EXPERIMENTAL COXSACKIE B3 VIRUS MYOCARDITIS IN GOLDEN HAMSTERS

Light and Electron Microscopy Findings in a Long-term Follow-up Study

HIROSHI MORITA, M.D.

Following intraperitoneal inoculation with coxsackie B3 virus all weanling Syrian golden hamsters (60 animals) developed severe myocarditis. In the acute phase, light and electron microscopy revealed massive cellular infiltration and myocytolysis in the myocardium which were most prominent on the 5th day and least obvious in 3 weeks. In the necrotic myocytes, mitochondria contained moderately electron dense inclusions different from the calcified granules seen in the myocardium of mice with coxsackie B3 virus myocarditis. Macrophages ingested and digested necrotic cell debris, leaving moderate fibrosis but no calcification in the myocardium. Viruses were isolated from the myocardium on the 3rd to 9th day, and virus particles were seen in a necrotic cardiocyte on the 9th day. In the chronic phase, most animals developed no cardiomegaly; light microscopy revealed minimal myocardial fibrosis; electron microscopy showed various degenerative changes in some cardiocytes. A few animals (two animals) developed significant cardiomegaly with moderate to marked myocardial fibrosis in the 6th and 14th month. In one heart there was marked biventricular dilatation. In these animals with cardiomegaly, ultrastructural changes of the myocardial cells were similar to those described in congestive cardiomyopathy in man. Golden hamsters appear to be a unique model for a study of the possible relationship between viral myocarditis and idiopathic cardiomyopathy in man.

Many virus infections can involve the heart. Among them, coxsackie viruses, especially group B, seem to be the most common agents responsible for viral myocarditis1-9 Recently, Cambridge et al10 and Kitaura11 reported serological studies showing that high neutralization titers to coxsackie B viruses were more common in patients with congestive cardiomyopathy than in controls. Therefore, it has been postulated that in some cases idiopathic cardiomyopathy may be the result of previous viral myocarditis.

In order to test the viral infection theory of idiopathic cardiomyopathy, morphological, im-

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munological and virological studies of coxsackie virus myocarditis have been carried out in mice by a number of investigators\textsuperscript{[15–18]} including the author and co-workers\textsuperscript{[19–23]} In these mice, histopathological changes of the myocardium did not resemble those in human viral myocarditis in that the histological picture of both the acute and the chronic phase of viral myocarditis in mice is characterized by calcified foci in the myocardium. Calcification of the myocardium has not been documented in human coxsackie virus myocarditis, except in a very few reports\textsuperscript{[24,25]} We thought that the morphological difference between humans and mice might be due to species-specificity. Therefore, we attempted to find other experimental animals in which viral myocarditis is not associated with calcification in the myocardium. In our preliminary study on Syrian golden hamsters\textsuperscript{[26–29]} coxsackie B3 virus (Nancy strain) did cause definite myocarditis without calcification in the myocardium.

A 14-month follow-up study on light and electron microscopic changes of the myocardium was performed in Syrian golden hamsters infected with coxsackie B3 virus. The results are presented here and discussed in terms of a possible relationship between viral myocarditis and idiopathic cardiomyopathy in man.

MATERIALS AND METHODS

Animals. Male Syrian golden hamsters, 24 ± 2 days of age, were obtained from the Japan CLEA Laboratory. These hamsters weighing 34.3 ± 3.9 g (mean ± SD) were fed water and standard chow ad libitum.

Virus. Coxsackie B3 virus (Nancy strain) was serially propagated in monolayer cultures of FL cells in Eagle’s medium with 1% calf serum. The virus was harvested by several consecutive freezing and thawing cycles after the cytopathic effect had developed. The fluid was pooled and centrifuged at 3,000 rpm for 15 minutes. The supernatant was stored at −20°C until inoculation. The stock virus concentration was 10\textsuperscript{8.0} TCID\textsubscript{50}/ml.

Experimental Infection. One hundred and ten hamsters were arbitrarily divided into 2 groups: 60 received an intraperitoneal injection of 0.8 ml inoculum of the stock virus, and 50 which were injected with the same dose of a medium of uninfected cell culture, served as controls. The animals were housed in 20 cages, with 6 infected or 5 control hamsters in each cage. During the 14 month period of the experiment, hamsters from each cage and each group were sacrificed on the 3rd, 5th, 7th, 9th, 15th, 22nd and 29th postinoculation day and 3, 6 and 14 months after inoculation. (In this study, the first postinoculation day corresponds to the day of inoculation). Prior to sacrifice, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg) and weighed to the nearest 0.1 gram. ECGs (lead II) were recorded, and blood was collected for virus isolation from a throat incision. After the thoracic cage was opened, the heart was excised above the origin of the great vessels, blotted on dry filter paper and weighed on a precision balance that was accurate to 1 mg. The ratio of heart weight to body weight (HW/BW) was estimated. The differences in measurements were analysed by Student’s t test and were considered significant when the p value was lower than 0.05. Data were expressed as mean ± standard deviation (SD).

Morphological Study. The heart was hemisected transversely at the midportion of the ventricle; the apical part was used for morphological study and the basal part was processed for virus isolation. Some small pieces (cubes of 1 mm) from the left ventricular free wall of the apical part were prefixed in 3% glutaraldehyde at 4°C for ordinary electron microscopic study and others were placed in 10% neutral buffered formalin for light microscopic study.

Light Microscopic Observation. After fixation, the heart specimens were dehydrated with a series of ethanol and embedded in paraffin. Four μm thick sections were cut transversely from several places separated from one other by approximately 500 μm, stained with hematoxylin and eosin and Mallory-azan. Periodic acid-Schiff (PAS) staining and alizarin red S staining for calcium salts were also performed. There was no significant topographical difference in severity and extent of the myocardial changes. Histopathological findings of the myocardium in the infected animals were categorized and graded semi-quantitatively, in accord with the criteria for human cardiac biopsies proposed by Noda\textsuperscript{30}

In this report, myocardial necrosis and fibrosis were graded as follows: focal and minimal, (1+); widespread, covering more than half the area of both ventricles and interventricular septum, (4+). PAS positive substance was observed in the myofibers and if present was called (+), Grade (−) signified no visible change. The data in Table
<table>
<thead>
<tr>
<th>Postinoculation</th>
<th>Heart Weight (g)</th>
<th>HW/BW (×10⁻³)</th>
<th>Myocardial Lesions</th>
<th>Virus Isolation***</th>
<th>No. of Viruses (TCID₅₀/ml)</th>
<th>Average Value</th>
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<tr>
<td></td>
<td>Incidence*</td>
<td>Necrosis</td>
<td>Cell infiltration</td>
<td>Fibrosis</td>
<td>PAS positive substance</td>
<td>Heart</td>
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<tr>
<td>3rd day</td>
<td>infec. 0.141 ± 0.013</td>
<td>3.51 ± 0.30</td>
<td>6/6</td>
<td>1+</td>
<td>1+</td>
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<tr>
<td></td>
<td>cont. 0.145 ± 0.009</td>
<td>3.34 ± 0.17</td>
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<tr>
<td>5th day</td>
<td>infec. 0.175 ± 0.018*</td>
<td>3.93 ± 0.42*</td>
<td>6/6</td>
<td>4+</td>
<td>4+</td>
<td>6/6</td>
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<tr>
<td></td>
<td>cont. 0.151 ± 0.004</td>
<td>3.16 ± 0.10</td>
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<tr>
<td>7th day</td>
<td>infec. 0.194 ± 0.011*</td>
<td>3.98 ± 0.41*</td>
<td>5/5</td>
<td>4++</td>
<td>4+++</td>
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<tr>
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<td>cont. 0.161 ± 0.018</td>
<td>3.08 ± 0.03</td>
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<td>9th day</td>
<td>infec. 0.207 ± 0.011*</td>
<td>3.68 ± 0.24*</td>
<td>6/6</td>
<td>2+</td>
<td>3+++</td>
<td>6/6</td>
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<tr>
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<td>cont. 0.175 ± 0.014</td>
<td>3.19 ± 0.15</td>
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<td>15th day</td>
<td>infec. 0.221 ± 0.033</td>
<td>3.71 ± 0.25*</td>
<td>5/5</td>
<td>2+</td>
<td>2+</td>
<td>0/5</td>
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<td>cont. 0.194 ± 0.012</td>
<td>2.93 ± 0.15</td>
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<td>22nd day</td>
<td>infec. 0.214 ± 0.016</td>
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<td>5/5</td>
<td>1+</td>
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<td>0/5</td>
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<td>Chronic Phase</td>
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<td>29th day</td>
<td>infec. 0.239 ± 0.012</td>
<td>2.87 ± 0.16</td>
<td>5/5</td>
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<td>2+++</td>
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<td>cont. 0.238 ± 0.009</td>
<td>2.80 ± 0.16</td>
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<td>3rd mo.</td>
<td>infec. 0.361 ± 0.031</td>
<td>2.66 ± 0.09</td>
<td>5/5</td>
<td></td>
<td>1+</td>
<td>0/5</td>
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<td>cont. 0.341 ± 0.007</td>
<td>2.68 ± 0.19</td>
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<td>6th mo.</td>
<td>infec. 0.416 ± 0.048</td>
<td>2.79 ± 0.27</td>
<td>5/6</td>
<td></td>
<td>1+++</td>
<td>0/6</td>
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<tr>
<td></td>
<td>cont. 0.417 ± 0.004</td>
<td>2.63 ± 0.15</td>
<td>1/6</td>
<td></td>
<td>2+</td>
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<td>14th mo.</td>
<td>infec. 0.509 ± 0.064</td>
<td>3.17 ± 0.36</td>
<td>4/5</td>
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<td>1++</td>
<td>0/5</td>
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<tr>
<td></td>
<td>cont. 0.433 ± 0.021</td>
<td>2.76 ± 0.15</td>
<td>1/5</td>
<td></td>
<td>2+ 3+</td>
<td></td>
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* Denominator indicates number of sacrificed animals; numerator, incidence of myocardial lesions.
** Smaller than given grade.
*** Denominator indicates number of sacrificed animals; numerator, incidence of virus isolation.
Mean ± SD, #: p < 0.05, #: p < 0.02, *: p < 0.01 for infected (infec.) versus control (cont.) group.
Fig.1. Small focus of myocardial necrosis with scantly mononuclear cell infiltration on 3rd day after inoculation. Hematoxylin and eosin stain. The scale bar in Figs. 1–4 and 6 represents 20 μm. (Figs. 1–6 light micrographs.)

Fig.2. 7th day after inoculation. Many necrotic myofibers and infiltration with numerous mononuclear cells. Note no definite mineralization in necrotic areas. Hematoxylin and eosin stain.

I express the mean value of the grades in the specimens of sacrificed animals at each interval of time after inoculation.

*Electron Microscopic Observation.* After 2 hours of fixation in glutaraldehyde, the specimens were washed in 0.1 M phosphate buffer at pH 7.2 overnight, postfixed in 1% osmium tetroxide, dehydrated and embedded in epon. Semithin sections were cut from the epon block, stained with toluidine blue and scanned with a light microscope to determine the most suitable area for electron microscopy. Subsequently, ultrathin sections were cut from the same block, stained with uranyl acetate and lead citrate, and studied with a Hitachi HU-11Ds electron microscope.

Viral Myocarditis in Hamsters: Follow-up Study

Fig. 3. 29th postinoculation day. Some myofibers replaced with fibrotic scars. Some remaining myofibers are hypertrophic or atrophic. Canalized capillaries in connective tissue scar. Hematoxylin and eosin stain.

Fig. 4. a) PAS positive substance (glycogen granules) increased in myocardial fibers, 6 months after inoculation. Periodic acid-Schiff stain.
   b) Myocardium from age-matched control animal. No positive PAS stain in myocardial fibers. Periodic acid-Schiff stain.

Virus Isolation. The specimens were homogenized with phosphate buffered saline at pH 7.4, and 10% suspensions were prepared. They were centrifuged at 10,000 rpm for 20 minutes, and the supernatant was used for virus isolation. Virus concentration was calculated by a 50% infective dose method (TCID₅₀) in FL cell culture tubes with serial 10-fold dilutions, which were examined for virus cytopathic effects for 7 days during incubation at 37°C.
Fig. 5. Histological sections of ventricles transversely cut at midportion of heart. Mallory-azan stain.

a) 14th month after inoculation. Right and left ventricular dilatation in one of the 5 infected animals. Multiple foci of moderate to marked myocardial fibrosis (arrows) in left ventricular free wall and interventricular septum, especially in middle to subendocardial portions. LV, left ventricle; RV, right ventricle. Interruption in LV free wall caused by cutting for electron microscopic study.

b) Age-matched control hamster. No ventricular dilatation or myocardial fibrosis.

Fig. 6. Enlarged portion from free wall of left ventricle of Fig. 5a. In residual myocardial lesions increased collagen fibers intermingling with single myofiber or myofiber groups, some with scarcity of myofibrils. Vacuoles of varying size in sarcoplasm of some degenerated myofibers. No evidence of inflammatory cellular infiltration in myocardium. Hematoxylin and eosin stain.

RESULTS

General Observations

Of the 60 infected hamsters, 3 animals died by the 9th day, 2 more by the 22nd day and one in the 9th month. None of the control hamsters died. Both the control and infected surviving animals appeared healthy throughout this experiment. The infected animals were rarely discernible by their general appearance, since neurological disturbances, peripheral edema and respiratory distress were not noted. Electrocardiography revealed that none of the infected or control animals had A–V block or extrasystoles throughout the experimental period.

Gross Inspection of the Heart

No animals developed abnormal gross pathological changes of the heart. In the 14th month, however, one of the 5 infected animals revealed marked enlargement of the heart with dilatation of both ventricles.

Heart Weight and Ratio of Heart Weight to Body Weight (HW/BW Ratio) (Table I)

The HW/BW ratio was significantly increased in the infected groups from the 5th to the 15th day, because their heart weights were greater and their body weights slightly lower than those of the controls. Thereafter, there was no statistical difference between the 2 groups. In the infected group 2 animals showed increased heart weight in the 6th and 14th months: 0.502 g (control group, 0.417 ± 0.004 g) and 0.618 g (control group, 0.433 ± 0.021 g), respectively, indicating cardiomegaly because the heart weights were over the range of 3SD.

Light Microscopic Findings

The histopathological findings were divided into the acute phase (up to the 22nd day) and the chronic phase (after the 29th day) (Table I).
The acute phase was characterized by necrosis and cellular infiltration in the myocardium and the chronic phase by myocardial fibrosis.

a) Acute Phase

In all infected animals, myocardial damage appeared on the 3rd day. Some groups of several myofibers underwent eosinophilic degeneration, and a small amount of cellular infiltration was present (Table I, Fig. 1). Occasionally, a few mononuclear cells surrounded the capillaries. The infiltrated cells appeared to consist mainly of lymphocytes, large mononuclear cells, eosinophils, plasma cells and a small number of polymorphonuclear leukocytes. On the 5th day, numerous foci of marked myocardial necrosis and massive cellular infiltration had developed throughout the myocardium of both left and right ventricles (Table I, Fig. 2). In the affected myofibers, the sarcoplasm appeared pale, striations were not discernible and nuclei were irregular in outline, frequently shrunken but sometimes enlarged. Necrotic myofibers were invaded mainly by large mononuclear cells and underwent lysis. A number of punched out empty spaces also appeared in necrotic foci. The empty spaces or vacuoles did not contain PAS positive material. Mononuclear cell infiltration in the myocardial necrotic areas appeared to be contiguous with that in the pericardium, but neither the valves nor the coronary arteries were affected. On the 9th to the 22nd day, many necrotic foci decreased rapidly in size, and fine moderate fibrotic replacement was observed in and around the foci without calcification (Table I).

b) Chronic Phase

On the 29th day, almost no necrotic foci or inflammatory cell reactions were seen (Table I). The remaining myofibers in the foci were atrophic or sometimes hypertrophic. In the fibrotic areas, some capillaries were canalized (Fig. 3). After the 3rd month, PAS positive substance (glycogen granules) appeared to be increased in the sarcoplasm of some myofibers (Fig. 4).
Fig. 9. 5th postinoculation day. Macrophages (Mac) containing phagocyted debris of disorganized myocardial cells. The basement membrane (BM) of the sarcolemma appears not to have been phagocyted and remains in the interstitial space (is). At right, part of a viable myocardial cell (MC) adjacent to a necrotic cell at an intercalated disc (ID). Uranyl acetate and lead citrate stain.

Fig. 10. 7th postinoculation day. Affected myocytes disorganized and phagocyted by macrophage (Mac), hemi-desmosomes (HD) and hemi-fasciae adherentes (HFA) of intercalated disc on a remaining viable myocyte (MC). Disorganized myofibrils appear to be connected with hemi-fasciae adherentes in a viable cell. Uranyl acetate and lead citrate stain.

the 6th month, the areas of myocardial fibrosis had become smaller (Table I). In the 6th month, one animal had much larger fibrotic areas in the myocardium than did the remaining animals (Table I); this animal did not develop ventricular dilatation. In the 14th month, 4 of 5 animals showed only a few minimal scars in the myocardium; these fibrotic scars had the appearance of fine lattice work or islets among apparently intact myofibers. The remaining one of the 5 animals had cardiomegaly with right and left ventricular dilatation; multiple foci of moderate to marked myocardial fibrosis were frequently seen in the middle to subendocardial portions of the left ventricular free wall and septum (Table I, Fig. 5). In many foci of the myocardium in this animal, increased collagen fibers appeared mainly as strands of connective tissue encircling and separating individual myofibers. Some myofibers showed scarcity of myofibrils. Vacuoles of varying size were seen in the myocardial sarcoplasm, which appeared to be markedly degenerated. No inflammatory cellular infiltration or proliferation of the capillaries in the interstitium was seen (Fig. 6).

In the control animals, there was no histological evidence of myocarditis or residual myocardial change throughout the experiment.

Electron Microscopic Findings
a) Acute Phase

In the infected animals, a number of myocardial cells underwent a variety of necrotic changes, and the cell debris was phagocytosed by numerous macrophages and leukocytes which had infiltrated the necrotic foci (Figs. 7-10). These changes were already present on the 3rd day (Fig. 7), were most prominent on the 5th day (Figs. 8 and 9), and were least conspicuous by the 22nd day.

Necrotic myocardial cells were often in contact with apparently uninjured myocardial cells at the intercalated discs (Figs. 8 and 9). These cell junctions were often partially dissociated and disrupted at various sites. Hemi-desmosomes and semi-fasciae adherentes remained only on the
side of the apparently viable cells, which were deformed with indentations of the sarcolemma (Fig. 10). In the necrotic myocardial cells, mitochondria were often swollen with or without loss of cristal membranes. Moderately electron dense amorphous inclusions often occurred in the matrix of these mitochondria (Figs. 7 and 8). Myofibrils often underwent lysis with loss of striations. The sarcolemma was also often disrupted or entirely obscured so that disorganized intracellular organelles appeared to be free in the interstitial spaces (Figs. 7–9).

In the remaining viable myocytes, myofibrils were often seen arranged in haphazard fashion, especially those adjacent to dissociated intercalated discs. The myofibrils were also occasionally scarce and disorganized. The tubules of the sarcoplasmic reticulum were occasionally proliferated and dilated. Some mitochondria were swollen but did not contain moderately electron dense amorphous inclusions (Figs. 8–10).

Numerous macrophages and some polymorphonuclear leukocytes were found in the interstitial space. These cells had phagocytosed the debris of myofibrils, mitochondria and other intracellular organelles derived from necrotic myocytes (Figs. 7–10). The basement membranes of necrotic myocardial cells appeared not to have been phagocytosed (Fig. 9). Abundant collagen fibrils were seen in the interstitial spaces in the necrotic foci.

On the 9th day, crystalloid structures in an orderly arrangement were found in the sarcoplasmic debris phagocytosed by the macrophages. The subunit was approximately 130 Å in diameter, and so could be a virus particle (Fig. 11). So far, however, these structures have been seen in only one cell among the numerous myocytes surveyed by electron microscopy in this study.
b) Chronic Phase

In this phase, myocardial cells in and around the residual foci often showed a variety of degenerative changes (Figs. 12–16) and were surrounded by numerous collagen fibrils. In some degenerated myocardial cells, myofibrils underwent lysis and disarray. They were scarce in the periphery of the sarcoplasm and partially connected to the subsarcolemmal Z-band like substance associated with the free sarcolemma of myocardial cells. Dilatation of the sarcoplasmic reticulum was seen. Mitochondria varied moderately in size. The β-type glycogen granules often appeared to have increased in amount in the sarcoplasm, corresponding to an increase in PAS reaction by light microscopy (Fig. 12). In some degenerated myocardial cells and more often in apparently normal myocardial cells, lysosomes, lipid droplets and β-type glycogen granules were occasionally increased in number (Fig. 13). Some degenerated myocardial cells appeared to have lost their connections with adjacent myocardial cells. The dissociated cell surface had a markedly irregular contour and partially thickened basement membrane. Intracytoplasmic junctions were also seen occasionally in some degenerated myocardial cells (Fig. 12).

In the 6th to 14th months, some degenerated myocardial cells also contained a number of peculiar fine filamentous structures (Fig. 14). These structures ran in various directions in the sarcoplasm where no myofibrils were seen. There was no apparent periodicity of substructure. At higher magnification the filamentous structures revealed a tubular appearance measuring approximately 50 to 80 Å in diameter, and were tightly tangled together in the sarcoplasm. These filamentous structures resembled those seen in some cases of idiopathic cardiomyopathy in man previously reported by Takatsu et al.\textsuperscript{32} and Kawamura and Hayashi.\textsuperscript{33}

In the hamster which had developed dilated cardiomegaly with moderate to marked fibrosis in the 14th month, a number of myocardial cells showed a variety of degenerative changes similar to those described in other animals. In addition,
these myocardial cells occasionally contained intracellular, partially collapsed, vesicles similar to those described by Vega et al.\textsuperscript{34} in some biopsies from patients with primary cardiomyopathy (Fig. 15). Some degenerated myocardial cells were connected with one another at irregularly convoluted cell junctions and/or extensive side-to-side junctions (Fig. 16).

In the control animals, the myocardium appeared normal except for minimal interstitial fibrosis.

Virus Isolation (Table 1)

From the blood and the heart of the infected animals sacrificed on the 3rd day, coxsackie B3 virus was isolated. Viremia was no longer present, except in one animal, on the 5th day. On the 9th day, only the heart contained virus. On and after the 15th day, virus was not isolated from the heart. The number of viruses isolated from the heart was highest on the 5th day, $10^{7.6}$ TCID$_{50}$/ml on the average.

Virus was not isolated from the blood and the heart of the control animals throughout the experiment.

DISCUSSION

In this study, all the infected golden hamsters developed severe myocarditis characterized by massive cellular infiltration and myocytolysis in the acute phase. It should be emphasized that no calcification was found in the myocardium in either the acute or the chronic phase, and the prominent myocardial lesions decreased in extent and severity more rapidly after the 7th day than those in mice with coxsackie B3 virus myocarditis.\textsuperscript{22,23} In the lytic myocardial cells in the acute phase, numerous mitochondria contained moderately electron dense amorphous inclusions, but they did not contain the very electron dense spicular or granular inclusions described in necrotic myocardial cells in mice with coxsackie B3
virus myocarditis. In these mice, very electron dense intramitochondrial inclusions were verified by micro X-ray analysis to contain significant amounts of calcium. These calcified mitochondria were phagocytosed by macrophages, and calcified granules remained undigested and appeared to be aggregated in a large mass so that they were easily identified as calcified foci even under the light microscope. By contrast, in the golden hamsters, no mitochondrion contained any calcified granules in the lytic myocardial cells. The absence of calcium deposition in mitochondria accounts for the lack of calcified foci in the myocardium in golden hamsters. These non-calcified mitochondria and cell debris appeared to be phagocytosed and easily digested by macrophages. This activity accounts for the remarkable healing process of the myocarditis in this animal.

In the chronic phase of the myocarditis, areas of replacement fibrosis gradually decreased in size and were minimal in extent in most of the infected animals in the 14th month. In a few animals (2 animals), however, the fibrotic areas were large in the 6th and 14th months. These animals developed cardiomegaly with or without dilatation of both ventricles. This cardiac state was considered to be postcardiopathic cardiomegaly by Okada. Kitaura reported that in some mice with severe coxackie B3 virus myocarditis in the acute phase the residual foci of myocardial fibrosis again increased in size after the 6th month. A similar situation might occur in the animals which had developed cardiomegaly without or with dilatation in the 6th and 14th months. On pathologic anatomy the typical heart of congestive cardiomyopathy has greatly dilated ventricles and ventricular wall of normal thickness. Light microscopically the increased collagen fibers appeared mainly as strands of connective tissue encircling and separating myocardial fibers, which has been termed interfibr
Viral Myocarditis in Hamsters: Follow-up Study

Fig. 16. Degenerated myocytes of hamster ventricle shown in Fig. 5a. The cells appear to be connected with one another at irregularly convoluted cell junctions and extensive side-to-side junctions (J). Near these junctions are spherical microparticles and Z-band-like materials (z'). The myofibrils (Mf), showing lysis and disruption, seen to be partially attached to subsarcolemmal Z-band-like substance (x). Uranyl acetate and lead citrate stain.

fibrosis or intermyocardial fibrosis. This type of fibrosis has been noted to be a histological feature of the congestive type of idiopathic cardiomyopathy. Electron microscopically, some myocardial cells showed a variety of degenerative changes, some of which resembled those seen in some viable myocardial cells in the acute phase of the myocarditis. The other changes, including increased glycogen granules and lysosomes, peculiar fine filamentous structures in the sarcoplasm, subsarcolemmal Z-band-like substances accumulated at the periphery of the myocytes, and intracytoplasmic junctions, characterized the ultrastructural changes of the chronic phase. Many of these changes in the myocardial cells resemble those described in myocardial biopsies obtained from many patients with idiopathic cardiomyopathy, especially the congestive type of cardiomyopathy.

Intracellular vesicular structures seen in the animal with cardiomegaly were similar to those documented by Vega et al., who claimed that they were most extensive in primary cardiomyopathy in humans. These common myocardial changes suggest a similar disease process in the myocardium of patients with cardiomyopathy and of the golden hamsters described in this report.

Moreover, we found that these animals with cardiomegaly had poor cardiac function, including reduced contractility and disturbed relaxation of the left ventricle, as determined from pressure curves obtained by a transmurally punctured micro-tip catheter (PC-350, Millar Co.) prior to sacrifice. The details of this experiment will be published elsewhere. It is not obvious how myocardial fibrosis affects cardiac performance. It has been well documented that some patients with coxsackie virus myocarditis or infection showed definite cardiac dysfunction several weeks to years following the acute infection. If the initial episode of infection had not been determined to be viral in origin,
these patients might have been diagnosed as having idiopathic cardiomyopathy. The same may apply to the infected animals, since virus could no longer be isolated from the myocardium and there were no inflammatory findings in the myocardium in the 6th and 14th months. So, the dilated ventricles and the residual pathological changes may belong to the category of congestive cardiomyopathy.

The morphological evidence in this study and the functional evidence in our preliminary reports\(^{27,28}\) suggest a similar disease process in infected hamsters and in humans with viral myocarditis, and some cases of the congestive type of cardiomyopathy in man may represent postcardiotic cardiomyopathy. The golden hamster appears to be a unique model for a study of the possible relation between preceding viral myocarditis and idiopathic cardiomyopathy in man.

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