rat islets also suggests that this pathway is important in insulin release. Intracellular levels of this metabolite are increased by increasing the glucose concentration and by the addition of tolbutamide, citrate, glucagon, or theophylline. These agents also enhance insulin release from islets. Epinephrine, which inhibits the release of insulin induced by glucose, decreases the level of 6-phosphogluconate in the islet (152). Additional support for this hypothesis is found in the toadfish islet, where the reduced pyridine nucleotides (NADH and NADPH) have a marked stimulant effect on insulin release (218).

Other observations on the metabolic functions of islets support the hypothesis that glucose metabolism and insulin secretion by islets may be causally related. Thus labeled CO_2 production from labeled glucose by islets of the fish (101) and rat (77) increases as the glucose concentration rises. In both fish and rat islets a greater total amount of C-1-labeled than C-6-labeled glucose is converted to labeled CO_2, and hence there must be considerable activity of the hexose monophosphate shunt. However, two dyes, *p*-phenylenediamine and phenazine methosulfate, which stimulate hexose monophosphate shunt activity in other tissues, do not stimulate insulin release from rabbit pancreas (27). Glucose oxidation by isolated mouse islets increases as the glucose concentration increases (4). The relationship between glucose concentration and glucose oxidation in mouse islets is identical to that found with insulin secretion from rat pancreatic pieces (127).

Mannoheptulose, which blocks insulin secretion, can also completely inhibit glucose oxidation by isolated islets of the mouse (4). Oxygen consumption by isolated islets of the obese hyperglycemic mouse increases as the glucose concentration is raised (70). Mannoheptulose, which inhibits glucose-induced insulin secretion in other species, reduces oxygen consumption in this system. On the other hand, mannose and fructose, which have a variable stimulatory effect on insulin secretion in a number of species, increase oxygen consumption only slightly.

Pyruvate, fumarate, and glutamate slightly enhance glucose-stimulated insulin secretion *in vitro* (27). Pyruvate stimulates secretion from the pancreatic explant of the fetal rat (A. E. Renold, personal communication) only when caffeine is present in the medium. In this preparation, only a small effect of glucose is observed unless caffeine is present. Citrate stimulates insulin secretion from pieces of pancreas of the duck (176) and rat (58) and from isolated rat islets (154).

Acetoacetate and β-hydroxybutyrate stimulate secretion of insulin *in vivo* in dogs (140) but have no effect in sheep (72). Ketone bodies either have no effect (27, 174) or inhibit (177) insulin secretion by the rabbit pancreas *in vitro*; but in the rat, stimulation of insulin release by β-hydroxybutyrate has been reported (117). In sheep and cows insulin secretion appears to be regulated by propionate and butyrate rather than by glucose (72, 130, 131). Since ruminant metabolism centers around fatty acids rather than carbohydrate, the regulation of secretion by propionate and butyrate is not unexpected. Octanoate has been shown to stimulate (186) or have no effect (214) on insulin release from pieces of rat pancreas, but secretion induced by glucose from isolated rat islets is enhanced by octanoate and butyrate (154).

Experimental elevation of free fatty acids in the plasma of dogs by infusion of heparin and lipid is associated with an increase in serum insulin and a decrease in glucagon (112). Similar observations have been made in man (164).

B. Amino acids and ions

Although glucose is clearly the major stimulus for insulin secretion, alternate physiological mechanisms are also present in the mammalian islet. In ruminants fatty acids are the major metabolic fuel and exert marked effects on insulin secretion. In other species, notably fish (207), amino acids may serve as the major stimulus for insulin secretion. Certain persons also exhibit an increased sensitivity to amino acids to the extent that they may develop hypoglycemic episodes. Such cases are rare but can be troublesome if the cause is not recognized and correctly diagnosed. About 30 cases of leucine-induced hypoglycemia have been described (25), and the fall in blood glucose associated with leucine or protein feeding is accompanied by a rise in blood insulin levels (228).

Several amino acids, whether given together or individually, increase insulin levels in human plasma (48, 49). A group of 10 amino acids given together proved the most potent stimulant *in vivo*, but individual acids have effects in decreasing order of potency from arginine, to lysine, phenylalanine, and leucine, to methionine, valine, histidine, threonine, tryptophan, and isoleucine. In diabetics and subclinical diabetics, the increase in plasma insulin induced by these 10 essential

amino acids or arginine alone is significantly less than in healthy persons. Insulin secretion from rat pancreatic pieces induced by glucose (100 mg/100 ml) is further enhanced by addition of leucine or arginine. In this system, other amino acids have less stimulant action (phenylalanine, lysine, tryptophan, and glycine) or no effect (histidine, valine, and methionine) (117).

Concentrations of inorganic ions appear to be involved in the release of insulin *in vitro*. Zinc forms a complex with insulin (30), and its concentration in *beta* cells decreases after stimulation of insulin release by glucose (105, 135). This fact suggests that zinc may be involved in secretion, but injection of zinc does not promote insulin release (105). Calcium ions are required for both glucose and tolbutamide to stimulate insulin secretion in the perfused rat pancreas (33, 64). Magnesium does not appear to be essential if calcium is present (64). On the basis of experiments with pieces of rabbit pancreas, Milner and Hales (147) have suggested that sodium and calcium may have an interrelated role in the mechanism of insulin release. In this tissue, a high level of magnesium inhibits insulin release (146).

In the perfused rat pancreas, elevation of the potassium level from 4 to 8 mEq/liter stimulates insulin release in the absence of glucose (64). This stimulation is equivalent to that seen in the presence of 200 mg glucose/100 ml. Milner and Hales (146a) have reported that potassium will also stimulate secretion from the rabbit pancreas *in vitro*, yet others have failed to obtain any effect with infusions of potassium into dogs (137). Effects of the various ions on insulin secretion suggest that an ion-membrane phenomenon may be involved. Substances that are known to initiate insulin release from isolated islets also alter the membrane potential of such preparations (36).

C. Effect of enteric hormones

That insulin secretion might be influenced by an intestinal hormone was first considered by Moore *et al.* in 1906 (155). An extract of duodenum was administered to several diabetics in an attempt to alleviate their symptoms, but the results were inconclusive. In 1964 two lines of evidence indicated a possible role for a humoral factor from the gastrointestinal tract in insulin release. It was shown that intrajejunal or oral administration of glucose increases plasma insulin levels more than a comparable intravenous infusion despite the fact that the blood glucose often rose higher after intravenous than after intrajejunal administration (45, 50, 138). Crude secretin-containing extracts of duodenal mucosa in man accelerated the rate of disappearance of intravenously administered glucose (42) and increased plasma insulin-like activity (43). Purified preparations of secretin increase insulin secretion from pieces of dog and rabbit pancreas (139, 167). In anesthetized dogs, purified secretin, pancreozymin-cholecystikinin, and gastrin increase insulin release, but after an immediate rapid rise in the concentration of insulin in the pancreaticoduodenal vein, a gradual decline in the insulin concentration is observed (211). Both pancreozymin (113) and secretin (37) increase peripheral insulin levels in man. Although the factor or factors responsible have not been clearly defined, it does appear that the process of glucose

TABLE 3

Effect of drugs on release of insulin

Agent	Condition	Effect on Insulin Secretion	Species	References
Epinephrine	*In vivo*	Inhibits	Man	170, 171
	In vitro	Inhibits	Rat, rabbit	27, 129
Norepinephrine	*In vitro*	Inhibits	Rat	129
Isoproterenol	*In vivo*	Stimulates	Man	169
	In vitro	Inhibits	Rat	123, 129
Acetylcholine	*In vitro*	Stimulates	Rat	129, 136
	In vitro	No effect	Rabbit	27
Carbachol	*In vitro*	Stimulates	Rat, rabbit, dog	129, 136
Methacholine	*In vivo*	Stimulates	Man, dog	80, 84
Bethanechol	*In vivo*	Stimulates	Dog	84
Theophylline	*In vivo*	Stimulates	Rat	209
	In vitro	Stimulates	Rat	66, 123
Glucagon	*In vivo*	Stimulates	Man, dog	21, 29, 89, 92, 184
	In vitro	Stimulates	Rat, rabbit, duck	38, 66, 123, 208
Secretin	*In vivo*	Stimulates	Man, dog	37, 211
Pancreozymin	*In vivo*	Stimulates	Man, dog	113, 211
Tolbutamide	*In vivo*	Stimulates	Man, dog	94, 114, 166, 229
	In vitro	Stimulates	Rat, rabbit	27, 65, 124, 201
Diazoxide	*In vitro*	Inhibits	Rat	53, 189
Alloxan	*In vivo*	Cytotoxic	Rat	103, 109
Streptozotocin	*In vivo*	Cytotoxic	Rat	3, 46, 79, 173

absorption in some way increases the responsiveness of islets to hyperglycemia. The effect of these and other agents mentioned below are summarized in Table 3.

D. Sulfonylureas

Sulfonylureas have been used for a number of years in treating the "maturity onset" diabetic. These drugs have a hypoglycemic action which appears to be produced by two mechanisms—stimulation of insulin release from the pancreas and less clearly defined extrapancreatic effects. The absence of hypoglycemic effect of the sulfonylureas in pancreatectomized dogs suggested to Loubatières that these compounds may directly stimulate insulin release (107, 108). This hypothesis is supported by numerous studies in man and experimental animals. Tolbutamide and other sulfonylureas cause degranulation of the pancreatic *beta* cells in a number of species and decrease extractable insulin in the pancreas (75, 104, 221). Administration of tolbutamide leads to a detectable increase in plasma-insulin in man and other species (114, 166, 229) and stimulates insulin release *in vitro* from the pancreas of the rabbit (27) or dog (94) and an explant of rat fetal pancreas (99). Tolbutamide also stimulates insulin release both from pieces of rat pancreas (124) and from the perfused rat pancreas (65, 201) in the presence or absence of glucose. In explants of fetal rat pancreas, tolbutamide stimulates insulin release in the presence of glucose. If caffeine is added, tolbutamide stimulates better whether glucose is present or absent (99).

In the perfused rat pancreas and in pieces of rat pancreas a transient effect of tolbutamide is observed (65, 124). Its persistence when the perfused pancreas is treated with puromycin suggests that the insulin initially released by tolbutamide is derived from stored rather than newly synthesized hormore (65). Pancreatic islets also appear to become refractory after stimulation with sulfonylureas. Bellens *et al.* (10) found in dogs that after a prolonged infusion of sulfonylurea the pancreas no longer responds normally to a second dose of the drug or to glucose. However, after prolonged hyperglycemia, produced by infusing glucose, the dog's pancreas can still be stimulated with sulfonylureas. A similar observation has been made *in vitro* with pieces of rat's pancreas (124). Tissue incubated in glucose and tolbutamide for 1 hr fails to respond to a second tolbutamide stimulation and exhibits a reduced response to glucose.

The mode of action of tolbutamide is not known, but it does appear to differ from that of glucose. The stimulant effect of tolbutamide is often weak, and it has less effect than high concentrations of glucose. The effect is transient and, as mentioned above, pancreatic tissue becomes refractory to stimulation by both tolbutamide and glucose. Stimulation by tolbutamide in rat pancreatic pieces is not enhanced by theophylline, nor is it inhibited by mannoheptulose or diazoxide. On the other hand, the stimulant effects of glucose and tolbutamide can both be inhibited with epinephrine. Unlike other agents that release insulin, tolbutamide can stimulate secretion in the absence of glucose.

The benzothiadiazines have been known for some years to produce hyperglycemia in man and animals (39, 220). The most thoroughly studied agent in this group, diazoxide, is used to relieve spontaneous hypoglycemia (141, 182) and as a research tool in the study of insulin secretion (47, 189). *In vivo* diazoxide releases catecholamines (97), and this might influence insulin release, but diazoxide inhibits insulin secretion also *in vitro* (53, 189). A recent study showed that insulin secretion induced by glucose from pieces of rat pancreas is inhibited by diazoxide but is not affected by chlorothiazide, another benzothiadiazine (116). The inhibition is not affected by addition of an *alpha* blocker (phenoxybenzamine), which prevents the inhibition produced by epinephrine. The inhibition produced by diazoxide is counteracted by addition of tolbutamide, whereas the inhibition produced by epinephrine is not. The above findings indicate that, while diazoxide inhibits glucose-induced secretion, this inhibition is independent of its ability to release catecholamines *in vivo*.

IV. ENDOCRINE REGULATION

A. Catecholamines

The catecholamines have been known for some time to induce hyperglycemia due, it was thought, to increased hepatic glycogenolysis. Recent studies indicate that catecholamines have a second action. Infusion of glucose alone into man causes hyperglycemia and an increase in plasma insulin but, when epinephrine is infused, there is a gradual rise in the blood glucose and the insulin level does not change (171). If the epinephrine infusion is then stopped, there is an immediate rise in the insulin level, followed by a decline in blood glucose. This action indicates

that epinephrine inhibits insulin release. This occurs in dogs *in vivo* (95) and in the pancreas of the rabbit (27) and rat (129) *in vitro*.

The mode of action of the catecholamines is suggested by effects obtained with the use of adrenergic blocking agents. These are summarized in Table 4. If an *alpha* blocker (phentolamine) is infused with epinephrine into man or the rat, serum insulin levels rise with the blood glucose (170, 209). In the pancreas of the rat *in vitro*, an *alpha* adrenergic blocker prevents the inhibition of insulin release produced by epinephrine (129). Infusion of isoproterenol, predominantly a *beta* agonist, leads to a modest increase in serum insulin levels in man (169). In the presence of an *alpha* blocker, this agent stimulates secretion from rat pancreas *in vitro* at glucose concentrations of 100 mg/100 ml or more (123). These observations indicate that the catecholamines can, under proper conditions, exert different effects on insulin secretion; *alpha* stimulation can inhibit secretion and *beta* stimulation can promote insulin release.

Several observations have led to the hypothesis that the adenylcyclase system

TABLE 4

Effect of adrenergic agents on release of insulin

Drugs		Conditions (Species)	Insulin Secretion	Cyclic AMP Level*	Reference
Catecholamine	Blocker				
Epinephrine		*In vivo* (man)	Slight decrease		
Epinephrine	Phentolamine		Increase		170
Epinephrine	Propanolol		Slight decrease		
Epinephrine		*In vivo* (rat)	Slight decrease	Decrease	
Epinephrine	Phentolamine		Increase	Increase	210
Epinephrine	Propanolol		Decrease	Decrease	
Isoproterenol		*In vivo* (man)	Increase		
Isoproterenol	Propanolol		No effect		169
Epinephrine		*In vitro*	Decrease		
Epinephrine	Phenoxybenzamine		No effect		
Epinephrine	Phentolamine	Glucose (100 mg%) stimulated	No effect		123
Epinephrine	Nethalide		Decrease		129
Epinephrine	Propanolol		Decrease		
Isoproterenol			Decrease		
Isoproterenol	Phenoxybenzamine	(Rat)	Increase		
Isoproterenol			No effect		
Norepinephrine			Decrease		

* Level obtained in relation to that found in the presence of theophylline.

is involved in insulin secretion. Various agents (*beta* adrenergic stimulants, theophylline, and glucagon) that are thought to act through the adenylcyclase system in liver and adipose tissue (19) also stimulate insulin secretion *in vivo* (89, 92, 169 209) and *in vitro* (123, 129). Recent studies (211) have demonstrated the presence of 3′,5′-cyclic adenosinemonophosphate (cyclic AMP) in pancreatic islets of Langerhans of the rat. Theophylline, which enhances insulin secretion *in vivo* (209) and *in vitro* (123), also increases the cyclic AMP level in pancreatic islets. Glucagon, in the presence of theophylline, further enhances the level of cyclic AMP. Epinephrine, in the presence of theophylline, prevented accumulation of 3′,5′-AMP, but when an *alpha* adrenergic blocker (phentolamine) was added, accumulation of the nucleotide occurred normally; *beta* adrenergic blockage (propanolol) did not antagonize the action of epinephrine (210). This fact suggests that production of cyclic 3′,5′-AMP is decreased to a very low level when an *alpha* adrenergic stimulation is applied. These observations, taken in conjunction with parallel effects upon insulin secretion *in vivo* and *in vitro*, suggest that the cyclic 3′,5′-AMP level in *beta* cells can modify the rate of insulin release.

The mechanism by which the adenylcyclase system affects insulin release is not known, but it has been suggested that cyclic 3′,5′-AMP could activate glycogen phosphorylase in the *beta* cells (123). This suggestion is based on the following observations. Pancreatic tissue from normoglycemic rats, containing no detectable glycogen in the islet tissue, does not secrete insulin in the absence of glucose, and the stimulant effects of glucose and theophylline are reduced or abolished by mannoheptulose or 2-deoxyglucose. Tissue obtained from rats made hyperglycemic with infusions of glucose, and containing glycogen within the islet tissue, responds to theophylline even in the absence of glucose. This stimulant effect is inhibited by 2-deoxyglucose but not by mannoheptulose, a result that suggests that the glucose normally used by isolated pancreatic tissue is extracellular. In tissue from the hyperglycemic rats, theophylline could stimulate glycogen phosphorylase (as it does in the liver) and so provide an intracellular source of glucose from stored glycogen. Since no such glycogen store is present in normal islets, this could not be the only operative mechanism. To explain this action of theophylline under normal conditions, one would have to implicate an action elsewhere in the glycolytic or oxidative pathways of glucose metabolism.

B. Glucagon

Glucagon stimulates insulin release in man (37, 89, 183) and dogs (21, 92). Since this stimulation in man precedes any increase in blood sugar level, glucagon appears to stimulate secretion directly, and indeed glucagon added *in vitro* increases secretion of insulin from the pancreas of the rat (38, 123), rabbit (208), and duck (175). Turner and McIntyre (208) have suggested that glucagon has a greater stimulant effect at higher glucose concentrations. Glucose appears to be necessary for glucagon to stimulate insulin secretion by pancreas (123), but other studies indicate that glucagon can initiate insulin secretion *in vitro* in the absence of glucose (38, 66, 202). Glucagon does not appear to stimulate secretion through a *beta* receptor, as the catecholamines do, since addition of propanolol *in vitro* has no effect on its action (123).

C. Adrenal corticoids and adrenocorticotropin (ACTH)

Corticoids, added *in vitro* to pancreas from the rabbit (27) and rat (126), do not alter insulin secretion. After adrenalectomy the pancreas of the rat *in vitro* has a decreased responsiveness to glucose; administration of cortisol *in vivo* restores the normal response to glucose *in vitro* (126). An excess of corticoids, as seen in hyperadrenocorticism or in persons receiving therapeutic doses (15, 165), is accompanied by an elevated insulin response to glucose. Cortisol administration to sheep also leads to an increased serum insulin level (6). ACTH administered to intact dogs (161) or perfused through the isolated rat pancreas (202) leads to increased release of insulin. At a high glucose concentration (300 mg/100 ml), ACTH stimulates insulin release from rat pancreatic pieces, but it has no effect at lower glucose concentrations (123). ACTH has no effect on insulin release from rabbit pancreas *in vitro* (27).

D. Growth hormone

Clinical studies in which plasma levels of insulin and growth hormone have been measured suggest that there is an inverse relation between the concentrations of these substances (35, 59). After glucose loading, the level of insulin rises while growth hormone levels tend to fall. In fasting or prolonged starvation, low levels of insulin and high levels of growth hormone are observed. In either case, hormone concentrations appear to be regulated in response to glucose.

That growth hormone administration to experimental animals over prolonged periods will produce permanent diabetes (20, 230) has suggested a direct effect of the hormone on insulin secretion and islet function. However, growth hormone added *in vitro* does not initiate insulin release from either the rat (121) or the rabbit (27) pancreas. In persons deficient in growth hormone, serum insulin responses to glucose or arginine are depressed (57, 142); subsequent administration of human growth hormone to these persons increased insulin release in response to glucose. Insulin release *in vitro* in response to glucose is also depressed in hypophysectomized rats, and administration of growth hormone *in vivo* increases the responsiveness of the pancreas to glucose but does not restore the response to normal. Chronic administration of growth hormone to normal rats for 4 days has no effect on the response of pancreatic pieces, but pancreatic tissue from rats bearing growth-hormone-producing tumors is hyperresponsive to glucose (121). Administration of growth hormone to dogs and to people (228, 231) and the high endogenous levels of growth hormone in acromegalics (14, 228) are accompanied by increased serum insulin levels. Campbell and Rastogi (20) have suggested that growth hormone causes a readjustment of the homeostatic control of pancreatic islets such that there is increased insulin release at any particular level of glucose.

E. Thyroid hormones

Thyroxine *in vitro* has no effect on insulin release from pancreatic tissue from normal or thyroidectomized rats (125). Pancreatic tissue from thyroidectomized rats has a decreased response to glucose when incubated *in vitro*, and administration of thyroxine *in vivo* restores the response. Administration of the thyroxine *in vivo* to normal rats for 4 days leads to a decreased insulin response to glucose

in vitro. Thyrotropin stimulates insulin release from rat pancreatic pieces at a high glucose concentration (300 mg/100 ml) but has no effect at lower concentrations (123).

This evidence suggests that the above hormones (corticoids, growth hormone, and thyroxine) do not alter insulin release when added *in vitro*. When they are given *in vivo* to deficient subjects (adrenalectomized, hypopituitary, or thyroidectomized), they restore to normal the response of pancreatic tissue to glucose. An increased level of these hormones *in vivo* is associated with increased (corticoids or growth hormone) or decreased (thyroxine) response of the pancreas to glucose. These findings suggest that the above hormones do not have sufficient time to act when added *in vitro* but, given *in vivo*, they modify the responsiveness of pancreatic tissue to glucose. On the other hand, ACTH and thyrotropin have direct effects on insulin secretion *in vitro*.

F. Oxytocin and vasopressin

Oxytocin and vasopressin (lysine vasopressin and arginine vasopressin) stimulate insulin release in the anesthetized dog (86). Oxytocin appears to stimulate secretion by a direct action on the islet since the plasma insulin level rises before the blood glucose. In rat pancreatic pieces, these two hormones do not stimulate insulin release (123).

G. Pregnancy and sex hormones

Lund and Weese (110) have noted that 44% of women who had previously delivered heavy infants developed impaired carbohydrate tolerance during a subsequent pregnancy. The impaired glucose tolerance would appear to be directly related to a defective insulin response during the initial ½ to 1 hr after glucose administration (81). When the subject is not pregnant, a normal response is usually observed, but impaired glucose tolerance and a reduced plasma insulin response may be reproduced by pretreatment with a glucocorticoid. It is possible that the altered response in pregnancy is associated with production of a growth hormone-like substance (placental lactogen) by the placenta (7, 88).

A marked elevation in plasma insulin in response to a standard glucose load has been reported in normal women treated for 33 weeks with Enovid (195) although the glucose tolerance was essentially unchanged. The increased plasma insulin response could not be correlated with either a family history of diabetes or weight gain. A second study (168) reported no change in glucose tolerance or plasma insulin after only 17 to 20 days of therapy with oral contraceptive preparations. Additional reports on possible effects of oral contraceptives on glucose tolerance and insulin release tend to be equivocal at best and do not permit any definitive conclusions regarding the action of these substances on islet function (194, 196, 198). Although changes in glucose tolerance have been noted during the menstrual cycle (76), parallel studies on insulin release have not been performed.

H. Obesity

In obese persons, levels of plasma insulin tend to be high in the fasting state and rise to abnormally high values after oral glucose (90, 96, 163). Such hyperin-

sulinism has been attributed to overproduction (96), but it could also be due to increased sensitivity of the islets to a natural stimulus such as glucose. In experimental forms of obesity, hyperplasia of the islets has been reported in the obese spiny mouse (61), and increased sensitivity of the islets to glucose has been found *in vitro* with pancreatic tissue from the hereditarily obese diabetic mouse ($C57BL/K_s$). As these latter obese mice grow older, the insulin content of their pancreatic tissue falls, and their secretory response to glucose *in vitro* falls (118). If normal islets are transplanted into obese littermates of this strain, further gain in body weight is reduced and blood-sugar concentration is returned to normal (200). Whether these experimental obese syndromes are associated with lesions analogous to those occurring in the obese human subject is not yet known, but they do provide useful tools for research in this field.

V. NEURAL REGULATION

In the early 1920's, several studies were done on the role of the vagus nerves in insulin secretion. It was indicated that repeated vagal stimulation definitely changes the histologic appearance of the islets of Langerhans, and there were conflicting reports as to whether insulin secretion was stimulated or inhibited. At that time methods to measure secretion of insulin quantitatively or histologically were not available. With present methods for assaying insulin, investigators are now reexamining the possible "neurogenic" control of secretion. Present studies on epinephrine's inhibitory effect on insulin release indicate a possible role for the adrenergic system, and several studies indicate that the cholinergic system may also play a role in regulating secretion.

Frohman *et al.* (56) have shown that stimulation of the vagus nerve in anesthetized dogs is followed by an increase in serum immunoreactive insulin. Vagotomy alone is accompanied by a decrease in insulin levels and no change in blood sugar. Stimulation of the cut distal end of the vagus leads to a prompt rise in portal insulin and a small rise somewhat later in peripheral plasma insulin. Stimulation of the right and left vagus produce the same effect, but when they are stimulated in succession, the first stimulation always produces an increase greater than the second. The rise in insulin was blocked by atropine. Kaneto *et al.* (85) have made similar observations in the dog, but they stimulated the cut vagus at different levels. An immediate increase in pancreatic vein insulin was found by stimulating the right or left cervical vagi or the dorsal vagus at the level of the diaphragm. The femoral venous plasma insulin also rose somewhat later than the level in the pancreatic vein. Stimulation of the ventral vagus trunk at the diaphragm had no effect on insulin secretion. Nelson *et al.* (159) under similar conditions found no increase in insulin in dogs after stimulation of the vagi at the cervical and diaphragmatic levels. Daniel *et al.* (34) found that stimulation of the cut right vagus of the baboon is followed by an appreciable increase in insulin in the inferior vena cava and a moderate increase in the splenic vein. Vagal stimulation did not affect the blood sugar, and it was therefore concluded that this was a direct effect of the nerve on secretion. Studies in the vagotomized dog and monkey indirectly indicate that cholinergic innervation of the islets may regulate insulin release (22, 145).

Additional evidence for the role of cholinergic involvement in insulin secretion has been obtained with acetylcholine and other cholinergic agents given *in vitro* and *in vivo*. Acetylcholine and carbachol enhance the release of insulin by glucose (100 mg/100 ml) from pieces of rat pancreas (129, 136), and this effect is blocked in the presence of atropine. Carbachol, added *in vitro*, stimulates the glucose-induced release of insulin from the pancreas of the rabbit and the dog (136). *In vivo*, plasma-insulin levels are increased in man by the injection of methacholine (80) and in dogs by injection of methacholine or bethanechol (84). The stimulant effect observed in dogs is blocked by pretreatment with atropine. In view of the above evidence, it appears that cholinergic innervation of the islets could play a role in the regulation of insulin secretion.

VI. CLINICAL APPLICATIONS

Several drugs and hormones known to influence insulin secretion have been used for diagnostic or therapeutic purposes in man. In essence, they are used either to assess the insulin secretory potential of islet tissue or to modify the rate of endogenous insulin secretion for therapeutic purposes.

A. Diagnosis

As already mentioned, it is not possible to make direct measurements of endogenous insulin secretion in man. Indirect methods are therefore employed to assess insulin secretory activity of the human pancreas.

To assess the degree of a patient's insulin insufficiency, glucose is most commonly used as the secretory stimulant, the response of the pancreatic islets being inferred from the succeeding changes in blood-sugar concentration. Thus, when glucose (1.75 g/kg ideal body weight) is given by mouth to the normal subject, the blood-sugar concentration should not exceed 100 mg/100 ml in the fasting state and should not rise above 160 mg/100 ml at 1 hr, 140 mg/100 ml at 90 min, and 120 mg/100 ml at 2 hr after the glucose load (24). The dose of glucose may be varied and different standards for a normal response may be set in different centers, but in each case insulin insufficiency is inferred from an excessive rise in blood-sugar concentration after the oral load of glucose. Where no abnormality can be detected by this means, a mild degree of insufficiency can sometimes be detected by first administering a dose of glucocorticoid (24). Misleading responses may be obtained in the presence of abnormal gastro-intestinal function such as may occur in pregnancy (11) or thyrotoxicosis (44) or after gastrectomy (178). Under these conditions more reliable evidence of impaired insulin secretory activity may be obtained by administering the glucose intravenously (52). Using these relatively simple but crude methods for the assessment of pancreatic function, it has proved possible to classify patients into various categories from the earliest to the most profound stages of islet failure (24).

Intravenously injected tolbutamide has also been used to assess pancreatic insulin secretory activity. In the very early stages of juvenile diabetes, for example, tolbutamide has been used to demonstrate that the pancreas is still capable of secreting some insulin; this ability is lost as the disease progresses (9). In

normal subjects, a single dose (1.0 g in 1.0 ml water) will reduce blood-sugar concentration in 20 min to a level which is less than 75% of the pre-injection value (212). In conjunction with a glucocorticoid, tolbutamide has been used to detect minor degrees of insulin insufficiency (106). It may also be employed in the diagnosis of hypoglycemia. Islet-cell adenomas or carcinomas can often be diagnosed on clinical grounds (219), but it has also been shown that in many such cases the plasma-insulin level will rise at an abnormally rapid rate after intravenous tolbutamide (51, 180). This is not invariably so, for the most marked rises appear to be associated with tumors that have high insulin contents, and no increase may be seen when the tumor is devoid of stored hormone (199). In reviewing their experiences with tolbutamide, Samols and Marks (179) emphasized that excessive responses are not specific for islet-cell tumors and concluded that the intravenous tolbutamide test is a "valuable adjunct in the recognition and differential diagnosis of hypoglycemia provided that its limitations are appreciated."

In normal subjects, oral leucine has little or no effect upon blood-sugar concentration or the level of circulating insulin unless a sulfonylurea has been administered previously (50). In some children, however, leucine can induce profound hypoglycemia associated with marked elevation in plasma-insulin concentration (25, 228).

B. Therapy

When the pancreas shows evidence of insulin secretory activity, sulfonylureas can sometimes be used for therapy. In cases of hyperinsulinism and some other clinical syndromes, a variety of means has been used to prevent onset of potentially dangerous attacks of hypoglycemia.

Many diabetics, and especially those who develop the disease in later life, are able to synthesize and secrete insulin and can sometimes survive without developing ketosis. If selected with care, such patients are likely to profit from therapy with one or other of the sulfonylureas (40). Of those who fail to respond in the face of apparently adequate treatment with sulfonylureas, some are found to benefit from additional therapy with a biguanide. Phenformin, which has no effect upon insulin secretion, could potentiate the action of the sulfonylurea by inhibiting gluconeogenesis and possibly increasing anaerobic metabolism of glucose (41). Sulfonylureas are also being tried in patients who show no evidence of insulin insufficiency, but it is still too early to assess the prophylactic value of these drugs in subjects who may be genetically prone to develop diabetes.

For the treatment of recurrent attacks of hypoglycemia, a variety of techniques has been employed but none has proved universally satisfactory. Alloxan, which is extensively used to induce diabetes in experimental animals (109), has proved of little use in cases of islet tumors in man (103). Streptozotocin, a compound isolated from the media used to cultivate *Streptomyces achromogens*, has a similar specific destructive effect upon pancreatic *beta* cells in animals (3, 46, 79, 173) but has yet to be used in man. Glucagon and epinephrine in long-acting forms have been given clinically in attempts to reduce the severity and frequency of hypoglycemic attacks but with little success. More recently, however, it has been

shown that diazoxide has a marked beneficial effect in the control of hypoglycemic attacks among patients with hypoglycemia of infancy, glycogenoses, and islet-cell adenomas and carcinomas (193). It is not free of undesirable side effects, but it promises to be of real value in the treatment of these distressing conditions.

REFERENCES

1. ALLISON, S. P., PROWSE, K. AND CHAMBERLAIN, M. J.: Failure of insulin response to glucose load during operation and after myocardial infarction. Lancet 1: 478–481, 1967.
2. ANDERSON, E. AND LONG, J. A.: The effect of hyperglycemia on insulin secretion as determined with the isolated rat pancreas in a perfusion apparatus. Endocrinology 40: 92–97, 1947.
3. ARISON, R. N., CIACCIO, E. L., GLITZER, M. S., CASSARO, J. A. AND PRUSS, M. P.: Light and electron microscopy of lesions in rats rendered diabetic with streptozotocin. Diabetes 16: 51–56, 1967.
4. ASHCROFT, S. J. H. AND RANDLE, P. J.: Glucose metabolism and insulin secretion by pancreatic islets. Lancet 1: 278–280, 1968.
5. ASHCROFT, S. J. H. AND RANDLE, P. J.: Glucose-6-phosphatase activity of mouse pancreatic islets. Nature (London) 219: 857–858, 1968.
6. BASSETT, J. M. AND WALLACE, A. L. C.: Influence of cortisol on plasma insulin in the sheep. Diabetes 16: 566–571, 1967.
7. BECK, P. M., PARKER, AND DAUGHADAY, W. H.: Radioimmunologic measurement of human placental lactogen in plasma by a double antibody method during normal and diabetic pregnancies. J. Clin. Endocrinol. 25: 1457–1462, 1965.
8. BECK, L. V., ZAHAREO, D. S., ROBERTS, N., MCNEIL, T., KING, C. AND BLANKENBAKER, R.: Insulin assay by combined use of I^{131}-labeled insulin, anti-insulin serum and insulinase. Life Sci. 3: 545–550, 1964.
9. BELLE, R. DE, BELMONTE, M. M. AND COLLE, E.: Effect of intravenous tolbutamide in juvenile diabetes mellitus. Diabetes 16: 215–218, 1967.
10. BELLENS, R.: Contribution a l'étude des mécanisme d'action des drogues hypoglycemiantes. Acta Endocrinol. (Copenhagen) 61: 1–243, 1961.
11. BENJAMIN, F. AND CASPER, D. J.: Oral versus intravenous glucose tolerance tests during pregnancy. Amer. J. Obstet. Gynecol. 94: 566–570, 1966.
12. BERSON, S. A. AND YALOW, R. S.: Immuno-assay of protein hormones. *In* The Hormones, ed. by J. Pincus, K. V. Thimann, and E. B. Astwood, Vol. IV, pp. 557–630, New York, Academic Press, Inc., 1964.
13. BERSON, S. A., YALOW, R. S., BAUMAN, A., ROTHSCHILD, M. A. AND NEWEYLY, K.: Insulin-I^{131} metabolism in human subjects; demonstration of insulin binding globulin in the circulation of insulin treated subjects. J. Clin. Invest. 35: 170–190, 1956.
14. BODEN, G., SOELDNER, J. S., STEINKE, J. AND THORN, G. W.: Serum human growth hormone response to I.V. glucose: diagnosis of acromegaly in females and males. Metabolism 17: 1–9, 1968.
15. BOOKMAN, J. J., DRACHMAN, S. R., SCHAEFER, L. E. AND ADLESBURG, L. E.: Steroid diabetes in man. Diabetes 2: 100–111, 1953.
16. BOUMAN, P. R. AND BOSBOOM, R. S.: Effects of growth hormone and of hypophysectomy on the release of insulin from rat pancreas *in vitro*. Acta. Endocrinol. (Copenhagen). 50: 202–212, 1965.
17. BROLIN, S. E. AND BERNE, C.: Enzymatic activities of the initial glycolytic steps in pancreatic islets and acini. Metabolism 16: 1024–1028, 1967.
18. BROWN, E. M., DOHAN, F. C., FREEDMAN, L. R., DE MOOR, P. AND LUKENS, F. D. W.: The effects of prolonged infusion of the dog's pancreas with glucose. Endocrinology 50: 644–656, 1952.
19. BUTCHER, R. W.: Cyclic 3′,5′-AMP and the lipolytic effects of hormones on adipose tissue. Pharmacol. Rev. 18: 237–241, 1966.
20. CAMPBELL, J. AND RASTOGI, K. S.: Augmented insulin secretion due to growth hormone. Diabetes 15: 749–758, 1966.
21. CAMPBELL, J. AND RASTOGI, K. S.: Effects of glucagon and epinephrine on serum insulin and insulin secretion in dogs. Endocrinology 79: 830–835, 1966.
22. CHOWERS, I., LAVY, S. AND HALPERN, L.: Effect of insulin administered intracisternally on the glucose level of the blood and the cerebrospinal fluid in vagotomised dogs. Exp. Neurol. 14: 383–389, 1966.
23. COCHRAN, B., MARBACH, E. P., POUCHER, R., STEINBERG, T. AND GWINUP, G.: Effect of acute muscular exercise on serum immuno-reactive insulin concentration. Diabetes 15: 838–841, 1966.
24. CONN, J. W. AND FAJANS, S. S.: The prediabetic state. A concept of dynamic resistance to a genetic diabetogenic influence. Amer. J. Med. 31: 839–850, 1961.
25. CORNBLATH, M. AND SCHWARTZ, R.: Disorders of Carbohydrate Metabolism in Infancy, W. B. Saunders Company, Philadelphia, 1966.
26. COORE, H. G. AND RANDLE, P. J.: Inhibition of glucose phosphorylation by mannoheptulose. Biochem. J. 91: 56–65, 1964.
27. COORE, H. G. AND RANDLE, P. J.: Regulation of insulin secretion studied with pieces of rabbit pancreas incubated *in vitro*. Biochem. J. 93: 66–78, 1964.
28. COORE, H. G., RANDLE, P. J., SIMON, E., KRACIER, P. F. AND SHELESNYAK, M. C.: Block of insulin secretion from the pancreas by D-mannoheptulose. Nature (London) 197: 1264–1266, 1963.
29. CROCKFORD, P. M., PORTE, D., WOOD, F. C. AND WILLIAMS, R. H.: Effect of glucagon on serum insulin, plasma glucose and free fatty acids in man. Metabolism 15: 114–122, 1966.

30. Cunningham, L. W., Fischer, R. L. and Vestling, C. S.: A study of the binding of zinc and cobalt by insulin J. Amer. Chem. Soc. 77: 5703–5707, 1955.
31. Curry, D. L., Bennett, L. L. and Grodsky, G. M.: Dynamics of insulin secretion by the perfused rat pancreas. Endocrinology 83: 572–584, 1968.
32. Curry, D. L., Bennett, L. L. and Grodsky, G. M.: Multiphasic insulin secretion by the isolated perfused rat pancreas. Fed. Proc. 27: 496, 1968.
33. Curry, D. L., Bennett, L. L. and Grodsky, G. M.: Requirement for calcium ion in insulin secretion by the perfused rat pancreas. Amer. J. Physiol. 214: 174–178, 1968.
34. Daniel, P. M. and Henderson, J. R.: Effect of vagal stimulation on plasma insulin and glucose levels in the baboon. J. Physiol. 192: 317–327, 1967.
35. Daughaday, W. H. and Kipnis, D. M.: Growth promoting and anti-insulin actions of somatotropin. Recent Progr. Hormone Res. 22: 49–92, 1966.
36. Dean, P. M. and Matthews, E. K.: Electrical activity in pancreatic islet cells. Nature (London) 219: 389–390, 1968.
37. Deckert, T.: Stimulation of insulin secretion by glucagon and secretin. Acta Endocrinol. 57: 578–584, 1968.
38. Devrim, S. and Recant, L.: Effect of glucagon on insulin release *in vitro*. Lancet 2: 1227–1228, 1966.
39. Dollery, C. T., Pentecost, B. L. and Samaan, N. A.: Drug-induced diabetes. Lancet 2: 735–737, 1962.
40. Duncan, L. J. P. and Baird, J. D.: Compounds administered orally in the treatment of diabetes mellitus. Pharmacol. Rev. 12: 91–158, 1960.
41. Duncan, L. J. P. and Clarke, B. F.: Pharmacology and mode of action of the sulphonylureas and diguanides. Annu. Rev. Pharmacol. 5: 151–162, 1965.
42. Dupré, J.: An intestinal hormone affecting glucose disposal in man. Lancet 2: 672–673, 1964.
43. Dupré, J. and Beck, J. C.: Stimulation of release of insulin by an extract of intestinal mucosa. Diabetes. 15: 555–559, 1966.
44. Elrick, H., Hlad, C. J. and Arai, Y: Influence of thyroid function on carbohydrate metabolism and a new method for assessing response to insulin. J. Clin. Endocrinol. 21: 387–400, 1961.
45. Elrick, H., Stimmler, L., Head, J. C., Jr. and Arai, Y.: Plasma insulin response to oral and intravenous glucose administration. J. Clin. Endocrinol. Metab. 24: 1076–1082, 1964.
46. Evans, J. S., Gerritsen, G. C., Mann, K. M. and Owen, S. P.: Anti-tumor and hyperglycemic activity of streptozotocin (NSC-37917) and its cofactor (U-15,774). Cancer Chemother. Rep. 48: 1–6, 1965.
47. Fajans, S. S., Flory, J. C., Knopf, R. F., Rull, J., Guntsche, E. M. and Conn, J. W.: Benzothiadiazine suppression of insulin release from normal and abnormal islet tissue in man. J. Clin. Invest. 45: 481–492, 1966.
48. Floyd, J. C., Jr., Fajans, S. S., Conn, J. W., Knopf, R. F. and Rull, J.: Stimulation of insulin secretion by amino acids. J. Clin. Invest. 45: 1487–1502, 1966.
49. Floyd, J. C., Jr., Fajans, S. S., Conn, J. W., Thiffault, C., Knopf, R. F. and Guntsche, E.: Secretion of insulin induced by amino acids and glucose in diabetes mellitus. J. Clin. Endocrinol. Metab. 28: 266–276, 1968.
50. Floyd, J. C., Fajans, S. S., Knopf, R. F. and Conn, J. W.: Evidence that insulin release is the mechanism for experimentally induced leucine hypoglycemia in man. J. Clin. Invest. 42: 1714–1719, 1963.
51. Floyd, J. C., Fajans, S. S., Knopf, R. F. and Conn, J. W.: Plasma-insulin in organic hyperinsulinism: comparative effects of tolbutamide, leucine and glucose. J. Clin. Endocrinol. 24: 747–760, 1964.
52. Franckson, J. R. M., Ooms, H. A., Bellens, R., Conard, V., and Bastenie, P. A. Physiological significance of the intravenous glucose tolerance test. Metabolism 11: 482–500, 1962.
53. Frerichs, H., Gerber, R. and Creutzfeldt, W.: Insulin secretion *in vitro* II. Inhibition of glucose induced insulin release by diazoxide. Diabetologia 2: 269–276, 1966.
54. Frerichs, H., Reich, U. and Creutzfeldt, W.: Insulinsekcretion *in vitro*. 1. Hemming der glucoseinduzierten Insulinalgabe. Klin Wochensch. 47 to 43: 136–140, 1965.
55. Froesch, E. R., Burgi, H., Ramseier, E. B., Bally, P., and Labhart, A.: Antibody suppressible and nonsuppressible insulin-like activities in human serum and their physiological significance. An insulin assay with adipose tissue of increased precision and specificity. J. Clin. Invest. 42: 1816–1834, 1963.
56. Frohmann, L. A., Ezdinli, E. Z. and Javid, R.: Effect of vagotomy, and vagal stimulation on insulin secretion. Diabetes 16: 443–448, 1967.
57. Frohman, L. A., MacGillivray, M. H. and Aceto, T., Jr.: Acute effects of human growth hormone on insulin secretion and glucose utilization in normal and growth hormone deficient subjects. J. Clin. Endocrinol. Metab. 27: 561–575, 1967.
58. Gagliardino, M.: Studies on the mechanism of insulin release. Metabolism 15: 1068–1075, 1966.
59. Glick, S. M., Roth, J., Yalow, R. S. and Berson, S. A.: The regulation of growth hormone secretion. Recent Progr. Hormone Res. 21: 241–269, 1965.
60. Gomori, G., Friedman, N. B. and Caldwell, D. W.: Beta cell changes in guinea pig pancrease in relation to blood sugar level. Proc. Soc. Exp. Biol. Med. 41: 567–570, 1939.
61. Gonet, A. E., Stanffacher, W., Pictet, R. and Renold, A. E.: Obesity and diabetes mellitus with striking congenital hyperplasia of the islets of Langerhans in spiny mice (*Acomys cahirinus*). Diabetologia 1: 162–171, 1966.
62. Grodsky, G. M., Batts, A. A., Bennett, L. L., Vcella, C., McWilliams, N. B. and Smith, D. F.: Effects of carbohydrates on secretion of insulin from isolated rat pancreas. Amer. J. Physiol. 205: 638–644, 1963.
63. Grodsky, G. M. and Bennett, L. L.: Insulin secretion from the isolated pancrease in the absence of insulinogenesis. Proc. Soc. Exp. Biol. Med. 114: 769–771, 1963.
64. Grodsky, G. M. and Bennett, L. L.: Cation requirements for insulin secretion in the isolated perfused pancreas. Diabettes 15: 910–913, 1966.
65. Grodsky, G. M., Bennett, L. L., Smith, D. and Nemechek, K.: The effect of tolbutamide and glucose on the

timed release of insulin from the isolated perfused pancreas. *In* Tolbutamide ... after ten years, ed. by W. J. H. Butterfield and W. Van Westering pp. 11-21, Excerpta Medica Foundation, New York, 1967.

66. GRODSKY, G. M., BENNETT, L. L., SMITH, D. F. AND SCHMID, F. G.: Effect of pulse administration of glucose or glucagon on insulin secretion *in vitro*. Metabolism 16: 222-233, 1967.
67. GRODSKY, G. M., AND FORSHAM, P. H.: Immunochemical assay of total extractable insulin in man. J. Clin Invest. 39: 1070-1079, 1960.
68. HALES, C. N., AND RANDLE, P. J.: Immunoassay of insulin with insulin-antibody precipitate. Biochem. J. 88: 137-146, 1963.
69. HELLERSTROM, C.: A method for the microdissection of intact pancreatic islets of mammals. Acta Endocrinol. (Copenhagen) 45: 122-132, 1964.
70. HELLERSTROM, C.: Effects of carbohydrates on the oxygen consumption of isolated pancreatic islets of mice. Endocrinology 81: 105-112, 1967.
71. HERBERT, V., LAU, K. S., GOTTLEIB, C. W. AND BLEICHER, S. J.: Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. 25: 1375-1384, 1965.
72. HORINO, M., MACHLIN, L. J., HERTELENDY, F. AND KIPNIS, D. M.: Effect of short-chain fatty acids on plasma insulin in ruminant and non-ruminant species. Endocrinology 83: 118-127, 1968.
73. HOWELL, S. L. AND TAYLOR, K. W.: Potassium ions and the secretion of insulin by islets of Langerhans incubated *in vitro*. Biochem. J.108: 17-24, 1968.
74. HUMBEL, R. E. AND RENOLD, A. E.: Studies on isolated islets of Langerhans (Brockmann Bodies) of teleost fishes. 1. Metabolic activity *in vitro*. Biochem. Biophys. Acta 74: 84-95, 1963.
75. JANES, R. G. AND SMITH, J. M.: Secondary failure of tolbutamide in the normal rat. Correlation between beta cell degranulation and blood sugar response. Diabetes 10: 312-315, 1961.
76. JARRETT, R. J. AND GRAVER, H. J.: Changes in oral glucose tolerance during the menstrual cycle. Brit. Med. J. 2: 528-529, 1968.
77. JARRETT, R. J. AND KEEN, H.: Glucose metabolism of isolated mammalian islets of Langerhans. Lancet 1: 633-635, 1966.
78. JARRETT, R. J. AND KEEN, H.: Oxidation of sugars, other than glucose, by isolated mammalian Islets of Langerhans. Metabolism 17: 155-157, 1968.
79. JUNOD, A., LAMBERT, A. E., ORCI, L., PICTET, R., GONET, A. E. AND RENOLD, A. E.: Studies of diabetogenic action of streptozotocin. Proc. Soc. Exp. Biol. Med. 126: 201-205, 1967.
80. KAJINUMA, H., KANETO, A., KUZUYA, T. AND NAKAO, N.: Effects of metacholine on insulin secretion in man. J. Clin. Endocrinol. Metab. 28: 1384-1388, 1968.
81. KALKHOFF, R. K., RICHARDSON, B. L. AND STODDARD, F. J.: Plasma insulin response during prednisolone glucose tolerance tests in subclinical diabetic mothers of heavy infants. Diabetes 17: 37-47, 1968.
82. KALKHOFF, R., SCHALCH, D. S., WALKER, L., BECK, P., KIPNIS, D. M. AND DAUGHADAY, W. H.: Diabetogenic factors associated with pregnancy. Trans. Ass. Amer. Phys. 77: 270-280, 1964.
83. KANAZAWA, Y., KUZUYA, T., IDE, T. AND KOSAKA, K.: Plasma-insulin responses to glucose in femoral hepatic and pancreatic veins in dogs. Amer. J. Physiol. 211: 442-448, 1966.
84. KANETO, A., KAJINUMA, H., KOSAKA, K. AND NAKAO, K.: Stimulation of insulin secretion by parasympathomimetic agents. Endocrinology 83: 651-658, 1968.
85. KANETO, A., KOSAKA, K. AND NAKAO, K.: Effects of stimulation of the vagus nerve on insulin secretion. Endocrinology 80: 530-536, 1967.
86. KANETO, A., KOSAKA, K. AND NAKAO, K.: Effects of the neurohypophysial hormones on insulin secretion. En docrinology 81: 783-790, 1967.
87. KANSAL, P. C. AND BUSE, M. G.: The effect of adrenergic blocking agents on plasma insulin and blood glucose during urethan or epinephrine induced hyperglycemia. Metabolism 16: 548-556, 1967.
88. KAPLAN, S. L. AND GRUMBACH, M. M.: Immunoassay for human chorionic "growth hormone-prolactin" in serum and urine. Science 147: 751-753, 1965.
89. KARAM, J. H., GRASSO, S. G., WEGIENKA, L. C., GRODSKY, G. M. AND FORSHAM, P. H.: Effects on selected hexoses, of epinephrine and of glucagon on insulin secretion in man. Diabetes, 15: 571-578, 1966.
90. KARAM, J. H., GRODSKY, G. M. AND FORSHAM, P.: Excessive insulin response to glucose in obese subjects as measured by immuno-chemical assay. Diabetes 12: 197-204, 1963.
91. KEEN, H., SELLS, R. AND JARRETT, R. J.: A method for the study of metabolism of isolated mammalian islets of Langerhans, and some preliminary results. Diabetologia 1: 28-32, 1965.
92. KETTERER, H., EISENTRAUT, A. M. AND UNGER, R. H.: Effect upon insulin secretion of physiologic doses of glucagon administered via the portal vein. Diabetes 16: 283-288, 1967.
93. KILO, C., DEVRIM, S., BAILEY, R. AND RECANT, L.: Studies *in vivo* and *in vitro* of glucose-stimulated insulin release. The effects of metabolizable sugars, tolbutamide and 2-deoxyglucose. Diabetes 16: 377-385, 1967.
94. KILO, C., LONG, C. L., BAILEY, R. M., KOCH, M. B. AND RECANT. L.: Studies to determine whether glucose must be metabolized to induce insulin release. J. Clin. Invest. 41: 1372-1373, 1962.
95. KOSAKA, K., IDE, T., KUZUYA, T., MIKI, E., KUZUYA, N. AND OKINAKA, S.: Insulin-like activity in pancreatic vein blood after glucose loading and epinephrine. Endocrinology, 75: 9-14, 1964.
96. KREISBERG, R. A., BOSHELL, B. R., DIPLACIDO, I. AND RODDAM, R. F.: Insulin secretion in obesity. New Eng. J. Med. 276: 314-319, 1967.
97. KVAM, D. G. AND STANTON, H. C.: Studies on diazoxide hyperglycemia. Diabetes 13: 639-644, 1964.
98. LACY, P. E. AND KOSTIANOVSKY, M.: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 16: 35-39, 1967.
99. LAMBERT, A., VECCHIO, D., GONET, A., JEANRENAUD, B. AND RENOLD, A. E.: Organ culture of fetal rat pancreas:

effect of tolbutamide, glucagon and other substances. *In* Tolbutamide . . . after ten years, ed. by W. J. H. Butterfield and W. Van Westering, pp. 61–82, Excerpta Medica Foundation, New York, 1967.

100. Laszlo, J., Harlan, W. R., Klein, R. F., Kirshner, N., Estes, H. and Bogdonoff, M. D.: The effect of 2-deoxy-D-glucose infusions on lipid and carbohydrate metabolism in man. J. Clin. Invest. 40: 171–176, 1961.
101. Lazarow, A.: Functional characterization and metabolic pathways of pancreatic islet tissue. Recent Progr. Hormone Res. 19: 489–546, 1963.
102. Lazarow, A., Dixit, P. K., Lindall, A., Moran, J., Hosteller, K. and Cooperstein, S. J.: Enzyme specialization of islet tissue. *In* The Structure and Metabolism of the Pancreatic Islets, ed. by S. E. Brolin, B. Hellman and H. Knutson, pp. 251–265, The Macmillan Co., New York, 1964.
103. Lazarus, S. S., Barden, H. and Bradshaw, M.: Pancreatic beta cells and alloxan toxicity. Arch. Pathol. 73: 210–222, 1962.
104. Lazarus, S. S. and Volk, B. W.: Ultramicroscopic and histochemical studies on pancreatic beta cells stimulated by tolbutamide. Diabetes 11: 2–10, 1962.
105. Logothetopoulos, J., Kaneko, M., Wrenshall, G. A. and Best, C. H.: Zinc, granulation and extractable insulin in islet cells following hyperglycemia or prolonged insulin treatment. *In* The Structure and Metabolism of the Pancreatic Islets, ed. by S. E. Brolin, B. Hellman and H. Knutson, pp. 251–265, The Macmillan Co., New York, 1964.
106. Long, C., Kilo, C. and Recant, L.: Experience with a steroid-tolbutamide test for the detection of impaired pancreatic reserve. Diabetes 13: 127–134, 1964.
107. Loubatières, A.: Analyse du mécanisme de l'action hypoglycemiante du p-amino-benzene sulphamide thiodiazole (2254 R.P.) C. R. Séances Soc. Biol. 183: 766–767, 1944.
108. Loubatières, A.: Etude physiologique et pharmacodynamique de certains derives sulfamides hypolgycemiants. Arch. Int. Physiol. 54: 174–177, 1946.
109. Lukens, F. D. W.: Alloxan diabetes. Physiol. Rev. 28: 304–330, 1948.
110. Lund, C. J. and Wesse, W. H.: Glucose tolerance and excessively large babies in nondiabetic mothers. Amer. J. Obstet. Gynecol. 65: 815–832, 1953.
111. Madison, L. L., Combes, B., Unger, R. H. and Kaplan, N.: The relationship between the mechanism of action of the sulfonylureas and the secretion of insulin into the portal circulation. Ann. N.Y. Acad. Sci. 74: 548–556, 1959.
112. Madison, L. L., Seyffert, W. A., Jr., Unger, R. H. and Barker, B.: Effect of plasma free fatty acids on plasma glucagon and serum insulin concentrations. Metabolism 17: 301–304, 1968.
113. Mahler, R. J. and Weisberg, H.: Pancreozymin and serum-insulin levels. Lancet 1: 860–861, 1968.
114. Maingay, D., DeRuyter, H. A., Tonber, J. L., Croughs, R. J. M., Schopman, W. and Lequin, R. M.: Rapid rise of insulin concentration in plasma after intravenous administration of sodium tolbutamide. Lancet 1: 361–362, 1967.
115. Malaisse, W. J., Lea, M. A. and Malaisse-Lagae, F.: Effect of mannoheptulose on the phosphorylation of glucose and the secretion of insulin by islets of Langerhans. Metabolism 17: 126–132, 1968.
116. Malaisse, W. J. and Malaisse-Lagae, F.: Effect of thiazides upon insulin secretion *in vitro*. Arch. Int. Pharmacodyn. Thér. 171: 235–239, 1968.
117. Malaisse, W. J. and Malaisse-Lagae, F.: Stimulation of insulin secretion by noncarbohydrate metabolites. J. Lab. Clin. Med. 72: 438–448, 1968.
118. Malaisse, W. J., Malaisse-Lagae, F. and Coleman, D. L.: Insulin secretion in mice with hereditary diabetes. Proc. Soc. Exp. Biol. Med. 129: 65–69, 1968.
119. Malaisse, W., Malaisse-Lagae, F., Gerritsen, G., Dulin, W. E. and Wright, P. H.: Insulin secretion *in vitro* by the pancreas of the Chinese Hamster. Diabetologia 3: 109–114, 1967.
120. Malaisse, W. J., Malaisse-Lagae, F. and King, S.: Quantitative and qualitative aspects of islet function in the rat. J. Lab. Clin. Med. 71: 56–64, 1968.
121. Malaisse, W. J., Malaisse-Lagae, F., King, S. and Wright, P. H.: Effect of growth hormone upon insulin secretion. Amer. J. Physiol. 215: 423–428, 1968.
122. Malaisse, W., Malaisse-Lagae, F., Lacy, P. E. and Wright, P. H.: Insulin secretion by isolated islets in presence of glucose, insulin and anti-insulin serum. Proc. Soc. Exp. Biol. Med. 124: 497–500, 1967.
123. Malaisse, W. J., Malaisse-Lagae, F. and Mayhew, D.: A possible role for the adenylcyclase system in insulin secretion. J. Clin. Invest. 46: 1724–1734, 1967.
124. Malaisse, W. J., Malaisse-Lagae, F., Mayhew, D. A. and Wright, P. H.: Effects of sulfonylureas upon insulin secretion by the rat's pancreas. *In* Tolbutamide . . . after ten years, ed. by W. J. H. Butterfield and W. Van Westering, pp. 49–60, Excerpta Medica Foundation, New York, 1967.
125. Malaisse, W. J., Malaisse-Lagae, F. and McCraw, E. F.: Effects of thyroid function upon insulin secretion. Diabetes 16: 643–646, 1967.
126. Malaisse, W. J., Malaisse-Lagae, F., McCraw, E. F. and Wright, P. H.: Insulin secretion *in vitro* by pancreatic tissue from normal, adrenalectomised, and cortisol treated rats. Proc. Soc. Exp. Biol. Med. 124: 924–928, 1967.
127. Malaisse, W., Malaisse-Lagae, F. and Wright, P. H.: A new method for the measurement *in vitro* of pancreatic insulin secretion. Endocrinology 80: 99–108, 1967.
128. Malaisse, W. J., Malaisse-Lagae, F. and Wright, P. H.: Insulin secretion *in vitro* by islets from insulin-deficient rats. Proc. Soc. Exp. Biol. Med. 126: 474–476, 1967.
129. Malaisse, W., Malaisse-Lagae, F., Wright, P. H. and Ashmore, J.: Effects of adrenergic and cholinergic agents upon insulin secretion *in vitro*. Endocrinology 80: 975–978, 1967.

130. MANNS, J. G. AND BODA, J. M.: Insulin release by acetate, propionate, butyrate and glucose in lambs and adult sheep. Amer. J. Physiol. 212: 747–755, 1967.
131. MANNS, J. G., BODA, J. M. AND WILLES, R. F.: Probable role of propionate and butyrate in control of insulin secretion in sheep. Amer. J. Physiol. 212: 756–764, 1967.
132. MARTIN, D. B., RENOLD, A. E. AND DAGENAIS, Y. M.: An assay for insulin-like activity using rat adipose tissue. Lancet 2: 76–77, 1958.
133. MARTIN, F. I. R., STOCKS, A. E. AND PEARSON, M. J.: Significance of disappearance rate of injected insulin. Lancet 1: 619–620, 1967.
134. MARTIN, J. M. AND BAMBERS, G.: Insulin secretion in glucosamine-induced hyperglycemia in rats. Amer. J. Physiol. 209: 797–802, 1965.
135. MASKE, H.: Interaction between insulin and zinc in the islets of Langerhans. Diabetes 6: 355–357, 1957.
136. MAYHEW, D. A., GOLDBERG, A. M. AND WRIGHT, P. H.: The influence of cholinomimetics upon insulin secretion. Diabetes 17: 308–309, 1968.
137. MCCORMICK, J. R. AND EGDAHL, R. H.: Effect of hyperkalemia and hyperglycemia on insulin secretion. Surg. Forum 17: 42–45, 1966.
138. MCINTYRE, N., HOLDSWORTH, C. D. AND TURNER, D. S.: A new interpretation of oral glucose tolerance. Lancet 2: 20–21, 1964.
139. MCINTRYE, N., TURNER, D. S. AND HOLDSWORTH, C. D.: Intestinal factors and insulin secretion. Diabetologia 1: 73, 1965.
140. MEBANE, D. AND MADISON, L. L.: Hypoglycemic effect of ketone bodies. J. Clin. Invest. 41: 1383–1384, 1962.
141. MEREU, T. R., KASSOFF, A. AND GOODMAN, A. D.: Diazoxide in the treatment of infantile hypoglycemia. New Engl. J. Med. 275: 1455–1460, 1966.
142. MERIMEE, T. J., BURGESS, J. A. AND RABINOWITZ, D.: Influence of growth hormone on insulin secretion. Studies on growth hormone deficient subjects. Diabetes 16: 478–482, 1967.
143. METZ, R.: The effect of blood glucose concentration on insulin output Diabetes 9: 89–93, 1960.
144. MIALHE, P. AND MEYER, V.: Secretion d'insuline par le pancreas de rat *in vitro*. Compt. Rend. Hebd. Séances Acad. Sci. (Paris). 253: 1861–1863, 1961.
145. MILLER, R. E. AND WHITTENBERGER, J. L.: Effect of vagotomy on venous insulin and glucose concentrations in the conscious monkey. Fed. Proc. 27: 565, 1968.
146. MILNER, R. D. G. AND HALES, C. N.: Role of calcium and magnesium in insulin secretion from rabbit pancreas studied *in vitro*. Diabetologia 3: 47–49, 1967.
146a. MILNER, R. D. G. AND HALES, C. N.: Stimulation by potassium of insulin secretion from rabbit pancreas *in vitro*. Biochem. J. 105: 28, 1967.
147. MILNER, R. D. G. AND HALES, C. N.: Cations and the secretion of insulin. Biochem. Biophs. Acta. 150: 165–167, 1968.
148. MITCHELL, M. L. AND BYRON, J.: Use of enzyme proteolysis for the immuno-chemical measurement of insulin. Diabetes 16: 656–663, 1967.
149. MOLONEY, P. J. AND APRILE, M. A.: On the antigenicity of insulin : flocculation of insulin -antisulin. Can. J. Biochem. Physiol. 37: 793–800, 1959.
150. MOLONEY, P. J. AND COVAL, M.: Antigenicity of insulin: diabetes induced by specific antibodies. Biochem. J. 59: 179–185, 1955.
151. MOLONEY, P. J. AND GOLDSMITH, L.: On the antigenicity of insulin. Can. J. Biochem. Physiol. 35: 79–92, 1956.
152. MONTAGUE, W. AND TAYLOR, K. W.: Insulin secretion and the intracellular concentrations of glucose-6-phosphate and 6-phosphogluconate in isolated islets of Langerhans. Biochem. J. 109: 30–31, 1968.
153. MONTAGUE, W. AND TAYLOR, K. W.: Pentitols and insulin release by isolated rat islets of Langerhans. Biochem. J. 109: 333–339, 1968.
154. MONTAGUE, W. AND TAYLOR, K. W.: Regulation of insulin secretion by short chain fatty acids. Nature (London) 217: 853–855, 1968.
155. MOORE, B., EDIE, E. S. AND ABRAM, J. H.: On the treatment of diabetes mellitus by acid extract of duodenal muscosal membrane. Biochem. J. 1: 28–36, 1906.
156. MORGAN, C. R. AND LAZAROW, A.: Immunoassay of insulin: two antibody system. Plasma insulin levels of normal, subdiabetic and diabetic rats. Diabetes 12: 115–126, 1963.
147. MORTIMORE, G. E. AND TIETZE, F.: Studies on the mechanism of capture and degradation of insulin-I^{131} by the cyclically perfused rat liver. Ann. N.Y. Acad. Sci. 82: 329–337, 1959.
158. MOSKALEWSKI, S.: Isolation and culture of the islets of Langerhans of the guinea pig. Gen. Com. Endocrinol. 5: 342–353, 1965.
159. NELSON, N. C., BLACKARD, W. G., COCCHIARA, J. C. AND LABAT, J. A.: Influence of the vagus nerves on pancreatic insulin secretion. Diabetes 16: 852–857, 1967.
160. ORSKOV, H. AND CHRISTENSEN, N. J.: Disappearance rate of exogenous human insulin. Lancet 2: 701, 1966.
161. OSHAWA, N., KUZUYA, T., TANIOKA, T., KANAZAWA, Y., IBAYASHA, H. AND NAKAO, K.: Effect of administration of ACTH on insulin secretion in dogs. Endocrinology 81: 925–927, 1967.
162. PAULSEN, E. P., RICHENDERFER, L. AND WINICK, P.: Suppression of plasma immunoreactive insulin in rats given D-mannoheptulose. Nature (London) 214: 276–277, 1967.
163. PAULSEN, E. P., RICHENDERFER, L. AND GINSBERG-FELLNER, F.: Plasma glucose, free fatty acids and immunoreactive insulin in sixty-six obese children. Diabetes 17: 261–269, 1968.
164. PELKONEN, R., MIETTINEN, T. A., TASKINEN, M. R. AND NIKKILA, E. A.: Effect of acute elevation of plasma glycerol, triglyceride and FFA levels on glucose utilization and plasma insulin. Diabetes 17: 76–82, 1968.

165. PERLEY, M. AND KIPNIS, D. M.: Effect of glucocorticoids on plasma insulin. New Eng. J. Med. 274: 1237–1240, 1966.
166. PFEIFFER, E. F.: Dynamics of insulin secretion in normal, obese and diabetic subjects following beta cell stimulation. *In* Tolbutamide . . . after ten years, ed. by W. J. H. Butterfield and W. Van Westering, pp. 127–139, Excerpta Medica Foundation, New York, 1967.
167. PFEIFFER, E. F., TELIB, M., AMMON, J., MELANI, F. AND DITSCHUNEIT, H.: Letter to the editor. Diabetologia 1: 131–132, 1965.
168. PI SUNYER, F. X. AND OSTER, S.: Effect of an ovulatory suppressant on glucose tolerance and insulin secretion. Obstet. Gynecol. 31: 482–484, 1968.
169. PORTE, D., JR.: Beta adrenergic stimulation of insulin release in man. Diabetes 16: 150–155, 1967.
170. PORTE, D., JR.: Receptor mechanism for inhibition of insulin release by epinephrine in man. J. Clin. Invest. 46: 86–94, 1967.
171. PORTE, D., GRABER, A. L., KUZUYA, T. AND WILLIAMS, R. H.: The effect of epinephrine on immunoreactive insulin levels in man. J. Clin. Invest. 45: 228–236, 1966.
172. POZZA, G., GALANSINO, G., HOFFIELD, H. AND FOA, P. P.: Stimulation of insulin output by monosaccharides and monosaccahride derivatives. Amer. J. Physiol. 192: 497–500, 1958.
173. RAKIETEN, N., RAKIETEN, M., AND NADKARN, M. V.: Studies of the diabetogenic action of streptozotocin (NSC-37917). Cancer Chemother. Rep. 29: 91–98, 1963.
174. R-CANDELA, J. L., R-CANDELA, R., MARTIN-HERNANDEZ, D. AND CORTAZAR, T. C.: Beta-hydroxybutyrate sodium citrate as stimuli of the *in vitro* secretion of insulin. Nature (London) 195: 711–712, 1962.
175. R-CANDELA, J. L., R-CANDELA, R., MARTIN-HENRNANDEZ, D. AND T. CASTILLA-CORTAZAR, T.: Insulin secretion *in vitro*. *In* Perspectives in Biology, ed. by C. F. Cori. *et al* Elsevier Publishing Co., Amsterdam, 1965.
176. R-CANDELA, J. L., CASTRILLON, A. M., MARTIN-HERNANDEZ, D. AND CASTILLA-CORTAZAR, T: Citrate stimulation of insulin secretion from the pancreas of the duck. Med. Exp. (Basel) 11: 47–51, 1964.
177. ROJAS, J., MENESES, P. AND R-CANDELA, J. L.: Effect of glucose on insulin secretion *in vitro* by rabbit pancreas pre-incubated with beta-hydroxy-butyrate. Nature (London) 203: 758, 1964.
178. ROTH, D. A. AND MEADE, R. C.: Hyperinsulinism-hypoglycemia in the postgastrectomy patient. Diabetes 14: 526–528, 1965.
179. SAMOLS, E. AND MARKS, V.: Evaluation of the intravenous tolbutamide test in the recognition and differential diagnosis of spontaneous hypoglycemia. *In* Tolbutamide . . . after ten years, ed. by W. J. H. Butterfield, and W. Van Westering, pp. 147–156, Excerpta Medica Foundation, New York, 1967.
180. SAMOLS, E. AND MARKS, V.: Insulin assay in insulinomas. Brit. Med. J. 1: 507–510, 1963.
181. SAMOLS, E. AND MARKS, V.: Disappearance rate of endogenous insulin in man. Lancet 2: 700, 1966.
182. SAMOLS, E. AND MARKS, V.: The treatment of hypoglycemia with diazoxide. Proc. Roy. Soc. Med. 59- 811–814, 1966.
183. SAMOLS, E. G., MARRI, G. AND MARKS, V.: Inter-relationship of glucagon, insulin and glucose. Insulinogenic effect of glucagon. Diabetes 15: 855–866, 1966.
184. SAMOLS, E., MARRI, G. AND MARKS, V.: Promotion of insulin secretion by glucagon. Lancet 2: 415–416, 1965.
185. SAMOLS, E. AND RYDER, J. A.: Studies on tissue uptake of insulin in man using a differential immunoassay for endogenous and exogenous insulin. J. Clin. Invest. 40: 2092–2102, 1961.
186. SANBAR, S. S. AND MARTIN, J. M.: Stimulation by octanoate of insulin release from isolated rat pancreas. Metabolism 16: 482–484, 1967.
187. SCHALCH, D. S.: The influence of physical stress and exercise on growth hormone and insulin secretion in man. J. Lab. Clin. Med. 69: 256–269, 1967.
188. SELTZER, H. S.: Quantitative effects of glucose, sulfonylureas, salicylate and indole-3-acetic acid on the secretion of insulin activity into pancreatic venous blood. J. Clin. Invest. 41: 289–300, 1962.
189. SELTZER, H. S. AND ALLEN, E. W.: Inhibition of insulin secretion in "diazoxide diabetes." Diabetes 14: 439, 1965.
190. SELTZER, H. S. AND HARRIS, V. L.: Exhaustion of insulogenic reserve in maturity onset diabetic patients during prolonged and continuous hyperglycemic stress. Diabetes 13: 6–13, 1964.
191. SHAMBAUGH, G. E. AND BEISEL, W. R.: Insulin response during tularemia in man. Diabetes 16: 369–376, 1967
192. SIMON, E. AND KRACIER, P. F.: The blockade of insulin secretion by mannoheptulose. Israel J. Med. Sci. 2: 785-800, 1966.
193. SMITH, H. M.: Diazoxide and the treatment of hypoglycemia. Ann. N.Y. Acad. Sci. 150: 191–467, 1968.
194. SPELLACY, W. N., BUHI, W. C., SPELLACY, C. E., MOSES, L. C. AND GOLDZIEHER, J. W.: Carbohydrate studies in long term users of oral contraceptives. Diabetes 17: 344–345, 1968.
195. SPELLACY, W. N., CARLSON, K. L. AND BIRK, S. A.: Carbohydrate metabolic studies after six cycles of combined type oral contraceptive tablets. Diabetes 16: 590–594, 1967.
196. SPELLACY, W. N., CARLSON, K. L. AND SCHAHR, S. L.: Effect of a sequential oral contraceptive on plasma insulin and blood glucose levels after six months treatment. Amer. J. Obstet. Gynecol. 101: 672–676, 1968.
197. SPIRO, R. G. AND HASTINGS, A. B.: Studies on carbohydrate metabolism in rat liver slices. XI. Effect of prolonged insulin administration to the alloxan-diabetic animal. J. Biol. Chem. 230: 751–759, 1958.
198. STARUP, J., DATE, J. AND DECKERT, T.: Serum insulin and intravenous glucose tolerance in oral contraception. Acta Endocrinol. (Copenhagen) 58: 537–544, 1968.
199. STEINKE, J., SOELDNER, J. S. AND RENOLD, A. E.: Measurement of small quantities of insulin-like activity with rat adipose tissue. IV. Serum insulin like activity and tumor insulin content in patients with functioning islet cell tumors. J. Clin. Invest. 42: 1322–1329, 1963.

200. STRAUTZ, R. L.: Islet implants: Reduction of glucose levels in the hereditary obese mouse. Endocrinology 83: 975-978, 1968.
201. SUSSMAN, K. E., STJERNHOLM, M. AND VAUGHN, G. D.: Tolbutamide and its effect upon insulin secretion in the isolated perfused rat pancreas. *In* Tolbutamide . . . after ten years, ed. by. W. J. H. Butterfield and W. Van Westering, pp. 22-33, Excerpta Medica Foundation, New York, 1967.
202. SUSSMANN, K. E. AND VAUGHAN, G. D.: Insulin release after ACTH, glucagon, and adenosine-3'5'-phosphate (cyclic AMP) in the perfused isolated rat pancreas. Diabetes 16: 449-454, 1967.
203. SUSSMAN, K. E., VAUGHAN, G. D. AND TIMMER, R. F.: An *in vitro* method for studying insulin secretion in the perfused isolated rat's pancreas. Metabolism 15: 466-476, 1966.
204. TÄLJEDAL, I-B.: Electrophoretic separation of enzymes from mammalian pancreatic islets. Med. Pharmacol. Exp. 15: 603-608, 1966.
205. TÄLJEDAL, I. B.: Electrophoretic studies on phosphatases from the pancreatic islets of obese hyperglycemic mice. Acta Endocrinol. (Copenhagen) 55: 153-162, 1967.
206. TÄLJEDAL, J. S.: Stimulation of 5'-nucleotidase activity in the pancreatic islets of rats by glucagon. Endocrinology 82: 173-178, 1968.
207. TASHIMA, L. AND CAHILL, G. F. JR.: Effect of insulin in the toad fish, *Opsanus tau*. Gen. Comp. Endocrinol. 11: 262-271, 1968.
208. TURNER, D. S. AND MCINTYRE, N.: Stimulation by glucagon of insulin release from rabbit pancreas *in vitro*. Lancet 1: 351-352, 1966.
209. TURTLE, J. R. AND KIPNIS, D. M.: Stimulation of insulin secretion by theophylline. Nature (London) 213: 727-730, 1967.
210. TURTLE, J. R. AND KIPNIS, D. M.: An adrenergic receptor mechanism for the control of cyclic 3'5' adenosine monophosphate synthesis in tissues. Biochem. Biophys. Res. Commun. 28: 797-802, 1967.
211. UNGER, R. H., KETTERER, H., DUPRÉ, J. AND EISENTRAUT, A. M.: The effects of secretin, pancreozymin and gastrin on insulin and glucagon secretion in anesthetized dogs. J. Clin. Invest. 46: 630-645, 1967.
212. UNGER, R. H. AND MADISON, L. L.: A new diagnostic procedure for mild diabetes mellitus. Evaluation of an intravenous tolbutamide response test. Diabetes 7: 455-461, 1958.
213. VALLANCE-OWEN, J. AND WRIGHT, P. H.: Assay of insulin in blood. Physiol. Rev. 40: 219-244, 1960.
214. VANCE, J. E., BUCHANAN, K. D., CHALLONER, D. R. AND WILLIAMS, R. H.: Effect of glucose concentration on insulin and glucagon release from isolated islets of Langerhans of the rat. Diabetes 17: 187-193, 1968.
215. VECCHIO, D. AND GONET, A. F.: Culture d'organe de pancreas foetal de rat: I. Effects du glucose, d'autres composants du milieu de culture, et d'un sulfamide hypoglycemiant. Helv. Physiol. Pharmacol. Acta 25: 103-122, 1967.
216. VECCHIO, D., LUYCKX, A. AND RENOLD, A. E.: Culture d'organe de pancreas foetal de rat: II. Effects du glucose, d'un sulfamide hypoglycemiant et du glucagon sur la liberation de l'insuline. Helv. Physiol. Pharmacol. Acta 25: 134-146, 1967.
217. VECCHIO D., LUYCKX, A., ZAHND, G. R. AND RENOLD, A. E.: Insulin release induced by glucagon in organ cultures of the fetal rat pancreas. Metabolism 15: 577-581, 1966.
218. WATKINS, D., COOPERSTEIN, S. J., DIXIT, P. K. AND LAZAROW, A.: Insulin secretion from toadfish islet tissue stimulated by pyridine nucleotides. Science 162: 283-286, 1968.
219. WHIPPLE, A. O.: Islet cell tumors of the pancreas. Can. Med. Ass. J. 66: 334-342, 1952.
220. WILKINS, R. W.: New drugs for the treatment of hypertension. Ann. Int. Med. 50: 1-10, 1959.
221. WILLIAMSON, J. R., LACY, P. E. AND GRISHAM, J. W.: Ultrastructural changes in islets of the rat produced by tolbutamide. Diabetes 10: 460-469, 1961.
222. WRIGHT, P. H.: Experimental diabetes induced by insulin antibodies. *In* On the Nature and Treatment of Diabetes, ed. by G. A. Wrenshall and B. S. Leibel, pp. 354-360, Excerpta Medica Foundation, New York, 1965.
223. WRIGHT, P. H., KREISBERG, R. A., HALPERN, B. AND DOLKART, R. E.: Properties of insulin antibodies produced by the guinea pig, horse, sheep and man. Diabetes 11: 519-526, 1962.
224. WRIGHT, P. H., MAKULU, D. R., MALAISSE, W. J., ROBERTS, M. N. AND YU, P. Y.: A method for the immunoassay of insulin. Diabetes 17: 537-546, 1968.
225. WRIGHT, P. H., MAKULU, D. R. AND POSEY, I. J.: Guinea pig anti-insulin serum; adjuvant effect of *H. pertussis* vaccine. Diabetes 17: 513-516, 1968.
226. WRIGHT, P. H. AND MALAISSE,W. J.: Effects of epinephrine, stress and exercise upon insulin secretion by the rat. Amer. J. Physiol. 214: 1031-1034, 1968.
227. WRIGHT, P. H., RIVERA-CALIMLIM, L. AND MALAISSE, W. J.: Endogenous insulin secretion in the rat following injection of anti-insulin serum. Amer. J. Physiol. 211: 1089-1094, 1966.
228. YALOW, R. S. AND BERSON, S. A.: Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. 39: 1157-1175, 1960.
229. YALOW, R. S., BLACK, H., VILLAZON, M. AND BERSON, S. A.: Comparison of the plasma insulin levels following administration of tolbutamide and glucose. Diabetes 9: 356-362, 1960.
230. YOUNG, F. G.: Growth and diabetes in normal animals treated with pituitary (anterior lobe) diabetogenic extract. Biochem. J. 39: 515-541, 1945.
231. YOUNG, F. G.: Growth hormone and experimental diabetes. J. Clin. Endocrinol. 11: 497-581, 1951
232. ZAHARKO, D. S. AND BECK, L. V.: Studies of a simplified plasma insulin immunoassay using cellulose powder Diabetes 17: 444-457, 1968.