group 4, and possibly by some of group 2. Anti-*C* si inhibited, weakly, by L-glucose alone of the monosaccharides we have tried. Anti-*C* and anti-*E* are both inhibited (less strongly than is anti-*D*) by streptomycin and rutinose, whereas we have not found these latter two compounds to inhibit anti-*A*, anti-*B*, anti-*M* or anti-*N*. We would suggest tentatively that both *C* and *E* receptors may contain a sugar of Mäkelä's group 4 as a (non-terminal) unit, and that the terminal unit of the active portion of *E* may be a sugar of group 3. We can not at present identify the terminal unit of *C*.

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Glutamic Dehydrogenase of Mung Bean Mitochondria

GLUTAMIC dehydrogenase has been demonstrated in several higher plants[1] and is associated with mitochondria of pea[2] and oat[3]. Mitochondria isolated from mung bean (*Phaseolus aureus*) seedlings[4] were found capable of oxidizing glutamic acid (90 μl. oxygen/hr./mgm. (nitrogen). The assay of glutamic dehydrogenase in intact mitochondria is limited by a permeability barrier to pyridine nucleotides. Reduced diphosphopyridine nucleotide was oxidized by mitochondria suspended in 0·2 *M* sucrose, 0·05 *M* phosphate buffer, *p*H 7·4 (change in optical density at 340 mμ of 0·1 in 4 min.) and the addition of 0·1 per cent (v/v) of a non-ionic detergent, *O.P.C.* 45 (Petrochemicals Ltd., London) increased the oxidation rate by 240 per cent. The suspension of mitochondria was immediately clarified by the addition of *O.P.C.* 45. To assay glutamic dehydrogenase, intact mitochondria were broken by exposure to 0·1 per cent *O.P.C.* 45 at 0°C. and immediately centrifuged for 30 min. at 20,000 *g*. The supernatant was assayed for glutamic dehydrogenase using the system: 0·02 *M* potassium glutamate, 0·05 *M* phosphate buffer, *p*H 7·4 and 0·0001 di- or tri-phosphopyridine nucleotide and measuring reduction of the nucleotides at 340 mμ. The glutamic dehydrogenase activity released by this method accounted for all the glutamic acid oxidase activity of the whole mitochondria. Freezing and thawing the mitochondria suspended in 0·1 *M* potassium bicarbonate[5], released 61 per cent of total soluble glutamic dehydrogenase. Incubation of the potassium bicarbonate extract with 0·1 per cent *O.P.C.* 45 for 1 hr. at 0°C. was not found to inhibit the activity of glutamic dehydrogenase. Glutamic dehydrogenase reduced diphosphopyridine nucleotide four times as rapidly as it reduced triphosphopyridine nucleotide. Dual specificity may be due to the presence of a transhydrogenase which has been demonstrated in pea mitochondria[2]. Reduced diphosphopyridine nucleotide produced by glutamic dehydrogenase and glutamic acid was oxidised on the addition of α-ketoglutarate or ammonium chloride, indicating the reversibility of the system.

Rautanen and Tager[3] found glutamic dehydrogenase activity in the mitochondrial and supernatant fractions of oat coleoptiles. The presence of glutamic dehydrogenase in the supernatant fraction may have been due to the leaching of mitochondria by the preparative media. Mung bean mitochondria suspended in 0·2 *M* sucrose, 0·05 *M* phosphate buffer, *p*H 7·4, 0·005 ethylenediamine tetracetate for 30 min. at 0°C. were found to have lost 34 per cent of the total soluble glutamic dehydrogenase. This is one of the difficulties in determining intracellular localization of enzymes. In the case of mung bean, homogenates contain large amounts of free amino-acids which render the spectrophotometric assay of glutamic dehydrogenase impossible. An experiment to overcome these assay conditions involved the degradation of glutamic acid uniformly labelled with carbon-14.

TABLE 1.

System	Glutamate-U-^{14}C utilized*	$^{14}CO_2$ formed †	Initial glutamate-U-^{14}C used	Conversion of glutamate-U-^{14}C to $^{14}CO_2$
	(μmole/hr./ml. of homogenate)		(per cent)	
Homogenate	1·31	0·16	40	2·4
Mitochondria	3·58	0·66	72	3·7

Homogenate incubated with 0·2 *M* sucrose, 0·05 *M* phosphate buffer, *p*H 7·4, 0·005 *M* disodium ethylenediamine tetracetate, 0·01 *M* magnesium sulphate, 5×10^{-4} *M* adenosine triphosphate and tracer glutamate-U-^{14}C (500,000 c.p.m.). Mitochondria incubated under same conditions as homogenate, except 0·02 *M* potassium glutamate was present. Total volume in both cases was 2·0 ml. Duration of experiment, 1 hr.; temperature, 30°C.
* Glutamate-U-^{14}C measured using glutamic decarboxylase of *Clostridium welchii* (ref. 6).
† $^{14}CO_2$ trapped in 2 *M* sodium hydroxide and assayed (ref. 7).

Results are given in Table 1. The mitochondria can utilize glutamic acid more effectively than can the homogenate, which suggests that the homogenate contains an inhibitor of glutamic acid degradation or that glutamic acid cannot be oxidized because other substrates effectively compete for the electron transport system. The latter state probably does exist in homogenates as the addition of glutamic acid to an homogenate preparation does not stimulate oxygen uptake. Glutamic dehydrogenase appears to be associated with the mitochondrial fraction and the presence of the enzyme of other fractions seems likely to be due to the method of preparation.

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