Serum Lipoprotein Concentrations in Cystic Fibrosis

Abstract. *Two major classes of lipoproteins, low density and high density, are decreased in the serum of patients with cystic fibrosis; major apoproteins are also decreased. Since essential fatty acids and certain fat-soluble vitamins depend on lipoproteins for transport in the serum, knowledge of lipoprotein levels in cystic fibrosis patients could prove valuable in understanding (i) the basis for the abnormally low serum levels of these fatty acids and vitamins and (ii) the effects of therapies involving these molecules.*

Cystic fibrosis (CF) is an inherited disease which, among Caucasians, occurs once in 2500 live births (*1*). Fifty percent of CF patients die before 21 years of age (*2*). Although the molecular basis of CF is unknown, studies of blood serum from CF patients have revealed low concentrations of cholesterol (*3*) and linoleate (an essential fatty acid) (*4*). Symptoms of vitamin deficiencies (A, K, and E) have also been reported in CF patients (*5*). A common denominator to each of these observations is the fact that cholesterol, linoleate, vitamins K and E, and β-carotene, a precursor to vitamin A, all require lipoprotein for transport to various tissues (*6*).

We now report that two major classes of serum lipoproteins are significantly lower in CF patients (*7*) than in controls (*8*). Both low density (LDL) and high density (HDL) lipoprotein concentrations were decreased in CF patients.

Four different, nonsequential methods were used in our study. Electrophoresis on sodium dodecyl sulfate (SDS)–polyacrylamide gel was used to study serum proteins and the major apoproteins of HDL and LDL. Agarose gel electrophoresis, analytical ultracentrifugation, and refractometry were used to measure the concentrations of LDL, HDL, and very low density (VLDL) lipoproteins.

High resolution electrophoresis on SDS-polyacrylamide gel (*9*) was used to compare serum proteins of CF patients with those of controls. Gel patterns and densitometer scans are shown in Fig. 1. In this type of gel, protein migration distance is a function of protein molecular weight (*10*). We observed that the visual intensities of two bands, a high-molecular-weight band (250,000 daltons, apo B in Fig. 1) and a 28,000-dalton band (apo A-1 in Fig. 1), were consistently lower in patterns from CF patient serums. Similar molecular weights have been reported for the major apoproteins within LDL and HDL complexes (*11*). Purified fractions of HDL and LDL were prepared (*12*). We confirmed our identification of the two whole serum bands by running proteins of the HDL and LDL fractions in the same slab of gel as the whole serum proteins. The major apoproteins of LDL and HDL, apo B and apo A-I, respectively, had migration rates identical to the whole serum bands which differed between patient and control (Fig. 1). Comparison of the densitometer scans of the four gel patterns in Fig. 1 confirmed that the apo B and apo A-I peaks were lower in the CF patients than in the controls (*13*). No other CF gel band showed a consistent difference from normal, suggesting that one major difference between CF and normal serum involves lipoprotein concentrations. Lipoprotein concentrations of individual CF patients are shown in Table 1. We standardized the agarose data by comparing the concentrations of lipoproteins on agarose to those obtained by analytical ultracentrifugation of portions of the same serum (*14*); a high correlation was found between individual lipoprotein values derived from agarose gels and the ultracentrifugation—the correlation coefficient (r) for LDL was 0.91; for VLDL, 0.94; for HDL, 0.85. We then confirmed the standardization technique by conducting refractometry studies of the LDL's (*15*). Total cholesterol and total triglyceride levels were also determined (*16*).

The values for LDL's and HDL's determined with agarose gel electrophoresis were significantly lower ($P < .01$, F test) in CF patients than in controls (Table 1). The LDL and HDL values from each patient were assigned a percentile rank, which was determined by comparing a patient's value to histo-

Table 1. Individual serum lipoprotein concentrations in CL patients and mean lipoprotein concentrations in adolescent controls. Clinical data for CF patients are also shown. Abbreviations: LDL, VLDL, and HDL are low density, very low density, and high density lipoproteins, respectively; GI, ratings of gastrointestinal problems in order of increasing severity (*23*); V, multivitamins including vitamins A, K, and E; EN, pancreatic enzymes; A, antibotics; B, bile salts; S, anabolic steroids; M, male; F, female; N, total number. Group values are means ± standard deviation. A multiple comparison F test was used to compare control and normal values. The LDL and HDL values were ranked compared to control group values.

Patient	Lipoprotein concentration (mg/dl)					Clinical data				
	LDL	Rank (%)	VLDL	HDL	Rank (%)	Sex	Age (years)	Height and weight percentiles	GI	Therapy
3	188	2.4	120	221	4.1	M	2	3/3	1	V,EN,A
4	302	46	155	135	0	F	3	10/10	2	V,EN
5	150	0	130	223	4.2	M	7	25/60	3	V,EN
6	210	6.9	74	257	7.2	F	9	30/30	1	V,EN,A
7	271	33	142	242	5.6	F	9	3/1	1	V,EN
8	308	49	158	257	7.2	F	9	3/1	1	V,EN
9	242	17	178	187	1.8	M	9	10/10	1	V,EN,B
10	203	5	22	206	3.1	M	13	10/1	2	V,EN,A,S
11	281	39	38	92	0	M	13	10/10	1	V,S
2	198	3.5	44	197	2.5	M	15	60/30	3	V,EN,A
12	141	0	100	265	8.4	F	15	40/20	2	V,EN,A
1	194	2.8	21	136	0	M	16	30/10	4	V,EN,B,S
13	133	0	46	265	8.4	M	16	60/25	4	V,EN,A
14	245	19	132	195	2.3	M	20	30/50	1	V,A
15	267	31	26	183	1.5	M	28	3/3	1	V,EN,A
$N = 15$	222 ± 57		92 ± 56	204 ± 52			12.3 ± 6.6			
Controls $N = 33$	325 ± 86*		76 ± 47	380 ± 87*			14.4 ± 1.4			

*$P < .01$.

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grams of control group values. Six out of 15 patients had either an LDL or HDL value lower than in any of the 33 controls. All HDL levels in the patients ranked in the lower tenth percentile.

Slightly higher VLDL values were observed in the CF patients than in the controls. This is in agreement with higher triglyceride levels in the CF group (108 ± 52 as opposed to 57 ± 34 mg/dl), since VLDL's are a major carrier for serum triglycerides (*6*). Chylomicron concentrations in nonfasted CF patients and the fasted controls were low, as indicated by very low staining at agarose gel origins in both groups.

Table 2. Mean lipoprotein concentrations in CF carriers and adult controls.

Subject	*N*	Lipoprotein concentration (mg/dl)		
		LDL	VLDL	HDL
Male adult controls	40	416 ± 83	119 ± 65	243 ± 83
Male CF carriers	4	391 ± 69	174 ± 54	257 ± 70
Female adult controls	40	359 ± 93	67 ± 38	348 ± 113
Female CF carriers	5	448 ± 81	106 ± 28	310 ± 93

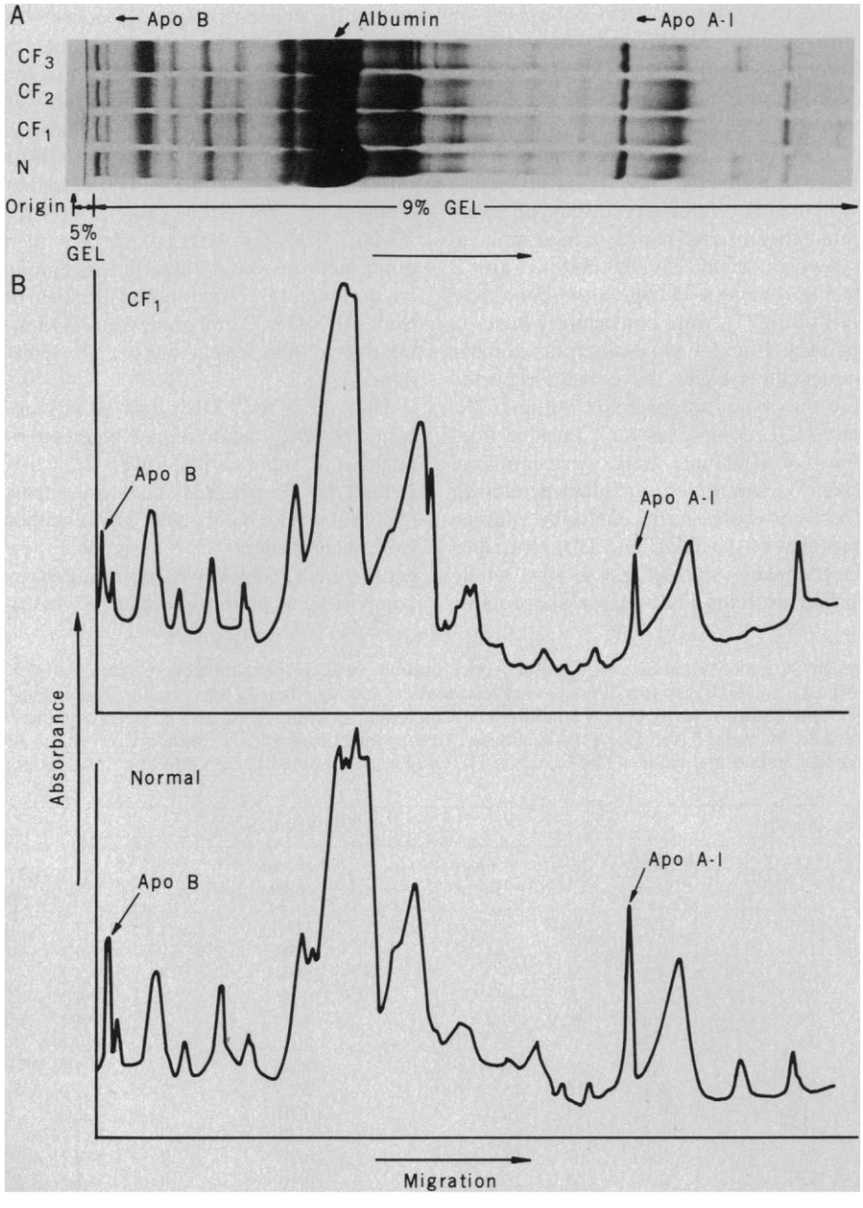

Fig. 1. (A) Gel patterns of serum proteins from three patients (CF_1, CF_2, CF_3), and one normal control (N). (B) Densitometer scans for normal control and CF_1. Total gel length of the 9 percent separating gel was 28 cm. Serum (100 μl) was dissolved in 2 ml of 4 percent SDS, 5 percent glycerol, 5 percent β-mercaptoethanol, 0.064*M* tris-HCl, *p*H 6.8; 20 μl of this solution was used for electrophoresis (*9*). Approximately 70 μg of protein were loaded into each gel slot. Densitometer scans were obtained with a reflectance densitometer (model RFT, Transidyne). Relative peak areas for apo B and apo A-I were determined (*13*).

Cholesterol concentrations in our CF patients were 34 percent lower (121 ± 22 as opposed to 182 ± 29 mg/dl) than in controls (Table 1), a finding that confirms earlier reports (*3*). Since cholesterol values show little variation between the ages of 5 and 20 years (*17, 18*), the slight age differences between CF patients and controls are probably not a determining factor here. Also, we found no correlation between cholesterol and age in either the patient or the control group.

While it appears from our findings that a decrease in lipoprotein concentrations is characteristic of CF patients, we also examined other factors such as a difference in age, sex, diet, liver, and intestinal function for their possible contributions to the depressed LDL and HDL concentrations in CF patients.

The LDL concentrations did not correlate with age in either group, even with the 2-, 3-, and 28-year-olds included in the CF group. The HDL levels, in contrast to an absence of correlation with age in the CF subjects, correlated inversely ($r = -0.41, P < .05$) with age in the controls. Therefore, the higher mean age in the controls would tend to minimize HDL differences between the two groups. Other studies have found only minor variations in LDL and HDL values in subjects between 5 and 20 years of age (*17*). Therefore, it is very unlikely that the differences in lipoprotein levels between CF patients and controls are due to age differences.

Within our group of CF patients, we found no significant sex-related difference in lipoprotein levels; male and female CF values were therefore pooled for calculation of means (Table 1). In our adolescent control group, only the HDL values were significantly different between the sexes (males, 346 mg/dl; females, 431 mg/dl). This sex-related difference in HDL values within the control group does not change the conclusion that HDL values are depressed (204 mg/dl) in CF patients.

Autopsies of CF patients indicate fatty infiltration of the liver in many cases (*19*). To check whether our CF patients had signs of liver malfunction, levels of bilirubin and four specific serum proteins were examined in 10 of the 15 patients (*20*). Our results indicated (*20*) that five of the CF patients had all test values within the normal ranges, suggesting that the decreased concentrations of LDL

and HDL, seen in these and other CF patients, are probably not due to liver malfunction.

Primary malnutrition (inadequate intake of nutrients) as a basis for depressed LDL and HDL levels in CF patients can be excluded since patients were not on restricted diets and received supplements of multiple vitamins and pancreatic enzymes. In addition, HDL's showed no correlation with height or weight percentiles (Table 1). The LDL's correlated inversely with the height percentile ($r = -0.67$, $P < .01$)—the opposite to what one would expect if low levels of LDL were due to malnutrition.

Many CF patients have steatorrhea (fatty stools). Since low fat diets can reduce steatorrhea, CF patients might tend to eat less fat and more carbohydrates. Studies have shown that individuals on high carbohydrate (90 percent), low fat (10 percent protein, zero fat) diets have increased triglyceride and VLDL levels and depressed cholesterol, LDL, and HDL levels (*21*). Similarly, we found elevated triglyceride and VLDL levels and depressed cholesterol, LDL, and HDL levels in CF patients. The CF patients, however, were not placed on low fat diets and received enzymatic supplements which included lipases to aid fat digestion. Malabsorption, rather than diet, may be an important influence on CF lipid and lipoprotein levels. In studies of patients with various forms of malabsorption (on diets with 70 g of fat per day) very similar shifts have been reported in triglycerides, VLDL, total cholesterol, LDL, and HDL compared to controls (*22*). In the malabsorption studies cited (*22*), malabsorption was measured by fecal fat excreted, which correlated inversely with LDL levels in male malabsorbers ($r = -0.61$, $P < .05$). While we did not measure fecal fat in CF patients, gastrointestinal (GI) problems (including symptoms of malabsorption) were graded 0 to 4 in order of increasing severity (*23, 24*). Scores for individual patients are listed in Table 1 along with the therapy we instituted. In CF males, LDL and total cholesterol correlated inversely with GI scores ($r = -0.77$, $P < .01$; $r = -0.88$, $P < .001$, respectively). Since GI problems included malabsorption symptoms and since LDL values correlated inversely with the GI scores, low LDL levels may relate to malabsorption in the CF male. In general, the findings of low levels of LDL are in accord with malabsorption symptoms because (i) LDL is the major carrier for cholesterol and β-carotene (*6*), and (ii) decreased levels of cholesterol and β-carotene are indicators of possible malabsorption (*25*). In addition, normal lipid absorption appears to require apo B, which is synthesized and found in intestinal epithelial cells (*26*); therefore, from the decreased concentrations of apo B in the serum of male CF patients (Fig. 1), one could again argue that malabsorption is possible.

We found no correlations between HDL levels in CF patients and their clinical symptoms. However, HDL levels were uniformly depressed (lower tenth percentile) in all CF patients, in spite of great individual variations in growth rates, clinical symptoms, and therapies. Further research will be needed to determine whether these low HDL levels are indeed characteristic of CF.

The serum lipid and lipoprotein levels of nine CF carriers (heterozygotes) were found to be within the normal adult range (Table 2). Since CF carriers are free of any symptoms, the decreased cholesterol, LDL, and HDL concentrations seen in CF patients may be related to the phenotypic expression of the disease rather than some benign characteristic of CF heterozygotes and homozygotes.

Knowledge of lipoprotein concentrations in the CF patient, irrespective of the cause of depressed LDL and HDL, may prove important in CF therapy. For example, CF patients have been treated both intravenously and orally with preparations rich in linoleate (*27*). Since linoleate requires lipoproteins for transport in the serum, knowledge of lipoprotein concentrations could provide a better understanding of the effects of linoleate therapy. For the same reason, such knowledge could also help in evaluating vitamin A, K, and E therapy in CF patients.

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References and Notes

1. D. M. Danks, J. Allan, C. M. Anderson, *Ann. Hum. Genet.* **28**, 323 (1965).
2. P. A. DiSant'Agnese and P. B. Davis, *N. Engl. J. Med.* **295**, 597 (1976); *ibid.*, p. 481.
3. H. F. Wiese, M. J. Bennet, I. Braun, W. Yamanaka, E. Coon, *Am. J. Clin. Nutr.* **18**, 155 (1966); P. T. Kuo and N. N. Huang, *J. Clin. Invest.* **44**, 1924 (1965).
4. M. L. Rosenlung, H. K. Kim, D. Kritchevsky, *Nature (London)* **251**, 719 (1974); R. Caren and L. Corbo, *J. Clin. Endocrinol. Metab.* **26**, 470 (1966).
5. C. C. Lobeck, *The Metabolic Basis of Inherited Disease* (McGraw-Hill, New York, 1972), pp. 1605–1626.
6. V. P. Skipski, in *Blood Lipids and Lipoproteins*, G. J. Nelson, Ed. (Wiley, New York, 1972), pp. 471–583; R. J. Kutsky, *Handbook of Vitamins and Hormones* (Van Nostrand Reinhold, New York, 1973), p. 36; N. I. Krinsky, D. G. Cornwell, J. L. Oncley, *Arch. Biochem. Biophys.* **73**, 233 (1958); E. C. McCormick, D. G. Cornwell, J. B. Brown, *J. Lipid Res.* **1**, 221 (1960).
7. With each CF patient, the diagnosis was based on abnormally high concentrations of Na^+ and Cl^- in the sweat (determined with the use of pilocarpine and ionophoresis), and a clinical picture compatible with the disease. The CF patients (homozygotes) were all ambulatory and did not rigidly observe low fat diets. Serum was taken from nonfasting individuals.
8. Controls were fasting high school student volunteers from the St. Louis, Mo., area and included 20 males and 13 females. Determining the ideal control group for CF patients is difficult: CF patients are often burdened with different degrees of lung and gastrointestinal problems leading to slower growth and development so that chronological age matching is of questionable importance. Therapy with different combinations of antibiotics, anabolic steroids, and digestive enzymes compound the difficulty of finding suitable controls.
9. W. J. Vaughan and M. Calvin, *Gerontology* **23**, 110 (1977); G. Ferro-Luzzi Ames, *J. Biol. Chem.* **249**, 634 (1974).
10. K. Weber and M. J. Osborn, *J. Biol. Chem.* **244**, 4405 (1969).
11. J. D. Morrisett, R. L. Jackson, A. M. Gotto, *Annu. Rev. Biochem.* **44**, 879 (1975).
12. F. T. Lindgren, L. C. Jensen, F. T. Hatch, in *Blood Lipids and Lipoproteins*, G. J. Nelson, Ed. (Wiley, New York, 1972), pp. 181–274.
13. Relative peak areas (percentage of control) determined by reflectance scanning densitometry for apo B are 59, 72, 70, for apo A-1 are 52, 67, 71, and for CF patients 1, 2, and 3, respectively. These values are peak areas and are not a measure of quantitative differences in apo B and apo A-I in various patterns. Rather, our intention is simply to demonstrate that these protein levels are depressed in CF serums. We make the assumption that proteins other than apo B and apo A-1 that may also comigrate are of insignificantly low concentrations.
14. F. T. Hatch *et al.*, *J. Lab. Clin. Med.* **81**, 946 (1973).
15. Total LDL values obtained with analytical ultracentrifugation can be checked by the refractometry of total LDL fractions isolated by preparative centrifugation, provided that chylomicron levels are low [see (*12*)]. For example, the mean total LDL concentrations of CF patients as determined by ultracentrifugation (sum of VLDL and LDL in Table 1) was 254 ± 94 (± standard deviation), compared with the refractometry value of 289 ± 100. Also, a high correlation ($r = 0.83$) was found for individual total LDL concentrations determined by these two methods.
16. Total cholesterol was determined by using Liebermann-Burchard reagent directly on serum (Hycel). Triglycerides were determined with a Gilford 3500 autoanalyzer.
17. J. Dyerbery and N. Hjörne, *Scand. J. Clin. Lab. Invest.* **31**, 473 (1973); S. R. Srinivasan, R. R. Frerichs, L. S. Webber, B. Berenson, *Circulation* **54**, 309 (1976).
18. B. C. Johnson, F. H. Epstein, M. O. Kjelsberg, *J. Chronic Dis.* **18**, 147 (1965); A. Lopez-S, W. A. Krehl, R. E. Hodges, *Am. J. Clin. Nutr.* **20**, 808 (1967).
19. E. H. Oppenheimer and J. R. Esterly, in *Perspectives in Pediatric Pathology*, H. S. Rosenberg and R. P. Bolande, Eds. (Year Book, Chicago, 1975), vol. 2, pp. 241–278).
20. Concentrations of globulin, glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase, and lactic dehydrogenase, as measured by SMAC (Technicon), were measured in CF patients 1, 2, 5, 6, 9, 10, 11, 12, 13, and 15. The values found were within the normal range for patients 1, 6, 9, 11, and 12.
21. D. E. Wilson, and R. S. Lees, *J. Clin. Invest.* **51**, 1051 (1972).
22. G. R. Thompson and J. P. Miller, *Clin. Sci. Mol. Med.* **45**, 583 (1973).
23. Evaluation of the severity of the gastrointestinal problems in the CF patients at the time of plasma sampling was done by a single investigator (J.B.W.). This investigator used a grading system similar to one devised by Smith *et al.* (*24*), in which problems were graded 0 to 4 in order of increasing severity. At the time of grading, the investigator had no knowledge of the lipoprotein levels. Scores reflected the degree of appetite, abdominal cramps and pain, abdominal distention, the number of stools per day at initial diagnosis, steatorrhea, and the response to diet fats after pancreatic enzyme replacement thera-

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

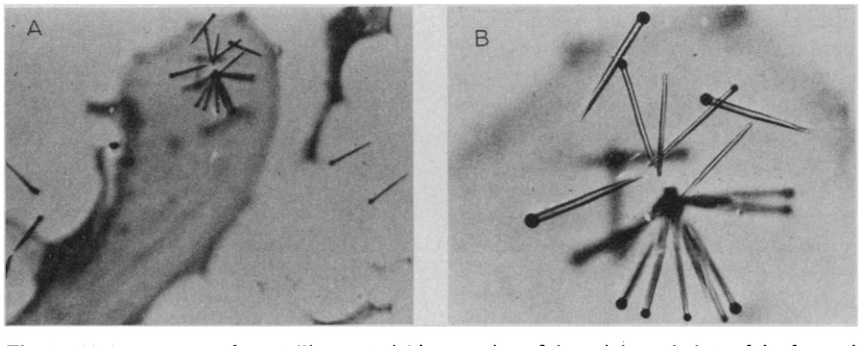

Fig. 1. (A) Appearance of crystalline material in a section of the epiphyseal plate of the femoral head. The proximal and distal epiphyseal plate were processed for histological examination as described by Gomori (*8*). (B) Close-up of the largest crystal cluster.

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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