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FATTY ACID SYNTHESIS IN CELL-FREE PREPARATIONS OF HUMAN ADIPOSE TISSUE

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SUMMARY

Comparison of normal human omental adipose tissue with the lipoma has shown that both contain the same fatty acids. The soluble enzymes of both require the same cofactors for fatty acid synthesis and incorporate radioactive acetate mainly into myristic and palmitic acids. Avidin inhibits the synthesis of fatty acids by both tissues and this inhibition is reversed by biotin. However, the lipoma synthesizes more fatty acids per gram of protein than adjacent normal subcutaneous fat. It is concluded that the lipoma and normal adipose tissue synthesize fatty acids by a pathway similar to that found in liver and yeast involving the formation of malonyl-CoA. In both normal adipose tissue and the lipoma, only the mitochondrial fraction was found to be capable of incorporating [1-^{14}C]acetate into a fatty acid which had the retention time on gas–liquid chromatographic analysis of an octadecatrienoic acid.

INTRODUCTION

It has been demonstrated within the past few years that adipose tissue is a very active site of lipid synthesis. Fat metabolism has been extensively studied in the rat-epididymal fat pad[1,2]. Cell-free preparations of the locust fat body[3], and slices of human adipose tissue[4] have also been shown to convert acetate into long-chain fatty acids. A comparison of the synthesis of lipids by slices of human normal subcutaneous adipose tissue and the benign fatty tumor, the lipoma, indicated that the rate of acetate incorporation into lipids was greater in the lipoma than in normal adipose tissue although the same lipids were synthesized by both tissues[4]. The mechanism of this synthesis was not determined.

Although the mechanism of fatty acid synthesis proceeds through the malonyl-CoA pathway in various species and tissues (liver, brain, mammary gland, rat-epididymal fat, locust fat body), demonstrable differences in the cofactor requirements, the chain length of the fatty acids synthesized and the intracellular site of fatty acid synthesis are evident[5–8]. It was therefore of interest to attempt to elucidate the mechanism of fatty acid synthesis in human adipose tissue and the cellular control mechanisms regulating the different rates of synthesis in normal subcutaneous adipose tissue and

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lipoma. This paper describes the results of studies on the synthesis of long-chain fatty acids by homogenate fractions prepared from such human adipose tissues. It has been found that the soluble enzymes of both normal omental adipose tissue and lipoma have the same cofactor requirements and synthesize fatty acids by a mechanism involving the formation of malonyl-CoA. The mitochondria and microsomes of human adipose tissue also participate in the biosynthesis of lipids. Compared to the adjacent normal subcutaneous tissue, a larger proportion of the cellular protein of the lipoma is specialized for the synthesis of fat. Only the mitochondrial fraction was found to be capable of incorporating [1-^{14}C]acetate into a fatty acid which had the retention time on gas–liquid chromatographic analysis of an octadecatrienoic acid. This acid may be linolenic acid or its positional isomer. Preliminary accounts of this work have been presented[9,10].

METHODS

Preparation of cell-free systems

Omental fat was obtained from 16 surgical patients undergoing abdominal operations for various reasons. Lipomas were removed from 8 patients under block local anesthesia. When sufficient adjacent subcutaneous tissue was available this was dissected immediately after removal of the lipoma. The tissues were kept at room temperature for an average of 20 min until ice-cold 0.25 M sucrose was added prior to homogenization. The remaining procedures were carried out at 0–4°. The tissue obtained from each patient was homogenized in a Waring blendor for 1 min at top speed in 1.5 vol. of 0.25 M sucrose. The homogenate was filtered through gauze and was then centrifuged at 2000 × *g* for 15 min to remove cell debris. The supernatant solution (with the floating fatty layer removed) was designated the nuclei-free homogenate. This nuclei-free homogenate was used for the initial incubation experiments and subsequent purification steps.

A soluble supernatant solution was obtained by centrifuging the nuclei-free homogenate in a Spinco preparative ultracentrifuge for 60 min at 100000 × *g*. This fraction was further purified by adding solid ammonium sulfate, with constant stirring, to bring the solution to 70 % saturation. The protein precipitate was dissolved in a solution that was 0.001 M with respect to cysteine and 0.04 M with respect to potassium bicarbonate, and dialyzed for 2 h against 4 l of the same solution. This fraction was designated the 0–70 % ammonium sulfate-precipitated soluble supernatant solution (AMS sol. spt.). In order to demonstrate the requirements for nucleotide-containing cofactors for fatty acid synthesis, 1 ml of Dowex-1-Cl was added to each 10 ml of the AMS sol. spt. and stirred for 15 min. The Dowex was then spun down in a clinical centrifuge and the supernatant enzyme solution was designated Dowex-treated AMS soluble supernatant solution (Dowex-AMS sol. spt.).

To obtain subcellular particles the nuclei-free homogenate was centrifuged at 8000 × *g* for 30 min at 0–4°. The precipitated mitochondria were washed 3 times with 0.25 M sucrose and then resuspended in sucrose. To obtain microsomes the 8000 × *g* supernatant fraction was centrifuged in the Spinco preparative ultracentrifuge at 100000 × *g* for 60 min.

The protein concentration of each subcellular fraction was determined, following Kjeldahl digestion, by the Nessler method. The protein concentration varied greatly among the tissue samples.

Incubation and extraction procedures

The various cell fractions of adipose tissue were incubated without shaking in stoppered glass tubes at 37° in $N_2 - CO_2$ (95:5). Unless otherwise specified, the pH was made 6.5 with phosphate buffer and the final volume brought to 3.0 ml. After the incubation period the reaction was stopped with 3 ml of a 36% potassium hydroxide solution in methanol. The mixture was heated in a boiling-water bath for 60 min, and the free fatty acids were extracted by previously described methods after the solution was acidified with sulfuric acid to pH 2 (see ref. 11). It was ascertained in control studies, by previously described methods[12], that less than 2% of the radioactivity from [1-^{14}C]acetate was incorporated into non-saponifiable compounds.

Gas–liquid chromatography and counting

Methyl esters of the free fatty acids were prepared to put on the Barber–Coleman gas–liquid chromatography apparatus (Model 10) by a modification of the method of METCALFE AND SCHMITZ[13] or of STOFFEL *et al.*[14]. Concentrated sulfuric acid was used in place of dry hydrochloric acid. The chromatography columns were 4 mm wide, U-shaped, 6 or 8 ft long and were packed with 16–17% ethylene glycol succinate on 80–100 mesh celite. A radium detector and argon gas were used. Known standards were run on each column to identify the fatty acid methyl ester peaks. In order to determine the distribution of radioactivity among fatty acids of different chain-lengths, individual fatty acid methyl esters were collected from the column in cartridges-containing siliconized anthracene[15]. The radioactivity was measured in a scintillation counter. A detailed description of these techniques has been reported[16].

Spectrophotometric studies

Each enzyme fraction was assayed in phosphate buffer for glucose-6-phosphate dehydrogenase and isocitric dehydrogenase activity according to previously described methods[17,18]. The soluble supernatant enzyme fractions were also assayed for DPN kinase and transhydrogenase activities by the methods of WANG[19] and COLOWICK *et al.*[20].

MATERIALS

[1-^{14}C]Acetate was purchased from New England Nuclear Corporation. ATP, TPN, DPN, and glucose 6-phosphate were bought from Sigma Chemical Corporation, isocitrate from Mann Biochemical Corporation and crystalline glucose-6-phosphate dehydrogenase from Boehringer and Soehne, Mannheim. Avidin (2.5 units per mg) and biotin were purchased from Nutritional Biochemical Corporation.

RESULTS

Cofactor requirements for fatty acid synthesis

The cofactor requirements of various fractions of normal omental adipose and lipoma tissues are shown in Table I. CoA, ATP and manganese and magnesium ions were requirements for fat synthesis. DPN or TPNH or DPNH could partially substitute for TPN in the Dowex-treated AMS soluble supernatant fraction. The require-

ment for DPN and TPN was variable if the enzymes were not pretreated with the resin. Maximal synthesis was obtained when cysteine, isocitrate and bicarbonate were also present. Fatty acid synthesis was inhibited 70% when the enzyme was prepared in sucrose and incubated in a bicarbonate-free reaction mixture with an atmosphere of nitrogen. Gassing with $N_2 - CO_2$ (95:5), however, almost completely restored activity. Mitochondrial synthesis of fatty acids was uninfluenced by the absence or presence of bicarbonate ions. Replacement of isocitrate by glucose 6-phosphate depressed synthesis about 75%. Addition of crystalline glucose-6-phosphate dehydrogenase to the system containing glucose 6-phosphate did not significantly stimulate fatty acid synthesis. When glucose 6-phosphate was added with isocitrate there was no significant inhibition of fatty acid synthesis although it might be expected that acetate formed from the glucose 6-phosphate *via* the Embden–Meyerhof pathway would dilute the [1-^{14}C]acetate added to the reaction mixture. Fatty acid synthesis was sharply dependent on CoA concentration as shown in Fig. 1.

Effect of incubation time and pH

The incorporation of [1-^{14}C]acetate increased with time for at least 3 h (Fig. 2). A slight lag presumably resulted from slow temperature equilibration to 37° since the tubes were not shaken. A 2-h incubation period was used in all other experiments. The optimum pH for fat synthesis was found to be between 6.5 and 7.

TABLE I

COFACTOR REQUIREMENTS FOR INCORPORATION OF [1-^{14}C]ACETATE INTO FATTY ACIDS

Percent of total counts/min incorporated. The complete system contained 200 μmoles of phosphate buffer (pH 6.5), 0.1 μmole CoA, 15 μmoles ATP, 2 μmoles TPN, 2 μmoles DPN, 20 μmoles cysteine, 20 μmoles DL-isocitric acid, 1.0 μmole Mn^{2+}, 5.0 μmoles Mg^{2+}, 10 μmoles $KHCO_3$, 7.5 μmoles sodium [1-^{14}C]acetate, $3 \cdot 10^6$ counts/min and 1.0 ml of enzyme solution in a final volume of 3.0 ml. 2 μmoles DPNH or TPNH were substituted where indicated. 20 μmoles of glucose 6-phosphate were substituted for isocitrate. All tubes were incubated for 2 h in an atmosphere of N_2–CO_2 (95:5), except pure nitrogen was used to show the requirement for $KHCO_3$. The values given are averages of 2–6 separate experiments.

System	*Normal omental fat*			*Lipoma*	
	AMS sol. spt.	*Dowex-AMS sol. spt.*	*Sol. spt. + microsomes*	*Dowex-AMS sol. spt.*	*Sol. spt. + microsomes*
Complete	100	100	100	100	100
−CoA	0–3	0–1	0–1	0–1	0–7
−ATP	0–3	0–2	0–1	0–1	0–3
−TPN, −DPN	3–80	2–5	40	—	27
−TPN	—	62	92	—	63
−DPN	—	88	95	—	94
−TPN, −DPN, +DPNH	35	35	—	—	—
−TPN, −DPN, +TPNH	—	39	—	—	—
−Cysteine	20	—	81	10	56
−Isocitrate	2	—	3	25	9
$-Mn^{2+}$, $-Mg^{2+}$	—	3	0–1	—	3
$-Mn^{2+}$	—	38	13	—	18
$-Mg^{2+}$	—	31	66	—	68
$-KHCO_3$	—	—	22	—	33
−Isocitrate +Glc-6-*P*	25	—	—	—	—

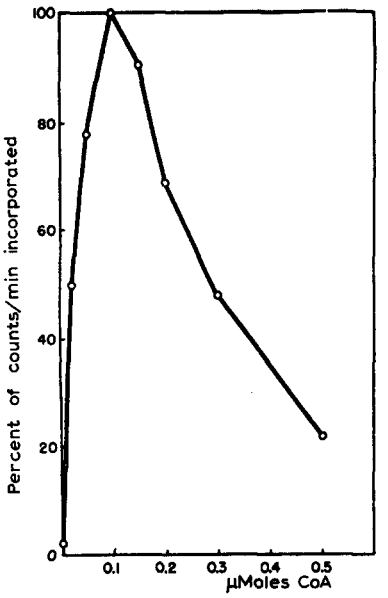

Fig. 1. Effect of CoA concentration on [1-^{14}C]-acetate incorporation into fatty acids. Reaction conditions as described in Table I except for variation in CoA concentration.

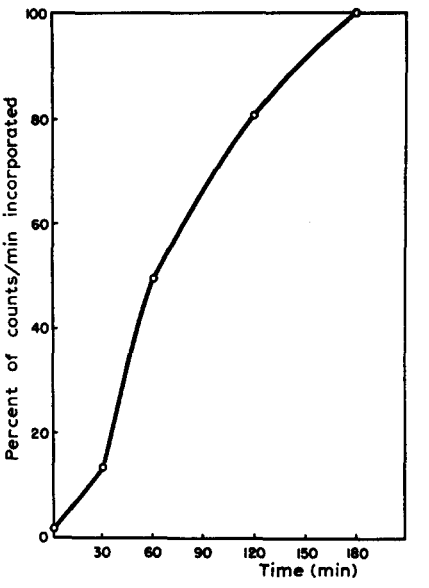

Fig. 2. Effect of the time on [1-^{14}C]acetate incorporation. Reaction conditions as described in Table I.

Fatty acid synthesis by the homogenate fractions

Since the patients used in this study were uncontrolled with respect to diet, weight, anesthesia and genetics, there was considerable variation from specimen to specimen in the specific activity of the fatty acid-synthesizing system (μmoles of acetate converted to fatty acids per gram protein). Table II shows the range of activity for different cell fractions of normal omental fat and lipoma tissue. The specific activity was increased by precipitation of the soluble supernatant solution with ammonium sulfate. The soluble supernatant solution, the soluble supernatant solution plus microsomes, and the mitochondria, were each capable of incorporating [1-^{14}C]acetate into long-chain fatty acids. Microsomes alone had no activity. About 25 % of the fats synthesized by the mitochondria were in the form of triglycerides whereas the other homogenate fractions incorporated at least 95 % of the radioactivity into free fatty acids. The addition of extra microsomes to the soluble supernatant solution of the lipoma increased the synthesis of fat although a different proportion of microsomes added to the omental supernatant solution had no demonstrable effect.

DPN kinase and transhydrogenase

Since, as shown in Table I, DPN could partially substitute for TPN, the possibility of the interconversion of these coenzymes was considered. However, no significant DPN kinase nor transhydrogenase activity could be demonstrated in any homogenate fraction of lipoma or normal adipose tissue. A small amount of kinase activity could be demonstrated in the nuclei-free homogenate of the rat epididymal fat pad. A change in absorbancy of 0.067/min/mg protein occurred.

During the kinase determinations it was observed that when the human enzyme

TABLE II

SPECIFIC ACTIVITY OF HOMOGENATE FRACTIONS OF OMENTAL FAT AND LIPOMA

The incubation system is described in Table I. Specific activity expressed as μmoles of acetate converted to fatty acids per gram protein. In experiments 6a and 12a the soluble supernatant plus microsomes was frozen 2–4 days before testing.

Expt. No.	*Nuclei-free homogenate*	*AMS sol. spt.*	*Dowex-AMS sol. spt.*	*Sol. spt. + microsomes**	*Mitochondria*	*Sol. spt.*
			Omental fat			
1	3	24	—	—	—	—
2	82	190	155	—	—	—
3	113	288	—	—	—	—
4	639	771	1817	—	—	—
5	791	1988	2028	—	—	—
6	—	—	—	942	118	847
6a	6* plus 0.031 mg microsomal protein			1017	—	920
			Lipoma			
7	13	82	83	—	—	—
8	54	—	—	20	155	—
9	66	—	—	46	—	—
10	103	—	—	74	—	—
11	329	—	—	271	153	—
12	—	—	—	19	—	—
12a	12* plus 0.012 mg microsomal protein			54	—	3

fractions were incubated with both DPN and ATP a significant increase in the 340-mμ absorption occurred. For the soluble supernatant plus microsome fraction this increase was 1.5 and 10.4 per mg protein after 60 min. The identity of the endogenous substrate for this possible reduction of DPN is not known.

Glucose-6-phosphate dehydrogenase and isocitric dehydrogenase

All the homogenate fractions studied, except the mitochondria and microsomes, contained both TPN-linked glucose-6-phosphate and isocitric dehydrogenases. The mitochondria lacked glucose-6-phosphate dehydrogenase and the microsomes had no demonstrable amounts of isocitric dehydrogenase activity. In the other homogenate fractions the rate of TPNH formation was 2- to 3-fold greater with isocitrate as substrate than with glucose 6-phosphate (Table III). No significant amount of DPN-linked isocitric or glucose-6-phosphate dehydrogenase activity was found in any of the adipose tissue extracts.

Products of the reaction

The fatty acids synthesized from [1-^{14}C]acetate by the homogenate fractions of both the lipoma and omental fat are shown in Table IV. The AMS sol. spt., with or without Dowex-1-Cl treatment, synthesized mainly palmitic (66 %) and myristic (22 %) acids. The soluble supernatant fraction together with the microsomes gave a somewhat different distribution of the counts with radioactivity also occurring in stearic (7 %) and linolenic (5 %) acids. The major fatty acids synthesized by the mitochondria were in the area tentatively identified as linolenate (25 %), and in stearate (18 %), oleate (12 %), palmitate (9 %), myristate (11 %), and laurate (12 %). In order

TABLE III

ISOCITRIC AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITIES IN HOMOGENATE FRACTIONS OF NORMAL FAT AND LIPOMA

The dehydrogenases were compared on the basis of change in absorbancy per mg protein per min at 340 mμ.

Fraction	*Isocitric dehydrogenase / glucose 6-phosphate dehydrogenase*	
	Normal fat	*Lipoma*
Nuclei-free homogenate	1.8	2.7
Soluble supernatant plus microsomes	2.3	2.2
Soluble supernatant	2.1	—

TABLE IV

PER CENT OF INCORPORATION OF [1-^{14}C]ACETATE INTO THE FATTY ACIDS SYNTHESIZED BY HOMOGENATE FRACTIONS OF LIPOMA AND OMENTAL FAT

The individual fatty acids eluted from the gas chromatography column were collected in vials containing anthracene. The values given are the average of 2–5 separate experiments. The same results were obtained with lipoma and omental fat tissues and combined data are given here.

	Fatty acid											
Fraction	*C 10*	*C 12*	*C 14*	—	*C 16*	*C 16-1*	—	*C 18*	*C 18-1*	—	*C 18-2*	*C 18-3*
AMS-soluble supernatant	—	1 ± 0.7	22 ± 5	3 ± 3	66 ± 10	3 ± 0.9	0	2 ± 1	1 ± 0.6	0	1 ± 0.3	1.0 ± 0.6
Soluble supernatant plus microsomes	1 ± 0.4	1.5 ± 0.4	30 ± 3	2 ± 0.3	46 ± 5	3 ± 0.2	1 ± 0.5	7 ± 2	2.5 ± 0.4	—	1 ± 0.6	5 ± 2.6
Mitochondria	2	12	11	2	9 ± 2	3	2	18 ± 4	12 ± 2	1	3	25 ± 3

to check the retention time, pure linolenate carrier was added to a duplicate aliquot of the extracted mitochondrial lipids and the column effluent corresponding to the linolenate peak was collected and counted. This peak contained 70 % of the presumed linolenate radioactivity.

Effect of avidin

Avidin inhibited the synthesis of fatty acids by homogenate fractions of omental, normal subcutaneous and lipoma fat. A typical experiment is shown in Fig. 3. Preincubation of the enzymes with 20 μg of biotin completely prevented any avidin inhibition. In the absence of avidin, biotin had no effect on the rate of fatty acid biosynthesis.

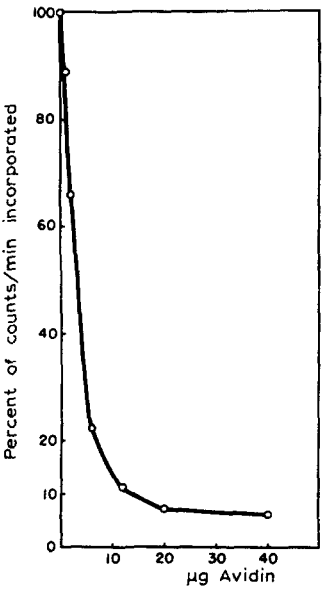

Fig. 3. Inhibition of [1-^{14}C]acetate incorporation into fatty acids by avidin. Conditions as described in Table I except for addition of avidin. Omental sol. spt. plus microsomes = 0.212 mg protein per tube. 100 % incorporation equals 942 μmoles acetate incorporated per gram protein.

Comparison of lipoma and adjacent normal subcutaneous fat

The fatty acid-synthesizing ability of the soluble supernatant solution plus microsome fraction of the lipoma was 3 times greater than that of adjacent normal fat in terms of incorporation of acetate per gram wet weight of tissue, and 7 times

TABLE V

INCORPORATION OF [1-^{14}C]ACETATE INTO LONG-CHAIN FATTY ACIDS BY SOLUBLE SUPERNATANT PLUS MICROSOMES OF LIPOMA AND NORMAL SUBCUTANEOUS FAT

Incubation conditions as in Table I.

Tissue	*μmoles incorporated per gram tissue*	*μmoles incorporated per gram protein*
Lipoma	0.0319	271
Normal	0.0102	39

greater on the basis of incorporation per gram of protein (Table V). The lipoma contained 2.6 times more isocitric dehydrogenase and 3.3 times more glucose-6-phosphate dehydrogenase per gram protein than the subcutaneous fat. The change in absorbancy per min per mg protein was 5.3 for isocitric dehydrogenase and 2.1 for glucose-6-phosphate dehydrogenase in the lipoma.

DISCUSSION

The biosynthesis of fatty acids by cell-free preparations of normal human adipose tissue and lipoma has been shown to require ATP, CoA, TPN or DPN, Mn^{2+}, Mg^{2+}, isocitrate, cysteine and bicarbonate. These cofactor requirements are similar to those reported for lipid synthesis by avian- and rat-liver soluble supernatant enzyme systems[21-23]. Since neither DPN kinase nor transhydrogenase is present in human adipose tissue soluble enzyme fractions, the lack of specificity for DPN or TPN is apparently not the result of the transformation of one pyridine nucleotide into the other. A similar substitution of DPN for TPN has been shown to occur in liver[24-26] and mammary-gland enzyme systems[27]. DPNH could not substitute for TPNH, however, in the soluble supernatant system from the rat epididymal fat pad[2].

In the human fat system glucose 6-phosphate cannot substitute for isocitrate although both glucose-6-phosphate and isocitric dehydrogenases are present and have herein been shown to generate TPNH from added TPN and their respective substrates. This suggests that the oxidation of isocitrate may do more than supply TPNH for fatty acid synthesis. ABRAHAM *et al.*[28] suggests that isocitrate may be involved in the carboxylation of acetyl-CoA to form malonyl-CoA. MARTIN AND VAGELOS[29] have shown that citrate, and to a lesser extent isocitrate, directly activate acetyl-CoA carboxylase. The formation of malonyl-CoA from acetyl-CoA, a biotin-requiring enzymic step which is inhibited by avidin[30], requires manganese and bicarbonate ions[31] and is involved in the synthesis of long-chain fatty acids[32-34]. The soluble fraction of human adipose tissue forms malonyl-CoA, as shown by the inhibition of fatty acid synthesis when avidin is added to, or manganese or bicarbonate ions are omitted from the incubation medium.

The soluble supernatant solution plus microsome fraction of human adipose is also inhibited by avidin and has essentially the same cofactor requirements as the purified soluble supernatant except that there is a less striking requirement for cysteine. WAKIL *et al.*[11] and BRADY *et al.*[35] have shown that the enzymes for fatty acid synthesis contain sulfhydryl groups which are protected by cysteine. The diminished requirement for cysteine in the presence of microsomes suggests that (a) the microsomal protein is protecting the –SH groups of the soluble enzyme or (b) there is an additional microsomal synthesis of fatty acids which does not require cysteine. Several investigators have demonstrated that the addition of microsomes stimulates fatty acid synthesis by the soluble enzymes[6,36-38] but none has observed synthesis from acetate with microsomes alone. ABRAHAM *et al.*[38] have recently demonstrated that the stimulatory effect of the microsomes occurs over only a narrow range of concentrations. This may explain the diverse effects resulting from the addition of different amounts of microsomes to our lipoma and normal omental fat-soluble enzyme systems.

The washed mitochondria of our human adipose tissues do not require CO_2 for fat synthesis. WAKIL *et al.*[39] have shown that liver mitochondria and the soluble enzymes synthesize lipids by separate pathways, and BARRON AND STUMPF[40] have demonstrated that the mitochondrial fraction of the avocado is associated with the synthesis of unsaturated fatty acids *via* a different pathway than that used in saturated fatty acid biosynthesis.

As in other mammalian systems, three fractions of human adipose tissue are active in the synthesis of fatty acids. The soluble enzymes synthesize mainly palmitic and myristic acids. Addition of microsomes results in a slightly different incorporation of ^{14}C into the long-chain fatty acids which may be caused by contamination with mitochondria. The washed mitochondria synthesize mainly octadecanoic acids as well as some laurate, myristate and palmitate. The major acid formed by the mitochondria appears in the linolenate region of the gas chromatographs. It should be noted that it is not yet known whether this acid was completely synthesized *de novo*, nor has a separation of the positional isomers been attempted. Linolenic acid comprises 0.3–0.5% of the total fatty acids in normal subcutaneous adipose tissue in man[41]. Other tissues and organisms also contain and can synthesize linolenic acid. Labeled linoleic plus linolenic acids were found after incubating whole blood with radioactive acetate[42,43]. Two highly labeled octadecatrienoic acids (one of which was linolenic) were isolated from the tissues of rats after feeding [1-^{14}C]methyl *cis*-12-octadecanoate[44]. The fungus *Trichoderium viride* also synthesizes linoleic and linolenic acids of relatively high specific activity from [1-^{14}C]acetate[45]. GETZ AND BARTLEY[46] found that mitochondria contain about 44% of the total polyunsaturated fatty acids of rat liver. Thus the synthesis of the unsaturated long-chain fatty acids may be the exclusive property of the mitochondria.

The comparison of normal human omental adipose tissue with the lipoma has shown that both contain the same fatty acids, require the same cofactors for fatty acids synthesis, and incorporate radioactive acetate into the same long-chain fatty acids. Both are inhibited by avidin and this inhibition is reversed by biotin. However, although lipid synthesis is greater in omental fat than in subcutaneous adipose tissue[47], the lipoma synthesizes more fatty acids per gram of protein than adjacent normal subcutaneous fat. This suggests that the lipoma contains a larger proportion of proteins which are active in the synthesis of fat than does normal subcutaneous adipose tissue, and corroborates the statistical study of GELLHORN AND MARKS[4]. Their work demonstrated that the rate of acetate incorporation into the long-chain fatty acids of lipoma was greater than in the adjacent normal adipose tissue. It may be concluded that the soluble fraction of the lipoma and of normal adipose tissue synthesize long-chain fatty acids by a pathway similar to that found in the rat-epididymal fat pad[2], in liver[26,34,48] and in yeast [49].

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