Uric Acid Biosynthesis and its Disorders

R. W. E. WATTS, MD, FRCP

Division of Inherited Metabolic Diseases, Medical Research Council Clinical Research Centre, Watford Road, Harrow

Uric acid is the end product of the catabolism of the purine moiety of the ribonucleotides that have been synthesised *de novo* and those that are derived from the diet. The biochemical reactions that constitute the overall process of purine metabolism are conveniently grouped as follows: biosynthesis *de novo*, nucleotide interconversion reactions, degradative reactions, and purine salvage reactions. Clinical disorders of purine metabolism arise from abnormalities involving each of these four main stages, as well as from the catabolism of excessive nucleic acid loads derived either from the diet or from excessive endogenous tissue breakdown.

URIC ACID FORMATION

Purine biosynthesis *de novo* begins with the reaction of phosphoribosylpyrophosphate (PRPP) and glutamine to form phosphoribosyl-1-amine (Fig. 1, reaction 2). This reaction forges the link between the nitrogen atom that will ultimately be N_9 of the purine ring and the carbohydrate part of the molecule. Phosphoribosylpyrophosphateamidotransferase (EC* 2.4.2.14) catalyses this first specific, or committed, reaction on the pathway of purine biosynthesis *de novo*, and is the biochemical site of action of regulatory mechanisms that affect the overall rate of the whole biosynthetic pathway. The biosynthetic sequence consists of a further eight steps, each of which is catalysed by a specific enzyme, and which ends with the production of inosinic acid (hypoxanthine ribonucleotide, or inosine-5′-phosphate). Carbon atoms 4 and 5 and nitrogen atom 7 of the purine ring are derived from an intact glycine molecule, carbon atoms 2 and 8 are derived from a 1-carbon unit designated 'formyl' and carbon atom 6 is derived from bicarbonate.

The purine interconversion reactions (Fig. 1) convert inosinic acid to adenylic and guanylic acids which are required for polynucleotide formation and other functions. The degradative reactions convert purine ribonucleotides in excess of the organism's need to uric acid (Fig. 1). Conversely, the purine salvage reactions (Fig. 1, reactions 4 and 5) provide a mechanism for purine conservation by

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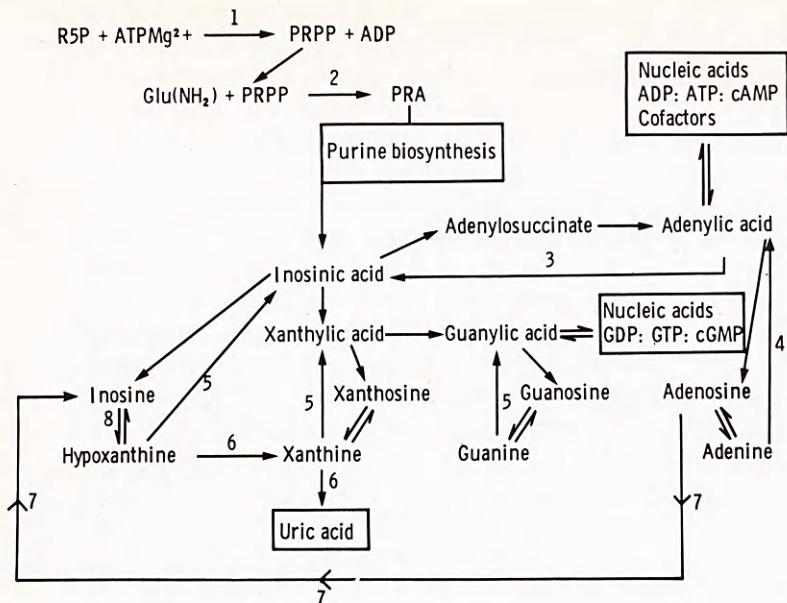* Enzyme Commission Classification

Fig. 1. The purine ribonucleotide interconversion reactions and the sites of action of the principal enzymes referred to in this article (redrawn and adapted from Watts, 1974).

R5P = ribose-5′-phosphate
PRPP = phosphoribosylpyrophosphate
PRA = phosphoribosyl-1-amine
1   = PRPP-synthetase (EC 2.7.6.1)
2   = Phosphoribosylpyrophosphateamidotransferase (EC 2.4.2.14)
3   = Adenylic acid deaminase (EC 3.5.4.6)
4   = Adenine phosphoribosyltransferase (APRT) (EC 2.4.2.7)
5   = Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (EC 2.4.2.8)
6   = Xanthine oxidase (EC 1.2.3.2)
7   = Adenosine deaminase (EC 3.5.4.4)
8   = Purine nucleoside (inosine) phosphorylase (EC 2.4.2.1)

converting them directly to their corresponding ribonucleotides by direct reaction with PRPP.

Dietary and tissue nucleoproteins are degraded by proteolytic enzymes, nucleases and phosphodiesterases to mononucleotides that are converted to nucleosides by nucleotidases and phosphatases. The nucleosidases and phosphorylases convert the nucleosides to their corresponding free purine bases. These, like the purines derived from newly synthesised nucleotides, can be conserved by the purine salvage enzymes.

REGULATION OF THE RATE OF PURINE BIOSYNTHESIS DE NOVO

The activity of phosphoribosylpyrophosphateamidotransferase and, hence, the

rate of purine biosynthesis *de novo* is regulated by the relative intracellular concentrations of PRPP and purine ribonucleotides. Increasing the PRPP concentration promotes disaggregation of the inactive dimeric form of the enzyme into the active monomeric form, increasing the purine ribonucleotide concentration has the opposite effect (Holmes *et al.,* 1974; Kelley and Wyngaarden, 1974). Cytotoxic drugs such as 6-mercaptopurine, which are converted to ribonucleotides by the purine salvage enzyme hypoxanthine guanine phosphoribosyltransferase (HGPRT), also inhibit purine synthesis *de novo* in this way. Biochemical abnormalities that increase phosphoribosylpyrophosphate synthesis, or cause its accumulation, because of non-utilisation, accelerate purine biosynthesis. It may well be that the allosteric regulation of the activity of PRPP-synthetase (EC 2.7.6.1) and, therefore, of PRPP production is as important a factor controlling the rate of purine production as alteration in the sub-unit structure of PRPP-amidotransferase. This appears to be the case in Ehrlich ascites tumour cells *in vitro* (Bagnara *et al.,* 1974). Ammonia can replace glutamine in the phosphoribosylpyrophosphateamidotransferase reaction, and this reaction can be short-circuited altogether by the direct reaction of ammonia with ribose-5-phosphate (Reem, 1972), which utilises one of the substrates of PRPP-synthetase. These interrelationships also offer possibilities for the fine regulation of purine biosynthetic rates by competing biochemical reactions, and the relative importance of the individual metabolic pathways may be different in different tissues.

GOUT AND HYPERURICAEMIA

Gout is the syndrome resulting from the crystallisation of monosodium urate monohydrate *in vivo* due to the body fluids being supersaturated with the salt. The results of studies of the incorporation of isotopically labelled glycine into urinary uric acid led to cases of gout being classified as due to uric acid overproduction (i.e. excessive synthesis *de novo*), under-excretion, or to both of these abnormalities (Seegmiller *et al.,* 1963). Table 1 lists the specific enzyme

Table 1. Known enzyme defects which are associated with increased purine biosynthesis *de novo* leading to uric acid overproduction, hyperuricaemia, hyperuric aciduria, gout and uric acid urolithiasis

1. Hypoxanthine guanine phosphoribosyltransferase (HGPRT) (EC 2.4.2.8) deficiency
    (*a*) Complete*
    (*b*) Partial*
2. Glucose-6-phosphatase deficiency (EC 3.1.3.9) (Type I glycogenesis)
3. Glutathione reductase (EC 1.6.4.2) variant with increased activity
4. Phosphoribosylpyrophosphate synthetase (EC 2.7.6.1) variant with increased activity

* See text for further definition of these terms in this context

abnormalities that have been associated with hyperuricaemia. In addition, primary abnormality of phosphoribosylpyrophosphateamidotransferase, making it abnormally resistant to inhibition by purine ribonucleotides or abnormally sensitive to activation by PRPP, has been widely proposed. However, no firm evidence for the existence of such an abnormality has yet appeared. The proof of such a metabolic lesion would require a detailed investigation of the kinetic and other properties of the purified enzyme isolated from the patient's tissues. It seems unlikely that such studies would be practicable at the clinical level. Increased hepatic xanthine oxidase (EC 1.2.3.2) activity was reported in a group of gout patients (Carcassi *et al.*, 1969). Similar changes were not found in jejunal mucosa (Raivio-Sforza *et al.*, 1969) and the most recent evidence indicates that the original observation was due to enzyme induction and not to an inherited abnormality of xanthine oxidase (Marcolongo *et al.*, 1974). The acceleration of purine biosynthesis by deficiency of hypoxanthine guanine phosphoribosyl-transferase [HGPRT (EC 2.4.2.8)] and glucose-6-phosphatase (EC 3.1.3.9) activity, and by the increased activities of PRPP-synthetase and glutathione reductase (EC 1.6.4.2) can be explained by the effects of these enzyme abnormalities on the intracellular concentration of PRPP. Deficiency of HGPRT decreases PRPP utilisation and reduces the supply of hypoxanthine and guanine nucleotides that inhibit activity of PRPP-synthetase (Bagnara *et al.*, 1974). Glucose-6-phosphatase deficiency leaves more glucose-6-phosphate available for metabolism by way of the phosphogluconate (pentose-phosphate) shunt pathway with increased generation of ribose-5-phosphate, which is the substrate for PRPP-synthetase.

The hyperuricaemia that is observed in patients with glucose-6-phosphatase deficiency and which causes gout and urinary stones in early adult life, is partly due to increased purine biosynthesis *de novo* and partly to urate retention caused by lactic acid inhibiting the renal tubular excretion of uric acid. Evidence for different primary abnormalities causing increased PRPP-synthetase activity has been advanced in two families with gout (Sperling *et al.*, 1972a,b; Becker *et al.*, 1973a,b). A high incidence of gout was demonstrated in a population in which an abnormal gene directing the synthesis of glutathione reductase (EC 1.6.4.2) was segregating (Long, 1967). The abnormal enzyme had increased activity with respect to glutathione reduction and a consequent increase in the intracellular $NADP^+/NADPH$ ratio; this is thought to increase pentose phosphate shunt activity with increased ribose-5-phosphate and, hence, PRPP production, as shown in Fig. 2.

Abnormal levels of blood and urinary uric acid are usually the only indication that the clinician is dealing with a disorder of purine metabolism, and Table 2 lists the causes of hyperuricaemia. Most hyperuricaemic patients with or without gout or uric acid stones have still to be classified as having essential hyperuricaemia. Only about 25 per cent of gout patients have an increased rate of purine
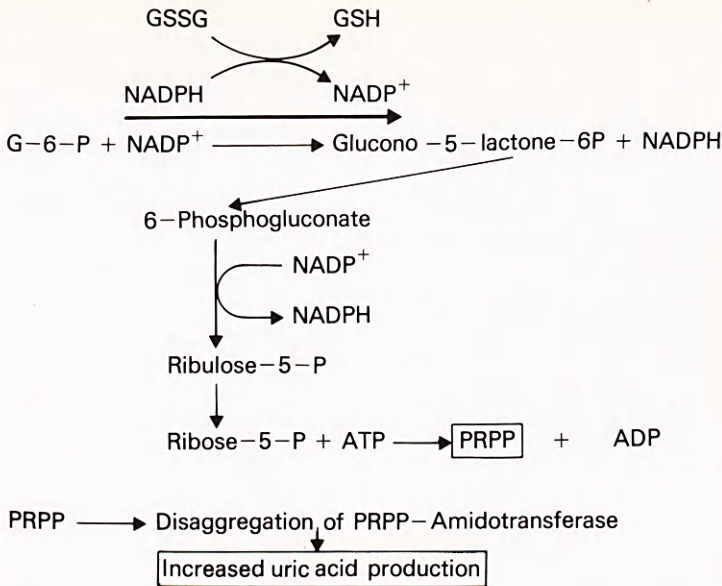
$$\text{GSSG} \qquad \text{GSH}$$

$$\text{NADPH} \qquad \text{NADP}^+$$

$$\text{G}-6-\text{P} + \text{NADP}^+ \longrightarrow \text{Glucono}-5-\text{lactone}-6\text{P} + \text{NADPH}$$

$$6-\text{Phosphogluconate}$$

$$\text{NADP}^+$$
$$\text{NADPH}$$

$$\text{Ribulose}-5-\text{P}$$

$$\text{Ribose}-5-\text{P} + \text{ATP} \longrightarrow \boxed{\text{PRPP}} \quad + \quad \text{ADP}$$

$$\text{PRPP} \longrightarrow \text{Disaggregation of PRPP}-\text{Amidotransferase}$$

$$\boxed{\text{Increased uric acid production}}$$

Fig. 2. The proposed mechanism whereby excessive glutathione reduction with increased generation of NADP$^+$ increases purine biosynthesis *de novo* and, therefore, uric acid production. The increased availability of NADP$^+$ increases the metabolism of glucose through the phosphogluconate shunt pathway and, therefore, the production of ribose-5-phosphate which, in turn, gives rise to the phosphoribosylpyrophosphate (PRPP).

biosynthesis *de novo,* and therefore potentially have a specific enzyme defect of purine metabolism as opposed to a reduced net renal tubular excretion of urate as the primary abnormality. This figure is probably an over-estimate. Patients with gout are not ordinarily examined for the four enzyme abnormalities listed in Table 1, or to determine if they have an increased rate of purine biosynthesis *de novo* with uric acid overproduction. Measuring the urinary uric acid excretion underestimates the proportion of uric acid overproducers in the hyperuricaemic population because of extra renal disposal of uric acid (e.g. into the gastro-intestinal tract) and the fact that some individuals have both an excessive rate of purine synthesis *de novo* and a functional renal abnormality promoting urate retention (Seegmiller *et al.*, 1961). The contribution of the four enzyme defects listed in Table 1 to the total gouty population is not known but it is unlikely to be more than 1 or 2 per cent of those with an increased rate of *de novo* purine synthesis. The identifiably renal or presumed renal causes of hyperuricaemia (Table 2) are particularly liable to cause gout if the patient is already genetically predisposed thereto. Patients with chronic renal failure rarely develop gout unless the renal failure is due to urate nephropathy.

Table 2. Causes of hyperuricaemia

1. Essential hyperuricaemia: overproduction, underexcretion, or both
2. Increased nucleic acid turnover:
    (a) Myeloproliferative disorders
    (b) Psoriasis
    (c) Chronic haemolysis
    (d) Secondary polycythaemia
    (e) Infectious mononucleosis
3. Specific enzyme defects believed to increase the intracellular concentration of PRPP at the sites of purine synthesis *de novo* (*see* Table 1)
4. Infantile hyperuricaemias other than the Lesch-Nyhan Syndrome (e.g. Hooft type (Hooft *et al.*, 1968), Nyhan type (Nyhan *et al.*, 1969), Coleman type (Coleman *et al.*, 1974)).
5. Factors causing urate retention by an effect on the renal tubule transport of urate:
    (a) Drugs: pyrazinamide, diuretics, salicylates (low doses)
    (b) Poisons: lead
    (c) Organic acids: lactic, β-hydroxybutyric, acetoacetic
    (d) Hypertension
    (e) Hyperparathyroidism
    (f) Idiopathic hypercalciuria
6. Overall renal failure
7. Down's syndrome
8. Hypothyroidism

In patients who are being treated by chronic haemodialysis, the plasma urate concentration may change quite widely between dialyses and they sometimes experience attacks of acute arthritis resembling acute gouty arthritis. However, these appear to be more often related to particular metastatic calcifications consisting of apatite than to a crystal synovitis. About 15 per cent of Down's syndrome patients are hyperuricaemic. This is reflected in the higher mean plasma urate concentrations observed when a population of Down's syndrome patients is compared with age and sex-matched controls in the same institution (Howell *et al.*, 1973). Patients with Down's syndrome have not been reported to be especially prone to gout.

Unsuspected hyperuricaemia is a fairly common incidental finding on routine biochemical screening. Provided that a careful clinical history and examination supported by the appropriate radiological and biochemical investigation reveals no evidence of gouty arthritis or renal damage, asymptomatic hyperuricaemia does not need treatment unless it is consistently greater than 9.0 mg/100 ml (0.535 mM). The risk of developing gouty arthritis increases with the height and duration of the hyperuricaemia, and becomes very high in this group (Hall *et al.*, 1967).

Gout is statistically associated with obesity, hypertension, arteriosclerosis, hypertriglyceridaemia and alcoholism. However, the hypertension, arteriosclerosis

and hypertriglyceridaemia are associates of obesity and not of hyperuricaemia itself. There is no excess of hypertension or hypertriglyceridaemia in hyperuricaemic populations when they are matched with non-hyperuricaemic populations of the same body weight (Myers *et al.,* 1968; Gibson and Graham, 1974). Coronary artery disease is more common than average in the overtly gouty patients who are also more likely to be obese, but not in asymptomatic hyperuricaemic subjects (Hall, 1965). In addition, the arteriosclerotic lesions do not contain urate crystals. There are, therefore, no cardiovascular grounds for treating asymptomatic hyperuricaemia more vigorously than outlined above.

Weight reduction is an important aspect of the management of gouty patients, but starvation ketosis should be avoided because this can precipitate attacks of acute gouty arthritis. Alcoholism and hyperuricaemia are correlated with one another as judged by the results of population studies, and several pathophysiological mechanisms are probably involved. Acute inebriation causes hyperlactic acidaemia with hyperuricaemia due to interference with uric acid secretion by the renal tubules. There is also evidence that the continued ingestion of alcohol (100 g daily) may lead to increased uric acid production with hyperuric acidaemia and hyperuric aciduria which return to normal after several days' abstinence (MacLachlan and Rodman, 1967). These observations suggest a direct effect on the rate of purine synthesis. Alcoholism may lead to hypertrigylceridaemia and this could increase urate binding to serum lipoproteins favouring supersaturation of the plasma with urate. Lead contamination of illicitly distilled spirits causing nephropathy may be a factor predisposing to hyperuricaemia in chronic alcoholics in some areas.

Dietary nucleoproteins are converted directly to uric acid and make only a minor contribution to the purine nucleotide pools which participate in the purine nucleotide interconversion reactions. Dietary purine restriction is not important in the clinical management of gout, because even a completely purine-free diet that would be unacceptable as a therapeutic regime lowers the serum uric acid by only about 1 to 2 mg/100 ml at the most (Seegmiller *et al.,* 1961; Zöllner and Griebsch, 1973). The relationship of dietary factors to hyperuricaemia and the complications thereof, however, have to be reconsidered because of the likely use of so-called single cell proteins as dietary supplements in industrialised countries and as a major source of dietary protein in regions where protein malnutrition is now a major problem. Different polynucleotides affect the serum uric acid to different degrees. This is important in relation to the proportions of ribonucleic acid (RNA) and of deoxyribonucleic acid (DNA) in the protein supplement. Also, a given amount of dietary purine affects the plasma uric acid concentration more than the urinary uric acid excretion in hyperuricaemic individuals, and this argues for renal factors being important in the causation of hyperuricaemia in gout patients (Zöllner *et al.,* 1972; Zöllner and Griebsch, 1974; Griebsch and Zöllner, 1974).

The increasing use of parenterally administered carbohydrates such as fructose and polyols (e.g. sorbitol and xylitol), makes it important to consider these nutritional factors in relation to serum uric acid levels. They increase uric acid production and excretion by mobilising liver nucleotides (Woods and Krebs, 1973; Fox and Kelley, 1974), and probably by increasing the rate of purine synthesis through indirect and at present rather ill-understood biochemical mechanisms (Perheentupa and Raivio, 1967; Mäenpää et al., 1968; Frank and Müller, 1974; Emmerson, 1974; Donahue and Powers, 1974; Nairns et al., 1974).

Before treating a case of gout, the physician should consider the possibility that there may be a treatable cause for the hyperuricaemia, or one that affects either the personal prognosis for the patient or the genetic prognosis for the family. He should also consider the possibility that an attack of acute gouty arthritis may have been precipitated by recent treatment with allopurinol or uricosuric drugs, local trauma, a surgical operation or an acute infection.

RENAL COMPLICATIONS OF URIC ACID OVERPRODUCTION

The renal complications of hyperuricaemia were numerous before it could be controlled by drug treatment. Thus, the aggregated data from several series of patients, where the hyperuricaemia had been of sufficient degree and sufficiently prolonged for them to have presented with gout, show that between 20 and 40 per cent had albuminuria, the same proportion were hypertensive, about 25 per cent died from renal disease, and between 80 and 100 per cent showed postmortem evidence of renal damage attributable to prolonged hyperuricaemia. About 20 per cent had uric acid urolithiasis and incidences as high as 1,000 times that in the general population have been reported (Wyngaarden, 1965).

The renal lesions of sodium urate nephropathy (gouty nephropathy or chronic hyperuricaemia nephropathy) include interstitial deposits of sodium urate, particularly in the pyramid regions; these produce segmental loss of renal tissue, interstitial fibrosis and epithelial necrosis. The changes of complicating chronic pyelonephritis and renal hypertension are also seen. About 20 per cent who present with uric acid stones are hyperuricaemic and 10 per cent have overt gout. It should be emphasised that these complication rates do not refer to a group of statistically defined hyperuricaemic patients detected on routine biochemical screening. They emphasise the importance of effective and energetic treatment of gout, but are not arguments for the treatment of asymptomatic hyperuricaemia unless it is of the degree defined above.

About 5 per cent of all urinary stones in the United Kingdom consist of uric acid, and the present position with respect to drug therapy in the management of patients with uric acid stones and sodium urate nephropathy merits consideration. It should be borne in mind that renal failure itself will raise the serum urate, and reduce uric acid excretion, so that, in the case of sodium urate nephropathy, cause

and effect may be impossible to unravel by the time the patient is seen. Patients with uric acid stones and hyperuric aciduria should be treated with allopurinol. Normo-uricaemic uric acid stone formers should be treated, at least in the first instance, with sufficient fluid to maintain a urine volume of 3 litres per 24 hours and to wake the patient during the night for micturition, when he should take another drink. He should also take sufficient sodium bicarbonate to keep the urine alkaline (pH $>$ 7), and be taught to check the pH of his urine with indicator paper. That component of the progressive loss of renal function in sodium urate nephropathy, which is attributable to renal deposits of sodium urate, can be arrested, but not reversed, by allopurinol treatment.

Increased uric acid excretion or the presence of uric acid crystals in the kidney with renal failure clearly indicate allopurinol treatment. If the uric acid excretion is normal in a patient with hyperuricaemia and impaired renal function, allopurinol is recommended only if the serum uric acid concentration exceeds 9.0 mg/100 ml (0.535 mM). The uricosuric drugs (e.g. probenecid and sulfin-pyrazone) increase the risk of stone formation and are inappropriate treatment for gouty patients with a history of stone formation or with hyperuric aciduria. If they are employed in patients who lack an increased uric acid excretion, they should be given with a high fluid intake and sodium bicarbonate, as described above.

Acute uric acid nephropathy may complicate the treatment of diffuse malignant disease, particularly the reticuloses, with cytotoxic drugs and/or irradiation. The dissolution of a large mass of tumour tissue liberates a heavy load of nucleic acid for catabolism. The uric acid excretion rises suddenly, and uric acid precipitates in the collecting tubules in the kidney as well as in the renal pelves and ureters. Acute oliguric renal failure ensues. This complication can be avoided by giving allopurinol prophylactically several days before beginning the treatment. The established condition requires ureteric catheterisation, and measures to produce a vigorous diuresis of alkaline urine. It is possible that other factors than the size of the uric acid load predispose to acute uric acid nephropathy. These include the secretion of an acid urine, increased urate clearance produced by some drugs and metabolites, dehydration, and genetic factors.

LESCH-NYHAN SYNDROME

The Lesch-Nyhan syndrome (Lesch and Nyhan, 1964; Nyhan, 1973) merits further consideration. It is an X-linked disorder in which the affected males do not reproduce, therefore one-third of the cases should arise from new mutations. This agrees with its considerable degree of clinical and genetic heterogeneity. The main features of the syndrome are: hypotonia, delayed motor development (apparent by 3 to 4 months of age), choreoathetosis and spasticity (apparent by about 1 year of age), compulsive self-mutilation (usually beginning by the age of 1.5 to 2 years), aggressive behaviour, mental retardation, megaloblastic anaemia,

hyperuricaemia and hyperuric aciduria with gout and renal complications. The syndrome is associated with virtually complete absence of HGPRT activity from erythrocytes and other tissues, although traces of activity may be detectable in cultured fibroblasts or in red cells if the assay is modified (Kelley and Meade, 1970; Emmerson and Thompson, 1973). Arnold *et al.* (1972) reported that the enzyme protein was always detectable immunochemically, but Upchurch *et al.* (1975) showed this to be wrong, more specific tests failing to reveal HGPRT protein in haemolysates from 14 cases of congenital HGPRT deficiency. Experience has shown that 'complete' HGPRT deficiency (Lesch-Nyhan syndrome) and 'incomplete' HGPRT deficiency form a continuum, and the degree of neurological deficit does not parallel the level of residual HGPRT activity, at least as measured in the erythrocytes (Emmerson and Thompson, 1973). Patients with the 'incomplete' HGPRT deficiency syndrome have sex-linked gout and/or urolithiasis with, in some cases, minor neurological abnormalities (e.g. mild spinocerebellar ataxia) which may or may not be accompanied by some intellectual impairment (Kelley *et al.,* 1969).

The degree of mental impairment in the Lesch-Nyhan syndrome is sometimes less than the degree of motor disability (Scherzer and Ilson, 1969; Watts *et al.,* 1974). This point requires special consideration when the education of these children is being planned.

The heterozygous carrier state can be identified by demonstrating mosaicism with respect to the presence of HGPRT. This can be done in cultured fibroblasts, or by studying the enzyme activity of individual hair follicles. McKeran *et al.* (1975) have recently reviewed this subject, and reported evidence that a case of the Lesch-Nyhan syndrome had arisen from a new mutation.

SOME POSSIBLE INTERRELATIONSHIPS BETWEEN PURINE METABOLISM AND NEUROLOGICAL FUNCTION

The mechanism whereby HGPRT deficiency causes the unique pattern of neurological dysfunction seen in the Lesch-Nyhan syndrome has excited considerable interest and speculation. Two theories have been advanced: the toxic metabolite theory, and the guanylic acid (GMP) deficiency theory. A toxic metabolite has not been identified in the blood or cerebrospinal fluid of these patients. The oxypurine (hypoxanthine and xanthine) concentrations in the cerebrospinal fluid are greater than normal in the Lesch-Nyhan syndrome, but higher concentrations occur in blood in xanthinuria (congenital xanthine oxidase deficiency) and there are no neurological abnormalities in that disorder. The cerebrospinal fluid oxypurine concentrations are raised in cases of incomplete HGPRT deficiency in which there are mild, if any, neuropsychiatric manifestations. Large doses of caffeine (1,3,7-methylpurine) and of theophylline (1,3-methylpurine) cause self-mutilation in rats and rabbits, whereas theobromine (3,7-methylpurine) does not do so. This apparently specific association of the

1-methyl and 1,3-methyl configuration with the experimental production of the most characteristic component of the Lesch-Nyhan syndrome has been used as an argument for the view that a specific toxic metabolite might be produced as a by-product of the gross purine overproduction that occurs in the disorder (Nyhan, 1973).

The metabolic lesion of the Lesch-Nyhan syndrome causes a continuous leakage of hypoxanthine and guanine and, therefore, of inosinic and guanylic acids from the purine ribonucleotide pools; the important biochemical functions that might be expected to be impaired by lack of GMP merit emphasis. These are: nucleic acid synthesis, guanosine triphosphate (GTP) dependent protein synthesis, GTP-dependent glycoprotein synthesis due to impaired activity of the GTP-dependent mannosyl transferase, impaired cell growth and neurotransmission due to lack of cyclic GMP (cGMP), and impaired GMP-dependent neurotubule formation. Brain cells have little or no capacity for purine biosynthesis *de novo* (Howard *et al.,* 1970), and therefore depend on the purine salvage enzymes for the maintenance of effective concentrations of purine ribonucleotides in their purine ribonucleotide metabolic pools. They would therefore be expected to be especially vulnerable to a leak of guanine as in the Lesch-Nyhan syndrome. One would also expect them to be most vulnerable at the stage of organogenesis when the brain cells are forming and differentiating most rapidly, namely in the perinatal period. In addition to this possible effect on perinatal morphogenesis, guanine deficiency could theoretically impair brain function by limiting the supply of cGMP that acts as an intracellular second messenger in the cholinergic and and 5-hydroxytryptaminergic neurones. Guanylate cyclase (EC 4.6.1.2), the enzyme catalysing the production of cGMP from GTP, is stimulated by acetyl choline and by 5-hydroxytryptamine (Weight *et al.,* 1974; Sandler *et al.,* 1975). These considerations offer a biochemical and pharmacological basis for the hypothesis that an imbalance between the 'ergotrophic' or arousal sympathetic nervous system and the diencephalic 'trophotrophic' or quieting parasympathetic system, for which 5-hydroxytryptamine is the neurotransmitter, underlies the autoaggressive behaviour in the Lesch-Nyhan syndrome. The report that 5-hydroxytryptophan was therapeutically effective in controlling the auto-aggression in the Lesch-Nyhan syndrome (Mizuno and Yugari, 1974) could not be confirmed (Frith *et al.,* 1975, 1976).

It has been possible to explore some of these suggestions experimentally using lymphocytes that have been stimulated to undergo blast transformation by phytohaemagglutinin in the presence of azaserine as a model of the rapidly dividing brain cells during the perinatal period (McKeran and Watts, 1976). Azaserine specifically blocks the pathway of purine biosynthesis *de novo,* but does not affect the purine salvage enzymes. DNA synthesis as judged by [^{14}C] thymidine incorporation, is less efficient in azaserine-treated transformed lymphocytes from Lesch-Nyhan patients than in identically treated lymphocytes

from control subjects. RNA synthesis is similarly affected. It has also been shown that Lesch-Nyhan patients' lymphocytes are more susceptible to azaserine inhibition of blast transformation than are normal cells (Allison *et al.*, 1975). These observations indicate that in the absence of the purine biosynthesis *de novo* pathway, as in the azaserine treated lymphocyte, and by analogy in brain cells, there is impaired response to a powerful mitotic stimulus, such as presumably initiates and maintains the perinatal spurt of brain growth.

It seems that we may be nearer to identifying the way in which a specific enzyme defect damages the brain in the Lesch-Nyhan syndrome than in any other type of mental retardation. It is possible that the study of this disorder may have implications beyond its own immediate confines.

HYPOURICAEMIA

Hypouricaemia is a less common marker of metabolic disease than is hyperuricaemia. The known causes of a significantly low plasma urate concentration, <2.0 mg/100 ml (0.119 mM), are shown in Table 3. Low xanthine oxidase

Table 3. Causes of hypouricaemia. Plasma uric acid <2.0 mg/100 ml (0.119 mM)

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1. Low xanthine oxidase activity
    (*a*) Congential (xanthinuria)
    (*b*) Iatrogenic (drug induced inhibition)
2. Congenital low PRPP-synthetase activity
3. Renal tubule reabsorption defects
    (*a*) Congenital
        (i) Isolated
        (ii) Associated with the Fanconi syndrome
    (*b*) Acquired
        (i) Radiocontrast agents
        (ii) Toxic
4. Neoplastic disease
5. Extensive liver damage
6. Syndrome of hypouricaemia, hypercalciuria and osteoporosis
7. Congenital purine nucleoside phosphorylase deficiency (Wadman *et al.*, 1976)

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activity may be due to congenital xanthine oxidase deficiency (xanthinuria) or to treatment with unnecessarily large doses of the xanthine oxidase inhibitor allopurinol. Congenital absence of phosphoribosylpyrophosphate synthetase activity is another cause of a markedly low plasma urate concentration. Hypouricaemia due to a deficiency of urate net reabsorption from the glomerular filtrate as it traverses the renal tubule is a less well recognised cause of hypouricaemia. This can accompany other renal tubule reabsorption defects, as in the Fanconi syndrome, or it can occur as an isolated phenomenon (Praetorius and Kirk, 1950; Greene *et al.*, 1972; Simkin *et al.*, 1974). The autosomal recessive

syndrome of hypouricaemia, hypercalciuria and decreased bone density reported by Sperling and his colleagues (1974) also appears to be due to a defect in the renal tubular reabsorption of uric acid. Occasional patients with neoplastic disease show hypouricaemia; the mechanism of this is unknown but may well be renal in some cases (Weinstein *et al.*, 1965). Extensive parenchymal liver damage is sometimes associated with hypouricaemia, which could be due to reduced xanthine oxidase activity or to reduced purine biosynthesis *de novo*, the liver being the major side of the latter process and purines being transported to other tissues as ribonucleotides in red blood cells.

Congenital PRPP synthetase deficiency has recently been recorded in an infant with mental retardation, megaloblastic anaemia, hypouricaemia, hypouric aciduria and an increased urinary excretion of orotic acid (Wada *et al.*, 1974).

XANTHINURIA

There are now about 25 well-documented cases of congenital xanthine oxidase deficiency (xanthinuria) reported in the literature, and the subject has been reviewed recently (Watts, 1976). About one-third of the patients have had one or more radiotranslucent xanthine stones. Most cases are detected by the incidental finding of a low plasma uric acid concentration that is subsequently shown to be accompanied by a reduced urinary uric acid excretion with increased levels of hypoxanthine and xanthine in the blood and urine. The enzyme defect has been demonstrated in liver tissue, jejunal mucosa, colostrum and milk. Three patients have been reported with xanthinuric myopathy, this being associated with the accumulation of hypoxanthine and xanthine in the skeletal muscles.

The metabolic lesion in xanthinuria (*see* Fig. 1) affects both the oxidation of hypoxanthine to xanthine and of xanthine to uric acid. Xanthine usually accounts for more than 70 per cent of the total amount of hypoxanthine and xanthine excreted in the urine in xanthinuria, presumably because hypoxanthine is converted more actively to inosinic acid by HGPRT than xanthine is converted to xanthylic acid by the same enzyme.

It has recently been shown immunochemically that a xanthinuric patient's milk lacks xanthine oxidase protein as well as xanthine oxidase enzyme activity (Gibbs *et al.*, 1975). This observation is compatible with the condition being due to a non-sense mutation as opposed to mis-sense mutation, and is similar to the most recent findings in the Lesch-Nyhan syndrome where the immunochemical evidence also suggests a non-sense mutation in most cases.

SPECIFIC ASSOCIATIONS BETWEEN SOME BIOCHEMICAL DISORDERS OF PURINE METABOLISM AND DIFFERENT TYPES OF IMMUNOLOGICAL DYSFUNCTION

Evidence is now accumulating that disorders of purine metabolism are specifically associated with certain types of immunological dysfunction. Thus, adenosine

deaminase (EC 3.5.4.4) deficiency is associated with severe combined immune deficiency (Giblett *et al.*, 1972; Dissing and Knudsen, 1972; Yount *et al.*, 1974). Purine nucleoside phosphorylase (EC 2.4.2.1) deficiency is associated with defective T-cell function, B-cell function being normal (Giblett *et al.*, 1975). Conversely, HGPRT deficiency is associated with a minor impairment of B-cell function (Allison *et al.*, 1975). It is reasonable to suppose that the ability to vary purine supplies and, hence, nucleotide production rates, may be closely related to the ability to initiate the production of new protein molecules which is necessary in order to mount an immunological response.

It is of interest that the reactions catalysed by adenosine deaminase, inosine phosphorylase and HGPRT are sequential steps on a pathway for the conversion of adenine nucleotides to guanine nucleotides by way of inosinic acid, this being an alternative to the direct deamination of adenylic acid which is catalysed by adenylic acid deaminase (EC 3.5.4.6), and which occurs, for example, during muscle contraction (*see* Fig. 1). These observations on the enzyme lesions associated with disorders of lymphocyte function suggest that purine nucleoside interconversions are as important as the nucleotide interconversion reactions that occur by means of xanthylic acid, which have received more attention in relation to human disease. This may be especially important if rapid modulations in the rate of polynucleotide and protein synthesis are needed, as when an immunological response is mounted. Another recent observation relevant to this is the very early production of PRPP which is needed for the HGPRT catalysed reaction as well as for purine biosynthesis *de novo*, when lymphocytes are exposed to the mitogen phytohaemagglutinin (Hovi *et al.*, 1975). It may be that further studies of the enzymes that catalyse the purine biosynthetic and interconversion reaction will provide a better biochemical understanding of other immune deficiency states.

CONCLUSION

William Heberden (1816) showed remarkable prescience when he wrote: 'The gout affords a striking proof of the long experience and wary attention necessary to find out the nature of disease and their remedies', because the clinical study of gout has been the starting point for the wide ranging investigations that are reviewed here.

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COMPLAINTS OLD AND NEW

The Medical and Physical Journal of 1810 reviewed modern medicine and said 'we cannot look with indifference, nor without apprehension, on the spirit that impels to the multiplication of books. The art, perhaps, peculiar to modern times of manufacturing literary wares, is extending with incredible rapidity into every branch of human knowledge. Books are easily formed out of books; and the scissars is the magical instrument that promulgates science and philosophy. When the plagiarism is not thus direct, and the pen has actually been employed, a flippant flourish of style, splendor of paper, neatness of type, and elegance of decoration, render superfluous the labors of investigation and the toil of research. Medical literature has not escaped from this delusion of the age. When we look on the fearful multitude of medical quartos, octavos, and duodecimos, that have issued from the British press in the present year, can it be doubted to what extent this modern art has been exercised?'