Distribution of Radioactive and Nonradioactive Vitamin B₁₂ in the Dog*

JACK M. COOPERMAN, A. LEONARD LUHBY, DAVID N. TELLER, AND JAMES F. MARLEY

From the Hematology and Nutrition Laboratory, Department of Pediatrics, New York Medical College, New York 29, New York

(Received for publication, September 8, 1959)

Correlation between the tissue distribution of radioactive and nonradioactive vitamin B₁₂ is important for the acceptance of Co⁶⁰-vitamin B₁₂ as a physiological tracer. Previous work has been limited to an analysis of one or two visceral tissues or body fluids (1-7). As a prelude to the study of the placental transfer of vitamin B₁₂ and its relative tissue distribution in malignancy, the comparative distribution of nonradioactive and radioactive vitamin B₁₂ after administration of tracer doses of Co⁶⁰-vitamin B₁₂ was studied in the dog. It was found that the distribution of radioactivity closely paralleled the total vitamin B₁₂ content of the viscera, and that the pituitary, kidney, and pancreas were the sites of the greatest vitamin B₁₂ concentration.

EXPERIMENTAL

Materials

The two littermate mongrel dogs, a male weighing 6 kg and a female weighing 5 kg, were studied. Each dog received daily, on 5 successive days by subcutaneous injection into the upper left thigh, a solution of 0.135 μg of Co⁶⁰-vitamin B₁₂ containing 0.0967 μg of Co⁶⁰ in 0.13 ml of 0.9% sodium chloride solution. The dogs were scanned daily for radioactivity over the hepatic area and site of injection with a probe type NaI scintillation counter as previously described (8). The dogs were killed 14 days after the last injection, at which time a reasonably good distribution of the vitamin should have occurred, since the radioactivity over the hepatic area reached a maximum within a week after the last injection. The organs and tissues were then carefully dissected out, cleaned, weighed, and frozen until prepared for assay.

Methods

Tissue Preparation—The organs and tissues were individually homogenized in a Waring Blender with carefully measured amounts of distilled water. Two aliquots of each homogenate were added to 57- x 27-mm screw top glass vials which were previously tared and counted for radioactivity. Small organs, such as the pituitary and adrenals, were minced in entirety and suspended as uniformly as possible in agar in 50- x 16-mm screw top glass vials.

Radioactivity Counting—The radioactivity of homogenized samples were determined in a well type scintillation counter containing a 3-inch thallium-activated sodium iodide crystal with a 11/ x 11/ -inch well. Each specimen was counted for a minimum of 10,000 counts. Two aliquots of each organ sample were counted in duplicate and the results averaged. Radioactivity standards were prepared from carefully measured amounts of Co⁶⁰-vitamin B₁₂ from the same batch used for injection of the experimental animals. The Co⁶⁰-vitamin B₁₂ standards consisted of 0.00512 and 0.00102 μg of vitamin B₁₂ containing 0.00365 and 0.000729 μC of radioactivity, respectively. To provide equivalent geometry, these were diluted in sufficient distilled water to fill the 57- x 27-mm and 50- x 16-mm glass vials used for specimen counting. Background and standardization counts were obtained before and after each specimen count, or every 2 hours when many specimens were counted consecutively. The radioactivity of the specimens was corrected for physical decay of the Co⁶⁰ and the results were calculated as μC of radioactivity per g of wet tissue and per total organ weight. Microcuries of Co⁶⁰ were converted to micrograms of Co⁶⁰-vitamin B₁₂ from the radioactive specific activity of the Co⁶⁰-vitamin B₁₂ employed.

Microbiological Assay—To extract vitamin B₁₂ from the tissue samples, a homogenate of 1 g of tissue with 100 ml of extracting solution was prepared and autoclaved for 15 minutes at 15 pounds pressure. The extracting solution consisted of 1.29 g of disodium phosphate, 1.1 g of citric acid, and 0.1 g of sodium metabisulfite per 100 ml of water (9). After being cooled and centrifuged, the supernatant from the homogenate was neutralized and diluted as necessary with triple distilled water and assayed for vitamin B₁₂ as described below.

A second aliquot of each supernatant was adjusted to pH 12 and autoclaved for 30 minutes at 15 pounds pressure, cooled, neutralized, and also assayed for vitamin B₁₂. The residual microbiological activity is caused by nucleosides (10). The value obtained after alkali treatment, which decomposes vitamin B₁₂, was subtracted from that of the first aliquot to give true vitamin B₁₂ activity.

The glassware used throughout the microbiological procedure was washed with detergent and triple distilled water, soaked in a 0.125% solution of ethylenediaminetetraacetate of pH 7.5 and rinsed several times with fresh triple distilled water to eliminate vitamin B₁₂ that may have contaminated the glassware. Vitamin B₁₂ was assayed microbiologically employing Lactobacillus lactis Dormer ATCC 8000 by the method of Cooperman et al. (11) with the following modification which permits reading the assay at the end of 16 hours instead of the original 40 hours, and increases the sensitivity so that as little as 3 μg per ml can be measured. A total of 2 ml of liquid, consisting of one ml of

* Aided in part by grants from the Playtex Park Research Institute, the National Institute of Arthritis and Metabolic Diseases (A-1071), and the Division of Cancer Control and Research, New York City Department of Health.
medium and one ml of sample plus triple distilled water, was added to 13- X100-mm test tubes and autoclaved for 6 minutes at 15 pounds pressure. After cooling, the tubes were inoculated and allowed to incubate at 37° for 16 hours. Growth was calculated from turbidity read in a colorimeter with a 660-mM filter.

RESULTS

The data for the distribution of radioactivity and microbiologically assayed vitamin B₁₂ are presented in Table I. It is apparent from these data that the liver contained the largest amount of both vitamin B₁₂ and radioactivity. Next in order was the gastrointestinal tract, which contained only about one-third as much as the liver. Surprisingly, more than half the vitamin B₁₂ and radioactivity of the gastrointestinal tract resided in the stomach. The heart and kidneys had appreciable quantities; and the vitamin B₁₂ content of the heart was comparable to that of the kidney.

Of the brain sections studied, the cerebrum had about 10 times the vitamin B₁₂ and radioactivity of other areas of the brain, i.e. cerebellum, medulla, and pons. In one dog, the corpus cal-

| Table I |

Comparison between total content of visceral organs with tissue concentration of microbiologically assay vitamin B₁₂ and radioactivity from parenterally administered C₁⁴-vitamin B₁₂ in dog

Visceral organ systems have been listed in order of greatest vitamin B₁₂ content.

<table>
<thead>
<tr>
<th></th>
<th>Total B₁₂</th>
<th>Total radioactivity</th>
<th>B₁₂ concentration</th>
<th>Radioactivity concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/organ</td>
<td>µg/organ</td>
<td>µg/t</td>
<td>µg/g</td>
</tr>
<tr>
<td>Liver</td>
<td>13.25</td>
<td>58,020</td>
<td>0.072</td>
<td>315.1</td>
</tr>
<tr>
<td>Gastrointestinal tract:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>2.96</td>
<td>12,451</td>
<td>0.060</td>
<td>251.0</td>
</tr>
<tr>
<td>Lower ileum</td>
<td>0.91</td>
<td>4,099</td>
<td>0.017</td>
<td>74.5</td>
</tr>
<tr>
<td>Duodenum and jejunum</td>
<td>0.96</td>
<td>3,431</td>
<td>0.022</td>
<td>78.5</td>
</tr>
<tr>
<td>Upper ileum</td>
<td>0.36</td>
<td>2,172</td>
<td>0.011</td>
<td>66.9</td>
</tr>
<tr>
<td>Colon, caecum, and rectum</td>
<td>0.34</td>
<td>1,601</td>
<td>0.013</td>
<td>92.0</td>
</tr>
<tr>
<td>Esophagus</td>
<td>0.25</td>
<td>356.4</td>
<td>0.021</td>
<td>30.4</td>
</tr>
<tr>
<td>Total</td>
<td>5.53</td>
<td>23,664</td>
<td>0.013</td>
<td>130.7</td>
</tr>
<tr>
<td>Heart</td>
<td>3.00</td>
<td>10,303</td>
<td>0.076</td>
<td>261.7</td>
</tr>
<tr>
<td>Kidneys</td>
<td>2.61</td>
<td>7,797</td>
<td>0.099</td>
<td>302.2</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.25</td>
<td>3,703</td>
<td>0.091</td>
<td>588.5</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.95</td>
<td>4,742</td>
<td>0.014</td>
<td>69.9</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrum</td>
<td>0.41</td>
<td>4,092</td>
<td>0.013</td>
<td>130.7</td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>0.16</td>
<td>1,748</td>
<td>0.014</td>
<td>108.6</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.07</td>
<td>678.0</td>
<td>0.011</td>
<td>101.2</td>
</tr>
<tr>
<td>Medulla and pons</td>
<td>0.07</td>
<td>639.0</td>
<td>0.013</td>
<td>114.9</td>
</tr>
<tr>
<td>Total</td>
<td>0.64</td>
<td>7,427</td>
<td>0.014</td>
<td>56.4</td>
</tr>
<tr>
<td>Cervical spinal cord, section</td>
<td>0.012</td>
<td>34.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoracic spinal cord, section</td>
<td>0.014</td>
<td>34.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.64</td>
<td>5,074</td>
<td>0.044</td>
<td>347.8</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>0.75</td>
<td>1,748</td>
<td>0.009</td>
<td>68.2</td>
</tr>
<tr>
<td>Tongue</td>
<td>0.22</td>
<td>1,012</td>
<td>0.011</td>
<td>53.9</td>
</tr>
<tr>
<td>Thigh muscle, section</td>
<td>0.012</td>
<td>34.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neck muscle, section</td>
<td>0.011</td>
<td>36.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parotid and salivary glands</td>
<td>0.10</td>
<td>523</td>
<td>0.025</td>
<td>126.3</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.078</td>
<td>630.0</td>
<td>0.023</td>
<td>184.8</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.030</td>
<td>313.4</td>
<td>0.041</td>
<td>186</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.005</td>
<td>51.0</td>
<td>0.013</td>
<td>121.5</td>
</tr>
<tr>
<td>Pituitary</td>
<td>0.0014</td>
<td>16.6</td>
<td>0.323</td>
<td>1,410</td>
</tr>
<tr>
<td>Cervical lymph node, section</td>
<td>0.025</td>
<td>145.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoracic lymph node, section</td>
<td>0.026</td>
<td>158.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovaries</td>
<td>0.010</td>
<td>102.7</td>
<td>0.025</td>
<td>238.7</td>
</tr>
<tr>
<td>Testes</td>
<td>0.47</td>
<td>2,247</td>
<td>0.030</td>
<td>144.8</td>
</tr>
<tr>
<td>Epididymis</td>
<td>0.13</td>
<td>323.2</td>
<td>0.044</td>
<td>111.5</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.021</td>
<td>140.1</td>
<td>0.020</td>
<td>136.0</td>
</tr>
<tr>
<td>Vagina</td>
<td>0.030</td>
<td>134.8</td>
<td>0.019</td>
<td>78.8</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>0.05</td>
<td>155.1</td>
<td>0.017</td>
<td>49.7</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>0.007</td>
<td>49.0</td>
<td>0.012</td>
<td>81.7</td>
</tr>
<tr>
<td>Bile</td>
<td>0.0038</td>
<td>12.5</td>
<td>0.0012</td>
<td>11.1</td>
</tr>
<tr>
<td>Ascending aorta, section</td>
<td>0.012</td>
<td>59.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>28.48</td>
<td>124,373</td>
<td>146,564</td>
<td></td>
</tr>
</tbody>
</table>
losum and the cerebrum were assayed independently; greater amounts of microbiologically determined vitamin B₁₂ and radioactivity were found in the cerebrum.

Although the kidney and pancreas of the dog have a larger concentration of vitamin B₁₂ and radioactivity per gram of tissue than the liver, as we (8) and Glass and Merseheimer (12) have previously reported, the liver contains a higher total content because of its larger size.

In Table II are listed the eight organs; i.e., liver, gastrointestinal tract, heart, kidneys, pancreas, lungs, brain, and spleen, which accounted for over 95% of the total vitamin B₁₂ and radioactivity of the viscera assayed. The liver possessed about 50% of this total amount.

Of particular importance is the demonstration, presented in Table II, that the distribution of radioactivity in the eight major visceral stores of the dog paralleled that of the vitamin B₁₂ content. This was also true for the other tissues studied.

The vitamin B₁₂ content and the radioactivity of the entire musculature were not determined. However, representative samples of muscle indicated that the average B₁₂ content was 0.01 μg per gram and the radioactivity 40 μμC per gram. If the assumption that approximately 40% of the animal's weight is muscle (13) is accepted, then the total musculature of Dog 1 contained 20 μg of vitamin B₁₂ and 80,000 μμC of radioactivity; of Dog 2, 24 μg of vitamin B₁₂ and 96,000 μμC of radioactivity.

Recovery of injected radioactive vitamin B₁₂ in the viscera of Dog 1 and Dog 2 was 28% and 30%, respectively. If one includes the estimated content of the musculature, the recovery was 42% for Dog 1 and 50% for Dog 2. For the purposes of this study no attempt was made to estimate the amounts of administered Co₆₀-vitamin B₁₂ excreted or residing in such other areas as bone, skin, blood, and bone marrow.

A comparison of the vitamin B₁₂ content and the radioactivity of the various glands in the dog revealed that the pituitary was by far the most concentrated source of vitamin B₁₂ as well as of radioactivity. In order to determine whether pituitaries of other species also had a high vitamin B₁₂ concentration, specimens were obtained from rats, rabbits, and a human infant at autopsy by Comar and Davis (15, 16) on the distribution of radioactive inorganic cobalt (1, 4–6, 7). Similarly, by comparing the increase in radioactivity and microbiologically determined vitamin B₁₂ in the urine of rats after administration of Co₆₀-vitamin B₁₂, it was found that changes in the radioactivity varied directly with changes in the vitamin B₁₂ content (2, 3).

It would appear reasonable a priori to expect that a similar relationship exists for other body tissues, but this might not necessarily be the case. One of the purposes of this study was to analyze all the viscera of an experimental animal after the administration of Co₆₀-vitamin B₁₂ for both radioactivity and microbiologically determined vitamin B₁₂ in order to determine whether the distribution of radioactivity parallels the distribution of nonradioactive vitamin B₁₂. Our studies establish for the first time that the radioactivity of Co₆₀-vitamin B₁₂ distributes itself in all the viscera in a fashion analogous to that of the distribution of vitamin B₁₂. That this parallelism is not due to a coincidentally similar distribution of vitamin B₁₂ and inorganic cobalt appears clear from the data presented by Comar and Davis (15, 16) on the distribution of radioactive inorganic cobalt in the cow, swine, and rabbit.

After parenteral administration of inorganic Co₆₀ salts, a pattern of distribution distinctly different from that of vitamin B₁₂ was found. For example, the brain and pituitary had little or no radioactivity; the thyroid and large intestine had very large concentrations of radioactivity; the stomach had very little. In contrast, when radioactive Co₆₀-vitamin B₁₂ was administered to the dog, the pituitary had the greatest concentration of radioactivity of all the viscera, and the brain and stomach had a very large concentration, whereas the thyroid and large intestines were very low in radioactivity.

### Table II

<table>
<thead>
<tr>
<th>Species</th>
<th>Total B₁₂ of organs assayed</th>
<th>Total radioactivity of organs assayed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dog 1</td>
<td>Dog 2</td>
</tr>
<tr>
<td>Liver</td>
<td>46.4</td>
<td>54.0</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>19.4</td>
<td>15.5</td>
</tr>
<tr>
<td>Heart</td>
<td>10.5</td>
<td>7.9</td>
</tr>
<tr>
<td>Kidneys</td>
<td>9.2</td>
<td>7.7</td>
</tr>
<tr>
<td>Pancreas</td>
<td>4.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Lungs</td>
<td>3.3</td>
<td>2.7</td>
</tr>
<tr>
<td>Brain</td>
<td>2.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.2</td>
<td>2.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>97.7</td>
<td>95.9</td>
</tr>
</tbody>
</table>

### Table III

<table>
<thead>
<tr>
<th>Species</th>
<th>B₁₂ concentration µg/t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>0.491</td>
</tr>
<tr>
<td>Dog</td>
<td>0.420, 0.323</td>
</tr>
<tr>
<td>Rat</td>
<td>0.270</td>
</tr>
<tr>
<td>Human infant</td>
<td>0.203</td>
</tr>
</tbody>
</table>

Attempts to establish that the cobalt of Co₆₀-vitamin B₁₂ does not dissociate from the remainder of the vitamin molecule, thus making Co₆₀-vitamin B₁₂ a valid physiological tracer, have been made in a limited number of viscera or body excretions by determining the relationship between vitamin B₁₂ and radioactivity in those materials. Analysis of kidney, liver, urine, and feces of animals and humans who were given Co₆₀-vitamin B₁₂, by comparing the mobility of the radioactivity with that of vitamin B₁₂ in paper chromatograms, indicates that the radioactivity in those materials has a mobility similar to that of crystalline nonradioactive vitamin B₁₂ rather than to that of inorganic cobalt (1, 4–6, 7). Similarly, by comparing the increase in radioactivity and microbiologically determined vitamin B₁₂ in the urine of rats after administration of Co₆₀-vitamin B₁₂, it was found that changes in the radioactivity varied directly with changes in the vitamin B₁₂ content (2, 3).
Thus it appears reasonable to conclude that determination of radioactivity in any visceral organ after administration of Co$^{58}$ vitamin B$_{12}$ reflects the distribution of vitamin B$_{12}$ in that tissue.

It is of interest that of all the viscera examined, the pituitary contained approximately 4 times as much concentration of vitamin B$_{12}$ as the kidney. After the pituitary, the kidney tissue in the dog contained the greatest concentration of vitamin B$_{12}$ per gram. Very high pituitary concentrations of vitamin B$_{12}$ were also found in three other species, the human, the rat, and the rabbit (Table III). The significance of the great pituitary concentration of vitamin B$_{12}$ is still to be determined. The concentration of vitamin B$_{12}$ in the adrenal was relatively high, whereas that in the thyroid was surprisingly very low. It would be of interest to determine whether the amount of vitamin B$_{12}$ concentration of a tissue is related to the mitochondrial density of the cell where the vitamin B$_{12}$ is located, or to the particular metabolic rate or function of the tissue.

There has previously been little emphasis on the fact that the heart is among the organs which are highest in vitamin B$_{12}$ concentration per gram of tissue was well as on a total organ basis. In the dog the concentration per gram was approximately 7 to 8 times that of skeletal muscle.

The modification of the microbiological assay for vitamin B$_{12}$ with the use of Lactobacillus Dornor as described in this paper increases the usefulness of this assay for the determination of the vitamin B$_{12}$ content of tissues and body fluids by increasing the sensitivity and rapidity of the assay as originally described (11). The sensitivity, at least 5 times greater than that of the previous procedure, brings this assay into the sensitivity range of the Euglena and Ochromonas techniques (17). It has a practical superiority over the latter methods in that conditions for performing the assay are less critical and the procedure requires only 16 hours to obtain results. Specificity of the Lactobacillus assay for vitamin B$_{12}$ in serum is excellent because the nonspecific stimulators in serum are of such low magnitude that they do not materially affect results (9). By the use of an alkaline hydrolysis “control” (10), specificity in tissues is attained because pseudovitamin-B$_{12}$ occurs essentially in fermentation broths.

**SUMMARY**

The relative distribution of radioactivity in the viscera of the dog after administration of Co$^{58}$-vitamin B$_{12}$ is directly proportional to their vitamin B$_{12}$ content. This further establishes the validity of Co$^{58}$-vitamin B$_{12}$ as a physiological tracer.

Comparative distribution of vitamin B$_{12}$ in the viscera showed the pituitary gland to contain the greatest concentration per gram of tissue. Next in order were the kidney, pancreas, liver, and heart. In order of greatest total organ vitamin B$_{12}$ content were the liver, gastrointestinal tract, heart, kidneys, pancreas, lungs, and brain.

An improvement of the Lactobacillus lactis microbiological assay for vitamin B$_{12}$ is described, which increases its sensitivity and decreases the time required for completion.

**Acknowledgments**—We wish to thank Dr. Robert Feldman for assistance in dissection of the dogs, and Julia M. Herrero for invaluable technical assistance. The encouragement of Dr. Lawrence B. Slobody, Director, Department of Pediatrics, is gratefully appreciated.

**REFERENCES**

Distribution of Radioactive and Nonradioactive Vitamin B$_{12}$ in the Dog
Jack M. Cooperman, A. Leonard Luhby, David N. Teller and James F. Marley


Access the most updated version of this article at http://www.jbc.org/content/235/1/191.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/235/1/191.citation.full.html#ref-list-1