Catalytic hydrogenation of crystalline vitamin $B_{12}$ yields a crystalline compound possessing an absorption spectrum and a chromatographic mobility pattern different from those of the original vitamin. This product has been designated as vitamin $B_{12j}$. West, in a personal communication cited by Kaczka et al., reported that the parenteral administration of 25 $\mu$g of this compound to a patient with pernicious anemia brought about a hematologic response considered to be 30 per cent of maximal. Studies on growth rates in chicks, rats and lactobacilli indicated that vitamin $B_{12j}$ was less active than vitamin $B_{12}$. Woodruff and Foster reported that both purified liver extracts and fermentation broths from $S$. griseus contain a substance with the microbiologic and chromatographic characteristics of vitamin $B_{12j}$. Some of the purified liver extracts tested contained considerably more vitamin $B_{12j}$ than vitamin $B_{12}$ and they also noted formation of vitamin $B_{12s}$ from vitamin $B_{12}$ during paper chromatography. Consequently, it appeared to us to be of practical as well as of theoretical interest to determine the relative hematopoietic activity of vitamin $B_{12j}$ in pernicious anemia.

 METHODS

This communication describes the results of the use of vitamin $B_{12j}$ in observations on 9 patients with the blood, bone marrow, and clinical abnormalities characteristic of Addisonian pernicious anemia. In some instances comparisons with the results following $B_{12}$ were made in the same patient. Both vitamins were administered in sterile solutions containing 1, 2 or 4 $\mu$g per milliliter. Hemoglobin, hematocrit and red blood cell determinations were made two or three times a week on venous blood samples, using balanced oxalate as the anticoagulant. Daily reticulocyte counts were made on capillary blood samples.
employing coverslips previously coated with a film of dried brilliant cresyl blue as in the usual preparation and staining of a blood smear. The method of serial reticulocyte responses was employed for the evaluation of the relative hematopoietic potencies of substances given to patients in uniform daily amounts during consecutive periods of ten or more days each.

All patients receiving parenteral therapy, with the exception of Case 122, were allowed a regular hospital diet. This patient, as well as 2 others receiving

![Table 1.—Effect of Daily Intramuscular Injection of Substances Indicated](image-url)
test preparations by mouth (Cases 124 and 125), was maintained on a diet containing no meat, fish or eggs. Cases 124 and 125 received tea, toast and jelly at 4 p.m., and no food thereafter until breakfast the following morning. The oral preparations tested were given daily at 8 p.m.

### Table 2.—Effect of Daily Oral Administration of Substances Indicated

<table>
<thead>
<tr>
<th>Days of Treatment</th>
<th>Case 124</th>
<th>Case 125</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R.B.C. (Mls.)</td>
<td>R.B.C. (Mls.)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td>2.4</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>2.4</td>
</tr>
</tbody>
</table>

**Results**

Vitamin B₁₂, exhibited potent anti-pernicious anemia activity, accompanied by rapid clinical improvement, when given by intramuscular injection in daily dosage of 1 μg. in Cases 119 to 123, inclusive, of 2 μg. in Case 118 and of 4 μg. in Case 117 (see table 1). During the second periods of observation of Cases 119, 121 and 122, when 1 μg. of vitamin B₁₂ was given daily by intramuscular injection, only Case 119 showed a second rise of reticulocytes, and this was of small magnitude. Thus, it appeared that vitamin B₁₂ did not possess significantly greater hematopoietic activity than did vitamin B₁₂₆. During the first periods
of observation of Cases 124 and 125, 5 μg. of vitamin B₁₂ were administered orally each day in 50 ml. of water. In both cases a small but definite reticulocyte rise occurred. During the second periods of observation of these 2 cases, however, when the same amount of vitamin B₁₂ was given daily in 50 ml. of neutralized normal human gastric juice, there occurred a second and greater rise of reticulocytes, followed by a prompt increase in red cell numbers (see table 2).

Mild subacute combined degeneration of the spinal cord, manifested by a positive Romberg’s sign, absence of vibratory perception below the hips, and paresthesias in the lower extremities, was present in Case 123 on admission to the hospital. After the daily intramuscular administration of 1 μg. of vitamin B₁₂ for sixteen days, Romberg’s sign had become negative and the subjective numbness of the legs had diminished.

**Discussion**

The vitamin B₁₂ employed in these observations was crystallized from the reaction mixture after catalytic hydrogenation of crystalline vitamin B₁₂. A compound designated vitamin B₁₂ has been crystallized from cultures of *S. aureofaciens,* and an apparently identical substance has been prepared from liver extract. At the beginning of these observations the presently established identity of vitamin B₁₂ and vitamin B₁₂ was not apparent. In fact, the information then available indicated distinct differences in their microbiologic and animal activities, and in their absorption spectra. A recent report, however, suggests that these differences may have been caused by variations in the experimental conditions. Thus, the similar hematopoietic activity of vitamin B₁₂ and vitamin B₁₂ upon parenteral administration in pernicious anemia in our hands apparently confirms the conclusions of Lichtman et al. with vitamin B₁₂. Anslow et al. have reported the crystallization of additional members of the vitamin B₁₂ group and have termed them vitamin B₁₂ and vitamin B₁₂. After preliminary clinical trials these two new compounds were reported to be as active as vitamin B₁₂ when tested as hematopoietic substances in patients with pernicious anemia.

The chemical difference between vitamin B₁₂ and vitamin B₁₂ (or vitamin B₁₂) is that vitamin B₁₂ contains one cyano group bound coordinatively to the cobalt atom, while vitamin B₁₂ lacks this group. As was the case with vitamin B₁₂ and with vitamin B₁₂ the hematopoietic activity of vitamin B₁₂ is here shown to be potentiated by simultaneous oral administration with normal human gastric juice. Ternberg and Eakin have reported a stoichiometric combination between gastric juice and vitamin B₁₂ of such a nature that the vitamin B₁₂ is no longer available for the nutrition of certain bacteria. However, some extracts of hog stomach mucosa exhibiting such binding power for vitamin B₁₂ do not potentiate its activity upon oral administration in pernicious anemia. This binding power is therefore not the specific property of the gastric juice that is concerned in the patient. Admixture with gastric juice, moreover, does not detectably alter the spectroscopic properties of vitamin B₁₂. There is thus no direct evidence, though it may be possible that chemical combination with gastric juice is responsible for the enhanced hematopoietic activity of vitamins of
HEMATOPOIETIC ACTIVITY OF VITAMIN B₁₂₅

the B₁₂ group which is induced by simultaneous oral administration with normal human gastric juice. Because vitamin B₁₂₅ lacks the cyano group characteristic of vitamin B₁₂, the cyano configuration cannot be directly concerned in the phenomenon, whatever its nature.

SUMMARY

1. Vitamin B₁₂₅, derived from vitamin B₁₂ by catalytic hydrogenation, is as potent a hematopoietic agent as vitamin B₁₂ when administered parenterally to patients with pernicious anemia in relapse.

2. The hematopoietic activity of vitamin B₁₂₅, like that of vitamin B₁₂, and of vitamin B₁₂₅₆, is potentiated by simultaneous oral administration with normal human gastric juice.

3. Observations on one patient suggest that vitamin B₁₂₅, like vitamin B₁₂, will arrest the progress of subacute degeneration of the spinal cord in pernicious anemia.

REFERENCES

Observations on the Etiologic Relationship of Achylia Gastrica to Pernicious Anemia: XIII. Hematopoietic Activity of Vitamin B12a (Vitamin B12b)

ROBERT F. SCHILLING, JOHN W. HARRIS and WILLIAM B. CASTLE