hancing effect in the production of essential fatty acid deficiency.

Summary. The presence of only trace quantities of arachidonic acid in the CEFA of gerbil serum is at variance with the hypothesis of a negative correlation between arachidonic acid levels in CEFA and susceptibility to atherosclerosis. Fasting for 18 hours resulted in a 66% increase of arachidonic acid at the expense of linoleic acid in CEFA of the rat, while in the gerbil, CEFA remained unchanged. In both species, palmitoleic and oleic acids were the major acids associated with cholesterol transport. The CEFA pattern in cholesterol-fed animals suggests an incipient essential fatty acid deficiency.

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Feedback Control of Cholesterol Biosynthesis in the Mouse.* (27360)

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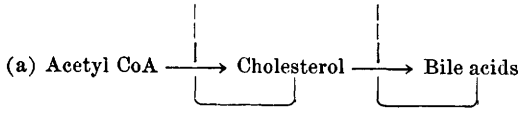
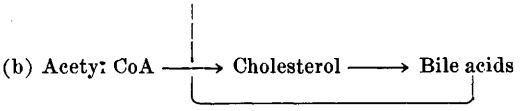
The effects of cholesterol and of the end products of cholesterol metabolism on rate of cholesterol biosynthesis have been the subject of several investigations(1). It is known that cholesterol itself lowers the rate of hepatic cholesterol synthesis, but it appears to have little effect on cholesterol synthesis in extrahepatic tissues. Bile acids also have been shown to control the rate of hepatic cholesterol metabolism through a feedback reaction(2). Two schemes have been proposed(2,3) to explain the mechanism of this feedback:



In this set of reactions bile acids, major end products of cholesterol metabolism, which are present in the enterohepatic circulation, reduce the rate of their own synthesis, causing an accumulation of small amounts of cholesterol at the intracellular level. This in turn reduces the rate of cholesterol biosynthesis.



In this scheme, the bile acids directly reduce the rate of cholesterol synthesis from acetyl CoA, thus reducing the amount of substrate available for conversion to bile acids.

Two approaches have been used in the study of the bile acid feedback reaction. Bergström and Danielsson(4) and Myant

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and Eder(3) have shown that bile acid synthesis is increased in bile fistula rats several hours after operation. The latter authors demonstrated an increased rate of hepatic cholesterol synthesis *in vitro* in these animals. We previously found(5) that bile acid synthesis in mice is inhibited by cholic acid and its conjugates. This study was made on animals with accumulated liver cholesterol, and under this condition, hepatic cholesterol synthesis is nearly at zero level. No decision between mechanisms *a* and *b* could be reached from the results of any of these experiments.

The present investigation was designed to study the bile acid feedback reaction and to locate the position or positions of feedback in normal mice maintained under normal conditions.

Methods. Forty-eight female albino mice of the Webster strain, weighing 24-30 g, were placed on a synthetic cholesterol-free diet(6) for a 2-week observation period. The diet of one-half of the mice was then supplemented with 0.5% cholic acid. Both diets were fed *ad lib.*

After 3 weeks the control and cholic acid fed mice were each divided into 3 groups of 8. For determination of hepatic cholesterol synthesis rates, a group of controls (Group I) and a group of cholic acid treated animals (Group II) received intraperitoneal injections of 5 μc sodium acetate-1-C^{14} (0.191 mg) in 0.9% saline. One hour later the animals were sacrificed and livers removed for analysis of cholesterol and cholesterol-4-C^{14}(7).

In the fecal steroid excretion studies, controls (Group III) and treated animals (Group IV) were injected intraperitoneally with 1 μc mevalonic acid-2-C^{14} (0.196 mg) in 0.9% saline. Mevalonic acid was used in this study since it has been shown that over a wide range of hepatic cholesterol biosynthesis rates, this substance (in limited quantities) is equally incorporated into liver cholesterol. An additional group of control (Group V) and treated mice (Group VI) received intraperitoneal injections of 1 μc cholesterol-4-C^{14} (17 μg) in a fine suspension. This suspension was prepared by dissolving cholesterol-4-C^{14} in absolute ethanol, homogenizing it with a suitable amount of 0.9% saline, and removing the ethanol by evaporation.

Both the mice injected with mevalonic acid (Groups III and IV) and those injected with cholesterol (Groups V and VI) were placed in metabolic cages. Feces were collected daily for 7 days. The mice were then sacrificed, and cholesterol and β-sterol-C^{14} levels determined in liver and carcass as previously described(8). Fecal steroids were fractionated (5) by extraction with ethanol, followed by hydrolysis with aqueous sodium hydroxide. $\alpha + \beta$ sterols were removed from the hydrolysate by petroleum ether extraction. The aqueous phase was acidified, and extracted with petroleum ether to remove fatty acids. Bile acids were recovered by further extraction with ethyl ether. The petroleum ether fraction containing $\alpha + \beta$ sterols was washed with dilute alkali to remove fatty acid contamination. The ethyl ether fraction containing the bile acids was washed with water to remove impurities, including mevalonic acid. Suitable aliquots of the washed extracts were plated in cup planchets and counted. All C^{14} activities were measured in an automatic windowless flow counter.

Results. Results of the study on hepatic cholesterol synthesis as affected by cholic acid are presented in the upper half of Fig. 1. Cholic acid caused a 10-fold reduction in the rate of acetate-1-C^{14} incorporation into liver cholesterol.

The time pattern of fecal steroid excretion was studied in control and cholic acid fed mice following single intraperitoneal injections of mevalonic acid-2-C^{14} or cholesterol-4-C^{14} (Table I). In control animals which received mevalonic acid (Group III), C^{14} activity was equally distributed between the $\alpha + \beta$ sterol and bile acid fractions during the first 24 hours; while in subsequent periods, activity in the former fraction was about double that in the latter one. Comparison of Groups III and IV (Table I) indicates that cholic acid did not significantly alter either the time course of C^{14} excretion, or the total activity excreted in the 2 fractions.

After a single injection of a fine suspension of cholesterol-4-C^{14}, in the control group (V) bile acid-C^{14} excretion was 40% lower than

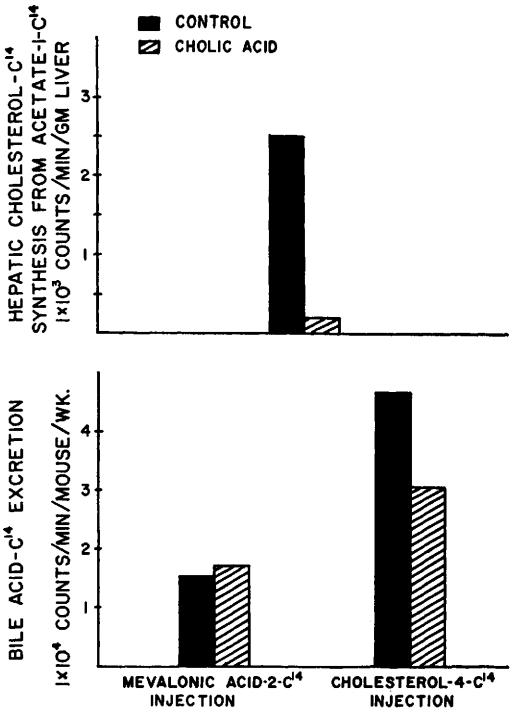

FIG. 1. Effect of cholic acid on hepatic cholesterol and bile acid synthesis.

the $\alpha + \beta$ sterol-C^{14} throughout the collection period. C^{14} excretion in both fractions was fairly constant after the first 24 hours. In the cholic acid treated group (VI), the fecal bile acid-C^{14} was uniform during the 7-day period, but after the first 24 hours, was reduced $\cong$ 40% with respect to the controls (Group V). Total excretion of bile acid-C^{14} was 36% lower in the treated animals than in the controls (Table I). The rate of $\alpha + \beta$ sterol excretion was not altered by cholic acid.

After sacrifice of the injected mice, β-sterol-C^{14} activity remaining in the livers and carcasses was determined (Table II). In animals injected with mevalonic acid-2-C^{14}, total β-sterol-C^{14} activity in the livers of cholic acid treated animals (Group IV) was higher than in controls (Group III). Carcass β-sterol-C^{14} and total C^{14} activity accounted for, was the same in these 2 groups.

When cholesterol-4-C^{14} was injected, cholic acid treated animals (Group VI) retained a significantly larger amount of hepatic β-sterol-C^{14} than controls (Group V). There was no difference in carcass sterol-C^{14} in the 2 groups (V and VI).

Liver cholesterol levels were increased significantly in the cholic acid groups (IV and VI), whereas carcass cholesterol levels remained constant.

Discussion. The experimental results reveal several interesting points. In normal mice the distribution of C^{14} activity in fecal steroids was approximately 60% in the $\alpha + \beta$ sterol fraction and 40% in the bile acid fraction, regardless of whether the steroids originated from intraperitoneal injections of mevalonic acid-2-C^{14} (Group III) or from cholesterol-4-C^{14} (Group V). Studies in normal rats(9,10), on the other hand have shown that 80-90% of the activity of cholesterol-4-C^{14} injected intravenously or intraperitoneally, is excreted in the fecal bile acid-C^{14} fraction. It seems that a species difference may exist between mice and rats in this distribution; this point is being investigated.

Excretion of fecal steroid-C^{14} in both control (Group III) and treated (Group IV) mice receiving mevalonic acid-2-C^{14} intra-

TABLE I. Effect of Cholic Acid on Time Course of Fecal Steroid-C^{14} Excretion in Mice.

	Mevalonic acid-2-C^{14}				Cholesterol-4-C^{14}			
	Group III, control		Group IV, cholic acid		Group V, control		Group VI, cholic acid	
Collection period, days	$\alpha + \beta$ sterols	Bile acids	$\alpha + \beta$ sterols	Bile acids	$\alpha + \beta$ sterols	Bile acids	$\alpha + \beta$ sterols	Bile acids
	Counts/min./mouse/24 hr							
1	7,821	7,577	8,619	9,336	7,549	4,040	4,525	4,531
2	6,010	3,071	5,486	3,220	15,663	10,130	12,893	6,456
3	3,227	1,666	4,878	1,512	18,143	11,151	20,429	6,051
4	2,830	1,234	3,741	1,027	18,447	13,110	24,751	7,471
5-7	2,783	1,490	1,713	1,987	17,044	8,475	15,305	5,734
Total excretion	22,671	15,038	24,437	17,082	76,846	46,906	77,903	30,243

TABLE II. Effects of Cholic Acid on Fecal and Tissue Steroid-C^{14} and Steroid Levels 7 Days after C^{14} Injection.

Source	Mevalonic acid-2-C^{14}		Cholesterol-4-C^{14}	
	Group III, control	Group IV, cholic acid	Group V, control	Group VI, cholic acid
	Counts/min./mouse			
Fecal steroids-C^{14}, total excreted	37,709	41,519	123,752	108,146
β-Sterol-C^{14} in liver	14,994	21,090	77,285	180,552
" in carcass*	45,585	44,864	330,510	323,080
Total C^{14} activity accounted for	98,288	107,473	531,547	611,778
	mg/g tissue			
Liver cholesterol	5.34 ± .67	7.53 ± 2.54	4.19 ± .97	7.52 ± 3.08
Carcass " *	2.24 ± .39	2.30 ± .39	2.06 ± .35	2.20 ± .38

* Carcass wt = body wt minus liver wt. ± stand. dev.

peritoneally was at a maximum during the first 24-hour interval and declined rapidly through the fourth day. In the case of cholesterol-4-C^{14} injection (Groups V and VI), after the first 24-hour interval, during which C^{14} excretion was low, rate of steroid-C^{14} excretion increased and then remained relatively constant. The difference between these groups was due to the slow rate of absorption of cholesterol-4-C^{14}, which was administered not as a solution but as a fine suspension, in an effort to maintain a constant hepatic cholesterol-4-C^{14} specific activity.

Certain data which need to be reconciled are shown graphically in Fig. 1. Total excretion of fecal bile acid-C^{14} in control and cholic acid treated mice which received mevalonic acid-2-C^{14} was not significantly different; thus it might appear that cholic acid has no effect on bile acid synthesis. However, Fig. 1 also shows that hepatic cholesterol synthesis was reduced 10 times in these animals. These findings might seem incompatible because:

A. Liver cholesterol level is slightly higher in treated animals than in controls; and yet B. Cholesterol synthesis rate is decreased 10 times in cholic acid treated animals; while C. Bile acid-C^{14} excretion rate proceeds at control rate. It is conceivable that in cholic acid treated mice the liver cholesterol level could be maintained *via* an extrahepatic source. This explanation, however, is inconsistent with the results because there was no significant difference in carcass β-sterol-C^{14} levels in controls and in cholic acid treated animals at the end of 7 days (Table II). Therefore cholesterol *could not* have entered the liver from the carcass. Furthermore, extrahepatic tissues have been shown to be poor sources of plasma cholesterol(11,12).

Consideration of the kinetics of hepatic cholesterol metabolism offers an explanation of the experimental results. If non-labeled cholesterol molecules are supplied to a liver containing a number of C^{14}-labeled cholesterol molecules, and all of these molecules are subject to the same chance of being metabolized to bile acids, the chances that a cholesterol-C^{14} molecule will be degraded in a given interval will be inversely related to the rate of supply of unlabeled cholesterol molecules. Keeping this fact in mind, it is possible to set up two situations relating cholesterol and bile acid synthesis rates, in order to determine which one agrees with the experimental data:

1. If non-labeled cholesterol synthesis were reduced 10 times, thus increasing the ratio of labeled/unlabeled cholesterol to be metabolized, one would expect a many-fold increase in bile acid-C^{14} excretion in 24 hours as compared with controls, even though bile acid synthesis was kept constant.

2. On the other hand, if *both* non-labeled cholesterol synthesis and bile acid synthesis were reduced 10 times, the ratio of labeled/unlabeled cholesterol would remain unchanged, and so no appreciable change in bile acid-C^{14} excretion would be expected per 24 hours, as compared with controls.

(Obviously these conditions would not necessarily apply in the first hour or two after introduction of labeled cholesterol.)

Since our data (Table I) on mice injected

with mevalonic acid show no significant difference between excretion of bile acid-C^{14} in control and cholic acid treated groups, the second of the above sets of conditions would apply, and it would follow that cholic acid must reduce both cholesterol and bile acid syntheses in normal mice. The reduction of bile acid synthesis by cholic acid is demonstrated (Fig. 1 and Table I) in the cholesterol-4-C^{14} injected mice in which the specific activity of liver cholesterol-C^{14} was maintained at a relatively constant level in control and cholic acid treated mice. It is now clear that both the rates of conversion of acetate to cholesterol and of cholesterol to bile acid are lowered in mice by cholic acid.

Since 5-8 mg of cholic acid was administered per day to each treated mouse, it seemed possible that this addition could have diluted the pool of bile acids in the enterohepatic circulation. If per unit of time a fixed fraction of the circulating pool had been removed *via* the fecal excretion, the amount of bile acid-C^{14} in the feces would have been reduced even though the rate of bile acid-C^{14} synthesis remained constant. However, Table II shows that such a dilution does *not* explain the inhibition of bile acid-C^{14} excretion in the treated animals. At the end of the experiments, the cholic acid treated animals retained more cholesterol-C^{14} in their livers than the controls. Since the excretion of sterol-C^{14} was identical in control and treated animals, liver cholesterol-C^{14} retention in cholic acid treated mice must have been due to inhibition of the conversion of cholesterol to bile acid by cholic acid. Kinetic considerations similar to those outlined above, show that inhibition of bile acid synthesis must have been very strong.

It was previously demonstrated(5) that cholic acid inhibits bile acid synthesis in mice whose hepatic cholesterol synthesis is blocked by cholesterol elevation. This showed that cholic acid can inhibit bile acid synthesis independently of its effect on rate of cholesterol synthesis. It follows that the rate of hepatic cholesterol synthesis in mice is controlled by cholic acid and its conjugates(13) through a double feedback reaction. Since taurocholic acid is the chief bile acid found in mouse bile (14), this substance must be important as a regulator in the homeostatic control of mouse hepatic cholesterol metabolism.

Summary. Effects of cholic acid on biosynthesis of hepatic cholesterol and bile acids were studied. Mice maintained on a basal diet, or this diet supplemented with 0.5% cholic acid, received single intraperitoneal injections of mevalonic acid-2-C^{14} or cholesterol-4-C^{14}. Feces were collected at 24-hour intervals, fecal steroid-C^{14} was isolated and fractionated. $\alpha + \beta$-sterol-C^{14} and bile acid-C^{14} were determined. In control mice the distribution of C^{14} activity in fecal steroids was 60% in the sterol fraction and 40% in the bile acid fraction. This ratio was constant with time after the first 24-hour interval, regardless of the substance injected. It was shown that cholic acid brings about equal decreases in the rates of both hepatic cholesterol and bile acid biosyntheses. Since a preceding study had shown that the rate of bile acid synthesis in mouse liver is inhibited by cholic acid independently of its effect on cholesterol synthesis, it was concluded that the rate of hepatic cholesterol biosynthesis in mice is controlled by cholic acid and its conjugates by means of a double feedback reaction.

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Influence of Plasma Filtrate Containing Erythropoietic Factor on Intestinal Iron Absorption in Rats.* (27361)

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It has been shown that acute exposure to altitude increases intestinal iron absorption in men(1,2). These findings suggested that the increased intestinal absorption of iron was associated with increased red cell production rather than lowered oxygen saturation of the blood or oxygen tension at the intestinal level. The close relationship between the level of red cell production and iron absorption suggests that the regulating mechanisms of iron absorption during altitude changes must take place in intimate relation to or through a mechanism similar to that controlling erythropoiesis. To test this hypothesis the effect of plasma filtrate obtained from rabbits submitted to simulated high altitude on intestinal absorption of iron in the rat was studied.

Material and methods. Thirty-three white, adult, male rats, weighing 150 to 200 g, were divided into 3 groups: 1) 11 rats were given plasma filtrate obtained from "altitude" rabbits; 2) 11 rats received plasma filtrate from "sea level" rabbits; 3) 11 rats were injected with saline.

The plasma filtrate was obtained from "sea level" rabbits and from rabbits exposed to a simulated altitude of 22,000 feet for 24 hours in a low presure chamber. Following Borsook's method(3), the plasma was acidified to pH 5.5 and heated in boiling water for 5 minutes; the suspension was filtered and frozen until ready for assay.

The test solutions were administered by the intraperitoneal route daily for 6 days, in a dose of 4 cc/day, 2 cc in the morning and 2 cc in the afternoon. On the fifth day 0.5 cc of a solution of ferrous sulfate (about 0.5 mg of metallic iron per kg of body weight) labelled with Fe^{59} was given by mouth under light pentothal anesthesia by means of a tuberculin syringe fitted with a curved No. 16 needle. The tip of the needle was blunted and smoothed to prevent damage to the esophagus. All the recipient animals were starved for 2 days prior to injection and remained without food for 3 days after injection. The day before receiving iron and 2 days after, the rats were fed with milk to restore normal intestinal function after the starvation; this food was chosen because of its low content of iron. From this time until the end of experiment they received a normal rat diet.

Blood samples were taken by heart puncture 7 days after the rats were given the iron solution. Feces were carefully collected for 5 days to determine the unabsorbed portion of iron. The total amount of feces from each rat was ground and treated with sulfuric acid. These solutions were kept in containers of uniform geometry for 3 to 4 days, then water was added to achieve a standard 500 cc volume. Radioactivity of Fe^{59} was measured in 2 cc samples of blood and in the whole samples of the stools in a gamma scintillation counter. In determining the total amount of absorbed iron appearing in red blood cells a blood volume of 7% of body weight was estimated according to previous determinations carried out in our colony of rats.

Results. Rats that received plasma from rabbits subjected to a simulated altitude of 22,000 feet showed a higher percentage of radioiron in their blood than other rats which were injected with either plasma from "sea level" rabbits or saline (Fig. 1). There was a corresponding decrease in unabsorbed iron

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