REFERENCES

1. D. G. McDevitt, *Post-grad. med. J.* **52** (suppl. 4), 157 (1976).
2. A. D. Toft, W. J. Irvine, I. Sinclair, D. McIntosh, J. Seth and E. H. D. Cameron, *N. Engl. J. Med.* **298**, 643 (1978).
3. D. G. McLarty, B. E. W. Brownlie, W. D. Alexander, P. D. Papapetrou and P. Horton, *Br. med. J.* **2**, 332 (1973).
4. D. R. Hadden, T. K. Bell, D. G. McDevitt, R. G. Shanks, D. A. D. Montgomery and J. A. Weaver, *Acta endocr. Copenh.* **61**, 393 (1969).
5. S. Biran and E. Tal, *J. clin. Pharmac.* **12**, 105 (1972).
6. R. G. Shanks, D. R. Hadden, D. C. Lowe, D. G. McDevitt and D. A. D. Montgomery, *Lancet* **1**, 993 (1969).
7. L. Wartofsky, R. C. Dimond, G. L. Noel, A. G. Frantz and J. M. Earll, *J. clin. Endocr. Metab.* **41**, 485 (1975).
8. J. Nauman, A. Nauman and K. Roszkowska, *Materia. med. Pol.* **6**, 178 (1974).
9. L. E. Murchison, P. D. Bewsher, M. I. Chesters and W. R. Ferrier, *Br. J. clin. Pharmac.* **3**, 273 (1976).
10. G. Lotti, G. Delitala, L. Devilla, S. Alagna and A. Masala, *Clin. Endocr.* **6**, 405 (1977).
11. A. D. B. Harrower, J. A. Fyffe, D. B. Horn and J. A. Strong, *Clin. Endocr.* **7**, 41 (1977).
12. W. M. Wiersinga and J. L. Touber, *J. clin. Endocr. Metab.* **45**, 293 (1977).
13. E. S. Williams and H. S. Jacobs, *Lancet* **2**, 829 (1970).
14. E. Tal, S. Biran and F. G. Sulman, *J. Endocr.* **53**, 503 (1972).
15. F. Azizi, A. G. Vagenakis, J. E. Bush and L. E. Braverman, *Metabolism* **23**, 525 (1974).
16. A. Goulding, R. McChesney and R. D. H. Stewart, *J. Endocr.* **71**, 399 (1976).
17. P. L. Altman and D. S. Dittmer, *Metabolism* p. 101. Federation of American Societies of Experimental Biology, Bethesda, Maryland (1968).
18. C. H. Bastomsky, J. M. Wyse and P. V. N. Murthy, *Clin Biochem.* **9**, 89 (1976).
19. N. M. Alexander and J. F. Jennings, *Clin. Chem.* **20**, 1353 (1974).
20. R. I. Gregerman, *Endocrinology* **72**, 382 (1963).
21. G. Morreale de Escobar and F. Escobar del Rey, *Recent Prog. Horm. Res.* **23**, 87 (1967).
22. J. J. Ballantine and L. Oliner, *Proc. Soc. exp. Biol. Med.* **127**, 388 (1968).
23. M. Saberi, F. H. Sterling and R. D. Utiger, *J. clin. Invest.* **55**, 218 (1975).
24. J. H. Oppenheimer, H. L. Schwartz and M. I. Surks, *J. clin Invest.* **51**, 2493 (1972).
25. A. Hayes and R. G. Cooper, *J. Pharmac.* **176**, 302 (1971).

Biochemical Pharmacology, Vol. 28, pp. 445–446.

0006-2952/79/0201-0445 $02.00/0

Increased activity of alkaline phosphatase in leukemic cells from patients resistant to thiopurines

(*Received* 25 *February* 1978; *accepted* 7 *June* 1978)

Thiopurines have an important role in the treatment of leukemia and certain other neoplastic diseases. 6-Mercaptopurine (6-MP), along with several other drugs, is used in the maintenance therapy of childhood acute lymphocytic leukemia (ALL) [1], and 6-thioguanine (6-TG) is often combined with cytosine arabinoside in the therapy of acute myelogenous leukemia of adults [1]. Both of these compounds as well as other thiopurines must be metabolized to the 5'-nucleotide form before they can be active as antitumor agents. The formation of the 5'-nucleotide is catalyzed by the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT) [2]. Although resistance to the thiopurines has been attributed usually to absence of or alteration of the HGPRT enzyme [3], there have been several reports where an increased degradation of the mononucleotide may account for the development of resistance to these drugs [4–6]. Wolpert *et al.* [7] provided evidence that, in a line of murine ascites cells (S-180) resistant to thiopurines, the increase in catabolism of these nucleotides was due to an elevated activity of a particulate bound alkaline phosphatase. Further, Rosman *et al.* [8] found that in several leukemic patients an increase in alkaline phosphatase activity was responsible, at least in part, for insensitivity to 6-thiopurines. Thus, this enzyme is an important one in the catabolism of thiopurine nucleotides and probably plays an important role in the development of resistance to the purine analogs that are used clinically. In this report, we have assayed the activity of alkaline phosphatase in several patients with acute leukemia, some of whom have become insensitive or resistant to thiopurines. The enzyme was assayed in the white blood cells from the same patients both before and after they became resistant to thiopurine therapy. Our purpose was to determine if changes in the alkaline phosphatase activity could contribute to the development of resistance to these drugs in humans. Leukemic blood was collected in plastic syringes containing heparin as the anticoagulant. Patients at the Roger Williams General Hospital or Rhode Island Hospital with the diagnosis of acute leukemia were studied. Blood samples from the patients with acute myelocytic leukemia (AML) or acute myelomonocytic leukemia (AMML) were taken prior to drug treatment and all subsequently proved sensitive. Blood was also taken from some of these same patients after developing resistance to thiopurine therapy. White blood cells were prepared free of contaminating erythrocytes and platelets as described previously [9]. All cell counts were made in a Coulter model B counter.

Preparation of enzyme extracts. After being washed, the purified cells were suspended in water and disrupted sonically for 75 sec intermittently with a sonifier cell disruptor (Heat systems) set at a 10 watt output while the cells were being kept ice cold. Tris–HCl (1 M, pH 7.6) was added to bring the final concentration in the extract to 0.05 M. The extracts were dialyzed overnight against 4 liters of the suspending buffer to remove endogenous nucleotides. They could be stored over a period of several months at $-20°$ without significant loss of enzyme activity.

Measurement of alkaline phosphatase activity. The incubation procedure was a modification of the method used by Wolpert *et al.* [7]. The incubation mixture contained, unless

Table 1. Alkaline phosphatase activity in acute leukemic patients before and after the development of resistance to thiopurine therapy*

Patient	Diagnosis	P_i (nmol/min/mg protein) Before therapy	After development of resistance to thiopurines
D. I.	AML	1.5	5.5
A. S.	AML	3.6	9.3
B. P.	AMML	3.5	7.2

* Enzyme was assayed as described in the text. AML = acute myelocytic leukemia; AMML = acute myelomonocytic leukemia.

otherwise indicated: 100 mM carbonate-bicarbonate buffer (pH 9.4), 10 mM $MgCl_2$ and 8 mM GMP; the reaction was initiated by the addition of 30 μl of cell-free extract in a total volume of 100 μl. Incubations were carried out at 37° for 30 min in a shaking water bath. Reactions were terminated by the addition of 100 μl of 20% trichloroacetic acid. Precipitated protein was removed by centrifugation at 1600 *g* for 30 min and a 100-μl aliquot of the supernatant solution was assayed for Pi according to the method of Dryer *et al.* [10] at 345 nm. Conditions were such that saturating levels of substrate were present during the incubation and enzyme activity was linear with protein concentration over the time interval measured. Protein was measured by the method of Lowry *et al.* [11].

Table 1 shows the leukocyte alkaline phosphatase activity in three patients both before and after they became resistant to therapy with thiopurines. Activity is expressed as units/mg of protein. The results were similar when activity was expressed as units/10^{10} cells. With all three patients the blast content of the white blood samples was between 88 and 90 per cent, both before treatment and at the time of development of resistance. The one exception was patient A. S. whose white cells had a blast content of 84 per cent at the time of resistance. In patients D. I. and A. S., with acute myelogenous leukemia, the phosphatase activity increased approx. 3-fold after resistance was acquired which was over a period of 3–6 months. These patients were treated initially with cytosine arabinoside and 6-TG and went into remission. This was followed by a relapse over 3–6 months during which time the patients no longer responded to either thioguanine or mercaptopurine therapy.

In patient B. P. who had acute myelomonocytic leukemia, the phosphatase activity was 3.5 nmol P_i/min/mg of protein before therapy and this doubled to 7.2 after resistance developed to the drug thioguanine. As with the above two patients, resistance was determined on the basis of relapse of the peripheral white cells after an initial response. Although GMP was used as the substrate in these studies, Wolpert *et al.* [7], using the enzyme from murine S-180 cells, have measured the increase with any one of several substrates.

The present study demonstrates an increased activity of the enzyme alkaline phosphatase in the white blood cells of patients who have become resistant to thiopurine chemotherapy. These results were from patients where the white cells were obtained from the same patient both before and after the development of resistance. Thus, they differ from the results of Rosman *et al.* [8] who also indicated that resistance to these drugs could at least be partially explained by the increased activity of this enzyme; however, their study simply compared the statistical means from a group of sensitive and a group of resistant patients. Therefore, our results may be more significant.

Acknowledgements—This investigation was supported by USPHS Grant CA 13943 and by the American Cancer Society institutional grant IN-45M. We would like to acknowledge the technical assistance of Mrs. Jo Anne Aspry.

Department of Medicine, ERIC M. SCHOLAR*
The Roger Williams General Hospital, PAUL CALABRESI
Providence, RI, and
The Division of Biological and Medical Sciences,
Brown University,
Providence, RI 02912, U.S.A.

* Correspondence should be addressed to Dr. Scholar, Department of Pharmacology, University of Nebraska Medical Center, 42nd St., and Dewey Ave., Omaha, NB 68105, U.S.A.

REFERENCES

1. G. A. LePage and T. L. Loo, in *Cancer Medicine*, p. 754. Lea & Febiger, Philadelphia (1974).
2. R. W. Brockman and E. P. Anderson, *A. Rev. Biochem.* **32**, 463 (1963).
3. R. W. Brockman, *Adv. Cancer Res.* 7, 129 (1963).
4. D. H. W. Ho, *Biochem. Pharmac.* **20**, 3583 (1971).
5. A. L. Bieber and A. C. Sartorelli, *Cancer Res.* **24**, 1210 (1964).
6. J. F. Henderson, I. C. Caldwell and A. R. P. Paterson, *Cancer Res.* **27**, 1773 (1967).
7. M. K. Wolpert, S. P. Damle, J. E. Brown, E. Sznycer, K. C. Agarwal and A. C. Sartorelli, *Cancer Res.* **31**, 1620 (1971).
8. M. Rosman, M. L. Lee, W. A. Creasey and A. C. Sartorelli, *Cancer Res.* **34**, 1952 (1974).
9. E. M. Scholar and P. Calabresi, *Cancer Res.* **33**, 94 (1973).
10. R. L. Dryer, A. R. Tammes and J. I. Rough, *J. biol. Chem.* **225**, 177 (1957).
11. O. H Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
12. F. Quagliata, D. Gaig, M. Conklyn and R. Silber, *Cancer Res.* **34**, 3197 (1974).
13. J. Lopes, D. Zucker-Franklin and R. Silber, *J. clin. Invest.* **52**, 1297 (1973).