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Metabolism of Tritium-Labeled Pyridoxine in Rats.* (27166)

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Earlier investigations on the metabolism of pyridoxine in animals have involved the study of degradation products by chemical methods and microbiological assay procedures (1-5). The present knowledge of the metabolism of Vit. B_6 (pyridoxine, pyridoxamine, and pyridoxal) in man and other animals, obtained by using these procedures, has been summarized by Snell(6). Low recoveries of ingested or injected pyridoxine in previous metabolic studies in animals have led to the speculation that a portion of the "missing" pyridoxine is excreted in conjugated forms as yet unidentified. An alternate hypothesis for the low recoveries would be that the ingested drug was stored in the tissues. This latter explanation was discounted by Rabinowitz and Snell(5) on the basis that no great storage of the vitamin would be expected in normal animals, and on the fact that excretion levels rapidly returned to base levels in human subjects. The present study of the turnover times and urinary and fecal excretion of H^3-pyridoxine was undertaken to investigate these hypotheses further.

Methods. Tritium- (H^3) labeled pyridoxine hydrochloride was obtained by exchange of pyridoxine borate in tritium water over platinum catalyst(7). Chemical and radioactive purity was established by paper chromatography. Specific activity of the H^3-pyridoxine hydrochloride was 308 μc/mg. The solution used for injection contained 2 mg (616 μc) of labeled and 18 mg of unlabeled pyridoxine hydrochloride/ml. All injections were intravenous *via* the jugular sinus route. The rats weighed between 250 and 350 g. Rat A received 184.8 μc H^3-pyridoxine · HCl. Rats B and C received 369.6 μc, while rats D through K received 616 μc H^3-pyridoxine · HCl. Following injection of the drug, the rats were placed individually in standard metabolism cages.

Rats A, B, and C had blood and urine samples taken at various time intervals post injection. Urine and fecal samples were collected from rats D to K for detailed urinary and fecal excretion and chromatographic studies.

Blood and urine specimens were measured for tritium using the liquid scintillation-dioxane system as described previously by Richmond *et al.*(8). The feces were homogenized in distilled water, decolorized with charcoal, and counted as above. The counting efficiency of each sample was determined with the use of an internal standard. All calculations were in absolute counting rates (dpm) and results expressed as % of injected dose.

All urine specimens obtained up to 5½ hours after injection were chromatographed by the descending method(9). One-tenth ml of urine was placed directly on No. 1 Whatman filter paper strips. In all instances, this was at least 5×10^6 dpm of H^3 activity. Eighty per cent propanol was the solvent system used. It was necessary to expose the chromatogram strips to X-ray film for at least 4 weeks to get satisfactory exposures on the film.

Results. Concentrations of H^3 activity in blood as a function of time after administration of labeled pyridoxine are shown in Fig. 1. The log of the % injected dose is plotted

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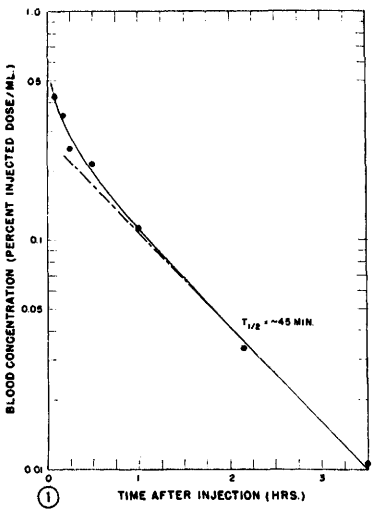

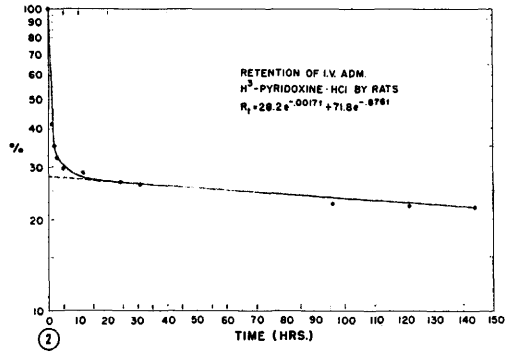

FIG. 1. Tritium activity in blood following intrav. administration of H³-pyridoxine hydrochloride in rats.

FIG. 2. Retention of H³-pyridoxine hydrochloride by rats following intrav. administration.

against time in hours. Excluding the early component of the blood clearance curve (*i.e.*, the first 15 minutes after injection), the turnover halftime of the activity in the blood was approximately 45 minutes during the first 3 hours after drug injection. Activity in the red blood cells was less than 1% of total activity in the blood.

The data in Tables I and II show accumulated urinary and fecal excretion, respectively. Seventy-five to 80% of the activity was excreted in the urine the first 24 hours. Average fecal excretion was negligible, being less than 0.03% in 96 hours.

Fig. 2 shows average retention of pyridoxine as a function of time after injection, assuming excretion was essentially all *via* the urine. The retention curve was fit by the following 2-component rate equation:

$$R_t = 28.2\ e^{-0.0017\ t} + 71.8\ e^{-0.876\ t}$$

in which R_t is retention in % of the injected dose, and t is time after injection in hours. Approximately 28% was retained with a half-time of 17 days and 72% with a half-time of only 47 minutes, corresponding to the blood clearance half-time of 45 minutes shown in Fig. 1.

One predominant band with R_f value of 0.65 ± 0.03 was present in all urine samples chromatographed. This R_f value corresponded to that of unchanged pyridoxine hydrochloride. A very faint trace of only one other band was present on all strips, R_f value of this unidentified band being 0.53 ± 0.03. The only degradation product of Vit. B_6 so far found in animals is 4-pyridoxic acid, a product which may be represented by this band.

Discussion. The urinary excretion results are in agreement with an earlier report(1) that 50-70% of pyridoxine was excreted essentially unchanged by rats within 24 hours, regardless of route of administration. Only one degradation product was found in very

TABLE I. Cumulative Urinary Excretion of Tritium Activity Following Intravenous Administration of H³-Pyridoxine • HCl in Rats.

Time after inj. (hr)	Rat No. & cumulative excretion (%) D	E	F	G	Avg
1.5	64.3	54.5	58.0	57.2	58.5
2.5	69.3	62.4	65.1	62.3	64.8
3.5	—	68.1	—	67.5	67.8
4.5	—	—	68.4	—	—
5.5	71.9	—	—	—	—
12	73.3	73.5	69.7	69.1	71.4
24	75.6	75.7	71.5	70.5	73.3
31	76.3	76.2	71.7	70.8	73.7
96	79.8	78.1	78.3	73.8	77.5
122	80.2	78.1	78.4	73.9	77.7
144	80.4	78.2	78.4	75.0	78.0

TABLE II. Cumulative Fecal Excretion of Tritium Activity Following Intravenous Administration of H³-Pyridoxine • HCl in Rats.

Time after inj. (hr)	Rat No. & cumulative excretion (%) H	I	J	K	Avg
12	<.001	<.001	—	<.001	<.001
24	.011	.020	.018	.008	.014
48	.016	.022	.023	.019	.020
72	.019	.027	.025	.022	.024
96	.028	.027	.026	.024	.026

small quantities. This was so for at least 5 hours after injection of the labeled material.

The majority of the pyridoxine (about 70%) was excreted unchanged with a half-time of about 45 minutes. There may be 2 reasons for rapid excretion of the unchanged drug: *in vivo* substrate concentrations of pyridoxine exceed the levels in which the tissue enzyme systems responsible for its oxidation can operate, and it may be a reflection of the inability of tissue fixation or conjugation. Retention of about 28% of the label with a half-time of 17 days may represent the portion which is fixed and stored in the tissue. It is possible that previous colorimetric and microbiological assay methods are not quantitatively refined to detect this slow turnover rate.

Only one degraded metabolite of pyridoxine could be detected in small amounts in the urine excreted within 5 hours after injection. At later times, the label was so dilute it was difficult to detect the tritium beta activity (18 kv).

It would appear from the present study that pyridoxine is excreted primarily as unchanged drug in the rat, and in small amounts as 4-pyridoxic acid. Also, the very slow excretion half-time of 17 days of about 28% of the drug would tend to support the hypothesis that the "missing" pyridoxine, referred to by Snell(6) as possibly being excreted in an unidentified conjugated form, may be stored in the tissue and excreted with a rather long half-time.

Summary. Urine, blood, and fecal samples from rats injected intravenously with tritium-labeled pyridoxine hydrochloride were collected at various time intervals post injection for excretion and chromatographic study. Approximately 70% of the administered label was excreted in the urine in 5 hours. Fecal excretion was less than 0.03% in 96 hours post injection. There were 2 rates of excretion: the initial rapid rate with a half-time of about 45 minutes, followed by a slower rate with a half-time of 17 days. Chromatographic studies of urine showed that the pyridoxine was excreted primarily unchanged with a small amount of only one metabolite, most likely 4-pyridoxic acid.

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Autoradiographic Studies on Intracellular Growth of *Brucella melitensis* and Thymineless *E. coli.** (27167)

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The biochemical basis of phagocytosis has received its greatest impetus from studies concerning the events immediately following ingestion of the particle by the phagocyte. For greatest clarity in understanding the biochemical data obtained, the use of inert particles has been fruitful in avoiding the con-

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