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Autoradiographic Studies on the Distribution of Quaternary Ammonium Compounds. III.[1] Distribution, Excretion and Metabolism of ^{14}C-Labeled Pancuronium Bromide in Rats[2]

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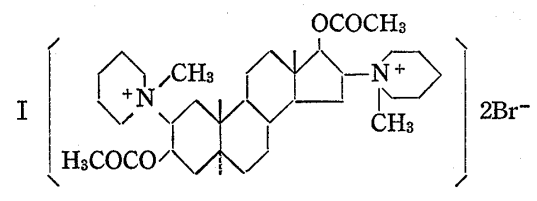
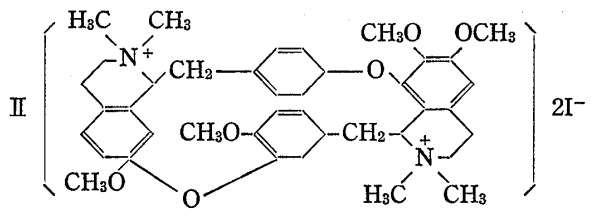
The distribution, excretion and metabolism of ^{14}C-labeled pancuronium bromide following intraperitoneal administration to rats were investigated by means of whole-body autoradiography and radioassay. The distribution of dimethyl-*d*-tubocurarine-^{14}C iodide was also investigated in mice for comparison. Pancuronium-^{14}C bromide was found to be accumulated rapidly in the liver, kidney and cartilage tissues such as the sternum, vertebra and trachea and slowly in the bone marrow and spleen, while no accumulation was observed in the muscular tissues and central nervous system. The accumulation in the liver continued for a long period and almost disappeared after 30 days survival. Counting of the liver radioactivity revealed that approximately 8, 2.5, 1.2 and 0.5% of the dose remained in the liver after 1, 6, 20 and 30 days, respectively; the concentration decreased with half-life of about 11.2 days after an initial decline with half-life of about 1.2 days. It was confirmed that pancuronium bromide is excreted mainly *via* urine, mostly in the unchanged form. Dimethyl-*d*-tubocurarine-^{14}C iodide showed a distribution pattern similar to that of pancuronium-^{14}C bromide.

The distribution pattern of pancuronium ion was thus quite similar to that of decamethonium ion, except that the latter accumulates in the muscular tissues. This is considered to be related to the fact that decamethonium is a depolarizer at the neuromuscular junctions, while both pancuronium and *d*-tubocurarine is a competitive depressant. It was also indicated that the extent of accumulation of bis-onium structure in the liver is determined not by the lipophilic character of the molecule, but primarily by the distance separating the two quaternary nitrogens.

In the previous paper,[1] the distribution of ^{14}C-labeled deca-, hexa- and dimethonium in mice were investigated on a comparative basis and a high accumulation of these substances in the cartilage tissues has been found as a characteristic feature common to the bis-quaternary structure. The accumulation in the liver was found to be the highest in decamethonium and decreased markedly in hexamethonium, while almost no accumulation was observed in dimethonium. The accumulation in the skeletal muscle was shown only by decamethonium and this was discussed in relation to its mode of pharmacological action. In the present paper, the distribution of ^{14}C-labeled pancuronium bromide (I),[4] another muscle relaxant possessing





1) Part II: H. Shindo, I. Takahashi and E. Nakajima, *Chem. Pharm. Bull.* (Tokyo), **19**, 1876 (1971).
2) This work was presented at the 92nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April, 1972.
3) Location: *Hiromachi 1-chome, Shinagawa-ku, Tokyo.*
4) Pavulon®, 2β,16β-dipiperidino-5α-androstane 3α,17β-diol diacetate dimethobromide, N.V. Organon.

the bis-onium structure, was studied by means of whole-body autoradiography and radioassay. The distribution of dimethyl-*d*-tubocurarine iodide was also investigated for comparison.

Material and Method

Labeled Drug——Pancuronium(methyl-^{14}C) bromide was supplied by Organon Laboratories. The specific activity was 108 μCi/mg and the radiochemical purity was ascertained to be 99.8% by thin-layer chromatography (TLC). Dimethyl-*d*-tubocurarine(methyl-^{14}C) iodide with the specific activity of 112 μCi/mg was purchased from New England Nuclear Corporation. Unlabeled pancuronium bromide and its 3-hydroxy, 17-hydroxy and 3,17-dihydroxy derivatives were also supplied by Organon Laboratories.

Whole-body Autoradiography——Male rats of Wistar-Imamichi strain weighing about 100 g were used. Pancuronium-^{14}C bromide was dissolved in physiological saline at a concentration of 100 μg/ml and 0.5 ml of the solution was injected intraperitoneally. The dose was 54 μCi/500 μg/kg body weight. The animals were kept in individual cages and sacrificed after 30 and 60 min, 6 and 24 hr and 3, 6, 20 and 30 days by immersing the body into the mixture of solid carbon dioxide and hexane at −75°. Dimethyl-*d*-tubocurarine-^{14}C iodide was dissolved in water and administered intraperitoneally to male mice of ddY strain in a dose of 0.5 μCi (4.5 μg)/0.15 ml/18 g mouse. The animals were sacrificed after 15, 30 and 60 min and 1, 3 and 6 days.

For the animals sacrificed before 3 days survival, the autoradiography was performed according to the method of Ullberg.[5] 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 with a heavy sledge microtome (Yamato Type 1111) in a freezing room and dried at −15°. The dried sections were brought to contact with Sakura Type-N X-ray film and exposed for a constant period of 40 days. For the animals sacrificed 3 days and afterward, the sawing method by Kalberer[6] was adopted because of a large weight gain of the animals. The frozen animals were cut into the whole-body slices of about 2 mm thickness with a round saw of 300 mm diameter with 96 teeth (MASIBA Apparatebau AG, Basel) in a freezing room. The slices, after removing the tissue dusts from the surface, were exposed onto X-ray film for 40 days in a freezer at −30°.

Determination of Tissue Concentration——Male rats of Wistar-Imamichi strain weighing about 180 g were used, three animals to one group. Pancuronium-^{14}C bromide was injected intraperitoneally in a dose of 5.4 μCi/50 μg/rat. Each animal was placed in an individual metabolic cage and food and water were given *ad libitum*. Thirty and 60 min, 6 and 24 hr, 3, 6, 10, 20 and 30 days after administration, the animals were sacrificed by bleeding from the carotid and the blood sample was collected in a test tube precoated with heparine. The liver, kidney, brain and skeletal muscle (femoral) were removed by dissection and weighed. The blood and tissue samples were solubilized in 30% KOH by warming at 80° for 3 hr and an aliquot of the solution was assayed for radioactivity. The carcasses were solubilized in an equal amount of 30% KOH by warming at 80° overnight.

Determination of Urinary and Fecal Excretion——The urine was collected for the periods of 1, 6 and 24 hr, 6 and 20 days after administration. In collecting the urine from the groups for the period shorter than 24 hr, the urinary bladder was removed by dissection, the contents drained, the bladder washed and the washings were combined with the urine. For the groups of 6 and 20 days periods, the urine was collected periodically at 2, 4, 6, 12 and 20 days after administration. The collected urine was centrifuged and the supernatant was assayed for radioactivity. The feces were collected for 1 and 7 day periods, homogenized with water and an aliquot was assayed for radioactivity.

Separation of Urinary Metabolites——An aliquot of the 24 hr urine was treated with appropriate amounts of unlabeled pancuronium bromide and its three deacylated products as carriers and was analyzed by TLC. The chromatography was performed on Kiesel-gel F_{254} plate (Merck, 0.25 mm) with the upper phase of the system; *n*-butanol/pyridine/acetic acid/20% ammonium chloride (15 : 10 : 3 : 12; v/v/v/v), as the solvent system[7] and developed by the ascending method for 18 hr in an open system. The radioactive spots were detected by autoradiography as well as by Dragendorff's reagent. Each spot was quantitatively transferred into the counting vials by careful scraping with a spatula, followed by the estimation of radioactivity.

Radioactivity Measurements——All samples were counted in a Beckmann LS-250 liquid scintillation spectrometer using a counting medium consisting of 2,5-diphenyloxazole (PPO) 8 g, 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP) 0.2 g, toluene 500 ml and methylcellosolve 500 ml. The counting efficiencies were determined by ^{137}Cs external standard method and the counts were converted to disintegration per minutes (dpm) with Olibetti Programma 101 computor. The tissue samples dissolved in alkali, usually 0.5 ml, were added with 2 g BBS-III solubilizer, neutralized with acetic acid and added with 15 ml of the liquid scintillator. Blood samples were measured in this same way after decolorization with a few drops

5) S. Ullberg, *Acta Radiol. Suppl.*, **1954**, 118.
6) F. Kalberer, *Advances in Tracer Methodology*, **3**, 139 (1966).
7) F. Van der Veen, Unpublished Results from Organon Laboratories (1971).

of 30% H_2O_2. For the feces samples, the liquid scintillator containing 4% Cab-O-Sil was used. The counting efficiencies were 60—70% and 75—85% for feces samples and all other samples, respectively.

Results

Whole-body Autoradiography of Pancuronium-^{14}C Bromide in Rats

The whole-body autoradiograms after intraperitoneal injection of pancuronium-^{14}C bromide to rats are shown in Fig. 1 and 2. Thirty minutes after injection (Fig. 1-A), some radioactivity remained in the peritoneal cavity, but high radioactivity was shown in the kidney, urinary bladder, intestinal contents and the cartilage tissues such as that of the vertebra, sternum and trachea. In the liver a high radioactivity was distributed in a spotted pattern and a concentration higher than the blood level was noted in the connective tissues in the skin and skeletal muscle. No radioactive uptake was detected in the central nervous system nor in muscular tissues such as the cardiac and skeletal muscles. An appreciable concentration of radioactivity was detected in the adrenal medulla, lung and spleen, while no radioactivity in the thymus and bone marrow.

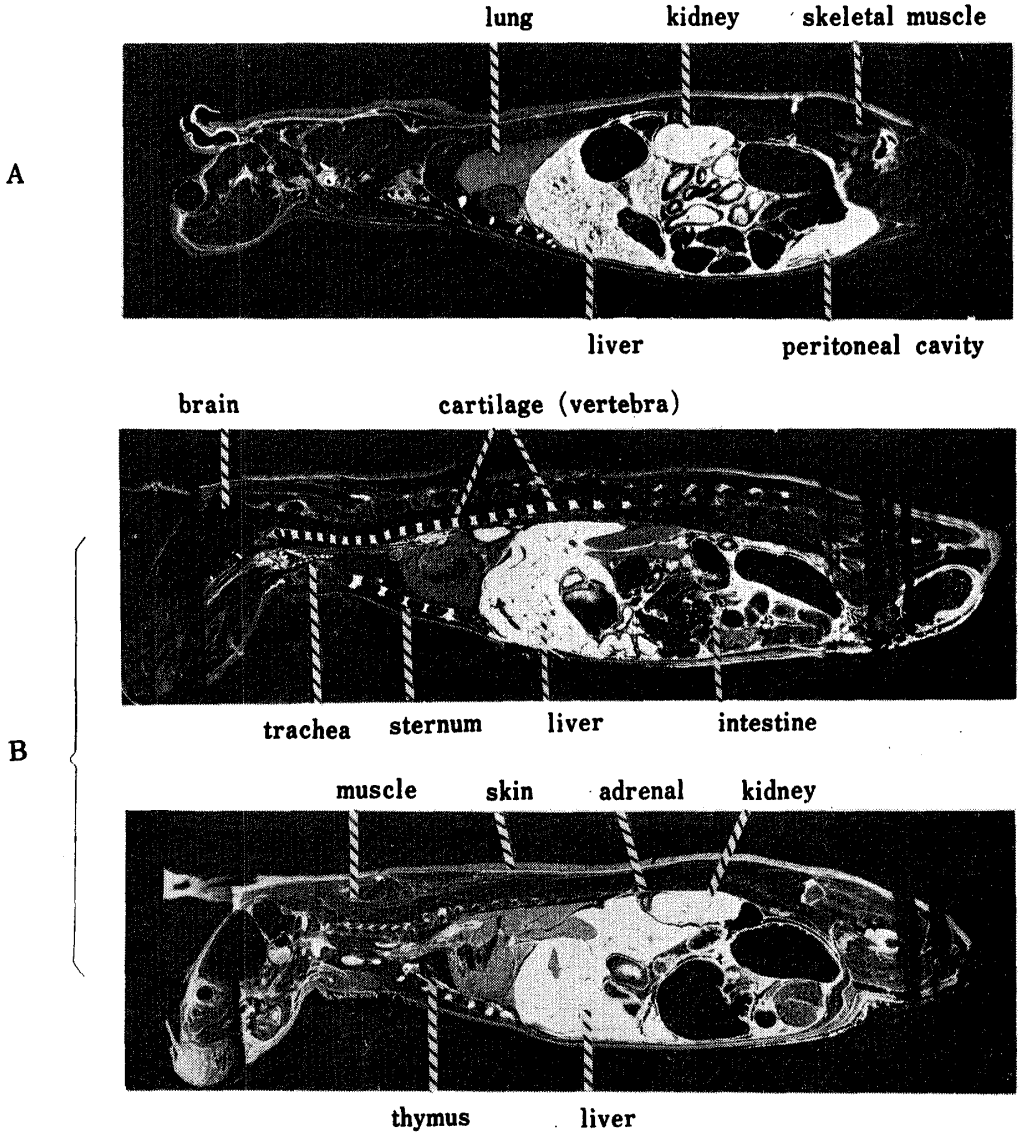


Fig. 1. Autoradiograms from Rats 30 min (A) and 1 hr (B) after Intraperitoneal Administration of Pancuronium-^{14}C Bromide

One hour after injection, the highest accumulation of radioactivity was observed in the liver and kidney and a high accumulation in the cartilage tissues, as can be seen from Fig. 1-B. The accumulation of radioactivity in connective tissues in the skin and skeletal muscle became more apparent and an uptake was also observed in the hair follicles and meninges. This time, an appreciable radioactive uptake was noted in the bone marrow, while no radioactive uptake was detected in the central nervous system and skeletal muscle fibers. It was noted that the blood concentration continues for a rather long period.

After 6 and 24 hr (Fig. 2-A and B), the radioactivity in the blood had disappeared almost completely and very high radioactivity remained only in the liver, kidney and intestinal contents. The radioactivity in the cartilage tissues disappeared, while an appreciable radioactivity was retained in the bone marrow, spleen and meninges.

The high accumulation of radioactivity in the liver and kidney was found to continue for more than 3 days. As shown in Fig. 2-C, high radioactivity was observed only in the liver and the renal cortex and an appreciable radioactivity was still present in the bone marrow, intestinal contents and spleen. After 10 and 20 days survival, appreciable radioactivity

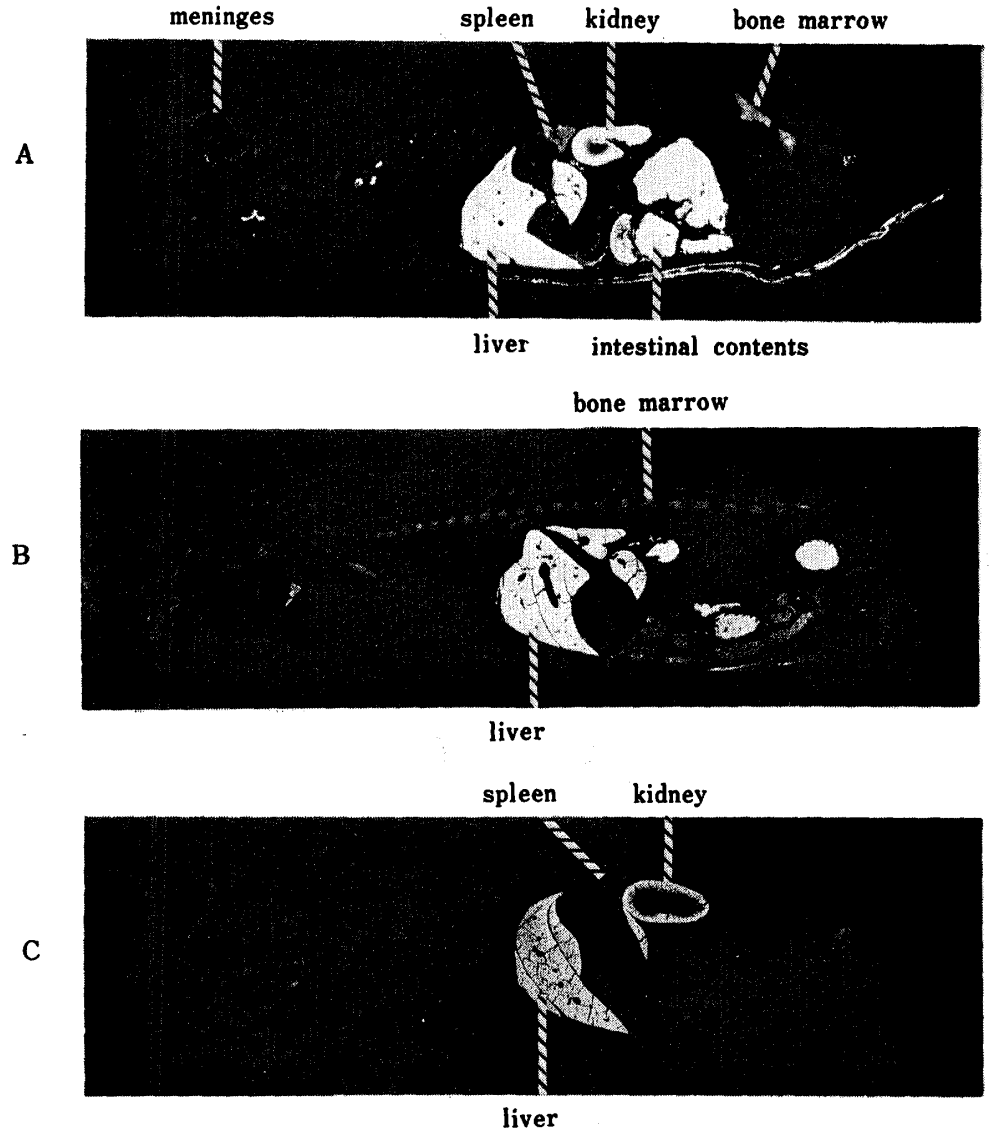


Fig. 2. Autoradiograms from Rats 6 hr (A), 24 hr (B) and 3 days (C) after Intraperitoneal Administration of Pancuronium-^{14}C Bromide

was still detected in the liver and the renal cortex and the radioactivity disappeared almost completely after 30 days. Enlargement of the autoradiogram 6 days after administration indicated that the site of residual radioactivity in the liver corresponds well to the peripheral part of the individual liver lobule.

Tissue Concentration and Excretion of Pancuronium-^{14}C Bromide in Rats

The urinary and fecal excretion of radioactivity after intraperitoneal administration of pancuronium-^{14}C bromide in rats are shown in Table I. During the first 1 hr, approximately 47% of the dose was recovered in the urine, indicating that the drug is rapidly absorbed from the peritoneal route. During a 24 hr period, approximately 75 and 12% of the dose was recovered in the urine and feces, respectively, indicating that the excretion of the drug proceeded mainly through the urinary route. In a separate experiment, the bile was collected for a 24 hr period from the cannulated rat wherein pancuronium-^{14}C bromide was administered intramuscularly. The result indicated that only about 6% of the dose was recovered in the bile during 24 hr, indicating that the biliary excretion does not participate significantly.

Table I. Urinary and Fecal Excretion and Retention in Liver and Kidney after Intraperitoneal Administration of Pancuronium-^{14}C Bromide to Rats

Hours or days	% to dose ± standard error ($n=3$)[a]					
	Urine	Feces	Liver	Kidney	Carcass	Recovery
0.5h	—[b]	—	7.56±1.19	2.40 ±0.56	—	—
1 h	47.8[c]	—	11.8 ±1.08	1.48 ±0.15	—	—
6 h	69.2±13.6	—	11.4 ±0.63	0.49 ±0.02	—	—
1 d	75.0±3.90	11.8 ±1.55	8.18±0.86	0.72 ±0.01	6.17[c]	101.8±0.91
3 d	—	—	2.84±0.06	0.30 ±0.02	—	—
6 d	73.1±5.10	14.9 ±3.50	2.40±0.10	0.15 ±0.02	—	—
10 d	—	—	1.82±0.27	0.16 ±0.03	—	—
20 d	88.9±9.70	9.18±1.41	1.16±0.05	0.11 ±0.03	0.65±0.07	100.0±8.16
30 d	—	—	0.48±0.05	0.003±0.00	—	—

a) n=number of experiments b) not determined c) $n=1$

The radioactivity in the whole liver, kidney and the carcass was also determined as included in the table. In the liver, the peak concentration was reached at 1 hr after administration and maintained at the same level until 6 hr, accounting for approximately 12% of the dose. The concentration decreased gradually afterwards and accounted for 8, 2.4, 1.2 and 0.5% of the dose after 1, 6, 20 and 30 days, respectively. All of the radioactivity administered was recovered in this way (Table I), indicating that no excretion of radioactivity occurs by expiration.

The blood and tissue concentration of radioactivity are shown in Table II. Radioactivity in the blood reached the maximum after 30 min and declined rapidly up until 6 hr after administration, followed by a much slower decline with a half-life of about 5 hr, reaching an undetectable level after 24 hr. The semi-logarithmic plots of the liver concentration revealed that, as shown in Fig. 3, the radioactivity decreased biphasically with a half-life of about 1.2 days for the first 3 days and, thereafter, much more slowly with a half-life of about 11.2 days. Thus, the extrapolation of the line indicated that the liver radioactivity diminishes to below 0.2% of the dose after about 45 days. In the kidney, the highest concentration of radioactivity which was about 3 times higher than the blood concentration was reached 30 min after administration and declined rapidly. The concentration in the skeletal muscle was only 1/6 to the blood concentration 30 min after administration and was undetectable after 6 hr, thereby confirming that no appreciable uptake occurs by the muscle fibers. No radioactive uptake was detected in the brain.

Table II. Blood and Tissue Concentration of Pancuronium-^{14}C Bromide after Intraperitoneal Administration to Rats (Dose: *ca.* 300 μg/kg)

Hours or days	μg/ml blood or g tissue[a] ± S.E. (n=3)[b]				
	Blood	Brain	Skeletal Muscle	Liver	Kidney
0.5h	0.229±0.015	0.003±0.000	0.036±0.004	0.455±0.048	0.715±0.170
1 h	0.083±0.029	0.003±0.000	0.023±0.003	0.855±0.075	0.565±0.095
6 h	0.020±0.005	—[c]	—	0.755±0.005	0.156±0.005
1 d	0.002±0.000	—	—	0.695±0.100	0.239±0.003
3 d	—	—	—	0.326±0.010	0.114±0.010
6 d	—	—	—	0.206±0.006	0.044±0.005
10 d	—	—	—	0.108±0.012	0.045±0.010
20 d	—	—	—	0.055±0.004	0.028±0.006
30 d	—	—	—	0.020±0.003	0.001±0.000

a) Radioactivity was converted to μg equivalents of pancuronium bromide.
b) n=number of experiments
c) No significant radioactivity

On the autoradiogram of thin-layer chromatogram of the rat urine collected for 24 hr after administration of pancuronium-^{14}C bromide, only one radioactive spot was detected at the position corresponding to unchanged pancuronium bromide. The counting of the spot as well as the areas corresponding to its deacylated products revealed that over 90% of the urinary radioactivity was unchanged pancuronium, along with, if any, approximately 5 and 2% of the monoacetyl and diol metabolite, respectively.

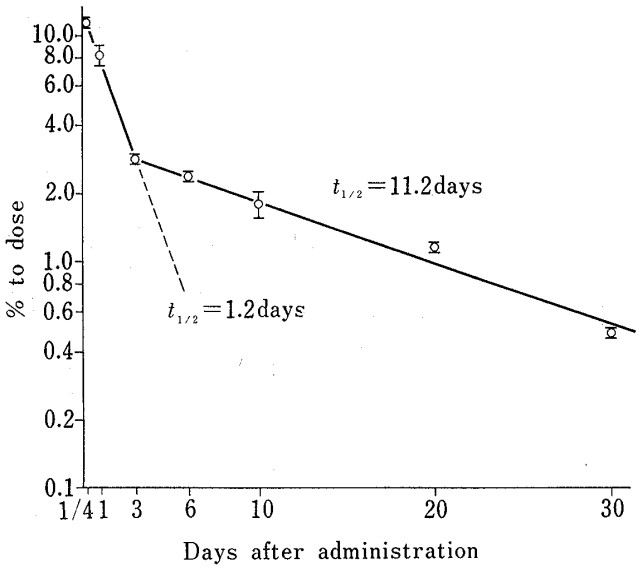


Fig. 3. Semi-logarithmic Plots of Radioactivity in Liver after Intraperitoneal Administration of Pancuronium-^{14}C Bromide to Rats

Each plot represents the average from three experiments ± standard error.

Whole-body Autoradiography of Dimethyl-*d*-tubocurarine-^{14}C Iodide in Mice

The whole-body autoradiograms of dimethyl-*d*-tubocurarine-^{14}C iodide after intraperitoneal injection to mice are shown in Fig. 4. After 30 min, the radioactivity disappeared mostly from the peritoneal cavity and a high concentration was observed in the kidney and urinary bladder as well as the circulating blood. A high accumulation of radioactivity was observed in the cartilage tissues such as that of the vertebra, trachea and sternum. In the liver, on the other hand, the concentration was only slightly higher than the blood concentration, while a much higher concentration was shown in the gall bladder, as shown in Fig. 4-A. The distribution pattern of radioactivity in the muscular tissues was the same to that of pancuronium-^{14}C; the radioactivity was distributed only in the extracellular spaces and no uptake was detected in the muscle fibers.

One hour after administration, the blood concentration decreased to a very low level and high concentrations were observed only in the liver, kidney, cartilage tissues and the urinary and gall bladders. Thus, dimethyl-*d*-tubocurarine appears to accumulate in the liver more slowly than pancuronium. No radioactivity exceeding the blood level was observed in the spleen and bone marrow, in contrast to the distribution of pancuronium. After 24 hr,

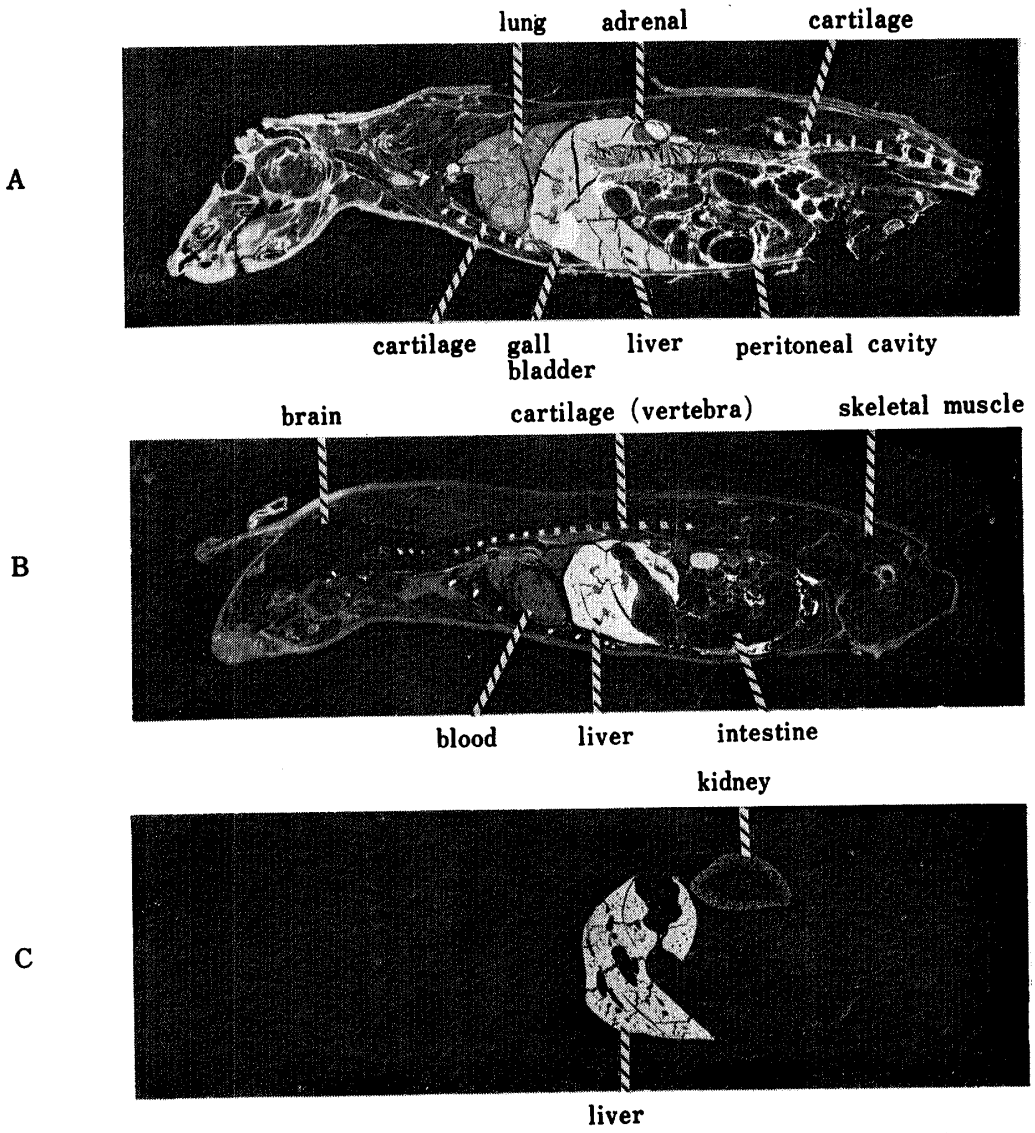


Fig. 4. Autoradiograms from Mice 30 min (A), 60 min (B) and 24 hr (C) after Intraperitoneal Administration of Dimethyl-*d*-tubocurarine-^{14}C Iodide

a persistent high radioactivity was observed only in the liver and appreciable radioactivity in the renal cortex, as can be seen in Fig. 4-C. Appreciable radioactivity was shown to remain in the liver after survival periods of 3 and 6 days.

Discussion

In the preceding paper,[1] the distribution between deca-, hexa- and dimethonium was compared after intraperitoneal injection to mice and the following was found: i) the bis-onium structure shows a completely different distribution pattern from that of mono-onium structure, ii) all compounds show a rapid accumulation in the cartilage tissues, iii) the rate and the extent of accumulation in the liver increases in the order of di-$<$hexa-$\ll$deca-methonium, and iv) only decamethonium, which acts as a depolarizer at the neuro-muscular junctions, shows an accumulation in the muscular tissues.

Pancuronium bromide is another muscle relaxant of bis-onium structure containing a steroid nucleus and currently in widespread clinical use. In the present investigation, the distribution of pancuronium-^{14}C bromide has been studied in order to see how a large change

in the structure of the remaining part of the bis-onium molecule can affect the distribution pattern. The compound is of particular interest because it has a nine carbon rigid chain between the two quaternary nitrogens and is expected to have a considerably higher lipophilic character than decamethonium. Furthermore, its mode of action has been clarified[8] to be a competitive action at the neuro-muscular junctions. Dimethyl-*d*-tubocurarine, a typical competitive depressant, has also studied for comparison.

One of the present results that both pancuronium and dimethyl-*d*-tubocurarine are rapidly accumulated in the cartilage tissues indicates that the accumulation is common characteristic to bis-onium structures irrespective of the structure of the remaining part of the molecule. It has been demonstrated in this laboratory[9] that when rat whole-body freeze-dried sections were incubated *in vitro* with ^{14}C-labeled bis-onium compounds the followed autoradiography revealed that a strong adsorption of radioactivity occurs selectively at the cartilage tissues. *In vitro* binding of decamethonium with chondroitin sulfate has been proved by Asgher, *et al.*[10] It is plausible, therefore, that the same binding of bis-quaternary ammonium ions to chondroitin sulfate, probably in a bridged structure, is also occurring *in vivo* when they are administered.

A high accumulation of pancuronium bromide in the liver has already been noted in dogs by Van der Veen,[7] approximately 35 and 11 to 24% of the dose being retained in the liver 1 and 5 days after intravenous administration, respectively. Complete elimination of radioactivity from the liver, however, has not been confirmed. In the present study, a high accumulation and its long retention in the liver was also demonstrated in rats by both autoradiography and radioassay, although the extent appears to be appreciably lower than that in dogs. Approximately 12% of the dose was accumulated after 1 hr and the concentration decreased to approximately 8, 2.4, 1.8 and 0.5% of the dose after 1, 6, 10 and 30 days, respectively. The radioactivity was almost undetectable on the autoradiogram after 30 days and the radioassay indicated that pancuronium bromide accumulated in the liver can be eliminated completely after about 45 days survival. Since an appreciable radioactivity continued to be observed in the urinary bladder on the autoradiograms, the radioactivity accumulated in the liver is eliminated gradually through the blood into the urine rather than into the bile. It has been reported that[11] the biliary excretion of pancuronium is insignificant in rat isolated perfused liver. The distribution pattern of dimethyl-*d*-tubocurarine-^{14}C was found quite similar to that of pancuronium-^{14}C, except that the rate and the extent of accumulation in the liver appears to be lower than pancuronium and that the biliary excretion appears to play an appreciable role.

Pancuronium bromide has a carbon chain almost comparable to that of decamethonium between the two quaternary nitrogens, while dimethyl-*d*-tubocurarine is expected to have a somewhat shorter N–N distance than that in pancuronium, being rather comparable to that in hexamethonium. On the other hand, the lipophilic character of pancuronium bromide is certainly considerably higher than that of decamethonium, while that of *d*-tubocurarine has been shown to be appreciably higher than that of pancuronium.[11] Therefore, it is suggested that the rate and the extent of accumulation of bis-onium ions in the liver is determined primarily by the distance between the two quaternary nitrogens, while almost independent of the lipophilic character of the molecule. It should be noted here that in the *in vitro* experiment of incubating the rat whole-body sections with decamethonium-^{14}C, no binding phenomenon was detected in the liver.[9] It might be most plausible, therefore, that the accumulation

8) W.L.M. Baird and A.M. Reid, *Brit. J. Anaesth.*, **39**, 775 (1967); W.R. Buckett, C.E.B. Maajoribanks, F.A. Marwick and M.A. Morton, *Brit. J. Pharmacol.*, **32**, 671 (1968).
9) H. Shindo, E. Nakajima and E. Shigehara, *Chem. Pharm. Bull.* (Tokyo), to be published.
10) K. Asgher and L.J. Roth, *Biochem. Pharmacol.*, **20**, 3151 (1971).
11) D.K.F. Meijer and J.G. Weitering, *Europ. J. Pharmacol.*, **10**, 283 (1970).

of bis-quaternary ammonium ions in the liver is caused from an active transport mechanism[12] and a structural specificity with respect to the distance separating the two quaternary nitrogens is involved.

The present result that both pancuronium bromide and dimethyl-*d*-tubocurarine iodide, unlike decamethonium, showed no accumulation in the muscular tissues is in good accord with the fact that both compounds have no depolarizing action at the neuro-muscular junctions, but exhibit a competitive action.[8] In the previous paper,[1] it was found that the uptake and accumulation of radioactivity in the muscular tissues was observed only by decamethonium, while not by di- and hexamethonium and tetraethylammonium, and this was considered to have a direct connection to the appearance of pharmacological effect of decamethonium as a depolarizer at the neuro-muscular junctions. In fact, microautoradiographic work by Creese, *et al.*[13] indicated that ^{3}H-decamethonium is localized in the muscle fibers mainly in the area of neuro-muscular junctions of mouse diaphragm and, more recently, Mackay, *et al.*[14] demonstrated *in vitro* that the uptake is proceeded through a carrier-mediated transport mechanism. It might be deduced from the present results that the uptake of decamethonium by the muscular tissues must be due to a structural factor other than the lipophilic character.

Thus, it can be finally pointed out that the distribution pattern of pancuronium bromide is quite similar to that of decamethonium with respect to the uptake by the cartilage tissues and the accumulation in the liver, but dissimilar with respect to its lack of uptake by the muscular tissues. Another difference noted between the two compounds was that decamethonium showed a slow but an appreciable accumulation in the thymus, while pancuronium showed a slow but an appreciable accumulation in the bone marrow. These characteristics will be discussed in a subsequent paper[9] in relation to the binding of bis-quaternary ammonium ions to desoxyribonucleic acid (DNA) in these tissues.

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12) C.B. Christensen, *Acta Pharmacol. Toxicol.*, **28**, 215 (1970).
13) R. Creese and J. Maclagen, *J. Physiol.*, **210**, 363 (1970).
14) D. Mackay and D.B. Taylor, *Europ. J. Pharmacol.*, **9**, 195 (1970).