Differential responses to fasting and subsequent feeding by microsomal systems of rat liver: 6- and 9-desaturation of fatty acids

CAROL A. INKPEN,* ROBERT A. HARRIS,‡ and FORREST W. QUACKENBUSH
Department of Biochemistry, Purdue University, Lafayette, Indiana 47907

ABSTRACT Hepatic microsomal preparations from nonfasted, fasted, and fasted–fed rats were employed, together with cofactors, in studies of 9-desaturation of stearate-1-^{14}C and 6-desaturation of linolenate-1-^{14}C. Prior fasting sharply reduced 9-desaturation but did not affect 6-desaturation; feeding restored 9-desaturation. Position of desaturation was determined by permanganate–periodate oxidation and separation of the dicarboxylic acids. Feeding after fasting stimulated both desaturase systems but either DL-ethionine or actinomycin D prevented this. Dietary carbohydrate or saturated fat increased 9-desaturation and dietary protein increased 6-desaturation. Insulin treatment of nonfasted rats increased 9-desaturation but not 6-desaturation. High dietary unsaturated fat (20% safflower oil) stimulated 6-desaturation but inhibited the 9-desaturation response to feeding.

The results indicate that the two desaturases are distinct and are inducible in response to different substances.

SUPPLEMENTARY KEY WORDS actinomycin D · DL-ethionine · insulin · safflower oil · hydrogenated coconut oil

HEPATIC microsomes have the enzymic capability for oxidative desaturation of palmitic, stearic, oleic, linoleic, and linolenic acids (1–4). The enzymic activity responsible for desaturation of stearate is decreased in alloxan diabetic (5–7) and starved animals (8–11), and its activity rebounds on insulin treatment of the diabetic animals or on feeding a fat-free diet after starvation.

Evidence obtained with microsomal preparations from fat-deficient rats (12, 13) indicates that oleic, linoleic, and linolenic acids compete for a common 6-desaturase and that another desaturase is responsible for 9-desaturation of stearic acid. In the alloxan diabetic rat the 6-desaturase activity (14) and the 9-desaturase activity (5–7) are both restored by insulin treatment. The purpose of the present investigation was to probe further the mechanism of control of 6- and 9-desaturation. We found that 9-desaturation of stearic acid and 6-desaturation of linolenic acid respond differently to nutritional factors. In addition, evidence is presented which supports the thesis (10, 15) that a hepatic deficiency of essential fatty acids increases the activity of long-chain fatty acyl desaturases for 9-desaturation, perhaps via adaptive enzyme formation (16).

MATERIALS AND METHODS

Preparation of Cell Fractions

The rats (Wistar, male, 150–250 g) were killed by cervical dislocation and their livers were immediately removed and placed in ice-cold 0.25 M sucrose. The livers from two to four rats were pooled and forced through a Harvard tissue press into 3 volumes of 0.25 M sucrose. The suspension was homogenized in a glass vessel with a loose-fitting Teflon pestle and centrifuged at 750 *g* for 15 min; the pellet was discarded. After

A preliminary report of this work was presented at the 50th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., 1966. This paper is Purdue University Journal Paper No. 3500 AES.

Abbreviations: 20-SUP, 20,000 *g* supernatant fraction; 105-SUP, 105,000 *g* supernatant fraction; 105-SED, 105,000 *g* pellet; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; EFA, essential fatty acid; SFO, safflower oil; HCO, hydrogenated coconut oil. Fatty acids are designated by chain length: number of double bonds.

* Present address: 531 Ventura Court, Ann Arbor, Mich. 48103.
‡ Present address: Institute for Enzyme Research, University of Wisconsin, Madison, Wis. 53705.

centrifugation at 20,000 *g* for 15 min, the mitochondrial pellet and floating lipid layer were discarded. To minimize mitochondrial contamination, we again centrifuged the residual solution at 20,000 *g* for 15 min. The resulting supernatant fraction, designated 20-SUP, was used as the source of enzymic activity in some studies. In other studies a homogenate in phosphate-buffered sucrose (17) was centrifuged at 8000 *g* for 30 min and the supernatant fraction was then separated into a microsomal fraction, 105-SED, and a soluble fraction, 105-SUP, by centrifugation at 105,000 *g* for 60 min. The long-chain fatty acyl desaturase activity is localized in the microsomes (4). However, we included 105-SUP in the assay system used in this study to insure that the fatty acid-activating enzyme was not a limiting factor.

Protein was estimated by the method of Lowry, Rosebrough, Farr, and Randall (18).

Substrates

The 1-^{14}C-fatty acids (stearic, 19 mc/mmole, New England Nuclear Corp., Boston, Mass.; and linolenic, 22 mc/mmole, Nuclear–Chicago Corporation, Des Plaines, Ill.) were checked for radiopurity by TLC and GLC of their methyl esters. The acids were solubilized in a 1% aqueous solution of BSA (bovine serum albumin, defatted, Calbiochem, Inc., Los Angeles, Calif.) as follows. A toluene solution of the fatty acid with 1% BSA was shaken for 30 min at 37°C, and the toluene was then removed in a stream of nitrogen.

Assay for 6- and 9-Desaturation Capability

Desaturation of the 9-position of stearate and the 6-position of linolenate by 20–SUP was assayed as follows. 1 ml of 20-SUP containing 18–22 mg of protein was incubated in an open 25 ml Erlenmeyer flask with 0.1 ml of the labeled fatty acid (0.05 μc in 1% BSA) and 1.0 ml of 0.08 M potassium phosphate buffer (pH 7.4) containing 7.5 μmoles of ATP, 1.5 μmoles of NADPH, 0.2 μmole of coenzyme A, and 5 μmoles of $MgCl_2$. Boiled 20-SUP was used to provide blanks and all flasks were shaken for 1.5 hr at 37°C. Reactions were stopped by addition of 10–15 ml of methanol containing 5 mg of carrier fatty acid and 0.5 μg of hydroquinone. Incubations were performed in duplicate.

Lipid extraction, upon completion of an incubation, was preceded by overnight saponification with 100–200 mg of KOH at room temperature. After acidification with concentrated HCl the lipids were extracted with chloroform–methanol 2:1, the single phase was broken by addition of water, and the chloroform layer was washed with water and evaporated under reduced pressure. The residual lipid was dissolved in ether and portions were used for permanganate–periodate oxidation (19), or for methylation with diazomethane (20) before separation by TLC or GLC.

For TLC of the methyl esters from stearate incubations, plates coated with silica gel containing 5% silver nitrate and 0.2% rhodamine-6G were used with a solvent system of benzene–hexane 1:1. Fractions were examined under UV radiation and identified with the aid of standard methyl esters on the same plate. Portions of the thin layer containing saturated and monounsaturated fractions were scraped into ether, the eluates were evaporated in scintillation vials, and radioactivity was determined.

For GLC of the polyunsaturated esters, a 180 cm glass column (6 mm I.D.) containing 20% diethyleneglycol adipate polyester was used in an F & M model 500 unit with a thermal conductivity detector (column temperature 205°C; injection port 315°C; detector 250°C; helium flow rate 60–80 ml/min). Fractions were collected manually by the method of Dutton(21), and their retention times were compared with those of the methyl esters in standard mixtures. Since no carrier methyl ester was available to mark the elution position of the octadeca-6,9,12,15-tetraenoic acid, its elution time was determined by collecting samples for half-minute periods after collection of the linolenate, and determining the radioactivity of each. Retention times were 11.5, 14.0, and 17.0 min for the three 9-unsaturated esters, 18:1, 18:2, and 18:3, respectively, and 18.6 min for the 6-unsaturated ester, 18:4. Similar fractions collected from the boiled blank contained less than 4% of the total added radioactivity (10,000 cpm) at the chosen time; experimental values for desaturation were corrected by appropriate subtraction of this amount of radioactivity.

Radioactivity was determined by scintillation counting in 0.01% *p*-bis[2-(5-phenyloxazolyl)]benzene and 0.4% 2,5-diphenyloxazole in toluene with a Packard Tri-Carb instrument. Radioactivity data are subject to a ±5% counting error. Desaturation is expressed as percentage conversion of radioactive substrate, corrected by subtraction of the percentage radioactivity in the corresponding fraction of the blank.

RESULTS AND DISCUSSION

Effect of Fasting and Subsequent Feeding on Hepatic Microsomal Capability for 6- and 9-Desaturation

Preliminary experiments established that the extent of desaturation by 20-SUP was much greater for linolenate than for oleate or linoleate, as might be predicted from affinities of these acids for the enzyme (12). Linolenate therefore was chosen as substrate for 6-desaturation and stearate as substrate for 9-desaturation. The data presented in the following tables and figures represent

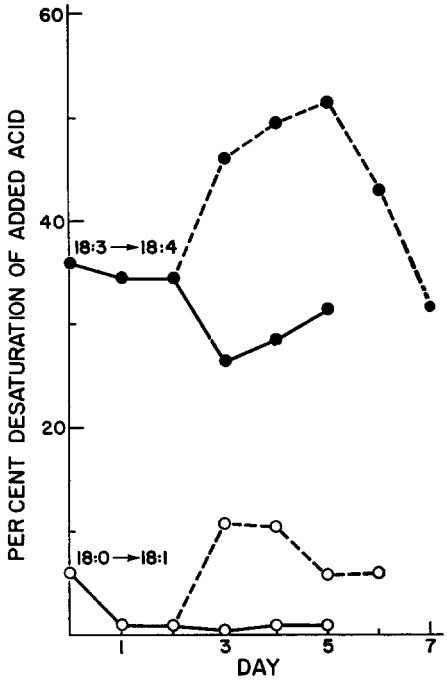

FIG. 1. Effect of fasting and subsequent feeding on desaturation of stearate-1-^{14}C (○) and linolenate-1-^{14}C (●) by 20-SUP. Feeding of colony diet (- - -) began after 2 days of fasting(——). Fatty acids are designated by chain length:no. of double bonds.

TABLE 1 DISTRIBUTION OF RADIOACTIVITY IN DICARBOXYLIC ACIDS AFTER PERIODATE–PERMANGANATE OXIDATION OF THE UNSATURATED FATTY ACID OBTAINED AFTER INCUBATION OF STEARATE-1-^{14}C AND LINOLENATE-1-^{14}C

Substrate	Microsomal Treatment	Distribution of Radioactivity in Dicarboxylic Fragments					
		C_5	C_6	C_7	C_8	C_9	$>C_9$
		cpm					
Stearate	Boiled	6	12	11	16	108	205
"	None	14	71	60	173	3570	450
Linolenate	Boiled	22	34	110	443	5330	660
"	None	73	1070	144	348	2460	670

After incubation of the substrate with 20-SUP, the total fatty acids extracted from the saponified incubation mixture were subjected to periodate–permanganate oxidation. The oxidation products were extracted with ether and methylated, and the mono- and dicarboxylic acid methyl ester fractions were separated by TLC in hexane–ether–acetic acid 70:30:1. The dicarboxylic acid methyl esters were then separated by GLC as described in the text, except that the helium flow rate was reduced to 40–50 ml/min. Each value is the mean of two analyses.

single experiments that are typical of the many that were performed under similar and varied conditions.

Incubation of stearate-1-^{14}C with 20-SUP from normal rats for 1.5 hr resulted in approximately 10% recovery of the label on the TLC plate as a monounsaturated fatty acid later identified as 9-octadecenoic acid. The desaturation rate was fast initially and became linear within 1 hr. When the animals were fasted for 24 hr before liver removal, the microsomal system produced only a trace of unsaturated acid (Fig. 1). However, when fasted animals were fed the colony diet, marked stimulation of 9-desaturation occurred within 24 hr.

Incubation of linolenate-1-^{14}C with 20-SUP from normal rats resulted in recovery of 36% of the label in a GLC fraction of the fatty acid esters which was later identified as the 6-desaturation product. In contrast to 9-desaturation, the 6-desaturase activity was not altered significantly by fasting (Fig. 1); both were increased temporarily by feeding after a period of fasting.

Proof of Position of Unsaturated Linkages Introduced Enzymically

When the radioactive unsaturated fatty acids resulting from 20-SUP action on stearate-1-^{14}C were oxidized with permanganate–periodate, more than 80% of the recovered label was in the C_9 dicarboxylic fragment (Table 1). In parallel experiments, in which a boiled microsomal preparation was used for the incubation, recovery of label as dicarboxylic acids was insignificant. From linolenate-1-^{14}C, more than 22% of the label was recovered in the C_6 dicarboxylic acid fragment and more than 50% in the C_9 fragment. As expected, after incubation with the boiled system more than 80% of the label was recovered as the C_9 dicarboxylic fragment. From these data, it was evident that the microsomal preparations were effecting 9-desaturation of stearate and 6-desaturation of linolenate.

Desaturation by Fractions Prepared at 105,000 g

The decrease in 9-desaturation activity after fasting reflected a change in the microsomes (Table 2). Combining 105-SUP prepared from the livers of nonfasted rats with 105-SED prepared from fasted rats gave the same results as the 20-SUP prepared from fasted rats. Likewise, the increase in 6- and 9-desaturation capability after feeding was shown to be a function of the microsomal fraction. When 105-SUP from nonfasted rats was combined with 105-SED from nonfasted rats, essentially the same results were obtained as with 20-SUP prepared from the livers of nonfasted rats. Thus, enzymes ancillary to the desaturation process were not affected measurably under the experimental conditions. It appears unlikely that the variation in capability for microsomal desaturation of stearate and linolenate reflects fluctuations in the concentration of fatty acid available for desaturation, since fractionation of total lipid extracts of the incubation mixtures revealed no variation in the percentage of the label recoverable as glycerides or sterol esters, or in the level of the available free fatty acids. These observations

TABLE 2 DESATURASE ACTIVITY OF RECOMBINED MICROSOMAL AND SUPERNATANT FRACTIONS, 105-SED AND 105-SUP

Fractions Combined		% of Substrate Desaturated	
Supernatant	Microsomal	Stearate-1-^{14}C	Linolenate-1-^{14}C
Nonfasted	Nonfasted	8.0	24.6
Nonfasted	Fasted	3.7	29.4
Nonfasted	Fasted–fed	42.6	37.7

Animals were fasted for 48 hr, then fed the colony diet; liver samples were taken 48 hr later. The 105-SED fractions from the livers were resuspended in phosphate-buffered sucrose. Each incubation flask contained 1 ml of 105-SED (equivalent to 0.55 g of liver) and 1.5 ml of 105-SUP (equivalent to 0.38 g of liver) together with 0.1 ml of the labeled fatty acid (0.05 μc in 1% of BSA), 7.5 μmoles of ATP, 1.5 μmoles of NADPH, 0.2 μmole of coenzyme A, 26 μmoles of $MgCl_2$, 5 μmoles of *sn*-glycerol-3-phosphate, 5.2 μmoles of glutathione, 105 μmoles of nicotinamide, 525 μmoles of KCl, and 875 μmoles of sucrose in potassium phosphate buffer (pH 7.0), total volume 3.6 ml. Flasks were flushed with oxygen–carbon dioxide 9:1, stoppered, and shaken for 1.5 hr at 37°C, and then assayed as described in the text.

suggest that fatty acid desaturation per se responds to fasting and subsequent feeding.

Effect of Varying Dietary Components on the Response of Desaturation Activity to Feeding

A semipurified diet (22) was employed for studies on the response of the long-chain fatty acyl desaturases to individual dietary energy sources (Table 3). Animals previously fed the colony diet were fasted and subsequently fed different modifications of the semipurified diet before liver removal. When glucose was the only caloric source in the diet, 9-desaturation was stimulated and 6-desaturation was suppressed, when compared to the normal nonfasted state. When casein was the only source of calories in the diet, these responses were reversed. Glucose and casein together stimulated both desaturase systems. When saturated fat as hydrogenated coconut oil (HCO) was the only caloric source, 9-desaturation was stimulated. However, this fat did not alter the responses of the 6-desaturase to either the glucose or casein although it did stimulate 9-desaturase activity when fed with casein. In contrast, incorporation of 20% of an unsaturated fat as safflower oil (SFO) into the diet clearly inhibited the response of the 9-desaturase to feeding but did not prevent the increase in 6-desaturase activity. In view of this evidence that enzymic activities for 6-desaturation and 9-desaturation respond differently to alimentation, one concludes that different enzyme complexes are involved and that these enzyme complexes are under the influence of different control mechanisms.

TABLE 3 EFFECT OF DIFFERENT DIETARY COMPONENTS UPON THE MICROSOMAL CAPABILITY FOR 9-DESATURATION OF STEARATE AND 6-DESATURATION OF LINOLENATE

Treatment	% of Labeled Substrate Desaturated	
	Stearate-1-^{14}C	Linolenate-1-^{14}C
None	7.8	36.6
Fasted	0.4	37.5
Fasted, fed: colony diet	29.6	63.8
Fasted, fed: 93 G*	35.6	23.2
Fasted, fed: 95 C	2.2	58.7
Fasted, fed: 79 G + 16 C	45.2	81.8
Fasted, fed: 87 HCO	23.6	41.5
Fasted, fed: 3 HCO + 90 G	34.5	28.2
Fasted, fed: 62 HCO + 29 C	30.6	62.9
Fasted, fed: 20 HCO + 55 G + 18 C	43.8	68.2
Fasted, fed: 20 SFO + 55 G + 18 C	9.7	67.9

Before the 48 hr fast all animals received the colony diet (4% fat, 60% carbohydrate, 23% protein, 13% noncaloric). One group was fed the colony diet for 48 hr before liver removal; others were fed modifications of a semipurified diet (19) in which the ratio of total calories to noncaloric components was held constant. 20-SUP prepared from pooled livers of two rats from each treatment was the source of enzymic activity.

* Diets are identified by percentages of glucose (G), casein (C), hydrogenated coconut oil (HCO), and safflower oil (SFO).

TABLE 4 EFFECTS OF INSULIN ON DESATURATION BY HEPATIC MICROSOMAL PREPARATIONS

Treatment	% of Substrate Desaturated	
	Stearate-1-^{14}C	Linolenate-1-^{14}C
None	6.4	30.4
Insulin injected	16.6	25.8
Insulin in vitro	6.3	23.2

Glucagon-free zinc insulin (10 U) and 10 ml of 5% glucose in saline were injected subcutaneously at different sites into normal rats twice daily for 2 days and then 20 U and 10 ml of the glucose were injected about 2 hr before livers were removed. Microsomal preparations (20-SUP) and incubations are described in the text. Insulin (0.4 U) was added in vitro to 20-SUP from normal rats that had received no insulin.

Further independent evidence for this conclusion was found with insulin-injected rats (Table 4). Injection of insulin into normal rats resulted in a marked stimulation of the 9-desaturase activity but no stimulation of the 6-desaturase.

Effect of Actinomycin D *and* DL-*Ethionine upon 6- and 9-Desaturase Activity*

Both actinomycin D and DL-ethionine injected at the end of a fast prevented the usual increase in microsomal capability for 6- and 9-desaturation when the animals consumed the diet (Table 5). Actinomycin D injected after fasting prevented a 9-desaturase response to feeding and appeared also to limit the level of 6-desaturase activity. In a different series of experiments DL-ethionine also limited the desaturation response to feeding. The animals were active and consumed their food readily. From these experiments it is apparent that the levels of

TABLE 5 EFFECT OF INHIBITORS OF ADAPTIVE ENZYME FORMATION UPON DESATURATION BY 20-SUP

Treatment	% of Substrate Desaturated			
	Stearate-1-^{14}C		Linolenate-1-^{14}C	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
None	13.2	10.0	24.3	16.3
Fasted	3.4	0.8	25.1	—
Fasted, fed	22.7	39.8	45.4	—
Fasted, fed; given actinomycin D	—	0.8	—	7.3
Fasted, fed; given DL-ethionine	4.1	—	29.6	—

Intraperitoneal injections of actinomycin D (200 μg/kg body wt) or DL-ethionine (250 mg/kg body wt) in saline were given at the time feeding (colony diet) was begun, 24 hr before liver removal. Other procedures are described in the text.

6-desaturase and 9-desaturase activity were fixed at the time of injection of the two substances. Since actinomycin D and DL-ethionine prevent adaptive enzyme formation in vivo (23, 24), the results suggest that adaptive enzyme formation accounts for the increase in 6- and 9-desaturation activity upon feeding.

Evidence from several sources is now available to support the thesis that adaptive enzyme synthesis plays an important role in the regulation of cellular capacity for fatty acid desaturation. Gellhorn and Benjamin (5–7) found that stearate desaturation by epididymal fat tissue of alloxan diabetic rats and starved rats is subnormal and that insulin injection and feeding, respectively, reestablish the enzymic activity in 48–72 hr. This response to insulin and feeding was effectively blocked by actinomycin D or puromycin. Allmann, Hubbard, and Gibson (10) have demonstrated that marked changes occur rapidly in the fatty acid composition of liver lipids of rats after a short period of fasting followed by feeding; fasting decreased the relative quantity of palmitoleate and oleate and feeding increased the quantity of both acids to extraordinarily high levels. Puromycin was established as effective in preventing these responses to fasting and feeding (16). In the present study actinomycin D and DL-ethionine, injected in vivo, were both found to be effective in preventing the postprandial rise in enzymic activity for desaturation of stearate and linolenate.

Evidence that Essential Fatty Acids Exert Control Upon the Enzymic Activity for 9-Desaturation and Lipogenesis

The failure of an increase in 9-desaturation in response to feeding with 20% fat as SFO is in sharp contrast to the increase observed with 20% fat as HCO (Table 3).

Responses of the 9-desaturase to feeding with mixtures of HCO and SFO (Fig. 2) were inversely related to the proportion of SFO present in the 20% of fat fed. The results indicate that this desaturase is sensitive to dietary unsaturated fat and suggest that linoleate, which constitutes 70% of the fatty acid of SFO, may exert a negative feedback action upon desaturation. One would predict that hepatic microsomes from rats deficient in essential fatty acids (EFA) would exhibit a relatively high 9-desaturase activity. To test this experimentally, we fed weanling rats an EFA-deficient, semipurified diet (22, 25) for 10 wk; the classical EFA deficiency syndrome (26) was observed. Under conditions in which microsomes prepared from the livers of normal rats desaturate 10–15% of the added stearate-1-^{14}C, the microsomes from the EFA-deficient rats were found to desaturate 40–50% of the added substrate. Allmann et al. (10) have reported that short-term fasting followed by feeding a fat-free or EFA-free diet produced an apparent EFA deficiency of the liver, and that feeding linoleate reduced the amount of palmitoleate and oleate in the liver. Brenner and Peluffo (12) have suggested that the 9-desaturase may have an active center capable of reacting with unsaturated acids, since a large excess of either oleic or linoleic acid diminished the desaturation of saturated fatty acids in their system. The evidence strongly suggests that linoleate or derivatives thereof may regulate or modify the enzyme activity for 9-desaturation.

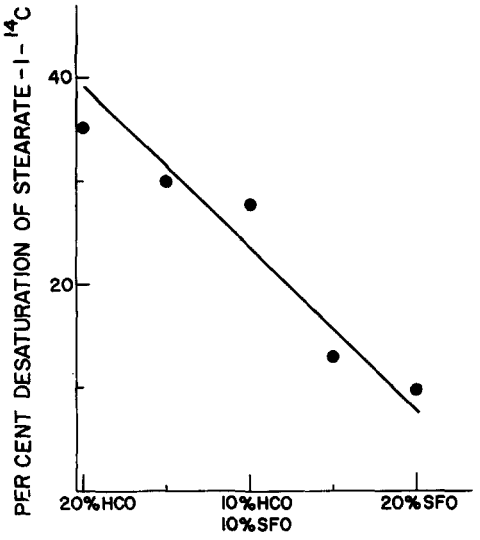

FIG. 2 Inhibitory effect of dietary safflower oil (SFO) upon 9-desaturation of stearate-1-^{14}C by 20-SUP. Rats fasted for 48 hr were fed five diets, each of which contained 20% fat, for 48 hr. Hydrogenated coconut oil (HCO) was replaced by 5% increments of SFO.

Many of the hepatic enzymes that play a role in the conversion of glucose to triglycerides (lipogenesis) are depressed by fasting but become elevated beyond normal levels in response to subsequent feeding (27). These include the acetyl CoA carboxylase and fatty acid synthetase (10, 28), which are the two enzyme systems that catalyze the synthesis of long-chain saturated fatty acids

from acetyl CoA via malonyl CoA. In addition, the citric acid cleavage enzyme (29), liver glucokinase (30), glucose-6-phosphate dehydrogenase (31), and, as confirmed by direct assay in the present study, the long-chain fatty acyl desaturase responsible for desaturation of saturated fatty acids (9–11), all respond to fasting and subsequent feeding. Allmann and Gibson (15) and Allmann et al. (10) have established that the level of hepatic EFA is a factor in the mechanism that regulates enzyme activity in lipogenesis. An inverse correlation was demonstrated between the relative amount of linoleic acid among the liver fatty acids and the activity of the enzymes concerned with lipogenesis. By utilizing fasting and feeding as a method for producing a shortage of hepatic EFA, we were able to demonstrate directly that the capability for 9-desaturation of long-chain fatty acids by microsomes is suppressed by dietary linoleate. These studies suggest that hepatic linoleate is a key factor in the cellular control mechanism for lipogenesis.

Summary of Evidence for Differentiation of 6- and 9-Desaturase Enzymes

In this communication evidence has been presented that 9-desaturation of saturated fatty acids and 6-desaturation of unsaturated fatty acids are accomplished by different enzymes, and that the activity of these enzymes is controlled by separate mechanisms. The evidence includes: (*a*) decreased 9-desaturase activity but not 6-desaturase activity during fasting; (*b*) increased 9-desaturase activity but not 6-desaturase activity after insulin injection of normal rats; (*c*) increased 9-desaturase activity but not 6-desaturase activity after feeding with carbohydrate or fat; and (*d*) increased 6-desaturase activity but not 9-desaturase activity after feeding with protein.

Actinomycin D was a gift from Merck Sharp & Dohme, Rahway, N. J.; hydrogenated coconut oil, from Procter & Gamble Co., Cinncinati, Ohio; and safflower oil, from Pacific Vegetable Oil Corp., Richmond, Calif.

This investigation was supported by U.S. Public Health Service Grants HE-08424 and T1 GM 1195.

Manuscript received 8 October 1968; accepted 16 January 1969.

References

1. Stoffel, W. 1961. *Biochem. Biophys. Res. Commun.* **6:** 270.
2. Nugteren, D. H. 1965. *Biochim. Biophys. Acta.* **106:** 280.
3. Nugteren, D. H. 1963. *In* Biochemical Problems of Lipids. A. C. Frazer, editor. Elsevier Publishing Co., Amsterdam. **1:** 460.
4. Marsh, J. B., and A. T. James. 1962. *Biochim. Biophys. Acta.* **60:** 320.
5. Gellhorn, A., and W. Benjamin. 1964. *Biochim. Biophys. Acta.* **84:** 167.
6. Gellhorn, A., and W. Benjamin. 1964. *Science.* **146:** 1166.
7. Gellhorn, A., and W. Benjamin. 1966. *Biochim. Biophys. Acta.* **116:** 460.
8. Elovson, J. 1965. *Biochim. Biophys. Acta.* **106:** 291.
9. Elovson, J. 1964. *Biochem. J.* **92:** 42P.
10. Allmann, D. W., D. D. Hubbard, and D. M. Gibson. 1965. *J. Lipid Res.* **6:** 63.
11. Butterworth, P. H. W., R. B. Guchhait, H. Baum, E. B. Olson, S. A. Margolis, and J. W. Porter. 1966. *Arch. Biochem. Biophys.* **116:** 453.
12. Brenner, R. R., and R. O. Peluffo. 1966. *J. Biol. Chem.* **241:** 5213.
13. Brenner, R. R., and R. O. Peluffo. 1967. *Biochim. Biophys. Acta.* **137:** 184.
14. Mercuri, O., R. O. Peluffo, and R. R. Brenner. 1966. *Biochim. Biophys. Acta.* **116:** 409.
15. Allmann, D. W., and D. M. Gibson. 1965. *J. Lipid Res.* **6:** 51.
16. Hicks, S. E., D. W. Allmann, and D. M. Gibson. 1965. *Biochim. Biophys. Acta.* **106:** 441.
17. Harris, R. A., E. R. Rivera, C. L. Villemez, Jr., and F. W. Quackenbush. 1967. *Lipids.* **2:** 137.
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. *J. Biol. Chem.* **193:** 265.
19. VonRudloff, E. 1956. *J. Amer. Oil Chem. Soc.* **33:** 126.
20. Schlenk, H., and J. L. Gellerman. 1960. *Anal. Chem.* **32:** 1412.
21. Dutton, H. J. 1961. *J. Amer. Oil Chem. Soc.* **38:** 631.
22. Quackenbush, F. W., and M. D. Pawlowski. 1960. *J. Nutr.* **72:** 196.
23. Gellhorn, A., and W. Benjamin. 1966. *In* Advances in Enzyme Regulation. G. Weber, editor. Pergamon Press, Inc., New York. **4:** 19.
24. Farber, E., K. H. Shull, and S. Villa-Trevino. 1963. *In* Advances in Enzyme Regulation. G. Weber, editor. Pergamon Press, Inc., New York. **1:** 259.
25. Harris, R. A., J. E. MacNintch, and F. W. Quackenbush. 1966. *J. Nutr.* **90:** 40.
26. Burr, G. O., and M. M. Burr. 1929. *J. Biol. Chem.* **82:** 345.
27. Gibson, D. M., S. E. Hicks, and D. W. Allmann. 1966. *In* Advances in Enzyme Regulation. G. Weber, editor. Pergamon Press, Inc., New York. **4:** 239.
28. Hubbard, D. D., R. E. McCaman, M. R. Smith, and D. M. Gibson. 1961. *Biochem. Biophys. Res. Commun.* **5:** 339.
29. Abraham, S., L. Kopelovich, and I. L. Chaikoff. 1964. *Biochim. Biophys. Acta.* **93:** 185.
30. DiPietro, D. L., and S. Weinhouse. 1960. *J. Biol. Chem.* **235:** 2542.
31. Tepperman, H. M., and J. Tepperman. 1958. *Diabetes.* **7:** 478.