ferences were found in weight gains, organ weights, carcass composition or serum cholesterol levels. Serum polyunsaturated fatty acid levels were not changed by feeding fatty acids in the different forms. Corn oil did elevate the polyunsaturated acid levels in the serum above those observed in the lard-fed group of rats.

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Effects of Exercise on Coxsackie A9 Myocarditis in Adult Mice.* (29696)

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A previous study of experimental viral myocarditis in adult mice suggested that spontaneous exercise of a mild degree and infection with Strain 13 of Coxsackie A9 virus each increases the relative weight of the heart and that these effects are additive(1). The present study was designed to determine the effects of forced, severe exercise under otherwise comparable conditions.

Materials and methods. A total of 108 male C_3H mice, 10 months old and weighing 23.9 to 41.6 g were housed 6 to a cage and fed standard Purina Rat Chow. They were divided into 4 groups: A. (36 mice) inoculated with virus and exercised; B. (36 mice) inoculated with virus, not exercised; C. (18 mice) not inoculated with virus, but exercised; and D. (18 mice) not inoculated with virus and not exercised. The exercise consisted of swimming in aquaria filled with water at 33°C. The mice in groups A and C were exercised for increasing periods,—once daily for 5, 15, 30 and 60 minutes on days 7, 6, 5 and 4, respectively, and twice daily for 60 minutes on the last 3 days *before* they were inoculated with virus. Following the inoculations, the swim period was 45 minutes twice a day for the first 3 days. After that, because the animals experienced difficulty toward the end of the swim periods on the third day, the period of exercise was shortened to 40 minutes twice daily until the day of sacrifice. All animals were weighed to the nearest 0.1 g before the training was begun, on the day of inoculation and immediately before they were sacrificed; surviving animals were also weighed on days 3, 6 and 9 after inoculation.

Viral infection. Mice of groups A and B were given an intraperitoneal injection of 10^6 $TCID_{50}$† of the third rhesus kidney passage of strain 13, Coxsackie A9 virus(2) in a volume of 0.03-0.05 ml. Mice in groups C and D were given an intraperitoneal injection of uninfected tissue culture media containing

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† $TCID_{50}$ is the dose of virus which infects 50% of inoculated tissue culture tubes.

debris of rhesus kidney tissue in a similar volume.

Pathology. One-third of the mice of each group was sacrificed by exsanguination(2) under light ether anesthesia at 24 hours, 4 days and 9 days after inoculation, and the blood was collected for virus isolation. Two aliquots of calf muscle and the entire heart, after severing the great vessels 2 mm from the base, were obtained from each mouse. The heart was grossly dried of blood by rolling on sterile filter paper, weighed to the nearest 0.2 mg, and divided into 3 portions. To estimate the water content of the myocardium, the largest portion (about one-half of the whole heart) was weighed to the nearest 0.01 mg as a measure of its "wet weight"; this portion was then dried in an oven at 90°C and weighed at intervals of 48 hours until there was a stable "dry weight." A second portion of the heart—cut to include parts of all 4 chambers—and one aliquot of skeletal muscle were fixed in 10% formalin, imbedded in paraffin, sectioned, and stained with haematoxylin and eosin. Two observers working independently read each slide as an unknown and recorded the number and character of the lesions.

Virology. The blood obtained by exsanguination and the remaining portion of heart and skeletal muscle were used for virus isolation in rhesus kidney tissue culture, as described previously(2). All specimens that yielded virus were titered for plaque-forming units(3). Paired 50 mm petri dishes with complete sheets of rhesus kidney tissue were inoculated each with 0.2 ml of a decimal dilution in Hanks' balanced salt solution of either the supernate of the original 1:10 suspension of tissue or with a 1:10 dilution of blood. After incubation for 30 minutes at 37°C, the tissue in each petri dish was overlaid with nutrient agar and incubated at 37°C in an atmosphere containing 5% CO_2. Unstained plaques of dead cells were counted after 4 days, at which time surviving cells were stained by overlaying with a 1:10,000 dilution of neutral red.

Five mice died less than 24 hours after inoculation and each was found to have blood in the peritoneal cavity. These deaths occurred in all 4 groups; they were attributed to trauma during inoculation and the animals were excluded from the experiment. Two drownings occurred because the animals were the last to be retrieved among several that were simultaneously experiencing difficulty in swimming on the third day after the inoculations; however, only one of them had been inoculated with virus. These 2 animals will be considered separately in the discussion of the results.

TABLE I. Recovery of Virus.

Days after inoculation	Source	No. yielding virus/No. of mice sacrificed A*	B	A+B	C	D
1	Blood	7/11	8/11	15/22	0/4	0/5
	Heart	2/11	4/11	6/22	0/4	0/5
4	Blood	1/12	2/12	3/24	0/6	0/6
	Heart	6/12	1/12	7/24	0/6	0/6
9	Blood	0/11	0/12	0/23	0/5	0/6
	Heart	4/11	1/12	5/23	0/5	0/6
Total	Blood	8/34	10/35	18/69	0/15	0/17
	Heart	12/34	6/35	18/69	0/15	0/17

* A, infected intraperitoneally with 10^6 $TCID_{50}$ of Coxsackie A9, Strain 13 and exercised; B, infected, not exercised; C, not infected, exercised; D, not infected, not exercised.

Results. Virology. None of the uninfected mice (groups C and D) yielded virus from either blood, muscle or heart (Table I).

Viremia was demonstrated in the majority of infected animals sacrificed 1 day after inoculation, but in only 1 or 2 mice of the groups sacrificed after 4 days, and in none of those sacrificed 9 days after infection. In this respect there was no significant difference between the mice that were exercised and those that were not. Except for 2 specimens of blood obtained on day one, the titers of virus in the blood were all <100 PFU/ml (Table II).

Different results were obtained with the cultures of cardiac tissue (Table II). Among the animals sacrificed after 1 day, virus was recovered from a smaller proportion and in lower titers among those exercised (group A) than from those that were not (group B). The reverse was true among the animals sacrificed 4 and 9 days after the infections; at both of these times virus was recovered in a greater proportion and in much higher titers from the hearts of mice that had been exer-

TABLE II. Results of Plaque Titrations of Virus-Positive Specimens.

Days after inoculation	Group*	Blood: No. pos	Blood: PFU†/ml	Heart: No. pos	Heart: PFU/ml
1	A	7	2,000 800 <100(5)‡	2	200 <100
	B	8	<100(8)	4	1,700 600 200 <200
4	A	1	<100	6	120,000 61,000 60,000 30,000 24,000 2,600
	B	2	<100(2)	1	7,000
9	A	0		4	15,000 4,500 400 <100
	B	0		1	<100

* A, mice inoculated with virus and exercised; B, inoculated with virus, not exercised.
† PFU, plaque-forming units.
‡ No. of specimens with same titer is shown in parentheses.

cised. Combining the results on days 4 and 9, virus was recovered from 10 of 23 exercised, as compared with 2 of 24 non-exercised, virus-inoculated animals. The probability that this difference is due to chance is <0.02 by the chi-square test, using Yates' correction(4).

Of the 2 animals that drowned on day 3, one had been infected and virus was isolated from its heart in a titer of 8,000 PFU/ml. If this mouse is counted with the others of group A, the total number with virus-positive hearts on days 3, 4 and 9 would be 11 among the 24 animals in the exercised group, and the significance of the difference from the animals that were not exercised would be enhanced ($P<0.01$).

Virus was isolated from the skeletal muscle of only one animal; this mouse had been exercised and was sacrificed on the fourth day. The titer of virus was <200 PFU/ml in the muscle and 30,000 PFU/ml in the heart, but none was demonstrated in the blood.

Pathology. Inflammatory infiltrates were seen in the muscle of 3 mice upon microscopic examination. These animals were all from group A, exercised and virus inoculated, 2 having been sacrificed on the fourth day and the other on day 9.

In the heart of one mouse of group A sacrificed on day 9, a 1-mm white plaque suggesting calcification was visible grossly over the apex. Microscopically this heart showed a large area of necrosis with an inflammatory infiltrate and 2 small foci of inflammatory cells. The titer of virus recovered from this heart was 400 PFU/ml. Similar lesions were not seen grossly in any other hearts, but many, including some from each of the 4 groups, showed microscopic deposits of calcium.

Although isolated small foci of inflammatory cells were found in hearts of some infected and non-infected mice, only the following histologic changes were considered significant: any large infiltrate; more than one small infiltrate; or an infiltrate accompanied by necrosis of muscle. By these criteria, significant lesions were seen in only 5 of 67 hearts from the mice sacrificed and examined on days 4 and 9 (Table III). The heart of one animal in control group D, sacrificed on day 4, had a large infiltrate in the right atrium; this was the first specimen in which an atrial infiltrate was noted in this laboratory in a mouse with or without Coxsackie A9 myocarditis. The remaining significant histological lesions occurred in single mice of the exercised and non-exercised infected animals (groups A and B) sacrificed on the fourth day and in 2 of the virus-inoculated swimmers (group A), sacrificed on the ninth day. The low incidence of significant histological lesions permits no conclusions other than to

TABLE III. Occurrence of Significant Histopathology in the Heart.

Group*	No. with pathology/No. examined: Day 4	Day 9
A	1/11	2/10
B	1/12	0/12
C	0/6	0/4
D	1/6	0/6

* A, mice infected and exercised; B, infected, not exercised; C, not infected, exercised; D, not infected, not exercised.

TABLE IV. Effects of Coxsackie A9 Infection and of Exercise on Weight* on Day 9.

Group		A	B	C	D
Virus inoculated		+	+	0	0
Exercised		+	0	+	0
No. of mice		11	12	5	6
B.W., g	Mean	34.0	34.5	34.0	34.1
	S.D.	1.3	1.9	1.3	1.8
H.W., mg	Mean	154.8	138.1	145.5	130.5
	S.D.	8.0	11.5	10.0	13.7
Significance, P:	cf. D	<.0005	<.20	<.05	
	C	<.05	<.20		
	B	<.0005			
$\frac{\text{D.H.W.}}{\text{H.W.}} \times 100$	Mean	23.8	24.0	24.0	25.2
	S.D.	2.9	1.8	1.2	1.2
$\frac{\text{H.W.}}{\text{B.W.}} \times 1000$	Mean	.454	.402	.427	.382
	S.D.	.019	.023	.013	.025
Significance, P:	cf. D	<.0005	<.10	<.005	
	C	<.01	<.025		
	B	<.0005			

* B.W., body wt; H.W., wet heart wt; D.H.W., dry heart wt.

indicate that they occurred more often among the exercised animals.

Weight. The means of the body weights of the 4 groups of mice varied until the sixth day after inoculation; this reflected primarily the losses of weight from the pretraining values which were greater and persisted longer in the exercised animals than in those that were not exercised. By the sixth day after inoculation, however, the body weight of all groups stabilized at about their original levels. The mean and standard deviations of wet heart weights (for the whole heart) and of the percent dry heart weight (for the portion that was weighed before and after drying) were calculated for each of the groups of mice sacrificed on days 1, 4 and 9. There were no significant differences in these values on days 1 and 4. On day 9 (Table IV) the mean heart weight of exercised mice, whether infected or not, was greater than that of the respective non-exercised group ($P<0.0005$ and $P<0.05$), by the t test(5). In addition, within the exercised groups, the mean heart weight of infected animals was significantly greater than that of the non-infected ($P<0.05$). On day 9, the ratio of dry heart weight to wet heart weight also did not differ among the groups (Table IV). The means of the ratios of heart weight to body weight were also calculated at the time the animals of each group were sacrificed. On days 1 and 4, before the body weights stabilized, these ratios were erratic. However, on day 9 the mean of the ratio of heart weight to body weight was greater in the exercised animals than in the non-exercised animals (Table IV) both in the infected and non-infected groups. Furthermore, in the exercised groups, the relative heart weight was significantly greater in the infected animals ($P<0.01$). Thus, on day 9, there was not only an increased heart weight and relative heart weight associated with exercise, but also a significant additive effect of virus infection on both the heart weight and relative heart weight of exercised animals.

Discussion. It has long been recognized clinically that rest is beneficial for patients with acute viral illness in general and for those with myocarditis in particular. The demonstration of isolated myocarditis in adult mice infected with Coxsackie A9 virus Strain 13(2) presented an opportunity to investigate the effect of exercise at the organ level during virus infection. The recovery of virus from a greater proportion of infected mice that were exercised than from those that were not exercised suggests that strenuous exercise facilitated the multiplication of

virus in the myocardium. The mechanism of this effect is not clear, but a number of possibilities may be suggested: 1) The virulence of the virus could have been increased as a result of changes in the host substrate resulting from exercise. This does not seem to be a likely explanation. 2) The resistance of the host may have been lowered as a result of changes in the heart resulting from a metabolic depletion during exercise. For example, a switch to anaerobic metabolism might lower the cellular resistance to one or more of the many steps in adsorption, replication and release of virus particles. Thus, Pearce and Lange(6) showed that many stresses leading to myocardial anoxia could induce histologic myocarditis in rabbits otherwise not susceptible to virus. 3) A loss of resistance could occur as a result of an increase in circulating cortico-steroids or in certain metabolites mobilized during exercise which might adversely effect the susceptibility of myocardial cells. Kilbourne *et al*(7) reported a greater incidence of histological lesions in the heart among mice inoculated intraperitoneally with Coxsackie B3 after they had received cortisone subcutaneously, but there was no concomitant increase in the titers of virus. Naude *et al*(8) gave intraperitoneal steroids to newborn mice prior to intracerebral inoculation of Coxsackie B3 and also failed to show an increase in titers of virus in the myocardium. 4) Finally, the apparent decrease in resistance of myocardial tissue may be secondary to an effect of exercise upon the local resistance within the peritoneal cavity, leading to an alteration in rate and/or duration of seeding of the blood stream with virus. This is unlikely because of the similar occurrence of viremia in the exercised and non-exercised mice.

Which one or which combination of these or other possible mechanisms of decreased host resistance is operative in the mice exercised during Coxsackie A9 infection cannot be determined from the available data. Unlikely as it may seem, the possibility that the training of the mice before inoculation affected the replication of virus must also be considered.

It was previously shown that microscopic myocarditis may appear at the time when detectable virus in the tissue is waning(9). The 20% incidence of significant microscopic changes on the ninth day in the virus-infected and exercised mice in the present study is less than in an earlier study, in which 38% of spontaneously exercising infected mice sacrificed on days 6 and 9 had microscopic lesions in the myocardium(1). It is possible that, for the mice used in the present study, the 10^6TCID_{50} of the third rhesus kidney tissue culture passage of Strain 13, Coxsackie A9 virus was less virulent than the 10^5TCID_{50} of the *second* rhesus kidney tissue culture passage of the same strain used to inoculate the mice of the previous study. Comparison of the frequency of virus isolation in the 2 studies supports this view; for, although the exercised groups are not comparable because the conditions of exercise were different in the 2 studies (forced exercise in the present study and spontaneous exercise in the former), the non-exercised groups should be comparable in this respect. Actually, however, virus was isolated from only 1 of 12 inoculated, non-exercised mice on each of days 4 and 9 in the present study, whereas, in the comparable group in the earlier study, virus was isolated from 8 of 12 mice sacrificed on days 6 and 9. This may be taken as evidence that the virus infection was indeed less virulent in the present study.

That exercise should have been associated with cardiac hypertrophy was to be expected (10). An additive effect of virus infection on both heart weight and heart weight relative to body weight was found on day 9. Since the increment in weight apparently was not due to either water or the sparse inflammatory infiltrates, it must be attributed to hypertrophy or hyperplasia of myocardial cells. One might postulate that some myocardial insufficiency accompanies myocarditis and induces compensatory cardiac hypertrophy in the absence of clinically detectable heart failure. If fever accompanies these infections in mice, this could augment the cardiac output by decreasing peripheral resistance, but it seems unlikely that this alone would induce comparable cardiac hypertrophy. A similar augmentation of relative heart weight

in exercised animals was observed in this laboratory in experimental *Trypanosoma cruzi* myocarditis of mice(11).

Summary. A cardiotropic strain of Coxsackie A9 virus was inoculated intraperitoneally into groups of adult C_3H mice, one-half of which were vigorously exercised daily by swimming. Uninfected controls were studied in parallel. Among the infected mice, the virus was isolated in high titer from the hearts of a significantly greater proportion of those that were exercised. The possible mechanisms by which exercise could augment replication of virus in the myocardial tissue were discussed. On the ninth day after inoculation, the weight and relative weight of the heart were significantly greater in the exercised mice than in those that were not exercised. Among the exercised animals, the the weight and relative weight of the heart were significantly greater in those that were infected with virus than in those that were not infected. The increase in weight could not be attributed to increased water content or to inflammatory infiltrate.

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Failure of Actinomycin D to Inhibit Antitoxin Production to a Challenging Injection of Antigen.*† (29697)

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Protein synthesis requires the presence of a specific mRNA, coded directly from DNA, which in bacteria appears to have a rapid rate of turnover(1). This is in contrast with studies in higher forms which suggest the existence of a long lived mRNA(2-6). Antibody is also synthesized by an mRNA coded from DNA(7,8,9) but it appears to require a specific antigen to stimulate its production. In addition, the antigen establishes an "immunological memory" which leads to an augmentation of the gamma globulin production upon re-exposure. This augmentation must be due either to a rapid assembling of protein synthesizing units or to an accumulation of such units in the cells. Since nucleic acids may be involved, it is possible to determine their role by the use of nucleic acid inhibitors. Actinomycin D has been shown to inhibit DNA-dependent RNA synthesis(10). It was therefore of interest to determine if such an inhibition would affect antibody production in response to an injection of specific antigen in immunized mice.

Materials and methods. The animals used were adult BDF^1/Jax mice, weighing approximately 25 g. They were immunized by 7 weekly subcutaneous injections of tetanus toxoid. The last sensitizing injection was given 9 months prior to the onset of drug treatment in order to obtain animals with a

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