

VIRUS-INDUCED DIABETES MELLITUS  
XVIII. Inhibition by a Nondiabetogenic Variant of  
Encephalomyocarditis Virus

BY JI-WON YOON, PATRICK R. McCLINTOCK, TAKASHI ONODERA, AND  
ABNER LOUIS NOTKINS

*From the Laboratory of Oral Medicine, National Institute of Dental Research, National Institutes of  
Health, Bethesda, Maryland 20205*

The M variant of encephalomyocarditis (EMC)<sup>1</sup> virus produces a diabetes-like syndrome in mice by infecting and destroying pancreatic beta cells (1-3). The severity of the diabetes correlates with the degree of virus-induced beta cell damage (4, 5). Only certain inbred strains of mice develop diabetes, and susceptibility to EMC virus-induced diabetes is inherited as an autosomal recessive trait (2, 6-8). The genetic factors controlling susceptibility operate at the level of the beta cell, and whether a particular strain of mouse develops diabetes appears to be related to differences in the permissiveness of beta cells to infection with EMC virus (9, 10).

Previous experiments (5) showed that when mice were inoculated with a high concentration of mouse-passaged EMC virus (10<sup>6</sup> plaque-forming units [PFU]), fewer animals developed diabetes than when inoculated with a low concentration of the same virus (10<sup>2</sup> PFU). Moreover, the diabetogenic capacity of the virus was markedly diminished after passage in mouse fibroblast cultures, but was restored when passaged in mice. This raised the possibility that the stock pool of EMC virus was made up of two populations of virus: one that had a tropism for beta cells and produced diabetes and the other that did not have a tropism for beta cells and was nondiabetogenic (5).

The present investigation was initiated to see, first, whether our stock pool of the M variant of EMC virus was made up of a mixture of diabetogenic and nondiabetogenic virus and, second, whether the nondiabetogenic virus inhibited the development of diabetes.

#### Materials and Methods

*Mice.* Unless otherwise indicated, SJL/J male mice, 5-6 wk old, obtained from The Jackson Laboratory, Bar Harbor, Maine, were used in all experiments. Animals were inoculated with virus by the intraperitoneal route.

*Pancreatic Beta Cell Cultures.* Pancreata were aseptically removed from suckling SJL/J mice, and beta cell cultures were prepared as described previously (9). The cultures were refed at 2-d intervals, and at 6 d the monolayers were used to passage virus. Staining of the monolayers with fluorescein isothiocyanate-labeled antibody to insulin indicated that 40-70% of the cells were beta cells (9).

*Virus.* The M variant of EMC virus (1), prepared as described elsewhere, was passaged five

<sup>1</sup> *Abbreviations used in this paper:* EMC, encephalomyocarditis; FITC, fluorescein isothiocyanate; IRI, immunoreactive insulin; PFU, plaque-forming units; SME, secondary mouse embryo; VSV, vesicular stomatitis virus.

times in beta cell cultures (9). The virus then was passaged five additional times in SJL/J male mice and harvested from hearts. Virus titer was determined by plaque assay on secondary mouse embryo (SME) cells (9).

*Plaque Purification.* The M variant of EMC virus, prepared as described above, was diluted to a concentration of 1 PFU/ml, and 0.1-ml aliquots were allowed to adsorb to monolayers of SME cells grown in 16-well cluster dishes (9-mm-diameter wells). After adsorption for 1 h at 37°C, cultures were overlaid with 1.5% (wt/vol) methylcellulose in Eagle's minimal essential medium that contained 5% fetal bovine serum. Monolayers were stained 2 d later with a 1:20,000 dilution of neutral red. When examined 24 h later, 7 of the 240 wells contained a single clone. The medium and the remaining cell monolayers were removed from these seven wells, diluted with fresh medium, and aliquots were inoculated onto confluent monolayers of SME cells. 2 d later, the monolayers showed extensive cytopathology, and the medium and cells were harvested. Six of the seven clones were tested for their ability to produce diabetes in mice and two of these clones, one diabetogenic and the other nondiabetogenic, were plaque-purified two additional times. In brief, material from the original clones was diluted, inoculated onto confluent monolayers of SME cells, overlaid with methylcellulose medium, and stained with neutral red. Monolayers showing a single distinct circular plaque were selected, and plaques from these plates were harvested with a micropipette. This material was diluted in medium, passaged in SME cells, and tested in mice. The plaque purification procedure was repeated a third time.

*Glucose and Insulin Assays.* Glucose levels (plasma) were measured, and glucose-tolerance tests were performed as described previously (7). Nonfasting glucose levels were measured 7 and 14 d after infection, and single-point 60-min glucose-tolerance tests were performed 10 and 17 d after infection. The data obtained on these 4 d were used to calculate the glucose index (7) for each mouse. The mean glucose index of 110 uninfected mice was  $145 \pm 19$  mg/dl. Any mouse with a glucose index  $>240$  mg/dl (5 SD above the mean) was scored as diabetic. In some experiments, only nonfasting glucose levels were determined. The mean nonfasting glucose of 69 uninfected mice was  $143 \pm 21$  mg/dl. In these experiments, any mouse with a nonfasting glucose  $>248$  mg/dl (5 SD above the mean) was scored as diabetic. The concentration of immunoreactive insulin (IRI) in the pancreas and plasma of infected and uninfected mice was measured by radioimmunoassay (11, 12).

*Fluorescein Isothiocyanate (FITC)-labeled Anti-EMC Virus Antibody.* Mice were immunized intraperitoneally every week with increasing doses of EMC virus beginning with  $2.0 \times 10^5$  PFU and ending with  $2.0 \times 10^6$  PFU. Serum obtained after five or more injections was precipitated by ammonium sulfate, passed through a DEAE cellulose column, and the IgG fraction collected and labeled with FITC (4, 13). Tissue sections were prepared, stained and examined as described previously (5). Sections from uninfected animals served as controls, and the specificity of the labeled immunoglobulin was established by inhibition tests with unlabeled anti-EMC virus serum.

*Interferon Assay.* Interferon in the supernatant fluids of cell cultures or sera from mice was assayed as described previously (14). In brief, monolayers of L-929 cells in microplates were incubated for 5 h at 37°C with serial twofold dilutions of samples in a 0.1-ml vol. The cells then were challenged with 30–40 PFU of vesicular stomatitis virus (VSV). At the end of 1 h, the virus inoculum was removed, the cells were washed three times, and refed with media supplemented with 0.7% methylcellulose. Plaques were counted 48 h later. The interferon titer was expressed as the reciprocal of the highest dilution that reduced VSV plaques by 50%.

## Results

*Selection of Variants.* To see whether the original virus pool contained a mixture of diabetogenic and nondiabetogenic virus, individual plaques were selected, cloned, and inoculated into mice. The data in Table I show that two clones (82 and 108) produced diabetes, two (5 and 16) produced only minimal changes in glucose, and two (125 and 162) gave intermediate results. Clones 108 and 16 were plaque-purified two more times and clones 108-D2 (designated D variant) and 16-B1 (designated B variant) were studied in greater depth. These latter viruses have now been passaged

TABLE I  
Isolation of Diabetogenic and Nondiabetogenic Variants

Virus	Glucose index* (Mean $\pm$ SD)	Diabetes %
EMC-M	252 $\pm$ 136	47
Clones‡		
5	191 $\pm$ 44	15
82	447 $\pm$ 75	95
125	213 $\pm$ 89	45
162	209 $\pm$ 43	30
16	174 $\pm$ 45	10
A	170 $\pm$ 64	15
B	159 $\pm$ 57	5
C	163 $\pm$ 59	25
D	166 $\pm$ 53	15
E	176 $\pm$ 68	20
F	170 $\pm$ 60	10
B-1	164 $\pm$ 12	0
B-2	170 $\pm$ 12	0
B-3	166 $\pm$ 15	0
108	441 $\pm$ 49	100
A	349 $\pm$ 135	75
B	390 $\pm$ 145	77
C	332 $\pm$ 121	70
D	430 $\pm$ 126	100
E	292 $\pm$ 120	55
F	414 $\pm$ 126	90
G	198 $\pm$ 75	21
H	164 $\pm$ 52	10
D-1	372 $\pm$ 61	100
D-2	430 $\pm$ 51	100
D-3	368 $\pm$ 65	95
Uninfected	145 $\pm$ 19	0

\* The mean glucose index of 110 uninfected SJL/J male mice was 145  $\pm$  19 mg/dl. Any mouse with a glucose index >240 mg/dl, which was 5 SD above the mean, was scored as diabetic.

‡ Each clone was inoculated into ~20 mice.

at least eight times in SME cells and have retained their diabetogenic and nondiabetogenic properties.

*Metabolic Alterations.* Fig. 1 shows that when mice were inoculated with the D variant of EMC virus, the mean glucose level reached 500 mg/dl within 4 d after infection and remained elevated for the duration of the experiment. In contrast, mice inoculated with the B variant showed no elevation of blood glucose as compared with uninfected controls. As seen in Fig. 2 A, the concentration of IRI in the pancreata of mice infected with the D variant was inversely related to the glucose level. At 3 d after infection, pancreatic IRI was approximately one-tenth that of uninfected animals or animals infected with the B variant. Plasma IRI of mice infected with the D variant showed an initial increase at 2 d after infection (Fig. 2 B), presumably a result of the release of insulin from virus-infected beta cells. This was followed by a marked

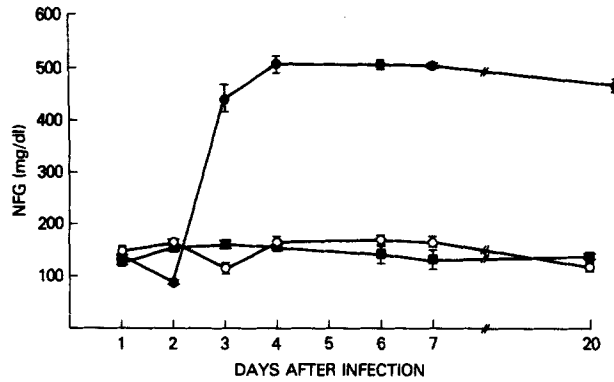


FIG. 1. Blood glucose levels of mice infected with the D or B variant of EMC virus. Each mouse received  $10^6$  PFU of virus. At the times indicated, the mice were bled, and nonfasting glucose (NFG) levels were determined. Each point represents the mean of 10 animals; vertical bars are the SEM. D variant (●), B variant (○), and uninfected controls (■).

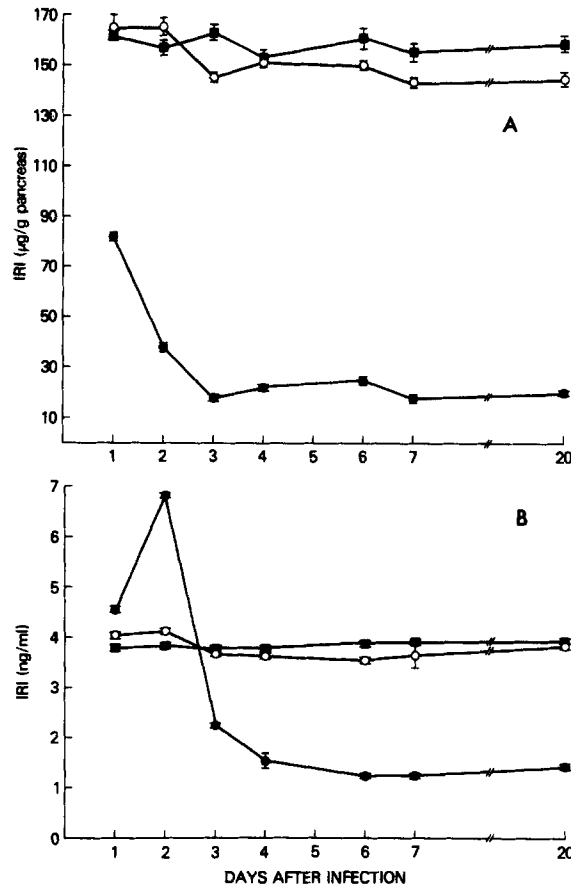


FIG. 2. IRI in the pancreas (A) and plasma (B) of the mice infected with  $10^6$  PFU of the D or B variant of EMC virus. Each point represents the mean of 10 mice; vertical bars are the SEM. D variant (●), B variant (○), and uninfected controls (■).

decrease in IRI to approximately one-third that of uninfected animals or animals infected with the B variant. The IRI in the pancreas and plasma remained depressed throughout the experiment.

*Histopathologic Changes.* To see whether the differences in glucose and insulin levels were related to the degree of virus-induced histopathology, sections of pancreata were prepared at different times after infection and examined microscopically. In general, islets from mice infected with the B variant showed little pathologic change (Fig. 3 A). In contrast, islets from mice infected with the D variant showed extensive destruction of beta cells (Fig. 3 B). Approximately 60% of the islets from mice infected with the D variant showed lymphocytic infiltration and moderate to severe destruction of the islets of Langerhans. Another 20% of the islets showed mild alterations. In contrast, <10% of the islets from mice infected with the B variant showed mild inflammatory changes.

*Viral Replication.* Evidence that the destruction of the islets by the D variant but not by the B variant was a result of differences in the capacity of these viruses to infect and replicate in beta cells, was obtained by immunofluorescence (Figs. 3 and 4). When beta cells from mice infected with the D variant were stained with fluorescein-labeled antibody to EMC virus, ~60% of cells in the islets contained viral antigens, as compared with <5% of the cells in islets from animals infected with the B variant. Moreover, by measuring infectious virus, 10–100 times more virus was recovered from islets of mice infected with the D variant as compared with the B variant (Fig. 5 A). Similar differences were found in the hearts of infected mice (Fig. 5 B).

*Immunologic Response.* To see whether the differences in viral replication might be related to the time of appearance or titer of neutralizing antibody, mice were infected with the B or D variants, and antibody titers were determined at different times after infection. Fig. 6 shows that neutralizing antibody could be detected within 4 d after infection, but there was no difference in the time of appearance or titer of antibody when the responses to the B and D variants were compared. Moreover, the B and D variants are antigenically quite similar (Table II). Antibody made against the D variant neutralized the D and B variants to about the same degree. Similarly, antibody made against the B variant neutralized the B and D variants equally well.

*Inhibition by the B Variant of the Induction of Diabetes by the D Variant.* To see whether the nondiabetogenic B variant had any effect on the induction of diabetes by the D variant, the two viruses were mixed at varying ratios and inoculated into SJL/J mice. The data in Table III show that as little as  $10^2$  PFU of the D variant induced diabetes in 77% of the mice. Between 95 and 100% of the animals developed diabetes when inoculated with  $\geq 10^4$  PFU. In contrast, none of the animals developed diabetes when inoculated with up to  $10^5$  PFU of the B variant. When, however, the B variant was inoculated with the D variant, at a 1:1 ratio, only 60% of the mice developed diabetes, as compared with 95% with D alone. When the B and D variants were mixed at a ratio of 9:1,  $\leq 11\%$  of the mice developed diabetes, and none developed diabetes when the B and D variants were mixed at a ratio of 99:1.

In other experiments, mice were first inoculated with the B variant ( $5 \times 10^5$  PFU) and then given the D variant ( $5 \times 10^5$  PFU) at different times thereafter. The data in Table IV show that none of these animals developed diabetes.

*Mechanism of Inhibition of the Induction of Diabetes.* The B variant might inhibit the

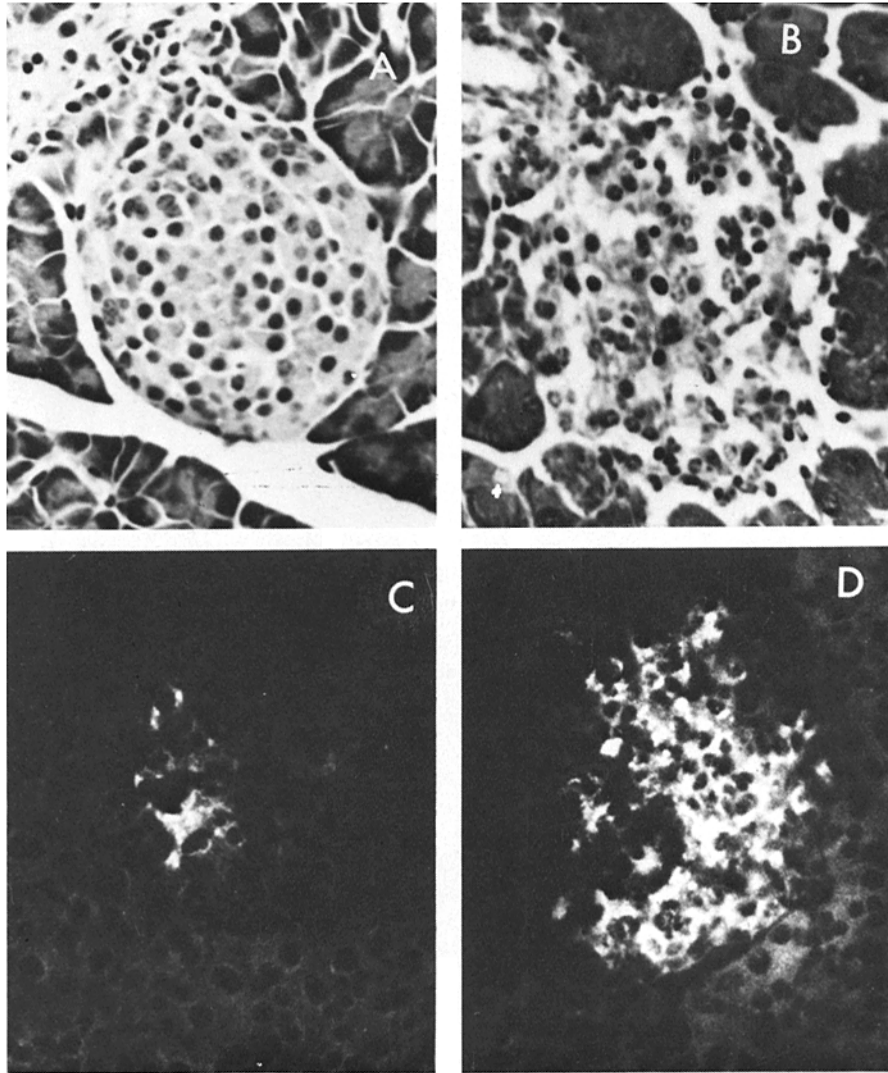


FIG. 3. Sections of pancreas from mice infected with the B or D variant of EMC virus. (A) Section taken 7 d after infection with the B variant showing little or no inflammation in the islets of Langerhans. Hematoxylin and eosin stained.  $\times 550$ . (B) Section taken 7 d after infection with the D variant showing extensive inflammatory infiltrate in the islets of Langerhans and beta cell necrosis. Hematoxylin and eosin stained.  $\times 550$ . (C) Section obtained 3 d after infection with the B variant and stained with FITC-labeled anti-EMC virus antibody. Only a few cells in the islets contain viral antigens.  $\times 340$ . (D) Section obtained 3 d after infection with the D variant and stained with FITC-labeled anti-EMC virus antibody. The majority of the cells in the islets contain viral antigens. The surrounding acinar cells show little fluorescence.  $\times 340$ .

induction of diabetes by the D variant if the immune response to the former occurred more rapidly or resulted in higher antibody titers. The data in Fig. 6 and Table II show that this is not the case. Further evidence that the immune response to the B variant is not responsible for inhibition of diabetes by the D variant comes from cell culture experiments that bypass the immune response. SME cells were infected with

## VIRUS-INDUCED DIABETES MELLITUS

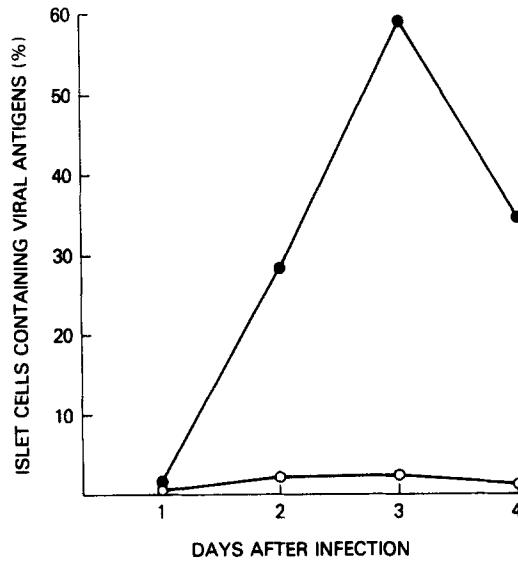


FIG. 4. Percentage of cells containing viral antigens in the islets of Langerhans of mice infected with the B or D variant of EMC virus. Mice were infected with  $5 \times 10^6$  PFU of virus, and at different times thereafter, sections of pancreas were stained with FITC-labeled anti-EMC virus antibody, and the percentage of islet cells containing viral antigens was determined. At each time point, between 1,500 and 2,500 cells were examined. D variant (●) and B variant (○).

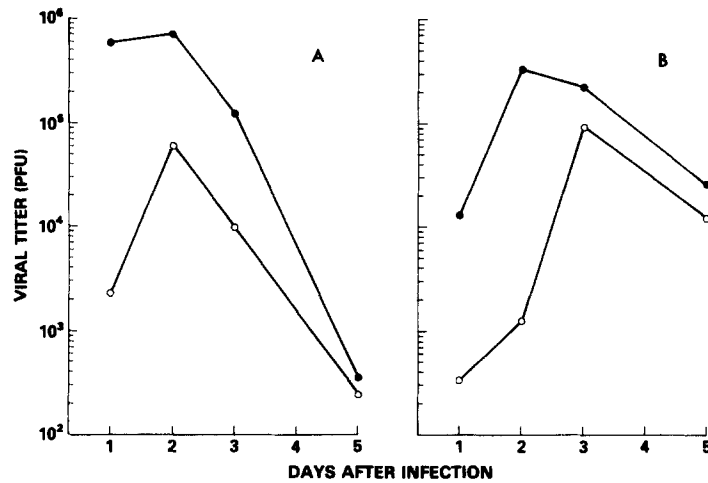


FIG. 5. Comparison of viral replication in the islets of Langerhans (A) and heart (B) of mice infected with the B or D variant of EMC virus. Mice were inoculated intraperitoneally with  $10^6$  PFU of virus. At the times indicated, pancreata were removed from two mice, the islets were isolated, homogenized, and assayed for infectious virus on SME cells (9). Similarly, hearts were removed from the same mice, homogenized, and assayed for infectious virus. The data represent the mean of the individual determinations. D variant (●) and B variant (○).

the B variant, the D variant, or a 1:1 mixture of the B and D variants. As seen in Fig. 7 A, close to 50 times more virus was produced in cultures infected with the D as compared with the B variant. Moreover, the mixture of the D and B variants produced considerably less infectious virus than the D variant alone. When the supernatant

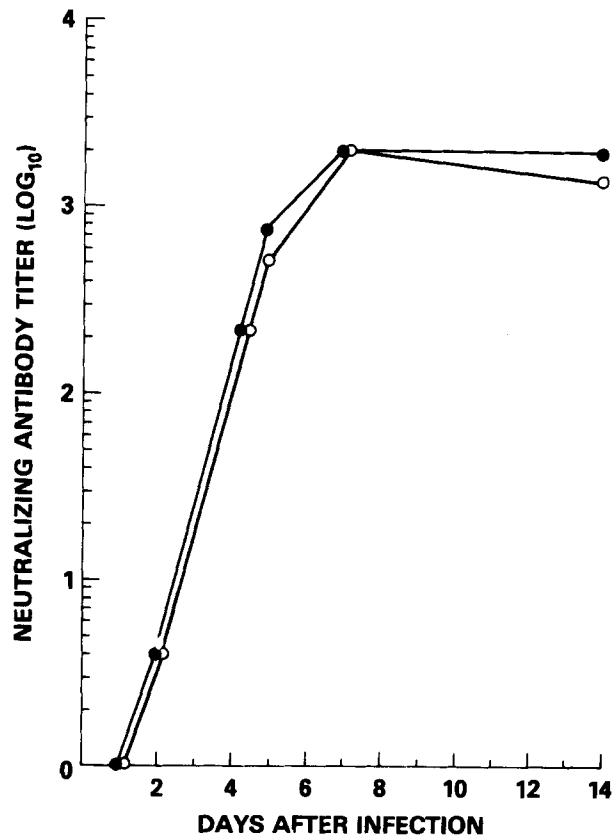


FIG. 6. Titer of neutralizing antibody in the serum of mice infected with the D or B variant of EMC virus. Mice were inoculated intraperitoneally with  $10^6$  PFU of virus. At the times indicated, three mice from each group were bled, the sera pooled, and antibody titers were determined. The data represent the mean of the two separate experiments. D variant (●) and B variant (○).

TABLE II  
*Serologic Relationship of the D and B Variants of EMC Virus*

Antiserum*	Variants (Neutralizing Antibody Titer)‡	
	D	B
Anti-D	2,560	1,280
Anti-B	5,120	2,560

\* For the preparation of antiviral antibody, rabbits were immunized intraperitoneally every week with increasing doses of the D or B variants beginning with  $5 \times 10^6$  PFU and ending with  $5 \times 10^7$  PFU. Sera were obtained after seven injections.

‡ Neutralization titers are expressed as the reciprocal of the highest dilution of serum that inhibited plaque formation by 50%.

fluids were examined for interferon (Fig. 7 B), the D variant induced little if any interferon ( $<10$ ), whereas substantial amounts of interferon were induced within 12 h by the B variant. The mixed pool, which contained both the B and D variants, induced intermediate amounts of interferon. Although there was a marked difference



TABLE III  
Inhibition of Virus-Induced Diabetes by the Nondiabetogenic B Variant\*

Virus		B:D ratio	Glucose index‡ (Mean ± SD)	Diabetes
D	B			
<i>PFU/mouse</i>				%
$1.0 \times 10^6$			461 ± 79	100
$1.0 \times 10^5$			439 ± 67	100
$1.0 \times 10^4$			396 ± 79	95
$1.0 \times 10^3$			342 ± 97	80
$1.0 \times 10^2$			318 ± 79	77
	$1.0 \times 10^5$		155 ± 16	0
	$1.0 \times 10^4$		159 ± 19	0
$5.0 \times 10^4$	$5.0 \times 10^4$	1	312 ± 108	60
$5.0 \times 10^3$	$5.0 \times 10^3$	1	242 ± 86	50
$1.0 \times 10^4$	$9.0 \times 10^4$	9	189 ± 89	11
$1.0 \times 10^3$	$9.0 \times 10^3$	9	176 ± 29	5
$1.0 \times 10^2$	$9.0 \times 10^2$	9	163 ± 17	0
$1.0 \times 10^3$	$9.9 \times 10^4$	99	156 ± 15	0
$1.0 \times 10^2$	$9.9 \times 10^4$	999	152 ± 9	0
Uninfected			152 ± 16	0

\* SJL/J mice were inoculated with the D variant alone, the B variant alone, or a mixture of B and D at different ratios.

‡ Each group contained between 10 and 20 mice.

TABLE IV  
Inoculation of D Variant at Different Times After the B Variant

Days after B variant*	Glucose index‡ (Mean ± SD)	Diabetes
		%
1	146 ± 20	0
2	164 ± 14	0
3	153 ± 20	0
4	152 ± 14	0
5	150 ± 15	0
6	148 ± 22	0
7	149 ± 11	0
D variant alone	405 ± 84	100
B variant alone	143 ± 35	0
Uninfected	151 ± 12	0

\* SJL/J mice were inoculated with  $5 \times 10^5$  PFU of the B variant and at different times thereafter challenged with  $5 \times 10^5$  PFU of the D variant.

‡ Each group contained 10 mice.

in the induction of interferon by the B and D variants, both of these variants were equally sensitive to the antiviral action of mouse interferon when tested on SME cells (data not shown).

To see whether the B and D variants induced different amounts of interferon *in vivo*, mice were infected, and, at various times thereafter, sera were drawn and assayed for interferon. Fig. 8 shows that substantial amounts of interferon were induced by the B variant within 10 h. In contrast, peak interferon titers were not reached until ~30 h after infection with the D variant, and the maximum titer was approximately

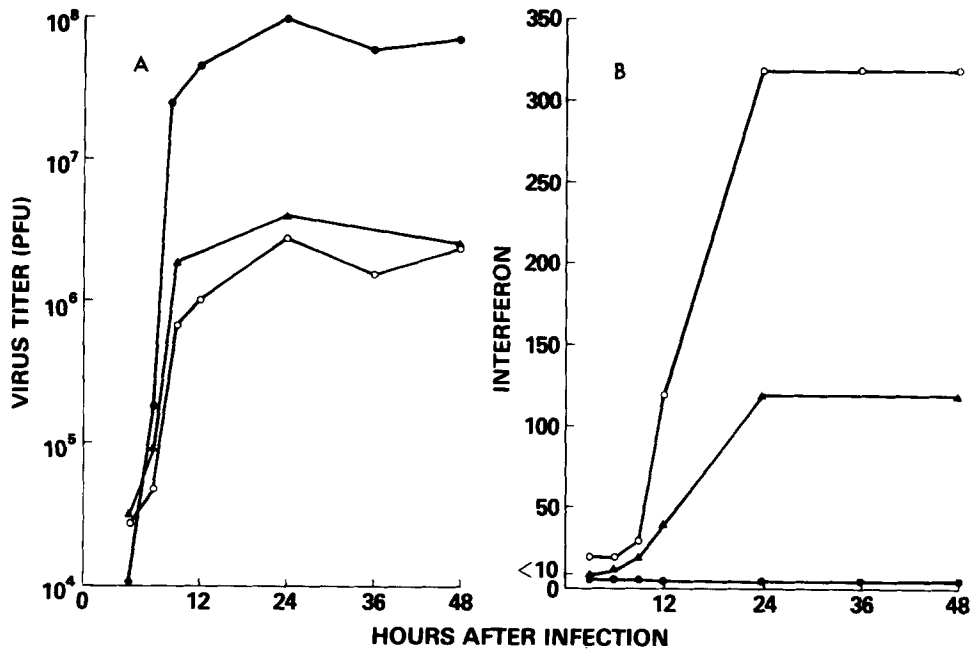


FIG. 7. Viral replication and interferon titer in cultures infected with the B or D variant of EMC virus. Confluent monolayers of SME cells in 60-mm plates were inoculated with the D variant ( $5 \times 10^7$  PFU), the B variant ( $5 \times 10^7$  PFU), or a 1:1 mixture of the two ( $2.5 \times 10^7$  PFU of each). The multiplicity of infection was  $\sim 10$ . At the end of 1 h, the monolayers were washed, and at different times thereafter, supernatant fluids were assayed for infectious virus (A) or interferon (B). Each point represents the mean of duplicate plates. D variant (●), B variant (○), and mixture of B and D (▲).

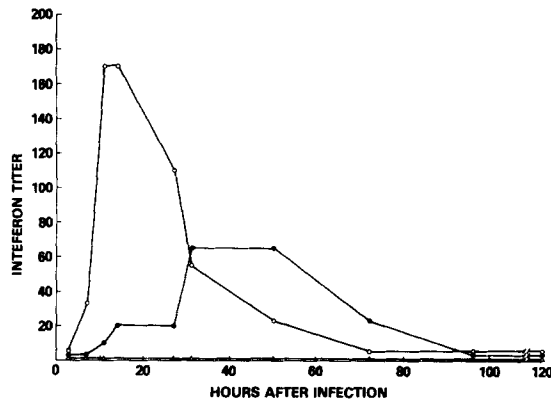


FIG. 8. Interferon levels in the circulation of mice infected with the B or D variant of EMC virus. Mice were infected with  $5 \times 10^5$  PFU of virus. At the times indicated, three mice from each group were bled, sera pooled, and interferon titers were determined. The data represent the mean of two experiments. D variant (●), B variant (○), and uninfected controls (Δ).

one-third that with the B variant. These findings suggest that induction of interferon by the B variant may inhibit replication of the D variant, thereby preventing the development of diabetes.

*Host Differences in the Development of Diabetes.* Earlier studies showed that only

certain inbred strains of mice developed diabetes when infected with the M variant of EMC virus (2, 6-8). The demonstration here that the M variant represents a mixture of the B and D variants raised the possibility that the D variant alone might have a different host spectrum. To test this possibility, different inbred strains of mice were infected with the D variant, and blood glucose indexes were determined. Table V shows that certain strains of mice developed diabetes, whereas others did not. The spectrum of host susceptibility, in general, was similar to that observed previously with the M variant (6-8), except the glucose indexes and the percentage of animals developing diabetes were higher with the D variant. In previous experiments, the A/J strain of mice showed abnormal glucose tolerance tests but were not diabetic, whereas in the present experiment ~50% of the A/J mice developed diabetes.

### Discussion

The experiments reported here show that our stock pool of EMC virus contains two variants: one diabetogenic and the other nondiabetogenic. The diabetogenic D variant infects and destroys pancreatic beta cells, thus resulting in hypoinsulemia and hyperglycemia, whereas the nondiabetogenic B variant causes little, if any, beta cell damage.

Why the D variant, but not the B variant, infects and destroys pancreatic beta cells is still not known. One possibility is that differences exist in the capsid polypeptides of the B and D variants. Viral receptors on the surface of beta cells may recognize these differences allowing the D but not the B variant to infect beta cells. Another possibility is that the host responds immunologically more rapidly to the B than to the D variant, thereby eliminating the infection before substantial beta cell damage has occurred. However, our experiments indicate that the time of appearance and magnitude of the immune response to the D and B variants are quite similar. A third possibility, presently under investigation, is that the higher interferon titers induced by the B variant in the circulation of mice actually protects beta cells from infection by the B variant.

TABLE V  
*Induction of Diabetes by the D Variant in Different Inbred Strains of Mice\**

Strain‡	Glucose index (Mean ± SD)	Diabetes %
SWR/J	436 ± 102	100
DBA/2J	376 ± 96	95
DBA/2J (♀)	177 ± 32	25
NIH/Swiss	259 ± 81	73
NFS/N	230 ± 78	52
A/J	234 ± 101	50
C3H/HeJ	171 ± 30	14
C57BL/6J	143 ± 25	0
AKR/J	129 ± 11	0
CBA/J	148 ± 20	0
LP/J	135 ± 13	0
CE/J	152 ± 14	0

\* Mice were injected with  $10^5$  PFU of the D variant. Each group contained ~20 mice.

‡ All mice were males, except where indicated (♀).

Antigenically, the B and D variants cannot be distinguished by the sensitive plaque-neutralization assay. Competition radioimmunoassays also have failed to find any major differences between these two variants, although small antigenic differences have not been excluded (P. R. McClintock. Unpublished results.). Thus far, molecular hybridization studies with a [<sup>3</sup>H]cDNA probe of the D variant have failed to distinguish the D and B variants (G. S. Aulakh. Personal communication.). Nonetheless, these variants differ biologically not only in their capacity to produce diabetes, but also in the titer of virus produced and the amount of interferon induced. Current RNA-fingerprinting studies may prove more sensitive in identifying differences in the nucleotide sequences of the two variants.

Of particular interest was the observation that the nondiabetogenic B variant inhibits the induction of diabetes by the D variant. The most likely explanation is that the rapid induction of interferon by the B variant protects beta cells from infection by the D variant, especially when both viruses are inoculated at the same time. The B variant also inhibited the induction of diabetes if inoculated from 1 to 7 d before the D variant (Table IV). Because, in our experiments, interferon disappeared from the circulation within 4 d after inoculation of the B variant (Fig. 8), the protection observed after 4 d is most likely a result of the presence of cross-reacting neutralizing antibody. Thus, interferon and antibody, acting alone or in combination, appear to contribute to the B variant-induced inhibition of diabetes.

It has been known for some time that only certain inbred strains of mice are susceptible to EMC virus-induced diabetes (2, 6-8). The present study with the D variant supports this observation. Moreover, the hyperglycemia caused by the D variant was often more severe than that observed in earlier experiments with our stock EMC virus pool, which suggests that, in those experiments, the B variant may have inhibited the development of diabetes. It also has been proposed that the genetic differences in susceptibility might be related to the number or type of EMC virus receptors on the surface of cells (15, 16). The inhibition of diabetes by interferon raises still another possibility: that genetic factors might control the host's interferon response to infection with the D variant. Ongoing experiments, however, indicate that the time of appearance and amount of interferon produced in response to the D variant is approximately the same in diabetes-resistant C57BL/6J mice and diabetes-prone SJL/J mice. Interferon, nonetheless, may be important if beta cells from C57BL/6J mice, as compared with SJL/J mice, are more sensitive to the antiviral action of interferon and are thus rendered resistant to infection and the subsequent development of diabetes. Differences in the sensitivity of certain cells to the antiviral action of interferon have been demonstrated in other systems (17, 18).

Whether viruses play anything more than a minor role in the etiology of insulin-dependent diabetes mellitus remains to be established (3, 19, 20), but the experiments with D and B variants illustrate some of the potential difficulties that might be encountered in attempting to isolate and identify diabetogenic viruses in the human population. First, by neutralization tests, it was not possible to distinguish the diabetogenic from the nondiabetogenic variant of EMC virus. Similar problems might very well be encountered when searching for diabetogenic variants of common human viruses by standard serologic techniques. Second, because our pool of EMC virus

contained a mixture of diabetogenic and nondiabetogenic variants that could not be distinguished antigenically or morphologically, each virus plaque had to be tested individually for diabetes in animals. If the nondiabetogenic particles in the pool had greatly outnumbered the diabetogenic particles, the chance of isolating the diabetogenic variant would have been greatly reduced. In our EMC virus pool, the two variants appear to have been present in nearly equal amounts; and, once the diabetogenic variant was plaque purified, it remained stable on passage in SME cells. In contrast, attempts to isolate and plaque purify a diabetogenic variant from a pool of Coxsackie virus B4 that produces diabetes in mice (21) has thus far been unsuccessful (A. Toniolo and G. W. Jordan. Unpublished data.). Presumably, the nondiabetogenic virus is in great excess. Third, as demonstrated in the present report, diabetogenic variants in virus pools may not always be detected when inoculated in animals because of interference produced by nondiabetogenic variants. Moreover, passage in certain tissue-culture cell lines of virus pools that contain a mixture of variants might favor replication of the interfering or non-beta tropic variant. This may account for the poor, and sometimes changing, diabetogenicity of some of our virus pools (5). Lastly, the studies with EMC virus have shown that only certain inbred strains of mice will develop diabetes and that susceptibility is genetically controlled (6–10). Although the strains of mice that are diabetes prone or diabetes resistant are known for EMC virus, other strains of mice or species may be required to demonstrate the potential diabetogenicity of human isolates.

### Summary

Plaque purification of the M variant of encephalomyocarditis (EMC) virus resulted in the isolation of two stable variants: one diabetogenic and designated D and the other nondiabetogenic and designated B. When the D variant was inoculated into SJL/J male mice, hypoinsulinemia and hyperglycemia developed in >90% of the animals. In contrast, none of the mice inoculated with the B variant developed diabetes. Histologic examination of pancreata from mice infected with the D variant revealed insulinitis and necrosis of beta cells, whereas islets from mice infected with the B variant showed little, if any, change. When islets were assayed for infectious virus, ~10 times more virus was recovered from animals inoculated with the D as compared with the B variant. Moreover, ~60% of islet cells from mice infected with the D variant contained viral antigens when stained with fluorescein-labeled anti-EMC virus antibody, whereas <5% of islet cells from animals infected with the B variant contained viral antigens.

Co-infection experiments showed that the induction of diabetes by the D variant was inhibited by the B variant. When the B and D variants were mixed together at B:D ratios of 1, 9, and 99, diabetes developed in 60, 11, and 0% of the mice, respectively. Tissue-culture experiments revealed that the B variant induced considerably more interferon than the D variant, and studies in animals showed that interferon appeared earlier and in greater amounts in the circulation of mice infected with the B as compared with the D variant. These studies suggest that the induction of interferon by the B variant is, at least in part, responsible for the inhibition of diabetes by the D variant.

The authors thank Tecolia Brown, Salvatore Selvaggio, and James Wheeler for technical help and Edith Rian for editorial assistance.

Received for publication 6 June 1980.

### References

1. Craighead, J. E., and M. F. McLane. 1968. Diabetes mellitus: induction in mice by encephalomyocarditis virus. *Science (Wash. D. C.)* **162**:913.
2. Boucher, D. W., and A. L. Notkins. 1973. Virus-induced diabetes mellitus. I. Hyperglycemia and hypoinsulinemia in mice infected with encephalomyocarditis virus. *J. Exp. Med.* **137**:1226.
3. Notkins, A. L. 1979. The causes of diabetes. *Sci. Am.* **241**:62.
4. Hayashi, K., D. W. Boucher, and A. L. Notkins. 1974. Virus-induced diabetes mellitus. II. Relationship between beta cell damage and hyperglycemia in mice infected with encephalomyocarditis virus. *Am. J. Pathol.* **75**:91.
5. Yoon, J. W., T. Onodera, and A. L. Notkins. 1977. Virus-induced diabetes mellitus. VIII. Passage of encephalomyocarditis virus and severity of diabetes in susceptible and resistant strains of mice. *J. Gen. Virol.* **37**:225.
6. Boucher, D. W., K. Hayashi, J. Rosenthal, and A. L. Notkins. 1975. Virus-induced diabetes mellitus. III. Influence of the sex and strain of the host. *J. Infect. Dis.* **131**:462.
7. Ross, M. E., T. Onodera, K. S. Brown, and A. L. Notkins. 1976. Virus-induced diabetes mellitus. IV. Genetic and environmental factors influencing the development of diabetes after infection with the M variant of encephalomyocarditis virus. *Diabetes.* **25**:190.
8. Onodera, T., J. W. Yoon, K. S. Brown and A. L. Notkins. 1978. Evidence for a single locus controlling susceptibility to virus-induced diabetes mellitus. *Nature (Lond.)* **274**:693.
9. Yoon, J. W., and A. L. Notkins. 1976. Virus-induced diabetes mellitus. VI. Genetically determined host differences in the replication of encephalomyocarditis virus in pancreatic beta cells. *J. Exp. Med.* **143**:1170.
10. Yoon, J. W., M. A. Lesniak, R. Fussganger, and A. L. Notkins. 1976. Genetic differences in susceptibility of pancreatic B cells to virus-induced diabetes mellitus. *Nature (Lond.)* **264**:178.
11. Davoren, P. R. 1962. The isolation of insulin from a single cat pancreas. *Biochim. Biophys. Acta.* **63**:150.
12. Hales, C. N., and P. J. Randle. 1963. Immunoassay of insulin with insulin-antibody precipitate. *Biochem. J.* **88**:137.
13. Kawamura, A., Jr. 1977. *Fluorescent Antibody Techniques and Their Applications*. Second edition. University of Tokyo Press, Tokyo. 37.
14. Ida, S., J. J. Hooks, R. P. Siraganian, and A. L. Notkins. 1977. Enhancement of IgE-mediated histamine release from human basophils by viruses: role of interferon. *J. Exp. Med.* **145**:892.
15. Chairez, R., J. W. Yoon, and A. L. Notkins. 1978. Virus-induced diabetes mellitus. X. Attachment of encephalomyocarditis virus and permissiveness of cultured pancreatic B cells to infection. *Virology.* **85**:606.
16. McClintock, P. R., L. Billups, and A. L. Notkins. Receptors for encephalomyocarditis virus on murine and human cells. *Virology*. In press.
17. Hanson, B., H. Koprowski, S. Barson, and C. E. Buckler. 1969. Interferon-mediated natural resistance of mice to arbo B virus infection. *Microbios.* **1B**:51.
18. Haller, O., H. Arnheiter, J. Lindenmann, and I. Gresser. 1980. Host gene influences sensitivity to interferon action selectively for influenza virus. *Nature (Lond.)* **283**:660.

19. Yoon, J. W., M. Austin, T. Onodera, and A. L. Notkins. 1979. Virus-induced diabetes mellitus: isolation of a virus from the pancreas of a child with diabetic ketoacidosis. *N. Engl. J. Med.* **300**:1173.
20. Jenson, A. B., H. S. Rosenberg, and A. L. Notkins. Virus-induced diabetes mellitus. XVII. Pancreatic islet cell damage in children with fatal virus infections. *Lancet*. In press.
21. Yoon, J. W., T. Onodera, and A. L. Notkins. 1978. Virus-induced diabetes mellitus. XV. Beta cell damage and insulin-dependent hyperglycemia in mice infected with Coxsackie virus B4. *J. Exp. Med.* **148**:1068.