Heberden oration 1979

Human aberrations of purine metabolism and their significance for rheumatology

J. EDWIN SEEGMILLER

University of California, San Diego, La Jolla, California 92093, USA

It is an honour and privilege to be invited to address you this day. Returning to the city of my grandfather's birth is always a great personal pleasure. This relationship is symbolic also of the close scientific and cultural heritage that we in America owe to this land. The origin of many of our important medical and scientific concepts today stem from the germinal ideas and observations of British scientists and physicians of past ages who have contributed substantially to building the scientific base of the clinical medicine of today.

Not the least of these contributions has come from detailed study of patients afflicted with very specific types of arthritis. Although it was the Swedish chemist, Scheele, who first isolated uric acid from a concretion of the urinary tract in 1776, it was the British chemist, Wollaston, a nephew of William Heberden, who first demonstrated in 1798 the presence of the same substance in a gouty tophus which he is purported to have removed from his own ear. The subsequent demonstration of hyperuricaemia in patients with gouty arthritis in 1848 by Alfred Baring Garrod provided the impetus for much more detailed study of the chemical factors causing disease, which has proliferated remarkably in the ensuing 132 years and continues today at an even more accelerated pace.^{1 2}

From detailed studies of patients with another heritable form of arthritis, alcaptonuria with ochronosis, Archibald Garrod carried his father's concepts to a more fundamental basis with formulation of a revolutionary new concept to account for the accumulation of large amounts of homogentistic acid in these alcaptonuric patients. In his Croonian lectures in 1908 he proposed that the accumulation and excretion of homogentistic acid in this disease is a direct result of a hereditary deficiency of the enzyme for its further processing within the body.³ This concept of one gene being responsible for one enzyme was thus formulated over 30 years before its more detailed investigation

by the microbiologists Beadle and Tatum.⁴ From these studies he also formulated the concept of inborn errors of metabolism and thus laid the groundwork for modern-day developments of our concept of hereditary diseases. The younger Garrod⁵ classified gouty arthritis as one of the inborn errors of metabolism, but unlike other such disorders no single enzyme defect has been found to account for the disease. However, the absence of the enzyme uricase in man and the higher apes, coupled with a remarkably inefficient mechanism for renal excretion of uric acid, results in a mean plasma concentration of urate in adult males of 5.1 mg/dl—a value not far from the theoretical limit of solubility of urate in serum of 6.4 mg/dl. The lower mean serum urate of women of 4.1 mg/dl, until after the menopause, may well account for the far lower incidence of gout in women. The supersaturation of all hyperuricaemic plasma sets the stage for the deposition of needle-shaped crystals of monosodium urate monohydrate in and about the joints and in the parenchyma of the kidney which account for the major pathology in this disease, as proposed by the elder Garrod over a century ago.² Although his concepts languished for over half a century, new evidence of the past 2 decades has validated his major proposals.^{6–9} Only in the past decade and a half have specific enzyme defects in purine metabolism responsible for this disease been identified.

Today I will review some of the circumstances involved in the discovery of these enzyme defects and also review the recent work in which other specific inborn errors of purine metabolism have been found to be associated with deficiency of the immune function and the new insight into possible regulatory mechanisms of the immune system that may well be affected by these enzyme defects. I want to acknowledge the major contributions of the many young investigators who have worked with me in these studies. I also hope to enlist your support in a systematic approach to the classification of our

gouty patients that will greatly facilitate, not only our rational management of their problems by selection of the most appropriate therapeutic agents, but will also allow us to work together in their further biochemical and genetic characterisation. Since it is a lifetime of treatment to which we are committing the patient, the extra time it takes for classification seems warranted and can also be conveniently performed while awaiting for the patient to recover from an acute attack of gouty arthritis and before institution of treatment with a drug known to lower the serum urate concentration.

Classification of gouty patients

The genetic heterogeneity of the gouty population is readily revealed by the simple test I am proposing we use on all our gouty patients—the determination of the 24-hour excretion of uric acid for each of 3 days after the patient has been equilibrated on a diet virtually free of purines.¹⁰ The mean value found in normal adult males is 425 mg/24 hours (Fig. 1) with a spread that places the upper range of normal at 600 mg per day. The population of gouty patients showed a mean value only slightly higher but the spread was much larger with around one-quarter of the gouty population showing production of excessive quantities of uric acid in the 24-hour period. As might be expected, the patients who had substantial renal damage excreted smaller amounts of uric acid than was found in the normal, indicating the basis for the well-known acceleration in rate of development of pathological changes of gout when renal impairment supervenes.

Knowledge of the classification of the gouty patient in regard to the degree of production of uric acid can also be of practical help to the clinician in the selection of the most appropriate drugs for management of the particular patient's disease. We know that allopurinol, in addition to its primary action in blocking the enzyme, xanthine oxidase responsible for the synthesis of uric acid, also has a secondary beneficial effect in shutting off excessive purine synthesis.¹¹ It therefore becomes the drug of choice for patients who have evidence of an excessive rate of purine synthesis, uric acid lithiasis or impaired renal function.

Although some of the patients who show a uric acid excretion in the normal range may have modest degrees of overproduction of uric acid that is obscured by increased extrarenal disposal into tophi or by intestinal uricolysis, a substantial portion of such patients show a diminished ability of the kidney to excrete uric acid as the cause of their hyperuricaemia.^{8 10 12} Therefore, uricosuric drugs correct the primary abnormality and provide a rational therapeutic approach for controlling their disease.

We have recently been using a total of 6 days on the purine-free diet with collection of urine during the last 3 days as an abbreviated procedure more acceptable to the patients. Even this degree of co-operation is often difficult to elicit. For detection of patients with the most extreme degrees of purine overproduction, a simple ratio of uric acid to creatinine in the morning urine suffices. However, it is not nearly as discriminating as the 24-hour collection when properly performed.¹³ A further

Fig. 1 Uric acid excretion by normal and gouty patients after equilibration for 5 days on a diet virtually free of purines. From Seegmiller et al.¹⁰

modification has been proposed in which the mg of uric acid excreted per ml of glomerular filtrate is obtained by dividing the above ratio by the serum creatinine.¹⁴

The origin and disposition of uric acid in the normal and gouty patients is shown in Fig. 2. Purines which give rise to uric acid can originate from purines in the diet or from those synthesised endogenously in all nucleated cells. From either source the purines can be incorporated into cellular nucleotides such as ATP and nucleic acid. The metabolic turnover of these purine-containing compounds gives rise eventually to uric acid. Around two-thirds to three-quarters of the uric acid formed each day is excreted unchanged in the urine, while the remainder is excreted in intestinal secretions where it is degraded by the action of uricolytic enzymes of the gut bacteria.^{9 10 12 15}

The hyperuricaemia required for development of gouty arthritis results from an expansion of the urate pool. In some patients a genetically determined excessive rate of production of uric acid is responsible for the hyperuricaemia. In others this excessive production arises from an enhanced cellular proliferation such as is found in myeloproliferative disorders with an increased turnover of nucleic acid, while in others, and this may well be the majority of patients, a diminished renal excretion of uric acid is responsible for hyperuricaemia.¹⁰ In some cases it has been traced to an associated lactic acidosis with competition of lactic acid or other organic acids

such as ketoacids in diabetic acidosis or in maple syrup urine disease for transport mechanisms.¹⁶ In type 1 glycogen storage disease, with its glucose-6-phosphatase deficiency, both a lactic acidosis and an increased rate of purine synthesis are found.¹⁷

Lesch-Nyhan disease with severe deficiency of hypoxanthine-guanine phosphoribosyl transferase (HPRT)

Clinical Presentation. The most extreme example of excessive uric acid production has been found in the paediatric practice rather than in the rheumatology clinic. A haematuria and uric acid crystalluria first brought a young boy with cerebral palsy to the attention of a medical student, Michael Lesch, and Dr W. L. Nyhan at Johns Hopkins Hospital.¹⁸ Both the patient and his brother had a severe neurological disorder consisting of choreoathetosis, spasticity, mental retardation, and a compulsive biting away of lips and tongue and amputation of the fingers by biting. Both children excreted in the daily urine over 6 times the normal quantity of uric acid. The affected children differed from most gouty patients in failing to diminish the rate of their purine synthesis in response to azathioprine, a 6-mercaptapurine derivative. Furthermore, their cells in culture failed to diminish the rate of purine synthesis when 6-mercaptapurine was added to the culture. We eventually traced this aberrant pharmacological response to a gross deficiency of the enzyme hypoxanthine guanine phosphoribosyl transferase

Fig. 2 Causes of hyperuricaemia. From Seegmiller et al.¹⁰⁰

(HPRT) normally present in all tissues of the body and required for converting 6-mercaptopurine to its metabolically active nucleotide form^{19 20} (Fig. 3).

Up until this time the HPRT enzyme had been assigned only the role of a salvage system for the thrifty reutilisation of purine bases by converting them to purine ribonucleotides at a cost of one ATP molecule as compared to at least 6 ATPs required for synthesis of the purine nucleotide *de novo*. Severe neurological disease and excessive uric acid production of the affected children was the first indication of an important role for this enzyme in both neurological function and in the control of the rate of purine synthesis.

X-linked inheritance. As more families were studied it was soon realised that this disorder affects only males and the pattern of inheritance with transmission

only through their mothers, suggested an X-linked pattern of inheritance. In order to demonstrate more conclusively the X-linked inheritance we made use of a theory first proposed by Dr Mary Lyon²¹ here in England, to account for the presence of Barr bodies in female somatic cells. She proposed their formation to result from the random inactivation of one of the two X chromosomes in female somatic cells at an early stage of zygote development. A consequence of such a random inactivation would be the presence of both normal and mutant phenotypes in cells of women heterozygous for an X-linked disorder, reflecting the inactivation of the X chromosome carrying either the mutant or the normal gene. The development of an analytical system for detecting the presence or absence of HPRT enzyme in single cells permitted the demonstration of these two cell

Fig. 3 Known enzyme defects in human purine metabolism. (1) Increased phosphoribosylpyrophosphate synthetase activity in patients with overproduction of uric acid and gout. (2) Gross deficiency of hypoxanthine guanine phosphoribosyltransferase in children with Lesch-Nyhan disease and partial deficiency of the same enzyme in patients with overproduction of uric acid and gout. (3) Adenine phosphoribosyltransferase deficiency in patients with kidney stones composed of 2-8 dioxyadenine that are often confused with uric acid stones. (4) Xanthine oxidase deficiency in patients with xanthinuria who are at increased risk for xanthine calculi of the urinary tract and in occasional patients myalgia from xanthine crystals in the muscle. (5) Adenosine deaminase deficiency associated with severe combined immunodeficiency disease. (6) Purine nucleoside phosphorylase deficiency associated with isolated defect in T cells. (7) Purine 5'-nucleotidase activity is low in lymphocytes of patients with agammaglobulinaemia that may be secondary to loss of B cells. (8) Adenosine kinase deficiency has so far been developed only in the human lymphoblast cell lines. Its counterpart in patients is yet to be identified.

populations predicted by Dr Lyon's hypothesis. The deficient enzyme is required for the first step in the incorporation of tritiated hypoxanthine into nucleic acid in cultured fibroblasts. Upon overlaying the washed cells with photographic emulsion, normal cells showed abundant ^3H incorporation, while cells from affected children showed virtually no incorporation. As predicted by the Lyon hypothesis, cells from the mother of an affected child showed populations of both normal and mutant cells, thus confirming very effectively the location of the gene for coding the HPRT enzyme on the X chromosome.²² The 2 populations are also detectable by enzyme assay of hair follicles.²³

Prenatal diagnosis. This same technique, which we applied to amniotic cells in culture, demonstrated for the first time the usefulness of biochemistry in determining genotype for an X-linked disease.²⁴ We have since monitored 21 pregnancies at risk for this disorder and have identified seven affected fetuses, each sufficiently early in gestation that the parents' desire to terminate the pregnancy could be met.^{25 26} At the present time prenatal diagnosis is theoretically possible for well over 50 serious hereditary metabolic diseases, thus introducing a new and dynamic role to genetic counselling, which permits individuals known to be at risk for producing children with certain specific serious hereditary defects to produce the normal children which they desire.

Screening test. Now that the Lesch-Nyhan disease is preventable through monitoring of pregnancies, the identification of affected children becomes important in order that their female relatives carrying the gene can be identified and informed of the need for monitoring of pregnancies. We have devised a screening test in which the automated determination of uric acid and creatinine in liquid urine samples or urine samples preserved by drying on filter paper is used to identify those patients in neurology clinics, genetics clinics, or cerebral palsy clinics in whom the more specific enzyme assay on erythrocytes should be performed.^{13 27} Female relatives who are carrying this gene can then be identified and alerted to the need to have their pregnancies monitored if they are to avoid producing affected children.

Neurological disorder. The mechanism by which the enzyme deficit in brain tissue leads to the severe neurological disease and compulsive behaviour is not well understood.²⁰ In glioma and neuroblastoma cell lines deficient in HPRT, monoamine oxidase activity is significantly diminished, suggesting the possibility of an imbalance also being created in the neurotransmitter system of the brain by this enzyme deficit.^{28 29} Hydroxytryptophan has been reported by Mizuno and Yugari³⁰ to ameliorate

the self-mutilating behaviour in children in Japan, but Frith *et al.*³¹ in this country and Ciaranello *et al.*³² in the United States were unable to confirm this observation. However, a combination of hydroxytryptophan and carbidopa (MK486) produced a transient cessation of compulsive self-mutilation in some patients which could not be sustained even by increased doses of the medications³³ (Nyhan, personal communication). A role for uric acid seems to be ruled out by the normal concentrations of uric acid found in the cerebrospinal fluid. However, hypoxanthine and xanthine were elevated in the cerebrospinal fluid and the methylated xanthines, caffeine, and theophylline have been shown to induce an analogous compulsive self-mutilation in rats and rabbits.^{20 33}

Gout with partial deficiency of HPRT

Clinical features. Understanding the enzyme defect of Lesch-Nyhan disease has substantially extended our understanding of other clinical disorders associated with excessive production of uric acid.³⁴ Less severe deficiencies of the same enzyme have been found in members of certain families with gout.^{35 36} Three gouty brothers, each showing a 3- to 4-fold increase over normal in daily uric acid production, had around 1% of normal HPRT enzyme activity in their erythrocyte lysates and no detectable neurological disease. In another family with a different mutation in the same enzyme showing around 0.5% of normal activity with guanine as a substrate both brothers showed an attenuated neurological disease consisting of a mild spastic quadriplegia in one young man and mental retardation and kidney stones in his 10-year-old brother. Additional neurological disorders have been found in other families, including seizures, dysarthria, mild motor difficulties, and mental retardation.^{20 36 37} Obviously, the clinical neurological signs would not permit such divergent clinical presentations to be classified together. Only the HPRT enzyme deficiency shared by these affected patients, has permitted the relationship to be established. As might be expected, all of the affected patients showed an excessive production of uric acid, a high incidence of renal calculi, and a progressive renal damage often beginning in childhood, with development of gouty arthritis at an earlier age than most gouty patients.

Apparently genetic heterogeneity is present even in defects involving the same gene, since different families carry different types of mutations. However, in general the severity of the clinical manifestations are proportional to the severity of the enzyme defect, although exceptions are known.^{20 38} Any patient

carrying the HPRT deficiency shows an aberrant response to allopurinol in which the deficit in uric acid production is made up by hypoxanthine and xanthine without the decrease in the total purine synthesis encountered in other gouty patients.³⁹

Mechanism of excessive purine synthesis in HPRT deficiency

The mechanism by which the deficiency of HPRT leads to an excessive rate of purine synthesis is now fairly well defined. Since the salvage activity of the enzyme produces purine nucleotides directly from free purine bases, its gross deficiency could conceivably result in a diminished intracellular concentration of purine nucleotides leading to a release of the normal feedback inhibition (Fig. 3). Although this is an attractive hypothesis, we have found no evidence to support it. The intracellular concentration of purine nucleotides in fibroblasts cultured from patients was the same as in those of normal fibroblasts.⁴⁰ Lymphoblasts carrying this enzyme deficit also showed no increase in adenine nucleotides, although an increased concentration of pyrimidine nucleotides was found.^{41 42} An alternative hypothesis was suggested by the fact that the presumed rate-limiting amidotransferase shares with the HPRT enzyme a common substrate, phosphoribosyl-1-pyrophosphate (PP-ribose-P). Therefore, the enzyme deficiency conceivably could increase the intracellular concentration of this substance. This, indeed, proved to be the case. HPRT-deficient fibroblasts showed 3- to 4-times greater than normal concentration of PP-ribose-P and in erythrocytes of affected children the PP-ribose-P was increased as much as 10-fold above normal.^{20 40}

Gout with increased phosphoribosyl pyrophosphate synthetase (PP-ribose-P)

Additional evidence in support of the important role of PP-ribose-P in determining the rate of purine synthesis came from studies of patients with gouty arthritis who had an excessive rate of purine synthesis de novo but normal HPRT activity. Their fibroblasts in culture also showed an increased concentration of PP-ribose-P.^{40 43} A different mutation was identified by Dr Becker while a post-doctoral fellow in our laboratory to account for this increased PP-ribose-P concentration.⁴⁴⁻⁴⁶ This mutation was most unusual in that it consisted of an increased activity, rather than a decreased activity, of the enzyme phosphorybosylpyrophosphate (PP-ribose-P) synthetase. It differed from a mutation described by Sperling, *et al.*⁴⁷ and Zoref⁴⁸ in a gouty patient in that this high activity was apparent at all

concentrations of inorganic phosphate with normal response to feedback inhibitors. Subsequent work by Dr Becker's laboratory has identified additional families, each with a different type of mutation in this same enzyme but all with a high activity. A child originally reported to show autistic behavior⁴⁹ was now found instead to be deaf and detailed examination showed both feedback resistant PP-ribose-P synthetase, and a high specific activity of this enzyme.⁵⁰ The presence of 2 different mutations, each leading to an accumulation of PP-ribose-P and an excessive rate of purine synthesis, provides strong evidence of the important role of the intracellular concentration of this substance as a determinant of the rate of purine synthesis de novo (Fig. 3).

Adjacent location of HPRT and PRPP synthetase on the X chromosome

Pedigrees of families carrying the gene for increased PP-ribose-P synthetase were compatible with an X-linked inheritance. Two cell populations showing normal and mutant enzyme have been found.^{51 52} In collaborative studies with Dr Goss at Oxford, using human-rodent hybrid cells, the genes for HPRT and PP-ribose-P synthetase were located near each other on the long arm of the X chromosome.¹⁰² This is the first example of genes for 2 successive enzyme reactions being found on the same mammalian chromosome.

Inosinic dehydrogenase deficiency as a potential enzyme defect

An increased purine synthesis accompanying a gross impairment of inosinic dehydrogenase activity has so far been studied only in cultured human cells exposed to pharmacological agents known to increase serum and urinary uric acid when administered in vivo. 2-ethyl amino 1, 3, 4-thiadiazole, an antitumour drug, when given to patients produced a marked increase in both serum urate concentration and in the excretion of urinary uric acid.⁵³ Its clinical administration greatly enhanced the incorporation of isotopically labelled glycine into urinary acid uric.¹⁵ Studies in vitro by Nelson *et al.*^{54 55} demonstrated a marked inhibition of the enzyme, inosinic dehydrogenase, through formation of an inhibitory thiadiazol analog of NAD. Ribavirin, an antiviral drug, also inhibits inosinic dehydrogenase and in cultured human lymphoblasts produces a marked increase in excretion of hypoxanthine into the medium.⁵⁶ It thus becomes a model for an additional hereditary defect that remains to be diagnosed among our gouty patients who are producing excessive

quantities of uric acid. We can supply shipping vials with culture media for sending blood from potential candidates to our laboratory for study.

Other enzyme defects of purine metabolism

Adenine phosphoribosyltransferase deficiency. This deficit was first identified in heterozygote state by Kelley *et al.*¹⁰³ from our routine assay of this enzyme as a control in our HPRT studies. The homozygotes for this disorder excrete large amounts of adenine in the urine along with 2-8-dioxyadenine, which is very sparingly soluble and forms calculi in the urinary tract as the presenting symptom that are easily confused with uric acid⁵⁷⁻⁵⁹ (Fig. 3).

Adenosine kinase deficiency. This mutation has so far been described only in cultured human lymphoblasts from a mutation derived *in vitro*.⁶⁰ No enhanced rate of purine synthesis was found. Its clinical presentation remains to be described (Fig. 3).

Xanthinuria. A marked hypouricaemia with the replacement of uric acid in urine by xanthine and hypoxanthine was first described by Dent and Philpot⁶¹ in a child who passed a xanthine calculus in the urine. The gross deficiency of xanthine oxidase, the enzyme responsible for uric acid formation, was subsequently demonstrated by Ayvazian *et al.*⁶² and Watts *et al.*^{63 64} (Fig. 3). Patients are usually detected by finding a profound hypouricaemia in routine tests. Around one-third of the known cases have developed xanthine calculi of the urinary tract.⁹ Three patients who had myalgias showed deposits of xanthine crystals in frozen sections of the muscle biopsies and one such patient was found in a rheumatology clinic.^{65 66}

Defects of purine metabolism associated with immunodeficiency disease

In the past 3 decades remarkable progress has been made in our understanding of the role of various components of the immune system. The origin and role of T and B cells has been defined (Fig. 4) and many phenomena regulating the response in this 'black box' have been identified.⁶⁷ The recent biochemical identification of specific enzyme defects associated with immune dysfunction has now provided a crack in the black box to let us glimpse some of the biochemical processes involved in regulation of the immune response.

Enzyme defects. Gross deficiency of each of 3 sequential enzymes involved in the catabolic degradation of purine nucleotides have been reported in patients with various types of defects of the immune system (Fig. 5). The first enzyme defect identified was a gross deficiency of adenosine deaminase (ADA) noted in red cells of 2 children with severe combined immunodeficiency disease by Dr Gillelt and associates in 1972.⁶⁸

All patients with ADA deficiency show a uniform severe impairment of T-cell function and varying degrees of impairment of B-cell function, with a severe lymphopenia. Some 3 years later she reported the first patient with a gross deficiency of purine nucleoside-phosphorylase (PNP) associated with an isolated defect in T-cell function.⁶⁹ As might be expected, this patient excreted large amounts of the conventional substrates for the missing enzymes inosine and guanosine; but quite unexpected was the relatively large amount of the corresponding deoxynucleosides, deoxyinosine, and deoxyguanosine, amounting to around one-third of total nucleosides.⁷⁰

Fig. 4 The origin of T and B cells. From Waldmann and Broder.¹⁰¹

Fig. 5 Enzyme defects of purine nucleotide catabolism associated with various immunodeficiency diseases. From Seegmiller *et al.*⁸³

On a molar basis the total daily purine excreted was quite comparable to the excessive rate of purines excreted by patients with Lesch-Nyhan disease. Since such children are incapable of making hypoxanthine (or consequently uric acid) they show a profound hypouricaemia. Both of these disorders are definitely genetic in origin.

More recently Johnson *et al.*⁷¹ and Webster *et al.*⁷² have reported a markedly decreased activity of ecto-purine-5'-nucleotidase, the first enzyme in this sequence in lymphocytes of patients with adult onset agammaglobulinaemia and a similar reduction in enzyme activity has been reported by Edwards *et al.*⁷³ in lymphocytes of patients with X-linked agammaglobulinaemia. However, the enzyme ecto-5'-nucleotidase is most abundant in normal mature B cells and is virtually absent from these cells at birth, suggesting that it may also be a marker for B-cell maturation, since it is also low in activity in many patients with chronic lymphocytic leukaemia.⁷⁴⁻⁷⁶ The low activities reported in peripheral lymphocytes may therefore reflect merely the reduced number of functioning B cells present in these hypogammaglobulinaemic states. Against this view are reports of low activity in T cells in patients with X-linked agammaglobulinaemia.^{77 78}

Mechanisms of immunodeficiency

The concept of a severe impairment of the immune system being the consequence of a single enzyme deficit was quite foreign to the thinking of most immunologists. One of the proposals made to account for it was that the genetic locus for ADA might reside in close proximity to the locus for immune response genes and that both were lost with a

genetic deletion of these 2 regions of the chromosome. Such deletions, however, are quite rare and over 2 dozen families have now been described in which this association has been found. Furthermore, the gene for adenosine deaminase has now been located on chromosome 20 and that for the immune response genes is chromosome 6, thus making the concept quite untenable.

Enzyme distribution. Knowledge of the distribution of the enzyme adenosine deaminase shown in Table 1 might well have allowed us to anticipate the consequences of its deficiency. The enzyme is most active in thymus, the site of origin of T cells, and next highest in spleen, lymph nodes, and other lymphoid organs.^{79 80}

Metabolic consequences of ADA deficiency

The unexpectedly large amounts of deoxynucleosides produced by children with PNP deficiency provides evidence that the degradative pathway for purine ribonucleotides shown in Fig. 5 serves also for degradative metabolism of deoxynucleosides as well. Although one might expect a substantial accumulation of both adenosine and deoxyadenosine in

Table 1 Enzyme activities in human tissues

Tissue	Deoxy-adenosine kinase*	Adenosine deaminase*	Purine nucleoside phosphorylase*
Thymus	0.78	282.8	23.3
Spleen	0.20	12.4	54.0
Brain	0.14	5.0	10.3
Kidney	0.07	1.8	100.0
Liver	0.07	1.1	36.2
Lung	0.06	0.8	38.0
Small intestine	0.08	14.2	63.9
Heart	0.08	2.1	32.2
Peripheral lymphocytes	0.32	20.7	114.7
Peripheral granulocytes	0.05	11.9	121.4

Human tissues were obtained from one baby who died during parturition, and peripheral lymphocytes and granulocytes were isolated from the blood of a normal adult.

*Activities are expressed as nmoles of product per minute per milligram of protein at a substrate concentration of 300 μM . From Carson *et al.*⁸⁰

METABOLIC FATES OF ADENOSINE AND DEOXYADENOSINE

Fig. 6 Alternate pathways of metabolism for adenosine and deoxyadenosine. From Seegmiller *et al.*⁸³

ADA-deficient patients, only deoxyadenosine accumulates to any large extent and is excreted in the urine in increased amounts.^{81 82} Potential pathways for further metabolism of adenosine and deoxyadenosine are shown in Fig. 6. The failure of these children to excrete any appreciable amounts of adenosine could be related to either the high affinity of adenosine for its kinase or to use of an alternative pathway (not shown) for breakdown of adenylic acid by the enzyme adenylic acid deaminase.⁸³

Adenosine deaminase deficiency

Effect of adenosine and deoxyadenosine on cell growth.
The availability of a number of inhibitors of ADA,

the most potent of which is deoxycoformycin with a K_i of $2.5 \times 10^{-12}M$ and erythro-9-(2 hydroxyl-3 nonyl) adenine (EHNA) which has a K_i of 1.3×10^{-9} , has allowed the study of the metabolic consequences of impaired ADA function in a variety of cell types. Adenosine, at relatively low concentrations, is lethal for cultured mammalian cells of lymphoid origin where it produced a gross deficiency of pyrimidine nucleotides which was reversed by uridine, suggesting that this was the main action of adenosine toxicity.⁸⁴ A similar type of adenosine toxicity in human lymphoblast lines treated with EHNA was reversed only in part by uridine. The failure of uridine to restore either mitogen responsiveness of lymphocytes of ADA-deficient children when studied in vitro or immune function when given in vivo argues strongly against this mechanism being a valid model of the disease.⁸⁵⁻⁸⁷ As shown in Fig. 7, deoxyadenosine inhibited proliferation of lymphoblasts at lower concentrations than did adenosine, and a T-cell line was far more sensitive to this inhibition than was a B-cell line. As shown in Table 2 the inhibition of the T-cell line was accompanied by a marked accumulation of deoxyATP. This greater accumulation in the

Fig. 7 Effect of adenosine and deoxyadenosine on the growth of a human leukaemic T-cell line and B-cell line. All cultures contain $5 \mu M$ EHNA. From Carson *et al.*⁹⁹

Table 2 Effect of deoxyadenosine on deoxy-ATP and ATP concentrations in T and B cell lines

Cell line	Deoxy-adenosine (μM)	EHNA (μM)	ATP (pmoles/ 10^6 cells)	Deoxy-ATP (pmoles/ 10^6 cells)
T cells			2100 $\pm$ 78 (SEM)	<50
T cells	20	5	1900 $\pm$ 165	607 $\pm$ 207
B cells			2753 $\pm$ 1054	<50
B cells	20	5	2075 $\pm$ 582	<50

From Carson *et al.*⁹⁹

Fig. 8 Breakdown of dATP by T- and B-cell lines. The T-cell lines ($\bullet$ left) and the B-cell line ($\circ$ right) at a density of 5×10^6 cells/ml were incubated for three hours with $1.0 mM$ deoxyadenosine without deoxycoformycin and then washed and placed in fresh medium. At various time points thereafter the cells were washed and extracted and deoxyATP concentration were determined by DNA preliminary assays. From Carson *et al.* *Proc Natl Acad Sci USA* 1979; 76: 2430-3.

T-cell line was associated with a far less rapid disappearance of deoxyATP upon removing deoxyadenosine from the media than was seen in B-cell line (Fig. 8) suggesting a major role for degradative enzymes in accounting for this difference in response. In broken cell preparations, shown in Table 3, deoxyATP, deoxyGTP and thymidine triphosphate were all degraded far more slowly by T-cell than B-cell lysates. This slower rate of degradation was correlated also with a substantially lower activity of ecto-purine-5'-nucleotidase in the T-cell line (Table 4).

The addition of deoxycytosine and deoxyadenosine to phytohaemagglutinin-stimulated lymphocytes from normal peripheral blood also results in a substantial elevation in deoxyATP concentration under conditions where ^{14}C leucine incorporation is grossly inhibited. As shown in Fig. 9, this inhibition was substantially overcome by addition of various other single deoxynucleosides and a combination of either thymidine or deoxycytidine with deoxyguanosine completely overcame the inhibition.⁸⁸

Since normal T cells from peripheral blood also showed a substantially lower activity of ecto-purine-5'-nucleotidase than B cells, as shown in Fig. 10, in patients with ADA deficiency, a similar

Fig. 9 Inhibition of PHA-induced proliferation of human lymphoblast by $10\ \mu\text{M}$ deoxyadenosine and $1\ \mu\text{M}$ deoxycytosine with reversal by addition of deoxynucleosides ($10\ \text{M}$). From Bluestein *et al.*⁸⁸

Table 3 Deoxyribonucleoside triphosphate catabolism in broken cells

Cell lines	Substrate		
	dATP	dGTP	dTTP
T	11.1 ± 7.6	10.0 ± 5.2	4.0 ± 1.2
B	102.3 ± 32	67.3 ± 18	84.9 ± 12

Three leukaemic T cell lines and 3 B cell lines were lysed by freezing and thawing and incubated with $300\ \mu\text{M}$ tritiated dATP, dGTP, or dTTP. After 30 min for the B cell lines or 120 min for the T cell lines, nucleotides were separated from nucleosides and bases by thin-layer chromatography. Activities are expressed as pmol of product (nucleoside + base)/min per 10^6 cells $\pm$ SD ($n = 1$ method). From Carson *et al.* *Proc Natl Acad Sci* 1979; 76: 2430-3.

Table 4 5'-Nucleotidase activity in T and B cell lines

Cell lines	Whole cells		Broken cells	
	-AMP- (CH_2)P	+AMP- (CH_2)P	-AMP- (CH_2)P	+AMP- (CH_2)P
T	3.07 ± 1.4	3.07 ± 1.4	46 ± 8.5	42 ± 15
B	387 ± 319	14 ± 7.0	330 ± 225	62 ± 15

To three T cell and 3 B cell lines at densities of $1.5\text{--}3.0 \times 10^6$ cells per ml in a buffer containing $8.6\ \text{mM}$ magnesium chloride and $50\ \text{mM}$ Tris-HCl at pH 6.9 was added $215\ \mu\text{M}$ ($8\text{--}^{14}\text{C}$) inosine monophosphate (specific activity $6\ \text{mCi/mmol}$, Amersham/Searle) in a final volume of $35\ \mu\text{l}$, either with or without $2.86\ \text{mM}$ AMP- $(\text{CH}_2)\text{P}$. After $15\text{--}60$ min at 37°C the reaction was terminated by the addition of $5\ \mu\text{l}$ of 8M perchloric acid, and the products inosine and hypoxanthine were separated from inosine monophosphate by thin-layer chromatography. Activities are expressed as pmol of product (inosine + hypoxanthine) min per 10^6 cells $\pm$ SD for the 3 T and the 3 B cell lines. From Carson *et al.* *Proc Natl Acad Sci USA* 1979; 76: 2430-3.

greater sensitivity of T cells to growth inhibition and a greater accumulation of deoxyATP might be expected.⁸⁰ Both erythrocytes and lymphocytes of ADA-deficient children show marked increases in deoxyATP which decrease after therapy of the patients with irradiated erythrocytes.⁸⁵⁻⁸⁹⁻⁹¹

Mechanism of immunosuppression of T cells and excessive purine synthesis in PNP deficiency

A similar mechanism of inhibition of T-cell function by accumulation of deoxyGTP has been proposed for patients with PNP deficiency as a result of accumulation of deoxyguanosine in this disease.⁸⁹⁻⁹⁰ At the present time the accumulation of deoxyGTP has been shown only in erythrocytes of affected children.

The common denominator to account for the excessive rate of purine synthesis in children with PNP deficiency and HPRT deficiency is the failure of children in both these diseases to reutilise hypoxanthine. Children with HPRT deficiency are missing the enzyme for utilising hypoxanthine while those with PNP deficiency are unable to make hypoxanthine. Studies of human cells studied in vitro show

Fig. 10 Ecto-5'-nucleotidase activity in lymphocytes from normal subjects and patients with X-linked agammaglobulinaemia and their female relatives. From Thompson *et al.*⁷⁶

that the rate of synthesis of purines *de novo* in normal cells can be accelerated to values quite comparable to that seen in cells from children with Lesch-Nyhan disease by removing all hypoxanthine from the medium.⁹²

Proposed mechanism of suppression of T-cell proliferation in ADA and PNP deficiency

A mechanism by which deoxyATP accumulation can arrest cellular proliferation is provided by the very well documented allosteric inhibition of all activity of the enzyme ribonucleoside diphosphate reductase by deoxyATP^{93 94} (Fig. 11). This enzyme constitutes the only known route of deoxyribonucleotide synthesis from ribonucleotides. Consequently its inhibition could result in cessation of

DNA synthesis from an inadequate supply of the deoxyribonucleoside triphosphate substrates. The ability of mixtures of deoxynucleosides to overcome the toxicity of deoxyadenosine could readily be explained by their providing a source for the formation of the other deoxynucleotides that are in short supply. An alternative explanation could be an inhibition of deoxyATP formation due to the other deoxynucleosides competing with deoxyadenosine in the deoxynucleoside kinase or subsequent phosphorylation reactions.

An analogous mechanism could well be operating for the immunodeficiency seen in patients with PNP deficiency. DeoxyGTP is also a known allosteric inhibitor of ribonucleoside diphosphate reductase and it specifically inhibits the formation of deoxyCTP and TTP.^{93 94} Deoxyguanosine toxicity for mouse

Fig. 11 Postulated mechanism of inhibition of lymphocyte proliferation in genetic deficiency of ADA or PNP.

T-lymphoma cells has been reversed by the addition of deoxycytidine (and hypoxanthine) to the culture medium.⁹⁵

The reversal of deoxyadenosine or deoxyguanosine toxicity in cultured cell systems by the addition of other deoxynucleosides suggests the possibility that these deoxynucleosides, particularly deoxycytidine, may be useful in treatment of patients with ADA deficiency or PNP deficiency. A few clinical attempts have been made to accomplish such a reversal, but no remarkable therapeutic benefit was produced. Success will depend on whether or not the patient has an adequate population of viable stem cells that can respond to differentiation into immunocompetent cells and whether appropriate routes of administration for the deoxynucleosides are used to deliver them to the stem cells at sufficiently high concentration. Here in England, as well as in the United States, cautious evaluations are under way to apply this new knowledge to the clinical control of T-cell leukemia by use of deoxycytosine.⁹⁶⁻⁹⁸

Ecto-5'-nucleotidase activity in X-linked agammaglobulinaemia

In a recent study Drs Thompson and Boss⁷⁶ in our laboratory separated T cells from B cells in peripheral blood by rosetting them with neuraminidase-treated sheep erythrocytes. As shown in Fig. 10, normal nonrosetting (B) cells, in agreement with Rowe *et al.*,⁷⁴ showed at least 3 times the activity of normal rosetting (T) cells. Monocytes and null cells showed negligible activity. In 5 patients with X-linked agammaglobulinaemia the nonrosetting (B) cells showed less than 5% of normal activity and failed to show any B cells with surface immunoglobulin. Of special interest was the finding of only 56% of normal activity in their rosetting (T) cells. However, the significance of this finding is not clear at this time.

A summary of the known enzyme defects in purine metabolism is shown in Fig. 3.

Conclusion

I have reviewed today for you the valuable insight into a variety of biological processes that have been provided by human mutations in purine metabolism, many of them in patients with rheumatic disorders. These mutations provide a dissecting probe and spotlight into biology for unravelling the complex interrelations involved in biological systems. Detailed studies of patients with alcaptonuria led Garrod to formulate the gene-enzyme hypothesis, which in turn provided the key for understanding a myriad other hereditary diseases. Once the abnormal gene product has been identified substantially more insight

into biological process of medical interest can be obtained. In many cases, as we have seen today for Lesch-Nyhan disease, what was previously a curious or even tragic clinical problem quite beyond our capability of understanding now becomes a fascinating 'experiment of nature' which with proper study is capable of revealing a rational sequence by which the abnormal gene product gives rise to the clinical expression and disease, and frequently rational approaches to therapy. The identification of enzyme defects associated with immunodeficiency diseases have already provided us with valuable information on biochemical differences in T and B cells, which could be used as the basis for development of more specific pharmacological agents for fine tuning correction of aberrations of the immune system which we now realise are responsible for a wide range of diseases, including many of our rheumatic diseases.

But the work has only begun. The enzyme defects identified so far in patients with gouty arthritis are but a start, since they account for less than 5% of patients with clinical gout seen in most clinics. However, with this knowledge we have identified in cell culture other defects in purine metabolism that should be found as enzyme deficits in the gouty population. The abnormal gene products responsible for the hereditary tendency for development of many other common rheumatic diseases, such as degenerative joint disease and chondrocalcinosis, and the mechanism by which they produce pathology remain to be identified. A similar need exists for identification of additional genetic factors responsible for susceptibility to other of our common diseases of familial nature, including cardiovascular disease, hypertension, neuromuscular diseases, and mental illness.

Perhaps the greatest promise of this new knowledge of genetics is the potential it holds for early detection of specific disease-prone states and for the formulation of a rational therapeutic intervention. This knowledge, in turn, can shift our emphasis in the future practice of medicine from merely awaiting the clinical presentation of advanced pathology to a whole new orientation of preventive medicine in which we identify disease susceptibility at birth or even before birth and then use a lifetime of preventive approaches to its control. For this vision of medical practice of the future to become a reality we must provide more opportunities and incentive for our most gifted and inspired younger physicians to become immersed in research endeavours at both basic and clinical levels. The potential savings can be great in cost of medical care, in effective use of medical manpower and most important in the health of our citizens.

The work was supported in part by National Institutes of Health grants GM 17702 and AM 13622 and a grant from the Kroc Foundation.

References

- Garrod A B. Observations on certain pathological conditions of the blood and urine in gout, rheumatism and Bright's disease. *Trans Med-Chir Soc (London)* 1848; **31**: 83-98.
- Garrod A B. *The Nature and Treatment of Gout and Rheumatic Gout*. London: Walton and Maberly, 1859.
- Garrod A E. The Croonian lectures on inborn errors of metabolism. Lecture II, Alkaptonuria. *Lancet* 1908; **2**: 73.
- Beadle G W, Tatum E L. Genetic control of biochemical reactions in *Neurospora*. *Proc Natl Acad Sci USA* 1941; **27**: 499.
- Garrod A E. *Inborn Errors of Metabolism*. London, Oxford. 1923.
- McCarty D J. Phagocytosis of urate crystals in gouty synovial fluid. *Arthritis Rheum* 1961; **4**: 425-6.
- McCarty D J Jr. Mechanisms of the crystal deposition diseases—gout and pseudogout. *Ann Intern Med* 1973; **78**: 767-71.
- Seegmiller J E, Howell R R, Malawita S E. Inflammatory reaction of sodium urate: Its possible relationship to genesis of acute gouty arthritis. *JAMA* 1962; **180**: 469-75.
- Seegmiller J E. Diseases of purine and pyrimidine metabolism. In: Bondy P K, Rosenberg L E, eds. *Metabolic Control and Disease*, 8th ed. Philadelphia: Saunders, 1980: 777-937.
- Seegmiller J E, Grayzel A I, Laster L, Liddle L. Uric acid production in gout. *J Clin Invest* 1961; **40**: 1304-14.
- Rundles R W, Wyngaarden J B, Hitchings G H, Elion G B, Silberman H R. Effects of a xanthine oxidase inhibitor on thiopurine metabolism, hyperuricemia, and gout. *Trans Assoc Am Phys* 1963; **76**: 126-40.
- Benedict J D, Forsham P H, Roche M, Soloway S, Stetten D W Jr. The effect of salicylates and adrenocorticotrophic hormone upon the miscible pool of uric acid in gout. *J Clin Invest* 1950; **29**: 1104-11.
- Kaufman J M, Greene M L, Seegmiller J E. Urine uric acid to creatinine ratio—A screening test for inherited disorders of purine metabolism. *J Pediatr* 1968; **73**: 583-92.
- Simkin P A, Hoover P L, Paxson C S, Wilson W F. Uric acid excretion: quantitative assessment from gout, midmorning serum and urine samples. *Ann Intern Med* 1979; **91**: 44-7.
- Seegmiller J E, Grayzel A I, Liddle L, Wyngaarden J B. The effect of 2-ethyl-amino-1, 3, 4-thiadiazole on the incorporation of glycine into urinary purines and uric acid in man. *Metabolism* 1963; **12**: 507-15.
- Schulman J D, Lustberg T J, Kennedy J L, Museles M, Seegmiller J E. A new variant of maple syrup urine disease (branched chain ketoaciduria): Clinical and biochemical evaluation. *Am J Med* 1970; **49**: 118-24.
- Alepa F P, Howell R R, Klinenberg J R, Seegmiller J E. Relationships between glycogen storage disease and tophaceous gout. *Am J Med* 1967; **42**: 58-66.
- Lesch M, Nyhan W L. A familial disorder of uric acid metabolism and central nervous system function. *Am J Med* 1964; **36**: 561-70.
- Seegmiller J E, Rosenbloom F M, Kelley W N. Enzyme defect associated with a sex-linked human neurological disorder and excessive purine synthesis. *Science* 1967; **155**: 1682-4.
- Seegmiller J E. Inherited deficiency of hypoxanthine-guanine phosphoribosyltransferase in X-linked uric aciduria (the Lesch-Nyhan syndrome and its variants). In: Harris H, Hirschhorn K, eds. *Advances in Human Genetics*. Plenum Publishing Corp., New York; Plenum, 1976; **6**: 75-163.
- Lyon M F. Gene action in X chromosome of the mouse (*Mus musculus* L). *Nature* 1961; **190**: 372-3.
- Rosenbloom F M, Kelley W N, Henderson J F, Seegmiller J E. Lyon hypothesis and X-linked disease. *Lancet* 1967; **2**: 305-6.
- Gartler S M, Scott R C, Goldstein J L, Campbell B, Sparkes R. Lesch-Nyhan syndrome: Rapid detection of heterozygotes by use of hair follicles. *Science* 1971; **172**: 572-4.
- Fujimoto F Y, Seegmiller J E, Uhlendorf B W, Jacobson C B. Biochemical diagnosis of an X-linked disease in utero. *Lancet* 1968; **2**: 511-2.
- Boyle J A, Raivio K O, Astrin K H, et al. Lesch-Nyhan syndrome: preventive control by prenatal diagnosis. *Science* 1970; **169**: 688-9.
- Van Heeswijk P J, Blank C H, Seegmiller J E, Jacobson C B. Preventive control of the Lesch-Nyhan syndrome. *Obstet Gynecol* 1972; **40**: 109-13.
- McInnes R, Lamm P, Clow C L, Scriver C R. A filter paper sampling method for the uric acid: creatinine ratio in urine. Normal values in the newborn. *Pediatrics* 1972; **49**: 80-4.
- Skaper S D, Seegmiller J E. Hypoxanthine-guanine phosphoribosyltransferase mutant glioma cells: Diminished monoamine oxidase activity. *Science* 1976; **194**: 1171-3.
- Breakefield X O, Castiglione C M, Edelstein S B. Monoamine oxidase activity decreased in cells lacking hypoxanthine phosphoribosyltransferase activity. *Science* 1976; **192**: 1018-20.
- Mizuno T, Yugari Y. Prophylactic effect of L-5-hydroxytryptophan on self-mutilation in the Lesch-Nyhan syndrome. *Neuropadiatrie* 1975; **6**: 13-23.
- Frith C D, Johnstone E C, Joseph M H, Powell R J, Watts R W E. Double-blind clinical trial of 5-hydroxytryptophan in a case of Lesch-Nyhan syndrome. *J Neurol Neurosurg Psychiatr* 1976; **39**: 656-62.
- Ciaranello R D, Anders T F, Barchas J D, Berger P A, Cann H M. The use of 5-hydroxytryptophan in a child with Lesch-Nyhan syndrome. *Child Psychiatry Hum Dev* 1976; **7**: 127-33.
- Nyhan W L. Behavior in the Lesch-Nyhan syndrome. *J Autism Child Schizophr* 1976; **6**: 235-52.
- Becker M A, Seegmiller J E. Genetic aspects of gout. In *Annual Review of Medicine*. Annual Reviews, 1974; **25**: 15-28.
- Kelley W N, Rosenbloom F M, Henderson J F, Seegmiller J E. A specific enzyme defect in gout associated with overproduction of uric acid. *Proc Natl Acad Sci USA* 1967; **57**: 1735-9.
- Kelley W N, Greene M L, Rosenbloom R M, Henderson J F, Seegmiller J E. Hypoxanthine-guanine phosphoribosyltransferase deficiency in gout. A review. *Ann Intern Med* 1969; **70**: 155-206.
- Greene M L. Clinical features of patients with the 'partial' deficiency of the X-linked uric aciduria enzyme. *Arch Intern Med* 1972; **130**: 193-198.
- Emmerson B T, Thompson L. The spectrum of hypoxanthine-guanine phosphoribosyltransferase deficiency. *Q J Med* 1973; **42**: 423-40.
- Kelley W N, Rosenbloom F M, Miller J, Seegmiller J E. An enzymatic basis for variation in response to allopurinol. *New Engl J Med* 1968; **278**: 287-93.

- ⁴⁰ Rosenbloom F M, Henderson J F, Caldwell I C, Kelley W N, Seegmiller J E. Biochemical bases of accelerated purine biosynthesis de novo in human fibroblasts lacking hypoxanthine-guanine phosphoribosyltransferase. *J Biol Chem* 1968; **243**: 1166-73.
- ⁴¹ Brenton D P, Astrin K H, Cruikshank M K, Seegmiller J E. Measurement of free nucleotides in cultured human lymphoid cells using high-pressure liquid chromatography. *Biochem Med* 1977; **17**: 231-47.
- ⁴² Nuki G, Astrin K, Brenton D, Cruikshank M, Lever J, Seegmiller J E. Purine and pyrimidine nucleotide concentrations in cells with decreased hypoxanthine-guanine phosphoribosyltransferase (HGPRT) activity. In: *Advances in Experimental Medicine and Biology*, Vol. 76A. (Series: *Purine Metabolism in Man—II: Regulation of Pathways and Enzyme Defects*). Muller, Kaiser, Seegmiller eds. New York: Plenum Press, 1977: 326-39.
- ⁴³ Becker M A. Patterns of phosphoribosylpyrophosphate and ribose-5-phosphate concentration and generation in fibroblasts from patients with gout and purine overproduction. *J Clin Invest* 1976; **57**: 308-18.
- ⁴⁴ Becker M A, Kostel P J, Meyer L J, Seegmiller J E. Human phosphoribosylpyrophosphate synthetase: Increased enzyme specific activity in a family with gout and excessive purine synthesis. *Proc Natl Acad Sci USA* 1973; **70**: 2749-52.
- ⁴⁵ Becker M A, Meyer L J, Wood A W, Seegmiller J E. Purine overproduction in man associated with increased phosphoribosylpyrophosphate synthetase activity. *Science* 1973; **179**: 1123-6.
- ⁴⁶ Becker M A, Meyer L J, Seegmiller J E. Gout with purine overproduction due to increased phosphoribosylpyrophosphate synthetase activity. *Am J Med* 1973; **55**: 232-42.
- ⁴⁷ Sperling O, Eilam G, Persky-Brosch S, de Vries A. Accelerated erythrocyte-5-phosphoribosyl-l-pyrophosphate synthesis. A familial abnormality associated with excessive uric acid production and gout. *Biochem Med* 1972; **6**: 310-316.
- ⁴⁸ Zoref E, de Vries A, Sperling O. Mutant feedback-resistant phosphoribosylpyrophosphate synthetase associated with purine overproduction and gout. Phosphoribosylpyrophosphate and purine metabolism in cultured fibroblasts. *J Clin Invest* 1975; **56**: 1093-9.
- ⁴⁹ Nyhan W L, James J A, Teberg A J, Sweetman L, Nelson L G. A new disorder of purine metabolism with behavioral manifestations. *J Pediatr* 1969; **74**: 20-7.
- ⁵⁰ Becker M A, Raivio K O, Bakay B, Adams W B, Nyhan W L. Superactive phosphoribosylpyrophosphate (PRPP) synthetase with altered regulatory and catalytic functions. *3rd International Symposium on Purine Metabolism in Man*, Madrid, Spain, 11-15 June 1979.
- ⁵¹ Zoref E, de Vries A, Sperling O. (1977). Evidence for X-linkage of phosphoribosylpyrophosphate synthetase in man. Studies with cultured fibroblasts from a gouty family with mutant feedback-resistant enzyme. *Hum Hered* 1977; **27**: 73-80.
- ⁵² Yen R C K, Adams B, Lazar C, Becker M A. Evidence for X-linkage of human phosphoribosylpyrophosphate synthetase. *Proc Natl Acad Sci USA* 1978; **75**: 482-4.
- ⁵³ Krakoff I H, Balis M E. Studies on the uricogenic effect of 2-substituted thiadiazoles in man. *J Clin Invest* 1959; **38**: 907.
- ⁵⁴ Nelson J A, Rose L M, Bennett L L Jr. Effects of 2-amino-1, 3, 4-thiadiazole on ribonucleotide pools of leukemia L1210 cells. *Cancer Res* 1976; **36**: 1375-8.
- ⁵⁵ Nelson J A, Rose L M, Bennett L L Jr. Mechanism of action of 2-amino-1, 3, 4-thiadiazole (NSC 4728). *Cancer Res* 1977; **37**: 182-7.
- ⁵⁶ Willis R C, Carson D A, Seegmiller J E. Adenosine kinase initiates the major route of ribavirin activation in a cultured human cell line. *Proc Natl Acad Sci USA* 1978; **75**: 3042-4.
- ⁵⁷ Cartier M P, Hamet M. A new metabolic disease: The complete deficit of adenine phosphoribosyltransferase and lithiasis of 2, 8-dihydroxyadenine. *CR Acad Sci (Paris)* 1974; **279**: 883-6.
- ⁵⁸ Simmonds H A, Van Acker K J, Cameron J S, Snedden W. The identification of 2, 8-dihydroxyadenine, a new component of urinary stones. *Biochem J* 1976; **157**: 485-7.
- ⁵⁹ Barratt T M, Simmonds H A, Cameron J S, et al. Complete deficiency of adenine phosphoribosyltransferase: A third case presenting as renal stones in a young child. *Arch Dis Child* 1979; **54**: 25-31.
- ⁶⁰ Hershfield M S, Spector E B, Seegmiller J E. Purine synthesis and excretion in mutants of the WI-L2 human lymphoblastoid line deficient in adenosine kinase (AK) and adenine phosphoribosyltransferase (APRT). In Muller Kaiser Seegmiller, eds. *Advances in Experimental Medicine and Biology*, Vol. 76A. (Series: *Purine Metabolism in Man—II: Regulation of Pathways and Enzyme Defects*). New York: Plenum Press, 1977: 303-13.
- ⁶¹ Dent C E, Philpot G R. Xanthinuria, an inborn error or deviation) of metabolism. *Lancet* 1954; **1**: 182-5.
- ⁶² Ayyazian J H. Xanthinuria and hemochromatosis. *N Engl J Med* 1964; **270**: 18-22.
- ⁶³ Watts R W E, Engelman K, Klinenberg J R, Seegmiller J E, Sjoerdsma A. The enzyme defect in xanthinuria. *Nature* 1964; **201**: 395-6.
- ⁶⁴ Engelman K, Watts R W E, Klinenberg J R, Sjoerdsma A, Seegmiller J E. Clinical, physiological and biochemical studies of a patient with xanthinuria and pheochromocytoma. *Am J Med* 1964; **37**: 839-61.
- ⁶⁵ Chalmers R S, Watts R W E, Bitensky L, Chayen J. Microscopic studies on crystals in skeletal muscle from two cases of xanthinuria. *J Pathol* 1969; **99**: 45.
- ⁶⁶ Isaacs H, Hefferon J J A, Berman L, Badenhorst M, Pickering A. Xanthine hypoxanthine and muscle pain. Histochemical and biochemical observations. *S Afr Med J* 1975; **49**: 1035-8.
- ⁶⁷ Eisen H N. *Immunology. An introduction to molecular and cellular principles of the immune responses*. Hagerstown: Harper and Rowe, 1974: 475.
- ⁶⁸ Giblett E R, Anderson J E, Cohen F, Pollara B, Meuwissen H J. Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity. *Lancet* 1972; **2**: 1067-9.
- ⁶⁹ Giblett E R, Ammann A J, Wara D W, Sandman R, Diamond L K. Nucleoside-phosphorylase deficiency in a child with severely defective T-cell immunity and normal B-cell immunity. *Lancet* 1975; **1**: 1010-3.
- ⁷⁰ Cohen A, Doyle D, Martin D W Jr, Ammann A J. Abnormal purine metabolism and purine overproduction in a patient deficient in purine nucleoside phosphorylase. *N Engl J Med* 1976; **295**: 1449-54.
- ⁷¹ Johnson S M, Asherson G L, Watts R W E, North M E, Allsop J, Webster A D B. Lymphocyte-purine 5'-nucleotidase deficiency in primary hypogammaglobulinaemia. *Lancet* 1977; **1**: 168-70.
- ⁷² Webster A D B, Asherson G L. Identification and function of T cells in the peripheral blood of patients with hypogammaglobulinemia. *Clin Exp Immunol* 1974; **18**: 499-504.
- ⁷³ Edwards N L, Magilav D B, Cassidy J T, Fox I H. Lymphocyte ecto-5'-nucleotidase deficiency in congenital agammaglobulinemia. *Clin Res* 1978; **26**: 513A.
- ⁷⁴ Rowe M, DeGase C G, Platts-Mills T A E, Asherson G L, Webster A D B, Johnson S M. 5'-nucleotidase of B and T lymphocytes isolated from human peripheral blood. *Clin Exp Immunol* 1979; **36**: 97.

- ⁷⁵ Boss G R, Thompson L F, Spiegelberg H L *et al.* Lymphocyte ecto-5'-nucleotidase activity as a marker of B-cell maturation. *Trans Assoc Am Phys* 1980; in press.
- ⁷⁶ Thompson L F, Boss G R, Spiegelberg H L *et al.* Ecto-5'-nucleotidase activity in T and B lymphocytes from normal subjects and patients with congenital X-linked agammaglobulinemia. *J Immunol* 1980; **123**: 2475-8.
- ⁷⁷ Edwards N L, Cassidy J T, Fox I H. Lymphocyte 5'-nucleotidase deficiency: clinical characteristics of the associated hypogammaglobulinemia. *J Clin Chem Clin Biochem* 1979; **17**: 404.
- ⁷⁸ Edwards N L, Gelfaud E W, Burk L, Dosch H -M, Fox I H. Distribution of 5'-nucleotidase in human lymphoid tissues. *Proc Natl Acad Sci USA* 1979; **76**: 3474-6.
- ⁷⁹ Adams A, Harkness R A. Adenosine deaminase activity in thymus and other human tissues. *Clin Exp Immunol* 1976; **26**: 647-9.
- ⁸⁰ Carson D A, Kaye J, Seegmiller J E. Lymphospecific toxicity in adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency: Possible role of nucleoside kinase(s). *Proc Natl Acad Sci USA* 1977; **74**: 5677-81.
- ⁸¹ Simmonds H A, Panayi G S, Corrigan V. A role for purine metabolism in the immune response: Adenosine-deaminase activity and deoxyadenosine catabolism. *Lancet* 1978; **1**: 60-3.
- ⁸² Kuttlesch J F, Schmalstieg F C, Nelson J A. Analysis of adenosine and other adenine compounds in patients with immunodeficiency diseases. *J Liq Chromatogr* 1978; **1**: 97-109.
- ⁸³ Seegmiller J E, Thompson L, Bluestein H, Willis R, Matsumoto S, Carson D. Nucleotide and nucleoside metabolism and lymphocyte function. In: Gelfand E W, Dosch H-M, eds. *Biological Basis of Immunodeficiency*. New York: Raven Press; 1980; 251-68.
- ⁸⁴ Ishii K, Green H. Lethality of adenosine for cultured mammalian cells by interference with pyrimidine biosynthesis. *J Cell Sci* 1973; **13**: 429-39.
- ⁸⁵ Polmar S H, Wetzler E M, Stern R C, Hirschhorn R. Restoration of *in vitro* lymphocyte responses with exogenous adenosine deaminase in a patient with severe combined immunodeficiency. *Lancet* 1975; **2**: 743-6.
- ⁸⁶ Polmar S H, Wetzler E M, Stern R C. Adenosine deaminase deficiency: Enzyme replacement therapy and investigations of the biochemical basis of immunodeficiency. In: Wamberg E, Guttler E, eds. *Inborn Errors of Immunity and Phagocytes*. Lancaster: NTP Press, 1978.
- ⁸⁷ Hirschhorn R. Defects of purine metabolism in immunodeficiency diseases. In: Schwartz R S, ed. *Progress in Clinical Immunology*. San Francisco: Grune and Stratton, 1977: 67-83.
- ⁸⁸ Bluestein H G, Willis R C, Thompson L F, Matsumoto S, Seegmiller J E. Accumulation of deoxyribonucleotides as a possible mediator of immunosuppression in hereditary deficiency of adenosine deaminase. *Trans Assoc Am Phys* 1978; **92**: 394-402.
- ⁸⁹ Cohen A, Gudas L J, Ammann A J, Staal G E J, Martin D W. Deoxyguanosine triphosphate as a possible toxic metabolite in the immunodeficiency associated with purine nucleoside phosphorylase deficiency. *J Clin Invest* 1978; **61**: 1405-9.
- ⁹⁰ Cohen A, Hirschhorn R, Horowitz S D *et al.* Deoxyadenosine triphosphate as a potentially toxic metabolite in adenosine deaminase deficiency. *Proc Natl Acad Sci USA* 1978; **75**: 472-6.
- ⁹¹ Coleman M S, Donofrio J, Hutton J J, *et al.* Identification and quantitation of adenine deoxynucleotides in erythrocytes of a patient with adenosine deaminase deficiency and severe combined immunodeficiency. *J Biol Chem* 1978; **253**: 1619-26.
- ⁹² Herschfield M S, Seegmiller J E. Regulation of *de novo* purine synthesis in human lymphoblasts: Similar rates of *de novo* synthesis during growth by normal cells and mutants deficient in hypoxanthine-guanine phosphoribosyltransferase activity. *J Biol Chem* 1977; **252**: 6002-10.
- ⁹³ Moore E C, Hurlbert R B. Regulation of mammalian deoxyribonucleotide biosynthesis by nucleotides as activators and inhibitors. *J Biol Chem* 1966; **241**: 4802-9.
- ⁹⁴ Reichard P. Control of deoxyribonucleotide synthesis *in vitro* and *in vivo*. *Adv Enzyme Regul* 1972; **10**: 3-16.
- ⁹⁵ Chan T-S. Deoxyguanosine toxicity on lymphoid cells as a cause for immunosuppression in purine nucleoside phosphorylase deficiency. *Cell* 14 1978: 523-530.
- ⁹⁶ Paine M R, Smyth J F, Harrap K R. Biochemical consequences of treatment with the adenosine deaminase inhibitor 2'-deoxycytosine. *J Clin Chem Clin Biochem* 1979; **17**: 434.
- ⁹⁷ Yu A, Kung F, Bakay B, Nyhan W L. Preliminary clinical trial of deoxycytosine in human T-cell leukemia. *J Clin Chem Clin Biochem* 1979; **17**: 451-452.
- ⁹⁸ Mitchell B S, Koller C, Mejias E, Kelley W N. Inhibition of adenosine deaminase by 2'-deoxycytosine in acute lymphoblastic leukemia. *J Clin Chem Clin Biochem* 1979; **17**: 427-8.
- ⁹⁹ Carson D A, Kaye J, Seegmiller J E. Differential sensitivity of human leukemic T cell lines and B cell lines to growth inhibition by deoxyadenosine. *J Immunol* 1978; **121**: 1726-31.
- ¹⁰⁰ Seegmiller J E, Laster L, Howell R R. Biochemistry of uric acid and its relation to gout. *N Engl J Med* 1963; **268**: 712-6.
- ¹⁰¹ Waldmann T A, Broder S, Schwartz R S, ed. Suppressor cells in the regulation of the immune response. In: Schwartz R S ed. *Progress in Clinical Immunology*. New York: Grune and Stratton, 1977; 3.
- ¹⁰² Becker M, Yen R C K, Itkin P, Goss S J, Seegmiller J E, Bakay B. Regional localization of the gene for human phosphoribosylpyrophosphate synthetase on the X-chromosome. *Science* 1979; **203**: 1016-9.
- ¹⁰³ Kelley W N, Levy R I, Rosenbloom F M, Henderson J F, Seegmiller J E. Adenine phosphoribosyltransferase deficiency: a previously undescribed genetic defect in man. *J Clin Invest* 1968; **47**: 2281-9.