De novo synthesis and elongation of fatty acids by subcellular fractions of monkey aorta

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ABSTRACT Subcellular fractions of aorta of squirrel monkey (*Saimiri sciureus*) were examined for their ability to synthesize and elongate fatty acids. High-speed supernate (HSS) incorporated substantial quantities of malonyl CoA into fatty acids while acetyl CoA was much less effectively utilized. Acetyl-CoA carboxylase activity exceeded the amount of acetyl CoA incorporated into fatty acids and thus does not account for the low incorporation of this substrate. Microsomes used malonyl CoA and acetyl CoA equally well; mitochondria incorporated either acetyl CoA or acetate. The amounts of substrate incorporated into fatty acids (mμmoles/mg of protein per hr) were 2.3 for HSS, 1.2 for microsomes, and 0.9 for mitochondria.

The synthesized fatty acids were separated by gas–liquid chromatography, radioassayed, extracted from the scintillation fluid, and decarboxylated. HSS completely synthesized palmitic and stearic acids from malonyl CoA. Microsomes and mitochondria utilized acetyl CoA to elongate endogenous fatty acids and gave mainly palmitic, stearic, and C_{18} and C_{20} monoenoic acids, with lesser amounts of other saturated and unsaturated fatty acids. A significant quantity of malonyl CoA was utilized by microsomes to yield a fatty acid tentatively identified as docosapentaenoic. Radioactive fatty acids are incorporated into various lipid classes by the particulate preparations.

These studies demonstrate that aortic tissue in a nonhuman primate is able to carry out several processes of fatty acid metabolism and that the aortic synthesis and elongation of fatty acids may play an important role in providing fatty acids for incorporation into aortic lipids.

KEY WORDS aorta · subcellular fractions · fatty acids · de novo synthesis · elongation · squirrel monkey · fatty acid extraction from scintillation fluid

IT IS BECOMING INCREASINGLY APPARENT that aortic tissue, like virtually all other tissues, does not just react passively to its environment, but also shares with other tissues the ability to carry out numerous metabolic reactions. The importance of lipids to arterial tissue in aging and atherosclerosis has long been recognized and reactions in which aorta metabolizes lipids are of paramount interest. These studies aimed at a better understanding of arterial synthesis and elongation of fatty acids that may later be incorporated into arterial lipids.

In addition to in vivo studies of aortic lipid metabolism (1, 2), work has been done on in vitro incorporation of acetate (3, 4), fatty acids (5–7), and lysolecithin (8) into lipids. Chernick, Srere, and Chaikoff (9), using whole rat arteries, and Feller and Huff (10), using rabbit aortic strips, found incorporation of radioactive acetate into a fraction isolated as fatty acids. Lofland, Clarkson, and their coworkers used minced aortic tissue (11) and perfused aortas of pigeon (12) to study the incorporation of acetate-1-^{14}C into the fatty acids of several lipid fractions. Whereat clearly showed that rabbit aortic strips (13) incorporate radioactivity specifically into fatty acids and later established that the aortic mitochondria are capable of chain elongation with acetate (14). These studies, while contributing to the picture of an active lipid metabolism in aorta, have left numerous questions unanswered, such as: what contributions towards fatty acid synthesis are made by the various subcellular fractions; are substrates that are more physiological than acetate utilized; and do the fatty acids synthesized in situ make an important quantitative contribution to lipids of the aortic wall?

The work reported here leads to the conclusion that aorta is able to carry out the two major biosynthetic reactions yielding fatty acids, namely, de novo synthesis and elongation of fatty acids. These reactions are carried out

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Abbreviations: HSS, high-speed supernate; GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

by different subcellular fractions. The contribution made by these syntheses may be significant in the eventual composition of fatty acids of the aorta.

MATERIALS AND METHODS

Materials

"Fatty acid-poor" human serum albumin was made by the method of Goodman (15). Radioactive substrates, obtained from New England Nuclear Corp., Boston, Mass., had the following specific activities: malonyl-1,3-^{14}C CoA, 7.4 mc/mmole; sodium acetate-1-^{14}C, 2 mc/mmole; acetyl-1-^{14}C CoA, 39.6 mc/mmole; and $NaH^{14}CO_3$, 20.5 mc/mmole. Calculations of the amount of substrate incorporated into fatty acids from malonyl CoA were based on only one-half of the substrate being utilized for synthesis.

Carrier fatty acids for GLC were obtained from Applied Science Laboratories, Inc., Inglewood, Calif., and the carrier partial glycerides for TLC were a gift from Dr. F. H. Mattson, the Procter & Gamble Co., Miami Valley, Ohio. All other reagents and enzymes were obtained from commercial sources.

Tissue Preparation

Mature, 600–700 g, female Brazilian squirrel monkeys (*Saimiri sciureus*) were maintained on a stock ration. They were anesthetized with Sernylan (phenylcyclidine hydrochloride, Parke, Davis, & Co., Detroit, Mich.) and the aorta was opened in situ from the aortic arch to the femoral bifurcation and flushed with ice-cold 0.9% NaCl solution. After removal, each aorta was placed under a binocular microscope at 10 × magnification and the intima plus media was stripped away from the adventitia and adherent material. The yield of intima plus media was about 80–100 mg wet weight per aorta. Tissues from several aortas were combined and homogenized in 0.4–0.8 ml of 0.25 M sucrose per 100 mg of tissue in a Kontes glass homogenizer kept in ice, started at 12 rpm and increased to 40 rpm until no large particles remained.

In a comparison of the metabolism of aorta with that of smooth muscle from another source, a 5 cm segment of the upper large intestine was removed, the luminal surface was scraped free of mucosa, and adherent fat material was removed. Examination of this preparation under the binocular microscope showed it to consist only of two layers of smooth muscle with no visible contamination by mucosa or fat tissue. Subcellular fractions of this intestinal muscle and of liver were prepared in the same way as aorta.

Centrifugation

A minimum amount of 0.25 M sucrose was used for homogenization so that the highest possible concentration of supernatant protein was obtained. Since the volume of the homogenate was often so small that available centrifuge tubes (capacity less than 2 ml) would collapse upon high-speed centrifugation, a special plug (Fig. 1) was fitted into the tube (Beckman 2 ml cellulose nitrate centrifuge tube, in adapter No. 303376 for centrifuge rotor type No. 50, Model L Spinco preparative centrifuge). The stainless steel plugs were made in three different lengths and a plug was selected such that the top of the homogenate was about 1 cm from the bottom of the plug.

The homogenate from the intima plus media from only one aorta could be centrifuged in a 1 ml Autoclear round-bottom centrifuge tube No. 2822 (International Equipment Co.) resting in an adapter fitted to the Spinco centrifuge rotor type No. 50. The usable volume of this tube was 0.6 ml.

In order that the maximal amount of high-speed supernate (HSS) might be obtained, the homogenate was centrifuged at 100,000 *g* for 60 min, the pellet was removed, and the supernate was again centrifuged at 100,000 *g* for 60 min. The pellet from the first centrifugation was suspended in 0.25 M sucrose with a Tenbroeck

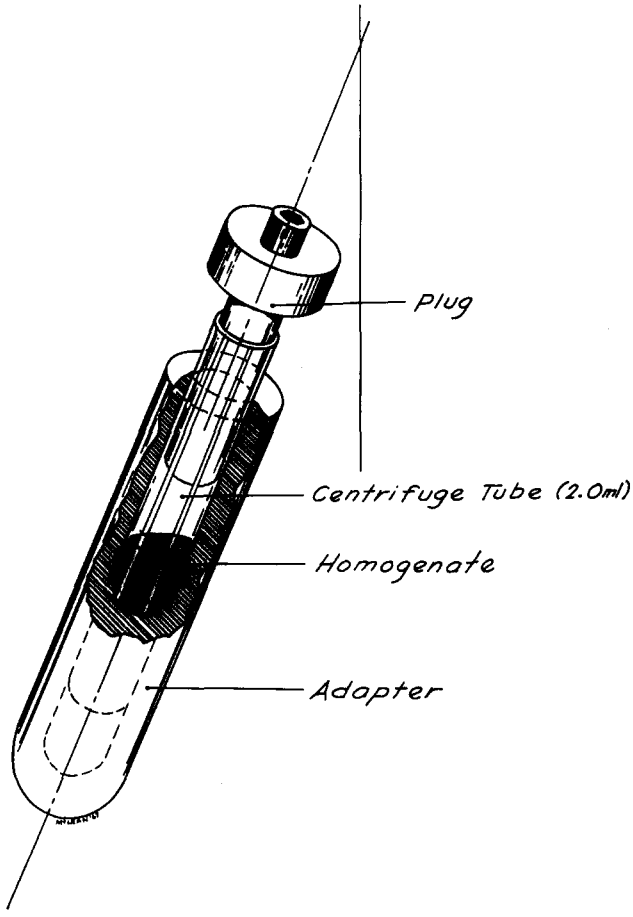

FIG. 1. Expanded view of centrifugation assembly used for small volumes (<2 ml) of homogenate.

homogenizer and centrifuged at 1000 *g* for 10 min in a refrigerated Phillips-Drucker centrifuge, and the debris was discarded. The 1000 *g* supernate was again centrifuged at 1000 *g* for 10 min and the supernatant fraction from this was centrifuged at 14,000 *g* for 10 min to yield a pellet containing the mitochondria. Even after the pellet had been washed with 0.25 M sucrose, electron microscopy revealed that a substantial portion of the total pellet consisted of elastin fibers. The supernate, after removal of the mitochondria, was centrifuged at 100,000 *g* for 60 min to yield the microsomal pellet. This aortic microsomal pellet had characteristics similar to those of liver microsomes (16). Protein was determined in all subcellular fractions by the method of Lowry, Rosebrough, Farr, and Randall (17). The incubation conditions and composition of each tube containing a subcellular fraction and radioactive substrate are given in Table 1.

Lipid Analysis

After incubation of the different subcellular fractions the composition of the total fatty acids synthesized as well as the distribution of ^{14}C amongst the lipid classes were determined for each tube. Unlabeled sodium acetate and sodium malonate, 200 μmoles of each, and carrier fatty acids were added to each tube. One-half of the reaction mixture was made 1.7 M with respect to NaOH, heated for 2 hr at 37°C, cooled, and extracted three times with petroleum ether (bp 40–49°C). These extracts were discarded, the aqueous contents were acidified with HCl, and the fatty acids were extracted twice with petroleum ether and then twice with *n*-hexane. The combined extract of fatty acids was washed with water containing a few drops of concentrated HCl and 250 μmoles of unlabeled acetate, and an aliquot was dried and radioassayed. The remaining portion of the fatty acids was methylated with diazomethane and subjected to gas–liquid chromatography (GLC).[1] About one-third of the GLC effluent was passed through a flame ionization detector and the remainder through Pasteur pipettes containing glass wool wetted with scintillation fluid to trap the methyl esters. Separate pipettes were used for each known fatty acid peak as well as the intervals between peaks. The pipette contents were rinsed into vials with scintillation fluid and radioassayed. Fatty acids were extracted from the scintillation fluid of certain vials for further analysis (18). Identification was based on cochromatography of the known carrier fatty acids.

Carrier lipids were added to the remaining half of the reaction mixture contents and the total lipid was extracted with chloroform–methanol (19). Thin-layer chromatography (TLC) was carried out on Chromagram sheets, which are flexible sheets coated with silica gel (Eastman Kodak Co., Rochester, N. Y.). The extract was applied as a spot to Chromagram sheets and developed in benzene–cyclohexane–acetic acid 30:30:1, and the spots were made visible with iodine vapor. The spots of the different classes of lipids were cut out of the Chromagram sheet and put in vials; scintillation fluid was added for radioassay. The Chromagram sheet did not affect the efficiency of the radioassay.

[1] The instrument used was a Barber-Colman gas–liquid chromatograph, series 5000, containing a glass U tube 6 ft × 6 mm I.D., packed with 15% HI-EFF 2BP (ethylene glycol succinate) on Chromosorb W(AW), 80–100 mesh (Applied Science Labs.). Temperature was 185°C and carrier N_2 flowed at about 200 ml/min.

TABLE 1 INCORPORATION OF DIFFERENT SUBSTRATES INTO FATTY ACIDS BY SUBCELLULAR FRACTIONS OF MONKEY AORTA

Substrate	Concn	HSS	Microsomes	Mitochondria
	mM	μμmoles substrate incorporated/mg of protein/hr*		
Malonyl-1,3-^{14}C CoA	0.27	2042–2546	1465, 1058	39–74
Acetyl-1-^{14}C CoA	0.10	20–40	1270, 1151	241–919
Acetate-1-^{14}C	6.3	20–86	47–75	448, 893

Incubation conditions: all tubes contained 131 mM glycylglycine, pH 7.4; 6.6 mM glutathione; 1.4 mM NADPH; 6.6 mM glucose 6-phosphate; 0.33 units/ml glucose 6-phosphate dehydrogenase; 1.6 mM NADH; 12.9 mM potassium isocitrate; and 2.5 mM niacinamide. Except for HSS + malonyl-1,3-^{14}C CoA, the tubes also contained 5.2 mM $MgCl_2$; 6.5 mM ATP; 0.65 mM $MnCl_2$; 6.5 mM creatine phosphate; 0.6 units/ml creatine phosphokinase; and 20 mM D,L-glycerol 3-phosphate. Defatted albumin, 4 mg/ml, was present in the HSS tubes, and 0.3 mM CoA was included in the HSS tube containing acetate-1-^{14}C and in all tubes with microsomes and mitochondria. Nonradioactive acetyl CoA, 36 μM, was present in the HSS when malonyl-1,3-^{14}C CoA was substrate and 33 mM $KHCO_3$ was used when acetyl-1-^{14}C CoA and acetate-1-^{14}C were substrates. Total volume was 0.75 ml and incubation was at 37–38°C for 1.5 hr with shaking.

* Values separated by a dash are the extremes of ranges obtained in four separate experiments, whereas those separated by a comma are values from two experiments.

Extraction and Decarboxylation of Fatty Acids

Fatty acids were extracted from the scintillation fluid by hydrolyzing the fatty acid methyl ester with 2.5% KOH in 95% EtOH at 37°C for 2 hr. Water was added to yield two phases, the fluor-containing toluene phase was removed, and the fatty acids from the acidified aqueous layer were extracted with petroleum ether (18). Part of the fatty acid was decarboxylated (20) and the ratio of total dpm to carboxyl dpm was determined.

RESULTS

Incorporation of Substrates into Fatty Acids

The data in Table 1 represent several experiments. Malonyl CoA was the only substrate significantly incorporated into fatty acids synthesized by the HSS. The addition of albumin as receptor (21) caused only a 1.1-

to 1.6-fold stimulation in the incorporation of radioactivity into fatty acids (e.g., see Table 3).

In the de novo synthesis of fatty acids by a cytoplasmic fraction, acetyl CoA is incorporated into fatty acids after carboxylation to malonyl CoA (22). It was now necessary to establish that acetyl CoA could serve as a precursor for malonyl CoA in this system.

Table 2 shows that HSS of aorta, liver, and intestinal smooth muscle can carboxylate acetyl CoA. Aortic HSS fixation of $^{14}CO_2$ was far greater than the amount of acetyl CoA incorporated into fatty acids (Table 1). This same effect, i.e. carboxylase activity > incorporation of acetyl CoA into fatty acids, was also found for muscle and, to a much lesser extent, liver (unpublished observations). The product of the reaction of acetyl CoA and $HC^{14}O_3^-$ was identified by incubation of the aortic HSS as in Table 2 except that the $NaH^{14}CO_3$ used (21 $\mu c/\mu$mole) was not diluted with carrier bicarbonate. The reaction mixture was saponified with KOH and acidified, and most of the salts and protein were precipitated with 40 ml of cold acetone. The supernate was dried, taken up in the least possible quantity of acetone, and applied to Whatman No. 1 chromatogram strips, which were developed in isoamyl alcohol saturated with 4 N formic acid (23). A radioactive peak (Model 7201 Packard radiochromatogram scanner) cochromatographed with malonic acid, which was made visible with bromophenol blue. The malonic acid spot was cut out and radioassayed in scintillation fluid. The amount of radioactive carbonate incorporated to form malonic acid was calculated to be 414 $\mu\mu$moles/mg of protein per hr (third value for aortic HSS in Table 2). As can be seen, there is good agreement between values of carboxylase activity calculated by the two different methods. Thus the carboxylation of acetyl CoA does lead to malonyl CoA in quantities sufficient for utilization in fatty acid synthesis.

Microsomes utilize both malonyl CoA and acetyl CoA for fatty acid synthesis (Table 1). Coenzyme A was included in the incubation system to aid in esterification reactions known to occur in microsomes (24, 25). Esterification of endogenous and nascent fatty acids removes inhibitors from the synthesizing complex (26). However, it was later found in studies with liver microsomes that the presence of this concentration of coenzyme A causes some inhibition in the utilization of acetyl CoA and, to a much lesser degree, malonyl CoA. Thus the amounts of acetyl CoA incorporated may have been greater than the values given in Table 1. Substantial amounts of acetate were incorporated only by the mitochondria, which also utilized acetyl CoA.

Distribution of Radioactivity in Biosynthesized Fatty Acids

When the products of fatty acid synthesis contained more than 300 dpm, they were separated by GLC, trapped, and radioassayed. The results are listed in Table 3. HSS or HSS + albumin always gave > 90% of the radioactivity in palmitic and stearic acids from malonyl CoA. Attempts were made to measure the distribution of radioactivity in the fatty acids synthesized from acetyl CoA by aortic HSS. Since the total radioactivity available for GLC, as well as that subsequently recovered, was low, the results are not as unequivocal as those obtained with large quantities of radioactivity. About half of the radioactivity was associated with palmitic and myristic acids, and the shorter-chain acids contained another 15% of the total (unpublished observations). The GLC techniques were such that fatty acids eluting before myristate were not quantitatively analyzed.

The products obtained from microsomes and mitochondria contained a variety of saturated and unsaturated fatty acids. About 50% of the acetyl CoA and acetate incorporated by these fractions was found in the C_{16} and C_{18} acids with an additional 15% in the C_{20} acids. Incubation of microsomes and malonyl CoA resulted in 13–40% of the radioactivity appearing at positions corresponding to the acids[2] 24:0 and 24:1. Two runs with these components are listed in Table 3 and show how the percentage of the other fatty acids was affected by the variable amount of radioactivity at 24:0 and 24:1. The major change was that fatty acids eluted with oleic acid increased when those at 24:0–24:1 decreased. Most of the radioactivity at 24:0–24:1 was identified as attributable to docosapentaenoic acid by extraction, hydrogenation (27), and rechromatography; 70–80% of the radioactivity recovered had a carbon number of 22. It can be calculated that 22:5 will chromatograph on Hi-Eff 2BP in the region of 24:0–24:1 (28). When acetyl CoA was the microsomal or mitochondrial substrate, 10–12% of the radioactivity appeared in this position on GLC and the majority could be accounted for as 22:5 fatty acid.

TABLE 2 CARBOXYLASE ACTIVITY IN TISSUES

Tissue	Activity
	$\mu\mu$moles $^{14}CO_2$ incorporated/mg of protein/hr
Aorta	584, 235, 414*
Liver	5460, 8240, 4294
Intestinal muscle	780

Incubation medium: 2.5 mM ATP; 0.14 mM acetyl CoA; 0.7 mM $MnCl_2$; 45 mM potassium isocitrate; 99 mM potassium phosphate buffer, pH 6.5; 10 mM $KH^{14}CO_3$ (4 $\mu c/\mu$mole); 0.4 ml final volume; incubation for 3 min at 37°C. Activity assayed as described by Waite and Wakil (41) except for the * value (see text).

[2] Acids are designated by chain length: no. of double bonds. Positions of the double bonds are indicated by superscript numerals.

TABLE 3 DISTRIBUTION OF RADIOACTIVITY AMONG FATTY ACIDS SYNTHESIZED BY AORTIC SUBCELLULAR PREPARATIONS

	Percentage Incorporation*						
	Malonyl CoA				Acetyl CoA		Acetate
Fatty Acids†	HSS	HSS + Albumin	Micros		Micros	Mito	Mito
14:0	1	1	1	1	5	2	4
14:1	1				2	1	1
16:0	70	72	5	7	12	14	14
16:1	tr.	tr.	3	3	3	5	7
	tr.		1	5	5	4	3
18:0	25	24	16	14	23	18	20
18:1	tr.	tr.	11	32	11	16	16
				1	1	1	1
18:2	tr.	tr.	2	5	2	3	3
						tr.	
							4
20:0	tr.	tr.	2	1	5	4	
20:1, 18:3	tr.		5	8	10	13	10
(20:3)‡	tr.	tr.	7	4	8	7	8
22:0	tr.		1		tr.	1	1
(20:4)‡		tr.				tr.	
22:1			1	1	1	2	2
	tr.	tr.	3	3	3	3	4
24:0	tr.	tr.	4	3	3	1	2
24:1			36	10	7	3	2
Radioactivity recovered (dpm)*	2,750	2,585	1,118	1,176	14,631	5,680	713
Enzyme activity§	2,252	2,546	1,058	1,156	1,151	919	448

Micros, microsomes; Mito, mitochondria.

* The percentage incorporation is the percentage of the total dpm collected from the GLC effluent fatty acids in 14:0 through 24:1. "tr." indicates less than 0.6%.

† Fatty acids listed are those added as carrier for cochromatography with the radioactive fatty acids.

‡ Enzyme activity = $\mu\mu$moles substrate incorporated per mg of protein per hr.

§ 20:3 and 20:4 were not added as carrier but would be located as indicated.

Decarboxylation of Synthesized Fatty Acids

Table 4 presents the ratio of total to carboxyl radioactivity determined in fatty acid biosynthesized by aortic preparations. These ratios indicate whether substrate was incorporated throughout the chain, as occurs during complete de novo synthesis, or by elongation of pre-existing fatty acids. The ratio is 8 for palmitic acid if radioactive two-carbon units are used throughout in the de novo synthesis and 7 if a nonradioactive acetyl unit is on the methyl end of the fatty acid. A ratio of 1 is found when only elongation occurs since all of the radioactivity of the fatty acid is contained in the carboxyl group.

Aortic HSS synthesizes palmitic and stearic acids completely from malonyl CoA (Table 4), whereas both mitochondria and microsomes elongate fatty acids with acetyl CoA, since the ratios are all about 1. The ratio of 1.6 for 22:0 could indicate elongation by two acetyl units in the synthesis of a major portion of this fatty acid.

The 22:5 fatty acid described in the previous section was decarboxylated and gave a ratio of 1.0; it could arise by the elongation of a C_{20} unsaturated fatty acid. Similarly, the 18:1 acid sometimes obtained in large quantities from microsomes plus malonyl CoA gave a ratio of about 1 and could therefore arise by elongation of 16:1 or by a combination of elongation and desaturation.

TABLE 4 DECARBOXYLATION OF FATTY ACIDS SYNTHESIZED BY AORTIC SUBCELLULAR FRACTIONS

	Ratio $\frac{\text{total dpm}}{\text{carboxyl dpm}}$			
	Malonyl CoA		Acetyl CoA	
Fatty Acid	HSS	Microsomes	Mitochondria	Microsomes
14:0			1.2	1.0
16:0	6.7	0.9	1.1	1.1
16:1			1.0	1.0
18:0	7.6	1.2	1.0	1.0
18:1		0.9	1.0	1.0
20:0			1.3	1.0
22:0			1.7	1.6

Distribution of Synthesized Fatty Acids in Lipids

The percentage of radioactivity that appeared in several classes of lipids is shown in Table 5. The presence of albumin in the HSS fraction did not radically change the compounds into which radioactivity was incorporated; free fatty acids were the main product. Incubation of

TABLE 5 Distribution of Radioactivity among Lipid Classes after Incubation of Aortic Subcellular Fractions with Malonyl-1,3-^{14}C CoA and Acetyl-1-^{14}C CoA

Lipid Class	Malonyl CoA				Acetyl CoA	
	HSS	HSS + Albumin	HSS + Micros	HSS + Mito	Micros	Mito
	% of radioactivity recovered					
Cholesteryl esters			1	1	1	1
Triglyceride			10	15	6	11
Fatty acids	95	89	21	27	23	28
Diglyceride	3	3	13	13	11	17
Monoglyceride			21	12	13	23
Phospholipid	1	6	35	32	45	20

Micros, microsomes; Mito, mitochondria.

HSS with a particulate preparation causes the radioactivity from malonyl-1,3-^{14}C CoA in fatty acids to be distributed throughout the different lipid classes. Analysis of the total fatty acids synthesized by this system gave much the same pattern of radioactive fatty acids as listed in Table 3 for microsomes or mitochondria alone. When microsomes or mitochondria were incubated alone with acetyl-1-^{14}C CoA, the percentage of free fatty acids was again less than in the HSS system, and the radioactivity was distributed among the several lipid classes, most notably the phospholipids.

DISCUSSION

These data demonstrate that subcellular fractions of aortic intima plus media are capable of the two major mechanisms for fatty acid synthesis, i.e., de novo synthesis and elongation.

High-speed supernatant preparations totally synthesize the saturated fatty acids, palmitic and stearic, from malonyl CoA. Fatty acid synthetase complex is present in the soluble supernate since the primary product (70%) is palmitic acid; malonyl CoA is utilized; and de novo synthesis, as judged by the decarboxylation ratio, occurs. Supernatant systems of liver (20, 22), brain (29), and adipose tissue (30) act similarly. The utilization of acetyl CoA by HSS for fatty acid synthesis is lower than that of malonyl CoA, but the ratios of efficiency for these two substrates are similar to those found in liver (31).

It was definitely established that aortic HSS does fix radioactive carbon dioxide and the product is malonic acid. Carboxylase activity is some 10–20 fold greater than the amount of acetyl CoA incorporated into fatty acids. These results are not unexpected for several reasons. Firstly, even at the rates for carboxylase measured here, the amount of malonyl CoA produced by the carboxylating enzyme would give only a small fraction of the concentration of malonyl CoA used in the synthetase measurements. Secondly, a spatially organized enzymatic sequence has probably been disrupted by the cellular fractionation. Thus acetyl CoA must be carboxylated to malonyl CoA which may then have to diffuse, in the in vitro preparation, and then be utilized by the synthetase. Malonyl CoA, on the other hand, need only react with the organized synthetase complex, being incorporated while on the complex into the measured fatty acid products. Further evidence that malonyl CoA is normally incorporated to a much greater extent than acetyl CoA by the HSS systems is provided by Abraham, Lorch, and Chaikoff (31). In a liver HSS system they found that malonyl CoA was incorporated into fatty acids 70–190 times more actively than acetyl CoA. In the studies reported here, the ratio of malonyl CoA to acetyl CoA used for synthesis ranged from 55 to 200 in aorta (Table 1) and from 10 to 250 in companion studies with monkey liver (unpublished observations). All of these values are of similar magnitude and indicate that HSS cannot synthesize fatty acids as effectively from acetyl CoA as it does from malonyl CoA. Nevertheless, the current data do support the concept of fatty acid synthesis by aortic HSS involving carboxylation of acetyl CoA to malonyl CoA and its subsequent incorporation into long-chain saturated fatty acids.

Reactions for elongating fatty acids are primarily carried out in the particulate preparations (Table 4). No exogenous fatty acids were supplied to the reaction mixture, so that these fractions elongated fatty acids already present in the preparations. This results in the production of a variety of saturated and unsaturated fatty acids. The unsaturated fatty acids containing radioactivity can be produced by the elongation of preexisting unsaturated fatty acids, or the fatty acids may be elongated first and then desaturated (32–36).

Mitochondria use both acetyl CoA and acetate for elongation. Harlan and Wakil (37) propose that the reactions for de novo synthesis of fatty acids can also be carried out by mitochondria, but the only enzymatic reaction measured under the present conditions was that of elongation. Since the aortic mitochondria utilized only minute amounts of malonyl CoA, data are not available to compare the mitochondrial products from the two substrates.

An acetate thiokinase is present in mitochondria (38, 39) and it must also be present in aortic mitochondria since this was the only fraction that utilized acetate to any extent. A partial solubilization of this enzyme may have accounted for the low, but significant, quantities of acetate incorporated by HSS and by microsomes, a fact also noted by Whereat (14).

Aortic microsomes used both acetyl CoA and malonyl CoA (Table 1) to yield a pattern of saturated and unsaturated fatty acids similar to that found in mitochondria (Table 3). Harlan and Wakil (37) reported the in-

corporation of acetyl CoA into fatty acids by liver microsomes, but this finding was not borne out by the data of Guchhait, Putz, and Porter (40) or Nugteren (36). However, the elongation of fatty acids with malonyl CoA has been shown by these latter two groups using pigeon (41) and rat (36) liver, and a similar reaction occurs in aortic microsomes. The 22:5 acid formed when microsomes are incubated with malonyl CoA would be $22:5^{4,7,10,13,16}$ according to the gas-chromatographic data of Ackman, Burgher, and Jangaard (28). This could arise by the elongation and desaturation of arachidonic acid ($20:4^{5,8,11,14}$).

It is of some interest to determine whether the reactions measured in the present work can yield significant quantities of fatty acids that could be utilized for complex lipid formation by the normal aorta. Estimates can be made about the total ability of the aorta to synthesize fatty acids.

The amount of substrate incorporated in the HSS system is not equivalent to the amount of fatty acids produced. Of the average 2290 $\mu\mu$moles of malonyl-1,3-^{14}C CoA incorporated into fatty acids by the HSS (Table 1), 70% of the acids formed, or 1603 $\mu\mu$moles, is palmitic acid and 25%, or 572 $\mu\mu$moles, is stearic acid (Table 3). The incorporation of 7 malonyl units into palmitic acid actually represents a net synthesis of 230 $\mu\mu$moles of fatty acid; and by similar reasoning, 72 $\mu\mu$moles of stearic acid are produced, yielding a total of 302 $\mu\mu$moles of fatty acid synthesized per mg of HSS protein per hr. Since the enzymatic reaction of the mitochondria and microsomes is that of elongation by one two-carbon unit, the quantity of fatty acid produced is nearly equivalent to the amount of substrate incorporated. For microsomes, an average value is 1230 $\mu\mu$moles of fatty acids produced per mg of protein per hr when either malonyl CoA or acetyl CoA is utilized. The amounts of substrate incorporated into fatty acids by mitochondria are probably greater than the values would indicate, as a result of at least two factors: (*a*) the elastin content of this fraction could cause a discrepancy in the protein measurement that would yield lower values of enzymic activity, and (*b*) since mitochondria can use fatty acids in oxidation reactions, the amount of radioactive substrate incorporated represents a balance of the fatty acids synthesized over those catabolized. Because of these two factors, which would lower the values recorded, the higher average (see Table 1) of 900 $\mu\mu$moles of fatty acid synthesized per mg of mitochondrial protein per hr will be used in this discussion. This is somewhat higher than the value of 125 $\mu\mu$moles of acetate-^{14}C incorporated per mg of protein per 2 hr found for rabbit aortic mitochondria (14).

Approximate calculations were made of the yield of each of the subcellular fractions. These are minimal values because of losses incurred during handling. An average of 83 mg of wet weight aortic tissue (intima plus media) per monkey was obtained, and this gave average protein values of HSS = 2.12 mg, mitochondria = 0.23 mg, and microsomes = 0.24 mg. The total contribution by each fraction was then HSS 0.64, mitochondria 0.20, and microsomes 0.30, or a total of 1.14 mμmoles of fatty acids synthesized per aorta per hr.

According to these calculations, about half of the fatty acids synthesized in the aorta could arise de novo in the cytoplasmic fraction. The remainder of the fatty acids produced in the aorta would arise by elongation, not only by the mitochondria as shown by Whereat (14), but also by the microsomes.

The contribution of in situ fatty acid synthesis to the total fatty acids used by the aorta is not known. The flux of fatty acids between aorta and blood has not been studied, but Stein and Stein (7) have used aortic slices to demonstrate uptake and esterification of fatty acids from a medium. Their rates of incorporation of linoleic-1-^{14}C acid into neutral lipids and phospholipids ranged from 18 mμmoles/100 mg of dry, defatted tissue per 2 hr in baboon to a value of 50 in rabbit. Conversion of this to present units gives the range of 2–5 mμmoles fatty acid incorporated per 83 mg wet weight of aorta per hr. Since the protein recoveries were not quantitative in the present work, the value of 1.14 mμmoles of fatty acids produced per 83 mg of tissue per hr is a minimal value. Thus the contribution of in situ fatty acid synthesis and elongation to the total fatty acid content of the aorta may be quite significant.

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