get accurately for areas smaller than a whole state. Since LACIE encompasses many states and is to run for at least another 2 years, its forecasts can be carefully scrutinized for accuracy. If the experiment is successful, operational systems could come quickly. Landsat III, to be launched in 1978, will have a fifth multispectral channel, and NASA planners are already thinking about a new spacecraft and advanced scanners with more channels and greater resolution. The prospects are thus substantial that by the end of the decade the technological means to manage a global food watch, if not the wisdom, will be available.

—ALLEN L. HAMMOND

Diabetes (II): Model Systems Indicate Viruses a Cause

Some epidemiological and genetic evidence suggests that viruses may be instrumental in causing at least one type of diabetes in humans. That evidence, which was discussed in a previous article, does not prove the hypothesis, however, and it does not point to any one virus as the culprit. Much more convincing evidence, many investigators believe, is the demonstration that viruses can produce a diabetes-like disease in laboratory animals. The model systems produced in this fashion are not completely satisfactory because they do not reproduce many of the complications observed in human diabetics. Nevertheless, they do appear to provide a highly useful new way to study the initiation of diabetes, the progression of the disease, and the genetics of susceptibility.

The possibility that viruses may be linked to diabetes has for a long time intrigued investigators. Their interest was aroused by the common observation that many viruses replicate in the pancreas. Several viruses produce pancreatitis, that is, inflammation of the pancreas, and some occasionally produce more lasting damage. But until a few years ago, there was no evidence that such infections resulted in any symptoms of diabetes.

In the early 1960's, two Italian investigators, E. Barboni and I. Manocchio, reported that some cattle developed hyperglycemia (high concentrations of sugar in the blood) and lesions of the pancreas after they had been infected with foot-and-mouth disease virus, a small, RNA-containing virus belonging to the family known as picornaviruses. Their experiments were performed on only a few animals, however, and apparently no other investigators attempted to reproduce them, so the significance of their observation was not noticed.

A major breakthrough occurred a few years later when John E. Craighead of the University of Vermont discovered that a diabetes-like disease could be produced in certain strains of mice by encephalomyocarditis (EMC) virus, a picornavirus first isolated from the heart of a domestic pig dying of myocarditis (an inflammation of the muscular walls of the heart) in the Republic of Panama. Craighead's work was subsequently confirmed and extended by Abner L. Notkins and his associates at the National Institute of Dental Research.

The EMC virus, like many other picornaviruses, replicates primarily in the myocardium, central nervous system, lacrimal and parotid glands, and the pancreas of experimental animals. In the pancreas, many picornaviruses replicate in and often damage the acinar cells (see box), but the infection only rarely produces ill effects in the animals. But Craighead discovered one strain of EMC virus, known as the M variant, that replicates (in the pancreas) exclusively in the islets of Langerhans, the insulin-secreting cells of the pancreas. No other known virus, Craighead says, demonstrates this exclusivity.

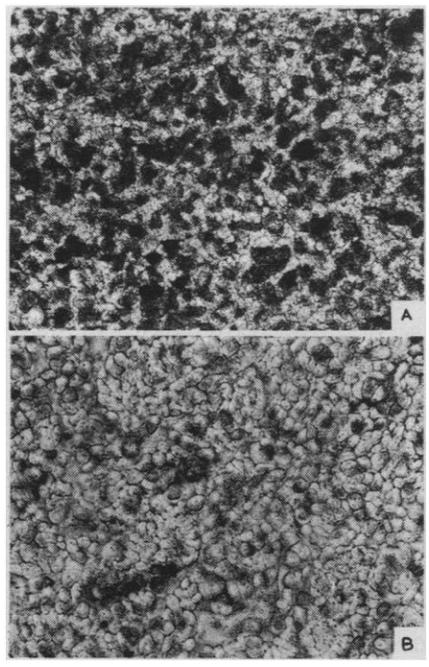

Fig. 1. Purified beta cells from two strains of mice infected with EMC virus. Beta cells from C57 black male mice (A) contain normal numbers of insulin-secreting granules, while those from SWR male mice (B) are largely degranulated. [Source: Abner L. Notkins and Ji Won Yoon, National Institute of Dental Research]

In certain strains of adult male mice, the M variant of EMC virus produces a transient infection in which virus can be recovered from the pancreas for as many as 18 days after inoculation. Notkins has shown by immunofluorescence techniques that viral antigens are found exclusively in the beta cells of the islets. The infection produces a spectrum of effects, ranging from mild or no alterations in glucose metabolism through transient hyperglycemia to a persistent diabetes-like syndrome. Symptoms of this syndrome include persistent hyperglycemia, glucose in the urine (glucosuria), excessive thirst and appetite, and decreased production of insulin—which are the usual criteria for defining diabetes. The more severe symptoms, Craighead says, are analogous to the juvenile or acute-onset form of human diabetes, while the milder symptoms are more like the maturity-onset form. Craighead has shown that the infection produces kidney lesions that are similar to those appearing as a side effect of human diabetes, but it is unknown whether other side effects also occur.

Both Craighead and Notkins have shown that the severity of the diabetic symptoms is directly related to the amount of damage to beta cells of the islets. This damage has also been described by K. F. Wellmann and his associates at the Kingsbrook Jewish Medical Center in Brooklyn and by H. Müntefering of the University of Dusseldorf in West Germany. Typically, the uniform architecture and arrangement of cells in the islets is distorted and there is swelling and accumulation of fluids in the pancreas. The beta cells begin to lose their granules—the organelles that actually produce insulin—and there is some death of beta cells

(necrosis); in the most severe cases, the number of beta cells is greatly reduced.

Necrosis of the beta cells has been shown by Wellmann to be accompanied by infiltration of the islets by immunoreactive lymphocytes and scavenger macrophages. There is thus speculation that part of the damage to the islets results from an autoimmune reaction mediated by these lymphocytes. There is little replacement of the damaged cells. Craighead has shown that there is some regeneration of islet cells by metaplasia of acinar cells at the periphery of the islets, but the regeneration is limited since the beta cells are tertiary cells that do not ordinarily undergo mitosis.

The factors that determine whether a mouse will become diabetic after the viral infection are still poorly understood, although probably there are many. It is clear that hormonal influences are important. In many of the strains of mice that are susceptible to pancreatic damage from EMC virus infection, the diabetic symptoms are much more severe in males than in females. If the testicles of these males are removed before inoculation of the virus, however, the diabetes is much less severe, even though the degree of beta cell damage appears to be the same as in noncastrated animals. Craighead has also found that necrosis of beta cells is greatly increased if the mice are given pharmacologic doses of corticosteroids. Certain types of stress also appear to play a role. Craighead has shown, for example, that the diabetic symptoms are more severe when the mice are made obese by the administration of gold-thioglucose, which causes an inhibition of appetite control mechanisms. Many of these factors are also thought to be important in the development of diabetes in humans.

As is suspected to be the case in humans, though, the most important factor in the development of diabetes in mice seems to be a genetic susceptibility to pancreatic damage resulting from the infection. Craighead, for instance, has studied the effects of the M variant of EMC virus in two carefully selected mouse strains, DBA/2 and C3H. By all tests, he says, the virus replicates to the same extent in the islets of both strains, and antibody is produced against the virus by both strains. But many of the DBA/2 mice develop pancreatic damage and diabetes, whereas very few of the C3H mice do.

Progeny obtained by cross-breeding the two strains develop diabetes at a rate that is only about one-third that of the DBA/2 strain. If the progeny are backcrossed with either of the parent strains, however, the incidence of diabetes in the new progeny is comparable to that in the original parental strain. Craighead interprets these results as an indication that the inherited predisposition to development of diabetes is controlled by more than one gene.

Similarly, Notkins has tested the M variant in 19 different inbred strains of male mice. Five of the strains developed hyperglycemia and abnormal glucose tolerance, and three other strains developed only abnormal glucose tolerance. The remaining 11 strains showed no abnormalities of glucose metabolism. In addition, he tested females from three strains in which males developed hyperglycemia and found that similar symptoms developed in only two.

It seems likely that there is a genetic basis for the differences among the strains, but to date Notkins has found no common denominator among either those strains that develop abnormal glucose metabolism or those that do not. It is possible that beta cells of the resistant strains are not susceptible to infection by EMC virus. For example, it is known that poliomyelitis virus, a related picornavirus, requires specific receptors on the surface of cells for infection. Notkins is now investigating this possibility for EMC virus. In any case it is apparent that the resistance of many strains to virus-induced damage of beta cells is partially responsible for the difficulties that were associated with the development of experimental diabetes in laboratory systems.

Other factors must have contributed to these difficulties. This is made clear by the contradictory experiences of many investigators who have tried to produce diabetes in laboratory animals by infecting them with coxsackie viruses, a family of picornaviruses that produce upper respiratory infections and a pattern of symptoms much like those of poliomyelitis except without paralysis. Because of the epidemiological evidence suggesting a link between human diabetes and infections by a type of coxsackie virus known as B4, it seemed reasonable that the virus would provide a good laboratory model for the human disease. But experience with coxsackie viruses has not met that expectation.

In the early 1950's, several investigators reported that another type of coxsackie virus, known as B1, caused extensive destruction of acinar tissue in pancreases of mice, but that it spared the islets of Langerhans. In 1971, George E. Burch and his associates at Tulane University School of Medicine reported electron microscope studies indicating that both coxsackie B1 and B4 viruses produce damage to the islets of

The Pancreas

The pancreas is a large gland, averaging about 70 grams in humans, and is attached to the duodenum, a portion of the small intestine connected to the stomach. The pancreas is composed of two main elements: exocrine tissue and endocrine tissue.

Exocrine tissue makes up the bulk of the pancreas. It is organized into a large number of saclike structures (acini) whose interiors are lined with cells (acinar cells) that secrete various enzymes important to the digestive process. These enzymes flow through passageways in the pancreas into a large duct through which they are released into the duodenum.

Scattered throughout the exocrine tissue are small, isolated pockets of endocrine tissue; these pockets are known as the islets of Langerhans. The islets may be composed of several different types of cells, but the predominant constituents are the alpha and beta cells, which generally occur in the ratio of four beta cells to each alpha cell. Both types of cells contain small organelles, known as granules, that have been identified as the source of the hormones produced by the pancreas. Granules in the beta cells produce insulin, while those in the alpha cells produce glucagon. Both hormones are secreted directly into the bloodstream.

Surgical removal of the pancreas leads, within 24 hours, to high concentrations of glucose in the blood. This hyperglycemia is followed in a short time by all the other symptoms of diabetes.—T.H.M.

Langerhans as well as damage to acinar tissue. A year later, Alyne K. Harrison and her colleagues at the Center for Disease Control in Atlanta reported that a third type, coxsackie B3, caused damage to the beta cells of newborn mice. They could isolate the B3 virus only from acinar cells, however. Neither Burch nor Harrison studied glucose metabolism in the infected mice.

The following year, D. Robert Gamble of the Public Health Laboratory at West Park Hospital in Epsom, Surrey, England, and K. W. Taylor of the University of Sussex in Brighton reported that they had induced diabetes in one type of mice (CD1) with a strain of coxsackie B4 virus. The experimentally produced diabetes has a slightly lower incidence and displays somewhat less severe symptoms, but otherwise is quite similar to that obtained by Craighead with EMC virus. The diabetic symptoms, Gamble and Taylor suggest, arise from a defect in the insulin-secreting capacity of the islets, even though there is only modest visible damage to islet cells. They also found that a strain of coxsackie B3 virus produces severe damage to acinar tissue, but no diabetic symptoms.

Coupled with the earlier epidemiological evidence, Gamble and Taylor's results would seem to provide a strong case for the hypothesis that coxsackie B4 viruses are implicated in the etiology of human diabetes. But no other investigators have been able to repeat their work. Notkins, for example, has examined more than 15 strains of coxsackie viruses and has found no evidence of beta cell damage or abnormalities of glucose metabolism, even when he used the same strain of virus used by Gamble and Taylor and the same type of mice.

Instead, Notkins found severe damage to the acinar cells of the pancreas similar to that reported by earlier investigators. Craighead, among others, has also not been able to reproduce Gamble and Taylor's results. There is as yet no explanation for this discrepancy. One suggestion is that the diet of the experimental animals might be involved, since R. E. Gleason and his associates at the Eliot P. Joslin Research Laboratory in Boston have shown that male CD1 mice spontaneously develop a diabetic syndrome when they are placed on a high fat diet. Another possibility is that the coxsackie virus used by Gamble and Taylor became attenuated between the time it was isolated and the time it was used by the American investigators. Gamble argues, however, that his mice were not exposed to a high fat diet. He suggests, instead, that there is probably significant genetic variability in the CD1 mice, a strain that is not an inbred strain. The resolution of this riddle will obviously require much further work.

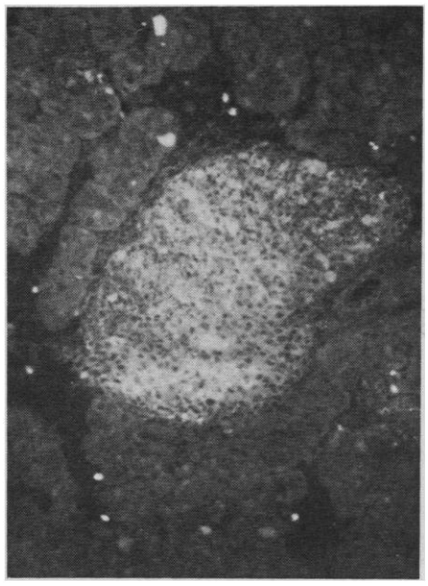

Fig. 2. A section from the pancreas of DBA/2 male mice 3 days after infection with EMC virus; the sample is stained with fluorescein-labeled antibody to EMC virus, which shows up as light areas. Viral antigens are observed in beta cells of the islets, but not in the surrounding acinar cells. [Source: Abner L. Notkins and Kozaburo Hayashi, National Institute of Dental Research]

A few other investigators have obtained some preliminary evidence linking diabetes in laboratory animals to viral infections. Bryce L. Munger and C. Max Lang of the Milton S. Hershey Medical Center, for example, have for 4 years been studying a form of "spontaneous" diabetes that they believe is infectious in at least two different types of guinea pigs. The disease was first observed in a colony of Abyssinian guinea pigs obtained from a Pennsylvania fancier. It is apparently spread by contact between the animals, but the infectious agent, presumed to be a virus, has not yet been isolated or identified.

The guinea pigs are afflicted with a mild form of diabetes characterized by glucosuria, hyperglycemia after fasting, abnormal glucose tolerance, and elevated concentrations of triglycerides in the blood plasma. Beta cells of the guinea pig islets lose some of their insulin-secreting granules, but no necrosis has been observed. The cytoplasm of the beta cells also contains small particles consisting of glycogen and granule cores, but the significance of these particles is unknown.

Munger and Lang have not, however, observed either damage to acinar cells or lymphocytic infiltration characteristic of an immune response to an infection. The cellular alterations of the guinea pig pancreases, Munger adds, are similar to those observed with the juvenile form of diabetes in humans.

Eliot Rayfield of the Mt. Sinai School of Medicine in New York has recently demonstrated that hamsters inoculated with Venezuelan equine encephalomyelitis (VEE) virus develop impaired glucose tolerance and impaired insulin release. The VEE virus is an RNA-containing virus of the arbovirus family. It causes inflammation of the brain and spinal cord in horses, humans, and some other animals. A recent epidemic (1972) of VEE in the southwestern part of the United States caused the deaths of large numbers of horses.

In hamsters the impairment of glucose tolerance and insulin release peaks about 24 days after inoculation with VEE virus. The glucose tolerance subsequently returns to normal, but the insulin release remains impaired. The virus apparently replicates in the pancreas, Rayfield says, but he has been unable to isolate it from there, and there is no evidence of tissue damage. The symptoms produced by the virus, he notes, are characteristic of the clinical condition known as prediabetes, which in humans generally progresses to diabetes. Rayfield is undertaking long-term studies to assess the effects of the virus more fully. He is also planning to determine whether the severity of the symptoms can be increased by subjecting the animals to stress conditions and to test the effects of the virus in other species, such as monkeys.

The significance of all these findings, as well as their relation to the human condition, is still rather uncertain. It is of interest that three of the viruses implicated in animal diabetes are members of the same family of viruses (picornaviruses) that has been suggested to be involved in human diabetes, but the evidence is still inconclusive. Certainly the model systems have not been shown to mimic some of the more severe complications of human diabetes, including arterial lesions and blindness, but most investigators agree that the systems are beginning to provide a more fundamental understanding of the etiology of this all-too-common affliction.

—THOMAS H. MAUGH II