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Autoradiographic Studies on the Distribution of Quaternary Ammonium Compounds. II.[1] Distribution of ^{14}C-Labeled Decamethonium, Hexamethonium and Dimethonium in Mice

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The distributions of ^{14}C-labeled decamethonium, hexamethonium and dimethonium in mice after intraperitoneal injection were compared by whole-body autoradiographic technique and the results discussed in relation to the progressive change in the structure and to the pharmacological effect. The rate and the extent of accumulation in the liver was increased in the order of di- $<$hexa- $\ll$decamethonium. Decamethonium was found to be accumulated in the liver soon after injection in an extremely high concentration, which was retained for more than 72 hr. The excretion was mainly through the urinary route and the rate was in the order of di-$>$hexa-$>$decamethonium. As a common feature for the bisquaternary ammonium, the all compounds showed a rapid accumulation and a long retention in the cartilage and hexa- and decamethonium in the meninges, possibly in the arachnoid. No penetration was detected in the brain parenchyma. The all bisquaternary structure did not show any accumulation in the salivary gland and other secretory glands, where monoquaternary structure appears to be accumulated. Only decamethonium, a muscle relaxant, showed a high accumulation in the muscular tissues, while hexamethonium, a ganglionic depressant, as well as dimethonium which is inert pharmacologically did not show any accumulation. These results and a finding that tetraethylammonium, another ganglionic depressant, did not show any accumulation might indicate a presence of a direct relation between the distribution of a drug in macro level and the appearence of the pharmacological action.

In the preceding paper,[1] the distribution of anisotropine methbromide-^{14}C, a quaternary derivative of atropine, in mice was compared to that of atropine-^{3}H and a marked change of the distribution pattern on the quaternization of tertiary nitrogen was pointed out. It was further clarified that the radioactivity was accumulated selectively in all the sites which are considered as the target organs of this anti-spasmodic agent. As far as a qualitative distribution pattern is concerned, monoquaternary ammonium structure appears to show generally a common feature.[3,4] As was pointed out in the preceding paper,[1] however, it seems also probable that some marked differences are still present in the distribution patterns depending upon the structure of the remaining part of the onium molecule.

The present investigations were performed in order to examine the general characteristics in the distribution pattern of quaternary ammonium compounds and their changes accompanying a progressive change of the structure in the remaining part of the molecule. In addition, the investigations were aimed to examine whether any relation is present or not between the appearence of any pharmacological effect and the distribution pattern of the drug in macroscopic level. In the present paper, the distribution of deca- (I), hexa- (II) and di-methonium (III) labeled with ^{14}C following the intraperitoneal injection into mice were compared by means of whole body autoradiographic technique.

$$(CH_3)_3\overset{+}{N}-(CH_2)_n-\overset{+}{N}(CH_3)_3$$

I: $n=10$; II: $n=6$; III: $n=2$

1) Part I: H. Shindo, I. Takahashi and E. Nakajima, *Chem. Pharm. Bull.* (Tokyo), **19**, 513 (1971).
2) Location: *Hiromachi 1-chome, Shinagawa-ku, Tokyo.*
3) R.M. Levine and B.B. Clark, *J. Pharmacol. Exp. Ther.*, **114**, 63 (1955); **121**, 63 (1957).
4) E. Hansson and C.G. Schmiterlöw, *Arch. Int. Pharmacodyn.*, **131**, 309 (1961).

Material and Method

Radioactive Compounds——^{14}C-Decamethonium dibromide (specific activity, 20.9 mCi/mmole) and ^{14}C-hexamethonium dichloride (specific activity, 1.87 mCi/mmole) were purchased from the Radiochemical Center, Amersham, England. ^{14}C-Dimethonium diiodide with specific activity of 3.51 mCi/mmole was prepared by the reaction of N,N′-tetramethyl-ethylenediamine with ^{14}C-methyl iodide in ethanol, followed by recrystallizations from methanol–water. All the compounds were ascertained to give single radioactive spot on the thin-layer chromatogram, prior to each experiment.

Autoradiography——Male mice of ddY strain weighing about 25 g were used. All the compounds were dissolved in a concentration corresponding to 12.5 μCi/ml physiological saline and 0.2 ml (2.5 μCi) of the solution was injected intraperitoneally into mouse. The amount of dose was about 2, 14.5 and 11.5 mg/kg for deca-, hexa- and dimethonium, respectively. Five, 15 and 30 min, 1, 3, 6, 12, 24, and 72 hr after injection, the mice were lightly anesthetized with ether and sacrificed by immersion in a mixture of hexane and solid carbon dioxide at about −70°. After a frozen animal was embedded on a microtome stage with aqueous carboxymethyl-cellulose gel, the sagittal 30 μ sections through the whole animal were cut[5] 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 a constant period of 23 days.

Result

Decamethonium

Five minutes after intraperitoneal injection of ^{14}C-decamethonium, the highest radioactivity was shown in the liver and kidney, while the blood concentration was relatively low. Although a considerable radioactivity was still remained in the peritoneal fluid, the results indicated that the drug is rapidly absorbed from the peritoneal route and is rapidly taken up by the tissues. The concentration which exceeded the blood level was observed also in the muscular tissues such as the lingual, cervical and cardiac muscles, and the cartilages, such as, of the vertebra, trachea and larynx.

Fifteen and 30 min after injection, the blood concentration was declined to almost undetectable level and the autoradiogram revealed a very characteristic distribution pattern of radioactivity, as shown in Fig. 1. The first characteristic was an accumulative uptake of decamethonium by the liver and kidney and the highest concentration of radioactivity was continued to be present in these two organs throughout the whole survival period investigated. A high radioactivity was shown in the urinary bladder, while the concentration in the gall bladder was much lower than that in the liver. These results and the lack of radioactivity in the intestinal contents suggest that the biliary excretion of the drug is not significantly occurring, but the excretion through the urinary route is predominant. The second characteristic was a high accumulation in the muscular tissues and a marked uptake of radioactivity was shown in the cardiac muscle, diaphragm, the lingual and cervical muscle and other skeletal muscle. In the stomach, a marked accumulation of radioactivity was shown only in the muscular layer, particularly of the nonglandular left side, and in the esophagal wall, but no radioactivity was detected in the mucosal layer of the stomach (Fig. 2). The muscular layer of the skin, Panniculus carnosus, also showed a marked uptake of radioactivity (Fig. 2). The third of the characteristics was an uptake of radioactivity by the cartilage and other connective tissues. A marked uptake was shown in the cartilages of the vertebra, trachea, bronchi and larynx and in the meninges surrounding the brain and spinal cord, probably the arachnoid (Fig. 2). It might be another characteristic that no uptake of radioactivity was detected in the parenchymal tissue of the central nervous system and that no appreciable radioactivity was shown in the bone marrow, spleen, pancreas, lung, salivary gland and brown fat. An appreciable radioactivity which exceeded the blood level was observed in the thymus, adrenal cortex and testis.

5) S. Ullberg, *Acta Radiol. Suppl.*, **118** (1954).

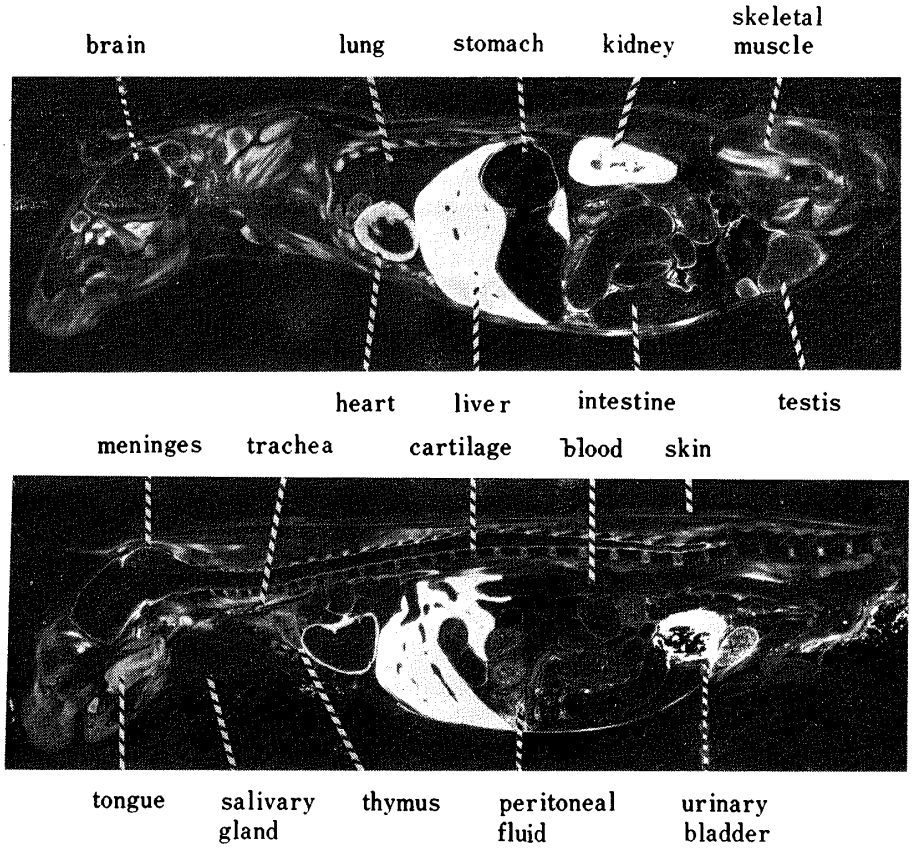

Fig. 1. Autoradiogram from a Mouse 30 min after Intraperitoneal Injection of ^{14}C-Decamethonium

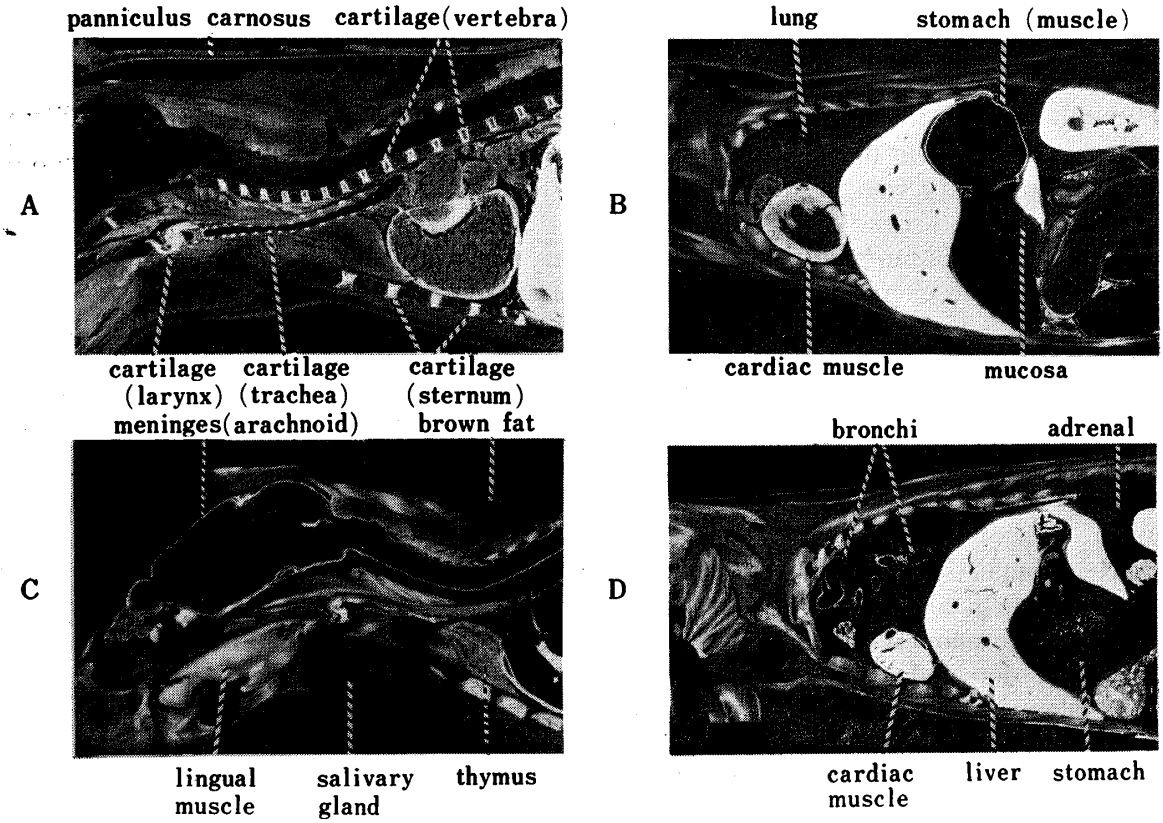

Fig. 2. Enlargements of Autoradiograms from Mice 15 min (A), 30 min (B), 3 hr (C) and 6 hr (D) after Intraperitoneal Injection of ^{14}C-Decamethonium.

During the period from 1 to 3 hr after injection, the distribution pattern of radioactivity showed only slight changes and almost the same characteristic pattern was continued for more than 6 hr. Six hours after injection (Fig. 3), an extremely high accumulation of radioactivity was still observed in the liver and in the kidney cortex and a high radioactivity was still remained in the cardiac and skeletal muscles, muscular layer of the stomach, esophagal wall, meninges, tracheal and broncheal cartilages, testis and epididymis. Radioactivity in the intervertebral cartilage seemed to be disappeared rather rapidly, before 3 hr after injection.

For the period between 24 and 72 hr after injection, a persistent high concentration of radioactivity was observed only in the liver (Fig. 4), indicating that the high accumulation of the drug in the liver is continued for a very long period. Some retention of radioactivity was also observed in the kidney cortex and in the cardiac and skeletal muscles. During this period, no radioactivity was detected in the gall bladder and no significant radioactivity in the intestinal contents, while an appreciable radioactivity was observed in the urinary bladder, indicating that a slow excretion of radioactivity was being proceeded through the urine, but not through the bile.

Hexamethonium

Five to 15 min after intraperitoneal injection of ^{14}C-hexamethonium into mice, a high level of radioactivity was observed in the circulating blood, indicating a longer duration of a higher blood level in hexamethonium as compared to that in decamethonium. No accumulation of the drug in the liver was observed and the concentration was appreciably lower than the blood level. In the lung, there was a concentration which was comparable to the

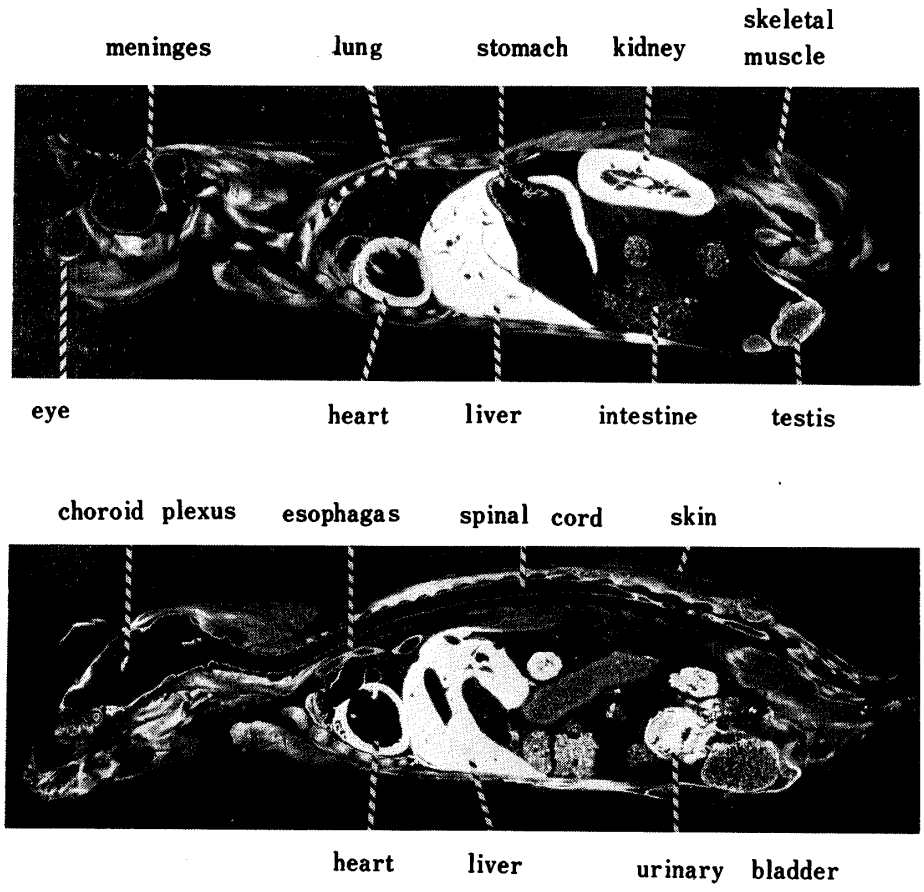

Fig. 3. Autoradiogram from a Mouse 6 hr after Intraperitoneal Injection of ^{14}C-Decamethonium

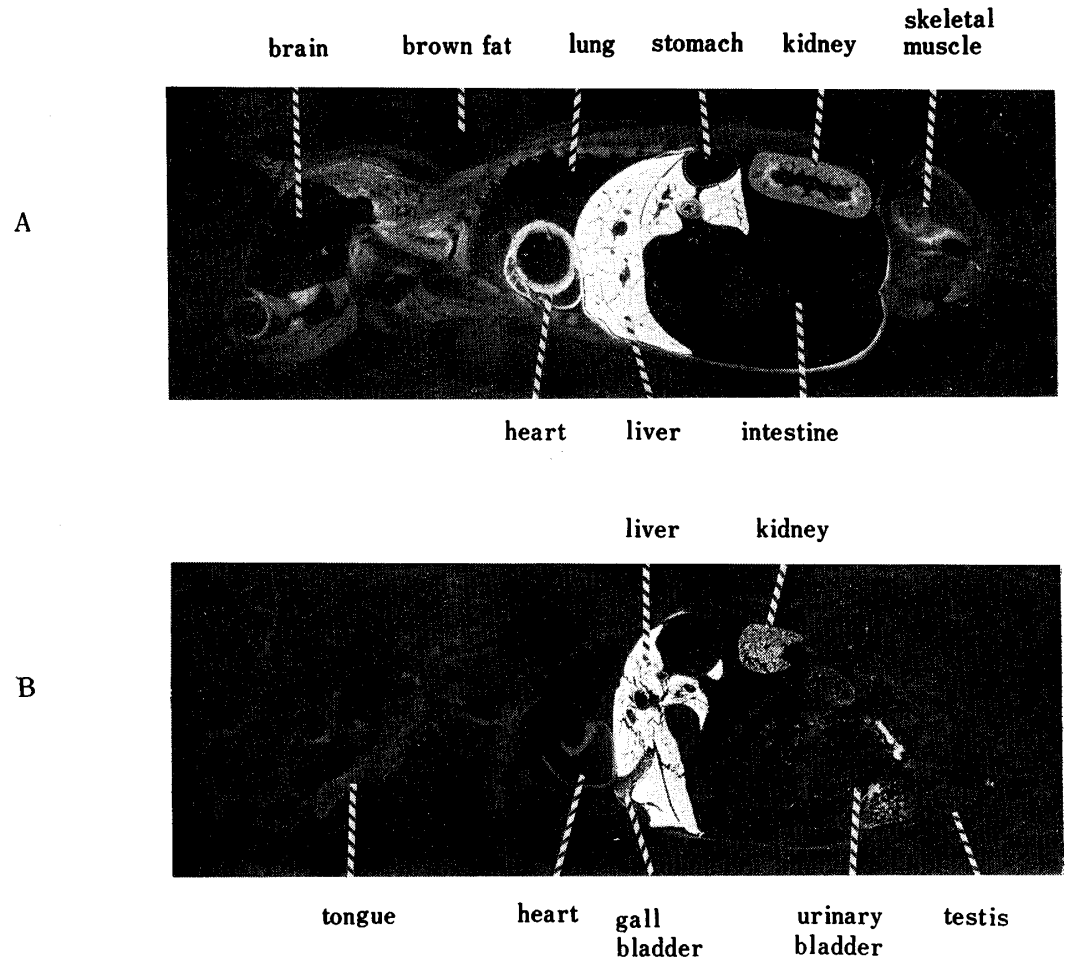

Fig. 4. Autoradiograms from Mice 24 (A) and 72 (B) hr after Intraperitoneal Injection of ^{14}C-Decamethonium

blood level. The highest concentration was shown in the kidney and urinary bladder, indicating a rapid excretion of the drug through the urinary route. A marked uptake of radioactivity was observed in the cartilages, such as, of the vertebra, trachea, sternum and larynx, but no uptake was observed in the meninges. In the muscular tissues and skin, only a weak and uniform distribution of radioactivity was observed.

Thus, 30 min after injection, hexamethonium revealed a distribution pattern of radioactivity which was significantly different from that of decamethonium, as can be seen from a comparison of Fig. 5 and 1. An increased concentration of radioactivity which exceeded the blood level was shown in the liver, but the highest radioactivity was still shown in the kidney and urinary bladder, indicating a much lower and slower accumulation of hexamethonium in the liver than that of decamethonium. A marked uptake of radioactivity was shown by the cartilages in a similar way as that in decamethonium and, after this survival period, an appreciable uptake was observed also in the meninges. No appreciable accumulation of radioactivity was shown, however, in the skeletal muscle and the concentration in the cardiac muscle was appreciably lower than the blood level. An appreciable uptake of radioactivity was noted in the mucosal layer of the stomach and intestine, while not in the muscular layer, being significantly different from the behavior of decamethonium.

During the period from 1 to 6 hr after injection, the blood level was declined to undetectable level and a high concentration of radioactivity was retained only in the liver and kidney, the most of radioactivity in other organs and tissues being disappeared rapidly before 6 hr (Fig. 6). The concentration in the liver showed a gradual decrease and almost disappeared

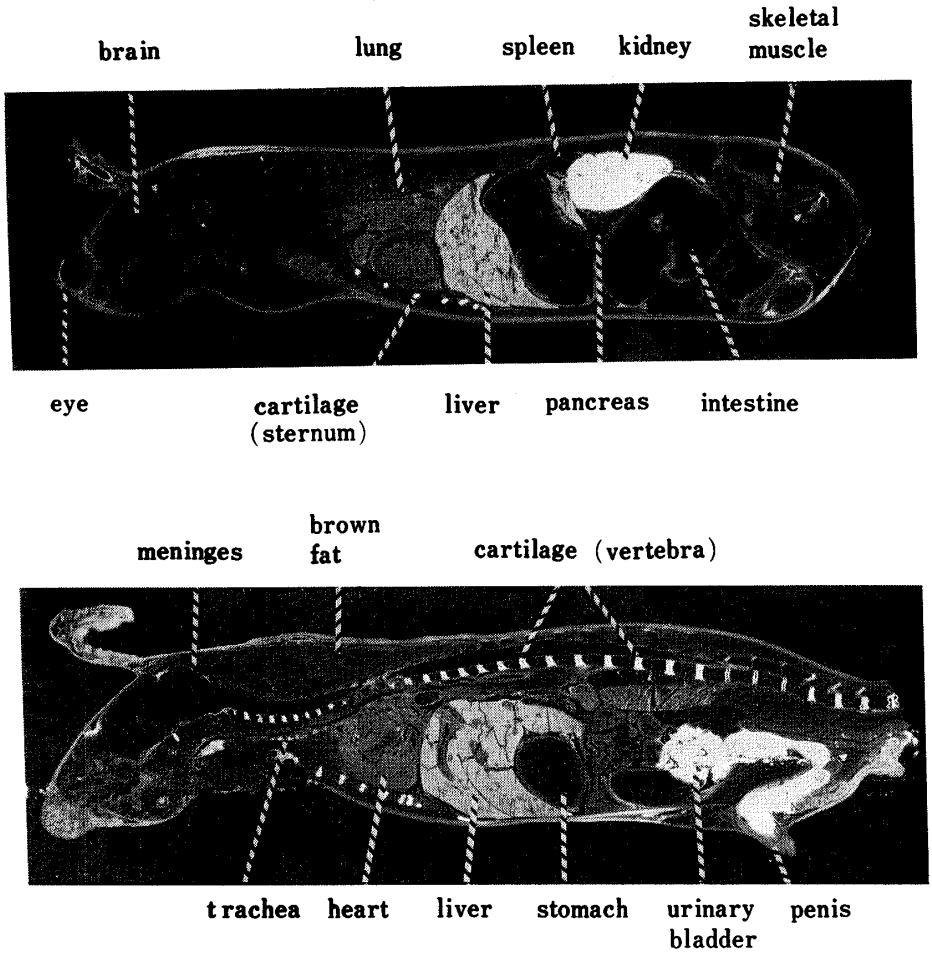

Fig. 5. Autoradiograms from a Mouse 30 min after Intraperitoneal Injection of ^{14}C-Hexamethonium

after 12 hr. Only a weak radioactivity was detected in the gall bladder and the intestinal contents, while a high radioactivity was continued to be present in the urinary bladder, indicating that the drug accumulated in the liver was gradually excreted mainly through the urinary route.

Dimethonium

Five and 15 min after intraperitoneal injection of ^{14}C-dimethonium, the highest radioactivity was observed in the urinary bladder and kidney and a high concentration in the circulating blood, revealing almost exclusively a pattern of rapid excretion of radioactivity through the urinary route. The radioactive uptake which exceeded the blood level was shown only by the cartilages. The lung showed a concentration comparable to the blood level, while the concentration in the liver was appreciably lower than the blood level.

Thirty minutes after injection, the blood level was declined to a low level and the radioactivity in the body appeared to be mostly concentrated in the urinary bladder, as shown in Fig. 7. Among other organs, only the kidney showed a concentration which exceeded the blood level. In the lung, the concentration was comparable to the blood level, while the liver and the muscular tissues did not show any appreciable uptake of radioactivity. Although an appreciable radioactivity was still remained in the peritoneal fluid, these results might indicate that the most part of dimethonium absorbed was directly excreted into the urine after circulation in the blood, without any appreciable uptake by the organs and tissues. The only characteristic which was common to the three bisquaternary compounds was a rapid

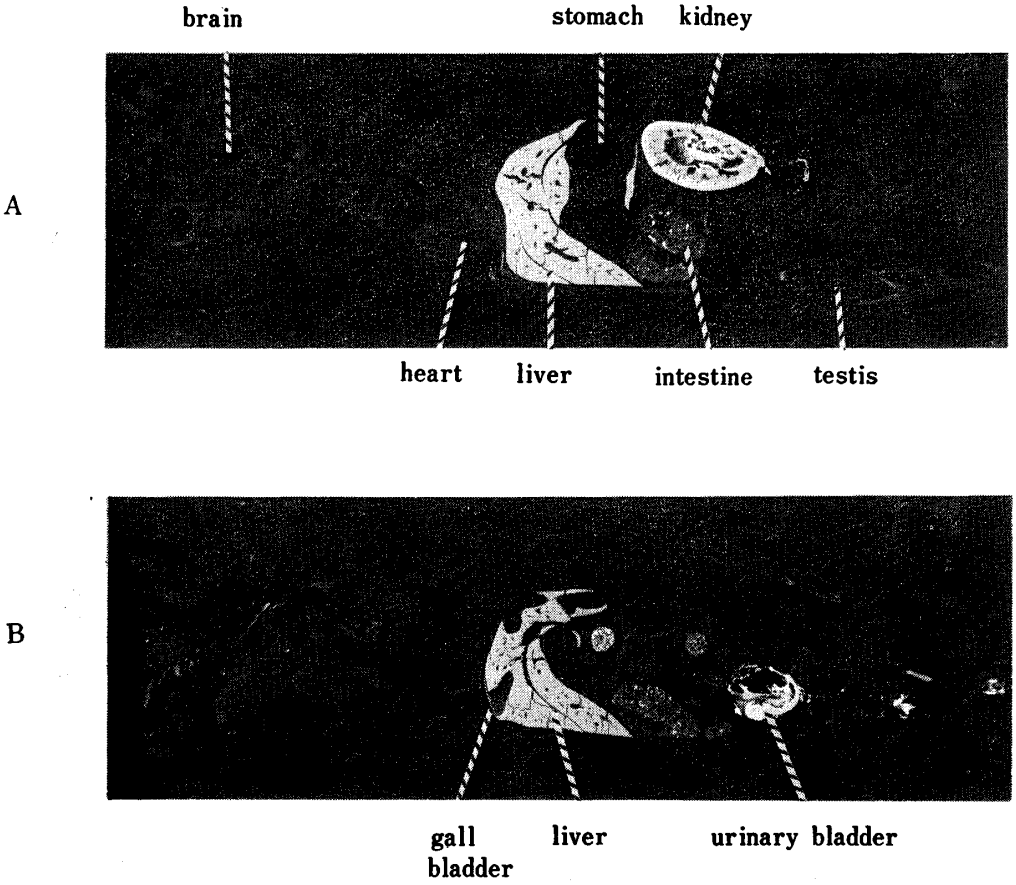

Fig. 6. Autoradiogram from Mice 3(A) and 6(B) hr after Intraperitoneal Injection of ^{14}C-Hexamethonium

uptake by the cartilage and a high radioactivity was observed in the cartilages of vertebra, sternum and trachea during the period from 5 min to 1 hr after injection of ^{14}C-dimethonium. In the meninges, however, no uptake of radioactivity was observed.

Three and 6 hr after injection, a high radioactivity was observed only in the urinary bladder and kidney and a trace of radioactivity in the liver and the intestinal contents, the most of radioactivity being disappeared from the body. It is thus evident that both the tissue uptake and retention of radioactivity is the far lowest for dimethonium among three compounds.

Discussion

Considering from a highly polar nature of the onium group, it was expected that the physicochemical nature of the quaternary ammonium compounds, thus possibly their distribution patterns, might not be affected so significantly by a structural change of the remaining part of the molecule. The present results revealed, however, that as far as di-, hexa- and decamethonium are compared the structural change gave rise to a large change in the distribution pattern so significantly that almost no common feature for the quaternary structure could be drawn. The same was true when the distribution of bis-quaternary compounds are compared to that of mono-quaternary structure, anisotropine methbromide.[1)]

Di-, hexa- and deca-methoniums are considered as a series of compounds with a progressive increase in the distance between two nitrogen atoms and in the partition coefficient of the molecule from aqueous to organic phases. The distribution patterns observed appear to have a close relation to these natures, as summarized in the followings.

i) The rate of uptake and the extent of accumulation in the liver were in the order of deca- ≫ hexa-＞di-methonium. Decamethonium showed an extremely high accumulation of radioactivity as early as 5 min after intraperitoneal injection and the same high concentration was retained for more than 72 hr. Hexamethonium showed the highest accumulation 1 hr after injection and the concentration was decreased considerably after 6 hr. Dimethonium did not show any concentration which exceeded the blood level for the whole period.

ii) The main route of excretion appears to be the urinary route for all three compounds, but the rate was in the order of di- ＞hexa-＞deca-methonium. Thus, the retention of the drug in the body was the longest for decamethonium, while the shortest for dimethonium.

iii) The uptake by the muscular tissues was observed specifically for decamethonium. A high and persistent radioactivity in the skeletal muscle and other muscular tissues was shown only after injection of ^{14}C-decamethonium.

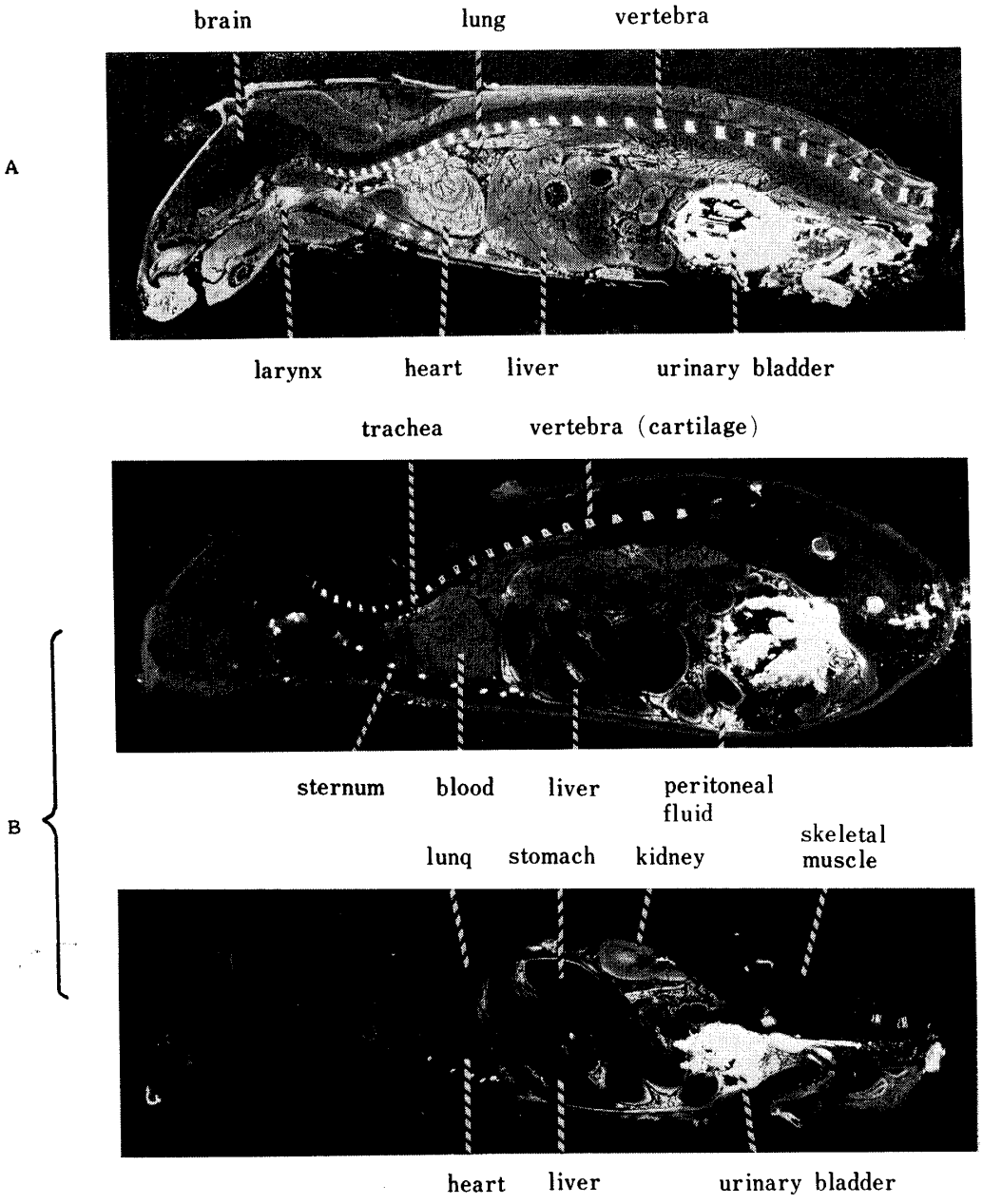

Fig. 7. Autoradiograms from Mice 15 (A) and 30 (B) min after Intraperitoneal Injection of ^{14}C-Dimethonium

iv) One of the common characteristic was the uptake by the cartilage and as early as 5 min after injection of any of three compounds a high radioactive uptake was observed in the cartilages of the vertebra, sternum, trachea and larynx.

v) The uptake by the meninges appears to be another common characteristic, but the rate and the extent appear to depend upon the structure. Decamethonium showed a high radioactivity in the meninges as early as 5 min after injection and the high concentration was remained for more than 6 hr. Hexamethonium showed an appreciable radioactivity 30 min after injection and the highest concentration after 1 hr. Dimethonium, on the other hand, did not show any appreciable concentration in the meninges.

A high accumulation of decamethonium in the mouse liver has been already reported by Christensen, *et al.*[6] and an active transport mechanism was suggested to be involved.[7] From the present results, it was further demonstrated that in a series of methonium compounds decamethonium is accumulated in the highest concentration with the longest retention in the liver, and it was suggested that the rate and the extent of the accumulation appear to be determined mainly by the length of carbon chain between two quaternary nitrogens, thus probably the lipophilicity of the molecule. It is of interest here to note that in spite of such a high accumulation in the liver almost no biliary excretion of the drug appears to occur and the cellular and subcellular localization of the drug remains to be investigated.

A high affinity of the methonium compounds to the cartilage might be a characteristic for the bisquaternary structure, since tetraethylammonium iodide[8] as well as anisotropine methbromide[1] did not show any uptake by the cartilage. It might be possible to consider that such a high affinity of the bis-quaternary structure is due to a binding of the quaternary ammonium ions to the sulfate ions in chondroitin sulfate, possibly, by forming a bridged structure. In the present results, the initial rate of the uptake appears to be the highest for dimethonium, suggesting that the affinity becomes weaker with increasing the distance between two quaternary nitrogens.

In the literatures, an appreciable uptake of some bisquaternary ammonium compounds by the central nervous system has often been suggested.[9] From the present investigations, however, it became clear that there is no penetration of both deca- and hexa-methonium into the brain parenchymal tissue, but both compounds are accumulated in high concentrations in the meninges and choroid plexus. A recent micro-autoradiographic work by Gosling, *et al.*[10] indicated that deca- and hexamethonium were localized in the arachnoid and choroid plexus in the mouse brain, in accordance with the present results.

It was noted to be an another characteristic of bisquaternary structure that they did not show any appreciable concentration in the salivary gland and other secretory glands. It was reported[1] that anisotropine methbromide showed a high concentration in the salivary gland and the gastric mucosa and the former was discussed of a possible relation to the side effect on the salivation. Tetraethylammonium iodide, another monoquaternary ammonium investigated,[8] also showed a high concentration in the salivary gland (Fig. 8). In the stomach, there was shown a sharp contrast between the distributions of anisotropine methbromide[1] and decamethonium, the former showing a high accumulation only in the mucosal layer, while the latter only in the muscular layer. Such clear differences in the distribution pattern depending upon the structure might be of great help in considering a modification of pharmacological or side effect in drug design.

6) C.B. Christensen and J. Holm, *Acta Pharmacol. et Toxicol.*, **27**, 17 (1969).
7) C.B. Christensen, *Acta Pharmacol. et Toxicol.*, **28**, 215 (1970).
8) H. Shindo, I. Takahashi and E. Nakalima, to be published.
9) R. Levine, *Nature*, **184**, 1412 (1959); T.C. Lu, J.A. Gosling and D.B. Taylor, *Europ. J. Pharmacol.*, **3**, 364 (1968).
10) J.A. Gosling and T.C. Lu, *J. Pharmacol. Exptl. Ther.*, **167**, 56 (1969).

The fact that the uptake and accumulation of radioactivity in the muscular tissues was observed only by decamethonium is condisered to have a direct connection to the appearence of the pharmacological effect of decamethonium as a depolarizer at the neuro-muscular junctions. In fact, microautoradiographic works by Creese, *et al.*[11] indicated that ^{3}H-decamethonium is localized in the muscle fibers mainly in the area of neuro-muscular junction of mouse diaphragm after intravenous injection. Hexamethonium is, on the other hand, a competitive inhibitor at the sympathetic and parasympathetic nerve ganglions and, therefore, the drug is not necessarily required to be present in the muscular tissue itself for the appearence of its pharmacological action. In accordance, hexamethonium did not show any appreciable accumulation in the muscular tissues. Dimethonium which has no pharmacological action[12] also did not show any appreciable uptake by the muscular tissues.

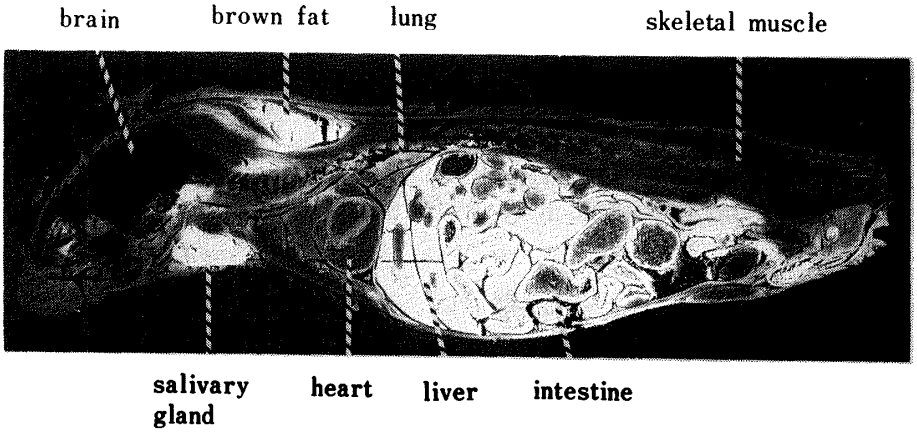

Fig. 8. Autoradiogram from a Mouse 30 min after Intraperitoneal Injection of ^{14}C-Tetraethylammonium

It is of interest here to compare the distribution of tetraethylammonium ion, because it is another ganglionic depressant and has eight alkyl carbons with respect to one quaternary nitrogen and, therefore, is regarded as a drug which is comparable to hexamethonium with respect to the pharmacological action, while is comparable to decamethonium with respect to the physicochemical nature such as the partition coefficient. From our unpublished results,[8] as examplified in Fig. 8, it was found that tetraethylammonium did not show any appreciable accumulation in the skeletal muscle, being similar to hexamethonium. A high accumulation was observed in the liver, on the other hand, being similar to decamethonium in this respect, providing a further evidence that the extent of accumulation in the liver is determined mainly by the physicochemical nature, possibly the lipophilic nature of the compound. These results might strongly suggest that there is a direct connection between the pharmacological effect of a drug and the distribution pattern of macroscopic level. This is considered to hold true generally when a rapid equilibration could be assumed of the drug concentration between the receptor compartment and the environmental tissue compartment.

Acknowledgement The authors express their deep gratitudes to Dr. G. Sunagawa, director of this laboratories, for his kind encouragement and to Prof. J. Tsurufuji of the University of Tokyo for his valuable advices and discussions.

11) D.B. Taylor, W.J. Dixon, R. Creese and R. Case, *Nature*, **215**, 989 (1967); R. Creese and J. Maclagan, *J. Physiol.*, **210**, 363 (1970).
12) W.D.W. Paton and E.J. Zaimis, *Pharmacol. Revs.*, **4**, 219 (1952).