Isotopically labeled uric acid has been injected intravenously into normal subjects and subjects with various diseases in order to determine the pool size and turnover rate of body uric acid. Such studies have been carried out both in this laboratory and in other laboratories (1, 2). It is the purpose of this communication to examine some of the assumptions used in these calculations and to present data which show that these assumptions are not always valid. The disappearance curves of urinary isotopic uric acid for several subjects and several therapeutic regimens will be discussed.

EXPERIMENTAL SUBJECTS

Normal controls with normal or elevated serum urate concentrations and patients with recognized clinical maladies served as the subjects. The patients were suffering from gout, acute gouty arthritis, rheumatoid arthritis, polycythemia vera or chronic myelogenous leukemia. The normal and hyperuricemic subjects were ambulatory. With but one exception all the other patients were hospitalized, the majority in a metabolism ward. In most instances the experimental subjects were on a constant diet of low purine content which was served from the diet kitchen. The fluid intake was maintained relatively constant but not precisely so. An abstract of the clinical history and physical examination of each subject is given in the protocols.

CHEMICAL PROCEDURES

The isotopically labeled uric acid employed in these studies was synthesized by the method of Cavalleri, Blair, and Brown (3). It contained N\(^14\) in the 1 and 3 positions and had an overall isotope concentration of approximately 16 atom per cent excess. In preparing a sample of the isotopic uric acid for intravenous injection, a quantity (25-100 mg.) of the purified uric acid was rinsed off a tared weighing glass with absolute alcohol into a previously autoclaved wide mouth glass jar. A few drops of alcoholic phenol red indicator were added. The jar was placed in an oven at approximately 70\(^\circ\) C. until the alcohol had evaporated, and a previously autoclaved plastic top was screwed onto the jar. At the time of the injection the uric acid was dissolved in 25-50 ml. of sterile (autoclaved) sodium hydroxide. Concentrated hydrochloric acid followed by dilute hydrochloric acid was added dropwise with stirring until the yellow color of the indicator appeared. The solution was immediately injected intravenously and the syringe rinsed several times through a three way stopcock with small volumes of isotonic saline solution. The neutral solution of uric acid was not allowed to stand more than five to ten minutes because of the tendency for uric acid to precipitate.

The sodium hydroxide solution was prepared in such a concentration that when neutralized with hydrochloric acid the resulting solution was isotonic in sodium chloride. Uric acid solutions prepared in this manner were found to be sterile and quantitatively accurate. No untoward clinical effects have thus far resulted from the injection of such a solution.

Urine was collected in four or six hour periods or at each voiding (ad libitum) for the first and second day of each experiment. Thereafter several samples were combined before processing.

Uric acid was isolated from fresh urine by precipitation with ammonium chloride and ammonium hydroxide (4). The precipitated ammonium urate was washed several times with ammonium sulfate-ammonium hydroxide wash solution and the drained precipitate was dissolved in concentrated sulfuric acid, the container being kept cool by immersion in a dry ice slurry. Distilled water was added and the uric acid that precipitated was centrifuged down. After the supernatant liquid was decanted, the uric acid was dissolved in sodium hydroxide. Norite-A was added, and the tube was chilled in the cold bath. The sodium urate solution was filtered through Whatman No. 3 paper by gravity. The filtrate was acidified with concentrated hydrochloric acid and the reprecipitated uric acid was centrifuged down. These processes were repeated until a white product was obtained. The uric acid was finally suspended several times in absolute alcohol and filtered by suction on Whatman No. 50 paper. Aliquots
of the purified uric acid samples were converted to ammonia by the Kjeldahl procedure according to the modification of Hiller, Plazin, and Van Slyke (5) and the ammonia converted to nitrogen by the hypobromite method of Sprinson and Rittenberg (6). The determinations of N\textsuperscript{15} were made on a Consolidated-Nier Isotope-Ratio Mass Spectrometer (Model 21-201).

The nitrogen content and the extinction coefficient at 290 m\AA of random samples of the isolated uric acid checked similar values of carefully purified reagent uric acid.

**EXPERIMENTAL DRUGS**

The three anti-rheumatic drugs used in these studies were colchicine, cortisone (Kendall’s Compound E) and ACTH (adrenocorticotropic hormone). The general procedure was to inject isotopic uric acid and collect urine samples for approximately one week. A drug was then administered for a period not exceeding three days. Isotopic uric acid was again injected.

Colchicine was given in hourly oral doses of 0.5 mg. until gastrointestinal distress developed. Cortisone or ACTH was administered twice daily in 50 mg. intramuscular injections. In some subjects the administration of cortisone or ACTH was continued through the experimental period. Any alteration in the size of the metabolic pool or velocity of turnover in the experiments following drug therapy must be interpreted in relation to acute experiments and does not presume to predict what might have happened if the drugs had been continued for a longer period of time and the pool determined at a later portion of the experiment. The experimental data from some of the control phases of the therapeutic periods were discarded because of technical discrepancies.

**THEORY AND ASSUMPTIONS**

In order to develop the simplest case concerning the behavior of injected isotopic uric acid, the assumptions enumerated below will be made tentatively. It is quite probable that none of the assumptions will be completely justified subsequently by experimental data but if the mathematical relationship finally derived tends to coincide with the observed findings, the assumptions will acquire some validity. Let us assume that:

1. All the uric acid in the body is freely diffusible and at the same concentration. This total amount of uric acid will be identified as the *body pool* of uric acid.
2. The size of this body pool (in milligrams or in millimols of uric acid) is homeostatically fixed (constant). This is equivalent to saying that the rates of origin and disposal are equal.
3. Intravenously injected, isotopically labeled uric acid mixes completely and immediately with all the uric acid in the body pool.
4. The molecules of isotopic uric acid are indistinguishable from the naturally occurring molecules insofar as metabolic processes or excretion are concerned.

(5) The uric acid synthesized \(^\text{8}\) by the body contains no N\textsuperscript{15} in excess of normal abundance. No catabolic fragments of enriched uric acid are reused for uric acid synthesis. Furthermore, no isotopic uric acid escapes from the body pool and re-enters this body pool at a later time after the isotopic concentration of the pool has changed.

(6) The concentration of isotope in the uric acid collected in the urine during a particular period is the same as the concentration of isotope in the uric acid of the body pool during that period.

(7) Uric acid is excreted in the urine at a constant rate. If this is not correct, then urine must be collected in short intervals. Otherwise uric acid collected during part of an interval will be diluted during the rest of the interval with a greater or lesser amount of uric acid of a different isotope concentration. To be precisely evaluated this would require the integration of the excretion curve. In practice, the excretion is assumed to be sufficiently constant, and the isotope concentration of an interval is placed at the midpoint of the interval. Of course, if uric acid excretion were constant only on a 24 hour basis, choosing the intervals as 24 hours might tend to smooth out the diurnal variations.

If all these assumptions are made then the equation describing the fall of the isotope concentration of urinary uric acid with time will be:

\[
-\frac{dI}{dt} = kI.
\]

This is because the probability of excreting isotopic molecules is directly proportional to their concentration in the mixture of isotopic and nonisotopic uric acid. As more isotopic molecules are removed, however, they are replaced by nonisotopic molecules (from synthetic processes) and the probability of withdrawing labeled molecules is decreased.

The differential equation integrates to the following:

\[
\ln I = kt + c.
\]

Thus when the logarithm of the isotope concentration of the urinary uric acid is plotted against time, a straight line should result.

By rearrangement of the fundamental differential equation it can be seen that:

\[
k = -\frac{dI}{dt}.
\]

In other words, k is the rate of change with respect to time of the isotope concentration, in terms of the prevailing isotope concentration. This is identified as the turnover rate constant and has the dimensions, reciprocal days. In the present situation it is equivalent to the turnover rate expressed as pools per day.

When \(t\) = 0, the integrated form of the equation can be solved for c (or the curve can be graphically extrapolated)

\(^{8}\) The word “synthesis” as here used means the formation of uric acid from any source whatsoever.
lated back to $t = 0$). The antilogarithm of this value can then be substituted into the isotope dilution equation to calculate the pool size at $t = 0$ or immediately after the injection of the labeled uric acid:

$$A = \text{Pool size (in same units as } a)$$

$$a = \text{Amount of injected uric acid}.$$  

$$A = a \left( \frac{I_1}{I_0} - 1 \right)$$

$I_1$ = Isotope concentration of injected uric acid.

$I_0$ = Isotope concentration of body uric acid immediately after mixing with injected uric acid.

**DISCUSSION OF POOL SIZE AND TURNOVER RATE**

Benedict, Forsham and Stetten (1) have employed the aforementioned equations to calculate pool size and turnover rate on three normal subjects and two gouty subjects. The normal pool size ranged from 1,145 to 1,341 mg. with turnover rates ranging from .533 to .757 pools per day. Their gouty subjects had pool sizes of 4,742 and 18,450 mg. and turnover rates of .524 and .463 pools per day, respectively.

Geren and coworkers (2) injected isotopic uric acid into a normal subject and determined the pool size to be 944 mg. with a turnover rate of .83 pools per day.

In this laboratory a number of human subjects has been injected intravenously with N$^{15}$ labeled uric acid. The results of 26 experiments are presented in Figure 1. The common logarithm of the isotope concentration of the urinary uric acid has been plotted against the time in days. It is apparent that the curves are essentially straight lines as had been predicted from the basic equations and assumptions. In some of the experiments, however, there is a tendency for one or more of the initial points to lie above the best straight line drawn through the latter points. This phenomenon has revealed itself in the present work probably because of the frequency of sampling during the initial periods of the experiments. Furthermore, not every subject shows it so that several subjects must be studied before it need appear. Some se-

![Graphs showing isotope concentration over time for different experiments](image-url)
lected curves of Benedict, Forsham, and Stetten (1) are slightly concave, suggesting the same phenomenon.

Calculation of the pool size is dependent upon determining the isotope concentration of the body pool immediately after injection of the isotopic uric acid since only at this time is the quantity of isotopic uric acid in the body accurately known. At a later time this amount will be decreased by any amount catabolized and excreted. Since it is not analytically feasible to determine the concentration of isotopic uric acid in the body immediately after the injection, one does the next best thing which is to extrapolate successive values back to the time of injection. This procedure has another advantage in that momentary irregularities in equilibration are minimized.

Extrapolation of the linear curves in Figure 1 can be performed easily, but extrapolation of the non-linear curves presents some difficulties. If the injected isotopic uric acid were assumed to be slowly equilibrating during the initial periods of these studies and if no isotopic uric acid were being lost by catabolism and excretion, one would be justified in extrapolating the latter straight portion of the curves to zero time. The pool size thus calculated might be called the “eventual pool” to distinguish it from the “immediate pool” or the amount of uric acid which equilibrates with the injected material within a few minutes. Since, however, it is obvious that isotopic uric acid is being continuously excreted and perhaps catabolized, it appears that the so-called “eventual pool” is a mathematical artifact except in those cases where the “eventual pool” and the “immediate pool” are

4 Folin, Berglund, and Derick (7) were unable to recover as urinary uric acid all of the uric acid they injected intravenously, thus suggesting another route of disposal. Stetten occasionally found some N\(^{15}\) in urinary urea after injecting N\(^{15}\) labeled uric acid (1). Presumably this could only arise by the catabolism of uric acid. It should be noted, however, that Geren and associates (2) recovered nearly quantitatively the N\(^{15}\) uric acid injected intravenously in one study on one subject.
FIG. 1. CONTINUED
identical, i.e., where the logarithmic curve is a straight line.

One method of calculating the pool size at any time involves a consideration of the amount of isotopic uric acid remaining in the body at that time with respect to the isotope concentration of uric acid in the body pool at that time. However, it is difficult to estimate the amount of uric acid remaining in the body at any time since this involves calculating the quantity of isotopic uric acid which has been disposed of prior to time \( t \). The calculation of the amount of labeled uric acid excreted as uric acid is usually based upon an inaccurate colorimetric method. Furthermore, there is the evidence already cited which suggests disposal of labeled uric acid by a catabolic pathway, although it is difficult to assess the magnitude of this process. It would be desirable if one could calculate the total amount of body uric acid with which injected labeled uric acid would eventually equilibrate because in the gouty subject, at least, it is usual to find uric acid widely distributed and in forms which might not equilibrate rapidly. The data, available here or indeed anywhere, are inadequate for such calculations.

The “immediate pool,” or the amount of body uric acid that equilibrates with injected uric acid, can be calculated from the extrapolated value of \( \ln I \) at \( t = 0 \). If the logarithmic curve is straight, this extrapolation is simple. If the logarithmic curve is not straight, its prolongation to the y axis, especially if accomplished free hand, may result in a considerable error because of the nature of the logarithmic units in this region. It has been found empirically that if one considers that a second but brief logarithmic process is superimposed upon the more stable disappearance process, the resultant of these two curves is a curve that closely resembles the observed curve. These two logarithmic straight lines can be extrapolated quite accurately and their y intercepts combined to approximate the intercept of the original curve. In practice this calculation can be carried out as in Figure 2. The common logarithms of the original points were plotted against time, producing a curve which was straight in the latter portion. This straight portion was extrapolated to zero time. The antilogarithm of each point on the original curve had subtracted from it the antilogarithm of the corresponding point on the extrapolated curve. When the logarithms of these differences were replotted at the proper time intervals, an approximately straight line lying below the other two curves was derived. The antilogarithms of the y intercepts of the two straight lines were added to give an estimate of the antilogarithm of the y intercept of the original curve. This estimate was then used in the isotope dilution equation to calculate pool size. Although the foregoing calculation is based upon a second additive logarithmic process, it should be emphasized that this method of approximation is only empirical and at present no interpretation is implied.

The turnover rate as calculated from the straight portion of the logarithmic curves was presumed to represent a definite concept so long as it was expressed in pools per day. Any attempt to multiply this value by the pool size in milligrams or millimols is valid only if both pool size and turnover rates are measured at the same time. This is obviously not true if the logarithmic curve is not linear near \( t = 0 \).

It may be concluded that in general the pool size can be estimated only immediately after the injection of the labeled uric acid and that even then caution is necessary in extrapolating the isotope disappearance curve. The turnover rate in pools per day is probably valid, but if expressed in milligrams or millimols of uric acid per day it is only valid if the equilibration of injected uric acid with body uric acid occurs immediately.

**RESULTS**

**Disturbance of uric acid metabolism and implications of therapy**

Table I lists the results of 26 determinations of pool size and turnover rate. Additional experiments were performed but the results were not considered to be acceptable technically. The values in the column entitled “Maximum Days for Equilibration” refer to the length of time before the logarithmic curves become straight. The immediate pool has been calculated according to the considerations already discussed. If more than one initial point deviated from the straight line, a second logarithmic curve was used in order to determine the y intercept of the original curve.
The “apparent pool” is included in order to show the magnitude of error involved in merely extrapolating the straight portion of the curve. The turnover rate is presumed to be accurate so long as it is expressed as pools per day, but may be multiplied by the pool size only under certain circumstances as noted previously.

The several patients with gout and the one patient with chronic myelogenous leukemia were the only ones that showed a marked alteration of uric acid pool size and turnover rate. The other subjects which included normal persons without an elevated serum urate concentration, persons otherwise normal except for idiopathic hyperuricemia, patients with rheumatoid arthritis, and patients with polycythemia vera were alike in regard to their uric acid metabolism. For the control periods of all non-gouty, non-leukemic subjects, the mean pool size was 1,057 mg. with a standard deviation of 161. The mean turnover rate in pools per day was .755 with a standard deviation of .134. In contrast, no gouty subject had a metabolic pool as small as that of the non-gouty subjects. Furthermore, the turnover rate in the gouty subjects, with but one exception, did not exceed .510 pools per day.

The subject with chronic myelogenous leukemia had a high serum urate level so it was not unexpected that his pool size was elevated. The lowered turnover rate may accompany an increased pool size.

Two normal subjects with hyperuricemia were included in this series. One subject, J. H. T., had had a concentration of serum uric acid above the normal for more than 16 years but had no familial history of gout. His metabolic pool size was somewhat below the mean as was also his turnover rate. The other hyperuricemic normal, E. S., had had no symptoms of arthritis, but had a brother with gout. The pool size of E. S. was the largest of all the subjects in the “normal” group and his turnover rate was nearly that of the gouty subjects. On the basis of these isolated observations it would appear that there are different types of hyperuricemia and that the hyperuricemia evidenced by relatives of gouty patients may well be a transition state between normal uric acid metabolism and the large pool size-low turnover

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A subject who consistently shows a serum uric acid concentration in excess of 6 mg. per 100 ml. by the colorimetric determination as performed in this laboratory is considered a hyperuricemic subject.
rate type of metabolism seen in patients with clinical gout. Whether the inability of the turnover rate to increase homeostatically with an increasing pool size might allow the pool size to increase progressively, or whether the lower turnover rate is a consequence of the enlarged pool size is a point for conjecture in the pathogenesis of gout.

Colchicine caused a dramatic decrease in the uric acid pool size, and a decrease in the turnover rate in one gouty subject, S. B. In another gouty subject the effect was equivocal. Colchicine administration was followed by a decrease in the uric acid turnover rate in two patients suffering from rheumatoid arthritis (C. C., K. S.). In one case the decrement was marked. Pool size was not greatly affected. Thus colchicine administration may lead to a decrease in the pool size of gouty subjects. Such a result would indicate a decrease in the rate of synthesis of uric acid either with or without a concomitant decrease in disposal rate such that the pool size would either remain constant or decrease. This is the first time that any definite action has been suggested for colchicine.

Cortisone was administered to three subjects. In one gouty subject, W. S., no metabolic effect was observed. In two rheumatoid arthritis subjects, however, the effect was marked especially on the turnover rate. In one patient, C. C., the turnover rate was increased from .806 to 1.133 and in the other patient, K. S., from .960 to 1.186.

The pool size was also decreased in both instances. Such a coincidence of effects would suggest increased excretion (or catabolism) of uric acid with no reduction (or at least no corresponding increase) in the amount of uric acid entering the metabolic pool. It is known that ACTH increases the urinary excretion of uric acid (8) and cortisone might be suspected of acting similarly. Clinically it is known that cortisone is a more potent therapeutic agent in rheumatoid arthritis than in gout. Perhaps the metabolic effect noted in the arthritic subjects and not in the gouty is a reflection of this fundamental difference in effect.

ACTH was administered to one gouty, one leukemic, and one hyperuricemic subject. In each subject the pool size and the turnover rate increased. This suggests an increase in the rate of synthesis of uric acid without a comparable increase in the rate of excretion. These results are not inconsistent with the suggested uricosuric action of ACTH (8) but coincident with this, one must postulate an even greater effect on uric acid synthesis since only in this way can the pool size increase. Thus ACTH and cortisone would appear to differ in their effect since ACTH most probably has an accelerating effect on uric acid synthesis while cortisone need not have such an effect.

It should be remembered that all drugs used in these studies were given for limited periods only.
If the therapy had been continued for several weeks the findings might have been different. The phenomena noted above are what might be called primary or initial effects.

SUMMARY

The pool size and turnover rate of body uric acid were studied by the intravenous injection of N\(^{15}\) labeled uric acid into subjects before and after therapy with colchicine, cortisone, and ACTH. The subjects included normal and hyperuricemic subjects, as well as patients with gout, rheumatoid arthritis, polycythemia vera, and chronic myelogenous leukemia.

In some experiments it was observed that injected labeled uric acid did not immediately equilibrate with body uric acid. This was evidenced by the fact that when the logarithm of the isotope concentration of the urinary uric acid was plotted against time, the curve did not become straight for many hours. In such cases it was suggested that only the amount of body uric acid that immediately equilibrated with the injected uric acid ("immediate pool") could be accurately calculated. An extrapolation procedure was suggested for such cases.

Two hyperuricemic subjects, one having a gouty brother, and the other with no familial history of gout, were examined. The former, on the basis of uric acid pool size and turnover rate, appeared to represent a transition state between the normal and the gouty. The other subject was quite normal.

Colchicine in several patients led to a decrease in the uric acid turnover rate and in one gouty subject caused a concomitant decrease in pool size. It was suggested that colchicine might decrease the rate of synthesis of uric acid.

Both ACTH and cortisone increased the turnover rate but the pool size tended to increase following ACTH and tended to decrease following cortisone. A uricosuric effect could be postulated for both drugs but ACTH must have increased the synthetic rate of body uric acid. Cortisone showed its greatest effect in patients with rheumatoid arthritis. The effect in the one gouty subject was minimal.

Since all drugs were given for a limited period, the results would only reveal the primary effects of such drugs and would not suggest what might occur after prolonged administration.

PROTOCOL

W. G., age 26, male. Has no past history of clinical significance. A normal concentration of serum urate was noted on several occasions. He was considered to be a normal subject.

J. H. T., age 48, male. Has no past history of joint disease and no pertinent clinical history. The concentration of serum uric acid when observed from time to time has been above normal for more than 16 years. No family history of gout. Considered to be a normal control with hyperuricemia only.

E. S., age 23, male. One brother has gout. E. S. has had no symptoms suggestive of acute or chronic gouty arthritis. He has had an elevation of the serum uric acid when determined. He is considered to be a normal person with hyperuricemia and a positive family history for gout.

G. D., age 43, male. Has had acute attacks of gouty arthritis for more than 10 years. He has extensive tophaceous deposits and is classified as suffering from moderately severe acute and chronic gouty arthritis.

S. B., age 44, male, a victim of acute attacks of gouty arthritis for 23 years. There have been no peripheral tophi and there are minimal X-ray changes in the joints suggestive of gouty arthritis. Has frequent attacks of acute gout and is considered to be a case of moderately severe recurrent acute gouty arthritis with minimal evidence of chronic gouty arthritis.

W. S., age 51, male. Has had symptoms of acute gouty arthritis for 17 years. He has had several peripheral tophi and X-ray evidence of osseous tophi. The diagnosis is intermittent acute gouty arthritis and chronic gouty arthritis, moderate.

M. B., age 50, male. Has had symptoms of acute gouty arthritis for 19 years. He has evidence of subcutaneous tophi and osseous tophi. The diagnosis is moderately severe acute and chronic gouty arthritis.

I. M., age 41, male. Has had symptoms of acute gouty arthritis for 10 years. There is no peripheral evidence of gout but X-rays show evidence of osseous tophi. He has mild chronic gouty arthritis.

C. C., age 26, male. Has had symptoms of rheumatoid arthritis for 14 years. There is evidence of extensive peripheral joint disease as well as rheumatoid spondylitis. He has a moderately severe crippling form of the disease.

K. S., age 36, male. Has had symptoms of rheumatoid arthritis for five years. There is evidence of moderate involvement of peripheral joints as well as moderate involvement of the spine. He has mild acute rheumatoid arthritis.

W. de R., age 35, male. A diagnosis of chronic myelogenous leukemia was made first in 1942. The patient had had many transfusions but none at the time these studies
were in progress. The white blood cell count was over 650,000 per mm. The red cell count was 2.7 million and the hemoglobin 7.5 gm. Serum uric acid was as high as 20 mg. per 100 ml. The patient died a few days after this study was terminated.

J. K., age 63, male. Patient had had symptoms related to polycythemia vera for more than two years. The red blood cell count was 7.8 million and the white blood cell count was 17,000. The oxygen capacity was 27.6 vols. per cent and the cell volume 75.2 per cent. The patient had received no specific treatment at the time these studies were completed.

H. M., age 73, female. Patient had had symptoms which were probably related to polycythemia vera for more than two years. The oxygen capacity was 26 vols. per cent. The red blood cell count was 9 million, the hemoglobin 17.9 gm., and the white blood cell count 27,000.

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