A Study of the Localization of Polynucleotide Phosphorylase within Rat Liver Cells and of its Distribution among Rat Tissues and Diverse Animal Species

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1. Rat liver polynucleotide phosphorylase was localized in the mitochondrion, but may also occur in the nucleus. 2. The mitochondrial enzyme was found in rat heart, kidney, liver, muscle and spleen. 3. Mitochondrial polynucleotide phosphorylase is also present in calf, chicken, guinea-pig and rabbit liver and in goldfish muscle. 4. A possible physiological role for the enzyme in the control of the intramitochondrial ADP concentration is suggested.

We have recently published the first unequivocal evidence for the presence of polynucleotide phosphorylase in eukaryotic cells (Fitt & See, 1970; See & Fitt, 1970, 1971). The enzyme was first isolated and partially purified from crude preparations of guinea-pig liver nuclei (Fitt & See, 1970; See & Fitt, 1970) and subsequently a similar enzyme was shown to be present in the inner membrane of rat liver mitochondria (See & Fitt, 1971). The mitochondrial enzyme has now been partially purified (See & Fitt, 1972), and the available evidence (See & Fitt, 1970, 1972) indicates that both the guinea-pig and rat enzymes have phosphorolytic activity only. In this respect, they resemble the enzyme from *Escherichia coli* Q13 (Thang *et al.*, 1969) rather than the better-known polynucleotide phosphorylases present in *Azotobacter vinelandii* (Grunberg-Manago *et al.*, 1956; Gajda *et al.*, 1970), *Clostridium perfringens* (Dolin, 1962; Fitt *et al.*, 1968; Fitt & Wille, 1969a,b), *E. coli* B (Littauer & Kornberg, 1957; Williams & Grunberg-Manago, 1964) and *Micrococcus lyso-deikticus* (Beers, 1957; Singer & Guss, 1962) or the very-low-molecular-weight enzyme from *Halobacterium cutirubrum* (Peterkin & Fitt, 1971), all of which catalyse the polymerization of nucleoside diphosphates and nucleoside diphosphate–orthophosphate exchange as well as the phosphorolysis of polyribonucleotides. The mitochondrial enzyme was associated with an exchange activity that was specific for adenine nucleoside di- and tri-phosphates, with a marked preference for ADP, but its behaviour during purification of the polynucleotide phosphorylase activity and its substrate specificity (See & Fitt, 1972) suggest that it was due to a separate enzyme.

Although preliminary results suggested that the enzyme was present in nuclei as well as in mitochondria (See & Fitt, 1971), the very close similarity in the properties of polynucleotide phosphorylases isolated from rat liver mitochondria and crude rat

liver nuclei, and a failure to detect the enzyme in purer nuclei, prompted a detailed study of its intracellular distribution in rat liver. In the present paper, we present evidence that polynucleotide phosphorylase, (i) although definitely mitochondrial may also be present in nuclei, (ii) is absent from other subcellular fractions of rat liver, (iii) is present in the mitochondria of several rat tissues, and (iv) is widely distributed among animal species.

Experimental

Materials

Animals and tissues were obtained from the following sources: Sprague–Dawley rats (350–450 g), Bio Breeding Laboratories, Ottawa, Ont., Canada; rabbits and guinea pigs, stock animals, Faculty of Medicine Animal House, University of Ottawa, Ont., Canada; bovine and chicken liver from freshly slaughtered animals, local suppliers. Goldfish were a gift from Dr. J. C. Fenwick, Department of Biology, University of Ottawa, Ont., Canada. Cytochrome *c* was purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. The sources of other chemicals and intermediates have been described (See & Fitt, 1972).

Buffers. The pH of buffers refers to the value at 25°C. Sucrose–tris buffer had the following composition: 0.25 M-sucrose–1 mM-2-mercaptoethanol–10 mM-tris–HCl, pH 7.4.

Methods

All operations were carried at 0–5°C.

Isolation of glycogen particles. Glycogen particles were isolated by a modification of the method of Luck (1961). Part of the supernatant, after removal of mitochondria from a crude liver homogenate in sucrose–tris buffer by 10000g centrifugation (See &

Fitt, 1972), was mixed with sufficient 2.2M-sucrose–1mM-2-mercaptoethanol–10mM-tris-HCl buffer, pH7.4, to increase the sucrose concentration to 0.44M. The samples (6ml each) of this mixture were layered over 2.1M-sucrose–1mM-2-mercaptoethanol–10mM-tris-HCl buffer, pH7.4 (5ml/sample). The pellets obtained by centrifugation for 1h at 105000g (Beckman L2-65B preparative ultracentrifuge; type 40 rotor) were suspended in sucrose–tris buffer (total vol. 1ml) and assayed for polynucleotide phosphorylase activity in the standard phosphorolysis assay medium without Triton X-100.

Isolation of rat liver microsomal fraction and soluble fraction. Rat liver (15g) was homogenized in sucrose–tris buffer (5vol.) in a motor-driven Potter–Elvehjem homogenizer. The nuclei and mitochondria were removed by centrifugation for 15min at 12000g. The supernatant was then centrifuged at 87000g (Spinco L2-65B centrifuge; type 30 rotor) for 90min. The pellet, suspended in sucrose–tris buffer (10ml), and the supernatant are referred to as 'the microsomal fraction' and 'the soluble fraction', respectively, and were assayed for polynucleotide phosphorylase activity in the standard phosphorolysis assay.

Isolation of mitochondria. Mitochondria were isolated as previously described (See & Fitt, 1972) from (i) bovine, chicken (cock), guinea-pig and rabbit liver, (ii) goldfish muscle and (iii) the following rat tissues: heart, kidney, liver, skeletal (thigh) muscle and spleen. They were suspended in sufficient sucrose–tris buffer to give a final concentration of mitochondrial protein of 3–4mg/ml and their polynucleotide phosphorylase activity was measured by the standard phosphorolysis assay.

Isolation of ^{32}P -labelled rat liver mitochondria. Mitochondria were labelled with ^{32}P in their phospholipids by the procedure of McMurray & Dawson (1969), by using the labelling period shown by these authors to give maximum incorporation into cardiolipin (inner membrane) together with a high specific radioactivity in other phospholipids. [^{32}P]P_i in dilute HCl (pH2–3) was neutralized with 1M-K₂HPO₄ to give a specific radioactivity of 1mCi/mg of P_i (final pH7.4). Male rats (340g) were injected intraperitoneally with the [^{32}P]P_i: two rats received 2μCi of [^{32}P]P_i/g body wt. and one rat 1μCi/g body wt. The animals were fed, except for the night before isolation of the mitochondria. They were decapitated 49h after receiving the injections and their livers were removed. The mitochondria were isolated as described above, and were then suspended in sucrose–tris buffer (50ml). Their radioactivity was determined by liquid-scintillation counting (Beckman LS-133 counter) of 50μl samples of the suspension dissolved in 10ml of a toluene-based scintillation fluid containing 0.4% (w/v) 2,5-diphenyloxazole and 10% (v/v) Beckman Biosolve BBS-3. The radioactive mitochondrial suspension was used for the estimation

of the mitochondrial contamination of nuclei prepared by the Chauveau *et al.* (1956) procedure (see the Results and Discussion section).

Isolation of rat liver nuclei. Liver nuclei from male fed rats were isolated by the following three methods. (i) The '2.2M-sucrose method' (Chauveau *et al.*, 1956) as described for the isolation of crude guinea-pig liver nuclei (See & Fitt, 1970), with 4ml of buffer/g wet wt. of liver. (ii) Isolation in buffer containing 0.25M-sucrose followed by further centrifugation through 2.2M-sucrose. The livers from six rats (about 90g wet wt. of tissue) were homogenized in 4vol. of sucrose–tris buffer with five strokes of a motor-driven Potter–Elvehjem homogenizer. The crude nuclear pellet obtained by centrifugation at 600g for 10min was resuspended by homogenization (Lourdes model MM1 homogenizer; 90s at full speed) in 2.2M-sucrose–1mM-2-mercaptoethanol–10mM-tris-HCl buffer, pH7.4, in a quantity of buffer equal to that used in the isolation of the crude nuclei. The homogenate was filtered through four layers of cheesecloth and the nuclei were then collected by centrifugation at 40000g for 1h. (iii) Large-scale modification of the method of Blobel & Potter (1966). Liver (about 90g wet wt.) was homogenized with 2ml of 0.25M-sucrose in 25mM-KCl–5mM-MgCl₂–1mM-2-mercaptoethanol–50mM-tris-HCl buffer, pH7.4 (TMK–ME buffer)/g, with a motor-driven Potter–Elvehjem homogenizer, and the homogenate was filtered through four layers of cheesecloth. Then 2.3M-sucrose in TMK–ME buffer (2vol.) was added and the suspension was mixed in a large graduated cylinder to give a final sucrose concentration of 1.62M. Equal volumes (90–125ml) were transferred to 250ml plastic, screw-cap centrifuge bottles and then underlaid with 2.3M-sucrose in TMK–ME buffer (80ml/bottle) by allowing the denser solution to run to the bottom of the bottle through a glass tube attached to a graduated separatory funnel. The capped bottles were centrifuged at 28800g_{max}. (14400g_{av}.) in an International model B-20 refrigerated centrifuge (IEC no. 872 angle rotor) for 5h. A white pellet of nuclei formed on one side of the bottom of each bottle, and a layer of cell debris extended across the top surface of the 2.3M-sucrose layer and down the side of the bottle opposite the nuclei. The bottles were stood upright and the layer of cell debris was gently detached from the sides with a spatula so that it floated to the surface of the liquid. The 1.62M-sucrose layer and cell debris were then aspirated through a glass tube attached to a water pump, followed by the 2.3M-sucrose layer. Traces of cell debris still adhering to the bottle were removed with absorbent paper. This method was also used for the preparation of guinea-pig liver nuclei.

Isolation of rat liver nuclear membranes. (i) Rat liver nuclei were isolated from 15g wet wt. of tissue by the Whittle *et al.* (1968) modification of the Blobel

& Potter (1966) method. The final nuclear pellet was suspended in 2.3 M-sucrose in TMK-ME buffer (2 ml) and the sucrose concentration of the suspension was lowered gradually by the addition at 10 min intervals of four 0.5 ml and two 2 ml portions of TMK-ME buffer lacking sucrose. The nuclei were then collected by centrifugation at 3600g for 5 min, washed twice with 0.25 M-sucrose in TMK-ME buffer (10 ml) and resuspended in 0.25 M-sucrose in TMK-ME buffer (10 ml) to give a final protein concentration of 1.22 mg/ml. The nuclear membranes were then isolated by the method of Berezney *et al.* (1970). Pancreatic deoxyribonuclease (50 µg/ml) was added to the suspension of nuclei and digestion was allowed to proceed for 18 h at 4°C. The membranes were collected by centrifugation at 3600g for 15 min and washed with 0.25 M-sucrose in TMK-ME buffer (10 ml) to give the crude membranes. The latter were resuspended in 0.25 M-sucrose in TMK-ME buffer (20 ml). A portion (1 ml) was centrifuged and the crude membranes were washed with sucrose-tris buffer (5 ml) and resuspended in sucrose-tris buffer (1 ml) to give the washed crude membranes. To the remainder of the crude-membrane suspension, solid MgCl₂ was added to a final concentration of 0.5 M. A large amount of protein was removed from the membranes by this treatment. The Mg²⁺-treated membranes were collected by centrifugation at 27000g for 15 min, washed twice with sucrose-tris buffer (10 ml) and resuspended in 1 ml of the latter to give the Mg²⁺-treated membranes. The supernatant from the Mg²⁺ treatment was dialysed overnight against 2 litres of 1 mM-2-mercaptoethanol-2 mM-EDTA buffer, pH 7 (ME-EDTA buffer). The precipitated protein was collected by centrifugation and resuspended in ME-EDTA buffer (2 ml) to give the Mg²⁺-soluble fraction. The washed crude membranes, the Mg²⁺-treated membranes and the Mg²⁺-soluble fraction were all assayed for polynucleotide phosphorylase activity in the standard phosphorolysis assay. (ii) A similar preparation was also carried out by using 1 mM-2-mercaptoethanol-10 mM-tris-HCl buffer, pH 7.4, instead of TMK-ME buffer at all stages, including the initial isolation of the nuclei, and the corresponding final fractions were assayed for polynucleotide phosphorylase activity by the standard phosphorolysis assay.

Isolation of rat liver plasma membranes. Rat liver plasma membranes were isolated from 15 g wet wt. of tissue by the method of Neville (1960) as modified by Emmelot *et al.* (1964). The purified membranes were suspended in sucrose-tris buffer (0.5 ml) and assayed for polynucleotide phosphorylase activity in the standard phosphorolysis assay.

Enzyme assays. Polynucleotide phosphorylase activity was measured by the standard phosphorolysis assay (See & Fitt, 1972). The assay medium (0.1 ml) contained: 0.1 M-tris-HCl buffer, pH 7.8 or

8.8 as indicated; 6 mM-MgCl₂; 1 mM-2-mercaptoethanol; 10 mM-K₂H³²PO₄ (50000-100000 c.p.m./µmol); poly(A), 1.5 mg/ml; 0.1% (v/v) Triton X-100; and enzyme as appropriate. Incubation was at 37°C for 1 h. The reaction was stopped with a charcoal-HClO₄ suspension and the charcoal was washed, dried and counted for radioactivity as previously described (See & Fitt, 1970). A unit is that amount of enzyme catalysing the incorporation of 1 nmol of [³²P]P_i into charcoal-adsorbable material/h. In the presence of 0.1% (v/v) Triton X-100, the results with whole or sonicated mitochondria were the same, and controls (i) without poly(A) and with mitochondria or (ii) with poly(A) and without mitochondria were identical within experimental error.

Cytochrome oxidase activity was determined by the method of Wharton & Tzagoloff (1967), by using a Zeiss PMQII spectrophotometer fitted with automatic zero and cell changer, a recorder and a thermostatically controlled cell holder. A unit is the amount of enzyme catalysing oxidation of 1 µmol of reduced cytochrome *c*/min.

DNA assay. DNA was assayed by the diphenylamine procedure (Burton, 1956) with calf thymus DNA as standard.

Protein assay. Protein was determined by the method of Lowry *et al.* (1951) in the presence of sodium dodecyl sulphate (See & Fitt, 1970), with bovine serum albumin as standard.

Phospholipid analysis. Phospholipids were extracted and identified as described by See & Fitt (1970).

Results and Discussion

Intracellular distribution of rat liver polynucleotide phosphorylase

Table 1 shows the distribution of poly(A) phosphorolysis activity in subcellular fractions of rat liver isolated by the methods described in the Experimental section. In all cases, the activities were assayed in the conditions found optimum for the membrane-bound rat mitochondrial enzyme before purification (See & Fitt, 1972) and also at pH 8.8, at which the guinea-pig liver enzyme is most active (See & Fitt, 1970). Polynucleotide phosphorylase could be detected only in the mitochondria and, possibly (see below) the nuclei.

Since nuclei isolated from either guinea-pig liver or rat liver by the 2.2 M-sucrose procedure (See & Fitt, 1970; Chauveau *et al.*, 1956) always yielded polynucleotide phosphorylase, whereas those prepared by the Blobel & Potter (1966) modification of the method of Chauveau *et al.* (1956) were free from the enzyme, a detailed study was made in an attempt to establish if this activity was genuinely nuclear or was due to mitochondrial contamination.

It was first determined that the nuclei isolated directly by homogenization and centrifugation in

Table 1. *Intracellular localization of rat liver polynucleotide phosphorylase*

Subcellular fractions were isolated as described in the Experimental section and their polynucleotide phosphorylase activity was measured by the standard phosphorolysis assay [in the presence of 0.1 % (v/v) Triton X-100]. The activity in nuclei was determined by using extracts prepared from the organelles by osmotic shock and subsequent dialysis to remove sucrose (See & Fitt, 1970), as described in Table 3.

Fraction	Activity			
	(units/mg of protein)		(units/g wet wt. of tissue)	
	pH 7.8	pH 8.8	pH 7.8	pH 8.8
Mitochondria	120-191	108-171	335-602	300-540
Nuclei	10-32	4-14	15-27	7-12
Glycogen particles, microsomal fraction, plasma membrane and soluble fraction	None detectable			

Table 2. *Comparison of the polynucleotide phosphorylase and cytochrome oxidase activities of rat liver nuclei*

Nuclei were isolated from rat liver (90 g) by either method (i) ('2.2M-sucrose') or method (ii) ('0.25M/2.2M-sucrose') (see the Experimental section), with buffers that lacked 2-mercaptoethanol, and were analysed for DNA content, cytochrome oxidase activity and polynucleotide phosphorylase activity as described in the Experimental section.

Expt. no.	Preparation of nuclei	Polynucleotide phosphorylase (units)	Cytochrome oxidase (units)	DNA (mg)	Polynucleotide phosphorylase/DNA ratio	Cytochrome oxidase/DNA ratio
1	2.2M-Sucrose	2284	29.6	5.1	438	5.80
2	0.25M/2.2M-Sucrose	684	3.92	3.7	184	1.06
Ratio Expt. 1/Expt. 2					2.4	5.5

2.2M-sucrose (Chauveau *et al.*, 1956; See & Fitt, 1970) contained 2-3% of the total liver mitochondria, in agreement with the degree of mitochondrial contamination reported (Berezney *et al.*, 1970) in nuclei isolated by a modified high-sucrose procedure. Two methods were used. In the first, radioactive rat liver mitochondria were prepared after being labelled *in vivo* by the procedure of McMurray & Dawson (1969). They were added to unlabelled rat liver from which nuclei were then isolated as usual in 2.2M-sucrose. After correction for dilution of the labelled mitochondria by the organelles in the unlabelled liver, 3% of the total liver mitochondria were found to be associated with the nuclei. In separate experiments, the separation of mitochondria and nuclei was followed by determination of the cytochrome oxidase activity in the crude rat liver homogenate in 2.2M-sucrose and in the nuclei obtained after high-speed centrifugation of the latter (in buffer without 2-mercaptoethanol). By this method, it was found that 2-3% of the cytochrome oxidase activity remained associated with the crude nuclear fraction, in good agreement with the degree of contamination found

independently with radioactively labelled mitochondria.

Table 2 describes two experiments in which the cytochrome oxidase and polynucleotide phosphorylase activity/mg of DNA present in nuclei isolated by two methods (in the absence of 2-mercaptoethanol) were compared. The nuclei with the least mitochondrial contamination (i.e. with least cytochrome oxidase activity) also contained the least polynucleotide phosphorylase. Further, when the nuclei isolated in 2.2M-sucrose alone were purified by suspension in the high-sucrose buffer and re-centrifugation, the purified nuclei contained no polynucleotide phosphorylase; however, the recovery of nuclei by this method was very poor. Nevertheless, the result agreed with that obtained with nuclei isolated in good yield by the modified large-scale Blobel & Potter (1966) procedure, which also lacked polynucleotide phosphorylase activity, whether the assays were performed on intact nuclei or extracts prepared by our usual method (See & Fitt, 1970). Finally, the enzyme isolated from rat liver nuclei by the procedure used (See & Fitt, 1970) to prepare guinea-pig liver

polynucleotide phosphorylase not only resembled the rat liver mitochondrial polynucleotide phosphorylase very closely, but contained small amounts of cardiolipin, which is considered to be present exclusively in mitochondria (Getz, 1970).

Thus, the evidence presented above is generally consistent with rat liver polynucleotide phosphorylase and, by extension, guinea-pig liver polynucleotide phosphorylase (Fitt & See, 1970; See & Fitt, 1970) being a mitochondrial enzyme found in crude nuclei only as a contaminant, and may be summarized as follows. (i) Highly purified nuclei contain no polynucleotide phosphorylase; (ii) the amount of polynucleotide phosphorylase present in nuclei decreases as the purity of the latter increases and the change roughly parallels the change in cytochrome oxidase activity in the preparation; (iii) the purified 'nuclear' enzyme from rat liver contains cardiolipin, typical of the mitochondria, and has enzymic properties apparently identical with those of the mitochondrial enzyme.

However, the possibility still remains that the enzyme is associated with nuclei as well as with

mitochondria. The results in Table 3 show that, even if all the polynucleotide phosphorylase present in mitochondria could be extracted by the osmotic-shock procedure used (See & Fitt, 1970) for preparation of the crude 'nuclear' enzyme, 25% of the mitochondria of rat liver would have to be present in the crude nuclei to account for the enzyme activity extracted from the latter. However, we have shown (see above) that a maximum of 3% of the mitochondria contaminated the nuclei. Further, extraction of isolated mitochondria by the procedure used for nuclei yielded (Table 3) only 50–60% of the total mitochondrial polynucleotide phosphorylase, and it could then only be purified with much greater losses than the enzyme from the crude nuclei, so that only 1.8–3.5% of the activity of the purified 'nuclear' enzyme could arise from contaminating mitochondria. It should be emphasized that these are the maximum degrees of contamination that we have observed in several experiments and that the yield of polynucleotide phosphorylase from mitochondria by osmotic shock is frequently much lower than that indicated, which is also the highest we have obtained.

Table 3. *Comparative isolations of rat liver polynucleotide phosphorylase from mitochondria and nuclei by osmotic shock*

Crude nuclei were isolated from rat liver (90g) by homogenization and centrifugation in 2.2M-sucrose–1 mM-2-mercaptoethanol–10mM-tris–HCl buffer, pH 7.4 [method (i), see the Experimental section]. Polynucleotide phosphorylase was extracted from the nuclei with 0.25M-sucrose–1 mM-2-mercaptoethanol–10mM-tris–HCl buffer, pH 7.4 (sucrose–tris buffer), and purified by pH 5.6 precipitation and DEAE-cellulose chromatography (See & Fitt, 1970). Mitochondria were isolated from rat liver (90g) as described by See & Fitt (1972). A small sample of the final mitochondrial suspension in sucrose–tris buffer was assayed for total polynucleotide phosphorylase activity and the remainder of the mitochondria were collected by centrifugation at 10000g. The pellet was resuspended in 10ml of 2.2M-sucrose–1 mM-2-mercaptoethanol–10mM-tris–HCl buffer, pH 7.4, and the suspension was left at 0°C for 20min. Sufficient 1 mM-2-mercaptoethanol–10mM-tris–HCl buffer, pH 7.4, was then added (all at once) to lower the sucrose concentration abruptly to 0.25M. The suspension was homogenized in a Dounce homogenizer with loose-fitting pestle, left at 0°C for 20min and then centrifuged (40000g; 10min). The pellet was re-extracted as described above and the supernatants were combined to give the fraction designated 'osmotic shock crude extract' in the Table. The polynucleotide phosphorylase activity was then purified as described for the nuclear enzyme. Polynucleotide phosphorylase activity was determined by the standard poly(A) phosphorolysis assay with 0.1% (v/v) Triton X-100 (see the Experimental section), except that incubation was for 3h.

Treatment	Phosphorolysis activity (units)		
	Mitochondria		Nuclei
	Expt. 1	Expt. 2	
1. Whole mitochondria	9108	8080	—
2. (i) Osmotic shock:			
(a) Crude extract	5260	4460	2066
(b) 3% of (a)	158	134	—
(ii) pH 5.6 precipitation	3680	—	3150
(iii) DEAE-cellulose column			
(a) Total recovery	208	—	350
(b) 3% of (a)	6.2	—	—

Finally, if the isolated mitochondria were first homogenized in 2.2M-sucrose, in the conditions used for preparation of the crude homogenate before isolation of the nuclei by our standard procedure (See & Fitt, 1970), and then submitted to osmotic shock by dilution of the medium to 0.25M-sucrose followed by homogenization in a Dounce homogenizer, the yield of polynucleotide phosphorylase was only 20% of that obtained by omission of the first homogenization step before dilution of the suspension. These results are difficult to reconcile with an exclusively mitochondrial location for rat liver polynucleotide phosphorylase and suggest that it may in fact be present in the nucleus. This implies that the methods necessary for the isolation of highly purified nuclei lead to a loss of polynucleotide phosphorylase.

We therefore conclude that polynucleotide phosphorylase, although definitely present in the mitochondrial inner membrane of rat liver (See & Fitt, 1971, 1972), may also occur in the nucleus. A rigorous proof of the latter possibility has not yet been obtained, but if the enzyme is present in nuclei it must be easily removed from these organelles by most procedures used for their extensive purification.

Distribution of mitochondrial polynucleotide phosphorylase in rat tissues

Table 4 shows the results of a study of the polynucleotide phosphorylase activity in mitochondria isolated from several tissues of the rat. Assays were performed at both pH 7.8 and 8.8, and in all cases the phosphorolytic activity was higher at pH 7.8. The specific activities of kidney and liver mitochondrial polynucleotide phosphorylases were similar, but kidney gave the highest yield of units/mg wet wt. of tissue. We have not studied the effect of the age or nutritional state of the animals on the polynucleotide phosphorylase activity in different tissues, so too much significance should not be attached to the

apparent relative abundance of the enzyme. However, it is clear that mitochondrial polynucleotide phosphorylase is present in significant amounts in all the tissues tested.

It should be emphasized that our previous studies (See & Fitt, 1971) have shown that 89% of the polynucleotide phosphorylase activity detectable in intact mitochondria in the presence of 0.1% (v/v) Triton X-100 is recoverable in isolated mitochondrial inner membranes and that it is specific for nucleoside diphosphates as products of the phosphorolysis of polyribonucleotides. The activity of whole mitochondria in the standard assay is thus a true measure of their polynucleotide phosphorylase content.

Occurrence of mitochondrial polynucleotide phosphorylase in different animal species

A comparative study was also made of the occurrence of mitochondrial polynucleotide phosphorylase in bovine, guinea-pig, rabbit, rat and chicken livers and in goldfish muscle. The results in Table 5 show that the enzyme was present in all cases and is therefore widespread amongst animal species. The assays were performed at both pH 7.8 and 8.8, which had been found in detailed studies to be the optima for the rat and guinea-pig mitochondrial enzymes respectively. The enzymes from all the other species tested so far resembled the guinea-pig mitochondrial polynucleotide phosphorylase rather than the rat enzyme and were more active at pH 8.8. However, it should be noted that the partially purified rat mitochondrial polynucleotide phosphorylase (See & Fitt, 1972) has a pH optimum of 8.2 and that at least part of the difference between the crude enzymes is due to the high phosphatase activity in the crude rat mitochondrial preparations (See & Fitt, 1972).

The widespread distribution of polynucleotide phosphorylase in the mitochondria of various animal

Table 4. *Distribution of mitochondrial polynucleotide phosphorylase in rat tissues*

Mitochondria were isolated from the indicated rat tissues as described by See & Fitt (1972) and their polynucleotide phosphorylase activity was determined by the standard phosphorolysis assay (see the Experimental section) in the presence of 0.1% (v/v) Triton X-100.

Tissue	Polynucleotide phosphorylase activity			
	(units/g wet wt. of tissue)		(units/mg of protein)	
	pH 7.8	pH 8.8	pH 7.8	pH 8.8
Heart	526	486	78	72
Kidney	1773	478	200	54
Liver	602	540	191	171
Muscle (thigh)	38	33	28	25
Spleen	248	67	18	5

Table 5. Occurrence of mitochondrial polynucleotide phosphorylase in various animal species

Mitochondria were isolated from the tissues indicated as described by See & Fitt (1972) and their polynucleotide phosphorylase activity was determined by the standard phosphorolysis assay (see the Experimental section) in the presence of 0.1% (v/v) Triton X-100.

Tissue	Polynucleotide phosphorylase activity			
	(units/g wet wt. of tissue)		(units/mg of protein)	
	pH 7.8	pH 8.8	pH 7.8	pH 8.8
Calf liver	1007	1215	146	176
Chicken (cock) liver	1298	1715	232	307
Goldfish muscle	64	78	30	37
Guinea-pig liver	2252	2964	235	309
Rabbit liver	486	721	209	310
Rat liver	602	540	191	171

species and rat tissues raises once again the problem of the uncertain biological role of the enzyme. Two suggestions have been made so far on the basis of studies with bacterial polynucleotide phosphorylase: (i) that the enzyme is responsible for the degradation of mRNA (Sekiguchi & Cohen, 1963; Andoh *et al.*, 1963; Futai *et al.*, 1966; Natori & Mizuno, 1967; Castles & Singer, 1969); (ii) that polynucleotide phosphorylase is involved in the control of the concentration of intracellular orthophosphate (Grunberg-Manago, 1963). The first function seems less likely since the demonstration (Morikawa & Imamoto, 1969; Morse *et al.*, 1969*a,b*) that mRNA is degraded sequentially starting from the 5'-terminus rather than in a 3'→5' direction, as would be required if polynucleotide phosphorylase were involved, but is not excluded, particularly in *Bacillus subtilis* (Duffy *et al.*, 1972; Chaney & Boyer, 1972). No evidence is available for assessment of the second possibility, but it too seems unlikely for the animal enzymes, which appear devoid of nucleoside diphosphate-polymerization activity (See & Fitt, 1970, 1972) and therefore do not catalyse a readily reversible reaction, unlike most of the bacterial polynucleotide phosphorylases. With the animal polynucleotide phosphorylases, their presence in the inner mitochondrial membrane, the site of the enzymes and electron carriers of the respiratory chain, suggests a further possible function: control of the concentration of ADP in the neighbourhood of the phosphorylation sites via a cycle involving (i) ATP; (ii) an ATP polymerase similar to those found in animal cells (Edmonds & Abrams, 1960, 1962; Burdon, 1963; Ventkataraman & Mahler, 1963; Klemperer, 1963, 1965; Hyatt, 1967*a,b*; Gurgo *et al.*, 1970; Dravid *et al.*, 1971), bacteria (August *et al.*, 1962; Gottesman *et al.*, 1962; Hardy & Kurland, 1966*a,b*; Payne & Boezi, 1970) and yeast (Twu & Bretthauer, 1971); (iii) poly(A) or an A-rich polyribonucleotide (see, e.g.,

Edmonds & Abrams, 1962, 1963; Hadjivassiliou & Brawerman, 1966; Lim *et al.*, 1969; Edmonds & Caramela, 1969) acting as a storage form of adenine nucleotides, as suggested by Edmonds & Abrams (1962); (iv) polynucleotide phosphorylase; (v) ADP; and (vi) the respiratory chain. ATP polymerase and poly(A)-rich polynucleotides have not yet been detected in mitochondria, although it has been claimed (Swanson, 1971) that intact mitochondria will incorporate polynucleotides, so a systematic search for the polymerase and the polymers in the isolated organelles would constitute a preliminary and comparatively simple test of this hypothesis.

Note added in proof. Jacob & Schindler (1972) have recently described the isolation of polyribadenylate polymerase (i.e. ATP polymerase) from rat liver mitochondria.

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