250-ml Erlenmeyer flask. The cultures (still) were held for 4 weeks at room temperature in the light. The entire culture contents were then freeze-dried at 150°F. This freeze-dried material was added to the diet for experiment 3.

DALE M. NORRIS
JOCELYN KING BAKER
Department of Entomology,
University of Wisconsin, Madison

References and Notes

1. H. Francke-Grosmann, in *Symbiosis*, S. M. Henry, Ed. (Academic Press, New York, 1967), vol. 2, pp. 141–205.
2. G. M. Chippendale and S. D. Beck, *Entomol. Exp. Appl.* **7**, 241 (1964).
3. J. L. Saunders and J. K. Knoke, *Science*, in press.
4. Potato-dextrose-agar medium.
5. Approved for publication by the director of the Wisconsin Agricultural Experiment Station. This study was supported in part by a research grant (AI 06195-03) from NIH.

3 April 1967

Urinary Metabolites in Congenital Hyperuricosuria

Abstract. *The excretion of oxypurine metabolites in urine of patients with congenital hyperuricosuria exceeds, on a creatinine basis, that observed in any previously recognized metabolic anomaly. The ratio of hypoxanthine to xanthine is from 2 : 1 to 3 : 1 and results from increased hypoxanthine excretion, in contrast to other hyperuricosuric conditions where ratios of less than one have been reported. Administration of allopurinol (a xanthine-oxidase inhibitor) reduces the excretion of uric acid but results in an equivalent increase in xanthine and hypoxanthine. These features appear to be unique to congenital hyperuricosuria.*

Lesch and Nyhan have recently described a familial disorder of purine metabolism in which hyperuricemia is associated with evidence of central nervous system dysfunction (*1*). Since the original report, many cases have been recognized and congenital hyperuricosuria now appears to be one of the more common "inborn errors of metabolism."

In our own experience, without making an intensive search for cases, four boys in four families have been identified within a year, and two additional close relatives probably have the disease. The mothers of two of the children are sisters, supporting the suggestion that inheritance is linked to the X chromosome (*2*).

The predominant oxypurine metabolites of nucleic acid metabolism have been measured in 24-hour collections of the urine in four of these childen (Table 1). Uric acid was measured by the uricase method. Hypoxanthine and xanthine were first partially purified by passing the urine through a column of Dowex 50 to remove uric acid and urea. The two oxypurines were separated on a second column of Dowex that had been calibrated with standard solutions of xanthine and hypoxanthine. Xanthine was eluted first with 0.15*N* HCl, and hypoxanthine was then eluted with 0.6*N* HCl. The concentrations were assayed with xanthine oxidase (*3*). The excretion of individual and of total oxypurines has been related to creatinine excretion for 24 hours. Body weight could also have been a standard without altering the interpretation.

The four normal children excreted somewhat larger amounts of uric acid per gram creatinine than adults, who usually excrete less than 500 mg (Table 1). However, the excretion of hypoxanthine and xanthine remains low with xanthine distinctly higher, as in adults. In the gout cases, and these may be considered representative (*4*), the excretion of uric acid is increased but that of the other oxypurines is normal. The same is true in the hyperuricosuria reflecting the rapid degradation of nucleic acids, as, for example, in granulocytic leukemia (Table 1).

The observations previously reported, and those we present, suggest that the major catabolic pathways to uric acid are from adenylic acid via adenosine and inosine to hypoxanthine, and from guanylic and xanthylic acids to xanthosine and xanthine. Tracer experiments indicate that normally adenine is the primary precursor for urinary hypoxanthine (*3*). Urinary xanthine exceeds hypoxanthine by severalfold in normal individuals, suggesting that xanthylic acid or guanylic acid (or both) may make the major contribution to uric acid. Support for this concept is found in patients with xanthinuria, in whom there is a congenital deficiency of xanthine oxidase (*3*). In addition to a sharp reduction in the synthesis of uric acid, the conversion of hypoxanthine to xanthine is reduced, and this permits a clearer interpretation of the relative rates of synthesis. Xanthine excretion is two or more times that of hypoxanthine. Possibly the difference in excretory rates of hypoxanthine and xanthine lies in the relatively more effective incorporation of adenine than of guanine into nucleic acids (*5*). This provides a mechanism for reutilization and conservation of the adenine moiety.

Thus the observations on the four children with congenital hyperuricosuria are simpler to intrepret. The children excreted amounts of uric acid far exceeding, based on creatinine excretion, those previously observed. They also excreted so much hypoxanthine that the usual ratio of hypoxanthine to xanthine was reversed. This anomaly could have resulted from a reduced efficiency in the reutilization of adenylic acid and its derivatives. An alternative hypothesis is that inosinic acid is synthesized in such large amounts that the bulk is converted to uric acid without being incorporated into nucleic acids. Some of this ino-

Table 1. Urinary metabolites. The oxypurines excreted are expressed as milligrams per gram of creatinine.

Patient	Allopurinol treatment (mg)	Oxypurines excreted		
		Uric acid	Hypoxanthine	Xanthine
		Hyperuricosuria		
C.W.*	None	2500	78	38
	100	1900	550	570
	150	1300	740	870
	200	550	670	910
	250	260	1100	2000
R.K.†	None	2700	70	28
	100	820	1000	700
J.A.†	None	2600	90	30
	100	970	1200	650
D.C.†	None	2800	100	43
		Leukemia		
S.W.(*7*)	None	900	3.2	17
	800	200	7.0	120
A.S.(*7*)	None	1300	4.7	11
	800	400	7.0	27
		Gout ‡		
	None	1290		
	300	500	190	
	None	1260	15	
	300	378	76	
		Normal children §		
	None	670 (475–880)	6 (3.5–9)	21 (10–33)
		Normal adults §		
	None	370 (270–580)	1.5 (.4–3.5)	30 (13–45)

* Average of two 24-hour urine collections. † Average of three 24-hour urine collections. ‡ Hypoxanthine and xanthine were not determined separately. Values in milligrams per 24 hours. Creatinine excretion in the adult approximates 1 to 1.5 g/24 hr. § Averages of four normal children (5 to 12 years) and five normal adults. Range of values in parentheses.

sinic acid overflows into a normally minor pathway to hypoxanthine.

The administration of the xanthine-oxidase inhibitor, allopurinol [4-hydroxypyrazolo(3,4-*d*)pyrimidine or Zyloprim] (*6*), reduced the excretion rate of uric acid. In C.W. (Table 1), the amount of reduction was directly related to the dose of allopurinol. Coincidentally, the amounts of urinary hypoxanthine and xanthine increased. The excretion of xanthine increased more than that of hypoxanthine, reducing the ratio of hypoxanthine to xanthine at times to less than one. This presumably reflects the accumulation of metabolites in the inosinic acid–hypoxanthine pathway, with a diversion of inosinic acid towards xanthylic acid and xanthine.

Another significant observation is that the total excretion of oxypurines —uric acid, hypoxanthine, and xanthine—remains unchanged in patients with congenital hyperuricosuria even when they received large doses of allopurinol. This is similar to the observation reported in one case of hyperuricosuria (*2*) but different from the response of patients with neoplastic disease where there is rapid turnover of nucleic acids (*7*) and different from the response in primary nontophaceous gout (*8*). In both of these conditions, the increased excretion of hypoxanthine and xanthine does not compensate for the reduction in uric acid, so that the total oxypurine excretion is reduced by over 20 to 30 percent (Table 1). It has been suggested that this results from the reutilization of hypoxanthine and xanthine for nucleic acid synthesis, and new purine synthesis is reduced as the result of "feedback" inhibition of 5-phosphoribosylpyrophosphate amidotransferase by the natural ribonucleotides (*9*).

A lack of regulatory control has long been considered the cause of increased uric acid excretion in gout and has also been suggested, though without evidence, as the fundamental defect in congenital hyperuricosuria. Our observations are consistent with, though not limited to, the interpretation that the regulatory mechanism is at least partially effective in gout, and is ineffective in hyperuricosuria with central nervous system dysfunction. Urinary excretion accounts for only 60 percent of the synthesized uric acid (*1*), so that care must be exercised in interpreting the urinary values alone.

Since our manuscript was submitted, it has been reported that these children are deficient in the enzyme hypoxanthine-guanine phosphoribosyltransferase, which is responsible for the conversion of hypoxanthine to inosinic acid (*10*). The increased urinary excretion of hypoxanthine may now be attributed to failure to re-utilize this metabolite. The loss of regulatory control of purine synthesis suggested by our data has thus been specifically delineated. Nyhan and Sweetman have made similar observations on urinary oxypurines in four patients with congenital hyperuricosuria.

M. Earl Balis, Irwin H. Krakoff
Peter H. Berman
Joseph Dancis
Sloan-Kettering Institute for Cancer Research, and *Pediatrics Department, New York University School of Medicine, New York*

References and Notes

1. M. Lesch and W. L. Nyhan, *Amer. J. Med.* **36**, 561 (1964).
2. D. S. Newcombe, S. L. Shapiro, G. L. Sheppard, F. E. Dreifuss, *J. Amer. Med. Ass.* **198**, 225 (1966).
3. J. H. Ayvazian and S. Skupp, *J. Clin. Invest.* **44**, 1248 (1965).
4. G. Elion, personal communication.
5. G. B. Brown and P. M. Roll, in *The Nucleic Acids*, E. Chargaff and J. N. Davidson, Eds. (Academic Press, New York, 1955), p. 341.
6. G. B. Elion, T. J. Taylor, G. H. Hitchings, *Int. Congr. Biochem. New York 6th Abstr.* **4**, 305 (1964).
7. I. H. Krakoff and M. E. Balis, *Ann. Rheumatic Dis.*, in press.
8. T. F. Yu and A. B. Gutman, *Amer. J. Med.* **37**, 885 (1964).
9. R. J. McCollister, W. R. Gilbert, D. M. Ashton, J. B. Wyngaarden, *J. Biol. Chem.* **239**, 1560 (1964); D. P. Nierlich and B. Magasanik, *ibid.* **240**, 358 (1965).
10. J. E. Seegmiller, F. M. Rosenbloom, W. H. Kelly, *Science* **155**, 1682 (1967).
11. W. L. Nyhan and L. Sweetman, unpublished.
12. We thank Dr. J. Hammond of the Willowbrook State School, Dr. M. Brust, and Dr. L. Greenspan for cooperation in the study of their patients. Support from the American Cancer Society (P330), PHS (CA 08748), and the National Foundation. The studies were done in the Special Clinical Unit of New York University Medical Center.

12 April 1967

Alarm, Defense, and Construction Behavior Relationships in Termites (Isoptera)

Abstract. *Evidence indicates that building behavior in termites is a direct consequence of low-level alarm stimuli and that its immediate function is defense. As in other forms of termite defense behavior, recruitment of nymphs and workers is accomplished by trail laying in conjunction with transmission of the alarm. The number recruited is related to the intensity of the input stimulus. Primary construction ceases when the original causal stimulus is eliminated by the effects of the actual building.*

The behavior of termites involved in building and in construction of nests has intrigued many workers over the years (*1*) and has been investigated principally by Grassé (*2*), and more recently by Howse (*3*). Grassé, on the basis of his observations of *Bellicositermes* and *Cubitermes* in Africa, has put forward a hypothesis to explain the social coordination involved in the building of a complicated termite nest. Briefly, his hypothesis of "stigmergy" is that building behavior is at first uncoordinated ("La phase d'incoordination"); when the construction at any one point reaches a certain critical density it attracts other termites topochemically. These focuses of the building material determine where the new pellets of earth used in the building are to be deposited. The constructions built thus act as new determinant stimuli for further construction. This hypothesis has not been universally accepted for a number of reasons (*4*). One drawback to the hypothesis which seems to have been overlooked, or at least not to have been emphasized, is that no adequate stimulus to stop a certain piece of construction has been shown to operate. My work supports some of the hypothesis of stigmergy, but it shows that the important initial phase is not haphazard, that it involves distinct directional cues, and that a feedback mechanism to halt building is present.

Howse (*3*) in his very recent work on construction behavior in *Zootermopsis angusticollis* (Hagen) and *Zootermopsis nevadensis* (Hagen) does not comment on the stigmergy hypothesis. He considers air movement to be the prime stimulus in nest building activity and feels it is this movement that attracts termites to minute openings made in their nest experimentally or naturally. He suggests that the tropical termites build around air currents that are set up in a developing nest, and he rejects the suggestion (*5*, *6*) that a sharp gradient in humidity or odor