THE ACTION OF IODIDE ON OXIDATIVE PHOSPHORYLATION*

by

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LOOMIS AND LIPMANN[1] have discovered that 2,4-dinitrophenol (DNP) uncouples oxidative phosphorylation in mitochondria. Later LIPMANN *et al.*[3] as well as MARTIUS *et al.*[6] have shown thyroxine to have a similar action. It seemed interesting to know what inorganic iodide would do in the same experiment. We find that in relatively low concentration it uncouples oxidative phosphorylation, that is, it suppresses completely the formation of $\sim$ P without inhibiting oxidation.

EXPERIMENTAL

Methods

Mitochondria were prepared from freshly-killed rats, young males of the Worcester strain (Charles River Breeding Laboratories) by the method of HOGEBOOM, SCHNEIDER AND PALLADE[7] and modified by KENNEDY AND LEHNINGER[8]. The chilled livers were pressed through silver wire gauze of mesh 0.5 mm and homogenized with a Sargent homogenizer in 0.25 *M* sucrose at 0° C. The sucrose volume was adjusted to 125 ml and centrifugation carried out at 600 *g* for 5 minutes at 0–2° C to remove debris, blood corpuscles, *etc.*, and this was repeated. The decanted supernatant was measured for volume, and to it one-tenth of its volume of 1.5% KCl was added, which agglutinated the mitochondria in 10 minutes, after which centrifugation at 2500 *g* brought down the mitochondria, in 20 minutes. The pellet was resuspended in 0.15% KCl in 0.01% $KHCO_3$, and centrifuged for 10 minutes, and finally resuspended in 0.15% KCl only for the last centrifugation for a period of 10 minutes. This last sediment was resuspended in 10 ml KCl solution, but in each run the dry weight of a measured volume of the suspension was taken, giving on the average a dry weight of 15 mg per 0.5 ml sample.

The mitochondrial sample was placed in Warburg flasks to measure oxidation and phosphorylation. The reaction mixture contained: glycylglycine 0.04 *M*; cytochrome *c* 4 μM; ATP 1 μM; KH_2PO_4 0.013 *M*; DPN 0.13 m*M*; B-hydroxybutyrate 0.01 *M*. $MgCl_2$ was in each control vessel, but in replacing chloride by iodide, this was replaced by MgI_2. This applied also to the KCl, 0.025 *M* in the control being replaced by KI in some flasks. In the side arm was 2.00 mg yeast hexokinase and 0.1 ml 1% glucose, forming the acceptor system. The total volume was 3.0 ml, pH 7.4, the center well containing 0.2 ml 20% KOH. Measurements were made at 28° C, equilibration time 10 minutes. After 10 minutes measurement of respiration rates, the side-arm contents were tipped in and the phosphate acceptor introduced.

After oxidation measurements were completed, the reaction was stopped by the addition of TCA, and the flask contents made up to 12 ml. An aliquot of this solution was taken for the estimation of inorganic phosphate. This was done by the Fiske-Subarrow method. The amount of phosphorus esterified was taken as the difference between the flask value and that of controls, into which TCA was introduced when respiration measurements were first begun.

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Results

The effect of replacing chloride by iodide in the reaction mixture is expressed graphically in Fig. 1. Keeping the total molar concentration of Cl + I at 0.04 *M*, iodide was added in increasing concentrations to finally replace all the chloride in the control reaction mixture.

As iodide replaces chloride, it can be seen that the effect upon respiration is slightly stimulatory, even when all the chloride has been replaced by iodide.

The effect upon phosphorylation is clear also, in that an increase of iodide concentration causes increased depression of phosphorylation (as measured on a control in which chloride alone is present in the reaction mixture), reaching a complete suppression at about 0.027 *M* iodide. The concentration of iodide needed to bring about some supression of phosphorylation is very small, being of the order of 0.01 *M*.

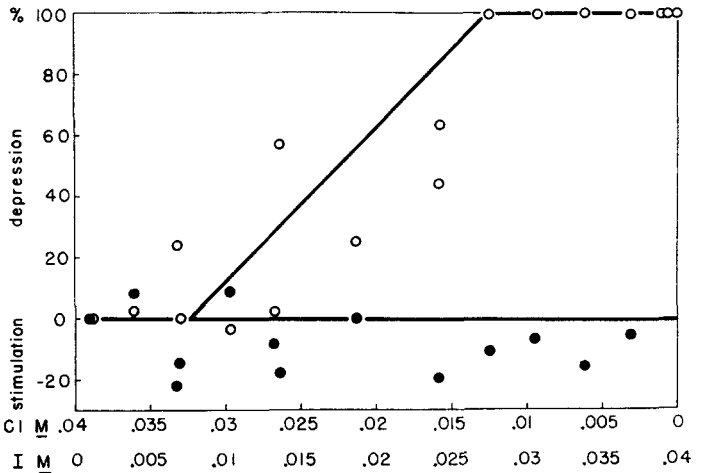

Fig. 1. Effect of replacing chloride by iodide in reaction mixtures on phosphorylation (open circles) and respiration (full circles) expressed in % of control in 0.04 *M* chloride.

This uncoupling of phosphorylation by iodide appears to have a close bearing on the observed effect of thyroxine on liver mitochondria under the same conditions. HOCH AND LIPMANN reported that the substrate on which the depression of phosphorylation was most marked was β-hydroxybutyrate, "demonstrating that thyroxine, like dinitrophenol, could disrupt energy transformations over the whole potential range of respiratory electron transfer".

DISCUSSION

The ability to suppress oxidative phosphorylation is a specific one and so if DNP, thyroxine and iodine share it, it is likely that they also share the same mechanism of action. So thyroxine might be looked upon as being essentially iodide linked to an organic radical which provides it with a specific reactivity and affinity, enabling it thus to exert its action in a low concentration at specific points. It can be hoped that a better understanding of the mechanism of uncoupling may lead to a better understanding of the mechanism of oxidative phosphorylation itself.

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As far as the authors are aware from personal communications, several researchers are inclined to explain the action of DNP by supposing that it competes with the physiological phosphate acceptors. This assumption has no experimental evidence and is made highly improbable by the uncoupling action of iodide which can hardly act as an acceptor. The action of iodide has to be explained by some specific quality of this ion. Iodine has various specific qualities. It stands with KSCN at the top of the Hofmeister series and shows a corresponding activity in colloidal reactions. This colloidal reactivity, however, cannot be invoked in the present case giving no satisfactory explanation for the activity of iodine built into the thyroxine molecule.

Another rather specific quality of iodide is its ability to quench fluorescence, that is quench the excited state of certain molecules by taking over and dissipating their energy of excitation. In fact iodide is the strongest quencher among inorganic anions. It follows that if iodide inhibits oxidative phosphorylation as a quencher, DNP and thyroxine also should be found to be quenchers. MC LAUGHLIN *et al.*[9] have actually shown that DNP and other nitrophenols do act as quenchers. They also showed that thyroxine acted likewise. Though their evidence was not conclusive it supports the assumption that nitrophenols, thyroxine and iodide suppress oxidative phosphorylation by quenching states of excitation and so, turning the argument round, its supports the assumption that such states of excitation play an important role in the biological energy transmission within mitochondria.

It seems not impossible that the lengthening of the active state in muscle by iodide, revealed by the experiments of CHAO[10], KAHN AND SANDOW[11], RITCHIE[13], and HILL AND MC PHERSON[12], may have an analogous explanation.

In these studies the action of bromide was found to be similar to that of iodide with the difference that it was weaker. In our experiments we found an analogous behaviour. Similarly to iodide, bromide uncoupled oxidative phosphorylation but did so only in a higher concentration. While iodide almost completely uncoupled in a 0.025 *M* concentration, bromide, in the same concentration, gave only a 3% inhibition and achieved complete uncoupling only in 0.04 *M*. This also supports the assumption that the active state in muscle is lengthened by iodide because the energy transmission within, or to, the excitatory mechanism was inhibited, possibly by the quenching of the state of electronic excitation of molecules involved in this process. This latter supposition finds further support in the observation that nitrophenols which uncouple oxidative phosphorylation also depolarise the membrane (HAJDU *et al.*[14], LÖWENSTEIN *et al.*[15]), which depolarising activity of various nitrophenols was found to go parallel with their quenching of fluorescence (MC LAUGHLIN *et al.*[9]).

SUMMARY

When iodide replaces chloride it suppresses oxidative phosphorylation in rat mitochondria. The results are discussed.

RÉSUMÉ

La substitution des iodures aux chlorures supprime la phosphorylation oxydative par les mitochondries du rat. Les résultats sont discutés.

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ZUSAMMENFASSUNG

Durch Ersetzung der Chloride durch Iodide wird die oxydative Phosphorylierung in Rattenmitochondrien unterbunden. Die Ergebnisse werden erörtert.

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