HUMAN PURINE METABOLISM AND BEHAVIOR

By William L. Nyhan

One genetic disease of human metabolism, which results in degradation of mental and neurological functions, has been traced to inactivity of the enzyme HGPRT.

Genetic disorders of purine metabolism have been known since antiquity. Gout was recognized by Hippocrates, who identified it as a disease of the adult male. It occurs occasionally in the female, but under these circumstances she is usually old enough to be postmenopausal. However, there are not any 100 percent rules in human biology or medicine, and it turns out that there are a number of disorders of purine metabolism that appear early in life. At least one and probably more of them has a characteristic pattern of abnormal behavior.

The Greeks considered gout to be a product, as it were, of Bacchus and Aphrodite. We now know that gout is not acquired but rather is an inherited disease. At the right is a partial list of some persons who have been important to the development of western civilization who have also had gout. The frequency of the disease in the general population is only two per thousand. The association of this disorder with the qualities of behavior that lead to success are too high to be fortuitous. There are other recent data which make the same kind of point. And I think this story hasn't been told completely yet.

These considerations with regard to classic gout involve intellectual superiority or perhaps behavioral successes. The patients we have studied, on the other hand, have been severely mentally retarded.

The first patient we studied with a disorder of purine metabolism in childhood was four years old and quite seriously mentally retarded. He had not learned to walk, and could not even sit without support. He kept his fists tightly clenched, which suggested that he had a neurological abnormality. All of the patient's muscles showed a severe degree of increased muscular tone—as did those of all of the patients we have studied. This is typical of a child or adult with a severe degree of spastic cerebral palsy.

Also, these children are in continuous motion, displaying the abnormal posturing that has been characteristic of all of the patients with this disease that we've studied. The movements are typical of the neurological abnormality called chorea and also of athetosis. So these patients have chore-athetosis. In this way a clinician builds up the picture of patients' behavior into what might be considered a syndrome. We now have some kind of information on more than 100 patients with this disease. In virtually all of them IQ's are less than 50. So these patients are mentally retarded, have spastic cerebral palsy, and have chore-athetosis; this, then, would be a clinical or neurological syndrome.

It was when we saw the brother of our first patient that we began to think we were dealing with a genetic disease. He was eight years old at the time and showed the same features we saw in the other patients. He couldn't sit up, and his legs tended to assume a scissor position, which is characteristic of patients with very severe degrees of cerebral palsy or spasticity.

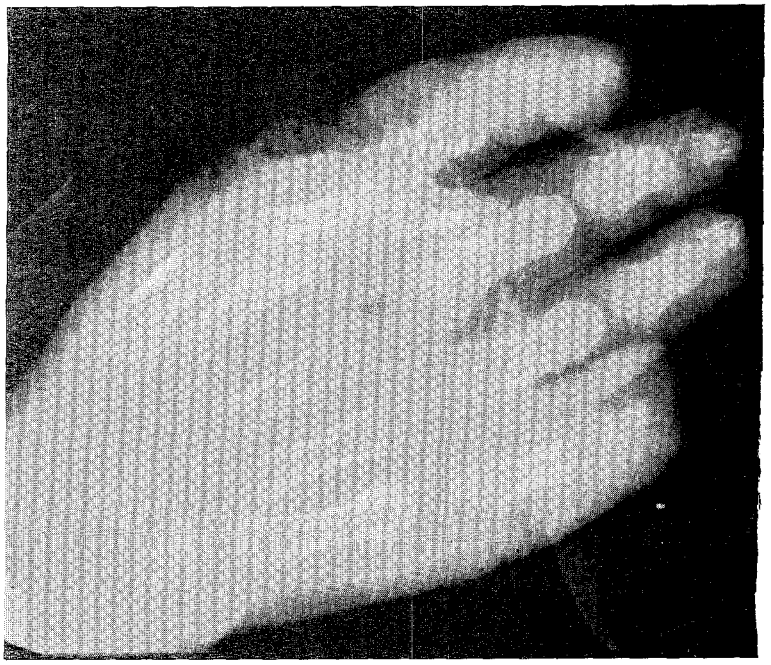
Many children with the disease look, I think, quite a bit smarter than someone with an IQ of about 50. Generally, as you see patients with the disease in the wards of institutions for the retarded, you get the feeling that they're smarter than the other children there. They seem to have a better capacity somehow in ways that we aren't very well able to test—possibly because they are prevented by

GOUT AND GREAT MEN

Alexander the Great	Thomas Gray
Isaac Newton	Goethe
Charles Darwin	James Russell Lowell
William Harvey	Alfred Tennyson
Benjamin Franklin	Edward Gibbon
Martin Luther	Henry Fielding
John Calvin	Horace Walpole
John Wesley	Samuel Johnson
Cardinal Wolsey	Lord Chesterfield
John Milton	Francis Bacon
Ben Johnson	Stendhal
William Congreve	Guy de Maupassant

Total incidence of gout: 2/1000 population

The enormous oversynthesis of purine is a basic metabolic defect which tends, to some extent, to establish the abnormal biochemical milieu under which these patients have had to develop and in which they must live day by day.



Self-mutilation is a typical syndrome of the disease. This Xray shows how one young patient actually bit off part of his little finger—bone and all.

their neurological abnormality from doing very well on a test procedure. The other thing you might see is that they have really nice smiles, an index of a sense of humor, which in turn is an index of intelligence.

All of our patients have related unusually well to people. In a hospital or in an institution it is very common for them to be the favorites of the nurses, the aides, and the attendants.

The most startling characteristic of the syndrome is that the patients often show self-mutilative behavior. Just recently, for example, at the University Hospital in San Diego we admitted a boy who had produced dramatic mutilation of a finger and thumb by biting. In fact, he managed to produce a partial amputation of a finger, bone and all.

We might think that a child who does this to himself would have a neurological abnormality in which he felt no pain. In fact, we have seen quite a number of children who have abnormalities of sensation. But those children are different. They look like pugilists, and might come in with a broken leg or jaw and not even be aware of it. Or they get burns around the hands because they put them in places they can't detect as dangerous. But they don't produce this very specific self-mutilation. Furthermore, we've now tested sensation extensively in children with this syndrome, and sensation is intact to anything we can test. These children don't like this behavior any more than we do or their parents do. We often have to wrap a patient's hands to protect them. If we slowly remove the wraps, immediately it looks to him as though he's no longer protected from himself; he becomes terrified. Nevertheless, if he isn't restrained, he begins to tear at his flesh with his teeth. These children cry when this goes on, but nevertheless, in an obsessive fashion, they still do it.

The clinical hallmark of the disorder is self-mutilative loss of tissue of the lower lip. The patient bites his lip to a point where he has a new mucocutaneous junction that is no longer accessible to his teeth. Interestingly, all of the damage in one patient might be to his lower lip; another might, for some reason, be dangerous to himself only in the upper lip, one thumb, or one finger.

The most dramatic example of the problem was first reported by Dick Haefnagel at Dartmouth, and we studied this patient on a number of occasions in Miami. A clinician seeing him first would probably think that he had been born with a hare lip and a cleft palate, but he was born completely intact. All of the loss of tissue was secondary to his own mutilative behavior. It points out, of course, that biting is not the only form of self-mutilating activity these patients engage in. He obviously did some of the damage with his fingers. We have seen children who have learned to lacerate themselves with braces or catch themselves in the spokes of a wheelchair. One patient who learned to get about in a wheelchair succeeded in scalding himself at a hot water faucet and on another occasion in producing burns with a dry ice and acetone mixture left by a dermatologist planning an experiment in the clinical research unit. The variety of this kind of behavior is in general limited only by the usually limited ability of these children to get about.

It has become apparent as we study older children that their behavior is not always addressed only against themselves. They'll bite other people; they'll hit other people. Doctors and nurses working with them count on losing a certain number of pairs of eyeglasses. The children learn how to use speech aggressively, particularly using words that they learn people find unacceptable. Many of them have learned to pinch nurses and other ladies, sometimes men too, in places that are not too acceptable socially.

The concentration of uric acid in the blood is probably the most frequently detected clinical expression of chemical abnormality in purine metabolism. Some years ago Dr. Jay Seegmiller in the *New England Journal of Medicine* indicated that most adult males have a serum uric acid concentration in the blood of about 5 mg/100 ml. A diagnosis of gout is supported by concentrations of over 6 mg/100 ml. Most of the patients we have studied have had uric acid concentrations in plasma that are in the vicinity of 9 to 11 mg/100 ml. But that doesn't indicate anything special about the condition, because in general a uric acid level of about 9 to 11 reflects the limits of solubility of urate in plasma.

When one studies an abnormality in metabolism and detects an elevated concentration of something in the blood, the next approach is to look at the excretion in the urine. We ran some tests in which the patient was allowed to ingest no exogenous purine; the urate excretion then was an index of how much purine was made by the body. Under those circumstances most adults have less than 500 mg of urate in the urine in a 24-hour period. Furthermore, adults with gout have about the same amount. This is one of the things that for many years led people to think that gout was a disorder of renal tubular excretion because there weren't large amounts of purine showing up in the urine. On the other hand, a smaller group of gouty adults, known as hyperexcretors, excreted more than 600 mg of urate in the urine in a 24-hour period.

Children tend to have somewhat less urate in the urine than adults, but that's because they're smaller. On the other hand, many of our 15-kilogram pediatric patients have so much extra urate in their urine that they could be called hyperexcretors even if they were 70-kilogram adults. This excretion, and hence production, of urate is enormous.

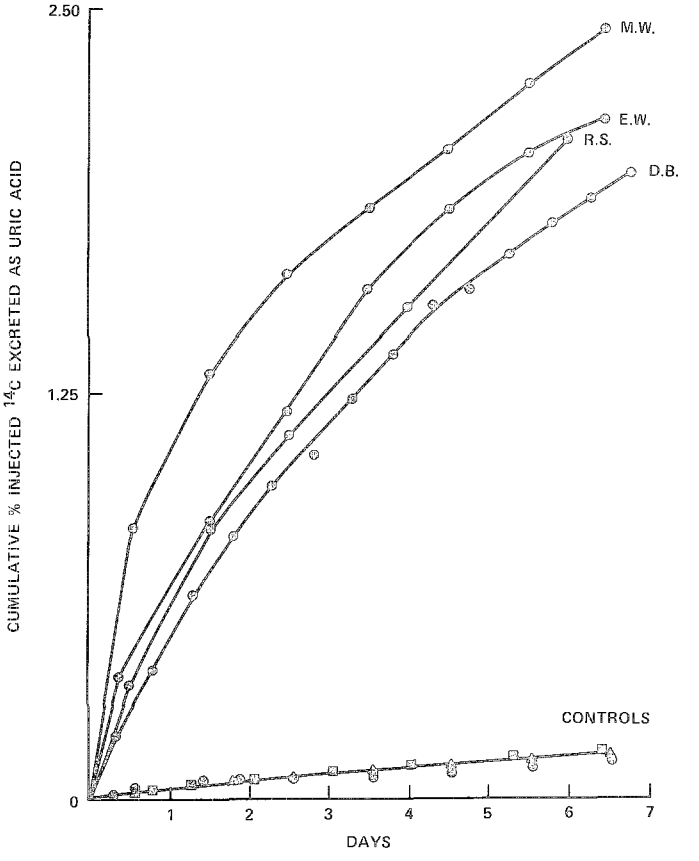
The classic way in which patients with gout have been studied chemically is to exclude exogenous purine from the diet and then to inject them on Day 0 with a small tracer dose of glycine labeled with carbon 14. In the body the glycine is synthesized into inosinic acid, which then shows up in the urine as uric acid. In a normal person about 1/10 of 1 percent of the glycine is excreted in uric acid in one week; our patients introduce somewhere around 2 percent of the glycine administered into the purine nucleus in the one-week period. Actually, most of the production goes on in the first 12 hours after injection. This enormous oversynthesis of purine is a basic metabolic defect which tends, to some extent, to establish the abnormal biochemical milieu under which these patients have had to develop and in which they must live day by day.

If our patients had large amounts of urate in their urine and blood, and had this enormous overproduction, one would then expect that they would have all of the

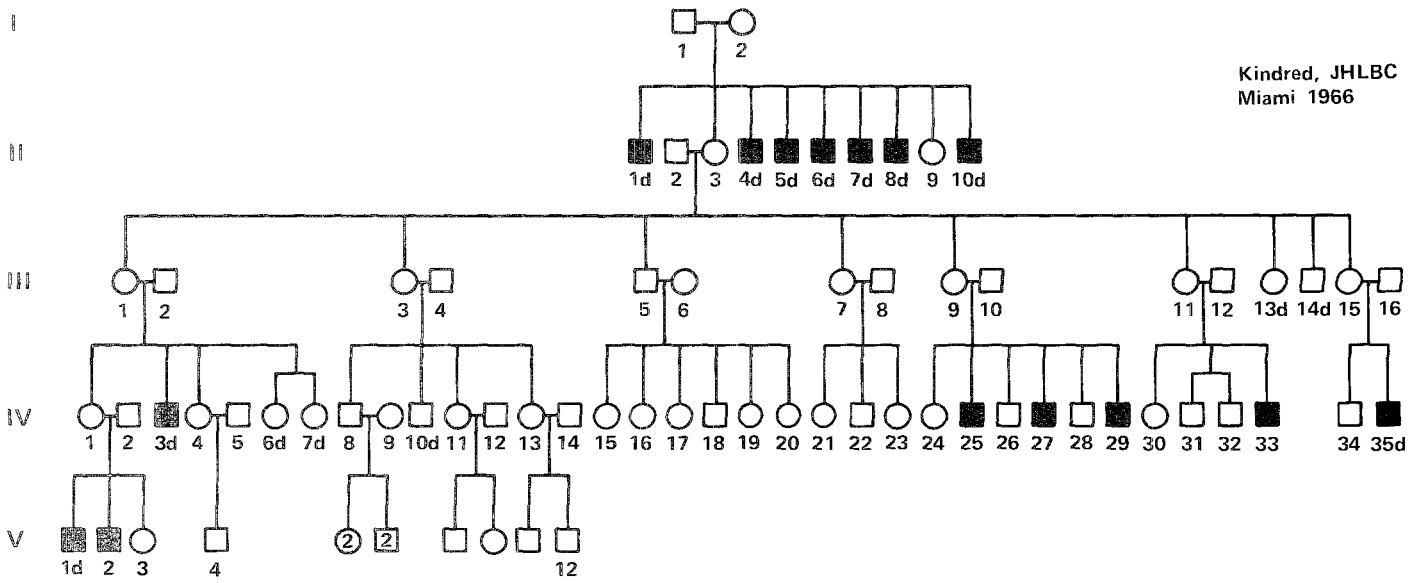
URIC ACID EXCRETION

	No.	MG/Day	Range	MG/KG/Day
CONTROL ADULTS	4	370	325-455	5.3
GOUTY ADULTS (Normal Excretors)	14	408	253-514	5.8
GOUTY ADULTS (Hyperexcretors)	4	754	587-1054	10.8
CONTROL CHILDREN	3	233	176-289	10.3
PATIENTS	2	690	669-712	45.3

The amount of urate in the urine of the children with the disease is almost as much as in the urine of hyperexcreting, gouty adults. A current hypothesis is that the abnormal behavioral characteristics represent the action of some chemicals that are a consequence of this enormous overproduction of purine.



When patients are put on purine-free diets and injected with radioactively labeled glycine (a simple amino acid), they produce about 20 times (2 percent versus 1/10 percent) the normal amount of purine in the urine in a one-week period.



This genetic chart of the family of the first patient studied clearly shows that over five generations the transmission of the disease has been through the females (circles) to the males (squares). The siblings under study are represented at the lower left.

clinical manifestations that one sees in adults with gout. In fact, they do.

Gouty arthritis is the caricature by which gout tends to be known. In general that symptom appears near age 50. Earlier in a patient who was to develop gout, somewhere around 15 to 20 years of age, there would be some elevation of serum uric acid. A urinary tract stone might occur at age 40. There are variations, but that's about the usual pattern. The children we study are producing much more purine than the average adult with gout, so the schedule is foreshortened. We have found stones in patients only a few weeks old. We have seen three teenagers with gouty arthritis.

The next chapter in this story involves the effects of the immunosuppressant azathioprine on purine metabolism. When we studied this agent in a man who had gout, he had a serum uric acid in the vicinity of 10 mg/100 ml. We gave him an enormous dose of azathioprine, which is the classic immunosuppressant used in transplanting kidneys and hearts. Sure enough, in about two weeks he was pretty well suppressed, and by that time his serum uric acid was down to normal. Azathioprine inhibits the synthesis of purine. Then we tried it on the types of children we've been discussing. The experiment was exactly the same as before, and we also started with a serum uric acid of about 10 mg/100 ml. We gave a similar dose of azathioprine. The uric acid level did not go down. These children are resistant to the action of azathioprine.

This was the key to the molecular basis of this disease, and it was first worked out by Drs. Seegmiller, Rosenberg, and Kelley, who at the time were at the National Institutes of Health. When azathioprine, which is a derivative of 6-mercaptopurine, is absorbed into the body, it undergoes a hydrolytic reaction, leaving 6-mercaptopurine to circulate. From experiments with leukemia on mice and men, it is known that 6-mercaptopurine is not active by itself, but must first be activated by an enzyme known as HGPRT. This enzyme is called hypoxanthine guanine phosphoribosyl tranferase, because it catalyzes the conversions of hypoxanthine and guanine to their respective nucleotides, inosinic and guanylic acids. It also converts 6-mercaptopurine to its ribonucleotide, after which it will work on cancer cells, and it will work as an immunosuppressant, and it will work on patients with gout. So one can say a patient resistant to the action of 6-mercaptopurine might have something wrong with this enzyme. This has been found to be the case. Analyses indicate that the enzyme HGPRT is 100 percent inactive in patients with this mutant gene.

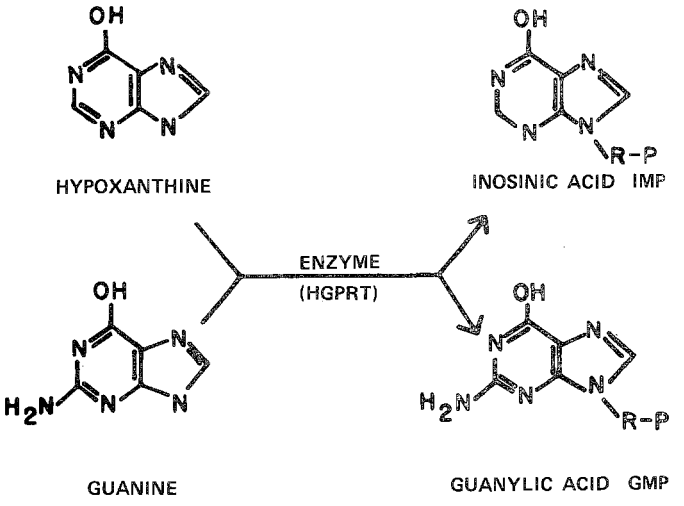
The genetics of this condition are that the transmission is through the female to the male, always in the pattern

of an X-linked recessive character. In studies with Drs. Barbara Migeon and Barton Childs of Johns Hopkins we studied the family of our first patient. The two brothers in the family both have the disease, and obviously the mother is a carrier because she produced them. It has now been shown that she does in fact have cells that carry the abnormal enzyme. We also know now that a female sibling, born after we studied the two boys, also carries the abnormal gene.

What might this girl, when she grows up, do to prevent the continuation of the disease in her progeny? If, in spite of carrying the gene, she is planning a family of her own, it is possible, were she to get pregnant, for us to obtain fluid containing amnion cells from the baby by an amniocentesis, or puncture of her uterus. We could then grow the cells in culture and detect whether or not the baby is carrying the abnormal enzyme. In this condition, and in a number of similar conditions, one can make a prenatal diagnosis of abnormality in time to permit a therapeutic abortion.

If such a woman were willing to go through this business of monitoring pregnancy and the risk of therapeutic abortion, one could guarantee the eventual production of a normal child. This, I think, is a positive approach to this type of problem.

The mechanisms for the neurological and behavioral manifestations of the disease are not known. Experience with patients with, say, phenylketonuria and other metabolic diseases suggests that something with a specific toxicity for the nervous system may accumulate as a result of the metabolic disease. One current operating hypothesis is that the abnormal behavioral characteristics represent the action of some chemical or chemicals that



The enzyme HGPRT ordinarily catalyzes the conversion of the purines hypoxanthine and guanine to their respective nucleotides. In patients with the disease, this enzyme is completely inactive.

What might this girl do, when she grows up, to prevent the continuation of the disease in her progeny?

are a consequence of the metabolic defect and particularly of the enormous overproduction of purine. It already appears clear that uric acid itself is not the offending agent. We have been able to control uric acid concentrations quite effectively with allopurinol, which is a superb drug for the treatment of gout. However, it in no way influences the central nervous system manifestations of the disease.

We are now looking at body fluids—cerebrospinal fluid as well as urine—in the search for purines that are different in patients with the disease from those found in control individuals. We have also initiated pharmacologic studies in an attempt to find an experimental model for the behavioral aspects of the syndrome.

The structure of uric acid compares closely with some rather well-known environmental purines, such as trimethyl purine (caffeine) and other methylated purines like theophylline, a drug commonly used in the management of patients with asthma. Both caffeine and theophylline are central nervous system stimulants. Two of our undergraduate students, Jacob Sage and Raymond McConald, have found that rats given large doses of caffeine and theophylline produce self-mutilation of their paws and abdomens. Both of these purines have the 1,3-methylated structure. A third similar purine, theobromine, which doesn't have that structure, produces no effect in the animals.

We settled on theophylline as a type compound with which to compare other chemicals, and we have since experimented with animals other than rats too. One of our most significant results came from a rat, treated with theophylline, who demonstrated self-mutilation worse than any of our patients. Most of the enormous destructive activity was around the mouth, which makes us think this may not be a bad model, and also that it might well reflect the possibility that purines are related to the kinds of abnormal behavior that we have had under study in man.

In conclusion, we have described what might be considered a possible molecular approach to behavior. We have gone from the clinical disease through the metabolic or biochemical abnormality to cell culture studies and enzymology. These studies have the important promise of a chemical understanding of human behavior.