Br. J. Pharmac. (1974), 52, 367-374

DISTRIBUTION OF LOCAL ANAESTHETICS: ACCUMULATION IN SOME ENDOCRINE POLYPEPTIDE-HORMONE PRODUCING CELL SYSTEMS

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1 After the injection of labelled procaine and lidocaine in mice, the location and concentration of radioactivity was demonstrated by autoradiographical methods.

2 An accumulation in some endocrine cells such as the pancreatic islets, the hypophysis, the adrenal medulla and certain cells of the thyroid (probably representing the calcitonin-producing parafollicular cells) was shown.

3 After the injection of [^{14}C]-procaine in chicks, an accumulation of radioactivity was observed in the ultimobranchial gland (which produces calcitonin in birds), but not in the thyroid.

4 Radioactivity was also shown to be strongly concentrated in structures containing melanin, such as the pigment of the eye, skin and hair and in some organs involved in the metabolism and excretion of these drugs.

Introduction

Recent studies have shown that drugs with local anaesthetic activity can affect the secretion of hormones from endocrine glands such as the pancreatic islets (Brisson, Malaisse-Lagae & Malaisse, 1971) and the adrenal medulla (Jaanus, Miele & Rubin, 1967). Information on the uptake and localization of local anaesthetics in the endocrine organs would be of value in explaining some of the mechanisms by which these drugs affect the production and/or release of these hormones. In the present investigation the distribution of procaine and lidocaine in the mouse and chick has been studied with special reference to accumulation in the endocrine glands. Micro- and whole-body autoradiographic techniques were used.

Methods

Labelled compounds

[^{14}C]-procaine (procaine-[carboxyl-^{14}C]-hydrochloride), sp. act. 10.2 μCi/mg was obtained from New England Nuclear Corp., Boston, USA. [^{14}C]-lidocaine (lidocaine-[carboxyl-^{14}C]-hydrochloride), sp. act. 0.89 μCi/mg and [^{3}H]-lidocaine, generally labelled, sp. act. 64 μCi/mg, were obtained from Astra Pharmaceutical Co., Södertälje, Sweden. All the radioactive substances were dissolved in 0.9% w/v NaCl solution (saline) and volumes of 0.2 ml were used for a single injection in the experimental animals.

Experimental animals

Mice Adult male albino mice of NMRI strain (weight 25 g) were used in the studies. In some experiments pigmented male mice (CBA strain, weight 20 g) were also employed. All the animals were kept on a complete pellet diet (AB Ewos, Malmö, Sweden) at room temperature (+25° C) and received water *ad libitum*.

Chicks The chicks used in the present investigation were 2 weeks old White Leghorn cockerels weighing about 130 grams. The birds were fed a standard growth diet (Forss, Sweden) and had free access to drinking water.

Whole-body autoradiography

Mice For the whole-body autoradiographic studies of [^{14}C]-procaine, 6 male albino mice and 2 male pigmented mice were used. Each mouse was injected intravenously with 5 μCi [^{14}C]-procaine. The survival intervals were: 2 min (one male albino mouse), 5 min (one male albino mouse), 20 min (one male albino mouse and one male pigmented mouse), 1 h (one male albino mouse), 4 h (one male albino mouse), 24 h (one male albino mouse) and 72 h (one male pigmented mouse).

For the whole-body autoradiographic studies of [^{14}C]-lidocaine, 5 male albino mice were used. Each mouse was injected subcutaneously with 5 μCi [^{14}C]-lidocaine. The survival intervals were 5 min, 20 min, 1 h, 4 h and 24 hours.

Chicks For the whole-body autoradiographic studies in chicks, 2 cockerels were used. Each cockerel received 10 μCi [^{14}C]-procaine intravenously in a radial vein. The survival intervals were 5 min and 30 minutes.

At the stated survival intervals the animals were anaesthetized with ether and killed by immersion in a mixture of hexane and solid CO_2 (−78° C). They were then embedded in a mixture of carboxymethylcellulose and water, sectioned on tape and subjected to autoradiography according to a technique described elsewhere (Ullberg, 1954; Ullberg, 1958).

For semiquantitative evaluation of whole-body autoradiograms, the radioactivity in different organs was densitometrically compared with autoradiograms of simultaneously exposed ^{14}C-isotope standard staircases (Berlin & Ullberg, 1963). Each standard staircase consisted of 11 squares of increasing isotope concentration in the geometric series 2^1 (2), 2^2 (4), 2^3 (8) ... 2^{11} (2048). The radioactivity for different localities was expressed as the relative isotope concentration of the staircase step with which it matched.

Microautoradiography

Four male albino mice were used for this experiment. Each mouse received 0.25 mCi [^{3}H]-lidocaine intravenously. Five min and 30 min after the injection (2 mice at each survival interval) the mice were killed by decapitation and pieces of the pancreas were quickly removed and immediately frozen in isopentane cooled with liquid nitrogen. The pieces were then freeze-dried under vacuum (10^{-4} mmHg) at about −40° C for five days. After freeze-drying the specimens were embedded in warm (60° C) paraffin *in vacuo* and subjected to a microautoradiographic technique for water soluble isotopes (Hammarström, Appelgren & Ullberg, 1965). At each time interval, several sections serving as controls were also subjected to conventional stripping film autoradiography (Doniach & Pelc, 1950), which includes deparaffinization in xylol and alcohol and floating of stripping film on to the sections in water.

Table 1 Semiquantitative evaluation of whole-body autoradiograms after intravenous injection of [^{14}C]-procaine in mice

Organ	*Time after injection of [^{14}C]-procaine*						
	2 min	*5 min*	*20 min*	*1 h*	*4 h*	*24 h*	*72 h*
Pancreatic islets	2048	2048	512	256	128	32	–
Thyroid	2048	2048	512	256	128	32	–
Adrenal medulla	2048	2048	512	256	128	64	–
Hypophysis	2048	2048	512	256	128	64	–
Liver	1024	1024	128	64	64	32	–
Kidney	2048	2048	512	64	64	32	–
Salivary glands	2048	2048	256	64	64	32	–
Brain (grey matter)	1024	1024	64	16	–	–	–
Lungs	512	512	128	64	32	16	–
Bone marrow	1024	1024	128	32	32	16	–
Spleen	1024	1024	128	64	64	–	–
Thymus	1024	1024	128	32	16	16	–
Myocardium	512	512	16	16	16	4	–
Pigment	–	–	2048	–	–	–	2048
Blood	128	128	32	16	8	–	–

The radioactivity in the different organs was compared with autoradiograms of simultaneously exposed ^{14}C-isotope staircases. These consisted of 11 steps of increasing isotope concentration in the geometric series 2^1 (2), 2^2 (4), 2^3 (8) ... 2^{11} (2048). The radioactivity for the different localities is expressed as the relative isotope concentration of the staircase step with which it matched.

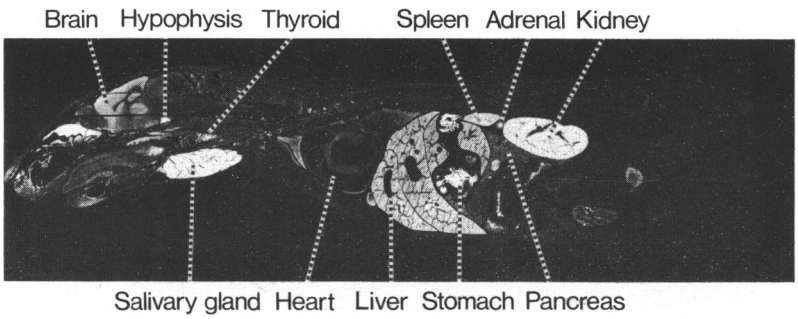

Fig. 1 Autoradiogram of an albino mouse 5 min after an i.v. injection of [^{14}C]-procaine. A high accumulation of radioactivity (light areas) can be seen in the brain, salivary gland, liver, spleen, kidney and gastrointestinal contents. In addition there is a high accumulation of radioactivity in the endocrine glands such as the hypophysis, the thyroid, the adrenal medulla and the pancreatic islets.

Results

Distribution of procaine and lidocaine in mice

The distribution of [^{14}C]-procaine obtained after intravenous injection in mice is summarized in Table 1. The distribution patterns seen after the subcutaneous injection of [^{14}C]-lidocaine were very similar to those of [^{14}C]-procaine. The difference observed seemed mainly to be due to the different ways of administration, and unless otherwise stated the distribution patterns will be described together.

The distribution was characterized by a high accumulation of radioactivity in endocrine organs such as the pancreatic islets, the hypophysis, the adrenal medulla and the thyroid. All these endocrine glands showed a high accumulation of label at the shortest survival intervals and a considerable activity could then be seen up to 4 h after the injection (Figures 1-5). Twenty-four hours after the injection of procaine, the radioactivity in the endocrine glands still remained the highest in the body with the exception of the excretory pathways. Unlike procaine, the concentration of isotope in the endocrine glands 24 h after the injection of lidocaine was rather low and did not exceed that of the blood. The accumulation in the different endocrine organs is described in more detail below.

Adrenals At short survival intervals there was a considerable accumulation of radioactivity in all cells of the medulla (Figures 1, 2 and 5). After long time intervals the most marked labelling seemed to be confined to the peripheral parts of the medulla.

Hypophysis The hypophysis showed a high concentration in all parts of the gland within 1 h after injection (Figure 4). Later on the activity disappeared gradually from the neurohypophysis while it remained high in the adenohypophysis (Figure 3).

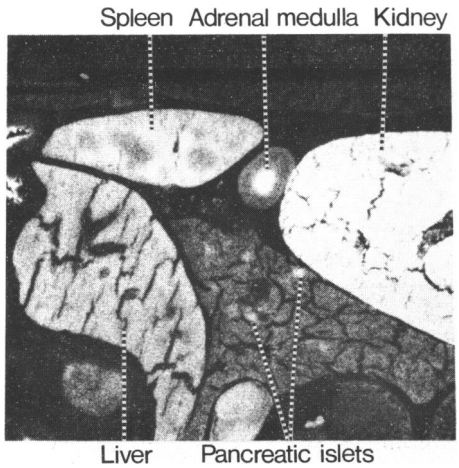

Fig. 2 Detail of Fig. 1, showing the accumulation of radioactivity in the adrenal medulla and the pancreatic islets 5 min after an i.v. injection of [^{14}C]-procaine.

Pancreas The radioactivity in the pancreatic islets was visible in most islets cells, possibly in all of them. At short survival intervals, some accumulation of radioactivity was also present in the exocrine part of the pancreas, but it was always lower than that in the islets (Figures 2 and 5).

The microautoradiograms obtained from the pancreas after the injection of [^{3}H]-lidocaine showed an even distribution of radioactivity over the whole pancreatic islet (Figure 6). The silver grains were present both over the nucleus and the

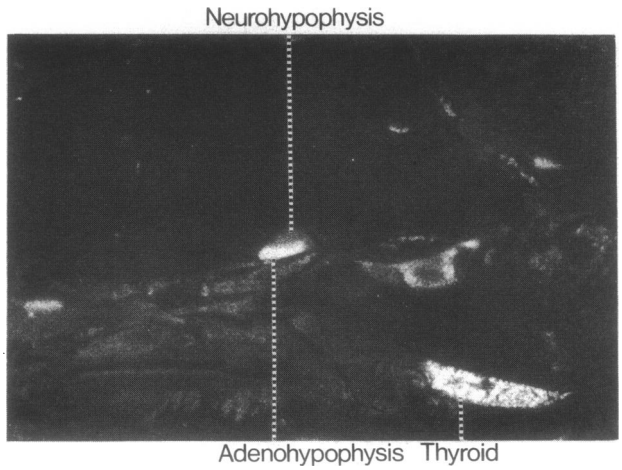

Fig. 3 Detail of a whole-body autoradiogram of an albino mouse 4 h after an i.v. injection of [^{14}C]-procaine. A high accumulation of radioactivity (light areas) can be seen in the thyroid and the hypophysis. The radioactivity in the thyroid has a spotty appearance and might correspond to labelled parafollicular cells. In the hypophysis the anterior part shows the highest radioactivity.

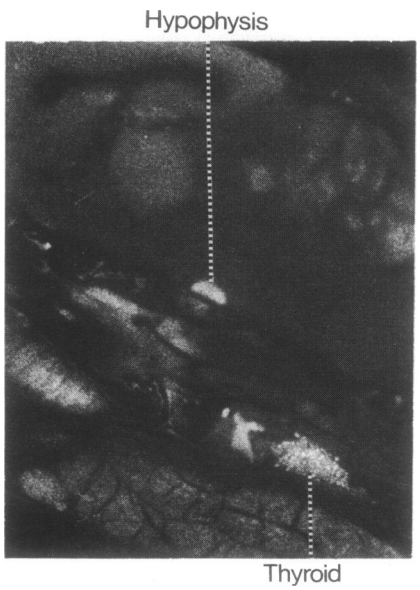

Fig. 4 Detail of a whole-body autoradiogram of an albino mouse 5 min after a s.c. injection of [^{14}C]-lidocaine. A high accumulation of radioactivity (light areas) can be seen in the thyroid and the hypophysis. The radioactivity in the thyroid is highest in certain spots, indicating an accumulation in the parafollicular cells.

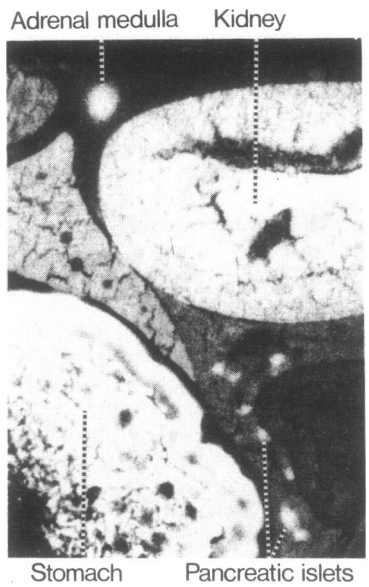

Fig. 5 Detail of a whole-body autoradiogram of an albino mouse 20 min after a s.c. injection of [^{14}C]-lidocaine. A high accumulation of radioactivity (light areas) can be seen in the adrenal medulla and the pancreatic islets. There is also a high radioactivity in the kidney and in the contents of the stomach.

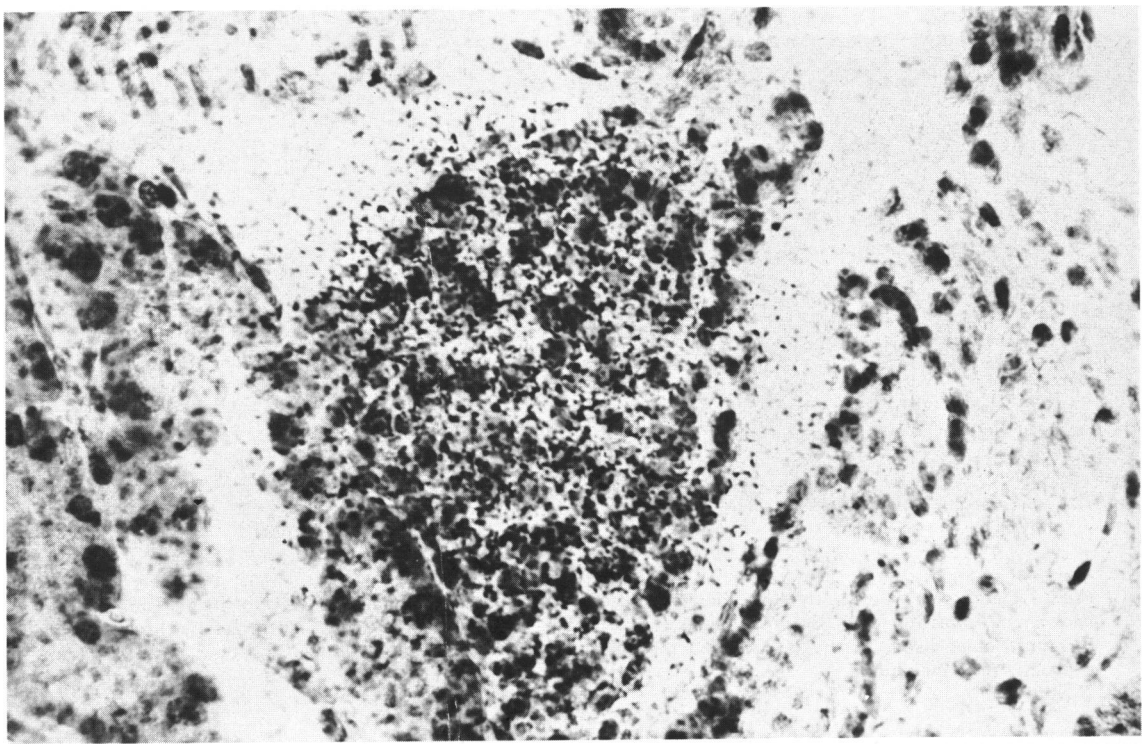

Fig. 6 Microautoradiogram of a pancreatic islet of an albino mouse 30 min after an i.v. injection of [^{3}H]-lidocaine. A high accumulation of radioactivity is distributed throughout the whole islet. The radioactivity in the exocrine pancreas is low. Ilford G5 nuclear plate. Haematoxylin-eosin (x225).

cytoplasm of the cells. In microautoradiograms subjected to the conventional microautoradiographic technique no specific labelling could be detected, illustrating the water-soluble state of the material.

Thyroid In the thyroid a high concentration of radioactivity was seen in some spots scattered all over the gland with a distribution pattern indicating that they might represent the calcitonin-producing parafollicular cells (Figures 3 and 4).

In addition to the endocrine organs, at short survival intervals a high accumulation of radioactivity was also observed in the kidneys, the salivary glands, the liver, the lungs, the brain (grey matter) and in lymphomyeloid tissues (Figure 1). After 4 and 24 h a moderate concentration could be detected in the liver, in the kidneys and in the salivary glands, probably reflecting the metabolic and excretory pathways of the drug. Retention of radioactivity was also observed in the organs of the lymphomyeloid system (Table 1).

Melanin accumulation In the pigmented mice procaine was strongly accumulated in melanin-containing structures such as hair, skin and uveal pigment as well as in the tissues already described. At 72 h, when no label was detectable in other tissues in the body, the accumulation in the eye pigment was still very pronounced.

Distribution of procaine in chicks

This study was performed in order to get more detailed information about the nature of the accumulation of radioactivity in the thyroid. In birds the calcitonin-producing cells, corresponding morphologically and functionally to the mammalian parafollicular cells, are located in a separate organ, the ultimobranchial gland (Almquist, Malmquist, Owman, Ritzén, Sundler & Swedin, 1971). This makes it possible to compare directly the uptake of radioactivity in the thyroid with that in the calcitonin-producing cells.

The whole-body autoradiograms of the chicks 5

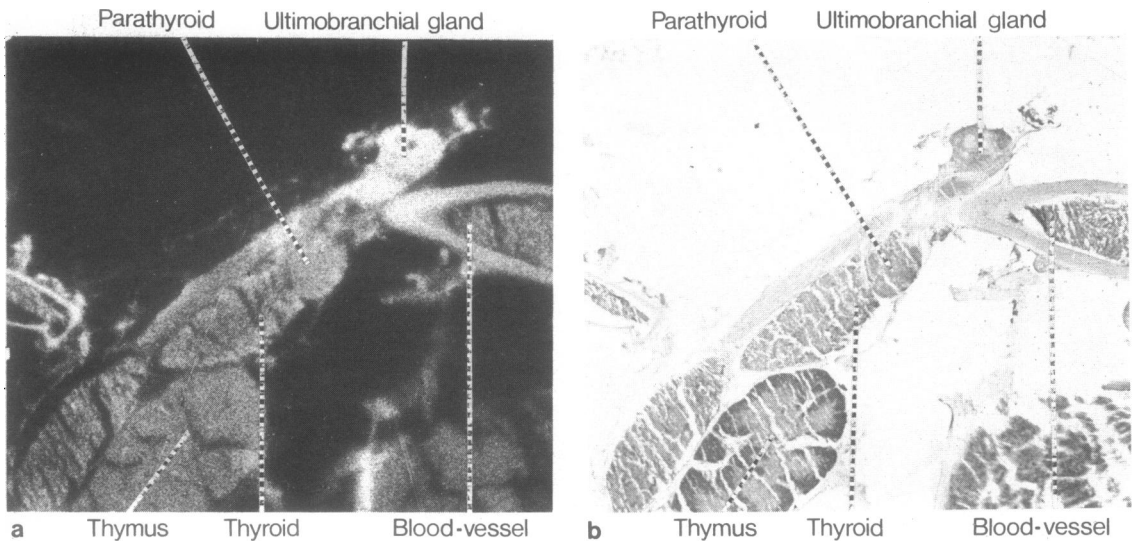

Fig. 7 Detail of a whole-body autoradiogram (a) with the corresponding stained section (b) of a chick 5 min after an i.v. injection of [^{14}C]-procaine. A high concentration of radioactivity (light areas) is present in the ultimobranchial gland. In the thyroid and the parathyroid the radioactivity is low and does not exceed the radioactivity in the blood.

and 30 min after injection of [^{14}C]-procaine showed a very low concentration of radioactivity in the thyroid, not exceeding that of the blood. In comparison a concentration of radioactivity about 4 times as high could be seen in the ultimobranchial glands (Fig. 7), indicating that it is mainly the calcitonin-producing cells which accumulate [^{14}C]-procaine.

Discussion

A characteristic feature in the distribution pattern of the local anaesthetics was the rapid and persistent uptake of radioactivity in some polypeptide-hormone producing cell systems, such as the hypophysis, the adrenal medulla, the islets of Langerhans and some cells in the thyroid representing probably the calcitonin-producing parafollicular cells. A high accumulation of radioactivity was also found in the calcitonin-producing ultimobranchial gland in chicks, while in the thyroid the radioactivity was low. The accumulation of radioactivity in the endocrine organs had already occurred at the shortest survival intervals which suggests that the radioactivity at least partly represents the unchanged local anaesthetics.

The specific and prolonged retention of the local anaesthetics in the endocrine cells indicates a possible direct interaction of these drugs with the production and/or release of the polypeptide hormones. Effects of local anaesthetics on the endocrine glands such as the adrenal medulla and the pancreatic islets have been reported (Jaanus *et al.*, 1967; Brisson *et al.*, 1971; Bressler & Brendel, 1971; Tjälve, Popov & Slanina, 1974).

The endocrine cells, in which the accumulation was observed, show common cytochemical characteristics in their ability to form and store dopamine and 5-hydroxytryptamine and in their possession of a positive reaction for true or pseudo-cholinesterase (Coupland, 1958; Falck & Hellman, 1963; Flack, Larsson, von Mecklenburg, Rosengren & Svenaéus, 1964; Ritzén, Hammarström & Ullberg, 1965; Cegrell, 1968; Pearse, 1968; Tjälve, 1971). It has recently been shown that some other drugs are also specifically accumulated in the same endocrine cells. This has been found for nicotine (Slanina & Tjälve, 1973), atropine (Albanus, Hammarström, Sundwall, Ullberg & Vangbo, 1968; Slanina & Tjälve, 1972) and alprenolol (Slanina, 1973). Dopamine and 5-hydroxytryptamine as well as these drugs have also been reported to have effects on the secretion of the hormones from at least some of the endocrine cells (Bisset & Walker, 1957; Malaisse, Malaisse-Lagae, Wright & Ashmore, 1967; Kaneto, Kajinuma, Kosaka & Nakao, 1968; Feldman & Lebowitz, 1970; Tjälve, 1971; Tjälve & Popov,

1973). The mechanism by which these drugs affect the release of the hormones is not clearly understood. An interference with the transport of calcium in the cells has been proposed (Jaanus *et al.*, 1967; Brisson *et al.*, 1971). Calcium is known to be a requisite for the secretory process (Douglas, 1968; Rubin, 1970; Brisson *et al.*, 1971). It might also be possible that the biogenic amines play a physiological role in the release of the hormones from the endocrine organs and that the drugs which are accumulated in the endocrine organs act by interference with the amines.

Another interesting finding in the present investigation was the accumulation of procaine in the melanin containing structures. An affinity for melanin has recently been shown for many drugs (Lindquist & Ullberg, 1972; Lindquist, 1973) and prolonged use of such drugs may cause side effects as for example ocular damage. Cocaine has also recently been shown to have an affinity for melanin. In ophthalmology more cocaine has to be applied to pigmented eyes than to non-pigmented, probably because the melanin affinity makes less drug available for anaesthetic action (Patil, 1972).

The concentration of the local anaesthetics in some other tissues, such as the brain, liver, salivary glands and kidneys has been observed in previous autoradiographic studies with [^{14}C]-lidocaine in rats (Katz, Gershwin & Hood, 1968) and with [^{14}C]-mepivacaine in mice (Kristerson, Hoffman & Hansson, 1965).

The prolonged high concentration of local anaesthetics and/or metabolites in the kidneys, the urine, the bile and the gastrointestinal contents probably reflects the main excretory pathways for these drugs. The results are in accordance with previous findings in various animal species and man (Katz *et al.*, 1968; Ahmad & Medzihradsky, 1971).

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(Received December 4, 1973)