EXPERIMENTAL COXSACKIE B VIRUS MYOCARDITIS IN MICE
18 Month Histopathological and Virological Study

YASUSHI KITaura, M.D.

In a series of experimental studies to test the hypothesis that idiopathic cardiomyopathy in man represents a sequela of virus myocarditis, coxsackie B 1, 3 or 5 virus was inoculated into ICR mice with two different amounts, that is, a small amount (0.1 ml of 10^{5.5} TCID_{50}/ml) and a large amount (0.1 ml of 10^{7.5} TCID_{50}/ml). Histopathological and immunofluorescent studies of the heart, analysis of the antibody titers in sera and evaluation of the virus concentration in various organs including the heart were carried out in an acute (up to the 21st day) and chronic phase (up to the 18th month) of the experiment. A small amount of coxsackie B 1 or 5 virus did not cause myocarditis, while a large amount of either virus rarely induced mild myocarditis. A small amount of coxsackie B 3 virus frequently caused mild myocarditis without obvious residual pathologic changes of the heart, while a large amount of the same virus always caused acute and severe myocarditis. In these animals, acute myocardial changes are almost in agreement with those in previous investigations except for capillary thrombi. The virus was isolated from the heart with higher titers than from other organs and identified in some cardiocytes by immunofluorescent study until the 14th day. Neutralizing antibody in sera appeared on the 7th day and remained for several months. Approximately two thirds of these mice left no significant myocardial lesions, whereas about one third of them which probably had extensive myocardial lesions in the acute phase developed significant myocardial fibrosis with calcification in the chronic phase. These lesions appeared to become larger after the 6th month. In and around the fibrotic lesions, atrophy, hypertrophy and/or disarray of myocardial fibers were observed. These hearts did not show hypertrophy or dilatation but their histologic findings resembled those seen in some cases of congestive cardiomyopathy except for severe calcification in the myocardium.

**Key Words:**
- Coxsackie B viruses
- Mouse
- Myocarditis
- Myocardial fibrosis
- Calcification

SINCE Saphir\(^1\) demonstrated that virus myocarditis is not rare, much attention has been paid to the heart in virus infections. Many viruses are known to cause myocarditis\(^2\)-\(^4\). Coxsackie B viruses and echoviruses are the most cardiotropic viruses\(^5\) and they often cause

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<td>(1)</td>
<td>1.8% (2/110)</td>
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<td>13.3% (12/90)</td>
<td>17.5% (14/80)</td>
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*: p < 0.05, **: p < 0.01, ***: p < 0.001 compared with Group D (control group).

common colds. The common cold is one of the most prevalent diseases, and if cardiac manifestations are not severe, myocarditis is easily overlooked at the time of infection but may remain as a sequela long after recovery from the cold per se. Many follow-up studies on acute virus myocarditis have shown that some virus species cause prolonged cardiac abnormalities, such as cardiomegaly, electrocardiographic changes and/or congestive heart failure in man7–17 and residual pathologic changes in the hearts of experimental animals18,19.

Moreover, several serological studies have shown that high virus antibody titers especially to coxsackie B viruses are more common in patients with idiopathic cardiomyopathy than in normal controls20–23. A similar study by this author suggests that previous infections not only with coxsackie B but also with herpes simplex, influenza and echo viruses are more common in patients with congestive cardiomyopathy and those with herpes simplex and coxsackie B 3 viruses are more common even in patients with hypertrophic cardiomyopathy than in normal controls23.

These results favor the virus infection theory of the etiology of idiopathic cardiomyopathy, however, they only suggest a possible correlation between those virus infections and the disease4. Moreover, if idiopathic cardiomyopathy represents a burnt-out virus myocarditis, virological investigations may not be able to demonstrate a causal relationship because a long time may have elapsed between the onset of the disease and the virological examination.

Therefore, in proving the hypothesis that idio-

pathic cardiomyopathy may represent a sequela of virus myocarditis, it will contribute much to follow a large number of patients with virus myocarditis and see if some actually develop cardiomyopathy. This is difficult, however, because virus myocarditis is rare in Japan. The long term study was performed in an animal model in which virus myocarditis was induced by coxsackie B 1, 3, or 5 virus, since the author has found that antibodies to these types are more common in patients with congestive cardiomyopathy than in normal controls23. Similar short-term studies have been performed by other investigators18,19 who have shown that mice with virus myocarditis have residual pathological changes in the heart.

The purpose of the present study is to confirm the results of the short-term investigations and to observe the later histopathological changes in the heart. Semiquantitative analysis for the myocardial lesions was attempted. In addition, the similarity and difference between the murine heart long after coxsackie B virus myocarditis and the findings in human cardiomyopathy are discussed.

MATERIALS AND METHODS

**Animals**

Female JCL®-ICR mice were obtained from The Japan Clea Laboratory. They weighed 12.5 ± 0.8 g (mean ± standard deviation) at 24 days of age.

**Viruses**

Coxsackie B 1 (Conn 5 strain), B 3 (Nancy

*Japanese Circulation Journal Vol. 45, July 1981*
### TABLE II  BODY WEIGHT, HEART WEIGHT AND HEART WEIGHT/BODY WEIGHT RATIO IN COXSACKIE B VIRUS INOCULATED GROUPS AND CONTROL GROUP

<table>
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<th>Days after inoculation</th>
<th>3 days</th>
<th>5 days</th>
<th>7 days</th>
<th>9 days</th>
<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
<th>2 mo.</th>
<th>3 mo.</th>
<th>6 mo.</th>
<th>12 mo.</th>
<th>18 mo.</th>
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<tr>
<td>Number of mice</td>
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<td>53±5</td>
<td>69±14</td>
<td>95±16***</td>
<td>99±7**</td>
<td>107±11**</td>
<td>123±6**</td>
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<td>147±6**</td>
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<td>426±18**</td>
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<td>423±11</td>
<td>424±12</td>
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<td>437±11</td>
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Groups B (2) and C (2) were omitted because mice of these groups did not develop myocarditis.

*: p < 0.05,  **: p < 0.01,  ***: p < 0.001 compared with Group D (control group).
strain) and B5 (Furukawa strain) viruses were used in tissue culture passages. Each strain of the stock viruses was prepared in a monolayer culture of FL cells in a Roux bottle with Eagle’s medium with 1% calf serum. The viruses were harvested by several consecutive freezing and thawing cycles after the cytopathic effect developed. The virus medium was centrifuged at 3,000 rpm for 15 minutes and its supernatant was diluted with Eagle’s medium so that it had a titer of $10^{7.5}$ TCID$_{50}$/ml. This stock virus was stored at $-20^\circ$C until inoculation.

**Experimental Infections**

A total of 840 ICR mice, 24 days of age, were divided into four major groups (Groups A, B, C and D). Groups A, B and C, 240 mice each, were inoculated with coxsackie B3, 1 and 5 virus, respectively. Each of these groups was subdivided into two groups (120 mice each). The mice of subgroups A (1), B (1) and C (1) were inoculated with 0.1 ml of the stock virus, while those of subgroups A (2), B (2) and C (2) were similarly inoculated, but with the concentration reduced to a $10^{-2}$ dilution. Group D, 120 control mice, were injected intraperitoneally with 0.1 ml of a supernatant of a virus free culture medium of FL cells. Each subgroup was further divided into 12 subsets of 10 mice each, and each subset was caged separately. A cage was selected arbitrarily, and the mice in it were sacrificed 3, 5, 7, 9, 14, 21 and 28 days, and 2, 3, 6, 12 and 18 months after inoculation. Prior to sacrifice, the mortality rate was calculated by dividing numbers of mice died by total numbers of mice of the remaining subsets (Table I). The mice were anesthetized with an intraperitoneal injection of sodium pentobarbital, 50 mg/kg and weighed to the nearest 0.1 g (Table II). Bipolar standard lead electrocardiograms were recorded with a Hewlett-Packard recorder system (Model 4588 D) at a paper speed of 200 mm/sec through needle electrodes placed in extremities up to the 28th day. Blood was sampled by heart puncture for titration of neutralizing antibodies. The chest was opened and the heart was excised and weighed to the nearest 1 mg after removal of blood (Table II). The mean values and their standard deviations of body weight, heart weight and the ratio of heart weight/body weight were calculated for each subset (Table II). The ventricles of the heart were cut transversely into three parts. The apical part was placed in n-hexan at $-76^\circ$C for immunofluorescent study.

The middle part between the apex and the base was fixed in 10% neutral formalin for light microscopy. In the case of mice in Group A (1), a small tissue block was obtained from the lateral wall of the left ventricle of the middle part was directly fixed in 3% glutaraldehyde for electron microscopy. The result of electron microscopic study will be published elsewhere. The basal part of the heart was kept at $-20^\circ$C for virus assay. The liver, brain, and intestine were also sampled and kept at $-20^\circ$C for titration of the virus.

**Light Microscopic Study**

Transverse section of the ventricles of the hearts were stained with hematoxylin and eosin, Mallory-azan, alizarin red S and von Kossa stain. In an arbitrarily selected transverse section of each heart, the thickness of the lateral wall of the right and left ventricles and of the interventricular septum was measured to the nearest 0.01 mm with an ocular micrometer disc provided with a linear scale. In the lateral wall of the right and left ventricles and in the interventricular septum, the shortest diameters of 100 consecutive cardiocytes of each heart were also similarly measured to the nearest 0.1 $\mu$m at the nucleated level. The data were expressed as mean value and standard deviation, and statistically evaluated by Student’s $t$ test.

In his preliminary observation on numerous histologic sections of the heart from mice with coxsackie B3 virus myocarditis, the author found that there was no obvious topographical difference in the distribution of necrotic lesion. In the present study, in an attempt to estimate the approximate extent of the necrotic or fibrotic lesions, the author photographed a histologic transverse section of the heart of each animal. Each section was printed at a final magnification of 20 $\times$ and on each print was superimposed a transparent sheet with a fine lattice. The intervals between the lattice lines was 2.0 mm, corresponding to 100 $\mu$m in the original section (Fig. 1). The ratio of the necrotic area to the total area of the transverse section of each heart was the number of crossing points of the lattice work falling on the lesions divided by the number of points falling on the total area of a cardiac section. The data were expressed as mean value and standard deviation and statistically evaluated by Student’s $t$ test (Table III).

**Virus Assay**

The heart, liver, brain and intestine from each

*Japanese Circulation Journal Vol. 45, July 1981*
Fig. 1. A transverse section of the ventricles, 12 months after inoculation with a large amount of coxsackie B 3 virus. The interval between neighboring lattice lines, either longitudinal or transverse, corresponds to 100 μm in the original section. Mallory-azan stain.

Fig. 2. Histological section of the myocardium, 7 days after inoculation with a large amount of coxsackie B 3 virus. A patchy necrotic lesion with severe calcification and moderate cellular infiltration is observed. Hematoxylin and eosin stain. scale: 25 μm
mouse were homogenized individually with phosphate buffered saline (pH 7.4) and centrifuged at 10,000 rpm for 30 minutes. The supernatant was used for virus titration which was performed by the 50% infective dose method in two culture tubes of FL cells. The cytopathic effect was observed until the 7th postinoculation day, and the titer was calculated by the Reed and Muench method.25

**Immunofluorescent Study**

The fluorescein isothiocyanate (FITC) conjugated antisera were the same as in the author's previous study on coxsackie B 1, 3 and 5 virus antigens in the myocardial biopsies from patients with idiopathic cardiomyopathy.23 Sections 4 µm thick were cut by a cryostat from a heart block fixed in cold acetone for 5 minutes. They were well washed with phosphate buffered saline (pH 7.2) and stained by the direct method. Slides were observed and photographed with a Carl-Zeiss RA fluorescent microscope.

**Measurement of Neutralizing Antibody Titer**

The test was performed by a constant virus-varying serum, 50% endpoint method with a microtest plate with FL cells. The plates with lids were placed in a humid 5% CO₂ incubator at 37°C. The cytopathic effect was observed until the 7th post-inoculation day, and the titer was calculated by the Reed and Muench method.25

**RESULTS**

**Group A**

(1) Inoculation with 0.1 ml of 10⁷-⁵ TCID₅₀ /ml (a large amount) of coxsackie B 3 virus

**Mortality Rate** The mortality rate was significantly higher than in the control group (Group D) after the 7th postinoculation day: 1.8% 5 days, 8.0% 7 days, 13.3% 9 days, 17.5% 14 days, 26.3% 28 days and 30.0% 12 months after inoculation (Table I). Between the 5th and 9th day approximately one third of the mice appeared to be ill and developed gait disturbances. These animals ate much less food than the control animals and looked to be poorly nourished. The death mostly occurred among these animals but after the 14th day the death usually occurred in the apparently helthy animals.

**Electrocardiograms** The mean values of the heart rate and its standard deviation were 510 ± 31/min, 511 ± 19/min and 506 ± 14/min, and the mean value of the PR interval and its standard deviation were 37 ± 3 msec, 36 ± 3 msec and 37 ± 2 msec on the 7th, 14th and 28th day, respectively. These data did not differ significantly from those of the age-matched control group. Atrioventricular block, premature contractions or obvious ST, T wave changes were not observed throughout the experiment.

**Body Weight, Heart Weight and Heart Weight/Body Weight Ratio** The body weight and heart weight decreased until the 7th day and then slowly increased until the 12th month. In the control group they always increased until the 12th month. Both body and heart weights were significantly smaller and the heart weight/body weight ratio was larger in the inoculated group than in the age-matched control group after the 5th day (Table II).

**Macroscopic Study** The heart appeared normal until the 5th day, when patchy or linear whitish calcified lesions developed on the surface of the heart in 4 of 9 mice. After the 7th day the similar lesions were seen in all the animals. These hearts did not show hypertrophy or dilatation of the ventricles, and pulmonary congestion, liver congestion or ascites was not present throughout the experiment.

**Microscopic Study of the Heart** Whether necrotic myocardial lesions were present or not, the experimental period was conventionally divided into the acute phase (up to the 21st day) and the chronic phase (beyond the 28th day).

**The Acute Phase** On the third day, scarcity of myofibrils and eosinophilic degeneration first developed in some cardiocytes. These cells often appeared to be connected with apparently intact myocardial cells. On the 5th day, patchy necrotic lesions of the myocardium became prominent. These lesions contained cell debris, inflammatory cells and granules of calcium salt which were identified by alizarin red S and von Kossa stains. In most of the animals cellular infiltrations were slight in these lesions. The infiltrated cells appeared to include lymphocytes, macrophages, neutrophiles, eosinophiles and plasma cells (Fig. 2). On the 5th, 7th and 9th days necrotic areas occupied approximately 10%, 25% and 10% of arbitrarily selected transverse sections of the ventricles, respectively (Table III). The total necrotic area varied markedly among the animals even of the same subset. After the 9th day the necrotic lesions decreased rapidly in size and were replaced by fibrosis, in and around which double nuclei were occasionally seen in...
<table>
<thead>
<tr>
<th>TABLE III</th>
<th>RATIO OF NECROTIC OR FIBROTIC AREAS/TOTAL AREA ON TRANSVERSE SECTION OF VENTRICLES IN COXSACKIE B VIRUS INOCULATED GROUPS AND CONTROL GROUP (MEAN ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days after inoculation</td>
<td>3 days</td>
</tr>
<tr>
<td>Group A (1)</td>
<td></td>
</tr>
<tr>
<td>Numbers of mice</td>
<td>9</td>
</tr>
<tr>
<td>Necrotic area (%)</td>
<td>0.1±0.1 ** 10.4±5.1 *** 24.5±6.4 *** 9.6±3.6 *** 0.8±0.4 ** 0.2±0.1 *** 0 0 0 0 0 0</td>
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<tr>
<td>Fibrotic area (%)</td>
<td>—</td>
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<tr>
<td>Group A (2)</td>
<td></td>
</tr>
<tr>
<td>Numbers of mice</td>
<td>10</td>
</tr>
<tr>
<td>Necrotic area (%)</td>
<td>0</td>
</tr>
<tr>
<td>Fibrotic area (%)</td>
<td>—</td>
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<tr>
<td>Group B (1)</td>
<td></td>
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<tr>
<td>Numbers of mice</td>
<td>10</td>
</tr>
<tr>
<td>Necrotic area (%)</td>
<td>0</td>
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<tr>
<td>Fibrotic area (%)</td>
<td>0.5±0.1</td>
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<tr>
<td>Group C (1)</td>
<td></td>
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<tr>
<td>Numbers of mice</td>
<td>10</td>
</tr>
<tr>
<td>Necrotic area (%)</td>
<td>0</td>
</tr>
<tr>
<td>Fibrotic area (%)</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>Group D</td>
<td></td>
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<tr>
<td>Numbers of mice</td>
<td>10</td>
</tr>
<tr>
<td>Necrotic area (%)</td>
<td>0</td>
</tr>
<tr>
<td>Fibrotic area (%)</td>
<td>0.5±0.1</td>
</tr>
</tbody>
</table>

Groups B (2) and C (2) were omitted because mice of these groups did not developed myocarditis.

* p < 0.05, ** p < 0.01, *** p < 0.001 compared with Group D (control group).
Fig. 3. Histological section of the myocardium, 14 days after inoculation with a large amount of coxsackie B 3 virus. In and around necrotic lesions double nuclei are occasionally seen in surviving myocardial cells. Left upper inset shows a higher magnification of the double nucleated myocardial cells. Arrows show the double nuclei. Hematoxylin and eosin stain. Scale: 50 μm

Fig. 4. Histological section of the myocardium, 12 months after inoculation with a large amount of coxsackie B 3 virus. In and around the severe fibrotic lesion, some myocardial cells show atrophy or hypertrophy. Hematoxylin and eosin stain. Scale: 100 μm
Coxsackie B Virus Myocarditis in Mice

moderate extent with calcifications and occasional mononuclear cell infiltrations in the interstitium. At this stage, total fibrotic lesions in arbitrarily selected transverse sections of the ventricles did not become larger (Table III). In the late stage of the chronic phase, approximately two thirds of the mice (11 of 16 mice) showed almost normal myocardium with minimal scar, whereas in the 6th month one of 6 mice, in the 12th month 2 of 6 mice and in the 18th month 2 of 4 mice had developed large fibrotic lesions in the myocardium with severe calcifications. In and around the lesions, myocardial cells had undergone atrophy or compensatory hypertrophy, especially in the lateral wall of the left ventricle and the interventricular septum (Figs. 1 and 4). Moderate disarray of myocardial fibers was frequently observed in these lesions. The mean ratio of the total fibrotic area to the area of an arbitrarily selected transverse section and its standard deviation were 2.5 ± 1.6%, 5.4 ± 3.0% and 9.7 ± 7.0% at the 6th, 12th and 18th months, respectively (Table III). The ratio had a tendency to increase between the 6th month and the 12th or 18th month (p < 0.1). In the control group (Group D), a total fibrotic area of the myocardium was extremely small throughout the experiment.

**Thickness of the Ventricular Walls and of the Interventricular Septum** The mean value of the thickness of the lateral wall of the right ventricle, left ventricle and of the interventricular septum and its standard deviation was 0.53 ± 0.08 mm, 1.19 ± 0.08 mm and 1.13 ± 0.06 mm on the 7th day and 0.56 ± 0.04 mm, 1.33 ± 0.15 mm and 1.54 ± 0.10 mm on the 12th month, respectively. These data did not differ significantly from those in the control group.

**Mean Diameters of Cardiocytes** The mean value and its standard deviation of the diameter of cardiocytes in the walls of the right ventricle, the left ventricle and the interventricular septum was 13.2 ± 0.32 μm, 13.8 ± 2.6 μm and 13.6 ± 2.6 μm on the 7th day, 15.2 ± 3.0 μm, 15.8 ± 3.4 μm and 15.8 ± 3.4 μm on the 28th day and 15.5 ± 3.8 μm, 18.2 ± 5.0 μm and 17.8 ± 5.0 μm in the 12th month in the inoculated subset, respectively. The diameters and their standard deviation for the subset of Group D (control group) were 14.0 ± 1.8 μm, 13.9 ± 1.6 μm and 14.4 ± 2.0 μm on the 7th day, 15.5 ± 2.0 μm, 15.6 ± 1.6 μm and 15.8 ± 2.2 μm on the 28th day and 15.2 ± 1.8 μm, 16.8 ± 1.8 μm and 16.0 ± 1.6 μm in the 12th month, respectively. There was no signifi-

![Table](image)

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some cardiocytes (Fig. 3), and calcified granules appeared to be tightly condensed so that calcified areas became smaller. Slight disarray of the myocardial fibers was seen in and around the lesions. On the 21st day, necrotic foci were rarely observed.

**The Chronic Phase** This phase was further divided into early stage (up to the 3rd month) and the late stage (beyond the 6th month). In the early phase of the chronic phase, approximately two thirds of the mice (14 of 20 mice) had recovered from acute myocarditis and left minimal scar without cellular infiltrations in the myocardium. But 2 of 7 mice on the 28th day and in the 2nd month, and 2 of 6 mice in the 3rd month had developed replacement fibrosis of

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*Japanese Circulation Journal Vol. 45, July 1981*
Fig. 6. Immunofluorescent study of the myocardium of a mouse inoculated with a large amount of coxsackie B 3 virus, 3 days after inoculation. When the myocardium was stained with FITC-labeled anticoxsackie B 3 virus globulin prepared in rabbits, positive fluorescence was observed in an individual myocardial cell. Scale: 25 μm.

Significant difference between the inoculated group and control one as to mean value of the diameter, but its standard deviation seemed to be larger in the inoculated group than in the control one, especially in the chronic phase.

Virus Assay The data are shown in Fig. 5. The virus was isolated from the hearts of all the mice until the 7th day. Concentration of the virus in the heart was highest on the 5th and 7th days, and much higher than in other organs. On the 14th day the virus was isolated from the heart of only one of the 8 mice and thereafter it was not isolated from any organ.

Immunofluorescent Study On the 3rd day, granular and/or patchy fluorescence first appeared in some cardiocytes and fluorescence positive cardiocytes were clearly demarcated from the adjacent fluorescence negative tissues (Fig. 6). On the 5th and 7th days intense fluorescence was seen in both the damaged and normally appearing cardiocytes which were located in and around the necrotic foci. On the 9th day 5 of 7 mice and on the 14th day 2 of 8 mice had patchy fluoroses in the myocardium and thereafter the result was negative in all the mice.

(2) Inoculation with 0.1 ml of $10^{5.5}$ TCID$_{50}$/ml (a small amount) of coxsackie B 3 virus

There was no significant difference in mortality rate (Table I) or electrocardiograms from the control group (Group D). On the 5th day, one of 10 mice developed slight gait disturbances. Body weight was significantly lighter than that of the control group on the 5th, 7th, 9th and 14th days and heart weight was lighter on the 5th, 7th and 9th days. The ratio of the heart weight/body weight was significantly larger than that of the control group on the 7th, 9th 14th and 21st and thereafter there was no statistical difference between the two groups (Table II). There was also no significant difference in the thickness of the lateral walls of the ventricles and of the interventricular septum compared throughout the experiment.

By light microscopy, necrotic lesions first appeared in the myocardium on the 5th day, and became larger until the 7th day. The necrotic lesions were the largest on the 7th day and 6 of 9 mice exhibited histological evidence of acute myocarditis. The total necrotic area did not occupy more than 10% of an arbitrarily selected transverse section of the ventricles (Table III). In these lesions, calcification of the myocardium was slight. After the 9th day the necrotic lesions became rapidly so small and scarce that there were hardly necrotic myocardial lesions on the
Fig. 7. Virus concentration in various organs (horizontal columns) and neutralizing antibody titers (NT) in sera of mice inoculated with a small amount of coxsackie B 3 virus. The virus was more frequently recovered with higher titers from the hearts than from other organs on the 3rd, 5th and 7th days. On the 9th day it was recovered from the heart of one of 9 mice, but not from other organs. Neutralizing antibodies were first detected on the 7th day and were highest on the 9th and 14th days but could not be detected in the 12th month.

Fig. 8. Virus concentration in various organs (horizontal columns) and neutralizing antibody titers (NT) in sera of mice inoculated with a large amount of coxsackie B 1 virus. The virus was recovered most frequently from the brain on the 3rd, 5th and 7th days, whereas it was rarely recovered from the heart and was not isolated after the 9th day. Neutralizing antibodies were first detected on the 7th day and were highest on the 14th day. In the 12th month they could not be detected.

21st day. In the chronic phase, all of the mice had no obvious replacement fibrosis of the myocardium, and there was no significant difference in extent of the fibrotic lesions between the inoculated and the control groups (Table III). Hypertrophy, atrophy and/or disarray of the myofibers were not seen in any of the mice, and there was no significant difference in mean value of diameter of cardiocytes and its standard deviation between this group and the control one.

The virological data are shown in Fig. 7. On the 3rd, 5th and 7th days, the virus was isolated from the heart of approximately 70%, with higher titers than from other organs. On the 9th day virus was isolated from the heart of only one of 9 mice and thereafter none was isolated. The results of the immunofluorescent study were similar to those in mice inoculated with a large amount of the same virus, but the extent and frequency of fluorescence in the myocardium was less than in mice inoculated with a large amount of the same virus. Neutralizing antibodies in sera were first detected on the 7th day and their titers were highest on the 9th day and 14th days. They could not be detected in the 12th month.

Group B
(1) Inoculation with 0.1 ml of $10^{7.5}$ TCID$_{50}$ /ml (a large amount) of coxsackie B 1 virus
There was no significant difference in mortality rate (Table I), electrocardiograms, body and
isolated (Fig. 8). In these hearts, immunofluorescent study exhibited a weak and patchy fluorescence in several groups of myocardial fibers. Neutralizing antibody was first detected on the 7th day in sera of 2 of 10 mice and its titer was the highest on the 14th day. In the 12th month it was not detected in all the animals (Fig. 8).

(2) Inoculation with 0.1 ml of 10^{-5.5} TCID_{50}/ml (a small amount) of coxsackie B 1 virus

There was significant difference from the control group as to mortality rate (Table I), electrocardiograms, body and heart weights (Table II), thickness of free lateral walls of the ventricles and of the interventricular septum or microscopic findings of the heart. None of the mice showed histological evidence of acute myocarditis and the myocardium appeared normal throughout the experiment.

The virus was isolated from the hearts of 2 and one of 10 mice on the 3rd and 5th day, respectively and thereafter it was not isolated. In these hearts, weak and patchy fluorescence was occasionally seen in a few cardiocytes. Neutralizing antibody was first detected on the 7th day and its titer was the highest on the 14th day.

Group C

(1) Inoculation with 0.1 ml of 10^{-7.5} TCID_{50}/ml (a large amount) of coxsackie B 5 virus

There was no significant difference between this group and control one as to mortality rate (Table I), electrocardiograms, body and heart weights (Table II) and thickness of lateral walls of the ventricles and of the interventricular septum.

In macroscopic and microscopic study, all hearts appeared normal throughout the experiment except for one mouse which showed a small perivascular necrotic lesion in the myocardium with mononuclear cell infiltrations on the 7th day. The necrotic lesions were so small and rare that there was no significant difference in the ratio of necrotic or fibrotic area to total area from a control section (Table III). The mean value of diameters of the cardiocytes were normal throughout the experiment.

The virus was isolated from the hearts of 2 of 10 mice on the 3rd day, and one of 10 mice on the 5th and 7th days and thereafter it was not
(2) Inoculation with 0.1 ml of $10^{5.5}$ TCID$_{50}$/ml (a small amount) of coxsackie B 3 virus

There was no significant difference from the control group as to mortality rate (Table I), electrocardiograms, body and heart weights, thickness of the lateral walls of the ventricles and of the interventricular septum. None of the mice showed histological evidence of acute myocarditis and the myocardium appeared normal throughout the experiment.

The virus was isolated from the heart of one of 10 mice on the 5th and 7th days. The results of immunofluorescent study were similar to those in mice of Group B (2). Neutralizing antibody titers were highest on the 14th day and they became less than 1:4 in the 3rd month.

**Group D**

The results were referred to in Tables I, II and III, and in the contexts of Groups A, B and C. None of the mice developed acute myocarditis and the myocardium appeared normal throughout the experiment. The virological findings revealed negative.

**DISCUSSION**

Many viruses cause acute myocarditis and it is usually benign.$^{1-5}$ But recently, many follow-up studies on virus myocarditis in man have shown that the myocarditis occasionally left cardiac abnormalities long after recovery.$^{2-17}$ Several serological studies on virus antibodies in patients with idiopathic cardiomyopathy suggest that the patients have experienced more virus infections than have normal controls.$^{20-23}$ These findings appear to favor the hypothesis that idiopathic cardiomyopathy in man represents a sequela of virus myocarditis. Although there have been many studies of virus myocarditis in experimental animals, most have investigated the acute phase of the disease and only a few have dealt with its chronic phase.$^{18,19}$

In order to test the virus theory of idiopathic cardiomyopathy, long term studies were conducted on virus myocarditis induced by coxsackie B 1, 3 or 5 virus. These viruses were chosen because serum antibodies to them were more common in patients with idiopathic cardiomyopathy than in normal controls in my previous study.$^{23}$ Mice inoculated with a large amount of coxsackie B 3 virus always developed acute and severe myocarditis, while mice inoculated with the same dose of coxsackie B 1 or 5 virus rarely developed mild myocarditis. Coxsackie B 3 virus was the most virulent for post-weaning ICR mice, although any type of coxsackie B viruses can induce myocarditis in mice.$^{26}$ When mice were inoculated with a small amount of coxsackie B 3 virus, a majority of them developed mild myocarditis. These results suggest that the incidence and severity of myocarditis are determined not only by the type but also by the dose of virus inoculated. Moreover, the severity of myocarditis varied markedly among the mice even when the same dose of coxsackie B 3 virus was given. This suggested that sensitivity to the virus varies considerably even among mice of the same strain. This may be analogous to virus infections among humans.

In the clinical diagnosis of myocarditis, electrocardiographic findings are considered to be very helpful$^{27}$ but in this study there were no obvious differences between the mice with severe myocarditis and normal controls. Thus electrocardiography is not useful in the detection of myocarditis in mice.

An absolute decrease in body and heart weights in the acute phase of myocarditis was seen in mice inoculated with a large amount of coxsackie B 3 virus. There was also a prolonged delay in regaining body and heart weights after the acute phase. Miranda et al.$^{28}$ observed similar changes in body weight of coxsackie B 5 virus-inoculated baby mice and speculated that a severe infection at a critical stage of growth would cause a marked delay in development. Widdowson and McCance$^{29}$ reported that malnutrition in the neonatal period could have long-term effects on growth. In this study, both severe infection and malnutrition due to anorexia appeared to cause a prolonged delay in regaining body weight. Thus the ratio of heart weight/body weight was significantly larger than in the controls, although the heart underwent massive necrosis and replacement fibrosis in the acute phase.

In the acute phase, histological findings of the heart with coxsackie B 3 virus myocarditis were almost identical with those described by other investigators.$^{4,18,19,30}$ except for capillary thrombi in the myocardium. The capillary thrombi first appeared on the third day and were frequently seen on the 5th to 9th days, when myocardial necrosis was most prominent. A similar vascular lesion has been documented in coxsackie B 4 virus myocarditis in mice.$^{31}$ Disseminated intravascular coagulation (DIC) will
not be related with these thrombi because they were formed with platelets, for in case of DIC thrombi are formed with fibrin\[32\].

In the pathogenesis of acute necrosis of myocardial cells in coxsackie B 3 virus myocarditis of mice, multiplication of virus in the myocardial cells would play an important role because the total area of the necrotic lesions of the myocardium appeared to parallel chronologically virus concentration in the heart. In the present study, the virus was identified in the damaged myocardial cells by immunofluorescent study. In addition, previously the author and his colleagues confirmed the same virus with crystalline arrays in a myocardial cell of mice with coxsackie B 3 virus myocarditis by electron microscopy\[32\]. Ischemia due to capillary thrombi might also play a role in the production of the myocardial lesions, because anoxia induces a variety of degenerative changes in myocardial cells\[34\] though it may be impossible to distinguish ischemic changes of the myocardium from those caused directly by virus multiplication.

In this study, in approximately two thirds of the mice inoculated with a large amount of coxsackie B 3 virus, necrotic lesions of the myocardium was not extensive in the acute phase. In these mice the lesions rapidly decreased in size and frequency so that the myocarditis appeared to heal without significant replacement fibrosis. This vigorous recovery from severe myocarditis was striking. In almost all patients with virus myocarditis studied by the author and Morita\[17\] there were only mild histological changes in the biopsied myocardium shortly after the acute phase of the myocarditis in spite of the fact that some of the patients even suffered from severe congestive heart failure. A similar recovery from idiopathic myocarditis of man was emphasized by Sekiguchi et al\[35\] in their study of serial endomyocardial biopsies from the patients. Shozawa et al\[36\] speculated that regenerative process of myocardial cells might play a role in recovery from diphtheric myocarditis in experimental animals, in which double nuclei were frequently seen in myocardial cells. Double nuclei of myocardial cells were also frequently observed in the acute phase of this study. This may support the above speculation, although double nuclei were also occasionally seen in myocardial cells of age-matched control animals. On the other hand, phagocytotic activity of macrophages may also play a crucial role in recovery from myocarditis without significant fibrosis. By electron micro-

scopy, the author and his colleagues\[17,37,38\] have observed that many macrophages had phagocytosed much cell debris and/or calcified granules in the necrotic lesions of the myocardium of mice and hamsters with coxsackie B 3 virus myocarditis. In hamsters, acute myocardial necrosis appeared more severe, but the healing process was more prominent than in mice\[17,37,38\]. In the necrotic lesions of the myocardium of the hamster, mononuclear cell infiltration including that of macrophages was more abundant than in those of the mouse\[17,37,38\].

On the other hand, approximately one third of the mice inoculated with a large amount of coxsackie B 3 virus exhibited residual pathologic changes characterized by patchy fibrosis with calcification and slight cellular infiltration in the early stage of the chronic phase. In the late stage of the chronic phase, fibrotic foci in the myocardium have become more extensive. In and around these lesions, some myocardial fibers had undergone atrophy or hypertrophy. The myocardial changes in the early stage of the chronic phase probably represent merely a replacement fibrosis following massive acute myocardial necrosis, but the mechanism of the progressive myocardial changes in the late stage of the chronic phase remains to be clarified. In their six-month study on murine hearts with coxsackie B 3 virus myocarditis, Wilson et al\[18\] observed residual pathologic changes of the heart after the acute myocarditis and speculated that immune response initiated by new antigens produced by necrotic myocardial cells might have some relation to the production of myocardial lesions in the chronic phase. Although Feinstein et al\[19\] observed similar residual myocardial changes, they could not demonstrate delayed hypersensitivity to cardiac antigens by the macrophage migration inhibition method. In this study, the progression of the myocardial fibrosis in the late stage of the chronic phase was not correlated with aging, because a similar fibrosis was not seen in age-matched control animals. These myocardial changes appeared to be observed only in mice which had developed large myocardial fibrosis in the early stage of the chronic phase. The early fibrotic and/or calcified lesions may damage myocardial cells in their vicinities and cause compensatory hypertrophy and atrophy. These myocardial cells might then replaced by further fibrosis. Although there has been no report on cardiac function in mice with coxsackie B virus myocarditis, large fibrotic lesions
of the myocardium would be expected to depress cardiac function. In hamsters, there is evidence that contractility and relaxation of the left ventricular myocardium is significantly depressed even after acute coxsackie B3 virus myocarditis; however, histological changes of the myocardium appeared to be much less prominent in this phase in hamsters than in mice. In mice with virus myocarditis, therefore, myocardial dysfunction may be caused even without obvious microscopic abnormality. This myocardial dysfunction might become more apparent with the advance of myocardial fibrosis in the late stage of the chronic phase.

Except for severe calcification, the histological findings of the myocardium in the late stage of the chronic phase resemble those of congestive cardiomyopathy of man particularly the post-carditic cardiomyopathy postulated by Okada. The occurrence of calcified foci in the myocardium of mice differs from the cardiomyopathy of man, but calcification does not seem to characterize coxsackie B3 virus myocarditis. In hamsters, the same virus caused severe myocarditis, no report is available on calcified foci in the myocardium, whereas in human fetus, the same virus causes myocarditis with calcification. The reason for the difference in the calcification of the myocardium among these varied conditions remains to be investigated.

Acknowledgement

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REFERENCES

1. SAPHIR O: Myocarditis: A general review with analysis of 240 cases. Arch Path 33: 1000, 1941; 33: 88, 1942
2. ABELMANN WH: Virus and the heart. Circulation 44: 950, 1971
5. HIRSCHMAN SZ, HAMMER GS: Coxsackie virus myopericarditis: A microbiological and clinical review (review). Am J Cardiol 34: 244, 1974
7. SMITH WG: Adult heart disease due to the coxsackie virus group B. Br Heart J 28: 204, 1966
12. KOONTZ CH, RAY CG: The role of coxsackie group B virus infections in sporadic myopericarditis. Am Heart J 82: 750, 1971
carditis in mice. J Path 109: 175, 1973


33. MIALE JB: Laboratory Medicine, Hematology (5th ed). CB Mosby Co., St Louis, 1977, p 965

34. JENNINGS RB, GATONE CE: Structural changes in myocardium during acute ischemia. Circ Res 34 and 35 (Suppl III): 156, 1974


36. SHOZAWA T, TAKASHINA R, KAWAMURA K, OKADA E: Histopathological study on idiopathic cardiomyopathy: I. Histopathological classifica-


38. DEGUCCI H, MORITA H, KITAURA Y, KAWAMURA K, TAKATSU T: Experimental coxsackie B 3 virus myocarditis in hamsters and mice: An electron microscopic and X-ray micros


41. OKADA R: Myocarditis and idiopathic myocardio
dopathy. Lung and Heart 24: 83, 1977
