TABLE I. Effect of Cadmium, Chromium, and Lead on Serum Cholesterol of Male Rats (mg/100 ml).

	Control	Cadmium	Chromium	Lead
No. of animals	20	20	20	20
Mean (mg/100 ml)	101.7	76.1	90.5	71.3
Range	82.5–128.6	54.6–102.5	61.8–115.3	54.0–87.7
Stand. dev.	±14.2	±14.8	±16.6	±13.2
Stand. error of mean	± 4.5	± 4.7	± 5.2	± 4.2
p (control)	—	<.01	N.S.	<.01
p (cadmium)	—	—	N.S. (<.1)	N.S.
p (chromium)	—	—	—	±.01
Duration of exp. (mo)	12	12	10	10

N.S. = Not significant.

groups. Chromium concentrations were 0-0.2 μg/g in the controls, 0.15-0.37 μg/g in the lead-fed, and 0.18-0.27 μg/g in the cadmium-fed groups. No chromium-fed animals died in the first year of the experiment.

Hepatic cholesterol concentrations were measured in pairs of male animals matched for age and dying spontaneously. No animals were killed as they are part of a larger experiment involving life-time studies. No obvious changes were evident. Controls averaged 335 mg/100 g, cadmium-fed 333 mg/100 g and lead-fed 325 mg/100 g tissue (wet weight).

Discussion. We cannot explain the lowering of serum cholesterol in male rats by small amounts of lead and cadmium in drinking water. Both of these metals accumulate in mammalian tissues; hepatic and renal concentrations in these rats approximated those present in young adult human beings in the United States(3,4). To our knowledge, no influence on synthesis or catabolism of cholesterol by these metals has been demonstrated. Chromium, on the other hand, might be expected to have some effect(1); none was demonstrable. Hepatic deposition of excesses was not shown. The mechanisms involved are obscure.

Summary. Male rats on a diet and environment controlled as to trace metals were given 5 ppm. cadmium, chromium and lead in drinking water for 10 to 12 months. Lead and cadmium appeared to lower serum cholesterol levels significantly; chromium was without demonstrable effect. Hepatic levels were apparently unchanged.

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Comparison of Serum Cholesterol Esters in Gerbil and Rat. (27359)

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The findings of Swell *et al.*(1) and Sinclair (2) suggest that a deficiency of arachidonic acid may be an important factor in the etiology of atherosclerosis. Swell(1) reported various serum-cholesterol-ester fatty acid (CEFA) patterns for 8 species, which did not appear to be related to dietary intake of fatty acids. The data suggested an inverse correlation between susceptibility to atherosclerosis of these species and content of arachidonic

acid in CEFA of their sera. In the rat, which is extremely resistant to production of atherosclerosis by artificial means, 50% of the CEFA of serum was arachidonic acid, whereas those species (goose, chicken, rabbit, guinea pig) having small proportions of arachidonic acid in the CEFA of serum were susceptible to atherosclerosis and developed the disease spontaneously(1).

It was reported that the Mongolian gerbil (*Meriones unguiculatus*), on a cholesterol-supplemented diet for prolonged periods, rapidly developed hypercholesteremia(3) but showed no atheromata or other vascular changes indicative of lipid infiltration(4,5). This apparent resistance of the gerbil to formation of atheromata prompted us to investigate the CEFA composition in the serum of the gerbil in comparison with the rat, under various dietary conditions.

Methods and materials. All animals used were males raised in our own colonies: rats (Sherman E strain), 150-250 g, and gerbils, 50-70 g. The diet consisted of Purina Laboratory Chow and water *ad libitum;* gerbils received escarole leaves daily instead of water. In diets containing 1.5% cholesterol and 0.5% cholic acid, cholesterol dissolved in ether and the dry cholic acid were spread evenly over ground chow, and the solvent was allowed to evaporate under continuous mixing. The effect of starvation was studied by removal of feed 18 hours prior to sacrifice. Animals were decapitated and the blood collected. Blood from 3 or 4 gerbils was pooled for each determination. Lipid extract of 2 ml serum samples was prepared according to the procedure described by Wycoff and Parsons(6) scaled up to the corresponding volumes. Total cholesterol was determined by the saponification and extraction procedure of Trinder(7) and the colorimetric analysis of Zak *et al.*(8). Cholesterol esters were separated by silicic acid chromatography(6) and interesterified in sodium methoxide-methanol(9). The methyl esters were sublimed according to the method of Stoffel *et al.*(10). Gas-liquid chromatography was carried out on a Barber-Colman Gas Chromatograph, Model 10, equipped with a 3.12 m $\times$ 6 mm I.D. column, 5% diethylene glycol succinate on Chromosorb W, 80-100 mesh, at 180°C and 39 p.s.i. argon gas.

Results. Data on the serum CEFA of rats and gerbils following 18 hours starvation are given in Table I (Group A). In the rat the major fatty acids present are linoleic and arachidonic acids comprising almost 79% of total CEFA. In contrast, the gerbil presents a strikingly different pattern; arachidonic acid was found only in trace quantities while oleic and linoleic acids represented the major fatty acids comprising approximately 88% of total CEFA.* Under these fasting conditions, in the rat 55% of the cholesterol in CEFA is esterified with arachidonic acid while in the gerbil, 65% is esterified with linoleic acid. In both, linolenic acid was present in only trace quantities.

The CEFA pattern in serum of rat and gerbil in the normal animal with food offered *ad libitum* is presented in Table I (Group B). In the rat linoleic acid and arachidonic acid were present in roughly the same concentrations, comprising 62% of total CEFA. The decrease in arachidonic acid concentration was associated with an increase in palmitoleic and linoleic acid. In the gerbil, the CEFA pattern was remarkably similar to the fasting state. Again only trace quantities of linolenic and arachidonic acids were detected.*

Data on the CEFA pattern following 5 days feeding of 1.5% cholesterol and 0.5% cholic acid is presented in Table I (Group C). Under the stress of cholesterol feeding, pronounced changes in the relative concentrations of CEFA were found in both rat and gerbil. A comparison of this group with normal animals (Group B) showed no significant changes in palmitic acid concentration, while palmitoleic acid concentration increased. Oleic acid was the predominant fatty acid associated with cholesterol transport showing an elevation in the cholesterol-fed rat whereas linoleic acid concentration was decreased. The most dramatic change occurred in the arachidonic acid level when it dropped from 33.5% in the normal rat to

* Similar results were reported to us earlier, in a personal communication from Dr. Leon Swell.

TABLE I. Composition of Serum Cholesterol Ester Fatty Acid (CEFA) of Rat and Gerbil.

	Group A		Group B		Group C	
CEFA*	Rat	Gerbil	Rat	Gerbil	Rat	Gerbil
Palmitic	7.3 ± .7†	5.4 ± .5	10.2 ± 1.1	5.6 ± .6	6.4 ± .6	4.2 ± 1.1
Palmitoleic	4.9 ± .3	6.8 ± .9	8.6 ± .7	10.1 ± .7	19.9 ± 1.6	17.5 ± 2.6
Oleic	8.8 ± .7	22.8 ± 2.0	10.0 ± 1.4	20.1 ± 2.0	35.3 ± 2.4	32.7 ± 1.2
Linoleic	23.2 ± 1.3	65.1 ± 1.7	38.8 ± 1.4	64.2 ± 2.9	28.2 ± 1.4	36.1 ± 2.3
Linolenic	T‡	T	T	T	3.7 ± .7	9.5 ± .4
Arachidonic	55.7 ± 1.4	T	33.5 ± 2.1	T	6.5 ± .6	T
Serum cholesterol, mg %	70 ± 3.1	148 ± 4.8	79.0 ± 1.5	150 ± 6.0	178.0 ±12.5	965.0 ±83.8

* Represents % of major fatty acids measured.
† Mean ± stand. error.
‡ Trace (less than 1%).

Group A: Animals after 18 hr starvation; 8 rats and 7 groups of gerbils (3 or 4 animals per group).
Group B: Food *ad libitum*; 7 rats and 8 groups of gerbils (3 or 4 animals per group).
Group C: Diet supplemented for 5 days with 1% cholesterol and 0.5% cholic acid; 6 rats and 3 groups of gerbils (3 animals per group).

6.5% in the cholesterol-fed group. Linolenic acid which had been present in only trace amounts in fasting and normal rats represented 3.7% of the total CEFA. In the gerbil the palmitic acid concentration remained unaltered, and arachidonic acid was still present in only trace amounts after cholesterol feeding. Palmitoleic and oleic acid levels were elevated, while linoleic acid decreased. As in the rat, linolenic acid concentration now represented a significant portion of total CEFA.

Discussion. The virtual absence of arachidonic acid in the serum of CEFA of the gerbil and its reported(4,5) resistance to formation of atheroma presents an exception to the hypothesis that the arachidonic acid level of an animal is related to its susceptibility to atherosclerosis(1). Thus it would appear that the proportion of arachidonic acid in the serum CEFA of an animal is a characteristic of the species.

Under conditions of fasting and cholesterol feeding the serum CEFA pattern of the gerbil appeared more resistant to change than in the rat. This is even more remarkable considering that the serum cholesterol of the rat increased by a factor of 2.5-while in the gerbil it was increased 6.5-fold. A comparison of CEFA patterns in cholesterol-fed animals with those in the fed controls is illuminating. In an examination of absolute quantities, palmitoleic and oleic acids increased 5.2- and 7.9-fold respectively in the rat, and 111- and 104-fold respectively in the gerbil. Hence, the major fatty acids associated with cholesterol transport in both species are palmitoleic and oleic acids.

In the rat, production of arachidonic acid from the essential precursor linoleic acid(11) is curtailed as the latter is now being used to meet the requirements for cholesterol absorption. Thus the essential fatty acids are in short supply because of demands for CEFA formation, and as a consequence, essential fatty acid deficiency is enhanced.

A relationship of essential fatty acids to cholesterol transport has been previously demonstrated(12,13). Conditions which are accompanied by hypercholesteremia, such as administration of dietary cholesterol, were found to accelerate essential fatty acid deficiency in the rat(14). The most striking change in the polyenoic acid pattern is the increase in content of trienoic acids and the concomitant decrease in dienoic and tetraenoic acids(14). An incipient essential fatty acid deficiency is suggested in the results of this study for the rat and perhaps in the gerbil, because here also there was a decrease in dienoic acid (linoleic) and an increase in trienoic acid (linolenic).

Under stress of increased cholesterol transport, additional quantities of polyenoic acids must be biosynthesized or liberated from tissue depots if homeostatic control is to be maintained. This suggests a mechanism by which dietary cholesterol could exert its en-

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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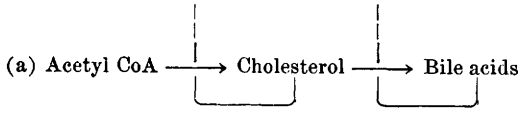
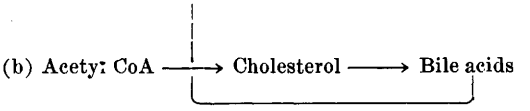
The effects of cholesterol and of the end products of cholesterol metabolism on rate of cholesterol biosynthesis have been the subject of several investigations(1). It is known that cholesterol itself lowers the rate of hepatic cholesterol synthesis, but it appears to have little effect on cholesterol 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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