# STEARATE DESATURATION BY MICROSOMES ON THE LOCUST FAT-BODY

A. TIETZ and N. STERN
*Israel Institute for Biological Research, Ness-Ziona*
*and the Department of Biochemistry, Tel-Aviv University, Tel-Aviv, Israel*

Received 28 February 1969

## 1. Introduction

It was shown in several laboratories that insects readily incorporated administered $^{14}C$-labeled acetate or glucose into long chain fatty acids. In agreement to results obtained with rats, it could be shown that when insects were grown under aseptic conditions only palmitic, palmitoleic, stearic and oleic acid were labeled. The conversion of $^{14}C$-labeled palmitate or stearate to the corresponding monounsaturated acids was also shown in these experiments [1–5]. Thus, insects like higher animals, can synthesize only saturated and monounsaturated fatty acids.

We have previously shown [6] that cell free extracts of the fat body of the migratory locust, *Locusta migratoria*, incorporated added $^{14}C$-acetate into fatty acids. The major fatty acid synthesized was palmitic acid. However, small amounts of unsaturated acids were also formed. It will be shown in the present communication that microsomes isolated from these extracts readily desaturated added $^{14}C$-stearic acid to oleic acid, when incubated aerobically in the presence of reduced pyridine nucleotides.

## 2. Methods

### 2.1. *Preparation of microsomes*

Adult male and female locusts, 8–15 days after the last molt were employed. The fat body was removed as previously described and collected in ice cold sucrose (0.3 M). Each 2 g of tissue were homogenized with 10 ml of sucrose (0.3 M) to which mercaptoethanol (0.01 M) was added. Free floating fat, cell debris and nuclei were removed by centrifugation at 400 *g* for 5 min.

Mitochondria were precipitated by centrifugation at 10,000 *g* for 10 min and microsomes at 140,000 *g* for 45 min. The particles were suspended in fresh buffer and assayed as soon as possible. In a few experiments microsomes were rapidly frozen and kept at −20° or freeze-dried and the dry powder kept at −20°. The dried powder was suspended in 1 M phosphate buffer, pH 7.2, before use [11].

### 2.2. *Assay of desaturase activity*

All incubations were done in glass stoppered tubes at 30° with constant shaking. The composition of the reaction mixtures is given in the table. At the end of the incubation 1 ml of 20% KOH in methanol was added and the tubes were kept over night at 40°. Fatty acids were extracted with hexane after acidification and methylated with diazomethane [7]. Saturated and unsaturated methyl esters were separated by thin layer chromatography on $AgNO_3$ impregnated plates [8] with benzene:hexane (40:60, v/v) and the corresponding spots were scraped directly into counting vials and counted [9].

To identify the $^{14}C$-labeled monoenoic acids produced, the unsaturated acids were isolated and purified as previously described [9]. The pure methyl esters were then subjected to oxidative cleavage according to Scheuerbrandt and Bloch's modification of the method of Van Rudlof [10]. The methyl esters of the dicarboxylic acids were separated by gas chromatography and counted [9].


*North-Holland Publishing Company – Amsterdam*

## 3. Results and discussion

When fat body homogenates or fractions obtained thereof by differential centrifugation were incubated with $^{14}$C-labeled palmitate or stearate only the microsomes showed desaturase activity. Addition of the 140,000 *g* supernatant to the microsomes reduced the extent of desaturation. The system preferentially desaturated $^{14}$C-stearate. The rate of desaturation of either palmitate or stearate did not change when the pH of the reaction mixture was changed from 6.8 to 7.8. The requirement for reduced pyridine nucleotides is shown in table 1. NADH seems more effective than NADPH and was therefore routinely added. 2-Amino, 4-hydroxy-6,7-di-methyltetrahydropteridine could not replace NADH or NADPH. Under our assay conditions the rate of the reaction was linearly dependent on the amount of protein and time of incubation over a limited range (fig. 1). The desaturase reaction showed an absolute requirement for oxygen;

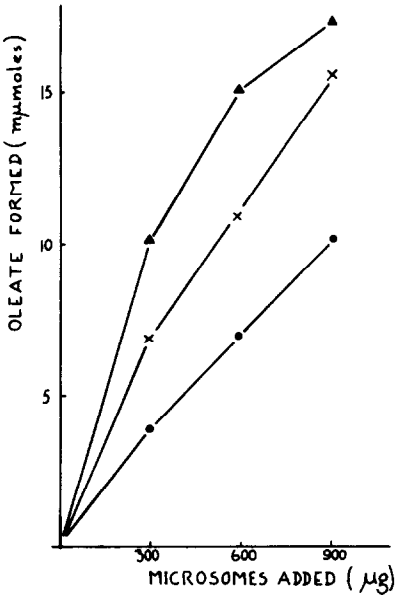


Fig. 1. Desaturation of $^{14}$C-stearate by microsomes: dependence on amount of protein added and time of incubation. The composition of the reaction mixture is described in table 1. (●—●) 5 min, (x—x) 10 min and (▲—▲) 20 min of incubation.

when incubated under nitrogen, desaturation of $^{14}$C-stearate did not occur. The system was very sensitive to cyanide; 50% inhibition was obtained at 0.5 mM KCN.

The unsaturated acids obtained from palmitate and stearate were isolated and identified as described under methods. 1-$^{14}$C-palmitate yielded exclusively $^{14}$C-hexadecenoic acid; 1-$^{14}$C-stearate, $^{14}$C-octa decenoic acid. On oxidative degradation of the monoenoic

Table 1
Requirements for the desaturation of $^{14}$C-stearate

| Substrate | Additions | Desaturation (mμmoles) |
|---|---|---|
| $^{14}$C-palmitate | NADPH | 4.1 |
| $^{14}$C-stearate | None | 0.5 |
| | NADP | 4.6 |
| | NADPH | 12.5 |
| | NAD | 9.6 |
| | NADH | 16.4 |
| | NADH, KCN (2 mM) | 1.6 |
| | NADH, KCN (0.5 mM) | 7.2 |
| | NADH, KCN (0.2 mM) | 13.2 |
| | NADH, Vac./air | 15.0 |
| | NADH, Vac./nitrogen | 1.6 |

The reaction mixture contained: phosphate buffer pH 7.2 50 mM; ATP 5 mM, $MgCl_2$ 5 mM; glutathione 5 mM, coenzyme A 0.1 mM, pyridine nucleotide 0.5 mM, microsomes 550 μg and 1-$^{14}$C-labeled stearate or palmitate 30 μmoles (100,000 disintegrations/min). NADPH was generated from NADP in the presence of glucose-6-phosphate 5 mM and glucose-6-phosphate dehydrogenase 10 μg. Total volume 1 ml. Incubated for 30 min at 30°. The last 2 incubations were done in Thunberg tubes. The tubes were connected for 2 min to a vacuum pump. One tube was filled with purified nitrogen, the other with air.

Table 2
Attempts to purify the microsomal desaturase

| Enzyme added | μg | Desaturation (mμmoles) |
|---|---|---|
| microsomes | 385 | 10.2 |
| | 770 | 18.6 |
| lyophilized microsomes | 730 | 9.0 |
| | 730 | 17.8 |
| soluble extract | 614 | 5.6 |
| red fraction | 410 | 17.4 |

The composition of the reaction mixture is described in table 1; time of incubation 20 min.

acids, 70–80% of the radioactivity was found in dimethyl azelaate, indicating that the $\Delta^9$ monoenoic acids were formed.

These results indicate that the properties of locust fat body microsomal desaturase are very similar to the properties of the particulate systems previously isolated from yeast [12] and rat liver [13,14]. The sensitivity to KCN has also been observed with rat liver microsomes [13]. In contrast to our results, Goldin and Keith [15] recently reported that mitochondria isolated from third instar larvae of *Drosophila melanogaster* incorporated $^3$H-acetate mainly into palmitic, palmitoleic and oleic acid. These mitochondria also converted added $^{14}$C-palmitate and stearate to the corresponding monoenoic acids. Under our experimental conditions, fatty acid synthesis and desaturation by mitochondria of the locust's fat body were negligible.

The intact microsomes rapidly lost activity on repeated freezing and thawing. When the microsomes were lyophilized and extracted with 1 M phosphate buffer pH 7.2 as described by Gurr et al. [11] the extract was found to be more stable than the microsomes. However, unlike the preparation described by Gurr, upon centrifugation at 140,000 *g* the "extract" separated into 3 fractions. Activity was concentrated in a reddish membrane like structure which accumulated on top (table 2).

## References

[1] M.L.Bade, J. Insect Physiol. 10 (1964) 333.
[2] E.N.Lambremont, Comp. Biochem. Physiol. 14 (1965) 419.
[3] E.N.Lambremont and A.F.Bennet, Can. J. Biochem. 44 (1966) 1957.
[4] A.D.Keith, Comp. Biochem. Physiol. 21 (1967) 587.
[5] A.D.Keith, G.Gauslaa and B.S.Anderson, Lipids 2 (1967) 429.
[6] A.Tietz, J. Lipid Res. 2 (1961) 182.
[7] H.Schlenk and J.L.Gellerman, Anal. Chem. 32 (1960) 1412.
[8] L.J.Morris, Chem. Ind. (1962) 1238.
[9] N.Stern, E.Shenberg and A.Tietz, European J. Biochem. accepted for publication.
[10] G.Scheuerbrandt and K.Bloch, J. Biol. Chem. 237 (1962) 1064.
[11] M.I.Gurr, K.W.Devey and A.T.James, FEBS Letters 1 (1968) 320.
[12] D.K.Bloomfield and K.Bloch, J. Biol. Chem. 235 (1960) 337.
[13] J.B.Marsh and A.T.James, Biochim. Biophys. Acta 60 (1962) 320.
[14] N.Oshino, Y.Imai and R.Sato, Biochim. Biophys. Acta 128 (1966) 13.
[15] H.H.Goldin and A.D.Keith, J. Insect Physiol. 14 (1968) 887.