

Autoradiographic Studies on the Distribution of Drugs. I. Distribution of ^{14}C -Anisotropine Methbromide in Mice

HIDEYO SHINDO, ISAO TAKAHASHI and EIICHI NAKAJIMA

Central Research Laboratories, Sankyo Co., Ltd.¹⁾

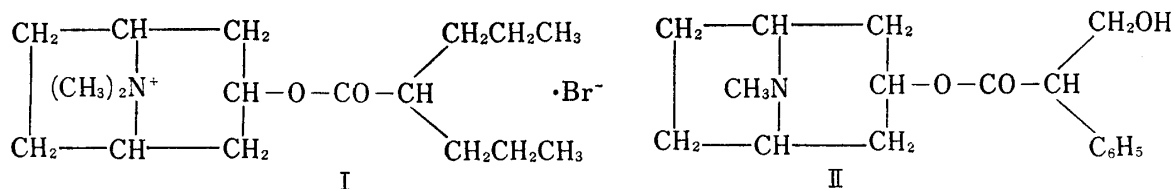
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The distribution of ^{14}C -anisotropine methbromide, a quaternary ammonium derivative of atropine, was studied in mice by whole-body autoradiographic technique. After *i.p.* and *s.c.* injections, the radioactivity was found to be mainly concentrated in the excretory organs: the liver, kidney, gall and urinary bladders, gastrointestinal tract and salivary gland, indicating a rapid excretion of the drug *via* both the urinary and biliary routes. In addition, the radioactivity was found to be accumulated specifically in the pancreas, gastric mucosa, walls of the spermatic and oviducts and uterus. Almost no accumulation was observed in the central nervous system, the muscular tissues and the endocrine organs. Autoradiography in mice of which the common bile duct was ligated prior to the injection showed an occurrence of a glandular secretion of the drug into the intestinal mucosa from the blood circulation. After oral administration, the radioactivity was found to be restricted very effectively to the gastrointestinal tract, indicating a limited absorption from the intestine, and the most part of the absorbed drug to be cleared back into the intestinal tract *via* the bile.

These characteristics are discussed with respect to the structural characteristic of quaternary ammonium structure and the possible relations are pointed out between the sites of the drug accumulation and those of the pharmacological action where the drug has been clinically applied as a peripheral anti-spasmodic agent with a lower side effect than has atropine.

Anisotropine methbromide (2-propylpentanoyl tropinium methylbromide, I)²⁾ is a quaternary ammonium derivative of atropine (II) and has been in wide use clinically as a potent anti-spasmodic agent with a weaker side-effect than atropine.³⁾

The tissue distribution of ^3H -atropine in mice has been investigated by autoradiographic technique by Albanus, *et al.*,⁴⁾ but that of the quaternary derivative of a closed structure has not yet been investigated. It seemed of interest, therefore, to compare the distributions of these two compounds in order to see how the difference in chemical structure, that is, tertiary and quaternary amines, can be related to that in the distribution pattern and to see how the specific sites of accumulation of the drug can be correlated to the sites of the pharmacological action.



In the present paper, the tissue distribution of ^{14}C -anisotropine methbromide was studied in mice by means of whole-body autoradiographic technique, after intraperitoneal and sub-

1) Location: *Hiromachi 1-chome, Shinagawa-ku, Tokyo.*

2) Valpin[®], Endo Laboratories, Inc.

3) H. Blumberg, H.B. Dayton, S. Carson and S.M. Gordon, *Federation Proc.*, **18**, 369 (1959).

4) L. Albanus, L. Hammarstrom, A. Sundwall, S. Ullberg and B. Vanbo, *Acta Physiol. Scand.*, **73**, 447 (1968).

cutaneous injections and oral administration. The distribution of ^3H -atropine in mice after subcutaneous injection was also studied in comparison.

Experimental

Anisotropine methbromide- $\text{N-}^{14}\text{CH}_3$ with a specific activity of $6.4 \mu\text{Ci}/\text{mg}$ ($2.32 \text{ mCi}/\text{mm}$) was prepared by treatment of anisotropine base with ^{14}C -methylbromide.⁵⁾ The isotopic purity was ascertained by thin-layer chromatography to be over 98%. The labeled drug was dissolved in physiological saline in a concentration of $5 \text{ mg}/\text{ml}$. Adult mice of ddY (male) and Swiss Albino (female) strains were used. Each animal weighing about 25 g was injected intraperitoneally or subcutaneously with 0.2 ml of the ^{14}C -anisotropine methbromide solution (*ca.* $40 \text{ mg}/\text{kg}$ body weight). The animals were killed at 15 and 30 min, 1, 3, 6, 24 and 72 hr after injection. In two male mice, the common bile duct was ligated before the subcutaneous injection and the animals were killed after 15 and 30 min. Two pregnant mice in late gestation state (*ca.* 40 g body weight) were injected subcutaneously with 0.32 ml of the solution and killed after 30 min and 3 hr. Two adult male mice were administered orally with 0.2 ml of the solution (*ca.* $40 \text{ mg}/\text{kg}$) by stomach tube and killed after 1 and 3 hr. In another experiment, 0.05 ml of the solution (*ca.* $10 \text{ mg}/\text{kg}$) was orally administered daily for 5 days and the animals were killed 15 min and 1 hr after the last administration. Two additional male mice were injected subcutaneously with 0.5 mg ($800 \mu\text{Ci}$) of ^3H -atropine⁶⁾ in 0.1 ml solution and killed after 15 min and 1 hr.

The autoradiographic technique employed was based on that described by Ullberg.⁷⁾ The mice were anesthetized with ether and sacrificed by immersion in a mixture of acetone and solid carbon dioxide at about -70° . After a frozen animal was embedded on microtome stage with aqueous carboxymethylcellulose gel, sagittal 20μ sections through the whole animal were cut by means of tape-sectioning (Scotch Magic Mending No. 810, Minnesota Mining) with a heavy microtome (Yamato Type 1111) in a freezing room and dried at -10° . The dried sections were brought into contact with Sakura Type-N X-ray film and exposed for 16 to 20 days. For the sections with ^3H , Nuclear Emulsion film, Sakura Type NR-EI, was used with the exposure time of 40 days.

Result

Distributions after Subcutaneous and Intraperitoneal Injections

Representative autoradiograms from mice 1, 3 and 6 hr after intraperitoneal injection of ^{14}C -anisotropine methbromide are shown in Fig. 1. One hour after injection (Fig. 1-A), the highest radioactivity was observed in the urinary bladder, renal medulla, liver and intestinal contents, indicating a rapid excretion of the drug *via* urinary route. Some radioactive uptake was noted in the salivary gland, brown fat, renal cortex, pancreas and gastric and intestinal mucosa, while no uptake was observed in the central nervous system and both the skeletal and cardiac muscles. Some radioactivity was still noticed in the peritoneal fluid, but almost no radioactivity was detected in the circulating blood, indicating that the drug is well absorbed through the peritoneal route and rapidly taken up by the tissues.

Three hours after injection, the gall bladder showed the highest concentration of radioactivity as well as the urinary bladder, indicating that the excretion through bile into the intestinal lumen occurs significantly in addition to the urinary excretion. A high concentration in the intestinal contents (Fig. 1-B) is thus considered to be mainly due to the biliary excretion of the drug. Among other tissues and organs, the liver accumulated the radioactivity in the highest concentration and a high accumulation was also noted in the walls of the spermatic ducts. Some but prominent uptake of the radioactivity was noted in the gastric and intestinal mucosa, renal cortex, pancreas, salivary and Harderian glands and brown fat, while almost no radioactivity was observed in the testis, spleen, adrenal and thymus. The concentration in the skeletal and cardiac muscles was very low and no radioactivity was detected in the central nervous system. No radioactivity

5) Purchased from le Commissariat a l'Energie Atomique, France.

6) Purchased from the Radiochemical Center, Amersham, England.

7) S. Ullberg, *Acta Radiol. Suppl.* **1954**, 118.

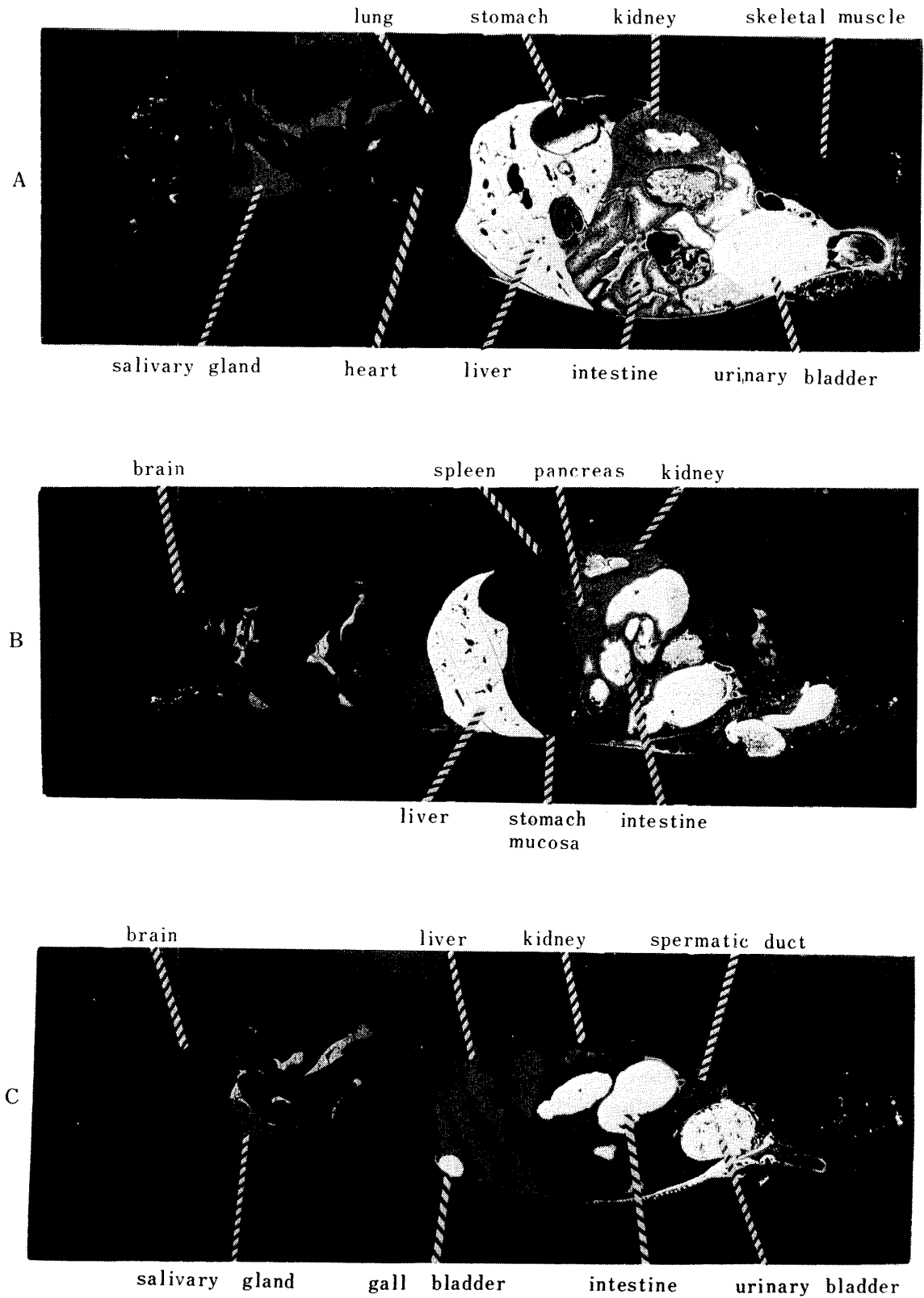


Fig. 1. Autoradiograms from Male Mice 1 (A), 3 (B) and 6 (C) hr after *i. p.* Injection of ¹⁴C-Anisotropine Methbromide

was found in the peritoneal cavity, indicating a complete absorption of the drug before 3 hr survival period.

After 6 hr (Fig. 1-C), the concentration of the radioactivity was found to be in the following order: the gall bladder, the intestinal contents, the urinary bladder and the renal medulla, indicating that the biliary excretion of the drug is more important than the urinary excretion at this time of the survival period. The concentration in the liver was decreased significantly as compared to that after 3 hr.

After 24 hr, the radioactivity has disappeared from the body almost completely, trace of radioactivity being observed only in the intestinal contents.

It can be stated, therefore, that on intraperitoneal injection of ^{14}C -anisotropine methbromide the radioactivity is taken up by the liver in a high concentration and the distribution is mostly restricted to organs mainly concerned with excretion of the drug such as the liver, kidney, gall and urinary bladder, gastro-intestinal tract and salivary gland.

After a subcutaneous injection, the distribution of the radioactivity was essentially the same to that after an intraperitoneal injection. Fifteen minutes after injection (Fig. 2-A), the highest concentration was observed in the liver, kidney, urinary and gall bladder and intestinal contents as well as the site of injection. A rather high level of radioactivity was found in the circulating blood. In the salivary gland and brown fat, there was an uptake which exceeded markedly the blood level. The lung, gastric and intestinal mucosa, pancreas and cartilage showed some radioactivity comparable to the blood level. In the muscular system

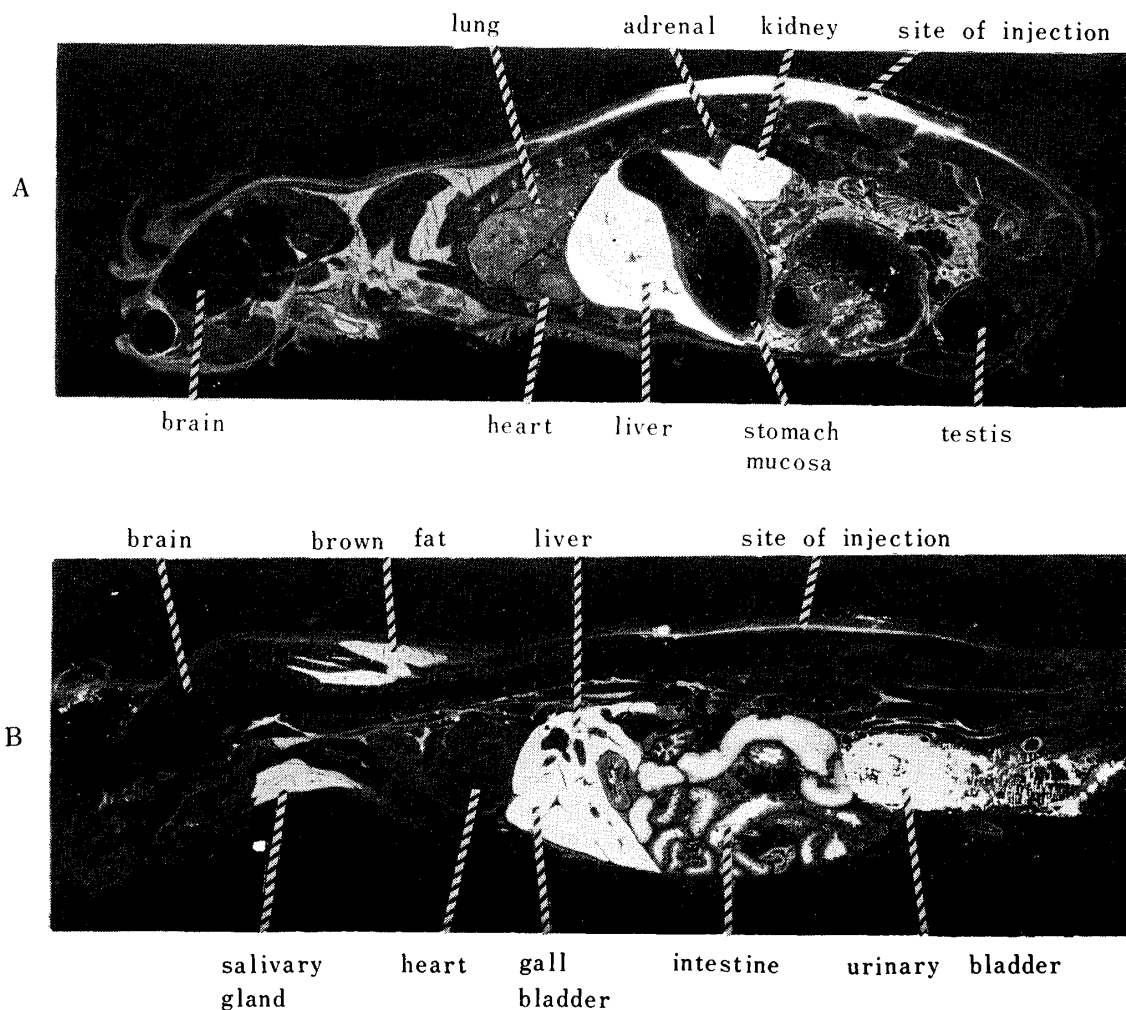


Fig. 2. Autoradiograms from Male Mice 15 min (A) and 1 hr (B) after *s.c.* Injection of ^{14}C -Anisotropine Methbromide

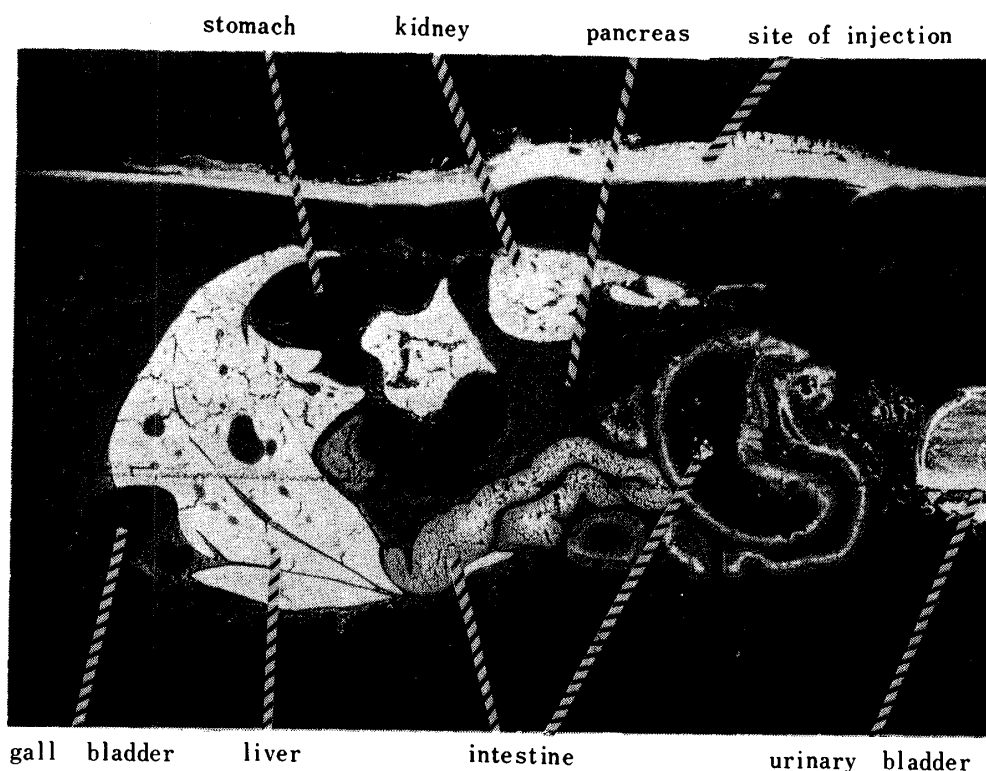


Fig. 3. Autoradiogram from a Male Mouse of which the Common Bile Duct was ligated, 30 min after *s.c.* Injection of ^{14}C -Anisotropic Methbromide

such as the cardiac and skeletal muscles, some radioactivity was observed, but the concentration was considerably lower than that in the circulating blood. The central nervous system showed no radioactive uptake. One hour after injection, the concentration in the blood was fallen down and as well those in the lung and muscular system also became very low. The radioactivity was thus concentrated in the excretory organs such as the liver, gall and urinary bladder, kidney and gastro-intestinal tract, with prominent but lower concentrations in the salivary gland and brown fat (Fig. 2-B).

In the animals of which the common bile duct was ligated prior to the drug administration, it was found that a high radioactivity appears in the intestinal mucosa and contents as early as 15 and 30 min after a subcutaneous injection (Fig. 3). In the normal mice there was a high accumulation of radioactivity in the gall bladder, while no noticeable radioactivity was found in the bile (gall bladder) of the ligated mice (Fig. 3). The results, therefore, indicate that there is involved a glandular secretion of the drug into the intestinal mucosa and contents directly from the circulating blood after subcutaneous administration.

In the female mice, the general distribution pattern of the radioactivity was the same to that observed in the male mice, but it was found to be a significant feature that a high accumulation of radioactivity was observed in the uterus 15 min after a subcutaneous injection and was retained for more than 3 hr. In Fig. 4, the abdominal part of the autoradiogram from a female mouse 3 hr after injection was compared with the corresponding tissue section stained with Hematoxylin-Eosin. From their comparison, it is quite evident that the accumulation of the radioactive substance in tissues and organs is very selective. The highest radioactivity is seen in the gall bladder, liver, renal medulla and intestinal contents. The pancreas shows a considerable uptake, while no radioactivity is observed in the circulating blood, spleen, lung, cardiac muscle and diaphragm. In the stomach, a marked accumulation of radioactivity is seen in the mucosal layer of the pylorus part where it remains for a long

time without the appearance of any appreciable amounts in the lumen, while the muscular layer shows no radioactivity. It is also evident that the uterus and the oviduct accumulate the radioactivity in a high concentration, while no radioactivity is seen in the ovary. In the kidney, the highest concentration is observed in the medulla and pelvis showing a rapid excretion pattern and a spotted appearance of high radioactivity in the cortex which might represent the concentration of radioactivity in the glomerulus.

In contrast to the selective accumulation observed in the distribution of ^{14}C -anisotropine methbromide, ^3H -atropine showed a much more uniform distribution pattern. As exemplified in Fig. 5, after a subcutaneous injection of ^3H -atropine in mice the highest radioactivity was

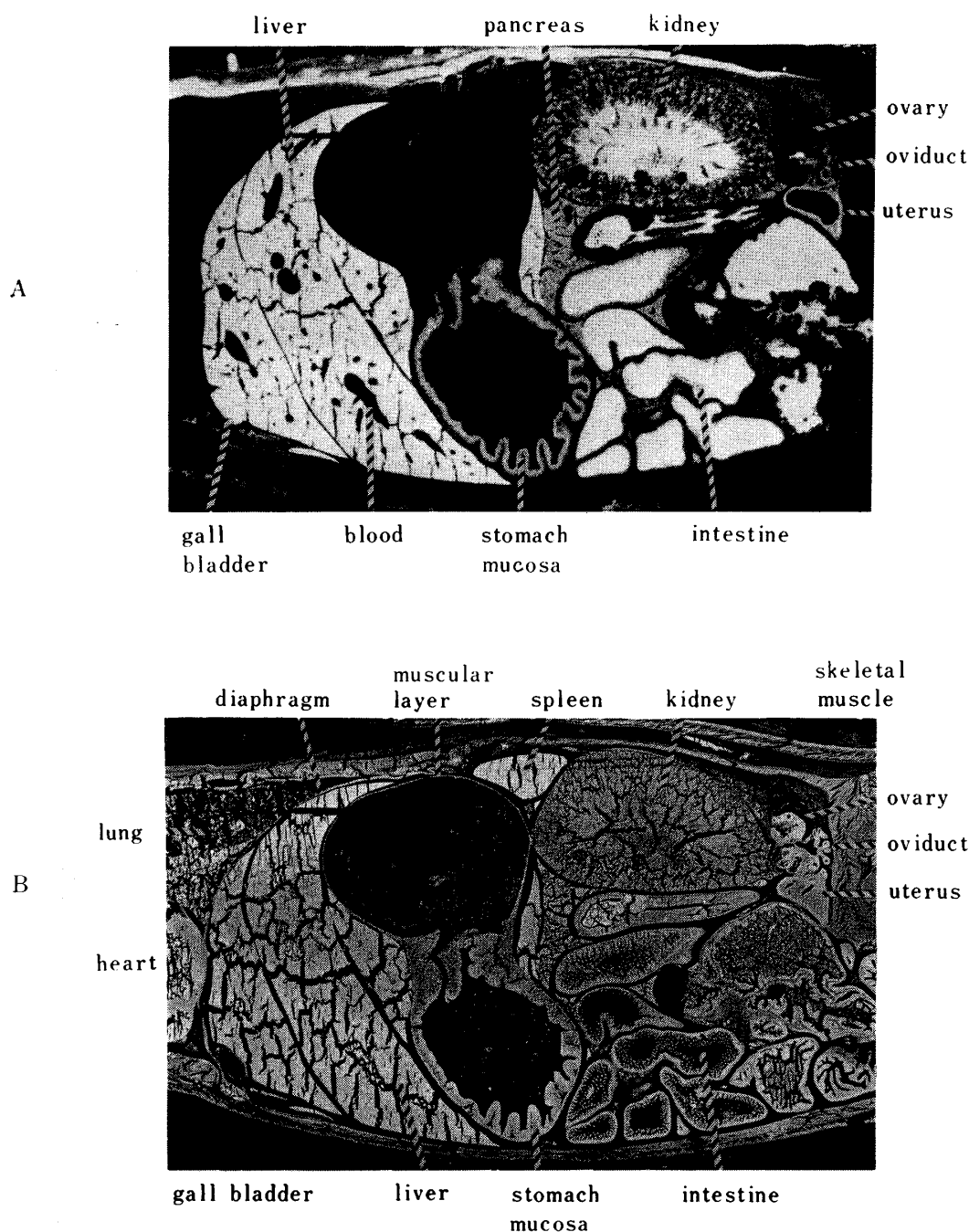


Fig. 4. Detailed Autoradiogram (A) and the Corresponding Tissue Section (B) of the Abdominal Part from a Female Mouse 3 hr after *s.c.* Injection of ^{14}C -Anisotropine Methbromide

seen in the urinary and gall bladders, renal medulla and intestinal contents as well as the site of injection, indicating a rapid excretion of the drug. The liver, however, did not show such a rapid and a high accumulation as was observed for ^{14}C -anisotropine methbromide, the brain showed a very low but an appreciable uptake of radioactivity and the skeletal muscles showed a uniform distribution of radioactivity. The salivary gland showed an accumulation of radioactivity almost as high as that in the liver. Furthermore, other organs such as the lung, spleen, bone marrow, testis and adrenal medulla where anisotropine methbromide did not show any appreciable concentration showed a concentration which exceeded that in the circulating blood.

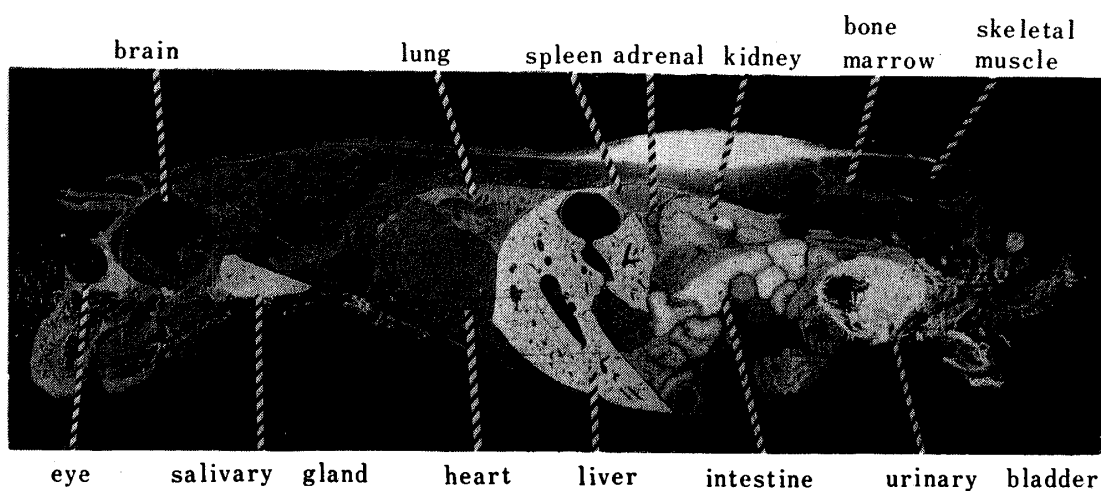


Fig. 5. Autoradiogram from a Male Mouse 1 hr after s.c. Injection of ^3H -Atropine

Distribution after Oral Administration

When ^{14}C -anisotropine methbromide was administered orally in mice, it was found that the radioactivity is kept restricted to the gastro-intestinal tract very effectively. As shown in Fig. 6, 3 hr after oral administration the most of the radioactivity was still located in the gastric and intestinal contents, indicating that the absorption of the drug from the intestine is very limited. An appreciable concentration was shown in the liver, while a high concentration in the gall bladder and a much lower concentration in the urinary bladder, indicating that the most part of the absorbed drug is cleared from the circulation by the liver and brought back into the intestinal lumen *via* the bile. In any other organs or tissues as well as the blood, almost no radioactivity was detected.

In a separate experiment, a low dose of the labeled drug was administered orally to a mouse daily for 5 days. The autoradiogram obtained 15 min after the last administration revealed that the radioactivity was observed only in the gastric and intestinal contents, indicating that there is no organ or tissue which accumulates the drug during a continued administration.

Distribution in Pregnant Mice

One hour after a subcutaneous injection of ^{14}C -anisotropine methbromide to pregnant mice in a late gestation state, no radioactivity was observed in the foetus, although the placenta showed a concentration of radioactivity which is comparable to that in the circulating blood. Three hours after injection, a very weak radioactivity could be detected in the liver of the foetus, but the concentration was extremely low as compared to that in the maternal liver. It can be said, therefore, that anisotropine methbromide does not pass the placental barrier easily as well as the brain barrier.

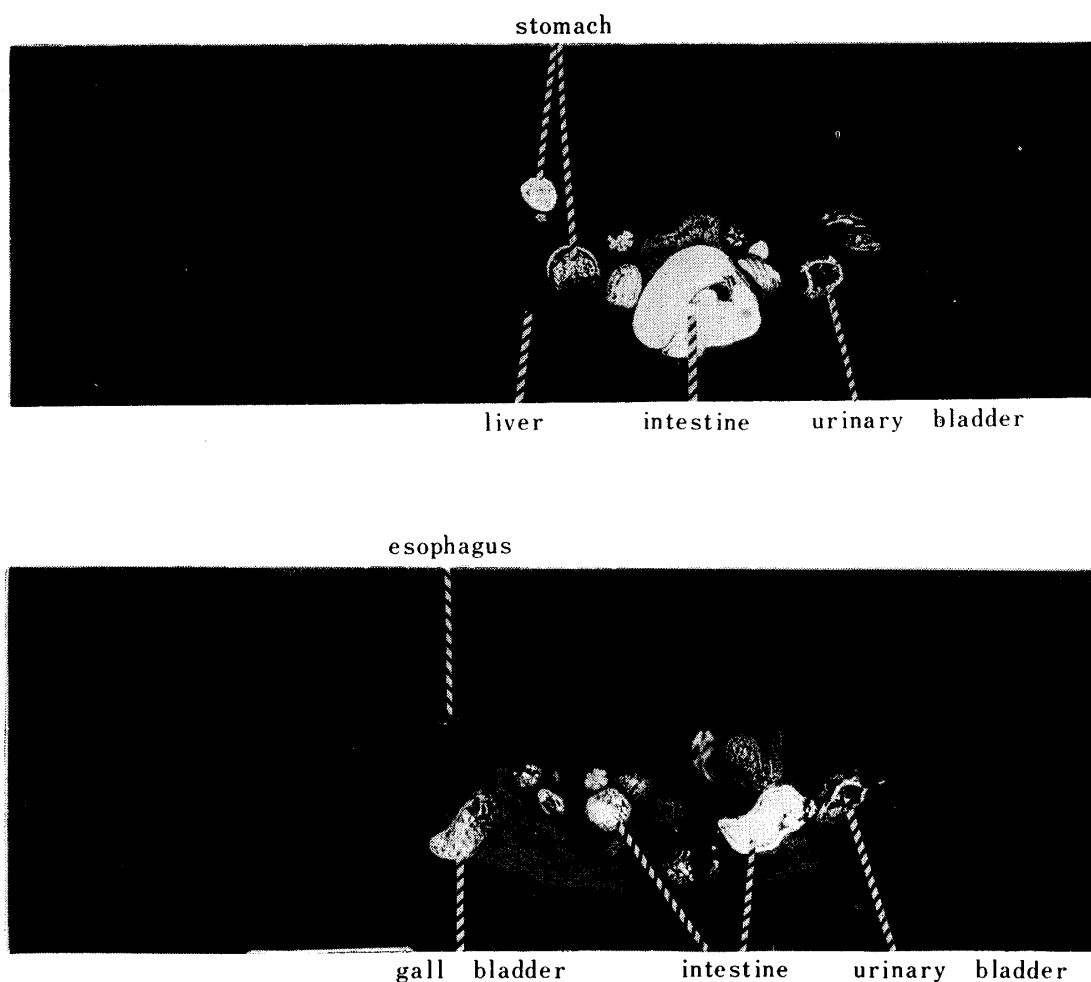


Fig. 6. Autoradiogram from a Male Mouse 3 hr after Oral Administration of ^{14}C -Anisotropine Methbromide

Urinary Metabolites

The urine from mice administered with ^{14}C -anisotropine methbromide subcutaneously was collected for a period of 24 hr and the radioactive substances were separated by thin-layer chromatography. Approximately 70% of the radioactivity administered was recovered in the urine during the first 24 hr and three radioactive spots were detected in addition to the spot corresponding to the original compound. The counting of the extracts from each spot revealed that, as shown in Table I, approximately 37% of the radioactivity excreted in the urine was unaltered anisotropine methbromide. Of the three metabolites, the structures are not determined.

TABLE I. Radioactive Metabolites in 24 hr Urine after Subcutaneous Injection of ^{14}C -Anisotropine Methbromide in Mice (50 mg/kg)

<i>R_f</i> value ^{a)}	% \pm S.E. (<i>n</i> =3)
0.41 (Original Comp.)	37.04 \pm 1.54
0.31	8.15 \pm 0.47
0.16	26.44 \pm 3.27
0.10	28.36 \pm 1.35

a) Kieselgel F-254 (Merck), solvent: *n*-BuOH: AcOH:H₂O (84:16:50)

Discussion

With respect to the fate and distribution of anisotropine methbromide, the followings were thought to be interesting: i) the differences in the distribution pattern between atropine and its quaternary derivative, ii) the difference in the distribution pattern of quaternary compound after a different route of administration, and iii) the relation between the sites of specific accumulation of the drug and those of the pharmacological actions.

From the present investigation, anisotropine methbromide was found to show a distribution pattern concentrated mainly in the excretory organs such as the liver, kidney, gall and urinary bladders and gastro-intestinal tract, after both subcutaneous and intraperitoneal injections in mice. The central nervous system, muscular tissues such as the skeletal and cardiac muscles and endocrine organs such as the adrenal did not show any appreciable uptake of the drug. Thus, the quaternary anisotropine methbromide showed a very distinct contrast in the distribution pattern to that of tertiary atropine which exhibited a much more uniform distribution pattern throughout the body, including the muscular tissues and endocrine organs. A low but an appreciable uptake of atropine was also detected in the central nervous system.

Hansson, *et al.*⁸⁾ compared the distributions of tertiary and quaternary phenothiazine compounds, Promethazine and Aprobil[®], and showed that the former passes through the blood-brain barrier and the placental barrier whereas the latter did not occur in the brain and foetuses. It was suggested that the change of the structure from a tertiary to a quaternary amine causes a significant difference in the biological fate of the molecule, which is responsible to the difference in the pharmacological action of the two compounds, that is, the sedative and non-sedative properties.

It may be generally true that the site of a high accumulation of a drug does not always correspond to the site of the pharmacological action, but it may be also assumed to be true that an appearance of any pharmacological effect requires a certain level of the drug concentration at the site of action. It is of interest, therefore, to see how the sites of accumulation of anisotropine methbromide is related to the sites to where this drug has been used clinically as a peripheral anti-spasmodic agent.

After oral administration of ¹⁴C-anisotropine methbromide it was found that the absorption from the intestine is limited and the radioactivity was kept restricted mostly to the gastrointestinal tract very effectively. This is in good accord with the fact that this drug has been used clinically as oral dose selectively for spasma in the gastrointestinal tract. It was found, furthermore, that the most part of the absorbed drug is cleared from the circulation by the liver and brought back into the intestinal lumen *via* bile and this behavior is considered to play an important role in the effectiveness of the drug against the peripheral parasympathetic nerves in the gastrointestinal tissues. A low absorbability of the drug from the intestine may be ascribed to a characteristic property of the quaternary structure and the formation of a complex with mucin has been pointed out.⁹⁾ A similar behavior of radioactivity has been reported after oral administration of ¹⁴C-detropine methiodide in mice as compared to tertiary detropine.¹⁰⁾

After subcutaneous and intraperitoneal injections, the radioactivity was mainly concentrated in the excretory organs such as the liver, kidney, urinary and gall bladders, intestinal tract and salivary gland. In addition to the excretory organs, however, specific accumulations of a high radioactivity were observed in the gastric mucosa, pancreas, walls of the spermatid and oviducts and uterus. These results are again in good accord with the fact that this drug has been used by injection for spasma in the female reproductive organs in addition to

8) E. Hansson and C.G. Schmitterlöw, *Arch. Intern. Pharmacodyn.*, **131**, 309 (1961).

9) R.M. Levine, M.R. Blair and B.B. Clark, *J. Pharmacol. Exptl. Therap.*, **114**, 78 (1955).

10) W. Hespe and H. Prins, *Biochem. Pharmacol.*, **18**, 53 (1969).

the gastrointestinal and urinary tracts. A high concentration in the salivary gland may be responsible to a side effect of the drug, blocking the salivary secretion. A high accumulation of atropine in the salivary gland has been pointed out by Albanus, *et al.*,⁴⁾ correlating it to the strong action of atropine. From a comparison of the distribution of the two drugs, however, it became evident that the concentration of atropine in the salivary gland relative to that in the liver is much higher than that of anisotropine methbromide, being again in accord with a much lower side effect of the latter.³⁾

Accumulation in the liver and a high participation of the biliary excretion seems to be a common feature to the mono-quaternary structure¹¹⁾ and an active excretion mechanism has been suggested for the excretion from the liver.¹²⁾ In the present results, the concentrations in the liver and gall bladder relative to those in other organs and tissues was found to be much higher for anisotropine methbromide than atropine, indicating a high accumulation in the liver and a high participation of the biliary excretion of the quaternary structure. A high concentration of radioactivity in the intestinal contents after intraperitoneal or subcutaneous injection is thus considered to be due to the biliary excretion of the drug. It cannot be drawn any conclusion, however, whether the radioactivity was derived from only the biliary excretion or the glandular secretion of radioactivity from the blood circulation was participating simultaneously. The result that soon after injection into mice of which the common bile duct was ligated prior to the administration a high radioactivity was seen in the intestinal walls indicates that a direct glandular secretion of the drug into the intestinal mucosa and the contents is also involved. It might be suggested, therefore, that an effective high concentration of the drug in the intestinal tissue might be achieved rapidly after the injection.

It might be assumed that the radioactivity distributed in the tissues during the first several hours after injection can be regarded as unaltered anisotropine methbromide and, probably, a metabolites retaining the tropinium structure, since in 24 hr urine approximately 40% of the radioactivity was found to be unaltered compound and no radioactivity was found to be appeared in the respiratory air,¹³⁾ indicating that the demethylation reaction does not occur to any significant extent.

As far as a general distribution pattern is concerned, it might be true that the mono-quaternary ammonium structure shows generally a common feature in the distribution pattern irrespective of the structure of the remaining part of the molecules, as has been pointed out by Levine, *et al.*¹¹⁾ However, when the distribution of anisotropine methbromide is compared with those of other mono-quaternary compounds so far reported, some distinct differences are noticeable. For example, Aprobit, a quaternary phenothiazine derivative, has been shown to exhibit a considerable radioactivity in the cardiac and other muscles after injection of the labeled drug in mice⁸⁾ and detropine methiodide a high concentration in the thoracic cavity after intraperitoneal injection.¹⁰⁾ In contrast to these observations, anisotropine methbromide did not show any appreciable accumulation in the muscular tissues and any appearance in the thoracic cavity. A further investigations are now in progress in order to see the variation in the distribution patterns of a series of mono- and diquaternary compounds and its relation to the structure of the remaining part of the onium structure.

Acknowledgement The authors express their deep gratitudes to Dr. G. Sunagawa, director of this laboratories, for his kind encouragement. Thanks are also due to Dr. E. Ohki of this laboratories for the preparation of the labeled drug.

11) R.M. Levine and B.B. Clark, *J. Pharmacol. Exptl. Therap.*, **114**, 63 (1955); *Idem, ibid.*, **121**, 63 (1957).

12) L.S. Schanker and H.M. Solomon, *Am. J. Physiol.*, **204**, 829 (1963).

13) A. Yasumura and T. Oshima, unpublished work in this laboratories.