of the Krebs' cycle. The increased level of citric acid in plasma and tissues of saturated fat fed animals may indicate either enhanced formation of citric acid in the tissues, or diminished utilization through Krebs' cycle as compared to that of unsaturated fat administered animals. The increased accumulation of ketone bodies in saturated fat fed animals may be caused by their diminished oxidation through Krebs' cycle.

Summary. Although the concentration of ketone bodies was found to be elevated in all fat fed animals, the saturated fat (butter fat, coconut oil and hydrogenated groundnut oil) adapted rats showed more accumulation of ketone bodies in plasma, liver, kidney and heart muscle than did those receiving unsaturated fats (groundnut oil and sesame oil). The citric acid level was also elevated in saturated fat fed rats whereas no such variation was observed in animals receiving unsaturated fat in the diet.

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Observations on the Course of Coxsackie A-9 Myocarditis in C3H Mice.* (28136)

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Acute myocarditis due to the Coxsackie viruses has been shown to occur in man in all age groups(1-3) and has been produced experimentally in suckling hamsters and, occasionally, in the adult mouse(4,5). As part of a general investigation of experimental myocarditis in the mouse, we have studied some effects of Coxsackie A-9 virus (strain 13) on adult mice.

Materials and methods. Male C3H mice,§ 8-9 months of age, weighing 28 to 40 g were chosen as experimental animals. Only mature mice were used because previous observations with the virus used in this study have demonstrated histologically evident myocardial lesions only in older mice(5). Female mice were excluded because of the effect of estrus on activity(6). The animals were housed 6

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per cage‖ and fed Standard Purina Rat Chow.

Coxsackie A virus type 9 (strain 13) at the second rhesus kidney tissue culture passage was employed. A single virus stock suspension was used for intraperitoneal inoculation of 0.03 to 0.05 ml, containing approximately 10^5 $TCID_{50}$, defined as that amount of virus producing infections in half of the inoculated mice.

A total of 95 mice were divided into 4 groups. Groups III (21 mice) and IV (33 mice) were inoculated, while Groups I (28 mice) and II (13 mice) served as controls.

Spontaneous activity in activity wheels was measured in Groups II and IV, while Groups I and III were not studied in this manner. The activity wheels were modified after Bruell (7).¶ The apparatus consists of 2 wire mesh drums 8 inches in diameter, mounted on roller bearings and attached to a single vertical support. The revolutions were counted photoelectrically. Infected and control mice were placed in the activity cages simultaneously for 3 two-hour periods, at intervals of 2 to 3 days. This initial period of exposure to the activity wheel is necessary for adequate training of the mice, following which 50-minute periods have been found to serve as a reproducible measure of activity in this laboratory and elsewhere(7). Activity was measured during four 50-minute periods before inoculation, at intervals of 1 to 2 days. Following inoculation, activity was measured for 50 minutes daily until day 6 and subsequently 2 to 4 times weekly to day 106.

The mice were weighed weekly and inspected almost daily. Animals from each of the groups were killed with ether on the 6th, 9th, 14th, and 106th day following inoculation. Some of the mice included in Group I were taken at different times but were comparable in age, sex, and weight to the animals of the other groups. The hearts were dissected free of mediastinal contents, the great vessels transected 2 mm from the base and the heart weighed to the nearest 0.2 mg. Heart plus tissue from hind limb, lung, liver, spleen and large bowel were formalin-fixed, embedded in paraffin and stained with hematoxylin and eosin.

In addition, on days 6 and 9, half of each heart and a piece of hind limb from both inoculated and control mice were tested for the presence of Coxsackie A-9 virus. On day 14, pooled sera were obtained from inoculated and control groups for subsequent determinations of neutralizing antibody levels. Virologic methods have been described(5).

Results. No difference in appearance was noted between the groups, and body weights were similar. Activity records did not differ in inoculated (Group IV) or control (Group II) animals during the acute experiment, nor in those mice followed for 106 days. Body weights and activity records of those animals in whom infection was documented by isolation of the virus and/or histologic demonstration of myocarditis are presented in Table I. These values did not change significantly following inoculation, nor did they differ significantly from simultaneous measurements in the uninfected control animals.

There were, however, 5 deaths in the inoculated animals which occurred between the 30th and 69th day. Three of these occurred in the same cage. Only one of these animals was suitable for autopsy, and the heart was normal.

Virology. Virus was isolated from the heart in 15 (60%) of the inoculated mice killed on day 6 and day 9 (Table II). Groups III and IV did not differ in the frequency with which virus could be isolated. Virus was recovered from the hind limb in only one mouse. In none of the control mice was virus isolated. Neutralizing antibodies were detected in pooled sera from the inoculated mice on day 14 at a titer of 1:20, none being measurable in similar sera from control mice.

Pathology. At time of autopsy all organs looked grossly normal. Measurements of heart weight on day 6, 9 and 14 were considered as a single group. Interesting differences were noted (Table III). There was a progressive increase both in absolute heart weight and in heart weight expressed as per cent of body weight from Group I through Group IV. There was a significant difference between

‖ Model LC-127, G. H. Wahmann Mfg. Co., Baltimore, Md.

¶ Harvard Apparatus Co., Inc., Dover, Mass.

TABLE I. Body Weight and Activity of Mice With Documented Infection (Group IV) and Their Controls (Group II), Before and After Inoculation with Coxsackie A-9 Virus.

				Body wt–g		Activity–R.P.M.	
			No. mice	Mean	S.D.	Mean	S.D.
Before inoculation		Infected	12	33.0	2.1	30.5‡	6.5
		Control	13*	32.1	2.0	33.1‡	4.4
After inoculation	1-5 days	Infected	12	31.9	2.5	30.8§	6.1
		Control	13	31.8	3.0	32.2§	4.1
	6-9 days	Infected	7	32.2	2.1	31.8‖	8.7
		Control	11†	33.0	3.2	33.8‖	4.8
	10-14 days	Infected	2	33.3	3.2	32.9¶	.6
		Control	9	33.3	3.5	33.2¶	3.6

* Body wt include only 12 animals.
† " " " " 10 "
‡ Mean of a group of mean scores derived from 4 separate days.
§ " " " " " " " " " 5 " "
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¶ " " " " " " " " " 3 " "

Group I and Group III ($p<0.01$ for heart weight; $p<0.02$ for $\frac{\text{heart weight}}{\text{body weight}}$) and Group II compared to Group IV ($p<0.01$ for $\frac{\text{heart weight}}{\text{body weight}}$). Furthermore, when Group I was compared to Group IV, the significance was of greater order ($p<0.001$ for heart weight; $p<0.001$ for $\frac{\text{heart weight}}{\text{body weight}}$). Thus, infection with Coxsackie A-9 virus produced an increase in heart weight, and periods of running in activity wheels were associated with a further increase in heart weight.

Seventeen mice from Groups I (4 mice), III (1 mouse), and IV (12 mice) were followed to the 106th day. Mean absolute heart weight in Group IV was 139.7 mg. This was less than the heart weight of animals of the same group sacrificed earlier, and similar to that of non-infected animals exposed to activity cages (Table III).

Histologic examination was negative except for cardiac muscle in the inoculated animals. Nine (36%) of the inoculated animals taken on days 6 and 9 showed myocarditis (Table II). In 9 mice (36%) virus was isolated, but myocarditis was not evident. On the other hand, in 3 animals, myocarditis was present, but virus was not isolated.

TABLE II. Evidence of Coxsackie A-9 Myocarditis in 25 Mice Sacrificed on Days 6 and 9 After Inoculation.

Virus isolated from heart	+	+	0	0
Myocarditis	+	0	+	0
Unexercised animals N = 12	3	5	1	3
Exercised animals N = 13	3	4	2	4
Total animals N = 25	6	9	3	7

Two of 14 mice on day 6 showed small areas of myocardial degeneration and necrosis with infiltration by lymphocytes, neutrophils and macrophages. On day 9, 7 of 14 mice demonstrated patchy necrosis, some cloudy swelling and infiltration in which macrophages predominated over lymphocytes and red blood cells (Fig. 1). Again although patchy, the areas involved appeared to be more extensive than on day 6. The myocardium in 2 of 10 mice sacrificed on the 14th day had small areas of necrosis with infiltration by macrophages and a few fibroblasts.

TABLE III. Effect of Coxsackie A-9 Myocarditis and of Exercise Upon Heart Weight.

Group	I	II	III	IV
Number	28	9	17	19
Inoculation	0	0	+	+
Exercise	0	+	0	+
Heart wt–mg				
Mn.	131.2	137.2	143.4	147.1
S.D.	11.9	11.8	17.3	15.3
Heart wt × 10^4/ body wt				
Mn.	40.0	41.4	43.5	45.1
S.D.	3.5	2.4	5.3	2.7

None of the 13 inoculated mice followed for 106 days showed pathologic changes in the myocardium. One mouse, however, showed endocarditis of the type known to occur spontaneously in the mouse(8).

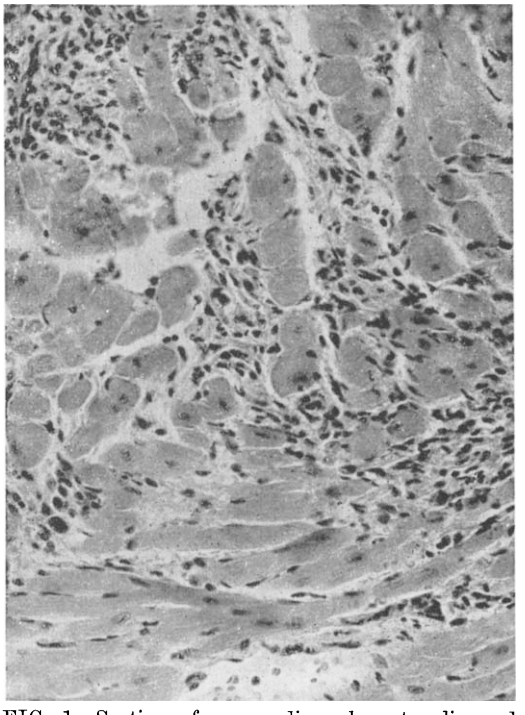

FIG. 1. Section of myocardium, hematoxylin and eosin stain, from mouse 9 days after inoculation with Coxsackie A-9 virus (strain 13) showing necrosis of muscle fibers and extensive infiltration.

Discussion. Inoculation of Coxsackie A virus type 9 (strain 13) into 8-month-old C3H mice resulted in acute myocarditis characterized principally by focal degeneration and necrosis. The histologic picture in the mature C3H mouse, although similar to that described in suckling mice inoculated with Coxsackie B virus(9), differs in that only the myocardium is affected. The finding of virus in tissues where no pathology is demonstrable and, on the other hand, the presence of myocarditis in hearts from which virus cannot be isolated (Table II), had been noted(9,10). Grodums(4) inoculated Coxsackie B-3 viruses into 12- to 56-day-old inbred albino mice and found myocardial degeneration and necrosis after one week. The incidence of lesions in his report was higher (100%) than in the present study (36%). Again, the lesions produced with Coxsackie A-9 virus are microscopic, while many of the mouse hearts in Grodum's report were grossly abnormal.

The most characteristic histologic feature of viral myocarditis in man is necrosis and inflammation of muscle. Coxsackie B infection in newborn infants is also characterized by necrotic inflammatory myocarditis, and not infrequently is associated with encephalitis(1,11). The histologic picture of adult myocarditis secondary to Coxsackie virus has not been reported to our knowledge.

It has been suggested that chronic myocardopathy in man may be the result of earlier viral myocarditis. In this regard, it is worth noting that the acute viral myocarditis in the present study healed completely, leaving no discernible histological trace nor affecting heart weight at 106 days.

Mice inoculated with Coxsackie A-9 virus had significantly heavier hearts within 2 weeks after infection than the control animals. Although histologic examination did not reveal diffuse infiltration or edema, it is not known whether this acute increase in heart weight represents inflammation with localized edema, hypertrophy, or both.

The acute myocarditis, so unmistakable microscopically, did not alter the appearance, body weight or spontaneous activity as measured and thus may be called sub-clinical. Measurement of activity was not designed to stress the heart. In fact, short periods of running were purposely chosen. Nonetheless, the data suggest that exercise may have affected heart weight.

Summary. Coxsackie A virus, type 9 (strain 13) inoculated into 54 8-month-old C3H mice resulted in acute sub-clinical myocarditis in 36% of the animals. The infected mice had significantly heavier hearts than controls. Thirteen inoculated mice followed to 106 days failed to reveal histopathologic changes in the myocardium.

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Studies in Choline Deficiency. Fate of Injected 1-C^{14}-Palmitic Acid and Fatty Acid Spectra in Fasting and Refed Rats.* (28137)

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The mode of action of choline as a lipotropic factor is obscure(1). Yoshida and Harper(2) have presented data indicating an increased synthesis of fatty acids in the liver *in vivo* and evidence of impaired transport of fat from the liver. On the other hand Lombardi and Recknagel(3) have suggested that "the hepatic triglyceride secretory mechanism" is not affected in choline deficient rats. The present work was undertaken in an effort to analyze the derangement in lipid metabolism in choline deficient rats. The approach has been to make a time study of the fate of intravenously injected albumin-bound 1-C^{14}-palmitic acid under 2 extremes of nutritional conditions, fasting and refeeding with carbohydrates. In these rats we also determined the detailed fatty acid composition of different lipid fractions of liver, blood and adipose tissue.

Materials and methods. Male Sprague-Dawley rats weighing about 180 g were maintained on an 8% casein, choline deficient diet. Control animals were maintained on the same diet supplemented with 0.5% choline chloride. After 10 days on the diet all animals were fasted for 24 hours and half of the animals in each group were then injected intravenously with albumin-bound 1-C^{14}-palmitic acid. The other half was offered 20% glucose in 0.45% saline *ad libitum* over night. Next morning each animal was given 5 ml of the above glucose solution by stomach tube 2 hours before the injection of label. The animals given glucose will be referred to as refed animals. Within each subgroup the animals were sacrificed 10, 30, 60, 90 and 180 minutes after the injection. Blood and liver lipids were extracted with chloroform-methanol and separated into the main lipid classes by silicic acid chromatography. The free fatty acids of blood were separated from the triglyceride fraction on Florosil. The carcass was digested in alcoholic KOH and an aliquot of the acidified digest extracted with ether-petroleumether. A piece of the epididymal fat pad was taken as a sample of adipose tissue for analysis of component fatty acids. Isotope determinations were performed with a Packard Tri-Carb Scintillation Spectrometer and gas-liquid chromatography of the methylated fatty acids on 2 Pye Argon Chromatographs with polyethyleneglycolsuccinate and Apiezon L columns. Calibration of the gas chromatography equipment permitted determination of the total amounts of long chain fatty acids in the plasma free fatty acid fraction. Triglycerides were determined according to Van Handel and Zilversmit(4). In a second experiment the amount of expired activity after a single intravenous injection of labeled palmitic acid was determined with an ion chamber.

Results. The fasting choline deficient rats show the changes in composition of liver and blood lipids which have long been attributed

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