Effect of Unsaturation in Fatty Acid-Induced Swelling of Rat Liver Mitochondria

The induced swelling of mitochondria by fatty acids is well known[1–4]. Avi-Dor[1] and Zborowski[4] showed that unsaturated fatty acids were more effective swelling agents than saturated fatty acids, but observed no difference between oleic and linoleic acid. Of the saturated fatty acids, those of intermediate chain length were the most effective in causing swelling of liver mitochondria. Although results vary widely, it appears that fatty acid composition of the dietary fat, especially relative to essential fatty acids, exerts an influence on the in vitro swelling properties of liver mitochondria under various conditions[5–8]. Houtsmuller et al.[5] investigated conditions whereby swelling amplitude of mitochondria could be used as an assay method for essential fatty acid activity. Inasmuch as free fatty acids are normal cell constituents[9], it appeared to be pertinent relative to the role of dietary fatty acid composition in membrane structure and function to investigate the effect of common unsaturated fatty acids on the swelling properties of liver mitochondria. Reported here are studies on the relative effects of 18:1, 18:2 and 20:4 acids as swelling agents under various conditions for rat liver mitochondria.

Methods. Weanling male rats of the Sprague-Dawley strain (Rolfsmeyer Co., Madison, Wisconsin) were raised on a basic sucrose-casein diet[10] and 10% by weight of cornoil. The animals were killed at ca. 3 months of age, the livers were excised, cooled to 0 °C, and homogenized in 10 volumes of 0.25 *M* sucrose containing 20 m*M* *Tris*-HCl buffer, pH 7.4. The mitochondrial fraction was preparated essentially according to the procedure of Sottocasa et al.[11] Microsomal contamination of the mitochondria was reduced to less than 10% as measured by their glucose-6-phosphatase content in this procedure. Contamination of the mitochondria with lysosomes was also minor as judged by the acid phosphatase activity. Fresh mitochondria (obtained less than 5 h after killing the animals) were used in the swelling experiments which were carried out using a Beckman DU spectrophotometer connected to a Haake thermostat to insure constant temperature. Rate and degree of swelling was followed by a decrease in absorbance at 520 nm at a temperature of 25 °C. Suspensions of 20 *mM*, pH 7.0, of the Na-salts of each of the fatty acids were prepared. The fatty acid-albumin complexes were prepared with 5% bovine serum albumin from Sigma (fat-free). Only fresh prepa-

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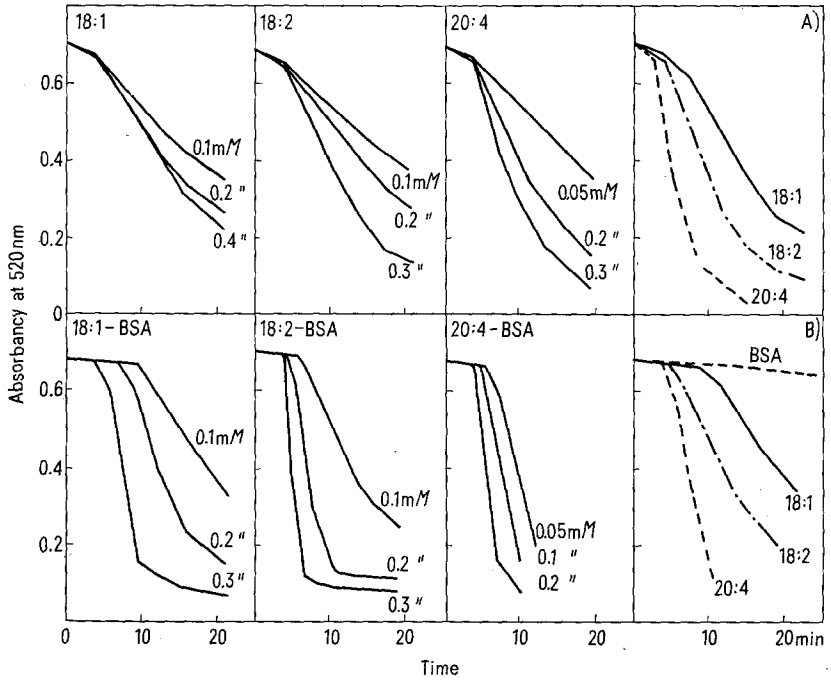

Fig. 1. Swelling of liver mitochondria induced by various concentrations of the sodium salts of oleic (18:1), linoleic (18:2), and arachidonic (20:4) acid (upper part), and of the bovine serum albumin (BSA) complexes of the same fatty acids (lower part). Incubation mixture: 0.25 *M* sucrose, 20 m*M* *Tris*-HCl buffer, pH 7.5, 0.1 to 0.4 m*M* of the sodium salt of the fatty acid, pH 7.0, or 0.05 to 0.3 m*M* fatty acid plus 5% bovine serum albumin, pH 7.0, and 0.1 mg mitochondrial protein, total volume 2.0 ml, temp. 25 °C. A) Comparison of the swelling amplitude of the 3 fatty acids at 0.3 m*M* concentrations. B) Comparison of the swelling amplitude of the three fatty acids at 0.1 m*M* concentrations (BSA complexes).

rations were used for the swelling experiments. Protein was determined by the method of LOWRY et al.[12].

Results and discussion. The effects of different concentrations of the sodium salts of oleic (18:1), linoleic (18:2), and arachidonic (20:4) acid on the swelling amplitude of rat liver mitochondria are shown in Figure 1 (upper part). The rate of swelling increased with fatty acid concentration employed in the range between 0.05 and 0.4 m*M*. Figure 1A shows that, at the same concentration, the rate of swelling was increased with increasing number of double bonds in the fatty acid. Any significant effect of chain length (C-18, C-20) can virtually be excluded in this experiment on the basis of the results of ZBOROWSKI et al.[4]. The increase in the swelling amplitude occurred in a linear fashion (Figure 2).

From the studies of AVI-DOR[1] and ZBOROWSKI and WOJTCZAK[4], it became apparent that mitochondria swelling differs with chain length (between C-10 and C-18) and possibly also with unsaturation which, however, was not investigated in greater detail. The present study shows that the swelling properties of isolated rat liver mitochondria can be affected by the degree of unsaturation of the fatty acid. The mechanism that triggers the swelling of the mitochondria may be due to a number of reasons such as a faster penetration of the higher unsaturated fatty acids through the mitochondrial membrane, a greater uncoupling effect of these acids[13–15], or a greater stimulating effect on the ATPase[16], all factors that have been known to be related to mitochondria swelling. The relationship between number of double bonds and degree of swelling induced by the sodium salts of the fatty acids was obvious but not very great (Figure 2), corresponding to the influence of individual fatty acids as uncouplers and stimulating agents of the ATPase activity[13–16].

The swelling characteristics of oleic, linoleic, and arachidonic acid as albumin complexes are shown in Figure 1 (lower part). As with the sodium salts, the rate of swelling increased at concentrations ranging from 0.05 to 0.3 m*M*. The major differences between the swelling properties of the sodium salts and the albumin complexes was the existence of a lag phase followed by a greater swelling rate with the latter. The lag phase or induction period appears to be related to the concentration as well as the degree of unsaturation of the fatty acid of the albumin complexes (Figure 1B). In case of 0.1 m*M* oleic acid, the lag phase lasted for about 10 min, exactly the time during which reserval of the oleic acid induced uncoupling by albumin was observed[16]. No swelling occurred with albumin alone (Figure 1B). Inasmuch as the effect of albumin has been attributed to its ability to combine with fatty acids[17,18], it seems probable that the ratio of the fatty acid-albumin complex may influence the extent of the lag phase. Thus, the inhibitory action of albumin on the fatty acid-induced mitochondria swelling, and hence the uncoupling effect may be altered by different albumin concentrations, thereby explaining some differences between results obtained by different investigators[15]. The plot of the percentage of swelling after 10 min versus the number of double bonds at constant fatty acid concentration (0.1 m*M*) gave a straight line with a slope steeper than for the salts of the fatty acids (Figure 2). From these data the function of albumin appears to be more complex, whereby part of its effect might be counteracted by an albumin denaturing effect of the unsaturated fatty acids, as has been shown for ovalbumin[19]. Nevertheless, the effect of free fatty acids on mitochondrial function could in vivo be basic in part to the effect of dietary fatty acid composition, inasmuch as dietary fat influences fatty acid composition of mitochondria[20,21] and undoubtedly the composition of the free fatty acid constituents of these organelles.

Zusammenfassung. In Gegenwart von Öl-(18:1), Linol-(18:2) oder Arachidonsäure (20:4) wurde eine konzentrationsabhängige, proportional zur Zahl der Doppelbindungen verlaufende Schwellung von Rattenleber Mitochondrien beobachtet. Die Fettsäure-Albumin Komplexe unterscheiden sich von den Natriumsalzen durch eine Lag-Phase vor Beginn der Schwellung gefolgt von einer stärkeren Schwellungsamplitude.

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23 January 1973.

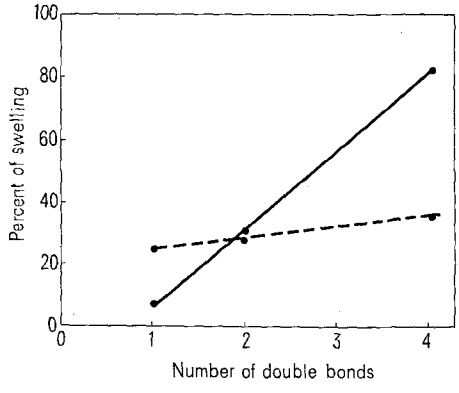

Fig. 2. Extent of swelling in percent of the unswollen sample in relation to the number of double bonds. Concentration of fatty acid: 0.1 m*M*, swelling time 10 min. Solid line, sodium salts; broken line, fatty acid-BSA complexes.

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