DONALD L. BUCHANAN*
JANE M. ROLLINS**

Radioisotope Service, V. A. Hospital, West Haven, Connecticut and Department of Biochemistry, Yale University School of Medicine

LACK OF CORRELATION BETWEEN GOUT AND THE INCORPORATION OF ISOTOPIC FORMATE INTO URIC ACID

Certain reports[1-4] have indicated that patients with gout incorporate the isotopic precursors of the purine ring into urinary uric acid to a greater extent than do non-gouty individuals. The possibility of utilizing this difference as a diagnostic test for gout has been explored. Carbon-14 labeled sodium formate was given orally or intravenously to 10 patients known to have gout, to 3 with hyperuricemia but no gouty symptoms, and to 16 non-gouty subjects without elevated serum uric acid levels. Three patients who had received oral formate were also given intravenous doses after the urinary uric acid specific radioactivity had become negligible. No correlation between the incorporation of isotope into urinary uric acid and the presence of gout or hyperuricemia was noted.

METHODS

For oral administration doses were prepared that contained 10μc. (0.6 mg.) of sodium formate-C^{14} mixed with 400 mg. of lactose. Enough labeled sodium formate for several doses was dissolved in a small quantity of water, added to dry lactose, and, after drying in a vacuum oven, the mixture was ground in a mortar and weighed into gelatin capsules on an analytical balance. The specific radioactivities of the mixture were found to be constant between capsules, and the radioactivity was shown to be virtually all formate by specific oxidation with mercuric ion.[5] For intravenous administration sodium formate-C^{14} was dissolved in a vial of sterile physiological saline to a concentration of 10μc. (0.6 mg.) in 3 ml. and autoclaved.

For experiments with oral dosage hospital patients who had just voided were given a capsule at 8:00 a.m. Breakfast was withheld until 10:00 a.m. and urine was collected until 4:00 p.m. The intravenous experiments were identical except that the dose contained no lactose.

After removal of a 5 ml. aliquot from the eight-hour urine specimen for uric acid determination the bulk of the remaining uric acid was precipitated as ammonium urate.[6] After the crude precipitate had settled it was concentrated by centrifugation, converted to uric acid with 3 ml. of 6 N hydrochloric acid, and washed with 3 ml. of

*Chief of Radioisotope Service, V.A. Hospital, and Associate Professor of Biochemistry, Yale University School of Medicine.
**Radioisotope Service, V.A. Hospital.
Received for publication 18 April 1961.

TABLE 1. INCORPORATION INTO URINARY URIC ACID OF ISOTOPIC FORMATE GIVEN ORALLY

Patient	Diagnoses	Serum uric acid		Excretion in 8 hrs.	Uric acid specific radioactivity	Total excreted radioactivity in uric acid
		Uricase mg. %	Folin mg. %	mg.	cts m/mg.	cts
CONTROLS						
A.B.	Intra-atrial septal defect	6.9	4.2	140	177	24,800
E.C.	Traumatic myositis	4.2	3.7 5.0	159	156	24,800
M.G.	Chronic lymphatic leukemia	5.2	3.7 5.0, 4.5, 4.6	194	113	21,900
M.B.	Essential hypertension	4.8	4.5 1.6, 1.5, 4.8 6.5, 5.1, 5.3, 4.7, 7.0	128	110	14,100
T.R.	Urticaria	—	—	190	72	13,700
T.C.	Essential hypertension, myocardial infarct	6.2 7.6	5.8 6.5	57	177	11,200
R.A.	Chronic lymphatic leukemia	5.3	4.9 4.6	162	44	7,130

GOUTS						
S.F.	Hyperuricemia, nephrolithiasis, chronic bronchitis No arthritis	5.5	5.5 7.7, 8.5, 8.3 6.0, 6.5	209	93	19,400
W.S.	Gout	5.8	5.7 7.7, 7.6, 20.0 11.2, 11.6, 10.8, 5.4, 8.0, 8.0 8.5, 8.0	131	105	13,800
G.C.	Gout, diabetes, sarcoma	7.4	8.2 7.0, 6.0, 6.1 6.8, 5.3	170	76	12,900
M.S.	Pylolithiasis, urate stone, no arthritis Hyperuricemia?	7.8	7.0 5.1, 4.5, 4.9 7.0, 7.3	204	61	12,200
S.M.	Gout, tophi	10.6	8.5 8.0, 9.5, 5.5 12.4, 10.8	182	52	9,460
L.K.	Gout, Glomer-ulonephritis	10.3	6.3 10.9, 7.2, 7.8, 5.2, 8.5, 7.6, 5.8, 6.3	206	27	5,360

water. It was then dissolved in a minimal quantity of N lithium hydroxide. After centrifuging to remove insoluble impurities, the supernatant was filtered by suction through a 0.5 by 5 cm. column of a half and half mixture of cellulose and charcoal. The column was poured on 2 cm. of pure cellulose to prevent the escape of charcoal into the filtrate. Uric acid was finally precipitated by acidification of the filtrate with glacial acetic acid. The centrifuge tube containing the sample was placed in boiling water for five minutes to convert a very flocculent precipitate to granular crystals. The product was twice recrystallized by solution in lithium hydroxide and precipitated with acetic acid. The purified uric acid was washed twice each in water, alcohol, and ether, and dried in a vacuum oven. The specific radioactivity of the recrystallized material was identical to that first precipitated with acetic acid. A high state of purity was confirmed spectrophotometrically.

Determination of the specific radioactivity was performed by proportional gas counting after sealed tube combustion.[7] Uric acid determinations of urine and some of serum were performed by the photometric uricase method of Kalckar[8] as modified by Praetorius.[9] Prepared tubes of buffered uricase* were used for most determinations. Serum levels were also performed by the hospital clinical chemistry laboratory with the Folin colorimetric method.[10]

The diagnosis of gout was established by history and the presence of arthritis typical of gout. It was confirmed in all but one instance by at least one serum uric acid level above 8 mg. per cent. The exception (A.C. Table 2) had a ten-year history of attacks of acute arthritis typical of gout and relieved by colchicine. Two gout patients had tophi. Three patients had elevated serum uric acid levels but no arthritis. Two of these had nephrolithiasis and the highest serum uric acid of one (M.S., Table 1) was 7.8 mg.%. Criteria for the absence of gout in the control series were: failure to elicit a history of arthritis resembling gout. absence of physical signs, and serum uric acid levels below 8 mg.

RESULTS

Table 1 gives the data on the patients who received oral doses and Table 2 gives the data on those getting labeled formate intravenously. The two patients with hyperuricemia and the one with questionable hyperuricemia and urate stone are listed with the gouts. The specific radioactivities of the uric acid isolated from the oral dose group were considerably higher than those in the intravenous group. Serum uric acid levels are given for each patient. The values first listed for each patient in Table 1 are those obtained from blood taken on the day of the study, and where the uricase method was employed, the Folin result given on the same line was obtained on the same serum. In general the uricase levels were slightly higher. The other values (all Folin) were obtained on various other occasions during the patients' hospitalizations. Uricase determinations were not performed on the sera of most of the patients of Table 2 and those few that were done

*Determatube-U, Worthington Biochemical Corporation, Freehold, New Jersey.

TABLE 2. INCORPORATION INTO URINARY URIC ACID OF ISOTOPIC FORMATE GIVEN INTRAVENOUSLY

Patient	*Diagnoses*	*Serum uric acid mg. %*	*Excretion in 8 hrs. mg.*	*Uric acid specific radioactivity cts/m/mg.*	*Total excreted radioactivity in uric acid cts*
CONTROLS					
W.M.	Infectious mononucleosis	4.5, 4.8	335	57	19,100
J.Z.	Duodenal ulcer	6.3	354	40	14,200
J.D.	Hemorrhagic cyst of thyroid	4.4, 3.9	272	51	13,900
E.C.	Traumatic myositis	3.7, 5.0, 4.2	194	48	9,310
M.G.	Chronic lymphatic leukemia	3.7, 5.0, 4.5 4.6, 5.2	182	33	6,000
A.Q.	Contact dermatitis	6.2	236	25	5,900
M.T.	Undiagnosed renal disease, elevated BUN	6.3, 6.0, 4.2 4.8, 4.6	264	20	5,280
V.T.	Duodenal ulcer	6.0, 4.3, 6.9	87	41	3,570
M.H.	Hypertensive cardiovascular disease, cerebrovascular accident	3.8	160	21	3,360
GOUTS					
G.O.	Gout	12.5, 7.1, 9.0 7.5, 8.0, 7.5, 6.8, 6.8, 8.5, 11.0, 11.3	268	32	8,580
G.C.	Gout, diabetes sarcoma	8.2, 7.0, 6.0 6.1, 6.8, 6.3, 7.4	211	39	8,230
A.K.	Gout, arteriosclerotic and rheumatic heart disease	9.0,6.1	147	51	7,500
F.F.	Gout, tophi	7.0, 7.3, 4.6. 5.3, 7.4, 6.5, 10.5, 6.5, 7.0	298	25.0	7,450
F.L.	Hyperuricemia, senile cataract, No arthritis	11.0, 9.3, 9.3	—	18.8	——
A.C.	Gout, chronic cellulitis	6.5, 4.7	240	18.2	4,370
J.F.	Gout, cerebrovascular accident	12.5, 5.8	—	11.0	——
J.G.	Gout, hypertensive cardiovascular disease	5.5, 9.2	268	6.85	1,840

are grouped with the results from the clinical chemistry laboratory. The uric acid content of each eight-hour urine is listed in each table.

In the oral dose series five non-gouty subjects showed a higher specific radioactivity of uric acid than any gouty subject, and the patient with the lowest value had gout. There was a four-fold spread of values among the non-gouty patients as well as among the gouty patients. When the total

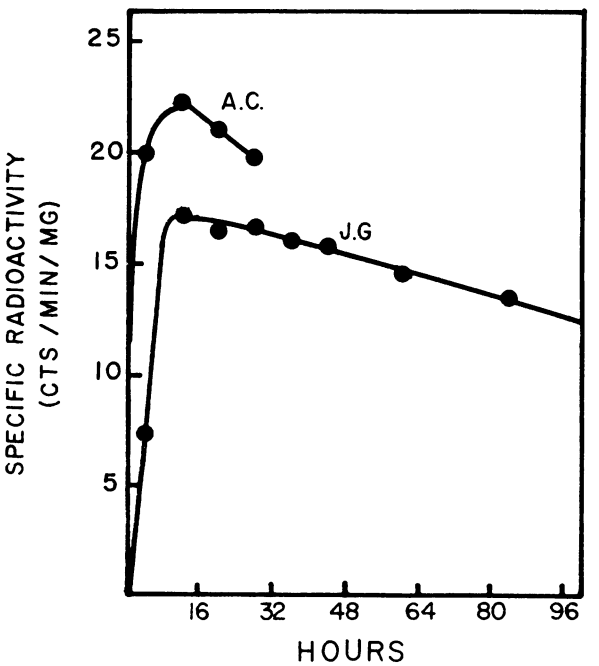

FIG. 1. Specific radioactivity of urinary uric acid from two patients with gout who received 10 μc. of formate-C^{14} intravenously.

counts in excreted uric acid were computed, the three highest values fell in the control group, and again the patient with the lowest value had gout.

The correlation between uric acid specific radioactivity and gout was equally as poor in the patients who received the labeled formate by vein. When the data were computed on the basis of total counts in excreted uric acid, the correlation was no better. Again the patient with the lowest incorporation by either method of computation had gout. Because these studies covered only an eight-hour period, urine from this patient (J. G.) and one other (A. C.) was collected over a longer period. The results are shown in Figure 1. In both patients the peak specific radioactivity occurred

in the sample collected between 8 and 16 hours but in neither did it rise to the level seen in the majority of the non-gouty control patients.

DISCUSSION

The work of Benedict *et al.*,[1,2] who administered N^{15} glycine, seemed to indicate that at least some patients with gout incorporate greater quantities of isotope into uric acid than do normal subjects. However, two of the four gouty subjects studied could not be distinguished from the two normal controls. Wyngaarden[4] postulated that the large chemical dose of glycine used might have masked the difference in incorporation, and with tracer doses of C^{14} glycine he found a high correlation between the extent of incorporation and gout. Each of seven gouty patients (one of these with asymptomatic hyperuricemia) incorporated more isotope into urinary uric acid than did three control subjects. Spillman[8] gave C^{14} formate to two patients with gout and two normal controls. The gouty patients had a six-fold greater peak specific radioactivity in the urinary uric acid than did the controls. He also found, as had Wyngaarden, that the peak specific radioactivity occurred much sooner in the patients with gout. These findings led us to believe that a short-term collection of urine would be more useful diagnostically than a longer time study with multiple samples. It would certainly be more convenient.

A subsequent report by Wyngaarden[11] includes data on two gouty subjects that did not incorporate more isotopic glycine than did the controls, and an accompanying paper by Seegmiller *et al.*[12] reported that two subjects without gout had a higher degree of incorporation than previously reported control subjects.

Other laboratories have reported similar studies which involved only one or two patients with one control in each study. In both experiments with a single gouty patient[13,14] the degree of incorporation was indistinguishable from that of the control. In the study involving two gouty subjects[15] the peak isotope content of uric acid in the control fell between those of the gouty patients.

In all of the reports that we have seen, 26 gouty patients (including asymptomatic hyperuricemia) and 14 control subjects have been studied. Because different compounds and somewhat different techniques were employed, it is difficult to reduce all data to a common basis for comparison. However, only 14 of the gouty subjects incorporated significantly more isotope than the control subjects of the same study.

Our study is in essential agreement with this composite picture except that we found the highest incorporation in patients of the control group.

When the data are considered in the light of information already available, it appears that there is great variability in the extent of incorporation of isotopic precursors into urinary uric acid in gouty as well as in non-gouty subjects. It would appear that a very extensive study and careful statistical analysis would be necessary if any significant difference between gouts and normals were to be uncovered by this type of study.

It is beyond the scope of this paper to review the evidence that gout is related to the overproduction of uric acid by a "shunt" mechanism,[16] but the results of isotopic studies have been given as the chief evidence to support this hypothesis. If all the studies cited are considered, the evidence in support of the overproduction theory seems weak indeed, and it is possible that the results of individual studies that have favored the overproduction theory occurred only fortuitously. The view that the hyperuricemia of gout may be related to a defect in the renal excretion of uric acid has been considerably strengthened by a report by Nugent and Tyler.[17] These workers pointed out that in earlier clearance studies serum levels of uric acid were not at the same levels in the gouty patients and in the controls. When they maintained elevated levels in the control subjects by dietary supplementation with ribose nucleic acid, the uric acid clearance rates were much higher in the non-gouty subjects than in those with the disorder. Latham and Rodnan[18] have very recently confirmed these findings by infusing lithium or sodium urate.

SUMMARY

Isotopically labeled formate was administered orally or intravenously to patients with gout or asymptomatic hyperuricemia and to control patients. Urinary uric acid was isolated from urine voided during the eight-hour period following the dose. No correlation between the presence of the disease and the specific radioactivity of the uric acid was found.

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