et al. [4], who showed that in HTC cell culture maximum DNA synthesis occurred 17 hr after mitosis and that the full cycle required 24–25 hr for completion. These data suggest that growth had been arrested at the G_1 rather than the S or G_2 phase of the cycle. Other data support this conclusion. Cytophotometric measurement of the DNA content of individual cells in cultures of human fibroblasts showed that in the presence of 0.4 mM indomethacin more than 90 per cent of the cells are in the G_1 phase of the cycle compared to 38–40 per cent for control cultures in exponential growth (B. M. Bayer, H. Kruth, M. Vaughan and M. A. Beaven, unpublished data). Other workers using similar techniques have reported that, in the presence of 750 μM indomethacin, 96 per cent of HeLa cells acquire a DNA content that corresponds to the G_1 phase of the cycle [5].

The above data also suggest that, even after complete arrest of growth, viability of the cells is not impaired. More direct evidence for this has been obtained in autoradiographic studies of HTC cultures grown in the presence of labeled thymidine for varying periods of time. These studies indicated that more than 98 per cent of the cells had incorporated thymidine into nuclei 22 hr after removal of the indomethacin (B. M. Bayer, H. Kruth, M. Vaughan and M. A. Beaven, unpublished data).

The mechanism by which the anti-inflammatory drugs inhibit cell growth is unknown. The drugs do not appear to have a direct inhibitory action on protein or nucleic acid synthesis [2] or amino acid transport (unpublished data). Although there is a parallelism in the ability of the drugs to inhibit culture growth and prostaglandin synthesis [2], the antiproliferative action of the drugs is not reversed by the addition of prostaglandins of the A, B, E and F series [3].

Other workers have noted that replication of HeLa cells [5], rat fibroblasts [6] and rat lymphocytes [7], like that of HTC cells and human fibroblasts [2], is inhibited by the non-steroid anti-inflammatory drugs. The ability of salicylates [8–10] and other anti-inflammatory drugs [11, 12] to inhibit lymphocyte blast transformation induced by phytohaemagglutinin [8–10], antigen [8], or allogenic lymphocytes [10] may be a related phenomenon. This action is also reversible [9], and the drugs appear to affect the early stages of transformtion. In addition to their ability to inhibit prostaglandin synthesis [13], the ability of anti-inflammatory drugs to inhibit proliferation and lymphocyte transformation could contribute to their therapeutic action.

Laboratory of Cellular Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20014, U.S.A.

BARBARA M. BAYER
MICHAEL A. BEAVEN

REFERENCES

1. V. Hial, Z. Horakova, R. E. Shaff and M. A. Beaven, *Eur. J. Pharmac.* **37**, 367 (1976).
2. V. Hial, M. C. F. de Mello, Z. Horakova and M. A. Beaven, *J. Pharmac. exp. Ther.* **202**, 446 (1977).
3. M. A. Beaven, M. C. F. de Mello, V. Hial and Z. Horakova, *Fedn Proc.* **36**, 1037 (1977).
4. D. Martin, G. M. Tomkins and D. Granner, *Proc. natn. Acad. Sci. U.S.A.* **62**, 248 (1969).
5. M. Adolphe, G. Deysson and P. Lechat, *Revue Eur. Étud. clin. biol.* **17**, 320 (1972).
6. G. Neupert and P. Müller, *Expl Path.* **11**, 1 (1975).
7. F. J. Beck, L. Levy and M. W. Whitehouse, *Br. J. Pharmac.* **49**, 293 (1973).
8. L. M. Pachman, N. B. Esterly and R. D. A. Peterson, *J. clin. Invest.* **50**, 226 (1971).
9. J. E. Crout, B. Hepburn and R. E. Ritts, *New Engl. J. Med.* **292**, 221 (1975).
10. G. Opelz, P. I. Terasaki and A. A. Hirata, *Lancet* II, 478 (1973).
11. G. R. Burgio and A. G. Ugazio, *Lancet* I, 568 (1974).
12. R. S. Panush, *Arthritis Rheum.* **19**, 907 (1976).
13. R. J. Flower and J. R. Vane, *Biochem. Pharmac.* **23**, 1439 (1974).

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Failure of propranolol to alter thyroid radioiodine uptake and serum concentrations of thyroxine and triiodothyronine in rats

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The beta-adrenergic blocking agent, propranolol, has been used for several years in the treatment of hyperthyroidism, e.g. with antithyroid drugs, before ^{131}I therapy has taken effect, in thyroid storm and in the preoperative preparation for thyroïdectomy (reviewed in Ref. 1). It has been used as the only drug before thyroidectomy [2] and even as the sole therapy [3]. Until recently, propranolol was thought to control peripheral manifestations of the disease without influencing thyroid function. Thus, it had no effect on thyroid radioiodine uptake [3–5], PBI [3, 6], PB[^{125}I] [5] or thyroid iodine release and peripheral thyroxine (T_4) turnover [7]. However, a number of recent reports [8–12] indicate that in hyperthyroid, euthyroid and hypothyroid T_4 maintained subjects propranolol treatment lowered serum triiodothyronine (T_3) concentrations. In some reports, serum T_4 was elevated [9, 11–13], while in others it was unchanged [8, 10] after propranolol administration. In three of four hyperthyroid patients treated with propranolol serum, PB[^{125}I] was elevated on day 8 after ^{125}I administration [4].

Three studies have been performed in rats [14–16]. In one study, propranolol induced striking increases in serum T_4 in both intact and hypophysectomized rats [14], while in the other two studies [15, 16] it had no effect. Propranolol did not influence the peripheral metabolism of T_4 and essentially did not affect thyroid ^{131}I uptake or the intrathyroid distribution of ^{131}I in iodoprotein [15]. Serum T_3 was not measured in any of these studies. The present report describes the failure of large doses of propranolol to influence serum T_4 and T_3 and thyroid ^{131}I uptake in rats.

Male Sprague–Dawley rats (Canadian Breeding Farms, St. Constant, Quebec), initially weighing 120–130 g, were divided into five groups, as indicated in Table 1. Group 1 was fed powdered Purina laboratory chow alone. Groups

Table 1. Effect of propranolol on body weight, thyroid ^{131}I uptake and serum T_4 and T_3 concentrations in normal and methimazole-treated T_4-injected rats*

Group	Additions to diet	T_4 injection	Body wt (g)	Thyroid ^{131}I uptake (% dose at 12 hr)	Serum T_4 (μg/100 ml)	Serum T_3 (ng/100 ml)
1	—	—	185.6 ± 2.9 (8)	6.07 ± 0.33 (8)	4.25 ± 0.36 (8)	42.7 ± 3.8 (8)
2	Propranolol	—	179.6 ± 2.3 (7)	5.47 ± 0.35 (7)	4.57 ± 0.21 (7)	39.0 ± 3.8 (7)
3	Methimazole	—	159.6 ± 4.6† (7)	0.60 ± 0.06† (7)	0.21 ± 0.07† (7)	0.9 ± 0.8† (7)
4	Methimazole	+	161.6 ± 3.4† (7)	0.05 ± 0.01†‡ (7)	11.41 ± 1.39†‡ (7)	110.4 ± 13.6†‡ (7)
5	Methimazole + propranolol	+	156.9 ± 4.3† (7)	0.07 ± 0.01†‡ (7)	13.63 ± 1.18†‡ (7)	134.3 ± 9.8†‡ (7)

* Data are expressed as the mean ± S. E.: the numbers in parentheses = number of rats. Diet: propranolol 0.02%; methimazole 0.07%. T_4 injection: 4 μg/100 g daily s.c.

† $P < 0.001$ vs group 1.

‡ $P < 0.001$ vs group 3.

3, 4 and 5 were fed the chow to which methimazole (Koch-Light Laboratories, Colnbrook, England) was added to a concentration of 0.07% (w/w) by thorough mixing in a mechanical blender. Ten 40-mg tablets of propranolol (Ayerst Laboratories, Montreal, Quebec) were pulverized with a mortar and pestle, and then blended with 2 kg of chow alone (group 2) or chow plus methimazole (group 4) to yield a concentration of 0.02% (w/w). Food intake was not measured, but rats of this size consume at least 10 per cent of their body weight daily [17]. This would amount to a daily intake of 20 mg/kg of propranolol in group 2. Since rats in group 5 weighed less, one may assume that their daily intake of propranolol was at least 10 mg/kg.

Groups 4 and 5 (methimazole without and with propranolol) were injected daily with T_4. T_4 (Sigma Chemical Co., St. Louis, MO), 1 mg/ml. was prepared as described previously [18]; 0.4 ml was added to 19.6 ml of 0.1% human serum albumin in 0.9% NaCl to give a concentration of 20 μg/ml. Four μg (0.2 ml) per 100 g body weight was injected s.c. daily at 20.00 hr. Eight days and 12 hr after the last dose of T_4 (i.e. at 8.00 hr), the experiment ended. Simultaneously with the last dose of T_4, all rats received 0.01 μCi ^{131}I in 0.2 ml 0.9% NaCl i.p. The rats were anesthetized with ether and blood was drawn from the abdominal aorta. The thyroid glands, attached to a segment of trachea, were removed. Their radioactivity and that of the dose of ^{131}I administered were determined in a well crystal scintillation counter and the 12-hr thyroid ^{131}I uptake (per cent of dose) was calculated. Serum T_4 concentrations were measured by competitive protein-binding analysis [18] and T_3 by radioimmunoassay according to a slight modification of the method of Alexander and Jennings [19]. T_3 antiserum was purchased from Diagnostics Biochem Canada (London, Ontario); cross reactivity with T_4 was 0.03 per cent in our hands. The statistical probability of differences between means was assessed by Student's t test.

The data are summarized in Table 1. Propranolol did not influence body weight in rats receiving Purina chow alone or methimazole enriched chow and T_4. Methimazole caused a significant reduction in weight, which was unaltered by T_4 or T_4 and propranolol administration. Propranolol did not alter thyroid ^{131}I uptake. In methimazole-fed rats, ^{131}I uptake was reduced greatly and T_4 injection lowered it even further, but in the latter propranolol treatment had no effect. Serum T_4 and T_3 concentrations were similarly unaffected by propranolol. In methimazole-fed rats, hormone concentrations approached zero; injection of T_4, 4 μg/100 g/day, a dose about three times the daily production rate of T_4[20], raised T_4 and T_3 concentrations to three times the values in untreated controls, but again propranolol did not influence their levels.

The present study did not demonstrate an effect of propranolol on thyroid function, assessed by thyroid ^{131}I uptake and serum T_4 and T_3 assays in normal and chemically thyroidectomized rats. For the latter purpose we chose methimazole, which has no effect on the peripheral degradation of T_4 [21–23] or its deiodination to T_3 [23], as opposed to propylthiouracil which inhibits T_4 degradation [21, 23, 24] and blocks its deiodination to T_3 [23, 24]. By excluding any thyroid contribution to serum T_4 and T_3, within the limits of our experimental design, we were unable to show any affect of propranolol on peripheral thyroid hormone metabolism.

Our data agree with the rat studies of Azizi *et al.* [15] and Goulding *et al.* [16], which showed no effects of propranolol or serum T_4 concentrations in normal [15, 16] or hyperthyroid [16] rats or upon the peripheral metabolism of T_4 or thyroid uptake and distribution of ^{131}I in thyroid iodoprotein in normal rats [15]. We measured serum T_3 as well, and again propranolol had no effect on its concentration. The reports cited above [15, 16], as well as our own data, are at variance with the data of Tal *et al.* [14]. They showed striking elevations in serum T_4 after propranolol treatment in intact and hypophysectomized rats. The reasons for this discrepancy are not clear. In view of the short half-life of T_3 in the rat, it is possible that we missed small changes in T_4 to T_3 conversion, but this seems unlikely. In all previous rat studies [14–16], propranolol was administered i.p., while we gave it in the diet. However, in rats given ^{14}C-labeled propranolol orally or i.v., the excretory pattern of ^{14}C (urine and faeces) was similar, indicating that oral doses were well absorbed and that faecal ^{14}C was excreted via the bile [25].

Similarly, we cannot reconcile our data with the numerous reports that propranolol lowers serum T_3 in euthyroid, thyrotoxic, or hypothyroid T_4-treated individuals [8–12]. Most of these subjects received 160 mg propranolol daily. We estimate that the daily consumption in our rats was 10–20 mg/kg, which would be equivalent to 700–1400 mg daily in a 70 kg man. We realize, however, that this comparison may not be valid owing to species differences in drug metabolism [25]. The authors of the human studies [8–12] suggest that propranolol may inhibit the peripheral conversion of T_4 and T_3 and, indeed, in some reports [9, 11, 13] serum T_4 was elevated significantly during propranolol therapy. There could be a species difference in propranolol action, but it seems to us that such a basic drug effect should be apparent in all mammalian species. Of note are the early reports, without T_3 assays, showing no effect of propranolol on thyroid function in man [3–7].

In summary, propranolol did not influence thyroid ^{131}I uptake or serum concentrations of T_4 and T_3 in normal or methimazole-treated T_4-injected rats.

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McGill University Clinic, Royal Victoria Hospital, Montreal, Quebec, Canada H3A 1A1

CHARLES H. BASTOMSKY
KUN-TSAN LIN

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).

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

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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