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INHIBITORS OF OROTATE PHOSPHORIBOSYLTRANSFERASE AND OROTIDINE-5′-PHOSPHATE DECARBOXYLASE FROM MOUSE EHRLICH ASCITES CELLS: A PROCEDURE FOR ANALYZING THE INHIBITION OF A MULTI-ENZYME COMPLEX*

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Abstract—Orotate phosphoribosyltransferase and orotidine-5′-phosphate decarboxylase, the last two enzymes in the pathway for *de novo* pyrimidine biosynthesis, exist as a multi-enzyme complex in mammalian cells. Because the two enzymes are not separable, assays for the phosphoribosyltransferase are normally dependent on the decarboxylase, and this has in the past produced misinterpretations in evaluating the effects of inhibitors of either enzyme activity. We have developed a procedure for calculating each enzyme activity independently of the other activity in the complex. Instead of 2 separate assays, one reaction vessel is used, (starting with orotate and phosphoribosyl pyrophosphate). The two products (i.e. orotidylate (OMP), the product of the transferase, and UMP, the product of the decarboxylase) are recovered separately, and the amounts recovered were used to calculate both enzyme activities. Purine or pyrimidine bases did not inhibit the decarboxylase activity, but the transferase activity is inhibited by uracil and uracil analogs, barbiturate, and most effectively by 5-F orotate. Both purine and pyrimidine nucleotides inhibit the decarboxylase activity, although di- and tri-phosphonucleosides were much less effective than monophosphonucleosides. XMP, UMP, and AMP were the most effective natural inhibitors of the decarboxylase, but the most potent inhibitors were 6-AzaUMP and 1-ribosylallopurinol-5′-phosphate. High concentrations of nucleotides inhibit both enzyme activities.

The two enzyme activities of Complex U, orotate phosphoribosyltransferase (EC 2.4.2.10) and orotidine-5′-phosphate decarboxylase (EC 4.1.1.23), catalyze the last two steps, reaction (1) and (2) respectively, of the *de novo* pyrimidine biosynthesis pathway:

$$\text{orotate} + \text{P-Rib-PP}\dagger \xleftrightarrow{\text{OPRTase, } Mg^{2+}} \text{OMP} + PP_i \quad (1)$$

$$\text{OMP} \xrightarrow{\text{ODCase}} \text{UMP} + CO_2 \quad (2)$$

The inhibition of the enzyme activities of Complex U is of interest because purine or pyrimidine base analogs used in the chemotherapy of cancer are converted by the transferase of this complex to a nucleotide that inhibits the decarboxylase of Complex U [1–3], and similar compounds used in the treatment of gout are converted to nucleotides that are potent inhibitors of the decarboxylase [4, 5].

Although these two activities are found in separable enzymes in yeast [6], there is now considerable support for the hypothesis advanced by Jones [7] that the transferase and the decarboxylase exist as a complex in mammalian cells [1, 8–14]. The fact that the two enzyme activities are inseparable can complicate the kinetic investigations of these two enzymes, and especially those concerned with orotate phosphoribosyltransferase. This is because OMP does not normally accumulate, due to the efficiency of the decarboxylase in converting OMP to UMP plus CO_2, and the fact that the decarboxylation is essentially irreversible (see reaction (2) above). However, if the decarboxylase is fully inhibited, the transferase reaction may appear to be inhibited since it is a reversible reaction whose equilibrium lies toward the substrates orotate and P-Rib-PP‡ [16].

In this paper we report a procedure for measuring both enzyme activities simultaneously, which we used to examine the specific site of action for inhibitors of these two enzyme activities that were found among purine and pyrimidine bases and their nucleotides, as well as some purine and pyrimidine analogs.

MATERIALS AND METHODS

Materials. Tetrasodium P-Rib-PP, dithiothreitol, and Tris were obtained from Sigma Chemical Co., St. Louis, MO. Carboxyl-labeled [7-^{14}C]orotate and [7-^{14}C]OMP were purchased from New England Nuclear, Boston, MA, while [6-^{14}C]orotate was supplied by Calatomic, Los Angeles, CA. PEI-Cellulose plates were obtained from Brinkman Instruments, Los Angeles, CA. Most of the bases, nucleosides, and nucleotides were purchased from Sigma Chemical Co.

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† Abbreviations used are: OPRTase, orotate phosphoribosyltransferase; ODCase, orotidine-5′-phosphate decarboxylase; P-Rib-PP, 5-phosphoribosyl-1-pyrophosphate; OMP, orotidine-5′-phosphate; allopurinol-5′-P, 1-ribosylallopurinol-5′-phosphate; PEI-cellulose, polyethyleneimine cellulose.

‡ Manuscript in preparation.

or from Calbiochem, La Jolla, CA. 5-oxo proline and 2-imidazolo-4-carboxylate were the gift of Dr. Alton Meister, Cornell University. Allopurinol, oxipurinol, and allopurinol-5'-phosphate were the gifts of Dr. Gertrude B. Elion, Burroughs-Wellcome Co., Research Triangle Park, NC.

Enzyme preparation

The two enzymes of Complex U were prepared from mouse Ehrlich ascites cells. Previous papers from this laboratory have reported the method of propagating these cells [11, 15], and the procedure for purifying these two enzymes [14]. In the present work, the protein fraction precipitating between 40 per cent and 65 per cent of saturation with ammonium sulfate was used as the source of enzymes; this corresponds to Fraction 4 of [14]. Protein content was determined by the method of Ross and Schatz [17] as described in [14].

Enzyme assays

Because the native enzyme complex catalyzes the conversion of OMP to UMP + CO_2 more rapidly than the synthesis of OMP from orotate and P-Rib-PP, assays of the orotate phosphoribosyltransferase activity normally follow a radioactive label from orotate to one of the two final products: UMP or CO_2. The enzyme activities can be measured by either of the two following assays:

Assay 1 ($^{14}CO_2$ assay). The decarboxylase activity was determined by measuring the release of $^{14}CO_2$ from carboxy-labeled OMP [15]. Assays were done at 37° and, following a 5 min preincubation with all components of the reaction mixture except substrate, the incubation time was 3 min. The standard reaction mixture contained in a final volume of 1 ml: Tris–HCl, 20 mM, pH 7.4, at 37°; dithiothreitol, 2 mM; and 30–40 μg enzyme protein (specific activity = 6–8 nmoles/hr/mg). The reaction mixture was started with [7-^{14}C]OMP (0.125 Ci/mole), 0.1 μM; reactions were stopped with $HClO_4$ and processed for scintillation counting as described [15].

Assay 2 (TLC assay). In this assay both enzyme activities were measured simultaneously by using thin layer chromatography to separate orotate, OMP and UMP on PEI-cellulose plates as described by Reyes and Guganig [13]. The standard reaction mixture contained in a final volume of 50 μl: Tris–HCl, 20 mM; dithiothreitol, 2 mM; $MgCl_2$, 5 mM; P-Rib-PP, 300 μM; and 2–5 μg enzyme protein (specific activity = 20–27 nmoles/hr/mg for OPRTase, 35–45 nmoles/hr/mg for ODCase). The reactions, which were run for 20 min, were started with [6-^{14}C]orotate (42.2 Ci/mole), 5 μM. The reaction was stopped by directly spotting 5 μl of the reaction mixture onto a 20 cm × 20 cm PEI-cellulose plate and drying the sample spot with hot air; total time for spotting and drying was 15–20 s. Up to 15 samples can easily be spotted at 1.3 cm intervals along a line near one end of the plate. Plates were developed by ascending chromatography for about 2 hr in 0.2 M LiCl, pH 5.5. To facilitate the detection of developed spots, every sample spot received 5 μl of a carrier solution containing orotate, OMP, and UMP, each at 5 mM. The developed spots were detected under a u.v. lamp, cut out of the plastic-backed PEI-cellulose plate, and counted directly in a toluene scintillation cocktail as described in [15].

Calculations of enzyme activities

Orotate phosphoribosyltransferase activity. This is the sum of OMP + UMP produced from orotate, and is expressed as a percentage of the uninhibited control enzyme activity.

Orotidine-5'-phosphate decarboxylase activity. This activity was always measured at "normal" OMP concentrations, i.e., the OMP formed from orotate via the transferase. Orotate was not depleted significantly during the assay (i.e., the OPRTase rate remains linear until 50 per cent of the initial orotate is converted to OMP*), but OMP levels could change considerably as a consequence of inhibiton of either one of the two enzyme activities, especially inhibiton of the decarboxylase. It was therefore necessary to calculate the average OMP concentration for the duration of the assay. In the absence of an inhibitor, the OMP concentration is established almost immediately, and maintained unchanged for the duration of the enzyme assay (see Fig. 2). In the presence of an inhibitor of decarboxylase activity, OMP formed by the transferase will accumulate and the potency of the inhibitor can only be assessed by comparing the inhibited decarboxylase activity to the expected activity of the normal enzyme at the elevated OMP concentration produced by an inhibitor of the decarboxylase. Thus, whenever $[OMP]_{sample}$ was less than or equal to $[OMP]_{control}$, that concentration of OMP was used directly to calculate the expected decarboxylase activity. When $[OMP]_{sample} > [OMP]_{control}$ an average $[OMP]_{sample}$ was calculated as 1/2 ($[OMP]_{sample}$

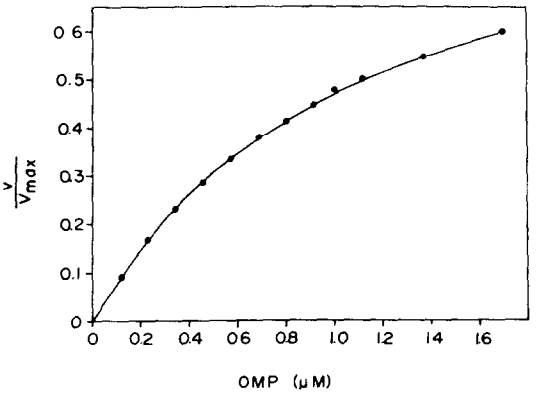

Fig. 1. Enzyme velocity curve for orotidine-5'-phosphate decarboxylase. The velocity of the decarboxylase activity was measured with Assay 1, and K_m = 1.1 μM, V_{max} = 5.8 nmoles/hr, for the partially purified enzyme preparation used here, at 0.15 mg protein/ml. This curve was used to calculate the expected decarboxylase activity under conditions where inhibitors of decarboxylase activity caused increases in the OMP concentration during the enzyme assay.

* We attribute this, in part, to the presence of an inorganic pyrophosphatase activity in our partially purified enzyme fraction. By hydrolyzing one of the products of the OPRTase reaction, PP_i, it helps to pull the reaction in the direction of OMP synthesis.

Table 1. Comparison of 2 methods for assaying the inhibition of orotidine-5′-phosphate decarboxylase activity

Inhibitor		Decarboxylase activity (% of control)	
	(mM)	Assay 1	Assay 2
Ribose-5-phosphate	10.0	4	1
	1.0	44	51
UDP	10.0	8	3
	1.0	44	42
	0.1	76	88
UTP	10.0	10	0
	1.0	67	71
	0.1	73	75

Assay 1 ($^{14}CO_2$ Assay) and Assay 2 (TLC Assay) have been described in "Methods". Assay 2 was used for the inhibition studies in Tables 2–4.

$- [OMP]_{control}) + [OMP]_{control}$. This average $[OMP]_{sample}$ was used to determine the *expected* decarboxylase activity from a graph of v/V_{max} vs [OMP] (Fig. 1), and the degree of inhibition was then calculated by comparing the *observed* activity (the actual amount of UMP formed) to the *expected* activity:

$$\% \text{ Activity} = \frac{\dfrac{[UMP]_{sample}}{[UMP]_{control}}}{\left(\dfrac{v}{V_{max}}\right)_{expected} \Big/ \left(\dfrac{v}{V_{max}}\right)_{control}} \times 100$$

To check the validity of this method for calculating activity, we measured the inhibition of the decarboxylase under similar conditions by means of the two assays described above. As shown in Table 1, our procedure for determining the decarboxylase activity (or its inhibition) is equal to the direct assay of activity as measured with Assay 1.*

RESULTS

Complex U is very efficient at converting orotate → OMP → UMP. As shown in Fig. 2, under normal conditions OMP does not accumulate; it quickly reaches a steady-state concentration which does not exceed 0.07 μM (when initial orotate is at 1–50 μM). This low OMP concentration becomes an important factor in interpreting the results of inhibition studies with the transferase activity of Complex U when Assay 1 is used.

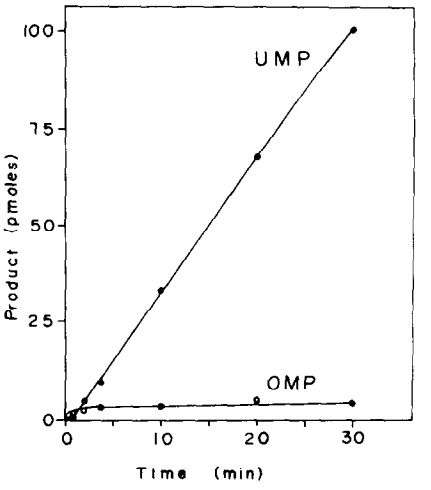

Fig. 2. Synthesis of OMP and UMP by complex U enzymes. Under standard assay conditions (Assay 2 in Methods), with initial orotate at 5 μM, OMP synthesis remains constant, and the concentration of OMP does not change throughout the duration of the assay, while UMP increases in a linear fashion. The OMP concentration is about 0.05 μM.

By using the TLC assay it was possible to measure the effect of an inhibitor on both enzyme activities simultaneously. Further, because OMP is measured, the assay of the transferase is not dependent on the decarboxylase activity with this procedure, and it was possible to obtain unambiguous results about the exact site of action for a variety of inhibitors.

Table 2 shows the inhibition produced by analogs of the pyrimidine base, orotate. Those compounds that show inhibition affect the transferase activity of Complex U. Of the natural analogs tested, only uracil and adenine were mild inhibitors; all uracil derivatives had a lower, but fairly similar, degree of inhibition. The two most effective inhibitors of the transferase were 5-F orotate and barbiturate.

Nucleotides and their analogs were the most effective inhibitors of the decarboxylase, as shown in Table 3 (at concentrations where they produced approximately 50 per cent inhibition). However, one nucleotide, GMP, inhibited both enzymes equally. We were able to repeat this observation with GMP from two different manufacturers, and at several concentrations. Every nucleoside monophosphate tested showed some inhibition of orotidine-5′-phosphate decarboxylase. The nucleosides diphosphate were approximately 10-fold less potent as inhibitors, and triphosphates had very little effect. Of the natural nucleotides, the most effective inhibitors were XMP, and the product of the decarboxylase, UMP. It is an interesting distinction between the two enzymes of Complex U, that while the first enzyme is fairly specific in its affinity for pyrimidine inhibitors, the second enzyme is readily inhibited by both purine and pyrimidine nucleotides. Also, the transferase has

* Two simple versions of the above formula may be used where speed of calculation is more desirable:

First approximation:

$$\% \text{ Activity} = \frac{[UMP]_{sample}/[OMP]_{sample}}{[UMP]_{control}/[OMP]_{control}} \times 100$$

Second approximation:

$$\% \text{ Activity} = \frac{[UMP]_{sample}/[\text{average OMP}]_{sample}}{[UMP]_{control}/[OMP]_{control}} \times 100$$

The first approximation simplifies calculations considerably, but this method is least exact, and will overestimate the degree of ODCase inhibition when OMP concentrations become more than 3 times greater than control levels (i.e., when OMP $\geq 0.2\,K_m$). The second approximation is quite good for most cases, but will overestimate the extent of ODCase inhibition when OMP concentrations approach K_m.

Table 2. Inhibitors of orotate phosphoribosyltransferase activity

Inhibitor	(mM)	Enzyme activity (% of control)	
		Transferase	Decarboxylase
Natural pyrimidines			
Uracil	1.0	71	100
Cytosine	10.0	64	100
Dihydro orotate	10.0	44	78
Uracil analogs			
5-Fluoro uracil	5.0	59	84
5-Chloro uracil	1.0	59	102
5-Bromo uracil	5.0	76	88
6-AzaUracil	1.0	87	81
Other pyrimidine analogs			
Barbituric acid	0.5	50	84
5-Fluoro orotate	0.05	26	85
Natural purines			
Adenine	1.0	42	80
Guanine	0.1	79	94
Xanthine	0.1	98	100
Uric Acid	0.1	103	109
Purine analogs			
Allopurinol	0.5	89	50*
Oxipurinol	0.5	86	64*
5 Carbon ring analogs			
5-Oxo proline	1.0	83	94
2-Imidazolo-4 Carboxylate	1.0	94	93
Nucleosides			
Adenosine	10.0	62	76
Orotidine	10.0	52	94
Uridine	10.0	62	96
Nucleoside analogs			
6-AzaUridine	10.0	58	98
6-AzaUridine Triacetate	10.0	60	97

* These values are attributed to the effect of the nucleotides which are formed from the bases during the enzyme assay.

Table 3. Inhibitors of orotidine-5'-phosphate decarboxylase activity

Inhibitor	(mM)	Enzyme activity (% of control)	
		Transferase	Decarboxylase
Nucleotides			
UMP	0.01	106	30
CMP	0.1	98	48
dUMP	1.0	84	44
TMP	10.0	111	25
XMP	0.001	110	60
AMP	0.05	92	43
IMP	0.1	85	34
GMP	1.0	66	67
UDP	1.0	95	42
ADP	1.0	94	47
UTP	1.0	89	71
ATP	1.0	103	93
TTP	1.0	87	64
Nucleotide analogs			
6-AzaUMP	0.0001	104	11
Allopurinol-5'-P	0.001	105	64
5-BromoUMP	0.1	97	41
Ribose-5-P	1.0	113	51

a greater affinity for orotate, the normal substrate, than for any other compound. The decarboxylase has a greater affinity for AzaUMP than for the substrate, OMP, and it binds to XMP and allopurinol-5′-phosphate about as effectively as to OMP.

The pattern of inhibition that appears in Tables 2 and 3 in general confirms earlier reports that some pyrimidine bases and their analogs are inhibitors of the transferase [1–3, 13, 18, 19], while some nucleotides and their analogs are inhibitors of the decarboxylase [8, 9, 13, 19, 20]. Exceptions to this pattern have been reported by Pausch *et al.* [21] who found that UMP had no inhibitory effect, and by Krooth *et al.* [22] who reported that CMP augmented the decarboxylase activity. In the former study [21], we feel that the ratio of inhibitor, UMP (1 mM), to substrate, OMP (0.34 mM), was too low for inhibition of decarboxylase activity to be detected. In the latter study [22] it was shown that CMP partially stabilizes this enzyme against loss of activity on dilution; it may be that the stabilizing effect of CMP under the conditions used in that study was greater than, and therefore able to mask, the inhibitory effect of CMP on the decarboxylase activity.

A recent paper by Tax *et al.* [23] reports the results of some inhibition studies with the enzymes of Complex U (from erythrocytes) that are completely opposite to our data: 8 natural purine and pyrimidine nucleotides were found to have no effect on the decarboxylase activity, while being inhibitors of the transferase activity. Their results were obtained with the $^{14}CO_2$ assay and illustrate how the TLC assay can be superior for multi-enzyme systems. Tax *et al.* first measured the effect of the various nucleotides (each at 5 mM) on decarboxylase activity, but since they used a high substrate concentration ($^{14}CO_2$-OMP at 0.3 mM), no inhibition of this enzyme could be detected. They then repeated the assay to measure inhibition of transferase activity by starting the assay with $^{14}CO_2$-orotate at 0.3 mM. However, when the assay starts with orotate, the OMP levels formed by the first enzyme are low (see Fig. 2), and now the ratio of inhibitor to OMP concentration is initially increased by at least 100-fold, and the inhibitors tested will markedly decrease the decarboxylase activity as shown by our data, Table 3. Unfortunately, since the $^{14}CO_2$ assay for transferase activity is dependent on the decarboxylase activity, then under the conditions used by Tax *et al.* (and by many investigators [4, 8, 10–12, 14–16, 21]), the inhibition of decarboxylase activity would be mistakenly interpreted as inhibiton of the transferase.

When we did similar inhibiton studies as reported in Table 3, the use of the TLC assay made interpretation of the results unambiguous, for labeled OMP was found to accumulate in the presence of the inhibitors, indicating inhibition of the decarboxylase, while the sum of the labeled OMP plus the very small amount of labeled UMP was equal to that of the control, indicating normal activity of the transferase!

Two compounds, orthophosphate and pyrophosphate, produced equal inhibition of each enzyme activity. Orthophosphate at 10 mM reduces the transferase activity to 53 per cent of the uninhibited control, and the decarboxylase to 30 per cent. Pyrophosphate at 0.5 mM reduces the transferase activity to 23 per cent and the decarboxylase to 47 per cent. In addition, certain nucleotides do have weak inhibitory effects on the first enzyme activity (Table 4), but this does not become apparent until very high ratios of inhibitor to substrate are used. Nonetheless, this secondary inhibition effect is worth noting, since it is often desirable to completely inhibit the decarboxylase in order to study the transferase, and the use of an inhibitor of the decarboxylase at too high a concentration could then have adverse effects on the orotate phosphoribosyltransferase [13, 24].

Table 4. Inhibitors of orotidine-5′-phosphate decarboxylase, that, at higher concentrations, can also inhibit orotate phosphoribosyltransferase

		Enzyme activity (% of control)	
Inhibitor	(mM)	Transferase	Decarboxylase
UMP	1.0	94	8
	10.0	34	2
AMP	1.0	74	28
	10.0	0	0
IMP	10.0	34	20
dUMP	5.0	63	31
	10.0	53	24
UDP	5.0	42	18
	10.0	10	3
ADP	10.0	0	0
UTP	10.0	0	0
ATP	10.0	1	0
TTP	10.0	11	5
6-AzaUMP	0.1	108	0
	1.0	84	0
	10.0	3	0

DISCUSSION

The inhibition studies illustrate the advantage of the TLC assay, an assay which measures the product of each enzyme activity in a multi-enzyme reaction. We also wish to emphasize the importance of the ratio of inhibitor to substrate in designing inhibition experiments for enzyme complexes. Although we have no exact values for orotate concentrations *in vivo*, we attempted to approximate physiological levels by setting our orotate concentation at 5 μM, which is quite low compared to other studies on the inhibition of Complex U; but this concentration may still be much greater than the true *in vivo* orotate concentration. One of the results of the inhibition studies was that a few natural nucleotides are effective inhibitors at approximate physiological concentrations. We have averaged values for nucleotide pools determined in liver [25], erythrocytes [26], and cultured fibroblasts [27] as being representative of the range of values that might include ascites cells. Therefore, we feel that both AMP (average cellular concentration of 0.06 mM) and UMP (average cellular concentration of 0.08 mM) are likely to play a role in regulating pyrimidine biosynthesis, since they are effective

inhibitors of the decarboxylase at these concentrations. Since most of the adenine and uridine nucleotides are present as the di- and tri-phosphates (ADP, 0.4 mM; ATP, 1.8 mM; UDP, 0.03 mM; UTP, 0.2 mM), cells are still able to maintain normal overall nucleotide pools because the di-phosphates and tri-phosphates of adenine and uridine are very poor inhibitors of Complex U.

The observed inhibition of orotidine-5'-phosphate decarboxylase by AMP may also help to explain the inhibition of cell growth as a consequence of the addition of adenosine to cell cultures [28-30]. Such added adenosine is readily phosphorylated and raises the levels of adenine nucleotides, while inhibiting the synthesis of pyrimidine nucleotides. Ishii and Green [30] have shown that orotidine and/or orotic acid accumulate in cells grown in the presence of adenosine. The orotidine would probably be derived from OMP, and it is not really clear which compound accumulates since both orotidine and orotate co-migrate on the thin layer chromatography system used by these authors. Thus, while Ishii and Green favor the explanation that orotate accumulates and that this represents the inhibition of orotate phosphoribosyltransferase, their data would also support the conclusion that seems likely from the results shown here, namely that OMP initially accumulates due to an inhibiton of orotidine-5'-phosphate decarboxylase by the AMP, which was made in excess in the cultured cells grown with adenosine. Obviously the purine nucleotides XMP and AMP are rather potent inhibitors of the decarboxylase, and are as likely to inhibit UMP formation from OMP (and therefore from orotate) as is UMP.

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REFERENCES

1. D. K. Kasbekar, A. Nagabhushanan and D. M. Greenberg, *J. biol. Chem.* **239**, 4245 (1964).
2. D. Kessel, J. Deacon, B. Coffey and A. Bakamjian, *Biochem. Pharmac.* **8**, 731 (1972).
3. P. Reyes, *Biochemistry* **8**, 2057 (1969).
4. W. N. Kelley, T. D. Beardmore, I. H. Fox and J. C. Meade, *Biochem. Pharmac.* **7**, 1471 (1971).
5. M. A. Becker, K. F. Argubright, R. M. Fox and J. E. Seegmiller, *Molec. Pharmac.* **10**, 657 (1974).
6. K. Umezu, T. Amaya, A. Yashimoto and K. Tomito, *J. Biochem.* **70**, 249 (1972).
7. M. E. Jones, in *Advances in Enzyme Regulation* (Ed. G. Weber) Vol. 9, p. 19. Pergamon Press, New York (1971).
8. D. Hatfield and J. B. Wyngaarden, *J. biol. Chem.* **239**, 2580 (1964).
9. S. H. Appel, *J. biol. Chem.* **243**, 3924 (1968).
10. G. K. Brown, R. M. Fox and W. J. O'Sullivan, *Biochem. Pharmac.* **21**, 2469 (1972).
11. W. T. Shoaf and M. E. Jones, *Biochemistry* **12**, 4039 (1973).
12. W. Grobner and W. N. Kelley, *Biochem. Pharmac.* **24**, 379 (1975).
13. P. Reyes and M. E. Guganig, *J. biol. Chem.* **250**, 5097 (1975).
14. P. R. Kavipurapu and M. E. Jones, *J. biol. Chem.* **251**, 5589 (1976).
15. K. Prabhakararao and M. E. Jones, *Anal. Biochem.* **69**, 451 (1975).
16. I. Lieberman, A. Kornberg and E. S. Simms, *J. biol. Chem* **215**, 403 (1955).
17. E. Ross and G. Schatz, *Analyt. Biochem.* **54**, 304 (1973).
18. J. E. Stone and V. R. Potter, *Cancer Res.* **17**, 800 (1957).
19. W. A. Creasey and R. E. Handschumacher, *J. biol. Chem.* **236**, 2058 (1961).
20. J. A. Fyfe, R. L. Miller and T. A. Krenitsky, *J. biol. Chem.* **248**, 3801 (1973).
21. J. Pausch, D. Keppler and K. Decker, *Biochim. biophys. Acta* **258**, 395 (1972).
22. R. S. Krooth, Y.-L. Pan and L. Pinsky, *Biochem. Genet.* **8**, 133 (1973).
23. W. J. M. Tax, J. N. Veerkamp, F. J. M. Trijbels and E. D. A. M. Schretlen, *Biochem. Pharmac.* **25**, 2025 (1976).
24. J. Veselý, A. Čihák and F. Šorm, *Biochem. Pharmac.* **17**, 519 (1968).
25. N. L. R. Bucher and M. N. Swaffield, *Biochim. biophys. Acta* **129**, 445 (1966).
26. E. M. Scholar, P. R. Brown, R. E. Parks, Jr. and P. Calabresi, *Blood* **41**, 927 (1973).
27. C. Colby and B. Edlin, *Biochemistry* **9**, 917 (1970).
28. R. S. Krooth, *Cold Spring Harbor Symp. Quant. Biol.* **29**, 189 (1964).
29. H. Green and T.-S. Chan, *Science, N.Y.* **182**, 836 (1973).
30. K. Ishii and H. Green, *J. Cell Sci.* **13**, 429 (1973).