

volved. It is a reasonable assumption that in all four fractions described here the labelled phosphorus was present as a mono-ester.

Hokin and Hokin<sup>5</sup> have obtained rapid labelling in phospholipid fractions which correspond neither to phosphatidic acid nor to the major phospholipids, and these authors produce evidence<sup>6</sup> which appears to eliminate lysophosphatidic acid. Rapid labelling with phosphorus-32 of two unidentified phospholipids in mitochondria has recently been reported<sup>7</sup>.

The metabolic significance of these minor components of the phospholipids cannot be assessed at the moment, but it is clearly of importance that their chemistry be elucidated.

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### Incorporation of Molecular Oxygen into *Trans*-L-Epoxy succinic Acid by *Aspergillus fumigatus*

SINCE its discovery in 1937<sup>1</sup> in appreciable yields in culture filtrates of two filamentous fungi, only a handful of studies, mainly by Sakaguchi, appear to have been made on the mycological formation of *trans*-L-epoxy succinic acid<sup>2</sup>. The mechanism of its biosynthesis has been studied by Nomura *et al.*<sup>3</sup> using compounds labelled with carbon-14 to trace the origin of the carbon atoms.

An outgrowth of earlier studies in this laboratory<sup>2</sup> was the idea that the epoxide oxygen atom in the *trans*-L-epoxy succinate molecule might be unique in being derived from molecular oxygen. This appears to be the case, and the following sheds some light on the pathway of biosynthesis of this uncommon dibasic acid.

Shaken cultures of *Aspergillus fumigatus* mycelium were prepared as described previously<sup>2</sup>. The filtered mycelium, 5.6 g wet weight, was suspended in 200 ml. of 3 per cent glucose solution in a 2-l. gas-tight suction flask. The atmosphere in the flask was replaced with air containing 80 per cent oxygen labelled with oxygen-18, and the suspension was then shaken continuously for 87 h at 30° C. The epoxy succinic acid was extracted from the culture filtrate with diethyl ether, yield *c.* 500 mg. It was purified by silica-gel column chromatography<sup>3</sup>, and recrystallized from acetone-chloroform mixture. The crystals had the correct melting point for *trans*-L-epoxy succinic acid (181° C).

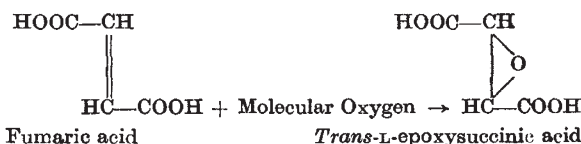
The oxygen isotopic composition of the pure acid was determined by the method of Rittenberg and Ponticorvo<sup>4</sup>. The results (Table 1) indicate that one atom of oxygen in the epoxy succinate was derived from molecular oxygen (assuming no exchange reaction). Since four of the five oxygen atoms in the acid are contained in carboxyl groups, we conclude that only one atom is labelled and that it must be the epoxide oxygen.

At the moment it is not possible to identify the oxygenation reaction generating epoxy succinate

Table 1

Exp.	Oxygen-18, Atom % excess in		Percentage of oxygen in epoxysuccinate derived from molecular oxygen
	O <sub>2</sub>	Epoxysuccinate	
I	5.466	1.086	19.87
II	4.906	1.056	21.52

because we have been unsuccessful in obtaining synthesis of this product in cell-free extracts of the mould. Nomura *et al.*<sup>3</sup> showed that the carboxyl carbons of epoxy succinate produced in the presence of <sup>14</sup>CO<sub>2</sub> had the same specific radioactivity as the mean values for carbons 1 and 6 in the citric acid formed in the same system, and oxalacetic acid was postulated as a common precursor of both acids. We have found that epoxy succinate is formed from malic, succinic or fumaric acid as the sole carbon source in suspensions of living mycelium. Based on the available facts we postulate the following reaction:



Such an oxygenase would be different from those described in the literature<sup>5</sup>, and investigations directed towards its isolation are now being carried out.

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### Oxidation of Vitamin A<sub>1</sub> and Vitamin A<sub>2</sub> Aldehydes to the Corresponding Acids by Enzymes from Pig and Rat Livers

VITAMIN A is stored in rat liver largely as its ester with small amounts of the alcohol, but is transported in the normal circulating blood in the latter form<sup>1</sup>. Although it was generally believed that the alcohol form is the more physiological state of the vitamin, since the work of Dowling and Wald<sup>2</sup>, it is being recognized that vitamin A acid and not the alcohol may be nearer to the 'active vitamin A'. If this were to be so, it would be important to demonstrate that a mechanism exists in the rat for the production of vitamin A acid from vitamin A alcohol through the intermediate, the aldehyde. Regarding the formation of the aldehyde, it has been well established that the alcohol dehydrogenase can bring about the conversion of vitamin A alcohol to retinene<sup>3</sup>. The presence of an