Orotidinuria Induced by Allopurinol

Abstract. *The administration of allopurinol to patients suffering from hyperuricemia and to normal subjects results in increased excretion of the pyrimidine nucleoside orotidine. Evidence is presented for the interference by allopurinol of the de novo biosynthesis of uridine 5'-phosphate in man.*

We present evidence that, in man, the synthesis in vivo of uridine 5'-phosphate (UMP) is interfered with by allopurinol [4,6-dihydroxypyrazolo(3-4d)-pyrimidine] and that there is, as a result, an abnormal excretion of the pyrimidine nucleoside orotidine. Allopurinol, which is an analog of hypoxanthine, is an inhibitor of xanthine oxidase; it is widely used in the control of hyperuricemia. Its influence on purine metabolism has been well established (*1*), but we believe that ours is the first evidence for its interference with pyrimidine biosynthesis de novo.

Normally orotic acid, a pyrimidine, is converted by a reversible reaction by the enzyme orotate phosphoribosyltransferase (E.C. 2.4.2.10) (reaction 1) to orotidine 5'-phosphate (OMP) which in turn is converted by orotidine 5'-phosphate decarboxylase (E.C. 4.1.1.23) (reaction 2) to UMP. A small amount of OMP is irreversibly dephosphorylated (reaction 3) to the metabolically inert orotidine, which is excreted in the urine. The normal excretion of orotidine has been reported as being about 2.5 mg per 24 hours (*2*).

After we detected two patients with hereditary orotic aciduria (*3*) manifested by deficiencies in the enzymes converting orotic acid to UMP, we used a colorimetric test for urinary orotic acid to detect heterozygous subjects (*4*). Using this procedure, we have consistently found a positively reacting substance in the urines of patients (and of rats) receiving allopurinol.

In the colorimetric test (*4*) orotic acid is converted to barbituric acid by bromination and reduction with ascorbic acid. Barbituric acid reacts with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde) to form an orange-yellow complex with an absorption maximum at 480 nm. On a molar basis we have found orotidine to be as reactive as orotic acid.

When normal urine is treated in this manner, an absorption peak at 412 nm with a plateau at 480 nm is produced (Fig. 1A). The addition of orotic acid or orotidine produces a secondary peak at 480 nm (Fig. 1B). Spectra obtained with urine from patients receiving allopurinol are shown in Fig. 1, C and D. The response indicated by the smaller 480-nm peak in Fig. 1C is typical of most patients (22 to date) examined, though three patients (Fig. 1D) have excreted considerably larger amounts of the reactive compound.

Allopurinol was administered to two normal subjects (300 mg/day), and daily urine samples were examined. Maximum development of the 480-nm peak had occurred by 6 to 8 days. A 24-hour urine sample was collected from one of these subjects who had been treated with allopurinol for 14 days. A 50-ml sample of the urine was concentrated to 15 ml, in a vacuum, and then subjected to ion-exchange chromatography on a Dowex-1 column (2 by 25 cm; formate). Elution was commenced initially with a gradient of 0.025 to 0.1*M* formic acid, then with a gradient of ammonium formate from 0.1*M* to 1*M*. At 0.3*M* ammonium formate a peak was eluted which had an ultraviolet spectrum identical with that of orotidine. A portion of this fraction was desalted by passage through a Dowex-50 column (2 by 5 cm; H^+), the eluate was lyophilized, and the residue was taken up in distilled water. The substance had an identical R_F value (0.59) with that of orotidine on thin-layer chromatography [cellulose MN-300HR, Macherey Nagel and Co.; solvent system, saturated ammonium sulfate, 1*M* sodium acetate, isopropanol (80:18:2)].

There was 40 mg of orotidine in the 24-hour urine sample, the estimation being made on the basis of an extinction coefficient of $9.6 \times 10^3 M^{-1}$ cm^{-1} at 267 nm (*5*). This agrees with estimates from the semiquantitative colorimetric reaction. Thus, the increase at the 480-nm peak, after patients received allopurinol, appears to be principally due to increased orotidine excretion. Tentative estimates of orotidine excretion, based on our experience with the colorimetric reaction are: normal subjects, 2 to 4 mg/day; persons receiving allopurinol (both normal subjects and patients), 30 to 60 mg/day. The group of three patients referred to above (see Fig. 1D) excreted much

Table 1. The following were incubated in phosphate buffer, *p*H 7.6, 0.06*M*, for 45 minutes at 37°C, in the presence of 0.1 ml of hemolyzed red cells, prior to the addition of [^{14}C]OMP. The final volume of the reaction mixture was 1.0 ml. The results are the average of triplicate determinations. PRPP, phosphoribosyl pyrophosphate.

$5 \times 10^{-6}M$ [^{14}C]OMP	$2.5 \times 10^{-4}M$ PRPP	$1 \times 10^{-4}M$ allopurinol	Decarboxylation OMP → UMP (count/min)	Inhibition (%)
+	−	−	4003	0
+	−	+	3989	0
+	+	−	4050	0
+	+	+	1055	74

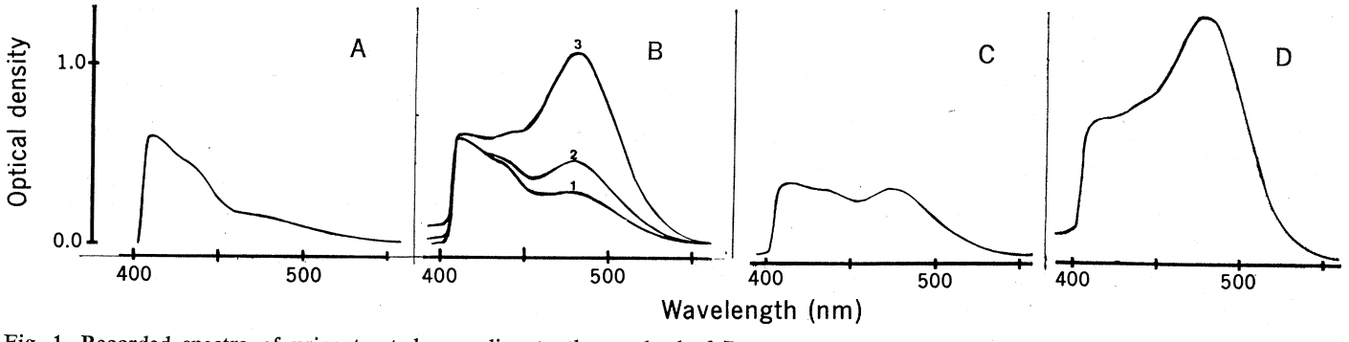

Fig. 1. Recorded spectra of urine treated according to the method of Rogers and Porter (*4*). (A) Normal urine; (B) normal urine containing orotidine; (1) 30 μg/ml, (2) 60 μg/ml, (3) 180 μg/ml; (C and D) urine from patients on allopurinol therapy.

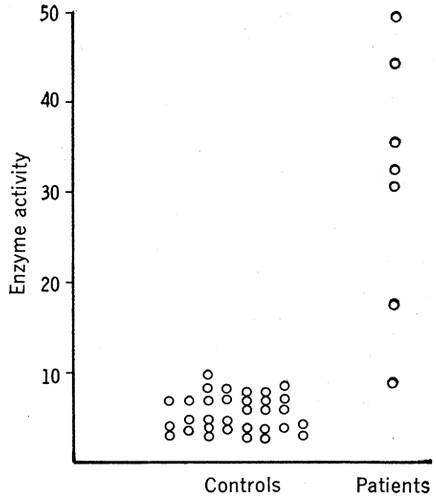

Fig. 2. Comparison of orotate phosphoribosyltransferase in the lysed red cells of normal subjects with that in patients receiving allopurinol. Enzyme levels are expressed as nanomoles of orotic acid converted to orotidine 5′-phosphate per 10^9 red blood cells per hour. Each circle represents a single estimation of activity in individual controls or patients.

greater quantities of orotidine, about 200 to 400 mg/day. These patients had renal insufficiency. Utilizing the thin-layer chromatographic system described above and the colorimetric test, we found that most patients receiving allopurinol also excrete excess orotic acid in their urine, but to a lesser degree than orotidine.

Subsequent investigations indicated that allopurinol is an inhibitor of uridine 5′-phosphate biosynthesis. Using the assay procedure of Smith *et al.* (*6*), we found that, in a lysed erythrocyte system, allopurinol appears to act as a competitive inhibitor (the inhibition constant K_i is about $10^{-5}M$) with respect to orotic acid in the phosphoribosyltransferase reaction. However, we were unable to detect any inhibition by allopurinol ($10^{-4}M$) of the decarboxylase reaction.

The results appear to be paradoxical insofar as the metabolite excreted in the urine is orotidine, the dephosphorylated product of the enzyme apparently inhibited. They are, however, somewhat analogous to results of Čihak and Šorm (*7*) with 5-azaorotate. These workers demonstrated that although 5-azaorotate itself was an inhibitor of orotate phosphoribosyltransferase, the inhibitor in vivo was the ribonucleotide. Thus orotate phosphoribosyltransferase converted 5-azaorotate to the ribonucleotide which then inhibited the decarboxylase reaction.

An experiment designed to test the possibility that a similar mechanism operated in the case of allopurinol (Table 1) indicated that, although allopurinol itself does not inhibit the decarboxylase reaction, prior incubation with phosphoribosyl pyrophosphate converts it to an inhibitor of this enzyme. This is most likely to be the ribonucleotide of allopurinol and presumably reflects the situation in vivo. Further investigation has revealed that the inhibition is competitive with respect to orotidine 5′-phosphate.

Thus we conclude that allopurinol in vivo is converted by a phosphoribosyltransferase to the ribonucleotide, which inhibits orotidine 5′-phosphate decarboxylase and leads to the accumulation of orotidine 5′-phosphate. The dephosphorylated product, orotidine, is then excreted in the urine.

We have also found that orotate phosphoribosyltransferase activity in the erythrocytes of patients receiving allopurinol increased severalfold (Fig. 2). This could arise from derepression of enzyme synthesis because of decreased formation of uridine 5′-phosphate during the period of erythrocyte maturation. However, Pinsky and Krooth (*8*) have demonstrated, in cultured human cells, increases of activity of orotate phosphoribosyltransferase when the cells are grown in the presence of 6-azauridine and other inhibitors of pyrimidine biosynthesis. They presented evidence that this increase in activity was due to accumulation of a precursor of uridine 5′-phosphate rather than depletion of this nucleotide.

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Endotoxin: Stimulation of Bone Resorption in Tissue Culture

Abstract. *Bacterial endotoxins can stimulate the release of previously incorporated calcium-45 and tritiated proline from fetal rat bone in tissue culture. Endotoxin from* Bacteroides melaninogenicus, *an organism regularly found in the gingival crevice of man, produces a response similar to parathyroid hormone and is effective at doses as low as 0.1 microgram per milliliter. This response is inhibited by serum and dependent upon the presence of albumin. Endotoxins may play a role in the bone loss characteristic of human periodontal disease.*

More teeth are lost in the adult population of the United States over 35 years of age as a consequence of chronic periodontal disease than as a result of dental caries (*1*). Resorption of the supporting bone that surrounds the roots of teeth is characteristic of human chronic periodontal disease (*2*). In epidemiological studies, the incidence of severity of bone destruction can be related to the amount of bacteria and microbial products adjacent to and in the gingival crevice (*1*). We, therefore, studied the effect of purified endotoxin from *Bacteroides melaninogenicus,* a microorganism regularly found in the gingival crevice of man (*3*), on bone resorption in tissue culture. It stimulated significant resorption at a dose as low as 0.1 μg/ml.

Resorption was assessed quantitatively by the release of previously incorporated ^{45}Ca from paired test and control bones from 19-day-old fetal rats, cultured in chemically defined medium (*4*). In one experiment, the pregnant rats were injected with 2 mc of tritiated proline instead of calcium on the day before they were killed. Endotoxin from two separate lots of *Bacteroides melaninogenicus*, strain CR-2A (*5*), as well as endotoxins from *Escherichia coli* and *Salmonella typhi* (*6*), could stimulate bone resorption in a manner similar to parathyroid hormone (Fig. 1). Morphologically, endotoxin caused a proliferation of osteoclasts and removal of matrix (Fig. 2). The endotoxin from *B. melaninogenicus* appeared to be more potent than that from the other bacteria and on a weight basis was similar to parathyroid hormone. However, with large doses of endotoxin (10 μg/ml) the response actually decreased, which suggests a possible toxic component. Several