occluded by the ligature applied, it is assumed that prevention of fibrillation occurs by back flow of blood into this area from adjacent coronary vessels. The high mortality in the control group is evidence that this flow is not adequate normally.

Stanton(2) postulates that better survivals, following experimental procedures designed to protect the heart, arise from increased fibrillation threshold which the performance of many of these procedures might induce. Since these dogs have had no operation prior to ligation, one concludes that radiation alone has produced sufficient vascularity to account for the observed differences in survival and increased fibrillation threshold.

Also of interest is the large percentage of radiated dogs which responded to defibrillation without removal of the ligature. Gregg (3) states that "experimentally, in dogs, it is rarely possible to revive a heart with an occluded coronary artery unless the occlusion is first removed and the ischemic area flooded with arterial blood." The experience with the control group tends to indicate that this situation is not as rare as described. However, the percentage difference in ability to defibrillate dogs between both groups and the ease with which defibrillation was performed in the radiated group is of great significance.

Summary. A method of creating increased myocardial vascularity by means of cardiac irradiation has been investigated. On the basis of mortality figures, there is a significant difference between control and irradiated dogs when acute complete ligation of the anterior descending branch of left coronary artery is performed. Ability to defibrillate radiated dogs successfully with the coronary ligature intact is strikingly evident.†

† Histological, physiological and electrocardiographic studies of the effects of cardiac irradiation, now in progress, will be reported.

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Effect of Dietary Cholic Acid on *in vivo* Cholesterol Metabolism.* (24502)

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The authors have presented evidence(1) which supported the view that effectiveness of dietary cholic acid in promoting accumulation of liver cholesterol in the rat, fed cholic acid and cholesterol simultaneously, is due to ability of bile acids to block cholesterol degradation. Furthermore, in the absence of dietary cholesterol, feeding cholic acid caused only a small accumulation of liver cholesterol, because of simultaneous and equal decreases in rates of liver cholesterol synthesis and mobilization. Inasmuch as in the rat, cholesterol is largely metabolized to bile acids before excretion, these effects could be of major importance in maintenance of cholesterol balances. However, several points must be investigated before this finding can be seen in its true perspective.

The present investigation concerns: 1. Effect of length of time of cholic acid-feeding period on rate of liver cholesterol synthesis; 2. Relationship of serum bile acid concentration to rate of cholesterol synthesis in liver; and 3. Effect of dietary cholic acid on kidney and intestine cholesterol synthesis.

Procedures. Forty-eight Sprague-Dawley female albino rats, weighing 250-280 g were divided into 2 groups. The control group was maintained on basal diet consisting of 97% Ground Rockland Rat Ration and 3% corn

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TABLE I. Effect of Dietary Cholic Acid on Serum and Liver Total Cholesterol, and on Serum Bile Acid, as a Function of Time.

Exp. period (days)	Serum total cholesterol		Liver total cholesterol		Serum bile acid	
	Control	Cholic acid*	Control	Cholic acid*	Control	Cholic acid*
	mg %		mg/g†		mg %	
1	95.4	99.4	2.41	3.03	2.53	3.14
3	81.6	89.2	2.51	2.75	1.96	5.39
5	89.4	85.7	2.69	3.16	2.41	4.16
7	86.6	91.5	2.58	2.88	2.37	4.05
10	90.4	84.2	2.44	2.85	2.73	5.13
16	83.3	94.7	2.53	2.62	3.13	7.66
21	86.0	90.9	2.68	2.72	2.54	5.38
Overall avg	87.5 ± 4.65	90.8 ± 5.18	2.54 ± .11	2.86 ± .18	2.52 ± .36	4.99 ± 1.43
P	.22		<.01		<.01	

* 0.5% cholic acid. † Wet wt. ± numbers are stand. dev.

oil; the experimental group received basal diet supplemented with 0.5% cholic acid. both groups were fed *ad lib.*, average daily food consumption being 18.1 ± 1.46 g for controls and 17.0 ± 1.32 g for treated rats. After 1, 3, 5, 7, 10, or 16 days on their respective diets, 3 control and 3 cholic acid-fed rats were injected intraperitoneally with 50 microcurie of sodium acetate-1-C^{14} in saline. Six hours after injection, blood was drawn by heart puncture, after which the animals were sacrificed and liver samples removed for analysis. In addition, 6 control and 6 cholic acid-fed rats, which had been maintained on their respective diets for 21 days, were injected as previously described. Half of these animals were sacrificed 30 minutes and the other half 6 hours after injection. Blood was drawn by heart puncture, and after decapitation, samples of liver, kidney and small intestine were removed for analysis. Serum cholesterol was determined by the method of Sperry and Webb(2); serum bile acid by a combination of methods of Minibeck and Wilken(3,4). Liver, kidney and intestine cholesterol was determined by a method described previously (5). For determination of radioactivity, tissue cholesterol was isolated as the digitonide and counted(1).

Results. Table I presents levels of serum total cholesterol, liver total cholesterol and serum bile acids. Dietary cholic acid caused no significant change in serum cholesterol concentration during the experiment. However, a small but highly significant increase in liver total cholesterol occurred after one day of cholic acid feeding. This increase remained almost constant for the balance of the 21-day period. One day of cholic acid treatment resulted in a slight increase in serum bile acid level. There was a further increase after 3 days treatment to a level that remained relatively constant for the rest of the experiment.

The effect of dietary cholic acid on incorporation of acetate-1-C^{14} into liver and serum cholesterol is shown in Table II. Feeding cholic acid for one day had no effect on rate of liver cholesterol synthesis. After 3 days of cholic acid feeding, rate of synthesis was reduced by approximately 65%. This reduction remained at about the same level for the rest of the experiment. The same pattern was found in the case of appearance of cholesterol-x-C^{14} in blood.

It is of interest to note that serum bile acid concentration reached a maximum level after 3 days of cholic acid feeding (Table I). This same interval was necessary for reduction of

TABLE II. Incorporation of Acetate-1-C^{14} into Liver and Serum Total Cholesterol in Control Rats and Rats Fed 0.5% Cholic Acid for Different Time Intervals.

Exp. period (days)	Liver total cholesterol		Serum total cholesterol	
	Control	Cholic acid	Control	Cholic acid
	counts/min./mg			
1	2,828	2,798	2,316	2,311
3	3,238	1,075	3,023	1,120
5	2,981	1,362	2,384	1,304
7	3,111	980	2,745	829
10	3,901	1,008	3,220	923
16	3,406	1,064	3,610	966
21	3,798	1,168	3,219	893

liver cholesterol synthesis (Table II). On the other hand, after one day of dietary cholic acid, liver cholesterol was slightly but significantly elevated. These findings suggest that the decrease in liver cholesterol synthesis is more closely related to serum bile acid concentration than to the slight increase in liver cholesterol level. However, a more detailed study is necessary to decide this point. Inhibition of cholesterol synthesis by cholic acid could very well be a feed-back reaction. Cholesterol degradation is hindered by excess of bile acids(1,6), which results in a slight increase of cholesterol concentration, and this in turn retards liver cholesterol synthesis(7).

Due to exchange of cholesterol between serum and tissues, difficulties are encountered in studying *in vivo* rates of cholesterol synthesis in tissues other than liver. Therefore incorporation of acetate-1-C^{14} in serum, liver, kidney and intestine total cholesterol was determined 30 minutes and 6 hours after injection in rats maintained on their respective diets for 21 days. There was no significant change in total cholesterol levels in serum or any tissues, due to dietary cholic acid (Table III). In case of the 6-hour acetate-1-C^{14} incorporation period (Table IV), the cholic acid-treated animals showed about 70% decrease in cholesterol-x-C^{14} concentration in serum and liver; about 50% decrease in kidney, and 20% decrease in the intestine. At end of 30 minute incorporation period, in the cholic acid-treated group, there was about 70% decrease in serum and liver cholesterol-x-C^{14} concentration. However, there was no change in kidney or intestine C^{14} activity.

No difference was observed in levels of liver cholesterol-x-C^{14} 30 minutes or 6 hours after injection of acetate-1-C^{14}. These results agree with the findings of Schwenk *et al.*(8) that metabolism of acetate is essentially completed within 30-60 minutes. In the case of serum cholesterol-x-C^{14}, although percentage decrease effected by cholic acid was the same in both incorporation periods, levels of cholesterol-x-C^{14} at 30 minutes were about one-half those at 6 hours. This is probably due to a finite rate of blood and liver cholesterol exchange.

TABLE III. Total Cholesterol Levels in Serum, Liver, Kidney, and Intestine following 21 Days on 0.5% Dietary Cholic Acid.

Tissue	Total cholesterol: Control	Cholic acid	P values
Serum, mg %	86.0 ±11.0	90.9 ±2.55	.49
Liver, mg/g*	2.68 ± .20	2.72 ± .23	.82
Kidney, " *	4.63 ± .32	4.75 ± .11	.58
Intestine, " *	2.30 ± .24	2.40 ± .24	.63

* Wet wt.

TABLE IV. Cholesterol-x-C^{14} Levels in Serum, Liver, Kidney and Intestine 30 Min. or 6 Hr after Injection of Acetate-1-C^{14} following 21 Days on 0.5% Dietary Cholic Acid.

Tissue	Cholesterol-x-C^{14}, counts/min./mg cholesterol: Control (30 min.)	Cholic acid (30 min.)	Control (6 hr)	Cholic acid (6 hr)
Serum	1,559	421	3,219	893
Liver	3,770	1,092	3,798	1,168
Kidney	71	69	303	141
Intestine	2,076	2,233	3,455	2,815

The exchange of cholesterol between blood and other tissues also explains the *apparent* effect of dietary cholic acid on rate of cholesterol-x-C^{14} synthesis in kidney and intestine at 6 hours, as contrasted with the lack of effect in the 30-minute period. This is more obvious with the kidney because of lower rate of cholesterol synthesis. Absence of any decrease in cholesterol synthesis in kidney and intestine due to dietary cholic acid correlates with lack of effect of dietary cholesterol in these tissues as shown by Gould(9).

Summary. 1. Dietary cholic acid reduced the rate of *in vivo* hepatic cholesterol synthesis within 3 days, as indicated by incorporated acetate-1-C^{14}. 2. Continued feeding of cholic acid produced no further decrease in hepatic cholesterol synthesis rate over the 21-day period. 3. Increased serum bile acid level correlated with decreased liver cholesterol synthesis rates at all time intervals. 4. There was a small but significant increase in liver cholesterol concentration caused by dietary cholic acid. This increase remained constant throughout the experimental period. 5. Dietary cholic acid had no effect on *in vivo* kidney and intestine cholesterol synthesis rates.

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Blood Glutathione and Alloxan Susceptibility in Inbred Mice.* (24503)

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Male mice of the strain Z(C_3H) have been reported(1) to be more resistant to a given intravenous dose of alloxan than are male mice of strain D_8. Degree of alloxan sensitivity of an experimental animal may possibly be related to the endogenous blood and tissue glutathione (GSH) levels, for GSH and other sulfhydryl compounds protect against the diabetogenic effect of alloxan when given prior to alloxan(2,3). Moreover, sodium deficient diet(4) and repeated acetoacetate injection (5) lower the GSH content of the blood and markedly increase susceptibility to alloxan (4,6). Likewise, such factors as cysteine feeding, thiouracil feeding, and thyroidectomy, which increase the liver and kidney GSH level, also increase resistance to alloxan (7). In view of this evidence that factors which alter the GSH content of the blood or tissues alter alloxan susceptibility, it was thought worthwhile to determine blood GSH levels in these 2 strains of mice. These levels were found to differ in the 2 strains, as did alloxan susceptibility, and therefore it seemed important to determine how these characteristics are transmitted to the F_1 hybrids resulting from the cross between these 2 strains.

Material and methods. Adult mice of strains Z(C_3H) and D_8 were used as well as hybrids resulting from crosses between D_8 female and Z(C_3H) male and between D_8 male and Z(C_3H) female. Male and female mice approximately 6 weeks in age were used for GSH determinations, but only male mice were used for alloxan susceptibility studies. Reduced GSH was determined by the alloxan 305 method as described by Patterson and Lazarow(8). Hematocrit levels were determined by the microhematocrit method of Van Allen (9). Glutathione is reported as mg of GSH/100 cc of red blood cells. In the susceptibility studies, alloxan was injected *via* the tail vein using a No. 26 needle, in doses of 40, 60, or 80 mg/kg body weight. Blood sugars were determined by the method of Folin-Malmros (10) at 48 hours and 7 days after the injection. The average blood sugar of normal mice was 135.6 $\pm$ 13.4 mg % 3 hours after the last meal. A level higher than 200 mg % 3 hours after the last meal was regarded as an indication of diabetes.

Results. Table I shows that mice of strain Z(C_3H) (alloxan resistant) had a significantly higher erythrocyte GSH level (39% higher) than those of strain D_8 (alloxan susceptible).

In determining erythrocyte GSH levels of both types of F_1 hybrids, it was found that, regardless of method of crossing; 1) erythrocyte GSH levels were not significantly different from those of Z(C_3H) (alloxan resistant) strain (see Table I), but 2) were significantly

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