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A COMPARATIVE AUTORADIOGRAPHIC STUDY ON THE DISTRIBUTION OF DEPTROPINE CITRATE AND DEPTROPINE METHIODIDE IN MICE

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Abstract—The distribution patterns of deptropine citrate-*N*-$^{14}CH_3$ and deptropine methiodide-*N*-$^{14}CH_3$ were studied in female mice by means of a macroautoradiographic technique.

The distribution of deptropine, both after oral and intraperitoneal administration shows a number of aspects which seem to be common for a group of structurally related basic drugs: a large volume of distribution of the radioactivity with highest levels in glandular tissue, lungs, lymphoid tissue, bone marrow, bile and specified zones in kidney and adrenals. The concentration of radioactivity in the brown fat and the high and prolonged level of radioactivity in the liver are related to *N*-demethylation and/or hydrolysis of deptropine-*N*-$^{14}CH_3$.

Compared with structurally related compounds studied earlier the deptropine radioactivity penetrates into the brain only to a small extent; remarkable are concentrations in the choroid plexus of the lateral and fourth ventricles, and sometimes in the walls of the former.

Quaternisation of the deptropine molecule, as in deptropine methiodide, leads to important changes in the distribution pattern of the radioactivity. After oral administration of deptropine methiodide-*N*-$^{14}CH_3$ the radioactive material is very effectively restricted to the gastrointestinal tract; most of the absorbed amount is cleared from the circulation by the liver and brought back into the intestinal tract via the bile. After i.p. administration, the shorter intervals showed not only a large amount of radioactivity in the peritoneal cavity but also in the thoracic cavity. Outside these cavities the salivary glands and thyroid gland show some accumulation of radioactivity. Bile, intestinal contents and especially the peritoneum show a long retention of the radioactivity.

DEPTROPINE CITRATE* has been in use for the treatment of chronic nonspecific respiratory diseases since its introduction in 1962.[1–3]

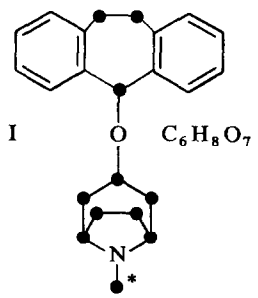
Results of studies on its metabolic fate have been published previously.[4] Although quaternisation of a tertiary amino group usually changes considerably the pharmacodynamic properties of a drug, the effect of quaternisation on metabolism and distribution of one particular drug is not so well documented. It seemed therefore interesting to combine further investigations into the metabolic fate of deptropine citrate with comparable studies of its methiodide, which had been found to have strong spasmolytic properties. The present report describes the preliminary results of such a comparison in which the distribution in mice was studied by means of an autoradiographic technique.

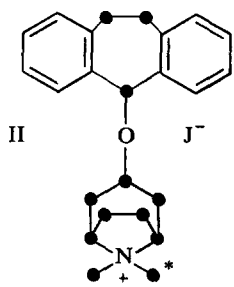
* Registered trade names: Brontine®, Brontina®, Su-Brontine®.

MATERIALS AND METHODS

Labelled compounds

For the autoradiographic distribution studies we used deptropine citrate-*N*-$^{14}CH_3$ (I) and deptropine methiodide-*N*-$^{14}CH_3$ (II). Compound I was synthesised by reductive methylation of *N*-demethyl deptropine with formaldehyde-^{14}C. Compound II was synthesised from I by coupling with methiodide. The compounds were purified until TLC indicated a satisfactory level of chemical and radiochemical purity. The corrected melting points of the two preparations were: 170–171° (I) and 223–224° (II).





Specific radioactivities: 1·5 mC/g (I) and 1·4 mC/g (II). For administration the deptropine was dissolved in a citrate-phosphate buffer (pH = 7·2) and the deptropine methiodide in saline, both in a concentration of about 5 mg/ml.

Animal experiments

Two groups of 4 virgin female mice (TNO-Swiss) of about 20 g of body weight were each dosed with one of the labelled compounds. A 50-mg/kg dose was given per gastric tube at the end of a 20-hr starvation period during which the mice had drinking water *ad libitum*. At the predetermined time intervals 1, 2, 8 and 24 hr after administration one mouse out of either group was killed, after a slight ether anaesthesia, by immersion in a solid carbon dioxide/acetone mixture. The frozen mice were immediately transferred to a freezing room (— 15°) and embedded on microtome stages in molds filled with an aqueous carboxymethylcellulose gel. The mounting medium was solidified by covering it with solid carbon dioxide.

In another experiment 2 groups of 5 non-starved female mice from the same stock received the compounds i.p. in a 25-mg/kg dose. Here mice of either group were killed at the intervals 15 min and 1, 2, 8 and 24 hr after administration.

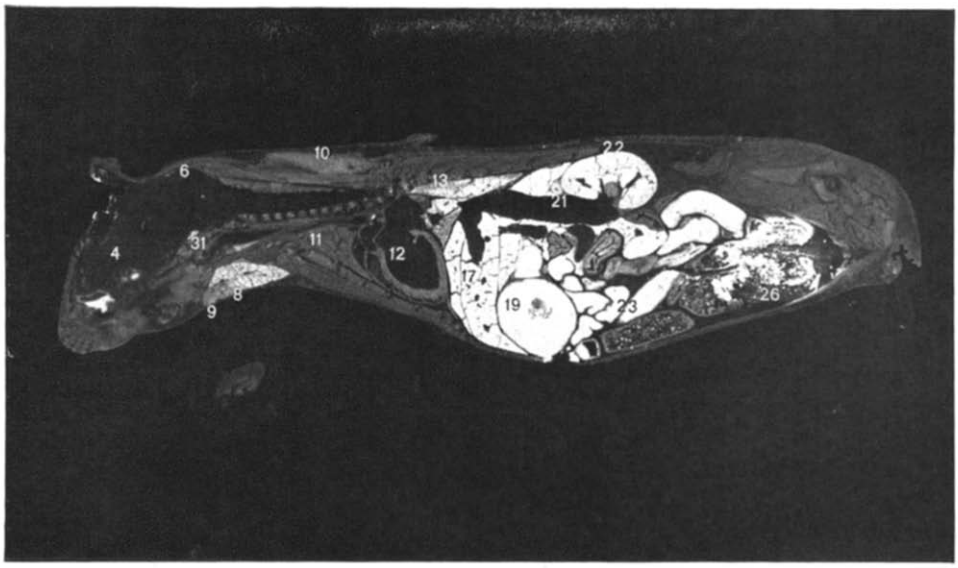
Autoradiographic procedure

The autoradiographic technique employed was that described by Ullberg:[5] in a freezing room 30-μ sections of the whole animals were taken by means of a heavy microtome (Jung Tetrander I). The dry sections were brought into contact with Gevaert Structurix D7 X-ray film. After sufficient exposure the autoradiograms were developed and prints were made for illustration purposes; consequently white areas in the illustrations correspond to high levels of radioactivity.

RESULTS AND DISCUSSION

Distribution following oral administration

The distribution patterns after oral administration of the two labelled compounds show very marked differences at each of the time intervals studied; this is illustrated



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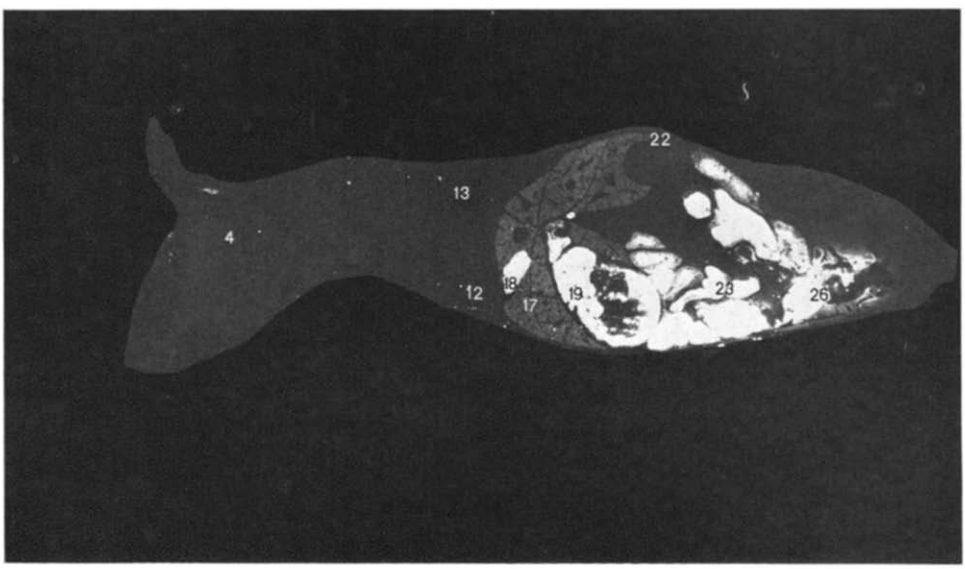


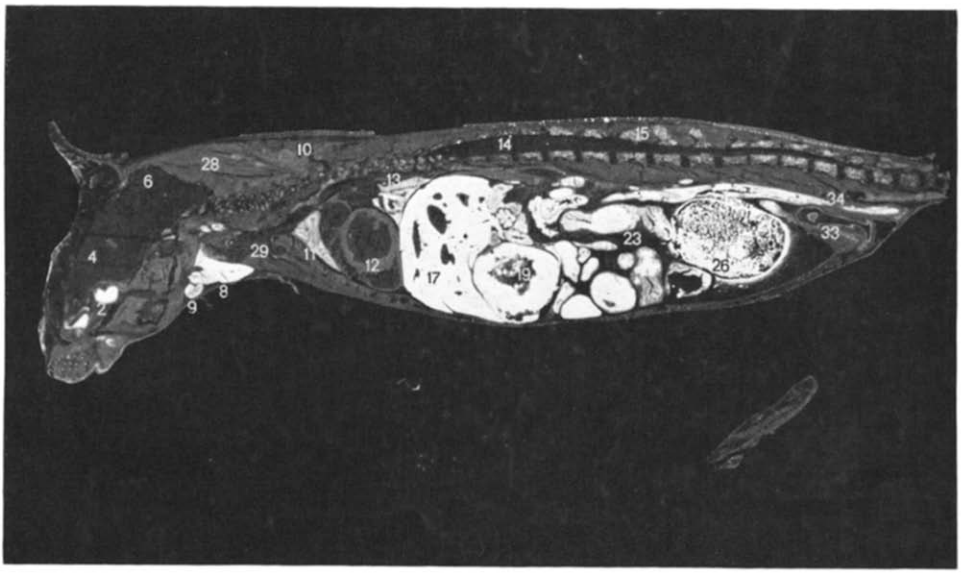
(b)

FIG. 1. Autoradiograms showing the distribution of radioactivity (light areas) 1 hr after oral administration of (a) deptropine citrate-*N*-$^{14}CH_3$ and (b) deptropine methiodide-*N*-$^{14}CH_3$

(Legend to Figs. 1–7)

1—eye	10—brown fat	19—stomach	28—skeletal muscle
2—Harder's gland	11—thymus	20—pancreas	29—blood
4—cerebrum	12—heart	21—adrenal gland	30—choroid plexus
5—hippocampus	13—lung	22—kidney	31—pituitary gland
6—cerebellum	14—spinal cord	23—intestines	32—bone marrow
7—tongue	15—vertebrae	25—pelvis	33—vagina
8—salivary glands	17—liver	26—urinary bladder	34—rectum
9—lymph node	18—gall bladder	27—faeces	

B.P.—*facing page 54*



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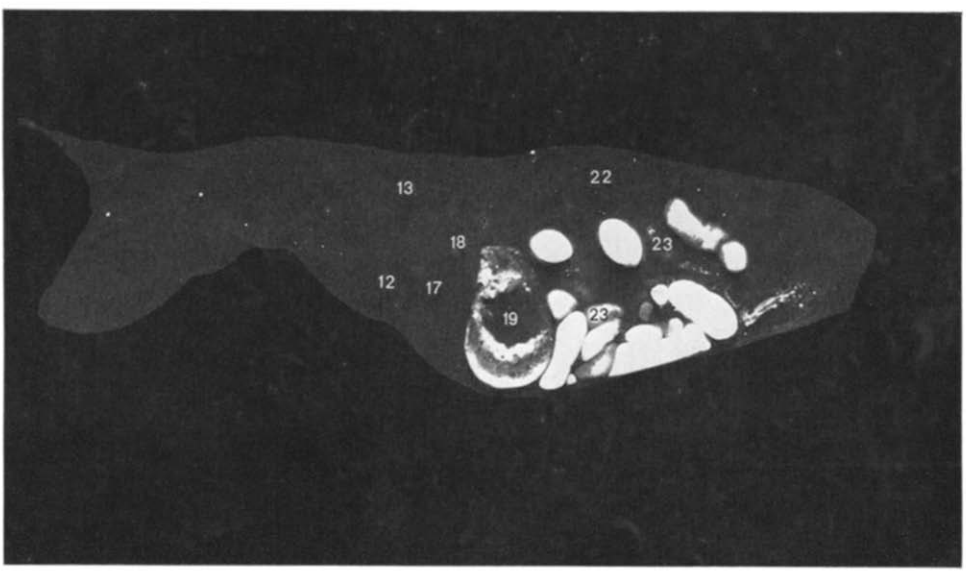
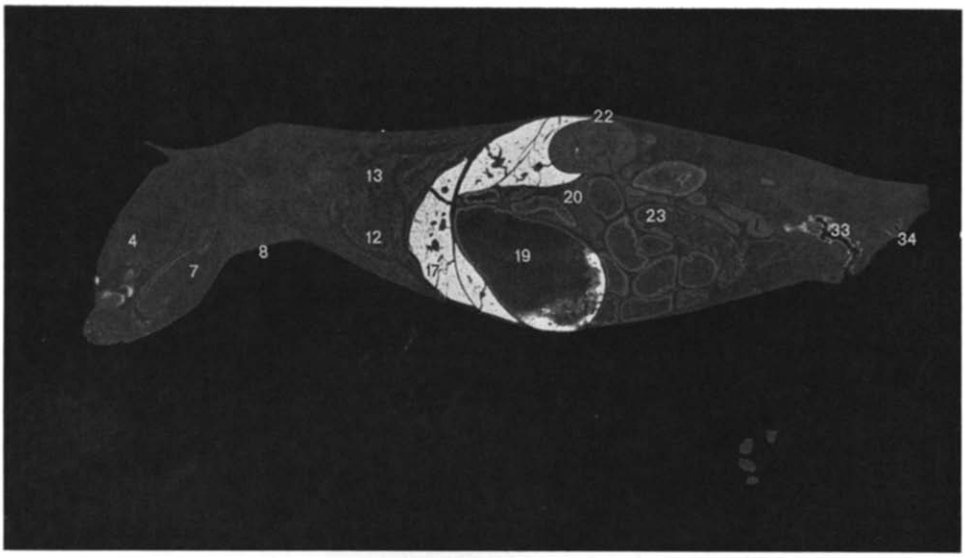


(b)

FIG. 2. Autoradiograms showing the distribution of radioactivity (light areas) 4 hr after oral administration of (a) deptropine citrate-*N*-$^{14}CH_3$ and (b) deptropine methiodide-*N*-$^{14}CH_3$. Legend as in Fig. 1.



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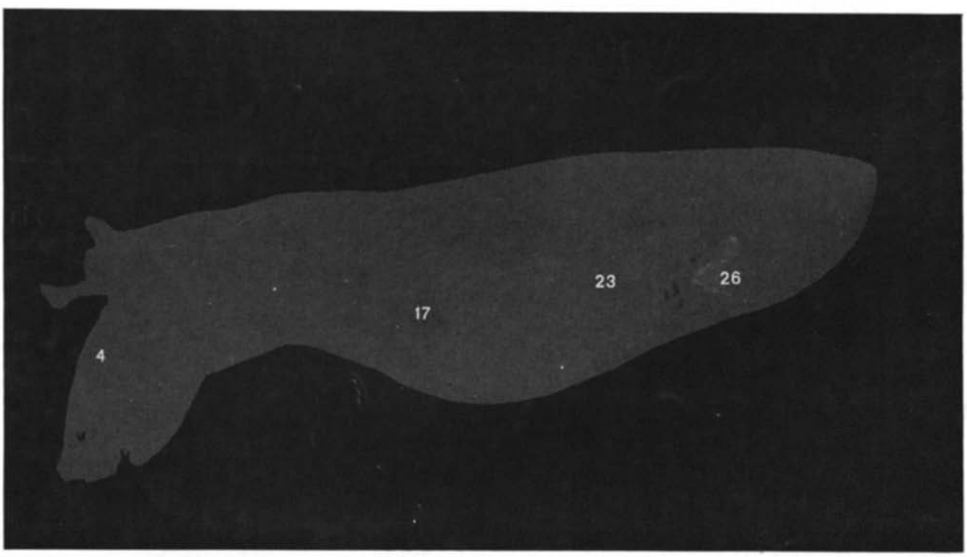


(b)

FIG. 3. Autoradiograms showing the distribution of radioactivity (light areas) 24 hr after oral administration of (a) deptropine citrate-*N*-$^{14}CH_3$ and (b) deptropine methiodide-*N*-$^{14}CH_3$. Legend as in Fig. 1.

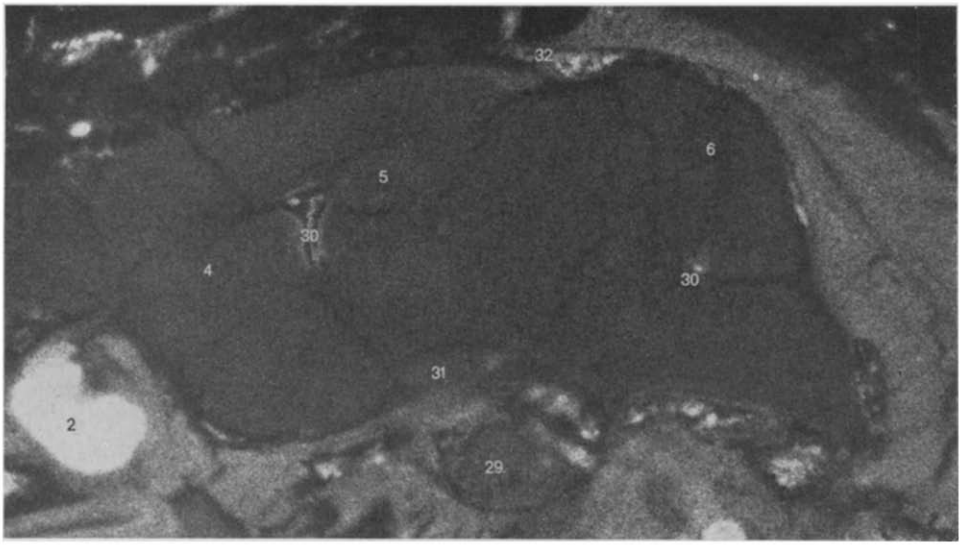
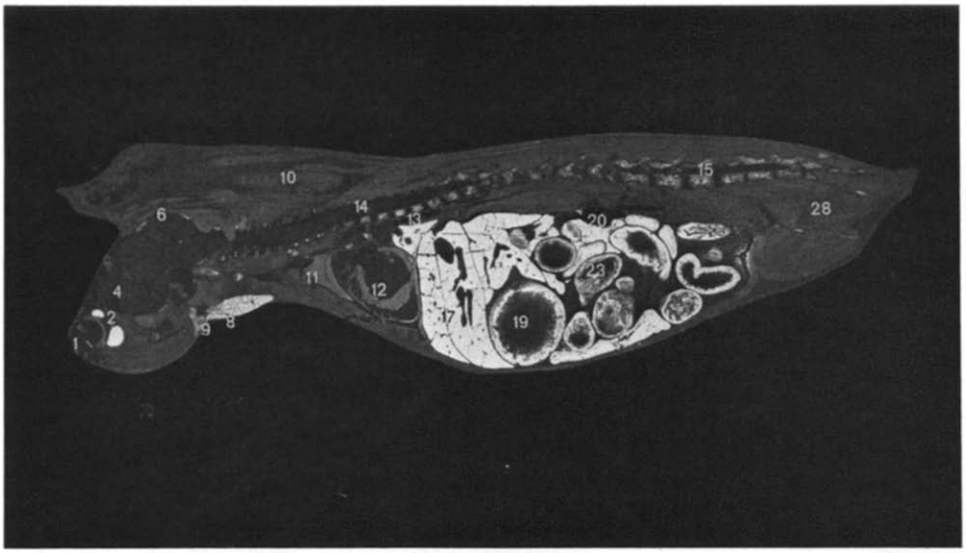


FIG. 4. Enlargement from an autoradiogram showing accumulation of radioactivity (light areas) in the choroid plexus and the tissue surrounding the ventricle 4 hr after oral administration of deptropine citrate-*N*-$^{14}CH_3$. Legend as in Fig. 1.



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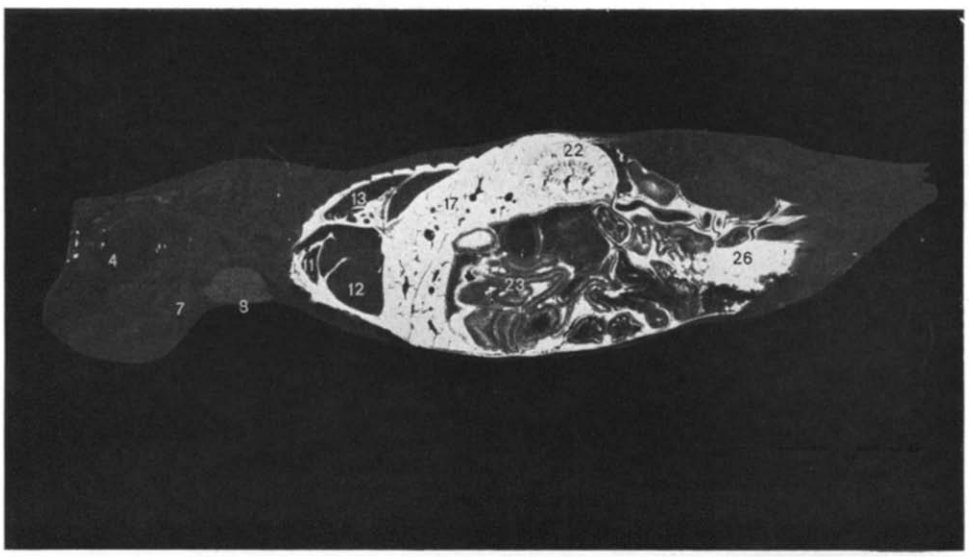
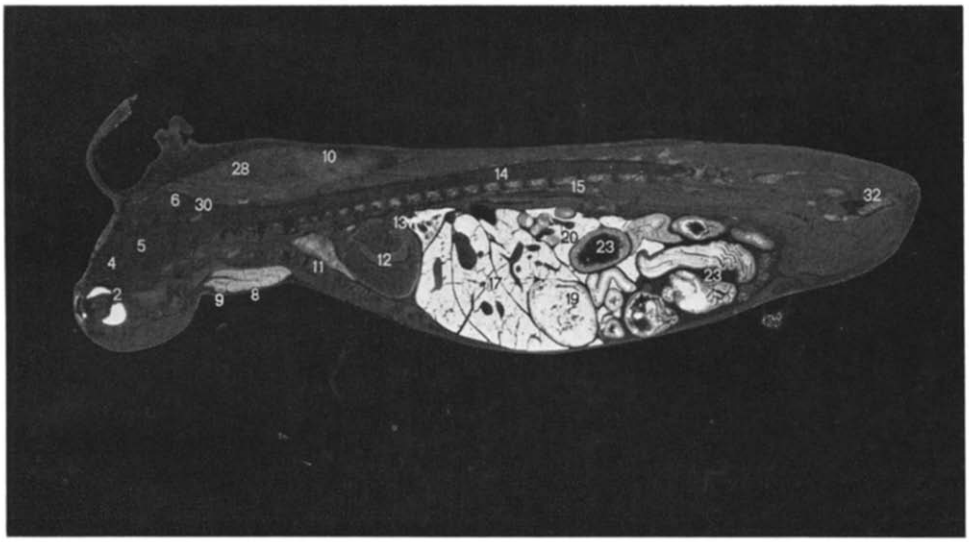


(b)

FIG. 5. Autoradiograms showing the distribution of radioactivity (light areas) 1 hr after i.p. administration of (a) deptropine citrate-*N*-$^{14}CH_3$ and (b) deptropine methiodide-*N*-$^{14}CH_3$. Legend as in Fig. 1.



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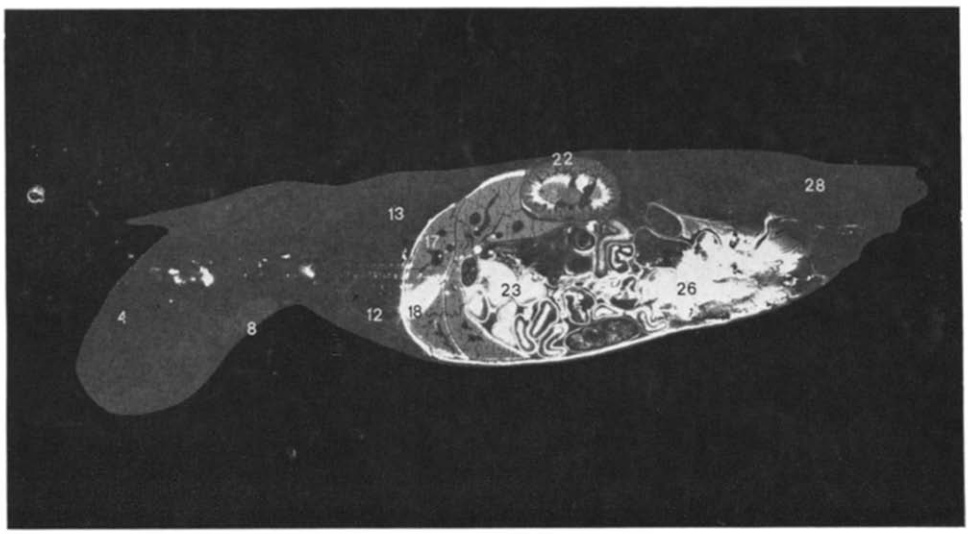
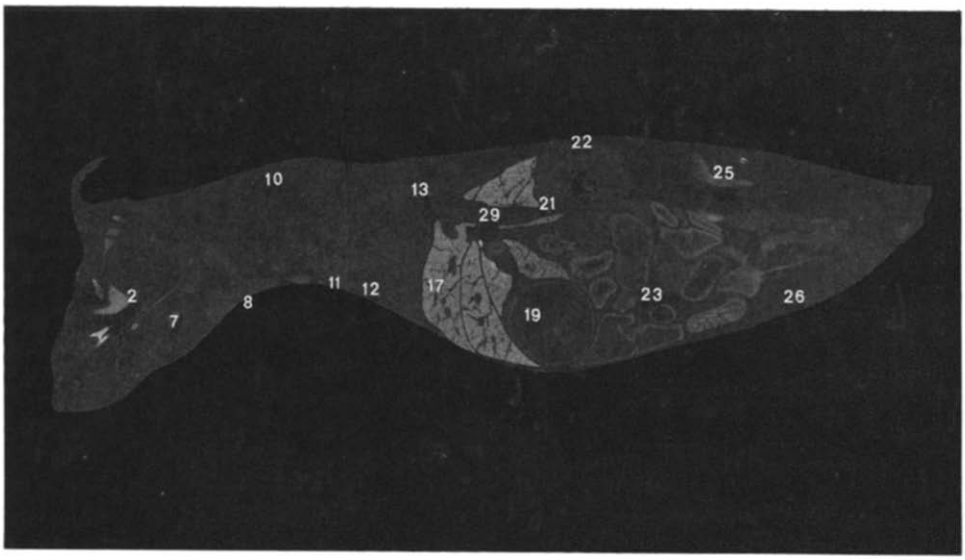


(b)

FIG. 6. Autoradiograms showing the distribution of radioactivity (light areas) 4 hr after i.p. administration of (a) deptropine citrate-*N*-$^{14}CH_3$ and (b) deptropine methiodide-*N*-$^{14}CH_3$. Legend as in Fig. 1.



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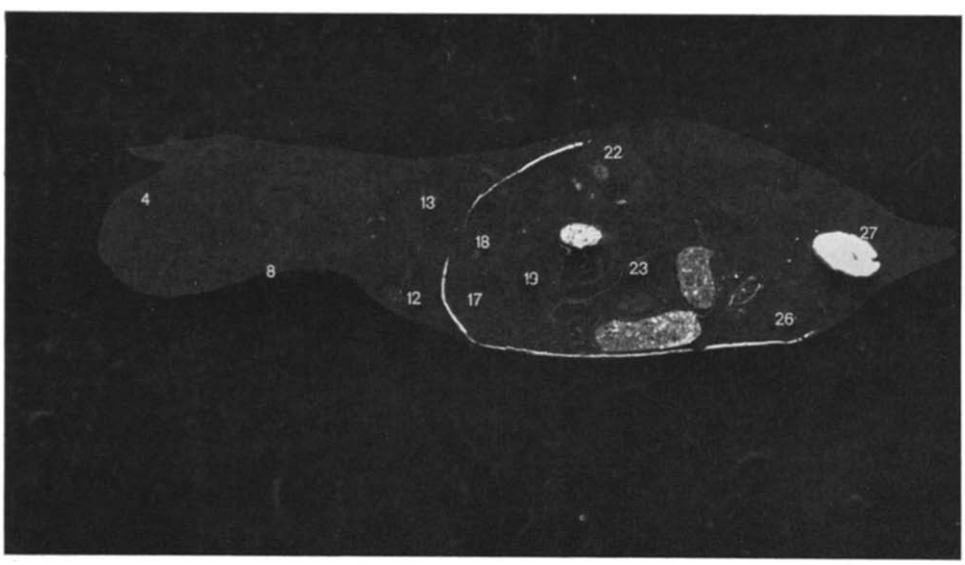

(b)

FIG. 7. Autoradiograms showing the distribution of radioactivity (light areas) 24 hr after i.p. administration of (a) deptropine citrate-*N*-$^{14}CH_3$ and (b) deptropine methiodide-*N*-$^{14}CH_3$. Legend as in Fig. 1.

in Figs. 1–3, which are representative examples of the autoradiograms obtained. The distribution following administration of deptropine shows a number of aspects which we also found in autoradiographic distribution studies of structurally related drugs:[6–8]

(1) A large volume of distribution of the radioactivity: nearly all the organs show a level of radioactivity which at the studied intervals exceeds that of the blood.
(2) A high level of radioactivity in glandular tissue: Harder's gland (Fig. 2(a)), salivary glands (Figs. 1(a), 2(a)), lacrimal gland (Fig. 2(a)), sebaceous glands, hypophysis (Fig. 1(a)), thyroid gland (Fig. 1(a)) and pancreas (Fig. 1(a)).
(3) A high concentration of radioactivity in the lungs (Figs. 1(a), 2(a)).
(4) A large concentration of radioactivity in the kidneys, especially in the inner cortex.
(5) A high level of radioactivity in the adrenals in a zone at the cortico-medullar border (Fig. 1(a)).
(6) A large concentration of radioactivity in the bile.
(7) A high level of radioactivity in lymphoid tissue (Fig. 2(a)).
(8) A rather high level of radioactivity in the bone marrow.

We have pointed out previously that these characteristics are common to the distribution patterns of a group of structurally related, basic pharmaceuticals.[9] The independence of these characteristics of the position of the label in the different compounds studied suggests that the intact compound and metabolites in which the original structure is still present to a large extent, e.g. the *N*-demethylated products and substituted derivatives, are mainly involved.

In addition to these general characteristics the distribution pattern of deptropine-*N*-$^{14}CH_3$ shows some aspects which occurred less frequently: (1) An accumulation of radioactivity in the brown fat (Fig. 1(a)). (2) A persisting high level of radioactivity in the liver. So far, these features have been found in cases where the compounds contain a ^{14}C-labelled NCH_3 group or where, regardless of the position of the label, hydrolysis *in vivo* affords labelled products of a relatively low molecular weight. Deptropine-*N*-$^{14}CH_3$ meets both requirements.

A rather extensive *N*-demethylation was observed in rats[4,10] and is likely to occur in mice as well. Since deptropine is also known to suffer hydrolysis after oral administration to rats, probably in the stomach,[4] it may be expected that after gastric administration of deptropine-*N*-$^{14}CH_3$ the hydrolytic product tropine-*N*-$^{14}CH_3$ will contribute, to some extent, to the distribution pattern in mice. We therefore assume that the two features mentioned above, are related to radioactive metabolic products originating either from *N*-demethylation or hydrolysis (tropine-*N*-$^{14}CH_3$ and its metabolites) or both. In comparison with structurally related compounds, the penetration of deptropine radioactivity into the brain is slight, which confirms our earlier metabolic studies of this compound.[4]

Remarkable are concentrations of radioactivity in the choroid plexus of the lateral and fourth ventricles and in the walls of the former (Fig. 4), which we observed at different intervals after administration. The hippocampus also shows a somewhat higher level of radioactivity compared with other brain areas, though less pronounced than in previous cases.[6,7]

Quaternisation of the deptropine molecule, as in deptropine methiodide, leads to profound changes in the distribution pattern of the radioactivity. The absorption becomes very limited (compare Figs. 1(b), 2(b) and 3(b) with 1(a), 2(a) and 3(a) respectively). One hour after administration radioactivity is found outside the gastro-

intestinal tract only in the liver and especially in the bile. At later intervals there is hardly any radioactivity left outside the gastrointestinal tract. A deposit of radioactivity in the nasal cavity in the animal killed 8 hr after administration probably is an artefact, resulting from the method of administration.

Absorption seems to take place mainly during the first hour after administration, which is in accordance with findings for quaternary compounds in general. According to the literature the limitation and short duration of the absorption should be connected with the formation of a complex of these quaternary compounds with mucine.[11] The high affinity to bile is also a characteristic which deptropine methiodide shares with other quaternary compounds of a related structure. It is known that an active transport mechanism is involved.[12]

In summary, it can be stated that on oral administration of deptropine methiodide-*N*-$^{14}CH_3$ the radioactive material is kept restricted to the gastrointestinal tract very effectively: the greater part of the absorbed amount is cleared from the circulation by the liver and brought back into the intestinal tract via the bile.

Twenty-four hours after administration hardly any radioactivity can be distinguished in the body of the mouse treated with deptropine methiodide-*N*-$^{14}CH_3$. It may be concluded that the elimination, mainly through the faecal route, is nearly completed by that time. The more prolonged levels of radioactivity in the deptropine citrate group, may, at least partly, be due to the inhibition of stomach emptying as a result of the atropine-like action of the drug.[13]

Deptropine methiodide, as was shown in both pharmacological and metabolic experiments with rats, has a relatively weak inhibitory action on stomach emptying. This is in accordance with our earlier observations showing that the absorption of a certain amount of an orally administered anticholinergic compound seems to be a condition for the inhibition of stomach emptying.[6] This inhibition is probably of a central origin, so that the lack of effectiveness of deptropine methiodide in this respect is not only due to the poor absorption but probably also to its inability to penetrate into brain.

Distribution following i.p. administration

Representative autoradiograms of the distribution of the two labelled compounds at different intervals after intraperitoneal administration are shown in Figs. 5–7. The deptropine-*N*-$^{14}CH_3$ distribution is largely similar to that after oral administration, with the exception of characteristics related to the route of administration. Nevertheless the i.p. group shows an unmistakable level of radioactivity in the stomach; from its increase with time (compare Figs. 5(a) and 6(a)) it can be concluded that a penetration of radioactivity into the stomach takes place on the level of the glandular part. There are also indications of a penetration of radioactivity into the intestinal lumen, although in more proximal parts this is difficult to ascertain in view of the high biliary excretion of the compound.

In contrast to the oral group, the i.p. group comprised the 15-min time interval after administration, too. At this time interval there are two localisations, which diverge a little from the general distribution picture described before: (1) The myocard shows, in contrast to other muscular tissues a rather high level of radioactivity. (2) The whole adrenal cortex shows the same high level of radioactivity, whereas at the other intervals studied, the radioactivity is mainly concentrated in one particular cortical zone.

These characteristics probably are rather common for the distribution pattern at early intervals after parenteral administration, as they were also observed in autoradiographic distribution studies with amitriptyline-$^{14}C^{14}$ and chlorpromazine-^{35}S.[15]

Both accumulations seem to be short of duration, since they were lacking at any of the other intervals studied. The zone of high radioactivity in the adrenals would thus represent an area of long retention rather than an area of particularly high uptake of labelled material.

The distribution of radioactivity following i.p. administration of deptropine methiodide-*N*-$^{14}CH_3$ shows differences both with the distribution after oral administration of this compound and with the distribution of i.p. deptropine-*N*-$^{14}CH_3$. At the shorter intervals a large amount of radioactivity is still located in the peritoneal cavity, while a striking feature is the large amount of radioactivity present in the thoracic cavity (Fig. 5(b)). There is probably a rapid penetration of radioactivity through peritoneum, diaphragm and pleura. Liver, bile, kidneys, urine and the intestinal contents show a high level of radioactivity. Outside the peritoneal and thoracic cavities some accumulation of radioactivity can be observed in the salivary glands and thyroid gland. Starting from the 1-hr interval there is a gradual decline of radioactivity levels within as well as outside the peritoneal and thoracic cavities.

Bile, intestinal contents and especially the peritoneum show a long persistence of the radioactivity.

After oral administration the excretion is mainly restricted to the faecal route. After i.p. administration the importance of the urinary excretion is increased considerably; it cannot be estimated which of the two routes prevails.

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