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# STUDIES ON THE DISTRIBUTION AND EXCRETION OF A METABOLITE OF GUANETHIDINE IN THE RAT

BY

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Guanethidine is an adrenergic neurone blocking drug which is used clinically as a hypotensive agent. Although a number of studies have been made of the distribution and excretion of guanethidine in the rat and in man (Dollery, Emslie-Smith & Milne, 1960; Kuntzman, Costa, Gessa & Brodie, 1962; Schanker & Morrison, 1965), very little is known about its metabolism. Evidence that guanethidine is metabolized by the rat was provided by Bisson and Muscholl (1962) who showed that rat urine and heart extracts contained a single metabolite as well as unchanged guanethidine. This metabolite was not isolated and no detailed studies of its formation, distribution or excretion have been reported.

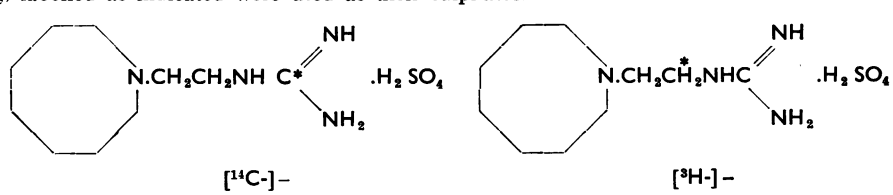
Because guanethidine has been used to study adrenergic mechanisms, the existence of a metabolite (or metabolites) could be of great significance in interpreting its mode of action. It therefore seemed highly desirable to clarify the metabolism of guanethidine.

Preliminary work on the formation of a polar metabolite by the supernatant fraction obtained when homogenates of rat and rabbit liver were centrifuged at 14,000 *g* and by microsomal preparations from the same tissues has recently been reported (Furst, 1967). The present paper describes the distribution and excretion in rats of the metabolite produced after the parenteral administration of isotopically labelled guanethidine. The results of previous workers on the distribution of guanethidine itself have also been extended by autoradiographic studies and tissue analysis.

## METHODS

(a) *Chemical*

[$^{14}$C-]-guanethidine (specific activity, 3.14 $\mu$c/mg) and [$^{3}$H-]-guanethidine (specific activity, 22.0 $\mu$c/mg) labelled as indicated were used as their sulphates.




[$^{14}$C-]– [$^{3}$H-]–

* denotes position of label

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The purity of both labelled compounds was established by their melting points (248°–250° C), infra-red spectra and by chromatography in two solvent systems. In isopropanol: concentrated ammonia: water (200:20:30) (System A) the $R_F$ value was 0.85 and in *n*-butanol: glacial acetic acid: water (120:30:50) (System B) 0.69, both of which values corresponded with an authentic sample of guanethidine. The chromatograms were run with descending development on Whatman No. 4 paper.

The percentages of guanethidine and metabolite in body fluids and tissue extracts were determined either by paper chromatography or a solvent extraction method. In the chromatographic procedure which was essentially that described by Bisson and Muscholl (1962) the two compounds were separated on paper-strips (25 cm × 1 cm) in system A and their relative amounts estimated by liquid scintillation counting.

In the solvent extraction method, described by Schanker and Morrison (1965), which has the advantage of greater sensitivity at low levels of radioactivity, tissue extracts were made strongly alkaline and counted before and after extraction with ten volumes of chloroform. By this procedure the guanethidine was removed into the chloroform phase while the metabolite remained in the aqueous alkaline residue. In recovery experiments from a number of tissues using a concentration of guanethidine of 0.3 μg/ml., 90–100% of the radioactivity could be removed in a single extraction.

(b) *Autoradiography*

The sections for whole-body autoradiography were prepared according to the method of Ullberg (1962) from albino mice (20 g) which had received [$^{14}$C-]-guanethidine sulphate (50 mg/kg) by intraperitoneal injection. The animals were killed with chloroform and frozen in a mixture of acetone and solid $CO_2$ (−70° C). The frozen animals were then covered with a 10% suspension of Sephadex G-10 which was allowed to set in the deep-freeze (−20° C). Sections 40 μ thick were cut on a base-sledge microtome (Leitz) and the exposure time was 35 days on Gevaert Fino X-ray film.

(c) *Distribution and Excretion Studies*

Male Wistar rats (200–300 g) received [$^{14}$C-]- or [$^{3}$H-]-guanethidine sulphate (20 mg/kg) by intraperitoneal injection of an aqueous solution (0.2–0.3 ml.). Three animals were killed with chloroform at each time interval. Blood was collected from the heart and inferior vena cava into centrifuge tubes containing heparin. Urine and faeces were separated in metabolic cages.

Tissues were quickly removed, rinsed, dried between sheets of paper tissue, weighed and homogenized with four to ten volumes of a 50:50 mixture of 5% trichloroacetic acid and 0.1 N-HCl, first in the Ultra-Turrax (Janke and Kunkel KG., Staufen i. Br., W. Germany) then with a Teflon pestle tissue-grinder. After homogenization the tissues were allowed to stand for 30 min before centrifuging at 11,000 *g* for 15 min to give a clear supernatant suitable for assay. The recoveries of added guanethidine (1 to 2 μg/ml.) taken through the same procedure ranged from 89% to 106%. Radioactivity was measured by a Packard liquid scintillation spectrometer.

(d) *Counting Procedure*

Aliquots (usually 0.1 ml.) of urine, plasma or tissue extracts were added to counting vials and dissolved in 5 ml. absolute ethanol and 15 ml. of a scintillator solution which contained BBOT (CIBA) 4 g/l. of toluene. Quenching was corrected for by the internal standard method.

For the assay of paper-strip chromatograms 1-cm squares were placed at the bottom of the counting vials and 1 ml. absolute ethanol and 10 ml. of scintillator solution added.

## RESULTS

### *Autoradiography*

The whole-body autoradiograph in Fig. 1 was prepared from a mouse killed 30 min after the injection of [$^{14}$C-]-guanethidine. It shows high concentrations of radioactive material in several tissues which have not previously been examined for the presence

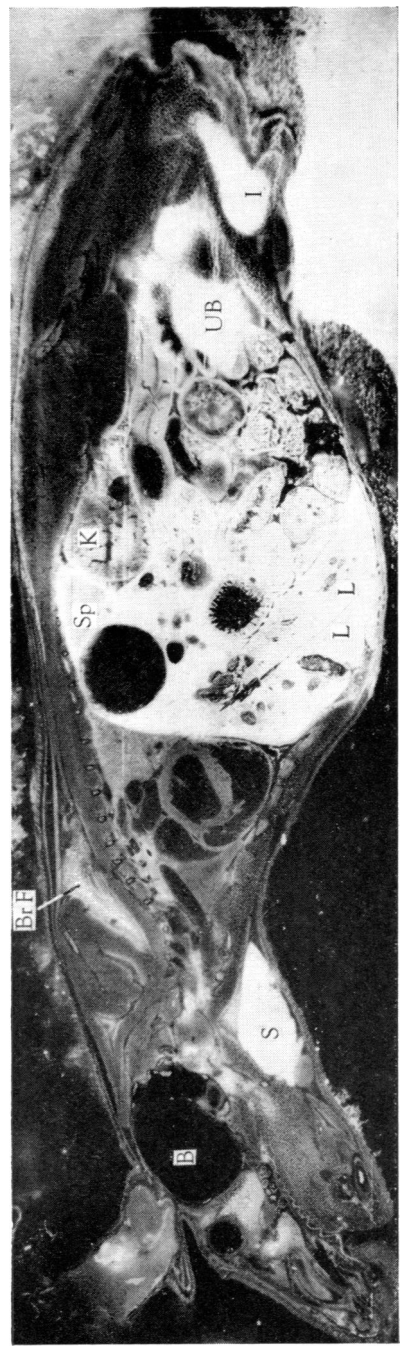


Fig. 1. Autoradiograph of a sagittal section of a mouse 30 min after guanethidine sulphate (50 mg/kg, I.P.). The light areas indicate the presence of radioactive material (guanethidine + metabolite). B, brain ; S, salivary gland ; Br.F., brown fat ; L, liver ; Sp, spleen ; K, kidney ; UB, urine bladder ; I, site of injection.

of guanethidine. The most prominent of these are the salivary glands and brown fat in the interscapular region, while smaller but still significant amounts of radioactive material can be seen below the orbit and in the nasal mucosa. High concentrations of radioactivity can also be seen in the liver, gastro-intestinal tract, spleen, kidney, urinary-bladder and at the site of injection.

The whole-body autoradiograph in Fig. 2 was prepared from a mouse killed 3 hr after the injection of [$^{14}$C-]-guanethidine. This median section shows clearly that neither guanethidine nor its metabolite have penetrated the blood-brain barrier. This finding agrees with that of Kuntzman *et al.* (1962) who showed that only trace amounts of guanethidine entered the rat brain after acute intravenous administration.

At this time when absorption from the site of injection was virtually complete the level of radioactivity in the heart wall seemed surprisingly low compared with that in the liver, gastro-intestinal tract or salivary glands. This is in contrast to the high affinity for the drug of the rat heart reported by other workers (Kuntzman *et al.*, 1962 ; Schanker & Morrison, 1965) and also in the present communication.

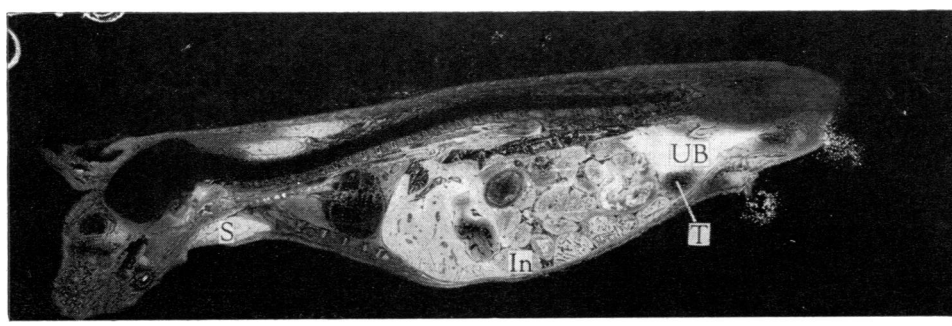


Fig. 2. Autoradiograph of a median section of a mouse 3 hr after guanethidine sulphate (50 mg/kg I.P.). The light areas indicate the presence of radioactive material (guanethidine + metabolite). No radioactive material is to be seen in the brain or spinal cord. S, salivary gland ; In, intestines ; T, testis ; UB, urine bladder.

*Distribution in Rat Tissues*

The results of the tissue distribution studies are given in Table 1. The tissues selected for the initial study were the heart, liver, kidneys and plasma and the percentage of the total radioactivity caused by the metabolite in these tissues were determined after chromatographic separation. In the case of the plasma the level of radioactivity was too low to estimate accurately the amount of metabolite present. The values obtained for the total radioactivity, however, provide a useful comparison with the tissue levels.

Later the level of metabolite in several other tissues was determined by the solvent extraction procedure. These tissues included the submaxillary glands, interscapular brown fat and three different skeletal muscles in order to compare the uniformity of distribution throughout this tissue.

TABLE 1

MEAN TISSUE CONCENTRATIONS OF GUANETHIDINE AND METABOLITE OBTAINED AFTER GUANETHIDINE SULPHATE (20 mg/kg I.P.)

The values for the heart, liver, kidney and plasma were obtained from one group of animals, those for the three skeletal muscles from a second group and those for the submaxillary gland and brown fat from a third group. Three animals were killed at each time interval. For the brown fat assays the tissues from all three rats were pooled because of the small amount of tissue available. The value (G) on the left of each column is the concentration of guanethidine (±S.E.M.) and that on the right (M) is the concentration of metabolite (±S.E.M.). n.m., not measured; * Total value only (guanethidine+metabolite).

| | Time (hr) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 12 |
| | Tissue conc. (μg/g) calc. as guanethidine sulphate | | | | | | |
| Tissue | G M | G M | G M | G M | G M | G M | G M |
| Heart | 21·4 (±0·1) 6·7 (±0·6) | 20·9 (±2·0) 9·4 (±0·3) | n.m. | 13·1 (±0·7) 7·3 (±0·4) | n.m. | 18·5* (±0·2) | n.m. |
| Liver | 12·8 (±2·0) 20·8 (±0·6) | 23·2* (±1·8) | n.m. | 4·7 (±0·0) 5·8 (±0·6) | n.m. | 10·0* (±0·3) | n.m. |
| Kidney | 11·0 (±0·4) 11·0 (±0·4) | 5·9 (±0·4) 7·9 (±0·7) | n.m. | 2·9 (±0·2) 3·3 (±0·1) | n.m. | 5·5* (±0·2) | n.m. |
| Submax. gl. | 21·2 (±1·0) 16·7 (±0·9) | n.m. | 12·9 (±0·6) 9·4 (±0·8) | n.m. | 10·5 (±0·7) 7·6 (±0·1) | n.m. | n.m. |
| Br. Fat | 10·1 9·7 | n.m. | 15·3 4·7 | n.m. | n.m. | n.m. | n.m. |
| Quadriceps M. | n.m. | n.m. | n.m. | n.m. | n.m. | 3·5 (±0·2) 1·3 (±0·0) | 1·3 (±0·2) 0·6 (±0·1) |
| Triceps M. | n.m. | n.m. | n.m. | n.m. | n.m. | 2·3 (±0·1) 1·5 (±0·3) | 2·0 (±0·1) 0·7 (±0·2) |
| Pectoralis M. | n.m. | n.m. | n.m. | n.m. | n.m. | 2·7 (±0·1) 1·6 (±0·0) | 1·4 (±0·1) 0·5 (±0·1) |
| Plasma (μg/ml.) | 3·9* (±0·0) | 2·7* (±0·1) | n.m. | 1·3* (±0·1) | n.m. | 0·9* (±0·1) | n.m. |

The results of earlier workers (Bisson & Muscholl, 1962; Kuntzman *et al.*, 1962; Schanker & Morrison, 1965) have shown that guanethidine is concentrated in the tissues with respect to the plasma. From the results in Table 1 it is evident that this is true also for the metabolite. While the level of metabolite approached that of guanethidine in the submaxillary glands and was higher in the liver and kidney the metabolite seemed to have a lower affinity for heart and skeletal muscle than did guanethidine.

In the three skeletal muscles the distribution of both guanethidine and the metabolite was relatively uniform. Thus after 6 hr the concentration of guanethidine ranged from 2.3 to 3.5 $\mu$g/g and after 12 hr from 1.3 to 2.0 $\mu$g/g while the metabolite concentration (expressed as guanethidine sulphate) at the same time intervals ranged from 1.3 to 1.5 $\mu$g/g and 0.5 to 0.7 $\mu$g/g. The levels of guanethidine indicated that this tissue as a whole could retain a significant proportion of the drug concentrated in the tissues.

*Rat Urinary Excretion*

After the injection of [$^{14}$C-]-guanethidine (20 mg/kg intraperitoneally) into the rat, radioactivity rapidly appeared in the urine. A paper-strip chromatogram revealed a large amount of metabolite ($R_F$ 0.35) and a smaller amount of unchanged guanethidine (Fig. 3).

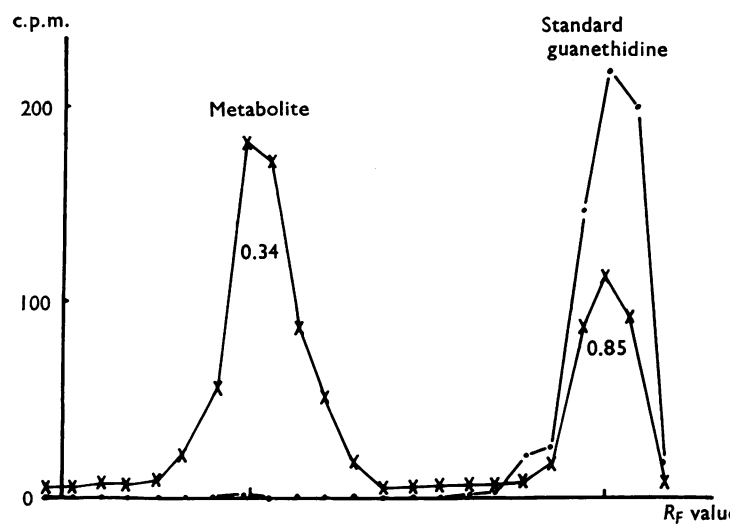


Fig. 3. Paper strip chromatogram of rat urine collected 4 hr after guanethidine sulphate (20 mg/kg I.P.). The distribution of radioactivity along the strip (c.p.m.) is compared with that of a reference sample of guanethidine run under identical conditions. The sample was run on Whatman No. 4 paper (25 cm × 1 cm) in system A with descending development. ×——×, Urine; ●——●, authentic guanethidine.

The time course for the excretion of guanethidine and the metabolite was followed in two rats after the intraperitoneal administration of guanethidine (20 mg/kg). The relative amounts of guanethidine and of metabolite were determined chromatographically and the results are shown in Fig. 4.

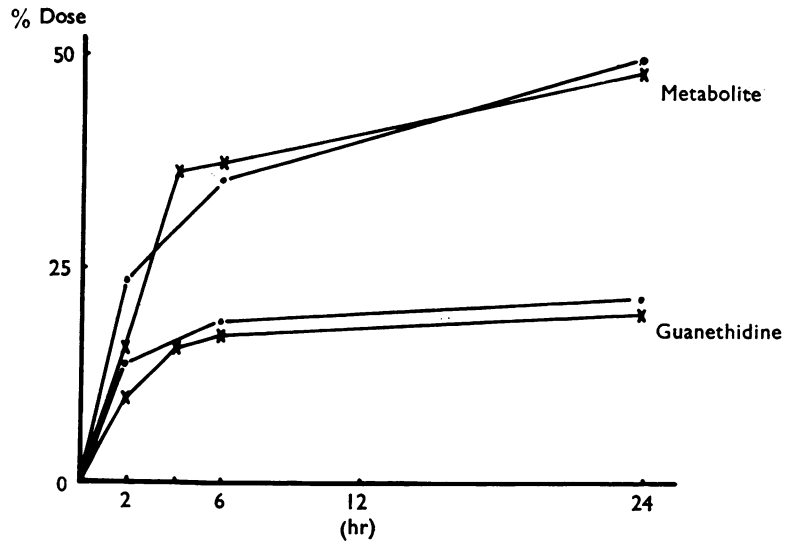


Fig. 4. Cumulative urinary excretion pattern of guanethidine and its metabolite obtained by chromatographic analysis of the urine of two rats following guanethidine sulphate (20 mg/kg I.P.).

These data show that guanethidine was rapidly metabolized and excreted after injection. Up to 39% of the total dose appeared in the urine within 2 hr. After 24 hr approximately 70% of the original dose had been excreted and of this about 70% was metabolized.

## DISCUSSION

The present results show that guanethidine is rapidly and extensively metabolized after injection in the rat. The examination for radioactivity of paper chromatograms of extracts of tissue and urine from rats given radioactive guanethidine showed in addition to unchanged guanethidine one other well defined peak which suggested the presence of a single metabolite as reported earlier by Bisson & Muscholl (1962).

Like guanethidine the metabolite was concentrated in all the tissues examined and this must be a significant contributory factor to the marked decline in the cumulative urinary excretion of both compounds that occurred between 4 and 6 hr after injection.

In this regard skeletal muscle seems capable of providing a large drug depot because it accounts for 40% to 45% of the total body weight of the rat. Assuming a relatively uniform distribution throughout this tissue it seems quite possible from the levels in the three different muscles examined that skeletal muscle alone could account for more than 25% of the total radioactivity remaining in the body 2 or more hr after injection.

The only two tissues in which the metabolite occurred in greater concentration than guanethidine were the liver, which is known to metabolize guanethidine (Furst, 1967) and the kidney. Significant concentrations of the metabolite were however also found in the heart, submaxillary glands and brown-fat, all of which have a rich sympathetic innerva-

tion. The level of metabolite in these tissues also fell more slowly than in the liver and kidney. This effect was most marked in the heart where the concentration of metabolite varied little between 1 and 4 hr after injection.

This is similar to the pattern exhibited by guanethidine itself as observed both in the present data and from that of Schanker & Morrison (1965). It is therefore tempting to speculate that the metabolite may be taken up by sympathetic nerve-endings as is guanethidine (Chang, Costa & Brodie, 1965; Boullin, Costa & Brodie, 1966). Guanethidine has been shown to block the sympathetic-neurones (Day, 1962) and to release catecholamines (Nash, Costa & Brodie, 1964) in *in vitro* experiments where no metabolite seems likely to have been present. Nevertheless the biochemical and pharmacological properties of the metabolite may be of importance.

SUMMARY

1. The metabolism of radioactive guanethidine has been studied in the rat.

2. On the injection of guanethidine sulphate (20 mg/kg intraperitoneally) the drug was rapidly and extensively metabolized. Approximately 70% of the dose was eliminated in the urine within 24 hr, mainly as a polar metabolite.

3. The metabolite was also found in varying amounts in all the tissues examined including the heart, submaxillary gland and brown-fat, which have a high content of catecholamines.

4. Both guanethidine and the metabolite were concentrated in the tissues with respect to the plasma.

5. The possible role of skeletal muscle as a drug-depot is discussed.

I wish to acknowledge the valuable technical assistance of Mr. T. Lowe.

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