

Comparison of the Mitochondria of the Small Intestine of Vertebrates

MITOCHONDRIA of the intestinal mucosa of the following vertebrates have been examined by electron microscopy: the rat (*Rattus norvegicus*), guinea pig (*Cavia percellus*), chicken (*Gallus domesticus*), lizard (*Tiliqua rugosa*), toad (*Bufo marinus*) and carp (*Carassius auratus*) (Fig. 1). The mitochondria in the intestinal cells of these species showed no major differences in structure from the other mitochondria that have already been described^{1,2}. The intestinal mitochondria from all these species swelled when they were isolated by differential centrifugation in 0.25 M sucrose (Fig. 2), but all except those from the rat exhibited considerable succinoxidase activity as measured manometrically (Table 1). The oxygen uptake of homogenates of the intestinal mucosa of rats was also low and variable compared with that of homogenates of intestinal mucosa prepared from guinea pigs. In contrast, the oxygen uptake (per mgm. dry weight) of whole intestinal cells of both the rat and the guinea pig was similar.

Homogenates of the intestinal mucosa of rats were found to inhibit the oxygen uptake of homogenates of the intestinal mucosa of guinea pigs. The inhibitor present in the rat intestinal homogenate was not destroyed by heating for 3 min. in a boiling water bath, or by adding hydrochloric acid to a final concentration of 2 N. The inhibitor was not dialysable, but it could be extracted with iso-octane. The iso-octane extract contained a substance that absorbed light strongly at wave-lengths below 250 m μ . This suggested the presence of long-chain unsaturated fatty acids. Chromatographic examination of the extract showed the presence of oleic acid and a smaller amount of linoleic acid. Small quantities of oleic acid

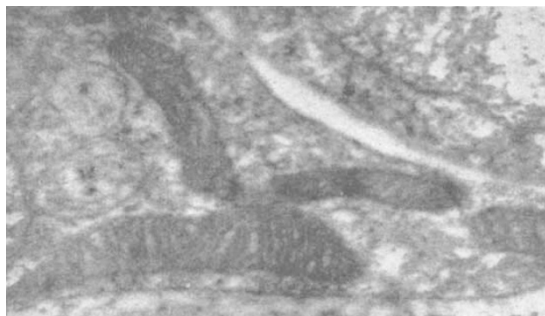


Fig. 1. Electron micrograph of a section of a cell of the intestinal mucosa of *Carassius auratus* showing the mitochondrial structure. $\times 35,000$



Fig. 2. Electron micrograph of a section of mitochondria isolated from the intestinal mucosa of *Carassius auratus*. $\times 37,500$

Table 1. SUCCINOXIDASE ACTIVITY EXPRESSED AS THE $Q_{O_2}N$ OF MITOCHONDRIA ISOLATED FROM THE INTESTINAL MUCOSA OF VARIOUS VERTEBRATES

Animal	$Q_{O_2}N$	Standard error
Rat	< 15	—
Guinea pig	420	13
Chicken	526	13
Lizard	467	13
Toad	212	25
Carp	356	13

(≈ 0.005 ml./mgm. mitochondrial N), or of the material recovered from the iso-octane extract, inhibited by 95 per cent the succinoxidase activity of mitochondria isolated from guinea pigs. The inhibitory effect of oleic acid on a succinoxidase preparation from ox-heart has previously been reported³. Nakamura *et al.*⁴ also ascribed the low succinoxidase activity of rat intestinal mucosa to the presence of fatty acids. That mitochondria from the intestine of the rat do contain succinic dehydrogenase can be shown cytochemically. When these mitochondria are incubated with 2 : 3 : 5 triphenyl tetrazolium chloride in the presence of succinate, deposits of formazan can be seen. No reduction of the dye occurs in the absence of succinate. As cytochrome oxidase has been shown⁵ to donate electrons to triphenyl tetrazolium chloride, it is suggested that the inhibitor of succinoxidase released on the rupture of rat intestinal cells acts between cytochrome oxidase and oxygen. Some evidence to confirm this has been obtained. The cytochrome oxidase activity (measured manometrically) of mitochondria isolated from the intestinal mucosa of the rat is less than one-tenth of that of intestinal mitochondria isolated from the guinea pig.

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¹ Sjostrand, F. S., *Nature*, **171**, 90 (1953).

² Zetterqvist, H., *The Ultrastructural Organization of the Columnar Absorbing Cells of the Mouse Jejunum* (Aktiebolaget Godvil, Stockholm, 1956).

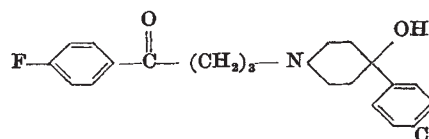
³ Edwards, S. W., and Ball, E. G., *J. Biol. Chem.*, **209**, 619 (1954).

⁴ Nakamura, M., Pichette, P., Broitman, S., Bezman, A. L., Zamcheck, N., and Vitale, J. J., *J. Biol. Chem.*, **234**, 206 (1959).

⁵ Nachlas, M. M., Morgulies, S. I., and Seligman, A. M., *J. Biol. Chem.*, **235**, 2739 (1960).

Combustion Analysis of Paper Chromatographic Spots

THE quantitative determination of paper chromatographic spots is usually carried out by using a variety of photometric techniques. In many instances, however, the known methods lack specificity, reliability and sensitivity.



Haloperidol (R1625)

For haloperidol¹⁻³, a new potent neuroleptic agent recently developed in this Laboratory, a satisfactory method of photometric analysis, suitable for the analysis of minute amounts in chromatographic spots, has not yet been found. I have therefore developed an alternative method, based on the quantitative micro-analysis of the organic fluorine content of the spots.