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Research Article

Quantitative Whole-Body Autoradiographic Determination of Tacrine Tissue Distribution in Rats Following Intravenous or Oral Dose

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Tacrine (1,2,3,4-tetrahydro-9-acridinamine) has been employed in diverse clinical situations but has recently been of considerable interest for the treatment of cognitive deficits associated with senile dementia (Alzheimer's disease). The present studies examined tissue distribution of radiolabeled tacrine by quantitative whole-body autoradiography. Tacrine radioequivalents were widely distributed to tissue following iv or peroral dose, with an apparently prolonged absorption phase following po dose. The presence of high levels of activity in kidneys and ureters indicates a major role for urinary excretion, but there is also evidence for biliary excretion and direct secretion of compound or metabolites into the intestinal lumen. Tacrine was rapidly taken up into the brain and demonstrated regional localization to cortex, hippocampus, thalamus, and striatum. Although the inhibition of acetylcholinesterase by tacrine is well documented, regional uptake in brain did not correlate consistently with distribution of the enzyme, supporting suggestions by others that the alleged action of tacrine in treatment of senile dementia may be by mechanisms other than cholinesterase inhibition.

KEY WORDS: tacrine; tissue distribution; acetylcholinesterase inhibitor; Alzheimer's disease; senile dementia; autoradiography.

INTRODUCTION

Senile dementia of the Alzheimer's type (SDAT) is characterized by regional loss of cholinergic activity associated with lowered levels of choline acetyltransferase (1), which may be ameliorated by treatment with acetylcholinesterase (AChE) inhibitors such as tacrine or physostigmine (2,3). Tacrine, 1,2,3,4-tetrahydro-9-acridinamine, CI-970, is a potent inhibitor of acetylcholinesterase (4,5), which has been used as an antagonist of morphine (6), in combination with morphine for the treatment of pain (7), to prolong the action of suxamethonium (8), and as an antidote for antidepressant overdose (9). Initial trials described by Summers *et al.* (10) showed improved performance on cognitive tests by Alzheimers patients treated with tacrine, when compared to controls. As a result of these findings there has been considerable interest in developing this compound for use in treating patients with SDAT.

There are few references to the distribution and metabolic fate of this compound. Recent work has shown that after a single dose of ^{14}C-tacrine to beagle dogs, very little of the radioactivity present in plasma or urine was unchanged drug (11). In rat, paper chromatographic analysis of urine indicated the presence of four metabolites but no unchanged drug following tacrine administration (12). Preliminary findings in our laboratories suggest that a major metabolite is 4-hydroxy tacrine. This compound has been shown to have a biochemical and pharmacological profile similar to that of tacrine (13).

The present study was designed to examine the distribution of radioactivity in rats following intravenous or oral dose of ^{14}C-labeled tacrine. Quantitative whole-body autoradiographic methods were chosen to provide detailed information on the distribution of the compound in all tissues, including consideration of regional localization in brain.

MATERIALS AND METHODS

Tacrine (9-[^{14}C], hydrochloride salt), specific activity 27.84 μCi/mg, was synthesized in the Radiochemistry laboratories at Parke-Davis, Ann Arbor, Mich. Radiochemical and chemical purity, 100 and 99.6% respectively, were determined by HPLC. For the iv experiment, jugular cannulae were inserted in male Wistar rats, which were allowed to recover for 24 hr following the surgery. After an overnight fast animals were dosed with 5 mg/kg ^{14}C-tacrine via the cannula. For the oral dose study, fasted rats were administered 20 mg/kg labeled compound by gavage tube. In both groups, animals were sacrificed in duplicate, by halothane anesthesia, at 30 min or 4, 8, or 12 hr postdose and rapidly frozen in a dry ice/hexane bath. Carcasses were embedded in methylcellulose ice and sectioned at −20°C in a PMV 2250

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cryomicrotome (LKB Instruments). Thirty to thirty-six sections per animal were cut at 50μm thickness, allowed to dry in the cryostat cabinet, and then exposed to Kodak SB-5 X-ray film at freezing temperatures for appropriate periods ranging from 1 to 12 weeks. Carbon-14 standards (American Radiolabeled Chemicals) were exposed simultaneously on representative films for subsequent calibration of the Eye-Com II image analyzer (Spatial Data Systems). Autoradiograms were digitized with the analyzer scanner and activity remaining in tissues was measured from the digital images. Total radioactivity (parent drug and radioactive metabolite[s]) is expressed as microgram equivalents tacrine free base per gram tissue. Elimination half-life values of total radioactivity for blood and various tissues were calculated for the iv dose. Elimination half-life was calculated as ln2/lambda z. Elimination rate constant, lambda z, was determined using linear regression of the natural logarithm of tissue concentration with time and was selected by inspection from apparent terminal elimination phase.

RESULTS

IV Studies

Thirty minutes postdose radioactivity was widely distributed to tissues (Table I, Fig. 1). Highest activity was found in the contents of the proximal intestines, ureters, and bladder, while concentrations were also detected in stomach contents and bile ducts. High activity was seen also in the vomeronasal organ, hair follicles, and preputial gland, a modified sebaceous-type gland. These structures remained among the most highly labeled over the course of these experiments. Moderate uptake was observed in the intestinal wall, at a level similar to that of spleen. Epididymus contained slightly more label than testis, where activity concentrated primarily in interstitium. A number of tissues demonstrated heterogeneous distribution of label. Cortical regions of thymus took up higher levels of radioactivity than medullary areas. Adrenal medulla contained considerably more label than cortex. In lymph nodes there was indication of higher activity in germinal centers, although this pattern was not seen at late time points and was not detected in animals dosed orally. Pancreas showed small areas of high activity distributed over the organ, indicating uptake of compound into pancreatic islets. This pattern was persistent at all time points examined. Activity was detected in the retina/choroid of the eye, and a slightly lower level was seen in the outer layer of lens.

Table I. ^{14}C-Tacrine Radioequivalents in Tissue Following IV Dose (μg equivalents/g)[a]

Tissue	Hours postdose				$t_{1/2}$[b]
	0.5	4	8	12	
Kidney	19.02	1.85	0.55	0.38	3.5
Liver	16.49	2.91	1.46	1.01	5.2
Pancreas	16.05	1.00	0.19	0.11	2.5
Lacrymal	15.99	2.50	0.96	0.64	4.1
Lacrymal-exorb.	15.99	1.78	0.89	0.57	4.9
Adrenal	11.64	2.34	1.10	0.62	4.2
Salivary	11.10	1.19	0.45	0.13	2.5
Marrow	10.32	0.72	0.28	0.15	3.5
Spleen	10.00	1.00	0.40	0.22	3.7
Lymph node	7.64	0.46	0.15	0.08	3.2
Thymus	7.05	0.77	0.26	0.11	2.9
Muscle	5.55	0.84	0.17	0.07	2.2
Harder's gland	5.37	0.43	0.14	0.09	4.0
Heart	4.99	0.47	0.17	0.09	3.4
Lung	4.80	0.47	0.16	0.10	3.6
Testis	3.61	1.14	0.44	0.16	2.8
Brown fat	2.89	0.47	0.15	n.d.[c]	—
Blood	1.75	0.30	0.13	0.07	3.8

[a] Mean, two animals.
[b] Calculated from 4 to 12 hr.
[c] No data.

Tacrine radioequivalents readily crossed the blood–brain barrier and distributed selectively to a number of structures at levels greater than that of blood (Table II and Plate 1). Highest concentrations of radioactivity in brain were localized in pituitary and to a lesser degree pineal, while lowest uptake was seen in white matter of brain and spinal cord. Activity in cortex was higher than most regions, and there was a suggestion that frontal cortex might have slightly more uptake than other lobes. Activity in hippocampus was differentially distributed among the layers. Highest uptake appeared in oriens, radiens, and dentate gyrus. Hippocampal molecular layer had activity only slightly greater than that of white matter and there was also indication of lesser activity in the pyramidal cell layer separating the oriens-radiens. Relatively low activity was observed in the olfactory bulb, although higher levels were evident in anterior olfactory nucleus. Uptake in cerebellum was substantially greater in gray matter. No activity was seen in ventricles or choroid plexus.

Four hours following iv dose radioactivity declined to 10–15% of that measured at 30 min, although relative distribution was similar to that seen at the earlier interval. Highest concentrations of activity were seen in contents of intestine, bladder, and ureters. There was also a relatively high concentration in the stomach lumen, esophagus, and mouth. Adrenal medulla maintained substantially greater activity than cortex, and pancreatic islets also remained readily distinguishable from surrounding parenchyma. The relatively high activity in adrenal medulla and pancreatic islets was persistent through the last time point studied. Activity in brain structures appeared to decline at a slightly more rapid rate than in other tissues. Pituitary and pineal glands remained the most highly labeled structures in the brain. Gray matter in cortex, hippocampus, striatum, thalamus, and olfactory nucleus demonstrated considerably more activity than white matter and other gray matter structures with the exception of cerebellar gray, which retained considerable activity.

Following the decline in activity seen between 30 min and 4 hr, there was a further but less rapid decline at 8 and 12 hr (Fig. 2) which was nearly linear on linear or semilog plots. Radioactivity in salivary gland, pancreas, and muscle appeared to decline somewhat more rapidly than that in other tissues. As in the earlier intervals, highest activity at 12 hr postdose was seen in the contents of the GI tract, although in more distal regions, and intense label could be seen in ureters and bladder. In tissue highest activity remained in preputial gland, hair follicles, vomeronasal organ,

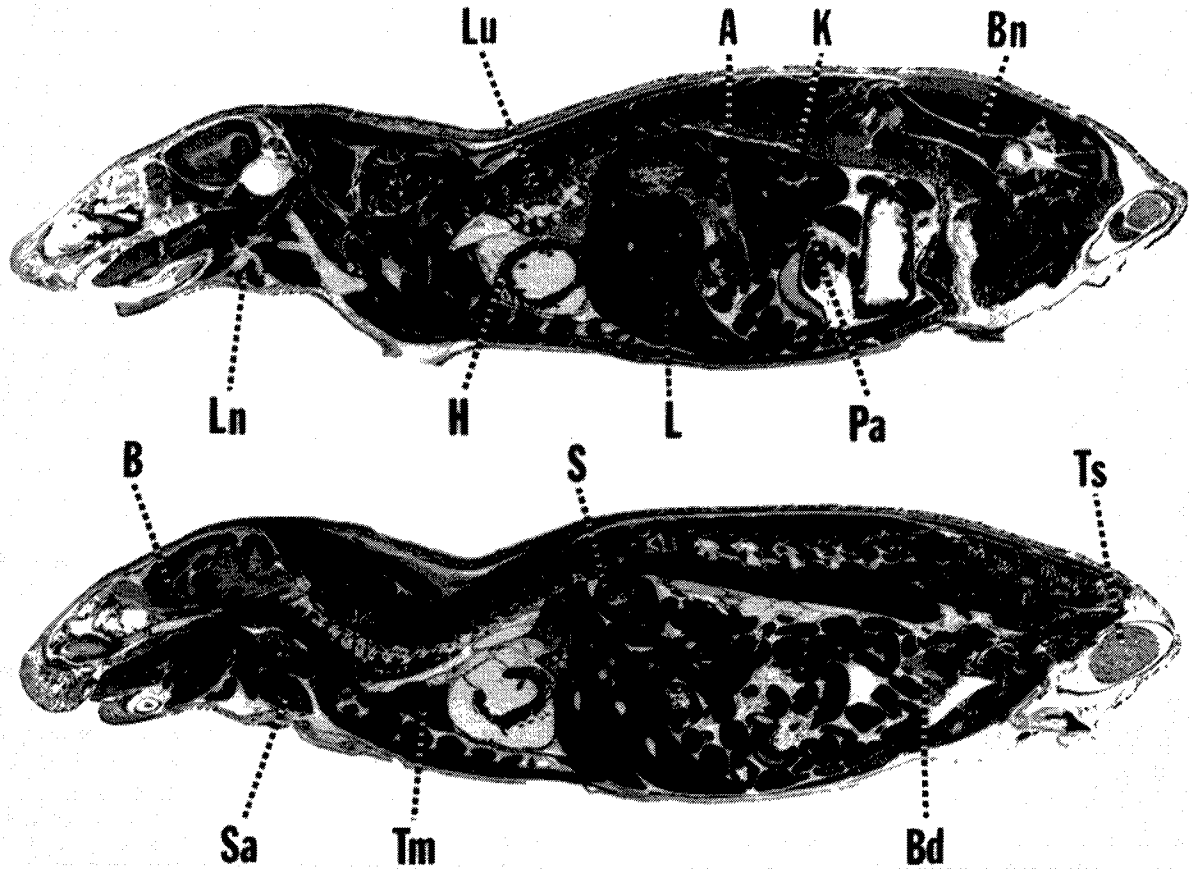

Fig. 1. ^{14}C-Tracine autoradiograms, 30 min post iv dose. One-week autoradiographic exposure. A, adrenal; B, brain; Bd, bladder; Bf, brown fat; Bn, bone marrow; H, heart; K, kidney; L, liver; La, lacrymal gland; Ln, lymph node; Lu, lung; Pa, pancreas; S, stomach, Sa, salivary; Sp, spleen; Tm, thymus; Ts, testis.

and adrenal medulla. Liver and lacrymal glands also maintained considerable activity. Spleen red pulp concentrated notably greater activity than white pulp, and thymic cortex had more label than medullary regions. Little activity was detected in choroid/retina of the eye, but there was considerable activity remaining in the lens. Activity in brain was well below blood level, but regional localization was still detectable.

Table II. ^{14}C-Tacrine Radioequivalents in Brain Following IV Dose (μg equivalents/g)[a]

Tissue	Hours postdose				
	0.5	4	8	12	$t_{1/2}$[b]
Cortex	5.71	0.44	0.12	0.04	2.3
Hippocampus	5.36	0.43	0.13	0.04	2.3
Thalamus	5.25	0.44	0.12	0.04	2.3
Striatum	5.00	0.40	0.12	0.04	2.4
Cerebellum	4.41	0.32	0.09	0.03	2.3
Olfactory bulb	4.31	0.32	0.08	0.03	1.8
Colliculus	4.17	0.26	0.06	bld[c]	—
Brain stem	3.70	0.20	0.05	bld	—
Pituitary	16.92	1.36	0.56	0.19	2.8

[a] Mean, two animals.
[b] Calculated from 4 to 12 hr.
[c] Below limit of detection.

PO Studies

Table III shows the results of video densitometry for animals given an oral dose of 20 mg/kg ^{14}C-tacrine. There was considerable interanimal variation in the amount of dose absorbed. At 30 min, distribution of radioactivity was remarkably similar to that seen at the same time point in the iv animals, with only bone marrow and testis showing noteworthy differences (Fig. 3). As expected, stomach contents contained the highest activity, and intense label was seen also in ureters and bladder. Other areas of high activity were the vomeronasal organ, preputial gland, and mucosa of the distal intestinal tract, most obvious in those sections where minimal activity was present in the lumen. There was also considerable activity in the esophagus which seemed to be associated with the superficial layer of the mucosa rather than in the lumen. Whether this is an artifact of dosing or represents some specific uptake is not clear. Brown fat concentrated more radioactivity in the orally dosed group than in the iv animals, and minor concentrations were also detected in body fat. As in the iv studies, high activity was seen in the choroid/retina of eye as well as some minor concentration in humoral fluid and in the periphery of lens. Uptake in brain was lower in the orally dosed animals than in the iv group at 30 min postdose, but the relative distribution to brain nuclei was similar (Table IV, Plate 2). As seen in the iv group, white matter concentrated considerably less activity than gray matter. Highest activity was seen in pituitary and pineal

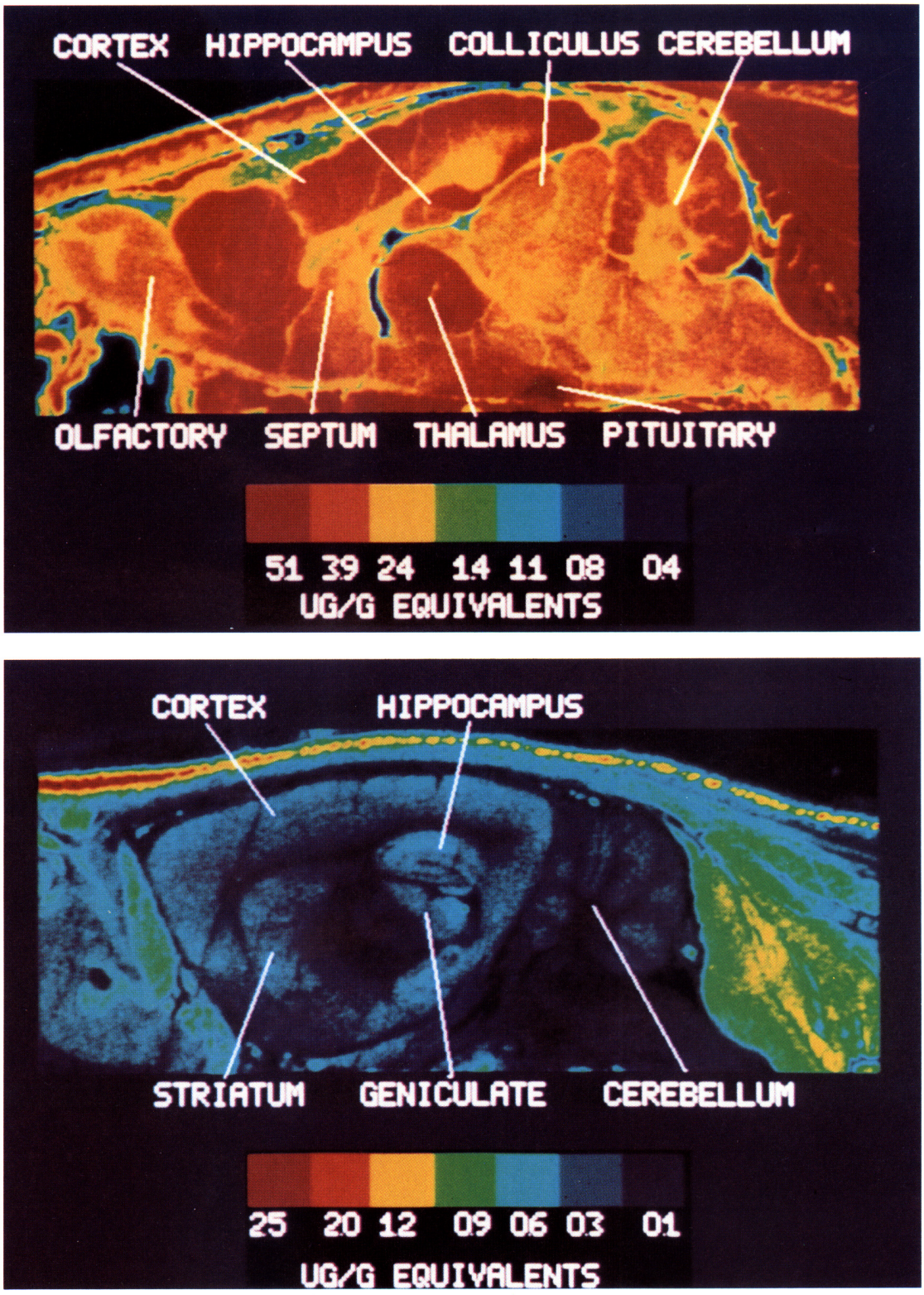

Plate 1. ^{14}C-Tacrine distribution in brain; computer-colored digital image. Top: 30 min post iv dose. Bottom: 4 hr post iv dose.

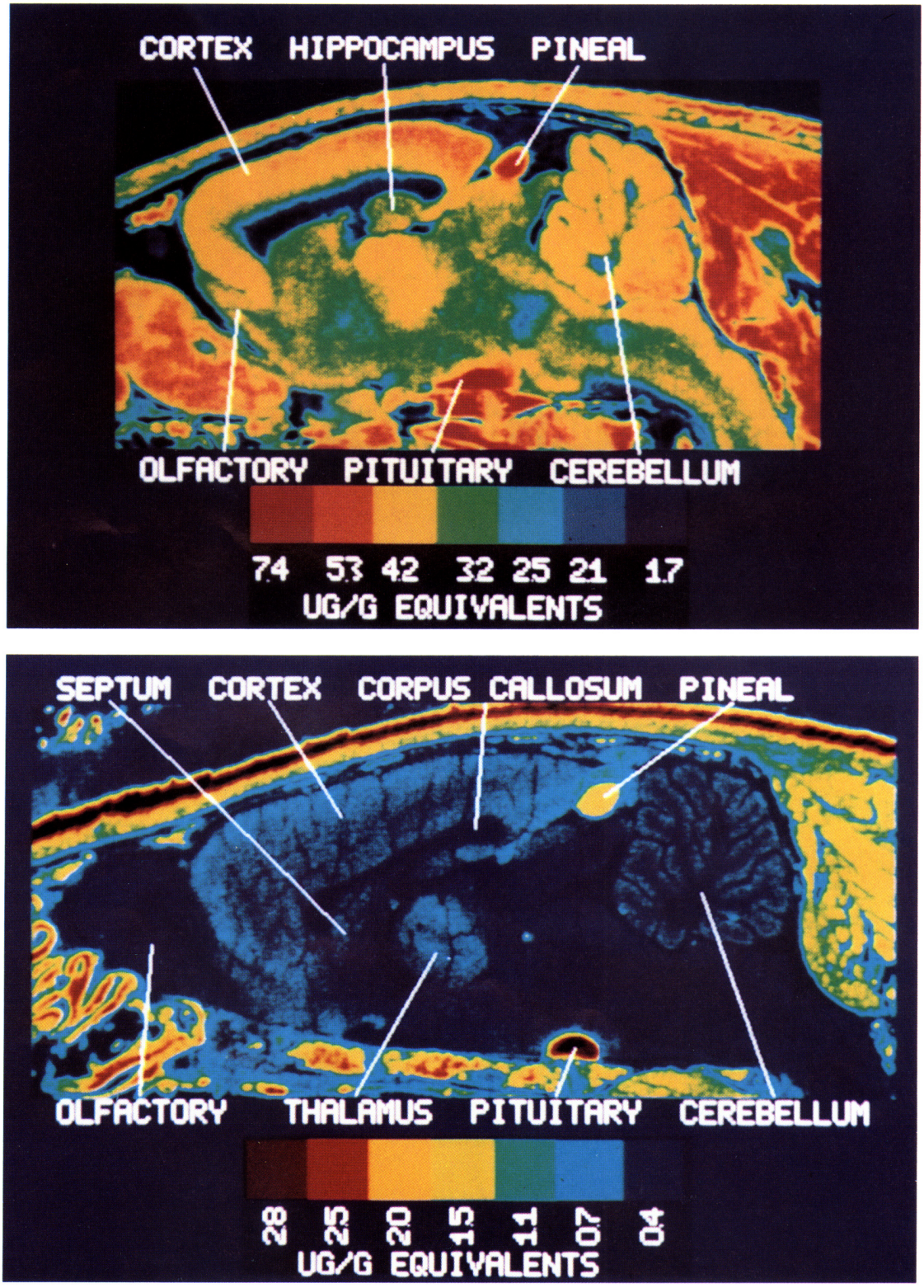

Plate 2. ^{14}C-Tacrine distribution in brain. Top: 30 min post oral dose. Bottom: 12 hr post oral dose.

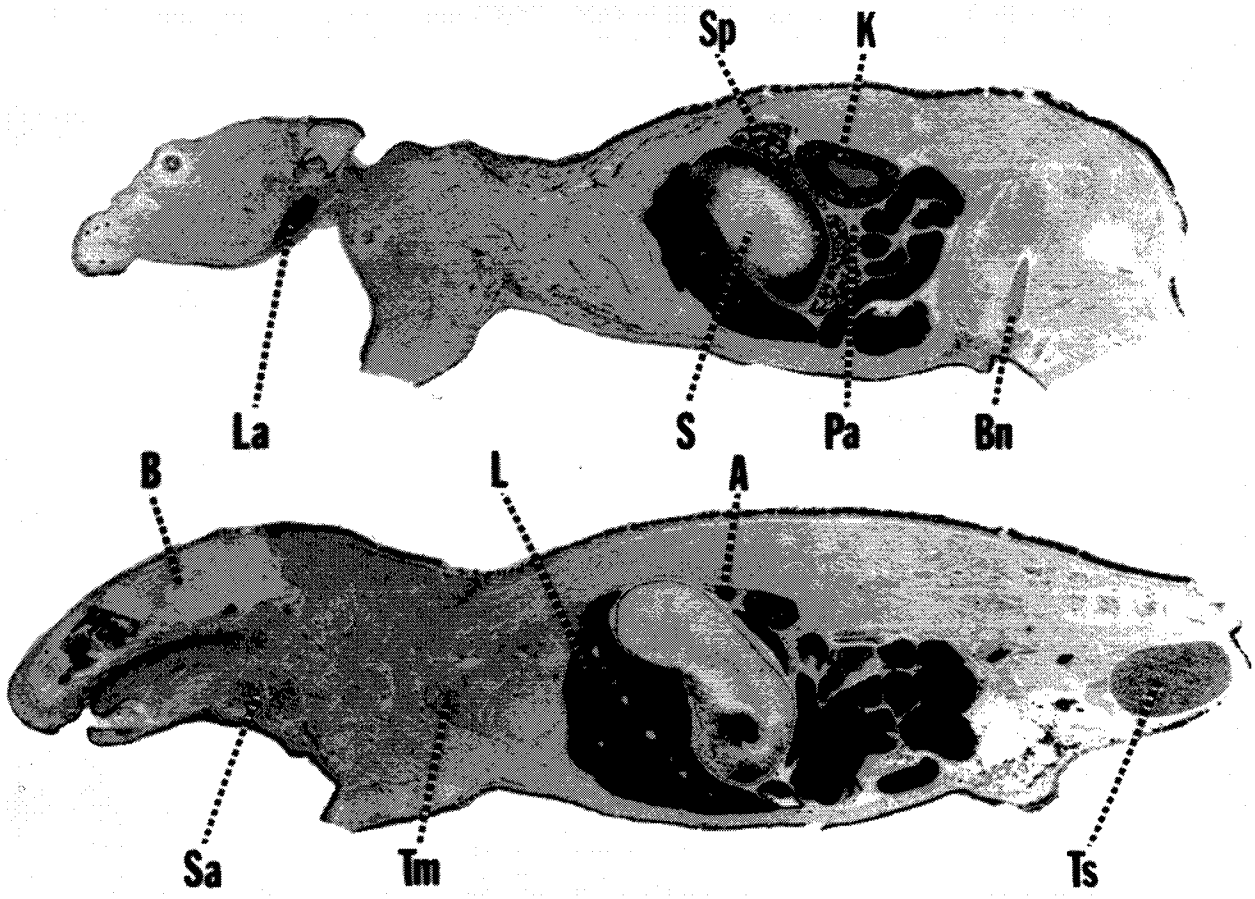

Fig. 2. ^{14}C-Tacrine autoradiograms, 12 hr post iv dose. Twelve-week autoradiographic exposure.

glands followed by cortex, thalamus, cerebellar gray, and striatum. Layers in hippocampus were well defined: activity in layers with higher levels was apparently equal to that in cortex.

Table III. ^{14}C-Tacrine Radioequivalents in Tissue Following Oral Dose (μg equivalents/g)[a]

Tissue	Hours postdose			
	0.5	4	8	12
Kidney	17.46	22.04	5.21	5.33
Liver	17.84	16.81	6.58	7.96
Pancreas	12.72	12.02	3.44	3.42
Lacrymal	10.34	10.69	4.60	4.68
Lacrymal-exorb.	13.30	13.99	5.40	3.64
Adrenal	14.01	17.15	6.61	8.04
Salivary	11.02	11.19	4.04	3.64
Marrow	5.84	11.93	2.60	2.51
Spleen	9.25	14.51	4.55	4.50
Lymph node	5.41	8.91	1.67	1.51
Thymus	4.03	12.20	2.37	2.22
Muscle	3.56	7.67	1.89	1.64
Harder's gland	4.90	4.79	1.27	1.23
Heart	5.05	6.01	1.69	1.78
Lung	4.29	5.63	1.35	1.43
Testis	1.06	9.22	2.85	2.69
Brown fat	4.70	5.44	1.42	1.72
Blood	1.86	2.66	1.06	1.07

[a] Mean, two animals.

Activity increased in most tissues at 4 hr postdose, although the increase in salivary glands was minor and there was a slight decline in liver, pancreas, and Harder's gland. In marrow, thymus, muscle, and testis the increase was greater than 100%. Highest concentrations remained in stomach and intestinal contents and in bladder. Preputial gland, hair follicles, and adrenal medulla were the most highly labeled tissues, along with kidney medulla. In spleen there was slightly greater uptake in red than in white pulp, and in thymus the cortical tissue concentrated more activity than medulla. Uptake in epididymis was slightly higher than that in testis, where activity was apparently localized to interstitium. There was evidence of higher uptake in pancreatic islets, as was seen in the iv-dosed animals. Mucosa of stomach and intestinal tract was highly labeled and there was considerable uptake in the mucosa of esophagus. Thyroid gland concentrated slightly greater activity than muscle. In the eye, uptake in choroid/retina was apparently lower than seen earlier, but distinct label could be detected in the periphery of lens. The increase in activity of cortex, hippocampus, striatum, thalamus, and olfactory nucleus in brain followed that in blood. Selective uptake to layers in hippocampus remained apparent.

After reaching apparent peak values at 4 hr, radioactivity in tissues declined at 8 hr postdose, in most cases to levels below those measured at 30 min, although relative distribution was similar at the later time points. At 12 hr postdose (Fig. 4) levels in most tissues remained about equal to or moderately declined from the 8-hr levels, although the

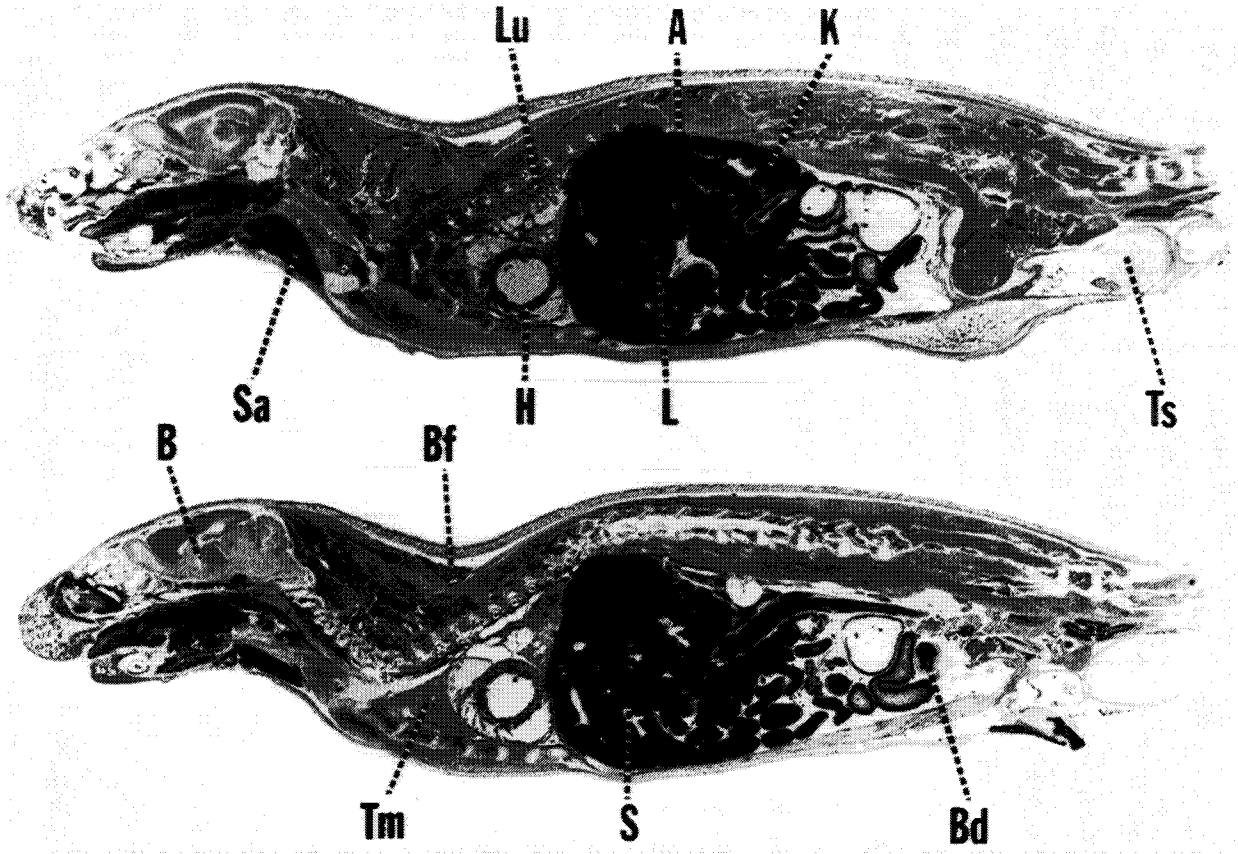

Fig. 3. ^{14}C-Tacrine autoradiograms, 30 min post oral dose. One-week autoradiographic exposure.

disparity in tissue concentrations seen in the two 8-hr animals made accurate assessment of decline difficult. At 12 hr, activity was extensively distributed in intestinal contents, and high levels were present in stomach, kidney medulla, ureters, and bladder. Hair follicles, preputial gland, adrenal medulla, and vomeronasal organ were among the most highly labeled tissues, and notable levels remained in glandular stomach mucosa and nasal turbinates. Activity in brain structures declined moderately between 8 and 12 hr, after a more acute decrease from 4 hr. The decline in brain was more rapid than in blood, and at 12 hr activity in most brain structures was lower than that of blood. Activity remained concentrated primarily in gray matter structures (Plate 2, bottom), although the well-defined distribution to the hippocampal layers seen at earlier time points was not as distinct.

Table IV. ^{14}C-Tacrine Radioequivalents in Brain Following Oral Dose (μg equivalents/g)[a]

Tissue	Hours postdose			
	0.5	4	8	12
Cortex	3.56	4.77	0.99	0.82
Hippocampus	2.94	4.72	0.97	0.75
Thalamus	3.50	4.51	1.01	0.73
Striatum	3.11	4.11	0.90	0.71
Cerebellum	3.15	3.56	0.71	0.57
Olfactory bulb	2.55	3.49	0.60	0.53
Colliculus	2.96	3.31	0.61	0.48
Brain stem	2.50	2.80	0.47	0.41
Pituitary	10.53	20.32	5.60	6.19

[a] Mean, two animals.

DISCUSSION

Tacrine radioequivalents were extensively distributed to tissues following a single iv dose. Clearance of radioactivity from tissues was more rapid between 30 min and 4 hr than between 4 and 12 hr, where activity declined in an apparent first-order process. Apparent elimination half-life in tissue ranged from 2.2 to 5.2 hr and was similar to that of blood, although in brain structures apparent elimination half-life was somewhat shorter. In orally dosed animals activity was also well distributed to tissues, where maximum levels were measured at 4 hr postdose. Activity dropped after 4 hr, but the rate of decline between 8 and 12 hr in most tissues did not appear to be as rapid. Tissue half-lives were longer for the orally dosed animals but interanimal variations in absorption make comparisons approximate. Nevertheless, these observations may reflect extended absorption of tacrine from the intestinal tract.

The high levels in kidney, ureters, and bladder seen at all time points in both treatment groups indicate that urinary excretion is a major route of elimination of tacrine, consistent with data from dogs, where 52 to 82% of administered radioactivity was recovered in urine (11). There is also biliary secretion indicated by the activity seen in bile ducts and

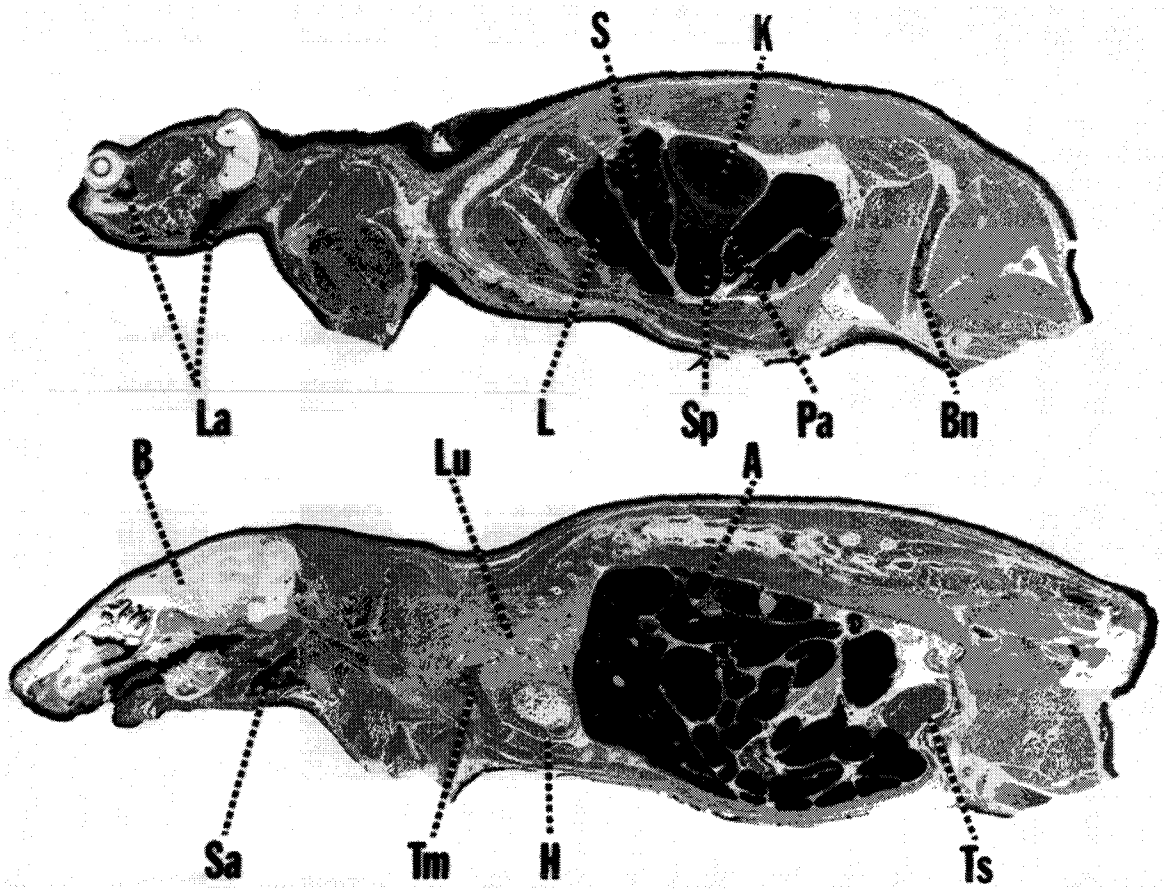

Fig. 4. ^{14}C-Tacrine autoradiograms, 12 hr post oral dose. Two-week autoradiographic exposure.

the appearance of label in the intestinal lumen following iv dose. Uptake was noted in the mucosa of the stomach and intestines in all the animals studied. Based on this observation, it is possible that radioactivity appearing in the GI tract results in part from direct secretion of drug or metabolites by the mucosa. Activity seen in the esophagus and oral cavity at all time points in the orally dosed animals and after 4 hr in the intravenously treated group, may have resulted from grooming behavior, especially in light of the high levels seen in urine. It is possible, however, that some of this distribution is due to salivary, lacrymal or nasal gland secretions, as all of these organs demonstrated relatively high activity, particularly at early time points.

Distribution of tacrine in brain was of particular interest since the compound has been shown, in the report by Summers *et al.* (10), to moderate the cognitive deficits associated with senile dementia. Liston *et al.* (14) have reported that at 10–120 min following intraperitoneal injection of 3.2 mg/kg to mice, brain concentrations of tacrine were 10 times higher than plasma levels and were well above the IC_{50} for AChE inhibition (220 n*M*). In the present study, tacrine radioequivalents readily penetrated the blood–brain barrier and concentrated in selective areas of gray matter, and while the brain/blood ratio was not as high as cited above, concentrations in several brain regions, notably in cortex, hippocampus, thalamus, and striatum, were also above the IC_{50} level. Higher uptake in pituitary and pineal glands probably reflects blood supplies outside the barrier. Although tacrine has been shown to be a potent inhibitor of acetylcholinesterase, the localization of tacrine radioequivalents in brain did not compare quantitatively with those areas which have been shown to be high in AChE by histologic or biochemical determination (15,16). Caudate-putamen, for instance, has been shown to have high levels of AChE, but while this area of the striatum was among the more highly labeled structures seen in these studies, the level of uptake was lower than might be expected based on reported concentration of enzyme. On the other hand, globus pallidus region of striatum has a lower enzyme level, which was reflected by lesser uptake and retention of radioactivity seen in autoradiograms. Uptake of tacrine radioequivalents in the cerebral cortex was similar to that in caudate-putamen, although enzyme levels in the cortex are considerably lower. Thus in the brain, neither a quantitative nor a consistently qualitative relationship appears to apply to the distribution of tacrine equivalents with respect to acetylcholinesterase. However, relatively high activity seen in cortex, hippocampus, and anterior olfactory nucleus may contribute to the efficacy of the compound, as these areas are implicated in the cognitive and behavioral deficits seen in SDAT (17). Several studies suggest that tacrine may exert its pharmacological activity by other mechanisms in addition to AChE inhibition. Davis *et al.* (18) have shown that tacrine demonstrates weak muscarinic antagonist properties, in contrast to other AChE inhibitors, and has little effect on local cerebral blood flow. A number of reports have shown that tacrine blocks potassium

channels in *in vitro* systems (19,20). Drukarch *et al.* (21) suggested that tacrine may stimulate the release of neurotransmitters by blocking slow K^+ channels, although this blockage occurred with concentrations in the micromolar range, which may not be achievable with pharmacological doses *in vivo*. In a later study this same group showed stimulation of release of norepinephrine and serotonin in cortex, and dopamine from neostriatum, and suggested that effects on monoamine neurotransmitters may contribute to the therapeutic activity of the compound in SDAT patients (22). The lack of strong correlation between tacrine distribution and AChE seen in the present studies supports to some extent the possibility of alternate mechanisms for the pharmacological effects of tacrine.

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