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Received May 27, 1968. P.S.E.B.M., 1968, Vol. 129.

Influence of Fasting on the Formation of Cholesterol Arachidonate by the Serum Cholesterol Esterifying Enzyme (33321)

LEON SWELL AND M. D. LAW

Veterans Administration Hospital and Department of Biochemistry, Medical College of Virginia, Richmond, Virginia 23219

Sperry (1) first demonstrated that human serum contains an enzyme that catalyzes the esterification of free cholesterol. The enzyme is present in other species (rat, dog, and chicken) and has been characterized as an acyl-transferase (2, 3). The cholesterol ester fatty acids are derived from the 2-position of lecithin and are principally polyunsaturated (2). Also the rat plasma esterifying enzyme appears to react preferentially with the high-density lipoproteins that are rich in polyunsaturated esters (4, 5). Portman and Sugano have shown that the types of cholesterol esters synthesized by the plasma-esterifying enzyme is dependent on the source of the plasma enzyme. Fresh rat plasma synthesizes esters rich in arachidonate, while human plasma synthesizes esters rich in linoleate. In the course of studies (7) on the synthesis of the individual serum and cholesterol esters, it was recently shown that the types of cholesterol esters synthesized by the different liver enzyme systems is dependent on the nutritional status of the animal. The liver particulate and soluble cholesterol ester synthetase systems from fasting rats synthesized considerably more arachidonate than those systems from the liver of fed rats. At the same time the serum cholesterol esters of fasting rats contained a much higher proportion of arachidonate and a lower proportion of linoleate than did those of fed animals. In view of these findings, it became of interest to ascertain what effect the nutritional state of the animal would have on the serum-esterifying system. The data presented show that the serum enzyme of the fasting animal synthesizes significantly more arachidonate than that of the fed animal. Also, there are marked changes in the fatty acid composition of the phosphatidyl choline and cholesterol ester fractions.

Methods and Materials. Male rats (Wistar strain), weighing 175–200 g, and fed on a stock pellet chow diet were used. Fed and fasting rats refers to those animals who either had access to food at all times or were fasted 18 hr prior to sacrifice. Blood was obtained from the abdominal aorta and immediately chilled at 2°, allowed to clot, and centrifuged at 2° to separate the serum. Two-milliliter aliquots of serum were incubated with 0.5 μCi (specific activity 131 μCi/mg) of cholesterol-4-^{14}C (added in 0.1-ml acetone) for 1 hr at 37° in a Dubnoff Incu-Shaker. Control digests with serum heated at 60° for 10 min or 20 μmoles *N*-ethyl maleimide gave no enzymatic activity. The incubated and unincubated serum fractions were extracted with 20 vol of 2:1 chloroform methanol (8). The free and esterified cholesterol fractions and phospholipids were separated by silicic acid column chromatography (9). The ^{14}C activity of the cholesterol fractions was determined by liquid scintillation counting. The free and esterified cholesterol concentration was determined by the method of Sperry and Webb (10). The ^{14}C activity distribution among the various cholesterol esters was determined by separating the cholesterol ester fraction into four major cholesterol ester classes (saturated, monounsaturated, linoleate,

TABLE I. Serum Cholesterol Ester Transferase Activity.

Group[a]	Cholesterol esterified mμmoles/hr/ml serum				
	Total	Saturated	Monounsaturated	Linoleate	Arachidonate
Fed	8.4 ± 1.3	0.9 ± 0.2	0.6 ± 0.2	2.1 ± 0.4	4.8 ± 0.7
Fasting	12.8 ± 2.3	1.0 ± 0.2	0.7 ± 0.3	1.7 ± 0.3	9.4 ± 1.5

[a] Values represent the averages of 6 rats per group ± SD. Two milliliters of rat serum was incubated with 0.5 μCi cholesterol-4-^{14}C (added in 0.1 ml of acetone) for 1 hr at 37°.

and arachidonate) by thin-lay chromatography on silica gel G impregnated with silver nitrate (11). The esters were visualized by spraying the plate with 2,7 dichlorofluoresceine and examining them under ultraviolet light. The respective ester zones were scraped from the thin-layer plates directly into liquid scintillation vials. Methanol was added to elute the esters and the ^{14}C activity of the ester fractions determined by liquid scintillation counting. The thin-layer chromatography procedure was checked with known pure cholesterol-4-^{14}C esters (12). The recovery of ^{14}C activity was determined for each ester when pure standards were analyzed singly and in mixtures and the recovery of each ester ranged from 93 to 99%. Another portion of the cholesterol ester fraction was transmethylated with BF_3 in methanol to produce methyl esters and free cholesterol (13). The fatty acid composition of the methyl esters was determined by gas-liquid chromatography (14). The phospholipid fraction was subjected to thin-layer chromatography on silica gel G impregnated with 1 m*M* sodium carbonate (15). The phosphatidyl choline fraction was eluted from the thin-layer plates (16); the fatty acid methyl esters were prepared (13) and analyzed by gas-liquid chromatography.

Results. In Table I are shown the cholesterol ester transferase activities of the serum obtained from fasting and fed rats. The serum of fasting rats had a significantly ($p < .05$) greater amount of cholesterol esterifying activity than that of fed rats. Examination of the individual esters synthesized indicates that the serum enzyme of fasting rats synthesized almost twice as much cholesterol arachidonate as did that of the fed animal. In Table II are shown the results obtained on the distribution of the ^{14}C activity in the newly synthesized cholesterol esters. The cholesterol esters formed by the serum enzyme of the fasting animal contained a significantly ($p < .05$) greater proportion of arachidonate and a lower percentage of linoleate than the esters synthesized by the serum enzyme from fed animals. In Table III are shown the cholesterol ester fatty acid compositions of the serum cholesterol esters and phosphatidyl choline. The serum cholesterol esters of the fasting animal contained a significantly ($p < .05$) high proportion of arachidonate and a lower proportion of linoleate than those of the fed animal. This same general trend was also present in the phosphatidyl fraction; there was a higher percentage of arachidonate in that fraction of the fasting animal than in that of the fed animal. Here again this was principally at the expense of linoleate. These differences were found to be significant ($p < .05$). A comparison of the data in Table II and III indicates

TABLE II. Distribution of ^{14}C-Activity in Synthesized Cholesterol Esters.

Group[a]	% of total ^{14}C-activity			
	Saturated	Monounsaturated	Linoleate	Arachidonate
Fed	10.7 ± 1.6	7.5 ± 2.8	25.3 ± 4.4	56.5 ± 7.0
Fasting	7.6 ± 1.5	5.2 ± 1.6	13.5 ± 1.3	73.7 ± 2.3

[a] Values represent the averages of 6 rats per group ± SD.

TABLE III. Fatty Acid Composition of Serum Cholesterol Esters and Phosphatidyl Choline.

Group[a]	% of total fatty acids			
	Saturated	Monounsaturated	Linoleate	Arachidonate
Cholesterol esters				
Fed	14.7 ± 2.8	10.7 ± 2.5	33.3 ± 3.5	41.3 ± 5.4
Fasting	11.8 ± 2.0	8.5 ± 2.0	18.8 ± 2.2	60.9 ± 4.5
Phosphatidyl choline				
Fed	52.1 ± 6.1	9.9 ± 3.5	28.5 ± 3.2	9.5 ± 3.1
Fasting	51.7 ± 5.5	9.5 ± 2.8	19.2 ± 2.9	19.6 ± 4.0

[a] Values represent the averages of 6 rats per group ± SD.

that the relative specific activity of the arachidonate ester was much higher in both the fasted and fed animals than the other cholesterol esters. Thus there would appear to be a preferential formation of cholesterol arachidonate by the serum enzyme of both types of animals. There was no significant difference in the serum cholesterol level of the two groups; the fed rats had a level of 35 ± 6 mg per 100 ml and the fasted rats 40 ± 8 mg per 100 ml.

Discussion. The present study has provided additional information on several parameters associated with the action of the serum cholesterol ester transferase enzyme. The serum of fasting rats was found to contain more cholesterol esterifying activity than the serum of fed rats. This increase was principally in the synthesis of cholesterol arachidonate at the expense of cholesterol linoleate. The pattern of esters synthesized in fasting and fed rats closely resembled the serum cholesterol ester fatty acid compostion of those rats. This increased formation of cholesterol arachidonate by the serum enzyme of fasted rats could be associated with the production of a greater amount of high-density lipoproteins by those animals. It has been shown in the rat (5) that the polyunsaturated cholesterol esters occur to the greatest extent in the high-density lipoproteins. These findings also correlate well with recent studies (7) obtained on the *in vivo* synthesis of the individual serum and liver cholesterol esters in fasted and fed rats. In that study, it was shown that the serum and liver cholesterol esters of the fasting rat contained a much higher proportion of arachidonate and a lower proportion of linoleate than the serum and liver esters of fed rats. The role of the cholesterol ester transferase enzyme has not been clarified. The increased level of the serum cholesterol esterifying enzyme of the fasted rat may be a reflection of increased cholesterol arachidonate synthesis in the tissues. This in turn could be associated with increased levels of the transferase and/or other enzyme systems involved in cholesterol arachidonate synthesis in the tissues and subsequent release of the transferase enzyme into the blood. The alternate possibility exists that the serum enzyme may play an important role in the synthesis of the serum cholesterol esters (2).

The studies of Glomset (2) have shown that the 2-position of phosphatidyl choline is the principal source of the fatty acids associated with the serum transferase enzyme reaction. These fatty acids are principally polyunsaturated. The present data suggest that fatty acid changes in the phosphatidyl choline fraction, particularly in the ratio of linoleate to arachidonate, may be an important factor in determining the relative proportions of the different cholesterol esters synthesized by the serum enzyme. However, in order to account for the much higher propertion of cholesterol arachidonate present in the mixture of esters formed by the enzyme than is present in the phosphatidyl choline unsaturated fatty acid mixture there must be a selective transfer of arachidonic acid from phosphatidyl choline to cholesterol. This could involve the preferential reaction of arachidonyl lecithin with cholesterol. The higher proportion of arachidonyl lecithin in

the serum of the fasting rat than in the fed rat and the increased serum enzyme activity of the fasted rat supports this type reaction. In man the serum enzyme forms principally cholesterol linoleate and the proportion of cholesterol arachidonate in the synthesized ester mixture is lower than is found in the unsaturated fatty mixture (2-position) of phosphatidyl choline (2, 6). This points up the marked differences in the specificity of the human and rat serum enzyme systems. The possibility also exists that specific phosphatidyl cholines of specific lipoproteins (4) are the principal reactants involved in ester formation by the serum enzyme of these two species.

Summary. The serum cholesterol ester transferase enzyme is more active in the serum of fasted than fed rats. This increase in activity is associated with principally one cholesterol ester, namely, cholesterol arachidonate. The serum cholesterol esters and phosphatidyl choline of fasted rats contain a higher proportion of arachidonic acid and less linoleic acid than those of fed animals. The findings indicate that the types of cholesterol esters synthesized by the serum enzyme are dependent, in part, on the nutritional state of the animal.

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Received June 3, 1968. P.S.E.B.M., 1968, Vol. 129.

Serologic Characterization of Adenoviruses Isolated from Chimpanzees Associated with Viral Hepatitis* (33322)

W. D. HILLIS,[1] A. W. HOLMES, AND V. DAVISON
(Introduced by F. B. Bang)

USAF Epidemiological Laboratory, Aerospace Medical Division, United States Air Force, Lackland Air Force Base, Texas; and Department of Microbiology, Presbyterian-St. Luke's Hospital, Chicago, Illinois

Chimpanzees and certain other nonhuman primates have been implicated in recent years in the transmission of viral hepatitis to their human caretakers and to other contacts (1–6). Mounting epidemiologic evidence for such transmission is documented by more than 100 human cases of the disease. This evidence supports the postulate that certain nonhuman primates act as carriers of the etiologic agent or agents of human viral hepatitis or of a disease so closely related as to be

* These studies were supported in part by Contract DA 49-193-MD-2721, United States Army Medical Research and Development Command.

1 Present address: Department of Pathobiology, The Johns Hopkins University School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore Maryland 21205.