DRUGS AND URIC ACID[1,2]

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The scope of this review will be limited to work published during the five years ending July, 1968. During this time five major conferences with published proceedings have been held to discuss topics related to drugs and uric acid: the "Conference on Problems Basic to Cancer Chemotherapy" [Rye, N.Y., Feb. 28 to March 2, 1963 (31)]; the "Conference on Gout and Purine Metabolism" [Princeton, N.J., Oct. 1 to 3, 1964 (32)]; the "Symposium on Gout and Other Disorders of Purine Metabolism" [Royal Society of Medicine, London, July 11, 1965 (208)]; the "Symposium on Allopurinol" arranged by the Heberden Society [Royal College of Physicians, London, June 8, 1966 (209)]; and the "Seminars on the Lesch-Nyhan Syndrome" [Stowe, Vermont, October 4 to 6, 1967 (198)].

As additional sources of general information, comprehensive reviews of gout and purine metabolism were published by Wyngaarden in 1965 and 1066 (229, 234). Gutman & Yü reviewed extensive chemical and physiologic information relating to uric acid metabolism in normal man and in primary gout in 1965 (79). A detailed and authoritative review of the uricosuric drugs, with special reference to probenecid and sulfinpyrazone, was presented by Gutman in 1966 (73). The principles of management of primary gout were reviewed by Talbott in 1964 (211) and by Yü & Gutman in 1967 (239). Finally, a short history of gout (34), a monograph dealing with uric acid lithiasis (9), and reviews of the biologic significance of uric acid from the standpoint of comparative biochemistry (74, 77) should be mentioned.

The area of major emphasis in the present review concerns the xanthine oxidase inhibitor, allopurinol, which was introduced in 1963 as a therapeutic agent for the treatment of gout, hyperuricemia, and urate stone formation (50, 182). Additional topics for briefer review or citation include new information which has appeared during the past 5 years concerning the ex-

[1] The survey of literature pertaining to this review was concluded in August 1968.

[2] The following abbreviations are used in this Review: A-PRTase (adenine phosphoribosyltransferase); HG-PRTase (hypoxanthine-guanine phosphoribosyltransferase); PP-Ribose-P (phosphoribosylpyrophosphate).

cretion and secretion of urate by the kidney in man, the pathogenesis of hyperuricemia, biochemical anomalies and unusual clinical manifestations of primary gout, studies regarding the pathogenesis of acute gouty arthritis in man, investigations of xanthinuria, and the recently discovered Lesch-Nyhan syndrome. A short introduction dealing with purine biosynthesis, degradation, and disposal is included for orientation and background.

Purine biosynthesis.—The end-product of purine metabolism in man is uric acid, a nonessential compound which serves no known function. Elevated concentrations of uric acid in body fluids are primarily of clinical significance since the limited solubility of the compound predisposes to the formation of crystalline deposits in the tissues, or urinary tract if concentrations rise above solubility limits. In some patients hyperuricemia may be a clue to the presence of renal (39), metabolic (1), or genetic disorders (133, 158). Abnormally low levels of uric acid in plasma or urine may occur in liver disease, bone marrow aplasia, drug toxicity, increased renal excretion of urate, xanthinuria, etc.

The body of the normal adult male contains an average of 1200 mg of uric acid, of which about 700 mg are replaced daily. Of this amount, two thirds is excreted via the kidney as uric acid, and one third via biliary, gastric, and intestinal secretions into the gastrointestinal tract (202) where it is degraded by bacteria to allantoin, allantoic acid, urea, CO_2, and NH_3.

Since several reviews of purine biosynthesis have appeared in recent years (25, 79, 229, 234), this account will emphasize newer knowledge of the regulatory mechanisms which govern the rates of operation of the pathways, and individual reactions which are subject to pharmacological control.

The purine ring is assembled in a complex series of reactions which begins with an irreversible step in which the amide group of glutamine displaces the pyrophosphate of α-5-phosphoribosyl-1 pyrophosphate, with simultaneous inversion of configuration, to yield β-phosphoribosyl-1-amine, the first specific and unique purine precursor. In phosphoribosylamine, one finds the elements of ribonucleotide structure, namely a base, sugar, and phosphoric acid, convalently linked. The amino group eventually becomes N-9 of the purine ring, after the sequential addition of atoms of carbon and nitrogen from the precursors glutamine, glycine, aspartic acid, formyl derivatives of tetrahydrofolic acid, and carbon dioxide. The first complete purine to be elaborated is inosinic acid (hypoxanthine ribonucleotide), which is the primary intermediate in the subsequent formation of all other purines, including uric acid.

The reaction generating the initial purine precursor, phosphoribosylamine, is assumed to be rate limiting for the entire sequence, although convincing demonstration that it is uniquely so has not been advanced. The enzyme catalyzing its production, glutamine phosphoribosylpyrophosphate amidotransferase, is subject to end-product regulation by adenyl and guanyl ribonucleotides (AMP and GMP) which bind independently at separate but

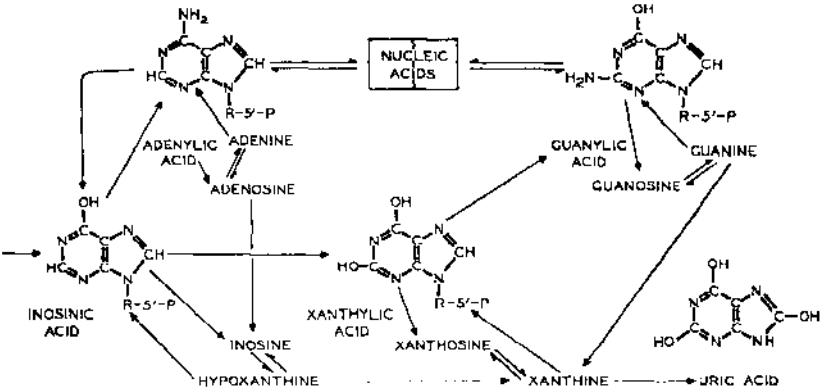

FIG. 1. Purine nucleotide interconversion pathways and biosynthesis of uric acid.

interacting sites (226). The activity of the reaction is therefore a complex function of the concentration of substrates L-glutamine and phosphoribosylpyrophosphate (PP-Ribose-P), the amount and intrinsic activity of the amidotransferase, and the absolute and relative concentrations of the various regulator ribonucleotides. As to the latter, the amidotransferase is inhibited by purine 5'-ribonucleotides, but not by purine 3'-ribonucleotides, ribonucleosides or free bases, deoxyribonucleotides or pyrimidine compounds (226). Metabolic processes which tend to remove ribonucleotides in macromolecule synthesis, or in degradative reactions, will tend to release the amidotransferase from inhibition and promote synthesis *de novo.* Also, an imbalance between 6-amino- and 6-hydroxy ribonucleotides, such as might result from interference with nucleotide interconversions, would tend to reduce the effectiveness of feedback control.

The second amidotransferase, glutamine-α-n-formylglycinamide ribonucleotide amidotransferase, in the pathway leading to synthesis of inosinic acid (IMP) is also inhibited by AMP and GMP, but its significance as a potential regulatory mechanism is unknown.

Inosinic acid is an obligatory precursor in the *de novo* synthesis of adenylic and guanylic acids (Fig. 1). The first reactions in the short pathways leading from IMP to AMP or GMP synthesis are irreversible, and unique to each pathway. They therefore have the characteristic locations and features of potential regulatory reactions. IMP and aspartic acid interact to form the intermediate adenylosuccinic acid, which on cleavage yields AMP and fumaric acid. GTP is required as an energy source in the initial condensation. This reaction is inhibited by AMP (228). In the formation of GMP, IMP is first oxidized to xanthylic acid (XMP) in reaction in which DPN serves as hydrogen acceptor. GMP is formed from XMP by an amination reaction in which glutamine serves as donor of the amide nitrogen. The dehydration of IMP is inhibited by GMP (143, 144).

Catabolism of purine ribonucleotides yields the free purine bases, adenine, guanine, hypoxanthine, and xanthine, via various degradative reactions summarized in Figure 1. Each of these bases may be returned to the ribonucleotide pool by a reaction with PP-ribose-P which involves simultaneous displacement of the α-pyrophosphate group and inversion to form the β-linked purine phosphoribosyl compound. Two phosphoribosyl transferases exist in mammalian tissues. Hypoxanthine-guanine phosphoribosyltransferase (HG-PRTase) reacts with 6-hydroxy compounds, including hypoxanthine, guanine, and xanthine, although the rate of reaction with xanthine is only 1 per cent of that with other bases (104). Adenine phosphoribosyltransferase (A-PRTase) reacts with 6-amino compounds, including adenine and amino-imidazole carboxamide. These phosphoribosyltransferases are widely distributed throughout mammalian tissues. The hypoxanthine-guanine phosphoribosyltransferase is 10 to 20 times as active in brain as in liver (178). Free adenine is not further degraded in mammalian tissues, which lack adenase. It may be oxidized to 2-hydroxyadenine, 8-hydroxyadenine, or 2,8-dihydroxyadenine *in vitro* by xanthine oxidase, but the reaction is very slow, and apparently does not occur *in vivo* unless large amounts of adenine are administered. Such adenine as is generated appears to be promptly reconverted to AMP. Guanine is readily deaminated to xanthine by guanase, an enzyme abundantly present in liver, brain, and skin. Hypoxanthine is oxidized to xanthine and the latter to uric acid by xanthine oxidase, which in man is present mainly in liver and in mucosa of the small intestine, and inconstantly in the marrow of children. Normally, nearly 90 per cent of the hypoxanthine generated each day is returned to the ribonucleotide pool; only 10 per cent is converted to uric acid (10–14, 23). No quantitative estimates of the turnover of other pools of purine bases exist, but it is clear from study of the turnover of hypoxanthine in the xanthinuric person (23), incapable of oxidizing hypoxanthine because of genetic lack of xanthine oxidase, and from purine excretion data in subjects lacking HG-PRTase (see below), that the turnover of free purine bases is much larger than previously supposed.

The PRTases are also regulated enzymes. Adenine-PRTase is inhibited by AMP, and HG-PRTase by GMP (59).

In summary, AMP and GMP regulate their own synthesis by three mechanisms. They cooperate in inhibiting the initial step of purine synthesis *de novo;* each further regulates its formation *de novo* from IMP by inhibiting the appropriate nucleotide interconversion. Finally, AMP and GMP also inhibit their respective purine phosphoribosyltransferases, A-PRTase and HG-PRTase, and thereby regulate their resynthesis from free purine bases.

Uric acid degradation and excretion.—In most mammals the filtered urate is reabsorbed by the renal tubules and converted by uricase, chiefly in the liver, to more readily soluble allatoin which is excreted in the urine. As a result of a genetic mutation in man's more immediate evolutionary forebears, uricase disappeared from the tissues and with it the main degradative

pathway of uric acid. The amount of urate in body fluids increased markedly, and new means for its excretion evolved by necessity. In man the combined rate of uric acid disposal via the kidney and gut suffices for normal physiological needs, but the newly established steady state must be regarded as a distinctly hyperuricemic level as compared to animals which still have uricase (*74, 76, 77*).

The major route of disposal of uric acid from the plasma is via the kidney. This involves a complex three-phase process consisting of filtration at the glomerulus, reabsorption of the filtered urate by the proximal convoluted tubule and secretion of urate by the distal tubule (*76, 153, 156, 235, 236*). Present knowledge suggests that virtually the entire filtered load is normally absorbed by the proximal tubules. Relatively little is known about the mechanism of the reabsorptive process. The tubular secretion of urate is markedly inhibited by pyrazinamide (*35, 36, 76, 206, 207, 235*). Competitive inhibition by a number of organic acids, including various uricosuric drugs, suggests the presence of an active transport system (*73, 167*).

Clinically, derangements of purine metabolism of major importance occur in patients with primary or secondary gout, in some hematologic diseases, and in patients who form urinary urate stones. Rarer diseases of investigative importance include the Lesch-Nyhan syndrome, mongolism (*91, 102*), and Type I glycogen storage disease (von Gierckе's). Children with the latter have severe hyperuricemia and may develop tophaceous gout (*1, 91, 97*). The persistent lactic acidemia inhibits the renal tubular secretion of urate, but there is also excessive production of purines, manifested by rapid turnover of the expanded uric acid pool, and excessive incorporation of labeled glycine into urinary urate (*1*). It has been suggested that excessive production of PP-ribose-P may underlie the excessive purine biosynthesis (*1, 97, 107*).

Primary gout.—The cardinal biochemical feature of this genetically determined disorder of purine metabolism is hyperuricemia. The serum urate in normal man averages about 5.0 mg per cent—only a little below the saturation point, 6.5 to 7.0 mg per cent. The solubility of urate in the plasma may be reduced in patients with gout and their kindred by the absence of an alpha$_1$-alpha$_2$ urate-binding globulin (*5–7*). The factor of immediate importance in the pathogenesis of gout, however, is over-production or impaired renal excretion of uric acid (*76, 157*). Balance, turnover, and tracer studies have documented excessive production of purines not only in the over-producers but in one half to two thirds of gouty subjects whose urinary urate excretion values are within the normal range (*232*). A limited number of the over-producers studied, representing perhaps 5 per cent of all patients with gout, have shown a partial deficiency of HG-PRTase in erythrocytes (*103, 108, 180*).

Hyperuricemia may remain asymptomatic for many years even though the serum uric acid concentration prevails well above the average saturation level (*78, 172, 231*). Acute gout may be precipitated in such individuals

by trauma, starvation, acidosis, or the use of drugs which reduce urate clearance by the kidney (21, 42, 48, 66, 92, 124, 142, 173). The incidence of urinary urate stones in gouty subjects is some 1000 times greater than in normal individuals (78, 240). Two factors seem to be responsible: the hyperuricosuria for one; and the persistently acid urine (150, 225, 240), associated with impaired ammonia synthesis by the kidney in gout, for another (75, 215). Tophaceous deposits tend to occur in various tissues in patients with long maintained hyperuricemia, and joint destruction or nephropathy, or both, develop insidiously over the course of years (78, 172). In unusual instances the disease may mimic rheumatoid arthritis (45, 69, 165); it may be associated with diabetes mellitus (17, 223), cystinuria (149), arterial hypertension (24, 28); or it may produce necrosis of the head of the femurs (93, 140), sacroiliac arthritis (146), or carpal tunnel nerve compression (164). Aminoaciduria (101) and impaired excretion of bromsulphthalein dye (188) are interesting additional anomalies in patients with gout.

In the treatment of primary gout two general approaches are possible: (*a*) measures to reduce the formation of uric acid, and (*b*) ways to increase its elimination. The exclusion of purines from the diet has been ineffective therapeutically, since most purines arise from endogenous rather than exogenous sources. The time-honored "specific" colchicine has no demonstrable effect on purine metabolism, although it is an exceedingly useful agent in aborting acute attacks of gout and in the prophylactic therapy of patients prone to develop acute arthritis (78, 145, 177, 239). Colchicine in some way or another reduces the formation of urate crystals or their phagocytosis, or both, by neutrophils in the joint fluid (26, 90, 136–138, 146, 147, 175, 190, 193, 195).

Well tolerated and effective uricosuric agents became available in 1950 (55, 73, 131, 177). These compounds increase the net excretion of uric acid by interfering with the tubular reabsorption of urate. With maintained uricosuric therapy using the most effective agents, probenecid and sulfinpyrazone, it was possible for the first time to control hyperuricemia in one half to two thirds of the gouty population (230). Joint disease was ameliorated, tophi reduced in size, etc. Increasing the amount of uric acid excreted in the urine, however, added to the risk of urinary stone formation and failed to improve renal function (73, 78). In fully one third of all patients with gout, uricosuric agents are of little or no benefit due to the severity of the basic disease, pre-existing or progressive impairment of renal function, adverse reactions, drug antagonisms (236), etc.

To reduce the production of uric acid, any point in the biosynthetic pathway for which a specific inhibitor existed would be a theoretical possibility. Both azaserine and DON (Diazo-oxo-norleucine), which block the conversion of formylglycinamide ribotide to the amidine (242), diminish urate production (70, 242). Two other antileukemic agents, azathioprine and 6-mercaptopurine, suppress purine synthesis (105, 159, 203, 204), presumably through feedback inhibition by 6-thioinosinate on the amidotrans-

ferase that controls the first step of purine biosynthesis (139). None of these drugs merits serious consideration in the therapy of gout, since, as antileukemic agents, they are useful primarily to suppress the growth of abnormal leukemic cells.

Inhibition of urate production at the ultimate step, xanthine → uric acid, has been found feasible, and has given rise to a new therapeutic agent, allopurinol, which is now widely used (182, 184–186, 16, 18, 41, 57, 62, 65, 80, 89, 109, 110, 112, 151, 163, 188, 200, 213, 217, 238, 239, 232).

Allopurinol.—Allopurinol (4-hydroxypyrazolo (3,4-d)-pyrimidine) is a xanthine oxidase inhibitor with a Michaelis constant some 20-fold lower than that of the substrate, xanthine (51, 51a, 51b, 218). Moreover, its metabolite, the corresponding analogue of xanthine (4,6-dihydroxypyrazolo (3,4-d)-pyrimidine, oxipurinol, oxoallopurinol, allo-xanthine) is also an inhibitor of the enzyme. Its Michaelis constant is somewhat higher than that of allopurinol and comparable to that of xanthine (51). Allopurinol may show substrate-competitive kinetics. Both allopurinol and oxipurinol produce pseudo-irreversible inactivation of xanthine oxidase: Inactivation occurs when allopurinol and enzyme are incubated in the absence of substrate, but enzyme activity can be restored by prolonged dialysis. Oxypurinol has no effect on enzyme alone, but inactivates it in the presence of xanthine (51). These experiments were carried out with molecular oxygen as hydrogen acceptor. Recently, Johns reported that allopurinol does not inhibit electron transfer from xanthine oxidase to acceptor dyes (99); similarly Westerfield had found semicarbazide and phenylhydrazine to inhibit electron transfer to 0_2 but not to methylene blue or DPNH (221, 222).

In man, there is no evidence for irreversible inactivation of xanthine oxidase, nor, for that matter, of enzyme induction. The maximum depression of the serum urate level is reached within a few days after beginning therapy, and remains relatively constant over prolonged periods (182, 186). Withdrawal usually results in a return to pretreatment serum urate levels within a few days and the exceptional more prolonged effects are clearly associated with delayed excretion of oxipurinol.

The possibility of inhibiting xanthine oxidase, *in vivo,* was first demonstrated with exogenous substrates, e.g., 6-mercaptopurine, 6-methylaminopurine, 6-chloropurine, in mice (49, 50). In man, inhibition of the oxidation of 6-mercaptopurine to 6-thiouric acid is achieved with relatively low doses of allopurinol (86, 182), and the resultant more efficient utilization of 6-mercaptopurine potentiates its activity by a factor of three to four (182).

Urate production in man is inhibited by allopurinol, also, as shown by a decrease in serum and urinary urate, and significant quantities of uric acid precursors, hypoxanthine and xanthine, appear in the urine as evidence of xanthine oxidase inhibition (86, 182). When 200 to 600 mg of allopurinol are given daily to patients with gout and other diseases, elevated serum uric acid levels ordinarily fall to normal within 3 to 5 days, and with appropriate

adjustment of dose, can be held as low as 2 to 3 mg per cent more or less indefinitely unless there is serious impairment of renal function (186). In patients who have normal or increased amounts of urate in the urine, the amount of uric acid excreted usually falls in parallel with the decline in serum uric acid concentration, and there is a concomitant but usually not equimolar increase in the amount of hypoxanthine and xanthine in the urine (163, 182, 186). From the therapeutic standpoint, urinary urate stone formation ceases for all practical purposes after allopurinol therapy has been instituted (3, 8, 9, 18, 57, 186, 216). When the serum urate concentration is maintained for some time below the saturation point, by the additional use of uricosuric agents in some patients, tophi begin to resolve, destructive arthritis improves and acute attacks of gout become less frequent and severe. The progression of gouty nephropathy, the pathogenesis of which is poorly understood (15, 29, 30, 47, 68, 152, 162, 176, 194, 207, 219) appears to halt in most patients (57, 134, 183, 186, 199, 218, 224).

Allopurinol is an exceptionally well-tolerated agent in many species (86, 87, 121), and can be given by mouth to man in doses two to four times that required to control hyperuricemia. Drug reactions are infrequent and their pathogenesis is obscure. Those that do occur subside promptly with cessation of drug administration (18, 96, 135, 186, 188, 224, 238).

Acceleration of tumor growth has not been observed clinically or experimentally (2, 122, 185, 186).

Secondary gout and hyperuricemia.—In many diseases such as acute leukemia, hemolytic anemia, polycythemia vera, chronic myelogenous leukemia and related myeloproliferative diseases, psoriasis, and pernicious anemia, the turnover of body cells is greatly accelerated. As a consequence, uric acid excretion may be increased some two to four times above normal. The likelihood of gouty complications is increased by the development of impaired renal function, the occurrence of acidosis, use of diuretic drug therapy, etc.

Krakoff and many others (33, 60, 72, 118, 119, 123, 126, 127, 220) have emphasized the frequency and seriousness of the hyperuricemia and hyperuricosuria occurring in patients with leukemia and lymphomas, particularly those treated by aggressive therapeutic regimens. Patients who have disease that is very sensitive to the effect of therapeutic agents may have a very rapid lysis of cells which can lead to the abrupt elevation of serum uric acid, hyperuricosuria, uric acid crystals, urinary stone formation, and urinary tract obstruction (126, 127). This has been reported to occur in perhaps 2 to 5 per cent of all patients with acute leukemia who were treated by aggressive regimens. The prophylactic use of allopurinol is generally advised now, with the agent being given for 1 to 2 days before starting therapy in patients particularly at risk. The amount of uric acid formed is greatly reduced and acute gouty or urinary obstructive complications virtually eliminated (40, 81, 118, 119, 122, 123, 125, 154).

Overproduction of uric acid, too, is a characteristic feature of the more chronic myeloproliferative diseases, particularly polycythemia vera, myeloid

metaplasia, and multiple myeloma (88, 117, 187). The incidence of secondary gout in these patients has been reported to be as high as 5 to 9 per cent. A particularly informative study was that of Yü, who reported that the mean interval between the development of polycythemia vera and secondary gout in 42 patients having both diseases was about 7 years. A comparison of these patients with a large number of individuals with primary gout showed that the age of onset in those with secondary gout was later, the incidence in females higher, acute attacks of gout less frequent, and the overall therapeutic management problem more difficult. Tophi developed earlier, the serum uric acid level was higher, and nephrolithiasis more frequent. Nephropathy and uremia were a common cause of death (237).

In this group of patients it has been recommended that allopurinol be given more or less indefinitely unless treatment of the underlying disease adequately controls the hyperuricemia and uricosuria (40, 119, 132, 187).

Oxypurine excretion.—With the inhibition of urate production by allopurinol, increased amounts of the precursors, hypoxanthine and xanthine, appear in the urine. The properties of these precursors have a direct bearing on the feasibility of long-term control of urate production through inhibition of xanthine oxidase. Nature has performed the ultimate experiment, essentially a complete deletion of the enzyme, xanthine oxidase, in patients with xanthinuria (43, 233). A few such patients have been studied in detail (10, 11, 13, 23, 58). The purine end-product they excrete is predominantly xanthine, with hypoxanthine being a minor constituent (23, 44, 58). Since such patients convert exogenous hypoxanthine to xanthine (11, 58), and since the route at the free purine level is blocked, this conversion must take place at the ribonucleotide level. By the administration of ^{14}C-oxypurines, Bradford et al. (23) showed that the metabolically active hypoxanthine pool in a xanthinuric patient was very large even though the daily excretion of hypoxanthine in the urine was only 6 per cent of this pool.

The natural occurrence of urinary xanthine stones in man is not unknown, although the number reported in the literature is small (20, 27, 43, 44, 63, 94, 95, 100, 130, 141, 148, 166, 174, 212, 233). In most instances the amount and constancy of the xanthinuria was not known. In only a few patients, has xanthinuria been definitely associated with a lack of xanthine oxidase (10, 23, 58, 233). In three of these patients no urinary calculi have been observed. However, a child with xanthinuria (44) did form a xanthine calculus. Children are known to exhibit a greater purine turnover than adults (159) on a body weight basis.

Renal xanthine deposits can be produced in animals by the administration of allopurinol (87). In fact, renal blockade by calculi consisting primarily of xanthine was the limiting factor in chronic toxicity testing in animals, and the intrinsic toxicity of allopurinol still is unkown. Calculus formation was dose-related, and occurred erratically when the urinary xanthine concentration greatly exceeded the solubility of xanthine. It has been noted that the apparent maximum tolerated dose of allopurinol increases with the size of the animal employed. Tolerability is correlated with the re-

ciprocal of purine end-product excretion, as a function of body weight and water flux. Thus, the total purine end-product of a 10 kg dog (allantoin) approximates that of a 70 kg man (urate) and is excreted in one fifth to one tenth the volume of urine. In a general way, total purine end-product per unit of body weight bears an inverse relationship to size (87, 93a). The administration of allopurinol results in the replacement (not necessarily stoichiometric, see below) of normal end-product by a mixture of xanthine and hypoxanthine, in the average (rather variable) ratio of about 2:1. In species with a high purine/water ratio the resultant xanthine can reach concentrations in the urine that exceed the solubility point several fold, depending on the extent of the xanthine oxidase inhibition.

The absence of xanthine calculi in patients treated with allopurinol hinges on several factors. In man, even aggressive therapy rarely diminishes urate production by more than 50 per cent, and the total amount of xanthine excreted may be of the order of 150 mg per day. The purines, in the resultant mixture of uric acid, hypoxanthine, and xanthine, exhibit independent solubilities so that, if the urinary volume is adequate, none reaches its saturating concentration (87, 112, 119, 134). Recently, in a child with Lesch-Nyhan syndrome treated with 9 mg of allopurinol per kg, xanthine precipitates were found in the urine (161, 201). Since children with this disease are tremendous over-producers of uric acid and are unable to reutilize hypoxanthine or xanthine (*vide infra*), inhibition of xanthine oxidase resulted in the excretion of as much as 800 mg of xanthine per day in this child (201).

When allopurinol is given, serum urate falls but serum oxypurines (hypoxanthine and xanthine) become elevated only slightly (53, 67, 238). The primary factor limiting their rise is rapid renal clearance (67). Earlier workers had found moderately low renal clearance values for hypoxanthine and xanthine, but were handicapped by the fact that the natural levels of these metabolites are close to the limits of the analytical methods, and by the fact that oxypurines are rapidly liberated in shed blood. Indeed, when Dickinson & Smellie found a high clearance for xanthine in their xanthinuric patient, they postulated a renal defect related to the defect in xanthine oxidase (44). The advent of allopurinol permitted accurate renal clearance determinations (67, 112). In normal and in gouty patients the clearance of xanthine approximates that of inulin.

Hypoxanthine and xanthine reutilization and feedback inhibition.—The replacement of urinary urate by oxypurines when allopurinol is given usually is less than stoichimetric (41, 87, 182, 184, 186, 238). The decrease in total purine end-product is roughly proportional to the degree of urate over-production present in the patients before therapy (87, 182, 186). In patients with rapidly resolving tumor masses following the use of chemotherapy or irradiation, the block in urate formation with allopurinol also appears to result in the excretion of considerably less than an equivalent amount of oxypurines (118, 119, 122, 123, 125). Similarly, when inosine was given to patients, urate accounted for 73 per cent of the purine adminis-

tered, but after allopurinol only 35 to 40 per cent was accounted for by the sum of the increments in urate and oxypurines (182, 186). The deficit appears to result from reutilization of hypoxanthine and xanthine. Thus, neither of these purines is salvaged very efficiently for nucleic acid purine synthesis when given alone, but both are extensively incorporated when given with allopurinol (168, 169). A xanthinuric patient incorporated hypoxanthine and converted it to derivatives of adenine and guanine (11). In some gouty patients (106, 112, 213, 214), and in children with the Lesch-Nyhan syndrome, the replacement of urate by oxypurines is stoichiometric (12, 155, 178, 194) for reasons which have recently come to light.

The clinical features of the Lesch-Nyhan syndrome, first described in 1964, include mental retardation, neurologic abnormalities (choreoathetosis and athetoid cerebral palsy), obsessive destructive behavior and extreme hyperuricosuria (133, 158, 160, 161). The latter frequently leads to hyperuricemia and the clinical features of gout. Most of the patients studied so far have had a serum uric acid concentration of around 10 mg per 100 ml and excrete 3 to 4 mg of uric acid per mg of creatinine—3 to 5 times as much as patients with gout (12, 179). Some patients have had a severe macrocytic, megaloblastic anemia unresponsive to therapy with vitamin B_{12} or folic acid, but a prompt hematologic response to adenine has been reported (241).

The condition has now been recognized in several dozen children, all males. All pedigrees indicate X-linkage, with full expression only in the hemizygote. Affected males show no activity of HG-PRTase in erythrocytes, but, curiously, activity values of the closely related enzyme, A-PRTase, are increased (82–85, 104, 108, 196).

In screening infants and children for the presence of this disease, suspects should include infants with unusual vomiting during the neonatal period, those having orange crystals in their urine or diapers, hematuria, urinary stones, polydypsia, and polyuria, as well as the major symptoms outlined above.

The administration of allopurinol to infants and children with this syndrome reduces the formation of uric acid and ameliorates the symptoms of secondary gout, but has no definite effect on the other manifestations of the disease (85, 155, 197). Children with the Lesch-Nyhan syndrome, having a complete deficiency of hypoxanthine-guanine phosphoribosyltransferase are unable to reutilize hypoxanthine, guanine, and possibly xanthine (104–6, 108, 178, 196). When uric acid production is suppressed in these children by allopurinol, an equimolar increase in the urinary excretion of xanthine and hypoxanthine occurs. A few patients with gout who are relatively deficient in HG-PRTase activity (79, 196) respond in a similar fashion (103, 106, 180). The failure of children with the Lesch-Nyhan syndrome to show a reduction in urate excretion when azathioprine is administered (105, 159, 196, 205), seems to be because of their inability to synthesize thioinosinate (105, 196). The excessive urate synthesis in patients with this defect suggests that normally there is a high turnover of the inosinate:hypoxanthine

pool, and that reutilization of free hypoxanthine is an important controlling mechanism. The "shunt pathway" would be viewed as escape of hypoxanthine from this pool with resultant conversion to xanthine and urate (203, 204, 227). The high proportion of xanthine in the urine of xanthinuric patients would suggest a high rate, possibly greater than normal, of reutilization of hypoxanthine. The route and rapid rate of hypoxanthine formation (presumably from inosinate) in the Lesch-Nyhan syndrome remain unexplained, and while these may reflect a unique abnormality a similar high turnover rate has been reported in a xanthinuric patient (14, 23).

Superficially, the Lesch-Nyhan syndrome resembles the lesion caused by the administration of 2-ethylaminothiadiazole (115, 189, 191). At any rate, the administration of this drug produces a high and uncontrolled rate of urate formation in a number of species (46, 116, 120, 191). The locus affected has not been identified. It obviously is not the same as that in the Lesch-Nyhan syndrome, since thiadiazole aggravates the condition, and furthermore thiadiazole uricogenesis is inhibited by mercaptopurine (120), nicotinamide (120, 195), and allopurinol (46, 123).

Allopurinol metabolism.—Allopurinol itself appears to have a renal clearance rate compatible with that of glomerular filtration but its metabolite, oxipurinol, is eliminated much more slowly (52, 53). The clearance of oxipurinol seems to be a small multiple of the urate clearance of the subject, and detailed analysis has shown it to be extensively reabsorbed like urate, and a competitor of urate in this process (i.e., a uricosuric) at high concentrations (53). Like urate, its clearance is increased by uricosuric drugs (52, 53). In therapy, oxipurinol persists at a level which is a direct function of the allopurinol dosage, and an inverse function of its renal clearance.

The biological half-life of allopurinol is a function of both its renal clearance rate compatible with that of glomerular filtration but its metabshort half-life (2 to 3 hr) (52). Since allopurinol and oxipurinol both inhibit the oxidation of allopurinol, the half-life of allopurinol increases as the level of xanthine oxidase inhibition increases. This may occur either through increasing dosage or the accumulation of oxipurinol, as in patients on continuing therapy (52, 210). In dogs given infusions of allopurinol at very high levels, the conversion to oxipurinol is almost completely inhibited (54).

Allopurinol and oxipurinol are distributed essentially equally in all body water (except brain which has about half the serum levels). Neither is bound to serum proteins. No evidence could be found for ribonucleotides or incorporation of either compound into nucleic acids. However, ribonucleosides of each were found as metabolites of allopurinol in man (52, 128). Allopurinol is a substrate for purine nucleoside phosphorylase, where it is an effective competitor of hypoxanthine, and yields the metabolite found in human urine, 1-ribosylallopurinol, a structural analogue of inosine (128). Oxipurinol is a poor substrate for this enzyme, but it does yield an analogue of xanthosine. This substance was not found among the urinary metabolites

of allopurinol (128). Oxipurinol, however, does give rise to a ribonucleoside, metabolically and by enzymatic synthesis, which is 7-ribosyloxipurinol (128). This substance is formed through the action of pyrimidine nucleoside phosphorylase; the ribosyl group is linked to a pyrimidine ring N-atom, and the product is, therefore, an analogue of uric acid-3-riboside, although it probably arises by a different mechanism (61, 128). The improbability of the formation of a ribonucleotide of either allopurinol or oxipurinol *in vivo*, receives support from studies of these as substrates for HG-PRTase. Oxipurinol shows negligible substrate activity, and allopurinol is a poor competitor of hypoxanthine with only one four-hundredth the capacity to bind the enzyme (129).

Although allopurinol is a much more potent inhibitor of xanthine oxidase *in vitro* than oxipurinol, it was thought that the latter compound, accumulating in the blood and being retained at a more constant and elevated level, might contribute significantly to the net therapeutic effect. The effect of oxipurinol was studied in three patients, two of whom having gout with nephropathy had had adverse reactions to allopurinol. The compound was well-tolerated and effective but the dose required appeared to be about twice that of allopurinol (185).

More detailed comparative studies in five additional patients showed similar dose/response relationships. Probenecid was shown to increase the urinary excretion of oxipurinol and it appeared that the kidney handles allopurinol like the oxypurines (hypoxanthine and xanthine) and oxipurinol more like uric acid. Evidence for the tubular secretion of oxypurinol was not obtained (53).

Soon after the clinical debut of allopurinol a flurry of concern arose about the possibility that it might interfere with the absorption or utilization of iron. The final consensus of a number of investigators seems to be that the agent has no such effect (22, 37, 38, 56, 64, 113, 114, 170, 171, 192).

The enzyme tryptophan pyrrolase is inhibited by allopurinol, (19) but another enzyme with substrates very similar to those acted on by xanthine oxidase, liver aldehyde oxidase, is not inhibited (98).

Finally, mention should be made of attempts to accelerate uric acid disposal through the administration of uricase. As a therapeutic measure, this procedure is limited by the availability of the enzyme, the quantity required, and by the quick termination of its effectiveness due to the formation of antibodies to the enzyme (4, 111, 132, 181).

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