Fatty acid synthesis in cell-free system from rabbit aorta

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ABSTRACT The objectives of this study were to identify the subcellular fraction responsible for fatty acid synthesis in rabbit aorta and to determine the effect of cholesterol feeding on the system. A method for homogenization of aorta is described which permitted the isolation of subcellular components of aorta, including mitochondria that were morphologically and functionally intact. Mitochondria were identified as the major site of fatty acid synthesis in this tissue. Cofactor requirements and products showed that the synthetic system operates by chain elongation.

Mitochondria from atherosclerotic aortas incorporated acetate into fatty acids faster than did mitochondria from control aortas. It is concluded that cholesterol feeding leads to alterations of aortic mitochondrial function and accelerates the fatty acid elongation pathway.

KEY WORDS aorta · homogenization · mitochondria · electron microscopy · fatty acid · biosynthesis · chain elongation · cholesterol feeding · atherosclerosis · rabbit · oxidative phosphorylation · nonprotein stimulants · NADH · Schmidt degradation.

It has previously been shown in this laboratory that the atherosclerotic rabbit aortic intima has a higher rate of oxygen consumption than does the control (1) and that fatty acid synthesis from acetate is significantly increased in atherosclerotic rabbit aortic intima (2). The differences in oxygen consumption and fatty acid synthesis were greatest in portions of the aorta where lesions were most advanced. Lofland, Moury, Hoffman, and Clarkson have recently shown that fatty acid synthesis is accelerated in atherosclerotic aorta from pigeons (3).

Abbreviations: GLC, gas–liquid chromatography. Fatty acids are denoted by carbon number and number of double bonds.

The objectives of the present study were to identify the subcellular fraction responsible for fatty acid synthesis in aorta, to characterize the system, and to compare the rates of fatty acid synthesis in cell-free systems from atherosclerotic and normal aortas. It was found that mitochondria were most active in fatty acid synthesis and that the synthetic rate was significantly higher in mitochondria from atherosclerotic as compared to control aortas.

METHODS

Male, white New Zealand rabbits, 2.5–4 kg in weight and 9–14 months in age were fed a control diet of Purina rabbit pellets to which was added 10% (by weight) cottonseed oil. The experimental animals were given the same diet to which 2% cholesterol was added. The rabbits had continuous access to food until 30 min before sacrifice. They were killed by pithing at approximately the same time of day for each experiment. The aortas were rapidly removed and divided longitudinally from 1 cm distal to the aortic valves down to the iliac bifurcation. The surrounding tissue and adherent fat were removed by dissection.

Preparation of Subcellular Fractions of Aorta

Obtaining a cell-free preparation of aorta is a difficult technical problem because of the toughness of the tissue. Stein, Stein, and Shapiro (4) have used an all-glass Kontes motor-driven homogenizer for this purpose. The method which, in our hands, gave the best material (both morphologically and functionally) was suggested by Dr. Niels Haugaard, to whom we are indebted. The isolated aortas were rinsed in 0.28 M sucrose (containing 5×10^{-5} M EDTA), blotted, and weighed quickly. A fine mince was made with small, curved scissors. About 200–300 mg of mince was placed in the upper bowl of an

all-glass TenBroeck homogenizer and 6–8 ml of 0.28 M sucrose (containing 5×10^{-5} M EDTA) was added to the barrel. Small amounts of mince were permitted to fall from the bowl into the barrel while the pestle was slowly rotated by hand. It took 4–6 min to obtain a fine, uniform suspension from each 200–300 mg. The homogenizers were made so that the diameter of the plunger was 11.2 mm and the internal diameter of the barrel was 11.7 mm. During the grinding, almost the entire homogenizer was kept under ice water.

Cellular debris was sedimented out of the homogenate at 800 × *g* for 6 min in a Servoll Superspeed Centrifuge. The supernatant suspension was centrifuged at 10,000 × *g* for 15 min. The mitochondrial pellet was washed by suspension in 12–15 ml of the original sucrose medium and was again sedimented at 10,000 × *g* for 15 min. The mitochondria were suspended in 0.04 M phosphate–0.094 M KCl buffer (pH 7.4). Average yields were 12.5 mg of mitochondrial protein per gram of wet weight of normal aorta, and 7 mg of mitochondrial protein per gram of wet weight of atherosclerotic aortas. The difference in yields is due largely to the increased lipid content in the latter group. Total extractable lipid is 2–4 times as high in the experimental as in the control group (per unit of dry weight).

For the isolation of microsomes, the supernatant fraction from the first mitochondrial separation was centrifuged at 100,000 × *g* for 1 hr in a Spinco Model E preparative ultracentrifuge. An aliquot from each cellular fraction was analyzed for protein with the aid of the biuret reagent (5).

A mitochondrial pellet prepared as described above was prepared for electron microscopy in the following way. The pellet was fixed in 1% osmic acid buffered with Veronal–acetate according to the technique of Caulfield (6). It was then dehydrated in graded alcohols and embedded in Ciba Araldite as described by Richardson, Jarett, and Finke (7). Sections were cut with glass knives and viewed with a Siemens Elmiskop IA.

The functional integrity of the mitochondria isolated and prepared in this fashion was examined by measuring P:O ratios. Respiration was measured in a 3 ml chamber with a stationary platinum microelectrode polarized at 0.6 v (8). Hexokinase and glucose were added to the mitochondrial suspension at the time of addition to the polarographic chamber. At zero time, at half time, and at the conclusion of oxygen measurement, aliquots were assayed for glucose-6-phosphate by measuring the generation of NADPH spectrophotometrically in the presence of glucose-6-phosphate dehydrogenase and NADP (9).

Incubation

The various cell fractions were incubated in a phosphate buffer; cofactors and isotopes were added as indicated in the tables. Nicotinamide was added to the incubation mixture to inhibit NAD nucleosidase (10). At the conclusion of the incubation, the entire mixture was hydrolyzed in methanolic sodium hydroxide. Nonsaponifiable lipid was extracted with ether. The alkaline hydrolyzate was acidified and extracted with hexane. The hexane extract was washed with 1% acetic acid and water until the washings were free from radioactivity. The extraction procedure was performed on boiled homogenates after the addition of various amounts of palmitate-1-^{14}C as a test of the reproducibility of the method. The recovery of added labeled palmitate was 90–95%. When acetate-1-^{14}C was added to the boiled homogenate, no significant amount of radioactivity was found in the hexane extract after the usual washings. For radioassay of the fatty acids the hexane extract was evaporated to dryness under a stream of nitrogen in glass counting vials. The residue was redissolved in toluene containing a mixture of 4.0 g/liter of 2,5-diphenyl oxazole and 0.05 g/liter of 1,4-bis[2-(5-phenyloxazolyl)]-benzene and counted in a Packard Tricarb Model 314 liquid scintillation spectrometer. Counting efficiency was approximately 60% and there was virtually no quenching from the sample.

Fatty Acid Composition

Methyl esters were prepared from the extracted fatty acids with diazomethane (11). The fatty acid methyl esters were analyzed by GLC in a Glowall Chromalab 310 gas chromatograph with ^{90}Sr ionization detector. Coiled glass columns, 3.3 m × 3 mm I.D., were packed with 10.5% EGSS-X (an ethylene glycol succinate polyester combined with a silicone, Applied Science Laboratories Inc., State College, Pa.) on Gas-Chrom P, 100–120 mesh, and operated at 185° C. Quantitative results with National Heart Institute Fatty Acid Standard agreed with the stated composition with a relative error of <5% for major components and <12% for minor components. Fatty acids were quantified by planimetry. The fatty acid isotope content was determined by collecting the effluent vapor in glass tubes, 24 cm length, which were changed as each mass peak appeared on the chart. The glass tubes were cooled gradually to room temperature according to the technique described by Schlenk and Sand (12) in order to avoid formation of noncondensable fog. A wad of glass wool on which a small amount of Apiezon had been coated was inserted near the outlet. The condensed methyl esters were washed from the collecting tube into glass counting vials with the scintillator solution. This method of collection gave consistent recoveries of 75–80% with a methyl palmitate-^{14}C standard (Applied Science Laboratories Inc., State College, Pa.) whose purity was determined by thin-layer

chromatography and subsequent autoradiography (13). Carboxyl-^{14}C was determined by means of a modified Schmidt reaction (14).

Enzymes (hexokinase and glucose-6-phosphate dehydrogenase) were obtained from Calbiochem, Los Angeles, Calif. NADH, NADPH, and substrates were obtained from Sigma Chemical Company, St. Louis, Mo. Avidin was purchased from General Biochemicals Div., North American Mogul Products Co., Chagrin Falls, Ohio. Acetate-1-^{14}C was purchased from New England Nuclear Corporation, Boston, Mass., and its radiopurity was checked by paper chromatography in a solvent system of 1-butanol saturated with 1.5 N NH_4OH; all of the radioactivity was contained in a single peak with the R_f of acetate.

RESULTS

Identification of Mitochondria

Fig. 1 illustrates the appearance of material found in the 10,000 × *g* pellet used for the fatty acid synthesis studies. Both electron photomicrographs have the same magnification (×28,000) and illustrate the morphology of the organelles used for assay of fatty acid synthesis rates. Fig. 1a shows mitochondria from the control aorta and 1b similarly obtained material from cholesterol-fed rabbits. The pellet consists almost exclusively of mitochondria, although some are bruised and fragmented.

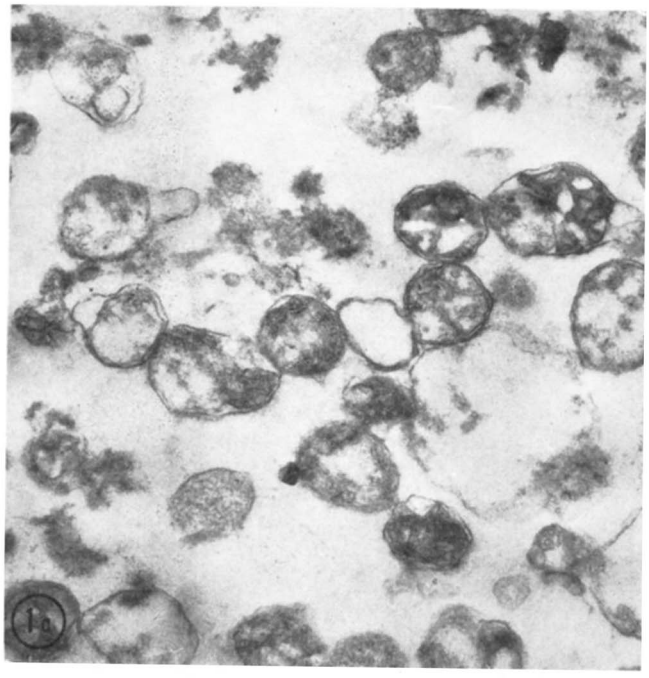

FIG. 1a. Electron micrograph of a portion of a mitochondrial pellet isolated from a homogenate of normal rabbit aorta. In addition to the intact mitochondria, there are some damaged mitochondria and some debris. ×28,000.

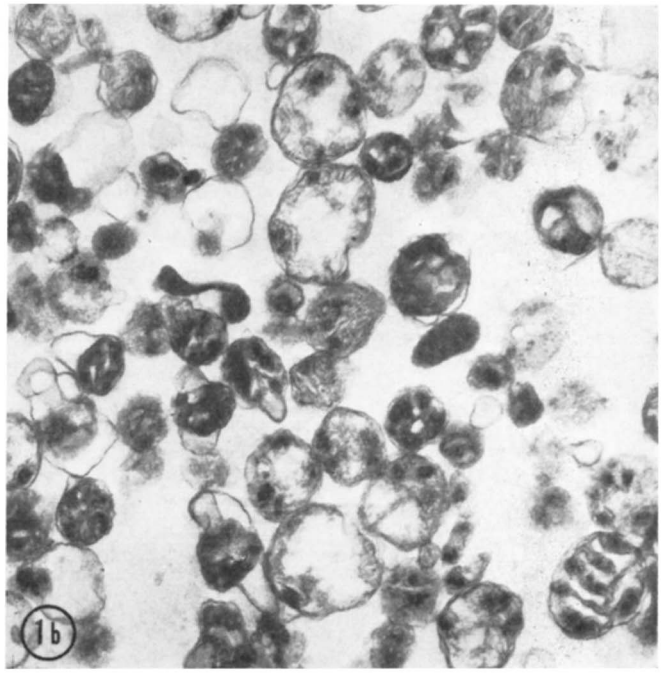

FIG. 1b. Electron micrograph of a portion of a mitochondrial pellet isolated from aorta of a cholesterol-fed rabbit. There are about the same proportions of damaged mitochondria and debris as in Fig. 1a. ×28,000.

Oxidative Phosphorylation of Aortic Mitochondria

P:O ratios were determined for aortic mitochondria from control and cholesterol-fed rabbits; succinate and glutamate plus malate were used as substrates (Table 1). The rates of oxygen consumption determined polarographically confirm our previously reported finding, with intact cells, that the rate of respiration is greatly increased in the cholesterol-fed rabbit aorta (1). ATP formation is not increased, so that P:O ratios are lower. Morphologically, the mitochondria from the cholesterol-fed rabbits (Fig. 1b) are no more damaged than those from the controls (Fig. 1a); no greater force was used in homogenizing the material from the cholesterol-fed animals.

Sites of Fatty Acid Synthesis

The medium originally used for homogenization contained reduced glutathione (15 mM) for the protection of any supernatant fatty acid-synthesizing enzymes. However, it was subsequently found that the supernatant solution contained virtually no activity and that glutathione inhibited by 50% or more the incorporation of acetate in each of the cell fractions (mitochondria, microsomes, and supernatant solution). Reduced glutathione or other mercaptans must be added to the buffer used for isolation of the soluble supernatant fatty acid-synthesizing systems from liver and adipose tissue in order to maintain the SH groups of component enzymes in the

TABLE 1 OXIDATIVE PHOSPHORYLATION OF ISOLATED RABBIT AORTIC MITOCHONDRIA

Diet	Substrate	Oxygen Consumption	ATP Formed	P:O
Control	Glutamate–malate	0.035	0.341	4.8
	Succinate	0.108	0.465	2.1
2% Cholesterol	Glutamate–malate	0.067	0.327	2.4
	Succinate	0.234	0.487	1.04

For each measurement aortas from seven rabbits were pooled to provide sufficient amounts of mitochondria. The medium contained 15 mM triethanolamine HCl (pH 7.4), 20 mM KCl, 10 mM potassium phosphate buffer (pH 7.4), 5 mM $MgCl_2$, 160 mM glucose, and 0.2 mg/ml of hexokinase (Calbiochem, Los Angeles, Calif.) in 0.25 M sucrose. One ml mitochondrial suspension in 0.25 M sucrose was added to 2 ml of the medium in a polarographic chamber (total volume 3 ml). Oxygen consumption and phosphorylation were measured after the addition of 20 μmoles of substrate and 10 μmoles of ADP. Control determinations in the absence of either mitochondria, substrate, or ADP gave zero oxygen consumption and phosphorylation.

TABLE 2 INCORPORATION OF ACETATE-1-^{14}C INTO LONG-CHAIN FATTY ACIDS BY CELLULAR FRACTIONS FROM NORMAL RABBIT AORTA

Fraction	Acetate Incorporation
	μμmoles/mg protein
Mitochondria	71.2
Microsomes	12.3
Supernatant fraction	16.4
Mitochondria	60.9
plus supernatant fraction	92.2*
" dialyzed supernatant fraction	89.5*
" boiled supernatant fraction	100.7*

Cellular fractions were isolated as described in the text. Each incubation flask contained 3 μc (1.5 μmoles) of acetate-1-^{14}C, 0.2 μmole of CoA, 1.0 μmole of Mg^{++}, 10 μmoles of ATP, 2.5 μmoles of nicotinamide, 20 μmoles of citrate, and 0.6 μmole of NADH in a volume of 2 ml. Incubations were for 1 hr at 36° C.

* per milligram of mitochondrial protein.

TABLE 3 COFACTOR REQUIREMENTS FOR ACETATE-1-^{14}C INCORPORATION INTO LONG-CHAIN FATTY ACIDS IN AORTIC MITOCHONDRIA

	Acetate Incorporated	
	Control	Atherosclerotic
	μμmoles/mg protein	
Complete system (2 ml)	125	252
minus ATP (7 μmoles)	22	19
" Mg^{++} (1 μmole)	56	15
" NADPH (0.25 μmole)	119	106
" NADH (0.25 μmole)	32	89
" CoA (0.20 μmole)	14	34
" nicotinamide (2 μmoles)	102	55
" HCO_3^-	126	250
plus avidin*	120	243

Mitochondrial suspensions prepared as described in text. Each incubation flask contained between 1 and 2 mg of mitochondrial protein, plus 3 μc (1.5 μmoles) of acetate-1-^{14}C, 25 μmoles of citrate, and all the other cofactors in the amounts indicated above. Incubations were for 2 hr at 36° C.

* Avidin (300 μg) was incubated for 10 min with mitochondrial suspension before cofactors were added.

reduced form (15) or else there is considerable loss of activity. Inasmuch as reduced glutathione inhibited acetate incorporation in the supernatant fluid from aortic homogenates, what little synthetic activity was present was probably due to particulate (i.e. mitochondrial) fragments or enzymes contaminating the supernatant fraction.

The individual cell fractions were incubated with cofactors and isotope (acetate-1-^{14}C) as indicated in Table 2. It can be seen that most of the fatty acid synthetic activity is present in the mitochondria. When supernatant fluid was added to mitochondria, there was some increase in fatty acid synthesis. A similar increase was seen when boiled supernatant fluid was added to the mitochondria. It is, therefore, likely that this modest enhancement in incorporation is due to a heat-stable factor in the supernant fluid which stimulated mitochondrial fatty acid synthesis. The microsomes did not contribute a significant stimulatory or inhibitory factor.

Cofactor Requirements

When acetate was substrate, ATP, CoA, Mg^{++}, citrate, and NADH were required (Table 3). The cofactor requirements are similar for mitochondria from atherosclerotic aorta. It is noteworthy that bicarbonate is not a requirement and avidin does not inhibit. The potency of the avidin was tested by incubating with a rat liver fatty acid-synthesizing system such as described by Wakil, Porter, and Gibson (15). Avidin (100 μg) inhibited incorporation of acetate-1-^{14}C CoA into long-chain fatty acids by this enzyme preparation (4.0 mg of protein) to 10% of control values. The optimal concentrations of cofactors were determined for the system and used in all subsequent experiments. Since the omission experiments indicated that NADH was a more strict requirement than NADPH, we studied the different adenine dinucleotides and combinations of them. Reduced NAD always gave the highest level of incorporation of acetate into fatty acids and this was not enhanced by combination with other adenine dinucleotides.

Fatty Acid Synthesis by Normal and Atherosclerotic Aortic Mitochondria

Aortic mitochondria from twelve rabbits from the control and cholesterol-fed groups were studied under identical conditions. There was a marked difference in the rate of acetate incorporation into fatty acid (Table 4) and the difference is highly significant ($P < 0.005$). In fact the difference in rates of synthesis is similar to that formerly demonstrated in the whole tissue (2). The in-

TABLE 4 FATTY ACID SYNTHESIS BY NORMAL AND ATHEROSCLEROTIC RABBIT AORTIC MITOCHONDRIA

	Number of Aortas	Acetate Incorporated
		μμmoles/mg protein
Normal	12	105.3 ± 5.95*
Atherosclerotic	12	443.6 ± 69.8*

Each incubation flask contained 1–2 mg of protein, 3 μc (1.5 μmoles) of acetate-1-^{14}C, 0.2 μmole of CoA, 10 μmoles of $MgCl_2$, 2.5 μmoles of nicotinamide, 10 μmoles of ATP, 20 μmoles of citrate, and 0.6 μmole of NADH. Incubations were for 2 hr at 36° C.
* SEM.

TABLE 5 EFFECT OF VARIOUS COMBINATIONS OF CELLULAR FRACTIONS ON LONG-CHAIN FATTY ACID SYNTHESIS FROM ACETATE

	Acetate Incorporated
	μμmoles/mg protein
Normal mitochondria	117
plus normal supernate	195
" atherosclerotic supernate	120
Atherosclerotic mitochondria	283
plus normal supernate	151
" atherosclerotic supernate	104

Incubations were performed under the same conditions as for Table 4.

creased rate of fatty acid synthesis by the tissue is, therefore, a consequence of altered mitochondrial function.

Fatty Acid Synthesis by Mixtures of Cell Fractions

The data given in Table 5 are from two rabbits, but similar data have been obtained from three similar pairs. From these recombination experiments it was determined that the increased incorporation in the atherosclerotic material is not due to a stimulatory factor in the atherosclerotic cytoplasm. The supernatant fraction from both normal and atherosclerotic aortas is inhibitory to atherosclerotic mitochondria. The normal supernatant fraction stimulates activity only when added to normal mitochondria.

Products of Synthesis

The fatty acid content (per milligram of protein) is reduced about 35% in the mitochondria from the cholesterol-fed group and increased about 67% in the cytoplasm from the same animals (Table 6). There is much more supernatant protein than mitochondrial protein in the aorta and therefore, the total cellular fatty acid content per gram of tissue is about twice as high in the cholesterol-fed aorta (2).

The percentage composition of the fatty acids found in mitochondria and cytoplasm from both normal and atherosclerotic aortas is illustrated in Table 6. Consistent quantitative differences between the fatty acid compositions of normal and atherosclerotic cell fractions have been observed in a large number of analyses. The largest percentage change between both groups (in both mitochondria and cytoplasm) is in the 20:2 acid. The identity of this acid has been confirmed (*a*) by its retention volumes on both polyester and Apiezon GLC columns, (*b*) by its disappearance from the chromatogram after bromination of the mixture, and (*c*) by isolation, hydrogenation, and demonstrated conversion to the 20:0 methyl ester.

Our particular interest in this 20:2 acid was a consequence of the distribution of the ^{14}C from acetate among the fatty acids (Table 7). It was necessary to collect the

TABLE 6 FATTY ACID COMPOSITION OF MITOCHONDRIAL AND CYTOPLASMIC LIPIDS FROM RABBIT AORTIC CELL FRACTIONS

	Mitochondria		Cytoplasm	
Fatty Acid	Normal	Cholesterol-Fed	Normal	Cholesterol-Fed
	area per cent of peaks			
14:0	1.4	1.4	1.4	2.2
16:0	21.4	22.6	15.7	17.2
16:1	3.3	2.5	2.9	2.8
17:0	0.6	0.4	1.6	3.5
18:0	13.4	10.8	18.3	17.2
18:1	24.6	26.3	23.2	22.5
18:2	20.0	28.0	16.3	22.3
20:0	0.4	0.3	1.5	1.1
18.3	2.7	1.4	2.1	1.6
20:2	0.1	1.5	0.1	0.9
22:0	5.2	2.3	4.4	3.1
20:4	4.2	2.5	6.3	1.5
>20:4*	0.8	0.0	1.7	0.5
	mg/mg protein			
	0.463	0.302	0.976	1.628

* Includes fatty acids with retention volumes greater than that of 20:4, up to and including 24:0.

TABLE 7 DISTRIBUTION OF ACETATE-^{14}C INCORPORATED INTO FATTY ACIDS FROM NORMAL AORTIC MITOCHONDRIA

Fatty Acids	Per Cent of Total Recovered Radioactivity
<16:0	4.5
16:0	2.3
16:1	1.7
18:0	8.2
18:1	5.6
18:2	0.0
20:0 + 18:3	14.4*
20:2	33.0*
22:0	0.0
20:4	4.6
>20:4	24.8*

The percentage figures of recovered radioactive fatty acids are averages of three separate GLC analyses. Two rabbit aortas were used for each of the three studies. The percentage values for the (*) fatty acids did not vary by more than 5% among the three analyses.

20:0 and 18:3 acids together for radioactive assay because there was some overlap of these peaks on this polyester column. Most of the recovered radioactivity was found in the collections designated 20:0 plus 18:3, 20:2, and >20:4. Even if no mass peaks could be discerned after 20:4, collection of effluent up to the time at which the 24:0 methyl ester would emerge demonstrated that a large amount of the radioactivity was in this material. From the distribution of label it appears that, most probably, acetate is added to preformed acyl units, with the elongation of 18:0 to 20:0, of 18:2 to 20:2, and of either 22:0 to 24:0 or C_{20} polyunsaturates to C_{22} polyunsaturates.

The total fatty acids were subjected to Schmidt degradation and the carboxyl-^{14}C:alkyl-^{14}C ratio was 10:1 (with average chain length calculated from the GLC data). This shows clearly that a chain elongation process was responsible for fatty acid labeling in this tissue.

DISCUSSION

The study of the metabolism of arterial tissue requires a satisfactory method for homogenization before cell fractions can be separated and individual enzymes isolated. The method described is demanding in terms of energy and time, but does permit mitochondria to be isolated that are, for the most part, structurally intact. This does not imply that morphologic integrity is essential for fatty acid synthesis. In fact treatment of these mitochondria by freezing and thawing or sonication does not destroy their ability to synthesize fatty acids (unpublished observations). This is not surprising inasmuch as it is well known that submitochondrial particles are capable of performing oxidative phosphorylation (16).

The reason for the reduction in P:0 ratio in the atherosclerotic mitochondria is not known. It has been reported that essential fatty acid deficiency will result in impaired phosphorylating efficiency in rat liver mitochondria (17). Whether or not cholesterol feeding causes a similar defect in coupling is not known. An alternative possibility is that oxygen may be used in reactions not coupled to phosphorylation, such as the peroxidation of polyunsaturated fatty acids.

Enzymes that synthesize fatty acids are found in the soluble supernatant fraction of the cell in those tissues with a large capacity for fatty acid synthesis, such as liver, mammary gland, and adipose tissue (18–20). Fatty acid synthesis in aorta occurs predominantly in the mitochondria. This is a distinction that the aorta shares with myocardium where, as Hülsmann has shown (21), the most active fatty acid synthesis occurs in sarcosomes.

The aortic mitochondrial system is an "elongation" system such as has been described by Wakil, McLain, and Warshaw (22). The failure of avidin to inhibit and the lack of requirement for bicarbonate indicate that malonyl CoA is not an intermediate. The carboxyl-labeling ratio and distribution of label suggest that acetate units are added to the carboxyl end of 16:0, 18:0, 18:2, and perhaps C_{20} unsaturated acids. The data indicate that 18:2 is the most active receptor of these acetate units and the resulting 20:2 has the greatest specific activity. In this regard it is worth noting that this 20:2 acid shows the greatest increment in percent composition when normal and atherosclerotic mitochondria are compared. Whether or not there is a correspondingly large change in rate of labeling of this acid in the atherosclerotic mitochondria is currently being evaluated.

A feature of this system is the failure of NADPH (alone or in combination with NADH) to give maximal incorporation. In all fatty acid-synthesizing systems which have so far been purified, one or both of the reductive steps have been shown to require NADPH. It is interesting, therefore, that NADH appears to be a better hydrogen donor in this system. Since these are intact mitochondria, NADH may not be the direct hydrogen donor. In the presence of ATP, transhydrogenation (NADH + NADP $\rightleftarrows$ NADPH + NAD) may occur to form NADPH inside the mitochondria and make it available at the intramitochondrial site of fatty acid synthesis.

We previously observed a faster rate of fatty acid synthesis in the atherosclerotic aorta than in the normal aortic tissue. The present study identifies the mitochondria as the subcellular fraction that is responsible for the accelerated synthetic rate. The supernatant fraction from normal aorta contains a heat-stable, nonprotein factor which stimulates the activity of normal mitochondria, but this fraction inhibits synthesis by atherosclerotic mitochondria, as does the supernatant fraction of atherosclerotic aorta. The mechanism by which cholesterol feeding alters the mitochondrial fatty acid synthetic rate is therefore not dependent simply on the presence of cholesterol in the cytoplasmic fluid. Since there are also significant changes in the rate of oxygen consumption and efficiency of phosphorylation in the atherosclerotic mitochondria, it appears that cholesterol feeding leads to fundamental alterations in mitochondrial function and perhaps structure. The mechanisms by which such changes are brought about are currently being studied.

The excellent assistance of Mrs. Margaret Orishimo and Dr. Franklin E. Hull is gratefully acknowledged. Electron microscopic examination and photographs were provided by Dr. Leonard Berwick of the Department of Pathology; oxygen electrode studies were done with the equipment and assistance of Dr. Ronald Estabrook in the Johnson Research Foundation; Schmidt degradations were performed by Dr. Joseph Rabinowitz of the Department of Biochemistry of the School of Dental

Medicine, all of this institution. The author is grateful to Drs. Howard Rasmussen and Julian B. Marsh for their helpful suggestions.

The author is recipient of a U.S. Public Health Service Research Career Program Award 5-K3-HE-7495 from the National Heart Institute. This work was supported by a Grant-In-Aid from the American Heart Association (62-G112) and by PHS Research Grants HE-5139, HE-08805-01 and HE-07290 from the U.S. Public Health Service.

Manuscript received 19 April 1966; accepted 2 June 1966.

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