SYNTHESIS OF POTENTIAL ANTICANCER AGENTS. II.[1] 6-MERCAPTO-9-β-D-RIBOFURANOSYLPURINE

Sir:

Although the antibacterial and antileukemic activities of 6-mercaptopurine are well known,[2,3] the mechanism of action of this drug is still not well understood. Since there is a distinct possibility that 6-mercaptopurine must be converted to its riboside or ribotide[4] before it becomes an effective drug, a number of workers have attempted the synthesis of 6-mercaptopurine riboside.[5,6]

For the synthesis of 6-mercaptopurine riboside, 6-chloropurine riboside[7] appeared to be a promising starting material. The reactivity of the chloro group of this compound was demonstrated by Brown and Weliky,[8] who treated 6-chloropurine riboside with ammonia at 100° to get a smooth conversion to adenosine in 86% yield. Since 6-chloropurine has been converted to 6-mercaptopurine by reaction with thiourea in ethanol,[9] the same reaction with 6-chloropurine riboside was the first one studied. In boiling ethanol, the reaction was quite rapid, and in spite of considerable cleavage to 6-mercaptopurine by the acid formed in the reaction, pure 6-mercapto-9-β-D-ribofuranosylpurine, micro m.p. 198–200° dec., could be isolated in low yield. The addition of calcium carbonate to the reaction mixture as an acid acceptor failed to improve the yields.

6-Mercaptopurine has also been prepared by reaction of 6-chloropurine with aqueous potassium hydrogen sulfide at 100° for seven hours.[10] The use of this reagent with 6-chloropurine riboside has the disadvantage that the imidazole moiety of nucleosides of this type is rapidly cleaved in the presence of aqueous alkali to give a 4-glucosylamino-5-formamido-6-chloropyrimidine.[11] However, under anhydrous conditions the 6-chloro group of a 6-chloropurine nucleoside can be replaced with methoxide without ring cleavage.[12] These observations suggested the use of methanolic sodium hydrogen sulfide as a reagent with which 6-chloropurine riboside might be treated without ring cleavage.

Anhydrous methanolic sodium hydrogen sulfide, prepared by saturating 10.5 ml of 1*N* methanolic sodium methoxide with hydrogen sulfide, was added to a refluxing suspension of 2.49 g. 6-chloro-9-β-D-ribofuranosylpurine in 40 ml. of methanol. The mixture was refluxed for 10 minutes, solution being complete in 7 minutes.[13] The solution was evaporated to dryness and the residue redissolved in 15 ml. of hot water and then acidified with acetic acid. On cooling, the solution deposited 2.00 g. (81%) of nearly pure 6-mercapto-9-β-D-ribofuranosylpurine, micro m.p. 196–201° dec. Recrystallization from 20 ml. of water afforded 1.70 g.(69%) of pure riboside as cream-colored needles; micro m.p. 198–200° dec., capillary m.p. 207–210° dec.; $[\alpha]^{23}D$ −73° (2.0% in 0.1 *N* NaOH); $\lambda_{max}^{pH\ 1}$ 322 mμ (a_M 22,500), $\lambda_{max}^{pH\ 6.7}$ 320 mμ (a_M 21,500), $\lambda_{max}^{pH\ 13}$ 310 mμ (a_M 22,130).[14] *Anal.* Calcd. for $C_{10}H_{12}N_4O_4S$: C, 42.2; H, 4.26; N, 19.7. Found: C, 42.2; H, 4.49; N, 19.8.

When tested against Adenocarcinoma 755 in the CBF_1 mouse, 6-mercaptopurine riboside profoundly inhibited tumor growth. The riboside administered at several dose levels was found to be equally as carcinostatic and equally as toxic as similar doses of 6-mercaptopurine on a molar basis.[15] Whether this compound is effective due to phosphorylation to the ribotide or due to enzymatic hydrolysis to 6-mercaptopurine and whether or not the riboside is effective against 6-mercaptopurine-resistant bacteria and leukemias is now under study in these laboratories.[15]

(1) For Paper I of this series cf. J. A. Montgomery, THIS JOURNAL, **78**, 1928 (1956). This work was supported by funds from the Kettering Foundation, the Sloan Foundation and the Black–Stevenson Foundation.

(2) G. B. Elion, G. H. Hitchings and H. VanderWerff, *J. Biol. Chem.*, **192**, 505 (1951).

(3) J. H. Burchenal, R. R. Ellison, M. L. Murphy, D. A. Karnofsky, M. P. Sykes, T. C. Tan, A. C. Mermann, M. Yuceoglu, W. P. L. Myers, I. Krakoff, and N. Alberstadt, *Ann. N. Y. Acad. Sci.*, **60**, 359 (1954).

(4) Preliminary experiments in this laboratory have shown that 6-mercaptopurine-8-C^{14}, when added to a growing culture of *Streptococcus faecalis* (ATCC 8043), is rapidly converted to a number of other compounds, as yet unidentified. On two dimensional chromatography several of these materials move to the same region of the chromatogram as do known nucleotides.

(5) The deoxy compound, 6-mercapto-9-(2′-deoxy-β-D-ribofuranosyl)-purine, has been synthesized enzymatically by M. Friedkin, *Biochim., Biophys. Acta*, **18**, 447 (1955).

(6) I. Goodman, G. B. Elion, and G. H. Hitchings, *Fed. Proc.*, **14**, 219 (1955), have synthesized 6-mercapto-9-β-D-glucopyranosylpurine in low yield, starting with 6-benzylmercaptopurine.

(7) Pure material was prepared in 28–30% yield by suitable modifications of the procedure of Brown and Weliky,[8] to be published.

(8) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953).

(9) A. Bendich, P. J. Russell, Jr., and J. J. Fox, THIS JOURNAL, **76**, 6073 (1954).

(10) G. B. Elion and G. H. Hitchings, THIS JOURNAL, **76**, 4027 (1954).

(11) Private communication from Drs. G. B. Brown and M. P. Gordon, Sloan-Kettering Institute, New York, New York. A similar observation has been made in this laboratory on treatment of 6-chloro-9-α-L-rhamnopyranosylpurine with aqueous sodium hydroxide (B. R. Baker and K. Hewson, unpublished results).

(12) B. R. Baker and K. Hewson, unpublished results.

(13) Inspection of the ultraviolet spectra of aliquots from a pilot run showed that the reaction was complete three minutes after all solid had dissolved. With longer reflux time, some decomposition of the mercapto compound takes place, as indicated by further changes in the ultraviolet spectrum.

(14) The 6-mercaptopurine riboside showed the following R_f values from ascending paper chromatography on 1-inch strips of Whatman No. 1 filter paper: R_f 0.64 in 0.1 *M* phosphate buffer, *p*H 6.9 R_f 0.62 in 5% ammonium sulfate–5% isopropyl alcohol in water.[5]

(15) F. M. Schabel, Jr., and H. E. Skipper, to be published.

(16) Affiliated with Sloan–Kettering Institute.

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ON THE MECHANISM OF ACTION OF PARATHORMONE[1]

Sir:

In the years since Dickens first reported a high bone content of citrate,[2] there has been a growing interest in the possibility of a relationship between calcium metabolism and serum citrate. A recent literature review[3] emphasizes the strong correlation between serum levels of the two ions and the re-

(1) This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

(2) F. Dickens, *Biochem. J.*, **35**, 1010 (1941).

(3) T. F. Dixon and H. R. Perkins, Chapter 11 in "The Biochemistry and Physiology of Bone," ed. by G. H. Bourne, Academic Press, New York, N. Y., 1956.

sponse of these levels to vitamin D and parathormone administration. However, no clear-cut mechanism for this relation has been suggested.

In the course of a series of investigations[4,5] on the solubility of the basic calcium phosphate system in the region of neutrality, convincing evidence was obtained that normal serum is supersaturated with respect to bone mineral. It was necessary, therefore, to postulate that a cellular mechanism (presumably under the control of the parathyroid glands) exists for maintaining a calcium ion gradient between blood and bone.

It was hypothesized that the cellular elements of bone normally secrete citrate (or citric acid) in response to parathyroid activity. This citrate carries complexed calcium to the serum where extraskeletal tissues (the kidney primarily[3]) oxidize much of the citrate leaving an excess of calcium ion in solution. If citrate were secreted as the acid, a local *p*H gradient would also contribute to the transport of ionized calcium to serum.

It was technically very difficult to test the hypothesis directly. However, by simply drilling a small hole in the spongiosa of the femur of an intact dog it was possible to collect blood directly from the spongiosal circulation for comparison with simultaneous arterial samples. While this sample was only "contaminated" with venous flow from the bone cells, the output of citrate from the bone was so great, clearcut analytical differences were easily shown. Furthermore, dramatic increases in citrate output from the bone were observed almost immediately following injection of parathyroid extract. Simultaneous measurements of the clearance of intravenously administered radiostrontium gave a measure of the proportion of collected sample which was actually venous flow from bone. A typical experiment is summarized in Fig. 1.

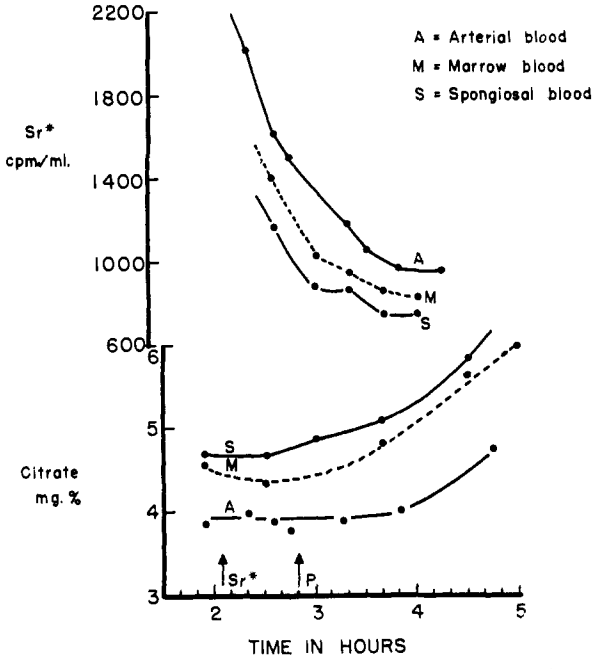

Fig. 1.—Curves showing the citrate production by bone and its response to parathyroid extract injection (arrow P, 1000 units, Eli Lilly Co.). The upper curves show the clearance of carrier-free radiostrontium injected at arrow Sr*). Note that the citrate level is inversely related to the clearance of radiostrontium indicating bone as the source of the citrate. Mixed venous blood from the general circulation, drawn at intervals throughout the experiment, exhibit citrate levels slightly below those of arterial blood.

(4) G. J. Levinskas and W. F. Neuman, *J. Phys. Chem.*, **59**, 164 (1955).

(5) B. Strates, W. F. Neuman and G. J. Levinskas, in preparation.

This direct substantiation of the initial hypothesis was given strong support by enzyme studies *in vitro*. It was found in confirmation of Dixon and Perkins[3] that mature bone lacks isocitric dehydrogenase which is needed for citrate utilization. Furthermore, parathyroid extract was shown spectrophotometrically to destroy the chromophoric group (340 mμ) of reduced Coenzyme II *in vitro* rendering it practically non-absorbent. The nature of this destruction is at present unknown, but consultation of current biochemical texts shows that blocking Coenzyme II-linked reactions shunts all of glucose metabolism ultimately to citrate production. This provides an intriguing biochemical mechanism by which a parathyroid-controlled citrate gradient maintains a steady but supersaturated level of ionized calcium in serum.

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ON THE CONFIGURATION OF CEVINE

Sir:

Evidence is advanced herewith for revision of the configuration at C_{16} in the generally-accepted formulation of cevine.[1,2] The argument also provides a firm basis for assignment of the configuration at C_{20} of cevine which we now propose be represented by formula I.

The susceptibility of the C_{16} acetate esters to base-catalyzed[3] methanolysis—a phenomenon which was regarded as presumptive evidence favoring the α(equatorial)-configuration[1,2]—appeared to us to be abnormally high even for an equatorial ester. This rate of methanolysis, indeed, was found to exceed that of an *unhindered* equatorial ester.[4] In the germine series the C_7 acetate, which is undoubtedly α(axially)-oriented,[5] exhibits comparable reactivity.[6] This behavior prompted us to postulate a facilitation of methanolysis by a hydroxyl group (the α-oriented C_{14} hydroxyl in this

(1) D. H. R. Barton, O. Jeger, V. Prelog and R. B. Woodward, *Experientia*, **10**, 81 (1954).

(2) D. H. R. Barton, C. J. W. Brooks and P. de Mayo, *J. Chem. Soc.*, 3950 (1954).

(3) W. J. Rosenfelder, *ibid.*, 2638 (1954).

(4) Whereas cevadine-D-orthoacetate 4,16-diacetate (A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **35**, 1942 (1952)) underwent methanolysis in 75% yield after 20 hours in dilute methanol, epiandrosterone 3-acetate was recovered largely (75%) unchanged after similar treatment in the presence of cevine to serve as the base (see ref. 3).

(5) S. M. Kupchan and C. R. Narayanan, *Chemistry and Industry*, in press.

(6) *E.g.*, the facile methanolysis of neogermitrine, J. Fried, P. Numerof and N. M. Coy, THIS JOURNAL, **74**, 3041 (1952).