ences/The Chicago Medical School; 20 February.

Lloyd Lowder, 52; professor of education, Pfeiffer College; 7 January.

Theodore K. Matthes, 41; associate professor of mathematics, University of Oregon; 10 February.

Kenneth Melville, 72; former chairman, pharmacology department, McGill University; 29 January.

Bethel S. Pickett, 92; former chairman, horticulture department, Iowa State University; 25 January.

W. Conway Pierce, 79; professor emeritus of chemistry, University of California, Riverside; 23 December 1974.

Leonard Reissman, 53; chairman, sociology department, Cornell University; 29 January.

Robert Robinson, 88; former professor of chemistry, Oxford University; 9 February.

Caroline B. Rose, 61; professor of sociology, University of Minnesota; 25 March.

R. Norris Shreve, 89, professor emeritus of chemistry, Purdue University; 17 February.

Nelson T. Spratt, Jr., 63; former chairman, zoology department, University of Minnesota; 16 February.

Katherine K. Stefic, 72; former professor of psychology, Catholic University; 21 January.

RESEARCH NEWS

Diabetes (III): New Hormones Promise More Effective Therapy

The traditional view of diabetes, and a view still held by most laymen, is that the disease is a rather simple metabolic disturbance resulting from impaired insulin production alone. This view resulted from two principal sets of experiments: the demonstration in 1889 by Joseph von Mering and Oscar Minkowski of the Halle Medical Polyclinic in Germany that removal of the pancreas produces diabetic abnormalities of glucose metabolism; and the 1922 demonstration by Frederick G. Banting and Charles H. Best of the University of Ontario that administration of insulin (in the form of a crude pancreas extract) could correct these abnormalities.

Subsequently most therapy of diabetes was based on the observations of Banting and Best. But even though the use of insulin has greatly increased the life-span of many diabetics, diabetes is still a major cause of death in the United States and its side effects have crippled many individuals. As clinical and research experience became more extensive, it became obvious that some factor in addition to impairment of insulin action is operative in diabetes; this has prompted increasing interest in the mechanism of the diabetes syndrome.

The prime focus of investigation in the molecular biology of diabetes during the last decade has been on glucagon, another hormone produced by the pancreas. Work by many different investigators has led to the conclusion, only now beginning to be accepted by a majority of scientists, that glucagon is as important a factor in diabetes as insulin. But whereas insulin is normally deficient in diabetes, glucagon is normally present in excess. This fact has made it quite difficult to study the role of glucagon, for while it is easy to add extra quantities of a hormone such as insulin, it has proved quite difficult to lower the concentration of glucagon.

It was thus a major breakthrough 2 years ago when several investigators discovered that another recently isolated hormone, somatostatin, could suppress the release of both insulin and glucagon. For the first time, then, it became possible to vary the concentrations of insulin and glucagon independently to investigate their effects. It quickly became apparent that glucagon plays a crucial role in the pathology of diabetes.

Glucagon was discovered in 1923 by John R. Murlin and C. P. Kimball of the University of Rochester; but as recently as 1969 most investigators thought that it played only an insignificant role in the regulation of glucose metabolism. It is a linear oligopeptide composed of 29 amino acid residues in the sequence shown in Fig. 1. Glucagon from all mammalian species thus far examined has this same primary structure; the only exception so far known is glucagon from turkeys, which differs by one amino acid residue.

Many investigators have shown that the primary site of release of glucagon is the alpha cells from the islets of Langerhans in the pancreas. Last year, however, Mladen Vranic of the University of Toronto and Sumer Pek of the University of Michigan reported a persistent, glucagon-like immunoreactivity in the blood of dogs that had been depancreatized. This observation has been confirmed by other investigators, including Tatsuo Matsuyama and Piero Foa of Wayne State University, James B. Field and his associates at the University of Pittsburgh, and Andrew V. Schally and his colleagues at the Tulane University School of Medicine.

Earlier this year, Roger H. Unger and his associates at the University of Texas Southwestern Medical School demonstrated that a substance which is immunologically, biologically, and physicochemically identical to glucagon is also released by tissues in the stomach and upper intestine of dogs. Lelio Orci of the University of Geneva in Switzerland has shown that these tissues contain cells that are morphologically identical to alpha cells of the pancreas.

But some apparently conflicting evidence was recently presented by W. A. Muller and his associates at the Elliott P. Joslin Research Laboratory in Boston. They showed that infusions of arginine, which normally produce an increase in the concentration of glucagon in the blood, produce no effect in humans in whom the pancreas was removed. This suggests that human stomachs do not produce glucagon. Vranic and Pek have shown, however, that release of glucagon by the pancreas and the stomach are subject to different controls. And Unger has shown that administration of insulin to depancreatized dogs will block the secretion of glucagon by the stomach (Muller's patients apparently received insulin). The situation in humans must thus be considered to be unresolved.

Three different lines of evidence, according to Unger and Orci, support the conclusion that glucagon has a role in the pathology of human diabetes: (i) An increase in the concentration of glucagon (hyperglucagonemia) has been observed in association with every type of increase in the concentration of sugar in the blood (hyperglycemia) of animals and humans. (ii) When the secretion of both glucagon and insulin are suppressed, hyperglycemia is not observed unless the concentration of glucagon is restored to normal by the concomitant administration of glucagon. (iii) The somatostatin-induced suppression of glucagon release in diabetic animals and humans restores blood sugar concentrations to normal and alleviates certain other symptoms of diabetes.

Glucagon and insulin appear to coexist in a delicate balance; high concentrations of insulin suppress glucagon release and low concentrations stimulate glucagon release. The latter situation has been particularly well documented. Unger and his associates have shown that hyperglucagonemia occurs in conjunction with insulin deficiency resulting from a variety of causes, including diabetes in man, chemically induced diabetes in dogs and rats, and diabetes induced in rats by injection of antibodies to insulin. Similar observations have been reported by K. D. Buchanan of the Queen's University of Belfast and A. M. McCarroll of Belfast City Hospital in Ireland; E. F. Pfeiffer and his associates at the University of Ulm in West Germany; Ellis Samols and his colleagues at the Medical College of Georgia; and John E. Gerich and his co-workers at the University of California Medical School in San Francisco. Unger has also observed hyperglucagonemia in association with the stress hyperglycemia caused by severe injuries, burns, certain types of heart attacks, and hemorrhagic shock. And Vranic and Pek, Matsuyama and Foa, and Field have shown that hyperglucagonemia is present in dogs that have been made diabetic by removal of the pancreas.

An increased concentration of glucagon accompanying hyperglycemia might, of course, be effect rather than cause. But there was no good way to examine this problem adequately until the discovery of somatostatin. Somatostatin, also called somatotropin-release inhibiting factor, was first isolated from sheep brains about 3 years ago by Paul Brazeau, Wylie Vale, Roger Guillemin, and their associates at the Salk Institute. As the name implies, the effect of somatostatin that was first observed was inhibition of the release of somatotropin (also called growth hormone) by the pituitary gland. It is produced by the hypothalamus.

Somatostatin is an oligopeptide containing 14 amino acid residues. Its structure (Fig. 2) was quickly deduced by Roger Burgus and Nicholas Ling of the Salk Institute, and they and Jean Rivier of the same institution synthesized relatively large quantities of the hormone and made it available to other investigators. Samuel S. C. Yen of the University of California School of Medicine in San Diego then showed that administration of somatostatin sharply reduces the amount of somatotropin that circulates in the blood of individuals afflicted with a form of gigantism known as acromegaly. It thus seems likely that somatostatin might be useful in the treatment of this hormonal disorder. Somatostatin has also been shown by Yen's and Guillemin's groups to inhibit the

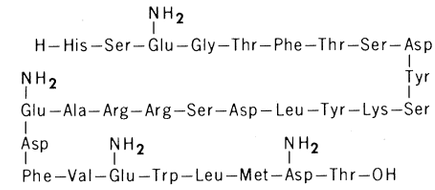

Fig. 1. The amino acid sequence of glucagon as determined in 1955 by Otto Behrens and A. Staub of Eli Lilly & Company, Indianapolis.

increased secretion of thyrotropin caused by thyrotropin-releasing hormone and, in certain circumstances, to inhibit the secretion of prolactin.

Extending these observations, John W. Ensinck, Charles J. Goodner, and their associates at the University of Washington School of Medicine noticed that the concentration of glucose in baboons' blood fell during the administration of somatostatin. They then discovered that, with appropriate doses of somatostatin, secretion of both insulin and glucagon could be completely and quickly inhibited. The inhibition of secretion of these hormones, they observed, is followed by a more gradual reduction in the concentration of glucose in baboon blood. The same effect was also observed in dogs.

The site of the action of somatostatin is the pancreas. Unger, Schally, and K. Lundbaek and J. Iverson of the Kommunehospitalet in Århus, Sweden, have each shown that infusion of somatostatin directly into isolated animal pancreases inhibits the release of either glucagon or insulin; Gerich has shown that it inhibits both. Gordon Weir of the Joslin Clinic has shown that somatostatin is present in the islets of Langerhans. But Suad Efendic and his associates at the Karolinska Hospitalet in Stockholm have reported that somatostatin does not inhibit secretion of insulin by islets isolated from the pancreas of rats. They suggest, however, that the islets might have been damaged by the enzyme collagenase during the isolation procedure. This possibility is supported by recent experiments of Ensinck in which he did observe suppression of hormone release by somatostatin in isolated islets.

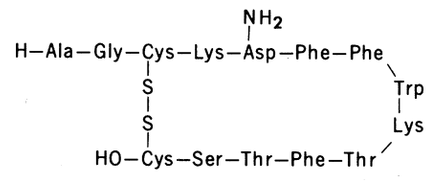

Fig. 2. The amino acid sequence of somatostatin as determined by Roger Burgus and Nicholas Ling of the Salk Institute. Pictured is the cyclic form; the linear form has the disulfide bridge reduced to two sulfhydryl groups.

The intriguing aspect of these observations is that suppression of insulin release does not produce hyperglycemia if it is accompanied by suppression of glucagon release. Unger has thus shown, for example, that a depancreatized dog does not develop hyperglycemia so long as it is maintained on somatostatin, although these experiments have been carried out for only a few hours. Ensinck and Goodner, Unger, Gerich, and G. M. Besser of St. Bartholomew's Hospital in London, and Reginald Hall of the Royal Victoria Infirmary in Newcastle upon Tyne, England, have shown, however, that hyperglycemia does develop in both depancreatized and healthy animals maintained on somatostatin if glucagon concentrations are restored to normal by infusion of glucagon in conjunction with somatostatin. If the glucagon infusion is halted while that of somatostatin continues, hyperglycemia disappears rapidly.

The rapid appearance of hyperglycemia accompanying the infusion of somatostatin and glucagon, Unger has shown, cannot be prevented by the simultaneous infusion of insulin. This result is in accord with earlier experiments by Vranic and by Paul W. Lacy of the Washington University School of Medicine; they infused glucagon (in the absence of somatostatin) into laboratory animals and observed a hyperglycemia that could not be abolished by insulin. These findings thus suggest that glucagon may be even more important than insulin in the maintenance of normal concentrations of glucose in the blood.

Meanwhile, in the experimental treatment of patients with acromegaly, Yen and Besser and Hall observed that administration of somatostatin reduces the concentration of insulin and of glucose in the blood. Subsequently, those two groups and Gerich demonstrated that somatostatin reduces the concentrations of glucagon, insulin, and glucose in the blood of healthy individuals, suggesting that the effects of somatostatin are much the same in humans as they are in experimental animals. All these results suggested that reduction of glucagon concentrations in the blood with somatostatin would alleviate the symptoms of diabetes, and the three groups of investigators have shown that to be the case.

Besser and Hall administered relatively low concentrations of somatostatin (78 micrograms per hour) to two insulin-independent diabetics and found a delay in the onset of the sharp increase in blood glucose concentrations that was characteristic of the patients' response to oral administration of glucose in tolerance tests. The somatostatin reduced the amount of glucagon in the patients' blood, but only de-

layed the increase in blood insulin that normally followed the glucose tolerance test.

They also infused the same low concentration of somatostatin into one insulin-dependent and one insulin-independent diabetic over a period of 28 hours. The release of glucagon was suppressed in both individuals throughout the infusion, but the secretion of insulin in the insulin-independent diabetic was only delayed after meals or after administration of oral glucose during the tolerance test. They further observed that the glucose tolerance of the insulin-independent diabetic—who was taking an oral antidiabetic agent—was markedly improved and that the insulin-dependent diabetic required much less insulin.

Yen and his associates gave larger amounts of somatostatin (100 micrograms initially plus 150 micrograms per hour) to three insulin-independent and two insulin-dependent diabetics. In the three insulin-independent diabetics, Yen observed a sustained 50 percent drop in the concentration of glucagon in the blood. The somatostatin also produced a 50 percent reduction in insulin concentrations and abolished the insulin increase normally seen in the patients in response to an oral glucose tolerance test or to a normal breakfast.

In the insulin-dependent diabetics (who were given insulin along with somatostatin), Yen observed no change in the blood glucagon concentration in one and only a modest decrease in the second. But the somatostatin abolished the hyperglycemia that normally followed breakfast or glucose tolerance tests in both patients; furthermore, it produced a gradual decline in blood glucose in one patient and a sharp decrease in the second. Yen thus suggests that there may be some glucoregulatory factor in addition to glucagon and insulin that is affected by somatostatin.

Gerich and his associates infused even higher concentrations of somatostatin (500 micrograms per hour) into ten insulin-dependent diabetics. They observed that the infusions reduced blood glucagon concentrations by an average of about 50 percent and blood glucose concentrations (during fasting) by about 25 percent. Additional tests in four of the patients showed that infusion of insulin and the same amount of somatostatin completely abolished hyperglycemia after a normal breakfast, and that the combination of the two hormones was more effective than insulin alone.

The Gerich group also assessed the effects of a normal breakfast on 12 insulin-dependent diabetics and 12 healthy controls. They found that the increase in blood glucagon concentrations after the breakfast was two to three times as great among the diabetics as among the controls. This enhanced glucagon response was not affected by administration of insulin. Infusion of somatostatin (500 micrograms per hour), however, abolished the glucagon response and diminished hyperglycemia after the meal by 60 percent. A combination of somatostatin and insulin produced a progressive decline in the concentrations of blood glucose, even after the meal.

All of these findings, taken together, strongly indicate that the presence of a relative or absolute excess of glucagon is an essential factor in the development of diabetes. The most reasonable hypothesis, Unger and Orci argue, is that the major role of insulin is regulation of the transfer of glucose from the blood to storage in insulin-responsive tissues such as liver, fat, and muscle. The role of glucagon, in contrast, is regulation of the liver-mediated mobilization of stored glucose so that the glucose can be used by vital tissues such as the brain in times of stress, including starvation. The principal consequence of insufficient quantities of insulin in the blood would thus be a reduced rate of removal of glucose from the blood, which would be manifested as hyperglycemia after meals. The principal consequence of high concen-

Somatostatin: The Search for Analogs

While research on somatostatin promises great advances in the therapy of diabetes, it is unlikely that somatostatin itself will find much use. The problems that this oligopeptide presents are probably too severe for any widespread use to develop. One problem is that somatostatin can generally be given only by infusion. If given orally, the oligopeptide is degraded before it can ever reach the bloodstream. If given by injection, it is degraded so rapidly by various enzymes in the blood and the liver that its effects persist only for minutes.

Perhaps even more serious is the fact that somatostatin suppresses the release of other hormones in addition to glucagon and insulin; the most crucial of these is growth hormone. It might thus not be advisable to use somatostatin in the treatment of youthful diabetics, even though these individuals might benefit the most from such therapy.

Investigators are thus searching for derivatives or analogs of somatostatin that might overcome these problems. Most of this search takes place at the Salk Institute, where Roger Guillemin, Wylie Vale, and their colleagues are investigating several different ways of modifying somatostatin activity. The goals of this research are:

- ▶To prolong the activity of somatostatin.
- ▶To dissociate its effect on insulin and glucagon from that on growth hormone and other hormones.
- ▶To find more potent forms of somatostatin.
- ▶To find antagonists of somatostatin activity.

The research has not progressed far, primarily because the effects of somatostatin have been recognized for only a short time. The investigators are taking several different approaches. They have shown, for example, that depot preparations in which the oligopeptide is bound to larger molecules will prolong its activity. Thus, Guillemin, Vale, Jean Rivier, and Paul Brazeau have demonstrated that a complex of somatostatin, zinc, and protamine (a protein), given by injection, will release somatostatin into the blood for as long as 6 hours in humans. They are also studying mechanisms by which the oligopeptide is degraded in the blood, with the goal of locating weak structural points which possibly can be strengthened.

But these approaches, in effect, are only stop-gap, since they do not provide any dissociation of activities. The investigators have thus begun the straightforward, albeit exceptionally tedious, process of systematically varying the composition of the oligopeptide. It was through this type of procedure, for example, that other investigators found separate polypeptides with vasopressin and oxytocin activities. Vale, Rivier, and Marvin Brown have already completed the alanine series, in which each of the 14 amino acid residues in somatostatin is replaced with alanine. None of these newly synthesized oligopeptides show any improved properties, however, so the team is proceeding with further substitutions.—T.H.M.

trations of glucagon would be the liver-mediated release into the bloodstream of inappropriately high concentrations of glucose, thereby producing a persistent hyperglycemia.

Glucagon also appears to regulate the oxidation of fatty acids in the liver and thus may be involved in the most severe short-term effect of diabetes—ketoacidosis. Ketoacidosis is the increased production of so-called ketone bodies, such as β-hydroxybutyric acid; release of these ketone bodies into the blood lowers its *p*H, inducing coma and, if the condition is not corrected, death. K. G. M. M. Alberti and his associates at the University of Southampton in England have shown that there is a definite correlation between the concentrations of glucagon and ketone bodies in patients with ketoacidosis.

Gerich and his associates have shown that withdrawal of insulin from insulin-dependent diabetics produces increases in the concentrations of many compounds associated with ketoacidosis—such as β-hydroxybutyric acid, free fatty acids, and glycerol—that can be correlated with the increase in the concentration of glucagon in the blood. At the University of Texas Southwestern Medical School, J. Denis McGarry and his associates have demonstrated that administration of glucagon to rats in the absence of food alters the metabolism of the rat livers so that the livers produce products characteristic of ketoacidosis. Ketoacidosis itself does not appear, however, because of the counterbalancing effects of insulin stimulated by the glucagon.

Conventional therapy for ketoacidosis is the administration of insulin. It now appears that the primary effect of the insulin may be suppression of the release of glucagon. Since insulin can mediate only a limited reduction in the concentration of glucagon, it seems likely that a more effective therapy would be administration of somatostatin. Gerich has recently demonstrated, in fact, that infusion of somatostatin prevents the development of ketoacidosis in insulin-dependent diabetics deprived of insulin.

Despite the remarkable preliminary successes with somatostatin in the therapy of diabetes, a great many problems must be resolved before there can be any substantial use of the new hormone. Perhaps the most difficult problem now is that somatostatin can be given only by infusion because its effects are so short-lived (see box). Investigators are now searching for analogs of the hormone that will persist longer in the bloodstream.

A severe problem for the long term is that somatostatin therapy might be inappropriate for youthful diabetics because it also inhibits the release of growth hormone. Many investigators are confident, however, that the development of somatostatin analogs will provide agents that are more selective in their action. It is possible also that somatostatin could be given to such individuals only in conjunction with meals; the agent would thus not interfere with growth hormone, which is released primarily during sleep.

A perhaps less important problem is that very few investigators can explore therapy in humans with somatostatin. Only Roger Guillemin has an Investigational New Drug permit from the Food and Drug Administration for use of somatostatin in humans. The practical consequence is that experimental therapy of humans with the hormone can be conducted only by the small group of investigators who work with him or by investigators in certain foreign countries.

And finally, many investigators have been concerned by reports by Donna J. Koerker of the University of Washington Medical School, who has suggested the possibility of an association between somatostatin administration and the deaths of several baboons. Koerker observed an unusual frequency of pulmonary and hepatic hemorrhaging in baboons that had been given frequent injections of the hormone. Subsequent experiments showed that somatostatin exerts a transient effect on the aggregation of platelets (which are responsible for clotting) comparable to that of aspirin. Koerker, furthermore, suggests that the effect is probably dose-related. The doses of somatostatin given to the baboons were about ten times as great as the highest dosages used by Gerich in humans, and Gerich has not been able to demonstrate the effect on platelets at the lower doses. Nonetheless, the possibility remains that arbitrary and empiric doses of somatostatin may affect nonendocrine cells.

Thus many years may pass before the results of this research will be used in human therapy. But it seems safe to predict that these developments will eventually result in a sharply improved therapy of diabetes and a reduction in the side effects that accompany the disease.

—THOMAS H. MAUGH II

Foundations of Mathematics: Ties to Infinite Games

Game theory has become increasingly important to the study of the foundations of mathematics. Strategies for winning one group of games, in particular, have been subject to intensive investigation by logicians. These games, as the logicians soon realized, are intimately connected to the structure of a collection of sets that are crucial to almost all theories in pure and applied mathematics. Exactly how the games and those sets are related was recently demonstrated by a proof that represents the first result in ordinary mathematics that requires the full power of set theory.

The games that have come to be so important to mathematicians were first introduced in 1953 by David Gale and F. M. Stewart, who were then at Brown University. Gale and Stewart analyzed these games in order to better understand other games rather than to study the foundations of mathematics. However, they discovered a connection between strategies necessary for winning these games and one of the axioms of set theory. This connection caught the attention of logicians who realized that it could lead to major results about the structure of sets.

The games introduced by Gale and Stewart are infinite games: that is, each of these games does not end until the players have made an infinite number of moves. Such a game is played by two participants who alternately pick the numbers 0 or 1 and record their choices at each turn. The game ends when the players have recorded an infinite sequence of 0's and 1's. The first player wins the game if this infinite sequence belongs to a collection of infinite sequences, called the payoff set, that was chosen before the game began. The second player wins if the infinite sequence does not belong to the payoff set. For example, the payoff set could consist of all those sequences produced when a 1 is recorded for the 1st move, 5th move, 7th move, 11th move, and so on. The first player could win a game that has this payoff set since he chooses all of the odd-numbered terms of the infinite sequence. Gale and Stewart then asked: Is every possible payoff set associated with a winning strategy for player 1 or player 2?