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Whole Body Autoradiographic Studies on the Distribution of ^{3}H-Labeled Penfluridol (^{3}H-TLP-607) in Mice and Rats

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The distribution of tritium-labeled Penfluridol (^{3}H-TLP-607), a potent and long-acting neuroleptics, was studied in mice and rats by the whole body autoradiographic technique.

In mice after intravenous administration the highest concentration of radioactivity was found in the lung, heart muscle, skeletal muscle, kidney and moderate in the central nervous system. In the brain the radioactivity in the cortices of the cerebrum and the cerebellum was higher than in the white matter, and a fair amount of radioactivity was observed also in the hippocampus. In the case of oral administration the highest radioactivity in various organs was observed 2 to 8 hours after the administration. In these periods the highest concentration was found in the lung, kidney and spleen, next to these in the thyroid and blood, and moderate in the central nervous system and sexual organs. In pregnant mice after oral administration, ^{3}H-TLP-607 and its radioactive metabolites passed through the placenta into the fetuses, but the concentration in the fetuses was lower than in the mother.

In rats the distribution pattern after oral administration was similar to that in mice; highest in the lung, kidney, spleen and moderate in the central nervous system. The distribution in the rat brain also resembled that in the mouse brain, but the uptake into the hippocampus was observed more remarkably. In female rats high radioactivity was found in the ovary, low in the mammary gland in earlier periods, and *vice versa* 16 hours later.

4-(4-Chloro-α,α,α-trifluoro-*m*-tolyl)-1-[4,4-bis(*p*-fluorophenyl)-butyl]-4-piperidinol (Penfluridol, TLP-607) is a new member of the potent and long-acting series of diphenylbutyl piperidine neuroleptics introduced by Janssen, *et al.*[2]

The present paper deals with the distribution of ^{3}H-TLP-607 in mice and rats studied by the whole body autoradiographic technique.[3]

Materials and Methods

Materials——^{3}H-TLP-607 (1.05 mCi/mg) was obtained from the Research Laboratories of Janssen, Beerse, Belgium. The radiochemical purity shown by thin-layer chromatography was higher than 98 percent. The compound was dissolved in 60% propylene glycol in the case of intravenous administration, or suspended in 0.2% carboxymethyl cellulose (CMC) solution in the case of oral administration.

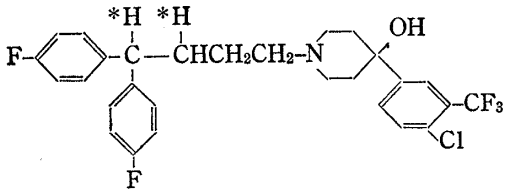

Chart 1 Chemical Formula and Labeled Position of Penfluridol (^{3}H-TLP-607)

Experimental Animals——Animals used were ddY male mice (body weight: about 20 g), pregnant mice at the 17th day of gestation (body weight: about 40 g), and Sprague-Dawley rats of both sexes (body weight: about 200 g). Mice were divided into 3 groups; *i.e.*, 4 male mice receiving ^{3}H-TLP-607 intravenously in a dose of 2 mg/kg (group 1), 7 male mice

1) Location: *Kawagishi, Toda-shi, Saitama.*
2) P.A.J. Janssen, C.J.E. Niemegeers, K.H.L. Schellekens, F.M. Lenaerts, F.J. Verbruggen, J.M. Van Nueten, and W.K.A. Schaper, *Europ. J. Pharmacol.*, 11, 139 (1970).
3) Y. Sato and K. Abe, *Pharmacometrics*, 3, 1 (1969); 3, 77 (1969).

orally in a dose of 20 mg/kg (group 2) and 4 pregnant mice orally in a dose of 10 mg/kg (group 3). Rats were also divided into two groups; *i.e.*, 2 male rats and 2 female rats both receiving ^{3}H-TLP-607 orally in a dose of 20 mg/kg (group 4 and 5). Before oral administration animals were fasted overnight but took tap water *ad libitum*.

Preparation of Autoradiograms[3]——At predetermined intervals from 1 minute to 120 hours after the administration of ^{3}H-TLP-607, the animals were killed, under a slight ether anesthesia, by immersion in a solid carbon dioxide-acetone bath; *i.e.*, 1, 4, 7, and 30 minutes in group 1; 30 minutes, 2, 4, 8, 24, 72, and 120 hours in group 2; 2, 4, 8, and 24 hours in group 3; 2 and 16 hours in group 4 and group 5. Whole body sagittal frozen sections of 40 μ in thickness were prepared by the microtome of type 1300 of Leitz. The freeze-dried sections were brought into contact with X-ray films (Sakura type N, noncoating) for about 9 weeks at 5°. The films were developed for 4 minutes with Konidol X (Konishiroku photography Industry Co., Ltd.) at 20°. Prints were made for illustration purposes; consequently white areas in the illustrations correspond to high levels of radioactivity.

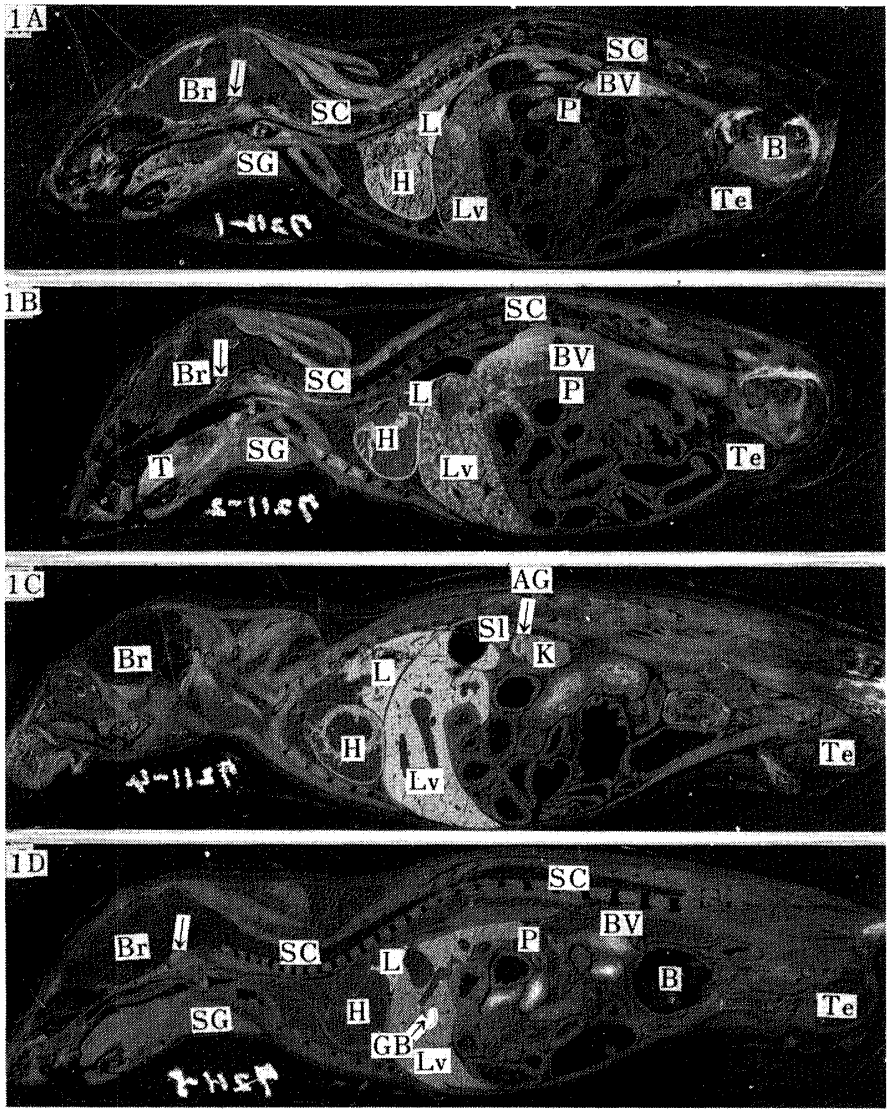

Fig. 1. Autoradiograms Showing Distribution of Radioactivity in Male Mice at Various Times after Intravenous Administration of ^{3}H-TLP-607 (42 μCi/0.04 mg/Mouse)

A, 1 minute; B, 4 minutes; C, 7 minutes; D, 30 minutes
abbreviations: Br, brain; SG, salivary gland; SC, spinal cord; H, heart; L, lung; Lv, liver; P, pancreas; BV, blood vessel; Te, testis; B, urinary bladder; T, tongue; Sl, spleen; AG, adrenal gland; K, kidney; GB, gall bladder; arrow, pituitary gland

Results

1. Whole Body Autoradiograms of Mice

1-1. Intravenous Administration——The distribution of radioactivity after intravenous administration of ^{3}H-TLP-607 is shown in Fig. 1. In the autoradiograms of 1 minute (Fig. 1A) and 4 minutes (Fig. 1B) after the administration, the highest radioactivity was observed in the lung, heart muscle, skeletal muscle, diaphragm, kidney and some endocrine organs such as pituitary, pineal, thyroid and adrenal glands. In the liver radioactivity was distributed unevenly like a "rosary" around the parenchyma. Moderate radioactivity was observed in the central nervous system. Relatively high radioactivity was observed in the blood immediately after the administration, but decreased rapidly after 4 minutes. In the autoradiograms of 7 minutes (Fig. 1C) to 30 minutes (Fig. 1D) after the administration, the patterns of distribution were similar to those described above, except the presence of high radioactivity in the liver at each time. ^{3}H-TLP-607 showed a marked accumulation in the posterior lobe of the pituitary gland (Fig. 2B), the envelope of the spleen (Fig. 3B) and the inner layer of the adrenal cortex (Fig. 3A).

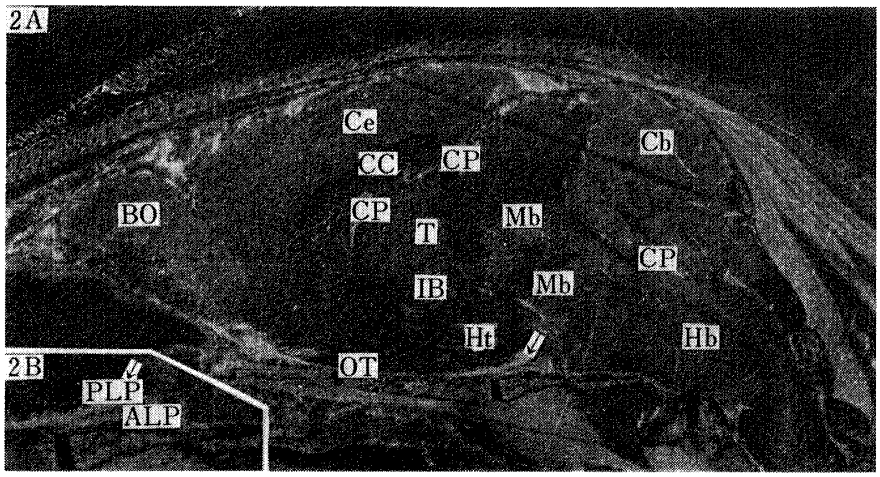

Fig. 2. Detail of Autoradiograms of the Brain (A) and the Pituitary Gland (B) at 1 Minute after Intravenous Administration of ^{3}H-TLP-607 to Mouse

abbreviations: Ce, cerebrum; Cb, cerebellum; BO, bulbus olfactorius; CC, corpus callosum; T, thalamus; CP, choroid plexus; Mb, midbrain; IB, interbrain; Hb, hindbrain; Ht, hypothalamus; OT, optic tract; PLP, posterior lobe of the pituitary gland; ALP, anterior lobe of the pituitary gland; arrow, pituitary gland

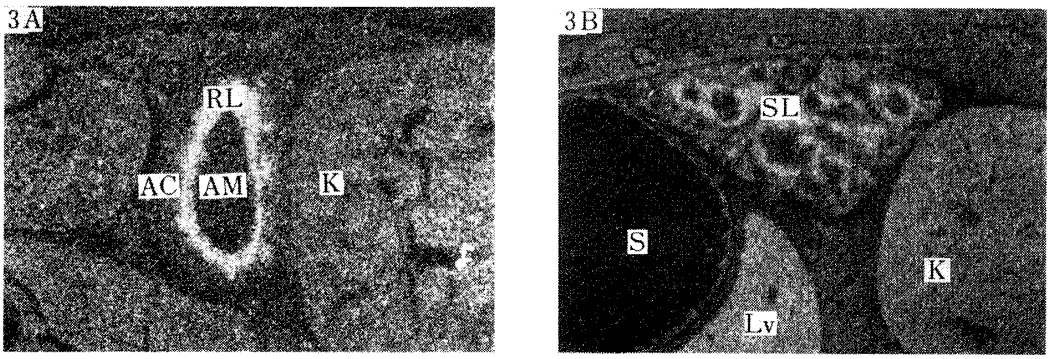

Fig. 3. Detail of Autoradiograms of the Adrenal (A) and Spleen (B) at 7 Minutes after Intravenous Injection of ^{3}H-TLP-607 to Mouse

abbreviations: AC, adrenal cortex; AM, adrenal medulla; RL, inner layer of the adrenal cortex; S, stomach; Lv, liver; Sl, spleen; K, kidney

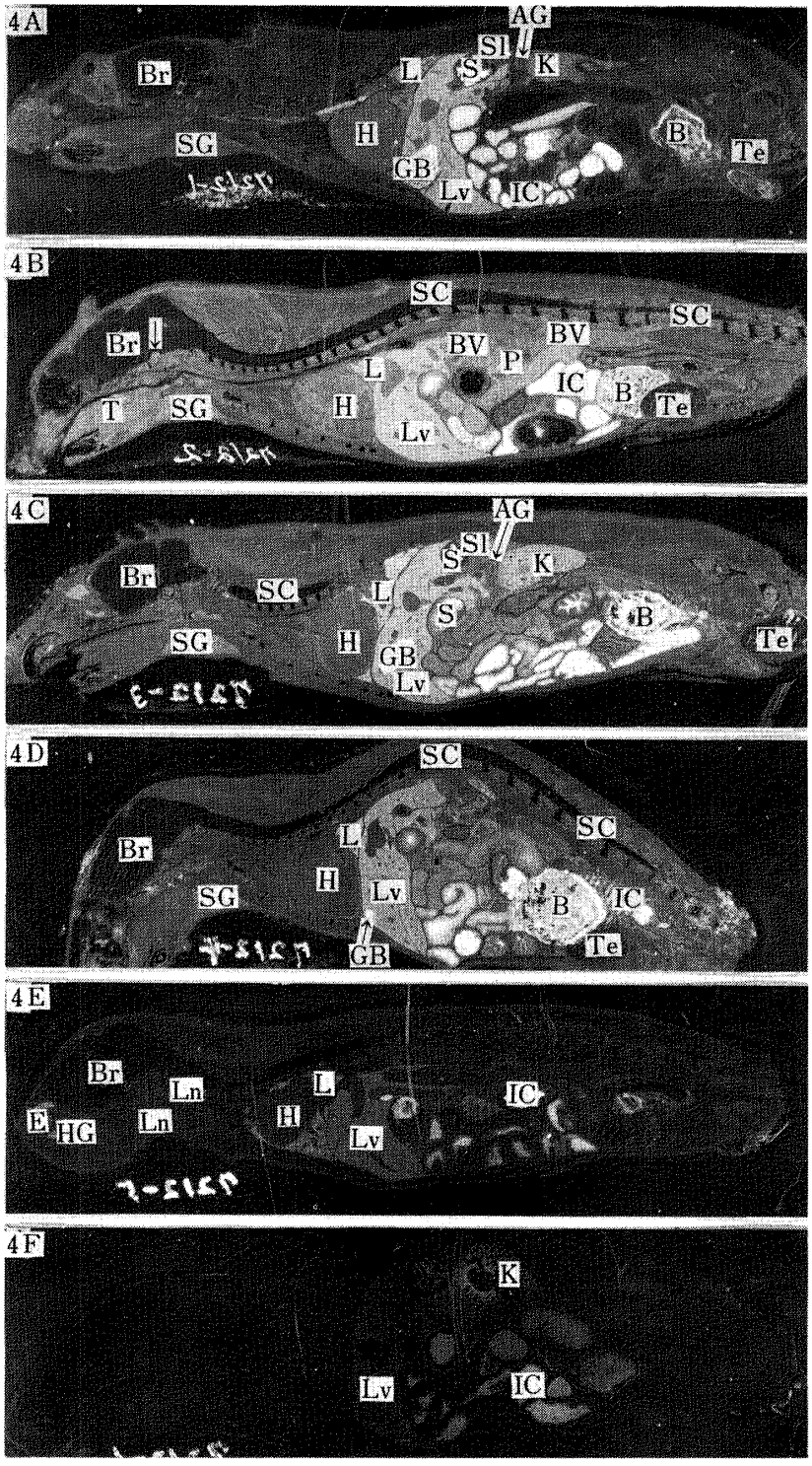

Fig. 4. Autoradiograms Showing Distribution of Radioactivity in Male Mice at Various Times after Oral Administration of ^{3}H-TLP-607 (420 μCi/0.4 mg/Mouse)

A, 30 minutes; B, 2 hours; C, 4 hours; D, 8 hours; E, 24 hours; F, 72 hours
abbreviations: Br, brain; SG, salivary gland; H, heart; L, lung; Lv, liver; GB, gall bladder; S, stomach; Sl, spleen; AG, adrenal gland; K, kidney; IC, intestinal contents; B, urinary bladder; Te, testis; T, tongue; BV, blood vessel; P, pancreas; SC, spinal cord; E, eye; HG, Harderian gland; Ln, lymph node; arrow, pituitary gland

1-2. Enlarged Autoradiograms of the Brain and Adjacent Areas——The autoradiogram at 1 minute after the administration of ^{3}H-TLP-607 showed marked radioactivity in the choroid plexus, intracranial blood vessels as well as the posterior lobe of the pituitary gland (Fig. 2). The uptake of radioactivity into the brain was moderate in the early periods after the administration. Its distribution pattern showed a considerable degree of localization; higher concentrations were found in the cortices of the cerebrum and the cerebellum and in the gray matter of the midbrain and pons in comparison with the corpus callosum and the white matter of cerebral peduncle. Radioactivity was also observed in the hippocampus.

1-3. Oral Administration——The distribution of radioactivity after oral administration of ^{3}H-TLP-607 is shown in Fig. 4. In the autoradiogram of 30 minutes (Fig. 4A) after the administration, the concentration of radioactivity in the liver, kidney and lung was higher than that in the blood, spleen and muscle. The most wide-spread distribution of radioactivity over the whole body was observed during the period of 2 to 8 hours after the administration (Fig. 4B, D), and in these periods, the highest radioactivity was seen in the lung, liver, kidney, lymph nodes, the envelope of the spleen, the inner layer of the adrenal cortex and the posterior lobe of the pituitary gland, and subsequently high radioactivity was seen in the salivary glands, thyroid glands, pineal gland, blood and bone marrow. Moderate radioactivity was observed in the central nervous system and sexual organs.

The radioactivity in most of the tissues and organs decreased with time. Radioactivity was scarcely detected at 120 hours after the administration, though a considerable amount of radioactivity was still observed in the outer layer of the renal medulla (Fig. 4F), uveal tract (Fig. 4E, F) and intestinal contents until 72 hours after the administration.

The Harder's gland and skin showed high radioactivity, but in the skin the concentration decreased relatively rapidly, while in the former the radioactivity was still observed at 24 hours after the administration (Fig. 4C, E).

1-4. Pregnant Mice——The distribution of radioactivity after oral administration of ^{3}H-TLP-607 to pregnant mice is shown in Fig. 5. Radioactive materials passed through

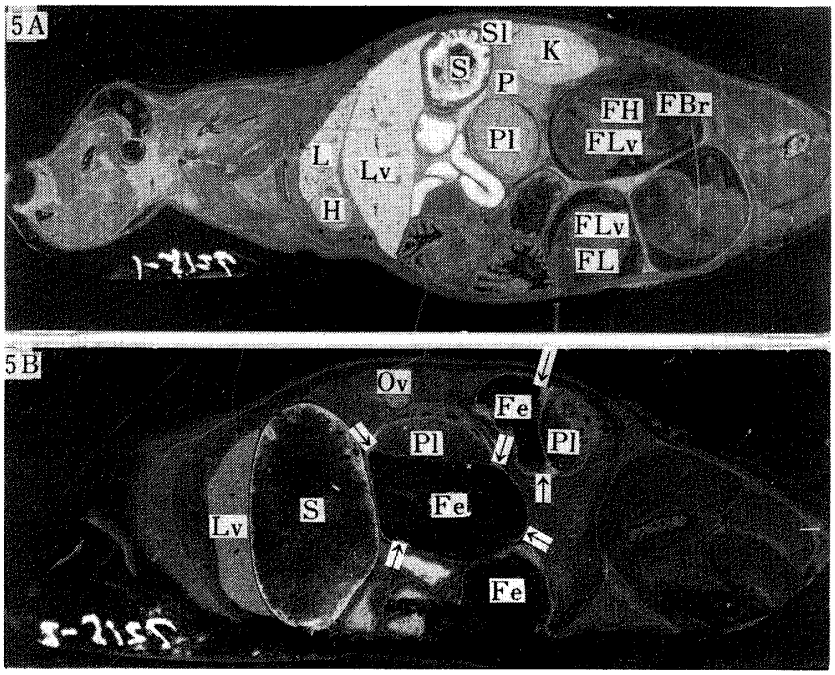

Fig. 5. Autoradiograms Showing Distribution of Radioactivity in Pregnant Mice at 2 Hours (A) and 24 Hours (B) after Oral Administration of ^{3}H-TLP-607 (420 μCi/0.4 mg/Mouse)

abbreviations: L, lung; H, heart; Lv, liver; S, stomach; Sl, spleen; K, kidney; P, pancreas; Pl, placenta; FBr, fetal brain; FH, fetal heart; FLv, fetal liver; FL, fetal lung; Ov, ovary; Fe, fetus; arrow, yolk sac adjacent to the chorioallantoic membrane

the placenta into the fetuses, and at 2 hours after the administration relatively high radioactivity was detected in the blood, liver and kidney of the fetuses. However, the level of radioactivity in the fetal organs was markedly lower than that in corresponding maternal organs.

The radioactivity decreased gradually with time, and was scarcely detected at 24 hours after the administration, whereas a considerable amount of radioactivity was found in the yolk sac adjacent to the chorioallantoic membrane (Fig. 5B).

2. Whole Body Autoradiograms of Rats

2-1. Male Rats——The autoradiograms obtained from male rats at 2 hours (Fig. 6A) or 16 hours (Fig. 6B) after oral administration were essentially similar to those obtained from the mice (Fig. 4B and 4E); *i.e.*, high concentrations of radioactivity were found in the lung, liver, kidney, spleen and some endocrine organs and moderate concentrations of radioactivity were seen in the central nervous system and sexual organs; a remarkable increase of the radioactivity was seen in such lymphatic organs as lymph nodes, thymus and spleen in the later period. In most tissues and organs of the rats, however, decreases of the radioactivity seemed to be slower than that in mice. Relatively high radioactivity was observed in the posterior lobe of the pituitary gland, but the radioactivity in the inner layer of the adrenal cortex was not so predominant in rats in both observation periods.

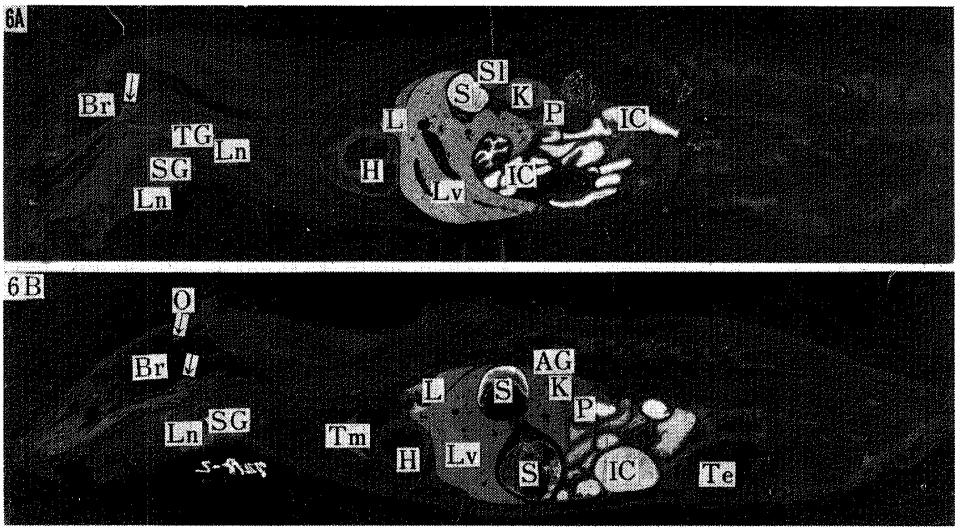

Fig. 6. Autoradiograms Showing Distribution of Radioactivity in Male Rats at 2 Hours (A) and 16 Hours (B) after Oral Administration of ^{3}H-TLP-607 (4.2 mCi/4 mg/Rat)

abbreviations: Br, brain; SG, salivary gland; Ln, lymph node; TG, thyroid gland; H, heart; L, lung; Lv, liver; S, stomach; SI, spleen; K, kidney; P, pancreas; IC, intestinal contents; Tm, thymus; AG, adrenal gland; Te, testis; arrow, pituitary gland; arrow with circle, pineal gland

2-2. Female Rats——The autoradiograms obtained from the female rats at 2 or 16 hours after oral administration are shown in Fig. 7. They were essentially similar to those in Fig. 6, except that the radioactivity was rather predominant in the autoradiograms obtained from the female rats than in those from the male rats all through the observation periods. In addition, the autoradiogram at 2 hours after the administration (Fig. 7A) demonstrated that high concentrations of the radioactivity were present in the ovary, especially in the lutein body (Fig. 8C), while the radioactivity was not so predominant in the mammary gland. At 16 hours, on the contrary, a high concentration of radioactivity was found in the mammary gland.

Enlarged autoradiograms of the brain are shown in Fig. 8, 9. A moderate amount of radioactivity was found also in the brain of both female and male rats. The distribution pattern of radioactivity was similar to that of the mouse brain. The radioactivity was obser-

ved in the cortices of the cerebrum and cerebellum and in the gray matter of the interbrain, midbrain, pons and hippocampus (Fig. 8A). The enlarged autoradiogram of the rat brain at 16 hours after oral administration showed that the radioactivity decreased considerably in the whole brain, but still remained in the hippocampus (Fig. 9).

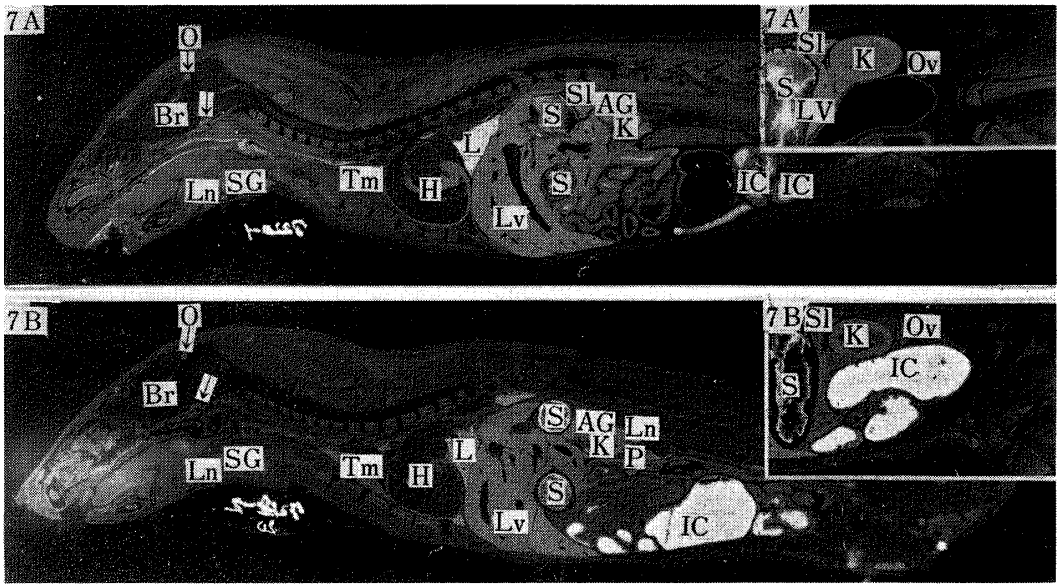

Fig. 7. Autoradiograms Showing Distribution of Radioactivity in Female Rats at 2 Hours (A, A′) and 16 Hours (B, B′) after Oral Administration of ^{3}H-TLP-607 (4.2 mCi/4 mg/Rat)

abbreviations: Br, brain; Ln, lymph node; SG, salivary gland; Tm, thymus; H, heart; L, lung; Lv, liver; S, stomach; Sl, spleen; AG, adrenal gland; K, kidney; IC, intestinal contents; Ov, ovary; P, pancreas; Arrow, pituitary gland; arrow with circle, pineal gland

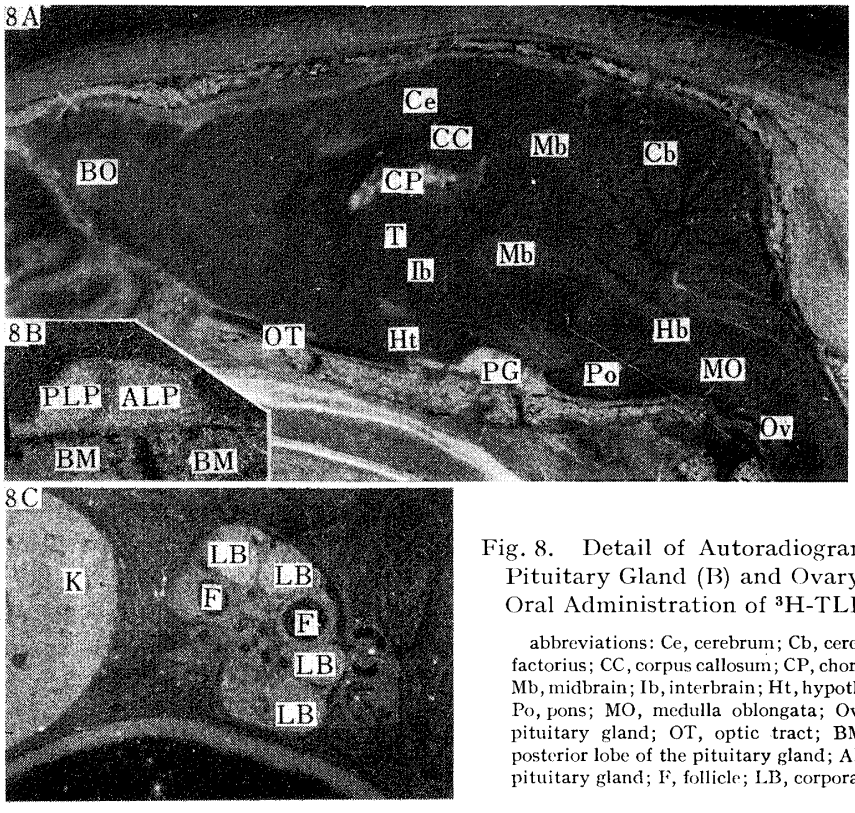

Fig. 8. Detail of Autoradiograms of the Brain (A), Pituitary Gland (B) and Ovary (C) at 2 Hours after Oral Administration of ^{3}H-TLP-607 to Female Rat

abbreviations: Ce, cerebrum; Cb, cerebellum; BO, bulbus olfactorius; CC, corpus callosum; CP, choroid plexus; T, thalamus; Mb, midbrain; Ib, interbrain; Ht, hypothalamus; Hb, hindbrain; Po, pons; MO, medulla oblongata; Ov, olivary nucleus; PG, pituitary gland; OT, optic tract; BM, bone marrow; PLP, posterior lobe of the pituitary gland; ALP, anterior lobe of the pituitary gland; F, follicle; LB, corpora lutea; K, kidney

Discussion

In mice and rats, oral administration of ^{3}H-TLP-607 as well as its intravenous application gave the highest radioactivity after 2 to 8 hours, suggesting that TLP-607 can be absorbed relatively well from the digestive tract. Autoradiographic examination in more detail, moreover, showed that the distribution pattern is very similar between oral and intravenous administration. Therefore it seems that the two different administration routes would bring about almost the same pharmacological action of TLP-607.

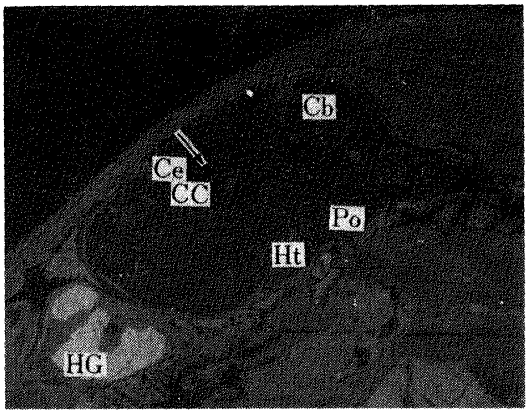

Fig. 9. Detail of Autoradiogram of the Brain at 16 Hours after Oral Administration of ^{3}H-TLP-607 to Female Rat

abbreviations: Ce, cerebrum; Cb, cerebellum; CC, corpus callosum; Ht, hypothalamus; Po, pons; HG, Harderian gland; arrow, hippocampus

The distribution of ^{3}H-TLP-607 immediately after intravenous injection showed a high and rapid uptake in the lung, kidney, heart muscle, skeletal muscle, diaphragm and certain endocrine organs. These patterns are similar to those of ^{35}S-chlorpromazine,[4,5] ^{14}C-amitriptyline,[6] ^{14}C-imipramine,[7] ^{14}C-FG5111[8] and many other labeled psychoactive agents.

The uptake of ^{3}H-TLP-607 into the central nervous system was moderate throughout the observation period up to 120 hours, irrespective of the route of administration. In the brain, higher concentrations were found in the cortices of the cerebrum and cerebellum compared with the corpus callosum and the white matter of cerebral peduncle, and especially in the hippocampus the radioactivity was still noticeable 16 hours after oral administration. It is interesting that the accumulation of radioactivity was found in the hippocampus, because many investigators have also reported such a peculiar distribution pattern sustained for rather a long time for many other psychoactive drugs.[3-8]

The high uptake of radioactivity was also seen in some endocrine organs such as the pituitary, adrenal and pineal glands. In earlier periods after the administration, the radioactivity in the posterior lobe of the pituitary gland was higher than that in the anterior lobe. However, with lapse of time the radioactivity became evenly distributed in the gland. Much the same distribution patterns in the pituitary gland were also observed for thyrotropin-releasing hormone (TRH).[9] The significance of this distribution pattern is difficult to assess. In the adrenal gland, the high uptake of radioactivity was seen in the inner layer of the cortex. This finding coincided with that obtained with ^{14}C-FG5111[8] in mice, but was not seen in other psychoactive agents.

Acknowledgement Thanks are due to Miss Misao Seki for her valuable technical assistance.

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