Biochemical Pharmacology, 1963, Vol. 12, pp. 85–93. Pergamon Press Ltd., Printed in Great Britain.

POTENTIATION BY INHIBITION OF DRUG DEGRADATION: 6-SUBSTITUTED PURINES AND XANTHINE OXIDASE

GERTRUDE B. ELION, SANDRA CALLAHAN, HENRY NATHAN, SAMUEL BIEBER, R. WAYNE RUNDLES and GEORGE H. HITCHINGS

The Wellcome Research Laboratories, Burroughs Wellcome & Company, (U.S.A.) Inc., Tuckahoe, N.Y.; and
Duke University, School of Medicine, Durham, N.C., U.S.A.

(*Received* 22 *August* 1962; *accepted* 4 *October* 1962)

Abstract—The administration of the xanthine oxidase inhibitor, 4-hydroxypyrazolo(3,4-d)pyrimidine, concurrently with 6-mercaptopurine, results in a marked decrease in the metabolic oxidation of the latter to 6-thiouric acid in both the mouse and man. The inhibition of metabolic degradation by this means results in several-fold potentiations of 6-mercaptopurine, 6-methylthiopurine, 6-propylthiopurine, and 6-chloropurine in trials against adenocarcinoma 755 and of 6-mercaptopurine and 6-chloropurine as inhibitors of the immune response of mice to sheep erythrocytes.

STUDIES on the absorption, distribution, and metabolism of 6-mercaptopurine in mice and in the human have shown that the disposition of the drug by degradative processes is rather extensive.[1, 2] 6-Thiouric acid appears early, accounts for a sizable proportion of the urinary excretory products,[1, 2] and is itself inactive as a tumor inhibitor.[3] The obvious route from 6-mercaptopurine to 6-thiouric acid is via oxidation catalyzed by xanthine oxidase, and evidence was soon forthcoming that this reaction takes place readily *in vitro*.[1, 4] Meanwhile studies of the effects of purine base analogs on xanthine oxidase-catalyzed reactions had discovered a substantial number of inhibitors of this enzyme.[5–10] It was of interest, therefore, to determine whether the oxidation of mercaptopurine to thiouric acid could be inhibited *in vivo*, and whether the apparent potency and possibly the chemotherapeutic index of mercaptopurine could be altered by this means. 4-Hydroxypyrazolo(3,4-d)-pyrimidine was chosen for trial on the basis of its high potency as a xanthine oxidase inhibitor*, and its moderate toxicity.[11] It is the purpose of this paper to report experiments, both in the mouse and in the human, which demonstrate that inhibition of xanthine oxidase activity can be attained *in vivo* when the pyrazolopyrimidine is administered, and that such inhibition does indeed result in a decrease in the metabolic destruction of 6-mercaptopurine and other 6-substituted purines.

* Unpublished data.[6]

MATERIALS AND METHODS

Compounds

All the compounds* used in the experiments were synthesized in these laboratories by published methods. ^{35}S-6-mercaptopurine was synthesized by the method previously reported[12] with the modification that the reaction of 6-chloropurine with ^{35}S-sodium hydrosulfide was carried out at room temperature for 48 hr rather than at 100° for 7 hr. The ^{35}S-6-thiouric acid was prepared by the action of xanthine oxidase on ^{35}S-6-mercaptopurine.[4]

Adenocarcinoma 755

Tests for antitumor activity against adenocarcinoma 755 were conducted in mice of the C57 BL/6 strain according to the protocol previously described.[13] The tumor weight index (TWI) = weight of experimental tumors/weight of control tumors. Groups of six animals were used in each experiment.

Antibody suppression

The compounds were tested in mice for their ability to suppress the formation of hemaglutinins to sheep red blood cells as previously described.[14] The antibody index is a ratio of the hemagglutinin titers for the treated animals to the titers in the untreated controls; an index of 0·6 or lower denotes a significant suppression of antibody formation.

Metabolism experiments

Mice. A dose of 6-MP of 10 mg/kg in the form of a solution of the sodium salt (1 mg/ml) was injected intraperitoneally into each of ten mice of the C57 BL/6 strain. In two sets of experiments ^{35}S-6-MP was used; in another set nonradioactive 6-MP was administered. In those groups receiving HPP the dose of 20 mg/kg (1·6 mg of the sodium salt/ml) was given immediately after the 6-MP. Each group was kept in a single metabolism cage without food but with water *ad libitum* for 24 hr. The urines were collected and filtered to remove any fecal contamination. The amounts of 6-MP and TU were determined on an aliquot of the urine by the isotope dilution technique. When the animals were given ^{35}S-6-MP, 100 to 200 μg of nonradioactive 6-MP and 200 μg of nonradioactive TU were added to a 50% aliquot of the urine. The 6-MP and TU were then separated on a Dowex-1 (formate) ion-exchange column and purified to constant specific activity by chromatography on a Dowex-50(H^+) column and paper chromatography in a butanol-acetic acid solvent. The amount of radioactive inorganic sulfate was determined by passing an aliquot of the urine through a Dowex-50(H^+) column and then treating the effluent with an excess of barium chloride to precipitate the sulfate as barium sulfate. The difference in radioactivity of the solution before and after precipitation was calculated as inorganic sulfate. When the animals were injected with nonradioactive 6-MP, approximately 124 μg of ^{35}S-6-MP and 164 μg of ^{35}S-TU were added to 50% aliquots of the urine respectively. An example of the complete method is given below in the experiment in man. No attempt was made to determine sulfate or other metabolic products of the nonradioactive 6-MP.

* The abbreviations used are as follows: 6-MP, 6-mercaptopurine; TU, 6-thiouric acid; SO_4^{2-}, inorganic sulfate; HPP, 4-hydroxypyrazolo(3,4-d)pyrimidine.

Man. A patient with chronic granulocytic leukemia, who had had 6-MP therapy for 1 week, except for the day prior to the metabolic experiment, was given a single dose of 150 mg of nonradioactive 6-MP. The urine was collected during the following periods of 0 to 12 and 12 to 24 hr; 48 hr after the first dose the patient received an oral dose of 150 mg of 6-MP and 300 mg of HPP, and the urine was again collected. In a second experiment after 6 weeks of therapy with 6-MP, when the patient's disease was essentially in complete remission, the drug was discontinued for 1 day; the patient was then given 150 mg of 6-MP together with 75 mg of HPP. The amounts of 6-MP and TU in the urine were determined by the addition of ^{35}S-labeled compounds, re-isolation of the compounds in pure form, and determination of the extent of isotope dilution. The method is illustrated below for the specimen collected 0 to 12 hr after the dose of 6-MP alone.

For the determination of the amount of 6-MP, 262 μg of ^{35}S-6-MP (specific activity = 4600 cpm/μg) was added to a 100-ml aliquot of the urine. The pH value of the urine was adjusted to 8; the urine was filtered and passed through a Dowex-1 (formate) column (20 mm in diameter $\times$ 38 mm in height). After the column had been washed with 50 ml of water, the 6-MP was eluted with 0·1 N formic acid. The specific activity of each fraction was determined by measuring the radioactivity and ultraviolet absorption spectrum of suitably diluted aliquots. The spectrum of 6-MP shows a λ max = 325 mμ, E_m = 19,000 at pH 1.[15, 16] The first 60 ml of eluate contained 74% of the added radioactivity and was used for further purification. This eluate was passed through a Dowex-50(H^+) column (10 mm in diameter and 20 mm in height) which was washed with 20 ml of water and 22 ml of 0·5 N hydrochloric acid. The radioactive 6-MP was then eluted with 1 N hydrochloric acid in 10-ml fractions. The third 10-ml eluate, which had the highest specific activity, was used for paper chromatography. An aliquot of 0·25 ml of this eluate was taken to dryness in an air stream. The residue was dissolved in 0·015 to 0·03 ml of water, spotted on paper, and the chromatogram developed by ascending flow using the butanol layer from a mixture of *n*-butanol:glacial acetic acid:water (40:10:50 v/v). The radioactive 6-MP, which ran to an R_f of 0·55 in this solvent, was eluted with 1·2 ml of water and the specific activity determined. (Whenever the ultraviolet absorption of the 6-MP was not that of pure material, the sample was rechromatographed on paper.) The eluate from the paper chromatogram showed a specific activity of 535 cpm/μg which represents a dilution of 8·6-fold from the original ^{35}S-6-MP added. The amount of unlabeled 6-MP in the urine was therefore calculated to be 1·99 mg/100 ml urine.

For the determination of 6-thiouric acid, a sample of 405 μg of ^{35}S-6-thiouric acid (specific activity = 820 cpm/μg) was added to a 50-ml aliquot of urine. The pH value was adjusted to 8, and the solution was passed through a Dowex-1 (formate) column (20 mm in diameter and 32 mm in height). The column was washed with 50 ml of water and 380 ml of 0·1 N formic acid (to remove 6-MP, uric acid, and other UV-absorbing materials). The TU was then eluted with 0·5 N formic acid, collected in 30-ml fractions. The third, fourth, and fifth fractions had identical specific activities (72 cpm/μg) and were pure according to UV-absorption spectrophotometry. TU has a spectrum with a λ max = 260, 355 mμ, E_m = 8100; 28,600, at pH 1.[4] This represented a dilution of 11·4-fold from the original TU and indicated the presence of 4·22 mg of TU per 50 ml of urine.

TABLE 1. EFFECTS OF PURINES ON THE GROWTH OF ADENOCARCINOMA 755 IN THE ABSENCE AND PRESENCE OF 4-HYDROXYPYRAZOLO(3,4-d)PYRIMIDINE (HPP)

Compound	Dose (mg/kg)	Without HPP TWI*	Without HPP Toxicity†	Dose of HPP (mg/kg)	With HPP TWI	With HPP Toxicity
6-Mercaptopurine	81		5/5			
	27	0·01	0/6	20	<0·001	0/6
	27	0·03	0/5	25	0·003	4/6
	9	0·05	0/6	20	0·001	1/6
	9	0·13	0/5	25	0·01	3/6
	3	0·50	0/6	20	0·02	1/6
	3	0·19	0/5	25	0·02	0/6
	3	0·19	0/6	50	0·06	0/6‡
	2	1·18	0/6	6·25	0·10	0/6
	2	0·75	0/6	25	0·11	0/6‡
	2	0·60	0/6	50	0·04	1/6§
	1	0·74	0/6	6·25	0·61	0/6
	1	0·74	0/6	12·5	0·40	0/6
	1	1·31	0/6	12·5	0·71	0/6
	1	1·5	0/6	20	0·005	0/6
	1	1·01	0/6	25	0·18	0/6
	1	0·24	0/5	25	0·08	0/6
	0·5	0·93	0/6	25	0·62	0/6
	0			12·5	0·93	0/12
	0			20	1·13	0/12
	0			25	0·85	0/12
	0			50	0·80	1/12‡
6-Chloropurine	150	0·05	0/6	20	0·02	0/6
	75	0·06	0/6	20	0·03	0/6
	75	0·02	0/6	20	0·03	0/6
	25	0·62	0/6	20	0·02	0/12
	25	0·47	0/6	20	0·26	0/6‡
	25			10	0·10	0/6‡
	25			5	0·42	0/6
	12·5	1·0	1/6	20	0·10	0/6
	6·25	1·4	0/6	20	0·31	0/6
	6·25	0·45	0/6	20 p.o.	0·01	0/6‡
	6·25			20	0·16	0/6
	3			20	0·85	0/6
	0			20	0·88	0/24
	0			20 p.o.	0·66	0/6
6-Methylthiopurine	200	0·30	0/6			
	100	0·40	0/6			
	50	0·33	0/6			
	25	0·63	0/12			
	10	0·77	0/6	25	0·03	2/6
	10	0·54	0/6	20	0·42	0/6
	0			20	0·48	0/6
6-Propylthiopurine	25	0·03	0/12			
	10	0·40	0/12			
	5	1·13	0/6	25	0·23	1/6
	5	0·26	0/6	25	0·04	0/6
	0			25	0·80	1/12

* Tumor weight index = average wt treated tumors/average wt control tumors.

† Toxicity = deaths/total no. of animals. All survivors showed a body weight index (BWI) greater than 0·85 unless otherwise indicated. BWI = weight gain of treated animals/weight gain of control animals.

‡ BWI = 0·80 to 0·82.

§ BWI = 0·68.

RESULTS

The inhibitory effect of 6-MP on the growth of adenocarcinoma 755 has been reported.[17, 18] With the tumor strain and dosage regimen used in these laboratories the minimum effective dose (MED) of 6-MP was between 2 and 3 mg/kg (Table 1). When the animals were injected with 6-MP together with HPP (20 mg/kg), the MED of 6-MP lay between 0·5 and 1 mg/kg (Table 1). This 3- to 4-fold increase in antitumor activity was seen over the full range of 6-MP concentrations, but toxicity did not increase proportionately; *e.g.* there were no deaths with 27 mg of 6-MP/kg and 20 mg of HPP/kg. (There was added toxicity, however, when 25 mg of HPP/kg was used.) It will be noted that the potentiation of antitumor activity cannot be ascribed to an additive effect of the activities of the two compounds since HPP was itself inactive against the tumor, even at its maximum tolerated dose (MTD) of 50 mg/kg. The effect of levels of HPP lower than 20 mg/kg upon the inhibitory effect of 6-MP were less marked. Thus, although 6·25 mg of HPP/kg rendered 2 mg of 6-MP/kg active, it was not so effective as the higher doses in demonstrating activity with 1 mg of 6-MP/kg.

TABLE 2. EFFECT OF HPP ON THE ANTIIMMUNE EFFECTS OF 6-MP AND 6-CHLOROPURINE

Compound	Dose (mg/kg)	Without HPP Antibody index	Dose (mg/kg)	With HPP Antibody index
6-MP*	0	1·0	20	0·71
	8·33	0·90	20	0·45
	25	0·55	20	0·36
	75	0·25	20	0·27
6-MP†	8·33		7	0·43
	8·33		20	0
	75	0·29		
	25	0·82	20	0·80‡
	75	<0·1		
6-Chloropurine†	30	0·85	7	0·69‡
	30		20	0·55‡
	90	0·76	7	0·59
	90		20	0·40
	270	0·71	7	0·46
	270		20	0·27‡

* Animals weighed 20 g and received 4 daily doses of drug.
† Animals weighed 30 g and received 5 daily doses of drug.
‡ One death occurred among 5 animals.

The activity of 6-chloropurine was increased even more than that of 6-MP (Table 1) by the simultaneous administration of HPP. The MED of 6-chloropurine alone was >25 and <75 mg/kg, whereas in the presence of HPP at 20 mg/kg it was 6·25 mg/kg. Moreover, there appeared to be no increase in toxicity to the host since 6-chloropurine at a dose of 150 mg/kg (the MTD) remained well tolerated even when HPP (20 mg/kg) was added. HPP at a dose of 10 mg/kg was as effective in increasing the activity of 6-chloropurine (25 mg/kg) as 20 mg/kg, but HPP at 5 mg/kg was ineffective. Of interest

also is the fact that HPP was at least as effective by mouth as intraperitoneally in potentiating the activity of 6-chloropurine.

With the alkylthiopurines, 6-methylthio and 6-propylthio (MED = 50 and 12·5 mg/kg respectively) the activity was likewise increased 5- and 2-fold respectively by the addition of HPP.

The activity of 6-MP in suppressing antibody formation has been reported.[14, 19–24] In the test that measures the hemagglutinin titer in mice 12 days after an injection of sheep red blood cells, 6-MP has been found to be markedly active at 75 mg/kg and to have borderline activity at 25 mg/kg. In the presence of HPP (20 mg/kg), activity was found when 6-MP was given at 25 mg/kg (except in one experiment) and even at 8·33 mg/kg (Table 2).

To verify the hypothesis that the potentiation of the activity of 6-MP by HPP is due to a suppression of the one of the principal reactions which inactivates 6-MP *in vivo*, namely the oxidation to thiouric acid by xanthine oxidase, the metabolism of 6-MP in the absence and presence of HPP was studied. In two separate experiments using ^{35}S-6-MP, HPP in mice was found to have a very pronounced effect on the amount of urinary thiouric acid derived from 6-MP. Thus, in one experiment the amount of TU was decreased 9-fold, in the second experiment 6-fold, while the amount of free 6-MP in the urine was increased about 3·5 times in each case (Table 3). Moreover, the amount of radioactive sulfate derived from 6-MP was markedly decreased by the administration of HPP.

TABLE 3. THE EFFECT OF THE ADMINISTRATION OF HPP UPON THE METABOLISM OF 6-MP IN THE MOUSE

6-MP (mg/kg i.p.)	HPP (mg/kg i.p.)	Products excreted in urine in 24 hr			
		Percent of dose			
		6-MP	TU	SO	Other
10*	0	21·4	18·9	29·5	
10*	20	69·5	2·2	3·9	
10*	0	11·9	12·2	13·4	10·5
10*	20	43·0	2·1	4·7	12·4
10	0	11·7	37·0		
10	5	22·7	14·5		
10	10	25·0	12·4		
10	20	40·3	10·0		

* ^{35}S-6-MP.

When mice were given nonradioactive 6-MP (10 mg/kg) and graded doses of HPP, the free 6-MP was increased 2-fold by HPP at 5 mg/kg and almost 4-fold at 20 mg/kg. The amounts of thiouric acid were markedly decreased in each case, with the greatest decrease evident at the highest dose of HPP. The ratios of the percentage of dose excreted as 6-MP/TU were 0·32, 1·56, 2·02, and 4·03 as the dose of HPP was increased from 0 to 5, 10, and 20 mg/kg.

Metabolic experiments using non-radioactive 6-MP were conducted in man, with and without simultaneous doses of HPP (Table 4). When the dose of HPP was 300 mg

there was a marked increase in the amount of free 6-MP in the urine (over 4-fold) and 8-fold decrease in the amount of TU excreted during the first 12 hr. (No 6-MP, and a negligible amount of TU, was found in the urine during the 12- to 24-hr period.) With the lower dose of HPP (75 mg) the amount of free 6-MP in the urine was likewise increased 4-fold, but the amount of TU was decreased only 2·5-fold as compared with the control. Thus the ratio of excreted 6-MP/TU was increased from 0·28 to 2·95 to 8·5 as the amount of HPP was increased from 0 to 75 to 300 mg. These results agree very well with those obtained in the metabolic experiments in the mouse.

TABLE 4. THE EFFECT OF ADMINISTRATION OF HPP UPON THE METABOLISM OF 6-MP IN MAN

6-MP (mg p.o.)	HPP (mg p.o.)	Time (hr)	Products excreted in the urine	
			Percent of dose	
			6-MP	TU
150	0	0–12	7·2	25·5
		12–24	0	0·96
150	300	0–12	29·2	3·43
		12–24	0	0·41
150	75	0–12	29·5	10·0

DISCUSSION

Work on the metabolism of 6-MP[1, 2, 4, 25] had demonstrated that 6-thiouric acid and inorganic sulfate are the principal metabolic products of this compound *in vivo*. The finding that 6-thiouric acid arises by the action of xanthine oxidase on 6-MP suggested that it might be possible to minimize this catabolic pathway by the use of a xanthine oxidase inhibitor. Previous reports have indicated that semicarbazide can inhibit xanthine oxidase and xanthine dehydrogenase activities *in vivo* as well as *in vitro*. However, the inhibition *in vivo* was less than 50% at doses that produced significant toxic effects.[26]

The activity of 4-hydroxypyrazolo(3,4-d)pyrimidine as a xanthine oxidase inhibitor *in vitro* was demonstrated in these laboratories as part of a program on the specificity of xanthine oxidase.[5, 6] Moreover, this compound appeared to be suitable for the proposed studies since it is itself inactive in the systems to be examined—*i.e.* adenocarcinoma 755 and antibody suppression. The present work demonstrates that this compound inhibits xanthine oxidase activity *in vivo* at nontoxic levels and that this inhibition can result in an increase in the effectiveness of a number of the 6-substituted purines. The close correlation between the results of the metabolic experiments in the mouse, with a 3- to 4-fold increase in the amount of free 6-MP excreted, and the 3- to 4-fold increase in antitumor effectiveness is striking. It is of some interest also that the amount of sulfate derived from 6-MP is also decreased. This suggests that sulfate may arise from 6-MP via thiouric acid or that HPP may be inhibiting some catabolic process in addition to the oxidation catalyzed by xanthine oxidase.

In the combination of 6-MP and HPP there appears to be an improvement in the chemotherapeutic index of 6-MP with regard to both the inhibition of adenocarcinoma

755 and the suppression of the immune response since toxicity to the host is not increased proportionately to toxicity to the tumor. That increased toxicity does not necessarily accompany increased antitumor activity is also demonstrated by the combinations of 6-chloropurine and HPP. 6-Chloropurine is known to be a substrate for xanthine oxidase, with the formation of 2,8-dihydroxy-6-chloropurine.[10] Moreover, *in vivo*, the latter is one of the prominent urinary metabolites of 6-chloropurine in the rat.[27] It was therefore to be expected that HPP would prevent the oxidation of 6-chloropurine in the same way as with 6-MP. It is significant that the activity against adenocarcinoma 755 can be increased almost 10-fold without any apparent increase in toxicity. This increase in therapeutic index may be the result of differential concentration of the HPP in the host and tumor tissues or to differences in xanthine oxidase levels which make the enzyme in the tumor particularly susceptible to the inhibitor. Alternatively it may mean that the limiting event in the toxicity of 6-chloropurine is a process (conceivably precipitation of 2,8-dioxy-6-chloropurine in the renal tubule) which is unrelated to that giving rise to tumor inhibition.

The metabolism of several of the 6-alkylthiopurines has been studied in mouse and man.[25, 28, 29] Among the products found were free 6-MP, thiouric acid, a 6-alkylsulfinyl-8-hydroxypurine and (in the case of 6-methylthiopurine) a glucuronide of 6-methylthio-8-hydroxypurine.[29] Since 6-methylthiopurine has been found to be converted to its 8-hydroxy derivative by xanthine oxidase* and since both the 8-hydroxy-6-alkylthiopurines and the 8-hydroxy-6-alkylsulfinylpurines are inactive against adenocarcinoma 755,[30] it appeared possible that HPP would have a potentiating effect on the antitumor activities of 6-methylthiopurine and 6-propylthiopurine. This has indeed proved to be so (Table 2).

With the principle established that the therapeutic effectiveness of the purines can be enhanced by the alteration of their metabolic degradation, experiments are in progress to determine whether the chemotherapeutic indices of these compounds in man can be improved in this way. The results of the metabolic experiment in man and their close resemblance to the results in the mouse lend some encouragement to this approach.

* Unpublished data.

Acknowledgements—The authors wish to acknowledge the valuable technical assistance of Dona Hooper.

REFERENCES

1. G. B. ELION, S. BIEBER and G. H. HITCHINGS, *Ann. N.Y. Acad. Sci.* **60**, 297 (1954).
2. G. B. ELION, S. BIEBER and G. H. HITCHINGS, *Proc. Amer. Ass. Cancer Res.* **1** (no. 2), 13 (1954).
3. D. A. CLARKE, G. B. ELION, G. H. HITCHINGS and C. C. STOCK, *Cancer Res.* **18**, 445 (1958).
4. G. B. ELION, S. MUELLER and G. H. HITCHINGS, *J. Amer. chem. Soc.* **81**, 3042 (1959).
5. D. C. LORZ and G. H. HITCHINGS, *Fed. Proc.* **9**, 197 (1950).
6. D. C. LORZ and G. H. HITCHINGS, *Abstracts, Amer. chem. Soc.* Meetings, Dallas, April 1956, p. 30c.
7. P. FEIGELSON and J. DAVIDSON, *Cancer Res.* **16**, 352 (1956).
8. J. B. WYNGAARDEN, *J. biol. Chem.* **224**, 453 (1957).
9. P. FEIGELSON, J. D. DAVIDSON and R. K. ROBINS, *J. biol. Chem.* **226**, 993 (1957).
10. D. E. DUGGAN and E. TITUS, *J. biol. Chem.* **234**, 2100 (1959).
11. H. F. SKIPPER, R. K. ROBINS, J. R. THOMSON, C. C. CHENG, R. W. BROCKMAN and F. M. SCHABEL, JR., *Cancer Res.* **17**, 579 (1957).
12. G. B. ELION and G. H. HITCHINGS, *J. Amer. chem. Soc.* **76**, 4027 (1954).

13. G. B. ELION, S. BIEBER, H. NATHAN and G. H. HITCHINGS, *Cancer Res.* **18**, 802 (1958).
14. H. C. NATHAN, S. BIEBER, G. B. ELION and G. H. HITCHINGS, *Proc. Soc. exp. Biol., N.Y.* **107**, 796 (1961).
15. G. B. ELION, E. BURGI and G. H. HITCHINGS, *J. Amer. chem. Soc.* **74**, 411 (1952).
16. G. B. ELION, *J. org. Chem.* **27**, 2478 (1962).
17. D. A. CLARKE, F. S. PHILIPS, S. S. STERNBERG and C. C. STOCK, *Ann. N.Y. Acad. Sci.* **60**, 235. (1954).
18. H. E. SKIPPER, J. R. THOMSON, G. B. ELION and G. H. HITCHINGS, *Cancer Res.* **14**, 294 (1954)
19. R. SCHWARTZ, J. STACK and W. DAMESHEK, *Proc. Soc. exp. Biol., N.Y.* **99**, 164 (1958).
20. R. SCHWARTZ and W. DAMESHEK, *Nature, Lond.* **183**, 1682 (1959).
21. R. SCHWARTZ, A. EISNER and W. DAMESHEK, *J. clin. Invest.* **38**, 1394 (1959).
22. J. ŠTERZL, *Nature, Lond.* **185**, 256 (1960).
23. R. M. CONDIE, W. I. MENNIS and C. MILLER, *Fed. Proc.* **20**, 26 (1961).
24. M. C. BERENBAUM, *Nature, Lond.* **185**, 167 (1960).
25. G. B. ELION, S. W. CALLAHAN, G. H. HITCHINGS, R. W. RUNDLES and J. LASZLO, *Cancer Chemother. Reports* no. 16, 197 (1962).
26. W. W. WESTERFELD, D. A. REICHERT and R. J. BLOOM, *J. biol. Chem.* **234**, 1889 (1959).
27. D. E. DUGGAN and E. TITUS, *J. Pharmacol. exp. Ther.* **130**, 375 (1960).
28. G. B. ELION, *Fed. Proc.* **20**, 156 (1961).
29. G. B. ELION, S. W. CALLAHAN and G. H. HITCHINGS, *Proc. Amer. Ass. Cancer Res.* **3**, 316 (1962).
30. G. B. ELION, S. W. CALLAHAN, G. H. HITCHINGS and R. W. RUNDLES, *Proc. VIIIth Int. Cong. Hematology*, Tokyo, 1961, **1**, 642.