INHIBITION OF MITOCHONDRIAL CONTRACTION BY A SOLUBLE MUSCULAR RELAXING FACTOR-PREPARATION

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Evidence that a soluble relaxing factor exists in muscle tissue has recently been obtained (Fuchs and Briggs, 1961, 1963; Baltscheffsky, 1964). The demonstration that very small amounts of relaxing factor-solution from a muscle homogenate can inhibit the superprecipitation of isolated actomyosin (Baltscheffsky, 1964; Baltscheffsky, Lagerkvist and Baltscheffsky, 1964) and the reported presence of actin- and myosin-like proteins in liver mitochondria (Ohnishi and Ohnishi, 1962; Neifakh and Kazakowa, 1963) prompted us to test the possibility that the relaxing factor-solution might influence contraction in isolated liver mitochondria.

It is well-known that ATP and Mg^{++} play a role in the contraction of isolated actomyosin. In liver mitochondria, swollen through the action of orthophosphate in a salt medium (Raaflaub, 1953), strong shrinking (contraction) may be obtained both by addition of agents necessary for oxidative phosphorylation (Beyer, Ernster, Löw and Beyer, 1955) and by addition of ATP + Mg^{++} (Baltscheffsky, 1957). The influence of changing metabolic states on mitochondrial structure has been examined by Packer (1960, 1961, 1962). Mitochondrial swelling and the contraction obtained with ATP + Mg^{++}, and several other agents, have been intensively studied by Lehninger (1959, 1962a, 1962b), who has developed the concept that mechanisms of "mechanochemical" activities in muscle and in

mitochondria may be very similar to or even identical with mechanisms of energy transfer in oxidative phosphorylation. Accordingly, this process and muscular activities may have more in common than is hitherto known.

EXPERIMENTAL. Liver mitochondria from guinea-pig were used following the earlier experience that they were more stable and gave a more complete contraction after addition of ATP + Mg^{++} than those from rat (Baltscheffsky, 1957). Actomyosin was prepared from rabbit muscle, and washed before its use, mainly according to Ebashi (1961). Relaxing factor was isolated from a rabbit muscle homogenate according to Baltscheffsky (1964) and the final supernatant was fractionated on Sephadex G-25. The fractions were tested for relaxing activity with the actomyosin system (Ebashi, 1961) and protein content. Fractions with high relaxing activity and low protein content were used in the mitochondrial experiments. The contraction of mitochondria was measured in a Beckman Model B spectrophotometer as increase with time in optical density of the suspension at 510 mμ, a method, which has been discussed in detail by Lehninger (1962a). The superprecipitation of actomyosin was measured according to the method of Ebashi (1961), but at a wavelength of 620 mμ.

RESULTS AND DISCUSSION. Fig. 1 shows that added relaxing factor--solution strongly inhibited the ATP + Mg^{++}-induced contraction of isolated guinea-pig mitochondria and that the time for addition influenced its action. If relaxing factor-solution was added when the contraction phase had already been initiated by addition of ATP + Mg^{++}, an as strong initial contraction as in the control could still occur. On the other hand, addition of relaxing factor-solution several minutes before the initiation of contraction resulted in a very efficient inhibition. This difference may be due to either a

limited penetration rate of the added agent to the site of its action or an influence of the different states of the contractile structures at the time for addition of relaxing factor-solution (presence and absence of added ATP + Mg^{++}).

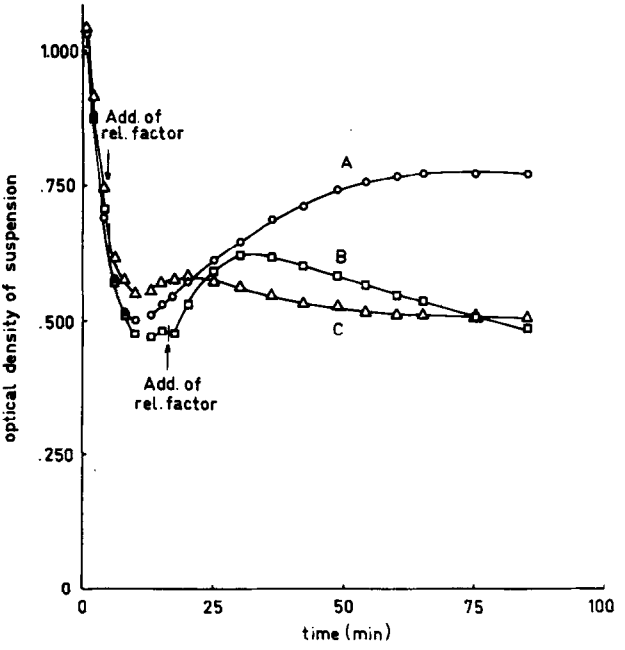

Fig. 1. Effect of relaxing factor-solution and the time for its addition on ATP + Mg^{++}-induced mitochondrial contraction. In the cuvettes A, B and C: 2.75 ml medium containing 105 mM KCl and 16 mM orthophosphate, pH 7.2, and 20 µl 0.2 M glutamate. Mitochondria (700 µg protein) were added to each sample at zero time. At the time interval where the curves are discontinued (10-13 min) 0.75 mM ATP and 7.5 mM $MgCl_2$ (small volumes, final concentrations) were added to each cuvette. 0.2 ml relaxing factor solution containing 10 µg of protein in 0.3 M sucrose was added to B and C at the times indicated. 0.2 ml of 0.3 M sucrose was added to the control (A) after 6 min. (Expt 99664)

Fig. 2 shows that relaxing factor-solution, which had been boiled for one hour gave a strongly decreased inhibition of mitochondrial contraction, which is in line with its strongly diminished relaxing action on the actomyosin system (Baltscheffsky, Lagerkvist and Baltscheffsky, 1964). This result clearly indicates that the same relaxing agent was active in both systems. The fact

that the effect of the unboiled relaxing factor-solution was here mainly a postponement of contraction, rather than an abolishment, renders a non-specific action on mitochondrial contraction very unlikely. Further evidence that the relaxing effect in mitochondria is not due to unspecific destruction of structural integrity by the added solution, was obtained by experiments, to be described elsewhere, showing that addition of relaxing factor-solution to phosphorylating mitochondria did not decrease the rate of phosphate esterification.

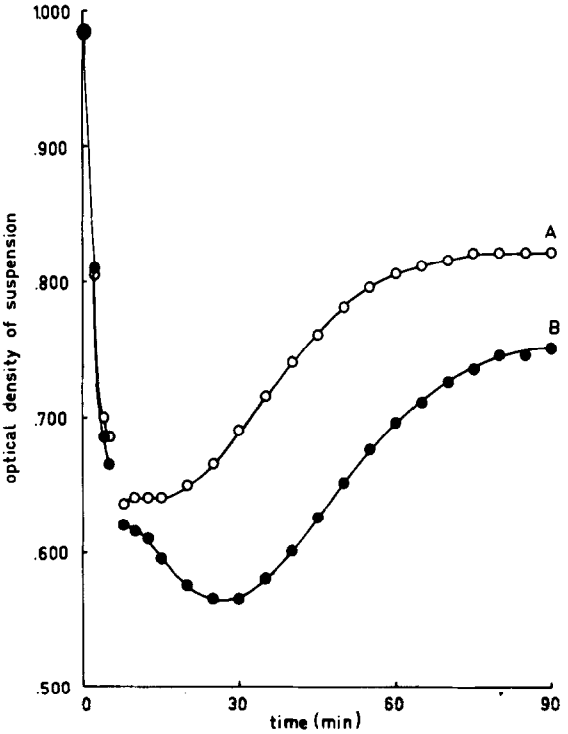

Fig. 2. Effect of prolonged boiling on the activity of relaxing factor-solution. Conditions as in Fig. 1, with the exceptions that the relaxing factor-solution was added immediately before the experiment and no glutamate was added. A = relaxing factor-solution boiled for 1 hr; B = unboiled relaxing factor-solution. (Expt 79664)

Also with isolated rat-liver mitochondria it was possible to obtain strong inhibition of contraction by addition of relaxing factor-solution. The demonstration that a soluble muscular relaxing

factor inhibits ATP + Mg^{++}-induced contraction in isolated liver mitochondria adds support to the concept that such a factor is indeed a participant in the physiological activities of contractile structures. The data presented also give evidence for functional resemblances between contractile elements in muscle fibers and mitochondria, such as would appear likely or at least possible in view of the above-mentioned, recently demonstrated structural similarities between the two systems. In view of some striking similarities between various reactions related to energy transfer in muscle and mitochondria (Lehninger, 1962b) and the occurrence of actin- and myosin-like proteins in mitochondria (Ohnishi, 1962) and chloroplasts (Ohnishi, 1964) it seems quite possible that this and similar approaches may become increasingly useful in the study of the mechanism of electron transport-coupled phosphorylation.

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