Biochemical Pharmacology, Vol. 31, No. 20, pp. 3317–3321, 1982.
Printed in Great Britain.

0006-2952/82/203317-05 $03.00/0

ASPARTATE CARBAMOYLTRANSFERASE ACTIVITY, DRUG CONCENTRATIONS, AND PYRIMIDINE NUCLEOTIDES IN TISSUE FROM PATIENTS TREATED WITH *N*-(PHOSPHONACETYL)-L-ASPARTATE*

E. COLLEEN MOORE†, JACQUELINE FRIEDMAN‡, MANUEL VALDIVIESO, WILLIAM PLUNKETT, JOSE R. MARTI‡, JOSEPH RUSS‡ and TI LI LOO

Department of Tumor Biochemistry (E.C.M.) and Developmental Therapeutics, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030, U.S.A.

(*Received* 17 *July* 1981; *accepted* 22 *March* 1982)

Abstract—Biopsy specimens were obtained from patients treated with *N*-(phosphonacetyl)-L-aspartate (PALA) in a phase I clinical trial. Activities of aspartate carbamoyltransferase (ACTase), the target enzyme, in ten specimens before treatment varied from 0.4 to 1.7 units/mg. PALA was measured in protein-free extracts of thirteen specimens by inhibition of rat ACTase. At 1.5 to 145 hr after doses of 1 to 6 g/m^2, PALA concentrations were 0.9 to 89 μg/g; at 4 hr or later the tissue concentrations were similar to those in plasma (five samples). The observed inhibition of ACTase (17–87%) correlated with the PALA concentrations. Pyrimidine nucleotides were decreased (relative to purine nucleotides) in nine of ten specimens, by 16–72%. ACTase partially purified from human spleen had a K_m for carbamoyl phosphate of 20.6 μM and the K_i for PALA was 0.011 μM. The results suggest that inhibition of ACTase by PALA affects the concentration of pyrimidine nucleotides in human tumors in a dose-dependent manner.

N-(Phosphonacetyl)-L-aspartic acid (PALA; NSC 224131)§ is an effective inhibitor of aspartate carbamoyltransferase (ACTase; EC 2.1.3.2), which catalyzes the second step in the biosynthesis *de novo* of the pyrimidine nucleotides [1, 2]. PALA was designed as a transition state analog, with structural resemblance to both substrates as well as the product, but it was found to be competitive only with carbamoyl phosphate [1]. Because it was active against an unusual spectrum of rodent solid tumors [1, 2], it is being tested clinically at several institutions.

Since in mice the antitumor effects of PALA are inversely correlated to the activity of ACTase in the tumors [3, 4], we were interested in confirming this finding in human tumors. We also wished to ascertain whether an inhibitory amount of PALA reached the tissues, how long the concentration was maintained, and whether the concentration of the pyrimidine nucleotides decreased. This was a part of our pharmacological studies of PALA during its Phase I trial [5, 6]. A preliminary report has appeared [7].

METHODS

Patients. Patients were selected from those entered on the Phase I trial of PALA [6, 8], who had at least three accessible lesions and gave informed consent. Doses for these patients were 5 or 6 g/m^2 (single dose), 3.6 g/m^2 daily for 1 or 2 days, or 1.0 g/m^2 daily for 5, 6, or 8 days. The drug was given as a 1-hr i.v. infusion. One lesion was removed by biopsy before treatment, and a second was removed at a selected time after the end of the infusion of PALA. The third was used for evaluation of clinical response. When treatment was given daily for 2–8 days, the second biopsy was done after the last treatment of the course, except for one case in which it was done after the first dose.

A specimen of spleen was obtained from a patient with chronic myelocytic leukemia who underwent splenectomy as a part of his treatment.

Materials. Alamine was obtained from the McKerson Corp. (Minneapolis, MN) and Freon TF from DuPont (Wilmington, DE). [U-^{14}C]Aspartic acid from the New England Nuclear Corp. (Boston, MA) was purified as described by Porter *et al.* [9] and diluted to 0.25 to 0.38 mCi/mmole. [Acetyl-^{14}C]PALA was obtained from The National Cancer Institute (Bethesda, MD) and purified by chromatography on Dowex-l-bicarbonate [5]. Carbamoyl phosphate was purchased from the Sigma Chemical

* Supported by Contract CM-87185 and Grant CA-05831 from the National Cancer Institute, NIH.

† Address reprint requests and correspondence to: Dr. E. C. Moore, Department of Tumor Biochemistry, M.D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave., Houston, TX 77030, U.S.A.

‡ Present address: J. Friedman, Biochemistry Laboratory, The University of Texas Graduate School of Biomedical Science at Houston, Houston, TX 77030, U.S.A.; J. Marti, Department of Surgery, State University of New York Downstate Medical Center, 450 Clarkson Ave., Brooklyn, NY 11203, U.S.A.; and J. Russ, 125 Hamilton St., Geneva, IL 60134, U.S.A..

§ Abbreviations: PALA, *N*-(phosphonacetyl)-L-aspartate, NSC 224131; and ACTase, aspartate carbamoyltransferase, EC 2.1.3.2.

Co. (St. Louis, MO); other chemicals were reagent grade. PCS, a phase-combining scintillation fluid, was obtained from the Amersham Corp. (Arlington Heights, IL). ACTase for use in the enzymatic measurement of PALA was partially purified from livers of normal or Novikoff tumor-bearing rats. The procedure described previously [10] was used except that for most batches diethylaminoethyl-cellulose chromatography was substituted for the gel filtration step. Measured under the conditions described [10], the specific activity was 2–3 nmoles per min per mg.

Analytical methods. Protein was determined with the Bio-Rad (Richmond, CA) protein assay kit. Radioactivity was measured in a Packard 2650 liquid scintillation spectrometer with automatic quenching correction. PCS from Amersham was used as the scintillation fluid.

Determination of ACTase activity. As soon as obtained, the specimen was immersed in ice-cold 0.9% NaCl. Specimens larger than about 0.5 g were divided into two or three portions. One portion was homogenized in buffer for the ACTase assay, one was used for nucleotide analysis, and the third was homogenized in cold 0.4 M $HClO_4$ for the measurement of PALA. The homogenizing buffer contained 0.05 M potassium phosphate buffer (pH 7), 10 mM $Mg(C_2H_3O_2)_2$, 1 mM EDTA, 1 mM dithiothreitol, and 3 mM L-glutamine. For each gram of tissue, 5 ml of buffer was used. The human tissue was minced with a scalpel blade and homogenized with a motor-driven Teflon pestle-glass tube homogenizer, or with a hand-operated all-glass Dounce homogenizer. In experiments with rat and mouse tissues, other homogenization techniques were also compared, but gave lower activity or higher blanks. The homogenates were centrifuged at 5000 *g* for 20 min; the supernatant fraction (S1) was stored at −80° until assayed.

The incubation mixture for assay of ACTase contained, in a total volume of 175 μl, 30 μl of tissue extract (S1) equivalent to 5 mg tissue, 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.4), 4.0 mM aspartate, and 1.0 mM carbamoyl phosphate. After a 30-min incubation, 1.5 ml of 0.2 M $HC_2H_3O_2$ was added and the product was separated by passing through Dowex-50 columns as previously described [10]. All assays were run in duplicate. A unit of activity is defined as 1 nmole product per min. Control incubations contained no carbamoyl phosphate; any activity observed was subtracted.

Rat liver and some other tissues, including some of the human specimens, showed enzyme activity which was detected in the ACTase assay (i.e. as ^{14}C from aspartate, which was not bound by a cation exchange resin) but was not dependent on carbamoyl phosphate and was insensitive to PALA. This spurious activity, which may be due to a transaminase plus its co-substrate, was not seen in extracts purified by ammonium sulfate precipitation, so it was not a problem in the PALA assay or the studies with the human spleen ACTase. However, it was necessary to subtract a blank without carbamoyl phosphate when ACTase was determined by this procedure in crude extracts of tissue. Dialysis of the extracts incurred the loss of ACTase activity from Lewis lung tumor and human spleen and, consequently, was impractical.

With the most active tumor extract, the activity was linear with time for at least 30 min. Only 14% of the carbamoyl phosphate and 3% of the aspartate were converted to product. Since the carbamoyl phosphate concentration was originally forty-eight times the K_m, it probably remained saturating.

Determination of PALA in tissue. When possible, a second portion of tissue was homogenized in 5 ml of cold 0.4 M $HClO_4$/g of tissue. After centrifugation, the extract was neutralized [11] by extraction with an equal volume of 0.4 M Alamine (a mixture of fatty amines) in Freon, and washed with 0.04 M Alamine in Freon, and then with Freon. If there was insufficient tissue for a separate $HClO_4$ extract, a portion of the buffer extract (S1) was precipitated by the addition of 0.1 vol. of 4.4 M $HClO_4$ and neutralized with Alamine in the same way. Control extracts or buffer samples that were precipitated with $HClO_4$ and neutralized with KOH were inhibitory, as was a control extract that was precipitated with trichloroacetic acid and extracted with ether.

PALA was assayed by the ACTase-inhibition method described previously [10] for the determination of PALA in plasma, using partially purified ACTase from rat liver. The substrate concentrations used earlier [10] were used for all PALA determinations. No more than 10 μl of neutralized $HClO_4$ extract was analyzed and an equal volume of extract from the pre-treatment tissue specimen was included in the controls and standards. The addition of larger amounts of a PALA free deproteinized extract, or buffer treated in the same way, inhibited ACTase and affected the PALA standard curve. When 10 μl or less was added, the inhibition was less than 18% and the PALA standard curve was only slightly less steep (slope 1.0 compared to 1.4). The results were calculated on the basis that 5 ml of neutralized extract corresponded to 1 g tumor; no correction was made for recovery. PALA in plasma was determined as described [10]; in some cases the value reported was interpolated from a plasma disappearance curve [5].

Kinetics of human ACTase. A 15-g sample of human spleen was homogenized and carried through the ACTase purification procedure [12] through the second ammonium sulfate precipation step, yielding enzyme with ACTase specific activity of 2.6 units/mg under our standard conditions. Substrate (carbamoyl phosphate) and PALA inhibition curves were obtained with this preparation using a 30-min incubation period, 4.0 mM aspartate, and the standard assay procedure. Kinetic constants were calculated by Cleland's computer program [13].

Determination of nucleotides. Solid tumor samples were weighed, diced with scissors, and homogenized in cold 0.4 M $HClO_4$. After centrifugation, the supernatant fluid was neutralized with KOH. The single myeloma sample was fractionated over a Ficoll Hypaque gradient, and the cell number was determined before $HClO_4$ extraction. The $KClO_4$ was removed by centrifugation, the volume of the supernatant fraction was determined, and this nucleotide-containing extract was frozen until analysis by high pressure liquid chromatography. Nucleotides were separated by standard procedures [14]

and were detected by their u.v. absorbance at either 254 or 280 nm. The change in pyrimidine ribonucleotides was measured as the ratio between pyrimidine and purine ribonucleoside triphosphates and is expressed as net change in this ratio following PALA treatment, assuming the same proportional losses of purines and pyrimidines during extraction or by dephosphorylation. Nucleotides recovered per gram of tissue sample varied with the specimens.

RESULTS

Extraction of PALA. To test reproducibility of the extraction, triplicate extracts from livers and Novikoff ascites tumor cells of a control rat and a rat treated with PALA (250 mg) 4 hr previously were analyzed for PALA by inhibition of ACTase. Only one of the extracts differed more than 9% from its replicates. Only one of twelve PALA-free control extracts inhibited more than 18% at the 10 μl level.

Recovery was determined with [^{14}C]PALA (250 mg, 9.3 μCi) administered intraperitoneally to a rat bearing both subcutaneously and intraperitoneally transplanted Novikoff ascites tumors. The liver, solid tumor, and ascites fluid were harvested and extracted 90 min after injection. Samples of the tissue and of the extract were combusted and counted as carbonate. The recovery was approximately 85% in the neutralized extracts from direct homogenization in $HClO_4$, and 75% in those prepared from buffer extracts. Similar results were obtained for liver and tumor extracts from a mouse 4 hr after PALA administration. The PALA concentration in rat ascites cells at 1.5 hr was only 8% (washed cells) to 44% (unwashed packed cells) of that in the ascites fluid, indicating incomplete equilibration at this time.

Kinetics of human ACTase. The ACTase preparation from human spleen had an activity of 2.6 units/mg under the high-substrate conditions. With the carbamoyl phosphate reduced to 100 μM, the activity was linear for at least 30 min, indicating that depletion or instability of substrate or instability of enzyme was not a problem with the partially purified enzyme. Substrate concentration curves for carbamoyl phosphate were determined at several concentrations of PALA, with 4 mM aspartate (Fig. 1). From these results, an apparent K_m of 24.1 ± 5.2 μM for carbamoyl phosphate and a K_i of 0.011 μM for PALA were calculated. From another experiment, with a lower range of carbamoyl phosphate concentration (5–200 μM), the K_m was 20.6 ± 1.3 μM. A similar experiment with the partially purified rat ACTase gave a K_m for carbamoyl phosphate of 46.8 μM and a K_i of 0.009 μM.

Biopsy specimens. Specimens from biopsies before and after PALA treatment were obtained from sixteen patients. ACTase activity was measured in specimens from ten of the patients. We determined PALA concentrations in eleven post-treatment specimens, and in six concurrent plasma specimens. Nucleotide concentrations were determined in ten pairs of specimens. The results are shown in Table 1.

The PALA concentrations in the tissues were relatively low at 1.5 and 2.75 hr, but by 4 to 4.5 hr it was 87–90% of the concurrent plasma level. The concentration decreased thereafter, remaining within 10% of the plasma concentration at 20 hr. Measurable PALA remained in the tissue even 6 days after a single dose of 3.6 g/m^2.

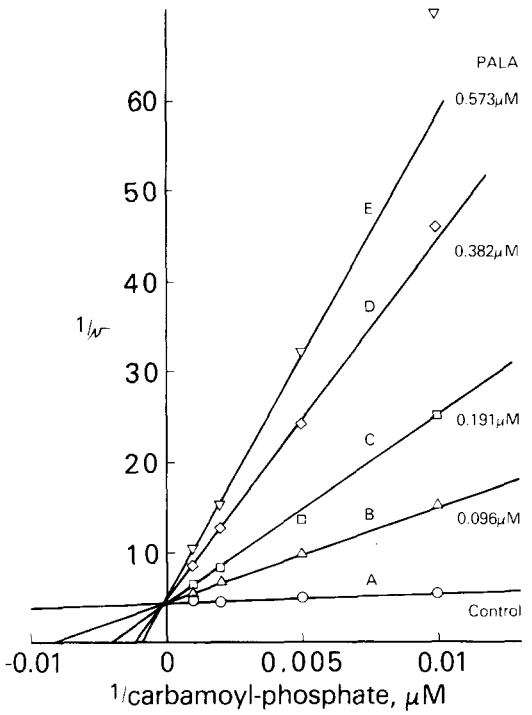

Fig. 1. Inhibition by PALA of partially purified ACTase from human spleen, and competition with carbamoyl phosphate. The aspartate was 4 mM in all cases, and the incubations lasted for 30 min. The lines were fitted by a computer program written by Cleland based on the equation for a hyperbola [13]. Abscissa: reciprocal of concentration of carbamoyl phosphate, μM. Ordinate: reciprocal of velocity, in nmoles/min. Curve A: control; B: 0.096 μM PALA; C: 0.191 μM PALA; D: 0.382 μM PALA; and E: 0.573 μM PALA.

The initial ACTase activity ranged from 0.435 to 1.746 units/mg for six melanomas and 0.531 to 1.21 units/mg for four other tumors, compared to 1.75 units/mg for Lewis lung tumor. Most of the individual values for these human tumors were published earlier [5], but they are included here for comparison with the other data.

The ACTase activity was lower in the specimens obtained after PALA treatment, as was expected. (Since the extracts were not dialyzed, the PALA in the tissue was present in the assay, diluted 35-fold.) Using the activity of the pre-treatment specimen as the uninhibited control, the percent of activity remaining was calculated and is listed in Table 1.

The pyrimidine nucleotide concentrations were decreased after PALA in nine out of ten pairs of specimens, and in five the decrease exceeded 50%. The greatest decreases were at the higher doses after 22–28 hr of exposure (patients 4–7). Only one of the four specimens from patients receiving 1 g/m^2 showed more than a 50% decrease in pyrimidine nucleotides. When the patients were classified according to their pre-treatment ACTase levels, the comparison shown in Fig. 2 was obtained.

Table 1. ACTase activity, PALA concentrations, and change in UTP in tumor specimens from patients

Patient No.	Diagnosis	PALA dose ($g/m^2 \times$ days)	Sampling time (hr)*	Initial ACTase (units/mg protein)	Final ACTase (% of initial)	PALA in tumor ($\mu g/g$)	PALA in plasma ($\mu g/ml$)	UTP† (% of initial)
1	Sq. Ca. lung	5.0 × 1	2.75	1.21	44	19		
2	Ad. Ca. lung	5.0 × 1	6.0			89	81	59
3	Melanoma	6.0 × 1	16.2	1.04	13			100
4	Neurofibrosarcoma	5.0 × 1	22			8.3		28
5	Melanoma	6.0 × 1	24	1.370	37	3.6		42
6	Ad. Ca. lung	3.6 × 2	1.5 (d. 2)			25	149	35
7	Ca. esoph.	3.6 × 2	4.0 (d.2)	0.531	19	75	86	33
8	Ca. breast	3.6 × 2	4.5 (d.2)			60	67	
9	Melanoma	3.6 × 1	20	0.435	53	12.9	12.8	
10	Melanoma	3.6 × 1	145			0.9		
11	Melanoma	1.0 × 8	4.0 (d.8)	1.75	35			79
12	Melanoma	1.0 × 8	5.5 (d.8)			10.1	11.1	
13	Melanoma	1.0 × 5	23 (d.5)	0.646	75	4.6		44
14	Mult. myel.	1.0 × 8	24 (d.1)	0.662	83			84
15	Sq. Ca. lung	1.0 × 5	48 (d.5)	0.673	51	4.2		71
16	Melanoma	1.0 × 6	96 (d.6)	0.502	77	2.7		

* Hours measured from the end of infusion, and day of treatment.

† CTP decreased by the same percentage as UTP, except for the specimens from patients 13 and 5 in which it was unchanged.

DISCUSSION

The assay method for PALA in tissues is reproducible to within about 10%. Since the values have not been corrected for recovery, they are probably 15–25% low. It is uncertain how much of the measured PALA is actually intracellular; no corrections have been made for extracellular fluid. The similarity of total tissue PALA and plasma PALA in patients at the later time points suggests, however, that the intracellular concentration is probably near the extracellular by 4 hr after exposure.

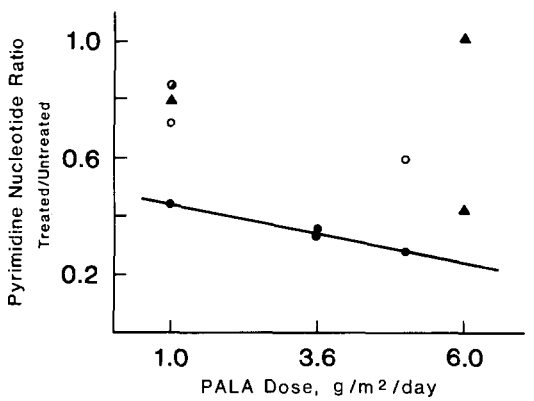

Fig. 2. Change in pyrimidine nucleotides with respect to PALA dose and initial level of enzyme. Circular symbols (○, ◑, ●) represent specimens from patients with pre-treatment ACTase levels below 0.7 units/mg or not measured; triangles (▲) represent those with pre-treatment ACTase above 1.0 units/mg. The two open symbols represent a specimen taken 6 hr after 5 g/m^2, and one 48 hr after the fifth dose of 1.0 g/m^2. All others were exposed to PALA for at least 16 hr, and sampled 24 hr or less after the last dose, except that the specimen indicated by ◑ was taken after only one dose of 1 g/m^2.

The initial ACTase activities varied widely among the melanoma specimens. No difference based on tumor type was apparent. The higher specific activities, up to 1.7 units/mg, were comparable to those found in Lewis lung tumor and of the same order of magnitude as those Jayaram *et al.* [3] reported for sensitive mouse tumors grown *in vivo* (1.8 to 6.6 units/mg). They were, however, much lower than those Johnson *et al.* [4] reported in cultured cells (19–29 units/mg for sensitive tumors).

The ACTase slightly purified from human spleen was inhibited competitively by PALA. The K_m for carbamoyl phosphate (20.6 μM) and the K_i for PALA (0.011 μM) agree well with those reported by Tsuboi *et al.* [15] for an extract of cultured human cells (10 μM, 0.0085 μM) and are similar to those reported for other mammalian ACTases [3, 16–18].

Our data indicate that the PALA concentration required for 90% inhibition was 0.265 $\mu g/ml$ when the carbamoyl phosphate concentration was 0.2 mM, and 1.5 $\mu g/ml$ (6 μM) when it was 1.0 mM. The higher value is lower than the 2.7 $\mu g/ml$ measured in tumor at 4 days after the last 1 g/m^2 dose in patient 16.

The observed decrease in ACTase activity in the biopsy samples after treatment with PALA was in many cases consistent with that predicted from the PALA concentration in the assay, suggesting that the inhibition can be attributed to the residual PALA in the tissue. In most of the other cases the inhibition was less than predicted. This may indicate synthesis of additional ACTase. However, results reported in the accompanying paper [19] suggest that certain other tissue components may interfere with inhibition by PALA. It is also possible that the metastases sampled before and after treatment were not strictly comparable.

The actual inhibition *in vivo* would depend not only on the sensitivity of the enzyme and the PALA concentrations in the cell, but also on the effective

concentration of carbamoyl phosphate at the enzyme. Since carbamoyl phosphate synthetase and ACTase occur together in an enzyme complex (together with dihydro-orotase, the third enzyme of the pathway) [12], this concentration is not easily determined. This question is discussed in the accompanying paper [19] and has been addressed by Kensler *et al.* [18]. Our experiments suggest that inhibition of ACTase *in vivo* is probably less effective than that observed *in vitro*.

We do, however, have indirect evidence of the effective inhibition in the depletion of the pyrimidine nucleotide pools. The decrease in the concentrations of pyrimidine nucleotides was also less than might have been expected from the PALA concentration or from the inhibition of ACTase activity as observed *in vitro*.

Other factors which may influence the UTP and CTP concentration are the rates of their degradation and consumption, the re-utilization of UMP and CMP from the breakdown of RNA, and the "salvage pathway" utilization of uracil or uridine from the blood. Jayaram *et al.* [3] reported higher uridine-cytidine kinase in some resistant tumors. The effect of such factors is probably seen in the results from patient 3, whose tumor showed no decline in pyrimidine nucleotides in spite of sufficient PALA in the tissue to produce an apparent 87% inhibition of ACTase *in vitro*, after dilution, at 16 hr after treatment, leaving one of the lowest residual ACTase activities in the entire group.

The changes in pyrimidine nucleotide pools showed a partial correlation with the original ACTase activity and with the dose and time of exposure (Fig. 2). The only specimens which showed more than 60% decrease in nucleotides were the three from patients having low initial ACTase activity, who were treated with 3.6 or 5 g/m^2, and sampled at 22–28 hr after treatment. A similar specimen at 6 hr had a 41% decrease. We tentatively conclude that an effective decrease in nucleotides requires more than 6 hr and is more likely if the starting ACTase activity is low and the PALA dose high. Unfortunately, our results are not sufficient to define accurately the timing of the decrease or recovery. Variability between tumors adds to the difficulty of such interpretation.

In patient 4, the only one of the group who had a decrease in tumor size (less than 50%), the decrease (72%) in pyrimidine nucleotides was the greatest. The results of Moyer and Handschumacher [20] indicate that a decrease of 90% or more in pyrimidine nucleotides may be necessary to inhibit growth of tumor cells completely.

We have shown that the tissue concentration of PALA is close to the plasma concentration at times from 4 to 20 hr, and that it is more than sufficient to produce substantial inhibition of ACTase under the usual *in vitro* assay conditions. The accompanying paper [19] suggests that there may be less inhibition *in vivo*. There was a substantial decrease in pyrimidine nucleotides in several cases, which was partially correlated with the starting ACTase activity and dose, and seemed greatest after about 24 hr. It appears that PALA does have a biochemical effect on human tumor, although it was not sufficient to destroy the tumors [6]. However, a 50–70% decrease in UTP and CTP might well be sufficient to alter the uptake or effect of other pyrimidine analogs in susceptible tumors, such as has been reported for 5-azacytidine [21] and 5-fluorouracil [22] in mouse tumors. Clinical trials of PALA in combination with 5-fluorouracil are in progress.

Acknowledgements—We thank Asa Shinkawa, Linda Lopez and Nadine Schultz for their preliminary experiments on animal tissues during the development of the methods. Dr. Daniel Martin contributed useful questions during a discussion about the manuscript.

REFERENCES

1. K. D. Collins and G. R. Stark, *J. biol. Chem.* **246**, 6599 (1971).
2. R. K. Johnson, T. Inouye, A. Goldin and G. R. Stark, *Cancer Res.* **36**, 2720 (1976).
3. H. N. Jayaram, D. A. Cooney, D. T. Vistica, S. Kariya and R. K. Johnson, *Cancer Treat. Rep.* **63**, 1291 (1979).
4. R. K. Johnson, E. A. Swyryd and G. R. Stark, *Cancer Res.* **38**, 371 (1978).
5. T. L. Loo, J. Friedman, E. C. Moore, M. Valdivieso, J. Marti and D. Stewart, *Cancer Res.* **40**, 86 (1980).
6. M. Valdivieso, E. C. Moore, A. M. Burgess, J. R. Marti, J. Russ, W. Plunkett, T. L. Loo, G. P. Bodey and E. J. Freireich, *Cancer Treat. Rep.* **64**, 285 (1980).
7. E. C. Moore, J. Friedman, M. Valdivieso, J. R. Marti, J. Russ and T. L. Loo, *Proc. Am. Ass. Cancer Res.* **20**, 221 (1979).
8. M. Valdivieso, E. C. Moore, T. L. Loo, G. P. Bodey and E. J. Freireich, *Proc. Am. Ass. Cancer Res.* **20**, 187 (1979).
9. R. W. Porter, M. O. Modebe and G. R. Stark, *J. biol. Chem.* **244**, 1846 (1979).
10. J. Friedman, E. C. Moore, S. W. Hall and T. L. Loo, *Cancer Treat. Rep.* **63**, 85 (1979).
11. J. X. Khym, *Clin. Chem.* **21**, 1245 (1975).
12. M. Mori, H.Ishida and M. Tatibana, *Biochemistry* **14**, 2622 (1975).
13. W. W. Cleland, *Nature, Lond.* **198**, 463 (1963).
14. W. Plunkett, L. Alexander, S. Chubb and T. L. Loo, *Biochem. Pharmac,* **28**, 201 (1979).
15. K. K. Tsuboi, H. N. Edmunds and L. K. Kwong, *Cancer Res.* **37**, 3080 (1977).
16. E. A. Swyryd, S. S. Seaver and G. R. Stark, *J. biol. Chem.* **249**, 6945 (1974).
17. N. J. Hoogenraad, *Archs Biochem. Biophys.* **161**, 76 (1974).
18. T. W. Kensler, G. Mutter, J. G. Hankerson, L. J. Reck, C. Harley, N. Han, B. Ardalan, R. L. Cysyk, R. K. Johnson, H. N. Jayaram and D. A. Cooney, *Cancer Res.* **41**, 894 (1981).
19. E. C. Moore, *Biochem. Pharmac.* **31**, 3315 (1982).
20. J. D. Moyer and R. E. Handschumacher, *Cancer Res.* **39**, 3089 (1979).
21. S. Grant, F. Rauscher, III, A. Jakubowski and E. Cadman, *Cancer Res.* **41**, 410 (1981).
22. S. Spiegelman, S. R. Sawyer, R. Nayak, E. Ritzi, R. Stolfi and D. Martin, *Proc. natn. Acad. Sci. U.S.A.* **77**, 4966 (1980).