The absorption of vitamin B₁₂ in normal and gastrectomized rats and the effect of some gastric extracts

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The advent of radioactive vitamin B₁₂ has made it possible to study quantitative absorption of the vitamin across the intestinal wall. In experiments on human beings, Heinle, Welch, Scharf, Meacham & Prusoff (1952) and Callender, Turnbull & Wakisaka (1954) based their results on the response to an administered dose of 0.5 μg vitamin B₁₂; the amount of vitamin absorbed was determined from the radioactivity of the faeces by simply subtracting the counting rate from that corresponding to the orally administered dose. With these techniques it has been possible to show that there is a diminution of absorption in patients with pernicious anaemia. The amount of vitamin excreted was about 80% of the amount administered, but the high level of excretion in these patients could be brought within the normal range, which has a mean of about 20%, by administering substances containing intrinsic factor.

It has also been shown that patients who have been subjected to total gastrectomy behave in their vitamin B₁₂ absorption in the same way as patients with pernicious anaemia (Swendseid, Halsted & Libby, 1953). This observation led us to carry out similar studies in gastrectomized rats, which obviously have great experimental advantages over human beings and might well serve for assay of intrinsic-factor preparations.

A brief preliminary report of part of the work described here has already been published (Clayton, Latner & Schofield, 1955).

EXPERIMENTAL

All the observations recorded in this paper were made on black-and-white adult rats of the Scott-Russ strain. Although some females were included, most of the animals were male. The ages of the animals ranged from 3 to 18 months and their weight from 300 to 400 g.

The experiments carried out included observations on the relationship of vitamin absorbed to the amount of vitamin administered in the normal rat. In addition to these observations, experiments have also been made on the change in absorption of
the vitamin in normal and gastrectomized rats when human or rat gastric juice, or alternatively a pig intrinsic-factor preparation of high potency, was administered. The materials were not mixed with the vitamin $B_{12}$ but administered from a separate pipette, the animal being allowed to sip alternately from the tip of each pipette.

For the study of absorption, animals were deprived of water for $16$ h and of solid food for $4$ h.

For observations other than those concerned with varying dosage, $16$ m$\mu$g vitamin $B_{12}$ labelled with $^{60}$Co dissolved in $1.5$ ml. tap water were administered with a pipette. It was found unnecessary to administer the material by stomach tube.

The animals drank the liquid rapidly and quantitatively. They were then immediately allowed to drink water, but solid food was not offered for a further $4$ h. The faeces were collected in a metabolism cage, designed to separate them from urine, for a period of $7$ days after administration of the radioactive vitamin $B_{12}$. All the faeces were separated into five approximately equal portions, and each portion was homogenized with sufficient water to bring the final volume to $50$ ml. The radioactivity of each portion was then determined with a scintillation counter. From the total radioactivity of the faeces it was simple to determine the percentage of radioactive vitamin absorbed, by comparing the results with those from $16$ m$\mu$g of the original $^{60}$Co-labelled vitamin $B_{12}$ also dissolved in $50$ ml. water. Animals were observed in this manner both before and after gastrectomy.

The dosage of vitamin employed for comparing normal and gastrectomized animals was, on a weight-for-weight basis, some three times greater than that used in similar experiments on man, so as to improve accuracy and to bring the readings well within the range of sensitivity available to us at the time. The counting accuracy of each observation was in the range of $\pm 1\%$.

Gastrectomy was carried out under ether anaesthesia. The oesophagus was divided just below the oesophago-gastric junction and the duodenum just distal to the pylorus. The duodenal stump was closed and the oesophagus anastomosed to the side of the duodenum $1$–$2$ cm from the closed end. After operation the animals were given nothing by mouth for $2$ days. During this period $10$ ml. of $0.9\%$ saline containing $5\%$ glucose and $20,000$ units of penicillin were injected subcutaneously night and morning. On the $3$rd day the animals were given milk and a low-residue carbohydrate diet made up according to the formula of Sognnaes (1948). After this they were gradually transferred to their normal diet of rat cubes, first powdered and later whole.

Apart from the test dose no attempt was made to control the weight of vitamin $B_{12}$ ingested in the normal diet. It was considered that the fasting period before and after the test drink provided a sufficiently uniform environment for consistent results and that the normal body vitamin $B_{12}$ level did not affect the amount of vitamin absorbed.

Gastric juice was obtained from the rats by the method reported by Shay, Sun & Grunstein (1954). The animals were given glucose saline only for $48$ h, and then the pylorus was tied with a silk ligature under brief ether anaesthesia. After $4$ h the animals were again anaesthetized with ether, the stomachs were removed and the accumulated gastric secretion was collected. It was centrifuged to remove debris, neutralized with $0.1\%$ NaOH and stored frozen at $-25\%$. 
RESULTS

Relationship of vitamin $B_{12}$ absorbed to vitamin $B_{12}$ ingested in the normal rat

By varying the administered dose it was possible to find the relationship between the amount ingested and that absorbed. The result is illustrated in Fig. 1, and was obtained by repeated observations on one animal only. It can be seen that the proportion of vitamin absorbed decreased markedly at the higher dose level, whereas at dose levels of 5 m$\mu$g, or less, there was complete excretion in the faeces. This last observation was confirmed in a number of animals.

Comparison of normal and gastrectomized rats

The range of absorption in normal and gastrectomized animals for a constant ingested weight of 16 m$\mu$g vitamin $B_{12}$ is given in Table 1. The reduction of absorption in the gastrectomized animals was established beyond doubt.

![Graph showing relationship of vitamin $B_{12}$ absorbed to vitamin $B_{12}$ ingested by a rat given different doses of the vitamin.](image)
Table 1. *Vitamin B₁₂* absorption in normal and gastrectomized rats

<table>
<thead>
<tr>
<th>Test substance</th>
<th>No. of experiments</th>
<th>Mean absorption with its standard error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats given 16 μg vitamin B₁₂ only</td>
<td>13</td>
<td>42.9 ± 1.0</td>
</tr>
<tr>
<td>Gastrectomized rats given 16 μg vitamin B₁₂ only</td>
<td>7</td>
<td>5.5 ± 1.1</td>
</tr>
</tbody>
</table>

Table 2. Absorption by normal and gastrectomized rats of vitamin B₁₂ when given simultaneously with either rat gastric juice, human gastric juice or a preparation of pig intrinsic factor

| Test substance | Normal rats | | Gastrectomized rats | | |
|----------------|-------------|----------------|-------------------|----------------|
| Vitamin B₁₂ and rat gastric juice | 2 | 6 | 40.9 ± 2.5 | 3 | 16 | 15.9 ± 1.2 |
| Vitamin B₁₂ and human gastric juice | 6 | 14 | 25.4 ± 2.5 | 3 | 4 | 15.5 ± 0.8 |
| Vitamin B₁₂ and pig intrinsic-factor preparation | | | | 2 | 4 | 6.5 ± 0.6 |

Table 3. Observations on individual gastrectomized rats which received rat gastric juice

<table>
<thead>
<tr>
<th>Material administered</th>
<th>Rat no.</th>
<th>No. of observations</th>
<th>Mean absorption of vitamin B₁₂ with its standard error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B₁₂ alone</td>
<td>1</td>
<td>6</td>
<td>3.3 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9</td>
<td>1.8 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>1.8 ± 0.9</td>
</tr>
<tr>
<td>Vitamin B₁₂ with rat gastric juice</td>
<td>1</td>
<td>5</td>
<td>13.4 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9</td>
<td>18.5 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>10.7 ± 0.7</td>
</tr>
</tbody>
</table>

Effect of administering gastric juices

In Table 2 the results of giving rat gastric juice and human gastric juice are shown. In normal animals rat gastric juice had little or no effect when administered in amounts between 0.5 and 4 ml. Given simultaneously with the vitamin, human gastric juice severely inhibited absorption of the vitamin in the normal animal (*t* = 6.4, *P* < 0.0001). There also appeared to be some significant reduction of absorption in the gastrectomized animal (*t* = 3.2, *P* < 0.01).

In sixteen experiments on three gastrectomized animals there was an increase in absorption when rat gastric juice was administered, though the mean value did not return to that of the normal rat (shown in Table 1). The results with these three gastrectomized animals are shown in somewhat greater detail in Table 3. It will be seen that each animal acted as its own control. In each there was a significant increase
Absorption of vitamin B₁₂ in rats

in absorption on administration of rat gastric juice. (The \( t \) values were 5·2, 7·6 and 8·5; for each \( P \) was <0·001.)

Effect of administering an intrinsic-factor preparation

A pig intrinsic-factor preparation was studied in the gastrectomized animals and showed no significant effect on absorption, as seen from Table 2.

DISCUSSION

The results obtained relating the amount of vitamin B₁₂ absorbed to the amount ingested indicate that there may be an absorption saturation level above which an increased weight of vitamin ingested does not result in further absorption. This finding would agree with those in human beings reported by Swendseid, Gastro & Halsted (1954) and would indicate that the intestinal mucosa has an absorbing power limited to a specific maximal amount. This limitation may be due to the relatively fixed amount of intrinsic factor available from the normal stomach or to factors affecting the absorbing power of the intestinal mucosa. Glass, Boyd & Stephanson (1954) and Baker & Mollin (1955) have produced evidence to indicate that such a limitation in intestinal absorbing power does exist in the human being. Their observations, however, were restricted to a study of absorption in relation to increasing administration of intrinsic factor in abnormal individuals with pernicious anaemia or after total gastrectomy.

It is interesting to note that with the ingested dose in the rat as low as 5 m\( \mu \)g there was no absorption at all, which must mean that in some way this amount of vitamin is rendered unavailable for absorption. It is possibly bound to intestinal contents and passes out with the faeces. This observation is not in accordance with the results of Watson & Florey (1955), who obtained appreciable absorption at this dosage level. To explain this difference, we have tried the effect of a diet made up according to the instructions of these workers, but the absorption results we obtained were closely similar to those already quoted. It may be that the difference in results is related to the difference in strains of rat employed.

It could be that at the 5 m\( \mu \)g dose level a significant portion of radioactivity excreted in the faeces resulted from traces of activity other than \(^{60}\)Co from the test dose. This possibility can be excluded, however, since we have carried out measurements on the radioactivity of the faeces of animals that did not receive any radioactive vitamin B₁₂ but were given the usual diet. At the time these experiments were performed, this source of error was not appreciable (less than 2% of activity obtained with the test dose of 15 m\( \mu \)g). We therefore thought it unnecessary to take any special precautions with the scintillation counter in relation to pulse height in order to increase selectivity.

The standard test dose of 16 m\( \mu \)g used in the other absorption experiments is seen (Fig. 1) to fall on the linear portion of the ingestion-absorption curve, where the absorbed dose is a constant percentage of that administered. This dose corresponds to three times the dose per unit body-weight given in the experiments on man by
Heinle et al. (1952). On our findings an equivalent dose would correspond to zero uptake as observed at the 5 μg level.

There seems little doubt that the absorption of vitamin B₁₂ by gastrectomized rats was significantly less than that by the normal animals; such a finding could be interpreted as indicating the presence of intrinsic factor in the rat stomach, but might on the other hand be due to disturbances of gastro-intestinal function caused by the severe operation. The fact that rat gastric juice increases absorption in the gastrectomized rat is in favour of the existence of a rat intrinsic factor produced in the stomach. Unlike other workers (Watson & Florey, 1955) we have been unable to restore absorption to normal. This may possibly be because we performed a more extensive gastrectomy operation and so produced greater disturbance of function, including perhaps a more hurried passage through the small intestine. This possibility has been discussed by Nieweg, Arends, Mandema & Castle (1956).

We attempted to raise the absorption in the gastrectomized animal by increasing the volume of rat juice given, but the results were not encouraging. It was difficult to persuade the animals to take quantities of juice greater than 3 ml. in addition to the vitamin solution, and absorption with this quantity was not consistently greater than with smaller quantities. In one experiment we used the equivalent of 6 ml. reduced in volume by freeze drying, but the absorption was not greater than with 1·5 ml. ordinary juice.

Further evidence for the existence of a rat intrinsic factor is produced by the observation that a rat-stomach extract increases vitamin B₁₂ absorption in a tied-off intestinal loop in an otherwise intact rat (Holdsworth & Coates, 1956).

Human gastric juice significantly inhibited the absorption of vitamin B₁₂ in the normal rat. This might mean that there is a species specificity of intrinsic factor and that the human substance acts as a blocking agent to an enzyme mechanism involved in rat intrinsic factor. Another explanation would be the presence in human gastric juice of a substance or substances that bind vitamin B₁₂ in such a way as to render it unavailable to the rat. Such an inhibitory substance has recently been prepared from pig mucosa (Latner & Merrills, 1956), and it is thus not surprising that the pig intrinsic-factor preparation we have used (Latner, Merrills & Raine, 1954) also showed an inhibitory effect in two experiments with intact animals, not reported in this communication. Rosenblum, Woodbury & Reisner (1954) and Chow, Quattlebaum & Rosenblum (1955) have also demonstrated a similar inhibition.

**SUMMARY**

1. Radioactive vitamin B₁₂ has been given orally to normal and gastrectomized rats and the absorption has been determined from estimation of faecal radioactivity.

2. The relation between absorbed and ingested weights of the vitamin has been studied in normal animals. Over the range studied the percentage weight absorbed did not remain constant; with doses of 5 μg or less there was no absorption at all.

3. For a constant dose of 16 μg vitamin B₁₂ normal rats showed a mean absorption of 42·9 ± 1·0%. In gastrectomized animals it was reduced to 5·5 ± 1·1%.
4. Rat gastric juice given simultaneously with the vitamin produced no change in the absorption in normal rats, but raised that of the gastrectomized rats to 15.9 ± 1.2%. Though this increase was highly significant, it was not possible to increase the absorption to normal levels with rat gastric juice administered orally.

5. Human gastric juice depressed the absorption in normal rats to 25.4 ± 2.5% and in gastrectomized rats to 1.5 ± 0.8%.

6. A pig intrinsic-factor preparation did not affect the absorption when given to the gastrectomized animal.

7. Our results are compatible with the production of an intrinsic factor by the rat stomach, but the total-gastrectomy preparation employed is not suitable for the testing of intrinsic-factor substances for human use.

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REFERENCES