Virus Persists in Beta Cells of Islets of Langerhans and Infection Is Associated With Chemical Manifestations of Diabetes

II. Morphologic Observations

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Persistence of lymphocytic choriomeningitis (LCM) virus in the islets of Langerhans was associated with mild hyperglycemia and abnormal glucose tolerance test results. Early histopathologic events consisted of occasional perivascular inflammatory mononuclear cells around both islet and acinar cells. Morphometric studies showed an increase in the size of islets from virus-infected mice. By electron microscopy, LCM virions were found within infected beta cells. Cytolytic injury of beta cells was minimal and did not account for the abnormalities of glucose metabolism. In contrast to the findings in islets, ultrastructural studies of acinar cells revealed LCM virions in abundance, vacuolar degeneration, and intracytoplasmic inclusions. This study extends the previous observation that LCM virus infection may persist in beta cells of the islets of Langerhans without causing structural injury but be associated with abnormalities resembling the chemical and histopathologic features of the early stage of Type II (adult-onset) human diabetes mellitus. (Am J Pathol 1985, 121:497–504)

IF DIABETES MELLITUS is caused by a virus, then it may be due to direct virus-induced destruction of beta cells in the islets of Langerhans, or the virus may induce an antiviral or autoimmune response directed against viral or self determinants on insulin-producing cells. Alternatively, a virus may persist in the beta cells and alter their ability to make or to release insulin. Epidemiologic and pathologic studies in humans have suggested that viruses may be important in the pathogenesis of Type I (juvenile-onset) diabetes mellitus.[1–4] In addition, a number of experimental animal models with acute virus infections have features in common with Type I diabetes mellitus.[5,6]

In contrast, there are few clues to the potential causes of Type II (adult-onset) diabetes mellitus. No relationship has been demonstrated between a physiologic defect in the absence of cytopathologic changes in islet cells and a viral infection. Recently we demonstrated[7] *in vivo* that a virus could persist in beta cells of the islets of Langerhans in certain strains of mice and be associated with metabolic and pathologic findings resembling those of Type II diabetes mellitus.

To extend this observation, in this paper we report a detailed histopathologic study using light and electron microscopy. We found lymphocytic choriomeningitis (LCM) virions within beta cells, but only minimal morphologic alterations of these cells had occurred. In contrast to the lack of injury to cells of the islets, cytopathic changes were observed in the acinar cells.

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Materials and Methods

Virus

The Armstrong (CA 1371) LCM virus was used. It was propagated in BHK-21 cell culture and cloned three times. The virus was quantitated by plaque assay in vero cells as described.[8]

Animal and Tissue Preparation

Thirty BALB/cWEHI (*H-2K^{d}D^d*) mice (Scripps colony) of both sexes were inoculated intracerebrally within the first 18 hours of life with 60 plaque-forming units of LCM virus. Age- and sex-matched noninoculated mice served as controls. Greater than 90% of the inoculated mice survived until sacrifice and were persistently infected with LCM virus. These animals carried 3.5 to 5.5 log units of infectious virus in their blood and organs.[9]

Animals were sacrificed at age 15 or 60 days by perfusion through the left ventricle with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). Under a dissecting microscope, both the head and tail portions of each pancreas were removed. Then each pancreas was cut into 15–20 1-mm blocks, dehydrated, osmicated, and embedded in Araldite. Sections 1 μ thick were placed on glass slides and stained with 1% toluidine blue. Selected blocks were trimmed, and ultrathin sections were stained with uranyl acetate and lead citrate for viewing with a Zeiss 10 electron microscope.

Morphometry

Selection of Slides

One slide was taken from each of the 15–20 blocks from each pancreas and numbered in sequential fashion. Infected and noninfected controls were numbered independently. This produced 120 slides from 15-day control mice, 125 slides from 60-day control mice, 182 slides from LCM virus-infected 15-day mice, and 196 slides from infected 60-day mice. Fifty slides from each of these four groups were selected by use of a random number table and used for all further morphometric studies.

Area of Individual Pancreatic Islets

Randomly selected slides containing one or more islets of Langerhans were projected with a Ken-a-Vision model X-1000-1 microprojector onto a digitized tablet (Science Accessories Corporation, Model 14). The area of each islet was determined digitally with a Hewlett-Packard 9825A microcomputer by connecting serial circumferential points. The computer program was tested on multiple projected images of different sizes and shapes and was found to give reliable and reproducible area measurements with an error of 1%. The area of every islet encountered in the sections was entered into the computer. The data were examined for statistical significance by the standard Student *t* test.

Ratio of Islet Area to Acinar Area

All of the 50 randomly selected slides from each of the experimental groups were used to determine the ratio of islet area to acinar area (slides not containing islet tissue were included). The slides were projected onto a 500-point checkerboard grid. A digitized Hewlett-Packard pen-point counter was used to determine, first, the relative cumulative area of islet cells and, second, the relative area of acinar cells. Capillaries and fibrous connective tissue projected on a crossing of the grid were not counted. Reproducibility testing showed an error of 1.5%.

The number of points falling on islet cells and on acinar cells were accumulated independently and expressed as a ratio of islet points per 1000 acinar points.

Further Morphometric Studies

To confirm the morphometric data, experiments were performed independently by one of us (M. Raitt). Two groups of BALB/c mice were used for these experiments: 4 mice were given an injection of LCM virus at birth, and 4 mice served as uninfected controls. Mice were sacrificed at age 15 days and prepared as above. Seven to 23 blocks were prepared from each pancreas, and a section was made from each block. Each section was projected onto paper; the islet and acinar areas were outlined, cut out, and weighed. The weights of the cuttings were used as a measure of the areas of the two components. Results were evaluated by the Student *t* test.

Results

Endocrinologic Studies

The biochemical aspects of glucose metabolism in mice persistently infected with LCM virus have been reported.[7] Briefly, these infected mice showed mild hyperglycemia and abnormal glucose tolerance test results but normal blood cortisol and insulin levels and normal growth hormone levels in the pituitary gland. Immunocytochemical studies with monoclonal antibodies[10] showed LCM virus nucleoprotein antigens predominantly in beta cells in the islets of Langerhans.[7] LCM virus antigens were also noted in acinar cells.

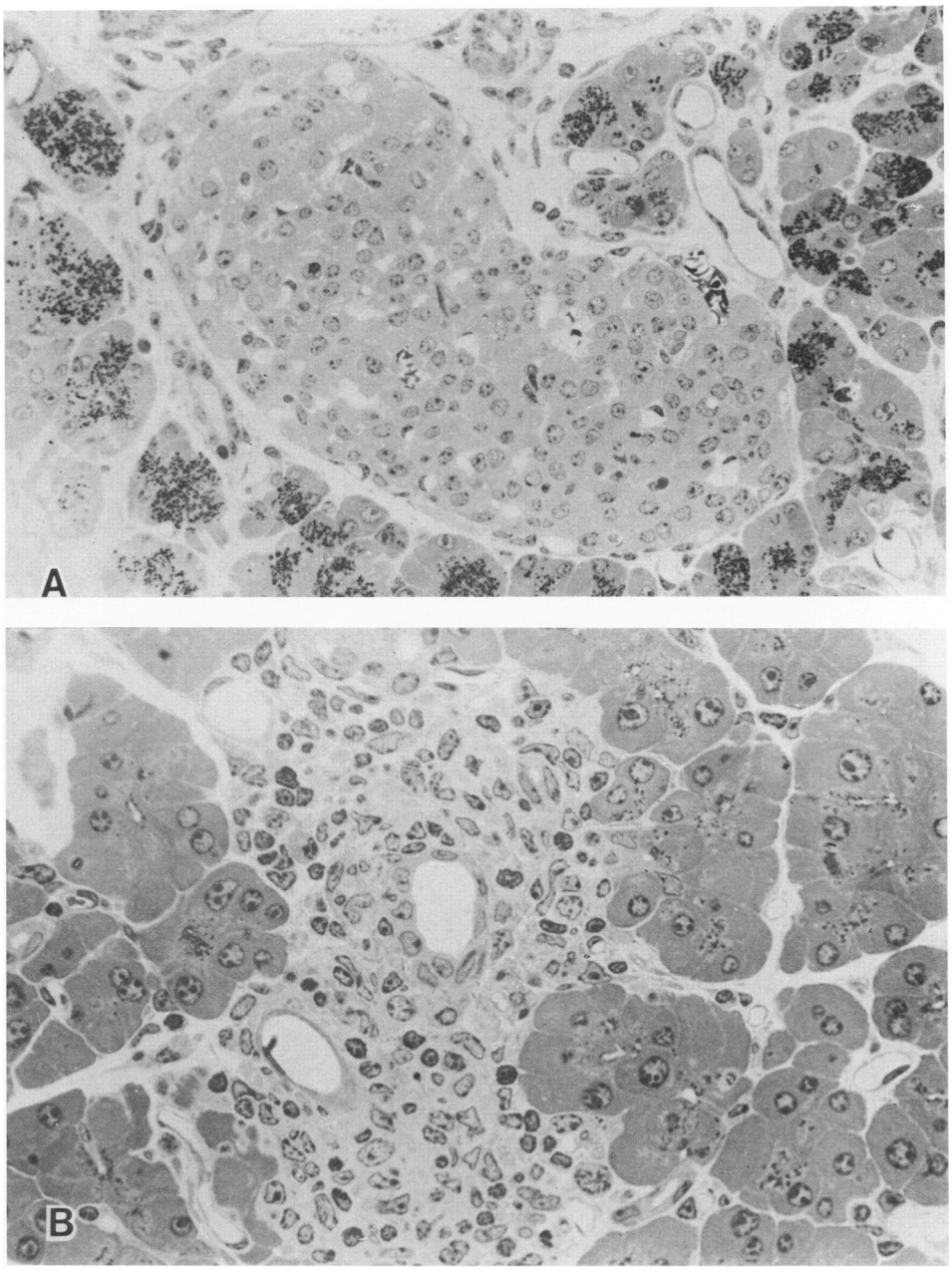


Figure 1A—Large islet from a mouse at Day 15 of LCM virus infection. Note the absence of morphologic alterations and surrounding acinar epithelial cells containing darkly staining intracellular secretory granules. (Toluidine blue, ×500) **B**—Inflammatory mononuclear cells surrounding a blood vessel and infiltrating into pancreatic tissue from a mouse at Day 15 of LCM virus infection. (×550)

Morphology (Light Microscopy)

Repeated observations on 1-μ-thick Araldite sections from infected pancreases showed the absence of abnormalities except for an apparent hypertrophy of individual islets (Figure 1A). The majority of sections showed no or only minimal morphologic abnormalities. A few sections demonstrated mononuclear cellular infiltrates surrounding both acinar and islet tissue. This was seen primarily in 15-day infected animals. The inflammatory cells were seen surrounding blood vessels (Figure 1B) and were observed in less than 2% of more than 300 microscopic sections studied. Hydropic changes, hyalinosis (amyloidosis), and regeneration were not seen in islet cells.

Ultrastructure

Acinar-Ductal Area

Electron microscopy revealed numerous LCM virions budding from the surface of acinar cells of the pan-

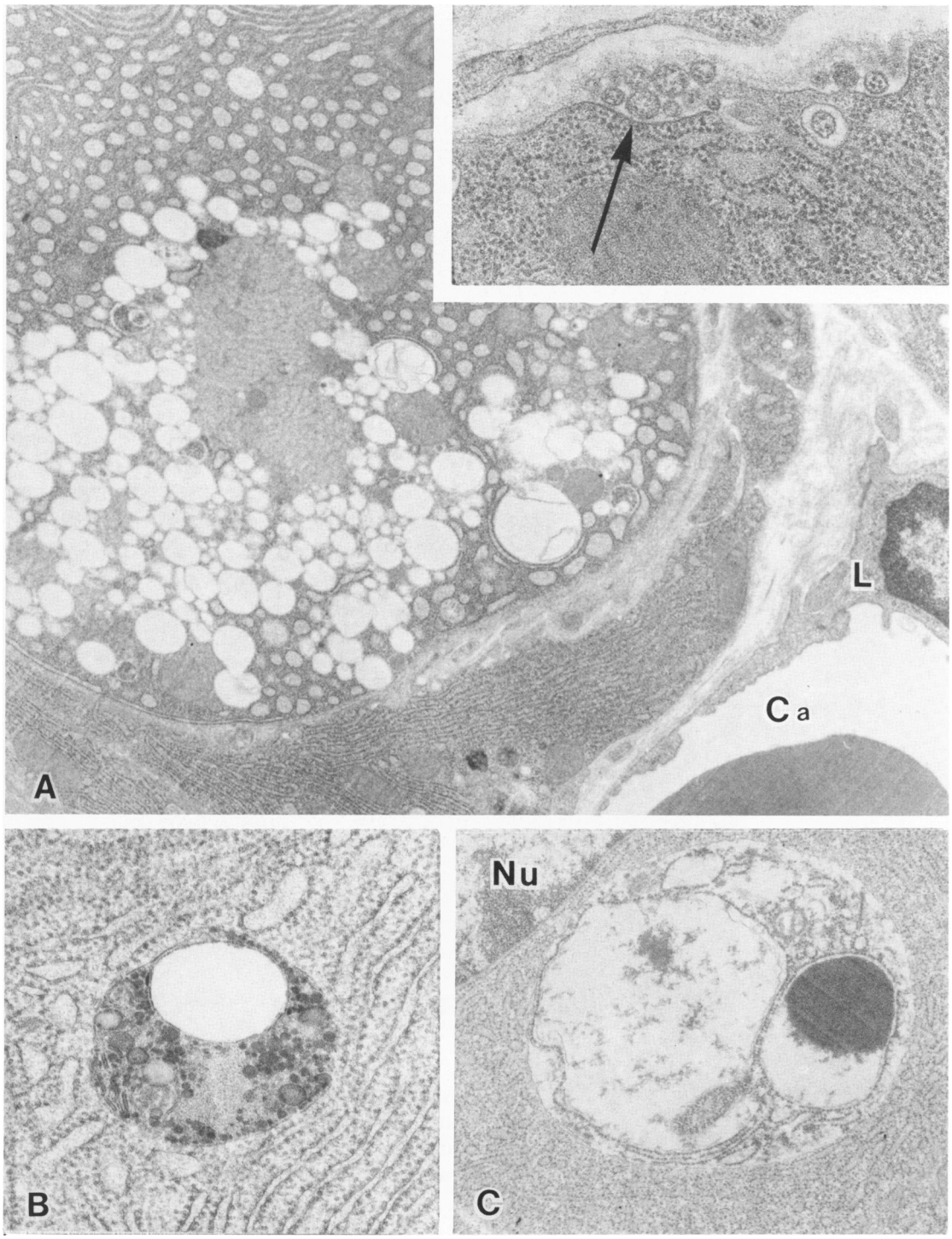


Figure 2—Morphologic abnormalities in pancreatic acinar cells of mice persistently infected with LCM virus. **A**—Tissue from a mouse at Day 15 of LCM virus infection. Note the infiltrating lymphocyte (*L*) migrating from the surface of a capillary (*Ca*). Infected acinar cell, showing vacuolar degeneration and necrosis. (×15,000) **Inset**—LCM virions (*arrow*) budding from surface of acinar cell into acinar lumen. (×64,000) **B**—Tissue from a mouse at Day 60 of LCM virus infection. Membrane-bound electron-dense inclusions are within the cytoplasm of an acinar cell. (×45,800) **C**—Tissue from a mouse at Day 60 of LCM virus infection. Membrane-bound vacuole with an electron-dense granular inclusion is present in an infected acinar cell. *Nu*, nucleus. (×16,700)

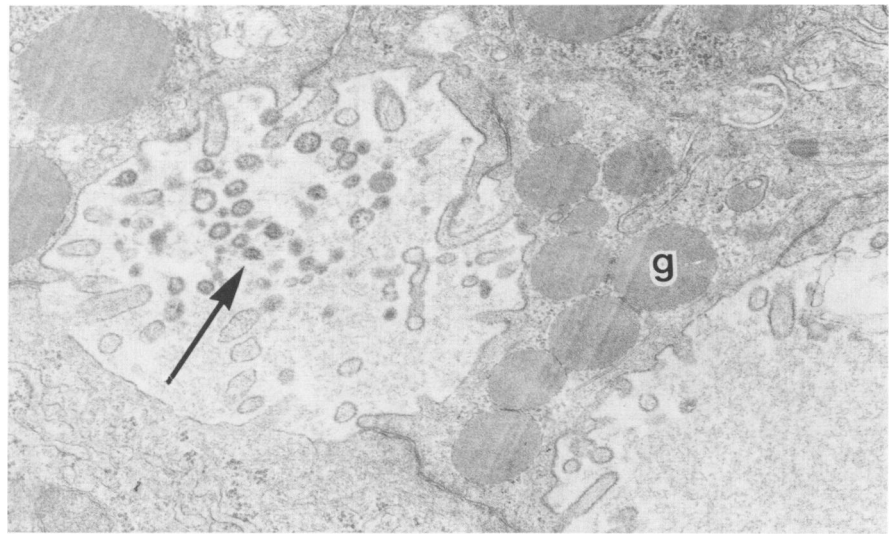
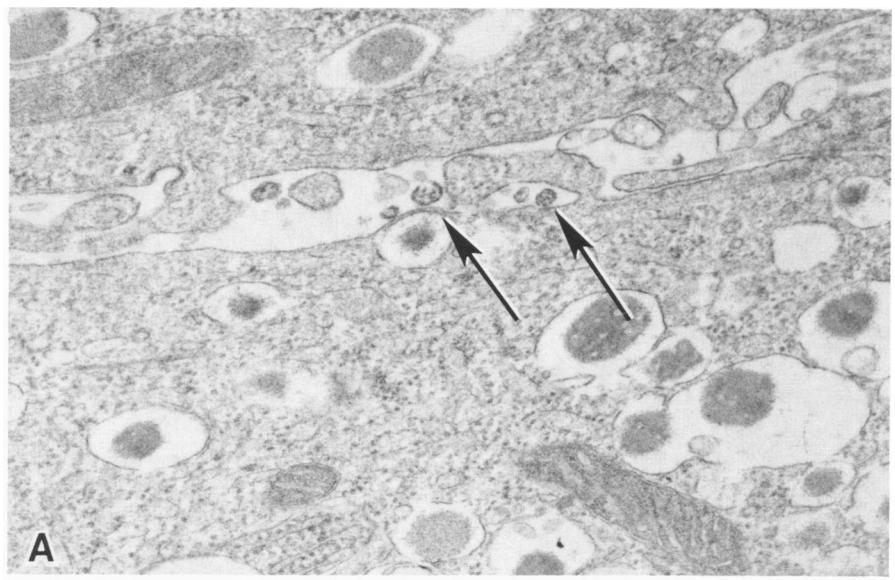


Figure 3—LCM virions (*arrow*) within the ductal lumen. Virus particles are budding from the surface of centroacinar cells. *g*, zymogen granule. (×24,600)



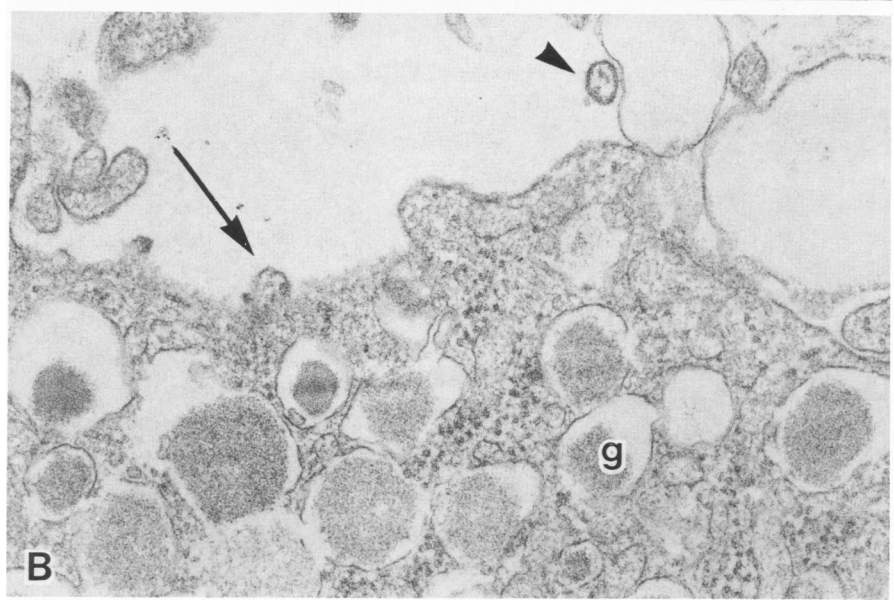


Figure 4—LCM virions within endocrine cells of islets of Langerhans from persistently infected mice. These mice had increased blood glucose values, compared with uninfected age- and sex-matched control mice. Infected mice had abnormal glucose tolerance test results but no abnormalities in blood cortisol or insulin levels or in growth hormone levels in the pituitary gland.[7] LCM virions budding from surface of infected beta (insulin-producing) cell characterized by granules with a clear, loose-fitting membrane (*g*). Note the LCM virus particles (*arrows* and *arrowhead*) with external radiating projections and internal ribosomelike particles. (×50,000)

creas (Figure 2A, inset). Virus particles were round or oval, with a diameter of approximately 120 nm. The virions had a membrane envelope with surface projections surrounding a lucent interior that contained varying numbers of ribosome-like granules. Virus particles were also seen within the lumens of capillaries and within the intercellular space. Acinar cells showed ultrastructural morphologic alterations consisting of vacuolar degeneration (Figure 2A) and intracellular inclusions (Figure 2B and C). The inclusions frequently were membrane-bound and contained electron-dense granules. Viruses were also seen budding from centro-

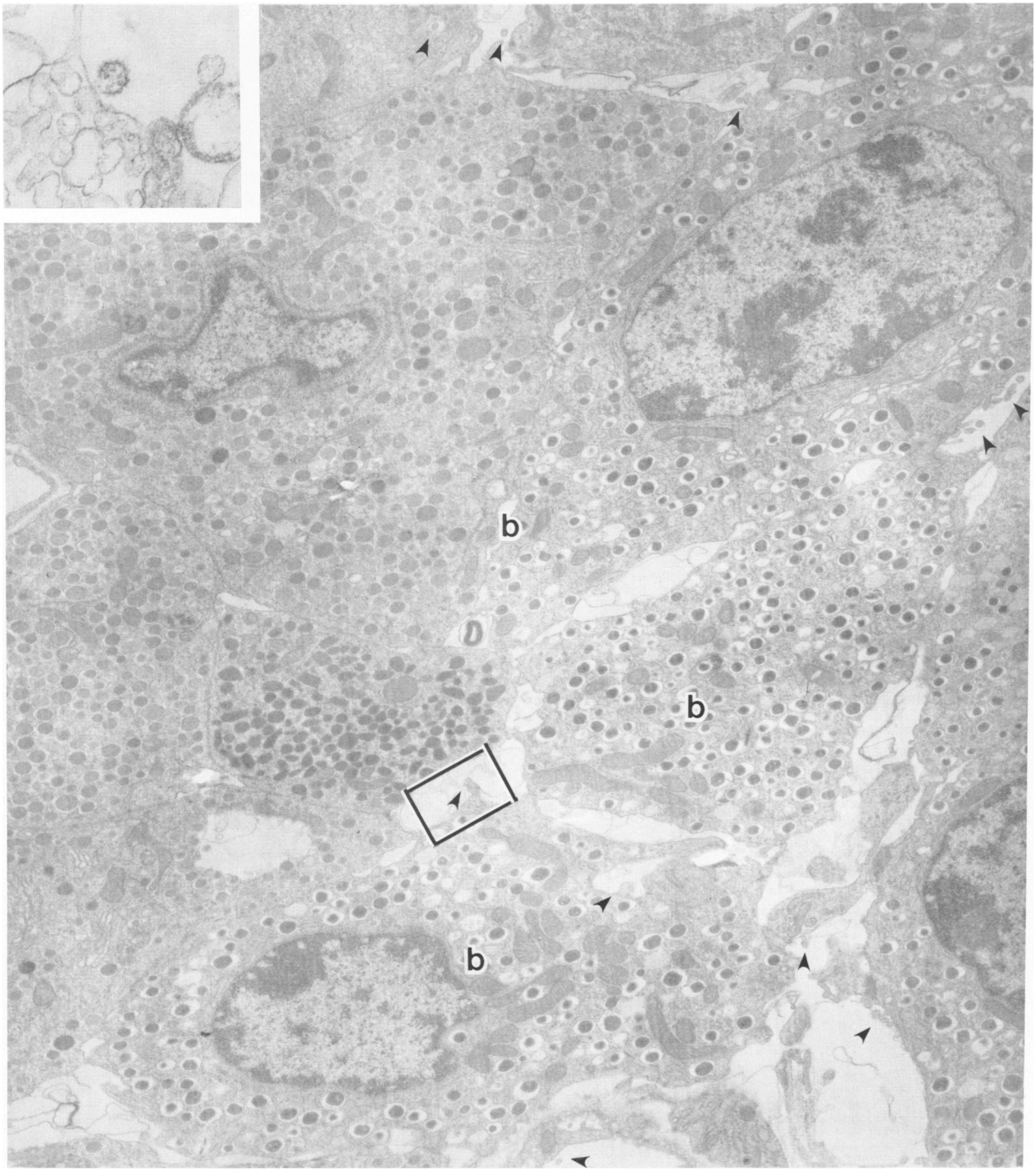


Figure 5—Beta (*b*) cells in islet of Langerhans from a mouse persistently infected with LCM virus (*arrowheads*). Note the abundant secretory granules and absence of structural abnormality. (×9300) **Inset**—Higher magnification of LCM virion. (×50,000)

acinar cells into the lumen of the acinus (Figure 3). Zymogen granules were abundant even though viruses were present.

Islets of Langerhans

Virus particles were seen budding from insulin-secreting beta cells, identified by their secretory granules with a surrounding clear halo (Figure 4). Occasional insulin granules contained a rectangular or polygonal crystalline core. Most beta cells showed no morphologic abnormalities (Figure 5). Abundant numbers of insulin-containing granules were present. Some beta cells appeared to be in a state of hyperactivity characterized morphologically by abundant endoplasmic reticulum, enlarged nuclei, and active degranulation. There was no vacuolar degeneration or hyalinization of beta cells. Most (>98%) islet cells were free of inflammatory infiltration. Rarely, virions were seen associated with alpha or delta cells of the pancreas.

Morphometry

The results of morphometric studies, detailed in Table 1, revealed a statistically significant increase in the mean area of individual islets from 15-day infected mice, compared with 15-day noninfected controls. The total islet-to-acinar area ratio in 15-day infected mice was 3.5 times greater than that in 15-day control mice. Subsequent morphometric studies using weight of cuttings showed the number of islets per unit area to be identical in both infected and uninfected mice. However, the mean islet size was 50% larger in the infected animals ($P < 0.05$). The morphometric data do not permit a conclusion as to whether the increased size of islets represents islet-cell hypertrophy or hyperplasia.

Table 1—Results of Morphometric Studies

Mice	n*	Area of individual islets† (sq mm)	Mean ratio of islet area to acinar area
LCM virus			
15-day	26	0.172 ± 0.024	0.063‡
60-day	28	0.105 ± 0.022	0.011
Control			
15-day	28	0.107 ± 0.014	0.018
60-day	30	0.102 ± 0.030	0.009

* n, number of islets studied.
† Shown as mean ± standard error.
‡ For difference from control, $P < 0.05$.

Discussion

The mice persistently infected with LCM virus had mild abnormalities of glucose metabolism compatible with diabetes mellitus.[7] Virus was observed budding from the surface of beta cells, but the cytomorphology of these infected cells most often was unremarkable. However, at day 15 of infection, the islets showed significant hypertrophy, compared with islets from uninfected mice, which suggests an increased attempt at synthesis of hormones. We detected abundant insulin granules in cells that showed budding LCM virions. Thus, the virus can persist in islet cells without causing the cells' destruction or attracting an inflammatory infiltrate. In contrast to the normal appearance of islet cells, vacuolar degeneration and cell death occurred in infected acinar cells. Thus, there appears to be a relative symbiosis between the virus and the infected beta cells of the islets of Langerhans despite the damage in surrounding ductal cells.

Previously, Oldstone et al.[11,12] and Rodriguez et al[13] had shown virus-induced alterations in the function of growth hormone-producing cells *in vivo*. Persistent LCM virus infection in C3H or CBA mice resulted in growth retardation and hypoglycemia, virus being expressed in growth-hormone–secreting cells of the pituitary. Virus was not detected in pituitary cells making prolactin, thyrotropin, or corticotropin; and normal levels of cortisol and insulin were found in the blood of infected animals.[12] Reconstitution of such infected mice with growth-hormone–producing cells returned growth to normal and corrected the glucose imbalance.[12]

A similar event may have occurred in the present experiment. Susceptible BALB mice showed persistent LCM virus infection in their islets of Langerhans. This was associated with increases in blood glucose concentration and abnormal glucose tolerance test results, findings similar to the early stages of human Type II diabetes mellitus. In both the experimental model reported here and in human Type II diabetes, in addition to aberrant glucose metabolism, beta cells show the presence of insulin granules, lack of cellular infiltrates, and a relative absence of cell injury.

Currently, we are evaluating whether insulin secretion is abnormal in morphologically normal virus-infected beta cells of the islet, whether antibodies to insulin receptor or enhanced peripheral resistance to insulin occurs, and whether there is an accompanying abnormality in glucagon or other hormones from the islets of Langerhans. The morphometric studies reported here show islet hypertrophy which suggests, in part, increased attempts at hormone synthesis; the pathogenesis of the destruction of acinar cells is unknown.

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