

Respiratory Metabolism of Parasitic Helminths without Participation of the Cytochrome System

LASER¹ has demonstrated high succinic dehydrogenase activity in extracts of muscle pulp of *Ascaris lumbricoides*. In the presence of succinate these preparations reduce methylene blue, but do not react with atmospheric oxygen. On the other hand, succinate stimulates the respiration of dialysed muscle mince of *Ascaris*². Furthermore, we have observed that homogenates of thoroughly washed *Ascaris* muscle oxidize succinate even in the absence of methylene blue. The activity of this preparation was markedly higher in 100 per cent oxygen than in air, and was increased when catalase and ethyl alcohol were added, indicating the production of hydrogen peroxide¹. No oxygen uptake was observed under similar conditions in the absence of succinate. Therefore, succinic dehydrogenase of *Ascaris* muscle can transfer hydrogen either to methylene blue or to an enzyme system, which, in turn, reacts with atmospheric oxygen. However, the latter is not identical with the cytochrome-cytochrome oxidase system considered to be the universal catalyst of all respiring cells³.

Addition of homogenates of unwashed *Ascaris* muscle did not increase the oxidation of succinate by succinoxidase of pig heart prepared according to Keilin and Hartree⁴. This preparation is deficient in cytochrome *c*, and as little as 3×10^{-10} moles of cytochrome *c* can be determined manometrically by this assay (*v_F*, 1.0 ml.; *v_G*, 4–5 ml.; pH 7.4). The activity of pig heart succinoxidase in the presence of succinate and of low concentrations of cytochrome *c* (6×10^{-7} molar) was not inhibited by the same homogenate of *Ascaris* muscle. On the basis of this assay, *Ascaris* muscle contains less than 2×10^{-9} moles of cytochrome *c* per gm. Furthermore, no cytochrome oxidase activity could be detected in *Ascaris* muscle homogenates when they were added to cytochrome *c* (molar concentration: 3.8×10^{-5}) and hydroquinone (molar concentration: 3.6×10^{-2}) (15° C.; pH 7.1). By contrast, cytochrome oxidase activity was observed when homogenates of rat lung were used. The latter contained five times less tissue than the *Ascaris* homogenates. Among mammalian tissues, lung has the lowest cytochrome oxidase activity⁵. The absence of cytochrome oxidase activity was not due to an inhibitory effect of this tissue on the enzyme, because the activities of cytochrome oxidase preparations^{4,6} were the same in the presence or in the absence of *Ascaris* muscle homogenates.

While succinate oxidation by washed *Ascaris* muscle preparations was increased by methylene blue (but not by two metalloporphyrin pigments recently isolated from *Ascaris* by Davenport⁷), no effect was observed with either cytochrome *c* or a cytochrome oxidase preparation containing no succinic dehydrogenase⁸, added alone or together. Therefore, succinic dehydrogenase of *Ascaris* does not react with the cytochrome system, the presence of which we were unable to detect in this organism. Possibly a flavine enzyme participates in the oxidation of succinate by *Ascaris* muscle.

Evidence has been obtained that the cytochrome system is not involved in the respiration of two other parasitic worms, the filarial nematode *Litomosoides carinii* and the trematode *Schistosoma mansoni*. The oxygen uptake of these two helminths is inhibited completely by cyanide (molar concentration: 2×10^{-4})⁸.

Yet, assay procedures similar to those used for *Ascaris* muscle revealed no cytochrome *c* nor cytochrome oxidase activities in the filariae. The schistosomes contained measurable cytochrome and cytochrome oxidase activities; but they accounted for less than 10 per cent of the overall oxygen consumption of these worms.

It is concluded that respiration in the above three helminths is mediated predominantly, if not exclusively, by respiratory catalysts which are not identical with the cytochrome system.

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E. BUEDING
B. CHARMS

Department of Pharmacology,
Western Reserve University,
School of Medicine,
Cleveland, Ohio.
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Influence of Folic Acid on Xanthine Oxidation *in vitro*

AN inverse relationship between liver xanthine oxidase and dietary folic acid has been demonstrated in the chick^{1,2}. Similar effects are reported for D-amino-acid oxidase activity^{2,3} and in glycine metabolism⁴. Since hydrogen peroxide, a product resulting from oxidations involving flavo-protein enzymes, is known to bring about destruction of haemoglobin⁵, these observations have been interpreted to mean that an excessively rapid rate of such oxidations may contribute to the breakdown of haemoglobin or haem-containing enzymes *in vivo* and thus explain a biochemical change in folic acid deficiency¹. However, recent reports would suggest that folic acid may not have a direct influence on catalase activity² or on liver xanthine oxidase in the rat⁶. Furthermore, earlier observations on the *in vitro* inhibition by folic acid of xanthopterin oxidase activity have now been ascribed to an aldehyde impurity which is a primary photo-fission product of the vitamin⁷.

The influence of folic acid on xanthine oxidation seemed therefore to merit re-investigation. Working with systems containing a milk xanthine oxidase preparation and catalase⁸, we have observed in various experiments some 10–40 per cent stimulation of xanthine oxidation *in vitro* in presence of aldehyde-free folic acid in concentrations of 60–300 μ gm. per cent, which correspond more nearly to the levels obtaining in normal plasma and liver than those used by previous workers. Folic acid does not exert any activation in absence of catalase, the influence of which was shown to be enzymic from experiments with heated catalase. In amounts of 1.5 gm. and more, folic acid inhibits xanthine oxidase activity by at least 20 per cent, presumably by competition with