A Case of Juvenile Pernicious Anemia: Study of the Effects of Folic Acid and Vitamin B₁₂

By David H. Clement, Charles A. Nichol and Arnold D. Welch

Pernicious anemia is a disease rarely encountered in persons under 30 years of age, in fact, in the majority of instances the diagnosis of juvenile pernicious anemia has not been established unequivocally. Until fairly recently such a diagnosis rested on the combination of a macrocytic anemia associated with a megaloblastic marrow, with or without gastric achlorhydria, in a child whose sustained remission was dependent on the continued parenteral administration of either liver extract or vitamin B₁₂. However a more nearly definitive diagnosis can now be made in such a patient through a demonstration of the absence of intrinsic factor activity in the gastric secretions and an inability to absorb a significant proportion of small amounts (0.25 to 10 µg.) of orally administered radioactive vitamin B₁₂, except when administered together with a source of intrinsic factor.

We have had an opportunity to study a four-year-old white boy with recurrent macrocytic anemia, glossitis, and megaloblastic marrow, in whom free hydrochloric acid was usually present in the gastric juice. Some light on the pathogenesis of this condition in the early years of life has been shed by studies (1) of the excretion of folinic acid-like materials in the urine (following the ingestion of folic acid); (2) of the absorption of orally administered vitamin B₁₂-cobalt; (3) of the effect of the patient’s gastric juice on the absorption of radioactive vitamin B₁₂ in a patient with classic Addisonian pernicious anemia; and (4) of the results of a gastric biopsy.

In adults, the disease is almost never seen in the presence of free hydrochloric acid in the gastric secretions, but in children the presence of gastric hydrochloric acid in individuals with otherwise classic manifestations of pernicious anemia has been observed previously. An hypothesis concerning the natural history of pernicious anemia has been offered that can account for this discrepancy. Some of the findings described in this paper have been referred to in earlier publications.

Materials and Methods

Microbial Assays: Streptococcus faecalis (ATCC 8043), grown in the medium described by Flynn et al. with pteroylglutamic acid as a reference standard, was used for the determination of folic acid activity by means of a turbidimetric assay. Similarly, Pediococcus cerevisiae (ATCC 8081), earlier known as Leuconostoc citrovorum, grown in the assay medium described by Sauberlich with synthetic folinic acid (leucovorin) as a reference standard, was used for the determination of folinic acid activity. In this paper the results of analyses for folinic acid are expressed on the basis of the natural form, taking into account the differing vitamin activities of the free and covalently bound forms.
account the presence of an inactive isomer in the synthetic material. Samples were autoclaved at 120° C. for 20 minutes in the presence of sodium ascorbate (2 mg./mL.) in order to insure conversion of certain heat-labile derivatives of tetrahydropteroylglutamic acid to the stable form, 5-formyl-tetrahydropteroylglutamic acid (folinic acid; CF). Thus the difference between the activity of heated and unheated samples provides an indirect measurement of the heat-labile reduced derivatives, resembling anhydroecovorin, which occur in human urine.8

Paper Chromatography: Strips of Whatman No. 3 paper, 1 inch in width, were loaded carefully with 0.05-mL portions of each sample and developed at 4° C. by a descending solvent (0.1 M. phosphate buffer, pH 6.0). The wet paper strips were placed directly on a solid assay medium that contained sodium ascorbate (2 mg./mL.) and was seeded with Pediococcus cerevisiae; long lucite trays provided with glass covers were used. Zones of growth observed after incubation overnight at 37° C. indicated the location of active compounds.

Case Report

C. E. K., a white boy aged 3 years and 9 months, was admitted to Grace-New Haven Hospital on March 17, 1955, because of pallor and lethargy. He was the result of his mother’s second normal pregnancy and at birth weighed 7 lb., 10 oz. The neonatal course was not remarkable. At four weeks of age the infant contracted thrush. When four months old he developed a localized, recurrent, erythematous tender lesion of the tongue.

At the age of one year (June, 1952) he was hospitalized near his home for two weeks because of weakness and anorexia of one month’s duration. His skin was lemon-yellow in color, but no jaundice was present. The tip of the tongue was red. There was no enlargement of liver or spleen. A severe normocytic, normochromic anemia (hemoglobin, 35 per cent), megaloblastic marrow, and absence of free hydrochloric acid on gastric analysis were noted. Treatment consisted of transfusions, oral folic acid, parenteral liver extract, and vitamin B12. Recovery was dramatic. Free hydrochloric acid was then found in the gastric juice. The patient received 3 ml. of crude liver extract intramuscularly at weekly intervals for three weeks.

The child did well for about sixteen months on oral vitamin B12, folic acid, ascorbic acid, and iron. On December 15, 1953, he was again admitted to the same hospital because of anemia (hemoglobin, 44 per cent). During the hospitalization he developed a bloody diarrhea which cleared. Gastric analysis again showed no free hydrochloric acid. Following a transfusion he was discharged on daily oral medications of folic acid 15 mg. and ascorbic acid 300 mg.

In the year preceding admission to our hospital, the child suffered from frequent respiratory infections which were treated with antibiotics. He received daily oral doses of vitamin B12, folic acid, ascorbic acid, iron, and multivitamin preparations. It is noteworthy that the child’s diet had been a varied one, like that of his parents. He was a good eater with few dislikes. Five weeks prior to admission he developed chickenpox and thereafter did poorly. He soon contracted bronchiolitis. Petechiae appeared on the cheeks with hard coughing.

The family history revealed that both parents were living and well in the age of 32 and a 7-year-old brother was well. The patient’s maternal grandfather, although never anemic and living to 71, did have completely gray hair at 22. The mother had a French father and English mother. The father’s parents were Czechoslovakian. There was no history of anemia among relatives.

Findings on Admission: When admitted to our hospital on March 17, 1955, the patient appeared pale and chronically ill but well-nourished. Positive physical findings included atrophy of the papillae of the tongue without redness and a grade 1 systolic murmur over the entire precordium. There was no enlargement of lymph nodes, spleen, or liver. Neurological examination disclosed no abnormalities.

Laboratory findings at the time of admission included the following: Hgb., 7.2 Gm. per
100 ml. of blood; RBC, 2,150,000 and WBC, 4,000 per cu.mm.; hematocrit, 22. Differential leucocyte count: polymorphonuclears 24, basophils 2, lymphocytes 72, monocytes 3, and eosinophils 2 per cent. The platelet count was 212,000 per cu.mm. of blood; the mean corpuscular volume was 105 cu, the mean corpuscular hemoglobin was 35.5, while the mean corpuscular hemoglobin concentration was 34 per cent. The erythrocytes showed moderate microcytosis and slight macrocytosis, slight variation in shape, slight hypochromia, occasional target cells, slight polychromasia, rare ovalocytes, and stippled cells. Some of the erythrocytes appeared to be well-filled with hemoglobin, and reticulocytes were 1 per cent. Marrow aspiration on admission gave the values shown in fig. 1. Nose and throat culture, stool examination, urinalysis, and butanol-extractable iodine level were not remarkable.

RESULTS

Course: The child was hospitalized for four weeks. Fever was present only during the first and part of the fourth weeks.

The therapeutic agents employed and the response of the blood and bone marrow values are depicted in figs. 1 and 2. It will be noted that, beginning on the sixth hospital day, the patient received, in three divided doses daily, 75 mg. of ascorbic acid for eight days and 15 mg. of pteroylglutamic (folic) acid for twenty days. Because the hemoglobin level fell from 7.2 Gm. to 4.8 Gm. per 100 ml. of blood on the ninth day without demonstrable blood loss, and the child had a temperature of 38.5° C. (rect.), he was given a transfusion of 210 ml. of sedimented red cells at that time. This elevated the hemoglobin level to 9 Gm. per 100 ml. of blood by the thirteenth day, but the reticulocyte value remained at only 0.3 per cent. Since the patient had received adequate amounts of ascorbic acid and folic acid daily for eight days (in addition to amounts of both vitamins that later were established as having been given regularly prior to admission to our hospital) without evidence of new blood formation, and because the clinical state of the child was clearly deteriorating rapidly, at this point he was given 30 g. of vitamin B1 intramuscularly. A reticulocytosis promptly ensued; after 36 hours, 3.9 per cent, and, after four days, a peak of 22.8 per cent was reached. The oral administration of folic acid, as indicated previously, was continued throughout.

On the basis of studies by Berk et al.10 and many subsequent workers, which demonstrated that vitamin B12 can be regarded as extrinsic factor whose absorption from the gastrointestinal tract is dependent upon the availability of intrinsic factor, studies of the capacity of the child to absorb vitamin B12 were carried out, at first according to the method devised by Schilling.11 Thus, about four months after the single parenteral dose of vitamin B12, the patient was given, by mouth, 0.5 μg. of vitamin B12-Co60 (0.06 μc.). Two hours following the above-described oral dosage, nonradioactive vitamin (1,000 μg.) was given

*Freshly obtained from the Research Laboratories of Merck and Co. through the courtesy of Dr. Charles Rosenblum. An earlier investigation of the absorption of vitamin B12-Co60, as influenced by intrinsic factor, was carried out in April, 1955, but this study was without success because the vitamin B12-activity of the material employed, as determined subsequently through the kind efforts of Dr. Rosenblum, had been lost completely. This deterioration has been attributed to bacterial degradation of the radioactive vitamin in a solution, the lack of sterility of which had not been appreciated.
Fig. 1.—Microscopic findings in bone marrow, and percentage of reticulocytes (RETICS) and hemoglobin levels (HGB) in peripheral blood, at time of admission and subsequent to therapy.
Fig. 2.—Total (WBC) and polymorphonuclear (PMN) leucocyte and platelet counts, in peripheral blood, as correlated with the therapeutic regimen.

Parenterally; urine was collected for 24 hours. Of the radioactivity administered, less than 1 per cent appeared in the collected urine. One week later, the test was repeated, except that the oral dose of radioactive vitamin, 0.5 μg., was administered together with 25 mg. of a potent source of intrinsic factor, i.e., the
fraction B described by Prusoff and his associates, again 1,000 μg. of non-radioactive vitamin B₁₂ were administered intramuscularly two hours subsequently. Urine was collected for 24 hours and was found to contain approximately 9 per cent of the radioactivity administered.

Approximately eighteen months later (January 14, 1957), during which time additional vitamin B₁₂ had not been given, the boy again was given 0.5 μg. (0.06 μc.) of vitamin B₁₂-Co⁶⁰ by mouth, but additional nonradioactive vitamin was not administered, and measurements of the fecal excretion of radioactivity were made according to the procedure of Heinle et al. These studies indicated that, within a very small experimental error, all the administered vitamin was contained in the stools. One month later the above-described test was repeated, except that 20 mg. of the previously mentioned concentrate of intrinsic factor was administered together with the oral dose (0.5 μg.) of radioactive vitamin B₁₂. Of the ingested radioactivity, only 28 per cent appeared in the stools, a finding indicating that as a result of the co-administration of intrinsic factor, approximately 70 per cent of the ingested vitamin had been removed from the gastrointestinal tract.

Just prior to the last administration of radioactive vitamin B₁₂, 0.1 mg. of histamine was given intramuscularly and 28 ml. of gastric juice were withdrawn. This material, very rich in "mucus," had a pH of 2.1; its behavior on titration with a dilute solution of sodium hydroxide indicated that it contained free hydrochloric acid. Similar observations were made in April, 1957, and evidence indicating the presence of both free hydrochloric acid and pepsin in normal amounts and concentrations was obtained. A gastric biopsy, kindly taken at this time by Dr. Howard Spiro, disclosed only a mild diffuse superficial gastritis and gave no indications of an anatomical reason for the failure to secrete intrinsic factor.

Studies on Urinary Folic Acid and Folinic Acid: During the period that the patient was receiving pteroylglutamic acid (PGA) (5 mg. every 8 hr.), samples of urine were obtained for determination of folic and folinic acid activity. At the time, these tests were considered exploratory in nature and, in retrospect, it is unfortunate that continuous 24-hour collections were not made. Urine was collected during 8-hour periods (from 6:00 a.m. to 2:00 p.m.) on March 26 and April 1, 1955, while the first dose of parenteral vitamin B₁₂ (30 μg.) was given on March 28. The samples were assayed, as described, for both folic acid and folinic acid activity. The results are presented in table 1. The marked differences in folinic acid activity in both the heated and the unheated portions of the samples collected before and after administration of vitamin B₁₂ are considered significant, since there was no appreciable difference in the total folic acid activity (S. faecalis assay) during each period of collection.

*The specificity of the requirement of Pediococcus cerevisiae 8081 (Leuconostoc citrovorum), for N⁵-formyl-tetrahydropteroylglutamic acid (folinic acid; CF) is only an apparent specificity related to the unique stability of the N⁵-formyl derivative and the assay technique. Of the various reduced derivatives of PGA, only that referred to as CF (and certain polyglutamate derivatives of it, e.g., the compounds containing two and three glutamic acid residues) withstands autoclaving temperatures in the usual microbial assay. The products of the metabolic reduction of PGA, however, include a substance (or sub-
words, an increase was observed in the proportion of the total folic acid activity that was excreted in the form of reduced derivatives of the vitamin (measurable as substances used by *Pediococcus cerevisiae*). Although it might be thought possible that an incomplete saturation of body tissues with reduced forms of folic acid existed at the time of the first collection of urine, this is quite unlikely, because PGA had already been administered by us for 8 days; in fact, the patient previously had been receiving regularly an oral vitamin supplement containing PGA (see history). In addition, the patient was undoubtedly “saturated” with ascorbic acid. Although the administration of supplemental ascorbic acid (25 mg. every 8 hr.) was discontinued on the day that vitamin B\textsubscript{12} was first injected, the significance of this variation in treatment, with respect to the folinic acid activity observed in the urine, is considered to be negligible. The presence in the urine of larger amounts of ascorbic acid would not have affected the observed differences.

At a subsequent time, when the patient had been without specific therapy for over two years and megaloblasts had reappeared in the bone marrow, PGA was administered in oral doses of 5 mg. every 8 hours, and a similar comparison was made of the folic acid and folinic acid activity of heated and unheated samples of urine. These were collected for consecutive 24-hour periods (Sept. 13 through Sept. 30, 1957) before and after the parenteral administration of vitamin B\textsubscript{12}. Unfortunately, under these conditions of partial relapse, the analyses disclosed no significant differences in activity between the two groups of samples.

The findings upon paper chromatography of the unheated samples of urine were of particular interest in relation to the initial administration of vitamin B\textsubscript{12} in this hospital (March, 1955). The samples described in table 1 were examined by paper chromatography and the location of compounds with folinic acid activity is shown in fig. 3. Having observed many chromatograms of the folinic acid derivatives in the urine of normal subjects receiving PGA, it was unexpected to find, in the unheated sample obtained before administration of vitamin B\textsubscript{12}, that the major portion of the activity remained at the origin (strip I, fig. 3). Similar treatment of the sample obtained after the administration of vitamin B\textsubscript{12} resulted in a distribution of activity similar to that seen in urine samples of normal subjects receiving similar amount of PGA (compare strips II and III, fig. 3). The compound that moved to \(R_f\) 0.6 can be identified as the heat-stable form of folinic acid: 5-formyl-5,6,7,8-tetrahydro-pteroylglutamic acid.\textsuperscript{3,7,14} However, chromatography of the duplicate heated samples indicated that the zones of growth at and near the origin were due to heat-labile compounds. In each case, heating in the presence of ascorbate increased the activ-

stances) which, when diluted and autoclaved with the medium in the usual microbial assay, is not measurable as CF unless the autoclaving is carried out in the presence of ascorbate (usually 1 or 2 mg./ml.) or another appropriate reducing agent. If the samples are not heated with ascorbate, but are added aseptically to the previously autoclaved medium (without added ascorbate), full CF activity is not recovered, since the precursor of folinic acid (possibly anhydroleucovorin) is not measured.\textsuperscript{5}
Fig. 3.—Tracings of paper chromatograms of unheated urine samples* showing zones of growth of *Pediococcus cerevisiae* 8081 (*Leuconostoc citrovorum*) in plates of solid medium.

*Indicates a sample obtained from the child two days before the initial parenteral administration of 30 μg. of vitamin B₁₂ (as described in the text). II indicates a similar sample obtained four days after this treatment. Oral therapy with PGA (5 mg., every 8 hr.) was maintained throughout this period. III indicates a urine sample from a normal male donor receiving similar treatment with PGA; this observation is representative of many similar chromatograms.

ity measurable as folinic acid (table 1); the size of the zone of growth at \( R_f \) 0.6 was also increased. Chromatography of these samples and examination in this manner were repeated three times and the same observations were made consistently.

On two separate occasions within the past year, the boy has developed glossitis and a decrease in the level of hemoglobin to approximately 10 grams percent 100 ml. of blood, in association with pinworm infestation (*Enterobius vermicularis*). Throughout this period he was being maintained on oral vitamin B₁₂ (15 μg.) together with a concentrate of intrinsic factor (15 mg.).* On

*Kindly furnished by Doctors L. Ellenbogen and W. L. Williams of the Lederle Laboratories Division of the American Cyanamid Company.
these occasions, with the latter medication maintained at its usual level, we treated only the pinworm infestation with piperazine. With the elimination of the parasites, the glossitis promptly cleared, as did the mild anemia. We have interpreted this observation as suggesting that the infestation upset in some way a marginal state of vitamin $B_12$ utilization or metabolism and created a deficiency of the vitamin. We are not aware of a similar association reported previously, although infestation with the fish tapeworm (*Diphyllobothrium latum*) has long been associated with the development of megaloblastic anemia.

**Discussion**

We have reported our findings in a young child with a juvenile pernicious anemia. His course and laboratory data present a number of noteworthy features, one or two of which contribute new knowledge to this syndrome. Although thrush in his mouth at the age of four weeks cannot confidently be regarded as evidence of mucosal atrophy, true glossitis was observed at the early age of four months, characterized by persistent redness and soreness which made feeding him difficult. By the age of one year his anemia was severe ($\text{Hgb} = 5.4 \text{ Gm. per cent}$) and it was megaloblastic, as shown by marrow examination. That the child responded only to parenterally administered vitamin $B_12$ and relapsed in spite of orally ingested vitamin $B_12$, folic acid, ascorbic acid, and iron is a most significant combination of circumstances. It strongly points to an inability to absorb vitamin $B_12$, presumably due to lack of intrinsic factor secretion; in other words, Addisonian pernicious anemia. The macrocytic anemia of the peripheral blood (mean corpuscular volume $= 105 \mu$ and mean corpuscular hemoglobin concentration $= 34$ per cent) and the megaloblastic marrow (40 per cent erythroid) also support the diagnosis.

But the diagnosis of pernicious anemia no longer can be said to have been proven by such data. Malabsorption syndromes and intestinal shunts may be associated with the foregoing findings. That our patient truly has pernicious anemia has been demonstrated by the fact that he failed to absorb orally administered radioactive vitamin $B_12$ alone, but did absorb the vitamin when it was given with intrinsic factor concentrate, and that his gastric juice, mixed with radioactive vitamin $B_12$ and fed to an adult with pernicious anemia in relapse, failed to enhance the latter’s absorption of the radioactive vitamin, as indicated by studies of the excretion of cobalt-60. The first two facts cited above were confirmed not only by measuring the quantity of the radioactive vitamin recovered in stools, but also by “flushing” it out in the urine with one milligram of parenterally administered conventional vitamin $B_12$ (Schilling test). Repeated relapses of the boy’s megaloblastic anemia in the absence of parenterally-administered vitamin $B_12$, or of the vitamin ingested with intrinsic factor concentrate, attest to his continuing need for such supplementation.

Of special interest is the presence of free hydrochloric acid and pepsin in the strongly acid ($\text{pH} = 2.1$) gastric juice and the essentially normal gastric mucosa, as demonstrated by biopsy. In adults with established pernicious anemia, achlorhydria and gastric atrophy are the rule. The failure to secrete acid and intrinsic factor were generally regarded as being secondary to atrophy.
of the gastric mucosa. That a child may have an anatomically normal stomach
and free hydrochloric acid in the gastric juice, in the absence of intrinsic factor,
suggests a reversal of the foregoing hypothesis. We believe that our patient's
course supports the concept recently proposed by others\textsuperscript{15,16,17} that the primary
inherited defect in pernicious anemia, presumably present from early life, is
inability to secrete intrinsic factor. This defect ultimately may lead to gastric
atrophy which, in turn, results in achylia gastrica. The atrophy eventually be-
comes irreversible. It should be possible to detect the absence of intrinsic factor
in patients with pernicious anemia before gastric atrophy occurs. Furthermore,
it seems reasonable to hope that in young patients such as ours, gastric atrophy
and achlorhydria may be prevented by the appropriate administration of vita-
mim B\textsubscript{12} throughout life.

The familial occurrence of pernicious anemia has been well-documented.
Three children with proven disease have had a family history of it.\textsuperscript{16} Our patient
had no anemic relatives, although his maternal grandfather had completely
gray hair at the age of 22, a sign often associated with the disease.

Neurologic signs, often observed in adults as combined system disease but
rarely seen in children, have been reported in two children with pernicious
anemia.\textsuperscript{16} At no time, however, did our patient show signs of central nervous
system involvement, despite the fact that his appreciation of vibratory sensa-
tion was regularly examined in both ankles.

Of potential importance to the concepts of mechanism of action of vitamin
B\textsubscript{12} and folic acid in pernicious anemia was the appearance in the urine of this
patient, prior to the injection of vitamin B\textsubscript{12} but while folic acid was being
continued, of a substance with chromatographic properties different from those
of folinic acid, per se (although, after heating in the presence of ascorbate, it
exhibited the microbial activity of the latter). The absence of this substance
from the urine obtained subsequent to the parenteral administration of vitamin
B\textsubscript{12} is of particular significance (fig. 3). Of additional importance is the marked
increase in the urinary excretion, following the injection of vitamin B\textsubscript{12}, of
substances with the microbial activity of folinic acid (table 1). It should be
emphasized in this connection that the patient absorbed efficiently the orally
administered folic acid. This is shown by the data of table 1, which demon-
strate that the urinary excretion of folic acid exceeded 60 per cent of the 8-hour
(5 mg.) dosages, both before and after the administration of vitamin B\textsubscript{12}. A
group of normal adults receiving roughly comparable amounts of oral folic
acid, on a weight basis (i.e., 50 mg. daily), excreted approximately 38 per cent
of the daily dose in the urine.\textsuperscript{19}

It is conceivable that, under certain circumstances, a really profound deficien-
cy of vitamin B\textsubscript{12} can lead to an alteration in the metabolism of folic acid and
the accumulation of a derivative (traces of which appear in the urine) that
is unable to catalyze a reaction critically concerned with normal erythropoiesis.
A logical extension of such an hypothesis would suggest that vitamin B\textsubscript{12} (or a
metabolically formed derivative of it) is involved in the conversion of a deriva-
tive of folic acid or tetrahydrofolic acid to a functional compound. Thus, re-
cent studies of the role of vitamin B\textsubscript{12} in the synthesis of the thymine-containing
precursor of deoxyribonucleic acid\textsuperscript{19} suggest that the effect is related to a tetra-
Table 1.—Effect of Vitamin B₁₂ on the Excretion of Folic Acid and Folinic Acid by a Child with Pernicious Anemia

<table>
<thead>
<tr>
<th>Urine samples*</th>
<th>Assay of activity in urine (S. faecalis)</th>
<th>Assay of activity in urine (Ped. cerevisiae)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before vitamin B₁₂</td>
<td>Total folic acid 4.1</td>
<td>Folinic acid 22.2</td>
</tr>
<tr>
<td></td>
<td>Unheated sample</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heated sample</td>
<td>3290</td>
</tr>
<tr>
<td>After vitamin B₁₂</td>
<td>Unheated sample</td>
<td>3120</td>
</tr>
<tr>
<td></td>
<td>Heated sample</td>
<td>3100</td>
</tr>
</tbody>
</table>

*The samples were obtained two days before and four days after the patient received a single injection of vitamin B₁₂ (30 μg. intramuscularly). The patient received pteroylglutamic acid (5 mg.) at 8-hour intervals throughout this period. Ascorbic acid was administered as described in the text. Portions of each sample were heated at 120° C. for 20 minutes after the addition of sodium ascorbate, 2 mg. ml.

†See discussion in accompanying text and footnote.

hydrofolic acid-dependent reaction concerned with the conversion of deoxyuridine 5'-phosphate to its methylated derivative, thymidylic acid. Furthermore, recent evidence obtained from the study of the formation or utilization of a derivative of tetrahydrofolic acid.²⁰,²¹

However, it remains to be demonstrated whether, in relatively simple systems, a sufficiently severe limitation on the supply of vitamin B₁₂ can cause an accumulation of a derivative of folic acid, as appears to have occurred in the present case. As has been suggested previously,²²,²³ there is steadily increasing evidence to suggest that the hematopoietic defect in pernicious anemia is a manifestation of a disturbance in the metabolism of folic acid that is secondary to the deficiency of vitamin B₁₂, which, in turn, is caused by an inadequate formation of intrinsic factor.

It is unfortunate that, upon withholding therapy with vitamin B₁₂ until the reappearance of anemia, a megaloblastic arrest of erythropoiesis in the bone marrow, and other manifestations of relapse, a repetition in the alteration of the pattern of excretion of folic acid-like compounds was not observed. In the absence of evidence to the contrary it may be suggested tentatively that the severity of the vitamin B₁₂-deficiency state which led to the first disturbances of folic acid metabolism studied by us was not, in the second instance, of sufficient magnitude to be reflected by comparable changes in the pattern of urinary excretion of metabolites of folic acid.

Whether or not the findings described have been interpreted correctly can be established only by additional investigation. Accordingly it is hoped that attempts will be made to discover other cases of very severe pernicious anemia in infants or young children, and that in them much more extensive studies will be made of the pattern of metabolic formation and urinary excretion of derivatives of folic acid before and after the parenteral administration of vitamin B₁₂.

**SUMMARY**

Observations on a 4-year-old boy with Addisonian pernicious anemia have been presented. Noteworthy clinical features included the onset of glossitis.
at the age of 4 months, followed by anemia severe enough to require hospital-
ization at the age of 1 year. Relapse occurred in the absence of specific therapy
with vitamin $B_{12}$ and was completely unaffected by the administration of folic
acid.

Studies with radioactive vitamin $B_{12}$ demonstrated that almost all of the
compound administered by mouth was unabsorbed and was recovered in the
stools. When the vitamin was given simultaneously with a concentrate of in-
trinsic factor, however, approximately 70 per cent was absorbed. Furthermore,
the child's gastric juice, when mixed with radioactive vitamin $B_{12}$ and
fed to an adult with pernicious anemia in relapse, failed to enhance the lat-
ter's absorption of the vitamin. The failure of our patient to absorb the vitamin
alone, but his ability to do so when it was administered with intrinsic factor
concentrate, was also confirmed by the “Schilling test,” in which a proportion
of the absorbed radioactive vitamin was “flushed” into the urine by parenteral
injection of one milligram of conventional vitamin $B_{12}$.

Of special interest was the occurrence in the urine of an unidentified deriv-
ate of tetrahydrofolic acid, derived from orally administered pteroylglutamic
acid. The presence of this compound in the urine was demonstrated chromato-
graphically when the patient was critically ill with his disease prior to treat-
ment with vitamin $B_{12}$. Subsequent to therapy with vitamin $B_{12}$, while the
administration of folic acid was continued, the abnormal metabolite of folic
acid could not be found in the urine. Similarly, the administration of folic
acid did not lead to the appearance of this metabolite in the urine at a time
when, after more than two years without specific therapy, a hematological
relapse occurred that was much less severe than that previously observed. The
implications of these observations, with respect to the metabolic interrelation-
ships of folic acid and vitamin $B_{12}$, are discussed.

Of further interest were the findings of strongly acid gastric juice containing
much mucus and free hydrochloric acid. A fairly normal gastric mucosa was
demonstrated by biopsy. The meaning of these unusual findings is discussed
and an hypothesis to account for them is offered. The probable sequence of
events in these patients from childhood to the development of anemia, usually
in later life, is set forth.

**Summario in Interlingua**

Es presentate observationes super un puero de 4 annos de etate. Notabile
tractos clinic include be inception de glossitis al etate de 4 menses, sequite al
etate de 1 anno per anemia satis sever pro requirer hospitalisation. Un recidiva
occurreva in le absentia de specific therapia a vitamina $B_{12}$ e esseva complete-
mente inafficite per le administration de acido folic.

 Studios con radioactive vitamina $B_{12}$ demonstrava que iste composito, ad-
ministrate per via oral, remaineva inabsorbite in su quasi-totalitate e poteva
esser retrovate in le feces. Tamen, quando le vitamina esseva administrate
simultaneemente con un concentrato de factor intrinsèc, aproximativmente
70 pro cento de illo esseva absorbite. Etiam, quando le succo gastric del pa-
tiente esseva miscite con radioactive vitamina $B_{12}$ e administrate a un adulto
con anemia perniciose in recidiva, illo non facilitava le absorption del vitamina
per le adulto. Le incapacità di nostre paziente di assorbire le vitamina sol, e suo capacità di assorbire lo quando illo essaeva administrare con concentrato de factor intrinsec essaeva etiam confirmate per le test de Schilling, in le qual un portion del absorbite vitamina radioactive essaeva "lavate" a in le urina per medio de un injection parenteral de 1 mg de ordinari vitamina B_{12}.

De interesse special esseva le presentia in le urina de un non-identificate derivato de acido tetrahydrofolic, le qual essaeva derivate ab oralmente administrate acido pteroglutamic. Le presentia de iste composito in le urina habeva esseva demonstrate chromatographically quando le patiente habeva essite criticamente malade ante le therapia a vitamina B_{12}. Post le inception del therapia a vitamina B_{12} le administration de acido folic essaeva continue, sed le metabolito anormal de acido folic non essaeva trovabile in le urina. Similemente le administration de acido folic non resultava in le reapparition de iste metabolito in le urina al tempore—post plus que duo annos sin therapia specific—quando ocorreva un recidiva multo minus sever que illo previemente observate. Es discutite le signification de iste observationes con reguardo al interrelationes metabolic de acido folic e vitamina B_{12}.

Etiam de interesse esseva le constatation de un fortemente acide succo gastric le qual contineva multe muco e libere acido hydrochloric. Un mucosa gastric satis normal essaeva demonstrate per medio de biopsia. Le signification de iste constatationes inusual es discutite, e un hypothese as offerite pro explicar los. Es presentate le probabile sequentia de eventos in le casos de patientes ab lor infantia usque al disveloppamento de anemia, generalmente a etates plus avantiante.

REFERENCES

1. Castle, W. B., and Minot, G. R.: Patho-
logical Physiology and Clinical Des-
description of the Anemias. Edited by H.
A. Christian. 205 pp. New York; Ox-
ford, 1936.
2. Reisner, E. H., Jr., Wolff, J. A., McKay,
R. J., Jr., and Doyle, E. F.: Juvenile
pernicious anemia. Pediatrics. 8:88-
106, 1951.
and folinic acids. In Symposium on
Vitamin Metabolism of the National
Vitamin Foundation, Nutrition Sym-
4. Welch, A. D.: Nutritional role of folic
5. Flynn, L. M., Williams, V. B., O'Dell,
B. L., and Hogan, A. C.: Medium for
assay of vitamins with lactic acid
acid upon the urinary excretion of a
growth factor required by Leucon-
ostoc citrovorum. J. Biol. Chem. 181:
467, 1949.
7. Cosulich, D. B., Smith, J. M., Jr., and
Broquist, H. P.: Diasteriomers of leucovorin. J. Am. Chem. Soc. 74:
4215, 1952.
8. Silverman, M., Ebaugh, F. G., and
Gardiner, R. C.: The nature of labile
citrovorum factor in human urine. J.
9. Albrecht, A. M., and Broquist, H. P.: Evidence for occurrence of 10-formyl-
tetrahydrofolic acid in human urine.
9. Albrecht, A. M., and Broquist, H. P.: Evidence for occurrence of 10-formyl-
tetrahydrofolic acid in human urine.
1956.
10. Berk, L., Castle, W. B., Welch, A. D.,
Heinle, R. W., Anker, R., and Ep-
stein, M.: Observations on the etiologic relationship of achylia gastrica to

David H. Clement, M.D., Associate Clinical Professor of Pediatrics, Yale University School of Medicine, New Haven, Conn.

Charles A. Nichol, M.D., Director of the Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, N. Y.

Arnold D. Welch, M.D., Chairman and Eugene Higgins Professor of the Department of Pharmacology, Yale University School of Medicine, New Haven, Conn.
A Case of Juvenile Pernicious Anemia: Study of the Effects of Folic Acid and Vitamin B\textsubscript{12}

DAVID H. CLEMENT, CHARLES A. NICHOL and ARNOLD D. WELCH