METABOLISM OF THEOPHYLLINE (1,3-DIMETHYLXANTHINE) IN MAN

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The intermediary metabolism of theophylline has been the subject of a number of studies, but its fate in the body is still unsettled. Kruger and Schmidt (1) working with dogs reported that the administration of theophylline to dogs is followed by the appearance of 3-methylxanthine in the urine. Myers and Wardell (2) and Buchanan, Christman, and Block (3) showed that ingestion of theophylline in man is followed by an increased urinary excretion of phosphotungstic acid-reducing material. The former investigators were unable to determine whether the increase in reducing material was due to uric acid or to a methyluric acid. The latter were able to show, by the use of uricase, that the increment in reducing material is not uric acid. They concluded that a large part of the theophylline is excreted as 1-methyluric but suggested that 3-methyluric or 1,3-dimethyluric acid may also be formed. None of these compounds was actually isolated in support of their hypothesis. Myers and Hanzal (4) working with the Dalmatian dog concluded, from the color-yielding value of the phosphotungstic acid-reducing material, that theophylline is excreted mainly in the form of 1,3-dimethyluric acid. In a preliminary report, Weinfeld (5) suggested that 1-methyl- and 1,3-dimethyluric acid are excreted in rabbit urine after feeding theophylline. The compounds were identified by their behavior on paper chromatograms.

The utilization of counter-current extraction and ion exchange chromatography have made it possible to obtain definitive information concerning a part of the metabolic pathway of theophylline. The present paper reports studies on the fate of theophylline in man and describes the isolation and characterization of 1,3-dimethyluric acid, a major transformation product.

Methods

Estimation of Theophylline in Urine—Theophylline is isolated for analysis from urine, to which has been added excess solid NaCl, by extraction at about pH 7 into 40 ml. of chloroform1 containing 3 per cent isoamyl alcohol. The solvents, reagent grade, are purified by successive washings with 1 N NaOH, 1 N HCl, and two washings with water.
alcohol. The NaCl serves to augment the completeness of the extraction. The chloroform phase is washed with 3 ml. of NaCl-saturated phosphate buffer, pH 7, to remove some normally occurring “blank” material. The drug is returned to an aqueous phase by shaking with 4 ml. of 2.5 N NaOH.

Theophylline exhibits a pronounced absorption peak at 277 mp. Some residual blank not removed by the buffer wash of the chloroform phase also absorbs light at this wave-length. Correction for this blank depends upon the observation that its optical density at 277 and 300 mp is almost the same, whereas that of theophylline decreases by about 95 per cent. The optical density of the alkaline solution of theophylline is determined at 277 and 300 mp in a spectrophotometer (Beckman). A standard solution of theophylline carried through the above procedure is measured at both wave-lengths. The theophylline concentration in the solution being measured equals \((U_a - U_b)/(S_a - S_b) \times C\), where \(U_a\) and \(U_b\) are the optical densities of the unknown at 277 and 300 mp respectively, \(S_a\) and \(S_b\) are the optical densities of the standard at these wave-lengths, and \(C\) is the concentration of theophylline in the standard.

Counter-Current Procedure—Counter-current distribution was carried out by means of a separatory funnel technique involving eight transfers (6). The immiscible solvent pairs were chosen so that the material under study was distributed about equally between the two phases. In each case the solvent pairs were previously saturated one with the other. After distribution, the partition ratio of the material in each separatory funnel was determined by measurement of the concentration of the material in each phase. Theophylline was measured in alkaline solution as described above. 1,3-Dimethyluric acid was measured spectrophotometrically in acid solution at 285 mp. Theophylline in the organic phases was transferred to an aqueous phase by extraction into alkali; 1,3-dimethyluric acid in the organic phases was transferred by evaporation of an aliquot of the solvent to dryness in a stream of air and dissolving the residue in acid solution.

EXPERIMENTAL

A human subject, on a diet free of coffee, tea, and other xanthine-containing beverages, was given 750 mg. of theophylline orally, in three divided doses, over a 5 hour period. Urine was collected for 18 hours following the last dose.

Identification of Theophylline in Urine—10 ml. of urine were adjusted to pH 7 with NaOH and shaken with 200 ml. of chloroform containing 3 per cent isoamyl alcohol. Theophylline-like material in the solvent was returned to an aqueous phase by shaking with 8 ml. of 0.1 N NaOH. The

\(^2\) About 4 per cent of the theophylline is removed by the buffer wash.
material was then subjected to an eight transfer counter-current distribution. The solvents used were chloroform containing 1.8 per cent isoamyl (1 volume) and 1 M phosphate buffer, pH 6.7 (1 volume). In Table I is shown the total amount of apparent theophylline present in each funnel, together with the fraction present in the chloroform phase. Except in the end funnels, which contained only a small percentage of the total material, the ratio of the amount of solute in the chloroform phase to the

<table>
<thead>
<tr>
<th>Separatory funnel No.</th>
<th>Amount per funnel</th>
<th>Fraction* in chloroform phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>γ</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>52.3</td>
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<tr>
<td>2</td>
<td>24.1</td>
<td>0.16</td>
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<tr>
<td>3</td>
<td>38.8</td>
<td>0.46</td>
</tr>
<tr>
<td>4</td>
<td>93.5</td>
<td>0.49</td>
</tr>
<tr>
<td>5</td>
<td>139.0</td>
<td>0.49</td>
</tr>
<tr>
<td>6</td>
<td>135.8</td>
<td>0.49</td>
</tr>
<tr>
<td>7</td>
<td>77.4</td>
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<tr>
<td>8</td>
<td>27.3</td>
<td>0.47</td>
</tr>
<tr>
<td>9</td>
<td>5.2</td>
<td>0.54</td>
</tr>
<tr>
<td>Total</td>
<td>593.4</td>
<td></td>
</tr>
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</table>

* When pure theophylline was distributed between the solvent pair, the fraction in the chloroform phase was 0.47.

total amount in both phases was constant and almost identical with that found for authentic theophylline measured at the same time, the same batch of equilibrated solvents being used. The material in Funnels 1 and 2 displayed no peaks in the ultraviolet and was, presumably, normally occurring material from the urine. These results indicate the presence of only one major component which possesses the solubility characteristics of theophylline.

Metabolic Transformation Products in Urine—The oxidation of theophylline in Position 8 would result in the formation of 1,3-dimethyluric acid. Oxidation, accompanied by the loss of a methyl group, would yield 1- or
3-methyluric acid. Conditions were examined for the isolation of these methyluric acids from urine. All three compounds can be extracted from acid solution into isobutyl alcohol. Adding petroleum ether to the alcohol phase and shaking with aqueous alkali returns the three compounds to an aqueous phase. Under these conditions uric acid is also extracted. Uric acid, however, can be destroyed by incubation with uricase at pH 9.2, without affecting the methyluric acids.

After the initial chloroform extraction (see above under theophylline) 150 ml. of urine were adjusted to about pH 9.2 by the addition of NaOH. 30 ml. of 0.8 M borate buffer, pH 9.2, were then added. The buffered urine was incubated for 2 hours at 45° with 7.5 gm. of uricase powder.3 The urine was then acidified with HCl and extracted twice with 5 volumes of isobutyl alcohol. 2 volumes of petroleum ether were added to the isobutyl alcohol and the organic solvent mixture was shaken with 0.05 volume of borate buffer, pH 9.2. The absorption spectrum of the aqueous phase was examined and revealed considerable amounts of material with an absorption peak at 295 mμ in alkaline solution, and at 285 mμ in acid solution. When normal urine was extracted as above, no material was found that had absorption peaks at these wave-lengths. These results suggested the presence of a methyluric acid.

The aqueous extract was then acidified and reduced to one-fourth its volume by evaporation at room temperature in a stream of air. The extract was adjusted to about pH 1 by the addition of concentrated HCl. A counter-current distribution was carried out with the immiscible solvent pair, isobutyl alcohol (1.5 volumes) and 0.1 N HCl (1 volume). The contents of Funnels 4 to 7 were found to have similar partition ratios. Funnels 8 and 9 contained a water-soluble material, approximately 15 per cent of the total. This material had no absorption peak in acid or alkaline solution. 3-Methyluric acid would have been carried into Funnels 8 and 9, as may be computed (7) from the measured partition ratio of the compound (Table II). It may be concluded, therefore, that little if any 3-methyluric acid was present in the urine. The contents of Funnels 4 to 7 were combined, 2 volumes of petroleum ether were added, and the acidic material contained in the isobutyl alcohol was transferred to the aqueous phase by shaking. The combined aqueous extract was then evaporated at room temperature in a stream of air to a volume of about 100 ml. Further purification of the material was effected by means of ion exchange chromatography. The solution was adjusted to pH 4 and the acidic material was adsorbed on an anion exchange column (Dowex 1-

3 The uricase preparation was a hog liver acetone powder, kindly donated by Dr. T. F. Yu.
The chromatogram was developed with water followed by 0.1 N HCl. The fractions which appeared soon after initiation of HCl development contained material with an absorption minimum at 260 m\(\mu\) and a maximum at 285 m\(\mu\). These fractions were pooled and concentrated to a volume of 40 ml. with a stream of air. The material was then subjected to a further counter-current distribution under the conditions previously described. Comparison of the partition ratios of the material in each funnel indicated that only one major component was present. The contents of Funnels 3 to 7 were pooled, and the phases separated and evaporated to dryness in a stream of air. The residue was dissolved in a minimal amount of hot water and allowed to crystallize. Two recrystallizations from water yielded 6.2 mg. of crystals which had a brownish tinge. A melting point was not taken, since methyluric acid derivatives decompose between 400-500\(^\circ\)

The solubility characteristics of the metabolite and of 1-methyluric, 3-methyluric, and 1,3-dimethyluric acids were compared. Table II shows the distribution of the compounds between isobutyl alcohol and water at various pH values. The solubility characteristics of the metabolite suggested that it was 1,3-dimethyluric acid, but did not definitely exclude 1-methyluric acid as a possibility. The ultraviolet absorption spectra of the metabolite and 1,3-dimethyluric and 1-methyluric acids were compared (Fig. 1). The spectra of 1,3-dimethyluric acid and the metabolite

<table>
<thead>
<tr>
<th>pH</th>
<th>1,3-Dimethyluric acid</th>
<th>1-Methyluric acid</th>
<th>3-Methyluric acid</th>
<th>Metabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.0</td>
<td>0.16</td>
<td>0.12</td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>7.0</td>
<td>0.26</td>
<td>0.16</td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>3.0</td>
<td>0.45</td>
<td>0.35</td>
<td>0.12</td>
<td>0.45</td>
</tr>
<tr>
<td>1.0</td>
<td>0.46</td>
<td>0.42</td>
<td></td>
<td>0.46</td>
</tr>
</tbody>
</table>

The column of Dowex I was washed successively several times with 1 N HCl, 1 N NaOH, and water. A large excess of 0.1 M phosphate buffer, pH 7, was then passed through the column. The excess phosphate was removed by washing with water.
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in 0.1 N HCl were the same but differed somewhat from that of the 1-methyl derivative, which had its absorption peak displaced about 4 my towards the shorter wave-lengths. At pH 8, the spectra of 1,3-dimethyluric acid and the metabolite were again the same, but in this case the 1-methyl derivative was markedly different. Conclusive evidence that the metabolite was 1,3-dimethyluric was obtained by comparison of infra-red spectra (Fig. 2).

The amounts of theophylline and 1,3-dimethyluric acid excreted in urine were measured subsequent to the administration of theophylline. Two human subjects were given 770 mg. of drug orally in three divided doses over a period of 5 hours, and the urine was collected for 18 hours following the last dose. The excretion of theophylline and its metabolite was negligible subsequent to this time. Only a small amount of the drug was excreted unchanged, in one case 54 mg. (7 per cent) and in the other 92 mg. (12 per cent), indicating that the drug is largely metabolized in the body.

The amount of 1,3-dimethyluric acid excreted in the urine was determined as follows: An aliquot of the urine was incubated with uricase and then extracted with isobutyl alcohol. The metabolite was returned to borate buffer of pH 9.2 and then subjected to an eight transfer counter-current distribution by use of the system isobutyl alcohol (1.5 volumes) and 0.1 N HCl (1 volume). After counter-current distribution the "apparent" 1,3-dimethyluric acid in both phases of Funnel 5 was determined spectrophotometrically, as described previously. The partition ratio was almost identical with that found for authentic 1,3-dimethyluric acid measured at the same time. It may be presumed, therefore, that all the material measured in Funnel 5 was 1,3-dimethyluric acid. The total amount of 1,3-dimethyluric acid present in the separatory funnels was calculated from the amount found in Funnel 5 by application of the binomial expansion in the manner described by Williamson and Craig (7). 1,3-Dimethyluric acid, equivalent to 51 and 38 per cent of the administered theophylline, was found to be present in the urine of the two subjects.

Action of Xanthine Oxidase on Theophylline—The possible catalytic effect of xanthine oxidase on the oxidation of theophylline to 1,3-dimethyluric acid was tested by using a method comparable to the technique of differential enzymatic spectrophotometry, described by Kalckar for the reaction xanthine → uric acid (9). The results indicate that xanthine oxidase, at least in vitro, does not catalyze the oxidation of theophylline to 1,3-dimethyluric acid.

6 Xanthine oxidase was prepared from raw cream according to the procedure of Horecker and Heppel (8). A preparation carried through the ammonium sulfate fractionation was kindly supplied by Dr. J. Rowen.
Fig. 1. Absorption spectra of synthetic 1,3-dimethyluric acid (solid line), synthetic 1-methyluric acid (dotted line), and metabolite isolated from urine (broken line). Concentration, 10 μg per ml. Cell thickness, 1 cm.

Fig. 2. Infra-red absorption spectra of 1,3-dimethyluric acid (upper) and compound isolated from urine (lower). 1,3-Dimethyluric acid (2.2 mg.) was mulled with 0.1 ml. of mineral oil. The unknown (1.6 mg.) was mulled with 0.12 ml. of mineral oil. Measurements were made with a Perkin-Elmer recording infra-red spectrophotometer (model 21).
The results obtained demonstrate that about 10 per cent of a large dose of theophylline administered to man appears unchanged in the urine and as much as 50 per cent as 1,3-dimethyluric acid. If any 1- or 3-methyluric acid is excreted, the amounts are small.

The finding that xanthine oxidase from milk is incapable of catalyzing the oxidation is in agreement with similar observations made with the enzyme on theophylline, caffeine, and theobromine (10–12). It is possible that the xanthine oxidase in tissues is different from that found in milk and can catalyze the reaction.

Theophylline may be oxidized in the body by a mechanism which does not require xanthine oxidase. It is conceivable that xanthine itself may also be oxidized, in vivo, by a similar mechanism.

We are indebted to Dr. A. A. Christman and Dr. R. F. Hanzal for the methyluric acids, and to Dr. J. Rowen for the infra-red absorption measurements.

SUMMARY

When theophylline is administered to man, only a small amount of the drug appears unchanged in the urine. A major pathway of metabolic transformation of theophylline is its oxidation to 1,3-dimethyluric acid. The oxidation of theophylline is not catalyzed in vitro by xanthine oxidase from milk.

BIBLIOGRAPHY

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