# DIETARY FAT AND HYPERCHOLESTEREMIA IN THE CEBUS MONKEY

## II. Esterification and Disappearance of Cholesterol-4-$C^{14}$*, ‡

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(Received for publication, June 24, 1957)

It was shown in a previous study (1) that the type of dietary fat has a striking influence on the serum levels of cholesterol and $\beta$-lipoproteins in the *Cebus* monkey fed 0.1 gm. of cholesterol per 100 calories of diet. This effect was observed regardless of whether the dietary protein was alpha protein or casein. Similar effects had been previously observed in numerous studies of man (*e.g.*, references 2, 3) and of animals (*e.g.*, references 4, 5).

There is, however, no conclusive evidence as to the active factors in fats that produce such large and rapid changes in the serum cholesterol levels. Most attention has been paid to various characteristics of the fatty acids. There is some direct (6) and a great deal of indirect evidence of a relationship between polyunsaturated fatty acids and cholesterol metabolism. It has furthermore been clearly shown in rodents, both by nutritional studies (7) and by isotopic methods (8), that linoleic and linolenic acids are not synthesized *in vivo*, although saturation and desaturation of single double bonded and saturated fatty acids respectively have been repeatedly proved (9, 10).

Several investigators (11) have advanced the hypothesis that different cholesterol esters are metabolized at different rates and that cholesterol esters of "non-essential" fatty acids may persist longer. Proof of this theory will require much more information about the fatty acid composition of sterol esters and about rates of turnover and exchange of such fatty acids (12, 13). The present information (13) indicates that the fatty acids esterified with cholesterol in the serum of man are composed of a high percentage of linoleic acid—apparently higher than that of any major lipide class. Some suggestive evidence that the type of dietary fat may influence the fatty acid composition of sterol esters exists (14).

Hellman *et al.* (15) have studied the turnover and esterification of endogenous and exogenous labelled cholesterol in humans and have ably summarized the present state

* Supported in part by grants-in-aid from the National Heart Institute (Nos. 136 and 2200), National Institutes of Health, Bethesda; Life Insurance Medical Research Fund, New York; Albert and Mary Lasker Foundation, New York; Nutrition Foundation, Inc., New York; John A. Hartford Memorial Fund and the Fund for Research and Teaching, Harvard School of Public Health.

‡ A preliminary report of this material was presented at the meetings of the Federation of American Societies for Experimental Biology, Chicago, April, 1957.

of knowledge concerning cholesterol esterification and absorption; *i. e.*, cholesterol is esterified (by classical conception in the gut lumen (16) ) before absorption; however, little is known with regard to the turnover of fatty acids of the sterol esters after absorption. Clinical data (17), as well as results with experimental animals, indicate that the maintenance of cholesterol–cholesterol ester ratios is an hepatocellular function. Thus the concept of a fairly rapid exchange of sterol fatty acids is suggested but not proved. The work of Lipsky *et al.* (13), however, would indicate that endogenous fatty acids esterified with sterols turn over slowly in the serum of man.

The present work had the following objectives: (*a*) a study of the rate of esterification and of disappearance of cholesterol-4-$C^{14}$ injected as an intravenous emulsion into normal and dietary hypercholesteremic *Cebus* monkeys, (*b*) a study of rates of disappearance and deesterification of cholesterol-4-$C^{14}$-stearate, (*c*) a measure of the long term effect of feeding different fats on the concentration of free and total cholesterol, lipide phosphorus, and total lipide of various tissues, and (*d*) a study in *Cebus* monkeys of the effect of small amounts of different fats and fatty acids used as vehicles for the intragastric administration of cholesterol-4-$C^{14}$ on the absorption and disappearance rates of cholesterol radioactivity from the sera.

## EXPERIMENTAL

A total of 22 *Cebus* monkeys weighing between 1200 and 1600 gm. were used in the several phases of this study. They were of both sexes but within individual experiments the proportions of each sex for experimental groups were kept constant. The animals were fed and housed as previously described (18, 1). Fat supplied 45 per cent of the dietary calories, and in some instances cholesterol was included at the rate of 0.1 gm./100 calories of diet.

*Radioactive Compounds.*—Cholesterol-4-$C^{14}$ [1] had a specific activity of 16.5 $\mu$c./mg., a melting point of 147–149°C., and an infrared absorption curve identical with that of authentic cholesterol. Cholesteryl-4-$C^{14}$-stearate[1] (19) had a specific activity of 9.77 $\mu$c./mg. (identical with the free cholesterol–specific activity on a molar basis), a melting point of 78.5–80°C., and an infrared absorption curve showing no hydroxyl bands (3700 to 3500 $cm.^{-1}$) and no carboxylic acid OH stretching vibrations 2700 to 2500 $cm.^{-1}$). When cholesteryl-4-$C^{14}$-stearate was added to free unlabelled cholesterol and free and total cholesterol were isolated and radioassayed (see below), it was shown that approximately 8 per cent of the total radioactivity was in the free fraction. When the labelled ester was added to a silicic acid column (20) with carrier cholesterol stearate and cholesterol, 95.7 per cent of the radioactivity was eluted with the cholesterol ester fraction.

*Method of Radioassay of Cholesterol-4-$C^{14}$.*—The specific activity of radiocholesterol in sera was determined using classical techniques. Free and total cholesterol were determined by the method of Sperry and Webb (21). Free and total cholesterol for radioassay were isolated by filtering cholesterol digitonide onto a filter paper disk, washing with 80 per cent ethanol, acetone, and ether, and counting to a mean error of $\pm 2$ per cent. Approximately 0.5 mg. of cholesterol was radioassayed for each sample, a quantity in the range of linearity for the sample geometry and counting device (G-M thin window tube) used.

*Preparation of Intravenous Emulsions of Radiocompounds.*—Individual ampules of radio-

[1] $C^{14}$-labelled compounds were obtained from the New England Nuclear Corp., Boston.

compounds for injection were made by adding 0.2 ml. of ether to dried compound (quantities equivalent to 5 μc.). One drop of tween 81[2] and 1.0 ml. of water were added and the ampule was closed and shaken for 10 minutes on a mechanical shaker. The ether was removed by warming in a water bath at 40°C. The ampule was then shaken again for 10 minutes.

*Preparation of Small Formula Meals Containing Radiocholesterol.*—Formula meals of the composition outlined by Ahrens *et al.* (22) and containing radiocholesterol were used in one experiment. Cholesterol-4-$C^{14}$ (0.66 mg.–10 μc.) was dissolved in 1.09 gm. of a selected fat or fatty acid which had been sufficiently warmed to become fluid. This oil was added to a mixture of 1.87 gm. lesofac, 2.07 gm. dextrose, and 15 ml. of water which had been previously mixed, and the oil was thoroughly suspended with a blender. Small portions were removed for radioassay and the remainders were administered to monkeys by gastric intubation.

*Fatty Acid Composition of Dietary Fats.*—Approximate fatty acid composition of the dietary fats used in these experiments were determined by calculation from the iodine value and thiocyanogen number (23). The calculated compositions in which only saturated acids, oleic, and linoleic acids are present in significant quantities have agreed well with the range of reported values based on more precise methods. Some values were corn oil: saturated—10.8, oleic—35.7; linoleic—53.5; lard: saturated—41.9, oleic—46.9, linoleic—11.2; safflower: saturated—6.8, oleic—19.3, linoleic—73.9; and coconut: saturated—92.5, oleic—5.6, linoleic—1.1.

*Extraction and Measurement of Lipide Components from Tissues.*—Tissues (liver, spleen, gastrocnemius, stomach and duodenum, kidneys, adrenals, thoracic aorta, and serum) were taken immediately after induction of anesthesia with intravenous sodium amytal and opening of the thoracic cavity. Organs were weighed, minced, and extracted with hot 95 per cent ethanol. The minced tissue was then continuously extracted (48 hours) with chloroform. The alcohol and chloroform extracts were then taken to dryness, and the residues were extracted several times with hot petroleum ether (b.p.— 60–70° C.). Aliquots of the petroleum ether were taken for the several determinations. Free and total cholesterol were determined by the method of Sperry and Webb (21), lipide phosphorus by the method of King (24), and total lipids by a gravimetric method similar to that of Sperry for brain (25). Routine serum total cholesterol determinations were performed by the method of Abell *et al.* (26).

## RESULTS

### *Effect of the Type of Dietary Fat on the Disappearance and Esterification of Intravenous Cholesterol-4-$C^{14}$.*—

Nine monkeys were used in the first experiment; 3 were fed diets including 45 per cent of the calories as corn oil without cholesterol, 3 were fed corn oil and 0.1 gm. cholesterol/100 calories of diet, and 3 were fed lard and sufficient additional cholesterol to give a concentration of 0.1 gm./100 calories of diet. At the end of 5 months when mean serum cholesterol values were 237, 268, and 601 mg. per cent respectively, each monkey was injected with an emulsion of cholesterol-4-$C^{14}$ as described above, and the disappearance of radiocholesterol from the sera was studied. The time-disappearance curves of serum radiocholesterol for these monkeys are indicated in Fig. 1.

It can be seen that the specific activities of radiocholesterol do not form any regular pattern as time passes during the first 3 days after injection, and that the maximum values are not obtained during the 1st day. This latter phenom-

[2] Tween 81 is polyoxyethylene sorbitan monooleate, Atlas Corp., Wilmington.

enon has been seen previously by others (27) and is undoubtedly related to the unphysiologic nature of the dispersion and resulting phagocytosis of the injected material. After the 3rd day the specific activities seemed to be reduced exponentially with time. When the biological half-lives were calculated (Fig. 1), it was seen that there was relatively little difference between the figures for

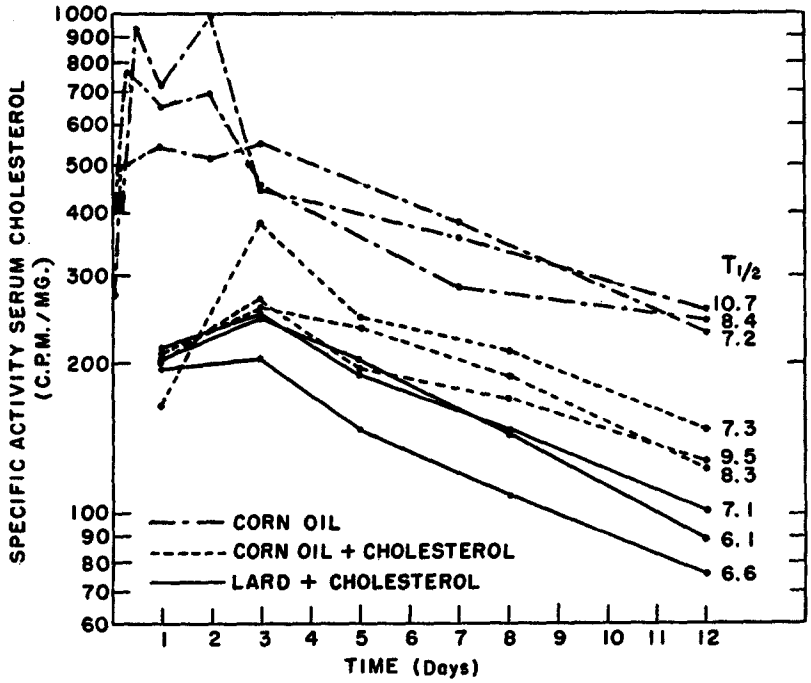


FIG. 1. Disappearance of a trace dose of cholesterol-4-$C^{14}$ (0.33 mg. to 5 $\mu$c.), injected as an intravenous emulsion, from the sera of *Cebus* monkeys. Monkeys had been fed diets containing 45 per cent of calories as fat for 5 months. One group was fed corn oil without cholesterol, one corn oil with cholesterol (0.1 gm./100 calories of diet), and a third lard with cholesterol; mean serum cholesterol values were 237, 268, and 601 mg. per cent respectively.

individual animals or groups of animals. Mean values for half-lives of serum radiocholesterol were 8.8 days for the corn oil group (mean cholesterol, 237 mg. per cent), 8.4 days for the corn oil–cholesterol group (mean cholesterol, 268 mg. per cent), and 6.6 days for the lard-cholesterol group (mean cholesterol, 601 mg. per cent). The monkeys with the higher serum cholesterol levels had somewhat accelerated rates of removal of serum radiocholesterol. The different absolute values for serum-specific activities suggested that the sizes of the pools in which the labelled cholesterol was diluted were consistent in relative size with the differences in measured values for serum cholesterol.

The rates of esterification of the injected radiocholesterol are portrayed, for the group of monkeys fed diets containing corn oil without cholesterol, in Fig. 2. The ratio of free cholesterol-specific activity to total cholesterol-specific activity is plotted against time. Thus immediately after administration of the trace dose this ratio was around 4.0, and then dropped progressively to a value of around 1.0 (free cholesterol-specific activity equal to total or ester-specific

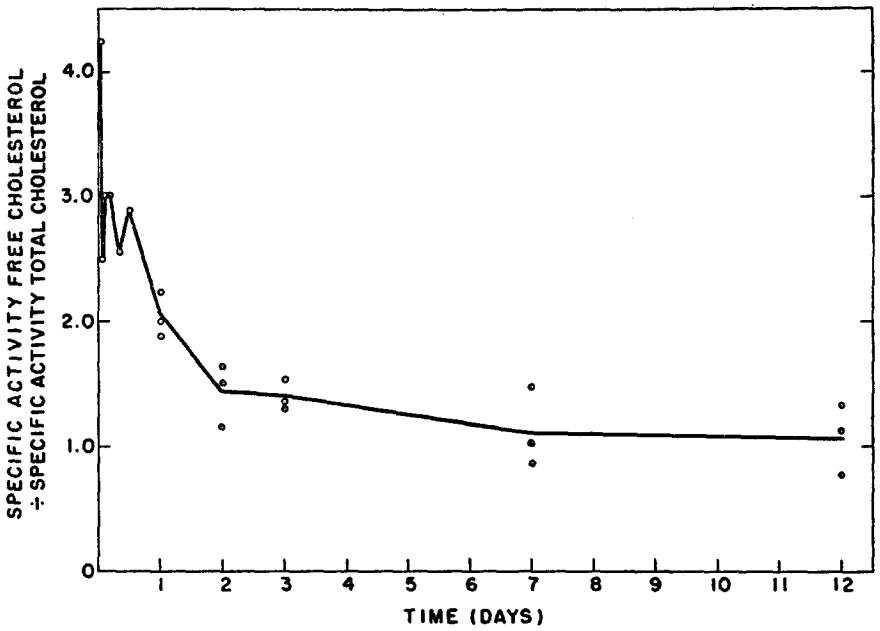


FIG. 2. The esterification of trace doses of cholesterol-4-$C^{14}$ injected as intravenous emulsions into 3 *Cebus* monkeys fed diets containing 45 per cent of calories as corn oil. The ratio of specific activity of free cholesterol to specific activity of total cholesterol is plotted as the ordinate.

activity). Fig. 3 shows similar values at 6 hours, 1 day, 2 days, and 3 days for *Cebus* monkeys fed diets including corn oil or lard and cholesterol. The ratio of specific activities of free and total serum cholesterol for monkeys fed corn oil and cholesterol had reached values slightly below 1.0 in 1 day, whereas all of serum-free: total cholesterol-specific activities for animals fed lard-containing diets were still well above 1.0 at 3 days. In spite of this apparent difference in esterification rates for the 2 groups shown in Fig. 3 the mean absolute free: total ratios of serum cholesterol were not different for the two groups of monkeys. It is not clear whether the lengthened time for equilibration of serum-free and total cholesterol-specific activity in the monkeys fed lard-

containing diets is causally related to the greatly elevated serum cholesterol values or whether it is a secondary reflection of the increased size of the cholesterol pool and load on the esterification system.

*Disappearance and Deesterification of Cholesterol-4-$C^{14}$-stearate.*—

Three monkeys were injected intravenously with an emulsion of cholesterol-4-$C^{14}$-stearate (0.51 mg. to 5.0 $\mu$c.). The rate of disappearance of activity from the serum total cholesterol fraction is indicated in Fig. 4.

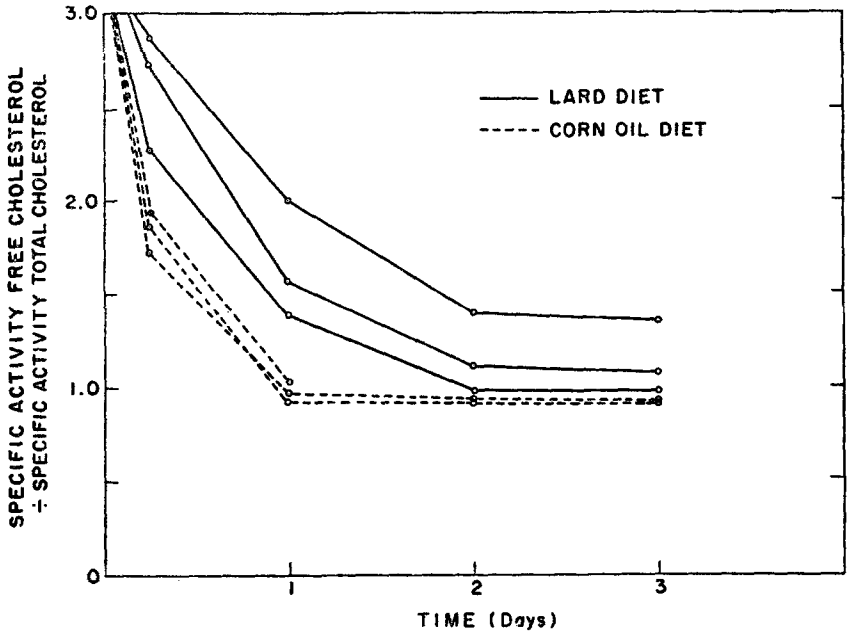


FIG. 3. The esterification of trace doses of cholesterol-4-$C^{14}$ injected as intravenous emulsions into *Cebus* monkeys fed diets containing 45 per cent of calories as corn oil or lard. Cholesterol was fed at 0.1 gm./100 calories of diet.

The log curves of specific activity (Fig. 4) do not appear to be linear at any phase of the experiment. The ratios of free to total serum cholesterol–specific activity for the three monkeys is indicated in Fig. 5. The specific activity of the total cholesterol was greater than that of the free only until 30 minutes after injection. From 1 hour until 7 days post injection the specific activity of serum-free cholesterol was greater than that of total cholesterol–specific activity. At 14 and 21 days the specific activity of free cholesterol was again greater than the total specific activity. It appears from this experiment and a comparison with the previous experiment using free radiocholesterol that the injected ester is largely deposited in tissues and released to the serum after deesterification.

*Effect of Long Term Feeding of Lard and Corn oil on Tissue Lipide Composition of Cebus Monkeys.*—

Six monkeys, 3 of which were fed diets containing corn oil with added cholesterol (0.1 gm./100 calories) and 3 of which were fed lard with added cholesterol for 7 months were sacrificed and lipide analyses were performed on various tissues. (This is the same series of animals used in the first experiment. Serum cholesterol levels of the lard group were 2½ times those of the corn oil group).

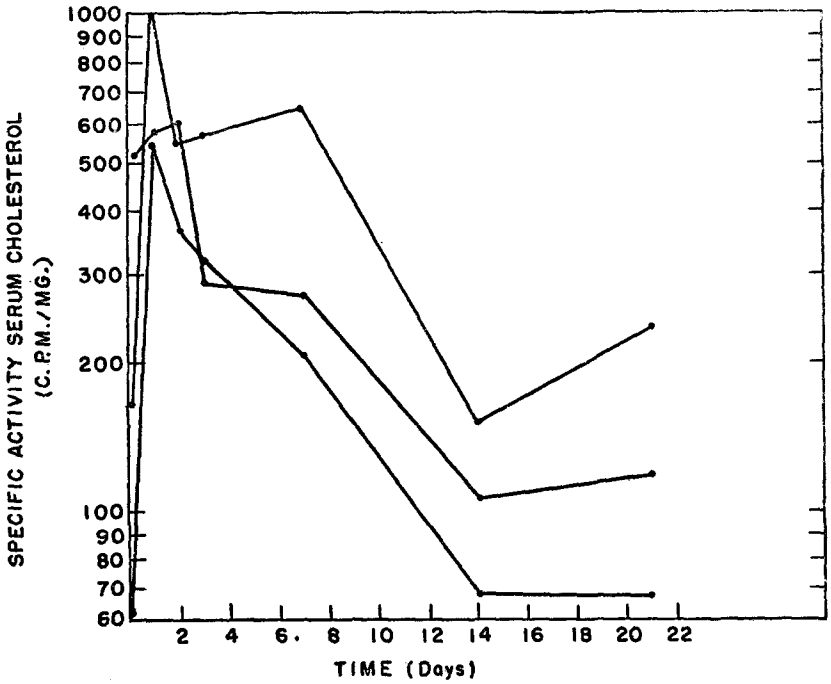


FIG. 4. Disappearance of digitonin-precipitable radioactivity from the sera of *Cebus* monkeys after the intravenous administration of emulsions of cholesterol-4-$C^{14}$-stearate (0.51 mg. to 5.0 μc.) into 3 *Cebus* monkeys. The animals had been fed diets containing 45 per cent of calories as corn oil and without cholesterol.

The results of this experiment are indicated in Table I. Few significant differences between the analyses of tissues of the monkeys fed the two diets were observed despite the prolonged differences observed in sera. The total lipide content (on a percentage basis) of adrenals from the monkeys fed diets based on corn oil were consistently double those found in monkeys fed diets based on lard. The average size of the adrenals from the corn oil group were also larger; therefore, the preponderance of absolute total lipide levels was even greater. It can also be seen that tissue lipides were not strikingly elevated above values for normal monkeys (fed low fat, cholesterol-free diets (18)). For

example, the highest value for total cholesterol of the liver was 460 mg. per cent and for total lipides of the liver, 6.5 per cent. The tissues of these monkeys showed no evidence of abnormal liposis by chemical analysis, gross appearance, or histologic examination. Although no difference between the two dietary groups was observed in the cholesterol analysis of lower thoracic aorta, a number of visible, shiny plaques were seen in the gross in the aortic arches of all three monkeys of the lard group and in the aorta of only one monkey in the corn oil group. The most diffuse involvement occurred, however, in this single

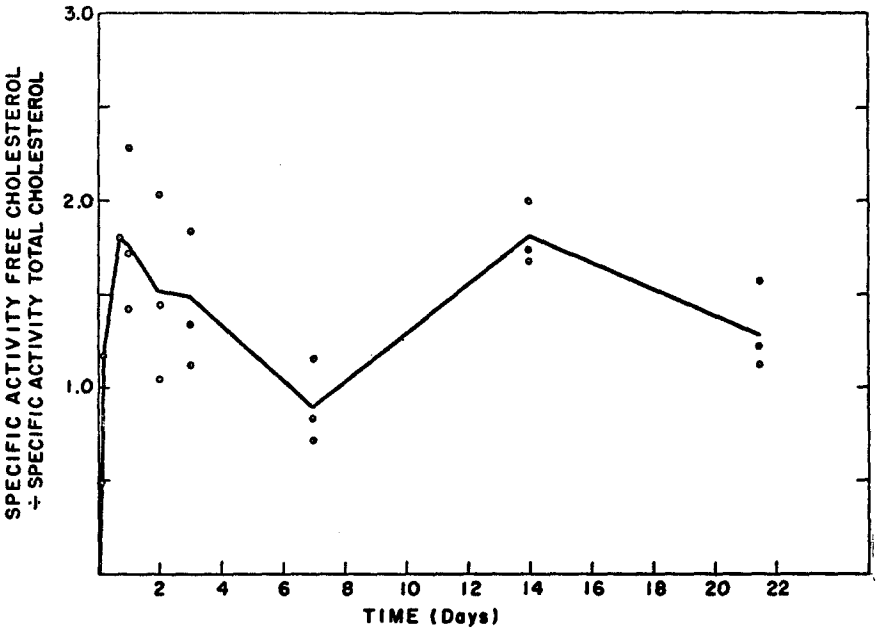


FIG. 5. Deesterification of a trace dose of cholesterol-4-$C^{14}$-stearate injected as an intravenous emulsion into 3 *Cebus* monkeys. The ratio of the specific activity of serum-free cholesterol to specific activity of total cholesterol is plotted as the ordinate.

monkey. A detailed histological study will appear later as part of another study.

*Effect of Fat or Fatty Acid Vehicle on the Absorption and Rate of Disappearance of Intragastrically Administered Cholesterol-4-$C^{14}$.*—

Ten *Cebus* monkeys which had been maintained on the same control diet (cholesterol-free; hydrogenated cottonseed oil supplied 45 per cent of calories; mean serum cholesterol was 240 mg. per cent) for 1 month were fasted for 24 hours, then given by intragastric tube a small formula meal including 1.07 gm. of fat or fatty acid in which cholesterol-4-$C^{14}$ (0.66 mg.–10 $\mu$c.) had been dissolved. The animals were fasted for another 24 hours and then returned to the original control diet.

Figs. 6 and 7 portray the rates of disappearance of specific activities of serum total cholesterol for the fat and fatty acid vehicles respectively. Within 12

hours after administration of the radiocholesterol specific activity of serum total cholesterol was slightly greater than specific activity of free cholesterol and remained so throughout the rest of the experiment. The maximum specific

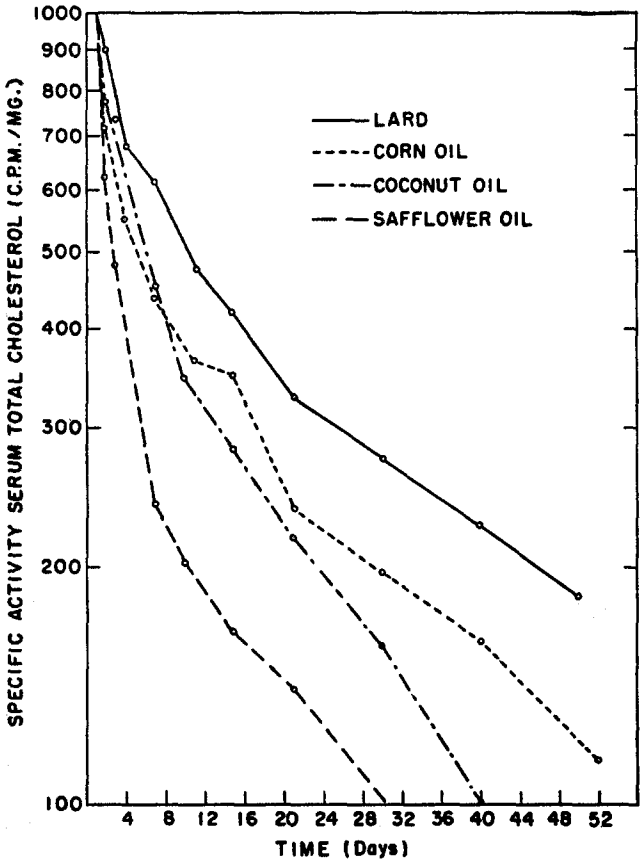


FIG. 6. Effect of the fat vehicle on disappearance of intragastrically administered cholesterol-4-$C^{14}$ from the serum total cholesterol of 4 male *Cebus* monkeys fed identical basal diets. Monkeys were fasted for 24 hours before and after intragastric administration of a test formula meal (22) containing 1.07 gm. of fat and 0.66 mg. (10 $\mu$c.) of radiocholesterol. The 1 day values were normalized to 1000 and the other values were correspondingly adjusted.

activities for serum total cholesterol of all animals were normalized to 1000 at 1 day postintubation for better portrayal and comparison of the different disappearance curves. Two distinct exponential components (Fig. 6) existed for the monkeys given different fats as vehicles for administration of radiocholesterol, an initial rapid phase (half-life, 1.9 to 6.6 days) followed by a slower secondary phase (half-life, 17–34 days). There was considerable variation in the values of the half-lives, perhaps depending on the type of fatty vehicle used. The choles-

TABLE I

*Analyses of Tissues of Cebus Monkeys Sacrificed after 7 Months on Diets Containing Corn Oil and Lard with Added Cholesterol (0.1 Gm. per 100 Calories of Diet)*

Monkeys 1-10, 1-24 and 1-26 received diets containing lard; monkeys 1-23, 1-25 and 1-27 received diets containing corn oil. Values are on a wet weight basis.

| Tissue | Animal No. | Tissue cholesterol | | Lipide P | Total lipide |
|---|---|---|---|---|---|
| | | Free | Total | | |
| | | *mg. per cent* | *mg. per cent* | *mg. per cent* | *per cent* |
| Liver | 1-10 | 180 | 414 | 86 | 6.5 |
| | 1-24 | 325 | 347 | 81 | 6.1 |
| | 1-26 | 230 | 460 | 96 | 5.1 |
| | 1-23 | 205 | 435 | 92 | 6.5 |
| | 1-25 | 158 | 208 | 63 | 6.0 |
| | 1-27 | 264 | 411 | 87 | 6.2 |
| Adrenals | 1-10 | 417 | 1030 | 81 | 12.4 |
| | 1-24 | 770 | 1290 | 163 | 16.9 |
| | 1-26 | 457 | 486 | 83 | 15.4 |
| | 1-23 | — | — | 280 | 32.0 |
| | 1-25 | 435 | 664 | 107 | 30.4 |
| | 1-27 | 385 | 748 | 98 | 30.3 |
| Duodenum-stomach | 1-10 | 100 | 113 | 41 | 7.6 |
| | 1-24 | 148 | 155 | 42 | 7.5 |
| | 1-26 | 142 | 259 | 58 | 9.1 |
| | 1-23 | 108 | 108 | 77 | 13.9 |
| | 1-25 | 68 | 161 | 76 | 5.9 |
| | 1-27 | 186 | 246 | 64 | 10.3 |
| Spleen | 1-10 | 289 | 420 | 71 | 4.8 |
| | 1-24 | 240 | 320 | 34 | 2.1 |
| | 1-26 | 140 | 457 | 65 | 2.9 |
| | 1-23 | 182 | 438 | 65 | 6.8 |
| | 1-25 | 171 | 253 | 68 | 2.0 |
| | 1-27 | 426 | 437 | 70 | 3.7 |
| Gastrocnemius | 1-10 | 100 | 113 | 34 | 2.2 |
| | 1-24 | 102 | 170 | 42 | 1.8 |
| | 1-26 | 48 | 62 | 22 | 2.4 |
| | 1-23 | — | 62 | 23 | 6.3 |
| | 1-25 | 50 | 75 | 50 | 2.2 |
| | 1-27 | 82 | 97 | 37 | 2.8 |

| Tissue | Animal No. | Tissue cholesterol | | Lipide P | Total Lipide | | | |
|---|---|---|---|---|---|---|---|---|
| | | Free | Total | | | | | |
| | | *mg. per cent* | *mg. per cent* | *mg. per cent* | *per cent* | | | |
| Kidneys | 1-10 | 383 | 400 | 85 | 5.7 | | | |
| | 1-24 | 225 | 279 | 44 | 5.1 | | | |
| | 1-26 | 259 | 339 | 57 | 4.6 | | | |
| | 1-23 | 182 | 203 | 72 | 8.3 | | | |
| | 1-25 | 219 | 276 | 75 | 4.2 | | | |
| | 1-27 | 360 | 420 | 89 | 4.6 | | | |
| Aorta | 1-10 | | 203 | | | | | |
| | 1-26 | | 233 | | | | | |
| | 1-25 | | 219 | | | | | |
| | 1-27 | | 214 | | | | | |
| | | | | | β-Lipoproteins | | | |
| | | | | | $S_f$0-11 | 12-20 | 20-35 | 35-100 |
| | | | | | *mg. per cent* | | | |
| Serum (terminal) | 1-10 | 115 | 462 | 10.0 | 412 | 100 | 8 | 0 |
| | 1-24 | 157 | 665 | 10.0 | 412 | 214 | 24 | 2 |
| | 1-26 | 184 | 679 | 16.2 | 456 | 334 | 34 | 2 |
| | 1-23 | — | 292 | — | — | — | — | — |
| | 1-25 | 51 | 286 | 7.5 | 142 | 96 | 15 | 2 |
| | 1-27 | 66 | 274 | 7.6 | 158 | 60 | 16 | 1 |

terol-4-$C^{14}$ disappearance curves (Fig. 7) for the 2 monkeys fed stearic acid meals and the 2 monkeys fed linoleic acid were not apparently different. However, the disappearance of radiocholesterol administered with oleic acid appeared to be slower in the latter phase and seemed to resemble the patterns observed with the natural fats except safflower oil.

Although it is clear that the maximum reading for cholesterol-specific activity in serum is not a certain index of the amount of cholesterol-4-$C^{14}$ absorbed, a comparison of these maxima for the various monkeys might give some indication of the relative value of the fats in promoting cholesterol absorption. Table II gives a listing of the monkeys with the relative size of the maximum specific activity divided by the number of counts intubated per unit of body weight. The highest figure obtained for any monkey was set equal to 1.00 with the remaining figures as fractions of 1. Thus the greatest apparent absorption was in the monkey administered cholesterol-4-$C^{14}$ with lard, the least absorption was in the two monkeys given stearic acid.

## DISCUSSION

The above experiments indicate that dietary hypercholesteremia in *Cebus* monkeys resembles essential hypercholesteremia in man in several important

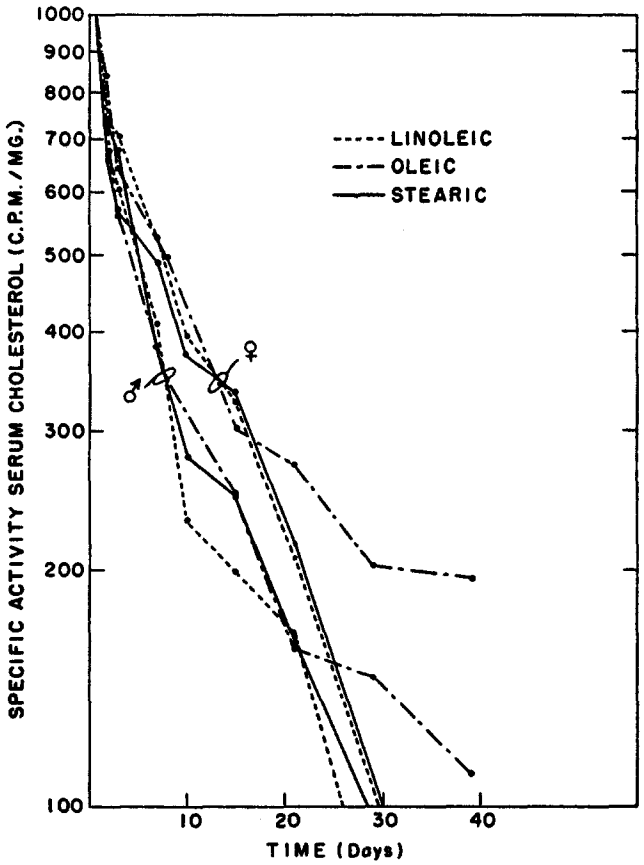


FIG. 7. Effect of the fatty acid vehicle on disappearance of intragastrically administered cholesterol-4-$C^{14}$ from the serum total cholesterol of *Cebus* monkeys. Monkeys were fasted for 24 hours before and after intragastric administration of a test formula meal containing 1.07 gm. of fatty acid and 0.66 mg. (10 $\mu$c.) radiocholesterol. The 1 day values were normalized to 1000 C.P.M./mg. cholesterol, and the other values were correspondingly adjusted.

respects: (*a*) serum cholesterols of 600 mg. per cent and greater are maintained for many months without the evidence of tissue liposis so characteristic of experiments with rodents (28), (*b*) the delayed rate of esterification of injected radiocholesterol in dietary hypercholesteremic monkeys is analogous to the finding of Hellman *et al.* (15) with endogenous radiocholesterol in patients with xanthoma tuberosum, and (*c*) the disappearance curves of labelled exogenous cholesterol (administered by gastric tube) in certain of the *Cebus* monkeys

were qualitatively and quantitatively very similar to those observed by Hellman *et al.* (15) in man.

It seems that the effects of different dietary fats on serum cholesterol levels of *Cebus* monkeys are not due to a difference in partition of cholesterol between serum and other tissues. For example, in monkeys fed diets containing lard and cholesterol, serum cholesterols were about 2½ times as high as those in monkeys fed corn oil and cholesterol, although analyses of other tissues were essentially indistinguishable.

On the other hand, the data presented indicate that there is a considerable

TABLE II

*Relative Efficiency in Cebus Monkeys of Various Fats and Fatty Acids in Promotion of Absorption of Cholesterol-4-C$^{14}$ Fed as a Single Test Meal*

Specific activity of serum total cholesterol at 24 hours postadministration divided by the number of counts administered per kilogram of body weight was taken as a measure of cholesterol absorption. The maximum figure thus obtained for any monkey was set equal to 1.00, and other values were set equal to a relative fraction of 1.00.

| Fat or fatty acid tested | Efficiency absorption cholesterol-4-C$^{14}$ |
|---|---|
| Lard | 1.00 |
| Safflower | 0.94 |
| Corn | 0.56 |
| Coconut | 0.44 |
| Oleic | 0.66 |
| | 0.79 |
| Linoleic | 0.47 |
| | 0.17 |
| Stearic | 0.055 |
| | 0.049 |

difference in the absorption and turnover of exogenous radiocholesterol depending on the fat or fatty acid vehicle used for administration. Our tests would indicate that the cholesterol esters of linoleic and oleic are well absorbed but that cholesterol stearate is poorly absorbed. It has been recognized that the long chain saturated fatty acid, stearic acid, is poorly absorbed. This does not appear to be associated with any defect in esterification with cholesterol but rather with a defect in absorption of cholesterol stearate (29). When radiocholesterol was fed with the test dose of coconut oil, whose principle fatty acid is lauric, it was well absorbed. There was, perhaps, a delayed elimination of radiocholesterol from the sera when oleic acid, as compared to linoleic or stearic, was the vehicle for intragastric administration. This was interpreted as a greater persistence of cholesterol oleate formed at the time of absorption, since the dose of oleic acid was too small to influence the metabolism of radiocholesterol after absorption.

Although only a relatively few fats have been previously evaluated in our laboratory for their effect on serum cholesterols in the *Cebus* monkey, it appears that the property characteristic of our hypocholesteremic fats is a high content of linoleic acid; the properties characteristic of the hypercholesteremic fats are a relatively low content of linoleic acid and a high content of oleic acid. The highest serum cholesterol levels yet obtained in the *Cebus* monkey were seen when 45 per cent of calories were supplied as triolein.[3] Hegsted and colleagues (30) had observed that triolein was one of the more potent of a large number of fats and combinations of fats tested in hypercholesteremic assays in rats.

## SUMMARY

A series of studies of cholesterol metabolism in the *Cebus* monkey were carried out in an attempt to understand the mechanisms responsible for the great differences in serum cholesterol levels when different dietary fats were used. Three groups of monkeys, one fed diets including 45 per cent of calories as corn oil, a second corn oil plus cholesterol (0.1 gm./100 calories), and a third lard plus cholesterol for 5 months (mean serum cholesterol values were 237, 268, and 601 mg. per cent, respectively) were injected with emulsions of cholesterol-4-$C^{14}$. The mean biological half-lives for the disappearance of serum radiocholesterol were 8.8, 8.4, and 6.6 days respectively. Esterification of radiocholesterol as measured by equilibration of specific activities of serum-free cholesterol and total cholesterol was delayed in the monkeys fed lard plus cholesterol. When cholesterol-4-$C^{14}$-stearate was given intravenously to a series of monkeys, an erratic non-exponential biological decay curve resulted. Specific activity for free serum cholesterol was greater than that for total cholesterol within 1 hour after the injection.

After 7 months on experimental diets including corn oil with added cholesterol and lard with added cholesterol the levels of lipides in most tissues were not different for the two dietary groups, nor were they appreciably elevated above previous control figures for monkeys not fed cholesterol. Total lipide levels in the adrenals of monkeys fed corn oil were twice those of monkeys fed lard.

Monkeys were fasted before and after intragastric administration of cholesterol-4-$C^{14}$ in small formula meals including various fats and fatty acids. The disappearance of total cholesterol from the serum consisted of a rapid followed by a slow exponential function. The type of fat and fatty acid appeared to influence the rate of disappearance of radiocholesterol. There was a broad range of apparent activity of the different fats and fatty acids in promoting cholesterol absorption.

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[3] The triolein used was a product of the Emery Industries, Inc., Cincinnati. Iodine number was 86.2; thiocyanogen number was 80.0. The material was fed without any type of purification. Several monkeys became extremely jaundiced while eating diets containing this oil.

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