THE EFFECT OF BENEMID (P-[DI-N-PROPSULFAMYL]-
BENZOIC ACID) ON URIC ACID METABOLISM IN ONE
NORMAL AND ONE GOUTY SUBJECT. 2

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(Submitted for publication February 23, 1951; accepted May 28, 1951)

Benzoic acid derivatives such as carinamide (4-
carboxy-phenyl-methane-sulfonanilide) have been
reported to be effective uricosuric agents, pre-
sumably by blocking renal tubular resorptions of
uric acid. Benemid (p-[di-n-propylsulfamyl]-
benzoic acid), another compound of this type, has
recently become available. In patients with tu-
cerculosis undergoing treatment with PAS (p-amino-
salicylic acid) the simultaneous administration of
Benemid tended to prevent the rapid renal excre-
tion of the PAS. Since this effect was presumably
due to some blocking of renal tubular activity, it
came of interest to test the effect of Benemid on
uric acid excretion.

The present study was carried out on two sub-
jects. One was an idiopathic hyperuricemic sub-
ject (J. H. T.) who appeared from previous stud-
ies to have a normal uric acid metabolism; the
other subject (W. S.) was known to be suffering
from gouty arthritis and had been studied pre-
viously in this laboratory.

Before and after a course of Benemid therapy,
N15 labeled uric acid was injected intravenously
for the determination of uric acid pool size and
turnover rate. Uric acid excretion was followed
quantitatively by the isotope dilution method.

EXPERIMENTAL PROCEDURES

Each subject was injected intravenously with N15
labeled uric acid (25-50 mg, 16 atom per cent excess) and
urine samples were collected for several days. The subject
then began the daily ingestion of 2 gm of Benemid.
On the third day of this regimen, labeled uric acid was
again injected. Benemid ingestion was continued until
the end of the study.

The determination of uric acid pool size and turn-
over rate employed the same materials and techniques
as previously reported (1). In addition, an aliquot of
each urine sample or combination of samples was taken
for quantitative uric acid determination by the isotope
dilution method. To this aliquot were added 25-50 mg
of purified N15 uric acid of approximately 1 atom per
cent excess dissolved in dilute sodium hydroxide solu-
tion. The molecules of added uric acid were presumed
to mix completely with the molecules of uric acid pres-
ent in the aliquot. A representative sample of this mixture
of uric acid molecules was precipitated and its isotope
concentration compared with that of a sample of uric acid
precipitated from the same urine without augmentation.
The amount of uric acid present in the original urine
was calculated by the isotope dilution principle.

From standard solutions of uric acid it was demon-
strated that this method under conditions comparable
to those used for urine, gave results accurate to within
2 per cent. This implied that for a daily excretion of 800
mg the determination would be accurate to within ±16
mg. It was believed that such results were considerably
better than could be expected from any of the usual meth-
ods for the quantitative determination of urinary uric
acid. Furthermore, the material determined as uric
acid was uric acid, not a mixture of uric acid and non-
uric acid chromogen as in the usual colorimetric pro-
cedures.

Serum uric acid concentrations were determined by
the method of Folin (2). Since these values were only
used as a clinical guide and not as the basis of further
calculation, it is not so necessary that they be highly
specific for uric acid.

RESULTS

Figure 1 is a graph of the isotope concentration
of the urinary uric acid of the non-gouty subject
(J. H. T.) in the period before the ingestion of
Benemid. On day 160, Benemid ingestion was be-
gun and was continued through day 166. Figure
2 shows the isotope concentration after the in-
jection of labeled uric acid on day 163 and during the
period of Benemid ingestion. The uric acid pool
size in the control period was calculated by assum-
ing a second additive process according to the method previously outlined (1). This was unnecessary in the Benemid period. In the control period the amount of body uric acid with which the injected uric acid immediately equilibrated was calculated to be 964 mg. The turnover rate was .666 pools per day. After Benemid therapy the immediate pool size dropped to 466 mg, and the turnover rate rose to 2.457 pools per day. These results are consistent with the interpretation that Benemid is an effective tubular blocking agent and inhibits the resorption of uric acid. Under such circumstances some uric acid from the body pool should be excreted, leading to a decrease in the pool size and an increase in the turnover rate. (If the rate of synthesis of body uric is constant then a decrease in the pool size is equivalent to an increase in the turnover rate in pools per day.)

In Figure 3, the daily uric acid output of the same subject has been represented by a bar graph. On the same graph has been plotted the serum uric acid concentration. The fall in serum uric acid concentration and the rise in urinary uric acid excretion during Benemid therapy is apparent.

The mean daily uric acid excretion during an 11 day control period which included all days of urinary collection before and after Benemid administration was 830 mg. The “extra” uric acid excreted during the seven days on Benemid was 2705 mg. This was several times the magnitude of the 498 mg decrease in the immediate pool size after Benemid administration. At least two explanations might be offered for this. One is that this “extra” uric acid is not derived exclusively from the immediate pool but from a more remote pool of uric acid. At present there does not seem
to be any good way of testing this hypothesis. Another explanation is that with the renal tubules blocked, more exogenous uric acid finds its way into the urine. This hypothesis cannot be rigorously proved, but there are isolated facts that tend to support it. One is that subjects on Benemid continue to excrete "extra" uric acid as long as they continue taking the drug. Since the decrease in the serum uric acid concentration and the decrease in pool size occur within a day or two of the beginning of therapy, it would seem that the body would soon reach a state of minimum uric acid content and that thereafter "extra" uric acid would of necessity arise from external or dietary sources. Normally some uric acid does arise from dietary sources since it has been shown in this laboratory (3) that different dietaries, specifically high-fat as against high-protein diets, cause a significant difference in uric acid excretion.

The labeled uric acid that was injected into the non-gouty subject was not completely recovered as urinary uric acid. By day 162 no more N15 uric acid from the first injection was recoverable from the urine; hence it was assumed that all the injected labeled uric acid had already left the body pool. Since 28.5 per cent of the injected dose had

FIG. 2. ISOTOPE CONCENTRATION OF URINARY URIC ACID OF SUBJECT J. H. T. DURING BENEMID INGESTION

FIG. 3. SERUM URIC ACID CONCENTRATION AND URINARY URIC ACID EXCRETION OF SUBJECT J. H. T. BEFORE, DURING, AND AFTER BENEMID INGESTION
not been accounted for as urinary uric acid, it was assumed that this amount had been disposed of by another route, probably catabolism since there has been scattered evidence for such a process (4, 5).

Before and during the second N\textsuperscript{15} uric acid injection period the subject had been taking Benemid. The injected uric acid was excreted quickly, and 89.6 per cent was recovered as urinary uric acid. This is consistent with the hypothesis that one or more alternate mechanisms is competing for the uric acid that would otherwise be excreted by the kidney. If the N\textsuperscript{15} concentration of the body uric acid pool is quickly reduced, the amount of the injected uric acid that can escape by the alternate routes will be reduced. Benemid does cause such a rapid decrease in the N\textsuperscript{15} uric acid concentration of the body pool, presumably by blocking resorption of urinary uric acid.

Further evidence for more than one pathway of uric acid disposal is presented in Figures 4 and 5. When the logarithm of the percentage of the dose apparently remaining in the body was plotted against time, the curve was not a straight line, neither during the control period (upper curve, Figure 4) nor during the Benemid period (upper curve, Figure 5). On the assumption that the amount of N\textsuperscript{15} uric acid remaining in the body pool should be calculated on the basis of only the uric acid excreted by the kidney, the data were recalculated. The amount of uric acid unaccounted for at the end of the study was subtracted from each datum and the logarithms of these values were again plotted in Figures 4 and 5 (lower curves). These new curves are straight lines. Thus the rate of urinary excretion of the labeled uric acid was directly related to the amount of the labeled uric acid actually left, not to the amount.
merely unaccounted for on the basis of urinary excretion.

The second subject of this study, W. S., although suffering from gouty arthritis, responded to Benemid therapy in much the same way as the first subject. Benemid ingestion was begun on day 181 and continued through day 186. As can be seen from Figure 6, the uric acid pool size in his control period was 2205 mg and the turnover rate was .484 pools per day. After Benemid therapy (Figure 7) his pool size fell to 1622 mg and the turnover rate rose to 1.006 pools per day. This paralleled a marked drop in serum uric acid concentration as shown in Figure 8. Yet the drop in the size of the immediate pool was only 573 mg while the "extra" uric acid excreted during the six days on Benemid was 4889 mg as can be seen from the same figure. It is quite possible, especially in the gouty subject, that some of this "extra" uric acid is derived from a pool much larger than the immediate pool. There is also the suspicion that at least part of the "extra" uric acid is derived from dietary sources.

One striking difference between the behavior of the non-gouty and gouty subjects was the amount of the injected dose of isotopic uric acid recovered as urinary uric acid. In the gouty subject this was 98.9 per cent as can be seen from Table I. This

subject was still excreting a small amount of labeled uric acid when the study was terminated, so that the ultimate recovery might have been even higher.

Since this subject apparently did not catabolize any uric acid, it became feasible to calculate his pool size at various times. In this study the ini-
tial dose minus the $^{15}$N uric acid excreted must equal the amount of $^{15}$N uric acid remaining in the body. These calculations were carried out in arbitrary units, since this simplified the calculations. The product of the number of milligrams of uric acid excreted during the first period and its isotope concentration in atoms per cent excess was subtracted from the product of the number of milligrams of uric acid in the injected dose and its isotope concentration. This new value, when divided by the estimated isotope concentration of the body pool at the end of this first period equaled the pool size in milligrams (neglecting the amount of uric acid contributed by the injected dose). The amount of isotopic uric acid excreted in the second period was similarly calculated and subtracted from the amount left at the end of the first period. This quantity divided by the estimated isotope concentration at the end of the second period gave the pool size at the end of the second period. As is apparent from Table I, the pool size increased constantly up to the point where it was no longer possible to make a satisfactory estimate of the isotope concentration of the body pool. When the pool size was plotted against time no asymptotic tendencies were evident so that an ultimate pool size could not be calculated. This may be due to the fact that in the body there are relatively large amounts of slowly equilibrating uric acid, perhaps in a solid phase.

In the Benemid period, the pool size started at a value closely approximating the pool size calculated by the graphical method and fluctuated down and up. This did not necessarily mean that there were not more than 1,600 mg of uric acid in the whole body at this time. It may be that no appreciable amounts of labeled uric acid had an opportunity to equilibrate with the less accessible depots (tophi, bound phases) of body uric acid because of the rapidity with which the $^{15}$N uric acid was excreted by the blocked tubules. The tendency of the uric acid pool size to increase toward the end of this study was not immediately explainable, but the last two or three values should

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**TABLE I**

**Uric acid pool size in gouty subject at successive intervals both before and during Benemid therapy**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time at end of Sample</th>
<th>Amount Left M g x 1</th>
<th>Estimated I at end of Period</th>
<th>Pool Size (mg uric acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>177.312</td>
<td>0</td>
<td>831** 100.0</td>
<td>2540</td>
</tr>
<tr>
<td>2-3</td>
<td>528</td>
<td>71</td>
<td>760 91.5 .299</td>
<td>2500</td>
</tr>
<tr>
<td>4</td>
<td>649</td>
<td>74</td>
<td>686 82.6 .274</td>
<td>2760</td>
</tr>
<tr>
<td>5-6</td>
<td>917</td>
<td>53</td>
<td>633 76.2 .229</td>
<td></td>
</tr>
<tr>
<td>7-8</td>
<td>178.271</td>
<td>55</td>
<td>578 69.5 .191</td>
<td>3020</td>
</tr>
<tr>
<td>9-10</td>
<td>531</td>
<td>61</td>
<td>517 62.3 .168</td>
<td>3080</td>
</tr>
<tr>
<td>11-13</td>
<td>179.003</td>
<td>73</td>
<td>444 53.4 .133</td>
<td>3340</td>
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<tr>
<td>14-15</td>
<td>281</td>
<td>21</td>
<td>423 50.9 .116</td>
<td>3640</td>
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<tr>
<td>16-17</td>
<td>545</td>
<td>28</td>
<td>395 47.5 .103</td>
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</tr>
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<td>36</td>
<td>359 43.2 .086</td>
<td>4180</td>
</tr>
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<td>21</td>
<td>338 40.7 .072</td>
<td>4700</td>
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<td>36</td>
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<td>62</td>
<td>233 28.0</td>
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<tr>
<td>36-43</td>
<td>183.264</td>
<td>30</td>
<td>203 23.4</td>
<td></td>
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<td>233</td>
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<td>1635</td>
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<td>264 21.2 .177</td>
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<td>48-49</td>
<td>184.024</td>
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<td>196 15.7 .140</td>
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<td>1350</td>
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<td>2000</td>
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<tr>
<td>65-71</td>
<td>187.240</td>
<td>18</td>
<td>14 1.1 .008</td>
<td>1750</td>
</tr>
</tbody>
</table>

* Sample collected immediately prior to initial injection of isotopic uric acid
** Initial injection: 50 mg x 16.618 atom percent excess = 831
*** Second injection: 25 mg x 16.618 atom percent excess = 416

$416 \times 203$ (remaining from first injection) $\times 619$
not be emphasized because the error of the isotope determination in this region is difficult to assess.

SUMMARY

One normal and one gouty subject have been injected intravenously with $^{15}N$ labeled uric acid. The isotope concentration of the urinary uric acid was determined and the amount of urinary uric acid excreted was determined by the isotope dilution method. After a suitable control period each subject received oral daily doses of 2 gm of Benemid (p-[di-n-propylsulfamyl]-benzoic acid), a potent uricosuric agent, and subsequently were re-injected with $^{15}N$ labeled uric acid.

In the normal and the gouty subjects the amount of body uric acid that would immediately equilibrate with injected $^{15}N$ uric acid decreased markedly after Benemid therapy, and the turnover rate of the body uric acid pool increased greatly.

The amount of "extra" uric acid that was excreted during the period of Benemid therapy was much greater than the amount that disappeared from the immediate pool. Some of this "extra" uric acid, especially in the gouty subject, might have come from a more remote depot of uric acid but other evidence suggests that the diet, under the conditions of this experiment, may have contributed to this "extra" uric acid.

In the control period of the non-gouty subject only 71.5 per cent of the injected $^{15}N$ uric acid was recovered as urinary uric acid up to the time when its excretion ceased. During Benemid therapy the recovery of a second dose of $^{15}N$ uric acid was 89.6 per cent. This was explained as being due to the more rapid excretion of the $^{15}N$ uric acid in the second period, thus decreasing the time during which the labeled uric acid could be disposed of by a competing catabolic or other disposal route. The recovery of injected $^{15}N$ uric acid in the total of both periods of the gouty subject was 98.9 per cent up to the time when excretion of the isotope had virtually ceased. This gouty subject apparently did not catabolize uric acid or dispose of it in any other way than by urinary excretion.

Since in the gouty subject all of the injected labeled uric acid was excreted as urinary uric acid, it was possible to calculate the uric acid pool size at succeeding time periods. The size of the pool in the control period of this subject rose from approximately 2,700 mg at the time of injection of the labeled uric acid to 4,700 mg within three days. It could not be reliably estimated thereafter. After Benemid therapy the pool size fluctuated around 1,600 mg.

Benemid was shown, both in the gouty and non-gouty subject, to be a potent uricosuric agent, this action being associated with a reduction in the serum urate concentration and a reduction in the pool size of body uric acid.

ACKNOWLEDGMENT

The authors acknowledge with pleasure the assistance of Dorthea Tamborski who performed many of the determinations, and Frank Stein and Ernest Lehmann of the Physics Department of the University of Buffalo, who contributed to the operation of the mass spectrometer.

REFERENCES

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