Analogues of Ribose 5-Phosphate and 5-Phosphoribosyl Pyrophosphate

THE PREPARATION AND PROPERTIES OF RIBOSE 5-PHOSPHOROTHIOATE AND 5-PHOSPHORIBOSYL 1-METHYLENEDIPHOSPHONATE

By A. W. MURRAY, P. C. L. WONG AND BEVERLY FRIEDRICH

*School of Biological Sciences, Flinders University of South Australia,
Bedford Park, S. Austral. 5042, Australia*

(Received 2 December 1968)

1. 5-Phosphoribosyl 1-methylenediphosphonate was isolated after reaction of ribose 5-phosphate and *O*-adenylyl methylenediphosphonate with 5-phosphoribosyl pyrophosphate synthetase from Ehrlich ascites-tumour cells. 2. The analogue reacted with adenine phosphoribosyltransferase, hypoxanthine phosphoribosyltransferase and nicotinamide phosphoribosyltransferase [K_m (analogue)/ K_m (5-phosphoribosyl pyrophosphate) 0.17, 0.19 and 6.3 respectively; V_{max} (analogue)/ V_{max} (5-phosphoribosyl pyrophosphate) 0.011, 0.26 and 1.1 respectively]. 3. The analogue was not a substrate for 5-phosphoribosyl pyrophosphate amidotransferase or orotate phosphoribosyltransferase. 4. Ribose 5-phosphorothioate was synthesized by allowing ribose to react with thiophosphoryl chloride in triethyl phosphate. The analogue was a substrate for 5-phosphoribosyl pyrophosphate synthetase from Ehrlich ascites-tumour cells. When this reaction was coupled to either adenine phosphoribosyltransferase or hypoxanthine phosphoribosyltransferase, adenosine 5'-phosphorothioate or inosine 5'-phosphorothioate was formed respectively.

It has been reported that AMPS* can be synthesized in high yield by allowing adenosine to react with thiophosphoryl chloride in triethyl phosphate (Murray & Atkinson, 1968); it was also shown that AMPS can mimic the effect of AMP as a regulator of yeast NAD-isocitrate dehydrogenase (EC 1.1.1.41), rat liver fructose 1,6-diphosphatase (EC 3.1.3.11) and muscle phosphorylase *b*. As an extension to this work the present paper describes the synthesis of ribose 5-phosphorothioate by a similar reaction of thiophosphoryl chloride with ribose; the new analogue was shown to react with PRPP synthetase (EC 2.7.6.1) from Ehrlich ascites-tumour cells to form 5-thiophosphoribosyl pyrophosphate.

Wong & Murray (1969) showed that AMP-PCP will react with PRPP synthetase in the presence of Mg^{2+} , phosphate and ribose 5-phosphate; the present paper reports the isolation of the product of the reaction, PR-PCP, and its reaction with some enzymes that utilize PRPP as a substrate.

* Abbreviations: AMPS, adenosine 5'-phosphorothioate; IMPS, inosine 5'-phosphorothioate; PRPP, 5-phosphoribosyl pyrophosphate; AMP-PCP, *O*-adenylyl methylenediphosphonate; PR-PCP, 5-phosphoribosyl 1-methylenediphosphonate.

EXPERIMENTAL

Substrates

PRPP was obtained from the Sigma Chemical Co. (St Louis, Mo., U.S.A.) and converted into the sodium salt by passage through a small column (20 mm. $\times$ 20 mm.² for 20 mg. of PRPP) of Chelex-100 (Na^+ form; Bio-Rad Laboratories, Richmond, Calif., U.S.A.). This material was 80% pure (based on ribose content; Dische, 1957) when assayed with orotate phosphoribosyltransferase (EC 2.4.2.10) as described by Kornberg, Lieberman & Simms (1955).

[8-¹⁴C]Adenine, [8-¹⁴C]hypoxanthine, [7-¹⁴C]nicotinamide and [8-¹⁴C]AMP were obtained from The Radiochemical Centre, Amersham, Bucks.

AMPS and IMPS were prepared as their sodium salts as described by Murray & Atkinson (1968).

AMP-PCP was obtained from Miles Laboratories, Elkhart, Ind., U.S.A.

Preparation and assay of enzymes

Adenine phosphoribosyltransferase (EC 2.4.2.7). The source of this enzyme was a crude extract from Ehrlich ascites-tumour cells prepared as described by Murray (1966). Assay mixtures contained 1 μ mole of $MgCl_2$, 20 μ moles of tris-Cl⁻ buffer, pH 7.8, 0.025 or 0.05 ml. of enzyme extract for assays with PRPP or PR-PCP respectively (0.31 or

Bioch. 1969, 112

0.62 mg. of protein), 3.2 nmol of $[8\text{-}^{14}\text{C}]\text{adenine}$ (0.12 μC) and the required amount of PRPP or PR-PCP, in a final volume of 0.4 ml. After incubation at 30° (for 2 min. or 8 min. for PRPP or PR-PCP respectively) reactions were stopped by addition of 0.01 ml. of 10M-HCl. Samples (0.05 ml.) were spotted on to Whatman 3MM paper together with internal markers of unlabelled adenine and AMP and developed in butan-1-ol-acetic acid-water (10:3:7, by vol.) for 4 hr. After the papers had been dried, radioactivity associated with the nucleotide area was measured by liquid-scintillation counting as described by Murray (1966).

Hypoxanthine phosphoribosyltransferase (EC 2.4.2.8). This enzyme was partially purified from Ehrlich ascites-tumour cells as described by Atkinson & Murray (1965). Assay conditions were as described above for adenine phosphoribosyltransferase but with 34.5 nmol of $[8\text{-}^{14}\text{C}]\text{hypoxanthine}$ (0.3 μC) instead of adenine. Assays with both PRPP and PR-PCP were carried out for 2 min. at 30° with 0.02 ml. of enzyme (0.46 mg. of protein). Reaction products were separated by chromatography as described above.

Nicotinamide phosphoribosyltransferase (EC 2.4.2.12). This enzyme was partially purified from rat liver as described by Dietrich, Fuller, Yero & Martinez (1966). Assay mixtures contained 2 μmol of MgCl_2 , 10 μmol of tris- Cl^- buffer, pH 7.3, 0.4 μmol of ATP, 0.02 μmol of $[7\text{-}^{14}\text{C}]\text{nicotinamide}$ (0.22 μC) and 0.02 ml. of enzyme (0.82 mg. of protein), in a final volume of 0.2 ml. After 30 min. at 37° reactions were stopped with 0.01 ml. of 5M-HCl and 0.05 ml. samples were spotted on to Whatman 3MM paper with nicotinamide and nicotinamide mononucleotide as internal markers. After chromatography for 4 hr. with 1M-ammonium acetate (adjusted to pH 5 with HCl)-95% ethanol (3:7, v/v), radioactivity associated with nicotinamide mononucleotide was measured as described above.

PRPP synthetase. The enzyme from Ehrlich ascites-tumour cells was partially purified and assayed as described by Murray & Wong (1967).

PRPP amidotransferase (EC 2.4.2.14). Crude extracts containing this enzyme were prepared from Ehrlich ascites-tumour cells as described by Tay, Lilley, Murray & Atkinson (1969). Assays were carried out by measuring the PRPP-dependent formation of $[^{14}\text{C}]\text{glutamate}$ from $[^{14}\text{C}]\text{glutamine}$ after electrophoretic separation (see Tay *et al.* 1969).

Orotate phosphoribosyltransferase. A preparation containing orotate phosphoribosyltransferase and orotidylate decarboxylase was prepared from baker's yeast. Dried yeast was extracted for 4 hr. at 37° with 6 vol. of 0.1M-tris- Cl^- buffer, pH 8.0; the extract was centrifuged at 20000g for 15 min. and the fraction precipitated at 70% saturation with $(\text{NH}_4)_2\text{SO}_4$ was dissolved in 0.1M-tris- Cl^- buffer, pH 8.0; the solution was dialysed against the same buffer and stored at -15° . Assay mixtures contained 100 μmol of tris- Cl^- buffer, pH 8.0, 2 μmol of MgCl_2 , 0.15 μmol of sodium orotate, enzyme (45 mg. of protein) and 0.15 μmol of PRPP, in a final volume of 3 ml. The reaction was followed by measuring the rate of decrease of E_{295} (see Kornberg *et al.* 1955).

RESULTS

Preparation of PR-PCP. The reaction mixture contained 150 μmol of magnesium chloride, 600 μmol of sodium phosphate, pH 8.0, 500 μmol of *N*-ethylmorpholine, pH 8.0, 30 μmol of GSH,

32 μmol of ribose 5-phosphate, 32 μmol of AMP-PCP and 5 ml. of PRPP synthetase (25 mg. of protein) in a final volume of 8 ml. After 1 hr. at 37° , the reaction mixture was chilled in an ice bath and mixed with 2 g. of Norit (SX2). The charcoal was removed by filtration at 2° through a Celite pad and washed with about 200 ml. of ice-cold water. Electrophoresis of the nucleotides at pH 4.5 after removal from the charcoal with aq. 50% ethanol-1% (w/v) ammonia and evaporation to dryness indicated the presence of 20.5 μmol of AMP and 12.3 μmol of unchanged AMP-PCP. The combined filtrate and washings containing PR-PCP were passed through a column (10 cm. $\times$ 1 cm. diam.) of Dowex 1 at 2° and the column was eluted with a linear gradient of 200 ml. of 1.5M-ammonium formate, pH 5, into 200 ml. of water. Elution was carried out at about 3 ml./min.; 10 ml. fractions were collected and 0.1 ml. portions assayed for pentose (Dische, 1957). Two pentose-containing peaks were obtained. The first peak (0.19-0.25M-ammonium formate) contained unchanged ribose 5-phosphate. Fractions of the second peak (0.49-0.57M-ammonium formate), containing PR-PCP, were pooled, and, after addition of 250 μmol of magnesium chloride and 3 vol. of ethanol, the preparation was kept at -15° overnight. The precipitate was collected by centrifuging at -10° , washed twice with cold ethanol and dried in air. The residue was dissolved in 0.5 ml. of water and passed through a small column (1 cm. $\times$ 0.5 cm. diam.) of Chelex-100 (Na^+ form) at 2° , and the column was washed with 1.5 ml. of water. The combined filtrate and washings were immediately neutralized with dilute hydrochloric acid and stored at -15° . Ribose determinations showed that 13 μmol of PR-PCP were recovered (41%

Fig. 1. Inhibition of adenine phosphoribosyltransferase by PR-PCP. Assays were carried out in the absence of PR-PCP (○) and in the presence of 3.23 μM (●) and 8.1 μM PR-PCP (△). v , nmol of AMP formed/min./mg. of protein.

Fig. 2. Reaction of PRPP (○) and PR-PCP (●) with hypoxanthine phosphoribosyltransferase. v , nmoles of IMP formed/min./mg. of protein.

yield based on conversion of AMP-PCP or ribose 5-phosphate). The preparation contained ribose and total phosphate in the ratio 1:3.09 and contained no detectable inorganic phosphate or acid-labile phosphate (10 min. at 100° in 0.5 M-sulphuric acid).

Reaction of PR-PCP with adenine phosphoribosyltransferase. PR-PCP was shown to react slowly with adenine phosphoribosyltransferase; assays were carried out as described in the Experimental section. The Michaelis constant, K_m , was 2.2×10^{-6} M [K_m (PRPP) is 13.3×10^{-6} M] and the maximum velocity, $V_{\max}$, was 0.096 nmole of AMP formed/min./mg. of protein [$V_{\max}$ (PRPP) is 8.9 nmoles of AMP formed/min./mg. of protein]. The high affinity of PR-PCP for adenine phosphoribosyltransferase suggested that it would be a powerful inhibitor of this enzyme; as shown in Fig. 1, PR-PCP was a competitive inhibitor with respect to PRPP. The value of the inhibitor constant, K_i , found (1.6×10^{-6} M) was similar to the K_m for PR-PCP (2.2×10^{-6} M).

Reaction of PR-PCP with hypoxanthine phosphoribosyltransferase. The reaction of PRPP and PR-PCP with hypoxanthine phosphoribosyltransferase is shown in Fig. 2. K_m (PRPP) and K_m (PR-PCP) were 23×10^{-6} and 4.3×10^{-6} M respectively; $V_{\max}$ values were 3.9 and 1.0 nmoles of IMP formed/min./mg. of protein respectively.

Reaction of PR-PCP with nicotinamide phosphoribosyltransferase. As shown in Fig. 3 PR-PCP was also a substrate for rat liver nicotinamide phosphoribosyltransferase. K_m (PRPP) and K_m (PR-PCP) were 19.8×10^{-6} and 125×10^{-6} M respectively; $V_{\max}$ values were 0.048 and 0.052 nmole of nicotinamide mononucleotide formed/min./mg. of protein

as substrate. Purified PRPP synthetase from *Salmonella typhimurium* has been reported to catalyse an exchange reaction between [^{14}C]AMP and ATP in the absence of ribose compounds, and an exchange between PRPP and [^{14}C]ribose 5-phosphate in the absence of AMP and ATP (cited by Switzer, 1968). Experiments to study the reversibility of Ehrlich-ascites-tumour-cell PRPP synthetase were complicated as the enzyme preparations were contaminated with an adenosine triphosphatase activity (see Murray & Wong, 1967) and the PRPP preparations commercially available contain ribose 5-phosphate. The reverse reaction of tumour-cell PRPP synthetase was demonstrated by measuring the incorporation of radioactivity into AMP-PCP after incubation of PR-PCP and [^{14}C]AMP with the enzyme; the PR-PCP preparation contained no ribose 5-phosphate and the ATP analogue formed was resistant to hydrolysis by adenosine triphosphatase.

Assay mixtures contained 5 μmoles of *N*-ethylmorpholine, pH 8.0, 0.5 μmole of GSH (neutralized with *N*-ethylmorpholine), 5 μmoles of sodium phosphate, pH 8.0, 2 μmoles of magnesium chloride, 0.24 μmole of [$8\text{-}^{14}\text{C}$]AMP (1.2 $\mu\text{C}/\mu\text{mole}$) and 0.25 μmole of PR-PCP. The reactions were started with 0.05 ml. of extract containing PRPP synthetase (Murray & Wong, 1967; 0.15 mg. of protein); the final volume of the assay mixture was 0.205 ml. After incubation for 30 min. at 37° the reactions were stopped with 0.01 ml. of 10M-hydrochloric acid and 0.05 ml. portions were subjected to chromatography in 66% (v/v) isobutyric acid adjusted to pH 4.2 with ammonia; radioactivity associated with AMP and AMP-PCP was measured by liquid-scintillation counting. In complete reaction mixtures containing PR-PCP, magnesium chloride, inorganic phosphate and [$8\text{-}^{14}\text{C}$]AMP, 4.4% of the AMP (53.5 nmoles) was converted into AMP-PCP, clearly demonstrating the reverse reaction of PRPP synthetase. There was significant incorporation of radioactivity into AMP-PCP in the absence of added phosphate or magnesium chloride, or both of these compounds, but the rate was only about 10% of that obtained with complete reaction mixtures.

Preparation of ribose 5-phosphorothioate. A 2-m-mole portion of ribose was dissolved in 5 ml. of triethyl phosphate; the solution was cooled rapidly to 0° and 4 m-moles of thiophosphoryl chloride were added. After 24 hr. at 2°, 20 ml. of aq. 10% barium acetate was added and the mixture was kept at 20° for 15 min. The solution was adjusted to pH 8 with triethylamine and the precipitate obtained on adding 60 ml. of 95% ethanol was washed with 70% ethanol (3 $\times$ 40 ml.) and extracted with water (3 $\times$ 50 ml.). The extract was passed through a column (20 cm. $\times$ 2.5 cm.²) of DEAE-cellulose

(HCO_3^- form); elution was carried out with a linear gradient of 250 ml. of 0.4M-ammonium hydrogen carbonate into 250 ml. of water. Fractions (12 ml.) were collected and 0.1 ml. portions assayed for pentose (Dische, 1957). The first major peak containing pentose (eluted between 0.09M- and 0.14M-ammonium hydrogen carbonate) was pooled, evaporated at 45°/25 mm., and the residue was again dried by evaporation with 50 ml. of ethanol, then twice with 1 ml. of triethylamine in 60 ml. of 80% ethanol and once with 50% ethanol. The residue was dissolved in 10 ml. of water and stored at -15°. Total material recovered (based on pentose content) was 174 μmoles (8.7% yield). With reaction times of 3.5 hr. and 48 hr. the yields were 0.5 and 5.1% respectively. Considerable pentose-containing material was eluted from the DEAE-cellulose column after the first major peak, but no attempt was made to characterize this material; the compounds present in these additional fractions did not react with PRPP synthetase (see below). Material from the first major peak contained ribose and total phosphate in the ratio 1:1 and contained no detectable inorganic phosphate or acid-labile phosphate. It was identified as ribose 5-phosphorothioate on the basis that it reacted to form AMPS and IMPS (see below), these compounds being fully characterized (Murray & Atkinson, 1968). On electrophoresis in 0.05M-citrate (in tris buffer, pH 4.5) material that reacted with PRPP synthetase (see below) had 40% greater anionic mobility than had ribose 5-phosphate (the ribose derivative was detected on the paper as described by Chernick, Chaikoff & Abraham, 1951). Two other components, with 115% and 95% of the anionic mobility of ribose 5-phosphate, were detected after electrophoresis; these compounds did not react with PRPP synthetase.

Reaction of ribose 5-phosphorothioate with PRPP synthetase. Ribose 5-phosphorothioate was a substrate for PRPP synthetase from Ehrlich ascites-tumour cells, assayed as described by Murray & Wong (1967) with 1mM-ATP and 12.5mM-magnesium chloride. Under these conditions the apparent K_m (ribose 5-phosphorothioate) was about $13 \times 10^{-3}\text{M}$ [K_m (ribose 5-phosphate) was $0.16 \times 10^{-3}\text{M}$ with the same batch of enzyme] and V_{max} (ribose 5-phosphorothioate) was 5.5 nmoles/min./mg. of protein [V_{max} (ribose 5-phosphate) was 71.5 nmoles/min./mg. of protein]. The value of K_m is an overestimate because of the apparent contamination of the ribose 5-phosphorothioate preparations with other ribose-containing derivatives.

Enzymic formation of AMPS and IMPS from ribose 5-phosphorothioate. Experiments were carried out to detect the formation of AMPS and IMPS in coupled assays with PRPP synthetase and either adenine phosphoribosyltransferase or hypoxanthine

phosphoribosyltransferase. For the synthesis of AMPS, assay mixtures contained 5 μ moles of magnesium chloride, 1 μ mole of GSH, 10 μ moles of *N*-ethylmorpholine, pH 8.0, 0.8 μ mole of ATP, 20 μ moles of sodium phosphate buffer, pH 8.0, 2 μ moles of ribose 5-phosphorothioate, 0.05 ml. of PRPP synthetase (see Murray & Wong, 1967; 0.15 mg. of protein), 0.02 ml. of adenine phosphoribosyltransferase (see the Experimental section) and 3.2 nmol of [8-¹⁴C]adenine (1.2 μ C) in a final volume of 0.44 ml. After incubation for 30 min. at 30°, reactions were stopped by addition of 0.01 ml. of 10 M-hydrochloric acid and 0.05 ml. portions were subjected to electrophoresis in 0.05 M-citrate (in tris buffer, pH 4.5). In this system AMPS has greater anionic mobility than has AMP (Murray & Atkinson, 1968), but in the present experiments a clear separation was not obtained. However, radioactivity associated with ADP and ATP was measured directly from the electrophoresis papers. Areas containing AMPS + AMP were eluted with water, concentrated and chromatographed in 66% (v/v) isobutyric acid adjusted to pH 4.2 with ammonia; radioactivity associated with AMP (R_F 0.51) and AMPS (R_F 0.31) (see Murray & Atkinson, 1968) was measured by liquid-scintillation counting. Under these conditions 0.65 nmole of AMPS and 1.05 nmol of AMP + ADP + ATP were formed. Similar assays were carried out but with mixtures containing 10 μ moles of sodium phosphate, 0.2 μ mole of ATP, 0.02 ml. of hypoxanthine phosphoribosyltransferase (see the Experimental section), 34.5 nmol of [8-¹⁴C]-hypoxanthine (0.3 μ C) and either 1 or 4 μ moles of ribose 5-phosphorothioate. In the isobutyric acid-ammonia system described above, IMP had R_F 0.20 and IMPS R_F 0.05 (see Murray & Atkinson, 1968). Assay mixtures containing 1 μ mole of ribose 5-phosphorothioate formed 5.4 nmol of IMPS and 1.9 nmol of IMP, and assay mixtures containing 4 μ moles of the phosphorothioate formed 9.5 nmol of IMPS and 3.7 nmol of IMP. Elution of areas containing either AMPS (see above) or IMPS and incubation of the extract with snake-venom 5'-nucleotidase (EC 3.1.3.5) resulted in the formation of adenosine and inosine respectively (detected by electrophoresis at pH 4.5). The formation of adenine nucleotides and IMP that was observed in these experiments could result either from breakdown of 5-thiophosphoribosyl pyrophosphate (formed from ribose 5-phosphorothioate) to PRPP, or from desulphurization of AMPS and IMPS. The former possibility is most likely, as in similar assays with ribose 5-phosphorothioate and adenine phosphoribosyltransferase, but with AMP-PCP replacing ATP, no AMPS was detectable but AMP, ADP and ATP were formed (about 0.48 nmole/30 min.).

DISCUSSION

Hypoxanthine phosphoribosyltransferase has been shown to have an ordered reaction mechanism with PRPP as the leading substrate (Henderson, Brox, Kelley, Rosenbloom & Seegmiller, 1968), and a similar mechanism has been suggested for adenine phosphoribosyltransferase (Gadd & Henderson, 1968). The present results show that both enzymes have a higher affinity for PR-PCP than for PRPP. The reaction between base and PRPP presumably involves nucleophilic displacement at the 1-position of ribose, and the lower V_{max} obtained with PR-PCP may partially result from the decrease in electronegativity on replacement of oxygen with a methylene group. Demonstration of the formation of AMP-PCP from AMP and PR-PCP, catalysed by PRPP synthetase, further indicates the usefulness of stable phosphonate analogues in studies with crude enzyme preparations (see also Wong & Murray, 1969).

The main aim of these studies was to determine the type of structural alterations that can be made to the substrates of enzymes that catalyse reactions leading to nucleotide formation. The results have shown that PRPP analogues containing modifications in either the 5'- or 1'-position can react with adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase; derivatives of ribose 5-phosphate substituted in the 5'-position are of particular interest, as these lead to the synthesis of nucleotide analogues. If ribose derivatives could be induced to enter cells either as such, or suitably substituted with lipophilic groups, they could then react with PRPP synthetase to form the corresponding analogue of PRPP; there is already some evidence for the entry of ribose 5-phosphate itself intact into Ehrlich ascites-tumour cells (Henderson & Khoo, 1965). It may then be possible to generate the desired nucleotide derivatives inside the cells by treatment with a purine base to react with the corresponding phosphoribosyltransferase and the PRPP analogue. For example, ribose 5-phosphorothioate would generate AMPS or IMPS if the cells were treated with adenine or hypoxanthine respectively. It has been shown that AMPS is relatively metabolically inert in that it is only a poor substrate for adenylate kinase, 5'-nucleotidase and adenylate deaminase, but that it can effectively mimic the regulatory effect of AMP on a number of allosteric enzymes (Murray & Atkinson, 1968). IMPS is a substrate for IMP dehydrogenase from *Aerobacter aerogenes*, with the resultant formation of xanthosine 5'-phosphorothioate (R. F. Elleway, M. R. Atkinson & A. W. Murray, unpublished work; Bayer, Brox, Gupta & Hampton, 1968). Although the possibility exists for selective chemotherapy, as only those cells with

high activities of the phosphoribosyltransferases (see Murray, 1966) would accumulate high concentrations of the nucleotide analogues, it seems likely that the ribose and PRPP analogues would themselves be toxic to cells in the long term. However, such a technique would be useful in studies on regulation by purine nucleotides in intact cells. The 5'-phosphorothioate derivatives may not be the most suitable analogues because of the possibility of desulphurization; the phosphonate derivative of ribose 5-phosphate (5'-deoxyribose 5'-methylphosphoric acid) has a stable $\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{PO}_3^{2-}$ grouping and would be of interest.

The authors are grateful to Mr B. S. Tay for carrying out the assays of 5-phosphoribosyl pyrophosphate amidotransferase and to Professor M. R. Atkinson for valuable criticism and discussion. This work was supported by grants from the Australian Research Grants Committee and the University of Adelaide Anti-Cancer Foundation.

REFERENCES

- Atkinson, M. R. & Murray, A. W. (1965). *Biochem. J.* **94**, 64.
 Bayer, M., Brox, L. W., Gupta, V. S. & Hampton, A. (1968). *Abstr. Pap. Amer. chem. Soc., 155th Meet., San Francisco*, no. N19.
 Chernick, S. S., Chaikoff, I. L. & Abraham, S. (1951). *J. biol. Chem.* **193**, 793.
 Dietrich, L. S., Fuller, L., Yero, I. L. & Martinez, L. (1966). *J. biol. Chem.* **241**, 188.
 Dische, Z. (1957). In *Methods in Enzymology*, vol. 3, p. 88. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
 Gadd, R. E. A. & Henderson, J. F. (1968). *Fed. Proc.* **27**, 639.
 Henderson, J. F., Brox, L. W., Kelley, W. N., Rosenbloom, F. M. & Seegmiller, J. E. (1968). *J. biol. Chem.* **243**, 2514.
 Henderson, J. F. & Khoo, M. K. Y. (1965). *J. biol. Chem.* **240**, 2349.
 Kornberg, A., Lieberman, I. & Simms, E. S. (1955). *J. biol. Chem.* **215**, 389.
 Murray, A. W. (1966). *Biochem. J.* **100**, 664.
 Murray, A. W. & Atkinson, M. R. (1968). *Biochemistry*, **7**, 4023.
 Murray, A. W. & Wong, P. C. L. (1967). *Biochem. biophys. Res. Commun.* **29**, 582.
 Switzer, R. L. (1968). *Biochem. biophys. Res. Commun.* **32**, 320.
 Tay, B. S., Lilley, R. McC., Murray, A. W. & Atkinson, M. R. (1969). *Biochem. Pharmacol.* (in the Press).
 Wong, P. C. L. & Murray, A. W. (1969). *Biochemistry* (in the Press).