

has been estimated to be 0.1 to 0.21 micron (7-9) in size, can pass through the arachnoid villi.

At the outset of the experiments we had

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expected that after intracerebral injection the virus would be readily demonstrable in the blood of the rabbit, in blood samples taken from the internal jugular vein. This assumption was not confirmed.

Summary. Fixed rabies virus was recovered from the blood stream of the mouse and rabbit during the first 3 hours following intracerebral inoculation.

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Metabolism of Radioactive Vitamin B₁₂ by the Rat.* (18606)

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Radioactive vit. B₁₂ labeled with Co⁶⁰ has been prepared by Rosenblum *et al.*(1) by fermentation. We have used this compound[†] to study the metabolism of vit. B₁₂ in the rat. Chow *et al.*(2) have reported that approximately 80% of the vitamin is excreted in the urine within 24 hours when injected subcutaneously, while at least 270 γ /300 g rat is needed to cause the excretion of the vitamin in the urine when administered orally.

In the present experiment we have studied the retention and excretion of radioactive vit. B₁₂ when given orally and when given by injection and also the form of the Co (whether as vit. B₁₂ or other) stored and excreted.

Experimental. Four adult white rats were used for this experiment. Rats A and B were used for qualitative work, and rats C and D for quantitative work. One ml of Co⁶⁰ labeled vit. B₁₂ solution containing approximately

20 γ of vit. B₁₂, approximately 1.3 microcuries of radioactivity, was administered to each rat. The vit. B₁₂ was injected subcutaneously into rats A and C and was given orally with the dry ration of ground checkers fed to rats B and D. The excreta were collected at 24-hour intervals for 3 days, at which time the animals were sacrificed. Paper strip chromatograms were run on the urine, feces, and extracts of the kidneys, liver and spleen of rats A and B. The solvent system was that used by Winsten and Eigen(3). Radioautographs were made of these chromatograms and of a pure radio-vit. B₁₂ chromatogram. A paper strip chromatogram was also

TABLE I. Radioautographs of Paper Strips.

	R _f (of spot)
Cobalt chloride	.0
Vit. B ₁₂	.05
Vit. B _{12b}	.08 to 0.13
Rat A (given B ₁₂ by inj.)	
24-hr urine	.13 (B _{12b})
Kidney	.095 (B _{12b})
24-hr feces	.0 (Co ⁶⁰ ++)
Liver	No spot
Rat B (given B ₁₂ orally)	
24-hr urine	.07 to 0.10 (B _{12b})
24-hr feces	0.0; .074 (Co ⁶⁰ ++ and B ₁₂)
Kidneys	No spots
Liver	
Spleen	

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1. Charet, L., Rosenblum, C., and Woodbury, D. T., *Science*, 1950, v111, 601.

[†] We wish to thank Dr. Charles Rosenblum of Merck and Co., Inc., Rahway, N. J., for the generous gift of 20 μ c of radioactive vitamin B₁₂.

2. Chow, B. F., Barrows, L., and Lang, C., *J. Nutrition*, 1950, v42, 405.

3. Winsten, W. A., and Eigen, E., *J. Biol. Chem.*, 1949, v181, 109.

TABLE II. Distribution of the Administered Radio-Vit. B₁₂. 1 ml of vit. B₁₂ solution gave 285000 $\pm$ 2850 counts/min.

Rat	Count/min.		% of total given	
	C (inj.)	D (orally)	C (inj.)	D (orally)
24-hr urine	265650 $\pm$ 2657	1500 $\pm$ 30	93.21	.53
48-hr "	2375 $\pm$ 48	300 $\pm$ 24	.83	.11
72-hr "	1500 $\pm$ 30	300 $\pm$ 24	.53	.11
24-hr feces	2200 $\pm$ 44	253825 $\pm$ 2538	.77	89.06
48-hr "	1950 $\pm$ 39	6325 $\pm$ 64	.68	2.22
72-hr "	1185 $\pm$ 24	1875 $\pm$ 38	.42	.66
Feed dish	—	450 $\pm$ 36	—	.16
Intest. cont.	1365 $\pm$ 27	1625 $\pm$ 33	.48	.57
Liver	1300 $\pm$ 26	1250 $\pm$ 25	.46	.44
Kidneys	1475 $\pm$ 30	1775 $\pm$ 36	.52	.62
Spleen	925 $\pm$ 19	800 $\pm$ 32	.32	.28
Blood	802 $\pm$ 16	625 $\pm$ 50	.28	.22
Body	—	5400 $\pm$ 216	—	1.89
Total	280727 $\pm$ 2960	276050 $\pm$ 3146	98.5 $\pm$ 1.04	96.86 $\pm$ 1.1

run on a non-radioactive CoCl₂ solution, the Co being detected by color. The feces, intestinal contents, blood, spleen, liver, and kidneys of rats C and D were ground and digested for 3 days at 37° with trypsin and pancreatin at pH 8.0. The urine samples and extracts of feces and tissues were concentrated to 25 ml. One ml of each sample and one ml of the vit. B₁₂ solution (diluted 1:1000) were taken for counting. The samples were dried in aluminum counting pans under infrared light and counted in a "Q-gas" counter.[†] The error due to self-absorption was overcome by using an aluminum shield to absorb the β -rays, the γ -rays only being counted.

Results. The Rf values of the spots observed on the radioautographs of the paper strip chromatograms are given in Table I. The distribution of the radioactive cobalt between urine, feces and various tissues of the rat following oral or intramuscular administration of radiovit. B₁₂ is given in Table II.

Discussion. From Table II it is observed that when the radiovitamin B₁₂ is given orally, approximately 92% of the radioactivity appears in the feces within 72 hours, while when given by injection approximately 95% was excreted in the urine in the same period of time. When injected there is a small excretion of Co⁶⁰⁺⁺ in the feces, and when given orally there is a small excretion of vit. B₁₂ in the urine. On the basis of the small amount of Co⁶⁰ excreted into the feces following injection of radiovit. B₁₂ it is apparent that the radioactivity of the feces following oral ingestion is due primarily to unabsorbed vit. B₁₂. However, on the basis of the chromatogram it appeared that about 60% of this unabsorbed vit. B₁₂ had been decomposed by bacterial action to yield radiocobalt.

Apparently the Co⁶⁰⁺⁺ excreted in the feces following injection is an end product of vit. B₁₂ metabolism in the body. The results indicate that some vit. B₁₂ is excreted in the urine even when a small dose is given orally but that most of it is unabsorbed. On the basis of the rat kidney chromatogram (Fig. 1) it appears that the Co⁶⁰ is stored in the tissues as vit. B₁₂, the small amount of ionic cobalt formed by metabolism being excreted via the intestine.

Summary and conclusions. Radioactive vit. B₁₂ has been given orally or subcutaneously to 4 adult white rats. The radioactivity (entirely B₁₂) was excreted primarily in the urine following injection, while the radioactivity (B₁₂ + inorganic cobalt) was excreted primarily in the feces following oral administration.

[†] Nuclear Instrument and Chemical Corporation, Chicago, Ill.