VIRUS-INDUCED DIABETES MELLITUS

I. HYPERGLYCEMIA AND HYPOINSULINEMIA IN MICE INFECTED WITH ENCEPHALOMYOCARDITIS VIRUS

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For nearly 75 yr it has been speculated that viruses might be one of the causes of diabetes mellitus in man. In 1899, Harris reported that diabetes developed in a patient shortly after a mumps infection (1). Since that time there have been numerous reports showing a temporal relationship between the onset of viral infections and the development of diabetes (2). A variety of viruses have been implicated in the etiology of diabetes with mumps being the most popular candidate. Since, however, the incidence of both diabetes and mumps is high, it is possible that the relationship between the two might simply be fortuitous. Recently, it has been suggested that Coxsackie virus B4 also might produce diabetes. Gamble et al. (3) found that the titer of neutralizing antibody to Coxsackie B4 was higher in newly diagnosed diabetic patients when compared with nondiabetic controls. The antibody titer to a variety of other viruses including mumps was not significantly different from controls. Although provocative, a number of other factors might account for these relationships, and proof that viruses cause diabetes in man is still lacking.

Evidence that viruses can produce diabetes in animals also is sparse. In the early 1960's a group of investigators from Italy observed that cattle infected with foot-and-mouth disease virus developed hyperglycemia and lesions in the pancreas (4, 5). Since only a small group of animals was studied these observations await confirmation. Other viruses also can produce lesions in the pancreas; but the islets of Langerhans often were not involved or the animals were not examined for evidence of diabetes (6-9). Recently, Craighead and co-workers reported that mice infected with the M variant of encephalomyocarditis (EMC) virus developed a diabeteslike syndrome (10, 11). Because of the potential value of animal models for understanding diabetes in humans, the present investigation was initiated to study in greater detail the characteristics and duration of the EMC-induced diabeteslike syndrome and to evaluate some of the factors responsible for its development.

1 Abbreviations used in this paper: EMC, encephalomyocarditis; IRI, immunoreactive insulin; MEM, minimal essential medium.

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Materials and Methods

Cells.—Primary mouse embryo cultures were prepared by trypsinization of minced 17–19-day old CAF-1 embryos. Cells were grown with Eagle’s minimal essential medium (MEM) supplemented with 10% calf serum, 100 U of penicillin, 100 μg of streptomycin, and 50 μg of neomycin per ml.

Animals.—DBA/2N and C57BL/6N mice were obtained from the breeding colony at the National Institutes of Health. BALB/CJ, CBA/J, and A/J mice were purchased from Jackson Laboratory, Bar Harbor, Maine. CD-1 mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. Mice were maintained on Purina NIH Rat and Mouse Ration containing 5.0% fat and 23.5% protein. Animals were allowed free access to food and water except where noted. Unless stated otherwise, male mice were used in all experiments.

Virus.—The M variant of EMC virus passaged in mice (MH22) was kindly supplied by Dr. J. Craighead (11, 12). 6-wk old DBA/2N mice were infected intraperitoneally (i.p.) with 0.1 ml of a 1:50 dilution of this material. 5 days later a 10% mouse heart suspension was prepared in Eagle’s MEM with 5% calf serum by homogenization in a TenBroeck all glass tissue grinder. A 10% suspension of hearts from uninfected mice was prepared in the same way. The virus titer was determined by inoculating 0.2 ml of appropriate dilutions of this material onto confluent monolayers of primary mouse embryo cells in 15-mm plastic Petri dishes. After adsorption for 1 h at 37°C, cultures were overlayed with Eagle’s MEM containing 5% calf serum and 2% methylcellulose (Methocel, 4000 cps; Fisher Scientific Co., Fairlawn, N. J.). Monolayers were stained 4 days later with a 1:10,000 dilution of neutral red and plaques were counted within 12 h. The titer of the stock virus pool by this method was 2.5 × 10^5 plaque-forming units (PFU)/ml.

For titration of virus in the pancreas a 10% suspension was prepared by homogenization as described above. To avoid possible inactivation of the virus by proteolytic enzymes released from the pancreas, the homogenate was titrated immediately for infectivity.

Analytical Methods.—Except where stated, 6–8-wk old male DBA/2N mice were inoculated i.p. with 0.1 ml of a 1:50 dilution of normal mouse heart suspension or stock virus containing 500 PFU of EMC virus. Mortality with this concentration of virus was 10–20%. Unless indicated otherwise blood was obtained from nonfasted mice by bleeding them from the retro-orbital plexus. Glucose was determined enzymatically by the glucose oxidase method (13) using o-dianisidine dihydrochloride as the reactive dye (Sigma Chemical Co., St. Louis, Mo.). Serum immunoreactive insulin (IRI) was measured by the solid-phase radioimmunoassay procedure (14) with porcine insulin as the standard (Pharmacia, Uppsala, Sweden). Glucose and ketones in the urine were measured using Tes-tape and Ketostix obtained respectively from Eli Lilly and Co., Indianapolis, Ind., and Ames Co., Div. of Miles Lab., Inc., Elkhart, Ind.

Glucose Tolerance Tests.—Mice were fasted for 18 h and then inoculated i.p. with a 20% aqueous solution (wt/vol) of dextrose, 2 mg/g body weight. Animals were bled immediately before injection of dextrose (0 time) and at 30, 90, and 120 min thereafter. The samples were then assayed for blood glucose.

RESULTS

Hyperglycemia and Glycosuria in EMC-Infected Mice.—The first experiment was designed to study the time-course and magnitude of the hyperglycemia induced by EMC virus. Male DBA/2N mice were infected with EMC virus, and at different times thereafter the animals were bled and blood glucose was determined. Mice inoculated with a 10% normal mouse heart suspension served as controls. Three separate experiments were performed with approximately
50 infected and 20 uninfected mice in each. The data in Fig. 1 represent the mean of these experiments. It can be seen that within 6–10 days after infection the mean blood glucose level rose to greater than 300 mg/100 ml. Blood glucose then gradually declined but remained above controls even at 170 days after infection. Some of these animals have now been hyperglycemic for over 300 days. In contrast, the mean blood glucose levels of mice inoculated with a normal mouse heart suspension ranged from 130 to 160 mg/100 ml. The mean glucose level of uninoculated mice was found to be 141 mg/100 ml with a standard deviation (SD) of ±29 and a standard error of the mean (SEM) of ±3. Less than 1% of these animals had blood glucose levels above 200 mg/100 ml. In this paper, animals were considered hyperglycemic if their blood glucose levels exceeded 200 mg/100 ml (i.e., 2 SD greater than the mean of uninfected animals).

A more detailed analysis of the above data, showing the percentage of animals with different levels of glucose, is illustrated in Table I. 7 days after inoculation of the virus, 68% of the animals were hyperglycemic while at 98 days only 22% were still hyperglycemic. No appreciable difference in mortality was found between the hyperglycemic and nonhyperglycemic groups.
TABLE I
Percentage of Animals with Hyperglycemia After Injection with EMC Virus*

| Group                          | Days postinfection | Animals |
|--------------------------------|-------------------|---------|
|                                | 7     | 21   | 31   | 63   | 98   |
| Hyperglycemic (>200 mg/100 ml) | %     | %    | %    | %    | %    |
| >400 mg/100 ml                 | 36    | 32   | 20   | 25   | 18   |
| 200-399 mg/100 ml              | 32    | 20   | 25   | 0    | 4    |
| Nonhyperglycemic (<199 mg/100 ml) | 32    | 48   | 55   | 75   | 78   |

* Data from Fig. 1 expressed as the percentage of animals with different blood glucose levels.

Data obtained by following blood glucose levels in individual animals revealed three general patterns. The most common pattern, found in approximately 60-80% of the animals, was a transient hyperglycemia lasting from a few days to several months. The second pattern occurring in 10-15% of the animals was a persistent hyperglycemia lasting 6 or more months. The third pattern found in 15-30% of the animals was characterized by the failure to develop hyperglycemia. The data in Fig. 2 represent these patterns in three animals studied over a 90 day period.

Infection of mice with EMC virus also resulted in glycosuria. The data in Fig. 3 show that in acutely infected mice there was a good correlation between hyperglycemia and glycosuria. Animals with blood glucose levels below 200 mg/100 ml had little or no sugar in the urine (0 to 1+), while the majority of animals with blood glucose levels above 300 mg/100 ml had substantial amounts of sugar in the urine (3+ to 4+). Marked discordance between glucose in the blood and urine was observed, however, in about 10% of the determinations. Glycosuria also was detected in hyperglycemic animals infected for 6 or more months (data not shown).

To see whether the hyperglycemia in EMC infection was associated with breakdown of fat deposits, the urine of infected mice was tested for ketones. The data in Table II show that although up to one-third of the infected mice had low levels of ketones in their urine, there was no correlation between ketosis and hyperglycemia. Ketones were not detected in the urine of uninfected mice.

Effect of Fasting on Blood Glucose Levels.—In the early phase of our experiments we observed that when animals were fasted overnight (18 h) the difference in blood glucose levels between infected and uninfected animals was obscured. To determine more precisely the rate at which elevated blood glucose levels returned to normal, glucose determinations were done at different times after removal of food. The data in Table III show that within 12 h the mean blood glucose level of the infected group approached that of the uninfected
Fig. 2. Representative blood glucose patterns in mice after infection with EMC virus. Each curve represents data from a single mouse. Persistent hyperglycemic, (Δ—Δ); transient hyperglycemic, (●—●); nonhyperglycemic, (○—○).

group. Only one of the infected animals had a blood glucose level above 200 mg/100 ml. As a result, nonfasted animals were used for most of our studies.

Food and Water Consumption in Infected Mice.—To study the effect of EMC virus infection on food and water consumption, mice were divided into groups on the basis of their nonfasting blood glucose levels. Animals were placed in individual cages and the amount of food and water consumed by each mouse over a 6 wk period was determined. The data in Table IV show that infected nonhyperglycemic animals consumed approximately the same amount of water and slightly more food than uninfected animals. However, infected hyperglycemic animals consumed almost twice as much water and significantly more food than the uninfected animals. There was no significant difference in weight gain among the three groups.

Impairment of Glucose Metabolism in Infected Mice.—The data in Table I and Fig. 2 showed that not all animals infected with EMC virus became severely hyperglycemic. Moreover, in a large percentage of the animals that did become hyperglycemic the blood glucose levels ultimately returned to normal. To see
Fig. 3. Temporal relationship between blood glucose and glycosuria. For each point blood and urine specimens were obtained within 4 h of each other. Data were obtained from 86 mice that had been infected for 12–15 days. Each point represents an individual animal. The line was computed by linear regression analysis (correlation coefficient, $r = 0.8$).

TABLE II

Ketones in the Urine of EMC-Infected Mice

| Time after infection wk | no. of mice tested | no. of mice with blood glucose >200 mg/100 ml | no. of mice with ketones in urine * | no. of ketotic mice with blood sugars >200 mg/100 ml | Ketone levels in the urine were determined with Ketostix strips and the amount was recorded as small (1+), moderate (2+), or large (3+). In all but two cases the amount of ketones in the urine did not exceed 1+. | Blood and urine were obtained within 4 h of each other. |
|------------------------|-------------------|---------------------------------------------|-----------------------------------|-----------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Control                | 30                | 30                                          | 19                                | 9                                             |                                                                                |                                                                                  |
| 1                      | 61                | 7                                           | 10                                | 4                                             |                                                                                |                                                                                  |
| 7                      | 28                | 7                                           | 10                                | 4                                             |                                                                                |                                                                                  |
| 12                     | 48                | 15                                          | 10                                | 5                                             |                                                                                |                                                                                  |
| 20                     | 54                | 7                                           | 11                                | 2                                             |                                                                                |                                                                                  |
| 26                     | 25                | 7                                           | 7                                 | 3                                             |                                                                                |                                                                                  |
TABLE III

| Group       | no. of mice | Blood glucose (mg/100 ml) | 0 | 4 | 8 | 12 |
|-------------|-------------|--------------------------|--|---|---|----|
| Uninfected  | 10          | 148 ± 151                | 144 ± 10 | 120 ± 22 | 96 ± 6 |
| Infected*   | 22          | 285 ± 20                  | 215 ± 13 | 200 ± 22 | 118 ± 9 |

* Animals had been infected for 42 days.
† Mean ± SEM.

TABLE IV

| Group       | no. of animals | Blood glucose (mg/100 ml) | Water consumption (ml/day) | Food consumption (mg/day) | Body weight (g) |
|-------------|----------------|--------------------------|---------------------------|--------------------------|-----------------|
| Uninfected  | 9              | 127 ± 8                  | 132 ± 5                   | 7.5 ± 0.1                | 21.2 ± 0.7      |
| Infected*   | 8              | 148 ± 7                  | 137 ± 8                   | 7.1 ± 0.8                | 22.6 ± 1.0      |
| Nonhyperglycemic | 12    | 509 ± 58                 | 463 ± 56                  | 13.8 ± 1.5               | 21.4 ± 0.8      |
| Hyperglycemic | 12    | 309 ± 60                 | 453 ± 56                  | 4.5 ± 0.2                | 26.1 ± 1.3      |

* At start of the experiment (0 wk) animals had been infected for 28 days.
† Mean ± SEM.
‡ P < 0.01 compared with uninfected animals.
¶ P < 0.01 compared with infected nonhyperglycemic animals.
** P < 0.05 compared with uninfected animals.

whether there was residual impairment of glucose metabolism in these animals, glucose tolerance tests were performed. As expected, the hyperglycemic animals (blood glucose levels above 200 mg/100 ml) displayed grossly abnormal glucose tolerance curves (Fig. 4). Abnormal glucose tolerance curves also were seen in infected animals that never had blood glucose levels greater than 200 mg/100 ml (Fig. 4A) and in infected hyperglycemic animals that now had blood glucose levels less than 200 mg/100 ml (Fig. 4B). The blood glucose levels in these animals were higher and remained elevated for a longer time than in uninfected animals. Thus, the ability of infected animals that were not hyperglycemic to handle a glucose load was impaired.

Insulin Levels in Infected Mice.—To see whether the impairment of glucose metabolism might be due to abnormal insulin levels, infected animals were divided into hyperglycemic and nonhyperglycemic groups. The amount of insulin in the plasma of fasted animals then was determined at different times after infection. The data in Table V show that the amount of insulin in the plasma of the infected nonhyperglycemic mice was not statistically different from that of the uninfected mice. The insulin in the plasma of infected hyper-
Fig. 4. Comparison of glucose tolerance tests in hyperglycemic and nonhyperglycemic mice. Mice were infected with EMC virus and divided into three groups: infected mice with blood glucose levels above 200 mg/100 ml, (●—●); infected mice with blood glucose levels below 200 mg/100 ml, (○—○); infected mice with blood glucose levels above 200 mg/100 ml during the acute phase of the infection, but below 200 mg/100 ml at 10 wk after infection, (□—□). Uninfected mice with blood glucose levels below 200 mg/100 ml (△—△) served as controls. Before the glucose tolerance tests, the animals were fasted for 18 h and their blood glucose levels determined (0 time). The mice then were injected i.p. with glucose (2 mg/g body weight) and bled at the times indicated. Each point represents the mean of four to eight animals. Animals had been infected for 5 wk (A) or 10 wk (B).

glycemic mice, however, was lower than that of uninfected mice at each of the times tested, with highly significant differences at 56 and 133 days.

The responsiveness of EMC-induced hyperglycemia to exogenous insulin is illustrated in Table VI. It can be seen that within 30 min after injection of insulin, there was a marked drop in blood glucose levels of both the infected and uninfected mice. Over the next 90 min the blood glucose levels in both groups began to rise; the blood glucose of uninfected animals returned to its preinsulin level, whereas the blood glucose in infected animals still was substantially below its preinsulin level.

Effect of Mouse Strain and Sex on Blood Glucose Levels.—The data in Table...
TABLE V

*Immunoreactive Insulin (IRI) in the Plasma of EMC-Infected Mice*

| Group                  | IRI (µU/ml)* | 3  | 8  | 14 | 28 | 56 | 133 |
|------------------------|--------------|----|----|----|----|----|-----|
| Uninfected             | 85 ± 5†      | 94 ± 10 | 92 ± 6 | 92 ± 6 | 97 ± 8 | 100 ± 9 |
| Infected               | 98 ± 6       | 87 ± 8 | 95 ± 10 | NT       | 84 ± 9 | 90 ± 9 |
| Nonhyperglycemic       | NT           | 77 ± 7 | 88 ± 7 | 73 ± 7 | 64 ± 9§ | 64 ± 6§,Ⅰ |
| Hyperglycemic          | NT           | 77 ± 7 | 88 ± 7 | 73 ± 7 | 64 ± 9§ | 64 ± 6§,Ⅰ |

NT = Not tested.
* Animals were fasted for 18 h before plasma was obtained for IRI determinations. Each group contained from 8–15 animals.
† Mean ± SEM.
§ P < 0.01 compared with uninfected animals.
Ⅰ P < 0.01 compared with infected nonhyperglycemic animals.

TABLE VI

*Changes in Blood Glucose after Injection of Exogenous Insulin* *

| Group                  | no. of mice | Blood glucose (mg/100 ml) |
|------------------------|-------------|----------------------------|
|                        |             | 0            | 30            | 60            | 120           |
| Uninfected             | 7           | 101 ± 2†     | 47 ± 10       | 71 ± 16       | 120 ± 20      |
| Infected (hyperglycemic)| 7           | 373 ± 41     | 81 ± 9        | 122 ± 19      | 163 ± 17      |

* Animals were inoculated i.p. with 0.06 U of porcine insulin. Blood for the 0 time determination was taken just before injection of the insulin.
† Mean ± SEM.

VII and Fig. 5 show that the development of hyperglycemia in EMC virus infection was dependent upon both the strain and sex of the animals. Of the six strains of male mice tested at 7 days after infection, only DBA/2N and CD-1 mice developed hyperglycemia (Table VII). Similar results were observed at 14 days after infection (data not shown). The low blood glucose levels noted in A/J mice may be related to the moribund state of these animals.

Comparison of blood glucose levels in EMC-infected DBA/2N male and female mice revealed the typical hyperglycemic response in males, whereas the mean blood glucose levels in females did not exceed 160 mg/100 ml (Fig. 5).

In another experiment with 80 infected female mice studied over a period of 35 days, only 4 out of 280 determinations gave blood glucose values above 200 mg/100 ml (data not shown).

*Title of Virus in the Pancreas.*—To see whether the failure of female mice to develop hyperglycemia was related to the degree of viral replication, the amount of infectious virus in the pancreas of male and female mice was determined.
TABLE VII

Blood Glucose Levels in Different Strains of Male Mice Infected with EMC Virus

| Strain      | Infected | no. of animals | Blood glucose* (mg/100 ml) | Percent hyperglycemic | Percent mortality* |
|-------------|----------|----------------|-----------------------------|-----------------------|-------------------|
|             |          |                |                             |                       |                   |
| DBA/2N      | -        | 10             | 147 ± 6§                   | 81                    | 0                 |
|             | +        | 21             | 326 ± 32                   | 0                     | 14                |
| CD-1        | -        | 29             | 123 ± 4                    | 0                     |                   |
|             | +        | 68             | 186 ± 12                   | 22                    | 3                 |
| CBA/J       | -        | 10             | 202 ± 7                    | 0                     |                   |
|             | +        | 39             | 192 ± 2                    | 0                     | 3                 |
| C57BL/6N    | -        | 14             | 110 ± 5                    | 0                     |                   |
|             | +        | 15             | 105 ± 4                    | 0                     | 25                |
| BALB/CJ     | -        | 10             | 115 ± 4                    | 0                     |                   |
|             | +        | 27             | 102 ± 7                    | 0                     | 33                |
| A/J         | -        | 10             | 160 ± 4                    | 0                     |                   |
|             | +        | 21             | 83 ± 5                     | 0                     | 48                |

* All mice were infected at 6 wk of age with 500 PFU of EMC virus. Blood glucose levels and percent mortality were determined 7 days after infection.

+ Animals were considered hyperglycemic if their blood glucose levels were 2 SD greater than the mean of uninfected animals of the same strain.

§ Mean ± SEM.

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**Fig. 5.** Blood glucose levels in DBA/2N male and female mice infected with EMC virus. Each point represents the mean of 30-40 mice. Male, (●—●); female, (○—○).
The data in Table VIII show that there was little difference in virus titer between the two groups. Infectious virus could not be recovered after 10 days even though hyperglycemia persisted in the males for over 6 mo.

**TABLE VIII**

*Titre of EMC Virus in the Pancreas of Male and Female Mice*

| Time after infection | Virus titre PFU (log10/g tissue) |
|----------------------|---------------------------------|
|                      | Male                             | Female                        |
| 2                    | $5.5 \pm 0.1$                   | $5.1 \pm 0.2$                 |
| 4                    | $6.0 \pm 0.1$                   | $5.7 \pm 0.3$                 |
| 6                    | $5.1 \pm 0.4$                   | $4.5 \pm 0.3$                 |
| 8                    | $3.4 \pm 0.4$                   | $3.5 \pm 0.1$                 |
| 10                   | <2.0                            | <2.0                           |
| 16                   | <2.0                            | NT                             |
| 26                   | <2.0                            | NT                             |
| 40                   | <2.0                            | NT                             |

NT = Not tested.

* Male and female mice were inoculated i.p. with 500 PFU of EMC virus. At each of the times indicated six male and three female mice were sacrificed, and their pancreases were removed, homogenized, and assayed for infectious virus on primary mouse embryo cells. The data represent the mean of the individual determinations.

**DISCUSSION**

Craighead showed that infection of mice with EMC virus produced a diabeteslike syndrome characterized by hyperglycemia and glycosuria (10). In the present paper we have confirmed and extended these observations. Our experiments showed that the metabolic abnormalities were most readily detected by measuring the blood glucose levels of nonfasted animals and by performing glucose tolerance tests on fasted animals. This was necessitated by the observation that the blood glucose levels of the infected hyperglycemic mice remained elevated for only a short time after removal of food, making it difficult to detect metabolically abnormal animals. Measurement of glucose in the urine was a useful screening procedure for detecting hyperglycemic animals, but it did not provide the fine quantitation necessary to distinguish animals on the basis of the severity of their disease.

Analysis of the blood glucose data from hundreds of EMC-infected animals revealed that the metabolic abnormalities fit a spectrum ranging from very mild alterations detected by glucose tolerance tests to very severe alterations detected by simply measuring the blood glucose levels of nonfasted animals. By dividing animals into groups on the basis of the severity of their hyperglycemia, it was possible to detect metabolic abnormalities that otherwise might have been obscured or completely missed. In addition to hyperglycemia and
glycosuria, the severe form of the diabeteslike syndrome is characterized by
polydipsia, polyphagia, and hypoinsulinemia. Low levels of ketones also were
found in the urine, but whether ketosis is part of the syndrome or a sequela
of the more generalized EMC infection is not clear. By comparing severely
hyperglycemic with nonhyperglycemic animals, it should be possible to more
accurately assess the various metabolic abnormalities and determine whether
the vascular changes often associated with human diabetes (e.g. glomerulosclerosis and retinopathy) occur in the animal model.

Several lines of evidence suggest that the metabolic abnormalities of the
diabeteslike syndrome are due to pancreatic damage and hypoinsulinemia.
First, infectious virus can be recovered from the pancreas during the acute
phase of the infection. Second, the infection results in diminution in the number
and size of the islets of Langerhans and marked degranulation of beta cells
(10, 11, 15). Third, decreased levels of IRI in the pancreas of infected mice have
been reported, but the effect of EMC infection on the IRI levels in the blood
has been difficult to evaluate because of wide variation in the data and small
numbers of animals (11). By using large numbers of animals and dividing them
into severely hyperglycemic and nonhyperglycemic groups, we have been able
to show that levels of IRI were significantly reduced in the blood of the hyper-
glycemic animals. The observation that the level of IRI was not as greatly
depressed in acutely infected hyperglycemic animals as compared with long-
term hyperglycemic animals (Table V) once again may simply reflect a dif-
ference in the population of animals sampled (i.e., we do not know what per-
centage of animals tested on days 8, 14, and 28 belonged to the transient as
compared with the more severe long-term hyperglycemic category). Finally,
the demonstration that the blood glucose levels of the hyperglycemic animals
could be lowered by injecting exogenous insulin supports the contention that
the EMC-induced diabeteslike syndrome is, at least in part, secondary to
insulin deficiency.

Why EMC virus produces severe and prolonged hyperglycemia in some
animals, transient hyperglycemia in others, and mild if any alterations in
glucose metabolism in still others is not known. Although the marked dif-
fERENCE IN INCIDENCE OF DIABETES BETWEEN STRAINS MIGHT BE EXPLAINED ON A GENETIC
basis, it is harder to explain the different responses observed in the DBA/2N
males since these are inbred animals. One possibility that is being investigated
is that subtle differences in the time of initiation and magnitude of the im-
une response may determine whether or not the virus produces severe or
mild damage to the pancreas. It does not appear that the low incidence of
hyperglycemia in female as compared with male DBA/2N mice can be ex-
plained simply on the degree of viral replication, since approximately the same
amount of infectious virus was recovered from the pancreas in both sexes.
Moreover, preliminary experiments have failed to reveal any significant dif-
fences in the titer of virus in the pancreas of infected hyperglycemic as com-
pared with nonhyperglycemic males at 4, 6, and 8 days after infection. The titer of virus in the homogenate of the whole pancreas, however, may not reflect differences in the degree of viral replication in the beta cells of these animals. Alternately, hormonal differences may make the pancreas in the male more susceptible to virus-induced injury. In this connection, it is known that EMC virus produces more severe disease and higher mortality in males than females (16). The observation that hyperglycemia persists in the males for many months after infectious virus can be recovered from the pancreas suggests that the infection produces acute beta cell damage that in some cases results in permanent impairment of function. The possibility does exist, however, that the virus may persist in the pancreas of these animals at low levels or in a latent form.

The demonstration that EMC virus can produce in laboratory animals a picture that metabolically resembles diabetes adds credence to the hypothesis that viral infections might be a cause of some forms of diabetes in man (2, 17). Viruses that subtly or grossly damage the pancreas of animals or man would be good candidates for further study. It is evident from our experiments, however, that the development of diabetes is not only dependent on the nature of the virus, but on the complex interactions between the virus and the host.

SUMMARY

Infection of DBA/2N male mice with encephalomyocarditis virus resulted in a diabetes-like syndrome characterized by hyperglycemia, glycosuria, hypoinsulinemia, polydipsia, and polyphagia. Blood glucose levels were elevated within 4 days after infection and reached a maximum mean level of 320 mg/100 ml within 12 days. Approximately 60–80% of the animals developed a transient hyperglycemia while 10–15% of the animals remained hyperglycemic for well over 6 mo. The remaining animals failed to become hyperglycemic but many had abnormal glucose tolerance curves. Hyperglycemia was most pronounced when animals were allowed free access to food, and the incidence of hyperglycemia was related both to the strain and sex of the animals, with few females developing hyperglycemia. The amount of immunoreactive insulin in the plasma of infected hyperglycemic mice was significantly lower than in appropriate controls, and injection of exogenous insulin resulted in a rapid drop in the blood glucose levels. Despite the fact that certain animals were hyperglycemic for many months, virus could not be recovered from the pancreas after the first 10 days of the infection.

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