PROPERTIES AND DISTRIBUTION OF VITAMIN B\textsubscript{12f}\textsuperscript{*}

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The existence of a new form of vitamin B\textsubscript{12} in the feces of rats was first noted during a study of the effect of dietary cobalt on the growth of rats employed for vitamin B\textsubscript{12} assay (1). When crude extracts of the feces were tested by the microbiological and rat assays, only a small fraction of the vitamin B\textsubscript{12} activity measured microbiologically could be detected by the rat assay. Upon chromatographing the fecal extracts, a vitamin B\textsubscript{12}-active compound was detected that possessed an $R_F$ value intermediate between those of vitamins B\textsubscript{12} and B\textsubscript{12b}. Fractionation of the feces yielded a red compound that possessed growth-stimulating properties for \textit{Lactobacillus leichmannii} but which failed to produce a growth response in the rat. For the sake of clarity when reference is made to the new compound, the notation vitamin B\textsubscript{12f} ("f" = feces) has been tentatively assigned to the substance.

While the paper referred to above was in press, Pfiffner et al. (2) reported the isolation of a vitamin B\textsubscript{12}-like compound produced by an anaerobic microorganism obtained from rumen contents. The compound, designated pseudovitamin B\textsubscript{12}, failed to support growth in the rat and chick but was active in this respect in \textit{L. leichmannii}. Recently (3), the notation cyano-$\beta$-cobalamin has been suggested for the substance. A comparison of the properties of vitamin B\textsubscript{12f} with those of pseudovitamin B\textsubscript{12} suggests that the two compounds are identical. However, final judgment must await chemical degradation of vitamin B\textsubscript{12f} to determine its exact relationship to this and other known forms of vitamin B\textsubscript{12}.

EXPERIMENTAL

Elimination of Vitamin B\textsubscript{12}—Although the vitamin B\textsubscript{12f} isolated from rat feces (1) was obtained as a crystalline material, it was not known with

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certainty whether the sample was homogeneous. Upon chromatographing on paper, with water-saturated n-butanol, three zones could be detected by the bioautographic technique (4, 5). The slow and fast moving spots corresponded in $R_F$ values to vitamins $\text{B}_{12\alpha}$ and $\text{B}_{12\beta}$, respectively. An intermediate zone represented vitamin $\text{B}_{12\gamma}$. To determine whether the vitamin $\text{B}_{12\gamma}$ and vitamin $\text{B}_{12\delta}$ were present as impurities or whether they arose from vitamin $\text{B}_{12\beta}$ during chromatography, the following procedure was carried out: 10 mgm. of vitamin $\text{B}_{12\gamma}$ were spotted on strips of Eaton-Dikeman paper No. 613\(^1\) and developed with water-saturated n-butanol for 72 hours at room temperature. The vitamin $\text{B}_{12\gamma}$-active materials were then located with duplicate pilot strips by the bioautographic technique. The vitamin $\text{B}_{12\gamma}$ microbiological assay medium of Thompson et al. (6) (plus 1.5 per cent agar) seeded with *L. Zeichmannii* ATCC 4797 was used in the plating procedure. Once the locations of the three forms on the paper strips were determined, the spots were cut out, eluted with water, and rechromatographed. The eluted fast spot, corresponding to vitamin $\text{B}_{12\delta}$, produced two zones possessing $R_F$ values identical with those of vitamins $\text{B}_{12\alpha}$ and $\text{B}_{12\beta}$. This conversion of vitamin $\text{B}_{12\gamma}$ to vitamin $\text{B}_{12\beta}$ during chromatography has been reported by Woodruff and Foster (7). The eluted slow moving zone when chromatographed produced only one zone, which corresponded to vitamin $\text{B}_{12\delta}$. The eluted middle spot (vitamin $\text{B}_{12\gamma}$) gave a vitamin $\text{B}_{12\alpha}$ zone and a very faint slow moving spot, but no vitamin $\text{B}_{12\beta}$ spot. This indicated that the vitamin $\text{B}_{12\gamma}$ noted in the vitamin $\text{B}_{12\gamma}$ samples was present as an impurity and did not arise from the vitamin $\text{B}_{12\alpha}$. Attempts were then made to separate the vitamin $\text{B}_{12\gamma}$ from the vitamin $\text{B}_{12\beta}$.

Separation by fractional crystallization or by use of ion exchange resins proved unsuccessful. By means of chromatography on alumina, however, a separation was accomplished. Columns of activated alumina mixed with Filter-Cel (2:1) were prepared with 95 per cent ethanol as the solvent. The vitamin $\text{B}_{12\alpha}$ samples, dissolved in 95 per cent ethanol, were applied to the columns and developed with more of the same solvent until the red band had moved a short way down the column. Movement was very slow with this developing agent, but substitution of 65 per cent ethanol resulted in rapid movement of the band and elution from the column. By repeated passages through such columns and by discarding the tailings of the band, the vitamin $\text{B}_{12\gamma}$ was eliminated from the vitamin $\text{B}_{12\alpha}$ preparations.

*Removal of Other Impurities*—The elimination of vitamin $\text{B}_{12\gamma}$ from the vitamin $\text{B}_{12\alpha}$ preparations failed to alter the absorption spectrum. How-

\(^1\) This paper can be purchased already cut into 3 inch strips, and the $R_F$ values obtained with it are comparable to those with Whatman No. 1 paper.
ever, in studying the behavior of vitamin B₁₂f toward ion exchange resins, it was noted that after passage through a column of IRA-400 (Rohm and Haas Company, Philadelphia) a strong maximum at 278 mµ was present. Previously this peak had not been detected (1), presumably because of absorption by masking impurities. Since only 1 to 2 mg. of vitamin B₁₂f was available, it is very likely that these small quantities were contaminated with traces of substances not completely removed by recrystallization. Fig. 1 shows the absorption spectrum of vitamin B₁₂f after treatment with the ion exchange resin. The vitamin B₁₂f has a much stronger maximum at 278 mµ than does vitamin B₁₂, but otherwise the spectra are very similar. The possibility is still not excluded that a trace of an impurity is producing this strong absorption in the 278 mµ region.

It is of interest to note that, while vitamin B₁₂f was not held by anion exchange columns, cation exchangers absorbed it so strongly that none could be detected in the effluent. This indicates a strongly basic group in the vitamin B₁₂f molecule. Vitamin B₁₂ is not absorbed by most anion and cation exchange resins (8).

Evidence for Cyanide Complex—Although the vitamin B₁₂ had been removed from the vitamin B₁₂f, a weak, slow moving zone could still be detected when chromatographed on paper. This slow moving compound possessed an \( R_F \) value very similar to that of vitamin B₁₂b, when chromatographed with \( n \)-butanol. However, when water-saturated sec-butanol (9) was employed, a definite difference in \( R_F \) values was observed. The slow spot present in the vitamin B₁₂f had an \( R_F \) value of 0.04, while the value for vitamin B₁₂b was 0.08. The values for vitamins B₁₂f and B₁₂

![Image of absorption spectra](http://www.jbc.org/)

Fig. 1. Absorption spectra in water at pH 7 of vitamins B₁₂f and B₁₂.
were 0.16 and 0.30, respectively. A mixture of 5 µgm each of vitamins B12 and B12f was chromatographed and four zones were detected. There was considerable tailing, but the four zones could be easily distinguished. These data are shown in Fig. 2.

While a 72 hour development period was required with n-butanol to effect a separation of these various forms of vitamin B12, only 20 hours were required for comparable results with sec-butanol. Descending development at room temperature was used. During the summer, with somewhat higher room temperatures, the \( R_f \) values increased slightly.

![Fig. 2. Chromatographic development of vitamin B12f with water-saturated sec-butanol.](image)

The presence of two spots in all preparations of vitamin B12f suggested the existence of a cyano-hydroxo relationship similar to that in vitamins B12 and B12h. 30 µ of vitamin B12f were irradiated with ultraviolet light for 45 minutes in water solution at pH 4. Under these conditions vitamin B12 is converted to vitamin B12h, and the conversion can be noted visually by a color change from red-violet to red-orange (10). Upon irradiation, vitamin B12f underwent a similar color change which could be reversed by the addition of KCN. When the irradiated samples were chromatographed, the slow moving spot was much more pronounced than in untreated vitamin B12f. However, the photolysis was not complete, since some vitamin B12f could still be detected in the irradiated samples. These results were compatible with a cyano-hydroxo relationship between the two spots.

Since an insufficient supply of vitamin B12f prevented the use of chemi-
cal degradation, further paper chromatographic studies were carried out. 10 μg samples of vitamin B₁₂ were chromatographed on paper with water-saturated sec-butanol for 20 hours. Duplicate pilot strips were used to locate the areas of active materials. Once located, the two spots from the vitamin B₁₂ were cut out and eluted with water. The slow spot was treated with 50 γ of KCN at pH 6.5 for 5 hours at room temperature and then resubmitted on paper strips. As a control, a sample of the eluted slow spot was resubmitted without any treatment. The vitamin B₁₂ spot was treated in the same manner. These results are summarized in Fig. 2. The untreated slow moving compound gave only one spot, as does vitamin B₁₂ (7). The eluted slow spot that had been treated with KCN produced two zones, the faster corresponding in Rₚ value to vitamin B₁₂, the slower to the untreated slow moving compound. Vitamin B₁₂ acts similarly when allowed to react with KCN, producing two zones, vitamins B₁₂ and B₁₂. The eluted, chromatographed vitamin B₁₂ spot gave two zones, and KCN had no effect on the Rₚ values obtained. Vitamin B₁₂ behaves in the same manner by always producing two zones when chromatographed.

It is concluded from these data that vitamin B₁₂, like vitamin B₁₂, is a cyanide complex and that the cyanide can be replaced to form, presumably, a hydroxy analogue which is the slow moving compound.

Stability to Acid and Alkali—The stability of vitamin B₁₂ is very similar to that reported for vitamin B₁₂ by Hartley et al. (11). No loss in activity was noted by standing at pH 6.5 for 1 month at room temperature in diffused light. Activity was determined by the L. leichmannii assay. Autoclaving at 115° for 30 minutes at pH 7 and 6 resulted in a loss of approximately 10 per cent of the activity. Complete inactivation resulted by autoclaving at 115° for 30 minutes at pH 9.

Animal and Microbiological Growth Response—Vitamin B₁₂ failed to give a growth response in the rat at a level of 0.9 γ per day for 1 week (1). 1 γ per day has been administered to rats, both orally (by stomach tube) and by injection, for 2 weeks without any noticeable increase in growth. The rat assay of Register et al. (12, 13) was employed. A maximum growth response is produced in this assay by 0.2 γ per day of vitamin B₁₂. These data are shown in Table I. The daily administration of a mixture of 1 γ of vitamin B₁₂ and 50 mg. of an intrinsic factor concentrate also failed to elicit a growth response in the rat.

Recently Hawk and Elvehjem (14) have reported that vitamin B₁₂ does not prevent the development of hemorrhagic kidneys in young rats on a low choline diet.

Previously it was reported that vitamin B₁₂ was capable of producing

a growth response in the chick. These results were obtained with preparations of vitamin B$_{12f}$ which contained some vitamin B$_{12}$. When the vitamin B$_{12}$ was finally removed from the vitamin B$_{12f}$, it was found to be completely inactive in promoting growth in the chick. The chick assays of the vitamin B$_{12f}$ (containing no vitamin B$_{12}$) were performed by P. H. Derse. The assay ration consisted mainly of a corn-soy bean meal diet supplemented with salts and vitamins. The vitamin B$_{12f}$ was mixed into the ration and fed for a period of 4 weeks. Sixteen birds per group were used. The growth data are summarized in Table I, from which it may be seen that vitamin B$_{12f}$ possessed no vitamin B$_{12}$ activity in the chick.

The growth-promoting ability of vitamin B$_{12f}$ in *Escherichia coli* (15) and *Euglena gracilis* var. *bacillaris* (16) was tested and found to be approximately the same as in *L. leichmannii*.

### Table I

**Growth Response of Rat and Chick to Vitamin B$_{12f}$**

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Rat</th>
<th>Chick</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average growth, 2 wks.</td>
<td>Average growth, 4 wks.</td>
</tr>
<tr>
<td>None</td>
<td>42 gm.</td>
<td>149 gm.</td>
</tr>
<tr>
<td>1 γ vitamin B$_{12}$ per day, oral</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>1 &quot; &quot; &quot; &quot; &quot; injected</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>1 &quot; &quot; &quot; B$_{12}$ &quot; &quot; oral</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>3 &quot; &quot; B$_{12f}$ per kilo ration</td>
<td></td>
<td>139</td>
</tr>
<tr>
<td>3 &quot; &quot; B$_{12}$ &quot; &quot; &quot;</td>
<td></td>
<td>176</td>
</tr>
</tbody>
</table>

**Solubility in Solvent Mixtures**—Vitamin B$_{12f}$ can be salted-out of water solution and made to dissolve in n-butanol by saturating the water with ammonium sulfate and extracting with n-butanol. This process was used with considerable success in the isolation of vitamin B$_{12}$ from liver (17). The procedure could be employed in the fractionation of rat feces only after a stage of purification had been reached at which no material was precipitated upon saturation with ammonium sulfate. Vitamin B$_{12f}$ was carried down almost quantitatively in such precipitates. Extraction with n-butanol from ammonium sulfate-saturated solutions was used with success with urine samples. Vitamin B$_{12f}$ can be extracted from water solutions with phenol and recovered from the phenol by the addition of ether.

**Excretion and Storage in Rat**—Dietary cobalt produced a large increase

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3 Research Laboratories, Wisconsin Alumni Research Foundation, Madison.
4 Performed by J. S. Chiao, Department of Biochemistry, University of Wisconsin.
in the amount of vitamin B₁₂-active compounds in the intestinal tract of
the rat (1), but these compounds were not detected in the liver or urine.
Since vitamin B₁₂ was found in the intestinal tract, its absorption and ex-
cretion were studied in an attempt to determine the reason for its absence
from the liver.

Male weanling rats were depleted of their vitamin B₁₂ stores by placing
them on a corn-soy bean meal diet (rat assay diet without iodinated casein)
for 2 weeks. Eight rats were used per group. At the end of the 2 week
depletion period, vitamin B₁₂ and vitamin B₁₅ were administered to the
animals both orally (by stomach tube) and by injection for another 2 weeks.
1 γ per day was given. During the last 3 days of supplementation, the
animals were placed in metabolism cages and the urine and feces collected
quantitatively. The rats were then sacrificed and their livers removed.
The urine, feces, and livers were analyzed microbiologically for vitamin
B₁₂ by the L. leichmannii assay. Correction for the presence of desoxy-
ribosides in the urine was made by the alkaline hydrolysis procedure (18).
The high desoxyriboside content of the urine was attributed to ration that
had fallen into the urine. The data are summarized in Table II and repre-
sent the average values obtained with eight animals.

No increase in vitamin B₁₂ activity of the urine and only a slight increase
in the liver was noted when vitamin B₁₂ was given orally. The fecal
excretion was considerably larger, however. When the vitamin was in-
jected, there was an increase in liver vitamin B₁₂ activity, but not as great
as with an equivalent amount of vitamin B₁₂. For the most part the
injected vitamin B₁₂ was eliminated through the kidneys. The pathway
of excretion of vitamin B₁₂ has been reported (19, 20) and the results
obtained in these experiments are in agreement with those reports.

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Average liver weight</th>
<th>Average weight of feces per day</th>
<th>Vitamin B₁₂ activity of liver, average</th>
<th>Vitamin B₁₂ activity per liver, average</th>
<th>Vitamin B₁₂ activity excreted in feces, average</th>
<th>Vitamin B₁₂ activity excreted in urine, average</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7.6</td>
<td>1.0</td>
<td>17</td>
<td>127</td>
<td>2.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Vitamin B₁₂, oral</td>
<td>8.1</td>
<td>1.6</td>
<td>50</td>
<td>404</td>
<td>3.8</td>
<td>6.3</td>
</tr>
<tr>
<td>&quot; injected</td>
<td>8.4</td>
<td>1.9</td>
<td>80</td>
<td>631</td>
<td>2.8</td>
<td>5.2</td>
</tr>
<tr>
<td>&quot; B₁₂, oral</td>
<td>6.6</td>
<td>1.3</td>
<td>23</td>
<td>156</td>
<td>3.8</td>
<td>4.8</td>
</tr>
<tr>
<td>&quot; injected</td>
<td>7.5</td>
<td>1.3</td>
<td>44</td>
<td>333</td>
<td>2.4</td>
<td>3.1</td>
</tr>
</tbody>
</table>

* Corrected for desoxyribosides.
† No detectable amount.
administered vitamin $B_{12}$ was excreted primarily by way of the feces, while, when injected, it was eliminated through the kidneys. However, an increase in liver vitamin $B_{12}$ was noted with both the oral and injected vitamin $B_{12}$. The excretion of vitamin $B_{12}$ is, therefore, very similar to that of vitamin $B_{12f}$ but there is a difference in liver storage of the two forms.

Attempts were made to determine whether it was actually vitamin $B_{12f}$ that was being stored in the liver when the material was injected. Unfortunately, the concentration of the vitamin $B_{12f}$-active compounds was so low that clearly separated zones could not be obtained when the liver samples were chromatographed.

The urine collected from the vitamin $B_{12f}$-injected rats was tested successfully by means of paper chromatography. A 3 day sample of urine was saturated with ammonium sulfate and then extracted with n-butanol. Enough of the butanol extract was spotted on the paper strips so that 5 mg/ml of vitamin $B_{12f}$-active materials were present on each strip. The strips were developed with water-saturated sec-butanol at room temperature for 20 hours. Only vitamin $B_{12f}$ and a faint zone corresponding to the slow moving compound could be detected in the urine.

Distribution in Natural Materials—Paper chromatography in conjunction with the bioautographic technique was applied to the study of the distribution of vitamin $B_{12f}$ in various natural materials. The solvent system used was water-saturated sec-butanol with descending development for 20 hours at room temperature.

Fecal samples were collected immediately after defecation and dried for 6 hours at 90°. They were then ground to pass a 200 mesh screen. Sheep rumen contents, obtained through a fistula, were dried and ground in the same manner as the feces. 10 gm. of the material were boiled with 100 ml. of water for 10 minutes to release and extract the vitamin $B_{12f}$-active materials. The mixture was filtered and concentrated under a vacuum to approximately 20 ml. A 1:10 homogenate was made of liver samples, which was then heated in a boiling water bath for 10 minutes, filtered, and concentrated. These preparations were assayed microbiologically with L. leichmannii for total vitamin $B_{12}$ activity and then spotted on the paper strips with a micro pipette so that 5 mg/ml of vitamin $B_{12f}$-active compounds were present on each strip. The results obtained are shown in Fig. 3. Table III contains the microbiological assay values of the fecal samples.

Vitamins $B_{12}$ and $B_{12f}$ gave sharp, slightly elliptical zones of growth with negligible tailing. The spots obtained with the natural materials were

*Supplied by W. G. Hoekstra, Department of Biochemistry, University of Wisconsin.
definite, but there was considerably more tailing. Vitamin $B_{12f}$ was found in the feces of the horse, sheep, cow, pig, chicken, guinea pig, and man.

![Figure 3. Distribution of vitamin $B_{12f}$ in various natural materials](image)

**Table III**

*Vitamin $B_{12}$ Content of Fecal Samples As Measured by *L. leichmannii*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Activity as vitamin $B_{12}$, dry weight $\gamma$ per 100 gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse feces</td>
<td>10</td>
</tr>
<tr>
<td>Pig feces</td>
<td>40</td>
</tr>
<tr>
<td>Chicken feces*</td>
<td>300</td>
</tr>
<tr>
<td>Cow feces</td>
<td>30</td>
</tr>
<tr>
<td>Sheep feces</td>
<td>12</td>
</tr>
<tr>
<td>Guinea pig feces</td>
<td>120</td>
</tr>
<tr>
<td>Human feces*</td>
<td>140</td>
</tr>
<tr>
<td>Rat feces*</td>
<td>180</td>
</tr>
<tr>
<td>&quot; cecal contents</td>
<td>240</td>
</tr>
<tr>
<td>Sheep rumen contents</td>
<td>25</td>
</tr>
</tbody>
</table>

*Vitamin $B_{12}$ in the diet. The values reported previously ((1) Table III) are for dry weight instead of wet weight.

From the area and density of the zones, there appeared to be a higher concentration of vitamin $B_{12f}$ and the slow moving compound than of vitamin $B_{12}$. No quantitative work was carried out in this respect, and this conclusion was drawn solely from the complete absence of the vitamin...
B₁₂ zone or, if present, from its relative size and density on the agar plate. The densest vitamin B₁₂ spot was obtained with the chicken feces, undoubtedly caused by the presence of a vitamin B₁₂ concentrate in the diet of the chickens. The other farm animals and guinea pigs received no dietary source of vitamin B₁₂. The human feces were obtained from individuals eating a normal mixed diet. While no definite statement can be made concerning the actual concentration of the various forms of vitamin B₁₂ in these fecal samples, the data do indicate that vitamin B₁₂f, or at least a compound with an Rₚ value similar to vitamin B₁₂f, is present in the intestinal tract of numerous types of animals.

Groschke et al. (21) have reported that fresh pig manure contained no vitamin B₁₂ activity for the chick, while incubated pig manure was highly active. Since vitamin B₁₂f and not vitamin B₁₂ was noted in fresh pig manure, it appeared of interest to determine the forms of vitamin B₁₂ present after incubation. Manure samples from pigs receiving no vitamin B₁₂ supplement were collected and divided in half. One portion was immediately dried at 90° for 6 hours and the other allowed to incubate for 4 days at 37°. The two samples were then analyzed by paper chromatography by the procedure described above. Only vitamin B₁₂f and the slow moving compound were detected in the fresh manure, while these two forms together with vitamins B₁₂ and B₁₂b were noted in the incubated manure. The fresh pig manure had a microbiological assay value of 40 γ per 100 gm., the incubated 160 γ per 100 gm. (dry weight).

Vitamin B₁₂f was found in the fecal matter of rats fed the corn-soy bean meal ration (1) whether or not they received additional cobalt. The new compound was also present in the feces of rats raised on a complete stock diet and in the fecal matter of rats fed a purified casein-sucrose diet. The rat fecal samples could not be collected absolutely fresh in sufficient quantities. To determine whether vitamin B₁₂f was actually produced in the intestinal tract and not synthesized after defecation, cecal contents of rats were removed, immersed in alcohol, and immediately dried. The dried material was extracted and chromatographed as described above. Under these conditions, only vitamin B₁₂f and the slow moving compound were detected. No vitamin B₁₂ was noted. Since the Rₚ values of the slow moving compound and vitamin B₁₂b are very similar, while the vitamin B₁₂f and vitamin B₁₂ zones are quite widely separated, the cecal contents were treated with KCN in order to convert any undetected vitamin B₁₂b into vitamin B₁₂. A water extract of the cecal contents (from rats fed the corn-soy bean meal diet) containing 3 γ of vitamin B₁₂ activity was allowed to react with 3 mg. of KCN at pH 6.5 for 5 hours at room temperature. 5 mμgm. of vitamin B₁₂-active materials were then spotted on strips and chromatographed. No vitamin B₁₂ zone was detected, but
the vitamin B₁₂f zone appeared more dense than in samples of untreated cecal contents. Therefore, it is assumed that these samples contained little if any undetected vitamin B₁₂f.

Sheep rumen contents and one sample of fish solubles were also found to be sources of vitamin B₁₂f. Only a small amount appeared to be present in the sample of fish solubles tested and the major portion of the vitamin B₁₂-active materials could be attributed to vitamins B₁₂ and B₁₂f. However, since only one sample of fish solubles was available for testing, these results cannot be considered representative. Rumen contents contained considerable vitamin B₁₂f and the slow moving compound together with some vitamin B₁₂. Vitamin B₁₂f could not be detected in either rat or beef liver, nor was it noted in Reticulogen. The rat liver was obtained from rats fed a stock diet containing whole liver powder.

DISCUSSION

Since vitamin B₁₂f has been found to be a cyanide complex, it can be assumed that it is not an analogue of vitamin B₁₂ formed by the replacement of the cyanide anion. The marked difference in the physiological action of the two substances also indicates that the structural difference is greater than simply a change in the anion coordinated to the cobalt atom. All of the anion analogues of vitamin B₁₂ that have been reported (22, 23) possess growth-stimulating properties in rats and chicks, but vitamin B₁₂f is inactive in this respect.

Although vitamin B₁₂f was found in considerable quantities in the fecal matter of numerous types of animals, no physiological function in higher animals has been noted. Furthermore, the fact that it is present in the intestinal tract and yet absent from the liver is puzzling. Under normal conditions, vitamin B₁₂f may be bound in the cells of the microorganisms which, presumably, synthesize it in the intestinal tract, and may, therefore, be unavailable to the host. This does not necessarily explain why orally administered vitamin B₁₂f is not detected in the rat's liver. Likewise, when vitamin B₁₂f is injected, the vitamin B₁₂ activity of the liver is less than when an equivalent amount of vitamin B₁₂ is given. It would appear that vitamin B₁₂f is broken down in some manner before or after reaching the liver. Assuming that vitamin B₁₂f and pseudovitamin B₁₂ are identical, an explanation may be that the vitamin B₁₂f, containing adenosine (24) instead of the ribazole moiety (25), is cleaved by certain ribonucleosidases (26) and does not remain intact within the body. It would be of interest to know whether ribonucleosidases are capable of inactivating vitamin B₁₂f and not vitamin B₁₂. If such an inactivation does take place, it is not complete, since some injected vitamin B₁₂f is detected unchanged in the urine.
The question also arises as to what actually is the active form of vitamin B\textsubscript{12}. Microorganisms appear to use both vitamin B\textsubscript{12} and vitamin B\textsubscript{12f} interchangeably, but it is not known whether one form must be converted into the other before it is physiologically active.

A comparison of the rat and chick growth data and the \( R_f \) values obtained with vitamin B\textsubscript{12f} and pseudovitamin B\textsubscript{12} suggests that the two are identical.\(^7\) The only difference noted is in their absorption spectra. While both possess an absorption maximum at 278 m\( \mu \), this peak is much larger in vitamin B\textsubscript{12f} than in pseudovitamin B\textsubscript{12}; otherwise, the spectra are nearly identical. Since such small quantities of vitamin B\textsubscript{12f} are being handled, it may be that a slight trace of an impurity which absorbs in the 278 m\( \mu \) region is still present and is responsible for the strong peak in vitamin B\textsubscript{12f}.

Hausmann (27, 28) has reported a bound form of vitamin B\textsubscript{12} in cow dung and in fish solubles which is inactive in pernicious anemia until digested with a proteolytic enzyme or treated with HCN. Since in the isolation of vitamin B\textsubscript{12f} both boiling and enzyme digestion are employed, it is unlikely that vitamin B\textsubscript{12f} is a bound form. Vitamin B\textsubscript{12f} has not been tested in pernicious anemia; however, pseudovitamin B\textsubscript{12} fails to cause a remission of symptoms. Wijmenga's vitamin B\textsubscript{12m*} from pig manure is active in pernicious anemia.

The growth-promoting property of vitamin B\textsubscript{12f} in the L. Leichmannii, E. coli, and Euglena assays indicates that caution should be used in analyzing materials in which bacterial action is known to have taken place.

**SUMMARY**

Vitamin B\textsubscript{12f}, isolated from rat feces, has been shown to be a cyanide complex. It is completely inactive in promoting growth in the rat and chick, but does possess growth-stimulating properties in Lactobacillus leichmannii, Escherichia coli, and Euglena gracilis.

In the rat orally administered vitamin B\textsubscript{12f} is excreted primarily by way of the feces, while, when injected, elimination is principally through the kidneys. A definite increase in vitamin B\textsubscript{12} activity of liver was noted with injected vitamin B\textsubscript{12f}, but, when orally administered, only a slight increase took place.

By paper chromatography a compound with an \( R_f \) value corresponding

\(^7\) An exchange sample of pseudovitamin B\textsubscript{12}, supplied by Dr. H. W. Dion, Parke, Davis and Company, Detroit, Michigan, was chromatographed according to our procedure. One major zone and a faint, slow moving zone were detected. These two spots corresponded exactly in \( R_f \) values to the two zones obtained with vitamin B\textsubscript{12f}.

\(^*\) Wijmenga, H. G., doctoral thesis, Utrecht, by personal communication. See also the review by Wijmenga and Veer (29).
to vitamin B$_{12i}$ was noted in the fecal matter of the cow, sheep, pig, horse, chicken, guinea pig, and man. Sheep rumen contents were also a source of the compound. No vitamin B$_{12f}$ was detected in rat or beef liver nor in Reticulogen.

Data are presented which suggest that vitamin B$_{12i}$ is identical with pseudovitamin B$_{12i}$.

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**BIBLIOGRAPHY**

PROPERTIES AND DISTRIBUTION OF VITAMIN B₁₂
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