# AUGMENTATION OF THE VIRULENCE OF MURINE COXSACKIEVIRUS B-3 MYOCARDIOPATHY BY EXERCISE*

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Certain strains of coxsackievirus A-9 induce minimal necrosis and interstitial inflammation of the murine heart (1, 2). Although affected mice appear well, cardiac size is increased. During the first 2 wk of infection, virus is present in the heart. When examined later, these myocardia are normal in size and are free of histologic change (3–5). Swimming increases myocardial replication of virus but does not alter the benignity of the process (6).

On the other hand, when weanling mice are inoculated with coxsackievirus B-3 (Nancy), a necrotic carditis involving 25–50% of the entire myocardium results. As with coxsackievirus A-9, coxsackievirus B-3 may be isolated only very early. Here, too, the infected baby mice continue to appear well. However, in the case of coxsackievirus B-3, healing is accompanied by myocardial fibrosis, deposition of calcium, and continuing inflammation. Since the continuing carditis is not associated with virus multiplication, we have called the entire process a myocardiopathy (7–9).

Human cardiac disease occurs, similar to that caused by the benign coxsackievirus A-9 and having lesions similar to the murine lesions caused by the virulent coxsackievirus B-3. The present experiments bring striking data to bear upon the role of the exercise induced by swimming upon murine coxsackievirus B-3 myocardiopathy.

## *Materials and Methods*

*Mice.*—Pregnant albino Swiss ICR mice were obtained at term. After delivery, each mother with its brood was housed in a separate cage. Nurslings were weaned at about 3 wk and, thereafter, were fed standard Rockland rat chow.

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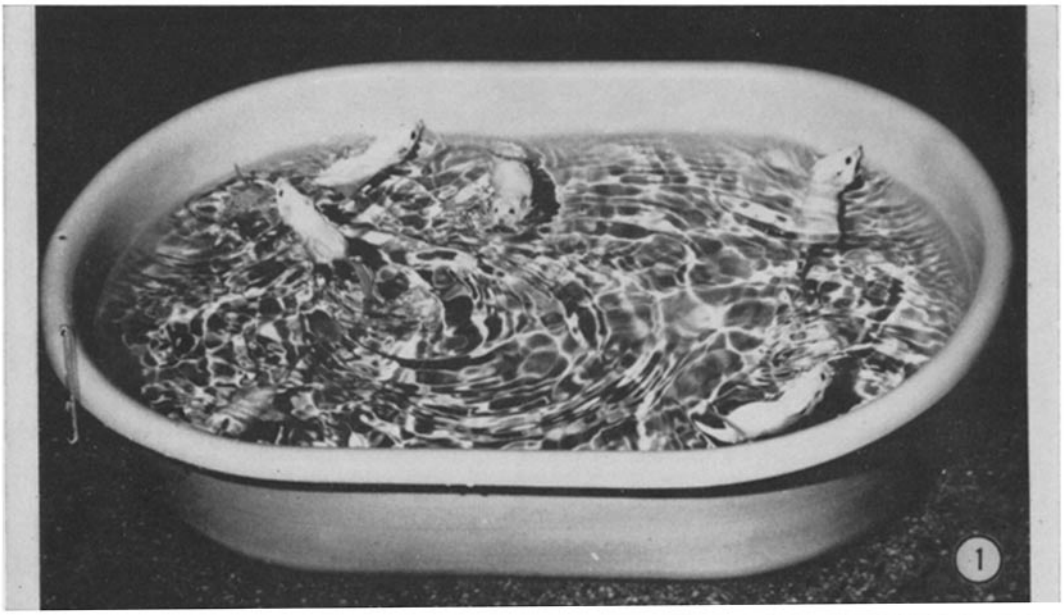


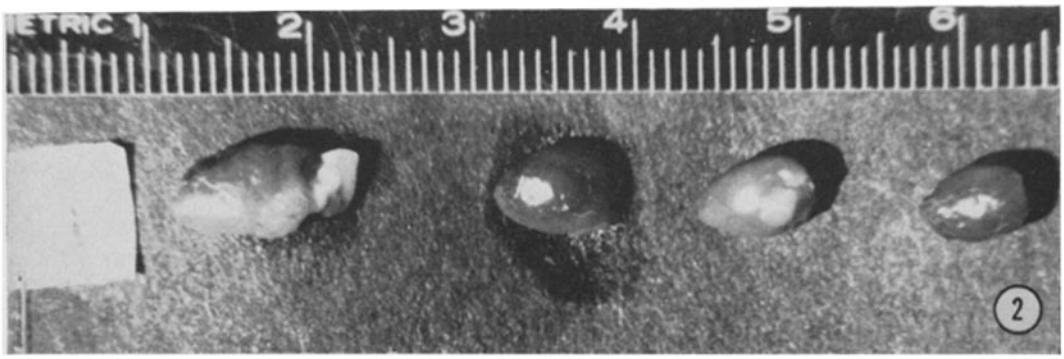




FIG. 1. Weanling mice, 39 days old, with coxsackievirus B-3 myocardiopathy swimming in a preheated pool (33°C) on the 25th day of disease.

FIG. 2. Relative size of hearts of 39-day old mice with coxsackievirus B-3 myocardiopathy on the 25th day of disease are shown. From left to right: group I, infected and made to swim throughout experimental infection; group II, infected but not made to swim; group III, not infected but forced to swim throughout experimental illness; and group IV, not infected and not forced to swim.

A preliminary experiment utilized 34 baby mice derived from 3 mothers. At 10 days they were forced to swim in a plastic pool containing warm water, 33°C, (Fig. 1). When exhausted and drowning, they were rescued by means of a net. Conditioning increased their tolerance and at 10 days mice could swim 30 sec; at 11 days, 1 min; at 12 days, 2 min; at 13 days, 3 min; at 14 days, 5 min; at 16 days, 15 min; and at 17 days, 20 min. On the 18th day of life, the

young mice swam for a full half hr. Thereafter, they swam 30 min in the morning and in the afternoon.

*Experimental Infections.*—Coxsackievirus B-3 (Nancy) was used (7, 10–12). After intracerebral, intraperitoneal, and subcutaneous murine passages, virus was carried twice in tube cultures of rhesus kidney and a pool of stock virus prepared. 0.5 ml samples of coxsackievirus B-3 were placed in screw-capped vials, and stored at −50°C until use. Each vial contained 15 × $10^6$ plaque forming units (PFU) per ml.

At 14 days of age, 6 groups of 36 baby mice were formed. Mothers were kept with their young until weaning at 21 days. Mice in group I were inoculated with 0.05 ml of a $10^{-2}$ dilution of virus stock intraperitoneally, and with another 0.02 ml intracerebrally. These mice were forced to swim as outlined above. Mice in group II were inoculated with coxsackievirus B-3 as above, but were not exercised. Nursling mice in group III were inoculated with similar volumes of uninfected fluids from rhesus kidney tissue cultures and were forced to swim. Others in group IV also received inoculations with uninfected fluids from tissue cultures, but were not exercised. Mice in group V were inoculated with cosackievirus B-3; forced swimming did not begin until 9 days later, when the late phase of the myocardiopathy ensued. Those in group VI received uninfected fluids; exercise began on day 9.

Unless they died earlier, six mice from each group were sacrificed by exsanguination under ether anesthesia on days 3, 6, 9, 13, 20, and 40 after inoculations. Animals which died within 24 hr after their intraperitoneal and intracerebral inoculations were considered traumatic and were excluded from the analysis. At death, dual samples of blood, heart, kidney (usually with perirenal fat and adrenal glands), liver, hind limb, and brain were placed in screw-capped vials containing 10% buffered formalin, and portions were also kept at −20°C for virological study.

Formalinized tissues of each organ were embedded in a single block. Hearts were placed so that portions of both ventricles and septum were sectioned. In most, portions of one or both atria and interatrial septum were also included. For each tissue 10 serial sections cut at distances of 6 μ and stained with hematoxylin and eosin were examined under code. At the time of reading, the pathologist had no knowledge concerning the donor animals. The degree of involvement of a tissue was estimated on the basis of a scan of all 10 sections. Grade zero indicated that there were no lesions. A grading of 1 was given when less than one-fourth of the myocardium contained lesions; with quarterly increments, the grades given were 2, 3 and 4. Special stains were made for calcium (von Kossa), iron (Prussian blue) and nucleic acids (methyl green–pyronin Y).

*Virology.*—As before, (7) tissues saved for virological study were allowed to thaw, minced, ground with Alundum, and 20% suspensions in Eagle's medium were made. Alundum was separated by centrifugation, and 0.1 ml of each of the supernates was inoculated into two tube cultures of rhesus kidney cells containing 1 ml of Eagle's medium and 2% fetal calf serum. Tubes were observed daily for the appearance of characteristic cytopathic effects. Supernates from cultures which showed questionable changes or were negative at 7 days were passaged into two additional tube cultures and observed for a second similar period. Amounts of coxsackieviruses in positive specimens were titered using an agar overlay method.

Suspensions of rhesus kidney cells from Parke Davis & Co., Detroit, Mich., were obtained through the courtesy of I. William McLean. Tissue cultures were prepared in 30 ml screw-capped plastic flasks.[1] 4 ml containing 100 × $10^4$ cells in Eagle's medium with 5% fetal calf serum and antibiotics[2] were placed in each flask. Medium was changed every 3rd day, until a confluent sheet of cells had formed. At this time fluid was decanted, and cultures washed

[1] These were obtained from Falcon Plastics, Los Angeles, Calif.

[2] Medium contained 100 μg of penicillin G and 50 μg of streptomycin per ml.

with 2 ml of warm Eagle's basal medium (37°C). 0.2 ml of appropriate dilutions of virus were added, and cultures were placed in an incubator (37°C) for 1 hr. During this period, flasks were gently agitated 10 times each 15 min to improve distribution of the inoculum. Two cultures were used per dilution.

After incubation, cultures were washed with another 2 ml of warm medium and finally overlaid with 2 ml of a twice-concentrated Eagle's medium with 10% fetal calf serum (37°C) and 2 ml distilled water with 1.5% Difco nutrient agar and 0.4% sodium bicarbonate (42°C). After 3 days of a further incubation at 37°C in the dark, a second similar overlay with the addition of neutral red at 1:40,000 was added. Plaques could be seen by the 5th day, and were read on day 7.

TABLE I

*Effect of Swimming on the Mortality of Weanling Mice with Coxsackievirus B-3 Myocardiopathy*

| Group No. | Infected | Forced to swim | Day after inoculations | | | | | | Total No. of deaths | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 3 | 6 | 9 | 13 | 20 | 40 | | |
| | | | | | | | | | | % |
| I | + | + | 1* | 6 | 4 | 4 | 0 | 0 | 15/30 | 50 |
| II | + | − | 0 | 0 | 1 | 0 | 1 | 0 | 2/36 | 5.5 |
| III | − | + | 0 | 0 | 0 | 0 | 0 | 0 | 0/36 | 0 |
| IV | − | − | 0 | 0 | 0 | 0 | 0 | 0 | 0/36 | 0 |
| V | + | +‡ | 0 | 0 | 0 | 4 | 1 | 0 | 5/36 | 13.9 |
| VI | − | +‡ | 0 | 0 | 0 | 0 | 0 | 0 | 0/36 | 0 |

* Numbers listed under "day after inoculations" represent the number of deaths.

‡ Swimming began on the 9th day after inoculations of viurs or its control of fluids from uninfected tissue cultures.

## RESULTS

*Clinical Findings.*—Of the 36 infected mice which were not forced to swim, one animal died on the 9th day and another on the 20th day after inoculations (Table I). This mortality (5.5%) contrasts to none among baby mice in groups III, IV, and VI which received no virus but which were forced to swim. Five mice in group V, infected and forced to swim beginning on the 9th day after inoculations, died. These mice were exercised only during the late phase of their cardiopathy. None expired before exercise was instituted, and all five died while swimming, four on the 13th day and one on the 20th. The overall mortality of this group was 13.9%.

Infected mice in group I were conditioned and forced to swim continually. One animal died on day 3; six on day 6; and four each on the 9th and 13th days after infection. No mouse of this group died after day 13. The majority (eight mice) died while swimming, six on the 6th day and two on the 9th day. The rest were found dead in their cages. In all, 15 of 30 of these mice died.

*Gross Findings.*—At sacrifice, hearts were larger in the exercised, uninfected mice (groups III and VI) than in similar uninfected mice which were not

forced to swim. Further increases in size occurred in infected mice which were forced to swim (groups I and V, Fig. 2). Hearts were largest in infected mice of group I which were forced to swim throughout their developing cardiopathy. In these animals, findings were most marked 9 days after infection when wrinkled gray-white streaks often discolored half of the heart. At section,

TABLE II

*Effect of Swimming on the Ratio of Mean Heart to Body Weight ($\times 10^6$) in Weanling Mice with Coxsackievirus B-3 Myocardiopathy*

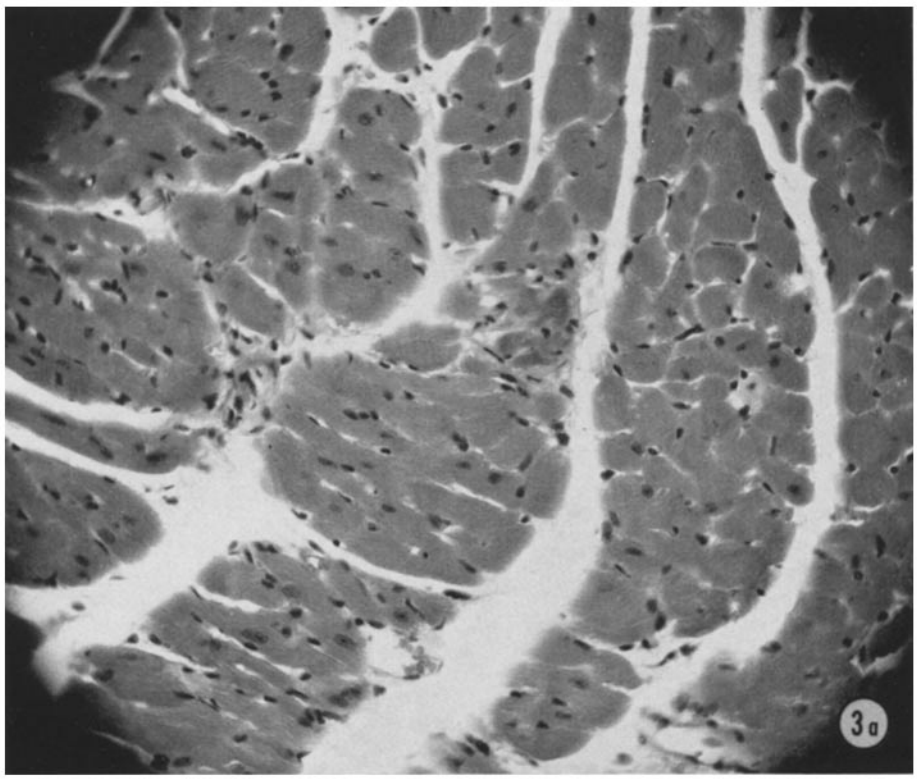
| Group No. | Infected | Forced to swim | Day after inoculations | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 3 | 6 | 9 | 13 | 20 | 40 |
| I | + | + | 788 (6)* | 840 (6) | 745 (6) | 732 (6) | 723 (3) | 674 (3) |
| II | + | − | 784 (6) | 801 (6) | 721 (6) | 722 (6) | 709 (6) | 662 (5) |
| III | − | + | 753 (6) | 763 (6) | 705 (6) | 700 (6) | 694 (6) | 654 (5) |
| IV | − | − | 682 (6) | 757 (6) | 695 (6) | 606 (6) | 549 (6) | 556 (5) |
| V | + | +‡ | 774 (6) | 779 (6) | 718 (6) | 673 (6) | 562 (6) | 540 (6) |
| VI | − | +‡ | 756 (6) | 775 (6) | 714 (6) | 630 (6) | 550 (6) | 558 (6) |

* Number in parenthesis indicates the number of mice included in calculation of means.
‡ Swimming began on day 9 after inoculations in groups V and VI.

chambers were dilated, hypertrophied, and necrotic. Moderate to severe edema was present in the lungs.

*Ratio of Heart to Body Weight (Table II).*—When compared to data from uninfected, nonexercised mice, mean ratios of heart to body weight were pro-

FIG. 3. Pathologic findings in coxsackievirus B-3 myocarditis are shown. The effects of swimming are included. *a.* A small focus of hyaline eosinophilia and scant mononuclear cell infiltrate is shown in the myocardium of a 54-day old weanling mouse sacrificed on the 40th day after infection. Swimming began on the 9th day of the cardiomyopathy (group V). Hematoxylin and eosin. × 135. *b.* There is focal myocardial fiber necrosis with granular sarcoplasm and an early mononuclear cell infiltrate in a 23-day old weanling mouse (group V) sacrificed on the 9th day after infection. An adjacent focus of hyaline eosinophilic fibers with slight granularity is seen. Hematoxylin and eosin. × 370. *c.* A focus of necrotic myocardial fibers is seen undergoing individual mineralization in a 27-day old mouse which was forced to swim throughout his 13 days of coxsackievirus B-3 cardiomyopathy (group I). Adjacent there is a marked mononuclear cell infiltrate. Hematoxylin and eosin. × 225. *d.* Another myocardium from a 27-day old mouse on the 13th day after infection of group I is shown. There is marked mineralization of individual myocardial fibers with extensive surrounding necrosis and mononuclear infiltration. Hematoxylin and eosin. × 90. *e.* There is necrosis and mononuclear cell infiltration in the adipose tissue and skeletal muscle from the hind limb of a 23-day old mouse from group I on the 9th day after inoculation of coxsackievirus B-3. Hematoxylin and eosin. × 135. *f.* In the adipose tissue and hind limb of a 20-day old weanling mouse of group V, 6 days after infection necrosis, mononuclear infiltrates and mineralization are seen. Hematoxylin and eosin. × 135.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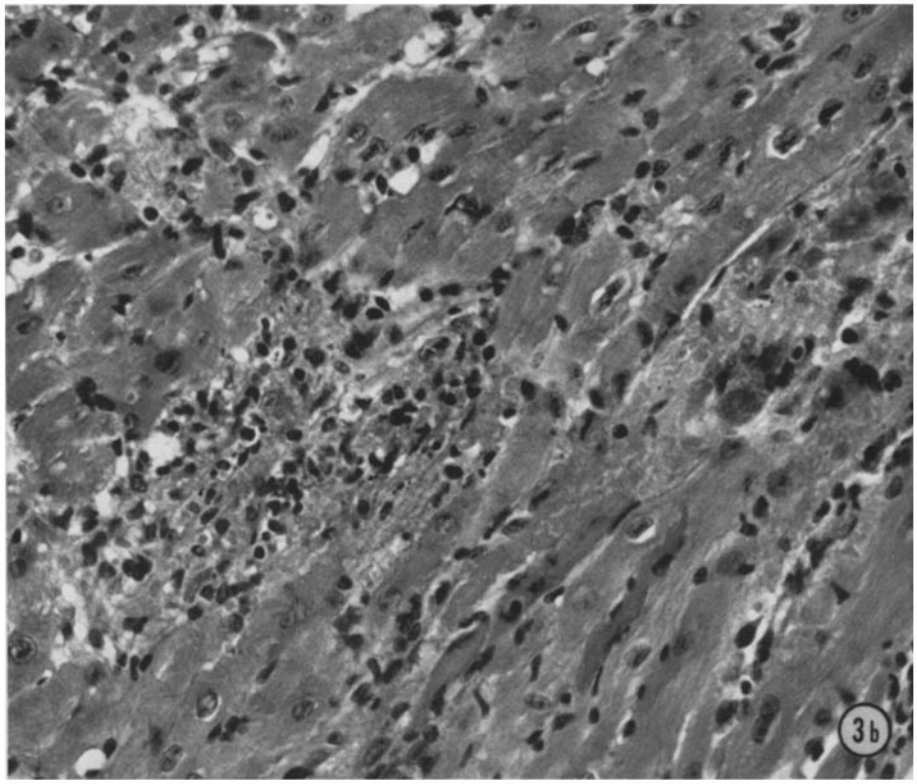
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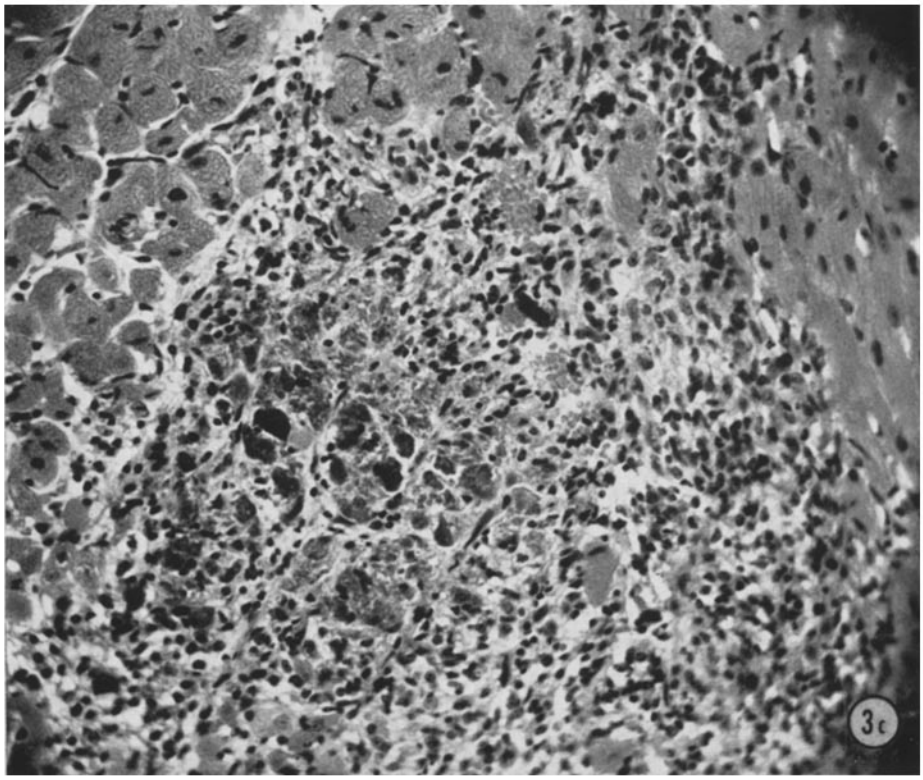
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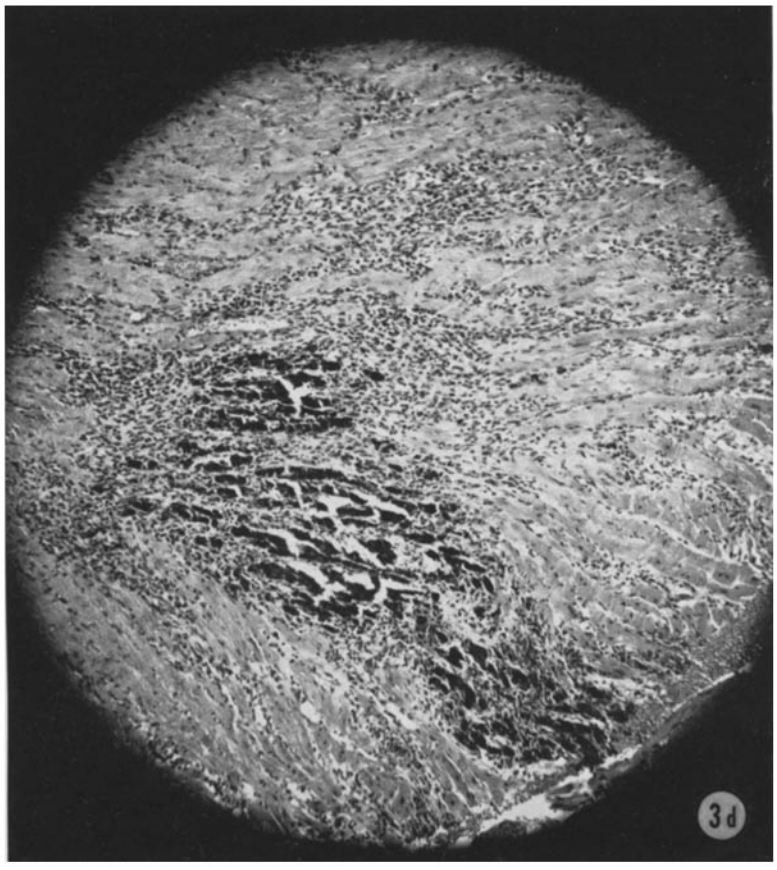
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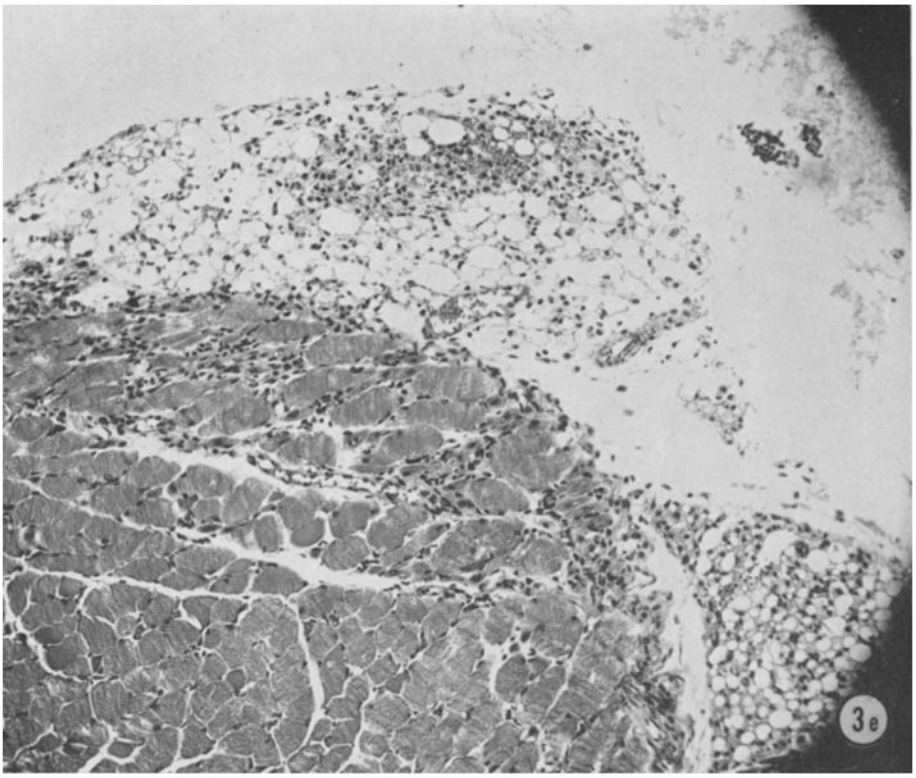
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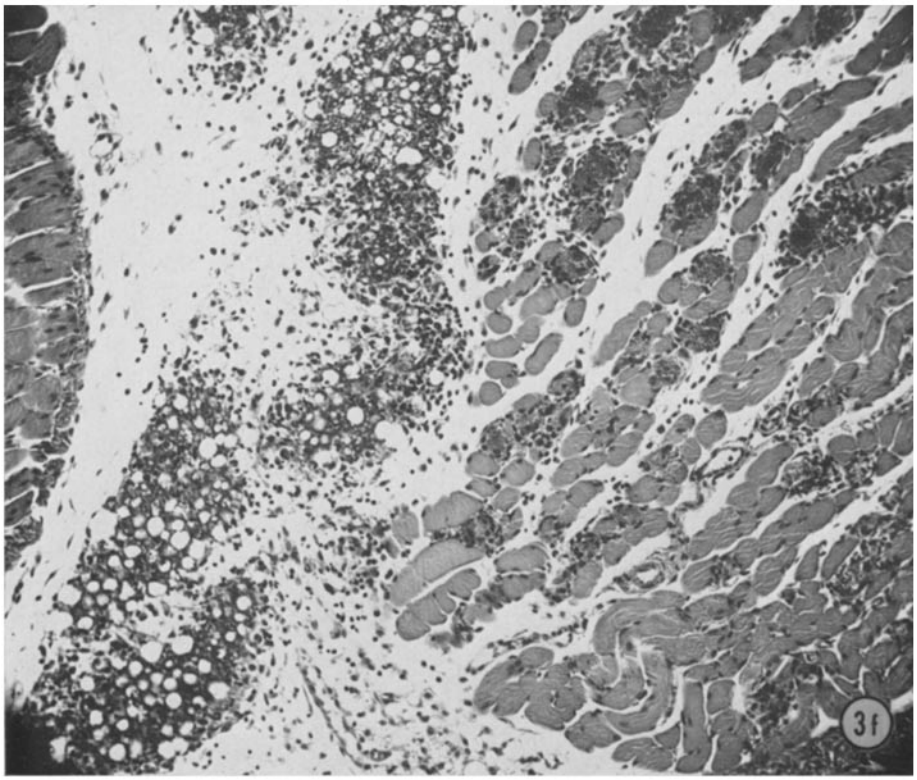
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gressively increased by swimming, infection, and the combination of both infection and swimming. Moreover, these differences in size of the heart persisted throughout the 40 days of this study. When swimming was begun on day 9 after inoculations, mean ratios of the heart to body weight did not increase in either the infected or uninfected mice.

*Microscopic Findings (Fig. 3).*—

*Heart:* The first evidence of disease of the heart was present by the 3rd day after inoculation (the first day in which animals were sacrificed). In these, a hyaline eosinophilic change affected small segments of some myocardial fibers

TABLE III

*Effect of Swimming upon the Histopathologic Findings in the Heart during Coxsackievirus B-3 Myocardiopathy*

| | Group | | | | | |
|---|---|---|---|---|---|---|
| | I Infected and immediately forced to swim | | II Infected, but not forced to swim | | V Infected and forced to swim, beginning 9 days later | |
| | | % | | % | | % |
| Pathologic findings in heart | 25/30 | 83 | 27/34 | 79 | 35/36 | 97 |
| Myocarditis involving 76–100% of the affected individual hearts (3–4+) | 20/25 | 80 | 5/27 | 18.5 | 9/35 | 26 |
| Myocarditis of 3–4+ severity noted on or after the 13th day of infection | 8/12 | 67 | 0/17 | 0 | 5/18 | 28 |

(Fig. 3 *a*). Changes were present singly and in clusters of up to four fibers. Occasionally, a few lymphocytes surrounded a nearby capillary or arteriole (Fig. 3 *b*). In animals sacrificed on the 6th day of their cardiopathy, affected segments appeared to be enlarged with coarse and fine granularity. The sarcoplasm was pale. Muscle nuclei in affected segments were irregular in outline, shrunken, and hyperchromatic. Necrotic foci were infiltrated and surrounded by moderate numbers of mononuclear cells, some of which had a plasmacytoid appearance (Fig. 3 *c*). Rarely by the 6th, but regularly by the 9th day after inoculation, a few fine hematoxylinophilic granules were found within the necrotic fibers. In the more advanced and later stages (Fig. 3 *d*) these granules enlarged and coalesced to fill segments of many of the necrotic fibers. Basophilic staining material gave positive stains for calcium and iron. Increasing mineralization was accompanied by a gradual decrease in the cellular infiltrates and often by fibrosis about individual fibers. Nuclei of some of the sur-

rounding muscle fibers in the severely affected animals were enlarged, multinucleate, and increased in numbers. New segmental involvement of myocardial fibers continued throughout the period of study, so that after the 12th day of coxsackievirus B-3 myocardiopathy, all phases were present. At all stages, the left ventricle showed greater and more severe changes than the right ventricle or atria. The epicardium, endocardium, or valves were not affected.

*Skeletal muscle:* A continuing sequence of similar pathologic changes occurred in some of the striated muscle taken from the lower limbs (Fig. 3 *e, f*).

*Adipose tissue:* Foci of necrosis with granularity and/or mineralization were found in the perirenal adipose tissue and that accompanying the muscle of the

TABLE IV

*Effect of Swimming upon the Histopathologic Findings in Hind Limbs during Coxsackievirus B-3 Myocardiopathy*

| | Group | | | | | |
|---|---|---|---|---|---|---|
| | I Infected and immediately forced to swim | | II Infected, but not forced to swim | | V Infected and forced to swim beginning 9 days later | |
| | | % | | % | | % |
| Pathologic findings in hind limb | 16/28 | 57 | 9/36 | 25 | 9/34 | 26 |
| Myositis involving 76–100% of the affected hind limb (3–4+) | 2/16 | 12.5 | 1/9 | 11 | 0/9 | 0 |
| Myositis of 3–4+ severity noted on or after the 13th day of infection | 0/11 | 0 | 0/18 | 0 | 0/18 | 0 |

lower limbs. The structural changes of the lesions suggested a single early episode of involvement (Fig. 3 *e, f*).

*Brain, kidney, liver, lungs and adrenals* were normal. In animals with severely affected hearts, acute passive hyperemia of the liver, sometimes with centrolobular necrosis, was present.

At least 80% of the mice inoculated with coxsackievirus B-3 developed myocarditis (Table III). Minimal to moderate lesions, involving 10 to 50% of the myocardial wall (1 to 2+), predominated among infected, nonexercised weanlings of group II (Table III). Only 5 of 27 (18.5%) hearts of this infected nonexercised group had severe, 3 to 4+ lesions. In these mice, no 3 to 4+ changes were seen after the 9th day.

In 9 of 35 (26%) affected hearts from mice whose swimming began on day 9 of the myocardiopathy (group V) 3 to 4+ changes were noted. Therefore, neither mortality (Table I) nor severity of the pathologic changes (Table III)

were as strikingly altered by swimming commencing on the 9th day after inoculations with coxsakievirus B-3.

On the other hand, 20 of 25 hearts of mice which were forced to swim during both phases of their developing myocardiopathy had 3 or 4+ changes. 12 of these severe lesions were noted at autopsies performed on the 6th or 9th days after infection.

Myositis was observed in 16 of 28 (57%) of the hind limbs of the immediately exercised mice, compared to 9 of 36 (25%) and 9 of 34 (26%) of mice which were not swam or whose swimming began on the 9th cardiopathic day, respectively (Table IV). Although incidence of myositis increased with swimming,

TABLE V

*Effect of Swimming upon the Histopathologic Findings in Perirenal and Pericardial Fat during Coxsackievirus B-3 Myocardiopathy*

| | Group | | | | | |
|---|---|---|---|---|---|---|
| | I Infected and immediately forced to swim | | II Infected, but not forced to swim | | V Infected and forced to swim beginning 9 days later | |
| | | % | | % | | % |
| Pathologic findings in perirenal and pericardial fat | 22/26 | 85 | 18/25 | 72 | 25/26 | 96 |
| Lesions involving 76–100% of the affected (3–4+) | 8/22 | 36 | 3/18 | 17 | 2/25 | 8 |
| Lesions of 3–4+ severity noted on or after the 13th day of infection | 3/10 | 30 | 0/15 | 0 | 1/15 | 6 |

in contrast to the heart, the extent and severity of the changes were similar in the affected groups. No 3 to 4+ changes were seen on day 13 or later. Since mice use their hind limbs for balance while swimming, and the severe test is to the forelimb, it is unfortunate that the severely exercised forelimbs were not examined.

Like the heart, perirenal and pericardial fat were regularly involved (Table V). Severe 3 to 4+ lesions were noted in 8 of 22 (36%) mice which were immediately forced to swim, but were noted less frequently in late or nonexercised mice. Severe lesions on or after day 13 were noted only in mice of group I which were forced to swim from the onset of infection.

*Virological Findings.*—From specimens taken on the 3rd and 6th days after inoculation (Table VI), coxsackieviruses were isolated from blood, heart, hind limb, kidney, and brain of infected mice in groups I, II, and V. More specimens from mice which were immediately forced to swim contained coxsackievirus B-3 than from any other group. Brain was least susceptible. Viremia

continued through day 6, but was no longer present on the 9th day after inoculation. Since virus was isolated from brain and kidney only during the viremia, and, since sections of tissue were normal, virus multiplication in these organs is unlikely. On the 9th day after infection, only the heart contained virus.

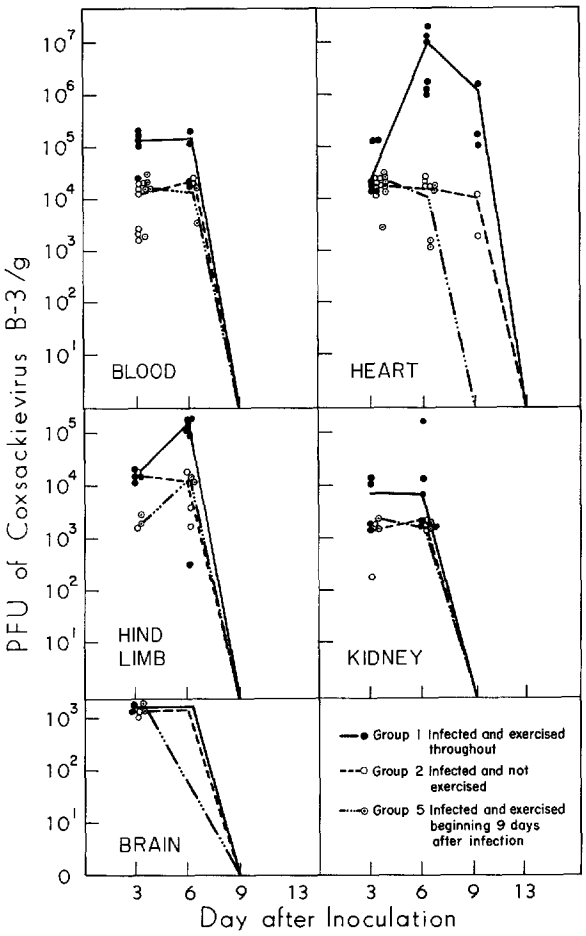


FIG. 4. Effect of swimming upon the multiplication of coxsackievirus B-3 in several tissues.

Quantitative titrations of coxsackieviruses from positive specimens are shown in Fig. 4. On day 3, titers of coxsackievirus in hearts of all infected mice, nonexercised and exercised, were equal. However, on days 6 and 9, hearts of mice which were immediately forced to swim had 530- and 100-fold increases in virus replication, respectively. When swimming was begun on day 9, myocardial virus replication was not augmented.

TABLE VI

*Effect of Swimming on the Isolation of Coxsackievirus B-3 (Nancy) from Several Tissues after Intraperitoneal and Intracerebral Inoculations into 14-Day Old Weanling Mice*

| Day(s) after inoculations | Group No. | Infected | Forced to swim | Isolation of coxsackievirus B-3 | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Blood | Heart | Skeletal muscle | Kidney | Brain |
| 3 | I | + | + | 6/6* | 6/6 | 4/6 | 4/6 | 2/6 |
| | II | + | − | 6/6 | 6/6 | 2/6 | 3/6 | 2/6 |
| | III | − | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | IV | − | − | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | V | + | +‡ | 5/6 | 6/6 | 2/6 | 3/6 | 2/6 |
| | VI | − | +‡ | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| 6 | I | + | + | 4/6 | 6/6 | 4/6 | 4/6 | 1/6 |
| | II | + | − | 2/6 | 4/6 | 3/6 | 3/6 | 1/6 |
| | III | − | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | IV | − | − | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | V | + | + | 2/6 | 4/6 | 2/6 | 3/6 | 0/6 |
| | VI | − | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| 9 | I | + | + | 0/6 | 3/6 | 0/6 | 0/6 | 0/6 |
| | II | + | − | 0/6 | 2/6 | 0/6 | 0/6 | 0/6 |
| | III | − | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | IV | − | − | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | V | + | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | VI | − | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| 13 | I | + | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | II | + | − | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | III | − | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | IV | − | − | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | V | + | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | VI | − | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| 20§ | I | + | + | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| | II | + | − | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | III | − | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | IV | − | − | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | V | + | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | VI | − | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| 40§ | I | + | + | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| | II | + | − | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | III | − | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | IV | − | − | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | V | + | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | VI | − | + | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| Total | I | + | + | 10/30 | 15/30 | 8/30 | 8/30 | 3/30 |
| | II | + | − | 8/36 | 12/36 | 5/36 | 6/36 | 3/36 |
| | III | − | + | 0/36 | 0/36 | 0/36 | 0/36 | 0/36 |
| | IV | − | − | 0/36 | 0/36 | 0/36 | 0/36 | 0/36 |
| | V | + | + | 7/36 | 10/36 | 4/36 | 6/36 | 2/36 |
| | VI | − | + | 0/36 | 0/36 | 0/36 | 0/36 | 0/36 |

* 6/6 indicates that six mice were autopsied and all the blood of six contained coxsackievirus B-3.

‡ Swimming began on day 9 in groups V and VI.

§ On days 20 and 40, three mice only from group I were sacrificed.

In blood, hind limb, brain, and kidney there were no significant differences in amounts of virus among the groups throughout the experiment.

## DISCUSSION

When poliomyelitis was prevalent in this country, it was often observed that paralysis was preceded by a period of exhausting physical exercise. Statistical study supports the impression that fatigue increases susceptibility to paralysis in persons already infected (13). In a controlled study of murine poliomyelitis, using the Lansing strain of poliovirus, Rosenbaum and Harford showed a small but significant increase in paralysis and death in exercised mice when compared to their rested controls (14). Their study did not include quantitative estimations either of virus multiplication or of the induced pathologic changes. The present experiments provide striking clinical and virological evidence of a marked aggravation of virulent coxsackievirus B-3 myocarditis by severe exercise. The data support the potential benefit of rest to the affected mouse and probably to man.

In the experiments presented, swimming increased mortality from 5.5% to 50%. Many of the affected mice died in congestive failure while swimming. Hearts were dilated and necrotic, and ratios of mean heart to body weight increased. Often virtually every myocardial fiber showed pathologic change. On the other hand, one-fourth to one-half of the myocardium was involved when infection was not accompanied by swimming. Concomitantly, virus multiplication in the heart was remarkably augmented by swimming.

When swimming was initiated while virus replication in the heart was waning, mortality was increased over their nonexercised controls to 13.8%. The duration of multiplication of virus was not increased by swimming.

Inflammatory lesions in pericardial and perirenal fat were increased in severity and somewhat prolonged in duration by swimming. Myositis in hind limbs was more frequent in mice which were forced to swim, but the severity of these changes was not increased.

The mechanism of marked increase in myocardial virulence induced by exercise remains to be determined. Similar worsenings of coxsackievirus infections have been initiated by cold (15), ionizing radiation (16), and corticosteroids (17). The roles of interferon, endogenous steroids, acidosis, or alterations in local host factors need to be considered.

## SUMMARY

Coxsackievirus B-3 myocardiopathy was induced in weanling mice by intraperitoneal and intracerebral inoculations of the Nancy strain. Acute mortality was 5.5%. The cardiomyopathy is characterized by an early phase lasting about 9 days with myocardial necrosis, associated inflammation, and

healing by fibrosis and calcification involving 25 to 50% of the contractile fibers in each affected mouse. Infectious coxsackievirus may be recovered from the heart during this phase. Continuing myocardial inflammatory lesions follow during the later phase, but infectious virus is no longer present.

When mice were forced to swim in a preheated pool (33°C) during both phases of their myocardiopathy, virulence was strikingly augmented. Fully half of the mice died of congestive failure, the majority while swimming. Hearts were dilated, hypertrophied, and grossly necrotic. The myocardium was transformed to a completely necrotic, inflammatory, calcifying mass.

At the peak of the infectious phase, myocardial replication of coxsackievirus was increased 530 times in nurslings which had been forced to swim. Myositis in hind limbs was more frequent, and inflammatory lesions in perirenal and pericardial fat were more severe in the mice which were forced to swim.

When swimming was begun on the 9th day after infection, the virulence and lethality (13.8%) of infection were moderately increased.

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