Oral Treatment of Pernicious Anemia with Small Doses of Vitamin B\textsubscript{12} Combined with Mucinous Materials Derived from the Hog Stomach

By George B. Jerzy Glass, M.D., and Linn J. Boyd, M.D.

A STUMBLING BLOCK in the oral treatment of pernicious anemia has been the necessity to administer both extrinsic and intrinsic hematopoietic factors in adequate dosage and proper mutual ratio. This was hampered for many years by ignorance regarding their nature. Therefore, the history of the oral treatment of pernicious anemia has followed closely the progress in processing both of these hematopoietic factors in a more and more pure and potent form.

As long as the nature of both hematopoietic factors was unknown, the oral treatment of pernicious anemia required the administration of organs or body fluids rich in these substances, such as crude liver\textsuperscript{4} or desiccated hog stomach\textsuperscript{5-11} and duodenum.\textsuperscript{12} Also, beef as a source of extrinsic factor administered with human normal gastric juice, as the source of intrinsic factor, produced adequate hematopoietic responses in patients with pernicious anemia.\textsuperscript{7,9} When vitamin B\textsubscript{12} (cyanocobalamin) was identified as at least one of the extrinsic hematopoietic factors,\textsuperscript{10,11} the original Castle experiment with beef and gastric juice could be repeated, and a satisfactory hematopoietic response was obtained when small doses of vitamin B\textsubscript{12}, ineffective alone, were given as a substitute for meat with normal human gastric juice.\textsuperscript{10,11,16}

Using animal sources of gastric intrinsic factor and vitamin B\textsubscript{12}, Bethell, et al.,\textsuperscript{17,19} Hall, et al.,\textsuperscript{19} and Meyer, et al.\textsuperscript{20} obtained suboptimal or optimal hematopoietic responses in over twenty patients with pernicious anemia when from 5 to 10 \textmu{g} of vitamin B\textsubscript{12} were given daily by mouth, together with from 10.0 to as little as 1.0 Gm. of desiccated hog pyloric or duodenal mucosa (site of intrinsic factor activity in hogs\textsuperscript{21,22}) or its extracts.

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Hall\textsuperscript{19} obtained an optimal hematopoietic response in one of his cases, and Spies\textsuperscript{23} a moderate effect in another, when a complex preparation containing only 0.33 Gm. of hog duodenal mucosa extract "binding" 9 \textmu g. of vitamin B\textsubscript{12} were given daily to their respective patients with pernicious anemia. The striking progress is evident when one compares this dose to that of 40 Gm. of desiccated stomach, which was required twenty years ago for therapy. However, it must be noted that the treatment periods were of very short duration in these foregoing studies\textsuperscript{17-19} (seven to eighteen days, and usually under two weeks) so that thus far no complete clinical or hematologic remission has been demonstrated with this form of therapy.

In our investigations on gastric mucous substances in man it occurred to us that the elusive intrinsic gastric hematopoietic factor of Castle could be related to one of the mucoproteins present within the heterogeneous complex of mucin dissolved in the human gastric juice.\textsuperscript{22} We became especially interested in the relationship of intrinsic factor to one of these mucoproteins which we named "glandular mucoprotein" because of its origin from the glands of the fundus and corpus of the stomach,\textsuperscript{24} the site of formation of intrinsic factor in man,\textsuperscript{21, 22} and the area which undergoes most severe atrophic lesions in pernicious anemia.\textsuperscript{26-29}

Because of its site of origin, glandular mucoprotein is entirely lacking (or present only in small amounts) in individuals with pernicious anemia,\textsuperscript{24, 30} even when the strongest stimulus for its secretion is applied.\textsuperscript{21} The absence of glandular mucoprotein from the stomach of patients with pernicious anemia contrasts with the regular occurrence of this substance in the gastric juice of patients with other nonpernicious anemias, as well as with the normal occurrence of other mucous substances of surface epithelial origin (visible mucus, dissolved mucoproteose\textsuperscript{32}) in the stomach of patients with pernicious anemia.\textsuperscript{24}

Considerable evidence points to the similarity of glandular mucoprotein to intrinsic factor; as for example, some of the physicochemical data and physiologic features of both these substances; these are reported at length elsewhere.\textsuperscript{32}

Direct evidence for intrinsic factor activity of a mucin fraction containing glandular mucoprotein was obtained in studies on patients with pernicious anemia\textsuperscript{22} and macrocytic nutritional nonpernicious anemia.\textsuperscript{33} In two patients with macrocytic nutritional nonpernicious anemia who had a normal concentration of glandular mucoprotein in the stomach, a good hematopoietic response was obtained with small doses (10 to 30 \textmu g.) of vitamin B\textsubscript{12} by mouth.\textsuperscript{34} In all of the nine patients with pernicious anemia, who lacked glandular mucoprotein in the stomach, no hematopoietic response was obtained under these conditions, except a trivial reticulocytosis without rise in erythrocytes in two of these cases. However, when a daily dose of from 50 to 200 mg. of glandular mucoprotein, processed from gastric juices of normal individuals or patients with duodenal ulcer, was added to the small doses of vitamin B\textsubscript{12}, seven out of nine cases had a suboptimal or optimal hematopoietic response, associated with disappearance of megaloblasts from the bone marrow, complete clinical remission, and rapid improvement of the neurologic signs of combined degeneration of the cord.\textsuperscript{32}

In view of this definite intrinsic factor activity of a mucin fraction of human gastric juice containing glandular mucoprotein, it occurred to us that the extracts from the mucosal lining of the hog stomach or duodenum used by Bethell
et al.\textsuperscript{17} and Hall, et al.\textsuperscript{19} as a source of intrinsic factor were active because they contained one or more mucoproteins exhibiting intrinsic factor activity. This becomes still more plausible when one considers the similarity of the techniques used for precipitation of the intrinsic factor containing mucinous materials from extracts of the animal stomach or duodenum\textsuperscript{17} and those used for processing gastric mucin from mucosal lining of the hog (Fogelson\textsuperscript{34}), or precipitation of mucoproteins from human gastric juice.*

We became interested in the various mucinous gastric fractions of animal origin in regard to their possible intrinsic factor activity, especially since the glandular mucoprotein from human gastric juice could not be processed in adequate amounts for large scale oral treatment of pernicious anemia. This report will deal with the assay of intrinsic factor activity of various mucinous materials of the hog stomach, and their applicability in association with small oral doses of vitamin \(B_12\) for the oral treatment of pernicious anemia.

**METHOD OF STUDY AND MATERIALS**

The study was conducted on twenty clinically and hematologically proven cases of pernicious anemia, of which ten were studied in the Eli Lilly Research Laboratories and Hospitals, and ten in the wards of the Flower and Fifth Avenue Hospitals and the New York Medical College Research Unit, Metropolitan Hospital Division, New York City. Of the latter ten cases, seven were in full relapse or in the initial period of full blown disease, and all of them had typical hematologic features of macrocytic anemia, megaloblastic bone marrow, absence of free acid in gastric juice after histamine stimulation, and more or less pronounced symptoms of combined degeneration of the cord.

The method of study complied in general with that recommended by the Anti-Anemia Preparations Advisory Board of the U. S. Pharmacopeia for the testing of products that are intended for the oral therapy of pernicious anemia patients. All of them were fed diets poor in vitamin \(B_12\), with elimination of liver, meat, fish, and allowance of only one egg, two glasses of milk, and one serving of cheese a day. In each case, daily reticulocyte counts and blood counts were performed from three to five times a week. Hematocrit values were determined twice a week, and bone marrow studies, two or three times during the experimental period. The same experienced hematologic technicians performed the reticulocyte and blood counts in each of their cases continuously so that comparable serial figures were obtained, and the error due to individual differences in counting technics largely eliminated.

In eight cases studied in the wards of the Flower and Fifth Avenue Hospitals and the Metropolitan Hospital Research Unit, a control period of from ten to fourteen days preceded the proper test. During this control period, vitamin \(B_12\) alone was given daily by mouth in a dose roughly similar to that to be used later during the actual therapeutic period.

Vitamin \(B_12\) was used as the source of extrinsic factor: Patients 1, 2, 3, 9a, and 10 were supplied with vitamin \(B_12\) in gelatin capsules in doses of 5 \(\mu g\) per capsule; Patients 9b, 13, 14, 15, and 16, during the control period, received tablets containing 5 \(\mu g\) Cyanocobalamin, U.S.P., employing a tablet granulate identical to the one employed for the mucous fractions.

* The fact that no search for intrinsic factor has been made so far among mucoproteins of the stomach is due, we believe, to the report\textsuperscript{35} of the separation of intrinsic factor from human gastric mucin by absorption of the latter (together with pepsin and rennin) on tricalcium phosphate or Lloyd's reagent. Since it is now known that gastric mucin is heterogeneous,\textsuperscript{31} possibly these authors precipitated only one part of mucoproteins with those reagents. This is suggested by the presence of as much as 26 per cent of initial total content of nitrogen in the filtrate and by formation of precipitate in this filtrate after full saturation with ammonium sulfate.

† Supplied by Organon Inc., through the courtesy of Dr. Kenneth W. Thompson, vice-president.
### Table 1—Hematopoietic Responses after Oral Administration of Vitamin B_{12} with Commercial Mucin Preparation

<table>
<thead>
<tr>
<th>Case Description</th>
<th>Daily oral treatment</th>
<th>Reticulocytes (per cent)</th>
<th>Red cell count (m. per cu. mm.)</th>
<th>Hematocrit (per cent)</th>
<th>Hemoglobin (Gm./100 cc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Duration days</td>
<td>Start</td>
<td>Maximal value % Day</td>
<td>Start</td>
</tr>
<tr>
<td>1. B. M., 83 yrs., female</td>
<td>10 μg. vit. B_{12}</td>
<td>14</td>
<td>0.2</td>
<td>0.5</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>3 Gm. comm. mucin</td>
<td>12</td>
<td>0.5</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>10 μg. B_{12} plus 3 Gm. comm. mucin</td>
<td>15</td>
<td>0.3</td>
<td>0.8</td>
<td>4.6</td>
</tr>
<tr>
<td>2. T. M., 40 yrs. male</td>
<td>10 μg. vit. B_{12}</td>
<td>13</td>
<td>1.0</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>3 Gm. comm. mucin</td>
<td>13</td>
<td>1.6</td>
<td>0.7</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>10 μg. B_{12} plus 3 Gm. comm. mucin</td>
<td>13</td>
<td>0.7</td>
<td>2.8</td>
<td>3.5</td>
</tr>
<tr>
<td>3. I. C., 85 yrs. female</td>
<td>10 μg. vit. B_{12}</td>
<td>13</td>
<td>1.2</td>
<td>0.5</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>3 Gm. comm. mucin</td>
<td>13</td>
<td>0.5</td>
<td>0.5</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>10 μg. B_{12} plus 3 Gm. comm. mucin</td>
<td>13</td>
<td>0.5</td>
<td>1.1</td>
<td>3.1</td>
</tr>
</tbody>
</table>
The following mucinous materials were used as sources of intrinsic factor:

1. Commercial mucin powder,* cases 1, 2, 3.

2. Mucus substances processed from the pyloric mucosa of hogs†: (a) lyophilized extracts prepared by extraction with 10 per cent acetic acid, cases 4, 5, 6; (b) lyophilized extracts prepared by extraction with 0.1 N HCl, precipitation by ammonium sulphate saturation, subsequent dialysis and filtration, cases 7, 8, 17, 18; (c) acetone-dried powders prepared by extraction with 0.1 N HCl, subsequent precipitation with two volumes acetone, and drying of the precipitate in vacuum, cases 11, 12, 19; (d) fractions prepared by the same technic as under 2b, but subject at a later stage to fractionation in the Kirkwood electroconvection apparatus, cases 9a and 10.

3. Bifacton‡ cases 9b, 13, 14, 15, 16, 20. Bifacton is a preparation containing “Vitamin B₁₂ with Intrinsic Factor Concentrate.”§

The presently marketed product provides in two tablets 1 U.S.P. oral unit, as defined by the Anti-Anemia Preparations Advisory Board. We are informed by Dr. K. W. Thompson of Organon, Inc. that in this preparation, as marketed, the amount of vitamin B₁₂ per U.S.P. oral unit (daily dose) is 15.0 µg together with a quantity of intrinsic factor concentrate derived by the approved process from a specified quantity of hog stomach tissue. Some of our studies of this preparation occurred before the unit value with specification of content of vitamin B₁₂ and intrinsic factor concentrate was assigned by the Board. Hence, in some of the charts no reference is made to units, but the actual quantities employed and the batch number of each are cited. We were also informed by Dr. K. W. Thompson that the process employed in the preparation of all these test materials was the same as that later approved by the Anti-Anemia Preparations Advisory Board, and hence we are designating these test materials as Bifacton. The content of total vitamin B₁₂ in these Bifacton test preparations was determined by Professor Paul Burkholder of Yale University who employed the U.S.P. method after heating the preparation to inactivate the “binding” effect of the intrinsic factor portion. The “free” B₁₂ was determined by incubating the material with a suspension of an E. coli mutant, which consumes free vitamin B₁₂. Thereafter, the bacteria were removed by centrifugation, and the remaining preparation was heated, so that the formerly “bound” B₁₂ was released; the vitamin B₁₂ was then determined by the microbiologic assay, employing L. leichmanii.

In our studies, various lots of Bifacton preparation were tested before their final composition became standardized. They differed from the final preparation released for trade in having various ratios of intrinsic factor concentrate to vitamin B₁₂, and by various weight equivalents of extracted material. The respective data available regarding the composition and quantities of the concentrate used in our work are cited in each of the case studies.

RESULTS

Commercial Mucin from the Hog Stomach

Commercial mucin is prepared by acid extraction of the hog gastric mucosa and subsequent precipitation with alcohol. Since such acid extracts have been shown to exhibit intrinsic factor activity,†,‡,§ it could be expected that commercial mucin would also show this activity, unless it had been destroyed during its processing.

In three patients with pernicious anemia in partial remission, the intrinsic factor activity of commercial mucin powder (Wilson) was tested. The experi-

* Supplied through the courtesy of the Wilson Co.
† Prepared for us by Eli Lilly Research Laboratories, Eli Lilly Co., Indianapolis, Indiana, through the courtesy of Drs. E. C. Campbell, A. H. Fiske, E. C. Kleiderer, and J. A. Leighty from the Biochemical and Research Divisions.
‡ Supplied by Organon Inc., through the courtesy of Dr. Kenneth W. Thompson, vice-president.
§ This generic name has been assigned to it by the Anti-Anemia Preparations Advisory Board, and the Council on Pharmacy and Chemistry of the American Medical Association.
Table 2.—Hematopoietic Responses after Oral Administration of Vitamin B₁₂ with Various Mucinous Materials from H. pylori Mucosa

<table>
<thead>
<tr>
<th>Case</th>
<th>Daily oral treatment</th>
<th>Reticulocytes (per cent)</th>
<th>Red blood cells (millions per cu. mm.)</th>
<th>Hemoglobin (Gm. per 100 cc.)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mucin fraction (mg.)</td>
<td>Days of oral treatment</td>
<td>Response to treatment</td>
<td>15th Day</td>
</tr>
<tr>
<td></td>
<td>Vitamin B₁₂ (µg.)</td>
<td>Before treatment</td>
<td>Day rise began</td>
<td>Maximal value day</td>
</tr>
<tr>
<td>41 H. Mc.</td>
<td>10% acetic acid extraction</td>
<td>125</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>5 R. W.</td>
<td></td>
<td>125</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>6 N. C.</td>
<td></td>
<td>250</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>7 (210751)</td>
<td>Dilute HCl extraction; ammon. sulf. precipitation</td>
<td>250</td>
<td>10</td>
<td>9</td>
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<tr>
<td>81 F. H.</td>
<td></td>
<td>500</td>
<td>20</td>
<td>20</td>
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<tr>
<td>17 J. D.</td>
<td></td>
<td>250</td>
<td>10</td>
<td>10</td>
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<tr>
<td>18 H. W.</td>
<td></td>
<td>250</td>
<td>10</td>
<td>22</td>
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ORAL TREATMENT OF PERNICIOUS ANEMIA

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Table 2.—(continued)

<table>
<thead>
<tr>
<th>Case</th>
<th>Mucin fraction (mg.)/Vitamin B_{12}(mg.)</th>
<th>Days of oral treatment</th>
<th>Reticulocytes (per cent)</th>
<th>Red blood cells (millions per cu. mm.)</th>
<th>Hemoglobin (Gm. per 100 cc.)</th>
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<td></td>
<td></td>
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<td>Before treatment</td>
<td>15th Day</td>
<td>Before treatment</td>
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<td>Response to treatment</td>
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<td>66th Day</td>
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<td>93rd Day</td>
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<td>96th Day</td>
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<td></td>
<td>99th Day</td>
<td>Indicated</td>
<td>Found</td>
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</tbody>
</table>

* Per cents of expected counts were calculated for reticulocytes and red blood cells. Expected counts were calculated on the basis of graphs of the maximal hematoipoietic response occurring at various levels of blood counts following massive parenteral treatment with vitamin B_{12} or liver extracts.

† Much objective and subjective improvement (better appetite, legs stronger, fever gone) on seventh day of oral treatment. Subsequently the patient was given massive parenteral treatment with vitamin B_{12}, and response obtained was similar to that obtained with oral treatment (see fig. 1).

‡ Mental confusion gone on ninth day of oral treatment, also marked physical improvement at that time. Subsequent parenteral treatment with massive doses of vitamin B_{12} resulted in suboptimal response.

§ Subsequently given massive parenteral treatment with vitamin B_{12}. Further data not available.

|| Subsequent parenteral treatment with massive doses of vitamin B_{12} for twenty-five days resulted in a maximal hematoipoietic response.
mental period was divided into three periods of from twelve to fifteen days each. During the first period only mucin was given in a daily dose of 3 Gm.; during the second period, 10 $\mu$g. vitamin B$_{12}$ alone was given daily by mouth; and during the third period, both substances were administered at the same dose simultaneously. Each day’s dosage of vitamin B$_{12}$ and mucin was divided in two parts and administered morning and evening at least two hours before or after food intake. The results are tabulated in table 1.

These observations indicate that commercial mucin, if given by mouth in a relatively large dose of 3 Gm. together with 10 $\mu$g. vitamin B$_{12}$ daily to patients with pernicious anemia in partial remission and blood counts around or above 3 million RBC produces a slight hematopoietic response as evidenced by some rise in reticulocytes and red cells. However, this response could not be interpreted because of high initial erythrocyte levels. The proper evaluation of the potency of the material used requires a study on patients with lower blood counts, for such cases are more sensitive to treatment.

**Mucous Substances Processed from the Pyloric Mucosa of the Hog**

Observations were made on ten cases of pernicious anemia treated with various mucinous materials prepared from hog pyloric mucosa, the site of origin of intrinsic factor in the hog. These cases were studied in the Eli Lilly Hospital* and the results are summarized in table 2.

In all cases shown in table 2, a period of treatment with vitamin B$_{12}$ alone did not precede the combined treatment with B$_{12}$ and mucinous extracts. The difficulties encountered in the assay work on critically ill patients with pernicious anemia were responsible for this situation in many of the cases listed in table 2. Because of the absence of the necessary control periods the results obtained in this series of cases must be evaluated cautiously, especially when daily doses of 10 to 20 $\mu$g. vitamin B$_{12}$ were given.

- **Extracts prepared by extraction with 10% acetic acid (cases 4, 5, 6, table 2).** These extracts were given to three patients with pernicious anemia in relapse in a daily dose of from 125 to 250 mg., together with 5 $\mu$g. of vitamin B$_{12}$ by mouth. In two of these cases (4 and 5), when a daily dose of 125 mg. of this material was given together with 5 $\mu$g. vitamin B$_{12}$ daily, a suboptimal reticulocyte rise was noted. This was followed by an optimal rise in red cells in Case 4, and a suboptimal rise in Case 5 (see fig. 1). In the third case, however, (Case 6) the rise in reticulocytes was only very slight and no increase in red cells was noted during the fifteen days of the treatment.

- **Mucinous material prepared by extraction with 0.1 N HCl, and precipitation by ammonium sulphate saturation (cases 7, 8, 17, and 18, table 2).** This material was given to four patients with pernicious anemia in relapse. In Case 7, this fraction was given in a daily dose of 250 mg. together with 10 $\mu$g. of B$_{12}$ during nine days. This resulted in an optimal rise in red cells, which was preceded by a suboptimal reticulocyte response. During the next twenty days, after the doses of both mucous material and B$_{12}$ were doubled, a second suboptimal rise in reticulocytes was observed followed by a suboptimal increase in red cells.

* These data were supplied through the courtesy of Dr. Otto K. Behrens, Director of Biochemical Research, Eli Lilly Research Laboratories.
In Case 8 a similar dose of 250 mg. mucinous material and 10 µg. vitamin B\textsubscript{12} failed to produce any response during the ten days of the treatment, although a marked physical improvement was noted on about the ninth day of the therapy. It is probable that the observation period was too short in this case.

In Case 18 the observation period was also very short (nine days) and during that time only very slight changes in reticulocytes, and red blood cells were observed.

The fourth case (Case 17) showed a suboptimal reticulocyte response but a supramaximal response of red blood cells with the same daily dosage of vitamin B\textsubscript{12} (10 µg.) and mucinous material (250 mg.). A complete clinical remission was obtained rapidly following this treatment. The detailed data on this case are shown in figure 2.

c. Mucinous material prepared by extraction with 0.1 N HCl and precipitation with acetone (cases 11, 12, and 19). This material was given to three patients with pernicious anemia in relapse. Two of them (Cases 11 and 19) were observed for only ten days. In the first, there was no reticulocyte response, but a suboptimal rise in red blood cells occurred, equivalent to about 50 per cent of the expected optimal value. In the second case (Case 19) no hematopoietic response was noted within the ten days of the treatment.

In the third case (Case 12), a suboptimal hematopoietic response was obtained,
characterized by a reticulocyte peak of 16 per cent on the eleventh day, followed by a rise in red blood cells to a level of about 70 per cent of the expected optimal value. The details of this observation are shown in figure 3.

d. Mucinous fractions prepared as described under b and further fractionated in Kirkwood electroconvection apparatus. Two fractions (F and S) were tested in two patients with pernicious anemia.

Case 9a: E. M., Puerto Rican male, 80 years old, was admitted to the Metropolitan Hospital as a known case of pernicious anemia; complaints: fainting on the street, exhaustion, dizziness, diffuse abdominal pain, and anorexia. Five months previously he had been discharged from the same hospital in complete remission after being treated for pernicious anemia, but he had not followed the maintenance treatment. Physical examination showed: skin pale with lemon color discoloration; tongue, smooth; absent knee reflexes and no vibratory sense in both great toes. Stool: negative for blood and parasites on five consecutive examinations. No free acid in gastric content found after fasting or one hour after administration of histamine. Icterus index 26. Blood sugar tolerance curve: fasting, 90 mg. per cent; after 1 hour, 150; 2 hours, 80; 3 hours, 85; 4 hours, 98; 6 hours, 95. No increased fat content in feces. Bone marrow: typically megaloblastic. The details of blood findings are shown in figure 3. No hematopoietic response was obtained when 10 μg. B₁₂ was administered daily by mouth but when 200 mg. of the S mucous fractions was added, a definite hematopoietic response ensued, characterized by a moderate reticulocyte peak on the tenth day, followed by a suboptimal rise in red blood cells. When the second course of oral treatment was given with 7.5 μg. B₁₂ and 250 mg. of mucous fraction daily, a second, almost optimal reticulocyte peak appeared, followed by another rise in
red blood cells, hemoglobin, and hematocrit values. Details of these observations are shown in figure 4.

Case 10: P. M., aged 75, male; typical case of pernicious anemia in third consecutive relapse; megaloblastic bone marrow; absence of HCl from the gastric juice after histamine; combined posterolateral degeneration of the cord. Hospital records show that liver treatment has effectively produced remissions. No hematopoietic response was obtained during the first week of treatment when 7 µg. of vitamin B₁₂ was administered daily by mouth. When 130 mg. of the mucous fraction S.F. was added to the same dose of 7 µg. of vitamin B₁₂ and given for nine days, a significant rise in reticulocytes was obtained on the fifth

![Graph](image)

**Fig. 3.**—Case 12, L. S. Eli Lilly Hospital. Suboptimal hematopoietic response in a case of pernicious anemia to the daily oral administration of 5 µg. vitamin B₁₂ with 100 mg. of mucinous material obtained by precipitation with acetone of the fluid extract prepared by extraction of hog pyloric mucosa with dilute hydrochloric acid.

day of this treatment. This was followed by a steady rise in erythrocytes, hematocrit values, and hemoglobin, and a marked clinical improvement. After an interval of two weeks another course of treatment was started. This time a single dose of 1000 µg. B₁₂ was given by mouth. This was followed by a trivial reticulocyte response and an insignificant rise in red cells and hemoglobin of short duration. After the red blood count leveled off, another dose of 1000 µg. B₁₂ was given by mouth, this time together with 1 Gm. of mucosal fraction S.F. A rise in red cells and hemoglobin followed immediately. After the blood count leveled off, two injections of vitamin B₁₂ in doses of 30 and 100 µg. each were given at intervals of two weeks. The rise in red blood cells was rather slow during the following month of observation (see fig. 5).

It can be stated safely that mucous substances extracted from hog pyloric mucosa by various technics will exhibit an intrinsic factor activity when given by mouth, together with small oral doses of vitamin B₁₂, to patients with perni-
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cious anemia. The intensity of this intrinsic factor activity depends, however, on several factors: (1) the method of preparation, the activity, and the dose of the intrinsic factor containing material; (2) the individual responsiveness of the patient; (3) the quantity of vitamin B₁₂ given jointly with the intrinsic factor containing material. It seems that the method of preparation of each of the mucinous materials described above is not yet optimal for the purpose of concentrating the intrinsic factor. The materials of animal source used in this

![Graph](image)

**Fig. 4.**—Case 9a, E. M., male, 80 years. Metropolitan Hospital Research Unit #6423/52. Partial hematopoietic response in a case of pernicious anemia to the daily administration of 7.5 to 10.0 μg. of vitamin B₁₂ together with 200 to 250 mg. of mucous fraction obtained by fractionation in the Kirkwood electroconvection apparatus of the mucinous gastric material. This mucinous material was obtained by precipitation with ammonium sulphate of the fluid extract prepared by extraction of the hog pyloric mucosa with dilute hydrochloric acid.

The above situation may explain to some extent the failure to obtain uniform results with various lots of material processed in a similar way. While cases 4 and 5 were treated with the same lot of material, and both responded well to the treatment, case 6, who failed to have a hematopoietic response, was treated with material processed in the same way, but prepared from another lot. Also, cases 7
and 8 were treated with materials prepared by the same technic but derived from different lots. They showed marked differences in their hematopoietic responses. On the other hand, cases 11 and 12, having been treated with the same lot of material, showed different intensities of hematopoietic response which was apparently dependent exclusively on individual responsiveness.

The data described below as well as previously reported results\textsuperscript{2} tend to indicate that the daily oral dose of vitamin B\textsubscript{12} required for obtaining a regular, consistent, and optimal hematopoietic response in patients with pernicious anemia in relapse should not be kept below 15 \textmu g. a day, if B\textsubscript{12} is given together with an adequate dose of intrinsic factor containing material. Since the daily dose of vitamin B\textsubscript{12} was only 5 \textmu g. in six tests described above, 7 \textmu g. in one, and 10 \textmu g. in five, the variable and suboptimal responses obtained in some of the cases may depend not only on the insufficiently active intrinsic factor preparation, but also on the inadequate dosage of vitamin B\textsubscript{12}.

Studies with Bifacton

Six patients with pernicious anemia were treated with this preparation. Because of the impressive results obtained, a description of cases and results is given as follows:

Case 9b: This patient was also described above as Case 9a. A period of three weeks had elapsed between the experimental periods. During the interval, the patient did not receive any medication and remained on the same restricted diet. No reticulocyte response and only a slight rise in red cells and hemoglobin were observed during the first control period of thirteen days when 5 \textmu g. of vitamin B\textsubscript{12} alone were given three times daily by mouth. No clinical improvement was observed during this period of time. Four weeks of Bifacton treatment followed with a daily dose of three tablets, each containing 6.5 \textmu g. vitamin B\textsubscript{12} (5 \textmu g. in "bound" form) and 51.0 mg. of the mucinous extract. On the fifth day, a reticulocyte peak of 14.2 per cent was observed. This was followed by a sharp rise in red blood cells, hemoglobin, and hematocrit readings. The reticulocyte response was considered optimal, and also the rise in erythrocytes was certainly optimal. Most impressive clinical remission was obtained, including subsidence of neurologic disturbances. The details of the observations are reported graphically in figure 6.

Case 13: S. B., age 63, male. Five months ago progressive weakness, loss of appetite, dyspnea, numbness of feet, and pain in abdomen began. Physical examination showed signs of Parkinson's disease, pale-yellowish discoloration of skin, subicteric sclerae, slight edema of the feet and ankles, smooth and pale tongue with atrophy on the edges, liver enlarged slightly, and signs of posterior column degeneration. Achlorhydria was complete after histamine. Bone marrow: typically megaloblastic. The hematologic data are listed graphically in figure 7. During a ten day control period of no treatment, no change in the blood status was observed. During the next thirteen days when 5 \textmu g. B\textsubscript{12} was given orally three times a day, a moderate reticuloctosis ensued. It was not followed by any rise in red cells, hemoglobin, or hematocrit values. One month of Bifacton treatment followed, during which one tablet was given three times a day, each tablet containing 5.06 \textmu g. vitamin B\textsubscript{12} (5.06 \textmu g. was in "bound" form) and 20.0 mg. of mucinous extract. During this treatment a second moderate reticulocyte peak was observed, followed by a sharp rise in red cells, hematocrit values, and hemoglobin concentration. The rise in red blood count was maximal. A complete clinical remission with gradual improvement of neurologic signs was obtained shortly after the end of this treatment. The blood response is shown in figure 7.

Case 14: E. McD., aged 68, Swedish woman, was admitted semiconscious, delirious, and incontinent. History was obtained from husband: Several months ago anorexia, nausea and vomiting, preumbilical pain, weakness, and malaise began. Four weeks prior to admission she fell and became bedridden. Gums bled for two days. Because the red blood
Fig. 5.- Case 10, M., male, 75 years. Metropolitan Hospital Research Unit. Hematopoietic response obtained in a case of pernicious anemia to the daily administration of 7.0 µg. vitamin B₁₂ together with 130 mg. of first mucous fraction processed in the Kirkwood electroconvection apparatus as described in figure 4, as well as to a single dose of 1000 µg. vitamin B₁₂ given alone and together with another mucinous material fractionated also in the Kirkwood apparatus.
count was only 600,000 per cu. mm., and the hemoglobin, only 2.0 Gm., three transfusions were given, in volumes of 500, 250, and 150 cc. respectively. Physical examination also showed semicoma, yellow-lemon like skin, bleeding gums, tongue coated with cobblestone-like bumps, Babinski and Hoffman's signs positive on both sides, incontinence, rales in both lungs. Blood: as shown in figure 8. Icteric index 10. Bone marrow: typically megaloblastic.

During the first twelve days of a control period, when 5 µg. of vitamin B₁₂ were given three times a day the red blood count fell to the level of 1,200,000 per cu. mm. Bifacton was then started in a dose of two tablets a day, each containing 6.55 µg. vitamin B₁₂ with 21.2 mg. of mucinous extract. On the sixth day of this treatment a rise in reticulocytes occurred with a peak on the eighth day (see fig. 8). Although a satisfactory rise in red blood cells followed, the general condition of the patient was still very poor and the dose of Bifacton was raised to three tablets a day. Following the administration of this increased dose the reticulocyte peak widened, and a further rise in red blood cells ensued. The mean corpuscular volume decreased markedly. The general condition of the patient improved very much, and at the conclusion of four weeks' treatment with Bifacton the patient showed signs of partial remission, including improvement of neurologic disturbances.

During the next twelve days we administered another lot of Bifacton tablets containing in each day's dose 16.5 µg. of vitamin B₁₂ and 49.5 mg. of mucinous extract. No further rise, but rather a trend to leveling off at the level of 2,500,000 to 2,700,000 red blood cells was noted. Still another Bifacton preparation was substituted, containing 15 µg. vitamin B₁₂ per tablet (out of which only 3.95 mg. was in "bound" form) and 12.35 mg. of mucinous extract. The daily dose of three tablets a day contained 45.0 µg. vitamin B₁₂ and 37.0 mg.

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**Fig. 6.**—Case 9b, E. M., male, 80 years. Nearly optimal hematopoietic response in a case of pernicious anemia to the daily administration of three tablets of an early Bifacton preparation, equivalent to 19.5 µg. vitamin B₁₂ and 153 mg. of the intrinsic factor concentrate per day.

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<th>DAILY ORAL TREATMENT</th>
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<td>19.5 µg. Vit. B₁₂</td>
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of mucinous extract. A new rise in the red blood cells occurred without any additional reticulocyte response. In spite of the continuation of this daily dose, another leveling off of the red cell counts was noted during the second week of this treatment. However, clinical improvement continued, and the patient, who had been entirely bedridden because of the posterolateral combined degeneration of the cord, became partially ambulant. Parenteral treatment with vitamin B₁₂ (30 μg, twice weekly) resulted in further subjective improvement, but no further rise in red cell and hemoglobin values was observed. The details are shown in figure 8.

![Graph showing hematopoietic response](image)

**Fig. 7.**—Case 13, S. B., male, 63 years. Metropolitan Hospital Research Unit #2907/37. Nearly optimal hematopoietic response in a case of pernicious anemia to the daily administration of three tablets of an early Bifacton preparation, equivalent to 15.2 μg. vitamin B₁₂ and 60.0 mg. of the intrinsic factor concentrate per day.

The results obtained in case 9b, and especially in cases 13 and 14, indicate that the daily administration of from two to three tablets of Bifacton, containing a daily dose of vitamin B₁₂ below 25 μg. and from 40 to 60 mg. of mucinous substances processed from the hog stomach, gives a satisfactory hematopoietic response and induces a clinical remission in patients with pernicious anemia. The response was optimal in two cases (9b and 13) and suboptimal in one (14). In the latter, the response could not be improved even by larger doses of Bifacton, and remained suboptimal also on parenteral treatment. It would appear from these data as well as from another case-study in progress that the daily administration of this preparation in tablet form can be considered as an effective oral treatment of pernicious anemia, and the doses used daily gave results that would be expected from approximately 1 U.S.P. oral unit. It is quite possible, however, that in some instances larger daily doses of the drug are needed, especially if the
disease is severe and if the individual responsiveness of the patient is below average.

The next case in the present study was treated with massive oral doses of this Bifacton preparation given once every seven to ten days.* This patient has shown a most dramatic response; the hematologic data are shown in figure 9, and the details are as follows:

Case 15: M. McC., female, age 64, admitted because of weakness, fainting, anorexia, sore tongue, throbbing sensation in ears, and numbness and tingling in hands and feet. Fifteen years ago she was found to have pernicious anemia and has taken liver extracts for twelve years, but for the past three years she has not taken any medication. Seven months ago she began feeling poorly and fainted on the street. Physical examination: lemon-yellow skin, smooth tongue with strawberry red edges, no palpable spleen or liver, absent tendon reflexes in lower extremities. Altered kinesthetic sense in both feet and ankles, and decreased vibratory sensitivity. Gastric juice after histamine showed no free HCl. Bone marrow: typically megaloblastic. Hematologic findings shown in figure 9.

Before the actual treatment was started, a massive dose of Bifacton, supposedly "inactivated" by heat, was given as a control. This dose comprised 150 μg. vitamin B₁₂, 75 μg. being in "bound" form, with 240 mg. of intrinsic factor containing mucous material. The tablet was crushed in a mortar, suspended in 50 cc. of distilled water, and heated to 95 to 97°C in a boiling water bath for 30 minutes. After cooling, the material was given to the patient three hours before dinner. It was expected that the intrinsic factor would have been inactivated and that this experiment would serve as a control to further study

* Through the courtesy of Dr. Kenneth W. Thompson, vice president, Organon Inc. specially made tablets were prepared, each containing a ten day dose of vitamin B₁₂ (150 μg.) and a five day dose of mucinous extract from hog stomach (240 mg.).
of the unboiled and fully active material. However, a maximal hematopoietic response was obtained. It was characterized by a maximal reticulocyte rise four and one-half days after the ingestion of the drug, and lasted until the twelfth day. A sharp optimal rise in red cells followed within eight days, associated with a rise in hemoglobin and hematocrit values and a tremendous subjective improvement (see fig. 9).

A second identical dose of a similarly boiled material was given two weeks after the first dose. A second reticulocyte rise was observed again four and one-half days after the treatment, and a new rise in red cells, hemoglobin, and hematocrit readings occurred. The third dose was given ten days following the second, and this time unboiled material was given. A third reticulocyte rise was noted five and one-half days after the administration of the drug, with a third rise in red cells, hemoglobin, and hematocrit values. There was also further rapid clinical improvement of the patient. The fourth dose was given after a shorter interval than the previous doses, since from the analysis of the hematopoietic responses to the first three single doses, it became obvious that the hematopoietic effect of those doses expired after about one week and the red cell counts showed a leveling off thereafter. Therefore, the fourth dose was given only one week after the third dose, and a fourth reticulocyte rise and a new rise in red blood cells occurred. A fifth dose was given only one week later, and this was followed again by a further rise in red blood cells. No further increase in blood count could be obtained with massive parenteral doses of vitamin B12. The results are shown in figure 9.

The treatment in this case was simply the administration of five single tablets, each at intervals of from one to two weeks. The total dosage of vitamin B12 was 750 µg. and of the intrinsic factor concentrate, less than 1.2 Gm. A maximal

![Graph showing hematological changes](image-url)
hematopoietic response and a maximally rapid and complete clinical remission, including the neurologic disturbances, occurred. The intervals between the first three doses should have been reduced to one week, and this would have hastened the completeness of the remission by at least ten days. The levelling off of red count, noticeable one week after the administration of each single dose, would have been prevented.

Two single orol doses of Bifacton, each comprising two capsules equivalent to 10 U.S.P. units

* 500 cc. blood.
† Died suddenly of a complicating gallbladder disease with peritonitis.

Fig. 10.—Case 20, A. R., female, 72 years. Flower and Fifth Avenue Hospitals 830/53. Submaximal hematopoietic response in a case of pernicious anemia to two single massive doses of Bifacton given orally at seven day intervals.

The next case in this series was also treated with massive oral doses of Bifacton given once every seven days. Also, here a maximal hematopoietic response was obtained, as shown in figure 10. The details of this observation are as follows:

Case 20: A. R., Italian woman, age 72, admitted because of weakness, dizziness, numbness in both legs, and complete anorexia of two months duration; bedridden for several weeks. Eight years ago was treated for pernicious anemia in Metropolitan Hospital. Physical examination: temperature 101 F., yellow-lemon colored skin, subicteric sclerae, smooth tongue, extensive edema of the feet and ankles, slightly enlarged liver, absence of kinesthetic sense in both legs, and definite pneumonic focus right lower lobe. Red cells, hemoglobin, and hematocrit readings as shown in figure 10. White blood cells: 4200, with 41 per cent neutrophiles and 59 per cent lymphocytes. Icterus index 30; bilirubin 3.13 mg. per liter. Bone marrow: highly megaloblastic. Achlorhydria was complete after histamine.

The patient received one single dose of Bifacton by mouth. The dose was equivalent to 10 USP units and contained 150 μg. vitamin B₁₂ with 500 mg. of intrinsic factor concentrate. The powder was given in two gelatin capsules two hours after lunch. Because of pneumonitis this patient also received a daily injection of 300,000 units of penicillin.
A maximal hematopoietic response was obtained following the first single dose of Bifacton. It was characterized by a sharp reticulocyte rise which started two and one-half days after administration of the drug and became maximal on the seventh day. This was followed by an optimal rise in red blood cell, hemoglobin, and hematocrit values, and improvement of patient’s general condition.

Shortly thereafter, however, a rise in temperature was noted, as well as progressive distension of the abdomen, jaundice, signs of acute infection of the biliary tract, and peritonitis. Massive doses of antibiotics and 500 cc. blood were given, as well as another single large oral dose of Bifacton. A new rise in the blood count followed. Laparotomy was considered, but the patient died suddenly before the preoperative decompression was achieved by means of Miller-Abbot tube.

These data tend to indicate that a single dose of 150 µg. of vitamin B₁₂ with 0.25 to 0.50 Gm. of the intrinsic factor concentrate as contained in Bifacton and administered by mouth at weekly intervals is roughly equivalent to the weekly injection of about 10 to 15 units of liver or 15 µg. of vitamin B₁₂. The usual ratio between the hematopoietic effect of parenteral vitamin B₁₂ to the oral is about 100:1, if conservatively estimated, and frequently much more. This ratio in Bifacton preparation appears to be only about 10:1, which indicates the relative potency of the intrinsic factor concentrate present in this preparation.

The studies with massive single doses of the oral preparation tend to suggest that the clinical response obtained is also similar to that seen after parenteral administration of massive doses of liver extract or vitamin B₁₂. The results obtained with these massive single oral doses of Bifacton in the above two cases appear to exceed those observed on administration of the same amount in divided daily doses. If these findings are reduplicated in further studies now in progress, it might be that the administration of large single doses of Bifacton at intervals of one week would be the treatment of choice for patients with severe pernicious anemia if a fast and maximal hematopoietic and clinical response is desired.

The optimal hematopoietic response in case 15 was obtained twice following the administration of a boiled massive dose of Bifacton. The data published by others indicate that significant hematopoietic responses have not followed single oral doses of vitamin B₁₂ below 1000 µg., and therefore it is most improbable that the response we observed was due solely to the 150 µg. of the thermostable vitamin B₁₂. Our results obtained in case 10 of the present series also seem to indicate that vitamin B₁₂ alone is not likely to produce such a striking response.

Since this finding would indicate thermostability of the Bifacton preparation, it was necessary to verify this by administering the Bifacton in small daily doses with amounts of vitamin B₁₂ not likely to induce a response. This was done in case 16, the description of which follows:

Case 16: W. B., aged 65, male of German descent, was admitted because of progressive weakness, loss of appetite, mental incoherence, and disorientation. The first symptoms of pernicious anemia appeared one month previously. Physical examination: lemon-yellow color of the skin, smooth and very pale tongue, absent reflexes in lower extremities, definite impairment of vibratory sensitivity in the middle part of the legs, and complete abolishment in the toes. He showed slight confusion in regard to time, space, and persons. Gastric juice: no free acidity on fasting or one hour after 1 mg. histamine. Bone marrow: megaloblastic. The hematologic data are shown in figure 11.

Five hundred cc. blood was given because the initial red count was only 750,000 per cu. mm., and the red cells rose thereafter to 1,500,000 per cu. mm. and hemoglobin to 4.2
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Gm. With a hematocrit value of 15, the mean corpuscular volume was high (100) after the transfusion. Another blood transfusion of 250 cc. was given after which a further rise in red cells and hemoglobin occurred. Now the control period was started, during which 15 \( \mu \)g B12 daily by mouth was given in two divided doses for a period of two weeks. On the fifth day, a moderate reticulocytosis was observed, and slight rise in red cells followed. No definite clinical improvement, however, was manifest, and the blood count which had risen slightly during the first week leveled off during the second week of the oral treatment with vitamin B12 alone and stayed so, unchanged, for a week.

Bifacaton treatment was then started twice daily in a total daily dosage of 15 \( \mu \)g B12 and 42.4 mg of the mucous material. Each dose of this preparation before administration was placed in a test tube containing 10 cc. water and dissolved. The tube was then placed in a boiling water bath for 45 minutes, and the actual temperature in the test tube was 97 \(^\circ\)C for this period. The test tube was cooled off, and frozen. A stock of these boiled, frozen Bifacaton materials were prepared for the entire period of treatment and each kept frozen until used. Before actual use the material was thawed at room temperature, and given to the patient in one-half glass of water at 10:00 A.M. and 10:00 P.M., at least three hours before or after food intake.

After beginning the treatment with boiled Bifacaton, another reticulocyte peak appeared; this showed a "spread character". It was followed by a sharp rise in red cells, hemoglobin, and hematocrit values. A definite clinical remission and rapid improvement of the neurologic disturbances ensued. The total rise in red cells during the first three weeks of the treatment with boiled Bifacaton can be considered as a submaximal hematopoietic response according to all standards. The administration of 30 to 50 \( \mu \)g vitamin B12 parenterally at weekly intervals did not cause any further rise in red cell values.

Thus, the observations in case 16 confirm those made with the massive doses...
ORAL TREATMENT OF PERNICIOUS ANEMIA

of boiled Bifacton in case 15. A conclusion can be drawn that the Bifacton preparation is thermostable, to the extent of retaining activity after boiling in water for 30 to 45 minutes.

**Discussion**

The data reported in this study suggest that various procedures of extraction, precipitation, and fractionation can be applied to hog stomach tissue to obtain preparations endowed with definite intrinsic factor activity. Such extracts are suitable to promote good hematopoietic responses on oral administration to patients with pernicious anemia if given together with small oral doses of vitamin B₁₂. The results of the process used, and the yield of active material, depend upon avoiding measures which cause losses of the intrinsic factor activity. Moreover, a proper selection and adjustment of the doses of vitamin B₁₂ and intrinsic factor concentrate is of great importance for obtaining optimal results with the oral treatment of pernicious anemia.

As little as 15 µg. (see case 16) of vitamin B₁₂ given orally alone without added intrinsic factor may cause, in some instances, a hematopoietic response. This is usually characterized by a moderate reticulocytosis of from 20 to 30 per cent of the optimal, with a slight rise in red blood cells of about 300,000 to 400,000 per cu. mm., and a slight clinical improvement.²⁹ Even as little as 10 µg. of vitamin B₁₂ alone may cause definite response,²⁵ although this is exceptional. More pronounced hematopoietic responses are observed with daily oral doses of as little as 5 µg. of vitamin B₁₂ (or even 1 µg.) when given together with materials containing intrinsic factor of human¹⁰,¹¹,¹⁴-¹⁶ or animal sources.¹⁷-²⁰

However, one must sharply differentiate between the marginal or irregular and exceptionally submaximal hematopoietic responses and consistently maximal hematologic and neurologic responses which are needed for treatment of severely ill patients with pernicious anemia in full-blown relapse. Here, a rapid effect of a reliable therapeutic agent is needed. Therefore, the doses of both vitamin B₁₂ and intrinsic factor must be larger than those which are employed in the detection of the intrinsic factor activity of an unknown material tested. As mentioned above, our experience tends to indicate that the daily oral dose of vitamin B₁₂ should be not lower than 15 µg., when given orally in conjunction with intrinsic factor concentrate, if uniformly consistent and maximal hematopoietic and clinical responses are to be obtained.

Similar considerations apply to the dose of the intrinsic factor concentrate which is needed for therapeutic purposes. Although Prusoff, et al.⁴³ have stated that as little as 0.6 mg. of material prepared from the human gastric juice has promoted hematopoietic activity of 5 µg. of vitamin B₁₂ on oral administration in one case of pernicious anemia their data have not been published in detail and cannot be accepted as sufficient evidence for this claim. For practical purposes, at present, it seems that the daily dose of intrinsic factor material need not be more concentrated than approximately 50 mg. of concentrate per day. This concentration is achieved in Bifacton, and approximately the same amount of mucoprotein from human gastric juice was required in our earlier observations in order to obtain a good hematopoietic response and complete clinical remission in patients with pernicious anemia if combined with small oral doses of vitamin
The similarity in dosage between these two concentrates of intrinsic factor activity from human and animal sources is striking.

Doubtless both these intrinsic factor concentrates can be further refined. Glandular mucoprotein processed from human gastric juice represents, on electrophoresis, a rather single homogenous peak, which is compatible with the assumption that it is a rather homogenous substance. However, the acetone precipitation process used to prepare it caused a certain loss of its intrinsic factor potency, and, thus, certainly can be improved. On the other hand, materials processed from the hog stomach (including Bifacton) are heterogeneous in their constitution. According to our electrophoretic data, which will be reported in more detail elsewhere, the concentrate employed in Bifacton shows four peaks on electrophoresis in phosphate buffers of pH 6.0 to 6.4, ionic strength 0.05 to 0.1, and at 0.7 to 1.25 per cent concentration. Their respective mobilities under these conditions are as follows: (1) $-3.60$ to $-3.90 \times 10^{-3}$; (2) $-2.45$ to $-2.60 \times 10^{-3}$; (3) $-1.20$ to $-1.40 \times 10^{-3}$ cm$^2$ sec$^{-1}$ volt$^{-1}$, whereby the second and the third peak are the largest. A very small fourth positively charged peak of a mobility $+3.3 \times 10^{-3}$ was found on some tracings in runs done in buffers of pH 7.0 to 7.7. The heterogeneity of the concentrate employed in Bifacton justifies the conclusion that this intrinsic factor preparation from the hog stomach can be further refined.

The thermostability of Bifacton can be correlated to the earlier observation of Hall et al. and Bethell et al. made in regard to another preparation of animal origin containing vitamin B$_{12}$ "bound" to intrinsic factor concentrate and which also appeared to be thermostable. If the boiling releases vitamin B$_{12}$ "bound" to intrinsic factor as has been stated then, following the heat-release in vitro, a new binding of B$_{12}$ to the boiled intrinsic factor must occur within the gastrointestinal tract of the patient with pernicious anemia.

It must be postulated, however, on the basis of these data, that as a result of the in vitro linkage of vitamin B$_{12}$ to intrinsic factor in Bifacton the intrinsic factor becomes thermostable under the conditions described. Another possibility is that the hematopoietic factor resulting from the binding in vitro of the intrinsic factor to vitamin B$_{12}$ as supplied in the preparation tested is thermostable, as contrasted to the thermolabile intrinsic factor alone.

**SUMMARY AND CONCLUSIONS**

1. Twenty patients with pernicious anemia were treated under rigidly controlled conditions by administering small oral doses of vitamin B$_{12}$ in combination with intrinsic factor containing mucinous materials processed from the hog stomach. This study was undertaken in view of our earlier observation concerning the relationship of one of the mucin fractions of the human stomach (glandular mucoprotein) to Castle's intrinsic factor. The following sources of intrinsic factor from animal stomach were used: (a) commercial gastric mucin; (b) acetic acid extracts of hog pyloric mucosa; (c) mucinous materials precipitated by acetone or by saturation with ammonium sulfate from hydrochloric acid extracts of the hog pyloric mucosa; (d) mucous fractions obtained by further fractionation in the electroconvection apparatus of the above mucinous materials precipitated with ammonium sulfate; (e) an intrinsic factor concen-
turate from the hog stomach in combination with small doses of vitamin B₁₂, processed under the trade name of Bifacton.

2. Results obtained in this study indicate the feasibility of attaining complete clinical remission and suboptimal or optimal hematopoietic responses in patients with pernicious anemia in relapse when small daily doses (below 20 µg.) of vitamin B₁₂ are given orally in combination with mucinous materials processed by various techniques from the hog stomach and containing intrinsic factor. The provisions are that: (1) the doses of both vitamin B₁₂ and intrinsic factor containing materials be adequate and optimal in regard to their mutual ratio; (2) the process involved in processing intrinsic factor containing materials not impair their activity and yield a product sufficiently concentrated.

3. The hematopoietic responses and clinical remissions obtained in six cases of pernicious anemia with Bifacton (vitamin B₁₂ with intrinsic factor concentrate) in different batches were uniform in our hands. The doses employed were marginal, in order to detect differences in potency, and although the reticulocyte responses were frequently suboptimal and protracted, the increases in red cells were roughly equivalent to those obtained with daily administration of approximately 1 unit of a standard oral antianemia preparation. Bifacton is supplied in strikingly smaller dosages than the standard liver or stomach oral preparations, and is fully active in a total daily oral dose of about 50 mg. of intrinsic factor concentrate with 15 µg. vitamin B₁₂.

4. Preliminary studies on two patients with pernicious anemia tend to indicate that the administration of massive single oral doses of Bifacton repeated at intervals of one week may imitate even more closely the results of parenteral treatment with injectable liver concentrates or vitamin B₁₂ than does the administration of this concentrate in small daily doses. The single dose of Bifacton which was given once a week to one of these patients contained 150 µg. vitamin B₁₂ with approximately 250 mg. of intrinsic factor concentrate. Five consecutive single doses administered at intervals of from seven to ten days induced a complete clinical and hematologic remission.

5. The studies on two patients with pernicious anemia indicate that Bifacton is resistant to boiling and that it preserves its hematopoietic activity after being boiled in water for from thirty to forty-five minutes at 95 to 100° C. This suggests either that the intrinsic factor of the animal stomach becomes thermostable after the interaction in vitro with vitamin B₁₂, or that the product of this binding is thermostable under conditions described.

6. The data reported here indicate that various extraction and precipitation procedures may be applied to hog stomach tissue to obtain materials which will exhibit definite intrinsic factor activity in patients with pernicious anemia. Although at present some such extracts are active orally in such small doses as from 40 to 50 mg. per day, still further refinement appears to be possible.

REFERENCES

G. B. J. GLASS AND L. J. BOYD

ORAL TREATMENT OF PERNICIOUS ANEMIA


Castle, W. B.: Personal communication.


Oral Treatment of Pernicious Anemia with Small Doses of Vitamin B₁₂ Combined with Mucinous Materials Derived from the Hog Stomach

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