BIOCHEMICAL STUDIES ON DIPHENHYDRAMINE
(BENADRYL*)

II. DISTRIBUTION IN TISSUES AND URINARY EXCRETION†

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Benadryl has been used extensively as an antihistamine agent, but little is known concerning its mode of action or metabolic fate. It is rapidly absorbed, as shown by earlier blood level studies (1-3) and the fact that clinical effects are observed in less than an hour (4). This paper presents observations on the distribution of Benadryl in the rat, chosen for its resistance to the action of histamine, and in the guinea pig, which is well known for its sensitivity to histamine. In addition, evidence is presented for the presence of a small amount of unaltered Benadryl in urine.

EXPERIMENTAL

Distribution of Benadryl in Rat—Benadryl hydrochloride in aqueous solution was administered subcutaneously to a series of rats which had not been fed for 18 hours, with a dosage of 2 mg. per 100 gm. of weight. Groups of three rats were sacrificed at intervals of 1, 2, 4, and 6 hours after administration of the drug. The tissues were removed immediately after death and frozen at -20°. Analyses were usually completed within a few days. Tissues were homogenized and extracted with heptane, and the Benadryl was transferred by extraction to acid and finally to ethylene dichloride for colorimetric analysis (5). All results were corrected for normal tissue blanks. The three analyses for each time interval were averaged, with the results presented in Fig. 1. By far the highest concentration of Benadryl was found in lung tissue. Spleen was next highest, followed by kidney, brain, liver, and muscle. Peak concentrations were observed in 1 to 2 hours after administration of the drug.

Other routes of administration were studied, with the results shown in Table I. In this series of experiments, the rats were given 0.5 mg. of Benadryl hydrochloride per 100 gm. of weight by the routes indicated in Table I, and the animals were sacrificed 30 minutes after administration.

* Benadryl hydrochloride, registered trade name for diphenhydramine hydrochloride.
† Reported in part at a meeting of the Federation of American Societies for Experimental Biology, March, 1948.
Fig. 1. Distribution of Benadryl in rat tissues. 2 mg. of Benadryl hydrochloride per 100 gm. of weight were administered subcutaneously. Each point represents the average results from three animals. Concentrations are expressed as micrograms of Benadryl base per gm. of tissue, corrected for normal tissue blanks.

Table I

Benadryl Concentration in Rat Tissues

0.5 mg. of Benadryl hydrochloride per 100 gm. of body weight was administered in aqueous solution by the routes indicated. The animals were sacrificed after 30 minutes, and the tissues analyzed for Benadryl. The figures express Benadryl concentrations as micrograms of free base per gm. of tissue.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Oral</th>
<th>Intraperitoneal</th>
<th>Subcutaneous</th>
<th>Intravenous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>0.6</td>
<td>4.6</td>
<td>5.7</td>
<td>13.7</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.9</td>
<td>7.7</td>
<td>3.8</td>
<td>11.3</td>
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<tr>
<td>Brain</td>
<td>0.1</td>
<td>2.0</td>
<td>2.2</td>
<td>8.7</td>
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<tr>
<td>Liver</td>
<td>1.5</td>
<td>1.5</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Heart</td>
<td>0.6</td>
<td>1.3</td>
<td>1.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Red cells</td>
<td>0.8</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

of the drug. The analyses again showed high concentrations of Benadryl in the lungs and spleen when Benadryl was administered parenterally. Oral administration resulted in elevated levels in the liver and spleen.
Fig. 2. Distribution of Benadryl in guinea pig tissues. 2 mg. of Benadryl hydrochloride per 100 gm. of weight were given subcutaneously. Each point represents the average results from three animals. Concentrations are expressed as micrograms of Benadryl per gm. of tissue, corrected for normal tissue blanks.

Table II

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Oral</th>
<th>Subcutaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>14.9</td>
<td>76.6</td>
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<tr>
<td>Spleen</td>
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<td>19.0</td>
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<td>Brain</td>
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<tr>
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<tr>
<td>Muscle</td>
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<tr>
<td>Heart</td>
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<td>6.7</td>
</tr>
<tr>
<td>Plasma</td>
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<td>1.7</td>
</tr>
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<td>Erythrocytes</td>
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<td>1.3</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td>1.9</td>
</tr>
</tbody>
</table>

Distribution of Benadryl in Guinea Pig—The distribution of Benadryl was also investigated in the guinea pig. A series of animals (average weight 370 gm.) was injected subcutaneously with 2.0 mg. of Benadryl.
hydrochloride per 100 gm. of weight. The animals were sacrificed in groups of three at 1, 2, 4, and 6 hours after administration of the drug. Tissues were analyzed for Benadryl, as described earlier (5), with the results presented in Fig. 2.

It will be seen from this experiment that the order of tissue concentrations of Benadryl is similar to that observed in the rat. Although the same dosage of Benadryl per unit weight of animal was administered to

![Graph](image)

**Fig. 3.** Counter-current analysis of human urine for Benadryl. Urine extracts were run through the twenty-four plate Craig apparatus, with equal volumes of heptane and 0.135 M phosphate buffer at pH 6.13 for the counter-current extraction. The total organic base in each plate was determined by the methyl orange reaction. A pure sample of Benadryl hydrochloride was analyzed in the same manner.

the rat (Fig. 1) and guinea pig (Fig. 2), the latter showed from 2 to 3 times the concentration of Benadryl found in rat tissues.

The differences between the oral and subcutaneous routes of administration in the guinea pig were also compared. A series of guinea pigs was given 1.0 mg. of Benadryl hydrochloride per 100 gm. of weight by stomach tube or by subcutaneous injection, and the animals were sacrificed 1 hour later. Tissues were analyzed for Benadryl concentration, with the results shown in Table II. Here again, as in the rat, the concentration of Benadryl in lungs and spleen remains high, regardless of the route of administration.

*Urinary Excretion of Benadryl*—Numerous studies have been made on the urinary excretion of Benadryl, indicating that 5 to 15 per cent of the
total dose is excreted (2, 3, 6) in 24 hours. Evidence is presented elsewhere (7) that non-basic degradation products are also present. The work described here demonstrates the presence of unaltered Benadryl in urine.\footnote{Since this work was completed, a paper by Hald (6) described the formation of a silicotungstic acid derivative of Benadryl from urinary sources.}

The identity of Benadryl in urine was established by counter-current extraction with the Craig technique (8), and by the ultraviolet absorption spectrum of the purified material. 750 ml. of urine were obtained from normal human subjects given 50 mg. of Benadryl hydrochloride orally a few hours prior to urine collection. The urine was made alkaline by the addition of NaOH and extracted with an equal volume of heptane (5). The heptane was shaken with 50 ml. of 0.1 N HCl, which was then separated, made alkaline, and reextracted with 9 ml. of heptane. 8 ml. of this extract were fractionated in a 24 plate Craig counter-current apparatus, with equal volumes of heptane and 0.135 M phosphate buffer at pH 6.13. After the counter-current extraction, 1.5 ml. of $\frac{1}{3}$ HCl were added to each plate and the mixture shaken, the Benadryl being transferred completely to the aqueous phase. Aliquots of the aqueous phase were made alkaline and extracted into ethylene dichloride for colorimetric analysis by the methyl orange procedure (5). A counter-current extraction was also performed with a known sample of Benadryl hydrochloride under the same conditions. The results are presented in Fig. 3.

The close correspondence of the peaks in Fig. 3 is strong presumptive evidence that they are due to the same substance (8). When examined for ultraviolet absorption with a Beckman spectrophotometer, the samples in the middle of the series (Plates 9 through 14) had a peak absorption at 258 m\(\mu\), with a minor peak at 253 m\(\mu\), identical with that of a known sample of Benadryl.\footnote{Spectroscopic examinations were made by Dr. J. M. Vandenbelt of the Research Laboratories, Parke, Davis and Company.} The presence of other organic bases more water-soluble than Benadryl is demonstrated by the methyl orange color reaction towards the end of the series.

DISCUSSION

The high concentration of Benadryl in lung and spleen is of great interest because of the role played by these organs in anaphylactic shock. There is not sufficient evidence to say that Benadryl accumulates at "receptor sites" in competition with histamine, although that possibility should not be overlooked. However, analysis of guinea pig skin (Table II) gave low concentrations of Benadryl, even though the drug is known to affect cutaneous reactions attributable to histamine (9). Also, brain
tissue, which normally has very little histamine present (10), showed fairly high concentrations of Benadryl on analysis (Figs. 1 and 2). The accumulation of Benadryl in various tissues apparently produces no pathological changes or blood dyscrasias (9), and toxic effects are promptly relieved by discontinuance of the drug, regardless of severity (11).

The concentration of Benadryl in the tissues of the rat and guinea pig showed significant differences (Figs. 1 and 2). In the rat, the level of Benadryl was one-half to one-third that shown by the guinea pig. This is perhaps better accounted for by the greater enzymatic activity of rat kidney and lung tissue than by differences in the rate of absorption or excretion of the drug (12).

We are especially indebted to Dr. A. C. Bratton, Dr. D. A. McGinty, and Dr. Graham Chen for their help and advice during the course of this work.

**SUMMARY**

Comparative data are presented on the levels of Benadryl in rat and guinea pig tissues at different time intervals following administration of the drug. After subcutaneous injection, the highest concentrations were found in the lungs, with progressively lower concentrations in the spleen, kidney, brain, liver, and muscle tissue. Peak concentrations were found in about 1 hour, with a fairly rapid drop towards normal levels in 6 hours.

The presence of a small amount of unaltered Benadryl was demonstrated in human urine by means of counter-current solvent extraction and ultraviolet absorption characteristics.

**BIBLIOGRAPHY**

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