Tissue Distribution of Parenteral Co^{60} Vitamin B_{12} in Mouse, Hamster, Rat and Guinea Pig.* (22654)

A. MILLER,† G. GAULL, J. F. ROSS,‡ AND H. M. LEMON.
(Introduced by B. S. Walker.)
Robert Dawson Evans Memorial, Massachusetts Memorial Hospitals, and the Department of Medicine, Boston University School of Medicine, Boston.

Species differences in the tissue distribution of parenterally administered Co^{60} vit B_{12} ($Co^{60}B_{12}$) have been suggested by recent studies. In the rat, the kidneys were found to be the main site of $Co^{60}B_{12}$ localization even at a time when urinary excretion was absent(1,2,3). In man, the greatest percentage of a parenterally injected dose of $Co^{60}B_{12}$ was found in the liver(4). In the chick(5) minimal localization of $Co^{60}B_{12}$ was found in the liver and kidney. In this study parenteral $Co^{60}B_{12}$ was administered to hamsters, rats, mice and guinea pigs in order to elaborate on possible species differences in its tissue distribution.

Methods. Experimental plan: Eight adult male Swiss white mice weighing 25-40 g, 8 adult hamsters (4 male and 4 female) weighing 85-120 g, 7 adult male Sprague-Dawley rats weighing 200-230 g, and 4 adult male guinea pigs weighing 450-600 g, were subcutaneously injected under ether anesthesia with $Co^{60}B_{12}$ (secured from Merck and Co., Rahway, N.J.). The dose was contained in a volume of 1.0 ml, except for mice, where 0.5 ml was given. The dose injected ranged from 0.10 to 0.40 μc of Co^{60}. Mice received 0.15 μg of B_{12}, hamsters 0.70 μg, rats 0.30 μg and guinea pigs 0.26 μg. After injection, rats and guinea pigs were housed in metabolic cages so as to allow separate urine and stool collections. Hamsters were housed in wire cages under which 2 thicknesses of Whatman filter paper #3 were spread. All stools fell on the filter paper while the urine was absorbed into the paper. Mice were housed in 2,000 ml beakers. The bottoms of the beakers were also lined with this filter paper on which urine and stools were deposited. Four days after the injection of the Co^{60} B_{12}, animals were anesthetized, exsanguinated by cardiac puncture, and the appropriate organs removed.

Preparation of specimens for analysis: (a) *Organs*. The liver, kidneys, spleen, heart, testes and portions of the hind leg musculature were removed and weighed. Organs were placed into flasks containing 10 ml of 2N NaOH, except for the livers where 20 ml was used. The organs were digested in alkali at room temperature for 24 hours. Then, if necessary, gentle heating for 30 to 60 seconds brought the gel-like digest into a liquid state. Measurements of radioactivity were made on duplicate 4 ml aliquots of these tissue digests. The total volume of the tissue digest solution was assumed to be equal to 10 ml when organ weight was less than 0.75 g as the volume contributed by the dissolved organ was negligible. For heavier organs the total volume of the digest was measured in a 25 ml graduated cylinder. (b) *Urine, stools, and blood.* Independent total urine collections were secured for the first 24 hours and for the following 3 days for the rats and guinea pigs. The total urine volume was measured in a 50 ml graduated cylinder. Radioactivity was determined on 4 ml aliquots of urine. For hamsters and mice, the filter paper on which the urine had dried was cut into small pieces and placed into 1¾ by 3⅛ inch glass bottles in which radioactivity was measured. The radioactivity in 4-day stool collections was also determined in 1¾ by 3¼ inch glass bottles. One to 4 ml of oxalated blood (hamsters and rats were only done) was used for radioactivity assay. (c) *Carcasses.* Mice and hamster carcasses were placed into 1¾ by 3¼ inch glass bottles while the rat carcasses were placed into 2½ by 6 inch glass bottles. Car-

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† Formerly U. S. Public Health Service Fellow. Present address: Boston Veterans Hospital, Boston.

‡ Present address: University of California Medical Center, Los Angeles.

TABLE I. Tissue Distribution and Excretion of $Co^{60}B_{12}$ in Mice, Rats, Hamsters and Guinea Pigs.

	Mice		Rats		Hamsters		Guinea pigs	
Organ	% of inj. dose*	% of dose* g wet wt	% of inj. dose	% of dose g wet wt	% of inj. dose	% of dose g wet wt	% of inj. dose	% of dose g wet wt
Kidney	6.2 ± .4	13.0 ± 1.4	16.2 ± 2.9	9.7 ± 1.9	7.2 ± .6	6.3 ± .1	2.4 ± .1	.3 ± 0
Liver	23.7 ± 2.5	11.2 ± 1.1	7.4 ± .4	.8 ± 0	18.0 ± 2.9	6.1 ± .1	29.7 ± 4.1	1.3 ± 0
Testes	1.5 ± .1	7.0 ± 1.1	1.0 ± 0	.6 ± 0	not done	—	.3 ± 0	.1 ± 0
Spleen	1.4 ± .2	1.7 ± .5	.8 ± 0	.6 ± 0	.3 ± 0	.8 ± 0	.2 ± 0	.3 ± 0
Heart	.4 ± .1	1.8 ± .1	.7 ± 0	.9 ± 0	.4 ± 0	.6 ± 0	.2 ± 0	.1 ± 0
Muscle	15.9 ± .9	1.3 ± 0	13.6 ± 1.1	.2 ± 0	7.7 ± 1.5	.3 ± 0	not done	—
Carcass	26.0 ± 2.9	—	34.5 ± 4.0	—	43.0 ± 3.8	—	" "	—
Urine	5.9 ± 1.8	—	7.4 ± 1.1	—	14.1 ± 1.0	—	12.5 ± .9	—
Stool	15.5 ± 1.8	—	18.5 ± 6.2	—	3.1 ± .9	—	5.5 ± .1	—
Recovery	106.1		100.7		95.1		50.8	

* Mean and stand. error of mean.

cass radioactivity measurements were made in these bottles. Because of their large size, the guinea pig carcasses could not be counted.

Radioactivity determination: A 1:100 dilution of the injected $Co^{60}B_{12}$ was made and varying aliquots were then used as standards for the varying conditions employed in the counting of organ digests, excreta and carcasses. By adding water as needed, the height of the standard in the glass bottles was always the same as that of the specimen being counted *i.e.*, stools, urine or carcasses. Blood, tissue and liquid urine samples were counted in a scintillation well-type counter (thallium activated sodium iodide scintillation crystal). The radioactivity of the organ samples usually counted 3-12 times background, except for the muscle, heart, blood and spleen which usually counted 1-3 times background. Samples were counted long enough to give a counting error of less than 2%, except for the slow-counting samples where a 5-8% counting accuracy was obtained. Duplicate samples usually agreed within 1-5%. All samples contained in the 1¼ by 3¼ inch glass bottles were counted by placing them on top of the above-mentioned sodium iodide well crystal. The diameter of these was just about equal to that of the diameter of the well. Under similar geometry, a standard was also counted. The 6 inch bottles containing the rat carcasses were marked into 3 equal 2 inch segments. With the bottle in the horizontal position, and directly in contact with a solid 1 by 1 inch thallium activated sodium iodide crystal, each 2 inch segment of the bottle containing the carcass was counted. Under similar geometry the bottle containing the standard was counted.

Calculations. (a) The percent radioactivity in organ =

$$\frac{\text{cpm per ml organ digest} \times \text{total volume digest}}{\text{cpm injected}} \times 100$$

In calculating the total percent uptake in the muscle mass, 40% of the animal's weight was assumed to be muscle. For the total percent uptake in blood, a blood volume was estimated based on the figure of 69 ml/kg body weight.

Results. The organ distribution and excretion of a parenteral dose of $Co^{60}B_{12}$ in the rat, guinea pig, mouse and hamster are shown in Table I.

Discussion. In the rat the tissue distribution of a parenterally injected dose of $Co^{60}B_{12}$ was similar to that found by Rosenblum *et al.* (1). Similar studies on the mouse, hamster and guinea pig are not available in the literature. Differences in the major organ sites of $Co^{60}B_{12}$ localization were noted in the 4 species studied. In the hamster, guinea pig and mouse, the liver was the chief organ of localization whereas in the rat the kidney was the chief site. The high $Co^{60}B_{12}$ uptake of the rat kidney is probably unrelated to its known role in the excretion of large doses of parenterally injected vit B_{12}(3,6,7), since the dose given the rat was comparable to that given the other 3 species. Furthermore, the other 3 species studied had comparable amounts of

radioactivity excreted in the urine without an increase in kidney $Co^{60}B_{12}$ uptake. At the time the high rat renal $Co^{60}B_{12}$ uptake was found, urinary excretion of the radiovitamin was negligible making it highly unlikely that the $Co^{60}B_{12}$ in the kidneys was there to be excreted. This is further supported by the observations of Harte(2), who found that high renal $Co^{60}B_{12}$ levels were maintained for as long as 90 days after injection despite the absence of urinary excretion after the first day. It would thus appear that the kidney represents an important site of $Co^{60}B_{12}$ localization in the rat rather than a mere storage site for further urinary excretion. The high $Co^{60}B_{12}$ concentration in the liver of the mouse, hamster and guinea pig is comparable to that found in man after the injection of labelled vit B_{12}(4). The rat, with the chief localization of injected $Co^{60}B_{12}$ in the kidney, is not analogous to man. It would, therefore, appear hazardous to relate to man the results of the experiments in the rat.

Differences in the routes of $Co^{60}B_{12}$ excretion were noted in the 4 species studied despite the administration of comparable amounts of vit B_{12} in relation to body weight. The stools were the chief routes of excretion in the rat and mouse, whereas the urine was the chief excretory pathway in the hamster and guinea pig. In previous studies in the rat (1,8) the urine was the major excretory pathway containing 50-90% of a parenteral dose of $Co^{60}B_{12}$. These results differ markedly from those found in this study and are probably due to the large doses of vit B_{12} (4-20 μg) used in these studies. There is no available data on the excretion of parenteral $Co^{60}B_{12}$ in the other 3 species studied. The present investigation suggests that the stools may be an important route for the excretion of vit B_{12} in the rat and mouse. Radioactivity as measured in this study merely indicates the presence of the Co^{60} moiety of the vit B_{12} molecule. Thus, the radioactivity may be due to vit B_{12} or to an intermediate degradation product of the vitamin. After parenteral administration of $Co^{60}B_{12}$ to rats, the radioactivity excreted in the urine was found to be due to vit B_{12}, whereas that in the stool was unassociated with vit B_{12} activity(1). The radioactivity in the kidneys was found to be due to vit B_{12} in a similar study(8). It appears likely that the radioactivity in the kidneys in this study is due to vit B_{12}. However, concerning the other organs, this question cannot be answered.

Summary. The excretion and tissue distribution of parenterally administered Co^{60} B_{12} has been studied in the rat, hamster, mouse and guinea pig. The liver contained more radioactivity than any other organ in the hamster, mouse and guinea pig, whereas the kidneys contained more radioactivity than any other organ in the rat. In the rat and mouse, more radioactivity was excreted in the stools than in the urine whereas in the guinea pig and hamster, the urine was the chief excretory pathway.

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