MSH-RF was not removed by the purification procedures.

The results of the present study give support to the idea that MSH-RF could be produced by cells having their soma in the paraventricular nucleus and their axons ending in the median eminence and neural lobe of the hypophysis. From these areas the neurosecretory material could reach the intermediate lobe of the hypophysis through the vessels which irrigate it. The vascularization of the intermedia lobe, described by Duvernoy(2), starting from the capillaries that initially run through the neural lobe and the median eminence gives anatomical support to this point of view.

Summary. The topographic distribution of MSH-RF in the hypothalamus was studied in the rat. MSH-RF was determined by measuring the capacity of hypothalamic extracts to deplete pituitary MSH concentration in recipient animals. The activity of extracts prepared from sections of hypothalamus taken in 3 different planes showed the highest concentration of the releasing factor in the area of the paraventricular nucleus. The median eminence was the second most important active zone. Extracts from neural lobe of the hypophysis were also active though to a lesser degree than those from hypothalamus and their effect did not depend on either oxytocin or vasopressin. The possibility that MSH-RF could be produced by neurons having their soma in the paraventricular nucleus and axons ending in the median eminence and neural lobe of the hypophysis is discussed.

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The Species Specificity of Cholestyramine in Its Effect on Synthesis Of Liver Lipids and Level of Serum Cholesterol. (31126)

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Cholestyramine, an insoluble quarternary ammonium anion exchange resin, has a strong affinity for bile acids *in vitro* and is not absorbed from the gastrointestinal tract(1). When given orally cholestyramine causes an increased fecal elimination of bile acids(2,3). The fecal excretion of neutral sterols is also enhanced by cholestyramine(2,3), probably because of a reduced concentration of bile acids in the intestinal lumen(4).

This increased fecal loss of steroidal substances during cholestyramine administration has been noted in all species studied. The loss is reflected by a decrease in plasma cholesterol levels in some species, notably the chicken(2), dog(2) and man(3,5); in other species, such as the rat(6), mouse* and pig (7) the feeding of cholestyramine does not lower plasma cholesterol levels. In the rat and pig the increased fecal loss of steroids caused by cholestyramine is accompanied by a large compensatory increase in *de novo* cholesterol biosynthesis(6,7). These observations suggest that the rat, mouse and pig can increase sterol synthesis sufficiently to maintain constant tissue sterol levels when cholestyramine is administered; species such as the chicken and man presumably cannot compensate to the same degree.

Data supporting this hypothesis were obtained by studying the effects of dietary cholestyramine on plasma and liver cholesterol levels and on the *in vitro* synthesis of lipids by homogenates of livers obtained from rats and chickens. The results showed that cholestyramine had relatively little effect on hepatic

* Gallo, D. G., Sheffner, A. L., unpublished observations.

TABLE I. Composition of Basal Diets.

Rats		Chickens	
	g/100 g		g/100 g
Casein, ANRC	18	Yellow ground corn	37.2
Coconut oil	10	Soybean oil meal (50% protein)	40.0
Corn oil	2	Coconut oil	10.0
Amidex*	61.65	Amidex*	5.0
Non-nutritive fiber	4	Steamed bone meal	3.5
Salt mix‡	4	Alfalfa meal (17% protein)	3.0
Vitamin mix§	.35	Salt (iodized)	.4
α-Tocopheryl acetate	.005	Vitamin mix†	.55
Oleum percomorphum	.015 ml	Methionine hydroxy analog	.35
		$MnSO_4$	.02

* Modified corn starch, Corn Products Co. Carrier for vitamin mix and methionine hydroxy analog.

† The amounts of vitamins added per kg diet were: A, 4000 I.U.; D_3, 400 I.C.U.; choline, 2 g; α-tocopherol acetate, 20 mg; niacin, 50 mg; inositol, 1 g; p-aminobenzoic acid, 20 mg; calcium pantothenate, 15 mg; riboflavin, 6 mg; pyridoxine hydrochloride, 5 mg; thiamine hydrochloride, 4 mg; folic acid, 4 mg; menadione, 750 μg; biotin, 200 μg; B_{12}, 20 μg.

‡ Jones, J. H., Foster, C., J. Nutr., 1942, v24, 245.

§ Sarett, H. P., Snipper, L. P., ibid., 1954, v52, 525.

lipid synthesis in chickens, while in the rat cholestyramine markedly stimulated the synthesis of lipids by liver tissue. The changes caused by cholestyramine were apparent within 24 hours after either the addition to, or removal of, cholestyramine from the diet.

Methods and materials. Male weanling rats of the Wistar strain† were maintained on a basal diet (Table I) for 2 weeks and then divided into 13 groups of 10 rats each on the basis of body weight and litter. Four groups were fed the basal ration, while 9 groups received the same basal ration containing 2% cholestyramine. After 4 weeks, cholestyramine was withdrawn from the diet of 3 of the latter groups. Groups of 10 animals were sacrificed at intervals during the 7-week study as indicated in the results.

Day-old White Leghorn cockerels, Kimber strain 137,‡ were maintained on a basal semisynthetic diet (Table I) until about 4 weeks of age. They were then divided on the basis of weight into 13 groups of 10 birds each. Dietary treatments were the same as outlined for the rats.

At sacrifice plasma and hepatic cholesterol levels were determined, following saponification and extraction, using the reagents of Abell *et al*(8). The liver, after removal of the sample for cholesterol analysis, was weighed and homogenized in 10% sucrose (3 ml per gram of liver) using the loose-fitting pestle recommended by Bucher(9). Each homogenate was filtered through gauze and an aliquot taken for determination of solids. A 5 ml aliquot of each filtrate was transferred to a 125 ml Erlenmeyer flask containing 5 ml of 0.1 M potassium phosphate buffer, pH 7.3, containing 0.006 M $MgCl_2$, 0.006 M succinate, 1 mg ATP, 1 mg DPN, 0.03 M nicotinamide and 4 μc (2 μmoles) acetate-1-C^{14}, sodium salt.§ The flasks were incubated in an oxygen atmosphere at 37°C with constant shaking for 4 hours.

The reactions were stopped by transferring the contents of each flask to a tube containing 0.5 ml 10 N H_2SO_4. The precipitated proteins were washed twice with distilled water by centrifugation and decantation. The lipids were extracted from the washed precipitate using 2:1 chloroform:methanol (v/v) and the extract washed by addition of 0.2 volumes of 0.02 N H_2SO_4. The lower chloroform phase was transferred to clean tubes and the solvent evaporated with a stream of nitrogen using moderate heat. One ml of 0.02 N H_2SO_4 was added to the lipid residue and the lipids extracted into redistilled hexane. This washing procedure eliminated contamination of the lipid extract by the radioactive substrate.

An aliquot of the hexane phase was taken and the solvent evaporated under nitrogen. The lipid residue was dissolved in chloroform and aliquots taken for determination of total lipid-C^{14} and for separation into lipid classes. The separations were accomplished by thin layer chromatography on silica gel G|| plates using 2 different solvent systems. Hexane:

† Harlan Industries, Cumberland, Ind.

‡ Farmer's Hatchery & Supply Co., Inc., Evansville, Ind.

§ New England Nuclear Corp., Boston, Mass.

|| Research Specialties Corp., Richmond, Calif.

TABLE II. Influence of Dietary Cholestyramine on Plasma and Liver Cholesterol Levels of Rats.

Treatment	Experimental day sacrificed					
	1	2	7	29	30	35
	Plasma cholesterol (mg/100 ml)					
Control	107 ± 7*		106 ± 5			108 ± 5
Cholestyramine	104 ± 5	108 ± 4	113 ± 5		97 ± 4	111 ± 4
Cholestyramine withdrawn†				113 ± 6	102 ± 4	‡ 96 ± 5
	Liver cholesterol (mg/g wet wt)					
Control	2.5 ± .04		2.7 ± .07			2.4 ± .07
Cholestyramine	2.5 ± .05	2.6 ± .05	2.6 ± .04		2.4 ± .03	2.5 ± .05
Cholestyramine withdrawn†				2.6 ± .04	2.6 ± .09	2.5 ± .05

* Mean ± standard error of 10 animals.
† Cholestyramine was withdrawn on day 28.
‡ Statistically lower than cholestyramine-treated group, $P < .05$.

diethylether (90:15) was used to separate triglycerides and lipids of lower polarity, while hexane:diethylether:acetic acid (50:50:1) was used to separate lipids more polar than triglycerides. With the latter solvent system phosphatides remained at the origin while monoglycerides were clearly separated from the origin. The chromatographic separations were monitored through the use of standard reference compounds, which were spotted on each plate. The separated lipids were visualized by spraying with a 1% solution of iodine in methanol. The desired areas were marked and the silica gel from each area scraped into separate counting vials. The C^{14} content of all samples was determined in an automatic Tri-Carb® Liquid Scintillation Spectrometer Model 314E¶ using a scintillator solution containing 3 g 2,5-diphenyloxazole, 0.5 g 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl) benzene, 100 ml redistilled absolute methanol and 900 ml redistilled toluene(10). Color quenching was corrected by the channels ratio method.

Results. Rats. In agreement with other reports(6,11), the feeding of cholestyramine at a level of 2% of the diet had no marked effect on plasma or liver cholesterol levels (Table II), or on weight gain or food consumption. The moderate reduction in liver cholesterol found 7 days after withdrawal of cholestyramine from the diet is not considered to have significant meaning.

In the animals which did not receive cholestyramine, the amount of acetate-C^{14} utilized for lipid synthesis was found to gradually increase during the 35-day experimental period (Fig. 1A). In this group, sterols and glycerides each comprised about 25% of the total radioactive lipids; from their chromatographic behavior most of the remaining radioactive lipids appeared to be phospholipids. No attempt was made to separate the individual phosphatides.

Addition of cholestyramine to the diet caused a significant elevation in total lipid radioactivity after 2 days of treatment (Fig. 1A); lipid synthesis continued to increase during the experiment, reaching a maximum level in about 30 days. On withdrawal of cholestyramine, a rapid reduction in lipid synthesis occurred; within 2 days, total lipid radioactivity was significantly lower than that found in the resin-treated animals, and after 7 days had returned to the level of the untreated control animals.

Both free and ester sterol synthesis were significantly increased after one day of cholestyramine feeding (Fig. 1B and 1C). The incorporation of radioactivity into the total sterol fraction increased more rapidly than into the other lipid fractions during the experimental period, rising from about 25% of the total lipid radioactivity in the untreated groups to approximately 60% in the cholestyramine-fed groups.

Within one day after withdrawal of dietary cholestyramine, there was a highly significant decrease in both free and ester sterol radioactivity. Sterol synthesis 2 days after cholestyramine withdrawal was only slightly greater than the control level, and after 7

¶ Packard Instrument Co., LaGrange, Ill.

days was indistinguishable from that of the controls.

Triglyceride synthesis was also stimulated by cholestyramine feeding, although because of high individual variation it was significantly greater than the control only at the seventh day (Fig. 1D). Because of the marked increase in sterol synthesis, the percentage of radioactivity present in the glyceride fractions was significantly lowered from about 25% in the untreated controls to about 10% in the treated animals. On withdrawal of cholestyramine there was a temporary increase in triglyceride synthesis, followed by a rapid decline to control levels within 7 days.

Squalene and mono- and diglyceride fractions were also separated and their radioactivity determined. Since the changes in squalene synthesis mirrored those seen in the sterol fractions, while mono- and diglyceride radio-

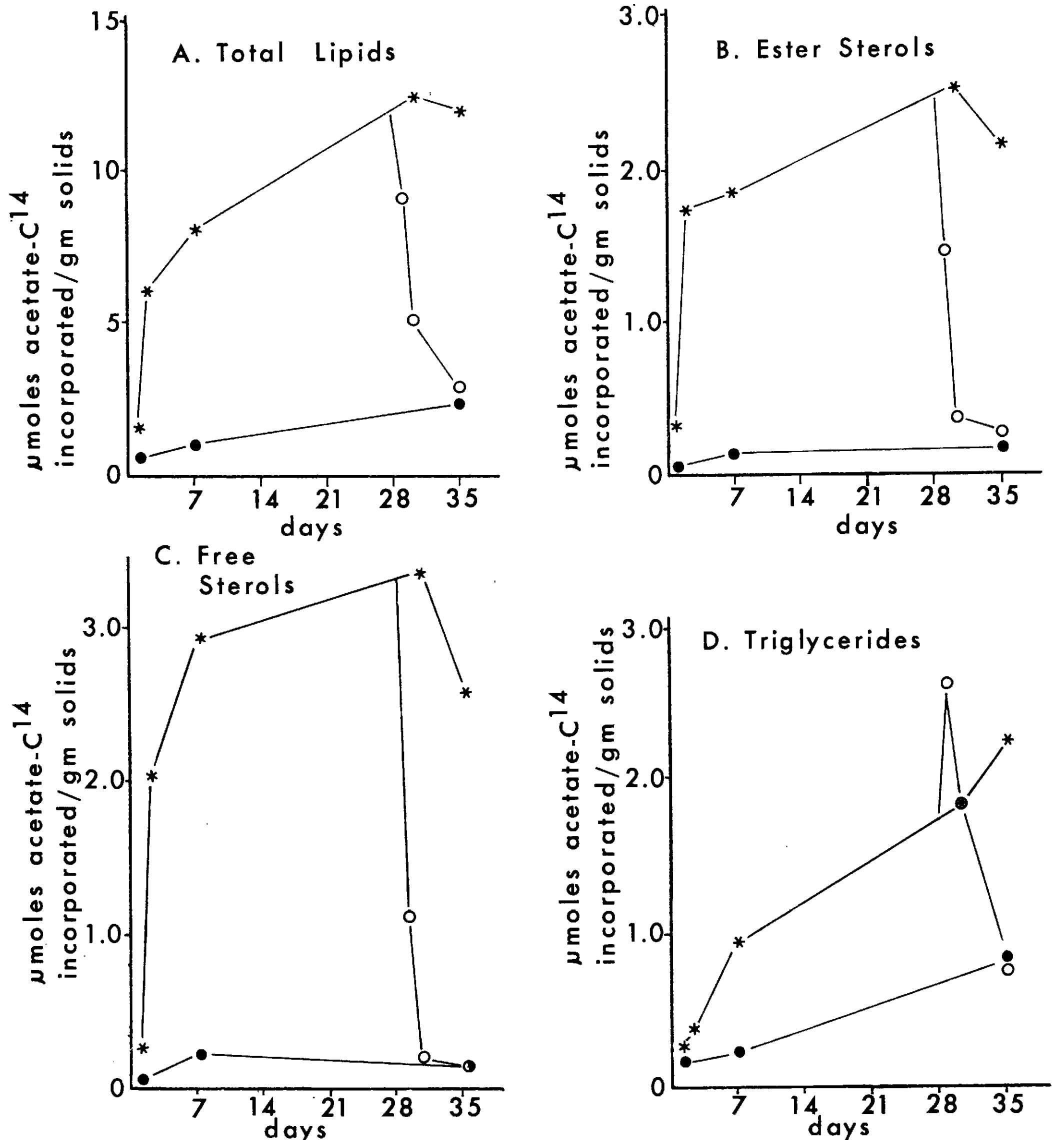

FIG. 1. Effect of cholestyramine feeding on liver lipid synthesis in rats. ●—●, no cholestyramine; *—*, cholestyramine treated; ○—○, cholestyramine withdrawn from diet on day 28. Each point represents the mean of data from 10 animals.

activity patterns were similar to those of the triglycerides, the data for these fractions are not reported.

Chickens. Plasma cholesterol levels in chickens were significantly lower following one day of cholestyramine feeding (Fig. 2), and decreased further on continued treatment reaching a minimum at 28 days. Withdrawal of cholestyramine resulted in a rapid return of plasma cholesterol to pretreatment levels. Cholestyramine treatment did not significantly alter body weight or food consumption, and had little effect on liver cholesterol.

The incorporation of acetate-C^{14} radioactivity into lipids decreased in the untreated control groups during the 35-day experimental period (Fig. 3A). The feeding of cholestyramine for one day significantly reduced total lipid radioactivity; however, lipid synthesis subsequently increased reaching a maximum at 28 days. Total lipid radioactivity was significantly elevated by cholestyramine only

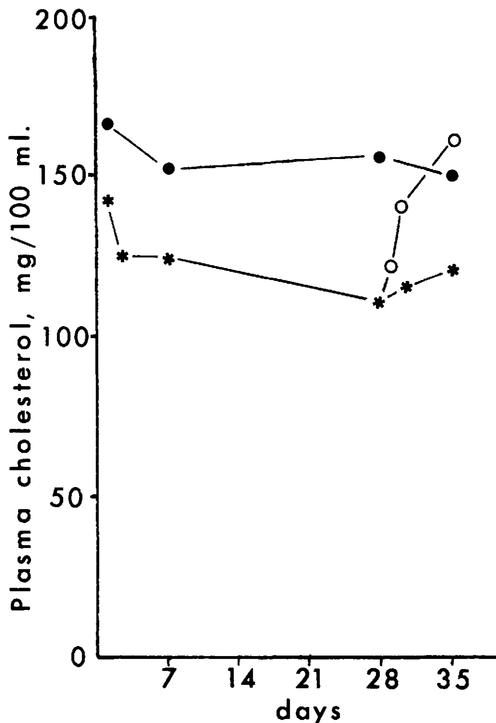

FIG. 2. Effect of cholestyramine feeding on plasma cholesterol levels in cockerels. ●—●, no cholestyramine; * — *, cholestyramine treated; O — O, cholestyramine withdrawn from diet on day 28. Each point represents the mean data from 10 animals.

after 35 days of feeding. Two days after withdrawal of cholestyramine lipid synthesis returned to control levels.

Ester sterol synthesis was significantly reduced following 2 days of cholestyramine feeding, but increased thereafter to levels about twice as great as those of the control groups (Fig. 3B). Radioactivity in the free sterol fraction was significantly increased after 7 days (Fig. 3C), and remained elevated during the remainder of the experiment. The stimulation of cholestyramine of both free and ester sterol synthesis was comparatively moderate and rapidly decreased after withdrawal of cholestyramine. Cholestyramine produced a temporary significant decrease in the incorporation of acetate-C^{14} into triglycerides (Fig. 3D); during the remainder of the experiment triglyceride radioactivity was slightly greater in the treated groups but no statistically significant differences were observed.

The C^{14}-labeled lipids synthesized by the untreated chickens contained about 10% sterols and 45% glycerides. The remaining radioactive lipids were primarily phosphatides. Cholestyramine treatment increased the sterol content of the labeled lipids to about 15%, with a concomitant reduction in glyceride content to about 35%.

Discussion. Huff *et al*(6) have shown that the feeding of cholestyramine to rats for 10 days or 9 weeks causes an approximately 12-fold increase in the *in vitro* conversion of acetate-C^{14} to digitonin precipitable sterols by liver homogenates. In the present study, a significant stimulation in sterol synthesis by rat liver homogenates occurred within 24 hours after addition of cholestyramine to the diet. The amount of acetate incorporated into sterols increased to a peak after about 30 days of treatment, with a greater increase occurring in the free sterol than in the ester sterol fraction.

The observed 12- to 15-fold increase in sterol formation by rat liver homogenates apparently compensated for the increased fecal loss of sterol and bile acids induced by cholestyramine, thus accounting for the lack of effect of the resin on plasma cholesterol levels in rats. The reason for the moderate stimula-

tion of glyceride synthesis in rats by cholestyramine feeding is not clear. Although the level of dietary fat can affect sterol and fatty acid synthesis from acetate-C^{14}(12), the amount of cholestyramine fed in this study should not have altered fat absorption sufficiently to account for the changes seen(13).

The chicken, a species in which cholestyramine does have a hypocholesteremic effect, showed some changes in hepatic lipid synthesis such as were found in rats but of a much smaller magnitude. Sterol synthesis by chicken liver homogenates was stimulated only about 3-fold, compared to the 12- to 15-fold increase found in the rat. Thus, cholestyramine induced a change in the pattern of lipid synthesis in the chicken which, while qualitatively similar to that found in rats, was quantitatively much smaller and apparently insufficient to replace the loss of steroidal substances induced by cholestyramine.

The changes in lipid synthesis induced by

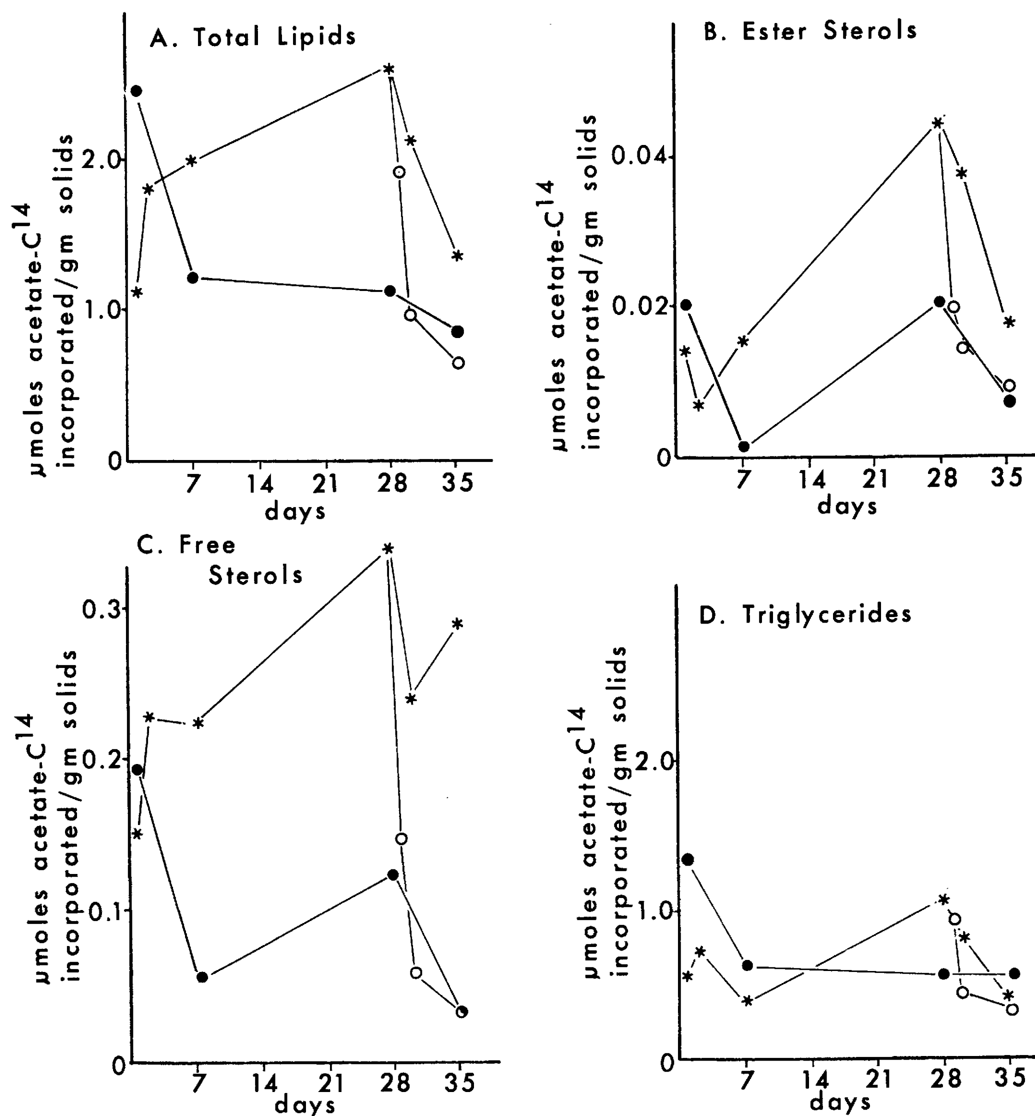

FIG. 3. Effect of cholestyramine feeding on liver lipid synthesis in chickens. ●—●, no cholestyramine; * — *, cholestyramine treated; ○ — ○, cholestyramine withdrawn from diet on day 28. Each point represents the means of data from 10 animals.

cholestyramine occurred rapidly. Statistically significant differences in sterol synthesis by rat liver homogenates occurred within 24 hours after either the addition or withdrawal of cholestyramine from the diet. The maximum stimulation of sterol synthesis in both species did not occur until cholestyramine had been fed for about 30 days. However, 48 hours after removal of the resin from the diet the rates of sterol synthesis returned to nearly normal control levels. Thus, reversal of the cholestyramine-induced stimulation of hepatic sterol synthesis occurred very rapidly.

The results obtained in this study indicate that a reduction in plasma cholesterol by cholestyramine can be prevented only if a marked stimulation of hepatic sterol synthesis occurs to offset the loss of steroidal substances caused by cholestyramine. Species such as the chicken, and presumably dog and man, are unable to increase hepatic sterol synthesis sufficiently to overcome this loss. Conversely, species such as the rat(6) and pig(7) apparently possess an adequate sterol synthesizing capacity. Since cholestyramine has been found to increase fecal bile acid excretion in every species studied, it would appear that the hypocholesteremic activity of cholestyramine in different species, and probably also individuals, is related to differences in their capacity to compensate for loss of cholesterol through increased sterol synthesis.

Summary. The effect of dietary cholestyramine on plasma and liver sterol levels, and on *in vitro* hepatic lipid synthesis from acetate-1-C^{14} were compared in the rat and chicken at intervals up to 35 days. The resin had no effect on plasma or hepatic cholesterol levels in the rat. In the chicken, plasma cholesterol levels were significantly reduced by cholestyramine although liver cholesterol was not changed.

Sterol synthesis by rat liver homogenates was significantly increased within 24 hours after addition of cholestyramine to the diet, and a significant reduction in sterol synthesis occurred within 24 hours after removal of cholestyramine from the diet. Cholestyramine also stimulated sterol synthesis by chicken liver homogenates, but the increase was of considerably lesser magnitude than in the rat.

The results are in accord with the hypothesis that cholestyramine significantly lowers plasma cholesterol levels in species which cannot increase hepatic sterol synthesis sufficiently to compensate for increased fecal loss of bile acids and other steroidal substances.

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