SIMILARITY OF EFFECTS OF IODINE AND THYROXINE UPON RAT LIVER MITOCHONDRIA

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The action of thyroid hormones upon oxidative phosphorylation (Lardy and Feldott 1951; Martius and Hess 1951) and upon the structural state of mitochondria (Tapley, Cooper and Lehninger 1955) have seemed to represent fundamental effects of these hormones. Whether thyroxine acts *in toto*, or whether it serves as a donor of I_2 (or I^+) is unknown. Since the latter possibility is open to investigation we have studied the effect of I_2 on certain aspects of mitochondrial structure and function.

Mitochondria were prepared from rat livers with certain modifications (Roche *et al.* in press) of the procedure of Schneider (Schneider 1948). Table I shows that I_2 caused swelling of mitochondria which, in certain respects, corresponded closely to the results seen with thyroxine (T_4). As little as 5×10^{-6} M I_2 caused demonstrable swelling. Strong sucrose solutions and albumin inhibited swelling from both I_2 and T_4, adenosine triphosphate (ATP) but not adenosine diphosphate caused contraction of mitochondria swollen by either T_4 or I_2. Cu^{++} (as cupric acetate) and potassium ferricyanide were chosen as oxidizing agents with redox potentials at pH 7.4 roughly similar to I_2. Ferricyanide was without effect on the size of mitochondria but Cu^{++} caused marked swelling. This swelling was not however reversed by ATP. Bromine (as Br_2) likewise caused swelling of mitochondria but was not reversed by ATP.

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TABLE I

Effect of Several Substances on Mitochondrial Size

	I_2	T_4	Cu^{++}	Br_2
Sucrose (0.75M)	—	—	—	—
Albumin (BSA, 1 mg/ml)	—	—		
Amytal (1.8×10^{-3}M)	+	—		
CN^- (10^{-3}M)	+	—		
Reversal with ATP (5×10^{-3}M)	Yes	Yes	No	No
Reversal with ADP (5×10^{-3}M)	No	No		
Reversal with DPNH (5×10^{-4}M)	No	No		

I_2, T_4, Cu^{++} and Br_2 produced swelling of mitochondria at concentrations of 5×10^{-6}M, 10^{-7}M, 10^{-5}M, and 10^{-5}M respectively in a medium of Tris 0.02M KCl 0.125M, pH 7.4. A + indicates that swelling was still produced by one of these substances in the presence of another material as indicated and a — that swelling was inhibited. Contraction of mitochondria swollen by these substances is indicated by yes or no. Size of mitochondria was determined by optical density at 520 mμ, and an initial O.D. of between 0.6 and 0.9 was employed.

Swelling by T_4 is prevented by pretreatment with amytal and cyanide (Lehninger and Ray 1957; Lehninger, Ray and Schneider 1959), but these reagents do not prevent swelling from I_2. In the case of CN^-, formation of ICN surely occurs. However, ICN itself is active as a swelling agent. Iodide at a concentration of 10^{-3}M is without effect upon mitochondria as previously reported (Lehninger 1961).

The contraction by ATP of the I_2 swollen mitochondria seemed of particular importance as it is a fairly specific action on T_4 swollen mitochondria (Lehninger 1959). It was therefore studied in more detail. Table II compares the effect of three compounds added to the mitochondria simultaneously with ATP upon the reversal seen with ATP alone. In these respects T_4 and I_2 swollen mitochondria react identically.

Figure 1 demonstrates the kinetics of swelling with I_2 and T_4. It can be seen that the effect of I_2, even at a concentration

TABLE II

ATP Reversal of Swelling by I_2 or T_4

	I_2	T_4
Arsenate (5×10^{-3}M)	not reversed	not reversed
DNP (2,4-dinitrophenol) (5×10^{-5}M)	reversed	reversed
CN^- (10^{-3}M)	reversed	reversed

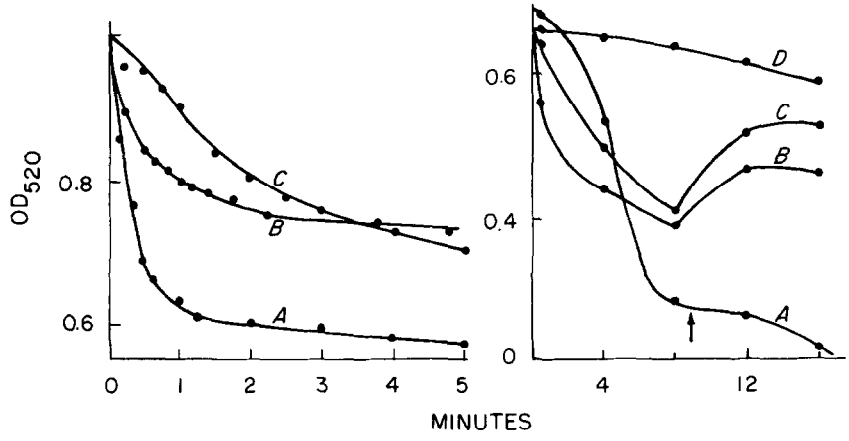

Fig. 1 and 2. On the left is depicted the decreases in O.D. at 520 mμ of mitochondria suspended in Tris 0.02M, KCl 0.125M, pH 7.4 upon various additions at time zero. O.D. of the tubes are normalized to zero time = 1.0. A = I_2 at a final concentration of 3×10^{-5}M; B = I_2, 2×10^{-5}M; C = thyroxine, 5×10^{-6}M.

On the right is depicted changes in O.D. under similar circumstances. The curves are not normalized. A = Cupric acetate, 2×10^{-5}M; B = I_2, 2×10^{-5}M; C = thyroxine, 10^{-5}M; D = control. At the arrow, ATP was added to each tube to give a final concentration of 10^{-3}M.

which produces submaximal swelling, is considerably faster than that produced by T_4. Figure 2 shows a typical experiment with I_2, T_4, Cu^{++} and the effect of ATP on the swollen mitochondria. I_2 as an oxidant will effect many reactions such as oxidation of sulfhydryl groups, aldoses, etc., and these non specific effects may mask at high concentrations more specific effects. Since I_2 rapidly oxidizes

DPNH (unpublished observations) it seemed possible that the effect of I_2 is mediated by the state of DPNH. However the addition of DPNH to I_2 or T_4 swollen mitochondria was without effect (see Table I) although the binding of DPNH by mitochondria may not have occurred.

Oxygen consumption was also studied using standard methods and a buffer previously described (Dickens and Salmony 1956). For determination of oxygen consumption mitochondria, in a concentration equal to one gram of liver per 3.3 ml of medium, were employed. Oxygen consumption was very sensitively related to the ratio of I_2 to mitochondria and an excess of I_2 depressed utilization of oxygen and small amounts were without effect. In both oxygen consumption and swelling experiments a higher concentration of I_2 seemed to be required to produce an effect on more concentrated mitochondrial suspensions. Several experiments using glutamate or succinate as a substrate have shown that 4×10^{-4}M I_2 caused a 15-20% increase in oxygen consumption of mitochondria, over that of control flasks containing equivalent amounts of KI. This is contrary to the findings of Klemperer who however, employed a 30 minute preincubation and a different concentration of mitochondria (Klemperer 1955). If the major effect of I_2 were to oxidize DPNH it might be expected to decrease total oxygen consumption. It appears at this time therefore, that I_2 has a specific effect on mitochondria not shared by other oxidizing agents and which is remarkably similar to the effects caused by thyroxine.

REFERENCES

Dickens, F., and Salmony, D., Biochem. Jour. 64, 645 (1956).
Klemperer, H. G., Biochem. Jour. 60, 122 (1955).
Lardy, H. A., and Feldott, G., Ann. N. Y. Acad. Sci., 54, 636 (1951).

Lehninger, A. L., J. Biol. Chem., 234, 2187 (1959).

Lehninger, A. L., Biochim. Biophys. Acta 48, 324 (1961).

Lehninger, A. L., and Ray, B. L., Biochim. Biophys. Acta 26, 643 (1957).

Lehninger, A. L., Ray, B. L., and Schneider, M., J. Biophys. and Biochem. Cytol., 5, 97 (1959).

Martius, C., and Hess, B., Arch. Biochem. Biophys., 33, 486 (1951).

Roche, J., Rall, J. E., Michel, R., Michel, O., and Varrone, S., Biochim. Biophys. Acta (in press).

Schneider, W. C., J. Biol. Chem., 176, 259 (1948).

Tapley, D. F., Cooper, C., and Lehninger, A. L., Biochim. Biophys. Acta 18, 597 (1955).