SC 2301

Induction of swelling of liver mitochondria by fatty acids of various chain length

Non-esterified fatty acids produce, in isolated mitochondria, manifold changes which may be summarized as follows: (a) stimulation of substrate oxidation in the absence of phosphate acceptor[1,2]; (b) activation of latent ATPase[3]; (c) inhibition of the ATP–P_i exchange reaction[4,5]; (d) uncoupling of oxidative phosphorylation[2,4]; and (e) induction of the so-called swelling of mitochondria[6,7]. Biochemical effects of fatty acids on mitochondria (points a to d) have been shown to be dependent upon the carbon chain length of the acids and upon the presence or absence of double bonds[3,8,9]. In contrast, little is known about the effects of chain length and of the presence of unsaturated bonds on the induction of mitochondrial swelling (point e), although a few data have been presented by AVI-DOR[7].

The present paper describes a study on the swelling effect exerted by fatty acids of the saturated series over the range of C_3 to C_{22} as well as by unsaturated acids and one hydroxy-monounsaturated acid.

Swelling of rat-liver mitochondria was measured photometrically at 520 mμ as described by LEHNINGER *et al.*[10].

Fig. 1 illustrates a typical experiment. It shows that the rate and the character of swelling was dependent on the chain length of the acids and on the presence of double bonds. The relationship between the swelling effect and the carbon chain

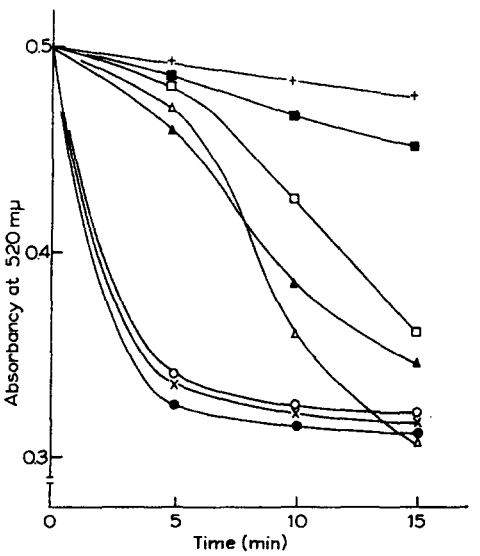

Fig. 1. Swelling of liver mitochondria induced by various fatty acids. Incubation mixture: 0.125 M KCl, 0.02 M Tris–HCl (pH 7.5), $1.33 \cdot 10^{-5}$ M sodium salts of fatty acids as indicated below, and rat-liver mitochondria containing about 5 mg protein; total volume, 7.5 ml; temperature, 20°. +—+, spontaneous swelling; ×—×, oleic acid; □—□, caproic acid (C_6); △—△, caprylic acid (C_8); ○—○, lauric acid (C_{12}); ●—●, myristic acid (C_{14}); ▲—▲, palmitic acid (C_{16}); ■—■, arachidic acid (C_{20}).

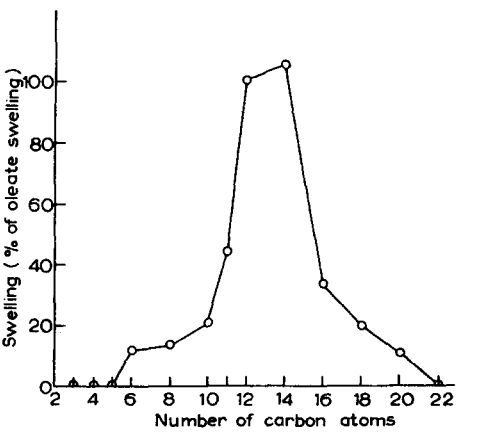

Fig. 2. Effect of saturated fatty acids on swelling of mitochondria from rat liver. Experimental conditions as in Fig. 1; incubation time, 5 min. Extent of swelling is expressed as per cent of the swelling produced by the same concentration of oleate during the same incubation time.

length of the saturated acids is seen in Fig. 2 where the swelling, during the first 5 min of incubation, is plotted against the number of carbon atoms. In order to facilitate comparison of results obtained from different experiments, the swelling produced by various acids is expressed as per cent of the swelling produced by the same concentration of oleate.

Fig. 2 shows that among the saturated acids the greatest swelling was produced by the acids of medium chain length (C_{12} and C_{14}). No swelling was obtained with fatty acids containing 5 carbon atoms or less, nor by behenic acid (C_{22}), even when these acids were used in a concentration ten times higher than that shown in Fig. 1.

A comparison of the swelling effect of saturated and unsaturated acids is shown in Table I. It is evident that *cis* unsaturated acids had a more pronounced swelling effect than saturated acids. Thus, oleic acid was a more potent swelling agent than stearic acid, and erucic acid was more active in this respect than behenic acid. On the other hand, the swelling effect of the *trans* isomer of oleic acid, namely elaidic acid, was almost the same as that of stearic acid. Additional unsaturation (linoleic acid) or hydroxylation (ricinoleic acid) or substitution of the triple bond for the double bond (stearolic acid) produced no further enhancement of the swelling effect as compared with oleic acid.

TABLE I

SWELLING EFFECT OF FATTY ACIDS

Experimental conditions as in Fig. 1.

Fatty acid		*Swelling after 5 min incubation (as per cent of oleate swelling)*
Name	*Shorthand designation**	
Stearic acid	18:0	20
Oleic acid	*cis* 18:1^{9}	100
Elaidic acid	*trans* 18:1^{9}	17
Linoleic acid	*cis cis* 18:2^{9,12}	98
Stearolic acid	18:1^{9}	99
Ricinoleic acid	*cis* 18:1^{9} hydroxy^{12}	111
Behenic acid	22:0	0
Erucic acid	*cis* 22:1^{13}	10

* The first number indicates chain length, the second indicates the number of double (:) or triple (:) bonds, and the index number the position of the unsaturated bonds.

The present results are in good agreement with those obtained by AVI-DOR[7], and reveal a striking similarity between the effect of various fatty acids on mitochondrial swelling and on the reactions of oxidative phosphorylation. Thus, PRESSMAN AND LARDY[3] found that the most potent activators of mitochondrial ATPase were acids of chain length from 12 to 16 carbon atoms (compare Fig. 2 in ref. 3 with Fig. 2 in this paper), and that *cis* unsaturated acids were more active than the saturated ones. A similar dependence upon the number of carbon atoms has also been observed with respect to the inhibition of the ATP–P_i exchange reaction[8], the most inhibitory acids being those of medium chain length. Finally, a stronger uncoupling action of unsaturated acids on the oxidative phosphorylation, as determined by the decrease in P:O ratio, has been reported by BORST *et al.*[9]. All these facts support the view[11]

that swelling of mitochondria is connected with the mechanism of coupling the electron transfer to the synthesis of ATP.

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A modified benzidine method for the chromatographic detection of sphingolipids and acid polysaccharides

Many sphingolipid and acid polysaccharide molecules of current biological interest can be detected by virtue of the reaction of their secondary amide group. When the Cl-substituted derivative of this amide is prepared and then combined with benzidine, a blue reaction product is formed[1] (Fig. 1). The modified method to be described differs chiefly from the foregoing method in two respects:

(1) The color reaction is more sensitive in practice (down to 5–10 μg).

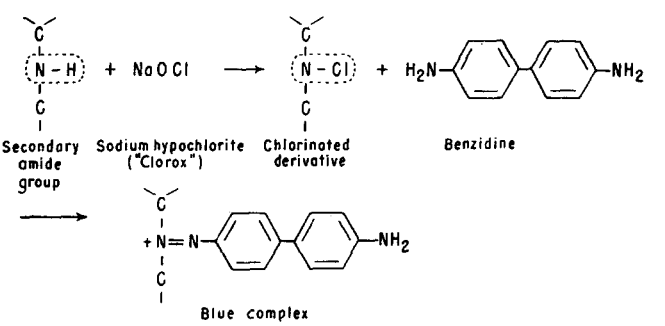

Fig. 1. One schematic simplification of the "Clorox–benzidine" method for detection of secondary amide groups. (This is but one of several theoretical possibilities and is thus neither the only possible nor the only correct reaction.)