

# THE EFFECT OF CERTAIN CHELATING SUBSTANCES (EDTA) UPON CHOLESTEROL METABOLISM IN THE RAT<sup>1</sup>

By RAY H. ROSENMAN AND MALCOLM K. SMITH

(From the Harold Brunn Institute, Mount Zion Hospital, San Francisco, Calif.)

(Submitted for publication June 27, 1955; accepted September 7, 1955)

There is increasing use in biology and medicine of certain salts of ethylenediamine tetraacetic acid (EDTA), substances which can form stable complexes with multivalent positive ions. Certain such chelating agents have been reported (1-3) to affect cholesterol metabolism. It therefore was believed of interest to study the nature of these possible effects.

## I. The Effect of Ingested Salts of EDTA on Plasma Cholesterol of Rats Fed Excess Cholesterol and Cholic Acid

### Methods

A series of 23 rats (I) was divided into two groups. Stock laboratory diet was fed to 15 rats while 8 rats were fed the same ration with added disodium EDTA<sup>2</sup> (4 per cent of diet).

Another series of 32 rats (II) was divided into four groups which were fed stock laboratory ration to which was added powdered cholesterol (2 per cent of diet) and cholic acid (1 per cent of diet), previously shown to induce chronic hypercholesteremia in rats (4, 5). Eight control rats ingested only this ration. The disodium salt of EDTA (4 per cent of diet) was added to the ration of 8 rats, tetrasodium EDTA (4 per cent of diet) to that of 8 other rats, and disodium calcium EDTA (4 per cent of diet) to the ration of the remaining 8 rats.

A third series of 30 rats (III) was fed stock ration with a larger amount of added powdered cholesterol (4 per cent of diet) and cholic acid (1 per cent of diet). The rats were divided into four groups. The basic ration was fed to 6 control rats while 8 rats in each of the three other groups ingested the same ration containing added salts of EDTA as in the preceding series of rats.

The daily food consumption of all rats was measured for 7 days. The rats of the first two series were bled after 14 days and those of the third series after 21 days. In each instance the plasma cholesterol was determined on an alcohol-acetone extract according to the method of Zlatkis, Zak, and Boyle (6).

<sup>1</sup> Aided by Grants from the American Heart Association and the San Francisco Heart Association.

<sup>2</sup> We wish to express our appreciation to Dr. Joseph A. Morrell of the R. J. Strassenburgh Company for supplying the EDTA salts used in this investigation.

## RESULTS

In Table I it can be seen that most rats ingesting diets with EDTA supplements exhibited moderate loss of weight. This could not be ascribed to diminished food intake since these rats each ingested from 16 to 21 grams of food per day, an amount essentially the same as that ingested by the control animals.

The ingestion of ration supplemented with cholesterol and cholic acid, as previously noted (4, 5), induced progressive chronic hypercholesteremia in the rats of Series II and III. As shown in Table I the magnitude of this hypercholesteremia was increased by the addition to the diet of each of the salts of EDTA, but was greatest in the animals fed disodium EDTA. In contrast, the rats ingesting ration containing disodium EDTA without added cholesterol or cholic acid (Series I) failed to exhibit any rise of plasma cholesterol.

## II. The Effect of Injected Disodium EDTA on Plasma Cholesterol of Rats Fed Excess Cholesterol

The following experiment was performed in order to determine if the above augmenting effect of *ingested* EDTA on the plasma cholesterol of rats fed excess cholesterol also occurred when EDTA was given parenterally and when cholic acid was omitted from the ration.

### Methods

A series of 24 rats was divided into three groups. Stock ration containing added powdered cholesterol (4 per cent of diet) was fed to all rats, but cholic acid was not added. One group of 8 rats (I) served as controls. Disodium EDTA (4 per cent of diet) was added to the ration of another group of 8 animals (III). The remaining 8 rats (II) ingested the cholesterol-supplemented ration and each rat was injected subcutaneously with a solution of 30 mg. of disodium EDTA daily (pH 7.25). For control purposes a final group of 8 rats (IV) was fed stock ration. After 14 days all rats were bled for determination of plasma cholesterol.

TABLE I  
Effect of ingested salts of EDTA on plasma cholesterol of rats fed excess cholesterol and cholic acid

| Series   | Dietary supplement          | No. of rats | Average weight   |                | Average plasma total cholesterol |                         |
|--|-----------------------------|-------------|------------------|----------------|----------------------------------|-------------------------|
|  |                             |             | Initial<br>(gm.) | Final<br>(gm.) | Day 14<br>(mg./100 ml.)          | Day 21<br>(mg./100 ml.) |
| I. Rats fed stock diet without added cholesterol or cholate    |                             |             |                  |                |                                  |                         |
|  | Control                     | 15          | 150              | 172            | 62<br>(48-73)*                   | —                       |
|  | Disodium<br>EDTA (4%)       | 8           | 155              | 134            | 57<br>(29-65)                    | —                       |
| II. Rats fed stock diet containing 2% cholesterol, 1% cholate  |                             |             |                  |                |                                  |                         |
|  | Control                     | 8           | 142              | 177            | 171<br>(71-305)                  | —                       |
|  | Disodium<br>EDTA (4%)       | 8           | 146              | 122            | 719<br>(486-880)                 | —                       |
|  | Tetrasodium<br>EDTA (4%)    | 8           | 153              | 124            | 286<br>(172-376)                 | —                       |
|  | Disod. calcium<br>EDTA (4%) | 8           | 158              | 178            | 211<br>(125-376)                 | —                       |
| III. Rats fed stock diet containing 4% cholesterol, 1% cholate |                             |             |                  |                |                                  |                         |
|  | Control                     | 6           | 150              | 166            | —                                | 319<br>(237-420)        |
|  | Disodium<br>EDTA (4%)       | 8           | 148              | 121            | —                                | 861<br>(377-1455)       |
|  | Tetrasodium<br>EDTA (4%)    | 8           | 154              | 131            | —                                | 669<br>(343-1165)       |
|  | Disod. calcium<br>EDTA (4%) | 8           | 172              | 152            | —                                | 598<br>(383-1160)       |

\* Range of values.

### Results

In Table II it can be seen that the addition of excess cholesterol alone to the diet (Group I) induced a slight rise of plasma cholesterol (average: 93 mg. per 100 ml.). A rise of plasma cholesterol of similar magnitude (average: 88 mg. per 100 ml.) occurred in the rats (Group II) fed the same ration and injected daily with disodium EDTA. In contrast, a significantly greater rise of plasma cholesterol (average: 190 mg. per 100 ml.) was induced by addition of disodium EDTA to the cholesterol-supplemented ration (Group III).

### III. The Effect of Disodium EDTA on Intestinal Absorption of Cholesterol

The previous demonstration (7) that exogenous cholesterol, after its intestinal absorption, is transported into the systemic circulation by the thoracic duct lymph provides a means of quantitating the

TABLE II  
Effect of injected disodium EDTA on plasma cholesterol of rats fed excess cholesterol

| Group  | Disodium EDTA supplement                                 | No. of rats | Average weight |             | Average plasma total cholesterol* (mg./100 ml.) |
|--|--|-------------|----------------|-------------|---|
|  |  |             | Initial (gm.)  | Final (gm.) |   |
| <i>Rats fed stock ration with added cholesterol (4%)</i> |  |             |                |             |   |
| I  | None   | 8           | 230            | 236         | 93<br>(81-107)†                                 |
| II   | Injection of disodium EDTA (30 mg. subcutaneously daily) | 8           | 229            | 226         | 88<br>(81-109)                                  |
| III  | Disodium EDTA added to diet (4%)                         | 8           | 215            | 204         | 190<br>(103-382)                                |
| <i>Rats fed stock ration</i>                             |  |             |                |             |   |
| IV   | None   | 8           | 250            | 270         | 60<br>(44-79)                                   |

\* Day 14.

† Range of values.

effect of the chelating agent upon intestinal absorption of cholesterol.

### Methods

As previously described (7) thoracic duct cannulation was performed in a series of rats following an overnight fast. The lymph was collected for 24 hours following the stomach feeding of 50 mg. of cholesterol dissolved in 1.5 ml. of olive oil. Twelve rats served as controls while 17 other rats received a similar stomach feeding but also containing 50 mg. of disodium EDTA. In each instance the cholesterol content of the lymph was then determined in the 24-hour sample of lymph.

### Results

In Table III it can be seen that the fraction of absorbed cholesterol was increased by feeding disodium EDTA with the test dose of cholesterol. Thus the control rats absorbed an average of 18.0 mg. of cholesterol in 24 hours while the rats also fed disodium EDTA absorbed an average of 26.0 mg. It is important to point out that an even greater difference may have been obtained if the EDTA would have dissolved in the oily solution of cholesterol.

#### IV. The Possible Effect of Disodium EDTA on Disappearance of Cholesterol from the Plasma

The previous studies indicated that the addition of salts of EDTA to the diet induced hypercholesteremia by augmenting the intestinal absorption of cholesterol. The following experiment was done to determine if the chelating agent also acted by impeding the usual disappearance of cholesterol from the plasma.

#### A. The possible effect of disodium EDTA on the disappearance of cholesterol from the plasma of dietary-induced hypercholesteremic rats

##### Methods

A series of 25 rats was fed stock ration containing added powdered cholesterol (2 per cent) and cholate (1 per cent). After 10 days all rats were bled for determination of plasma cholesterol and then were placed upon a fat-and-sterol-free diet. The rats were divided into 3 groups of which one group (I) of 6 rats served as controls, and a second group (II) of 11 rats was injected subcutaneously with 50 mg. of disodium EDTA daily. Disodium EDTA was added to the ration (5 per cent of diet) of the third group (III) of 8 rats. All rats were bled for determination of plasma cholesterol 7 days later.

##### Results

Table IV shows that hypercholesteremia again was induced by feeding excess cholesterol and cholate (4, 5). When the fat-and-sterol-free diet subsequently was fed for 7 days, the magnitude of the previously induced dietary hypercholesteremia progressively diminished. The rate of disappearance of the excess cholesterol from the plasma was not found to be diminished by the dietary or parenteral administration of disodium EDTA.

#### B. The possible effect of disodium EDTA on the disappearance of injected "physiological" cholesterol from the plasma

##### Methods

Hypercholesteremic rat plasma was prepared by ligating the bile ducts of a series of rats. For 3 days, each rat was starved except for a daily stomach feeding of 100 mg. of cholic acid. As previously found (8) such rats develop marked hypercholesteremia of endogenous

TABLE III  
*Effect of disodium EDTA on intestinal absorption of cholesterol in rats*

| Disodium EDTA supplement | No. of rats | Average weight (gm.) | Lymph (0 to 24 hours)* |                             |                             |
|--------------------------|-------------|----------------------|------------------------|-----------------------------|-----------------------------|
|                          |             |                      | Average volume (ml.)   | Average cholesterol         |                             |
|                          |             |                      |                        | Concentration (mg./100 ml.) | Content (mg./24 hr.)        |
| None                     | 12          | 249                  | 21.9<br>(15.0-33.0)†   | 84.0<br>(41.0-109.0)        | 18.0 ± 1.77‡<br>(12.5-30.0) |
| 50 mg.                   | 17          | 278                  | 28.0<br>(19.0-40.0)    | 94.0<br>(53.0-218.0)        | 26.0 ± 1.9<br>(13.0-48.0)   |

\* Following stomach feeding of 50 mg. cholesterol dissolved in 1.5 ml. olive oil.

† Range of values.

‡ Standard error of the mean.

TABLE IV  
*Effect of disodium EDTA on regression of previously induced\* dietary hypercholesteremia in rats*

| Group | Dietary regimen                         | Disodium EDTA injections    | No. of rats | Average weight |             | Average plasma total cholesterol |                     | Average decrease of plasma cholesterol (mg./100 ml.) |
|-------|---|-----------------------------|-------------|----------------|-------------|----------------------------------|---------------------|--|
|       |   |                             |             | Initial (gm.)  | Final (gm.) | Day 0 (mg./100 ml.)              | Day 7 (mg./100 ml.) |  |
| I     | Fat and sterol-free                     | None                        | 6           | 206            | 228         | 143<br>(109-201)†                | 94<br>(74-111)      | 49 ± 13.9‡   |
| II    | Fat and sterol-free                     | 50 mg. subcutaneously daily | 11          | 234            | 234         | 166<br>(100-285)                 | 112<br>(74-1637)    | 54 ± 10.8  |
| III   | Fat and sterol-free: disodium EDTA (5%) | None                        | 8           | 241            | 200         | 186<br>(132-342)                 | 110<br>(62-177)     | 76 ± 15.7  |

\* By feeding diet containing 2 per cent cholesterol and 1 per cent cholate for 10 days.

† Range of values.

‡ Standard error of the mean.

origin. The rats were bled, their plasma pooled, and the pooled plasma was found to contain 15 mg. of cholesterol per 3-ml. aliquot.

A series of 5 rats was subjected to cannulation of their lumbar veins with polyethylene tubes and then placed in restraining cages. Each rat was injected intravenously with 5 mg. of disodium EDTA and 3 ml. of the hypercholesteremic rat plasma containing 15 mg. of excess "physiological" cholesterol. Then, by means of a special apparatus, each rat received 6 ml. of a solution (pH 7.25) containing 50 mg. of disodium EDTA by constant intravenous infusion over an 18-hour period. All rats were bled for plasma cholesterol immediately after injection of the excess cholesterol load and again in 18 hours. Another series of 5 control rats was treated similarly except that 5 per cent glucose was used as the intravenous infusion fluid.

### Results

Immediately after injection of the excess cholesterol load, the experimental rats had an average plasma cholesterol of 201 mg. per 100 ml. (Range: 158 to 240) and the control rats averaged 192 mg. per 100 ml. (Range: 140 to 232). After 18 hours the average plasma cholesterol of the two groups were, respectively, 77 mg. per 100 ml. (Range: 72 to 82) and 76 mg. per 100 ml. (Range: 60 to 90). Thus the intravenous infusion of disodium EDTA was not found to impede the usual disappearance of the excess cholesterol from the plasma.

### C. The possible blocking effect of disodium EDTA on the egress of endogenously synthesized cholesterol from the plasma

### Methods

Previously it was shown that cholate (8, 9), Triton WR 1339 (10), hypothyroidism (11), and the induction

of nephrosis (12) in the rat are each capable of impeding the usual transfer of endogenously synthesized cholesterol from the plasma into the liver. In order to determine whether disodium EDTA also was capable of such "blocking" effects it was injected intravenously into hyperthyroid as well as control rats. The entrance of newly formed cholesterol into the plasma is markedly accelerated in the hyperthyroid state due to a considerably increased rate of hepatic synthesis of cholesterol (11). Thus the ability of any substance even to retard slightly the usual egress of newly synthesized cholesterol from the plasma would, of course, be more strikingly shown by administering it to animals with an accelerated turnover of plasma cholesterol such as occurs in hyperthyroid rats. A series of 6 rats was made severely hyperthyroid by adding powdered thyroid substance to their diet (0.3 per cent) for 3 weeks (11). A second series of 8 rats remained euthyroid. Disodium EDTA (30 mg.) was injected subcutaneously daily for 3 days into all rats and then 50 mg. of the chelating substance, contained in 7 ml. of solution, was given to each rat by continuous intravenous infusion over the ensuing 24-hour interval into a cannulated lumbar vein. At the end of this 24-hour interval all rats were bled for determination of plasma cholesterol.

### Results

The plasma cholesterol of the 8 euthyroid rats averaged 62 mg. per 100 ml. (Range: 48 to 73) and that of the 6 hyperthyroid animals averaged 59 mg. per 100 ml. (Range: 45 to 72). The failure of the plasma cholesterol to rise following the 24-hour intravenous injection of disodium EDTA in either group of rats indicates that the chelating agent does not prevent the usual egress of cholesterol from the plasma, even when the rate of synthesis of the plasma cholesterol is considerably increased (11).

### V. The Possible Effect of Disodium EDTA upon the Hepatic Deposition of Cholesterol

In order to determine if the chelating agent is capable of inducing hypercholesteremia by blocking the deposition of absorbed cholesterol into the liver, the following experiment was performed.

#### Methods

A series of 14 rats was fed stock ration containing added cholesterol (4 per cent) and cholate (1 per cent). Disodium EDTA (4 per cent) was added to the ration of 8 rats and 6 other rats served as controls. The animals were sacrificed after 21 days, at which time the concentrations of cholesterol in the plasma and liver were determined.

#### Results

In Table V it can be seen that the hypercholesteremia induced by feeding excess cholesterol and cholate again was considerably augmented by addition of disodium EDTA to the diet and again occurred in spite of the loss of weight induced by the chelating agent. The cholesterol concentrations of the livers of both groups of rats were markedly increased, the highest concentrations being observed in the rats whose ration contained the disodium EDTA. These rats exhibited a lower total liver cholesterol content due to their diminished liver weight which was associated with the loss of total body weight induced by the EDTA. It seems clear that the chelating agent did not impede the egress of cholesterol into the liver from the plasma.

### VI. The Effect of Disodium EDTA upon the Rate of Hepatic Synthesis of Cholesterol

The next experiments were performed in order to determine whether administration of the chelat-

ing agent was capable of significantly altering the rate of cholesterol synthesis by the liver. Two different techniques were used to study this problem.

#### A. Effect of disodium EDTA upon bile cholesterol content

##### Methods

As previously shown (13) the rate of excretion of biliary cholesterol provides an index of the rate of hepatic synthesis of cholesterol in the rat. The reasons underlying the validity of this technique have been fully discussed elsewhere (14, 15). This index fails to follow the rate of incorporation of  $C^{14}$  acetate with cholesterol in all cases. The discrepancy appears particularly great when cholesterol is fed (16). Fredrickson, Loud, Hinkelman, Schneider, and Frantz (17) also believed that a discrepancy existed between the rate of hepatic synthesis as suggested by the biliary index and that observed by their tracer measurements in the bile ligated rat. These authors so believed because they found the rate of hepatic synthesis of cholesterol to be increased as measured by tracer technics whereas the biliary index showed no such increase (8). However, our own later tracer measurements in this same condition (9) as well as those of Landon and Greenberg (18) agree with the biliary index in that they also found no increase in the rate of hepatic synthesis of cholesterol in bile ligated rats. These latter authors also found unequivocal evidence that the cholesterol present in bile was always newly synthesized as measured by tracer technics. Furthermore, after the period of active incorporation of  $C^{14}$  acetate into cholesterol had ceased, the cholesterol in bile was not radioactive although such labelled cholesterol was in abundance both in the blood and other tissues of the animal. All of this evidence, of course, suggests that the cholesterol found in bile is a portion or increment of cholesterol, newly synthesized in the liver. This in turn supports its usefulness as an index of the hepatic rate of cholesterol synthesis.

The bile ducts of 17 rats were cannulated and the 24-hour excretion of bile collected and analyzed for its

TABLE V  
Effect of disodium EDTA on hepatic cholesterol content of rats fed hypercholesteremic diet\* for 21 days

| Dietary regimen                   | No. of rats | Average weight |             | Average plasma total cholesterol (mg./100 ml.) | Average dry weight (gm.) | Liver   |                                   |
|-----------------------------------|-------------|----------------|-------------|--|--------------------------|---|-----------------------------------|
|                                   |             | Initial (gm.)  | Final (gm.) |  |                          | Average cholesterol Concentration (mg./100 gm.) | Average cholesterol Content (mg.) |
| Control*                          | 6           | 150            | 166         | 319<br>(237-420)†                              | 3.29<br>(3.12-3.58)      | 14,850<br>(14,050-16,400)                       | 488<br>(449-522)                  |
| Control*<br>disodium<br>EDTA (4%) | 8           | 148            | 121         | 861<br>(377-1455)                              | 2.20<br>(1.64-2.83)      | 18,070<br>(16,350-19,800)                       | 396<br>(287-463)                  |

\* Stock ration containing 4 per cent cholesterol, 1 per cent cholate.

† Range of values.

cholesterol content as previously described (13). Nine rats received a daily subcutaneous injection of 30 mg. of disodium EDTA for 3 days prior to collection of bile and during the 24-hour period of collection of bile each rat also received 7.2 ml. of solution (pH 7.25) containing 50 mg. of disodium EDTA by continuous intravenous injection into a cannulated lumbar vein. The remaining 8 rats served as controls and during the bile collection interval each rat received a continuous intravenous injection of 5 per cent glucose solution. Plasma cholesterol was determined in all rats at the end of the collection period.

### Results

As shown in Table VI, the subcutaneous and intravenous administration of disodium EDTA did not alter the plasma cholesterol levels. The concentrations of biliary cholesterol and the 24-hour biliary excretion of cholesterol also were not altered significantly by the administration of the chelating agent. Indeed the average daily excretion of cholesterol was of slightly lower magnitude in the rats treated with the chelating agent.

#### B. *The effect of disodium EDTA on the rise of plasma cholesterol in rats injected with Triton WR-1339*

The possible effect of the chelating agent on cholesterol synthesis by the liver also was studied by the following technique. Previously it was shown that the endogenous source of the plasma cholesterol is the liver (15) and that injection of a sufficient amount of Triton WR-1339 into the rat seriously impedes for at least 24 hours the usual egress from the plasma of any newly formed cholesterol following its discharge into the blood stream from the liver (10). Thus, the unique property of Triton to impede the normal rate of

egress of cholesterol from the plasma offers a means of estimating the amount of cholesterol discharged into the blood by the liver. This latter conclusion has been questioned by Frantz and Hinkelman (19) who found the rate of incorporation of tracer doses of  $C^{14}$  labelled acetate into cholesterol to be increased after administration of Triton. However, we note findings by Landon and Greenberg (18) opposed to those of Frantz and Hinkelman (19) concerning the rate of incorporation of  $C^{14}$  acetate into cholesterol in another condition, that of bile duct ligation in the rat. Furthermore, Frantz and Hinkelman (19) have cited findings obtained by others as well as themselves concerning the normal rate of synthesis of cholesterol by the rat which show this rate to be too low to account for the cholesterol rise in the blood after Triton administration. Other workers such as Hutchens, Van Bruggen, and West (20) and Landon and Greenberg (18) may be cited who found by similar technics more rapid normal rates of "synthesis" which are adequate to account for the blood cholesterol rise after Triton without the necessity of postulating large increases in synthesis rate. It also should be pointed out that there is agreement between the findings obtained in our laboratory (10) and that of Frantz and Hinkelman (19) concerning the quantitative amounts of cholesterol in plasma and liver before and after administration of Triton to rats. Both groups agree that no change occurs in the *total* amount of cholesterol present in the liver (or other tissues) while the amount of cholesterol present in the blood does increase substantially following Triton injection. The rise in blood cholesterol therefore affords a means for estimating the minimal net amount of cholesterol

TABLE VI  
*Effect of disodium EDTA upon biliary cholesterol excretion*

| Type of rat                 | No. of rats | Average weight (gm.) | Average plasma total cholesterol (mg./100 ml.) | Bile (0 to 24 hours) |                             |                        |
|-----------------------------|-------------|----------------------|--|----------------------|-----------------------------|------------------------|
|                             |             |                      |  | Average volume (ml.) | Average cholesterol         |                        |
|                             |             |                      |  |                      | Concentration (mg./100 ml.) | Content (mg./24 hours) |
| Control                     | 8           | 230                  | 62.0<br>(48-73)                                | 13.9<br>(10.8-18.1)† | 22.5<br>(10.5-30.0)         | 3.2<br>(1.4-5.4)       |
| Treated with disodium EDTA* | 9           | 216                  | 50.3<br>(49.0-59.0)                            | 13.0<br>(10.8-16.3)  | 23.3<br>(11.0-39.0)         | 2.7<br>(1.5-5.4)       |

\* See text.

† Range of values.

which was discharged into the blood during the period in which the rise occurred, and this is so regardless of the possibilities for free exchange of cholesterol molecules between plasma and liver shown by tracer studies. In other words, the finding of free exchange between liver and plasma cholesterol following Triton injection fails to explain the quantitative accumulation of cholesterol in the plasma which it induces and does not negate the conclusion that Triton blocks the normal metabolic removal of cholesterol. The actual amount of cholesterol discharged into the blood during the study period will be somewhat higher than indicated by the net rise in blood cholesterol because some is destroyed and excreted during the period of observation (10).

### Methods

Five of 10 rats (Av. wt.: 177 gm.) were injected subcutaneously with 30 mg. of disodium EDTA daily for 3 days and then each received through a lumbar vein cannula a continuous intravenous injection of 7 ml. of solution containing 60 mg. of the chelating agent (pH 7.25) during the ensuing 24-hour interval. At the same time 5 rats (Av. wt.: 181 gm.) served as controls, each receiving an injection of 7 ml. of 5 per cent glucose solution. Subsequent to cannulation of the lumbar vein each rat was injected intravenously with 100 mg. of Triton WR-1339 in a 10 per cent solution. All rats were bled for determination of plasma cholesterol 24 hours later.

### Results

The injection of Triton led to marked hypercholesteremia in the control rats, with an average plasma cholesterol concentration of 354 mg. per 100 ml. (Range: 285 to 428) (S.E. Mean:  $\pm 32.5$ ) after 24 hours. The rats which also received disodium EDTA exhibited an average plasma cholesterol of 393 mg. per 100 ml. (Range: 319 to 488) (S.E. Mean:  $\pm 34.6$ ). The difference between the average levels in the two groups does not appear to be significant.

### DISCUSSION

The preceding observations indicate that salts of EDTA are capable of augmenting to a considerable degree the otherwise slight hypercholesteremia which occurs in rats fed excess cholesterol. In the first experiment (Table I) it was found that the disodium salt of EDTA exerts a

much more striking effect in this regard than does the tetrasodium or disodium calcium salt of EDTA.

Previously it was shown that progressive chronic hypercholesteremia occurs in rats when cholesterol and cholic acid are added to the diet (4, 5), due to an increase in the fraction of absorbed cholesterol induced by cholate (9). Addition of disodium EDTA to a diet containing excess cholesterol (Table II) augmented the magnitude of the hypercholesteremia in a fashion similar to that of cholate. Much greater hypercholesteremia occurred in cholesterol-fed rats when cholate and the chelating agent were both added to the ration (Table I). On the other hand, hypercholesteremia was not induced by feeding disodium EDTA in the absence of excess dietary cholesterol (Table I) and the augmenting effect of ingested disodium EDTA upon the hypercholesteremia occurring in the cholesterol-fed rats was not similarly observed (Table II) when the chelating substance was given parenterally. This indicated that cholesterol absorption is increased by salts of EDTA, a conclusion which was substantiated by determining the effect of disodium EDTA upon the intestinal absorption of cholesterol (Table III).

These studies indicate that salts of EDTA markedly increase the hypercholesteremia which occurs in rats fed excess cholesterol, a propensity similar to that of cholic acid (5, 9). These chelating substances, like cholic acid, have surface active effects and are capable of increasing the detergency of surfactants. It seems possible that the effect of both substances upon cholesterol absorption may be related at least in part to their surface active properties. The difference in magnitude of the effects of the various salts of EDTA is probably due to differences in their pH, solubilities, and other properties.

It was of interest to determine if other phases of cholesterol metabolism also were altered by the EDTA salts in such a manner as to contribute to its hypercholesteremic effects. The effect of disodium EDTA upon the rate of hepatic synthesis of cholesterol, the primary source of the plasma cholesterol (15), was studied, the data indicating that the chelating agent did not alter significantly the rate of cholesterol synthesis by the liver. These results are contrary to the conclusion reached by Curran (3) who reported that the incorporation of labelled acetate into cholesterol

by small amounts of surviving liver obtained from two animals was increased by EDTA compared to his control value.

The effect of disodium EDTA upon the egress of cholesterol from the plasma also was studied. No evidence was found that the chelating agents block the usual transfer of cholesterol from the plasma. Thus it was shown that in the absence of excess dietary cholesterol the plasma cholesterol was not elevated either by oral (Table I) or subcutaneous (Table II) administration of disodium EDTA or by its intravenous infusion (Table VI), even when the rate of turnover of the plasma cholesterol was markedly increased by the presence of an hyperthyroid state (11). Furthermore, when hypercholesteremia was induced by dietary means, and subsequently allowed to regress by placing the animals on a fat-and-sterol-free diet, the rate of disappearance of the excess cholesterol from the plasma was not diminished either by oral or subcutaneous administration of disodium EDTA (Table IV). Moreover, it also was found that the chelating agent did not impede the usual disappearance from the plasma of an injected load of excess "physiological" cholesterol of endogenous origin. Finally, it was found that the disodium EDTA did not block the entrance of cholesterol from the plasma into the liver (Table V), also indicating that the hypercholesteremic effect of the chelating agent could not be ascribed to any "blocking" effect upon the usual egress of cholesterol from plasma into the liver. These results are not in agreement with the conclusions reached by Uhl, Brown, Zlatkis, Zak, Myers, and Boyle (2) who found that the subcutaneous administration of sodium EDTA augmented dietary-induced hypercholesteremia in rabbits in a fashion similar to that induced by its oral administration. However, the intake of food by their rabbits was not reported and the relatively small plasma cholesterol differences in their groups of rabbits were not subjected to statistical evaluation. These authors also observed that both oral and parenteral sodium EDTA protected against deposition of dietary-derived cholesterol in the rabbit's liver, an effect which we could not confirm in the rat. Our observations therefore do not substantiate their conclusion that the effect of the chelating agent upon the plasma cholesterol is mediated by interference

with the transfer of cholesterol from plasma into the liver.

#### CONCLUSION

The effects of various salts of EDTA upon cholesterol metabolism were studied in rats. It was found that the oral administration of these substances markedly increases dietary-induced hypercholesteremia by augmenting the intestinal absorption of cholesterol. The chelating agents were not found to alter the hepatic synthesis of cholesterol in rats or to interfere with the usual egress of cholesterol from the plasma or to block the deposition of cholesterol in the rat's liver.

#### ACKNOWLEDGMENT

We wish to thank Dr. Meyer Friedman and Dr. Sanford O. Byers for their helpful advice in these studies.

#### REFERENCES

1. Boyle, A. J., Uhl, H. S. M., and Brown, H. H., Effect of ethylenediamine tetraacetic acid on cholesterol metabolism in rabbits. *Am. J. Clin. Path.*, 1953, 23, 801.
2. Uhl, H. S. M., Brown, H. H., Zlatkis, A., Zak, B., Myers, G. B., and Boyle, A. J., Effect of ethylenediamine tetraacetic acid on cholesterol metabolism in rabbits: Preliminary report on effect of parenteral and oral administration of disodium and calcium salts. *Am. J. Clin. Path.*, 1953, 23, 1226.
3. Curran, G. L., Metal chelating agents and hepatic cholesterol synthesis. *Proc. Soc. Exper. Biol. & Med.*, 1955, 88, 101.
4. Page, I. H., and Brown, H. B., Induced hypercholesterolemia and atherogenesis. *Circulation*, 1952, 6, 681.
5. Rosenman, R. H., Byers, S. O., and Friedman, M., Role of cholate in dietary-induced hypercholesteremia of rats and rabbits. *Am. J. Physiol.*, 1953, 175, 307.
6. Zlatkis, A., Zak, B., and Boyle, A. J., A new method for the direct determination of serum cholesterol. *J. Lab. & Clin. Med.*, 1953, 41, 486.
7. Biggs, M. W., Friedman, M., and Byers, S. O., Intestinal lymphatic transport of absorbed cholesterol. *Proc. Soc. Exper. Biol. & Med.*, 1951, 78, 641.
8. Friedman, M., and Byers, S., Production and excretion of cholesterol in mammals. VI. Bile acid accumulation in production of hypercholesteremia occurring after biliary obstruction. *Am. J. Physiol.*, 1952, 168, 292.
9. Byers, S. O., Friedman, M., Biggs, M. W., and Gunning, B., Observations concerning the production and excretion of cholesterol in mammals. IX. The



- mechanism of the hypercholesteremic effect of cholic acid. *J. Exper. Med.*, 1953, **97**, 511.
10. Friedman, M., and Byers, S. O., The mechanism responsible for the hypercholesteremia induced by Triton WR-1339. *J. Exper. Med.*, 1953, **97**, 117.
  11. Rosenman, R. H., Byers, S. O., and Friedman, M., The mechanism responsible for the altered blood cholesterol content in deranged thyroid states. *J. Clin. Endocrinol. & Metab.*, 1952, **12**, 1287.
  12. Rosenman, R. H., Friedman, M., and Byers, S. O., The distribution of cholesterol and total lipids in the nephrotic rat. *J. Clin. Invest.*, 1955, **34**, 700.
  13. Byers, S. O., and Friedman, M., Production and excretion of cholesterol in mammals. VII. Biliary cholesterol: Increment and indicator of hepatic synthesis of cholesterol. *Am. J. Physiol.*, 1952, **168**, 297.
  14. Byers, S. O., Friedman, M., and Rosenman, R. H., Review: On the regulation of blood cholesterol. *Metabolism*, 1952, **1**, 479.
  15. Friedman, M., Rosenman, R. H., and Byers, S. O., Deranged cholesterol metabolism and its possible relationship to human atherosclerosis: A review. *J. Gerontol.*, 1955, **10**, 60.
  16. Gould, R. G., Lipid metabolism and atherosclerosis. *Am. J. Med.*, 1951, **11**, 209.
  17. Fredrickson, D. S., Loud, A. V., Hinkelman, B. T., Schneider, H. S., and Frantz, I. D., Jr., The effect of ligation of the common bile duct on cholesterol synthesis in the rat. *J. Exper. Med.*, 1954, **99**, 43.
  18. Landon, E. J., and Greenberg, D. M., Endogenous cholesterol metabolism in the rat studied with C<sup>14</sup>-labeled acetate. *J. Biol. Chem.*, 1954, **209**, 493.
  19. Frantz, I. D., Jr., and Hinkelman, B. T., Acceleration of hepatic cholesterol synthesis by Triton WR-1339. *J. Exper. Med.*, 1955, **101**, 225.
  20. Hutchens, T. T., Van Bruggen, J. T., and West, E. S., Fatty acid and cholesterol synthesis rates in the intact rat. *Arch. Biochem. & Biophysics*, 1954, **52**, 261.