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Mechanism of the Physiological Action of Rotenone

Rotenone has been widely used as a poison for the extinction of fish populations. Its action was earlier ascribed to a histolysis of the gills[1] and a blocking of the circulation[2] in the gills. Recently, one of us[3] has demonstrated that these effects are secondary changes due to advanced stages of poisoning.

Excised gill filaments from specimens of *Leuciscus rutilus* L. pretreated with rotenone ($3 \cdot 1 \times 10^{-7}$ *M*) for 8 min. show an inhibition of the respiration of 79 per cent (Warburg experiments, medium Krebs–Ringer solution). Addition of 3×10^{-3} *M* methylene blue to the medium restores respiration up to 83 per cent. Also in mouse liver slices rotenone causes an inhibition of the respiration, a concentration of 160×10^{-7} *M* giving an inhibition of 47 per cent.

In order to gain further information on the point of attack of rotenone, we studied its effect on the respiration of rat liver mitochondria, isolated according to Schneider and Hogeboom[4]. The medium was a sucrose–phosphate buffer *p*H 7·4 (2·0 ml.), containing 50 μmoles phosphate, 10 μmoles magnesium chloride, to which were added 2 μmoles adenosine diphosphate, 80 μmoles glucose, and an excess of hexokinase in order to secure maximum phosphorylation. Each experimental sample contained mitochondria corresponding to 350–400 mgm. wet weight tissue. Rotenone was dissolved in ethanol, the final concentration of which in the experimental samples did not exceed $8 \cdot 4 \times 10^{-3}$ *M*. Succinate or pyruvate was used as substrate, and added in amounts of 60 μmoles per sample. It appears from Table 1 that the respiration of mitochondria is inhibited by rotenone only when pyruvate is the substrate. This inhibition is reversed by methylene blue. In order to find out if the inhibition is caused by a block in electron transport or in oxidative phosphorylation we studied the effect of 10^{-4} *M* dinitrophenol on the inhibition caused by rotenone in the same mitochondrial system as above. This substance did not cause any change in the effect of rotenone upon respiration. Obviously rotenone inhibits electron transport, the block being situated on the substrate side of cytochrome *b*. Viewed against the generally accepted opinion that methylene blue acts as an acceptor of electrons from diaphorase, our results suggest that the block caused by rotenone is located between diaphorase and cytochrome *b*. Difference spectra (Fig. 1) between samples identical with those used in the respiration experiments with pyruvate as substrate, and kept under partial anaerobiosis, show, however, that cytochrome a_3, *a*, *c*, *b* and diaphorase are oxidized in the presence of rotenone. We will not comment at present on these results.

Table 1. Respiration of Rat Liver Mitochondria measured at 30° C. in the Warburg Apparatus. Gas Phase Air. For Medium, see Text

Substrate	Respiration μl. O_2/30 min.		
	Control	6×10^{-7} *M* rotenone	6×10^{-7} *M* rotenone + 2×10^{-3} *M* methylene blue
Pyruvate (sparker: 3 μmoles glutamate)	112·3	5·5	96·0
Succinate	205·0	217·2	—

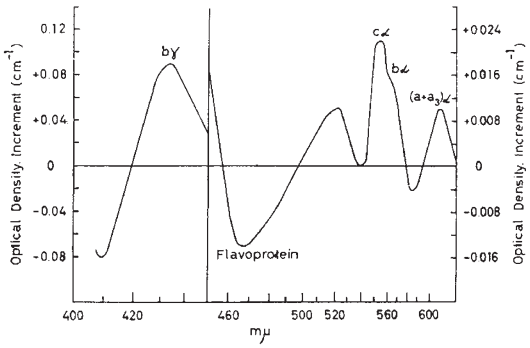

Fig. 1. Spectra representing the differences of absorption between two samples of mitochondria, one of which contained 6×10^{-7} *M* rotenone, registered with the Beckman spectrophotometer *DK*-2. The experimental and control cuvettes contained the reaction media with pyruvate as used in the Warburg experiments. Optical density along the ordinate: to the left for the spectral range 400–450 mμ, to the right 450–625 mμ. Temperature, 22° C. Optical depth of cuvettes, 10 mm.

A detailed report is being published elsewhere.

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Influence on the Effect of Vasoconstrictor Amines by Pretreatment with Reserpine

I have examined the influence of reserpine pretreatment (5 mgm./kgm./2 days, intraperitoneal) on the vasoconstrictor action of a series of sympathomimetic amines and of acetylcholine in high concentration, using the hind legs of a rat and Jackson's toad preparation.