these animals. Clearly, there are no standard data in the literature regarding the effects of thyroactive compounds on cholesterol metabolism, since dosages used, routes, and duration of administration have varied from study to study.

Summary. In a series of 5 separate experiments, rats were fed high fat and high fat-high cholesterol diets and were treated with various thyroactive compounds. Two dosages of 3,5,3′-triiodothyropropionic acid (Triprop) (0.03 mg and 0.15 mg/100 g body weight) were administered subcutaneously and, in general, were found to lower serum and liver cholesterol levels when compared with controls. Administration of D-thyroxine (0.05 mg/100 g body weight) and L-thyroxine (0.005 mg/100 g) also lowered serum and liver cholesterol levels. Among individual experiments there was a variation in magnitude, and in a few instances in the direction of effect.

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Decline in Rate of Cholesterol Synthesis During Maturation of Chicken Aorta.* (26908)

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While most of the cholesterol of the normal artery wall appears to arrive there by transfer from plasma, there has been reason to believe that local synthesis may contribute significantly(1). The experiments described here indicate, among other things, that cholesterol synthesis by avian aortic tissue falls off rapidly as the animal matures.

Materials and methods. White Leghorn cockerels, maintained on a commercial feed, were sacrificed by exsanguination. Aortas were removed, immersed in chilled buffer, freed of clots and connective tissue, and opened longitudinally. In some experiments the aorta was cut into thoracic and abdominal segments, and each of these was split longitudinally into 2 portions, one for incubation with acetate as substrate and the other with mevalonate. Each artery or piece of artery was weighed and placed into an incubation vessel containing 3.0 ml of Krebs-Ringer bicarbonate buffer (pH 7.4), 0.1 ml of 5% dextrose, and 0.1 ml of either sodium acetate-1-

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TABLE I. Sterol Synthesis from Acetate and Mevalonate by Chicken Aorta.

Exp. No.	Individual body wt, g	Mean aortic wt, g	Acetate as substrate: Before bromination — Cholesterol radioactivity, cpm/g tissue: Thoracic	Abdominal	Whole†	% conversion (whole aorta)	Loss of radioactivity* in bromination (whole aorta), %	Mevalonate as substrate: Before bromination — Cholesterol radioactivity, cpm/g tissue: Thoracic	Abdominal	Whole†	% conversion (whole aorta)	Loss of radioactivity* in bromination (whole aorta), %
1	1565 1460	1.048	268	933	611	.0158	—	252	187	219	.0269	96
2	1563 1410 1402	.871	319	166	241	.0055	76	298	180	228	.0221	92
3	1560 1363 1330	.639	732	455	595	.0098	—	173	217	192	.0137	—
4	1075 803 725	.568	341	2180	1325	.0173	85	283	355	326	.0230	98
5	547 506 504	.455	—	—	—	—	—	—	—	650	.1410	94
6	495 490 480	.491	—	—	1087	.0519	73	—	—	—	—	—
7	548 412 405	.451	—	—	—	—	—	—	—	1313	.2810	95
8	501 338 311	.369	—	—	4320	.1550	79	—	—	—	—	—
9	395 345 345	.443	—	—	—	—	—	—	—	672	.1420	96
10	385 383 380	.465	—	—	2170	.0977	83	—	—	—	—	—

In Exp. 1-4, each flask contained half of either a thoracic or abdominal aorta. In Exp. 5-10, each flask contained a whole aorta. In all experiments, cholesterol digitonide was isolated from the pooled aortic tissue of the 2 or 3 animals in the group.

* Corrected for losses of material (by weight) during bromination and recovery.

† In calculation of these figures for Exp. 1-4, correction was made for inequality in the sizes of the 4 "quarters" of each aorta. Hence, the figure is not identical to the mean of the thoracic and abdominal aorta radioactivities.

TABLE II. Production of $C^{14}O_2$ from Labeled Acetate and Mevalonate by Chicken Aorta.

		Acetate as substrate				Mevalonate as substrate			
		CO_2 radioactivity, cpm/g aorta ($\times 10^{-5}$)				CO_2 radioactivity, cpm/g aorta			
Exp. No.*	Wt of aorta, g	Thoracic	Abdominal	Whole†	% conversion (whole aorta)	Thoracic	Abdominal	Whole†	% conversion (whole aorta)
1	.963	3.33	2.65	2.98	7.9	797	1564	1203	.113
	1.132	1.73	2.11	1.94	4.8	555	1783	1176	.183
4	.498	5.34	5.03	5.19	7.2	3440	2510	2950	.168
	.589	2.86	4.14	3.45	6.0	2330	2310	2320	.123
	.590	2.90	2.03	2.46	3.4	1021	1027	1025	.077
5	.464	—	—	—	—	—	—	265	.058
	.460	—	—	—	—	—	—	374	.079
	.442	—	—	—	—	—	—	579	.123
6	.486	—	—	1.46	7.0	—	—	—	—
	.522	—	—	1.05	5.3	—	—	—	—
	.464	—	—	.93	4.1	—	—	—	—

In Exp. 1 and 4, each flask contained half of either a thoracic or abdominal aorta. In Exp. 5 and 6, each flask contained a whole aorta.

* Numbers correspond to those in Table I.

† In calculation of these figures for Exp. 1 and 4, correction was made for inequality in the sizes of the 4 "quarters" of each aorta. Hence, the figure is not identical to the mean of the thoracic and abdominal aorta radioactivities.

C^{14} (3.7 μc) or sodium DL-mevalonate-2-C^{14} (0.76 μc). Specific activity of both labeled compounds was 1 μc/μmole. Tissues were shaken for 3 hours at 37°C in an atmosphere of 95% O_2, 5% CO_2.

In some experiments, center well flasks were used to permit collection of CO_2. Each flask was stoppered with a serum vial cap, and flushed with the O_2-CO_2 mixture at the start of incubation *via* hypodermic needles inserted through the cap. At the end of incubation, 0.3 ml of 2 N NaOH was delivered into the center well through a long needle, followed by 0.1 ml of 10 N H_2SO_4 into the outer compartment. The flasks were shaken 10 minutes and then unstoppered. The solution in the center well was removed and added to 9-10 ml of 1 N NaOH, which was used to wash the container. To each sample were added 5 ml of 1 M $BaCl_2$. After standing 15 minutes, the precipitated $BaCO_3$ was centrifuged off and washed twice with water and twice with methanol.

Aortas or quarter-aortas from 2 or 3 animals were pooled in each experiment. The non-saponifiable material was obtained(2), and to it were added 12 mg of pure cholesterol as carrier. An aliquot of this solution was used for direct precipitation of cholesterol digitonide(2). The cholesterol in another aliquot was purified *via* the dibromide (3), and digitonide was prepared from this purified material.

Samples of $BaCO_3$ and cholesterol digitonide were mounted by filtration onto tared filter paper discs. Each sample was dried and weighed, and counted in an ultra-thin-window flow gas counter (Nuclear-Chicago Model D47) having an efficiency of approximately 30%. A minimum of 2,000 counts was recorded for each sample. All radioactivity data were corrected for self-absorption to a standard thickness of 5 mg/cm^2.

Results and discussion. These experiments reveal a striking diminution in the rate of aortic sterol synthesis with increasing body weight (Table I). This was observed with both acetate and mevalonate as substrate. The relationship to body weight was apparent whether the sterol precursors of cholesterol were included (non-brominated material) or removed by bromination. The decrease in sterol synthesis with increasing body weight and aortic size was not attributable to impermeability of the thicker aortas to substrate, inasmuch as production of $C^{14}O_2$ failed to exhibit a similar trend (Table II). Plots of the data suggest that there may be

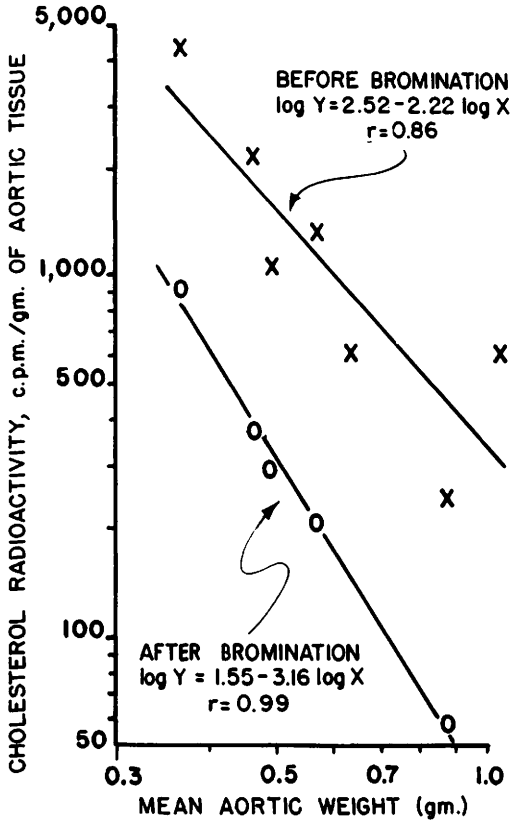

FIG. 1. Sterol synthesis from acetate by chicken aorta.

an inverse linear relationship between log cholesterol synthesis rate (c.p.m./g of tissue) and either log aortic weight or log body weight. This is particularly striking with regard to the data of the acetate experiments, using log aortic weight as abscissa (Fig. 1). A log-log plot for the mevalonate experiments (not shown in the figure) is less suggestive of a linear relationship, although a correlation coefficient of 0.85 is obtained between log cholesterol synthesis rate (c.p.m./g of tissue) and log aortic weight, using the data from the non-brominated samples.

The basis of these relationships is not completely elucidated by the present experiments. However, it seems likely that cholesterol synthesis in aortic tissue is largely related to tissue growth. It is also possible that the decrease in cholesterol synthesis during maturation may be a reflection of decreasing tissue cellularity. The data of the present experiments do not permit a definitive test of these possibilities.

The thoracic and abdominal aortic segments exhibit no consistent differences in rates of cholesterol synthesis, despite distinct differences in wall structure and in susceptibility to atherosclerosis(4).

As in other experimental systems(5), mevalonate was a more efficient precursor of sterol, and a poorer source of CO_2 carbon, than was acetate (Tables I and II). However, efficiency of sterol synthesis from mevalonate was still quite low—only about twice that observed with acetate—and almost all the radioactivity was removed by bromination. The presence of practically all the sterol radioactivity in the high-counting cholesterol precursors implies that the experiment was terminated relatively early, in comparison to the situation in the acetate experiment. However, existing information on cholesterol biosynthesis suggests that there is only one pathway for incorporation of acetate carbon into sterol, and that mevalonate or a closely related compound is an obligatory intermediate(5). These observations may be reconciled by postulating that aortic tissue is less permeable to mevalonate than to acetate, or that for other reasons mevalonate has slow access to cholesterol-synthesizing enzymes, and that sterol synthesis from mevalonate is consequently delayed in this *in vitro* system.

Summary. *In vitro* synthesis of labeled cholesterol by aortic tissue of cockerels was found to fall off rapidly with maturation, with either acetate-1-C^{14} or mevalonate-2-C^{14} as precursor. Production of $C^{14}O_2$ failed to show a similar relationship, indicating that the declining rate of cholesterol synthesis is not a consequence of decreasing permeability of the tissue to the substrate. In the system employed, mevalonate was only twice as efficient as acetate as a source of sterol carbon, and almost all the sterol radioactivity derived from mevalonate was removed by bromination. Thoracic and abdominal aorta displayed similar rates of cholesterol synthesis.

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Hepatic Clearance of Endotoxin Absorbed from the Intestine.* (26909)

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Endotoxin absorbed from the gut has been demonstrated in the blood of animals in 3 types of traumatic shock(1). In the normal animal the endotoxin is extracted from the blood and destroyed almost immediately by the reticulo-endothelial system (R.E.S.)(2). But in the shocked animal the capacity of the system to extract or destroy endotoxin is severely impaired(2). Thus endotoxin becomes free to injure the circulation and to prevent recovery. The amount of endotoxin absorbed varies with the size of the pool of the gram-negative flora in the gut(3). Additional evidence to this effect is presented below.

Method. New Zealand rabbits (average wt 2 kg) with normal intestinal flora, as indicated in each instance by culture from rectal swabs, were anesthetized with nembutal intravenously, and the abdomen opened with aseptic precautions through a midline incision. The intestine was gently exteriorized, and 7-10 ml of blood withdrawn from the portal vein into heparinized tubes. Similarly, blood was withdrawn from the inferior vena cava. These bloods were cultured and were found to be uniformly free of bacteria. They were also tested for the presence of endotoxin by the method of Smith and Thomas(4). A positive test consisted of multiple hemorrhages and death of at least 4 of 6 ten-day-old chick embryos, to each of which 0.1 ml of the same sample of blood had been administered. Death of not more than 2 of 6 embryos was considered evidence of the absence of endotoxin. Because the cultures from rectal swabs do not always accurately reflect the pattern of the intraintestinal flora, cultures from the lumen of ileum and colon were obtained at the end of each experiments as a check upon the preoperative cultural data from rectal swabs.

Five groups of experiments were performed. In Group 1 the procedure as described above was carried out. In Group 2 the procedure was the same, except that 3 hours preoperatively 100 mg of an *E. coli* $0111B_4$ endotoxin (MLD/100 = 3-4 mg/kg) in 30 ml of normal saline was given by gavage. In Group 3 the procedure was the same as in Group 2, but in addition 2.75 ml/kg of Thorotrast was given intravenously when the endotoxin was fed. In Group 4 the procedure was the same as in Group 1, except that the rabbits used were coliform-free by the cultural data from preoperative rectal swabs. In Group 5 the procedure was the same as in Group 4, except that these rabbits were given neomycin (250 mg/kg) by gavage daily for 3 successive days, and again 3 hours preoperatively on the 4th day.

Results are listed in Table I. *Group 1.* The data of Group 1 indicate the presence of endotoxin in the blood of the portal vein in one-half, and in the blood of the systemic veins in one-sixth of rabbits with a normal intestinal flora. There was no instance in

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