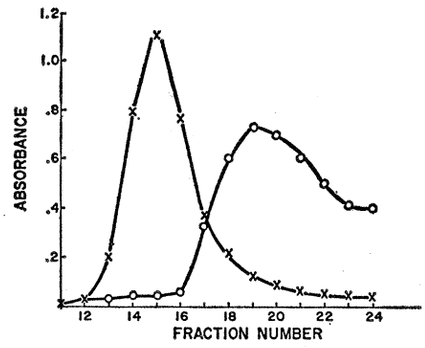

Fig. 1. Elution pattern of crystalline trypsin by gel filtration on Sephadex G-50. Void volume was 20 ml with blue dextran. Ribonuclease was determined by the orcinol method, and optical densities were recorded at 670 nm; X, 280 nm; ○, 670 nm (ribonuclease activity).

tained an amount of activity intermediate between the three samples. At a trypsin concentration of 100 μg/ml, 50 percent of the RNA, incubated at a concentration of 1 mg/ml, was solubilized within 30 minutes. Even at a concentration of trypsin of 10 μg/ml, 17 percent of the RNA was degraded in this time. Longer incubations with 10 μg/ml produced further RNA breakdown.

Since certain experiments require that trypsin be entirely devoid of ribonuclease activity, a procedure was devised which yields trypsin that contains no ribonuclease. The optimum purification was obtained with a column (45 by 1.5 cm) of Sephadex G-50 in 0.15*M* NaCl. One milliliter of solution containing 10 mg of trypsin in 0.15*M* NaCl was filtered at room temperature through the column at a flow rate of approximately 2 ml in 5 minutes. The effluent was collected in 2-ml fractions immediately after the column was loaded. Protein was determined from absorbance measurements at 280 nm, and ribonuclease activity was assayed. In several experiments with different trypsin preparations, the protein peak preceded the peak of ribonuclease activity by four tubes (Fig. 1). The tubes containing protein devoid of ribonuclease activity were pooled, dialyzed in the cold against distilled water, and lyophilized.

The recovered trypsin was compared to the original trypsin with respect to hydrolysis of RNA and the protein azocasein (*5*). Each sample of trypsin was diluted to the same concentration of protein as determined by absorption at 280 nm. Assays with RNA of the trypsin recovered from the column showed a lack of ribonuclease activity. Even at 2 hours of incubation when the original trypsin solubilized over 60 percent of the RNA, less than 5 percent degradation resulted. The activity of the protease was decreased somewhat in the process of removal of the contaminant ribonuclease. The rates of splitting azocasein were reduced by 30 percent in one experiment and 40 percent in a second experiment with a different trypsin preparation. However, the loss in specific activity can be compensated for by doubling the amount of trypsin without introducing significant amounts of the contaminant into the incubation mixture.

Trypsin incubated overnight at *p*H 8.5 lost most of its tryptic activity, but there was no decrease in the ability of the solution to hydrolyze RNA. This ribonuclease is thus similar to ribonuclease A in that neither enzymatic activity is abolished by incubation with trypsin (*6*). Therefore, if large amounts of trypsin of low proteolytic activity are used, large quantities of ribonuclease will be introduced into the incubation mixture.

Tests of all of the samples of trypsin for deoxyribonuclease activity by the diphenylamine method of Dische (*4*) revealed that this substance was not present.

In view of this finding, certain observations in complex biological systems and their interpretations which are based on the proteolytic activity of trypsin may have to be reexamined with respect to the possibility that ribonuclease action contributed significantly to the results.

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23 August 1968

Diabetes Mellitus: Induction in Mice by Encephalomyocarditis Virus

Abstract. *Hyperglycemia and lesions of the pancreatic islets of Langerhans developed in some, but not all, adult mice infected with a variant of the encephalomyocarditis virus. Large amounts of virus were recovered from the pancreas during acute stages of infection. At this time blood glucose concentrations were markedly elevated and the islets of Langerhans exhibited focal necrosis and degranulation of beta cells. Evidence of abnormal glucose metabolism persisted for varying periods after recovery from the infection. The islets of Langerhans of chronically hyperglycemic mice were distorted and decreased in size, and the beta cells were degranulated. Encephalomyocarditis virus appears to cause diabetes mellitus by reducing the mass of functional beta cells of the islets of Langerhans.*

Diabetes mellitus can be induced in animals with endocrine hormones and with chemicals such as alloxan. It occurs spontaneously in selected lines of inbred rodents and in mice fed high caloric diets. An infectious etiology has never been established, although cases have been described in man after mumps and in cattle after foot-and-mouth disease (*1*). We report here the induction of diabetes mellitus in mice with a variant of encephalomyocarditis (EMC) virus which causes lesions in the islets of Langerhans.

The source of the M variant of EMC has been recorded (*2*). Pools were prepared from both myocardial tissue of infected mice and cultures of L cells inoculated with virus that had been purified by plaque selection. Adult male mice, free of pathogens (*3*), were given 10 to 100 plaque-forming units subcutaneously and maintained in individual cages with Purina mouse chow and water freely available. All were tested routinely before inoculation to exclude glycosuria and hyperglycemia (fasting blood glucose $\geq$ 140 mg/100 ml); glucose determinations were made on blood from the orbital sinus (*4*). Glucose tolerance tests were carried out by bleeding animals (fasted for 15 hours) at intervals after the intraperitoneal injection of 1 mg of glucose per gram of body weight. Urine was tested by the glucose oxidase method.

Evidence of abnormal carbohydrate

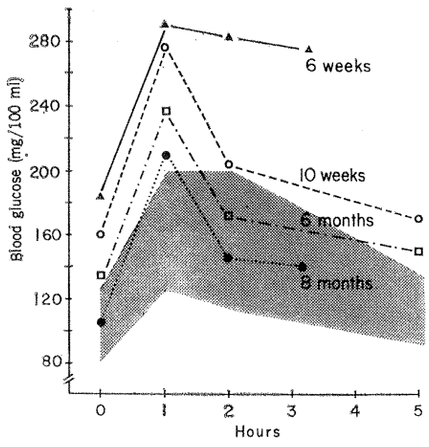

Fig. 1. Results of glucose tolerance tests on a selected mouse at intervals after inoculation of the M variant of EMC virus. Shaded area, mean of uninfected control mice ± 2 standard deviations.

metabolism was demonstrated in 36 percent of mice infected with the M variant of EMC in five separate experiments, each employing 25 to 80 animals. Blood glucose levels of 300 to 550 mg/100 ml were detected as early as 4 days after inoculation of virus and persisted for varying periods. In our studies 59 percent of the hyperglycemic mice survived for three or more weeks. Of these, 80 percent were hyperglycemic for a few days to several months, whereas the remainder exhibited elevated concentrations of blood glucose or glycosuria transiently. Animals with persistent hyperglycemia were killed at intervals for histologic studies. Thirteen were maintained for 2 months or longer. Most of the chronically affected mice appeared healthy and had a stable body weight; a few died spontaneously of unknown cause. Acetonuria was rarely observed. The number of affected mice, as well as the duration and severity of hyperglycemia, varied in different experiments. Abnormalities of glucose metabolism were not demonstrated in uninfected controls nor in animals inoculated with virus neutralized by antiserum against the Mengo strain of EMC.

Glucose tolerance tests were done on surviving animals with normal concentrations of blood glucose to document altered function of the islets of Langerhans during convalescence. These studies were carried out on two or more occasions from the 2nd to the 8th month after inoculation of virus. An abnormal response to the glucose challenge was found in ten mice that were transiently hyperglycemic during or after the acute infection. Figure 1 summarizes the results of serial studies on a mouse that was hyperglycemic from the 7th to the 23rd day. Although glucose tolerance curves initially were aberrant, the animal recovered the ability to metabolize glucose in a normal fashion over an 8-month period. Similar patterns were found in four additional animals.

Hyperglycemia during the acute stage of infection is associated with the presence of large amounts of virus in the pancreas and histologic changes in the islets of Langerhans. From 10^3 to 10^7 plaque-forming units are recovered from pancreatic tissue for 2 weeks after inoculation. Lesions first were noted in the islets on the 6th day (Fig. 2A). Scattered cells were necrotic and degranulation of beta cells was prominent (Fig. 2B). Lesions of acinar tissue were not seen. Subsequently, the islets decreased in size. By the 4th week the average diameter was reduced and the degranulated cells varied in size and shape. Islets of Langerhans are difficult to locate in the pancreases of chronically hyperglycemic animals. Invariably they were distorted and small (Fig. 2C) and contained only a few granulated beta cells (Fig. 2D). When cortisone (1 mg/day) was administered during acute stages of infection, the islets of Langerhans developed extensive necrosis (Fig. 2F) (*5*). Lesions were not observed in the pituitary, the adrenals, or the liver of the hyperglycemic mice we studied.

The evidence indicates that diabetes mellitus in our animals is caused by the M variant of EMC. Our findings suggest that the virus acts on the islets of Langerhans to reduce the mass of functional beta cells. Histologic changes in the pancreas and abnormal glucose metabolism fail to develop in many infected mice. It is unclear why some, but not all, animals are affected.

Encephalomyocarditis is one of a large number of human and animal pathogens classified as picornaviruses (*6*). At least three other members of this group (coxsackievirus group B and the viruses of foot-and-mouth disease and infectious pancreatic necrosis of fish) multiply in the pancreas and cause lesions of the acinar tissue and islets of Langerhans (*7*).

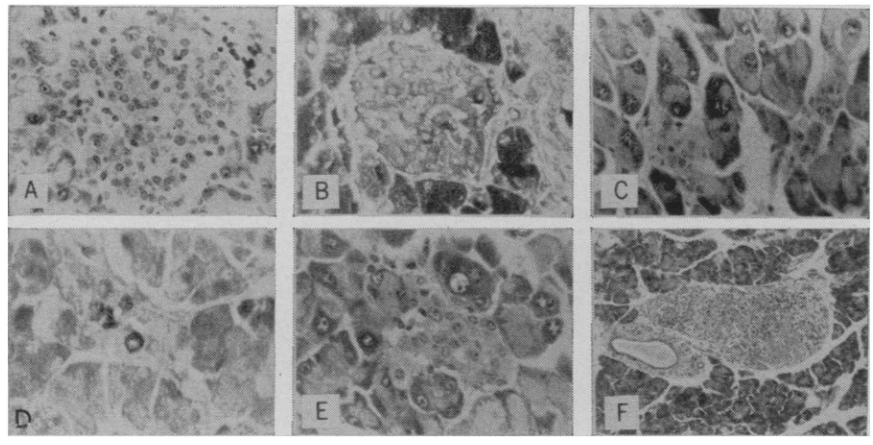

Fig. 2. Islets of Langerhans in hyperglycemic mice inoculated with the M variant of EMC virus. On the 6th day the organized arrangement of cells in the islets was lost and scattered cells were necrotic (A). Most beta cells were degranulated at this time (B). By the 3rd month the islets were small and distorted and varied in size and shape (C). Only a few of these cells were granulated (D). During the convalescent period proliferation of acinar tissue was seen at the margin of the shrunken islets (E). Extensive necrosis of the islets occurred by the 6th day in mice treated with cortisone (F). Photomicrographs were prepared from 5-μ sections stained with either hematoxylin and eosin (A, C, E, and F) or aldehyde-fuchsin (B and D).

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8. Supported by PHS grant AI 06481.

19 September 1968