that the mg/kg dose of succinylcholine diiodide on single intravenous administration was much lower (0.05 mg) in dog and cat (*4, 13*) than in man (0.5 mg) (*8*), and that the inhibition of neuromuscular transmission following a single dose also lasted longer in dog and cat than in man. Similarly, the mg/kg/min dose of succinylcholine diiodide (0.04–0.12) was also considerably greater in man (*8*) than in dog and cat.

According to Harvey, procaine prevents acetylcholine from acting, by interfering with the acetylcholine receptor mechanism (*14*). It has also been shown that procaine inhibits acetylcholine release at the neuromuscular junction (*15*). According to the present accepted theory of the mechanism of action of the different types of muscle relaxants (*16*), the suppression of acetylcholine release or prevention of its action should increase the effectiveness of the antidepolarizing muscle relaxants (curare, Flaxedil, etc.), but decrease the effect of the depolarizing agents (decamethonium). Inasmuch as succinylcholine has been classified as one of the depolarizing relaxants (*17, 18*), procaine would be expected to decrease its effectiveness. In contrast to this, it was found (Fig. 1) that in the dosage and manner used (2 mg/kg in dogs) procaine increased the intensity of neuromuscular blockade produced by succinylcholine. It is conceivable that under the experimental conditions reported, the effect of procaine on plasma cholinesterase activity was dominant over its antidepolarizing action (*14, 15*). Under other experimental conditions, it was possible to demonstrate that procaine is also capable of antagonizing the succinylcholine-induced neuromuscular blockade (*19*).

It was observed in patients and demonstrated in animal experiments that the intravenous injection of procaine during succinylcholine administration increased the inhibitory effect of succinylcholine on neuromuscular transmission. This resulted in an increased respiratory depression in patients, and a decreased response of the sciatic-gastrocnemius preparation to repeated single electrical stimuli in animals. *In vitro* hydrolysis studies demonstrated that substrate competition exists between procaine and succinylcholine for the plasma cholinesterase. The practical importance of this observation lies in the fact that procaine and succinylcholine might be employed simultaneously in anesthetized patients, and therefore the anesthesiologist has to be aware of possible additive effects of these two agents.

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Effects of Ferric Chloride and Bile on Plasma Cholesterol and Atherosclerosis in the Cholesterol-fed Bird[1]

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Because of its possible importance in atherogenesis, the level of plasma cholesterol has received considerable attention in recent years. Severe restriction of foodstuffs rich in lipides has been the most practical method so far proposed for lowering the concentration of circulating cholesterol. In this laboratory, however, we have been interested in procedures other than those involving dietary restrictions for the control of plasma cholesterol. We have recently shown that absorption of cholesterol from the intestinal tract stops completely in the absence of bile (*1*), and this observation suggested to us the feeding of ferric chloride, which is known to precipitate bile salts *in vitro*. It is shown here that the rise in plasma cholesterol, as well as the associated atheromata resulting from cholesterol feeding, can to a large degree be prevented by the feeding of ferric chloride.

Experiment I. Four-month-old White Leghorn cockerels, obtained through the courtesy of the Depart-

TABLE 1

COMPOSITION OF DIETS*

Expt.	Group	Diet	Wesson oil	Cholesterol	Ferric chloride	Bile concentrate
I	1	A	5	None	None	None
	2	B	5	1	"	"
	3	C	5	1	3	"
II	4	D	5	None	None	10
	5	E	5	1	"	10
	6	F	5	1	3	10
III	7	E as above				
	8	F " "				

* Percentage of Purina broiler chow (starter ration), with added constituents.

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[2] The technical assistance of Jacob V. Sherokoff and Donna M. Folliard is gratefully acknowledged.

ment of Poultry Husbandry of this university, were used. They were fed Purina broiler chow (starter ration) for a period of 4 weeks, during which time 2 samples of blood were taken for determination of plasma cholesterol. They were then divided in three groups of 3 birds each and treated as follows: Group 1 was fed Diet A; Group 2 was fed Diet B; and Group 3 was fed Diet C (Table 1). The birds were kept on the diets for about 5 months. Food consumption for each bird was measured daily (Table 2).

TABLE 2

AVERAGE FOOD CONSUMPTION
(Grams consumed per bird per day)

Expt.	Group	Months 1	2	3	4	5	6
I	1	114	98	87	53	57	......
	2	95	89	71	54	60	......
	3	100	89	83	50	......	......
II	4	94	99	84	65	65	71
	5	106	105	84	73	63	67
	6	85	79	78	59	59	48
III	7	89	67	62	70	......	......
	8	96	76	69	71	......	......

The total cholesterol of the plasma was measured on five occasions; the average values for the birds of each group are shown in Fig. 1. Plasma cholesterol

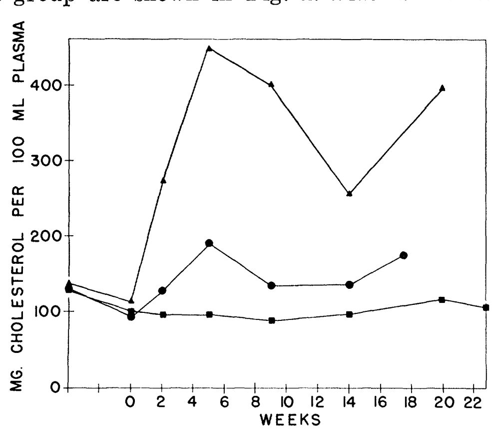

FIG. 1. Influence of ferric chloride feeding upon the cholesterolemia of the cholesterol-fed bird. ■: Group 1, regular diet; ▲: Group 2, regular diet + 1% cholesterol; ●: Group 3, regular diet + 1% cholesterol + 3% ferric chloride.

was determined by a modification of the method of Sackett (*2*).

The feeding of cholesterol plus fat resulted, as was to be expected, in a pronounced increase in the cholesterol concentration of plasma (Group 2). The highest value was observed about 1 month after the feeding of the diet was started, and at this time the average cholesterol value was 450 mg %.

It is clear from Fig. 1 that the addition of iron to the cholesterol-fat diet inhibited the rise in plasma cholesterol. The values for the iron-cholesterol–fed birds (Group 3) were higher than those found in the

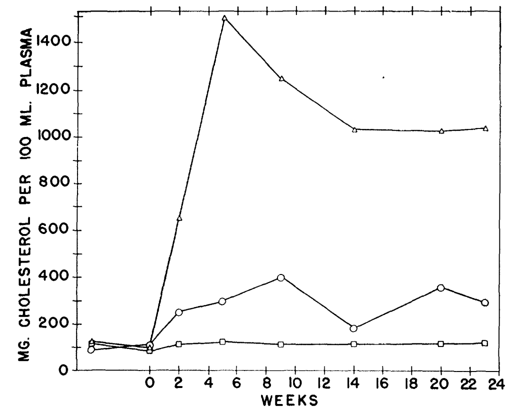

FIG. 2. Influence of ferric chloride feeding upon the cholesterolemia of the cholesterol-bile–fed bird. □: Group 4, regular diet + 10% bile concentrate; △: Group 5, regular diet + 1% cholesterol + 10% bile concentrate; ○; Group 6, regular diet + 1% cholesterol + 10% bile + 3% ferric chloride.

controls (Group 1), but in no case did the plasma cholesterol concentrations in the birds of Group 3 approach those observed in the birds fed cholesterol alone (Group 2).

At the termination of the study (17–23 weeks) autopsies were performed on all animals. The heart and aorta of each bird were dissected out and carefully examined for gross atheromata. Severity of the thoracic and abdominal aortic lesions was graded as follows:

0: No gross changes
0.5: Extensive coalescing intimal discoloration, lacking a grossly elevated intimal surface
1: Definite plaques less than 3 mm^2, white to cream-colored, with raised intimal surface
2: Definite plaques greater than 3 mm^2, cream to light yellow in color
3: Numerous plaques greater than 3 mm^2, yellow in color
4: Severe gross atheromatosis (lesions yellow in color, raised and calcified on gross examination)

Examination of the aortas at the end of the experiment indicated that there were fewer lesions in the birds that received ferric chloride plus cholesterol than in those fed cholesterol alone. The prolonged feeding of the large amounts of ferric chloride was not, however, without toxic action. Cataract formation, decreased food intake, and weight loss were observed about the 14th week of the experiment. For this reason we cannot conclude from this experiment that the ferric chloride per se was responsible for the lesser degree of atherogenesis in the birds of Group 3. But, as judged by the intake of food, gain in weight, and the general well-being of the bird, no toxic effects were encountered until the period of about 3½ months had elapsed. Table 2 shows that during these first 3½ months the iron-fed birds (Group 3) actually ingested slightly more food, and hence more cholesterol, than did the birds of Group 2. Despite this, however, plasma cholesterol in the birds of Group 3 remained lower than in those of Group 2.

Experiment II. In view of the role of bile in choles-

TABLE 3

Effect of Ferric Chloride upon Degree of Atherosclerosis in Cholesterol-fed Birds and Birds Fed Cholesterol Plus Bile

Expt.	Group	Diet			Bird	Aorta		Degree of Athero-sclerosis
		Cholesterol	Bile	$FeCl_3$		Thoracic	Abdominal	
					1	0.5	0	
	1	None	None	None	2	0	0	0.17
					3	0.5	0	
					4	0	0	
I	2	+	“	“	5	0	2	0.92
					6	0.5	3	
					7	0	0	
	3	+	“	+	8	0	2	0.33*
					9	0	0	
					10	0	2	
	4	None	+	None	11	0	0	0.33
					12	0	0	
					13	2	4	
II	5	+	+	“	14	2	3	2.50
					15	1	3	
	6	+	+	+	16	0	0	
					17	2	0	0.50
					18	3	2	
	7	+	+	None	19	3	2	2.67
					20	3	3	
III					21	†	†	
					22	0	2	
	8	+	+		23	0	3	0.62
				+	24	0	0	
					25	0	0	

* This value may be low since the birds of group 3 were sacrificed earlier than those of the other groups of this experiment.

† Bird died in cage and severe postmortem changes made gross grading impossible.

terol absorption we undertook a study of the effect of the simultaneous administration of cholesterol and bile upon plasma cholesterol levels and atherogenesis. Three groups of birds (3 in Groups 4 and 5, 2 in Group 6) were fed as follows:

Group 4 was fed Diet D (Table 1). The bile as obtained from the abbatoir was concentrated to approximately 1/7 of its volume by heating overnight at 100° C and passing a stream of air over its surface to facilitate evaporation. Group 5 was fed Diet E. Group 6 was fed Diet F.

The birds used in Expts. I and II were from a single hatch, and both experiments were run concurrently. The feeding in Expt. II was continued for about 6 months.

The food intake of the 8 birds used in Expt. II is given in Table 2. Their plasma cholesterol values are shown in Fig. 2, and the degree of atheromatosis in Table 3.

The addition of bile to the cholesterol diet resulted in an extraordinary rise in plasma cholesterol (*cf.* Group 2 in Fig. 1 with Group 5 in Fig. 2). At the end of 2 weeks these birds showed cholesterol levels ranging from 420 to 850 mg %, and by the time 5 weeks had elapsed the values were 1022–2017 mg %. The average values throughout the remainder of the 6-month period did not drop below 1000 mg %.

Good evidence that the degree of atheromatosis keeps pace with the level of plasma cholesterol is shown by the comparison of plaque formation in the birds of Group 2 (Expt. I) with those of Group 5

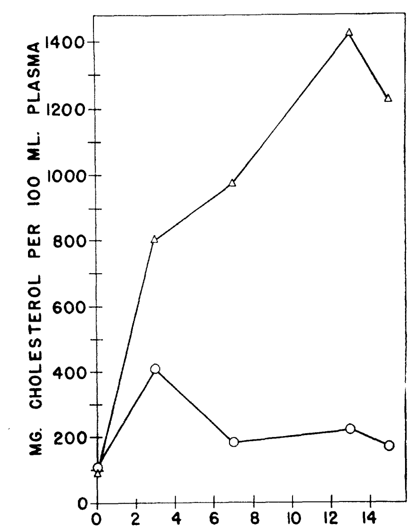

Fig. 3. Influence of ferric chloride feeding upon the cholesterolemia of the cholesterol-bile–fed bird. △: Group 7, regular diet +1% cholesterol +10% bile concentrate; ○: Group 8, regular diet +1% cholesterol +3% ferric chloride.

(Expt. II). Atheromatosis was 3 times as extensive in the birds of Group 5 as in those of Group 2.

The dramatic action of iron feeding in the prevention of the rise in plasma cholesterol is clearly shown in Fig. 2. The feeding of iron even in the presence of bile (Group 6) held plaque formation to a degree approximately 1/5 that observed in birds of Group 5. No toxic manifestations (cataracts or weight loss) were noted in the birds fed the ferric chloride-containing diet.

Experiment III. Two groups of birds (4/group) were studied in this experiment (Table 1). Group 7 was fed Diet E. Group 8 was fed Diet F.

The birds were 5 months old at the start of the feeding, and the experimental period was shortened to 4 months. During this period no untoward manifestations were noted in the birds fed the iron-bile-cholesterol diet.

The results of the experiment, shown in Fig. 3 and Table 3, are essentially the same as those of the second experiment. The degree of atheromatosis in the birds fed bile and cholesterol was approximately 4 times as great as that observed in the birds fed bile, cholesterol, and iron.

The interesting finding is that the feeding of ox bile along with cholesterol results in a greater degree of atherosclerosis, as well as a higher cholesterolemia, than does the feeding of cholesterol alone. The hypercholesterolemic effect of bile, which has been noted previously (*3, 4*), is probably the result of augmented cholesterol absorption. This view is supported by the observation that birds fed Purina chow supplemented with Wesson oil and bile but no cholesterol showed no greater cholesterolemia nor atheromatosis than control birds fed the Purina chow plus Wesson oil.

The enteral administration of ferric chloride reduced the extent of the rise of plasma cholesterol not only in birds fed cholesterol but also in those fed cholesterol plus bile. It is likely, although not yet proved, that the iron acted by precipitating bile salts in the intestinal tract.

The action of bile in facilitating the appearance of atheromata in the cholesterol-fed bird, as well as an earlier finding that bile controls the rate of cholesterol absorption by the intestine in the rat (*1*), raises an interesting point—namely, the part played by intestinal bile in the development of arteriosclerosis in man. Our findings suggest that the binding of bile salts in the intestinal tract, thereby suppressing cholesterol absorption, may offer a means of controlling the development of arteriosclerosis.

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Comments and Communications

Photoperiodicity in Animals: The Role of Darkness

RECENT results with quail (*1*) have been claimed as apparently "the first clarification of the importance of the dark period in the photoperiodic responses of any higher animal" (as opposed to results with plants, aphids, and snails). In a neat experiment, 5 groups of birds were exposed to artificial lighting daily: either as 17 hr (followed by 7 hr darkness) or as 9 hr plus 1 hr (the two periods separated by a 0-, 1½-, 3½-, or 7-hr dark period, and followed by another dark period to complete the 24-hr cycle).

The results are summarized by pointing out that stimulation (gonad and oviduct size) is roughly "inversely proportional to the length of the longest dark interval. . . . It is evident that the dark period is an inhibitory factor." The growth effects obtained with 17 hr light (and one 7-hr dark period) seem to have been about the same as with 10 hr total light (with two 7-hr dark periods daily).

These results might have been expressed in another way—that stimulation was roughly proportional to the length of day (above a threshold level); and that the "day" did not have to be continuous but could be broken by a dark interval. It is perhaps a mistake to think of darkness—which is merely the absence of light—as playing an active role.

Hart's demonstration (*2*) that ferrets would become oestrous if the dark period was broken was open to criticism (*1*). But experiments (*3*) at the Worcester Foundation, based on Hart's results, showed that only 7 hr illumination daily, if divided over a 12-hr period, will stimulate: so indeed (*4*) will 4 hr divided over 14 hr.

There seem to be differences between photoperiodic mechanisms of birds and mammals; but in both there is evidence that the length of the dark period affects the response to light, and not in an inhibitory fashion. In the weaverbird (*5*) an optimum length of day (and hence of night) has been postulated. In the ferret interpretation of experiments is complicated by what has been termed an "inherent rhythm" (*6*).

This rhythm may be due, or partly due, to the dissipation in darkness of inhibitory aftereffects of long days. Analogy with results on mink (*3, 7*) suggests this: stimulatory effects of light can be stored for long periods (*4*).

Hart (*2*) found a more rapid response to 16 hr