py. Pulmonary symptoms were considered in cases where they appeared to mask gastrointestinal symptoms.
24. F. R. Smith, B. A. Underwood, C. R. Denning, A. Varma, *J. Lab. Clin. Med.* **80**, 423 (1972).
25. D. N. Holvey, Ed., *The Merck Manual of Diagnosis and Therapy* (Merck, Rahway, N.J., 1972), p. 739.
26. J. I. Kessler, J. Stein, D. Dannacker, P. Narcessian, *J. Biol. Chem.* **245**, 5281 (1970); R. M. Glickman and K. Kirsch, *J. Clin. Invest.* **52**, 2910 (1973); R. M. Glickman, J. Khorana, A. Lilgore, *Science* **193**, 1254 (1976).
27. R. B. Elliott and P. G. Robinson, *Arch. Dis. Child.* **50**, 75 (1975); R. B. Elliott, *Pediatrics* **57**, 474 (1976); M. L. Rosenlund, J. A. Selekman, H. K. Kim, D. Kritchevsky, *ibid.* **59**, 428 (1977).
28. This work was supported in part by NIH grants HL-18574-01, AG 05046-01, and RR 05467 and the U.S. Energy Research and Development Administration. Control subject data were kindly made available by R. M. Burton and D. Goldring, Washington University Medical School, St. Louis, from a screening study of normal school children. We thank S. Pan for the computer analysis of data, G. L. Adamson for technical assistance, and R. Krauss and A. Nichols for their critical review of the manuscript.

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Saturnine Gout: Lead-Induced Formation of Guanine Crystals

Abstract. *Intravenous injection of a sublethal dose of lead acetate into a domestic pig resulted in a 4.5-fold increase of guanine in the urine, indicating an impairment in the conversion of guanine to xanthine. This impairment is probably due to the inhibition of guanine aminohydrolase (guanase), since the activity of this enzyme is inhibited by Pb^{2+} (the inhibition constant being 3.0 × 10^{-6}M). Postmortem histological examination revealed concretions of crystalline material in the epiphyseal plate of the femoral head. Extraction of the section containing the concretions showed that they were guanine. The relation of these findings to saturnine gout is discussed.*

That there is a relation between lead intoxication and gout has been known for more than a century (*1*), and a few clinical studies that distinguish saturnine from typical gout have been published (*2, 3*). Saturnine gout differs from typical gout in that (i) it occurs earlier in life, and is not hereditary; (ii) more women are afflicted, and often before menopause; (iii) acute attacks are less frequent; and (iv) often there is a history of lead intoxication in childhood. Other studies have shown that attacks of arthritis in saturnine gout are not well correlated with blood uric acid levels and that some precursor of uric acid may be involved (*4*). These observations suggest that saturnine gout and typical gout differ at the molecular level. Since saturnine gout is still present in modern times (*5*), we undertook a study of the etiology of the disease.

We found that the enzyme guanine aminohydrolase (guanase), which catalyzes the hydrolytic deamination of guanine to xanthine, was exquisitely sensitive to Pb^{2+}. Guanine is a highly insoluble purine, and the possibility that saturnine gout was caused by accretions of guanine resulting from inhibition of guanase was explored. The inhibition of guanine by Pb^{2+} is noncompetitive, and has a K_i of 3.0 × $10^{-6}M$. At 1.0 × $10^{-6}M$, the degree of inhibition was 36 percent; and, at 1.0 × $10^{-5}M$, it was 88 percent. By comparison, xanthine oxidase was not inhibited by Pb^{2+} when tested in concentrations up to 4.0 × $10^{-5}M$.

In order to test the hypothesis that Pb^{2+} causes accumulation of guanine in vivo, we chose the pig as our animal model because of its low reserve of guanase (*6, 7*). A Yorkshire sow (3½ years old) weighing 180 kg, in excellent health with no apparent joint pathology, was catheterized, and urine samples were collected prior to and after intravenous injection of 11 ml of 1*M* lead acetate dissolved in isotonic glucose. Urine samples, collected at 24 and 48 hours after injection, were analyzed for guanine and for creatinine. The guanine values were normalized to the creatinine concentration to correct for diurnal fluctuations in urine output. The guanine concentration rose 4.5-fold in 24 hours and remained elevated after 48 hours (Table 1).

After the 48-hour-period the pig was killed and immediately exsanguinated; the femoral head was then prepared for histological examination. Examination of the epiphyseal plate revealed crystalline accretions (Fig. 1). The accretions were needles with approximate dimensions of 3 by 30 μm having a round nodule at one end. The bone segment containing the crystals was deparaffinized and extracted with 0.1*M* HCl; and a small amount of [^{14}C]guanine was added as marker. The HCl extract was purified by a method similar to that used above for quantifying guanine in urine. The final product showed a spectrum identical to that of guanine with a peak at 249 nm and a shoulder at 270 nm. The absorption ratios at 280 : 260 and 250 : 260 nm were 0.87 and 1.31, respectively, which compares favorably with the values of 0.84

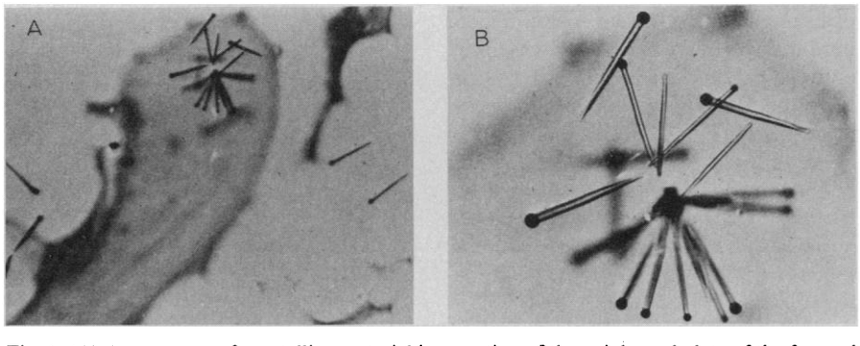

Fig. 1. (A) Appearance of crystalline material in a section of the epiphyseal plate of the femoral head. The proximal and distal epiphyseal plate were processed for histological examination as described by Gomori (*8*). (B) Close-up of the largest crystal cluster.

Table 1. Effect of Pb^{2+} on guanine concentration in urine. One hundred milliliters of urine were acidified to 0.01*M* with 12*M* HCl, and 3 nmole of [^{14}C]guanine (40 mCi/mmole) was added for monitoring guanine during purification and for calculating the recovery of guanine. The acidified urine was passed through a Dowex 50 H^+ column (2.5 by 40 cm) and washed with water until absorbance of the eluate dropped below 0.05. The guanine was then eluted with 1.5*N* HCl (310 to 400 ml). The guanine was concentrated by flash evaporation, and the *p*H was adjusted to 9.0; the sample was then adsorbed to a Dowex 1-formate column (1.5 by 13 cm). The column was washed with water, and the guanine was eluted with 1*M* formic acid (48 to 69 ml). The sample was taken to dryness and dissolved in 0.01*M* HCl. The guanine was readsorbed to Dowex 50 H^+ and eluted with a linear gradient (0 to 3*M* HCl). Guanine concentration was calculated from its absorbance at 249 nm. The creatinine concentration was determined in the clinical laboratories of The University of Tennessee Memorial Hospital. Recovery of guanine was of the order of 50 percent.

Time	Guanine (μmole/100 ml of urine)	Creatinine (mg/100 ml of urine)	Guanine (μmole/mg of creatinine)
Before injection	6.4	135	0.047
24 hours after Pb^{2+}	27.2	125	0.218
48 hours after Pb^{2+}	22.2	155	0.143

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and 1.37 for authentic guanine. When the enzyme guanase was added to the sample, the guanine peak and shoulder disappeared, and a single peak at 277 nm, characteristic of xanthine, was observed.

Additional proof that the compound extracted from the femoral head was guanine was obtained by comparing the fluorescence spectrum of the sample with that of authentic guanine. From 5 g of femoral head 100 μg of the guanine material was obtained. Similar sections that did not contain crystals were also extracted from the same animal, but no guanine was obtained. From a local meat-packing plant we obtained bones from healthy pigs of comparable age and processed the bones in an identical manner; no accretions of guanine were found. These data and observations are consistent with the hypothesis that Pb^{2+} inhibited the enzyme guanase and caused an increase of guanine concentration in vivo that resulted in the crystallization of this insoluble purine in the joints.

Finally, accretions of guanine have been described by Mendelson in swine tissue as a very rare occurrence. Investigators in the veterinary sciences who have attempted to repeat Mendelson's observation by examining large amounts of normal material obtained at slaughterhouses have been unsuccessful (*7*). It is possible that the meat products from animals in which guanine crystals have been observed were from animals suffering from lead poisoning, possibly due to ingestion of lead-based paint.

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References and Notes

1. A. B. Garrod, *Med. Chic. Tr.* **37**, 181 (1859); A. Magnus Levy, *Z. Klin. Med.* **36**, 353 (1898).
2. G. Lormier, *Br. Med. J.* **2**, 163 (1898).
3 .B. T. Emmerson, *Arthritis Rheum.* **11**, 623 (1968).
4. G. D. Ludwig, *Arch. Intern. Med.* **100**, 803 (1957); A. B. Gutman, *Ann. Intern. Med.* **39**, 1062 (1953).
5. G. V. Ball and L. B. Sorensen, *N. Engl. J. Med.* **280**, 1199 (1969).
6. W. Jones and C. R. Austrian, *Z. Physiol. Chem.* **48**, 110 (1906).
7. W. Mendelson, *Am. J. Med. Sci.* **95**, 109 (1888).
8. G. Gomori, in *Histochemistry Principles and Practice* (Univ. of Chicago Press, Chicago, 1972), vol. 40, pp. 120–136 and 162–165.
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Human Breast Cancer: Androgen Action Mediated by Estrogen Receptor

Abstract. *Growth of the human breast cancer cell line MCF-7 is enhanced by androgens, but only at pharmacological concentrations. Although physiological concentrations of androgens translocate the androgen receptor into the nucleus, no mitogenic effects are observed. By contrast, pharmacological androgens translocate not only the androgen receptor but also the estrogen receptor, and at these high doses significantly increase both DNA and estrogen-dependent protein synthesis. We therefore propose that androgens stimulate MCF-7 cell growth not through the androgen receptor but rather through the estrogen receptor.*

Lippman *et al.* (*1*) recently reported that the growth of MCF-7 human breast cancer cells is stimulated by androgens and concluded that this response is mediated by an androgen receptor. Since androgens are normally used to induce tumor regression in breast cancer patients, it is important to determine why the opposite effect is observed in human breast cancer cells in culture.

The requirement for very high pharmacological doses of androgen to stimulate MCF-7 cell growth provided a clue that some mechanism other than an androgen receptor might be responsible. It had been reported (*2*) that in the rat uterus pharmacological concentrations of androgens interact not only with androgen receptors but also with estrogen receptors, resulting in translocation of estrogen receptors into the nucleus. If pharmacological concentrations of androgens similarly affect the estrogen receptors in MCF-7 tumor cells this would provide a mechanism for the observed growth stimulation, since physiological doses of estrogen stimulate MCF-7 cell growth (*3*, *4*). We therefore in-

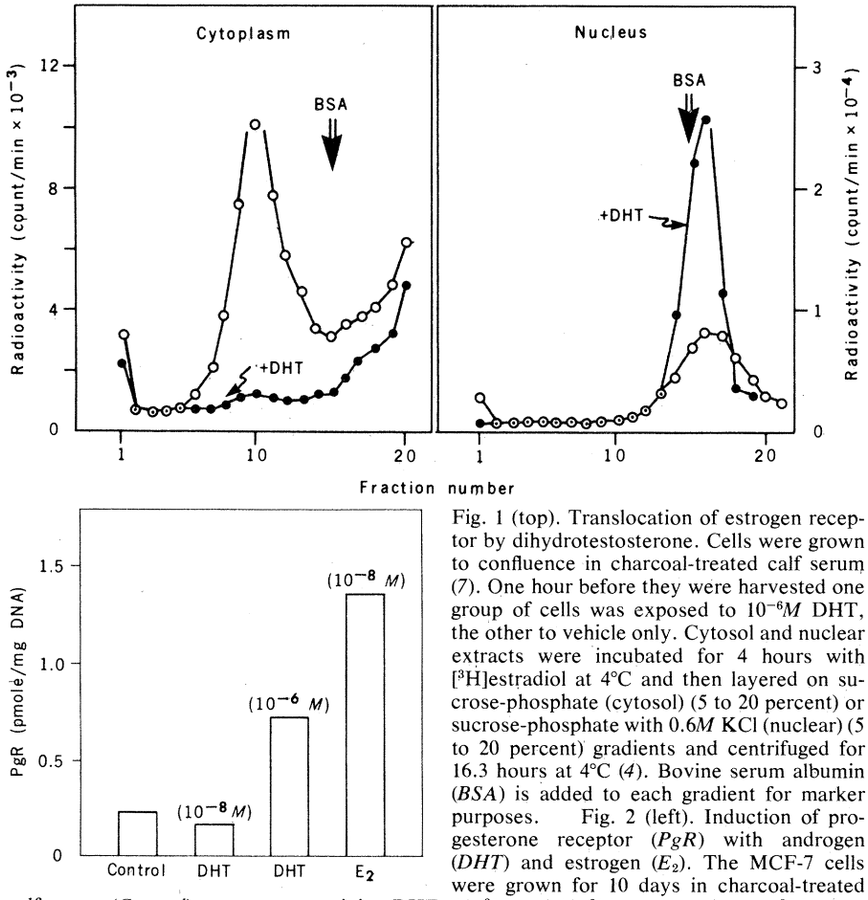

Fig. 1 (top). Translocation of estrogen receptor by dihydrotestosterone. Cells were grown to confluence in charcoal-treated calf serum (*7*). One hour before they were harvested one group of cells was exposed to $10^{-6}M$ DHT, the other to vehicle only. Cytosol and nuclear extracts were incubated for 4 hours with [^{3}H]estradiol at 4°C and then layered on sucrose-phosphate (cytosol) (5 to 20 percent) or sucrose-phosphate with 0.6*M* KCl (nuclear) (5 to 20 percent) gradients and centrifuged for 16.3 hours at 4°C (*4*). Bovine serum albumin (*BSA*) is added to each gradient for marker purposes. Fig. 2 (left). Induction of progesterone receptor (*PgR*) with androgen (*DHT*) and estrogen (E_2). The MCF-7 cells were grown for 10 days in charcoal-treated calf serum (*Control*) or serum containing DHT ($10^{-8}M$ and $10^{-6}M$) or estradiol ($10^{-8}M$). Progesterone receptor was measured by means of [^{3}H]R5020 (a synthetic steroid) and both the protamine sulfate and dextran charcoal methods previously described (*7*, *8*). Briefly, cytosol was incubated for 4 hours with [^{3}H]R5020 at 4°C and then treated either with protamine sulfate or dextran charcoal. No quantitative differences were noted between the two assays. Values represent the mean of duplicate determinations for the dextran charcoal assay.

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