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ACCELERATED TURNOVER OF PHOSPHORIBOSYLPYROPHOSPHATE, A PURINE NUCLEOTIDE PRECURSOR, IN CERTAIN GOUTY SUBJECTS *

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Existing tracer studies on the metabolic defect of primary gout have employed injection of labeled uric acid and measurement of the kinetic behavior of the uric acid pool by classical dilution technics (3, 4), or administration of a labeled precursor and measurement of its incorporation into uric acid (5, 6, 7, 8). The dilution studies have shown major increases in the miscible pool of uric acid in almost all gouty subjects studied. The incorporation studies have consistently demonstrated unequivocal abnormalities of uric acid production only in those gouty patients showing excessive basal urinary excretions of uric acid, a subgroup estimated to represent about 28 per cent of the gouty population (9). In the larger subgroup of gouty patients with normal basal urinary excretions of uric acid, abnormalities of turnover of uric acid or of incorporation of labeled precursor into uric acid have been encountered in only about one-half of the subjects studied (10). These abnormalities have frequently been of less decisive degree and at times have been demonstrable only after appropriate corrections have been applied for the greater extrarenal disposal and greater dilution of newly formed labeled uric acid found in gouty patients than in controls (11). In addition, subjects of both subgroups have shown abnormalities of intramolecular distribution of N^{15} in uric acid after administration of $N^{15}H_4Cl$ or of glycine N^{15} (12). The metabolic abnormalities encountered are consistent with a defective regulatory mechanism of purine synthesis de novo in the overexcretor gouty subject and possibly of more subtle degree in the normal excretor gouty subject also.

Data on labeling of urinary purine bases after oral administration of glycine 1-C^{14} gave suggestive evidence that the pathways of excessive uric acid synthesis in gout involved the same purine nucleotide intermediates as in normal subjects (13). When subsequently a potential feedback regulatory mechanism of purine synthesis was discovered in which the first specific step of purine synthesis was found to be controlled by adenyl ribonucleotides such as ADP and ATP (14), it was suggested that a defect of this regulatory mechanism might explain the metabolic aberrations of gout (10). The biochemical step in question is that in which 5-α-phosphoribosyl-1-pyrophosphate (PRPP) and glutamine react to form 5-β-phosphoribosyl-1-amine (15), the first unique and obligatory precursor of purine ribonucleotides. Hence, a necessary concomitant of a derangement of this feedback control mechanism would be an increase in turnover of PRPP and glutamine in patients showing accelerations of uric acid synthesis de novo.

Recently a second reaction has been described by which phosphoribosylamine may be formed. In this reaction ribose 5-phosphate and ammonia interact directly to form phosphoribosylamine without the intermediary formation of PRPP (16). It was first thought that this reaction was catalyzed by a specific enzyme. The reaction has now been shown to occur nonenzymatically (17).

For many reasons, therefore, it appeared interesting to investigate the role of PRPP in purine synthesis in gouty subjects. Among them is the desirability of appraising the results of previous studies which have included the end product, uric acid, by an independent method involving an early precursor of purines. The present paper reports an indirect approach toward study of the metabolic turnover of PRPP, based upon prior observations of other workers (18, 19). The approach is that of labeling PRPP by oral administration of glucose U-C^{14} and of trapping a portion of the PRPP by concomitant administration of

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imidazoleacetic acid (IAA), a natural metabolite of histidine which is converted to its ribonucleotide derivative by reaction with PRPP (20) and is subsequently excreted in urine as an isolable product, imidazoleacetic acid ribonucleoside (IAAR) (21).

MATERIALS AND METHODS

Imidazoleacetic acid hydrochloride was synthesized from DL-histidine hydrochloride according to Bauer and Tabor (22). The hydrochloride was removed by passage of the compound through Dowex-1-acetate (23).[1] Imidazoleacetic acid ribonucleoside, 90 mg,[2] was tritiated by the Wilzbach procedure at the New England Nuclear Corporation, Boston. This material was purified by sequential chromatography on Dowex-1-acetate and Dowex 50-hydrogen resins, and on paper in *t*-butanol-formic acid-H_2O, and finally by paper electrophoresis for 1.5 hours at 900 v in 0.025 M phosphate, pH 6.5 (18). The IAAR-H^3 recovered from paper after electrophoresis migrated as authentic IAAR in parallel and mixed paper chromatograms in several solvent systems (18). Final yield was 7 per cent of material exposed to tritium gas, and the specific activity of IAAR-H^3 was 0.96 mc per mg. Hydrolysis with bacterial hydrolase showed 27.4 per cent of the total H^3 to be in the ribose moiety of IAAR. Glucose C^{14}, uniformly labeled, 4 mc per mmole, was obtained commercially.[3] β-Riboside hydrolase was prepared from *L. delbrueckii* 9649,[4] according to Takagi and Horecker (24) through stage IV (the 50 to 80 per cent ammonium sulfate fraction). Xanthine oxidase of high specific activity, 15 to 16 U per mg of protein (25), required for assay of hydrolase activity, was prepared by submitting the commercial enzyme, 2 ± U per mg,[5] to chromatography on an hydroxyapatite column (26) according to the method of Carey, Fridovich and Handler (27). Uricase[5] was employed in the determination of uric acid in serum and urine by differential ultraviolet spectrophotometry (28).

Subjects. The subjects studied included seven control and nine gouty subjects, all males (see Table I). The control subjects were patients hospitalized for study of mild illness, who gave no personal or familial history of gout and who were found to have normal serum and 24-hour urinary uric acid values. The gouty subjects included three with elevated 24-hour urinary uric acid excretion values and six with normal 24-hour excretion values, as defined by Gutman and Yü (9). All gave histories of typical colchicine-responsive attacks of acute gout, except for one hyperuricemic member of a gouty family who was asymptomatic prior to study (J.E., Jr., Table I) but who has since experienced a colchicine-responsive gouty attack. The studies on J.E., Jr., and J.E., Sr., and the second study on J.B. were conducted while they received uricosuric drugs. Serum and urinary uric acid values had been obtained on these patients prior to the beginning of therapy.

Experimental procedure. All subjects avoided purine-rich foods for 2 to 3 days prior to the study. After an overnight fast each subject received 18 μmoles per kg of IAA together with an equivalent amount of $NaHCO_3$ in water orally. Immediately thereafter he was also given 15 to 25 μc of glucose U-C^{14} in water orally. (All results were subsequently normalized to an administered dose of 20×10^6 cpm.) Two hours later he was permitted to eat a normal breakfast and to resume normal hospital ward activity. Urine was collected either in five consecutive 2-hour samples or in a single 10-hour sample, each in 5 ml of glacial acetic acid. IAAR was then isolated from one-third of this urine sample according to Hiatt (19, 21) by employing Dowex-1-acetate × 4, 100–200 mesh, in columns containing resin beds 20 cm in height and 4.5 cm in diameter. Initially, IAAR was located in the 200 to 600 ml fractions of the 0.2 N acetic acid eluate by release of an orcinol reactive substance from an aliquot after hydrolysis with bacterial β-riboside hydrolase. In later experiments the position of IAAR in the eluate was located by employing a small quantity of IAAR-H^3 as a marker. IAAR-H^3, 25,000 counts, was added to the urine sample before adsorption onto Dowex-1-acetate resin, the H^3 peak located in the eluate, and IAAR analyzed in this fraction. In paired experiments the results were the same, but use of the tritiated marker shortened the procedure and permitted greater precision in selection of appropriate eluates for further analysis. (The concentrations of IAAR in the eluates are too low to permit localization by orcinol or periodate tests without preliminary reductions of volume of individual samples.)

The eluates from the Dowex-1-acetate column which contained IAAR were reduced to dryness at 30° in a flash evaporator, redissolved in 5 ml of water, and placed on Dowex 50-hydrogen × 8, 200–400 mesh, in a column containing a resin bed 8 cm in height and 1.0 cm in diameter. The IAAR was eluted by gradient elution (21). The separatory funnel reservoir contained 250 ml of H_2O and into this flowed 2N HCl. IAAR appeared in the 100 to 200-ml eluate.

The fractions containing IAAR were pooled, reduced to dryness in vacuo at 30° C, dissolved in water, and buffered to pH 6.0 with citrate buffer, 0.05 M; the IAAR was then hydrolyzed for 6 hours at 38° C with freshly prepared bacterial enzyme.[6] The reaction was stopped

[1] In later experiments imidazoleacetic acid was purchased from California Corporation for Biochemical Research.

[2] Obtained as a gift from Dr. G. A. Jamieson and Dr. Herbert Tabor of NIH, Bethesda, Md.

[3] Volk Radiochemicals, Chicago.

[4] Obtained from American Type Culture Collection.

[5] Purchased from Worthington Biochemical Corporation, Freehold, N.J.

[6] A minimum of 1.0 U of enzyme activity per mg of IAAR-ribose was employed, since this ratio of enzyme activity to IAAR was found to give quantitative hydrolysis in 4 to 5 hours. If the final yield of ribose indicated

TABLE I

Data on subjects of the present study

Subject	Age	Weight	Uric acid		Excretion of IAAR	C^{14} in IAAR	Specific activity of IAAR	Comment
			Serum*	Urine†				
	yrs	*kg*	*mg%*	*mg/24 hr*	*% of administered IAA*	*% of administered glucose U-C^{14}*	*cpm/mg ribose*	
					Control subjects			
J.D.	16	63.6	4.1	400	7.1	0.010	128	Idiopathic epilepsy
L.D.	26	75.0	6.0	400	4.7	0.013	251	Inactive rheumatic heart disease, Class IA
Jo.B.	28	89.1	5.2	226	4.4	0.021	439	Emotional disturbance; BUN‡ 15 mg%
J.W.	68	95.4	5.8	429	12.4	0.043	312	Non-toxic goiter
W.R.	38	81.8	5.6	413	19.3	0.047	217	Inactive rheumatic heart disease, Class IA
W.Y.§	32	81.8	6.5	629	21.6	0.074	306	Asymptomatic brother of gouty subject J.Y. (below)
R.G.	24	70.0	4.7	475	12.5	0.057	484	Normal college student
					Gouty subjects			
J.E., Jr.	15	61.4	11.4	287	10.0	0.012	127	Son of J.E., Sr., asymptomatic when studied, 1 yr later had gouty attack responsive to colchicine C_{inulin}, 116 ml/min; C_{PAH}, 583 ml/min; C_{urate}/C_{inulin} = 4.7% (29). Normal mean, 7.6±2.4% (9)
J.E., Sr.	39	65.9	10.9	397	1.57	0.058	331	Severe tophaceous gout; PSP‡ 62%/2 hours
H.H.	50	110.0	9.8	685	15.4	0.164	950	Non-tophaceous gout, 6 yrs; BUN 16 mg%; PSP 55%/2 hrs; incorporated glycine 1-C^{14} into uric acid excessively (30)
T.W.	35	102.0	8.5	973	12.1	0.200	1104	Recurrent podagra, 3 yrs; BUN 13 mg%; PSP 68%/2 hrs
J.B.	45	79.6	10.8	670	19.2	0.310	1220	Moderately severe tophaceous gout; 7-yr history; BUN 15 mg%; PSP 45%/2 hrs
M.C.	26	104.5	7.4	588	14.4	0.019	120	One attack of podagra, responding dramatically to colchicine
J.Y.	36	84.1	10.5	292	14.3	0.062	387	Typical gout attacks, 7 yrs
L.E.	36	90.0	10.0	352	7.3	0.019	242	Typical gout attacks, 6 yrs
J.L.	45	57.8	10.9	360	12.9	0.040	402	Three typical attacks of podagra, responding to colchicine, in previous year. Carcinoma of colon, loss of 10 kg

* Normal range, enzymatic method, 5.1±1.0 mg% (31).
† Normal range, 418±70 mg/24 hours (9).
‡ Abbreviations: BUN = blood urea nitrogen; PSP = phenolsulfonephthalein.
§ W.Y. is considered a control subject because his serum uric acid level falls within the normal range and he has had no symptoms suggestive of gout. He is, however, a member of a gouty family, and his 24-hour uric acid excretion value (single sample) is elevated. In any event, his PRPP turnover data are normal and in no way resemble the results found in the overexcretor gouty subjects.

with perchloric acid, $KClO_4$ removed from the neutralized chilled filtrate, and the supernatant solution then passed through a mixed-bed resin,[7] according to Hiatt (21). The eluates and washings were pooled and reduced in volume. Aliquots were then analyzed for ribose according to Albaum and Umbreit (32) and for C^{14} in ethanol-toluene-PPO-POPOP (33) in a liquid scintillation spectrometer. Samples containing both C^{14} and H^3 were counted at the optimal voltage for each isotope to permit calculation of the radioactivity attributable to each individually (34). All samples were subsequently recounted by employing an internal C^{14} standard to correct slightly variable quenching effects of the ions in the column eluates.

A number of control studies were performed to evaluate the specificity of procedures employed to isolate the ribose moiety of IAAR. The specific activity values of ribose were 50 to 132 per cent higher after rechromatography of the IAAR fraction on Dowex 50-H^+ than in the initial eluate from Dowex-1-acetate column. The increments were comparable in control, gouty normal excretor and gouty overexcretor patients. The major additional ribose-containing compound in the Dowex-1-acetate column eluate was shown by spectral and paper chromatographic methods to be uridine, which was unlabeled in the 0- to 10-hour period after glucose U-C^{14} administration. Uridine was separated from IAAR on Dowex

that a lower enzyme/IAAR ratio had been employed, another aliquot was hydrolyzed with more enzyme.

[7] MB-3, Fisher Company.

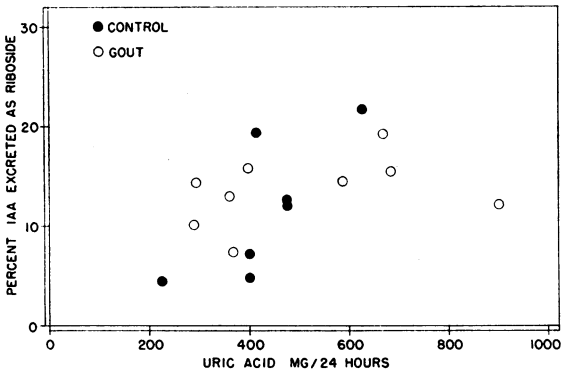

FIG. 1. EXCRETION OF IMIDAZOLEACETIC ACID RIBONUCLEOSIDE. The percentage of the test dose of imidazoleacetic acid excreted as the ribonucleoside derivative is plotted against the 24-hour urinary uric acid excretion for control and gouty subjects. The two values are not significantly correlated.

50-H^+, together with some labeled compounds, more acidic than IAAR, which were not further identified.

In a few instances the IAAR fraction obtained from Dowex 50-H^+ resin was reduced to dryness at 30° C in a flash evaporator, redissolved in water, and chromatographed on Whatman no. 1 paper by the descending method in isoamyl alcohol-phosphate buffer solvent (18). The IAAR was located by treating margin strips with periodate and benzidine (35). IAAR was eluted and hydrolyzed and the ribose recovered after passage through a mixed-bed resin. The specific activity of ribose was about 30 per cent higher after the additional paper chromatographic purification of IAAR, but this step was not employed routinely because of the losses of material encountered. The results reported in this paper are those obtained after hydrolysis of IAAR in the Dowex 50-H^+ eluate. A second point, that of the purity of ribose-C^{14} obtained from the mixed-bed resin column, was evaluated in selected samples by subsequent paper chromatography of ribose in butanol-acetic acid-H_2O (18). There was no change in the specific activity of ribose as a result of this additional procedure in the four samples studied and it also was not employed routinely. In paper chromatographic and electrophoretic work, IAA was located with diazotized p-nitroaniline (23) or diazotized sulfanilic acid (36), IAAR with periodate and benzidine (35), and ribose with aniline hydrogen phthalate (37).

RESULTS

Urinary excretion of imidazoleacetic acid ribonucleoside. The percentage of the test dose of IAA excreted as the ribonucleoside derivative in 10 hours in urine is listed in Table I and plotted for all subjects as a function of the 24-hour urinary uric acid excretion value in Figure 1. The values

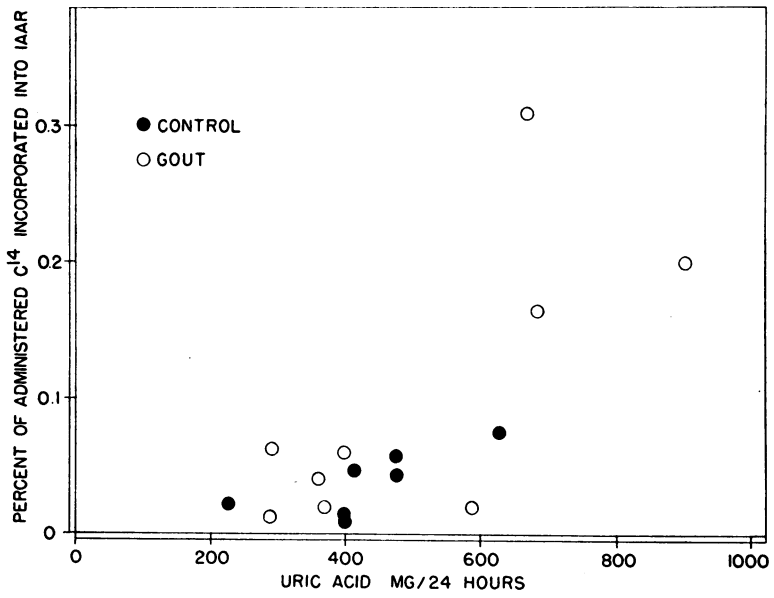

FIG. 2. INCORPORATION OF C^{14} INTO IMIDAZOLEACETIC ACID RIBONUCLEOSIDE. The percentage of the administered dose of glucose-U-C^{14} incorporated into imidazoleacetic acid ribonucleoside and excreted in urine in 10 hours is plotted against the urinary uric acid excretion. Note the three gouty subjects who show both excessive incorporation of C^{14} and excessive excretion of uric acid.

ranged from 4.4 to 21.6 per cent in seven control subjects (mean, 11.7 ± 6.8 per cent) and from 7.3 to 19.2 per cent in nine gouty subjects (mean, 13.5 ± 3.4 per cent). Among the gouty patients, the mean value of the six subjects with normal uric acid excretions was 12.4 per cent, and the mean value of the three subjects with excessive uric acid excretions was 15.6 per cent. None of these mean values is significantly different from the mean value of the control group. Furthermore, the positive correlation between the per cent of IAA excreted as IAAR and the 24-hour uric acid excretion in these 16 subjects was not statistically significant ($r = +0.465$, $p > 0.05$).

Incorporation of C^{14} into urinary IAAR. The percentage of the administered dose of glucose U-C^{14} appearing in urinary IAAR in 10 hours is listed in Table I and plotted in Figure 2 as a function of the uric acid excretion value of each subject. In the control subjects, incorporation values ranged from 0.010 to 0.074 per cent and in the gouty patients with normal uric acid excretions, from 0.012 to 0.062 per cent. In the three patients with clinical gout and excessive urinary uric acid excretion, the incorporation values were 0.164, 0.200, and 0.310 per cent. The values found in the gouty subjects with normal uric acid excre-

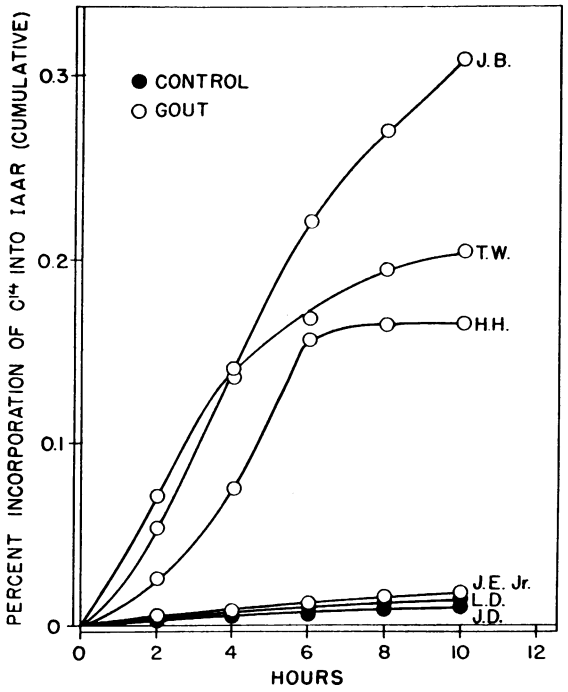

FIG. 3. CUMULATIVE INCORPORATION OF C^{14} INTO IMIDAZOLEACETIC ACID RIBONUCLEOSIDE. The cumulative incorporation of C^{14} into IAAR is presented for two control subjects, one gouty subject with a low normal value of uric acid excretion (J.E., Jr.), and three gouty subjects with excessive values of uric acid excretion (J.B., T.W., and H.H.).

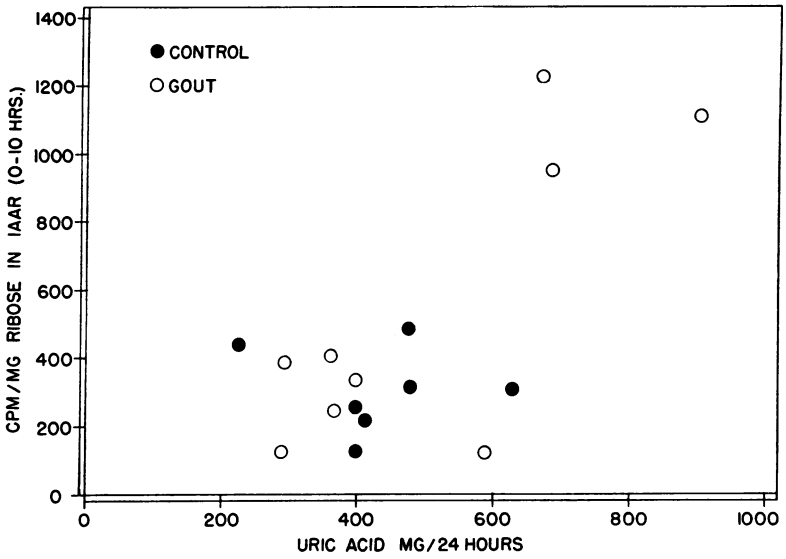

FIG. 4. SPECIFIC ACTIVITY OF THE RIBOSE MOIETY OF IMIDAZOLEACETIC ACID RIBONUCLEOSIDE. The specific activity value of the ribose moiety of IAAR during the 10-hour period after administration of IAA and glucose U-C^{14} is plotted against the uric acid excretion value of control and gouty subjects. Note the three gouty subjects who show both excessive specific activity values and excessive excretion of uric acid.

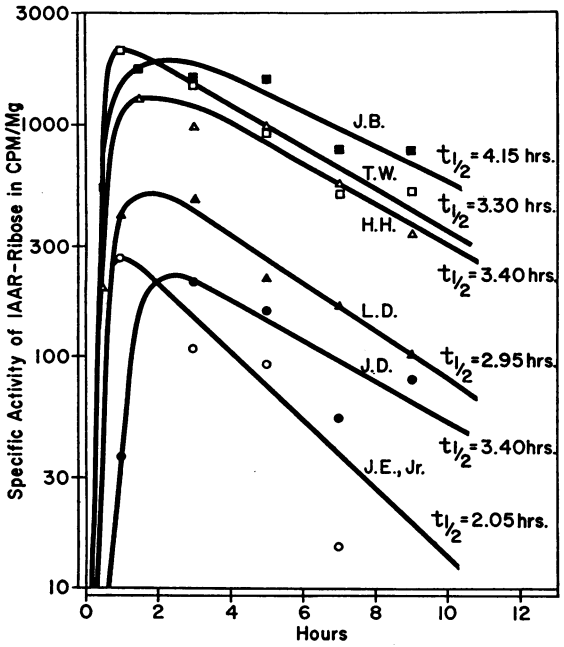

FIG. 5. TIME COURSE OF ENRICHMENT OF THE RIBOSE MOIETY OF IMIDAZOLEACETIC ACID RIBONUCLEOSIDE. The specific activity values of the ribose moiety of IAAR, in successive 2-hour urine samples of control and gouty subjects described in the legend of Figure 3, are plotted on semilogarithmic coordinates. The specific activity decay curves have been drawn and the half-times calculated, chiefly to show that the rates of decline of specific activity values are similar in the overexcretor gouty subjects (J.B., T.W., and H.H.) and the controls (L.D. and J.D.).

tions fall entirely within the control range and the mean values are very similar: controls, 0.038 per cent; gouty subjects with normal urate excretion, 0.035 per cent. In contrast, the incorporation values found in the overexcretor group of gouty patients are clearly elevated and well outside the control range.

The cumulative incorporation of C^{14} into IAAR was followed in consecutive 2-hour urine samples in two control and four gouty subjects. The gouty subjects included one with normal and three with excessive urinary uric acid excretion. The results are shown in Figure 3. The excessive incorporation of C^{14} into IAAR in the three overexcretor gouty subjects was marked by an acceleration of initial rate of isotope incorporation, most prominent in the first 4 to 6 hours after administration of glucose C^{14} and IAA.

Specific activity of the ribose moiety of IAAR. The mean specific activity of ribose in IAAR during the 10-hour experimental period is shown for all subjects in Table I and is plotted as a function of the 24-hour uric acid excretion in Figure 4. Specific activity values in the seven control subjects ranged from 128 to 484 (mean 305) cpm per mg of ribose; in the 6 gouty subjects with normal uric acid excretion the values ranged from 120 to 402 (mean 268) cpm per mg of ribose. There was no correlation of specific activity values with uric acid excretion in either group. In contrast, the three gouty patients with excessive uric acid excretion values showed strikingly elevated specific activity values, in keeping with the excessive incorporation of C^{14} reported above. The values were 950, 1,104, and 1,120 cpm per mg of ribose.

Specific activity values were obtained in consecutive 2-hour samples of the two control subjects and four gouty patients whose cumulative incorporation data were presented above. These data are shown in Figure 5. Peak enrichment values were attained during the first two hours in the gouty patients and in the two- to four-hour sample in both controls. The maximal value attained in the three overexcretor gouty subjects exceeded those found in control subjects and in the normal excretor gouty subjects by three-fold. However, the half-times of rates of decline in

TABLE II

Repetition of study in gouty overexcretor subject, J.B.

Date of study	Uric acid		Excretion of IAAR— Per cent of administered IAA	C^{14} in IAAR— Per cent of administered glucose-U-C^{14}	Specific activity of IAAR
	Serum	Urine			
	mg%	*mg/24 hrs*			*cpm/mg ribose*
2-29-60	10.8	670	19.2	0.310	1220
4-24-61	2.9*		30.5	0.270	1209

* Second study conducted without interruption of uricosuric drug; see text.

specific activity values were comparable in all subjects. Even though the data are insufficient to permit determination of precise half-time values (Figure 5), they were clearly not shorter in the overexcretor gouty group than in the control subjects.

Confirmatory study on gouty overexcretor subject. As a methodologic and biologic check of the abnormal results obtained in the overexcretor group of gouty subjects, the study on J.B., a 45-year-old man with severe tophaceous gout, was repeated after a 14-month interval. At the time of the first study he was receiving no drug treatment; at the time of the second he was receiving daily colchicine and sulfinpyrazone and had a serum uric acid value of 2.9 mg per 100 ml. The results of the two studies are shown in Table II and are taken to be in satisfactory agreement. Furthermore, in the second study rechromatography of IAAR on paper after elution from Dowex 50-H^+ resin (see Methods) demonstrated conclusively that the highly enriched ribose was in IAAR, and not some other ribose-containing compound. These results also indicate that the turnover of PRPP is not regulated by the serum level of uric acid, nor affected to any major extent by the drugs employed.

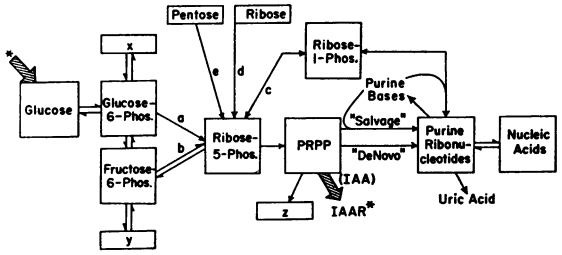

FIG. 6. MODEL OF HYPOTHETICAL METABOLIC POOLS AND KNOWN BIOCHEMICAL REACTIONS INFLUENCING ISOTOPIC LABELING OF RIBOSE IN THE PRESENT STUDY. The glucose pool was labeled by oral administration of glucose-U-C^{14}. The PRPP pool was sampled by oral administration of imidazoleacetic acid, which reacts with PRPP to form the corresponding ribonucleotide and is excreted in urine as imidazoleacetic acid ribonucleoside. Pathways *a*, *b*, *c*, *d*, and *e* represent routes of synthesis of ribose 5-phosphate (see text). Pools *x* and *y* represent other metabolic pools in equilibrium with glucose 6-phosphate and fructose 6-phosphate respectively; pool *z* represents all non-purine ribonucleotide pools to which PRPP may contribute, some of which may also be sources, ultimately, of ribose 1-phosphate.

DISCUSSION

The present study demonstrates that gouty subjects who excrete excessive quantities of uric acid in urine also convert a greater fraction of an administered dose of glucose-U-C^{14} to phosphoribosylpyrophosphate (PRPP) and attain a greater specific activity in PRPP than do control subjects or gouty subjects with normal uric acid excretion values. The data also suggest that gouty overexcretors achieve peak specific activity values in PRPP somewhat earlier than control subjects, but the rates of decline of specific activity values following the maxima are not more rapid, nor are the quantities of imidazoleacetic acid ribonucleoside formed and excreted larger than in control subjects.

The greater specific activity of PRPP signifies that the administered glucose-C^{14} has undergone less dilution with unlabeled substances in gouty overexcretors than in the other subjects. This could theoretically result from *1*) poor mixing of glucose C^{14} within the glucose pool, *2*) an unusually small pool of glucose, ribose 5-phosphate, or of any intermediate compound, or *3*) an increase in the fraction of PRPP derived from glucose in comparison with the fraction derived from other less highly labeled precursors.

The reaction model shown in Figure 6 has been constructed on the basis of known biochemical reactions of pertinence to the present study. It should be recalled that gouty overexcretors regularly show enhanced incorporation of labeled precursors into uric acid, whether the precursor is ammonium-N^{15} (12), glycine-N^{15} (5) or C^{14} (6, 7, 11, 30), formate-C^{14} (8), or 5-amino-4-imidazole-carboxamide-C^{14} (38). Thus, an increased activity of purine synthesis de novo is well established in this type of gouty subject.

The increased utilization of all reactants of an unbranched biosynthetic sequence is a necessary corollary of the increased utilization of any one, if the laws of stoichiometry are not to be violated. Hence, an increased utilization of PRPP would be anticipated if PRPP is indeed the sole precursor of phosphoribosylamine, the obligatory antecedent of purine ribonucleotides. This study was designed to test this point and, if possible, to assess the compensatory participation of alternative path-

ways for synthesis of ribose 5-phosphate, the immediate precursor of PRPP.

As shown in Figure 6, ribose 5-phosphate has five known pathways of synthesis: *a* represents the phosphogluconic acid oxidation pathway by which glucose 6-phosphate is irreversibly converted to ribose 5-phosphate; *b* represents the fructose 6-phosphate cleavage pathway via which fructose 6-phosphate and ribose 5-phosphate may be interconverted; *c* represents the phosphoribomutase reaction whereby ribose-1-phosphate, released from ribonucleosides by action of purine and pyrimidine nucleoside phosphorylases, is reversibly converted to ribose 5-phosphate; *d* represents the ribokinase reaction whereby free ribose, released by action of phosphatases on ribose phosphates, is phosphorylated to form ribose 5-phosphate; *e* represents a number of potential pathways, probably of trivial quantitative importance under normal circumstances, by which other pentoses may be converted to D-ribulose 5-phosphate and thence to ribose 5-phosphate (39).

Increased utilization of PRPP in purine synthesis will lead to an increased rate of renewal of PRPP from ribose 5-phosphate and subsequently of ribose 5-phosphate via one or more of its biosynthetic pathways. If the glucose 6-phosphate pool is preferentially labeled by administration of glucose C^{14}, the peak specific activity of ribose 5-phosphate will reflect the quanta of ribose 5-phosphate arising via *a* with respect to $a + b + c + d + e$, or if glucose 6-phosphate and fructose 6-phosphate rapidly equilibrate, then the specific activity of ribose 5-phosphate will reflect the quanta of ribose 5-phosphate arising via $a + b$ with respect to $a + b + c + d + e$. Pathways *c* and *d* cannot contribute isotope to ribose 5-phosphate unless ribose 5-phosphate first becomes labeled via *a* or *b* and is then hydrolyzed to free ribose, or is reversibly converted to ribose 1-phosphate via phosphoribomutase, or converted to ribose 1-phosphate via PRPP, and ribonucleotide and ribonucleoside intermediates.

The results obtained in the gouty overexcretor patients are best explained as consequences of increases in the rate of operation of the pathway of synthesis of purine ribonucleotides de novo, with an increase, pari passu, in the rates of synthesis of PRPP, and of ribose 5-phosphate, from glucose 6-phosphate, via either the phosphogluconic acid oxidation pathway or the fructose 6-phosphate cleavage pathway, or both. The data exclude at least one alternative consideration: a simple reduction of pool size of ribose 5-phosphate would give rise to a higher than normal peak specific activity value but would also result in an accelerated rate of decline of specific activity values. Poor mixing of glucose C^{14} within the glucose pool, or a small mixing pool of any intermediate prior to ribose 5-phosphate, is unlikely to have occurred only in the three gouty hyperexcretor patients, and in one of them twice at a 14-month interval, but it cannot rigorously be excluded. Nor can a disproportion of relative contributions of pathways *a* to *e* (e.g., a markedly reduced contribution of *c*) be excluded as a theoretical cause of increased labeling of ribose 5-phosphate, but such perturbations appear rather implausible.

The accelerated synthesis of PRPP from glucose, set forth as the best interpretation of the data, is consistent with a number of possible mechanisms for the metabolic aberration of gout and is indeed a necessary consequence of any of them. These possibilities include: *1*) a defect of nitrogen metabolism by which an increased quantity of one or more amino acids is shunted into purine synthesis (12, 40, 41); *2*) a disturbance of the feedback control mechanism governing activity of PRPP-amidotransferase leading to a primary excess of synthesis of purines; *3*) a catabolic defect causing a persistent "leak" of inosinic acid, for example, toward purine bases and uric acid, and a secondary increase in purine synthesis; or *4*) an increase in turnover of a metabolically active nucleic acid component. A primary increase in activity of the phosphogluconic acid oxidation pathway with resultant oversynthesis of PRPP might also be entertained, but such a defect, if responsible for the abnormalities of PRPP labeling reported herein, would have been anticipated to yield concomitant increases in the quantities of imidazoleacetic acid riboside in urine, and such were not found.

The arguments presented above rest upon the assumption that the pool of PRPP sampled in synthesis of imidazoleacetic acid ribonucleotide is identical with, or in rapid equilibrium with, the pool of PRPP available to PRPP-amidotransferase in the synthesis of phosphoribosylamine. Since an accelerated turnover of PRPP was dem-

onstrated in types of patients presenting abundant independent evidence for overproduction of purines de novo, this assumption appears to be a reasonable one. It is anticipated that the enhanced labeling of PRPP would also be reflected in other products synthesized from PRPP, e.g., diphosphopyridine nucleotide (42), orotidylic acid, the first ribonucleotide intermediate of pyrimidine synthesis (43), and purine ribonucleotides formed by direct condensation of purine bases released by degradative reactions with PRPP (44). All of these reaction sequences proceed actively in liver and might also give information on the turnover of PRPP if they derived their ribose moieties from the same intracellular pools of PRPP and if they could be sampled simultaneously with IAAR. As pointed out above, urinary uridine was unlabeled in the urine sample obtained 0 to 10 hours after administration of glucose C^{14} and therefore presumably represents uridine synthesized at an earlier time, perhaps arising from ribonucleic acid catabolism. Thus, no information of the degree to which the increased labeling of PRPP observed in overexcretor gouty subjects was reflected in other ribose containing compounds was obtained.

Since PRPP is involved in a number of reactions, a question arises whether a block in one sequence might provide an excess of PRPP for utilization in other sequences and whether overproduction of purines might arise as one consequence of such a block (45). Although this might be a theoretical possibility, a simple reapportionment of PRPP among its various fates would not lead to an increase of PRPP labeling and hence seems excluded as a consideration in the gouty overexcretor subject.

The normal labeling of PRPP in the group of gouty patients with normal uric acid excretion values again raises the question of heterogeneity of the gouty defect. It seems unlikely from the data of Figure 2 and Figure 4 that PRPP turnover (and by implication uric acid production) is a continuous variable in the gouty population; despite the limited number of patients studied, the two groups give every appearance of being discontinuous. The normal specific activity values of PRPP in this group, in whom other techniques of study frequently show abnormalities of isotope incorporation or distribution in uric acid, have several possible interpretations. *1*) The study of PRPP turnover may be inherently too gross at the technical or biological level to reveal minor differences which might nevertheless be of metabolic importance. *2*) PRPP turnover may actually be accelerated in this group, but the resultant increase in synthesis of the precursor, ribose 5-phosphate, may involve pathways *a, b, c, d,* and *e* in a balanced way so that the fraction of ribose 5-phosphate arising from glucose 6-phosphate is unchanged, and hence the specific activity of ribose 5-phosphate and PRPP is unchanged. *3*) Purine production may not be accelerated in these particular patients, and renal factors may be responsible for hyperuricemia. Independent incorporation studies were not done in this group; in only one patient (J.E., Jr.) were clearance studies performed, and low urate/inulin clearance ratios were found in him (*29*). Recent studies in other laboratories have refocused attention on the role of the kidney in the hyperuricemia of gout (46). There is evidence that both overproduction and underexcretion of uric acid may be involved (47) and that these mechanisms are not mutually exclusive but may operate in concert in certain patients.

SUMMARY

1. The turnover of 5 α-phosphoribosyl-l-pyrophosphate (PRPP), a key early intermediate in purine synthesis, has been studied in control and gouty subjects by an indirect approach involving administration of glucose-U-C^{14} to label the ribose moiety of PRPP and imidazoleacetic acid to sample PRPP in the formation of its ribonucleotide derivative. Urinary imidazoleacetic acid ribonucleoside (IAAR) was isolated and studied.

2. The incorporation of C^{14} into IAAR and the specific activity of IAAR were three times greater in three gouty subjects who overexcreted uric acid than in control subjects or in gouty subjects with normal urinary excretions of uric acid.

3. These results indicate that the turnover of PRPP is accelerated in the type of gouty patient in whom other studies give independent evidence for overproduction of uric acid. They further suggest that the accelerated turnover of PRPP is accompanied by an increase in rate of renewal of PRPP from glucose 6-phosphate.

4. Normal isotopic labeling of IAAR was found in gouty persons with normal uric acid excretion,

and this may signify that PRPP turnover is not accelerated in these patients or that this method of study is inadequate to detect increases of turnover of subtle degree.

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