BIOSYNTHESIS OF FATTY ACIDS FROM [CARBOXY-^{14}C] ACETATE BY SOLUBLE ENZYME SYSTEM PREPARED FROM RAT MAMMARY GLAND HOMOGENATES

by

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We have described the biosynthesis of short- and long-chain fatty acids (C_6–C_{18}) from acetate by homogenates prepared from the mammary gland of lactating rats and of sheep[1]. In the preparations made from the rat tissue three conditions were required for the activation of the fatty-acid synthesizing system: (a) aerobic incubation; (b) the addition of either of the three keto-acids, pyruvate, oxaloacetate or α-ketoglutarate; (c) and the addition of ATP. The highest incorporation of acetate into fatty acids was observed in the presence of oxaloacetate (0.02 *M*) and ATP (0.01 *M*) and when the gas phase was air instead of pure O_2.

We wish to report here the centrifugal fractionation of homogenates of rat mammary gland and which have yielded a soluble enzyme system actively synthesizing fatty acids from [*carboxy*-^{14}C]-acetate. The finely minced abdominal glands of lactating rats (7–14 days after parturition) were homogenized with 2 vols of ice-cold buffer (0.154 *M* KCl, 100 pts; 0.154 *M* $MgCl_2$, 10 pts; and 0.1 *M* potassium phosphate buffer, pH 7.4, 35 pts) in a glass homogenizer provided with a tightly fitting piston which was driven by an electric motor. The homogenate, filtered through four layers of gauze, was centrifuged at 0° and at 400 *g* for 10 min. A pad of fat which separated at the top was scooped off. The supernatant, filtered through a small pad of cotton wool, provided the "full homogenate". It was then centrifuged at 0° and at 25,000 *g* for 30 min. The clear, transparent and pink supernatant (Sp I) was taken off and filtered through a small pad of cotton-wool to remove a thin film of fat from the top. The sediment, designated as "mitochondria", was washed twice by dispersion in fresh buffer and sedimentation at 25,000 *g* for 15 min each. A sample of Sp I was centrifuged further at 2° and 104,000 × g for 30 min at the full speed of the centrifuge. The supernatant (Sp II), which in appearance was similar to Sp I, was taken off and the sediment, a pinkish-brown translucent pellett, designated as "microsomes", was washed once with buffer and sedimented at 104,000 *g* for 10 min at the full speed of the centrifuge. The "mitochondria" obtained from 5 ml of full homogenate were suspended with the aid of a glass homogenizer in 2.5 ml of buffer or with the same volume of Sp I or Sp II to provide 2 ml for one incubation and 0.5 ml for determination of dry weight.The "microsomes" obtained from 10 ml of Sp I were treated in the same way with buffer or Sp II. The incubations were made in WARBURG apparatus at 37.5° for 3 h. Each flask contained 60 μmoles of [carboxy-^{14}C]-acetate as the sodium salt (5 μc ^{14}C), 60 μmoles of potassium oxaloacetate, 30 μmoles of ATP (monosodium salt, L. Light & Co., Ltd. Colnbrook, England) and 2 ml of the preparations to be tested for enzyme activity; the final volume was 3 ml and the gas phase air. In order to obtain sufficient amounts of fatty acids for assay of ^{14}C, an ethanol-ether solution of the fat obtained during the centrifugal fractionation of the homogenates was added to all preparations, except the full homogenate, at the end of the incubations and before saponification. The fat added was equivalent to 10 mg of mixed fatty acids. The extraction of fatty acids and their purification from contaminating ^{14}C-acetate by a chromatographic technique has been described elsewhere[2]. The oxygen consumptions ($-Q_{O_2}$) reported represent total O_2 consumed (μl) per mg dry weight (corrected for salts and fat) of the preparations during the first hr of the experiment, and fatty acid synthesis is expressed as $1 \cdot 10^{-3}$ μmoles acetate used per 100 mg dry weight (corrected) of the preparations.

As can be seen from the results shown in Table I Sp I incorporated 5–10 times as much acetate into fatty acids as the full homogenate or the mitochondria. Moreover, when Sp I was combined with mitochondria fatty acid synthesis was reduced to the lower levels observed with the full homogenate. Sp II, which is a particle-free solution, was less active than Sp I but more active than the full homogenate in synthesizing fatty acids. The recombination of microsomes with Sp II did not restore the enzymic activity to the level observed in Sp I. Whether our inability to disperse the microsomes adequately in Sp II or a loss of some co-factor from the microsomes during washing is the explanation for this result cannot be decided at present. The relatively high O_2-consumption of Sp I and Sp II indicates the presence of respiratory enzymes. The O_2-uptake of the preparations cannot be ascribed to autoxidation of components since incubation of (*e.g.*) Sp II without ATP reduced the $-Q_{O_2}$ to 0.9. Spectroscopic analyses of Sp I and II indicated the presence of fairly high concentrations of cytochrome *c* and of DPN.

water content according to various authors: 1, 1½, 2 and 3 mols of water have been reported. The specific rotation values for the free acid derived from the barium salt also fluctuate from —10.7° to —14.4°[2,3,4].

In our investigation phosphoglyceric acid (PGlA) we observed that the presence of ammonium salts prevents the crystallization of the scantily soluble derivatives of D(—)PGlA. A method for rapid purification of the acid barium salt was based on this fact. The latter is soluble in warm ammonium salts and is precipitated from the aqueous solution of these salts in pure form, beautifully crystallized and without appreciable losses by methanol, ethanol or *iso*propanol. Any ammonium salt sufficiently soluble in the above alcohols, such as NH_4Cl, NH_4Br, NH_4CNS, $CH_3.COONH_4$, $CH_3.CHOH.CO_2NH_4$, ammonium salicylate etc., may suitably be used as solvents for the barium salt of PGlA. (Ammonium nitrate or succinate will also dissolve, but are unsuitable since they are precipitated as such by the addition of alcohols.)

The recovered barium salt does not contain any ammonium. The solubility in the ammonium salts is not due to some type of double transformation, nor to the mildly acid reaction of some of the ammonium salts. (Aqueous solutions of NH_4Cl and NH_4Br show a pH of 4.6. The solubilization phenomenon remains unaffected by neutralization of the ammonium salt solution with NH_4OH.)

1 g acid Ba–PGlA is heated with 25 ml 25% NH_4Cl. The barium salt is dissolved while the adhering impurities — the quantities of which will vary with the degree of purity of the raw material- remain undissolved. They are filtered off and the clear filtrate is added with 100 to 125 ml methanol or ethanol. The instantaneously appearing opaque precipitate condenses into a mass of crystals which microscopically viewed show platelets or sometimes tetrahedral crystal structures.

If *iso*propanol is used, an ammonium chloride solution of only 20% concentration should be applied, since higher concentration will result in the salting out of the *iso*propanol. The quantity of ammonium salt to be applied may vary according to purity of the barium salt.

The substance is filtered off by suction, washed first with 75% ethanol and subsequently with pure ethanol or methanol. The compound dried over $CaCl_2$ at 20 to 22° C to constant weight shows the composition $C_3H_5O_8PBa.2H_2O$. A higher crystal water content has never been observed by us. A product free from crystal water may be prepared by drying in the Abderhalden drying apparatus in a high vacuum at 80° C for 48 hours, with benzene as heating bath. The specific rotation of the dihydrate amounted to —13.86° as the average value of 5 determinations. The analytical data for the substance purified according to this new method coincide with the values previously obtained for the pure substance[1]. Deviating data should pertain to impure substances. KIESSLING AND SCHUSTER[4] finally found the same composition and rotation value as NEUBERG AND KOBEL.

It should be pointed out in this connection that the striking solubility in ammonium salts of insoluble derivatives, especially of alkaline earth salts, is also encountered among other types of compounds[5], *e.g.* such as 1,6-fructose-diphosphates, various salts of PGlA, Ca- and Mg-phytates, Ca-*n*-butyrate and the alkaline earth salts of glycerophosphoric acid.

REFERENCES

[1] C. NEUBERG AND M. KOBEL, *Biochem. Z.*, 260 (1933) 241, 263 (1933) 219, 264 (1933) 156;
C. ANTONIANI, *Biochem. Z.*, 267 (1933) 376; *Chem., Zentr.*, II (1935) 62;
C. CATTANEO, *Biochem. Z.*, 267 (1933) 456, 270 (1934) 382;
P. PRATESI, *Enzymologia, Neuberg-Festschrift*, 242 (1937).

[2] G. EMBDEN, H. J. DEUTICKE AND G. KRAFT, *Klin. Wochenschr.*, 12 (1933) 313;
G. EMBDEN AND H. J. DEUTICKE, *Z. physiol. Chem.*, 230 (1934) 1929.

[3] E. LEHNARTZ, *Z. physiol. Chem.*, 230 (1934) 90;
P. A. LEVENE AND A. SCHORMÜLLER, *J. Biol. Chem.*, 106 (1934) 598;
W.KIESSLING, *Ber. chem. Ges.*, 68 (1935) 244;
A. HAHN, H. OTTAWA AND E. MEHLER, *Z. Biol.*, 97 (1936) 373;
O. MEYERHOF and assoc., *Biochem. Z.*, 276 (1935) 241, 297 (1938) 61; *J. Biol. Chem.*, 145 (1942) 445, 179 (1949) 1373.

[4] W. KIESSLING AND P. SCHUSTER, *Ber. chem. Ges.*, 71 (1938) 127.

[5] C. NEUBERG AND S. SABETAY, *Biochem. Z.*, 161 (1925) 240;
C. NEUBERG AND H. LUSTIG, *J. Am. Chem. Soc.*, 64 (1942) 2722;
C. NEUBERG, *Arch. Biochem.*, 3 (1943) 93;
C. NEUBERG, H. LUSTIG AND M. A. ROTHENBERG, *Arch. Biochem.*, 3 (1943) 93;
C. NEUBERG AND I. MANDL, *Z. Vitamin-, Hormon- u. Fermentforsch.*, 2 (1948) 480.

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