Properties of Pea Seedling Uracil Phosphoribosyltransferase and Its Distribution in Other Plants

RAY A. BRESSAN, MICHAEL G. MURRAY, JAMES M. GALE, AND CLEON W. ROSS
Department of Botany and Plant Pathology, Colorado State University, Fort Collins, Colorado 80523

Received for publication July 28, 1977 and in revised form October 3, 1977

ABSTRACT

A uracil phosphoribosyltransferase (UMP-pyrophosphorylase) was found in several angiosperms and was partially purified from epicotyls of pea (Pisum sativum L. cv. Alaska) seedlings. Its pH optimum was about 8.5; its required approximately 0.3 mM MgCl2 for maximum activity but was inhibited by MnCl2; its molecular weight determined by chromatography on Sephadex G-150 columns was approximately 100,000; its Km values for uracil and 5-phosphorylribosyl 1-pyrophosphate were 0.7 μM and 11 nM, and it was partially resolved from a similar phosphoribosyltransferase converting orotic acid to orotidine 5'-phosphate. Enzyme fractions containing both uracil phosphoribosyltransferase and orotate phosphoribosyltransferase converted 6-azauracil and 5-fluorouracil to products with chromatographic properties of 6-azauridine 5'-phosphate and 5-fluorouridine 5'-phosphate. Uracil phosphoribosyltransferase probably functions in salvage of uracil for synthesis of pyrimidine nucleotides.

Although cytosine is metabolized slowly if at all by most organisms investigated, uracil and most other pyrimidines, purines, and their nucleosides can be converted into plant nucleic acids by so-called salvage pathway enzymes (10, 19, 21, 24). An extensive review of purine and pyrimidine metabolism in animals and microorganisms was published by Hartman (7), but he discussed almost none of the research dealing with plants. Two enzymes capable of adding a ribose moiety to uracil are known to exist in most organisms. One, uridine phosphorylase (EC 2.4.2.3), catalyzes the reversible addition of ribose from ribose-1-P, forming uridine and phosphoribose. The other, uracil phosphoribosyltransferase (EC 2.4.2.9) (UPRT), also catalyzes the following apparently irreversible reaction:

\[ \text{uracil} + \text{PRPP} \rightarrow \text{UMP} + \text{pyrophosphate} \]

Although UPRT occurs in certain bacteria (3–5, 11) and some animal cells (9, 17), its presence seems to be quite restricted (7). The only evidence for its existence in plants comes from work with pea and wheat seedlings by Wasilewska and Reifer (24, 25).

1 This research was supported by Grant GB 36480 from the National Science Foundation.
2 Present address: Michigan State University, ERDA Plant Research Laboratory, East Lansing, Mich. 48824.
3 Present address: Department of Plant Biology, Carnegie Institution of Washington, Stanford, Calif. 94305.
4 Present address: Department of Botany, University of Georgia, Athens, Ga. 30602.
5 Abbreviations: UPRT and OPRT: phosphoribosyltransferases acting on uracil and orotic acid; PMSF: phenylmethylsulfonylfluoride; PRPP: 5-phosphorylribosyl 1-pyrophosphate; OMp: orotidine 5'-phosphate; 5-fluorouracil; 5'-UMP: 5-fluorouridine 5'-phosphate; 6-aza-UMP: 6-azauridine 5'-phosphate.

and Mazuć and Buchowicz (13). In the last two of these reports the product UMP was demonstrated, but no characterization of UPRT from plants has yet been described. We wish to report its separation from OPRT (EC 2.4.2.10) in pea seedlings, its partial purification, some of its properties, its distribution in various parts of the seedling, and its presence in several other species.

MATERIALS AND METHODS

Plant Materials. Peas (Pisum sativum L. cv. Alaska) were dark-grown in glass trays containing moist vermiculite at about 22°C. The upper 3 cm of the epicotyl from 6-day-old seedlings were used unless noted otherwise. Other plants included pinto beans (Phaseolus vulgaris L. cv. Idaho 111), soybeans (Glycine max L. Merr. cv. Hawkeye), corn (Zea mays L. cv. Embro 49A), barley (Hordeum vulgare L. cv. Will), and oats (Avena sativa L. cv. Russell). These were either dark-grown for 10 days as described for peas or grown in the greenhouse for 17 days in soil. Entire above ground portions of both dark-grown and light-grown plants were harvested, except for pinto beans where only the upper 3 cm of the shoot from dark-grown plants and only the primary leaves from light-grown plants were taken.

Chemicals. The following were purchased from Sigma Chemical Co.: Na2PRPP, UMP, uridine, uracil, OMP, orotic acid, pyrophosphate, and yeast OMP decarboxylase. Chemicals obtained from Calbiochem included 6-azauridine, 6-azauracil, 6-aza-UMP, 5-fluorouracil, PMSF, [2-14C]-6-azauracil (6.2 mCi/mmol), and [2-14C]-5-fluorouracil (20 mCi/mmol). New England Nuclear Corp. supplied [2-14C]uracil (56.8 mCi/mmol) and [5-3H]UMP (22 Ci/mmol). Two lots of [6-14C]orotic acid were used, one from International Chemical and Nuclear Corp. (17.2 mCi/mmol) and another from Schwarz/Mann (47 mCi/mmol). All 14C-labeled compounds were chromatographed in at least one solvent to detect radioactive contaminants. By these methods, all compounds appeared to be at least 97.4% pure except for the first mentioned lot of orotic acid; it contained one contaminant possessing 3.6% of the total 14C. The 6-azauracil was 99.6% pure. Ultrapure biochemical grade ammonium sulfate, myoglobin, γ-globulins, and BSA were obtained from Schwarz/Mann.

Protein Measurement. The method of Lowry et al. (12) was used with BSA as a standard. Proteins were always precipitated with 5 to 10% (w/w) trichloroacetic acid and redissolved in 50 mM NaOH prior to analysis.

Measurement of Radioactivity. A Nuclear-Chicago Unilux-III liquid scintillation spectrometer was used. All measurements were made with a toluene scintillation mixture containing 4 g/l PPO and 50 mg/l POPOP. Ion exchange paper discs and sections from paper chromatograms were immersed directly in the scintillation fluid prior to counting. Background values were determined from appropriate blanks.

Chromatography. One-dimensional chromatography on Whatman 3MM papers (3.8 × 57 cm) was employed with the following solvents: BAW (1-butanol-acetic acid-H2O, 2:1:1, v/v), IFW (iso-

Extraction and Purification. All extraction and purification steps were carried out on ice or at about 2 °C. Enzyme was routinely extracted from all tissues (5-20 g fresh wt) by homogenization in 20 mM Tris-Cl buffer (pH 7.5) containing 50 µM PMSF and 0.02% (w/v) NaN3 using a Willems polytron at maximum speed for 1 min. The buffer volume to tissue fresh wt ratio was 3:1, except for data in Table 1 where a 6:1 ratio was employed. The buffered extract was passed through Miracloth and centrifuged at 25,000g for 20 min. The resulting supernatant solution was used as crude enzyme preparation. Since the crude or partially purified enzyme preparations lost most of their activity overnight at -20 C or at 4°C, extracts of fresh tissue were prepared prior to each experiment.

Crude enzyme was routinely brought to 30% saturation with (NH4)2SO4 by the slow addition of solid (NH4)2SO4 to the crude extract while it was continuously stirred at 2 °C. After chilling on ice for 30 min, the extract was centrifuged at 25,000g for 20 min. The pellet was surface-washed twice with homogenization buffer to remove residual salt, then redissolved in 1 to 4 ml of the same buffer.

Enzyme Assays. Routine assays for UPRT were made in reaction mixtures (0.3 ml) containing 0.1 mM PRPP, 5.8 µM [2-14C] uracil (0.1 µCi), 0.5 mM MgCl2, 20 mM Tris-Cl (pH 8.5), and 25 to 50 µl of enzyme from crude homogenates or (NH4)2SO4 precipitates, and 150 µl of Sephadex G-150 fractions. Reactions were performed at 30 °C for 30 min unless specified otherwise, after which 50 µl of 50 mM Na2EDTA were added to each reaction vessel (to chelate Mg2+) before placing it on ice. (Rates were constant for at least 45 min.) Aliquots of 50 µl were removed from reaction mixtures, and the UMP present in each aliquot was resolved from unreacted uracil and assayed by the DEAE paper disc method of Ives et al. (8) after subtraction of values for blanks lacking enzyme. Whatman DE81 and DE20 performed equally well for the separation of uracil from UMP. The formation of 14C-labeled UMP from [2-14C]uracil was verified by one-dimensional paper chromatography in IWF, IAW, MAA, and IBA. Chromatographic results indicated that the routinely used DEAE disc method underestimated the amount of UMP formed by about 10%, probably due to loss from the discs during washing, but corrections were not made for this loss.

Reaction mixtures used to determine the ability of 6-azauracil and 5-fluorouracil to serve as substrates for UPRT were the same as above, except that [2-14C]6-azauracil or [2-14C]5-fluorouracil was added in place of uracil.

Assays for OPRT were made with reaction mixtures containing 0.1 mM PRPP, 5 mM MgCl2, 42 mM potassium fluoride (to inhibit phosphatases), 50 mM K-phosphate (pH 7.5), either 3.7 µM or 9.7 µM [6-14C]orotic acid (0.05 µCi), and enzyme from crude homogenates, 30% (NH4)2SO4 precipitates, or Sephadex G-150 fraction in a final volume of 0.3 ml. Yeast OMP decarboxylase (0.1 unit) was also added to facilitate conversion of OMP to UMP in the experiments where Sephadex purification was used. The DEAE disc method did not separate orotic acid from OMP, so reaction components were separated by one-dimensional paper chromatography in BAW. This solvent resolved uracil, uridine, UMP, orotic acid, orotidine, and OMP.

Reversibility of UPRT. The reversibility of the reaction catalyzed by UPRT was examined using enzyme from the upper 3 cm of pea epicotyl. Extraction and purification were the same as previously described through the 30% (NH4)2SO4 precipitation step. Reaction mixtures contained 0.5 mM MgCl2, 0.17 mM pyrophosphate (or H2O), 50 mM Tris-phosphate (pH 8.5), 67 µM [5-3H]UMP (1 µCi), and 0.4 µg (25 µl) of enzyme protein. Radioactivity in 0.5-cm-wide sections of papers chromatographed in IWF and IAW was used to determine whether labeled uracil was formed from [5-3H]UMP.

RESULTS

Distribution in Pea Seedlings. When different parts of 6-day-old, dark-grown pea seedlings were analyzed using both crude extracts and 30% (NH4)2SO4 precipitates, the highest specific activity of UPRT occurred in the apical 3 cm of the epicotyls (Fig. 1). Lowest activity was found in the roots and oldest region of the epicotyl.

Partial Purification of UPRT. Initially, we determined that 0.5 mM DTT in the extraction buffer was 20 to 30% inhibitory to UPRT activity in crude epicotyl extracts, so no sulfhydryl compound was used thereafter. UPRT was readily detectable in crude extracts (approximately 0.4 nmol of UMP/mg of protein·30 min), although phosphatases hydrolyzing both substrate PRPP and product UMP were probably present. Preparation of a 30%-saturated (NH4)2SO4 solution of the crude supernatant routinely caused precipitation of about two-thirds of the UPRT activity and resulted in an apparent 2.5-fold enzyme purification. OPRT activity, on the other hand, remained largely unprecipitated (or was denatured) at that salt concentration. In one experiment the relative activities of UPRT and OPRT in the crude and 30% (NH4)2SO4 fractions were, respectively: 0.41 and 0.28 nmol of UMP formed/g fresh wt of epicotyl tissue·30 min; and OPRT, 25 and 4.1 nmol of UMP + orotidine formed/g fresh wt of tissue·30 min. (Orotidine was apparently formed in OPRT assays from a contaminating phosphatase acting on OMP, even though potassium fluoride was present. Because of orotidine formation, 14C in both UMP and orotidine was always summed to estimate OPRT activity. OMP was apparently rapidly decarboxylated and partially dephosphorylated, because no OMP was detectable in the reaction mixtures by paper chromatography in BAW.)

Chromatography of crude extracts on Sephadex G-150 columns

FIG. 1. Distribution of UPRT activity in 6-day-old pea seedlings. Apical regions of the epicotyl averaged 2.2 cm in length and included the youngest leaves. The subapical epicotyl zone was divided into two portions, each about 3 cm in length, and the upper half included the first node and its leaves. The width of each bar represents the relative length of that section. Shaded areas show the activity of crude extracts, while the top of each bar indicates activity in 30% (NH4)2SO4 precipitates. Protein concentrations in mg/g tissue fresh wt of crude extracts were: apex, 11.7; upper subapical, 3.3; lower subapical, 3.2; cotyledons, 19.8; roots, 6.2. Corresponding protein values in the 30% (NH4)2SO4 precipitates were: apex, 3.8; upper subapical, 0.6; lower subapical, 1.2; cotyledons, 3.9; roots, 2.1.
at 2°C allowed further purification of UPRT and its partial separation from OPRT (Fig. 2). In fraction 7, a maximum UPRT activity of 3.7 nmol of UMP/mg of protein·30 min was obtained, while the maximum OPRT activity in fraction 6 was 25.3 nmol of orotic acid converted/mg of protein 30 min. In subsequent attempts to further purify UPRT on DEAE-cellulose columns, no activity in any eluates could be detected.

The mol wt of UPRT was estimated in three experiments to be 100,000 to 110,000 by comparison of its $V_s/V_o$ to that of sperm whale myoglobin, BSA, and human $\gamma$-globulin marker proteins on Sephadex G-150 columns (2) (data not shown).

Chromatographic Characterization of Products. To confirm that $^{14}$CUMP was formed from [2-$^{14}$C]uracil during assays for UPRT activity, reaction mixtures incubated with enzyme from the 30% (NH$_4$)$_2$SO$_4$ precipitate were chromatographed in MAA, IBA, IAW, and IFW with unlabeled uracil, uridine, and UMP present as markers which were detected under UV light. Figure 3 indicates that the major product of uracil migrated with standard UMP in all four solvents. The small peak between UMP and uracil in IAW and IFW solvents matched the uridine marker in both solvents. This putative uridine presumably arose from phosphatase action on UMP.

pH Optimum. Tris-phosphate buffers varying at 0.5 pH unit intervals from 6 to 10.5 were used to determine the optimum pH for UPRT activity. Maximal activity occurred at pH 8.5, while the values at pH 6, 7, and 10 were, respectively, 12, 53, and 67% of the maximum (data not shown). Similar results were obtained with Tris-Cl buffers varying from pH 7 to 10. Comparable pH optima for the UPRT enzyme from mouse murine leukemic cells were pH 10 (9) or 10.5 to 11 (16).

Requirement for $\text{Mg}^{2+}$. UPRT from other organisms requires $\text{Mg}^{2+}$ for maximum activity, and Dahl et al. (6) reported that the molar ratio of $\text{Mg}^{2+}$ to PRPP should be 1 or greater to allow formation of an active complex between them. Data in Figure 4 are consistent with this interpretation. They indicate that a $\text{MgCl}_2$ concentration of 0.125 mM nearly maximized the reaction rate in the presence of 0.1 mM PRPP. Both $\text{MnCl}_2$ concentrations employed inhibited activity, and other experiments showed that concentrations as low as 10 $\mu$M were also inhibitory. Most investigators have not described $\text{Mn}^{2+}$ effects, although Reyes (16) found that $\text{Mn}^{2+}$, $\text{Ca}^{2+}$, and $\text{Co}^{2+}$ all slightly stimulated 5-fluoroUMP synthesis from 5-fluorouracil by an apparent UPRT from mouse leukemic cells. He also found that $\text{Mg}^{2+}$ was nearly three times as effective as $\text{Mn}^{2+}$ when each was studied at 6 mM.

FIG. 2. Fractionation of UPRT and OPRT on a Sephadex G-150 column. Ordinates on right (note different scales) indicate enzyme activities determined in a 30-min incubation period, while ordinate on left shows concentration of protein in each fraction. To a Sephadex column (2.7 x 23 cm) having a void volume of 38 ml, 3 ml of crude extract were added. Column was eluted with the Tris-Cl extraction buffer described under "Materials and Methods." The first 25 ml of eluate were discarded before collection began. Duplicate analyses of UPRT activity are shown.
Effects of Various Uracil and PRPP Concentrations. UPRT activity was measured at uracil concentrations varying from 0.1 μM to 11.8 μM with 0.1 mM PRPP. The results of three such experiments are in Figure 5. Data include two experiments for which the reaction was allowed to occur for 30 min, as usual (ordinate on right), and one for only 10 min (ordinate on left). The inset to Figure 5 shows reciprocal data for the lowest (10 min) curve, from which a $K_m$ value for uracil was calculated to be about 0.7 μM. Lindsay et al. (11) and Molloy and Finch (14) obtained values for bacterial enzymes to be 4 μM and 7.7 μM, respectively, while Kessel et al. (9) obtained far higher $K_m$ values of 5 mM for the mouse murine leukemic enzyme and 4 mM for the beef heart enzyme. The normal substrate for the last two enzymes appears to be orotic acid (9).

Figure 6 shows results obtained when the PRPP concentrations were varied from 3.1 to 100 μM at a uracil concentration of 5.8 μM. These and other data not shown indicate that the reaction rate was constant above 100 μM PRPP under these conditions. The inset to Figure 6 taken from data in the middle curve shows a reciprocal plot giving a $K_m$ value of 11 μM for PRPP. Comparable values for enzymes from Lactobacillus leichmannii (5), mouse leukemic cells (17), and Escherichia coli (14) were reported to be 44, 28, and 20 μM, respectively.

Reversibility. Earlier results (4, 5) indicated that the equilibrium for the UPRT-catalyzed reaction strongly favors UMP formation from uracil, but we are aware of no data in which the $K_m$ has been calculated. We performed two experiments with [5-14C]UMP to determine whether the reaction is reversible, using the 30% (NH₄)₂SO₄ pellet as an enzyme source. Although the enzyme converted 3.4 nmol of uracil to UMP/mg of protein· hr, no conversion of labeled UMP to uracil in the presence or absence of 167 μM pyrophosphate could be detected after separation of UMP, uridine, and uracil in IFW and IAW. These results suggest little if any reversibility of UMP synthesis. They are therefore consistent with the data for adenine phosphoribosyltransferase, but not for OPRT (7).

Presence of UPRT in Other Species. Two experiments were performed to determine whether UPRT exists in the shoot systems of other species, one involving 17-day-old, light-grown species and the other 10-day-old, etiolated plants. Data in Table 1 show large differences in activity in the species used. Light-grown barley and oat plants contained highest activities, while corn, the other monocot, displayed very little activity when light-grown and none when dark-grown. Most plants showed more activity when light-grown, although tomato was a notable exception that contained approximately 8-fold greater activity when grown in darkness. Strict comparisons of activities in various species is not feasible, because variations in growth patterns necessitated analysis of different amounts of the various tissue present. The apparent activities were probably significantly affected by the presence of variable quantities of phenolic or other inhibitors, and the inhibitor levels might have been affected by light. Nevertheless, evidence for the presence of UPRT in every species was obtained.

Metabolism of 6-Azauracil and 5-Fluouracil. The formation of 6-aza-UMP and 5-fluoro-UMP from their corresponding bases was also catalyzed by enzyme activity in eluates from Sephadex G-150, although the rates were much lower than for either UMP or OMP. These products were tentatively identified by paper chromatography in IFW, IAW, MAA, and IBA. Because a 5-fluoro-UMP standard was unavailable, its position was estimated by comparing the $R_f$ value of 5-fluorouracil to those of uracil, uridine, and UMP, which have paper chromatographic properties similar to the corresponding fluoro-compounds (3, 6). When labeled 5-fluorouracil was used as a substrate, the only detectable product had chromatographic properties in all solvents expected for 5-fluoro-UMP. When 5-fluorouracil was provided at 2, 4, 8,
16, and 80 μM, the corresponding rates of formation of putative 5-fluoro-UMP were 0.42, 0.8, 1.5, 2.5, and 10.9 nmol/mg of protein·30 min, indicating a relatively high Ka for this substrate.

When labeled 6-azaauracil was a substrate, a distinct peak of 14C matching the 6-aza-UMP marker spot was found in all solvents. No 14C-labeled 6-azaauridine was detected. Azaauracil concentrations of 6.7, 13.4, 27, 54, and 270 μM allowed the following rates of 6-aza-UMP formation: 0.13, 0.32, 0.48, 0.85, and 4.2 nmol/mg of protein·30 min, respectively. The rate was therefore proportional to a wide range of 6-azaauracil concentrations. These conversions of 5-fluoroauracil and 6-azaauracil were much slower than that of uracil by the same enzyme fraction. Thus, at 1.5, 2.9, and 5.8 μM concentrations of uracil, the rates of UMP formation were 2.8, 3.1, and 3.3 nmol/mg of protein·30 min.

**DISCUSSION**

The different response of UPRT and OPRT to (NH₄)₂SO₄ fractionation, their partial separation on a Sephadex column, and their opposite responses to Mn²⁺ (26) suggest that they are different enzymes. OPRT is considerably more active than UPRT, consistent with its participation in the de novo pathway of pyrimidine nucleotide synthesis. Although UPRT activity has repeatedly eluted from Sephadex G-150 columns at a position corresponding to a mol wt of about 100,000, there is a discrepancy in the behavior of OPRT. Data of Figure 1 suggest that OPRT is slightly larger than UPRT, but previous results from this laboratory with OPRT from cotyledons of Alaska peas (15) indicate that its mol wt is about 62,000. We cannot explain this difference, but wish to point out that Reyes and Guganig (17) found leukemia cell phosphoribosyltransferase activities for uracil, uracil, and 5-fluorouracil corresponding to a mol wt of 100,000 on G-150 columns of moderate size, but on a column with 3-fold greater area the apparent mol wt was only 55,000 to 60,000. They suggested that dilution on the larger column favored conversion to an enzyme form of lower mol wt. Shoaf and Jones (22) had earlier shown that OPRT from tumor cells behaves as though its mol wt is about 110,000 in sucrose gradients containing 30% dimethylsulfoxide and 5% glycerol, but only 55,000 to 60,000 in the absence of these compounds.

Our data show that 5-fluorouracil and 6-azaauracil can be converted slowly to their corresponding nucleotides by a phosphoribosyltransferase, but whether this is UPRT or OPRT was not determined. Others found similar PRPP-dependent conversions of 5-fluorouracil to 5-fluoro-UMP by enzymes from *E. coli (3)* and mouse leukemia cells (9, 16, 17). Apparently, a single enzyme metabolizes uracil, orotate, and 5-fluorouracil in the mammmals so far investigated, while bacteria seem to have separate enzymes, one acting upon orotate and the other on uracil, 5-fluorouracil, and 2-thiouracil (3, 11).

Early studies with 6-azaauracil in mammals and bacteria indicate that it is metabolized first to 6-azauridine by a phosphorylase enzyme dependent on ribose-1-P (23), rather than by a phosphoribosyltransferase. Both 6-azauridine and 6-azauridine-5'-P were formed when [2-14C]-6-azaauracil was fed to cocklebur leaf tissues (18), but those data allowed no conclusions about the initial anabolic reaction. The present results show that at least part of the 6-azaauracil converted to the 6-azauridine-5'-P that inhibits RNA synthesis and OMP decarboxylation (26) in plants is metabolized by a phosphoribosyltransferase.

The normal role of UPRT is presumably to salvage uracil formed during degradation of pyrimidine nucleotides and RNA for synthesis of new pyrimidine derivatives. Because it was found in all of the angiosperms we investigated, its distribution might well be widespread in the plant kingdom. There is also evidence for uridine phosphorylase in pea, wheat, barley, and *Lathyrus tingitanus* (13, 25), so there are apparently two mechanisms for uracil salvage in plants. However, UPRT appears to be considerably more active than the phosphorylase, and the latter enzyme was undetectable in pea cotyledons (20) and in mung bean seedlings (1).

**Acknowledgment.**—We appreciate the competent technical assistance of P. H. Bressan.

**LITERATURE CITED**

2. BLOXHAM P 1965 The gel-filtration pattern of enzymes related to their molecular weights over a wide range. Biochem J 96: 595-600
9. KEBBEL D, J DEACON, B COFFEY, A BALAMAN 1972 Some properties of a pyrimidine phosphoribosyltransferase from murine leukemia cells. Mol Pharmacol 8: 731-739
10. KING J, D WANG, ER WAYGOOD 1965 Biosynthesis of nucleotides in wheat. II. Pyrimidines from C-4 labeled compounds. Can J Biol Chem 43: 237-246
15. MURRAY MG, CW ROSS 1971 Molecular weight estimations of some pyrimidine-metabolizing enzymes from pea cotyledons by gel filtration. Phytochemistry 10: 2645-2648
18. ROSS CW 1964 Metabolism of 6-azaauracil and its incorporation into RNA in the cockleburr. Phytochemistry 3: 603-607
21. ROUX JM 1973 Nucleotide supply of the developing animal: role of the so-called "salvage pathways." Enzyme 15: 361-377
22. SHOAF WT, ME JONES 1973 Initial steps in pyrimidine synthesis in Ehrlich ascites carcinoma. Biochemistry 12: 4039-4051