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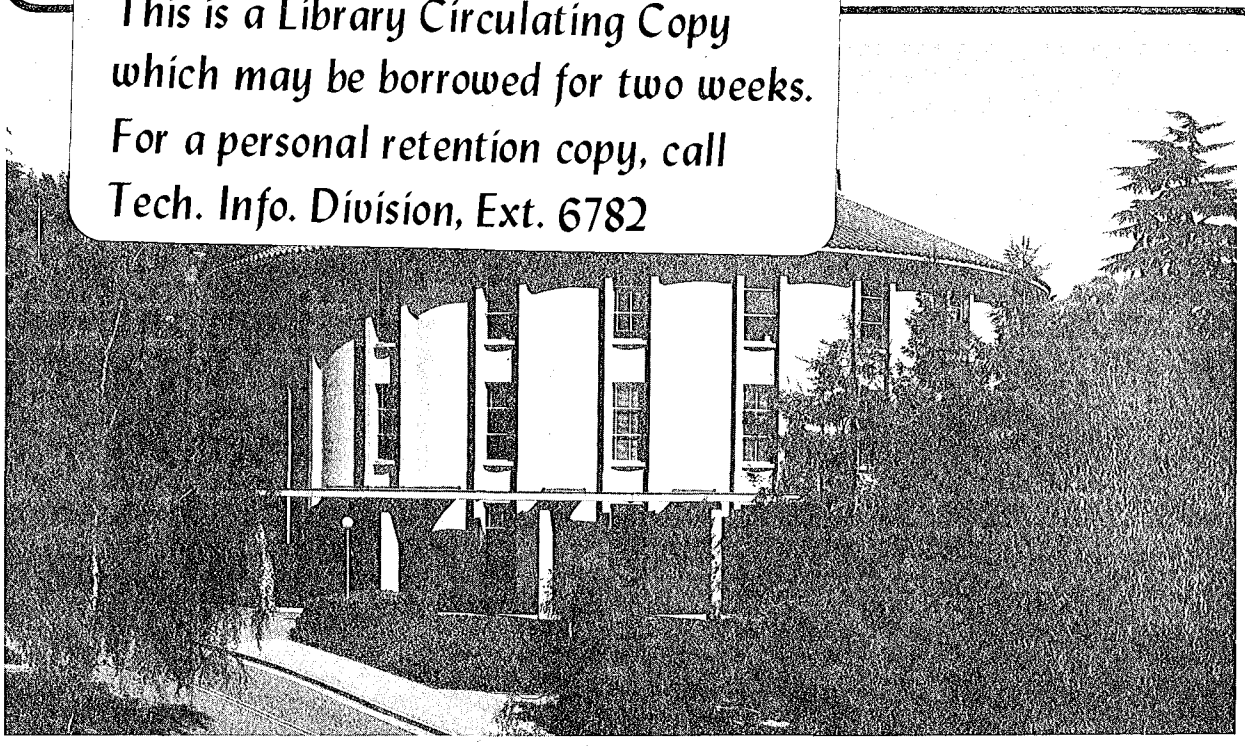

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UPTAKE OF [^{3}H]-COLCHICINE INTO BRAIN AND LIVER OF MOUSE, RAT, AND CHICK

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BENNETT, E.L., M.H. ALBERTI, AND J. F. FLOOD. Uptake of [^{3}H]-colchicine into brain and liver of mouse, rat, and chick. PHARMAC. BIOCHEM. BEHAV. xx, xxx-xxx (1980). - The uptake of [ring A-4-^{3}H] colchicine and [ring C-methoxy-^{3}H] colchicine has been compared in mice from 1 to 24 hr after administration. Less radioactivity was found in brain after administration of [ring A-4-^{3}H] colchicine than after administration of the methoxy-labeled colchicine. Three hr after administration of ring-labeled colchicine, 5% of the label was in liver and about 0.01% of the label was present in brain. Forty percent of the brain radioactivity was precipitated by vinblastine. The amount of colchicine entering mouse brain after subcutaneous injection is comparable to the minimum behaviorally effective dose when administered to the caudate. The metabolism of [ring C-methoxy-^{3}H]- and [ring A-^{3}H]-colchicine was also studied in rats. The general pattern was similar to mice; less radioactivity was found in brain after administration of the ring-labeled alkaloid than after administration of methoxy-labeled colchicine. Again, 40-50% of ring-labelled colchicine was precipitated by vinblastine. These experiments, together with behavioral experiments (6), support the hypotheses that structural alterations in synapses by recently synthesized proteins which are transported down the axons and dendrites may be an essential process for long-term memory formation.

Key words: Colchicine; tubulin, mice, rat, chick; [ring-labeled] colchicine; [methoxy-labeled] colchicine; colchicine, uptake in brain; colchicine, uptake in blood; colchicine and memory; axonal transport and memory.

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INTRODUCTION

Substantial evidence has been obtained that protein synthesis is essential for long-term memory formation (for reviews see 5,7). It has been suggested by Cronly-Dillon et al. (3) that microtubules may be involved in fast transport of the necessary neurochemicals from the cell body to the synaptic endings to bring about the local changes in membrane structure or cell morphology that result from learning and which are presumed necessary to establish long-term memory. Cronly-Dillon et al. showed that colchicine administered intracranially to goldfish impaired long-term memory formation. Crothers and McCluer demonstrated that intracranially administered colchicine impaired the axoplasmic transport of proteins in mice (4). The behavioral experiments, reported by Flood et al. in a companion paper (6) to this, were initiated after Rose and Sinha (14) reported that colchicine administered intraperitoneally to rats at a dose of 40 μg/kg impaired the accumulation of radioactively-labeled protein into synaptosomes.

The initial purpose of the biochemical experiments reported here was to estimate the amount of colchicine entering the mouse brain after its subcutaneous administration under the conditions of the behavioral experiments described by Flood et al. (6). During the course of these experiments, it became apparent that significantly less radioactivity entered the mouse brain than had been anticipated based upon the reports of Stewart and Rose (18) from experiments using rats. Subsequent experiments were then performed in rats, mice, and chicks to compare the effects of (a) label position and (b) species upon apparent uptake of colchicine. In addition, the tubulin content of mouse brain was determined by colchicine binding assays, and the ratio of colchicine uptake to tubulin was estimated. While only a small quantity of colchicine enters the brain of mice or rats, the amounts are

comparable with the dose shown to be behaviorally effective. (6).

GENERAL DESCRIPTION OF PROCEDURE

Animals and Reagents

Swiss-Webster male mice from Charles River Breeding Laboratories, Wilmington, MA were obtained at 6 weeks of age and maintained in our colony until 50-70 days of age, at which time they weighed 30-35 g. Wag-rij male rats in Experiments 1 and 12 were obtained from the Lawrence Berkeley Laboratory colony and were approximately 70 to 90 days of age. For Experiments 13 and 17, Wistar (Lewis) rats obtained from Charles River Breeding Lab were used. Animals were maintained on a 12 hr light-12 hr dark cycle and were housed singly for 2-3 days prior to the experiment. Fertilized White Leghorn chicken eggs were obtained from a local hatchery, and the chicks were hatched in the laboratory and were maintained in the incubator room at 37°C until sacrificed.

[Ring A-4-^{3}H] colchicine (in ethanol) was obtained from Amersham Corp. The specific activity of the three batches used in these experiments was 7.7 Ci/mmole. The colchicine was stored in the dark at -10°C and appeared to be stable during the course of the experiments. [Ring C-methoxy-^{3}H] colchicine, (in 9:1 benzene-ethanol) was obtained from New England Nuclear (NEN) and Amersham (Am). The specific activities of the lots were 10.1 and 19.6 Ci/mmole (NEN) and 5.0 Ci/mmole (Am). Immediately prior to use, an appropriate aliquot of the radioactive colchicine solution was evaporated to dryness by a N_2 stream, and redissolved in sterile 0.9% NaCl. The radioactive colchicine was not diluted for the *in vivo* experiments described in this report. For *in vitro* studies to estimate the amount of tubulin in mouse brain, non-radioactive colchicine was added to obtain a final specific activity of approximately 0.04 Ci/mM.

Vinblastine sulfate was obtained from Sigma Chemical Co., St. Louis, MO and Sephadex G-100 from Pharmacia, Piscataway, NJ. Anisomycin (ANI) was purchased from Pfizer Diagnostics, Clifton, NJ; cycloheximide was obtained from Calbiochem-Behring Corp., La Jolla, CA. Colchicine (0.5 mg/ml) was obtained in sealed ampoules from Eli Lilly and Co., Indianapolis, IN. The rpi counting cocktail 3a70B was obtained from Research Products International Corp., Elk Grove Village, IL. Other chemicals, reagent grade, were obtained from usual suppliers.

EXPERIMENTAL PROCEDURE

To conform to the drug design used in the behavioral studies (6), ANI was administered subcutaneously to mice at a dosage of 20 mg/kg in Experiments 3-10. Seventy-five min later, radioactive colchicine was administered subcutaneously in 0.3 ml of 0.9% NaCl. In subsequent experiments, ANI was not administered; results were unchanged. The mice were anesthesized by ether; a blood sample was taken, and then the animal was perfused with normal saline prior to dissection of liver and brain. These two organs were weighed. Typically, two brains (0.4-0.5 g each) were combined and homogenized in 1.0 ml of PMG buffer (10 mM sodium phosphate with 0.1 mM GTP and 5 mM $MgCl_2$, pH 6.8) (16). The homogenate was centrifuged for 30 min at 20,000 RPM (50,000 x g) at 4°C in a Sorvall RC2B centrifuge, SM-24 head. The precipitate was resuspended in 1.0 ml PMG buffer, recentrifuged, and the supernatants combined (S_1). The combined volume of S_1 was typically 1.6-1.8 ml and it contained approximately 25% of the brain protein. Radioactivity was determined on two-200 µl aliquots of S_1. The residual brain was homogenized in 9 ml of H_2O and 0.6 to 1.2 ml aliquots were counted.

To determine the vinblastine precipitable radioactivity, duplicate

200 µl aliquots of S_1 were incubated in a shaking water bath at 37°C with 0.5 mg vinblastine (final concentration 2.5 x 10^{-3}M) for 30 min and then centrifuged at 50,000xg for 30 min at 25°C (16). Radioactivity was determined in both the supernatant and the vinblastine precipitate of tubulin which was rinsed with 200 µl of H_2O (40°). To determine the trichloroacetic acid (TCA) precipitable radioactivity, 200 µl cold 12% TCA was added to an equal volume of S_1, and kept in ice for at least 30 min. The precipitate was sedimented in a clinical centrifuge, resuspended in 600 µl cold 6% TCA, and recentrifuged. Radioactivity was determined in supernatant, wash, and precipitate.

The biochemical procedures used for the rat experiments were similar to those used for mice, except that one rat was used for each experiment, no ANI was given, and colchicine was injected intraperitonally rather than subcutaneously to follow the procedure of Stewart and Rose (18). The volumes used to process a rat brain weighing approximately 2.0 g were twice those used for the mice. Chicks were administered 22 nM of [ring-A-4-^{3}H] colchicine in 50 µl of saline in the heart region following the general description of Cronly-Dillon, et al. (3). The chicks were sacrificed 1 or 3 hrs after administration of colchicine, and individual brains (800-900 mg) were analyzed by the same biochemical procedure as described for mouse brains.

Livers were homogenized at a concentration of 100 mg/ml in H_2O; 100 µl aliquots were counted in a gelled scintillator solution without further processing. Blood samples (10 µl) were also directly counted; it was assumed that the total blood volume of a mouse is 78 ml/kg (21), a rat 58 ml/kg (8) and chick 2.0 ml.

Tubulin Content of Brain

The tubulin content of mouse brain was determined by gel filtration following described procedures (16,20). The 50,000 x g S_1 supernatant was prepared as described above using homogenate concentrations ranging from 0.05 g brain/ml to 0.5 g/ml. The supernatant was incubated at 37°C for 60 min with 50 x 10^{-6} M colchicine, sp. act. 88,000dpm/nM. One ml of this incubation mixture was chromatographed thru a Sephadex G100 column, 1 x 9 cm, using 10 mM sodium phosphate-10 mM $MgCl_2$ buffer, pH 6.8. One ml aliquots were collected. Total radioactivity, vinblastine-precipitable radioactivity, and total protein (determined by the Lowry procedure with bovine serum albumin as standard), were determined on aliquots.

Determination of Radioactivity

Radioactivity was determined with a Packard Model 3385 scintillation counter using mini-vials. Counting efficiency, typically 30-40%, was determined from the AES ratio and checked by internal standards. The counting mixture normally contained 100-500 µl of sample, 1.2 ml of H_2O, and 3 ml of rpi complete counting cocktail 3a70B.

In each experiment, the corresponding tissue from a pair of mice, a rat, or a chick which had not been injected with radioactive colchicine was fractionated by the described procedure, and appropriate aliquots were used for background corrections. This was particularly important for brain samples, especially those from rat, since total counts were low and the blank correction was a significant fraction of the total counts.

RESULTS

The main objective of the first series of experiments was to determine the amount of colchicine entering mouse brain under the conditions of our behavioral experiments. A dose of about 4 nM/mouse was chosen because this approximated the behaviorally effective dose. Mice were sacrificed 3 hr after administration of ring-[^{3}H]-labeled colchicine since the behavioral experiments suggested that this period was critical for determining long-term memory formation in these experiments. The mice were administered ANI, but other experiments indicated that ANI had little effect on the uptake of ring-labeled colchicine.

The results, summarized in Table 1, showed that while substantial quantities of radioactivity were present in liver, and moderate quantities in blood, less than 0.01% of the administered radioactivity (0.4 picomoles equivalent colchicine) was found in brain. About 60% of this radioactivity was found in the S_1 supernatant, and 40% remained in the once-washed precipitate.

Approximately 40% of the radioactivity, equivalent to 0.14 pm of colchicine, was precipitated with tubulin by 2 x 10^{-3} M vinblastine. Approximately an equivalent amount of radioactivity (40-50%) was found in samples of brain supernatant dried in an N_2-stream. Little or no radioactivity was precipitated by 6% trichloracetic acid (Note that 4% of the brain supernatant activity is equivalent to about 200 dpm after administration of 30 µCi of colchicine). Little radioactivity is lost from brain between 3 and 24 hrs after administration of labeled colchicine (Tables 1 and 2).

In light of the report (18), indicating that much larger amounts of colchicine were found in rat brain after its administration, subsequent

experiments were designed to resolve the discrepancies between that report and our results. In the first of these experiments [ring C-methoxy-^{3}H] colchicine was used instead of ring A-labeled colchicine. Using [ring C-methoxy-^{3}H] colchicine, the proportions of the injected radioactivity retained in blood, liver, and brain of mice were all much higher than had been found with ring A-labeled colchicine (Table 2). However, the proportion (5%) of the radioactivity precipitated with the tubulin by vinblastine was approximately one-eighth that precipitated after administration of ring A-labeled colchicine. A similar small fraction of the radioactivity remained in N_2-dried samples of the brain supernatant. The best estimate of colchicine equivalent in mouse brain (normalized to a 4.0 nM dose) was approximately 0.30 pmoles in these experiments with methoxy-labeled colchicine. This is in good agreement with the estimate of 0.14 pmoles obtained from the first series of experiments. In addition, Experiment 10 M, where the dosage was increased by a factor of 8 to 12, showed that the uptake in brain was not saturated over this dose range, but was, instead, proportional to dose. Again, in contrast to the report of Stewart and Rose (18) essentially none of the radioactivity was precipitable by TCA.

The next series of experiments compared the uptake of both ring and methoxy-labeled colchicine in the rat at several time intervals (Table 3). Even a smaller proportion of the injected radioactivity was found in rat brain by 3 hr after administration than had been found with mice. Little, if any, of the radioactivity was precipitable by trichloroacetic acid. As with the mouse, more radioactivity was found in brain after the administration of methoxy-labeled colchicine than after the administration of ring-labeled material. Again, a smaller proportion of the methoxy-labeled drug was precipitated by vinblastine in the tubulin fraction than when ring

A-labeled material was used. In rat, the small amount of vinblastine-precipitable colchicine found in brain at any time precluded a determination of the time dependence of uptake.

The tubulin content of mouse brain was estimated to be a minimum of 3.4 mg/gm (uncorrected for decay) based on the bound radioactive colchicine precipitable after chromatography on a Sephadex column. This is equivalent to approximately 15 nM of tubulin/mouse brain or approximately 3% of the total brain protein. This concentration is somewhat less than that reported by Sherline, et al. (17) for rat brain, but is approximately twice that reported for rat occipital cortex. (10)

Inasmuch as Cherfas and Bateson (2) have reported behavioral effects of colchicine in young chicks, a third series of experiments was carried out to provide data concerning colchicine uptake into the young chick brain. In spite of administration directly into (or near) the heart, and the young age of the chick, the amount present in brain (Table 4) was similar to that present in the mouse.

DISCUSSION

The behavioral exeriments described by Flood et al. (6) were initiated after a report appeared showing that intraperitoneally administered colchicine can cross the blood-brain barrier of rats and impair axonal transport of newly synthesized protein (14). The biochemical experiments were carried out to address the question of colchicine uptake into mouse brain under conditions of the behavioral experiments.

On the basis of a limited number of studies, it is generally accepted that the blood-brain barrier completely excludes colchicine from the central nervous system. In an early study, Back et al. (1) used [^{14}C] colchicine and concluded "that labeled colchicine was not present in blood, brain, muscle,

and heart" of mice. However, the limitation of the low specific activity (27 uCi/g) of the colchicine should be borne in mind in evaluating their results. Subsequently, Hunter and Klaassen (9) studied the metabolism of [^{3}H] colchicine in a variety of species including rat. The majority of the administered colchicine was excreted in the bile, and a lesser amount was excreted in the urine. The investigators reported that the concentration of tritium in brain was the lowest of all tissues sampled, and was 1% of that of the liver 20 min after intravenous administration of colchicine. We found that the maximum quantity of [ring A-4-^{3}H] colchicine in mouse brain 3 hr after administration was about 0.01% of that administered. Approximately 40-50% of the radioactive material in brain was vinblastine precipitable, presumably bound to tubulin.

Stewart and Rose (18) found that a minimum of 0.1% of intraperitoneally administered [ring C-methoxy-^{3}H]-colchicine was present in rat cortex.* They reported that radioactivity in brain was largely precipitated by 10^{-2}M vinblastine or 6% TCA.

Our studies used both ring- and methoxy-labeled colchicine. The uptake of [ring-4-^{3}H] colchicine or [ring C-methoxy-^{3}H]colchicine in rat brain was substantially less than that in the mouse; much less than 0.01% of the administered dose was found in brain either 3 or 24 hr after administration. Thus, when 60 μCi of methoxy-labelled colchicine was injected, less than 20,000 dpm was found in brain. In the experiment using ring-labelled

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* These authors did not indicate the labeling position of the colchicine; however S.P.R. Rose affirmed that methoxy-labeled colchicine was used in their studies. (Personal communication)

colchicine, 120 μCi of colchicine was administered and typically less than 8,000 dpm was found in the brain homogenate. In experiment 1 R, 1.1 nM (8 μCi) of ring-labelled colchicine was administered, and less than 2000 dpm was detected in the brain. It thus appears unlikely that the low percentage uptake in our experiments is due to saturation. However, the apparent uptake of colchicine was somewhat influenced by the label position; again, more uptake was found when the methoxy-label colchicine was used. Up to 20 times as much radioactivity was found in mouse brain after administration of methoxy-labeled colchicine than after administration of ring-labeled drug. Schönharting, et al. (15) showed that rat and mouse liver microsomes demethylate colchicine. Hunter and Klaassen (9) showed that significant quantities of demethylated derivatives of colchicine are present in bile and urine of rats after administration of [^{14}C]-colchicine. Therefore, we suggest that much of the radioactivity in brain after administration of ring-C-methoxy colchicine may be derived from this liberated methyl group and may include $^{3}H_2O$. This suggestion is supported by the observation that the non-volatile radioactivity in the brain homogenate supernatant corresponded closely with vinblastine-precipitable radioactivity in the same fraction.

A significant fraction of the radioactivity in the brain supernatant after administration of ring-labeled colchicine was precipitated by vinblastine, but little or no radioactivity was precipitated by TCA. Furthermore, in our hands, [^{3}H] colchicine bound to tubulin in vitro by standard procedures (16) was dissociated from tubulin by TCA, but was precipitated as expected, by 10^{-3}M vinblastine.

Knowledge of the maximum amount of colchicine (0.14 pM, average concentration, 0.3×10^{-9}M) present in mouse brain after administration of 4 nM of colchicine combined with an estimate of the tubulin content of mouse

brain (7.5 nM) permits an estimate of the average colchicine/tubulin ratio after subcutaneous administration which has been demonstrated to be effective in the behavioral experiments of Flood et al, (6). This ratio is approximately 10^{-5}. However, much of the tubulin is present as microtubules in the brain. It is generally believed that colchicine interfere with microtubule formation by first binding to free tubulin. The tubulin-colchicine molecules subsequently are bound to the growing ends of microtubules and impair continued growth. In this way, relatively small concentrations of colchicine, resulting in colchicine-tubulin ratios, which are several orders of magnitude less than unity, can impair biological processes. The total amount of colchicine present is also similar to that used in the behavioral studies of Flood et al using intracerebral injections of colchicine. Crothers and McCluer have shown that colchicine remains localized near the site of injection (4).

Extremely low concentrations of colchicine interfere with numerous biological processes that involve tubulin and microtubules. Olmsted and Borisy (12) estimated that a colchicine to tubulin binding ratio of 1/25 will completely inhibit polymerization. Owellen et al, (13) calculated that colchicine inhibited tubulin polymerization by 50% as measured by viscosity at a molar concentration of 5×10^{-7} and a colchicine to tubulin ratio of about 1/250. Margolis et al. (11) showed recently that 1.3×10^{-7}M colchicine inhibits microtubule assembly under steady-state conditions in vitro by 50%. In these experiments, the colchicine to tubulin ratio was approximately 1 to 50. Vinblastine, an effective amnestic agent in the behavioral experiments of Flood et al., inhibits the assembly-disassembly reaction of tubulin at even lower concentrations (12,13,20). One striking example of a biological action at an extremely low concentration is the

demonstration by Taylor (19) that mitosis of human cells in tissue culture is completely arrested by a colchicine concentration of 5×10^{-8}M. Thus the observed effects of colchicine on memory formation in mice (6) are not ruled out by the low uptake of colchicine into brain as measured in the present series of experiments.

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Table 1

Radioactivity in Blood, Liver and Brain of Mice

After s. c. Administration of [Ring A-4-^{3}H] Colchicine

Expt. #	nM Adm	% in Blood*	% in Liver	% in Brain	% of Brain Supernatant (S_1) TCA Insoluble	% of Brain Supernatant (S_1) Vinblastine Precipitable	Colchicine Equivalent in Brain** (picomoles)
			1 Hr				
14R	3.6	.68%	5%	.009%	2%	44%	.16
15R	4.0	1.19	7	.007	4	42	.12
Average 1 hr		.94%	6%	.008%	3%	43%	.14
			3 Hr				
3R	5.4	.18%	5%	.009%	-	-	-
4R	3.6	.12	3	.009	-	54	.19
5R	3.9	.36	6	.010	4	37	.15
6R	3.9	.12	5	.007	3	54	.15
7R	4.2	.53	9	.008	4	32	.10
8R	3.5	.27	7	.011	3	35	.15
15R	4.0	.23	2	.010	1	26	.11
Average 3 hr		.26%	5%	.009%	3%	40%	.14
			24 Hr				
15R	4.0	.22%	0.3%	.012%	1%	10%	.05

Table 1 (cont'd)

*Assumed blood volume 78 ml/kg mouse. Liver averaged 2 g. Body weights were 34 ± 3 g.

**Colchicine equivalent calculated from percentage of brain supernatant precipitated by 10^{-3}M vinblastine multiplied by total radioactivity in brain normalized to a 4.0 nM dose of colchicine.

Table 2

Radioactivity in Blood, Liver and Brain of Mice

After s.c. Administration of [Ring C-methoxy-^{3}H] Colchicine

Expt #.	nM Adm.	% in Blood*	% in Liver	% in Brain	% of Brain Supernatant		Colchicine Equivalent in Brain** (picomoles)
					TCA Insoluble	Vinblastine Precipitable	
			1 Hr				
14 NEN-M***	12.0	1.75%	10%	.045%	1%	9%	.16
15 Am-M	4.0	2.11	10	.071	-	5	.14
Average 1 hr		1.9%	10%	.058%	-	7%	.15
			3 Hr				
8 NEN-M	5.8	2.26%	13%	.20%	1%	3%	.24
9 NEN-M	1.5	1.62	8	.17	1	5	.34
10 NEN-M	48.0	2.60	12	.29	1	5	.58
15 Am-M	4.0	1.01	4	.09	1	6	.20
16 NEN-M	0.8	0.82	6	.12	2	5	.26
16 Am-M	2.9	0.63	5	.08	1	6	.20
Average 3 hr		1.49%	8%	.16%	1%	5%	.30
			24 Hr				
15 Am-M	4.0	0.99%	0.7%	.09%	1%	4%	.15
16 NEN-M	0.8	0.65	2	.10	1	10	.41
16 Am-M	2.9	0.41	2	.10	2	10	.42
Average 24 hr		0.68%	2%	.10%	1%	8%	.33

Table 2 (cont'd)

* Assumed blood volume, 78 ml/kg mouse. Liver averaged 2 g. Body weights were 34 $\pm$ 3 g.

** Colchicine equivalent calculated from percentage brain supernatant precipitated by 10^{-3}M vinblastine times the total radioactivity in brain, normalized to a 4.0 mM dose of colchicine.

*** NEN indicates colchicine obtained from New England Nuclear Corp; Am signifies Amersham was the supplier.

Table 3

Radioactivity in Blood, Liver and Brain of Rats

After ip Administration of Colchicine

Expt. #	nM Adm	% in Blood*	% in Liver	% in Brain	% of Brain Supernatant: TCA Insoluble	% of Brain Supernatant: Vinblastine Precipitable	Colchicine Equivalent in Brain** (picomoles)
			[Ring A-4-^{3}H]-Colchicine				
				1 Hr			
13 R	14.8	0.36%	0.8%	.0018%	5%	52%	.15
17 R	16.4	0.50	8.5	.0020	7	56	.18
				3 Hr			
1R	1.1	0.22%	2.1%	.01%	-	-	-
12R	17.8	0.16%	5.4	.0062%	4%	4%	.04
17R	16.4	0.23	2.6	.0024	4	40	.15
				24 Hr			
13R	14.8	0.13%	0.08%	.0030%	1%	3%	<.02
17R	16.4	0.13	0.02	.0006	1	<10	<.02
			[Ring C-methoxy-^{3}H]-Colchicine				
				1 Hr			
13 Am-M	16.8	[0.07%]	0.4%	.0017%	0%	11%	.03
17 Am-M	12.1	0.30	0.4	.0096	1	8	.12
				3 Hr			
12 NEN-M	15.5	0.30%	5.0%	.0236%	3%	5%	.20
13 Am-M	16.8	0.18	1.1	.0020	5	18	.06
17 Am-M	12.1	0.33	1.0	.0071	4	12	.14
				24 Hr			
13 Am-M	16.8	0.20%	0.1%	.0052%	1%	3%	.02
17 Am-M	12.1	0.47	0.5	.0128	3	7	.14

*Assumed blood volume, 58 ml/kg rat. For Expt. 1, Wag-rij rat weighed 207 g, liver weighed 8 g. For Expt. 12, rats weighed 370 g and had 22 g livers. For Expt. 13, Wistar rats weighed 290 g, livers 15 g. For Expt. 17, rats weighed 220 g, livers 9 g.

Table 3 (cont'd)

**Colchicine equivalent calculated from percentage of brain supernatant precipitated by 10^{-3}M vinblastine multiplied by total radioactivity in brain normalized to a 16.0 nM dose of colchicine.

Table 4

Radioactivity in Blood and Brain of Chicks

after i.p. Administration of Colchicine*

Age of chick	Duration of colchicine administration	% injected found in Blood	% injected found in Brain
20 hr	1 hr	0.9%	0.034%
20 hr	3 hr	0.15%	0.020
	3 hr	not done	0.027
10 hr	3 hr	0.5	0.064
	3 hr	0.3	0.030

* 22 nM [Ring-A-4-^{3}H] colchicine in 50 µl saline was injected into the heart region of chicks. One or three hrs later chicks were decapitated.

60%-70% of the radioactivity in brain was precipitated by vinblastine or co-chromatographed with tubulin peak on a G-100 Sephadex column. These values were measured on the approximately 50% of the radioactivity in brain that extracted into S_1 by our procedures.

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