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Studies on the Metabolism of Oxazolam.[1] I. Distribution and Excretion Studies

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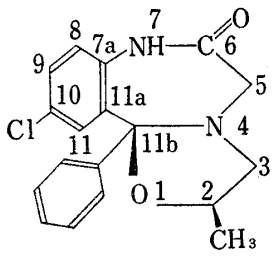
The distribution of orally and intravenously administered ^{14}C-oxazolam was studied in mice by means of whole-body autoradiography and in rats by scintillation counting of the organs and tissues. The radioactivity was found to be widely distributed in various tissues including the brain at 30 min after oral administration, reached the maximum levels within 1 hr and thereafter declined rather rapidly. The distribution patterns were similar for mice and rats, except that the brain uptake of the drug was appreciably higher in mice than in rats. A high affinity of oxazolam to the brain tissue was demonstrated by an extremely high and rapid uptake of radioactivity by the brain after intravenous injection in mice. The brain level reached its maximum at about 1 min after the injection and thereafter fell off rapidly, some retention of radioactivity being observed in the white matter of the brain-stem and in the trigeminal nerve. Autoradiographic studies in pregnant mice demonstrated that there is a slow and only a slight penetration of radioactivity through the placenta. A comparison of the excretion patterns of oxazolam after oral administration in mice, rats, dogs and man revealed that in mice and rats the excretion in the feces, mostly derived from the biliary excretion, was more important than that in the urine, while in dogs a larger part was excreted in the urine and in man the most part (*ca.* 80% of the dose) in the urine.

Oxazolam (10-chloro-2,3,5,6,7,11b-hexahydro-2-methyl-11b-phenylbenzo[6,7]-1,4-diazepino[5,4-*b*]oxazol-6-one, I) is a new minor tranquilizer agent synthesized in this laboratories.[3] It is an analogue of diazepam and chlordiazepoxide and has been shown to have a low toxicity and side effects such as locomotor ataxia.[4] In addition, oxazolam has been shown to be clinically very effective as an antianxiety agent.[5]



I

In the present series of investigations, the fate of oxazolam after oral administration has been studied with respect to the intestinal absorption, tissue distribution, rate and route of excretion and biotransformation. In the present paper, the distribution of ^{14}C-labeled oxazolam after oral and intravenous administrations have been studied by means of mouse whole-body autoradiography and by scintillation counting in rat organs. Excretion patterns have also been studied in rats, dogs and man.

1) Previous name: Oxazolazepam.
2) Location: *Hiromachi 1-chome, Shinagawa-ku, Tokyo.*
3) *a*) T. Miyadera, A. Terada, M. Fukunaga, Y. Kawano, T. Kamioka, C. Tamura, H. Takagi, and R. Tachikawa. *J. Med. Chem.* (1971), to be published. *b*) Sankyo Co., Ltd., Belg. Patent 724,478 (1969).
4) H. Takagi, T. Kamioka, S. Kobayashi, Y. Suzuki, and R. Tachikawa, *Nippon Yakurigaku Zasshi*, **66**, 107, 134 (1970).
5) F. Ohkuma, *Igaku no Ayumi* (Tokyo), **72**, 200 (1970); T. Satoh, *Shinyaku to Rinsho* (Tokyo), **19**, 819 (1970).

Material and Method

Radioactive Compound——^{14}C-Oxazolam uniformely labeled on 11b-phenyl ring was prepared from 2-amino-5-chlorobenzophenone-^{14}C which was prepared by Grignard reaction of ^{14}C-bromobenzene (Radiochemical Center, Amersham, England). The specific activity was 2.4 and 4.0 μCi/mg for the different preparations and the isotopic purity was ascertained by thin-layer chromatography to be over 98% for both preparations.

Whole-body Autoradiography——Five adult male mice of ddY strain weighing about 20 g were administered orally with 50 mg/kg (*ca.* 2.3 μCi/body) of ^{14}C-oxazolam dispersed in 0.5% Tragacanth solution and were sacrificed after 30 min, 1, 3, 6 and 24 hr. Two pregnant mice of Swiss albino strain in late gestation state weighing about 50 g were administered orally with 20 mg/kg (*ca.* 2.3 μCi/body) of ^{14}C-oxazolam and were sacrifised after 1 and 24 hr. One additional pregnant mouse in early gestation state weighing about 40 g was administered orally 10 mg/kg of ^{14}C-oxazolam daily for 7 days and was sacrificed at 24 hr after the last dose. The animals were killed after anesthetizing with ether by immersion in a mixture of acetone and solid carbon dioxide at about $-70°$.

For intravenous injection, the compound was dissolved in dichloromethane containing Tween-80, the solvent was evaporated and the residue solubilized in physiological saline. The final concentration of Tween-80 was 2%. Five male mice weighing about 20 g were injected intravenously with 25 mg/kg (*ca.* 2.0 μCi/0.3 ml/body) of ^{14}C-oxazolam and were sacrificed after 15 sec, 1, 2 and 5 min and 2 hr. The animals were killed by a rapid immersion in liquid nitrogen at about $-190°$.

The autoradiographic technique employed was based on that described by Ullberg.[6] After the frozen animal was embedded on microtome stage with aqueous carboxymethyl-cellulose gel, sagittal 20 μ sections through the whole animal were cut by means of tape-sectioning (Scotch Magic Mending No. 810, Minnesota Mining) with Yamato Type 111 microtome in a freezing room and were dried at $-10°$. The dried sections were brought into contact with Sakura Type-N industrial X-ray film and exposed for 20 to 30 days.

Experiments in Rats——Male rats of Wistar-Imamichi strain weighing about 125 g were administered orally with 30 mg/kg ^{14}C-oxazolam dispersed in 0.5% Tragacanth solution by stomach tube. The animals were sacrificed by bleeding from carotid at various time after the administration and the brain, lung, heart, liver, kidney, spleen, testis, fat (subcutaneous) and skeletal muscle (femoral) were removed. In the separate experiments, the urine and feces were collected over 2 days period after orall and intravenous administrations of ^{14}C-oxazolam. For intravenous injection, the compound was solubilized by dissolving in a small amount of 4.0N HCl and raising the pH to 3.0 with 1.0N NaOH.

Blood, feces and tissue samples were homogenized in 4 volumes of 60% aqueous acetone and after centrifugation the supernatants were assayed for radioactivity. The whole gastro-intestinal tract and the carcass were solubilized by warming in 30% KOH solution at 80° for 24 hr and the solutions were assayed for radioactivity.

All the extracts, solution and urine were counted in the Packard Tri-Carb liquid scintillation spectrometer Model 3240 using a counting medium consisted of 8 g PPO, 200 mg dimethyl-POPOP, 200 ml toluene and 800 ml dioxane. The counts were converted to disintegration per minutes (dpm) by the use of ^{14}C-toluene as an internal standard.

Experiments in Dogs and Man——Five healthy mongrel dogs weighing 8.5 to 13.5 kg were used in each experiment after fasting for about 18 hr. The dogs were administered orally with gelatin capsules containing either 20 or 200 mg oxazolam or intravenously with 25 mg oxazolam solubilized in 10 ml saline of pH 3.0 in the same way as described before. Blood samples heparinized were collected from the forearm vein at the following times: 1, 2, 4, 6 and 8 hr after oral administration and 5, 10 and 30 min and 1, 2, 4 and 8 hr after intravenous injection. The plasma samples were then obtained by centrifugation. The urine and feces were collected for 48 and 72 hr periods, respectively.

As will be described in the subsequent paper,[7] the following seven compounds have been isolated from the rat liver and urine as the main metabolites of orally administered oxazolam: N-desmethyldiazepam, oxazepam, 7-chlor-1,3-dihydro-5-(4-hydroxyphenyl)-2*H*-1,4-benzodiazepine-2-one, 2-(2-hydroxy-*n*-propylamino)acetylamino-5-chlorobenzophenone, 2-amino-5-chlorobenzophenone (ACB) and 2-amino-5-chloro-3-hydroxybenzophenone (ACHB) and its glucuronide. Among them, ACHB glucuronide was found to be excreted in the largest amount in the rat urine.[7] Oxazolam and all of its possible metabolites are, therefore, expected to be converted to either ACB, 2-amino-5-chloro-4′-hydroxybenzophenone (4′-OH-ACB) or ACHB by an acid degradation.[8] Thus, after an acid hydrolysis of samples, ACB and 4′-OH-ACB[9] were determined spectro-

6) S. Ullberg, *Acta Radiol. Supple.* **1954**, 118.
7) A. Yasumura, H. Murata, K. Hattori, and K. Kougo, *Chem. Pharm. Bull.* (Tokyo), to be published.
8) S.S. Walkenstein, R. Wiser, C.H. Gudmundsen, H.B. Kimmel, and R.A. Corradino, *J. Pharm. Sci.*, **53**, 1181 (1964); B.A. Koechlin, M.A. Schwartz, G. Krol, and W. Oberhansli, *J. Pharmacol. Exptl. Therap.*, **148**, 399 (1965).
9) 4′-OH-ACB was ascertained to give an absorption with an almost the same maximum and intensity as ACB.

photometrically after diazo-coupling reaction with N-diethyl-N′-naphthylethylenediamine using the absorption maximum at 550 mμ (method I). ACHB does not undergo the diazo-coupling reaction and was determined separately from the above using its absorption maximum at 398 mμ after glucuronidase treatment of the sample (method II). Recovery of ACB and ACHB from their equimolar mixture by these methods was 104±5.8 and 97.0±2.6% (n=5), respectively. The sum of the results obtained from the two methods was, therefore, regarded as a total amount of oxazolam and its metabolites.

After deproteinization of 1 ml plasma with 3 ml of 10% trichloroacetic acid and a followed reextraction with 5% trichloroacetic acid, the combined extract was heated at 100° for 90 min with 0.5 ml of 6.0N HCl and the solution was assayed after the diazo-coupling reaction (method I). Recovery of oxazolam added to mormal plasma was 101.0±4.8% (n=5). For determining ACHB in plasma, 1 ml plasma was incubated with 5 mg β-glucuronidase (NBC) at 37° overnight in 0.2M phosphate buffer (pH 6.5). The solution was extracted with 10 ml ethyl acetate at pH 5.0 and the extract was assayed by the method II. Recovery of ACHB added to normal plasma was 96.0±2.5% (n=5). One ml aliquots of urine were incubated with 5 mg β-glucuronidase at 37° overnight after the pH was adjusted to 6.5 and were extracted with two portions of 10 ml ethyl acetate at pH 7.0. After the solvent was evaporated to dryness, the residue was heated at 100° for 60 min with 3 ml of 1.0N HCl and the solution was assayed by the method I. Recovery of oxazolam added to normal urine was 97.4±1.4% (n=3). ACHB in urine was determined by treating 1 ml urine in the same way as for the plasma. Recovery of ACHB added was 99.3±4.0% (n=3).

The feces were extracted with 500 ml of 5.0 N HCl by warming at 50° for 10 min and the extract was treated in the same way as for the urine.

Six healthy men weighing 60 to 69 kg were administered with 10 mg oxazolam in a gelatin capsule with 180 ml water after a light breakfast and the whole urine was collected periodically at 2, 4, 6, 8, 12 and 24 hr after administration. Five ml aliquots of urine were treated in the same way as described above and assayned by the methods I and II.

Result

Distribution of ^{14}C-Oxazolam in Mice

Representative autoradiograms from mice 30 min, 1, 6 and 24 hr after oral administration of ^{14}C-oxazolam are shown in Fig. 1 to 3. At 30 min after administration (Fig. 1), a wide distribution of radioactivity was observed throughout the body tissues, while the blood concentration was relatively low, indicating that the drug is well absorbed from the gastro-intestinal tract and rapidly taken up by the tissues. The highest radioactively was shown in the excretory organs such as the urinary bladder, liver, kidney and gall bladder as well as the gastro-intestinal tract, indicating a rapid excretion of the drug. The Harderian gland and nasal mucosa also showed a high radioactivity. A prominent uptake of radioactivity was seen in the lingual and heart muscles, brown fat, salivary gland, pituitary, adrenal cortex and pancreas. The brain showed an appreciable uptake which exceeded the blood level. A low but uniform distribution was observed in the skeletal muscles and a slightly higher concentrations in the adipose tissues such as perirenal and subcutaneous fats.

At 1 hr after administration (Fig. 2), the tissue concentrations of radioactivity became more prominent and at 3 hr and afterward the concentrations appeared to be decreased with time, indicating that the tissue and blood concentrations of the drug reach their maxima at about 1 hr after oral administration.

At 3 and 6 hr after administration, the highest radioactivity was seen in the gall and urinary bladders and intestinal contents, indicating that the excretion through bile back into the intestinal lumen occurs significantly as well as the urinary excretion. At 6 hr after administration, the harderian gland and nasal mucosa still showed a high concentration of radioactivity, while some radioactivity was seen in the oesophagus and stomach contents, suggesting a possibility of some excretion of the drug through the lacrymal secretion. In the brain an appreciable radioactivity was still seen after 6 hr and it was noted that a slightly higher concentration appears to be persisted in the brain-stem, white matter of the spinal cord and trigeminal nerve.

At 24 hr after administration, some radioactivity was observed only in the intestinal contents and trace of radioactivity in the liver and Harderian gland, the most of radioacitvity having been disappeared from the body.

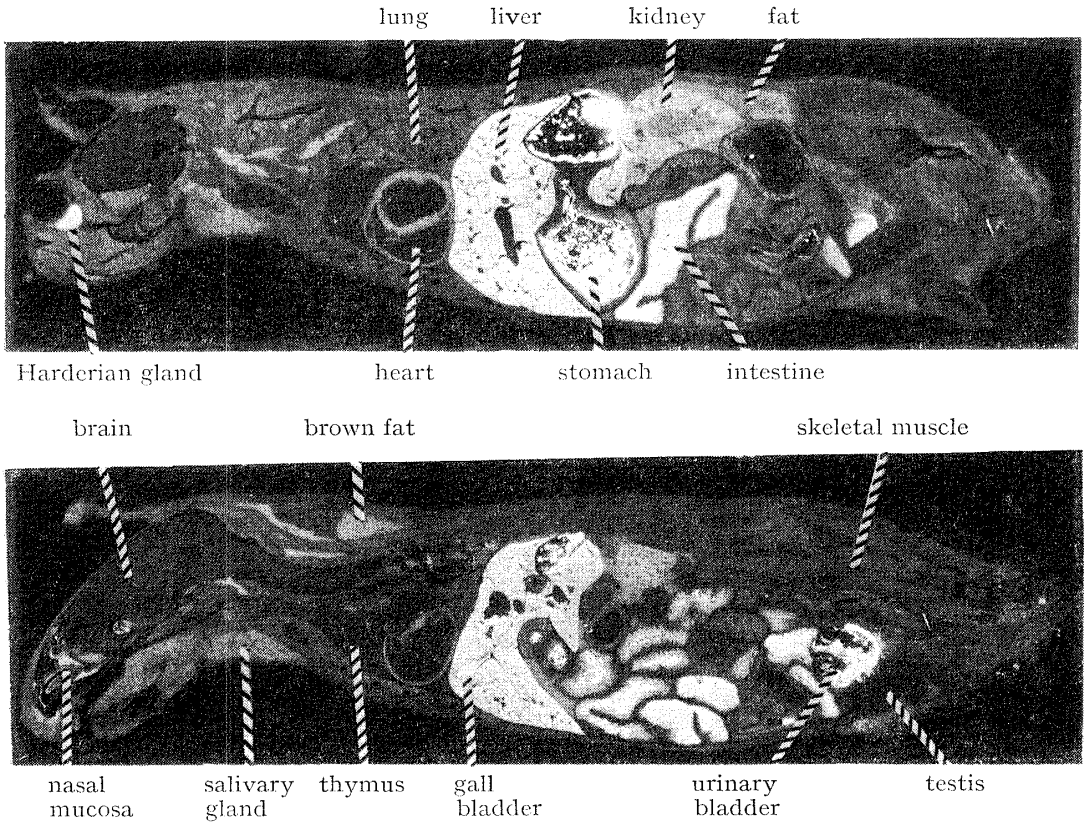


Fig. 1. Autoradiograms of Oxazolam-^{14}C in a Mouse 30 Minutes after an Oral Administration

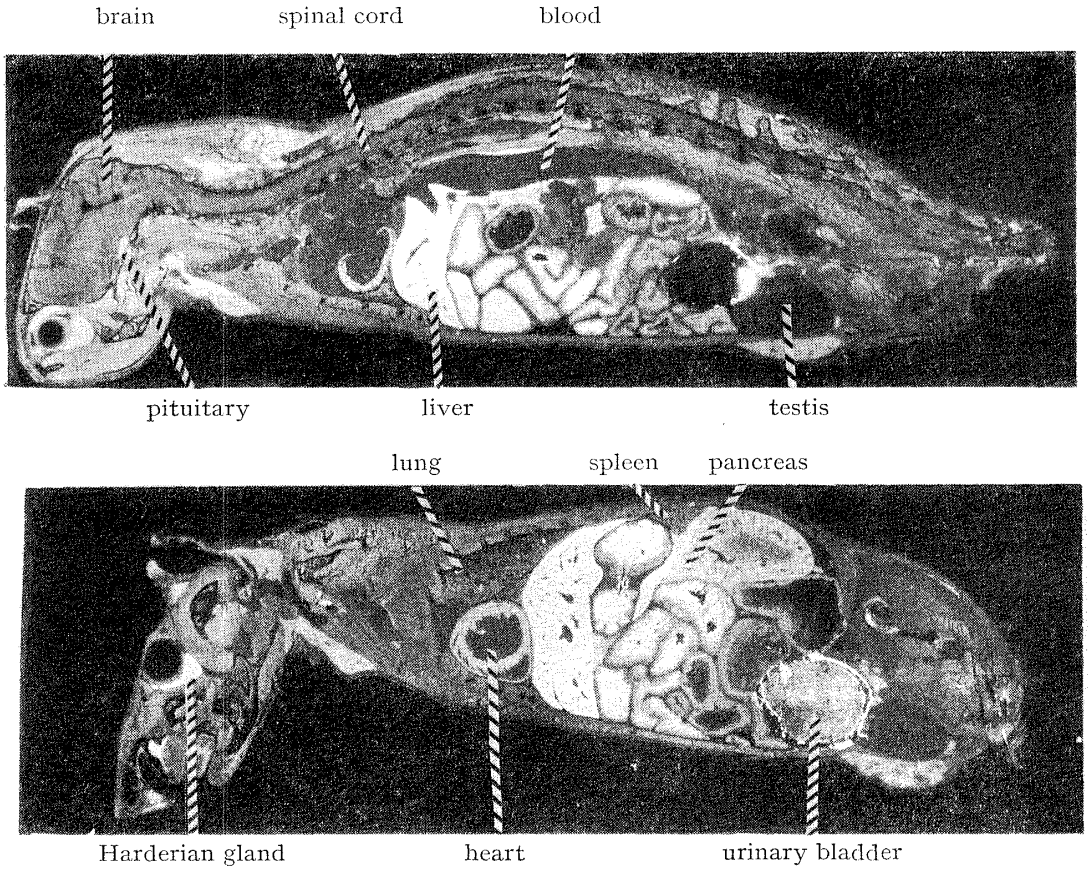


Fig. 2. Autoradiograms of Oxazolam-^{14}C in a Mouse 1 Hour after an Oral Administration

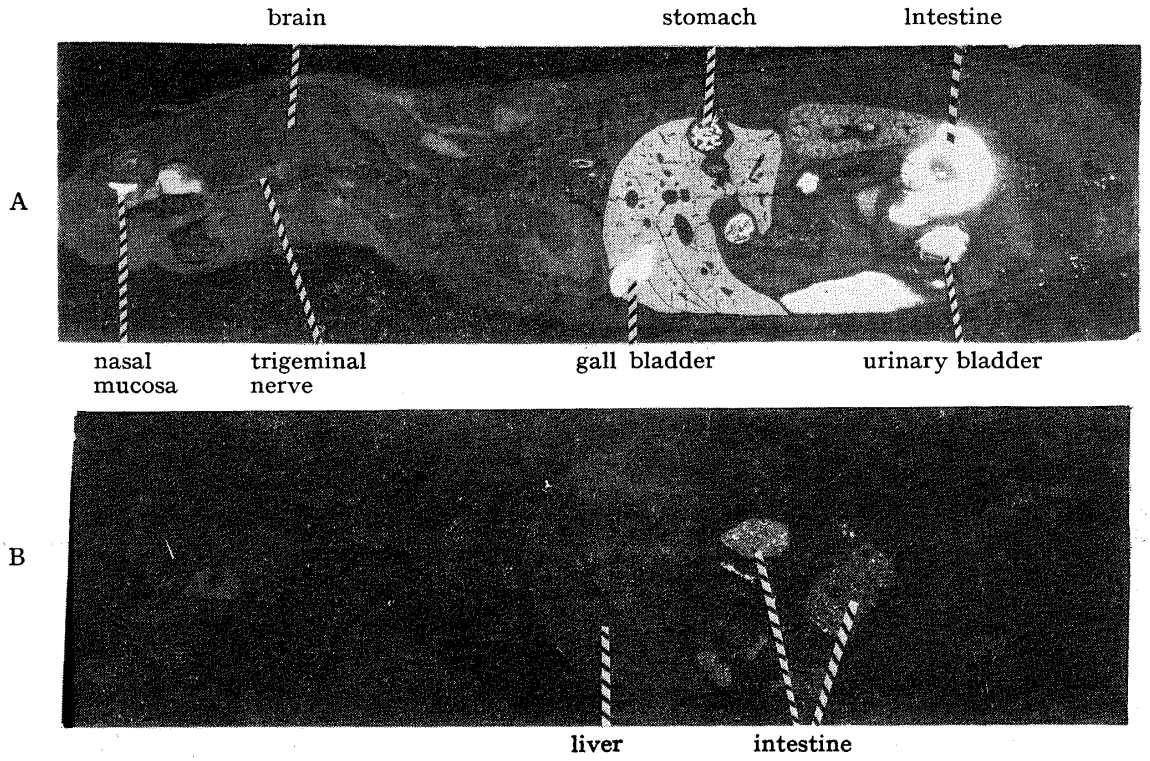


Fig. 3. Autoradiograms of Oxazolam-^{14}C in Mice 6 (A) and 24 (B) Hours after an Oral Administration

At 1 hr after oral administration of ^{14}C-oxazolam into pregnant mice (Fig. 4), some uptake of radioactivity which exceeded slightly the blood level was observed in the placenta, while an extremely low radioactivity was detected in the foetus. After 24 hr, the radioactivity was detected only in the excretory organs of the mother and completely disappeared from both the placenta and foetus. In a pregnant mouse administered orally with ^{14}C-oxazolam continuously for 7 days, no radioactivity was detected in both the foetus and placenta at 24 hr after the last administration, only trace of radioactivity being detected in the maternal liver and intestinal contents.

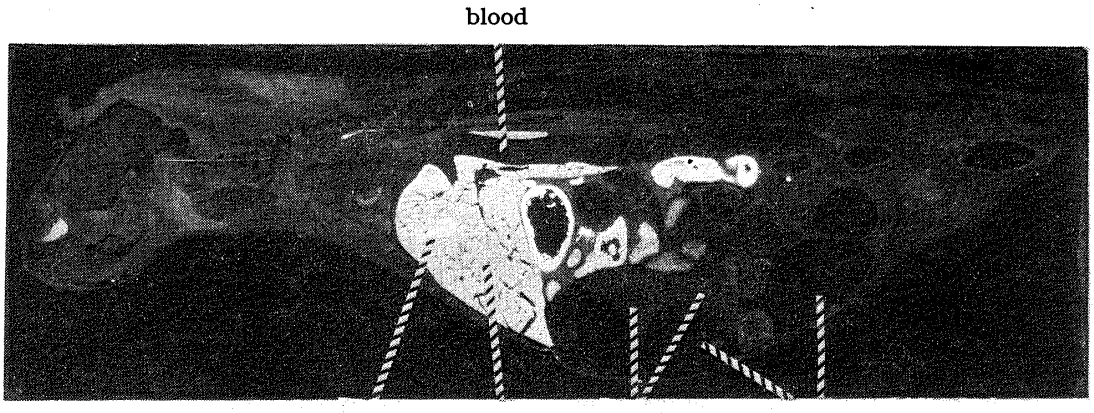


Fig. 4. Autoradiogram of Oxazolam-^{14}C in a Pregnant Mouse 1 Hour after an Oral Administration

The autoradiograms from mice administered intravenously with ^{14}C-oxazolam are exemplified in Fig. 5. As early as 15 sec after injection, a considerable uptake of radioactivity was observed in the brain and the concentration reached its maximum at about 1 min after injection. As can be seen in Fig. 5-A, at 1 min after injection the blood level was found to

be very low, while an extremely high uptake of radioactivity was observed in the brain, indicating a very high affinity of the drug to the brain tissues. High concentrations of radioactivity were also found in the myocardium, cervical and lingual muscles, lung, pituitary and adrenal cortex. The liver showed an uneven distribution of low radioacitvity, the kidney some uptake only in the cortex and the urinary and gall bladders no radioactivity, showing no sign of occurring the excretion of the drug.

At 2 min after injection and afterward, the concentration in the brain was declined rather rapidly with time. At the earliest survival period, the concentration appears to be

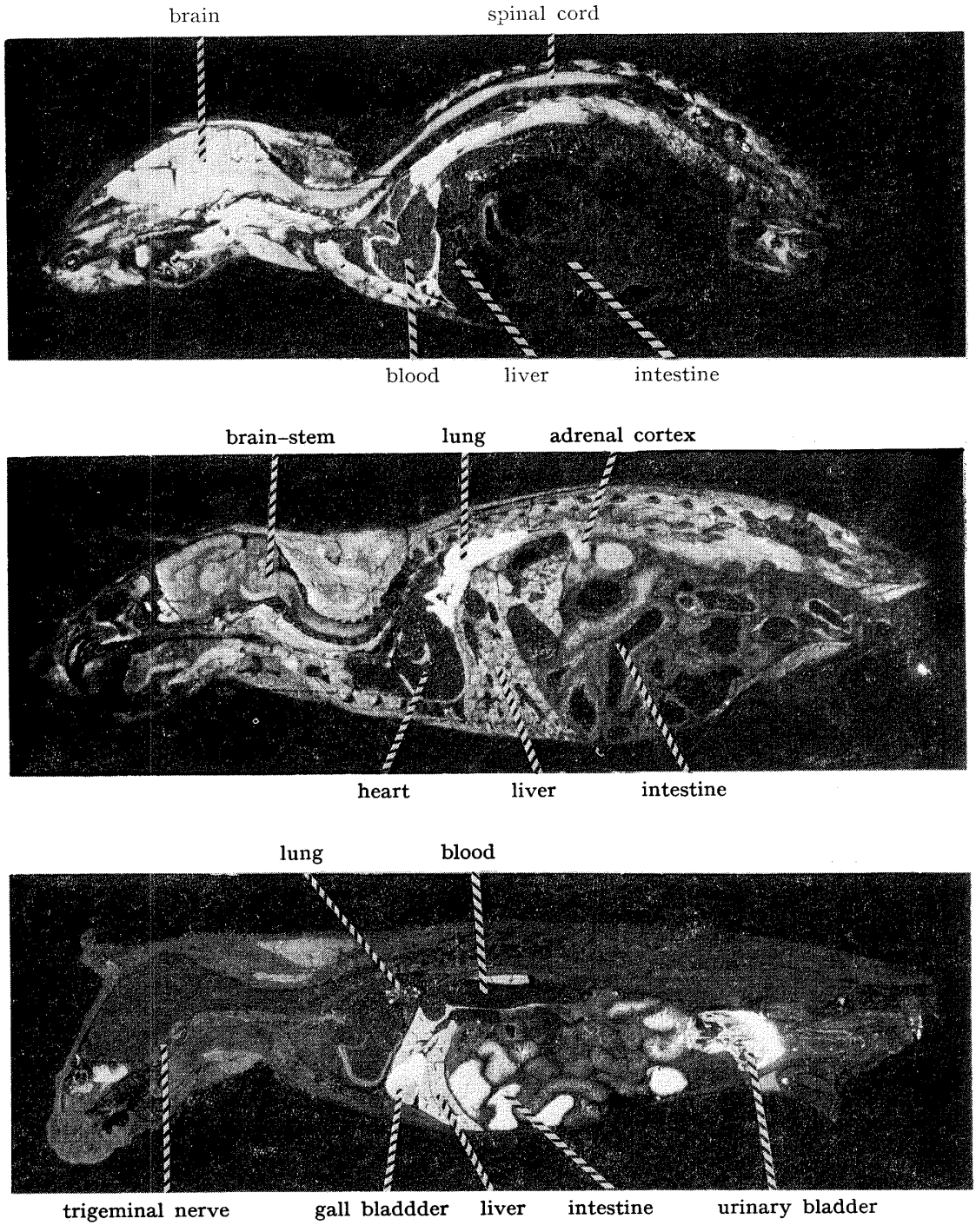


Fig. 5. Autoradiograms of Oxazolam-^{14}C in Mice 1 (A) and 5 (B) Minutes and 2 Hours (C) after an Intravenous Administration

slightly higher in the grey matter of the cerebral and cerebellar cortex, but after 5 min some selective retention of radioactivity was noticed in the white matter of the brain–stem and spinal cord and in the trigeminal nerve. The concentration in the liver was increased with time and a high radioactivity was detected in the gall bladder after 5 min, indicating that the biliary excretion of the drug starts within this survival period. In the urinary bladder, however, no radioactivity was seen yet at this time of survival period.

At 2 hr after administration, high concentrations of radioactivity were observed mainly in the excretory organs: the urinary and gall bladder, intestinal contents, Harderian gland, nasal mucosa and liver. A spotted pattern of a high radioactivity was observed in the lung. In the brain an appreciable concentration of radioactivity was still observed which exceeded the blood level appreciably and some retention of radioactivity was noticed in the trigeminal nerve and in the brain–stem (Fig. 5-C).

Distribution and Excretion of ^{14}C-Oxazolam in Rats

The concentrations of radioactivity in rat organs and tissues after oral administration of ^{14}C-oxazolam are presented in Table I, expressed as μg equivalents of oxazolam. In general, the blood and tissue concentrations appear to reach their maxima at about 1 hr after administration and thereafter to be declined rather rapidly. At 1 hr after administration, the ratio of the tissue to blood concentrations was found to be greater than 1 in all tissues except the brain and testis. The highest concentration was seen in the liver (14.8×blood), followed by the kidney (5.5×blood), subcutaneous fat (3.0×blood), heart (2.4×blood) and lung (2.2× blood). For the period from 30 min to 1 hr, the concentration of radioactivity in the brain was almost comparable to the blood level. After 24 hr the concentrations in the brain and blood and after 48 hr those in all tissues except the liver and kidney fell off to an undetectable level.

Table I. Distribution of Radioactivity in Rat Tissues after Oral Administration of ^{14}C-Oxazolam (30 mg/kg)

Tissue	μg/g wet tissue						
	0.5 hr	1 hr	2 hr	4 hr	7 hr	24 hr	48 hr
Blood[a]	6.27	7.00	5.67	2.03	0.99	—[b]	—
Brain	6.76	6.23	3.83	1.14	0.21	—	—
Lung	15.60	15.73	12.93	2.25	1.99	0.26	0.11
Heart	23.65	16.76	11.99	3.77	1.45	0.15	—
Liver	97.79	103.60	56.31	18.47	10.55	0.43	0.28
Kidney	34.16	38.29	36.97	11.47	6.25	0.54	0.45
Spleen	6.23	9.33	8.50	2.63	1.03	0.24	—
Fat[c]	19.14	20.65	20.83	6.42	1.78	0.30	—
Testis	4.77	6.16	5.11	2.01	1.09	0.06	—
Muscle[d]	9.52	10.77	8.95	3.51	1.26	0.06	—

Each value represents the mean of 2 animals.
Radioactivity was converted to μg equivalents of oxazolam based on the specific activity (2.40 μCi/mg).
a) μg/ml *b*) undetectable level *c*) subcutaneous *d*) thigh

The concentration of radioactivity in the blood and some organs after intravenous administration of ^{14}C-oxazolam in rats are presented in Table II. It was found that the concentration in the brain was very high as early as 3 min after injection and thereafter fell off rapidly. The ratio of the brain to blood concentrations was 6.5, 3.5 and 2.4 at 3, 15 and 30 min after injection, respectively. The results indicate that, in consistent with the finding in mice by autoradiographic technique, the drug passes the blood–brain barrier very easily and has a high affinity to the brain tissues. In the liver, on the other hand, the concentration appears

to reach its maximum sometime around 15 min after injection and thereafter to be decreased gradually, the behavior being again in consistent with that observed in mice.

The excretions of radioactivity in rats after oral and intravenous administrations of ^{14}C-oxazolam are shown in Table III. During 48 hr period after oral administration, approximately 30 and 60% of the dose were recovered in the urine and feces, respectively. It was noted that during the first 24 hr period 29.1% of the dose was excreted in the urine, while virtually no urinary excretion was observed during the subsequent 24 hr period. In the feces, on the other hand, 37 and 24.4% of the dose were excreted during the first and the subsequent 24 hr periods, respectively. At 24 hr after intravenous administration, a larger amount of radioactivity (50% of the dose) was found in the feces and gastro-intestinal tract as compared to the amount recovered in the urine (33%), indicating a significant secretion of radioactivity into the intestinal tract, most probably, through biliary excretion. Therefore, it seems probable that a considerable fecal excretion observed after oral administration was mostly derived from absorbed drug which was brought back into the intestinal lumen through biliary excretion.

Table II. Tissue Concentration of Radioactivity in Rats after Intravenous Administration of ^{14}C- Oxazolam (30 mg/kg)

Tissue	μg/g wet tissue					
	3 min	15 min	30 min	1 hr	2 hr	4 hr
Blood[a]	14.7	10.8	6.14	5.33	4.09	1.84
Brain	93.3	44.5	14.4	8.50	4.58	0.14
Liver	26.1	70.0	58.6	43.9	35.5	16.2
Kidney	51.1	47.1	39.6	34.3	24.0	11.4
Fat[b]	4.56	24.4	35.6	28.0	19.3	5.11

Each value represents the mean of 2 animals.
Radioactivity was converted to μg equivalents of oxazolam.
a) μg/ml *b*) subcutaneous

Table III. Excretion and Recovery of Radioactivity in Rats following Oral or Intravenous Administration of ^{14}C-Oxazolam (30 mg/kg)

Sample	% of dose		
	Oral administration		*i.v.* injection
	24 hr	48 hr	24 hr
Urine	29.1	29.9	33.3
Feces	37.0	61.4	31.5
G.I. tract[a]	23.1	3.7	18.9
Carcass	3.5	1.3	1.7
Recovery	92.7	96.3	85.4

Each value represents the mean of 2 animals.
a) tissue+contents

Blood Concentration and Excretion of Oxazolam in Dogs and Man

As shown in Fig. 6, the blood concentration of oxazolam and its metabolites in dogs, estimated by method I,[10] reached its maximum at about 2 hr after oral administration in either dose level of 20 or 200 mg/body. After intravenous administration, as shown in Fig. 7,

10) The amount of ACHB estimated by method II was found to be relatively small in the blood.

the blood concentration fell off very rapidly till about 1 hr after injection and thereafter decreased rather slowly with time.

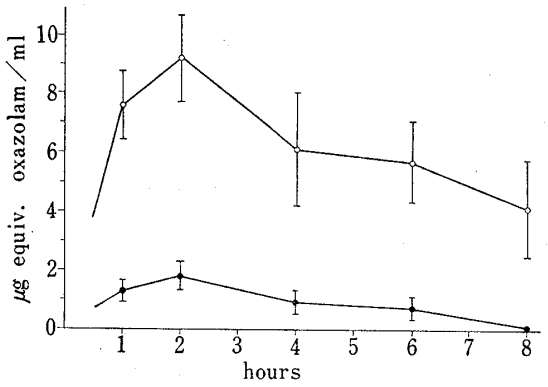


Fig. 6. Blood Concentrations of Oxazolam and Its Metabolites after Oral Administration in Dogs

Values are expressed as μg equivalents of oxazolam per ml ± S.E. ($n=5$), determined by method I. dose: 20 mg (—●—) or 200 mg (—○—)/body

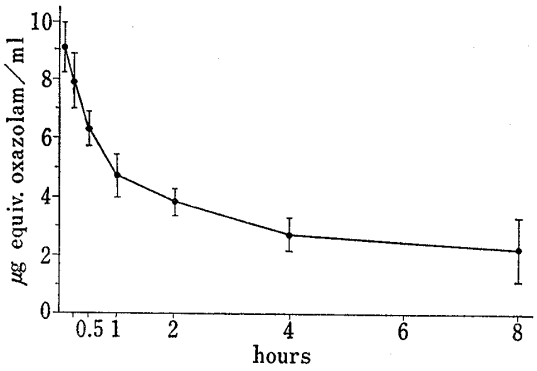


Fig. 7. Blood Concentrations of Oxazolam and Its Metabolites after Intravenous Injection in Dogs

Values are expressed as μg equivalents of oxazolam per ml ± S.E. ($n=5$), determined by method I. dose: 25 mg/body

The urinary and fecal excretion of oxazolam and its metabolites after oral and intravenous administrations in dogs are shown in Table IV. Oral administration of 20 mg oxazolam resulted in a complete recovery of the drug during 72 hr period and 69 and 37% of the dose were excreted in the urine and feces, respectively. Intravenous injection of 25 mg oxazolam also resulted in a complete recovery during 72 hr period and 59.3 and 38.5% of the dose were excreted in the urine and feces, respectively. These results indicate that the excretion of the drug into the intestinal lumen through biliary excretion is occurring also in dogs, although the extent appears to be less significant than that in rats. It was noted that ACHB, which was estimated by method II, was excreted to a significant amount in dogs, particularly, in the feces after both oral and intravenous administration.

Table IV. Excretion of Oxazolam in Dogs after Oral or Intravenous Administration

	% of dose ± S.E. ($n=5$)[a]			
	Oral administration (20 mg/body)		Intravenous injection (25 mg/body)	
Method	urine[b]	feces[c]	urine[b]	feces[c]
I	48.3±3.9	19.3±3.3	41.2±7.1	10.4±2.8
II	20.7±9.3	17.5±2.8	18.1±3.2	28.1±7.1
Total	69.0±5.6	37.2±5.5	59.3±5.4	38.5±3.3
Recovery	106.3±9.2		97.7±13.6	

a) determined as oxazolam equivalents *b*) 48 hr *c*) 72 hr

The urinary excretion of oxazolam and its metabolites after oral administration of 10 mg oxazolam in man is shown in Table V, expressed as mg equivalents of oxazolam. During the first 8, 12 and 24 hr periods, approximately 50, 63 and 80% of the dose were recovered in the urine, respectively, indicating that the excretion of the drug is mainly through urinary route in man. From the elimination rate constant, the biological half-life of oxazolam in man after oral administration was calculated to be 4.6 hr. It was noted that a considerable

amount, approximately 50% of the total urinary excretion, was found to be ACHB and/or its glucuronide.

Table V. Urinary Excretion of Oxazolam in Man after Oral Administration (10 mg/body)

Subject	Weight (kg)	mg equivalents of oxazolam[a]					
		2 hr	4 hr	6 hr	8 hr	12 hr	24 hr
T.H.	63	1.11	2.66	4.03	5.10	6.16	8.21
K.S.	69	0.97	3.50	4.78	5.63	6.51	10.32
Y.K.	60	1.12	2.24	3.52	4.11	4.99	5.88
S.F.	65	0	2.96	4.58	5.45	6.50	8.41
K.I.	60	1.07	2.61	3.56	4.27	5.91	7.12
T.S.	60	1.46	4.00	5.52	6.26	7.38	8.91
Mean±S.E.	method I	0.51±0.14	1.62±0.22	2.53±0.25	3.07±0.24	3.61±0.23	3.96±0.22
	method II	0.44±0.11	1.37±0.19	1.80±0.23	2.06±0.26	2.63±0.29	4.09±0.59
	total	0.96±0.20	3.00±0.26	4.33±0.32	5.13±0.33	6.27±0.32	8.07±0.65

a) cumulative amount of total excretion

Discussion

The present studies on the distribution of ^{14}C-oxazolam in mice and rats revealed that after oral administration the blood and tissue concentrations of radioactivity reached their maxima at about 1 hr after administration and thereafter declined rather rapidly, with no organ or tissue retaining radioactivity for more than 48 hr. After 1 hr the concentration of radioactivity in rat tissues was the highest in the liver, followed by the kidney, subcutaneous fat, heart muscle, lung, skeletal muscle, spleen, blood, brain and the lowest in the testis. The brain showed a concentration which is almost comparable to the blood level and this might be considered as indicating an appreciable uptake of the drug by the brain tissues, since the vascularity in the brain tissue is very low. The order of the tissue concentration in rats was in good accord with that observed in mice by autoradiographic technique, with an apparent exception that in mice the brain showed a concentration which exceeded appreciably the blood level. The higher uptake of oxazolam by the brain in mice than in rats is consistent with the observation that the pharmacological activity as antipentetrazol activity is much stronger in mice than in rats.[11]

A high affinity of oxazolam to the brain tissue was demonstrated in mice by whole-body autoradiographic technique and the brain showed an extremely rapid, high and selective uptake of radioactivity immediately after intravenous injection of ^{14}C-oxazolam. A rapid accumulation of diazepam and chlordiazepoxide in the brain after intravenous injection in mice has been demonstrated by the same technique[12,13] and it was further noted that [12]the concentration of ^{14}C-diazepam in the brain reached its maximum sometime before 1 min after injection, while ^{14}C-chlordiazepoxide increased progressively for 5 min after injection. In the present study, concentration of ^{14}C-oxazolam in the brain reached its maximum at about 1 min after injection. Therefore, it might be concluded that the penetration rate of oxazolam into the brain is slightly slower than diazepam, while appreciably faster than chlordiazepoxide. In this respect, it seems of interst to compare the lipophilic character of these compounds. *Rf*-Values on thin-layer chromatography, which might be regarded as a measure of the relative

11) H. Takagi, unpublished work in this laboratories.
12) E. Van der Kleijn, *Arch. int. Pharmacodyn.*, **178**, 193 (1969).
13) G.F. Placidi and G.B. Cassano, *Int. J. Neuropharmacol.*, 7, 383 (1968).

lipophilic character when neutral solvent systems are used, were found to be in the order: diazepam>oxazolam≫chlordiazepoxide (Table VI). This order parallels with the rate of penetration into the brain, suggesting that a passage of these drugs through the blood–brain barrier is proceeded by a passive diffusion process which depends on the lipid solubility of the compound.

Table VI. *Rf*-Values on Thin-Layer Chromatogram

Compound	*Rf*-value × 100	
	Solvent I	Solvent II
Diazepam	92	92
Oxazolam	89	83
Chlordiazepoxide	61	38

solvent I: benzene–ethylacetate–ethanol (18:6:1)
solvent II: chloroform–acetone (15:1)
silicagel F_{254} (Merck)

^{14}C-Oxazolam showed a rapid and high accumulation in the grey matter of the cerebral and cerebellar cortex in the earliest period after intravenous injection in mice, while some selective retention of the radioactivity was noted in the white matter of the brain-stem and spinal cord and in the trigeminal nerve, in the later period. These behaviors, which are closely similar to those observed in ^{14}C-diazepam,[12] are of special interest with respect to the appearence of pharmacological action, though the implications are not well understood at present. It should be added here that, as will be shown in the subsequent paper,[14] the radioactivity in the brain in the earliest period might represent mostly unchanged oxazolam, while that in a later period mostly N-desmethyldiazepam, which has been reported to have a high pharmacological activity.[15]

It was noticed as a characteristic feature of oxazolam when it was injected intravenously in mice that the radioactivity was accumulated in a high concentration in the lung, which persisted as a spotted pattern of a high radioactivity for more than 2 hr (Fig. 5-C). A spotted appearence of radioactivity in the lung has been reported, *e.g.*, in ^{14}C-DOPA and ascribed to its distribution in adrenergic nerves.[16] It might be more plausible here, however, that because of an extremely low water solubility of oxazolam and a high dose applied (30 mg/kg), a part of the labeled drug was precipitated to form aggregated particles which were trapped by the capillaries in the lung during the circulation.

As to the excretion pattern of oxazolam, autoradiographic studies in mice revealed that a biliary excretion of the drug contributes significantly as well as an urinary excretion. As early as 5 min after intravenous injection of ^{14}C-oxazolam, a high radioactivity was detected in the gall bladder and a persistent high concentration of radioactivity was observed in the gall bladder over a period from 30 min to more than 6 hr after oral administration. The fact that after intravenous injection in rats a larger amount of radioactivity was recovered in the feces and intestinal contents (*ca.* 50% of the dose) than in the urine (*ca.* 30%) indicates that the biliary excretion must be more important than the urinary excretion in rats. Therefore, the observation that after oral administration a major part (*ca.* 60%) of the dose was recovered in the feces, while a smaller part (*ca.* 30%) in the urine can be interpreted as indicating that oxazolam is well absorbed from the gastro-intestinal tract and the fecal excretion is mostly derived from the absorbed drug which was brought back into the intestinal lumen

14) H. Shindo, T. Komai, and K. Tanaka, *Chem. Pharm. Bull.* (Tokyo), to be published.
15) F. Marcucci, A. Guaitani, J. Kvetina. E. Mussini, and S. Garttini, *Europ. J. Pharmacol.*, **4**, 467 (1968).
16) J.M. Van Rossum, C.C. Wijffels, and N.V.M. Rijntjes, *Europ. J. Pharmacol.*, **7**, 337 (1969).

through the biliary excretion. A high absorbability of oxazolam from rat intestine and a high excretion through bile will be further demonstrated in the subsequent paper.[14] In dogs, on the other hand, 69 and 37% of the dose were recovered in the urine andfeces, respectively, after oral administration, indicating that the urinary excretion appears to be more important than the fecal, thus probably the biliary excretion. In man, it was clarified that oxazolam is well absorbed from the intestine and excreted mainly through the urinary route, approximately 80% of the dose being recovered in the urine during 24 hr after oral administration. It might be said, therefore, that the relative importance of the biliary and urinary route in the excretion of oxazolam varies significantly depending on the species and that the contribution of the biliary excretion appears to be decreased concomitant with increased importance of the urinary excretion in the order: mice and rats, dogs and man. The same trend of the species difference in the excretion pattern has also been reported in diazepam.[17] A species difference was also noted in the amount of ACHB excreted in the urine and a larger amount was found to be excreted as ACHB and/or its glucuronide in man (*ca.* 50% of the total urinary metabolites) than in dogs (*ca.* 30%).[18]

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17) M.A. Schwartz, B.A. Koechlin, E. Postma, S. Palmer, and G. Krol, *J. Pharmacol. Exptl. Therap.*, **149**, 423 (1965).

18) ACHB was found to be excreted mostly as the glucuronide conjugate in rats (ref. 7).