Arch. Dis. Childh., 1969, **44**, 773.

Plasma Uric Acid Levels in Children

R. A. HARKNESS and A. D. NICOL

From the Department of Paediatric Biochemistry, Royal Hospital for Sick Children, and Department of Clinical Chemistry, University of Edinburgh

Uric acid is a major end-product of purine and nucleic acid metabolism. Possibly as a result of increased nucleic acid turnover, hyperuricaemia has been found in a large number of diseases (Bywaters and Glynn, 1964). Inborn errors of purine metabolism are now known (Kelley *et al.*, 1968). Of these, the Lesch-Nyhan syndrome is the most remarkable and is frequently associated with high levels of urate in serum (Lesch and Nyhan, 1964). This condition is characterized by overproduction of uric acid as a consequence of deficiency of the enzyme hypoxanthine-guanine phosphoribosyl transferase (EC 2.4.2.8) (Seegmiller, Rosenbloom, and Kelley, 1967). From these considerations, it was felt that measurement of plasma urate in children in hospital might be of value as a general index of purine and nucleic acid metabolism, and might be closely connected with growth. Furthermore, Nyhan (1968) stressed the need for data on plasma urate levels in childhood.

The purpose of the present communication is to go some way towards fulfilling this need, with special reference to children attending hospital, and to assess whether or not plasma urate measurement can be used as a screening test for the many conditions in which abnormal urate levels have been described. The results of the survey have shown that plasma urate levels are of little or no value as a screening test for the majority of conditions in which abnormal levels have been described. Mean levels for the present hospital population have been obtained, and the results suggest that there is a fall in mean plasma urate levels at the time of the mid-growth spurt. In addition, high levels of urate have been found in some cases of unexplained abdominal pain.

Materials and Methods

Blood samples. The blood samples studied were unselected and were those sent for routine haematology; this source offered the widest range of conditions for which blood had been withdrawn as part of a justifiable routine screening. These samples were obtained by venepuncture and collected in polystyrene tubes containing EDTA. Plasma was removed from the cells after centrifugation and analysed for uric acid on either the day of collection or that following. All samples were stored at room temperature to avoid possible precipitation of urate.

Assay of plasma uric acid. Uric acid was estimated using the UV-spectrophotometric uricase method in which urate concentrations are proportional to enzymically catalysed decreases in absorption at 292 nm (Liddle, Seegmiller, and Laster, 1959). Recommendations made by Klein and Lafeber (1966) with regard to blanks, reference cells, and turbidity were incorporated in the assay. Hog liver uricase (Catalogue No. 5589t, Koch-Light Laboratories, Colnbrook, Bucks.) was used after further purification as outlined below.

Crude uricase in 0·07 M glycine buffer, *p*H 9·5, was applied to a column of Sephadex G-100 (height : diameter ratio, 40 : 1) equilibrated with the same buffer. On elution with glycine buffer, material with uricase activity emerged within the column void volume, as expected from the findings of Mahler, Hübscher, and Baum (1955). These workers reported an approximate molecular weight of 100,000 for uricase. They also reported an increase in the absorbance at 280 nm relative to the absorbance at 260 nm as enzyme purity increased. Accordingly, fractions in which the $\frac{\epsilon 280\text{ nm}}{\epsilon 260\text{ nm}}$ ratio was largest were pooled, stored frozen in sealed ampoules, and later used as 'working uricase'. Activities of new batches of partially purified uricase were checked against an aqueous uric acid standard, and an enzyme blank (enzyme+ buffer) was included with each set of uric acid analyses so that correction could be made for absorption at 292 nm by enzyme protein.

Whenever possible, samples with high or low urate levels were analysed again by either the uricase method or the Folin colorimetric phosphotungstate procedure (King and Wootton, 1956). For 21 duplicate analyses by the uricase method, the standard deviation was ±0·13 mg./100 ml. for a mean of 3·88 mg./100 ml. (coefficient of variation = 3·2%).

Treatment of data. For each individual an initial record was made of age, sex, and provisional diagnosis (or reason for attending the Royal Hospital for Sick Children, Edinburgh). Later, the information was

Received May 27, 1969.

checked and if necessary amended from the hospital records. In all cases the diagnosis recorded in the hospital records was used. Means, standard deviations, standard errors of the means and ranges were calculated according to the methods given by Moroney (1965), Henry and Dryer (1963), and Neumann (1968).

For the purposes of computing a 'normal range', results were excluded that came from children with diseases in which an increased frequency of hyperuricaemia has been reported. Examples are renal failure and acute leukaemia (Bywaters and Glynn, 1964).

The value of plasma urate as a screening test was assessed. Solely on the basis of the final diagnosis, each patient was assigned to one of three groups—low, normal, or high plasma urate—without knowing the actual levels. This clinical selection was made on the basis of reports in the literature of factors affecting plasma urate (for reviews see Bywaters and Glynn, 1964; Wyngaarden, 1966; Balis, 1967). In addition, factors such as food intake were taken into consideration. Grouping was then repeated, this time simply according to the plasma urate level recorded for each patient. The two groupings were compared and the expected grouping assessed against the observed grouping in respect of the number of successful predictions made.

Results

Distribution of plasma urate values. Of 251 plasma urate values in children between 0–13 years of age, only 171 were used in calculating a 'normal' plasma urate range for children attending this hospital. The other results were rejected, occasionally because they were repeat analyses on a patient, but more often because they were obtained on children with kidney disease, leucocytosis, liver disease, or conditions in which deranged purine metabolism was probably present.

The over-all distribution is shown in Fig. 1a; the distributions for 113 boys and 58 girls were found to have a similar appearance. Though these distributions were skewed positively, only the over-all distribution was subjected to logarithmic transformation (Fig. 1b) as a preliminary to obtaining the 'normal' plasma urate range by the graphical method described by Neumann (1968). Logarithmic transformation of the observed distribution to an essentially Gaussian distribution, together with processing by the probit method (Neumann, 1968), were necessary in order to reduce the disproportionate effect of a small number of extreme values. Statistical parameters are shown in Table I.

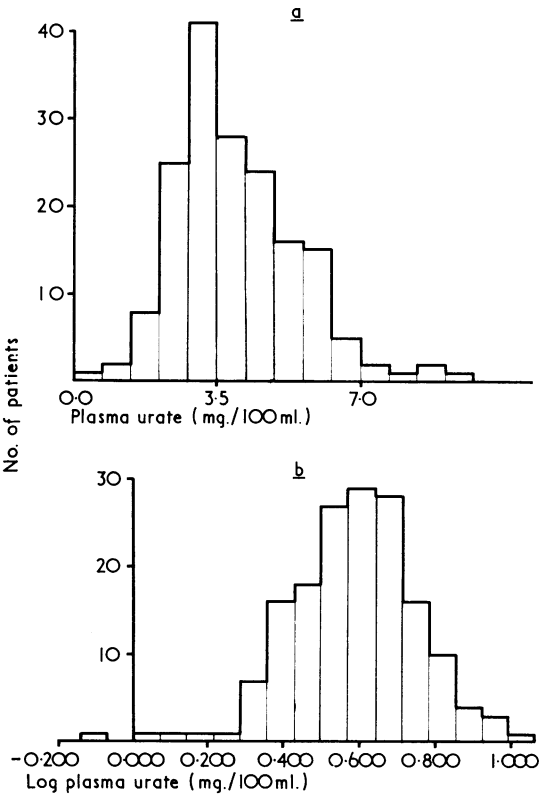

FIG. 1.—*Distribution of plasma urate levels in children attending hospital. (a) Actual levels; (b) Logarithmic transformation.*

TABLE I

Plasma Urate Levels in Children

	No.	Mean Plasma Urate (mg./100 ml.) ($\bar{x}$)	SD	SE of Mean	Plasma Urate Range (mg./100 ml.) ($\bar{x} \pm 2SD$)
Girls	58	4·1	±1·80	0·236	0·5–7·7
Boys	113	4·0	±1·40	0·132	1·2–6·8
Over-all (Fig. 1a)	171	4·0	±1·50	0·115	1·0–7·0
Logarithmic transformation (Fig. 1b)	171	3·7* (0·571)	1·47* (0·170)	0·103* (0·013)	1·7–8·1* (0·231–0·911)
'Probit' analysis according to Neumann (1968)	139	3·6	Not calculated	Not calculated	2·0–6·7

* Brackets show the parent logarithms.

For the over-all distribution, the arithmetic mean plasma urate is 4·0 mg./100 ml., whereas the geometric mean concentration is 3·7 mg./100 ml. The difference between plasma urate means for boys and girls was not significant when examined using the 'Student's t' test. The present results are similar to the normal range for children aged 4–14 years, which may be derived from the data of Mikkelsen, Dodge, and Valkenburg (1965). Their range calculated as the arithmetic mean ±2 SD was 1·8–6·2 mg./100 ml. Our lower limit for the 'normal range' obtained by the statistical treatment described by Neumann (1968) is 2·0 mg./100 ml., and the upper limit is 6·7 mg./100 ml. However, when examining the diagnostic value of plasma urate, the upper limit has been taken as 6·4 mg./100 ml., because this is the serum saturation level for monosodium urate (Wyngaarden, 1966). Fig. 2

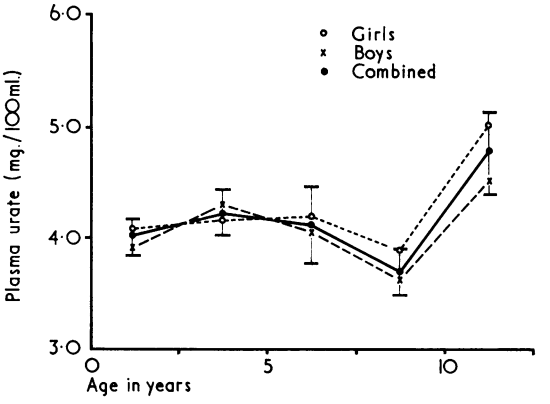

FIG. 2.—*Mean plasma urate levels in boys and girls aged 0–13 years. Vertical lines indicate a range of ±1 standard error of the combined mean.*

shows the change in mean plasma urate concentration with age, in the range 0–13 years. These data suggest that there is a decline in the mean plasma urate between 6–9 years of age, and a rise in serum urate levels around 10 years; this rise is similar to that found by Mikkelsen *et al.* (1965) in the Tecumseh survey.

Plasma urate as a diagnostic aid. Solely on the basis of clinical factors, mainly the final diagnosis, the patients were grouped into low (< 2·0 mg./100 ml.), normal (2·0–6·4 mg./100 ml.), and high (> 6·4 mg./100 ml.) plasma urate levels. If such predictions were frequently correct this could probably be taken as an indication that urate levels in plasma will often have diagnostic significance. On the other hand, if the success rate was low, the value of plasma urate measurements must be questionable.

Table II shows that it was possible to predict correctly 'normal' plasma urate levels in approximately 9 out of every 10 patients. However, only about 1 in 8 patients was correctly assigned to 'high' or 'low' groupings. From the results of this survey it seemed justifiable to conclude that plasma urate levels were of little value as a reliable screening test for any of the large number of conditions in which abnormal urate levels have been described. In the survey, those conditions included renal disease, leukaemia, Wilson's disease, reticulosis, and hepatic necrosis.

TABLE II

Evaluation of Plasma Urate in Screening; Actual and Predicted Numbers of Patients with Low, Normal, or High Levels.

Group	Low (<2·0 mg./100 ml.)	Normal (2·0–6·4 mg./100 ml.)	High (>6·4 mg./100 ml.)
Predicted numbers in each group ..	8	130	34
Number of correct predictions ..	1	116	4
Percentage of correct predictions ..	12·5%	89·2%	11·8%
Actual distribution observed within each group			
Low	1	4	3
Normal	7	116	27
High	0	10	4

Conditions associated with high or low plasma urate levels. Table III gives information on the 14 patients with high plasma urate concentrations. The most interesting finding was the very high levels found in 4 patients with unexplained abdominal pain (i.e. Cases 1, 3, 4, and 8). A total of 12 patients with unexplained abdominal pain was studied, and conventional causes for this symptom ruled out. The remaining 8 patients with abdominal pain had normal urate levels.

Of the patients listed in Table III, 4 (Cases 5, 6, 7, and 14) had renal insufficiency, and belong to a group of 11 patients with renal insufficiency in whom plasma urate levels were excluded from calculations of the normal range. The individual values for the 11 renal insufficiency patients were examined. Though application of 'Student's t' test indicated that the group mean of 5·3 mg./

100 ml. was significantly different from the 'normal' mean at the 5% level, 7 of the 11 patients had normal values. This is consistent with the statement that plasma urate concentrations only rise late in renal failure (Hawk, Oser, and Summerson, 1947).

Table IV shows the conditions in which plasma urate levels were found to be low, or at the lower end of the normal range (i.e., $\leqslant 2{\cdot}5$ mg./100 ml.). 23 of the 204 patients studied showed such levels.

TABLE III

Conditions Associated with High Plasma Urate Levels ($> 6{\cdot}4$ mg./100 ml.)

Final Diagnosis	Sex	Age			Plasma Urate (mg./100 ml.)
		Years	Months	Weeks	
1. Unconfirmed peptic ulcer	F	6	—	—	9·8
2. Gastro-enteritis—febrile fit	F	—	3	—	9·0
3. Unexplained colicky abdominal pain	F	7	—	—	8·9
4. Unexplained abdominal pain	M	3	—	—	8·4
5. Acute glomerulonephritis	F	8	—	—	8·1
6. Ureteric transplants	M	4	6	—	7·7
7. Renal failure	M	—	4	—	7·5
8. Rheumatic fever with abdominal pain	M	7	—	—	7·5
9. Height and weight below normal; frequent infections	M	—	5	—	7·3
10. Urticaria, egg allergy	M	10	—	—	7·0
11. Whooping cough and anaemia	F	—	7	—	7·0
12. '? Virus infection—apnoeic attack'	M	—	—	10	6·9
13. '? Virus infection—drowsy'	M	—	—	5	6·7
14. Renal hypoplasia	F	—	—	2	6·5

Though the conditions listed differ widely, it seems justifiable to suggest that diminished food intake or absorption would be present in at least 12 of the patients listed; these are the patients numbered 5, 6, 8, 9, 11, 12, 13, 14, 17, 18, 20, and 22 in Table IV.

Discussion

The results of the present survey of hospital patients are similar to those of Mikkelsen *et al.* (1965), who also used a uricase method. This group studied 90% of the population of Tecumseh, Michigan, U.S.A. The close agreement between the two sets of results suggests that the majority of values obtained in the present investigation are also 'normal'.

TABLE IV

Conditions Associated with Low or 'Low-Normal' Plasma Urate Levels ($\leqslant 2{\cdot}5$ mg./100 ml.)

Final Diagnosis	Sex	Age		Plasma Urate (mg./100 ml.)
		Years	Months	
1. Spiral fracture of right thigh	M	—	4	2·5
2. Transverse myelitis	M	2	—	2·5
3. Asthma	?	?	?	2·5
4. ? Neoplasm left orbit; ? viral infection	M	8	—	2·5
5. Diarrhoea/vomiting; cleft palate	F	—	5	2·4
6. Mandible injury	M	9	—	2·3
7. Ventricular septal defect	M	3	—	2·3
8. Mentally retarded; peritonitis; epilepsy	F	3	—	2·3
9. Anaemia and rickets	M	—	5	2·3
10. Baby for adoption	M	3 days		2·3
11. Coeliac disease	M	7	—	2·2
12. Infective hepatitis	F	6	—	2·2
13. Peritoneal anastomosis	F	1	6	2·1
14. Inguinal abscess drainage	M	8	—	2·0
15. Talipes equinovarus	M	6	—	2·0
16. Enlarged thymus—systolic murmur	F	1	—	2·0
17. Herpetic stomatitis	F	2	6	2·0
18. Appendicular abscess	M	3	—	1·9
19. Phaeochromocytoma	M	10	—	1·8
20. Hiatus hernia—post-operative review	F	7	—	1·6
21. Idiopathic thoracic scoliosis; some spinal compression with loss of bladder control	F	13	1	1·5
22. Haemophilus meningitis; subdural effusion; otitis media and pneumonia	M	9	—	1·3
23. Viral myalgia	M	10	—	0·7

The effect of sex upon urate concentrations is small. Our data on children indicate that the marked degree of skewing towards higher values is similar in boys and girls, though a more marked skewing towards higher values has been noted in adult females (Mikkelsen *et al.*, 1965); this is probably explicable on an endocrine basis.

More detailed comparison of the present results with those from the Michigan survey reveals that some 7% of the results on Tecumseh children between 4 and 14 years of age exceeded 6·0 mg./100 ml., whereas 11% of our 'normal' population (ages 0–13 years) were above this point. A number of possible causes for this difference can be suggested. For example, the majority of the children in the Tecumseh study were above 10 years of age. A clinically selected hospital population might also be expected to yield more abnormal values by inclusion of conditions not yet recognized as being associated with hyperuricaemia. In addition, slight differences

in methodology may be responsible for the disparity. The Michigan workers performed their analyses on serum that had been deep-frozen. The possibility exists that uric acid might occasionally be lost by adsorption or precipitation on to either the blood clot or the walls of the container (Wyngaarden, 1966).

Part of the present investigation was aimed at gauging the value of plasma urate levels in screening, that is, in providing unique or supplementary information on the cause of a patient's illness. The results in Table II indicate that isolated plasma urate measurements have less value than might be expected, and some results can be so different from the expected values as to be misleading. This is strikingly demonstrated by Table II where 34 results were predicted to exceed 6·4 mg./100 ml. Only 4 exceeded this level; the rest were 'normal', and 3 were actually below 2·0 mg./100 ml. In addition, the number of normouricaemic children with impaired renal function was surprisingly high, while normal results (2·3 and 3·3 mg./100 ml.) were obtained on both occasions in which serum urate was measured in a boy with Wilson's disease, where serum uric acid is stated to be low (Bearn, 1966). These and other results also show that day-to-day variations in the same individual can be marked.

There is some evidence (Fig. 2) that the mean plasma urate decreases between 6 and 9 years of age and starts to rise again around age 10 years; when different age intervals were used to plot Fig. 2, this decrease appeared earlier in girls. The decrease may be due to increased renal excretion of urate. Kaufman, Greene, and Seegmiller (1968) have recorded an increased urinary uric acid/creatinine ratio in children between 6 and 8 years of age. Since there is evidence of increased creatinine output in this age range (Clark *et al.*, 1951), renal excretion of uric acid must also be increased. It is of interest that this age period is the time of the mid-growth spurt (Tanner, 1962); fuller studies of this are needed.

Though fasting is generally recognized as causing hyperuricaemia (MacLachlan and Rodnan, 1967; Scott, McCallum, and Holloway, 1964), the diagnoses shown in Table IV suggest that low plasma urate levels could be associated with impaired nutrition and a low intake of exogenous purines. This could arise from diminished food intake due to gastro-intestinal disease or anorexia.

The relatively large number of children with unexplained abdominal pain who had very high plasma urate levels is of considerable interest and is currently being investigated.

Summary

Plasma urate levels have been measured in 204 children aged 0–13 years attending hospital. By appropriate statistical treatment, a mean of 3·6 mg./100 ml. and a normal range of 2·0–6·7 mg./100 ml. have been derived from the data in 171 children who did not have conditions normally associated with hyperuricaemia. The value of plasma urate determinations as a screening procedure in children has been shown to be very small. The data suggest that mean levels in normal children fall at about the time of the mid-growth spurt. In addition, 4 out of the 12 cases of unexplained abdominal pain studied had abnormally high levels of plasma urate.

We are grateful for the advice of Professor L. G. Whitby, Dr. E. F. Harding, and Dr. W. M. McCrae; and for the help of Mrs. M. Herkes, Mr. C. Paterson, and the staff of the Haematology and Records Departments, the Royal Hospital for Sick Children, Edinburgh.

References

Balis, M. E. (1967). Metabolism of oxypurines in man. *Advanc. clin. Chem.*, **10**, 157.

Bearn, A. G. (1966). Wilson's disease. In *The Metabolic Basis of Inherited Disease*, 2nd ed., p. 761. Ed. by J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson. McGraw-Hill, New York.

Bywaters, E. G. L., and Glynn, L. E. (1964). Connective tissue disorders. In *Biochemical Disorders in Human Disease*, 2nd ed., p. 809. Ed. by R. H. S Thompson and E. J. King. Churchill, London.

Clark, L. C., Jr., Thompson, H. L., Beck, E. I., and Jacobson, W. (1951). Excretion of creatine and creatinine by children. *Amer. J. Dis. Child.*, **81**, 774.

Hawk, P. B., Oser, B. L., and Summerson, W. H. (1947). *Practical Physiological Chemistry*, 12th ed., p. 513. Churchill, London.

Henry, R. J., and Dryer, R. L. (1963). Some applications of statistics to clinical chemistry. In *Standard Methods of Clinical Chemistry*, Vol. 4, p. 205. Ed. by D. Seligson. Academic Press, London.

Kaufman, J. M., Greene, M. L., and Seegmiller, J. E. (1968). Urine uric acid to creatinine ratio: a screening test for inherited disorders of purine metabolism. *J. Pediat.*, **73**, 583.

Kelley, W. N., Levy, R. I., Rosenbloom, F. M., Henderson, J. F., and Seegmiller, J. E. (1968). Adenine phosphoribosyltransferase deficiency: a previously undescribed genetic defect in man. *J. clin. Invest.*, **47**, 2281.

King, E. J., and Wootton, I. D. P. (1956). *Micro-analysis in Medical Biochemistry*, 3rd edition, p. 19. Churchill, London.

Klein, F., and Lafeber, G. J. M. (1966). Improvements of the uricase UV-method for the determination of uric acid in serum and urine. *Clin. Chim. Acta*, **14**, 708.

Lesch, M., and Nyhan, W. L. (1964). A familial disorder of uric acid metabolism and central nervous system function. *Amer. J. Med.*, **36**, 561.

Liddle, L., Seegmiller, J. E., and Laster, L. (1959). The enzymatic spectrophotometric method for determination of uric acid. *J. Lab. clin. Med.*, **54**, 903.

MacLachlan, M. J., and Rodnan, G. P. (1967). Effects of food, fast and alcohol on serum uric acid and acute attacks of gout. *Amer. J. Med.*, **42**, 38.

Mahler, H. R., Hübscher, G., and Baum, H. (1955). Studies on uricase. I. Preparation, purification, and properties of a cuproprotein. *J. biol. Chem.*, **216**, 625.

Mikkelsen, W. M., Dodge, H. J., and Valkenburg, H. (1965). The distribution of serum uric acid values in a population unselected as to gout or hyperuricemia: Tecumseh, Michigan 1959–1960. *Amer. J. Med.*, **39**, 242.

Moroney, M. J. (1965). *Facts from Figures.* Chap. 4, 6, and 10. Penguin, Harmondsworth.
Neumann, G. J. (1968). The determination of normal ranges from routine laboratory data. *Clin. Chem.*, **14**, 979.
Nyhan, W. L. (1968). Summary of clinical features. *Fed. Proc.*, (Seminars on the Lesch-Nyhan syndrome), **27**, 1034.
Scott, J. T., McCallum, F. M., and Holloway, V. P. (1964). Starvation, ketosis and uric acid excretion. *Clin. Sci.*, **27**, 209.
Seegmiller, J. E., Rosenbloom, F. M., and Kelley, W. N. (1967). Enzyme defect associated with a sex-linked human neurological disorder and excessive purine synthesis. *Science*, **155**, 1682.
Tanner, J. M. (1962). *Growth at Adolescence.* 2nd ed., p. 1. Blackwell, Oxford.
Wyngaarden, J. B. (1966). Gout. In *The Metabolic Basis of Inherited Disease*, 2nd ed., p. 667. Ed. by J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson. McGraw-Hill, New York.

Correspondence to Dr. R. A. Harkness, Department of Paediatric Biochemistry, Royal Hospital for Sick Children, 1 Rillbank Crescent, Edinburgh EH9 1LJ.

Erratum

Status Epilepticus in Infants and Young Children Treated with Parenteral Diazepam. By Sheilah McMorris and P. K. A. McWilliam. **44**, 609.

The authors have drawn our attention to an error in punctuation which has altered the sense. Page 609, left hand column, lines 18 and 19 should read . . . '10 mg. diazepam was given intravenously. 45 minutes later, he coughed' . . . etc.