tration in MEM had no effect on the initial growth rate but did cause a significant lowering of the final cell density. The relative acceptance of the three preparations for histidine and leucine are shown in Table 2. Clearly, incubation in Eagle's MEM has no effect on the relative histidine acceptance of tRNA, whether the cells are in the growing or the resting state. However, in cells that have been grown up to and maintained in the resting state in medium with 0.2 times the concentration of histidine in MEM, the relative concentrations of $tRNA^{His}$ doubled, whereas the relative concentrations of leucine tRNA ($tRNA^{Leu}$) were unchanged. These results show that the increase in relative histidine acceptance requires histidine deprivation and is not simply a nonspecific result of inhibition of cell growth.

Previous results from this laboratory (*2*) showed that isoleucine tRNA and noninitiator methionine tRNA concentrations in reticulocytes from different breeds of anemic sheep are correlated with the isoleucine and methionine contents, respectively, of the hemoglobin being synthesized. Those results suggested that specific tRNA levels are not directly programmed as part of the process of cell differentiation, but rather that they are controlled by a physiological adaptation to the pattern of amino acid utilization by the protein synthesizing apparatus. In this report, we have presented evidence that such a physiological adaptation may involve the extent of aminoacylation as a signal to control the relative levels of specific tRNA's. Whether specific tRNA levels are controlled by varying their synthetic rates (*13*) or by varying their rates of degradation or the rates of activation of inactive precursor tRNA's (*14*) cannot be determined from our data.

MICHAEL LITT
KRISTINA WEISER
Department of Biochemistry and
Division of Medical Genetics, School of Medicine, University of Oregon Health Sciences Center, Portland 97201

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Virus-Induced Diabetes Mellitus: Reovirus Infection of Pancreatic β Cells in Mice

Abstract. *Reovirus type 3, passaged in pancreatic β-cell cultures, produced an insulitis when inoculated into 1- to 2-week-old mice. By means of a double-label antibody technique, in which we used fluorescein-labeled antibody to reovirus and rhodamine-labeled antibody to insulin, reovirus antigens were found in β cells. By electron microscopy, viral particles in different stages of morphogenesis were observed in insulin-containing β cells but not glucagon-containing α cells. The infection resulted in destruction of β cells, reduction in the insulin content of the pancreas, and alteration in the host's capacity to respond normally to a glucose tolerance test.*

The possibility that viruses might be one of the causes of juvenile-onset diabetes mellitus by infecting and destroying pancreatic β cells has received considerable attention. Mumps and members of the coxsackie B group have been the viruses most often suggested as possible causes of juvenile diabetes, but proof that these viruses can actually infect β cells and produce diabetes has not been obtained (*1*). Recently, however, it was shown that at least in vitro, human β cells can be infected and destroyed by mumps virus (*2*) and coxsackie virus B3 (*3*).

Support for the hypothesis that viruses

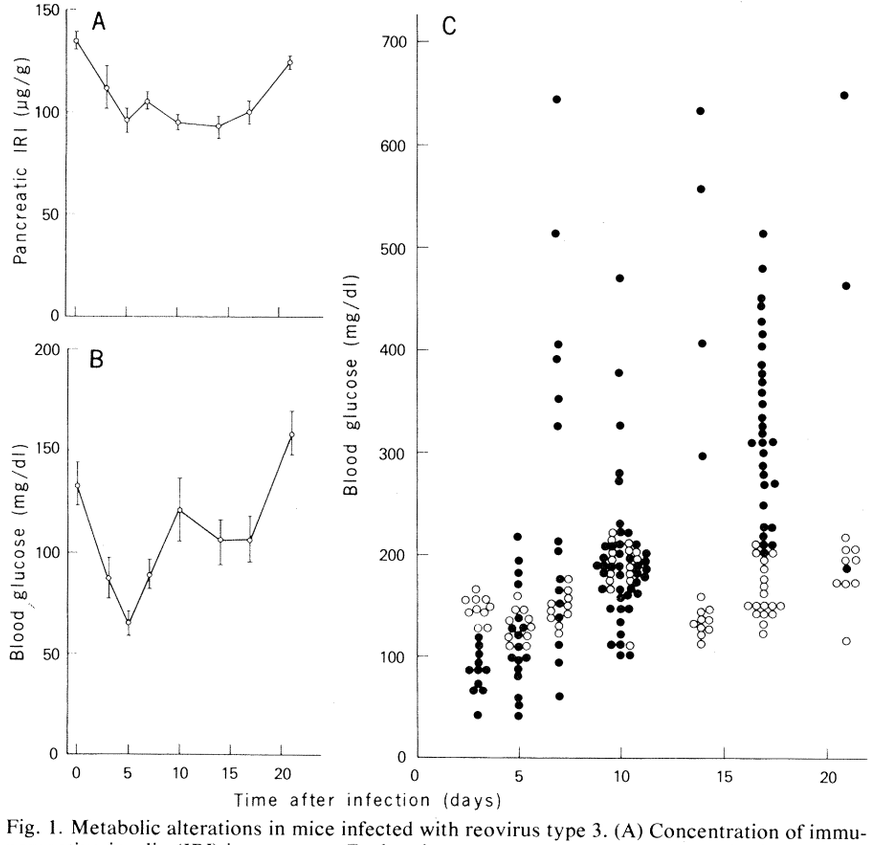

Fig. 1. Metabolic alterations in mice infected with reovirus type 3. (A) Concentration of immunoreactive insulin (*IRI*) in pancreas. Each point represents the mean and standard error of at least six mice. (B) Concentration of glucose in the blood. Each point represents the mean and standard error of at least six mice. (C) Glucose tolerance tests in infected (●) and uninfected (○) mice. The concentration of glucose in the blood was determined 60 minutes after the intraperitoneal administration of 2 mg of glucose per gram of body weight.

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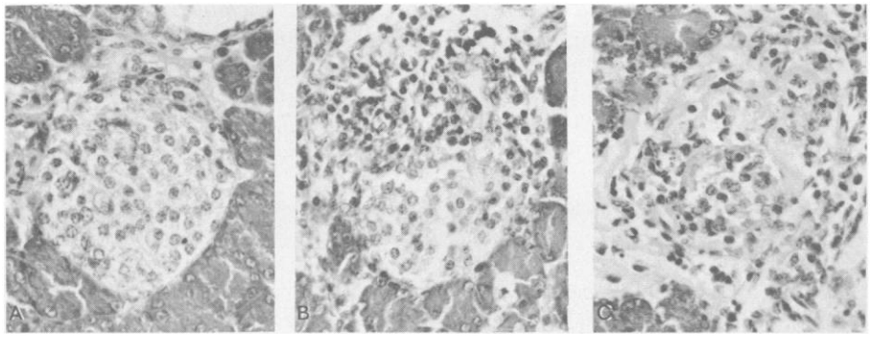
might be one of the causes of juvenile-onset diabetes also comes from studies in animals. In mice, the M variant of encephalomyocarditis virus (EMC) infects pancreatic β cells and produces a diabetes-like syndrome (*1*, *4*). Only certain inbred strains of mice develop EMC-induced diabetes, and susceptibility is genetically controlled (*5*). Attempts to produce diabetes in animals with other viruses have been largely unsuccessful. We now report that a virus which is widely disseminated in the human population, reovirus type 3, can infect mouse β cells and alter the host's capacity to handle glucose.

Reovirus type 3 (Abney strain; American Type Culture Collection) was passaged at least seven times in cultures of pancreatic β cells (*6*) from SJL/J mice (*7*) and assayed on L-cell monolayers (*8*). Mice aged 1 to 2 weeks (*7*) were inoculated intraperitoneally with 1.0×10^5 plaque-forming units of virus. Unless otherwise stated, β-cell-passaged virus was used in all experiments.

Antibody to reovirus was produced in guinea pigs, and the γ-globulin fraction was labeled with fluorescein isothiocyanate (FITC) (*9*). The γ-globulin fraction of antibody to porcine insulin (Miles Laboratories) was labeled with tetramethyl rhodamine isothiocyanate (TRITC) (*9*). Frozen sections of mouse pancreas were examined for reovirus-infected cells and insulin-containing β cells by a double-label antibody technique (*2*, *3*).

Procedures for determining the blood glucose levels, for the glucose tolerance tests (GTT), and radioimmunoassay for pancreatic immunoreactive insulin (IRI) were described previously (*3*, *10*). Pancreatic tissue for electron microscopy was prepared (*11*) and examined with a Philips 201 electron microscope.

Mice were infected with reovirus type 3, and at different times thereafter the concentrations of insulin in the pancreas and glucose in the blood were determined. Within 3 days after infection the concentration of IRI in the pancreas had declined and was reduced by approximately 30 percent at 5 days (Fig. 1A). The IRI remained depressed over the next 10 days and then began to return toward normal levels. Blood glucose levels (Fig. 1B) fell in parallel with the decrease in pancreatic insulin, presumably because of the rapid release of insulin into the blood from damaged β cells (*4*, *12*). Glucose levels then began to return toward normal, and at 21 days after infection were slightly above control values. No evidence of overt hyperglycemia was found. However, a number of the infected animals showed a distinctly abnormal response to a glucose load (Fig. 1C). Abnormal tolerance to glucose was first detected at 7 days after infection; glucose levels of uninfected animals rarely ex-



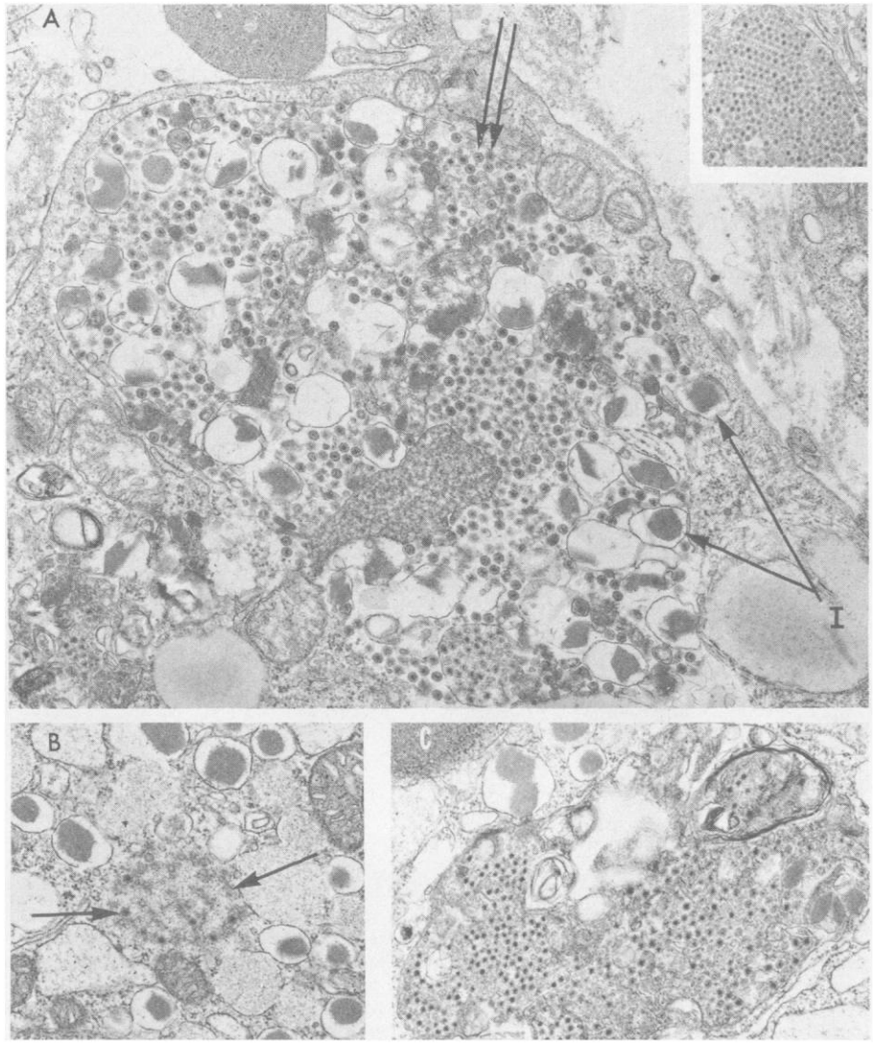


Fig. 2 (top). Pathologic changes in islets of Langerhans 7 days after infection with reovirus type 3. (A) Section of pancreas from infected mouse showing normal appearing islet surrounded by acinar cells. (B) Section of pancreas showing an islet with half of its architecture appearing normal and the other half showing degeneration and necrosis with infiltration of inflammatory cells. (C) Section showing extensive coagulation necrosis with a few cells remaining intact in the central portion of the islet (hematoxylin and eosin; ×550). Fig. 3 (bottom). Electron microscopic changes in β cells of mice 5 days after infection with reovirus. (A) A β cell with reovirus particles (double arrows) interspersed among characteristic insulin granules (*I*) (×31,500). Inset shows reovirus particles in crystalline array from another part of the same cell (×14,500). (B) Reticulogranular viral matrix (arrows) in β-cell cytoplasm characteristic of early stage of reovirus morphogenesis (×28,500). (C) A β cell with reovirus particles sequestrated in cytoplasm by focal cytoplasmic degradation (×28,500).

ceeded 220 mg/dl, whereas the glucose of infected animals often ranged from 250 to 650 mg/dl. Abnormal tolerance to glucose was still apparent in some animals at 17 and 21 days after infection.

Evidence that the metabolic alterations were secondary to reovirus-induced β-cell damage was obtained by light microscopy, immunofluorescence, and electron microscopy. Light microscopy showed that the infection resulted in damage to many, but not all, of the islets within the pancreas (Fig. 2, A to C). As early as 3 days after infection, interstitial edema was seen in areas surrounding the islets. Shortly thereafter, pycnotic nuclei and degenerative cytoplasmic changes were observed within islet cells. Within 7 days after infection, many islets showed focal necrosis (Fig. 2B) and some showed extensive coagulation necrosis with loss of islet architecture (Fig. 2C). Occasional neutrophils, but mostly mononuclear leukocytes, were seen infiltrating the interstitial connective tissue and islets (Fig. 2, B and C). In the exocrine portion of the pancreas, periductal inflammation, together with minimal focal but diffuse necrosis of acinar and ductal cells, were observed.

To see whether the pathologic changes found in the islets were due to replication of the virus in β cells, we used a double-label antibody technique (*2, 3*). Frozen sections of pancreas from animals at day 5 of infection were stained with FITC-labeled antibody to reovirus and TRITC-labeled antibody to insulin (see cover and legend to cover). When fluorescein filters were used, cells containing viral antigens appeared green (cover, left column). When the same fields were examined with rhodamine filters, insulin-containing β cells in the islets of Langerhans appeared orange (cover, middle column). By double-exposure photography (cover, right column), insulin-containing β cells infected with reovirus were readily indentified by their orange and green (or yellow) color. The photomicrographs on the cover represent sections of pancreas from five infected mice and were arranged from top to bottom to show a progressive increase in the number of β cells containing viral antigens. The double-exposure technique also shows that some noninsulin-containing cells (for example, acinar cells, ductal cells, or degranulated β cells) had become infected with reovirus.

Further proof that reovirus type 3 replicates in β cells comes from electron microscopic examination of the islets. Figure 3A shows numerous reovirus particles, 70 nm in diameter, interspersed among insulin granules and in crystalline array (inset) in the cytoplasm of a damaged β cell. A reticulogranular viral matrix, characteristic of active reovirus replication, was sometimes seen among insulin granules (Fig. 3B). Focal cytoplasmic degradation, encapsulating large numbers of reovirus particles, also was found in the cytoplasm of many infected β cells (Fig. 3C). Glucagon-containing α cells also were readily identified in many of the islets (not shown), but thus far viral particles have not been found in these cells.

Reoviruses produce a variety of lesions in newborn mice, including encephalitis, hepatitis, myocarditis, adrenalitis, and acinar pancreatitis (*11, 13*). Infection of the islets of Langerhans, however, had not been observed. By electron microscopy and immunofluorescence, we have now shown that mouse β cells are susceptible to reovirus infection. Moreover, by use of the double-label antibody technique, we have recently found that unpassaged reovirus infects β cells, but that passage of the virus in β-cell cultures markedly enhances this capacity (*14*). The precise explanation for this enhancement is not clear, but passage of the virus in β-cell cultures might have resulted in adaptation or selection of virus, or both, with increased tropism for β cells. The finding by electron microscopy of virus particles in β cells, but not adjacent α cells, suggests that either virus with a tropism for α cells was not selected during passage in culture or that there might be specific receptors for the virus on β cells. Recently, it has been postulated that the σ1 outer capsid polypeptide may be the component of the reovirion that binds to cell surface receptors, and that differences in σ1 may be the basis for differences in cell tropism and neurovirulence among reovirus strains (*15*). Whether the tropism of reovirus for β cells also is due to the σ1 polypeptide remains to be determined.

In mice infected with EMC virus, the severity of the hyperglycemia was shown to correlate with the degree of virus-induced β-cell damage (*16*). Mice infected with reovirus, however, did not become overtly hyperglycemic. This is undoubtedly due to the fact that a substantial reduction in β cells must occur before hyperglycemia becomes overt (*17*). In our experiments, the number of β cells destroyed by reovirus varied considerably, with some islets showing extensive β-cell destruction and others showing little, if any, β-cell destruction. The capacity of many of the animals to handle a large glucose load, however, was clearly impaired by the infection. The failure of reovirus-infected animals to develop more severe or prolonged problems in handling glucose also might be related to the greater generative capacity of β cells from young animals (*18*). It is unlikely that more severe metabolic alterations would occur in older mice, since they are less susceptible to reovirus infection (*19*).

In humans, infants and young children are particularly prone to infection with reoviruses. Seroepidemiologic studies have shown that 50 to 90 percent of the population have antibody to these viruses (*19, 20*). Although sporadic illness has been reported, the infection is generally thought to be asymptomatic or mild (*19, 21*). On the basis of our findings in mice, however, the possibility that reoviruses might infect and damage human β cells merits investigation.

TAKASHI ONODERA
A. BENNETT JENSON
JI-WON YOON
ABNER LOUIS NOTKINS
Laboratory of Oral Medicine, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014

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