that formalin fixation is a kind of histochemical method for the selective demonstration of noradrenaline in the adrenal medulla, a point now under more detailed study.

While this work was in progress, Hillarp and Hökfelt[3] described a technique for histochemical demonstration of noradrenaline. With this technique, using buffered potassium iodate, they also came to the conclusion that the two medullary catechol amines are stored in two different kinds of cells.

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O. Eränkö

Institute of Occupational Health,
Työterveyslaitos, Helsinki.
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Cyanide-resistant Mitochondria from the Spadix of an *Arum*

Highly active preparations of mitochondria have been obtained from the spadix of *Arum maculatum*[1]. These exhibited the ability to oxidize acids of the Krebs's cycle and pyruvic acid in their presence. No carrier needed to be added and the terminal oxidase responsible for the oxidations was not identified. The respiration of slices of spadix is not inhibited by cyanide[2], even though it is very rapid, with Q_{O_2} rising to 31·8. As mitochondria are generally supposed to promote respiratory oxidations through their contained cytochromes, the reaction of these aroid mitochondria to cyanide is of obvious interest.

Using the method of Hackett and Simon, we were able to prepare mitochondrial suspensions of high activity. The extraction and suspension medium contained 0·05 *M* phosphate buffer, *p*H 7·1; 0·2 *M* sucrose; and 0·001 *M* magnesium sulphate; before oxygen uptake was measured, 0·0004 *M* adenosine triphosphate was added. Endogenous respiration in this medium was virtually nil in all preparations. Addition of citrate, α-ketoglutarate or succinate at 0·02 *M* concentration caused rapid uptake of oxygen. 0·002 *M* Pyruvate in the presence of 0·002 *M* malate was also oxidized.

After two hours incubation at 20° C. the contents of the manometer flasks containing no substrate and with α-ketoglutarate added were examined for acid formation by paper chromatography. 1-ml. aliquots from each were deproteinized with 2 volumes of ethanol, and the dried supernatants, after acidifying with hydrochloric acid, were extracted with six lots of 10 ml. of ether. The ether extracts were run on two-dimensional chromatograms using ethanol-ammonia and mesityl oxide – formic acid as solvents[3]. After spraying the dried papers with bromo-phenol blue, no acids could be detected in the control experiment; but large amounts of citrate, malate, succinate and fumarate had been formed during the incubation with α-ketoglutarate, as shown in Fig. 1. All the α-ketoglutarate added had been oxidized.

Table 1. 1 ml. Mitochondrial Suspension = 2 gm. Fresh Weight of Tissue. Total Volume, 30 ml. T = 20° C.

Substrate	Oxygen uptake (μl.) in 15 min.	Hydrogen cyanide added
Nil	0	—
Citrate	150	151
α-Ketoglutarate	293	(291)
Succinate	388	365
Pyruvate + malate	115	—

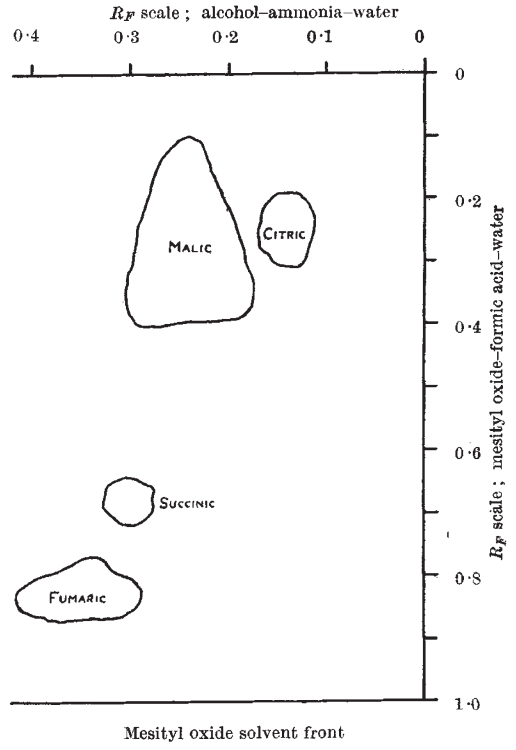

Fig. 1

Addition of 0·001 *M* hydrogen cyanide with the substrate caused no inhibition of oxygen uptake in the presence of citrate or succinate during the first 15 min. There was no inhibition in the presence of α-ketoglutarate, and the pattern of acid formation was not changed either; but here the cyanide would probably be removed as cyanhydrin of the keto-acid present in large excess.

After longer periods partial inhibition slowly developed in the presence of succinate. After two hours it amounted to about 60 per cent; but by this time the rate in the control sample had itself fallen away considerably. In the presence of citrate no inhibition or slowing down had appeared after 55 min.

These experiments appear to confirm the suggestion that the mitochondria of *Arum* spadix are able to catalyse a Krebs's cycle of reactions; but they also indicate that at least some of the oxidations on which it is based are not inhibited by 0·001 *M* cyanide. The partial inhibition that develops slowly in the presence of succinate is very different from the rapid and complete inhibition of the usual metallic oxidases.

W. O. James
Daphne C. Elliott

Department of Botany,
University of Oxford.
Oct. 29.

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