De Novo Purine Synthesis in Avian Liver

CO-PURIFICATION OF THE ENZYMES AND PROPERTIES OF THE PATHWAY

(Received for publication, October 3, 1977, and in revised form, June 16, 1978)

Peter B. Rowe,‡ Eric Mc Cairns, Gemma Madsen, Dorit Sauer, and Helen Elliott
From the Department of Child Health, University of Sydney, New South Wales, 2050, Australia

The enzymes of the de novo purine biosynthetic pathway have been partially co-purified from pigeon liver by a method dependent upon the use of the nonionic polymer polyethylene glycol for enzyme stabilization and cofractionation. Although the enzymes did not appear to constitute a large macromolecular complex it was evident that some particular inter-relationship between them was preserved during the purification procedure. Analysis of the end products and pathway intermediates was carried out primarily by sensitive high pressure liquid chromatographic techniques. Substrate and cofactor requirements were confirmed and optimal conditions of pH, temperature, and K⁺ ion activation established. At phosphoribosyl pyrophosphate (PP-ribose-P) concentrations below 0.3 mM the activity of the first pathway enzyme amidophosphoribosyltransferase was rate-limiting, and the inhibition of this enzyme by AMP regulated the rate of purine ring synthesis. At higher concentrations of PP-ribose-P, aminomimidazole ribonucleotide synthetase, the fifth enzyme of the pathway became rate limiting and was subject to inhibition by added AMP. It was evident that regulation of purine synthesis was quite complex and that AMP inhibition (perhaps reflected in a low adenylate energy charge) can be effected at different points on the purine pathway.

The regulation of de novo purine synthesis in eukaryotic cells is poorly understood. Allosteric inhibition of the first enzyme amidophosphoribosyltransferase (EC 2.4.2.14 ribosylamine 5-phosphate:pyrophosphate phosphoribosyltransferase) by nucleotide end products is considered to be the major regulatory mechanism, but substrate limitation at any of the 10 enzymatic reactions of the pathway could well prove to be rate-limiting under different conditions. Considerable emphasis has in fact, been placed on the availability of phosphoribosyl pyrophosphate, a substrate for the first enzyme, as a controlling factor in de novo synthesis (1). It is quite probable, moreover, that regulatory mechanisms will be complex and that AMP inhibition (perhaps reflected in a low adenylate energy charge) can be effected at different points on the purine pathway.

Experimental Procedures

Materials

Polyethylene glycols were obtained from British Drug Houses and purified prior to use (2). 5,10-Methylenetetrahydrofolate and 10-formyltetrahydrofolate were synthesized from tetrahydrofolic acid (Sigma) (3). A sample of formylglycinamide ribonucleotide was supplied by Dr. John M. Buchanan of Massachusetts Institute of Technology. [14C]FGAR and [14C]gymamide ribonucleotide were synthesized by the method of Lukens and Flaks (4) except that the enzyme preparation used was the 4 to 11% PEG fraction of pigeon liver described below. [14C]Formylglycinamide ribonucleotide was produced by cleavage of [14C]FGAR with alkaline phosphatase (5). [14C]Formylglycinamide ribonucleotide and [14C]aminomimidazole ribonucleotide were enzymatically synthesized from [14C]FGAR (6). The specific radioactivity of all of these intermediates was 5.0 Ci/mmol. Aminomimidazolecarboxylate ribonucleotide was a gift from Dr. Gordon Shaw of the University of Birmingham, England. Aminomimidazole carboxamidribonucleotide was synthesized from formamidinosamido ribonucleotide (7) generously provided by Dr. Joseph Goto of the University of Pennsylvania. [14C]Glycine, [U-14C]glutamine, [8-14C]hypoxanthine, [8-14C]inosine, [8-14C]adenine, [8-14C]adenosine, [8-14C]AMP, [8-14C]ATP, [8-14C]GMP, [15N] nicotinic acid, and [14C]formate were purchased from the Radiochemical Centre, Amersham. [8-14C]IMP was obtained from Schwarz/Mann. All nonradioactive purine bases and their ribonucleoside and ribonucleotide derivatives, PP-ribose-1′ and phosphonompyruvic acid were obtained from P-L Biochemicals, Inc. Pyruvate kinase (type III, free of adenylylate kinase, adenylylate deaminase, and 5′-nucleotidase), glutamine, and aspartic acid were purchased from Sigma Chemical Co. Azaserine was a Calbiochem product, and purified protein molecular weight markers were from Schwarz/Mann and Worthington Biochemical, Inc. Tetrabutylammonium hydroxide was purchased from Eastman Organic Chemicals.

Methods

De Novo Purine Synthesis

This was assayed by measuring the incorporation of [1-14C]glycine into IMP and its catabolites inosine and hypoxanthine. [1-14C]Glycine was purified by Dowex 50 chromatography (8) immediately prior to use. The reaction mixture contained [1-14C]glycine (specific radioactivity, 1.6 Ci/mol, 0.6 mM; PP-ribose-P, 1.5 mM; 5,10-methylenetetrahydrofolate, 1.5 mM; aspartic acid, glutamine, and sodium formate, 1.6 mM). The reaction was initiated by the addition of the enzyme preparation and the labeled products were separated from unreacted glycine by reverse phase liquid chromatography.

1 The abbreviations used are: FGAR, formylglycinamide ribonucleotide; PP-ribose-P, phosphoribosyl pyrophosphate; GAR, glycaminid ribonucleotide; FGAM, formylglycinamidine ribonucleotide; AIR, aminomimidazole ribonucleotide; CAIR, aminomimidazole carboxyamide ribonucleotide; SAICAR, aminomimidazole N′-succinocarboxamide ribonucleotide; AICAR, aminomimidazole carboxamide ribonucleotide; PEG, polyethylene glycol; FGA, formylglycinamide.

* This work was supported by grants from the National Health and Medical Research Council of Australia and the New South Wales State Cancer Council. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom communications should be addressed at Institute of Child Health, Royal Alexandra Hospital for Children, Camperdown, New South Wales, 2050, Australia.
each 2.0 mM; ATP, 2.5 mM; MgCl₂, 5.0 mM; KHCO₃, and phospho-
enolpyruvate, each 10.0 mM; pyruvate kinase, 50 units; KCl, 50 mM; purine synthetic enzyme fraction in Tris-Cl, 50 mM, pH 7.8, con-
taining 2% PEG 20 M and 5.0 mM β-mercaptoethanol in a final volume of
3.5 ml. After incubation at 38°C usually for 10 min, the reaction
was stopped by the addition of 3.5 ml of ice cold 2.0 M perchloric acid.
Protein was removed by centrifugation and the supernatant solution
divided into three equal fractions. Fraction 1 was hydrolyzed and
hypoxanthine extracted by the method of Hershko et al. (9). Fraction
2 was hydrolyzed for 1 h at 95°C, effectively cleaving all purine
derivatives to their bases and all purine pathway intermediates up to
potassium hydroxide and clarified by centrifugation. The products
of the reaction were identified and their concentration and radioactive
density determined by a combination of high pressure liquid and thin
layer chromatographic techniques.

High Pressure Liquid Chromatography

This provided the most accurate method for identification of not
only the purine end products of the overall reaction sequence but also
of the various pathway intermediates which accumulated under cer-
tain experimental conditions. The three major techniques used were
(i) paired ion reverse phase, (ii) anion exchange, and (iii) cation
exchange on a Spectra Physics model 3500 liquid chromatograph
equipped with a model 770 double beam variable wavelength spectro-
photometric detector and a Spectra Physics SP4000 computing inte-
grator. All chromatograms were run at 33°C.

Paired ion reverse phase chromatography was performed on a
Partisil 10-ODS-2 (Reeve Angel) column (25 cm × 4.6 mm) equili-
brated in a buffer consisting of 5.0 mM tetrabutyl ammonium hydrox-
ide (adjusted to pH 6.0 with phosphoric acid) and 10% (v/v) methanol.
Following sample injection the column was washed with this buffer
for 10 min at a flow rate of 1.0 ml/min. The concentration of methanol
was then linearly increased to 50% (v/v) over 15 min at the same flow
rate. Under these conditions the following retention times (min) were
reproducibly (+3%) observed: glycine, 3.5; FGAM, 4.5; AIR, 5.5; hypoxanthine, 7.5; inosine, 9.5; FGAR, 20.0; IMP, 24.0; CAIR, 25.0; AMP, 26.0; ADP, 28.5; ATP, 31.0. The eluate
was then linearly increased to 50% (v/v) over 15 min at the same flow
rate. Under these conditions the following retention times (min) were
reproducibly (+3%) observed: glycine, 3.5; adenosine, 3.5; adenine, 3.8;
guanine, 4.0; inosine, 4.3; guanosine, 4.5; hypoxanthine, 4.9; AMP, 6.1;
IMP, 20.5; XMP, 21.4; GMP, 22.4; adenosine, 42.7; ADP, 44.4; GDP,
46.7; ATP, 60.0; CTP, 72.8.

Anion exchange chromatography was performed on a Partisil
SCX (Reeve Angel) column (25 cm × 4.6 mm) with a programmed gradient
of 10 mM ammonium phosphate buffer, pH 2.7 (Buffer A) and 0.5 M
ammonium phosphate buffer, pH 3.8 (Buffer B) at a flow rate of 0.8
ml/min. Following sample injection, Buffer A was run for 15 min and
over the next 10 min the concentration of Buffer B was linearly
increased to 100% (v/v). Fractions (0.5 ml) were collected with an
autosampler data obtained by the liquid chromatography techniques.

(i) Cellulose precoated plastic sheets (Polygram Cel 300 UV, Mach-
erey Nagel and Co.) developed with 5% (w/v) disodium hydrogen
phosphate, pH 9.1, saturated with isomyl alcohol gave the following
RF values: 0.97, FGAR and adenylosuccinate; 0.93, glycine; 0.92; ATP;
0.95, IMP and XMP; 0.89; GMP; 0.86 ADP; 0.84, AICAR; 0.83, CAIR;
0.78, AMP and inosine; 0.73, xanthosine; 0.70; guanosine; 0.68, hypo-
xanthine; 0.51, adenosine; 0.44, guanine; 0.38, xanthine; 0.37, adenine.

(ii) DEAE-cellulose precoated plastic sheets (Polygram Cel 300
DEAE, Macherey Nagel and Co.) developed with 1% formic acid gave
the following RF values: 0.99, FGAR; 0.97, glycine, adenosine, adeny-
line; 0.90, hypoxanthine; 0.91, AMP, inosine, and guanosine; 0.70, AICAR;
0.75, xanthosine; 0.61, xanthine; 0.24; IMP; 0.16, ADP; 0.13, XMP; origin, ATP. (iii) Polyethyleneimine cellulose precoated
plastic sheets (Polygram Cel 18, Macherey Nagel and Co.) developed with 2% perchloric acid fluoresced with 2% medium chloride gave the following RF values: solvent front, glycine; 0.75; inosine; 0.64, adenosine; 0.59, GMP; 0.58, hypoxanthine; 0.50, guanosine and xanthosine; 0.52, GAT and AIR; 0.44, GFR; 0.43, xanthine; 0.39, adenosine; 0.36, guanine; 0.19, IMP and AICAR; 0.11, AMP; origin, ATP, ADP, GMP, XMP, and CAIR.

Pathway activity was determined by incorporation of purine ring syn-
thesis mg of protein / h. After a time lag of approximately 1.5 min (see Fig. 2), reaction velocity was linear and proportional to the
amount of enzyme to a point where 80% of the glycine was incorpo-
rated into purines.

Individual Enzyme Assays

(i) Amidophosphoribosyltransferase—This was assayed by meas-
uring the PP ribose-P dependent release of [14C]glutamic acid from
[14C]glutamate (10). This assay and all other individual purine path-
way enzyme assays were performed in the same buffer as the purine
biosynthetic assay.

(ii) GAR Synthetase (EC 6.3.4.13, Phosphoribosylglycinamide
Synthetase)—This was assayed by measuring the ATP-dependent
incorporation on [14C]glycine of GAR (13). This was determined by
anionic exchange chromatography (15).

(iii) FGAR Amidotransferase (EC 6.3.5.3, Phosphoribosylformyl-
glycaminidase synthetase)—This was assayed by measuring the con-
version of FGAR to AIR in a coupled enzyme system (12).

(iv) AIR Carboxylase (EC 4.1.1.21, Phosphoribosylaminomida-
zone Carboxylase)—This was measured in the reverse direction by
following the decarboxylation of CAIR at 295 nm in a recording
spectrophotometer (13).

(v) SAICAR Synthetase (EC 6.3.2.6, Phosphoribosylaminomida-
zone Succinocarboxamide Synthetase)—This was assayed by meas-
uring the production of the reaction, SAICAR, by the modified
Bratton-Marshall procedure (13).

(vi) Formyltetrahydrofolate Synthetase (EC 6.3.2.3, and Meth-
ethenyltetrahydrofolate Cyclohydrolase (EC 3.5.4.9)—These were mea-
sured spectrophotometrically (14).

(vii) Adenine Phosphoribosyltransferase (EC 2.4.2.7) and hypo-
xanthine Phosphoribosyltransferase (EC 2.4.2.8)—These were as-
sayed by measuring the incorporation of the radioactive purine into
their respective nucleotides (15).

Purification of Purine Pathway Enzymes

Freshly excised pigeon liver was homogenized in 1.5 volumes of a
buffer consisting of 0.25 M sucrose, 5.0 mM β-mercaptoethanol, 5.0 mM
MgCl₂, 25 ml KCl and 1.0 mM ATP in 50 mM Tris-Cl, pH 8.0, at
4°C and centrifuged for 6 x 10⁵ g x min. The supernatant solution
was adjusted to 11% (w/v) saturation of PEG 4000 by the dropwise addition, with continuous stirring of a 50% (w/v)
aqueous solution of PEG 4000. The 4 to 11% protein precipitate was
dissolved in a minimum volume of homogenizing buffer containing
2% (w/v) PEG 20 M instead of 0.25 M sucrose.

Controlled-pore glass (CPG-10-240, average pore diameter 25 Å, 80
to 120 mesh, Electro-Nucleonics, Inc., N.J.) was pretreated with PEG
20 M in order to minimize ionic adsorptive effects, equilibrated in the
buffer (degassed) used for dissolving the protein precipitate and
poured to form a column (172 x 1.5 cm). 1.0 milliliter of the enzyme
solution, approximately 50 mg of protein, was applied to the column
which was developed with the equilibrating buffer at 15 ml/h. Column
fractions (2.0 ml) were scanned spectrophotometrically at 260 nm.

The fractions containing the peak purine synthetic activity were
pooled and the enzymes precipitated with 15% (w/v) PEG 4000 and
redissolved in a minimal volume of column buffer. The addition of
glycerol to a final concentration of 50% (v/v) and storage at −20°C
prolonged the half-life of purine synthetic activity from 3 days to 4
weeks. Protein concentration was measured spectrophotometrically
(19) as PEG interfered with determination by fluorimetric or various
colorimetric methods.

Polyacrylamide Gel Electrophoresis—This was performed as previ-
ously described (11) with 5% acrylamide gels with a 30:1 ratio of
acrylamide to methylene bisacrylamide. When 1% PEG 20 M was
incorporated into the gels, they became opalescent, and setting time
was doubled.

Sucrose Density Gradient Ultracentrifugation—This was performed using hemoglobin, bovine \( \gamma \)-globulin, milk xanthine oxidase, and horse spleen apoferritin as molecular weight markers (18).

**RESULTS**

Purification Procedure—The co-purification of the enzymes of the pathway is summarized in Table I. Very little purine synthetic activity is lost, the final yield of 76% reflecting that obtained from the high-speed supernatant solution with an overall 200-fold increase in specific activity. The relative distribution of the activities of the overall pathway and a number of the individual pathway enzymes is shown in Table II. A number of significant points emerge from this.

Amidophosphoribosyltransferase activity generally parallels that of de novo synthesis and, as will be discussed later, is equal to the rate of incorporation of glycine into pathway intermediates and end products. This observation points up the obvious inadequacy of the GAR synthetase assay using preformed phosphoribosylamine in which the recorded activities were usually several orders of magnitude lower than that of amidophosphoribosyltransferase, particularly in the cruder enzyme fractions. Little weight, therefore, can be placed on the fractional distribution of this second enzyme of the pathway.

FGAR amidotransferase activity distribution was also similar to that of amidophosphoribosyltransferase although, in contrast, significant losses of activity occurred in the course of purification. It is evident, however, that the difference in specific activity between these two enzymes in the more purified protein fractions is not in fact reflected in measurements of overall pathway activity.

AIR carboxylase and SAICAR synthetase showed a similar type of fraction distribution although this was obscured by the apparent high losses of SAICAR synthetase activity during purification. Nevertheless, the activities of both of these enzymes were almost two orders of magnitude higher than that of amidophosphoribosyltransferase, and they clearly would not represent rate-limiting steps.

### Table I

| Purification of enzymes of de novo purine synthesis from pigeon liver |
|-------------------------|-------------------------|-------------------------|-------------------------|
| Step                    | Specific activity       | Total protein           | Recovery % activity |
| 1. Homogenate           | 56.0                    | 5,120                   | 100%                   |
| 2. High speed supernatant solution | 398.2                  | 544                     | 75%                     |
| 3. 4% PEG supernatant solution | 485.0                  | 397                     | 67%                     |
| 4. 11% PEG precipitate   | 1,288.4                 | 175                     | 79%                     |
| 5. Column pool           | 7,245.0                 | 34                      | 86%                     |
| 6. 15% PEG precipitate   | 12,672.0                | 18                      | 76%                     |

**Table II**

Differential distribution of purine synthetic and individual enzyme specific and total activities

Specific activity expressed as nmol mg \(^{-1}\) h \(^{-1}\); total activity (in parentheses) expressed as pmol h \(^{-1}\) except for AIR carboxylase and SAICAR synthetase values which are expressed as \( \mu \)mol mg \(^{-1}\) h \(^{-1}\) and \( \mu \)mol h \(^{-1}\).

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Purine synthesis</th>
<th>Amidophosphoribosyltransferase</th>
<th>GAR synthetase</th>
<th>FGAR amidotransferase</th>
<th>AIR carboxylase</th>
<th>SAICAR synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. High speed supernatant solution</td>
<td>298.2 (216)</td>
<td>1,060 (570)</td>
<td>0.29 (0.16)</td>
<td>2,250 (1,230)</td>
<td>176 (95.7)</td>
<td>112 (60.9)</td>
</tr>
<tr>
<td>2. 4% PEG precipitate</td>
<td>19.3 (4.6)</td>
<td>350 (80)</td>
<td>1.3 (0.31)</td>
<td>109 (30)</td>
<td>14 (4.1)</td>
<td>24.8 (5.9)</td>
</tr>
<tr>
<td>3. 11% PEG precipitate</td>
<td>1,288 (225.4)</td>
<td>2,770 (460)</td>
<td>1.6 (0.28)</td>
<td>2,640 (640)</td>
<td>636 (111)</td>
<td>200 (35)</td>
</tr>
<tr>
<td>4. 11% PEG supernatant solution</td>
<td>0</td>
<td>900 (130)</td>
<td>5.0 (0.76)</td>
<td>272 (40)</td>
<td>64 (9.7)</td>
<td>2.4 (0.36)</td>
</tr>
<tr>
<td>5. Column pool</td>
<td>7,245 (246)</td>
<td>12,900 (430)</td>
<td>216 (7.3)</td>
<td>1,940 (70)</td>
<td>3,430 (116)</td>
<td>460 (15.6)</td>
</tr>
<tr>
<td>6. 15% PEG precipitate</td>
<td>12,072 (217)</td>
<td>18,500 (350)</td>
<td>92 (1.6)</td>
<td>3,800 (70)</td>
<td>3,230 (58.8)</td>
<td>832 (15)</td>
</tr>
<tr>
<td>7. 15% PEG supernatant solution</td>
<td>48 (0.7)</td>
<td>9,100 (50)</td>
<td>358 (5.7)</td>
<td>0</td>
<td>580 (9.3)</td>
<td>0</td>
</tr>
</tbody>
</table>

The separation of individual enzyme activities was best demonstrated during the glass chromatography procedure (Fig. 1). The peak of purine synthetic activity coincided with that of an internal enzyme marker formyltetrahydrofolate synthetase, molecular weight 290,000 to 240,000 (19, 20), and with amidophosphoribosyltransferase, GAR synthetase, and FGAR amidotransferase. AIR carboxylase and SAICAR synthetase cochromatographed at a somewhat lower elution volume corresponding to the molecular weight of 350,000 reported for these two enzymatic activities from chicken liver (13), but their relatively high activity was reflected by their tailing into the region of the purine biosynthetic peak. Methylenetetrahydrofolate cyclohydrolase cochromatographed with formyltetrahydrofolate synthetase while hypoxanthine and adenine phosphoribosyltransferases cochromatographed immediately preceding the hemoglobin marker. In the absence of PEG from the elution buffer amidophosphoribosyltransferase, and accordingly purine synthetic activity, could not be

**Fig. 1.** Chromatographic elution profile of the 4 to 11% PEG fraction on a CPG-10-240 glass column (172 x 2.5 cm) showing absorbance at 280 nm (——) and glycine incorporation into purines (——) in \( \mu \)mol \( \mu \)mol h \(^{-1}\). Column markers (8) were tobacco mosaic virus (TMV), a void volume marker, horse spleen apoferritin (Fe), M, the formyltetrahydrofolate synthetase (F), M, = 220,000 to 240,000; and hemoglobin (Hb), M, = 64,000. The elution positions of other enzyme activities are indicated by S (AIR carboxylase and SAICAR synthetase) and PRTase (hypoxanthine and adenine phosphoribosyltransferases).
De Novo Purine Synthesis in Avian Liver

FIG. 2. The incorporation of glycine into purines (○) and purines together with pathway intermediates (■) as a function of time of incubation. Assays containing approximately 4 mg of protein from the 4 to 11% PEG fraction or 0.4 mg of protein from the final 15% PEG fraction were incubated under standard conditions and 1.5 mM PP-ribose-P. Points represent duplicates in the same study.

detected. The leading protein peak was broader and associated with a long tailing effect. The position of the various molecular weight markers and of formyltetrahydrofolate synthetase and the purine salvage enzyme activities was unaltered, but their peaks were broader and less clearly defined. This indicated that PEG minimized adsorbance to the glass beads as well as stabilizing enzymatic activity.

Alternative Molecular Weight Determinations—As ionic adsorptive effects may modify the behavior of proteins on glass beads, the final 15% PEG precipitate was subjected to sucrose density gradient centrifugation. The peak of purine synthetic activity again corresponded to a protein of molecular weight 240,000. The sample applied to the sucrose gradient contained 2% PEG but over 50% of the purine synthetic activity was lost during the centrifugation procedure. If PEG was omitted from the enzyme solution, all activity was lost. The movement of the protein molecular weight markers was unaffected by the presence of PEG in the enzyme solution.

Properties of the de Novo Synthetic Pathway—The properties discussed here relate to the most highly purified enzyme fraction, the final 15% precipitate. The same results, however, were obtained with the 4 to 11% PEG fraction. The only major difference occurred in the purine ring end product, which was largely IMP in the more purified fraction due to loss of IMP 5'-nucleotidase activity on column chromatography. The l-14C-carbon atom from glycine was not incorporated into any metabolite other than purine pathway-related compounds.

The pH optimum was broad with a maximum between 7.5 and 8.0 and a sharp fall above pH 8.5, consistent with the reported pH optima of between 7.0 and 8.0 for the individual pathway enzymes from different types of animal cells (21). The temperature optimum was 50°C with a sharp fall above 55°C. Specific substrate and cofactor requirements were confirmed by deletion experiments.

At all stages of enzyme purification, however, analysis of the reaction end products demonstrated that above 0.3 mM PP-ribose-P, the activity of the first enzyme amidophosphoribosyltransferase was not rate-limiting, and consequently a number of pathway intermediates accumulated. The incorporation of glycine greatly exceeded the rate of purine ring synthesis (Fig. 2), and the disappearance of labeled intermediates as a function of time paralleled the increase in purines. Extrapolation of the purine ring synthetic rate curve to the abscissa illustrates the 1/4-min time lag which was independent of the amount of enzyme activity added to the assay mixture (Fig. 3). This lag presumably was related to the time

FIG. 3. The rate of purine ring synthesis as a function of the amount of enzyme added and the incubation time under optimal assay conditions. The quantities (milligrams of protein) of 4 to 11% PEG fraction added were 3.2 (○), 4.0 (■), and 6.4 (△). Identical results were achieved with the final 15% PEG fraction.

and consequently the stained bands were broader. The relative migration of the protein molecular weight markers was, however, unaffected by the presence of PEG in the gel.
maximum synthetic rates. A series of studies was undertaken using phosphate buffers which produced lower synthetic rates, between these two enzyme activities.

The accumulation of intermediates as a function of PP-ribose-P concentration is shown on Fig. 4. Two of the intermediates have been identified chromatographically as FGAR and FGAM while a third intermediate has been tentatively identified as FGAR polyphosphate. This identification was based on the following studies: (i) the late elution from the reversed-ion reverse-phase chromatographic system (30 min) consistent with a highly charged molecule and (ii) acid hydrolysis of the intermediate isolated by preparative reversed-ion reverse-phase chromatography released [14C]glycine and alcaline phosphatase digestion produced a compound which cochromatographed with [14C]FGA riboside (5).

The accumulation of FGAR (and perhaps FGAR polyphosphate) and FGAM and the observation that this was related to K+ concentration (vide infra) suggested that AIR synthetase (EC 6.3.3.1, phosphoribosylaminimidazolyl synthetase) was the rate-limiting step. Both FGAR amidotransferase and AIR synthetase are K+ sensitive (21), and although there was significant loss of the former activity during purification (Table II) the pattern of intermediates would indicate a relatively lower activity for the latter enzyme. This was confirmed by direct assay of the conversion of FGAR to AIR where clearly AIR synthetase was rate-limiting (Table III). It must be emphasized that the relative rate limitation was the same with enzyme fractions at every stage of the purification procedure. This would suggest a direct functional correlation between these two enzyme activities.

Tris-HCl buffer proved to be the most effective in attaining maximum synthetic rates. A series of studies was undertaken using phosphate buffers which produced lower synthetic rates, only 50% of the rate in 50 mM Tris-HCl being achieved in 50 mM P. Not only was the rate of glycine fixation reduced, but there was a significant inhibitory effect on AIR synthetase resulting in a greater accumulation of pathway intermediates.

**PP-ribose-P, Glutamine and NH3 Requirements—**The rate of incorporation of [14C]glycine was proportional to the concentration of PP-ribose-P, 50% maximal velocity being achieved at approximately 0.3 mM (Fig. 4). Commercial PP-ribose-P was at best, 70% pure, and the concentration in these studies was based on that figure. Ribose-5-P was, however, a major contaminant and some PP-ribose-P could be derived from this. Ribose-5-P in fact could effectively substitute for PP-ribose-P in crude protein fractions, but there was apparently considerable loss of PP-ribose-P synthetase activity at the glass chromatography step.

Under standard assay conditions the rate of purine ring synthesis was maximal at 1.0 mM glutamine. There was clearly minimal diversion of glutamine into alternative reactions in purified protein fractions as virtually 100% of the glycine present could be incorporated into purines in the presence of stoichiometric quantities of glutamine (1:2). The replacement of 2.0 mM glutamine, however, with 2.2 mM NH3 resulted in 50% reduction in the rate of incorporation of [14C]glycine, but synthesis proceeded only as far as FGAR. This indicated that significant ammonia PP-ribose P aminotransferase activity was present but that FGAR amidotransferase could not utilize or compete for ammonia under these assay conditions. No glycine incorporation resulted from the substitution of ribose-5-P and ammonia for PP-ribose-P and glutamine indicating the absence of 5' phosphoribosylamine synthetase (EC 6.3.4.7) in, at least, the final 15% PEG precipitate.

**Glycine Concentration—**Studies with [14C]HCO3- and [14C]formate confirmed the absolute requirement for glycine for purine ring synthesis. An approximate Km of 8 μM for glycine was determined in the presence of saturating levels of other substrates.

**ATP Requirement—**The enzyme preparation readily degraded ATP in the absence of any purine synthesis. An ATP-regenerating system was essential to maintain ATP levels and to prevent the accumulation of potential inhibitors such as AMP, or a reduction in the adenylate energy charge. The pyruvate kinase added for this purpose was selected because of the low level of contamination by adenylate deaminase, adenylate kinase, and 5'-nucleotidase. These three latter enzyme activities were present in our enzyme preparations as

\[ \text{G. Toby and A. S. Bagnara, personal communication.} \]

### Table III

<table>
<thead>
<tr>
<th>mM</th>
<th>FGAR %</th>
<th>FGAM %</th>
<th>AIR %</th>
<th>AIR (Bratton-Marshall) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>20</td>
<td>60</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>2.0</td>
<td>20</td>
<td>60</td>
<td>11</td>
<td>15.5</td>
</tr>
<tr>
<td>3.0</td>
<td>17</td>
<td>83</td>
<td>0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

*Fig. 4. The incorporation of glycine into (i) purines together with pathway intermediates (x), (ii) total intermediates (●), (iii) purines (○), (iv) FGAM (▲), (v) FGAR (●), (vi) FGAR polyphosphate (▲) as a function of PP-ribose-P concentration. Assays were incubated for 10 min. As no pathway intermediates usually accumulated below 0.3 mM PP-ribose-P the curves for purines and intermediates (x) and purines (○) are coincident below this point.*
was evident indirectly from the analysis of the residual AMP levels at the end of the synthetic assay (Fig. 8). The purine synthetic rate was maximal at 1.5 mM ATP and was not affected by ATP concentrations as high as 10.0 mM. The ATP requirement was absolute and could not be met by other nucleoside triphosphates.

One-carbon Reduced Folate Derivatives—These were essential for purine synthesis. These were usually added in the form of 5,10-methylenetetrahydrofolic acid. Although the pH of the reaction resulted in the rapid nonenzymatic conversion of the methenyl derivative to the formyl derivative presumably the presence of methylenetetrahydrofolate cyclohydrolase in the enzyme preparation ensured an adequate supply of both one-carbon donors for the two formylation reactions of the purine pathway. Formate was not necessary for purine synthesis but was added in order to maintain a one-carbon regeneration system via the enzymes cyclohydrolase and formyltetrahydrofolate synthetase. The actual concentration of one-carbon folate derivatives required must be extremely low as sufficient reduced folate was present bound to the protein in the 4 to 11% PEG fraction to ensure maximal rates of purine synthesis in the presence of added formate. Studies with [14C]formate and tetrahydrofolic acid confirmed a 2:1 stoichiometry of formate to glycine incorporation into the purine ring. The omission of a one-carbon donor system resulted in the accumulation of GAR. In the presence of saturating levels of the other substrates the rate of synthesis of GAR was twice that of the control purine synthetic rate confirming the presence of a rate-limiting step distal to GAR synthetase.

K+ Concentration—This was critical for maximum rates of purine synthesis. This was achieved between 50 mM and 100 mM (Fig. 5) with inhibition of purine ring synthesis observed at 250 mM. The extrapolations of the velocity curves to the common abscissa intersect reflect the 1½-min time lag referred to earlier for attaining maximum velocity (Figs. 2 and 3). The decreased rate of purine ring synthesis at low K+ concentrations is clearly related to the activity of AIR synthetase and possibly of FGAR amidotransferase as shown in Fig. 6 where the accumulation of the intermediates, FGAM, and FGAR (and a small quantity of FGAR polyphosphate) is equivalent to the drop in purine synthetic rate. In this particular experiment all of the [14C]glycine has been incorporated into these pathway intermediates or into purines indicating that the K+ concentration did not affect the activities of amidophosphoribosyltransferase, GAR synthetase, or FGAR synthetase (EC 2.1.2.2). This was confirmed in other studies where the amount of residual glycine was not related to the K+ concentration.

Inhibition of FGAR amidotransferase with 1.0 mM azaserine resulted in the accumulation of FGAR (32% of [14C]glycine), GAR (8%), and FGAR polyphosphate (4%) under conditions where the control assay incorporated only 22% of the glycine into purines. This experiment again confirmed the presence of the rate-limiting step, distal to FGAR synthetase.

HCO3− Requirements—These were difficult to establish as the enzyme preparations all contained significant HCO3− despite degassing of buffers. The rate of purine ring synthesis in the absence of added HCO3− was 30% of the rate at the optimum concentration of 10 to 50 mM while AIR, FGAM, and FGAR accumulated proximal to the relative block. A small amount of FGAR polyphosphate was present, but in addition two more intermediates with similar chromatographic properties on the paired-ion reverse-phase system were also detected. It was possible that these were polyphosphate derivatives of AIR and FGAM as they produced

---

**Fig. 5.** The incorporation of glycine into purines as a function of the quantity of enzyme and the incubation time measured at variable K+ ion concentration: 2.0 mM (●), 10 mM (○), 15 mM (△), 50 mM and 100 mM (△), 250 mM (△). The assays were carried out under standard conditions with either the 4 to 11% PEG fraction or the final 15% PEG precipitate, the only variables being the K+ ion concentration and the addition of HCO3− as the Na+ salt instead of the usual K+ salt.

**Fig. 6.** The incorporation of glycine into purines (●) and total intermediates (FGAM, FGAR, and FGAR polyphosphate) (○) as a function of K+ ion concentration. These studies were carried out as indicated in Fig. 5, and the incubation time was 20 min at which time virtually no free glycine remained.
incorporation of glycine into these intermediates. CAIR, however, could not be identified despite use of both perchloric acid and 80% methanol to terminate the reaction. Presumably, therefore, this unstable intermediate decarboxylated to form AIR. There was, however, a much larger relative accumulation of "polyphosphate" intermediates which progressively increased with time of incubation. These intermediates were again acid-labile, releasing [14C]glycine on hydrolysis. In order to determine whether any of the polyphosphate intermediates were derived from AIR they were isolated by preparative paired-ion reverse-phase liquid chromatography. Unfortunately, folate derivatives have the same retention time in this analytical system, and the Bratton-Marshall reaction was therefore not sufficiently sensitive. Treatment with alkaline phosphatase resulted in the conversion of only 25% of the radioactivity to FGA riboside but did not effect the chromatographic behavior of the remainder. This indicated that the remaining compounds were either not polyphosphates or that they were resistant to the action of this enzyme.

Nou de novo synthesis of AMP, GMP, or XMP could be demonstrated in the more purified enzyme fractions, and this was due to the absence of adenylosuccinate synthetase (EC 6.3.4.4) and GMP synthetase (EC 6.3.4.1) although small amounts of IMP dehydrogenase (EC 1.2.1.14) activity were present.2 AMP Inhibition—the addition of AMP resulted in inhibition of purine synthesis. Due to the presence of adenylate kinase, an ATP-regenerating system, and adenylate deaminase, small amounts of added AMP were rapidly converted, largely to ATP, but also eventually to IMP. The loss of AMP was linear over the time course of the assays (usually 5 or 10 min), but the concentrations of AMP reported in these studies were derived from AIR they were isolated by preparative paired-ion reverse-phase liquid chromatography. Unfortunately, folate derivatives have the same retention time in this analytical system, and the Bratton-Marshall reaction was therefore not sufficiently sensitive. Treatment with alkaline phosphatase resulted in the conversion of only 25% of the radioactivity to FGA riboside but did not effect the chromatographic behavior of the remainder. This indicated that the remaining compounds were either not polyphosphates or that they were resistant to the action of this enzyme.

Aspartic Acid—This was absolutely essential for purine synthesis, and there was an accumulation of intermediates similar to that observed in the HCO3- deletion studies in its absence. Similarly, the deletion of aspartate did not affect the incorporation of glycine into these intermediates. CAIR, however, could not be identified despite use of both perchloric acid and 80% methanol to terminate the reaction. Presumably, therefore, this unstable intermediate decarboxylated to form AIR. There was, however, a much larger relative accumulation of "polyphosphate" intermediates which progressively increased with time of incubation. These intermediates were again acid-labile, releasing [14C]glycine on hydrolysis. In order to determine whether any of the polyphosphate intermediates were derived from AIR they were isolated by preparative paired-ion reverse-phase liquid chromatography. Unfortunately, folate derivatives have the same retention time in this analytical system, and the Bratton-Marshall reaction was therefore not sufficiently sensitive. Treatment with alkaline phosphatase resulted in the conversion of only 25% of the radioactivity to FGA riboside but did not effect the chromatographic behavior of the remainder. This indicated that the remaining compounds were either not polyphosphates or that they were resistant to the action of this enzyme.

No de novo synthesis of AMP, GMP, or XMP could be demonstrated in the more purified enzyme fractions, and this was due to the absence of adenylosuccinate synthetase (EC 6.3.4.4) and GMP synthetase (EC 6.3.4.1) although small amounts of IMP dehydrogenase (EC 1.2.1.14) activity were present.2 AMP Inhibition—the addition of AMP resulted in inhibition of purine synthesis. Due to the presence of adenylate kinase, an ATP-regenerating system, and adenylate deaminase, small amounts of added AMP were rapidly converted, largely to ATP, but also eventually to IMP. The loss of AMP was linear over the time course of the assays (usually 5 or 10 min), but the concentrations of AMP reported in these studies were derived from AIR they were isolated by preparative paired-ion reverse-phase liquid chromatography. Unfortunately, folate derivatives have the same retention time in this analytical system, and the Bratton-Marshall reaction was therefore not sufficiently sensitive. Treatment with alkaline phosphatase resulted in the conversion of only 25% of the radioactivity to FGA riboside but did not effect the chromatographic behavior of the remainder. This indicated that the remaining compounds were either not polyphosphates or that they were resistant to the action of this enzyme.

Aspartic Acid—This was absolutely essential for purine synthesis, and there was an accumulation of intermediates similar to that observed in the HCO3- deletion studies in its absence. Similarly, the deletion of aspartate did not affect the
were those determined at the end of the reaction. The relationship between added and final AMP concentrations is plotted in Fig. 8. The adenylate energy charge given by the ratio \((\text{ATP} + \frac{1}{2} \text{ADP})/(\text{ATP} + \text{ADP} + \text{AMP})\) equal to half the number of anhydride-bound phosphate groups per adenosine moiety (23) was proportionately reduced by the addition of increasing quantities of AMP (Fig. 8). The ATP concentration never fell below the optimum of 1.5 mM, and ADP concentrations were always low irrespective of the amount of AMP added. That inhibition was effected by AMP or by a reduction in the adenylate energy charge was confirmed by longer time course studies in which the rate of purine synthesis increased as the AMP level progressively fell and the adenylate energy charge rose. IMP, the major end product of adenine nucleotide breakdown was not an inhibitor of the biosynthetic pathway.

At low (0.3 mM) PP-ribose-P concentrations where the activity of amidophosphoribosyltransferase was rate limiting AMP inhibition occurred at this first step of the pathway, and no inhibition was observed at any later steps (Fig. 9). Fifty per cent inhibition was observed at a final AMP concentration of 0.5 mM (Fig. 9B) and an adenylate energy charge of 0.8 (Fig. 10B). At higher (1.5 mM) concentrations of PP-ribose-P the pattern of inhibition was more complex. A maximum inhibition of glycine fixation (presumably amidophosphoribosyltransferase inhibition) of 25% was achieved at 1.5 mM AMP (Fig. 9) and an adenylate energy charge of 0.6 (Fig. 10). Inhibition of purine ring synthesis, however, was more pronounced with 50% inhibition at 0.85 mM AMP at an adenylate energy charge of 0.75 (Figs. 9 and 10). This inhibition of purine ring synthesis was associated with a relative accumulation of FGAM, FGAR, and FGAR polyphosphate indicating that AIR synthetase was the affected enzyme. The derived inhibition plot for this enzyme is shown on Fig. 9B as a function of the AMP concentration. The inhibition was confirmed by direct assay of the conversion of \(^{14}\text{C}\)FGAR to \(^{14}\text{C}\)AIR (Table III) where 0.6 mM AMP produced significant inhibition of the conversion of FGAM to AIR while not affecting FGAR amidotransferase activity. AMP, 3.0 mM, produced almost complete inhibition of AIR synthetase based on the radioisotope data. The Bratton-Marshall reaction, however, indicated that some activity was still present. At this higher concentration of AMP there was also a suggestion of inhibition of FGAR amidotransferase.

![Fig. 9](image_url)

![Fig. 10](image_url)
AMP Inhibition of Amidophosphoribosyltransferase—In order to establish that AMP was in fact an effective inhibitor of the first enzyme, inhibition studies were performed in both Tris-HCl and P, buffers at low (0.3 mM) and high (1.5 mM) PP-ribose-P concentrations. At high PP-ribose-P concentrations in Tris-HCl a maximum inhibition of approximately 20% was achieved at 2.0 mM AMP confirming the AMP inhibition data for glycine incorporation in the purine biosynthetic studies (Fig. 9). At the low PP-ribose-P concentration the same level of AMP produced 65% inhibition again confirming the AMP biosynthetic data. In 50 mM P, buffer the activity of amidophosphoribosyltransferase was reduced to approximately 50% of that in 50 mM Tris-HCl. Sensitivity to AMP inhibition was increased in that 1.0 mM AMP produced the same inhibition as 2.0 mM AMP in Tris-HCl buffer.

The decreased activity and increased sensitivity to AMP inhibition of the first enzyme in P, buffer was reflected in studies of purine synthesis at both high and low PP-ribose-P concentrations. The pattern of inhibition was generally identical with that seen in Tris-HCl buffer (Figs. 9 and 10) except that equivalent inhibition of glycine into purines and into intermediates was achieved at approximately one-half the AMP concentration.

**DISCUSSION**

Several years ago the existence of a possible multienzyme complex for purine biosynthesis in pigeon liver was reported (24). The concept of such a complex, a group of enzymes linked by noncovalent bonds, catalyzing sequential reactions of a metabolite pathway, was particularly attractive from the point of view of the efficient vesticular catalysis of a series of unstable pathway intermediates. On the premise that such protein-protein interactions may well be disrupted by dilutional and high ionic strength effects during routine protein purification methods, we developed a procedure designed to obviate some of these problems. The nonionic polymer, polyethylene glycol, was finally selected for protein fractionation and stabilization. PEG 4000 (average molecular weight 4000) was employed for fractionation procedures as higher polymers were too viscous for this purpose at low temperatures. PEG 20 M was used for stabilization as its average molecular weight of 20,000 was toward the lower limit of the range of molecular weights for enzyme peptide chains (25). The concentration of 2% (w/v) or 1.0 mM was based on the calculated cytosol concentration of monomeric protein subunits of yeast cell glycolytic enzymes (26). PEG 20 M clearly promotes protein-protein interactions or polymerization as was evident from the protein elution profile on the glass chromatography column and from the behavior of the protein fraction on polyacrylamide gel electrophoresis. This property was not unique to PEG 20 M as in other studies, not reported here, polyethylene oxide (Polyox WSR N10, Union Carbide), average molecular weight 100,000, achieved the same effect at an equivalent molar concentration. Nonionic polymers were not required if water was replaced by deuterium oxide in the buffer solutions. While the stabilizing effect of deuterium oxide has been reported to be the result of polymer aggregation secondary to hydrophobic bond enhancement (27) other studies have suggested that the effect is related to hydrogen bond reinforcement (28). There is no evidence available to suggest that the PEG effect was due to either of these mechanisms. One outstanding property of these polymers is the exponential rise in the kinematic viscosity of their aqueous solutions below 5°C.

If a purine biosynthetic multienzyme complex does exist, clearly the protein-protein interactions are not strong as there was some differential partitioning of individual enzyme activities during purification and on glass-column chromatography. The distribution of the different activities on chromatography could, in part, be accounted for by what is known of the physical properties of some of the enzymes. Amidophosphoribosyltransferase from pigeon liver has a molecular weight of the order of 200,000 (29), while FGAR amidotransferase (chicken liver) has a molecular weight of 135,000 but can polymerize under certain conditions (30). GAR synthetase (Aerobacter aerogenes) has reported molecular weights in the region 34,000 to 48,000 (31), but there is a possibility that it exists as a higher complex with AIR synthetase in certain cell types (21). With the exception of this last enzyme all of these activities were identified as co-chromatographing with the peak of *de novo* purine synthetic activity. The combined activities of AIR carboxylase and SAICAR synthetase chromatographed at a somewhat higher molecular weight range, but their activities were so high relative to the other enzymes measured that their tailing peak would have provided sufficient activity for efficient synthesis at these two steps. SAICAR lyase (EC 4.3.2.2 adenylsuccinatylase from Neurospora crassa) has a reported molecular weight of 200,000 (32) while no figures are available for AICAR formylase (EC 2.2.2.3) and IMP cyclodrolase (EC 3.5.4.10) catalyzing the last two steps of the *de novo* pathway. These two activities are considered to reside on a single multifunctional protein in avian liver (33). The identical chromatographic behavior of formyltetrahydrofolate synthetase and methylenetetrahydrofolate cyclodrolase was consistent with their reported association in a multienzyme complex, molecular weight 218,000, from ovine liver (19). It would appear then that the co-purification of the purine enzymes could be the result of their possessing certain similar physical properties which were selected out by our current purification procedure. The bifunctionality of certain proteins, however, would suggest that, in *vivo*, there is perhaps some close physical inter-relationship between them. This was emphasized by the assays performed on the 4 to 11% PEG supernatant and the final 15% PEG supernatant solutions which contained significant quantities of the first two pathway enzymes (Table II) but which could not incorporate glycine into GAR in the presence of PP-ribose-P and glutamine. Furthermore, the activity of at least two enzymes, GAR synthetase and FGAR amidotransferase, was significantly lower with preformed substrates than their activity in the overall *de novo* synthetic sequence.

In these studies we have confirmed the overall pathway substrate and cofactor requirements. While amidophosphoribosyltransferase was largely responsible for the synthesis of phosphoribosylamin, PP-ribose-P amidotransferase activity was also present and could account for significant nitrogen fixation in the presence of high ammonia levels. At lower PP-ribose-P concentrations (0.3 mM) the activity of the pathway was limited by that of the first enzyme while AIR synthetase appeared to be rate limiting at higher concentrations. Concentrations of PP-ribose-P of the order of 10 to 100 mM have been reported for a number of mammalian cell types (34), but a concentration of 0.7 mM has been reported in avian liver cytosol (35). There are significant problems in estimating PP-ribose-P concentrations in different tissues (36), and the levels reported may be unrelated to the true concentration in the relevant enzyme microenvironment.

The observation that AIR synthetase activity was rate limiting at higher PP-ribose-P levels was not likely to be an artifact of the purification procedure as it was seen in all protein fractions. The rate limitation was distal to the second major point of nitrogen fixation and this may be important to uricotelic species.

The approximate $K_m$ for glycine of 8 $\mu$M was considerably
lower than that reported for bacterial GAR synthetase (37). This may reflect rapid utilization of GAR by the next pathway enzyme. Similarly HCO\textsubscript{3} is saturating at 10 mM although studies with partially purified avian AIR carboxylase indicated that the equilibrium of the carboxylation reaction was such that 50% conversion of AIR to CAIR could be achieved only at 0.3 mM HCO\textsubscript{3} (38). Later studies (39) reported a K\textsubscript{m} for HCO\textsubscript{3} of 2.1 mM for the more purified pigeon liver enzyme.

The identity of the three compounds accumulating as "polyphosphate" intermediates in the HCO\textsubscript{3} and aspartate deletion studies is not clear. FGAR polyphosphate would appear to be one of these intermediates but the insensitivity of the other two to the action of alkaline phosphatase cast some doubt on the polyphosphate component of the other two. While it may be possible that these compounds were polymers derived from AIR, equivalent to those which accumulate in mutants of Saccharomyces cerevisiae which lack AIR carboxylase or SAICAR synthetase (40), we have not as yet been able to achieve positive identification.

Formate and tetrahydrofolic acid in the presence of formyltetrahydrofolate synthetase and cyclohydrolase provided an effective source of one-carbon units for purine synthesis. In the light of the reported pH activity profile of cyclohydrolase and the pH-mediated interconversion of 10-formyltetrahydrofolate and 5,10-methylenetetrahydrofolate (14), the possibility of the former derivative acting as the one-carbon donor for both formylation reactions must be seriously considered.

The regulation by adenine nucleotides was complex. At low (0.3 mM) concentrations of PP-ribose-P, the inhibition of purine ring synthesis by AMP was a direct result of the inhibition of the first enzyme. Neither ADP or ATP were inhibitors, and it was unlikely that the fall in the adenylate energy charge but that the drop in charge resulted in a curtailment of energy-utilizing processes such as protein and purine synthesis (46). The regulation of PP-ribose-P synthetase has been proposed as being effected through the adenylate energy charge (47). In de novo purine synthesis, therefore, the lack of ATP would perhaps be reflected in decreased PP-ribose-P levels and a subsequent drop in purine synthetic rates. Whether or not this applies to the liver cell in a uricotelic species, in which purine synthesis serves a critical role in nitrogen waste product fixation and excretion, is not known. It is evident, however, that even if PP-ribose-P was preferentially diverted to the purine pathway in uricotelic cells under conditions of energy deprivation, that nitrogen fixation would take place at the levels of the 1st and 4th reactions, but the pathway would shut down effectively at the next step catalyzed by AIR synthetase.

In terms of regulation, therefore, it would appear that a number of controls could well operate at different levels. The relative significance of each of these controls is unclear but must be related overall to the supply of ATP and PP-ribose-P. The regulation of metabolic pathways in a cell with specialized functions must be considered in the light of the relative importance of each of these pathways not only in terms of the energy balance and survival of that particular cell type but of the animal as a whole.

REFERENCES
De Novo Purine Synthesis in Avian Liver

De novo purine synthesis in avian liver. Co-purification of the enzymes and properties of the pathway.

P B Rowe, E McCairns, G Madsen, D Sauer and H Elliott


Access the most updated version of this article at http://www.jbc.org/content/253/21/7711

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/253/21/7711.full.html#ref-list-1