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DEUTERIUM ISOTOPE EFFECTS IN THE METABOLISM OF N-ALKYLSUBSTITUTED AMPHETAMINES IN MAN

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SUMMARY

Deuterium isotope effects in the metabolism of deuterated amphetamine and N-substituted deuterated amphetamines were studied in man. The isotope effect is large when in the elimination process of the amphetamine from the body, the metabolic processes, deamination and N-dealkylation are the major pathways and the renal excretion is the minor one. It appeared to be probable, that in the deamination and N-dealkylation process the tertiary hydrogen atom is of importance.

INTRODUCTION

Within a series of N-alkyl substituted amphetamines it has been observed that the larger alkyl groups are more easily removed (N-dealkylation) than the smaller groups; i.e. isopropyl > ethyl > methyl. As a result of this dealkylation, amphetamine itself becomes the metabolite. Examination of the ratio of unaltered compound/amphetamine metabolite, when the excretion is maximal, indicates a fixed ratio between the two substances. These ratios are: 6 for methylamphetamine, 2 for ethylamphetamine and 0.6 for isopropylamphetamine. Within the same series, we are dealing with the energy difference of the C-H bond between the primary H of the methyl group, secondary H of the ethyl group and tertiary H of the isopropyl group. Whereas the dextro-isomers show the above decrease in ratio of compound/metabolite excreted, the levo-isomers show no change from one system to another, with all compounds exhibiting the same fixed ratio of 10:1. The physico-chemical parameters (e.g. pKa, lipid solubility) of both isomers (d+l) show only small differences, whereas the levo-isomer show no stimulant properties and are metabolized to a much lesser extent, indicating a sterical preference both for the stimulus receptor system and to the metabolizing enzyme system. Metabolic clearance of amphetamine derivatives involves three pathways: parahydroxylation, deamination and N-dealkylation. Dring has reported that, in man, the parahydroxylation is about 1–2% when the urinary excretion rate of the amphetamines is maximal. In view of the preceeding statement, it may be assumed that the metabolic clearance due to parahydroxylation is negligible and that the overall elimination of amphetamines is due to excretion of unaltered compound, deamination.
Fig. 1. Elimination pathways of amphetamine, phentermine and the deuterated amphetamines.

and dealkylation. We may therefore assume that the sum of the unaltered amphetamine excreted, plus deaminated and dealkylated product is equivalent to 100% of the ingested dose.

In this manner, the percentage of deaminated amphetamine, found after excretion of 70% unaltered compound, is 30% (see Fig. 1). Similar results are found when amphetamine and phentermine are compared. When these compounds are simultaneously administered to the same person, 100% of the phentermine and 70% of the amphetamine is excreted unaltered. The total amount of amphetamine excreted is dependent upon the ratio of metabolic and renal clearance constants, $k_m$ resp. $k_r$ which in turn are dependent upon the physico-chemical parameters of both the amphetamine and the enzyme system. Since from each amount of amphetamine absorbed or metabolically formed, 70% is excreted unaltered when amphetamine is a metabolite of some administered compound, the percentages of excretion, deamination and dealkylation of the original compound may be calculated (see Table I).

Phenylethylamine is excreted only 1–5% unaltered. Comparison of this result to that of amphetamine plus its derivatives suggests that the deaminating enzyme is hindered when the aliphatic side chain is branched and/or that the deamination requires a hydrogen atom. If the deamination did not require the presence of a proton,

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Deamination</th>
<th>% Dealkylation</th>
<th>% Excretion</th>
<th>$k_r$</th>
<th>$k_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ amphetamine</td>
<td>30</td>
<td>—</td>
<td>70</td>
<td>$k_r &gt; k_m$</td>
<td></td>
</tr>
<tr>
<td>+ methylamphetamine</td>
<td>20</td>
<td>15</td>
<td>65</td>
<td>$k_r &gt; k_m$</td>
<td></td>
</tr>
<tr>
<td>+ ethylamphetamine</td>
<td>30</td>
<td>30</td>
<td>40</td>
<td>$k_r = k_m$</td>
<td></td>
</tr>
<tr>
<td>+ isopropylamphetamine</td>
<td>45</td>
<td>45</td>
<td>10</td>
<td>$k_r &lt; k_m$</td>
<td></td>
</tr>
<tr>
<td>+ dimethylamphetamine</td>
<td>10</td>
<td>50</td>
<td>40</td>
<td>$k_r = k_m$</td>
<td></td>
</tr>
<tr>
<td>- amphetamine</td>
<td>10</td>
<td>—</td>
<td>90</td>
<td>$k_r &gt; k_m$</td>
<td></td>
</tr>
<tr>
<td>- methylamphetamine</td>
<td>10</td>
<td>10</td>
<td>80</td>
<td>$k_r &gt; k_m$</td>
<td></td>
</tr>
<tr>
<td>- ethylamphetamine</td>
<td>0</td>
<td>10</td>
<td>90</td>
<td>$k_r &gt; k_m$</td>
<td></td>
</tr>
<tr>
<td>- isopropylamphetamine</td>
<td>0</td>
<td>15</td>
<td>85</td>
<td>$k_r &gt; k_m$</td>
<td></td>
</tr>
<tr>
<td>- dimethylamphetamine</td>
<td>10</td>
<td>20</td>
<td>80</td>
<td>$k_r &gt; k_m$</td>
<td></td>
</tr>
</tbody>
</table>

* % of ingested dose, $k_r$ renal clearance constant, $k_m$ metabolic clearance constant.

then we would have expected some deamination of the phentermine. Since the latter is excreted essentially unchanged, it may initially be assumed that the hydrogen atom is of importance. The purpose of this investigation therefore was to determine the importance of the alkyl hydrogen with respect to dealkylation and deamination and whether its abstraction was the rate limiting step.

When proton abstraction (C–H bond rupture) is believed to be the rate limiting step in the reaction sequence between a particular molecule and an enzyme system, the use of deuterium in place of hydrogen at the suspected position of proton abstraction may demonstrate the validity of the hypothesis; that is to say, if a deuterium isotope effect can be demonstrated, the corresponding C–H bond exerts a determining role with respect to the rate limiting step. In accordance with this, the hydrogen atom in question was replaced by a deuterium atom. The occurrence of a primary deuterium effect indicates a slower rate of C–D bond rupture compared to C–H bond rupture.

RESULTS

The results obtained give clear evidence as to the involvement of the tertiary hydrogen in both the N-dealkylating and deaminating sequence. It should be recognized that, in the human body, several elimination mechanisms may mask a small deuterium effect.

The excretion rate, of the unaltered compound, which is maintained constant and maximal by maintaining the urine acidic, can be of importance. For example, when the hydrogen compound is excreted 50% unaltered and the deuterium compound is excreted 50.5% unaltered, the conclusions resulting from the cumulative excretion, may be that there is no isotope effect, whereas many observations may show a statistical significant effect. Other factors, such as the partition volume and the excretion rate of the deuterium and hydrogen compounds may be different. The partition volumes and the excretion rates both depend upon the pKa value of the compound and this value may differ maximal 0.05 between the 2 compounds. The deuterated compounds are more ionized than the hydrogen compounds at the pH of the blood (7.40). Since the nonionized form of amphetamine is reabsorbed in the renal tubulus, the deuterated compounds are, or can be, more rapidly excreted than the hydrogen compounds. If the latter is true then the difference in excretion rate must be constant during the whole period of excretion and the deuterium content of the urine samples must gradually decrease.

<table>
<thead>
<tr>
<th>Drug</th>
<th>pKa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>9.83 ± 0.01</td>
</tr>
<tr>
<td>Deuteroamphetamine</td>
<td>9.88 ± 0.01</td>
</tr>
<tr>
<td>Methylamphetamine</td>
<td>9.90 ± 0.02</td>
</tr>
<tr>
<td>Trideuteromethylamphetamine</td>
<td>9.95 ± 0.02</td>
</tr>
<tr>
<td>Isopropylamphetamine</td>
<td>10.31 ± 0.01</td>
</tr>
<tr>
<td>Deuteroisopropylamphetamine</td>
<td>10.36 ± 0.01</td>
</tr>
<tr>
<td>Deuteroisopropyldeuteroamphetamine</td>
<td>10.38 ± 0.01</td>
</tr>
<tr>
<td>Dimethylamphetamine</td>
<td>9.07 ± 0.01</td>
</tr>
<tr>
<td>Di-trideuteromethylamphetamine</td>
<td>10.08 ± 0.01</td>
</tr>
</tbody>
</table>
The retention time of the compounds on the Apiezon-KOH column reflects the lipid solubility of the compounds. The deuterium compounds tend to decrease the retention time and due to this observation it is unlikely that the lipid solubility and the related partition volume have increased.

When one is dealing with a deuterium effect in the metabolism, (N-deamination or the N-dealkylation), the enzymatic process must be slower for the deuterium compound due to the higher force constant of the C-D bond. Therefore, the gradual increase in the deuterium content to the hydrogen content in the urine at subsequent time intervals will be less, or even a decrease may be found.

In our study a continuous increase of the percentage deuterium in the samples was found indicating a necessary manifestation of the deuterium effect in the metabolism. The observation that the deuterated compound is excreted totally to a higher extent is secondary and is caused by factors other than the difference in metabolism. The increase of the percentage deuterium of the samples may be a large one, for instance with deuteroisopropylamphetamine or a small one, as with deuteroamphetamine.

(+) deuterated amphetamine vs. (+) amphetamine (Table III) (Fig. 2).

Observation of the deuterium percentage of amphetamine in the first urine sample after ingestion of both compounds (50% H, 50% D), and the deuterium content in one of the last urine samples (48 h) indicates an appropriate increase of 15% (Accuracy of the determination is ± 2%).

### TABLE III

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dosis</th>
<th>%D dosis</th>
<th>%D start</th>
<th>%D end</th>
<th>%H excr.</th>
<th>%D excr.</th>
<th>% excr.</th>
<th>% pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.G.</td>
<td>12 mg</td>
<td>50.8</td>
<td>45</td>
<td>60</td>
<td>79</td>
<td>68.9</td>
<td>10.1</td>
<td>15</td>
</tr>
<tr>
<td>F.H.</td>
<td>10 mg</td>
<td>50.1</td>
<td>50</td>
<td>68</td>
<td>71.9</td>
<td>90</td>
<td>18.1</td>
<td>18</td>
</tr>
<tr>
<td>F.H.</td>
<td>12 mg</td>
<td>50.4</td>
<td>53</td>
<td>62</td>
<td>70.7</td>
<td>88.4</td>
<td>17.7</td>
<td>9</td>
</tr>
<tr>
<td>H.F.</td>
<td>12 mg</td>
<td>50.0</td>
<td>54</td>
<td>61</td>
<td>54</td>
<td>75.3</td>
<td>20.3</td>
<td>7</td>
</tr>
</tbody>
</table>

From this data it can be concluded that there is a small deuterium effect. The composition of deuterium and hydrogen compound in the first urine samples determines the total cumulative excretion data. Foreman* has shown with deuterated amphetamine a distinct deuterium effect with respect to metabolism by homogenized rabbit liver. The rabbit and human metabolize amphetamine in the same way, but from Table I it can be seen that when the renal excretion kr is more important than the metabolic clearance km, the deuterium effect measured is small.

### TABLE IV

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dosis</th>
<th>%D dosis</th>
<th>%D start</th>
<th>%D end</th>
<th>%H excr.</th>
<th>%D excr.</th>
<th>% excr.</th>
<th>% pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>W.V.</td>
<td>7.7 mg</td>
<td>50.1</td>
<td>54</td>
<td>54</td>
<td>69</td>
<td>88</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>L.S.</td>
<td>7.0 mg</td>
<td>50.0</td>
<td>55</td>
<td>55</td>
<td>74</td>
<td>90</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>J.G.</td>
<td>7.5 mg</td>
<td>50.2</td>
<td>56</td>
<td>56</td>
<td>61.3</td>
<td>75.7</td>
<td>14.4</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 2. Renal excretion rate, urine pH, urine production and cumulative excretion of dextro-amphetamine and dextro-deuteroamphetamine. The deuterium content of the urine samples shifted from 50% in the first urine samples to 60% in the last ones (Table III). The difference in excretion rate between D- and H-amphetamine is small, due to the fact that the renal clearance of the unaltered compound is much more important than the metabolic clearance (Table I).

(+ ) trideuteromethylamphetamine vs. (+ ) methylamphetamine (Table IV)

With methylamphetamine, the deuterium content of the urine samples varies between 52 and 56% throughout the total period and essentially there is no shift in the percentage deuterium. The total amount of trideuteromethylamphetamine excreted is higher than the amount of methylamphetamine excreted.

Fig. 3. Renal excretion rate, urine pH, urine production and cumulative renal excretion of dextro-deuteroisopropylamphetamine and dextro-isopropylamphetamine. There is a remarkable deuterium effect, which begins 10 h after ingestion of the compounds.

(+)-di-trideuteromethylamphetamine vs. (+) dimethylamphetamine (Table V)

Dimethylamphetamine does not show a deuterium effect in the N-dealkylation of the first and second methyl group. The deuterium content of the urine samples varies but no deuterium shift is observed.

DEUTERIUM ISOTOPE EFFECTS IN MAN

TABLE V
DIFFERENCES IN METABOLISM AND EXCRETION BETWEEN (+) DIMETHYLMETHYLAMPHETAMINE AND (+) DITRI-DEUTEROMETHYLAMPHETAMINE

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dosis</th>
<th>%D dose</th>
<th>%D start</th>
<th>%D end</th>
<th>%H excr.</th>
<th>%D excr.</th>
<th>Δ% excr.</th>
<th>Δ% pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.G.</td>
<td>20 mg</td>
<td>50.1</td>
<td>50</td>
<td>60</td>
<td>18</td>
<td>27</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>J.B.</td>
<td>21 mg</td>
<td>50.0</td>
<td>50</td>
<td>61</td>
<td>18</td>
<td>24</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>J.H.</td>
<td>14.6 mg</td>
<td>50.1</td>
<td>50</td>
<td>57</td>
<td>6</td>
<td>9</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>M.H.</td>
<td>13.8 mg</td>
<td>50.2</td>
<td>59</td>
<td>60</td>
<td>17</td>
<td>25</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

(-- ) dimethylamphetamine and (-- ) ditrideuteromethylamphetamine.

M.H. 15 mg 50.0 55 55 18 26 8 0 5

The accuracy in %D by means of massfragmentometry is ± 5%.

TABLE VI
DIFFERENCES IN METABOLISM AND EXCRETION BETWEEN (+) ISOPROPYLAMPHETAMINE AND (+) DEUTERO-ISOPROPYLAMPHETAMINE

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dosis</th>
<th>%D dose</th>
<th>%D start</th>
<th>%D end</th>
<th>%H excr.</th>
<th>%D excr.</th>
<th>Δ% excr.</th>
<th>Δ% pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.V.</td>
<td>13 mg</td>
<td>50.0</td>
<td>48</td>
<td>90</td>
<td>13.7</td>
<td>14.2</td>
<td>0.5</td>
<td>42</td>
</tr>
<tr>
<td>J.G.</td>
<td>15 mg</td>
<td>49.5</td>
<td>48</td>
<td>89</td>
<td>9.35</td>
<td>8.75</td>
<td>2</td>
<td>41</td>
</tr>
<tr>
<td>J.S.</td>
<td>15.1 mg</td>
<td>49.5</td>
<td>48</td>
<td>82</td>
<td>8.75</td>
<td>10.14</td>
<td>3.19</td>
<td>34</td>
</tr>
<tr>
<td>J.G.</td>
<td>13 mg</td>
<td>50.0</td>
<td>48</td>
<td>90</td>
<td>5.9</td>
<td>6.5</td>
<td>2.15</td>
<td>32</td>
</tr>
<tr>
<td>C.V.</td>
<td>15 mg</td>
<td>50.0</td>
<td>48</td>
<td>80</td>
<td>12.1</td>
<td>12.4</td>
<td>0.30</td>
<td>32</td>
</tr>
</tbody>
</table>

(-- ) isopropylamphetamine and (-- ) deuteroisopropylamphetamine.

J.G. 15 mg 50.0 51 51 63.2 63.5 0.3 0 5

TABLE VII
DIFFERENCES IN METABOLISM AND EXCRETION BETWEEN (+) ISOPROPYLAMPHETAMINE AND DEUTERO-ISOPROPYL-DEUTEROA MPHETAMINE

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dosis</th>
<th>%D dose</th>
<th>%D start</th>
<th>%D end</th>
<th>%H excr.</th>
<th>%D excr.</th>
<th>Δ% excr.</th>
<th>Δ% pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.V.</td>
<td>44.1 mg</td>
<td>49.5</td>
<td>50</td>
<td>80</td>
<td>7.95</td>
<td>6.02</td>
<td>1.93</td>
<td>30</td>
</tr>
<tr>
<td>T.V.</td>
<td>43.0 mg</td>
<td>50.0</td>
<td>45</td>
<td>75</td>
<td>3.14</td>
<td>2.04</td>
<td>1.10</td>
<td>30</td>
</tr>
<tr>
<td>J.B.</td>
<td>42.3 mg</td>
<td>51.3</td>
<td>40</td>
<td>77</td>
<td>1.70</td>
<td>1.25</td>
<td>0.45</td>
<td>37</td>
</tr>
</tbody>
</table>

(-- ) isopropylamphetamine and (-- ) deuteroisopropyldeuteroamphetamine.

F.H. 41.6 mg 50.0 52 54 88 76 12 2 5

H.H. 40.4 mg 50.1 51 52 82 80 2 1 5

(+ ) deuteroisopropylamphetamine vs. (+ ) isopropylamphetamine (Table VI) (Fig. 3)

This compound elicits a rather large difference between the hydrogen and deuterium compound. The deuterium content increases from 48% to 90% in all the subjects, but in some subjects the deuterium shift begins or is measurable only 10 h after ingestion. When the urine is acidic, (pH 5) the total amount of isopropylamphetamine excreted in the first 10 h determined the total cumulative excretion and for this reason the differences between deuterium and hydrogen cumulative excretion are very small, in spite of the great deuterium shift. The dextro-isomer shows this effect, but the levo-isomer does not. From Table I it can be seen that for the dextro-isomer the metabolic processes of elimination are much more important than the renal excretion and that with levo-isopropylamphetamine the relationship between renal and metabolic clearance is reversed.

Fig. 4. Renal excretion rate, urine pH, urine production and cumulative renal excretion of dextro deuteroisopropldeuteroamphetamine and dextroisoproplamphetamine. There is a deuterium-effect of the same magnitude as mentioned in Fig. 3.
According to the results with deuteroisopropylamphetamine it was expected that the half-life time of deuteroisopropyldeuteroamphetamine would be doubled. The experiments, on the contrary, do not show a further increase in T 1/2 of the dideuteroisopropylamphetamine. The reason for this may possibly be the following:

The purpose of the experiments was to prove that both tertiary hydrogen atoms in isopropylamphetamine are chemically identical for the enzyme. Therefore they were replaced by deuterium atoms in order to show that the hydrogen atoms in question are involved and are rate limiting. We have compared H-isopropyl-with D-isopropylamphetamine and found an increase in T 1/2 from 2 h to 4 h for the deuterium compound. From above experiments 2 hypotheses may follow:

_Hypothesis I._ The enzymes deaminate and dealkylate the H- and D-isopropylamphetamine with the same affinity, thus, the difference in excretion rate and T 1/2 is caused by the C-D bond rupture.

_Hypothesis II._ The enzyme can perform one metabolic action with the compound and after that action the compound leaves the enzyme action site.

If both dealkylation and deamination occurred at the same time, no amphetamine would be found as metabolite. When two different enzymes are involved, then the dideuterated isopropylamphetamine must be metabolized at a slower rate than the monodeuterated isopropylamphetamine. When we are comparing isopropylamphetamine with the dideuteroisopropylamphetamine and take into account hypothesis II, then it does not matter whether the C-D bond of the amphetamine moiety is broken or the C-D bond of the isopropyl moiety is broken. When the isopropyl group has been removed, the metabolite shows the normal deuterium effect.

**EXPERIMENTS**

**Synthesis**

Deuterated dextro- and levo-amphetamine were synthesized according to Foreman. The rotations of the d- and l-deuterated amphetamine found were: \((\alpha)_{D} = +29.0^\circ C = 1.995\) in water (d-tartrate salt) and \((\alpha)_{D} = -28.0^\circ C = 2.005\) in water (l-tartrate salt).

_Di-trideuteromethylamphetamine._ 0.01 mole of d-amphetamine was treated with 0.1 mole deuterated formic acid and 0.05 mole of trideuteroformaldehyde. The reaction was followed by means of gas chromatography-mass spectrometry. Completion of reaction was evidenced by disappearance of the basepeak at 72 (H-dimethylamphetamine) and the appearance of the base peak 78 (D-dimethylamphetamine). The levo isomer was prepared in a similar manner.

_Trideuteromethylamphetamine._ 500 mg of the di-trideuteromethylamphetamine was oxidized by a tenfold excess of an alkaline permanganate solution and the reaction was followed by GLC-MS until the ditrideuteromethylamphetamine disappeared.

_Deuteroisopropylamphetamine._ 2 g of d-amphetamine were refluxed with 150 ml of aceton and 5 g of anhydrous magnesium sulphate. The reaction was followed gaschromatographically. The aceton was removed by vacuum and to the concentrated Schiff base 3 ml of D_2O and sufficient sodiumborodeuteride were added in small portions under reflux. The reduction was followed continuously by GLC-MS and stopped when the base peak of the Schiff base in the mass spectrum (m = 84) had dissappeared.
The mass spectrum of the deuteroisopropylamphetamine showed a base peak at m = 87. The levo isomer was prepared in the same way.

Deuteroisopropyl-deuteroamphetamine. 1 g of d-deuteroamphetamine is refluxed with 150 ml of acetone and 5 g of anhydrous magnesium sulphate. Due to the magnesium sulphate the yield of the reaction is quantitative.

The excess of acetone is removed under vacuum and sodiumborodeuteride and D₂O was added in small portions. The reduction was followed by GLC-mass spectrometry.

Full details of the reactions will be published elsewhere¹³.

METHODS

Apparatus
Gaschromatograph H P 402 with flame ionisation detection. Glass column, 1.80 m, 3 mm diameter, filled with 20% Apiezon L 5% KOH Diatoport-S-60-80 mesh. Oven temperature 160°, temperature injection block 200° and temperature detector block 220°. Nitrogen flow 30 ml/min, hydrogen flow 40 ml/min and air flow 300 ml/min.

Gaschromatograph-massspectrometer LKB 9000.
Glass column 1.20 m, 3 mm diameter, filled with 20% Apiezon L 5% KOH Diatoport-S-60-80 mesh. Oven temperature 140°, injection block 200° separator 220° and ion source temperature 250°.
Ionisation energy 70 eV, trapcurrent 60 mA, accelerating voltage 3.5 kV.

Detection methods
The concentration of the amphetamines in the urine of the subjects was measured by means of the gaschromatographic analysis with the internal standard method. The same samples were injected into the LKB 9000 system in order to determine the percentage of deuterium of the sample.

Determinations
Mixtures of equal amounts of amphetamine and deuterated amphetamine were ingested as HCl salts by male subjects (Table III-VII).

The urine was kept acidic, pH 5.0 ± 0.2, by the ingestion of 1.6 g of ammonium chloride four times a day. Each amount of urine was collected for 60 h or until such time that the excretion rate was 1% of the maximum excretion rate. The pH of the urine sample was measured upon receipt with a Copenhagen Radiometer. The average urine production was measured and the curves of average renal excretion rate were constructed. To 10 ml of the urine, 10 μg of the internal standard N-methyl benzylamine was added and the solution made alkaline to pH 13 with KOH pellets. Then the alkaline solution was extracted twice with 10 ml of freshly distilled ether, the combined ether layers extracted with 5 ml 2 N HCl. The acidic layer was made alkaline to pH 13 and extracted twice with 10 ml of ether. The combined ether layers were evaporated under a mild stream of dry air until 100 μl remained. 5–8 μl was injected into the gaschromatograph HP 402 and into the LKB 9000.

Detection methods
In order to be able to determine the ratio of deuterated–non deuterated amphet-

amine in a mixture of these compounds, we used the mass spectrum of this mixture, taking the ratio of the base peaks as a measure for the ratio of concentrations in the mixture. A calibration curve was made using mixtures of D- and H-amphetamines of known composition. The masses examined were: amphetamine-deuteroamphetamine, line intensities $\text{m/e}^+ 44$-$45$ methylamphetamine-trideuteromethylamphetamine, line intensities $\text{m/e}^+ 58$-$61$ isopropylamphetamine-deuteroisopropylamphetamine, line intensities $\text{m/e}^+ 86$-$87$ isopropylamphetamine-deuteroisopropyldeuteroamphetamine, line intensities $\text{m/e}^+ 86$-$88$ dimethylamphetamine-ditrideuteromethylamphetamine, mass fragmentometry ratio of peak areas of the masses 72 and 78.

For the calibration curves, line intensities at various concentrations in the gaschromatographic peak were measured. The average ratio of the line intensities was used. This method is allowed when the retention times of the H- and D-amphetamine are equal. When there is a difference in retention time, as is the case with dimethylamphetamine, the method of mass fragmentometry must be followed. For the other compounds both methods are followed but give the same calibration curve. The accuracy of the estimation of the percentage deuterium in the samples is $\pm 2\%$.

An example of a calibration curve is given in Fig. 5.

![Calibration curve of percentage deuterium isopropylamphetamine and the ratio of line intensities of the base peaks $\text{m/e}^+ = 86$ and $\text{m/e}^+ = 87$.](image)

**Fig. 5.** Calibration curve of percentage deuterium isopropylamphetamine and the ratio of line intensities of the base peaks $\text{m/e}^+ = 86$ and $\text{m/e}^+ = 87$.

**Determination of $pK_a$ value of some amines by means of second differential titration curves**

$\text{pH}$ measurements were carried out with Radiometer equipment, consisting of a $\text{pH}$ meter type PHM 26, equipped with a scale expander, a glass electrode type G 202 B combined with a calomel electrode, type K 401. The system was calibrated using standard buffers: for iso-$\text{pH}$-adjustment: 0.025 $M \text{KH}_2\text{PO}_4$ (3.40225 g/l) and 0.025 $M \text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$ 12 (8.95210 g/l) yielding: $15^\circ \text{pH} 6.900$

$20^\circ \text{pH} 6.881$

$25^\circ \text{pH} 6.865$

for electrode sensitivity adjustment: 0.05 M KHptalate (10.21150 g/l) with
pH = 3.999 at 15°
4.002 at 20°
4.008 at 25°
The titration vessel was thermostated at 22° and contained about 4 ml solution. The
titrant was carbonate free KOH 0.100 N (Titrisol, Merck). A microburette (max. 
volume 5 ml) was connected with a fine capillary, dipping into the solution. The plun-
ger type burette had a accuracy of about 0.1% per 1 ml titrant. The solution was stir-
red with a magnetic stirrer and kept under nitrogen. 0.1 mmole of the amine HCl salt 
was dissolved. The end point of the titration was determined by differentiating the 
 pH curve and taking d²pH/dV² in which V is the added volume titrant. The pKa 
value was determined by interpolating in the pH curve.

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