SC 2182

Properties of mitochondrial preparations from the small-intestinal mucosa of the guinea-pig

Little is known about the oxidative processes of mitochondrial preparations from the mucosa of the small intestine. Succinate oxidase activity has been determined in mitochondrial preparations from the mucosa of a few vertebrates[1,2] and the low activity in those from the rat is thought to be due to inhibition by the long-chain fatty acids present in the preparation[1,3]. Oxidative phosphorylation by mitochondrial preparations from the small-intestinal mucosa of the guinea-pig has been described briefly[1], with phosphorylation quotients (P/O ratios) of 0.98 and 3.15, using succinate and α-ketoglutarate, respectively, as substrates. During an investigation of the metabolism of the mucosa of the small intestine[2], substrate oxidations and esterification of inorganic phosphate by preparations from the guinea-pig intestine were examined and are described in more detail in this paper, together with some further observations.

Small intestines from 2 guinea-pigs were washed out by irrigation with cold isolation medium. The mucosa was obtained by placing segments of the small intestine on a cold glass plate and scraping with a wooden spatula. The mucosal scrapings were disrupted at 0° in a loosely fitting Potter–Elvejhem homogeniser (550 rev./min; 12 strokes up and down in 3 min) in 70 ml of 0.3 M sucrose containing 2 mM EDTA and enough $KHCO_3$ to give a final pH of 7.4. The final volume was made up to 200 ml. Mucus was precipitated by the addition of 1 ml of a 10% $BaSO_4$ suspension (containing 1% Na_2SO_4) to 100 ml of the homogenate[4]. The homogenate was stirred for 3 min and then centrifuged at 15000 $g \cdot$min to sediment mucous material, cell debris, and nuclei. The mitochondrial fraction was sedimented from the supernatant at 120000 $g \cdot$min and suspended in the isolation medium (8–12 mg/ml protein).

Measurements of oxidation and phosphorylation were made as indicated in the legends. Mitochondrial swelling was assayed as described by LEHNINGER[5].

Mitochondrial preparations from the mucosa of the small intestine of the guinea-pig rapidly oxidised citrate, α-ketoglutarate, succinate, glutamate, pyruvate, and fumarate (see Table I), with the simultaneous esterification of inorganic phosphate (see Table III). The oxidation of citrate was linear for at least 1 h. There was a fumarate-sparked oxidation of pyruvate though the rate of oxidation was much less than that with succinate or citrate as substrate. The mitochondrial preparations also showed respiratory control when the rate of oxidation of citrate and of succinate was measured in the presence and absence of an ADP-generating system (see Table II).

Though good P/O ratios were obtained, there were indications that the mitochondria were somewhat damaged. First, the oxidations were strongly dependent on the addition of cytochrome *c*. Secondly, added NADH was oxidised at a relatively high rate. "External" NADH is thought to be oxidised only by partially damaged mitochondrial preparations[6]. Thirdly, mitochondrial swelling could not be induced by the addition of Ca^{2+} or phosphate ions using conditions which caused a marked swelling of mitochondrial preparations from rat liver.

Dinitrophenol at concentrations of 10^{-5} M only partially uncoupled oxidative phosphorylation using fumarate as substrate. Furthermore, dinitrophenol at concentrations between 10^{-5} and 10^{-3} M did not increase the ATPase activity of intestinal mitochondrial preparations assayed according to KIELLEY[8].

TABLE I

OXIDATION OF TRICARBOXYLIC ACID CYCLE INTERMEDIATES

Oxygen uptake was estimated by the Warburg technique. The incubation medium was similar to that described by ALDRIDGE[7] and each flask contained μmoles (complete system): sucrose (144 μmoles), EDTA (2 μmoles), $MgSO_4$ (32 μmoles), glycylglycine (40 μmoles), KCl (184 μmoles), KH_2PO_4 (45 μmoles), AMP (3 μmoles), ATP (3 μmoles), glucose (50 μmoles), horse-heart cytochrome *c* (0.5 mg), bovine serum albumin (2.5 mg), and Sigma type III hexokinase (2.0 mg) in a final volume of 2.5 ml. pH 7.0. 3–6 mg of mitochondrial protein were used per flask. The substrates were added as indicated in the table. Fortified complete system refers to a medium which contained in addition to the substances listed above NAD^+ (1 μmole), $NADP^+$ (0.2 μmole), and CoASH (1 μmole) per flask. The flasks were incubated at 37° and the gas phase was air. Measurements were started 7 min after equilibration. The Q_{O_2} is expressed as μl O_2 per mg protein per h.

Substrates	*μmoles added*	*Additions*	Q_{O_2}
None	—	Complete system	4.5
Succinate	50	Complete system	114.0
Succinate	50	Cytochrome *c* omitted	8.0
Citrate	50	Complete system	80.0
Citrate	50	Fortified complete system	95.0
Citrate	50	Cytochrome *c* omitted	31.0
α-Ketoglutarate	50	Complete system	60.0
α-Ketoglutarate	50	Fortified complete system	70.0
Glutamate	50	Complete system	66.0
Glutamate	50	Cytochrome *c* omitted	27.0
Pyruvate	45	Complete system	5.6
Pyruvate + fumarate	45 + 5	Complete system	58.0
Fumarate	5	Complete system	38.0
NADH	10	Complete system	45.0

TABLE II

OXIDATIVE PHOSPHORYLATION AND RESPIRATORY CONTROL

The assay system was that described in Table I. P/O ratios were determined using two flasks. The rate of uptake of inorganic phosphate was measured by estimating the phosphate concentration in the first flask at 0 min, and in the second at 20 or 30 min after equilibration. Respiratory control was determined by estimating the rate of oxygen uptake in two flasks in the presence and in the absence of the ADP-generating system (glucose and hexokinase), respectively.

Substrate	*P/O ratio*	*Respiratory-control ratio*
Succinate	1.4	1.3
Citrate	2.6	2.8
Citrate*	2.1	—
α-Ketoglutarate	2.2	—

* Fortified complete system (see Table I).

The oxidation of a variety of substrates and the good P/O ratios obtained with mitochondrial preparations from guinea-pig small-intestinal mucosa are in contrast to the inability of preparations from some other species to oxidise substrates other than succinate and to esterify inorganic phosphate[2]. Since the method of preparation,

including the $BaSO_4$ treatment, was the same in all species, it seems unlikely that the mitochondria were altered by the addition of $BaSO_4$ to the homogenate. Furthermore, rat-liver-mitochondrial preparations so treated had an unimpaired capacity for oxidative phosphorylation and for swelling. Other factors, apart from the presence or absence of long-chain fatty acids, may be important. For example, autolytic destruction and in particular lipolytic action, though minimised by rapid preparation and low temperatures prior to incubation, may be a decisive factor and may be higher in some species than in others. Also the relative amount and type of bile acids present in the preparation may be an important factor.

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Phosphorus levels of normal and conditioned tobacco callus tissue

When higher-plant tissues are first isolated it is found that they possess an absolute requirement for auxin and a cell-division factor for continued growth in culture. Some of these tissues when grown for sometime in a culture medium containing an auxin such as indoleacetic or napthaleneacetic acid develop the capacity to grow indefinitely in an auxin-free medium[1–3]. These tissues have been termed habituated or conditioned. In an effort to characterize this type of auxin-independent growth phosphorus compounds in normal and conditioned tissues were investigated.

Conditioned callus tissue of *Nicotiana tabacum* var. White Burley was subcultured on a modified MOREL's medium[4] containing 0.5 g $Ca(NO_3)_2$, 0.5 g KH_2PO_4, 0.125 g KNO_3, 0.125 g $MgSO_4$, 20 g glucose, 0.5 ml Bertholet's solution, 10 mg cysteine hydrochloride, 1 mg aneurine and 5 g of agar per l. Normal tissue was subcultured on this medium supplemented with 100 ml/l coconut milk and a final concentration of 10^{-8} M naphthaleneacetic acid. Subcultures were made with pieces of tissue approx. 20 mg fresh wt. and were grown at 25° with a light intensity of 200 ft-candles. Phosphorus compounds of 4-week-old cultures were fractionated by the method of HOLDEN[5]. Orthophosphate and acid-soluble phosphorus were removed from 1-g samples of tissue with 10-ml aliquots of 0.2 N $HClO_4$ in the cold. Phosphorus soluble in ethanol – ether was extracted with three successive 5-ml aliquots of neutral