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Original Investigations · Originalarbeiten · Travaux originaux

Effect of N-Hydroxyethylpromethazine (Aprobit®) on the Distribution of ^{35}S-Chlorpromazine Studied by Autoradiography in Cats and Mice*

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Summary. The effect of a quaternary phenothiazine compound, N-hydroxyethylpromethazine (Aprobit®, NHP), on the distribution of ^{35}S-chlorpromazine (^{35}S-CPZ) was studied in mice and cats using an autoradiographic technique. NHP did not change the sedative action of CPZ or ether but it caused a strong relaxation of the nictitating membrane, mydriasis and shivering in all cats.

A pronounced accumulation of ^{35}S-CPZ was recorded in the inferior cervical ganglion of the cat during the entire 16 h period of observation. In addition to the lung parenchyma a strong activity was concentrated in the bronchial mucosa and excretions. The uptake was very high in the brain, eye, liver, kidney, pancreas, oesophagic excretion, in the gastro-intestinal contents, salivary glands and especially in the sublingual gland.

The NHP-pretreatment increased the radioactivity in the sympathetic ganglia, sublingual glands, lungs, liver and bone marrow. NHP delayed the penetration of ^{35}S-CPZ into the brain. At 16 h after the i.v. injection a stronger retention of the radioactivity in the cerebral tissue was observed in the NHP-pretreated animals. In pregnant mice NHP decreased the accumulation of ^{35}S-CPZ or its metabolites in the fetuses.

The possible mechanism whereby NHP increases the retention of ^{35}S-CPZ or its metabolites in the brain is discussed.

Key-Words: Chlorpromazine — Radioautographie.

A quaternary phenothiazine compound N-hydroxyethylpromethazine (Aprobit®, NHP) is an antihistaminic drug without sedative effect on experimental animals and in clinical use (CARLSSON *et al.*, 1960). Unlike such tertiary phenothiazine compounds as chlorpromazine (CPZ) (SJÖSTRAND *et al.*, 1965) or promethazine (HANSSON and SCHMITERLÖW, 1961) NHP does not accumulate in the brain and lungs or in the fetus. ALLGRÉN (1960) demonstrated that quaternary phenothiazine compounds are metabolized more slowly than the tertiary compounds. AHTEE and PAASONEN (1965) showed that NHP to some degree inhibits the hemolytic effect of CPZ. It is likely that NHP and CPZ are absorbed by the

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same mechanism at the cellular membranes. Only CPZ, however, reaches the intracellular sites of action to a considerable degree.

The aim of this investigation was to determine whether NHP could cause any differences in the distribution of CPZ or its metabolites, especially in organs like the brain and lungs and in the fetus. The mice and cats were pretreated with NHP and after the administration of ^{35}S-chlorpromazine the distribution was investigated by autoradiography.

Material and Methods

^{35}S-chlorpromazine was obtained from the Radiochemical Centre, Amersham, England. The substance (specific activity 22 μC/mg) was dissolved in saline and its radiochemical purity was tested by autoradiography of thin-layer chromatograms.

Eight cats (seven females) weighing 1.9—2.9 kg were given ^{35}S-CPZ by injection into the femoral vein under a slight ether anesthesia. The dose employed was 9 mg/kg, corresponding to about 0.4 mC/animal. Four cats were pretreated 20 min earlier with non-labelled NHP in a dose of 25 mg/kg given i.p.. The cats were sacrificed by exsanguination under ether anesthesia 20 min, 1, 6 and 16 h after the administration of CPZ. 22 different organs e.g. brain, eye, ganglion cervicale inferior, the thoracic part of the sympathetic trunk, salivary glands, lung, liver, pancreas, kidney, stomach and pieces of the intestine were sectioned and frozen as described by AIRAKSINEN and IDÄNPÄÄN-HEIKKILÄ (1967). The organs were then sectioned at −10°C and 50—90 μ thick specimens were taken on Scotch tape. The sections were dried for 24 h and then pressed on the Nuclear Plates (Illford) or Kodirex X-ray films.

A dose of 45 μg/g (1 μC/g) of ^{35}S-CPZ was injected into the tail vein of 6 male white mice weighing 20—21 g. Six pregnant white mice weighing 44—52 g were given a dose of 22.5 μg/g (0.5 μC/g). Three mice in both groups were pretreated with NHP 25 μg/g injected i.p. 20 min before the administration of ^{35}S-CPZ. The animals were killed by an overdose of ether 5 and 15 min and 1, 2, 4, and 16 h after i.v. injection, and frozen in alcohol cooled with solid carbon dioxide and embedded in carboxymethylcellulose. Sagittal sections were taken at various levels and autoradiography was performed as described above and by ULLBERG (1963).

To achieve as precise conclusions as possible from the autoradiograms the sections of control and NHP pretreated animals (at least 15 specimens of each) were exposed at −25°C for the same period of time. To avoid the maximal blackening of several points on the films varying exposure times (14—21 days) were tested. The films were developed simultaneously in a vessel for the same period. Further series of sections representing the all different animal groups were exposed on the same film as above. The degree of blackening on the films was estimated twice by eye and the

scale "0" to "+++++" was used. The figures in the present work were made by copying the negatives simultaneously side by side on the same paper.

Results

The distribution of radioactivity in mice and cats was nearly the same. In mice the fate and distribution of ^{35}S-CPZ were seen in the whole body sections; from cats a more detailed information was obtained from various organs.

Cats pretreated with NHP were given ^{35}S-CPZ i.v. under ether anesthesia, but no potensive sedative effect of NHP with CPZ or ether was recorded. About 5 min after the i.p. administration of NHP a strong relaxation of the nictitating membrane, mydriasis and shivering were observed in all cats.

Central Nervous System. The activity in the brain during the first 20 min was lower in NHP pretreated animals, especially in the cerebral cortex, hypothalamus and white matter (Fig. 1). Later on the total activity was nearly the same but in animals receiving only ^{35}S-CPZ the distribution remained more diffuse. There was also a difference in the hypothalamus and white matter which were more active in the controls. In the brains of animals pretreated with NHP the radioactivity was clearly higher at 16 h after the i.v. injection of ^{35}S-CPZ than in the control animals (Fig. 1).

Eye. The choroid, corpus ciliare, ciliary processes and iris exhibited high activity during the whole period of observation in both groups (Fig. 4). In the controls the radioactivity of the cornea and sclera reached its maximum at 1 h after the injection and then decreased subsequently. In NHP pretreated animals the increase of radioactivity in the cornea and the sclera was more sustained and the highest concentration was recorded at 16 h. In the lens, anterior chamber and vitreous body some activity was evident from 6—16 h after the i.v. injection in both groups.

Sympathetic Structure and Adrenals. A very high content of radioactivity was seen in the inferior cervical ganglion during the entire 16 h period of observation in the NHP pretreated animals (Fig. 2). In the controls the accumulation of activity was not so high, but a considerable amount also remained after 16 h (Fig. 2). A lower level of radioactivity was observed in the sympathetic fibres. The thoracic ganglions in both groups took up a moderate amount of labeled CPZ. The adrenal cortex displayed strong activity from 5—60 min in both groups. After that, the activity in the cortex subsided. In the medulla the concentration of radioactivity was lower but more constant. More detailed information on the adrenals will be published (Vapaatalo, Idänpään-Heikkilä, and Neuvonen, 1968).

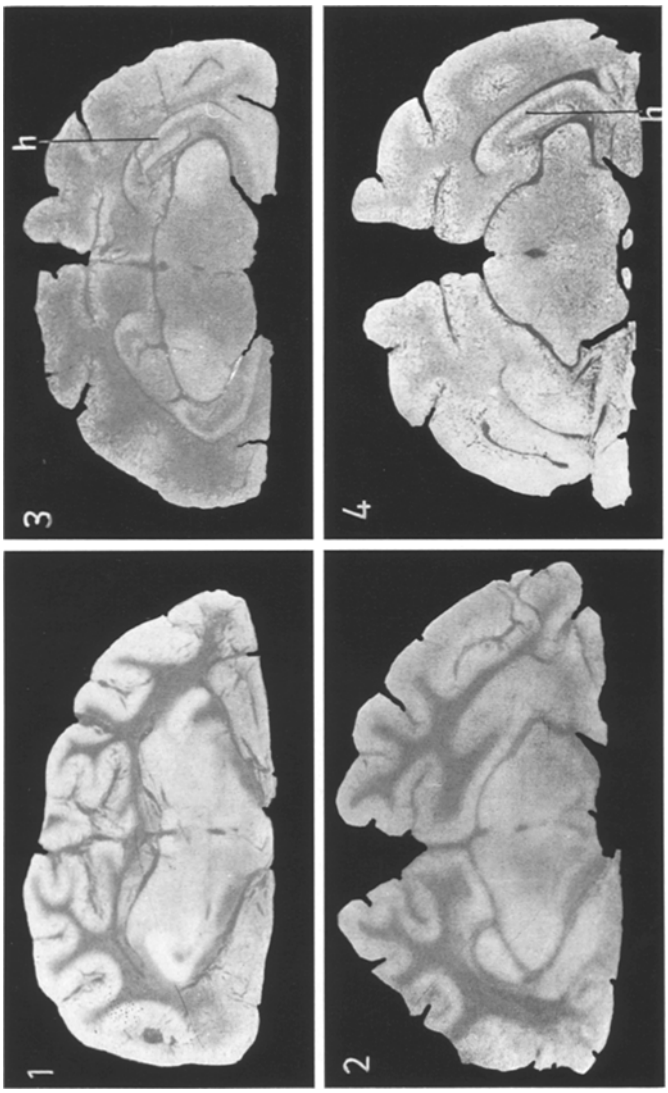

Fig. 1. The content of radioactivity (light areas) in the brain of the cats 20 min (1 and 2) and 16 h (3 and 4) after the i.v. injection of ^{35}S-CPZ. In Figs. 2 and 4 the cats were pretreated with NHP. *h* Hippocampus

Respiratory System. The lungs were one of the most active organs in both groups (Fig. 3). From 6—16 h the radioactivity in controls but not in NHP animals decreased. The paratracheal lymph glands, bronchial mucosa and especially the bronchial excretes contained a very high amount of activity (Fig. 4) at 1 h in controls and from 6—16 h in NHP pretreated animals.

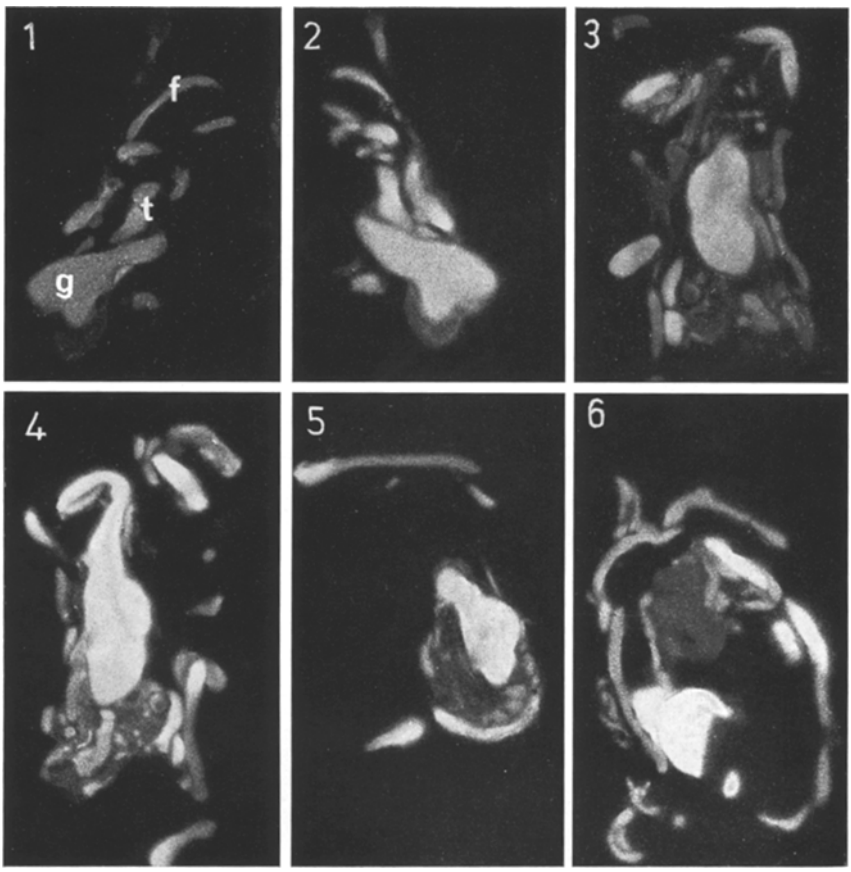

Fig. 2. The content of radioactivity (light areas) in the inferior cervical and thoracic ganglions and in the fibres of the sympathetic trunk of the cats. From left to right: 20 min, 6 h and 16 h after the i.v. injection of ^{35}S-CPZ. The upper line (1—3) without and the lower line (4—6) with NHP-pretreatment. *g* Inferior cervical ganglion, *t* Thoracic ganglions, *f* Sympathetic fibres

Cardiovascular System. A remarkable concentration of radioactivity was found in the myocardium at 20 min after the injection in control animals. In animals pretreated with NHP the accumulation in heart muscle was lower at 20 min but later on detectable differences in comparison to controls were not observed. In both groups a lower level of radioactivity was found in the aortic wall and only traces in aortic blood. Both in the myocardium and the aortic wall the activity diminished successively but was still detectable after 16 h.

The Endocrine Glands. The thyroid exhibited a rather high activity. At 16 h it was still present and a slightly stronger accumulation was found

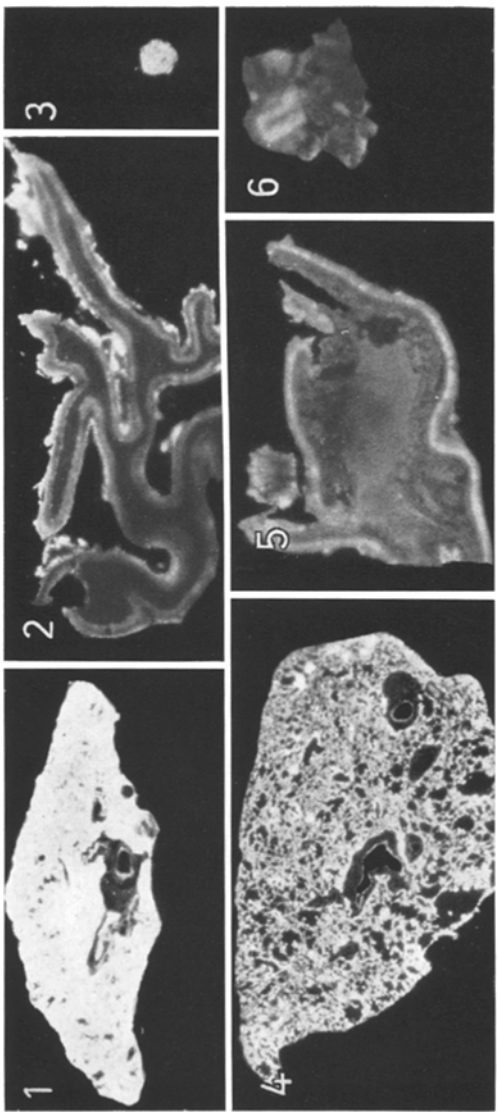

Fig. 3. Distribution of radioactivity (light areas) in the cats 20 min after the injection of ^{35}S-CPZ. From left to right: lung, stomach and bone marrow. The upper line (1—3) without and the lower line (4—6) with NHP-pretreatment

in NHP animals. The thymus was radioactive to almost the same degree. A strong activity was observed in the ovaries during the first hour after the injection. After that the radioactivity in animals treated only with ^{35}S-CPZ diminished gradually, but in NHP pretreated animals the same amount of activity was recorded up to 16 h (Fig. 5).

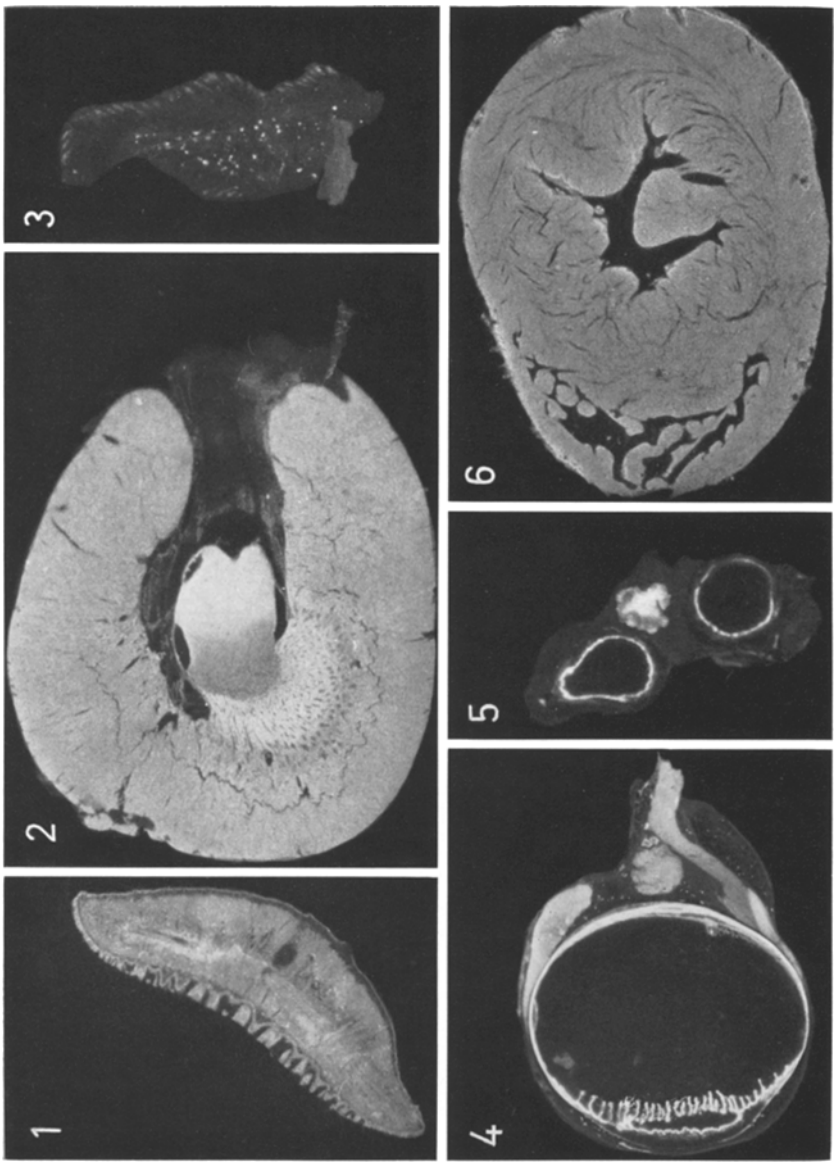

Fig. 4. Distribution of radioactivity (light areas) after the i.v. injection of ^{35}S-CPZ in some cat organs. 1 tongue (1 h), 2 kidney (1 h), 3 skin (1 h), 4 eye (20 min), 5 bronchi (6 h), 6 heart (1 h)

Liver and Pancreas. In both groups the liver showed a remarkable uptake of the radioactivity as soon as 5 min after the administration of the drug, and high activity remained longer in NHP pretreated animals. At 16 h the controls had lost a great deal of radioactivity, but in NHP animals it was still high (Fig. 5). The uptake in the gall-bladder increased

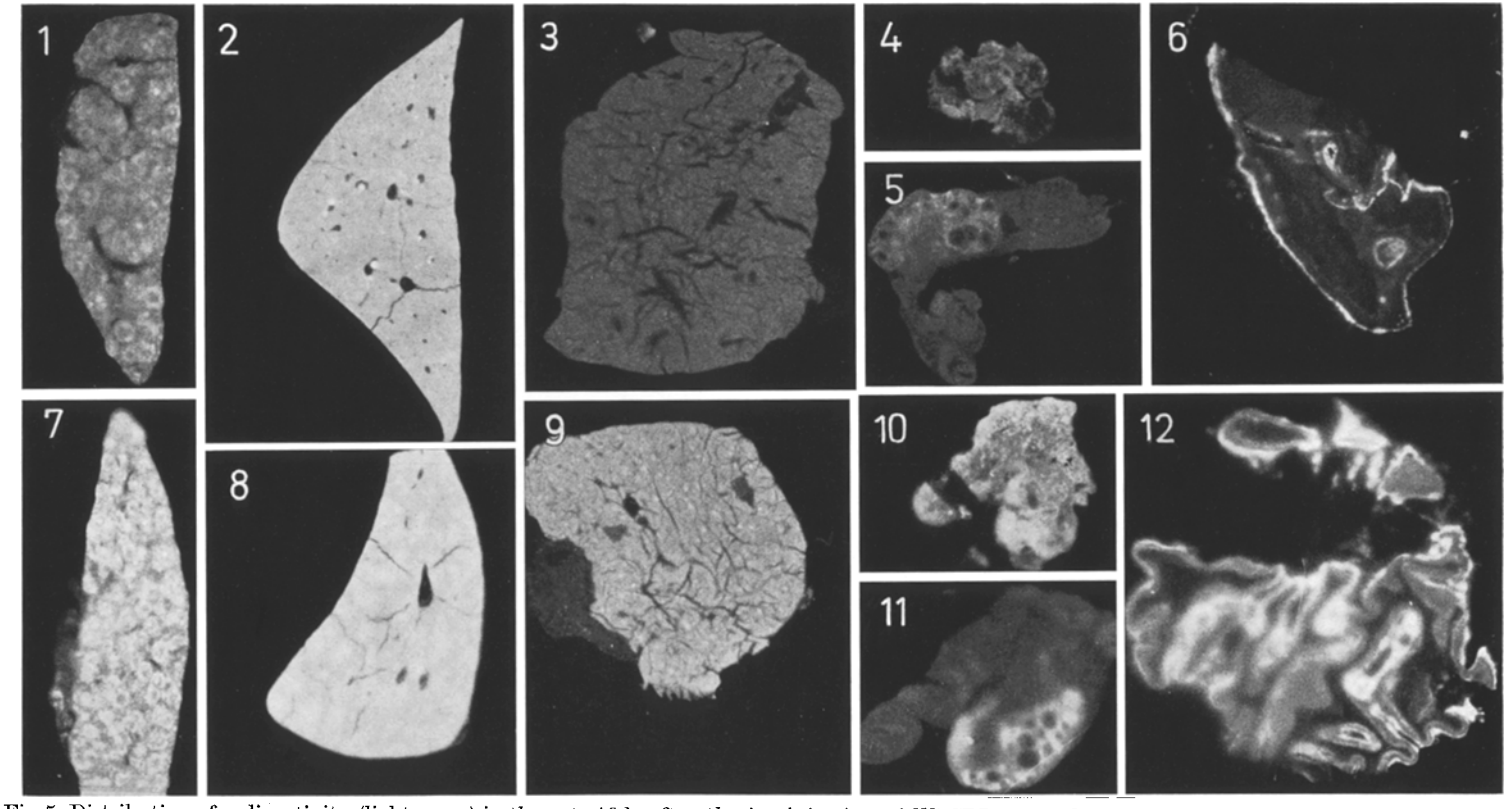

Fig. 5. Distribution of radioactivity (light areas) in the cats 16 h after the i.v. injection of ^{35}S-CPZ. From left to right: spleen, liver, pancreas, bone marrow (4 and 10), ovaries (5 and 11) and stomach. The upper line (1—6) without and the lower line (7—12) with NHP-pretreatment

from 5 min to 6 h and was highest at 16 h in NHP animals. At 20 min activity in the pancreas was nearly the same as in the liver but diminished subsequently and sooner in the control animals (Fig. 5).

Digestive Tract. CPZ or its metabolites accumulated in the salivary glands 5 min after the i.v. injection in both groups. The radioactivity was concentrated in the salivary secretory units and the saliva within the collecting ducts. In cats the submandibular and sublingual glands were investigated more thoroughly. At 20 min the concentration of radioactivity in the control animals was higher in both these glands. Activity in the sublingual gland was stronger, especially in the NHP pretreated animals. One of the submandibular and sublingual glands was taken for radioactive measurements and the same results were obtained (NEUVONEN, IDÄNPÄÄN-HEIKKILÄ, and VAPAATALO, 1968).

The oesophagic mucosa and secretions were surprisingly active. The stomach content was very active from 20 min to 16 h but the activity in the gastric mucosa and wall was lower (Fig. 3), except at 16 h in NHP pretreated animals which exhibited a moderate uptake of the radioactivity in the mucosa (Fig. 5). A higher activity was recorded in the duodenal contents of the control animals. In the large intestine the content was found to have at 16 h a remarkably lower concentration of radioactivity in the animals pretreated with NHP.

Two of the cats had intestinal parasites. The medullary parts of the worms showed a very high content of radioactivity.

Urinary System. The activity was concentrated to the kidney in both groups (Fig. 4). From 5—20 min more activity was located in the medullary zone of the NHP pretreated animals, with some activity in the tubules. At the same time the radioactivity of urine in the calyces was high; it decreased later to the level of the control animals.

Spleen and Bone Marrow. The activity in the spleen was moderate but there were no differences in the uptake of the spleen between these groups until 16 h. Thereafter the spleen in the pretreated animals was more active (Fig. 5). Bone marrow displayed a rather high uptake at 20 min but this was somewhat lower in NHP pretreated animals (Fig. 3). Later the activity in the controls remained at the same level but in NHP animals the concentration of the radioactivity in the bone marrow increased and was higher than in the control animals at 16 h (Fig. 5).

Fetuses. Both in the mice with and without NHP pretreatment the ^{35}S-CPZ rapidly penetrated the placental barrier. At 15 min the placentae and fetal membranes had an activity corresponding to the maternal salivary glands and spleen. The fetuses displayed a lower activity, especially in the NHP pretreated mice. The situation was the same at 2 h after the i.v. injection. At 16 h the fetal membranes were still very active, but the activity in the placentae had decreased. In the fetuses of the control

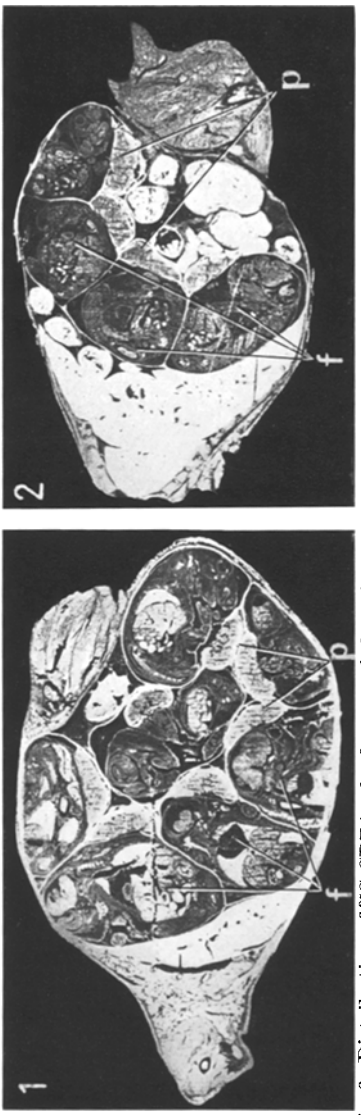

Fig. 6. Distribution of ^{35}S-CPZ in the fetuses of the mice without (1) and with NHP-pretreatment (2). Note the lower content of radioactivity (light areas) of fetuses in 2. *f* Fetuses, *p* Placentas

animals a high concentration of radioactivity was seen in the lung, the stomach and gut content, the liver, the brain and the bone marrow. In mice pretreated with NHP the total activity was much lower and there were no specific tissue accumulations, such as those seen in the control animals (Fig. 6).

Discussion

The method of this work is mostly directed towards determining the distribution of the drugs in animals. It is more difficult in autoradiography to ascertain whether one drug would modify the distribution pattern of another. We have formed the opinion that the methodological details applied in the present work would permit us to draw semiquantitative conclusions from the accumulation of the radioactivity in the various tissues of control and NHP pretreated animals.

The radioactivity showed a strong affinity to the sympathetic ganglia where NHP seemed to prolong retention. It is recognized that the site of accumulation of a drug does not necessary correspond to its site of action. Nonetheless, it may be possible that some side-effects of CPZ, like orthostatic hypotension, are related to the high and long retention of CPZ in sympathetic ganglions.

A considerable amount of radioactivity was recorded at 20 min in oesophagic and bronchial secretions in both groups. The same accumulation was observed in the mucosa of the oesophagus and the bronchus. A certain portion of the radioactivity in oesophagic mucus could be derived from the swallowed saliva. It is obvious that the slight ether anesthesia used before the exsanguination increased salivation and mucus production. The results may, however, allow the conclusion that ^{35}S-CPZ or its metabolites are excreted via the oesophagic and bronchial glands.

The high accumulation of CPZ or its metabolites in the salivary glands has been reported earlier by SJÖSTRAND *et al.* (1965). It seems probable that the salivary glands are sites of excretion of CPZ. In this work more activity was observed in the sublingual than in the submandibular glands. The salivary gland secretion is normally evoked by autonomic nerves. According to EMMELIN (1953) the cat's sublingual gland possesses an ability to secrete spontaneously without external stimuli. It is likely that a higher excretion of the drug is responsible for the greater accumulation of radioactivity in the sublingual gland.

In the NHP pretreated pregnant mice the fetuses had a lower activity than the control fetuses. The radioactivity in the placentae and fetal membranes, however, was about the same in both groups. It seemed that NHP altered in some way the placental barrier to the penetration of CPZ. In contrast to the brain, the placental barrier did not lose its ability to inhibit the passage of CPZ during the whole observation period.

N-hydroxyethylpromethazine seemed to modify the distribution and tissue uptake of ^{35}S-chlorpromazine. NHP delayed the accumulation of radioactivity in the brain at the beginning and, on the other hand, caused a longer retention. SJÖSTRAND *et al.* (1965) have reported that the radio-

activity in the tissues e.g. in the brain 20 min after the injection, mainly consists of unchanged ^{35}S-CPZ. After this the metabolites appear. Those results and our studies suggest that NHP may decrease the penetration of unchanged CPZ, but not its metabolites, into the brain. This may also mean that NHP delays the occurrence of the sedative effect of CPZ. Whether the longer brain retention of the radioactivity of ^{35}S-CPZ administered with NHP means that the central effects of CPZ last longer was not studied in this work. NHP has been reported to penetrate BBB and remain in the extravascular structure (NAIR, SCHMITERLÖW, and ROTH, unpublished 1965). On the other hand HANSSON and STEINWALL (1961), using autoradiographic technique, did not observe any accumulation of ^{35}S-NHP in the brain unless the BBB was disturbed by regional perfusion of $HgCl_2$, cyaninen dye 863 or NHP itself. Studies on the metabolism and excretion of ^{35}S-CPZ in cats in this laboratory (NEUVONEN, IDÄNPÄÄN-HEIKKILÄ, and VAPAATALO, 1968) showed that NHP may delay the elimination of ^{35}S-CPZ or its metabolites. The extravascular appearance of NHP in the brain and its effect on the elimination rate of ^{35}S-CPZ may explain some of the influences of NHP pretreatment on the behaviour of CPZ or its metabolites in the brain.

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