that the ventral nerve cord of another annelid, the leech, contains both 5-HT and catecholamine-containing cells (as revealed by fluorescence microscopy)[15]. Moreover, both ^{14}C-octopamine and ^{14}C-dopamine are formed during incubation (of leech nervous tissue) in media containing ^{14}C-tyrosine[15]. There was no evidence of production of noradrenaline[15]. If it can indeed be confirmed that little if any noradrenaline is present in annelid nervous systems, such an observation would conform well to the established pattern that invertebrate central nervous systems contain more dopamine than noradrenaline[16]. Another most important emerging pattern is the importance in invertebrate nervous systems of octopamine. While octopamine is found only in small amounts in vertebrate central nervous systems[1], it occurs in relatively large amounts in all the invertebrate groups so far examined. This now appears to be true for all the major groups of the *Protostomia*, i.e. the molluscs, the arthropods and the annelids. The high levels of octopamine in the *Protostomia* suggests that this amine plays an important role in these groups. Furthermore, the differences in octopamine content, in connection with the already-noted predominance of dopamine over noradrenaline in invertebrate nervous systems[16], may reflect a fundamental biochemical dichotomy between the nervous systems of *Protostomia* and *Deuterostomia*.

Summary. Octopamine has been found in very high concentrations in cerebral and subpharyngeal ganglia of the earthworm *Lumbricus terrestris* and may function as a neurotransmitter in the peripheral nervous system.

H. A. ROBERTSON[17]

Psychiatric Research Unit, University Hospital, Saskatoon (Saskatchewan, Canada S7N OW8), 25 March 1975.

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Presence of a Specific Uridine 5'-Monophosphate Pyrophosphorylase in Baker's Yeast

Uracil is readily incorporated into pyrimidine nucleotides and nucleic acids by many bacteria, apparently via its reaction with 5-phosphorybosyl 1-pyrophosphate (PRPP), catalyzed by UMP pyrophosphorylase[1-3].

GRENSON[4] showed that in yeast UMP liberates uracil in two steps, catalyzed by a phosphatase and by an uridine ribohydrolase respectively. In addition the same author observed that uracil could be converted to UMP by a *Saccharomyces cerevisiae* mutant lacking uridine kinase activity. These observations prompted us to check the possible presence of UMP pyrophosphorylase in yeast. The data reported in this communication show the presence of such activity in baker's yeast and contribute to the elucidation of its fundamental role in the UMP recycling.

In addition, aim of this report is also to furnish the suitable assay conditions, found for the first time on partially purified preparation, for a more detailed study of the enzyme.

Materials and methods [8-^{14}C] Adenine (50 mCi/mmol), [2-^{14}C] Uracil (50 mCi/mmol), [8-^{14}C] Hypoxanthine (50 mCi/mmol), [2-^{14}C] Cytosine (50 mCi/mmol) and [2-^{14}C] Thymine (50 mCi/mmol) were purchased from the Radiochemical Center, Amersham, England. PRPP as tetrasodium salt, bases, nucleotides, orotic acid, $MgCl_2$, orotidine 5'-monophosphate decarboxylase, orotidine 5'-monophosphate pyrophosphorylase and all other chemicals were purchased from Sigma Chemical Co. The actual concentration of PRPP was determined by its capacity to convert orotate to orotidine-5'-monophosphate[5].

The standard assay for the enzyme activity was performed as follows: 10 nmoles of PRPP, 0.6 μmoles $MgCl_2$, 8 nmoles of 2-^{14}C uracil, 10 μmoles of phosphate buffer (pH 7.8) were mixed with an appropriate amount of enzyme solution in a final volume of 0.2 ml. After incubation at 37 °C for 30 min 25 μl of 50% trichloroacetic acid were added.

After centrifugation, 10 μl of the supernatant were spotted onto cellulose thin layer (Eastman Kodak) together with an appropriate amount of UMP and uracil chromatographed for 30 min in a solvent system composed by butanol, water and acetic acid in the ratio 4:2:1 (v/v). After development of the chromatogram, it was dried and the spots, visualized by fluorescence, corresponding to uracil and UMP, were cut out and placed in scintilla-

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Table I. Purification of uridine 5'-monophosphate pyrophosphorylase

Step	Volume (ml)	Total proteins (mg)	Total activity (units)	Specific activity (units(mg)	Purification (fold)	Yield (%)
1 Crude extract	150	1395.0	5370.7	3.85	—	100
2 Ammonium sulfate fractionation (50–85% saturation)	6	242.8	5365.9	22.1	5.94	99.9
3 Agarose A 1.5	17	24.14	2710.9	112.3	29.17	50.5

Results are the average of 4 preparations each starting with 150 g of baker's yeast. Assay mixtures and conditions were as described under 'Materials and methods'.

tion vials with 12 ml of scintillation mixture (4 g PPO and 0.05 g dimethyl POPOP per litre of toluene) for counting in a liquid scintillation spectrometer.

Protein content was determined by the modified Folin method of LOWRY[6]. Enzyme activity unit was defined as the amount of the enzyme which catalyzes the formation of 1 nmole of UMP per 30 min.

Preparation of partially purified UMP pyrophosphorylase activity from baker's yeast was conducted starting from 150 g of baker's yeast Vulcania plasmolyzed according to KUNITZ[7]. After 18 h, the aqueous phase containing the cellular homogenate was collected and centrifuged for 20 min at 15,000 $\times g$; the precipitate fraction was discarded and any turbidity from the supernatant eliminated by filtration through Whatman No. 3 MM paper. The filtered supernatant was considered as the crude extract (Table I).

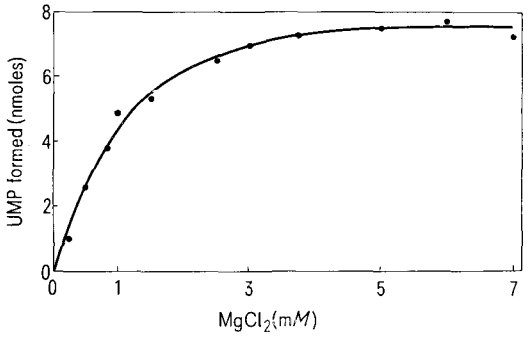


Fig. 1. Effect of $MgCl_2$ concentration upon baker's yeast uridine 5'-pyrophosphorylase activity.

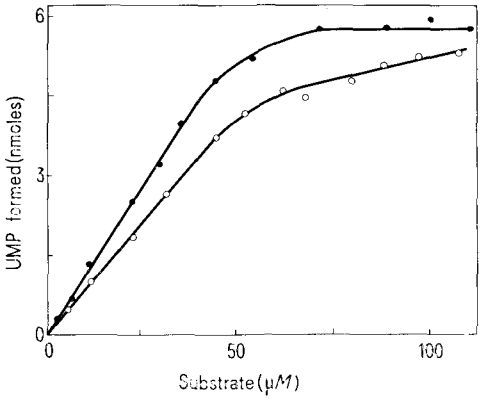


Fig. 2. Effect of PRPP and uracil concentrations on baker's yeast uridine 5'-monophosphate pyrophosphorylase activity. ●–●–●, PRPP; ○–○–○, uracil.

Table II. Substrate specificity of baker's yeast uridine 5'-monophosphate pyrophosphorylase

Substrate	Concentration (μM)	Activity (mononucleotide formed/30 min, nmoles)
[2-^{14}C] Uracil	0.04	6.5
[8-^{14}C] Hypoxanthine	0.12	0.0
[8-^{14}C] Adenine	0.10	0.0
[2-^{14}C] Cytosine	0.10	0.0
Orotate	0.20	0.0
[2-^{14}C] Thymine	0.10	0.0

For all substrates, except orotate, activity was determined as described under 'Materials and methods'. The enzyme (87 μg) used was from Agarose A 1.5 column (step 3 Table I). Orotate pyrophosphorylase activity was assayed as described by KORNBERG[5] using commercial preparation of orotidine 5'-monophosphate de carboxylase.

The crude extract was precipitated by adding solid ammonium sulfate up to 50% saturation. After centrifugation, to the supernatant fluid solid ammonium sulfate was slowly added till 85% saturation. The pellet was dissolved in 6 ml of 50 mM phosphate buffer pH 7.8 and dyalized 6 h against the same buffer. The dyalisate was placed onto a 2.7 × 85 cm Agarose A 1.5 (Bio-Rad) column equilibrated with the above buffer and the elution was performed by collecting 5 ml fractions at constant flow rate of 5 ml per h.

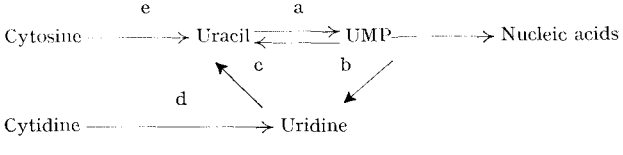
The enzyme activity was eluted between 200 and 250 ml volume elution and the ammonium sulfate fractionation was performed keeping the pH at value of 7 by addition of ammonia hydroxyde. All the operations were carried out at 4 °C.

Results and discussion. The pH optimum of the enzyme activity was found to be in the range of 7.8–8.0 using *Tris*-phosphate buffer 50 mM in the assay medium and 87 μg of the enzyme preparation (step 3, Table I). The concentrations of other components of the incubation mixtures were those described above. At the pH optimum, the enzyme is stable for 15 days at −20 °C if phosphate ions are present. Prolonged dialysis against buffers not containing phosphate, results in a complete loss of activity. Loss of activity was observed also if, during the purification, gel filtration through Agarose was performed in absence of phosphate (see above).

Figure 2 shows the dependence of the rate of reaction upon increasing concentrations of uracil and PRPP. The kinetic behaviour seems to be of michaelian type and the *Km* values for uracil and PRPP are 8.5×10^{-6} M and 2×10^{-5} M respectively.

UMP pyrophosphorylase activity is strictely dependent upon the presence of Mg^{++}. In Figure 1 the curve of the activity versus Mg^{++} concentration is shown. In absence of Mg^{++} uridine 5'-monophosphate pyrophosphorylase activity is zero and the maximum of activity could be achieved at 3 mM Mg^{++} concentration, whereas *E. coli*[8] and *L. bifidus*[1] enzymes are still active also in absence of Mg^{++} ion. As in the case of the enzyme from *L. bifidus* baker's yeast uridine 5'-monophosphate pyrophosphorylase seems to be distinct from orotidine 5'-monophosphate pyrophosphorylase. In addition the enzyme is completely inactive toward hypoxanthine, adenine, thymine and cytosine. These results are illustred in Table II. Among several nucleotides tested, only UMP affects the enzyme activity, exerting 62% inhibition at 0.42 mM concentration.

The presence of UMP pyrophosphorylase strongly suggests that in baker's yeast the utilization of preformed bases and nucleosides works according to the following scheme:



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a) UMP pyrophosphorylase; b) UMP phosphatase, probably a 5′-nucleotidase (unpublished observations); c) uridine nucleosidase. Thise enzyme has been purified to homogeneity in this laboratory and studied in detail by MAGNI et al.[9]. It is inhibited by ribose and glucose-6-phosphate. d) e) Cytidine deaminase and cytosine deaminase. They have been studied and characterized by IPATA et al.[10,11]. These enzymes are inhibited by several nucleotides, and show regulatory properties. This scheme is supported by the fact that in baker's yeast, uridine phosphorylase is absent[12,4] and uridine kinase activity is very low compared to uridine nucleosidase activity[12].

Summary. Uridine 5′-monophosphate pyrophosphorylase was found to be present in baker's yeast. The enzyme preparation, purified about 30-fold, shows a strict specificity toward uracil and requires Mg^{++} for its activity.

P. NATALINI, E. FIORETTI, S. RUGGIERI, A. VITA and G. MAGNI

Laboratory of Applied Biochemistry, University of Camerino, I-62032 Camerino (Italy), 7 April 1975.

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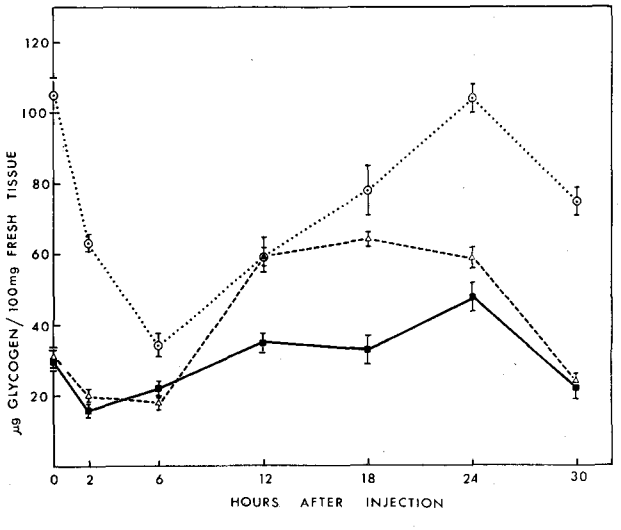
Glycogen Concentration in Isoproterenol-Stimulated Salivary Glands of Mice

The influence of isoproterenol (IPR) on the major salivary glands of rodents has been extensively studied and was recently reviewed[1]. IPR promotes glycogen breakdown through adenylate cyclase activation[2,3]. This sympathicomimetic drug was pointed out as the most efficient adenylate cyclase stimulator in the salivary gland cells[4]. Glycogenolysis in the rat submandibular gland was also observed after parasympathetic stimulation[5].

MALAMUD and BASERGA[6] reported a rapid decrease of glycogen concentration in the pooled parotid (P), submandibular (SM) and sublingual (SL) glands soon after IPR injection in mice. On the other hand, 18 h after this stimulation the glycogen concentration reached a value 5 times higher than the control one. After this peak, there was a new evident glycogenolysis process occurring simultaneously with DNA synthesis up to 30 h after the injection.

The 3 major salivary glands of the mouse, however, present different structures, metabolisms and functions. Therefore, the purpose of this investigation was to study the influence of a single IPR injection on the glycogen concentration of each gland separately.



Effects of isoproterenol on the glycogen concentration in the parotid (■ - - - - ■), submandibular (△ - - - - △) and sublingual (⊙ · · · · · ⊙) glands of mice. Values are means ± SEM. Each value is the mean of 6 to 10 different determinations.

Material and methods. A total of 140 white male adult mice (28–35 g) divided into 7 groups of 20 animals each were used. All animals were allowed regular diet and water ad libitum.

The animals were injected i.p. with a single dose of DL-isoproterenol-HCl (Sigma) solution (7 mg/30 g of body weight). The mice were killed by cervical dislocation at 0, 2, 6, 12, 18, 24 and 30 h after the injection. The sacrifice was always performed at 08.00 h.

Each salivary gland was dissected out, carefully cleaned and weighed. An amount of approximately 90, 60 and 80 mg of SM, SL and P gland respectively was used for each glycogen determination, using the method described by JOHAN and LENTINI[7].

Results and discussion. The glycogen concentration showed different features in the 3 major IPR-stimulated salivary glands. At time zero, the concentration was higher in the SL and similar in P and SM glands. No difference was observed between the uninjected control mice and the injected ones sacrificed immediately after IPR administration. 2 h after the IPR stimulation, the glycogen concentration decreased in the P gland, but still decreasing up to 6 h for the SM and SL glands. Glycogen concentration increases from 6 h after stimulation, returning to the control values in the P and SL glands. However, in the SM gland its accumulation is more evident, reaching twice the control value 18 h after the IPR injection.

Therefore, the interpretation of MALAMUD and BASERGA[6] for the increased glycogen concentration in the pooled major salivary glands must be changed. According to our results, only the SM gland presents a higher glycogen concentration relative to the control values.

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