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THE INCORPORATION OF GLYCINE-2-^{14}C INTO URINARY URIC ACID IN NORMAL AND PORPHYRIC HUMAN SUBJECTS*

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In 1953 Shemin and Russell[1] showed that δ-aminolaevulinic acid (ALA) could serve as a common metabolic precursor of porphyrins and purines, and so suggested the possibility of a relationship between purine and haem biosynthesis. Subsequent observations have strengthened this suggestion. Labbé *et al.*[2] and Talman *et al.*[3] showed diminished synthesis of uric acid by chick embryos rendered porphyric with allyl-isopropyl-acetamide. Gajdos and Gajdos-Török[4] have reported inhibition of porphyrin synthesis by the addition of purine nucleotides and nucleosides to the growth medium sustaining *Rhodopseudomonas spheroides*. Beneficial therapeutic effects of purine compounds on the clinical and biochemical manifestations of porphyria in animals and man have been reported by several authors.[3-8] Drugs such as orotic acid,[9] ethionine[10] and allyl-isopropyl-acetamide[11] are porphyrinogenic and all reduce hepatic ATP levels.

Relatively little work has been done on purine metabolism in human porphyria. Ludwig[12] and Taxay[13] reported low blood uric-acid concentrations in acute human porphyria although their observations may be explicable on the basis of renal loss of urate and haemodilution rather than from decreased purine synthesis. In view of the suggested inverse relationship between purine synthesis and porphyrin synthesis, we have conducted a series of experiments in which glycine-2-^{14}C was administered to human volunteers with porphyria.

Glycine-2-^{14}C may be incorporated into purines by one of two pathways: (*a*) It may be incorporated as part of the intact glycine molecule into position 5 of the purine nucleus (carbon atom 4 being derived from the carboxyl carbon of glycine, and nitrogen atom 7 from the amino nitrogen of glycine), or (*b*) it may be incorporated into the 5th carbon atom of ALA and thence into carbon atoms 2 and 8 of the purine nucleus (Fig. 1). If there were defective synthesis of purines from ALA without concomitant impairment of incorporation of glycine into positions 4, 5 and 7 of the purine nucleus, porphyric subjects should show diminished incorporation of radioactivity into carbon atoms 2 and 8 (C2 + 8) with relatively normal incorporation of radioactivity into carbon atoms 4 and 5 (C4 + 5) following the administration of glycine-2-^{14}C. The specific activity ratio C4 + 5 : C2 + 8 should be correspondingly higher in urinary uric acid excreted by porphyrics than by normal subjects.

MATERIALS AND METHODS

The experiments were performed with the informed consent of 3 control subjects (H.J., A.J. and A.M.), 3 patients with symptomatic porphyria (A.A., B.P. and W.B.) and 5 patients with variegate porphyria (A.v.R., M.N., M.de J., Z.M. and C.J.). The 3 control subjects were free of any disease that could have affected purine or glycine metabolism. The diagnosis of symptomatic porphyria and acute porphyria was established by the usual laboratory criteria. Relevant clinical and laboratory data are given in Table I.

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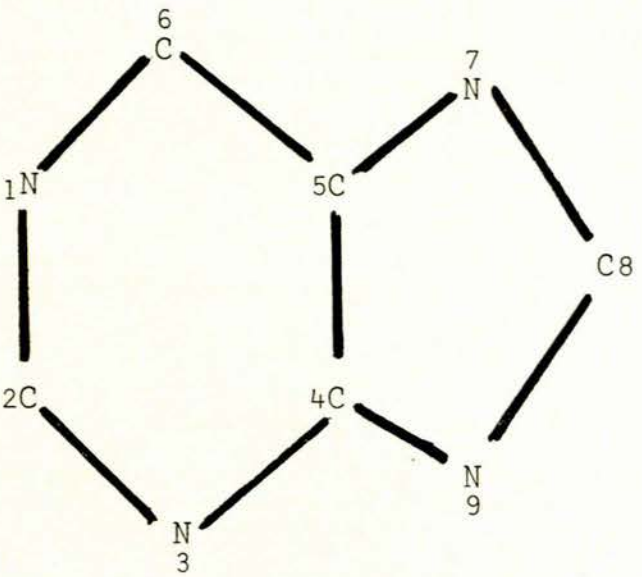

Fig. 1. Diagrammatic representation of the purine nucleus of the uric acid molecule. Uric acid (see text) was degraded in such a manner as to allow separate radioassay of carbon atoms 4 + 5 and 2 + 8.

At 8 a.m. on the first day of the experimental period each subject received an intravenous injection of an accurately weighed solution containing approximately 90 μc (2 mg.) of glycine-2-^{14}C. Twenty-four-hour urine samples were collected daily for 2 weeks for measurement and isolation of uric acid. Serum uric-acid concentrations were determined daily.

The concentration of uric acid in the serum and urine was determined by the enzymatic spectrophotometric method of Liddle *et al.*[14] Uric acid was isolated from the urine by the method of Hawke *et al.*[15] After isolation the uric acid was recrystallized twice by the method of Liddle *et al.*[14] and degraded as described by Korn[16] to yield carbon atoms 4 and 5 as glyoxylate, and carbon atoms 2 and 8 as urea. The glyoxylate was isolated for radioassay as the crystalline semicarbazone. The urea was degraded with urease to carbon dioxide which was isolated as barium carbonate for radioassay.

The uric-acid crystals, glyoxylate semicarbazone crystals and barium-carbonate powder were weighed accurately for radioassay into tared liquid scintillation vials and suspended in a scintillator of the following composition: 2,5-diphenyloxazole (PPO) 0·3 G; 1,4-bis-2-(5 phenyloxazole)-benzene (POPOP) 0·3 G; Cab-O-Sil (Packard) 4 G; toluene 1 litre. The samples were then counted on a Packard Tri-Carb Liquid Scintillation Spectrometer. Preliminary experiments indicated that there was no self-

TABLE I. SUMMARY OF RELEVANT DATA RELATING TO SUBJECTS RECEIVING GLYCINE-2-^{14}C

Subject	Sex	Age (yrs)	Diagnosis	Serum urate mg./100 ml. (range)	Urinary urate mg./24 hr (range)	Dose of glycine-2-^{14}C (d.p.m. × 10^{-6})
H.J.	M	48	Peripheral vascular disease. Renal function normal	6·0–7·0	458–625	181·8
A.J.	M	23	Post-traumatic Brown-Sequard syndrome	4·5–6·2	200–500	186·2
A.M.	M	32	Convalescent osteitis	2·8–4·4	500–709	199·6
A.A.	M	46	Symptomatic porphyria	3·7–5·9	471–639	199·5
B.P.	M	49	Symptomatic porphyria	4·1–4·5	449–795	203·4
W.B.	M	36	Symptomatic porphyria	2·7–5·0	491–688	191·3
A. v. R.	F	19	Variegate porphyria (remission)	3·4–4·1	363–665	197·6
M.N.	F	31	Variegate porphyria (acute attack)	2·5–5·4	279–539	178·2
M. de J.	F	26	Variegate porphyria (acute attack)	3·3–5·9	240–583	195·2
Z.M.	F	53	Variegate porphyria (acute attack)	4·8–8·6	288–527	192·0
C.J.	F	37	Variegate porphyria (acute attack)	1·8–3·3	317–536	191·8

absorption over the range of weights used in the counting vials. The counting rates were corrected to disintegrations per minute by re-counting the samples following addition of a known amount of standardized n-hexadecane-^{14}C.

RESULTS

In all subjects radioactivity could be detected in the urinary uric acid excreted during the first 24 hours. The specific activity of the excreted uric acid rose to reach a peak within the first 48 hours after administration of glycine-2-^{14}C, after which it declined slowly as an irregular and fluctuating plateau. Since an approximately similar fraction of the administered ^{14}C was excreted as urinary uric acid each day for 2 weeks, the cumulative excretion curve was linear in all subjects. The results of a typical experiment are shown in Figs. 2 and 3.

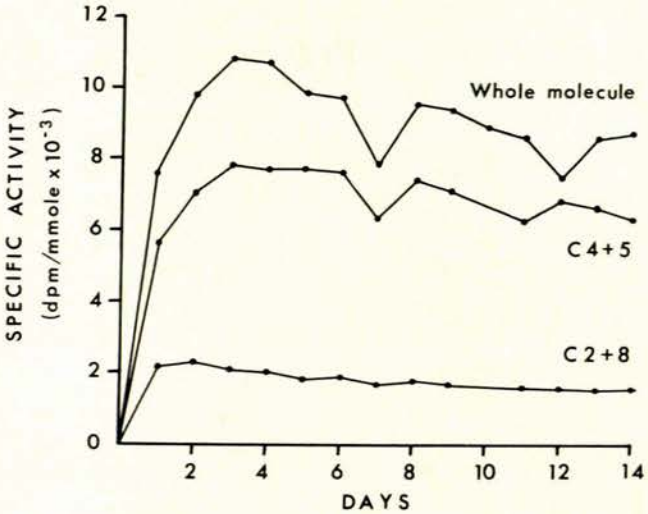

Fig. 2. Specific activity of urinary uric acid and carbon atoms 4 + 5 and 2 + 8 following approximately 90 μc of glycine-2-^{14}C given intravenously to patient W.B. with symptomatic porphyria. Note the rapid rise in specific activity followed by an irregular plateau.

The 3 normal subjects excreted an average of 0·172% of the dose as uric acid, 0·115% of the dose as C4 + 5, and 0·029% of the dose as C2 + 8. The 3 subjects with symptomatic porphyria excreted 0·228% of the dose as uric acid, 0·166% as C4 + 5, and 0·044% as C2 + 8. The subject with variegate porphyria in remission excreted 0·167% as uric acid, 0·123% as C4 + 5, and 0·033% as C2 + 8. The 4 subjects with variegate porphyria in acute attacks excreted 0·186% of the dose as uric acid, 0·113% as C4 + 5, and 0·028% of the dose as C2 + 8.

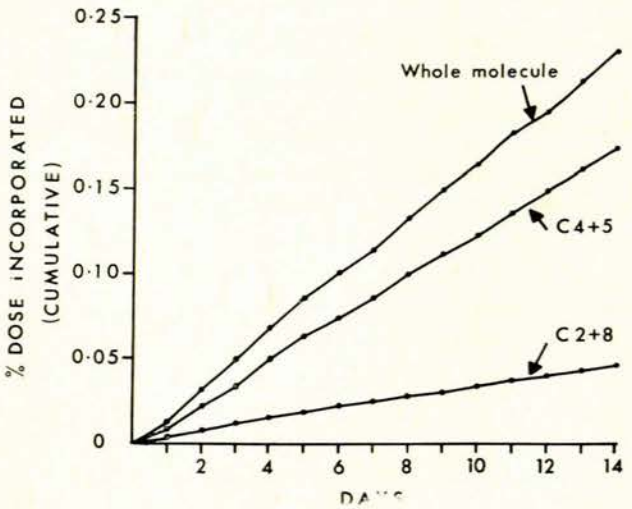

Fig. 3. Cumulative incorporation of radioactivity injected as glycine-2-^{14}C into urinary uric acid in patient W.B. with symptomatic porphyria. Note the virtually constant rate of cumulative incorporation for the duration of the study.

Detailed figures for each individual are presented in Table II. As can be seen from these data, the patients with symptomatic porphyria excreted a consistently higher fraction of the dose of glycine-2-^{14}C as uric acid. There was no significant difference between normal subjects and subjects with variegate porphyria in the excretion of glycine-2-^{14}C as urinary uric acid.

Degradation of the uric-acid samples for separate radioassay of C4 + 5 and C2 + 8 showed a daily variation in C4 + 5 : C2 + 8 specific activity ratios that differed in extent from subject to subject. Technical factors were presumably responsible for part of the variation, since it was wider in subjects where isolation of the urinary uric acid proved difficult or where only small amounts

of uric acid were available for degradation. Apart from a slight and inconsistent tendency for C4 + 5 : C2 + 8 ratios to be lower during the first 3 - 4 days of each experiment, no definite pattern of C4 + 5 : C2 + 8 ratio change with time emerged. The results of one experiment showing relatively little variation are shown diagrammatically in Fig. 2. Mean C4 + 5 : C2 + 8 ratios for all subjects with ranges are given in Table II. The normal subjects had a mean C4 + 5 : C2 + 8 ratio of 4·09 with relatively little scatter. Mean ratios found in porphyric subjects were 3·5 in symptomatic porphyria, and 4·02 in variegate porphyria. There were clearly no significant differences in this regard.

TABLE II. INCORPORATION OF GLYCINE-2-^{14}C INTO URINARY URIC ACID IN NORMAL AND PORPHYRIC HUMAN SUBJECTS

		Cumulative % dose excreted as urinary uric acid in 14 days			
Subject	*Diagnosis*	*Whole molecule*	*C4 + 5*	*C2 + 8*	*Ratio: C4 + 5 / C2 + 8*
H.J.	Control	0·210	0·137	0·035	3·91
A.J.	Control	0·187	0·124	0·033	4·04
A.M.	Control	0·119	0·084	0·020	4·32
	Mean	0·172	0·115	0·029	
A.A.	Symptomatic porphyria	0·239	0·164	0·049	3·51
B.P.	Symptomatic porphyria	0·216	0·161	0·039	4·17
W.B.	Symptomatic porphyria	0·230	0·173	0·046	3·82
	Mean	0·228	0·166	0·044	
A. v. R.	Variegate porphyria (remission)	0·167	0·123	0·033	3·68
M.N.	Variegate porphyria (acute attack)	0·200	0·160	0·025	6·42
M. de J.	Variegate porphyria (acute attack)	0·154	0·112	0·033	3·39
Z.M.	Variegate porphyria (acute attack)	0·127	0·065	0·019	3·31
C.J.	Variegate porphyria (acute attack)	0·262	0·116	0·034	3·30
	Mean	0·186	0·113	0·028	

DISCUSSION

Our finding of prompt labelling of urinary uric acid followed by a fluctuating plateau of specific activity is similar to the experience of others.[17-27] The early labelling of uric acid in non-gouty subjects has been regarded by Gutman and Yu[28] and Wyngaarden[29] as an overflow 'bypass' for the elimination of surplus inosine monophosphate and other ribonucleotides generated in excess of body needs. This idea is supported by the observation by Wyngaarden *et al.*[30] of prompt and striking labelling by isotopic glycine of urinary hypoxanthine in excess of that of other purine bases. The specific activity curves of hypoxanthine and uric acid in the experiment described bore a precursor-product relationship.

It is, however, also conceivable that the time of appearance of labelled uric acid in the urine is related to the biological life of the labelled purines from which the uric acid was derived. Messenger ribonucleic acid, for example, with a half-life of approximately 4 - 8 hours[31] would contribute to the appearance of early-labelled uric acid in the urine. DNA and RNA in hepatic,[31-33] myeloid[34,35] and lymphoid[35] tissues have different rates of turnover and they would be expected to contribute radioactive uric acid to the urine when degraded at a later stage after administration of an isotopic precursor. The superimposition of several curves of urinary uric-acid specific activity, each with a different time relationship, could well explain the irregular 'plateau' appearance of the uric-acid specific activity curve and the failure of urinary uric-acid specific activity to fall off exponentially with time following ^{14}C precursor administration.

Had there been any consistent aberration of purine synthesis in porphyria affecting one particular nucleic-acid pool, one might have expected a distortion of the uric-acid specific activity plateau characteristic of the biological life of the nucleic acid involved. This was not found in any of the subjects studied.

In normal subjects and those with variegate porphyria the percentage of the dose of glycine-2-^{14}C incorporated into C4 + 5 and C2 + 8 over 2 weeks did not differ significantly, indicating no major impairment of purine synthesis in the patients with porphyria in remission or in the acute phase. It should be noted, however, that no attempt was made to estimate the size of the uric-acid pool or the extent of faecal elimination of uric acid, so that these values cannot be regarded as an accurate reflection of the synthesis of uric acid from glycine. In the 3 subjects with symptomatic porphyria increased incorporation of glycine-2-^{14}C into urinary uric acid, C4 + 5 and C2 + 8 was noted. Since hyperuricaemia is not a feature of this disorder, there is no reason to presume that excessive synthesis of uric acid occurs in this disease. It is more probable that hepatic excretion of urate in this disorder where liver disease is invariably found is impaired and, as a consequence, a greater proportion of total urate excreted is eliminated by the kidney.

The C4 + 5 : C2 + 8 specific activity ratios were similar in normal and porphyric subjects, indicating no selective defect in the synthesis of the ureido carbons of uric acid from glycine-2-^{14}C. This observation is in accord with other studies from this laboratory[36] that failed to demonstrate impairment in the utilization of ALA-5-C for purine synthesis in porphyria.

It can, on the basis of these experimental data, therefore, be concluded that purine synthesis in porphyria is normal. This conclusion is supported by the observations of De Matteis *et al.*,[11] who found unchanged liver purine levels in experimental porphyria.

SUMMARY

Glycine-2-^{14}C was administered intravenously to 3 control subjects, 3 subjects with symptomatic porphyria and 5 subjects with variegate porphyria. Uric acid was isolated from daily urine samples for 2 weeks thereafter and the radioactivity assayed in the whole molecule and in carbon atoms 4 and 5, and 2 and 8 of the purine nucleus. There were no major differences in incorporation of radioactivity between normals and porphyrics. It is concluded that there is no major defect in purine metabolism in this disorder.

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