PERNICIOUS ANAEMIA TREATED ORALLY WITH "BIFACTON"*

REFRACTORINESS TO POTENT ANIMAL INTRINSIC FACTOR

BY

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The underlying defect in pernicious anaemia is a severe deficiency of intrinsic factor secretion by the glandular epithelium of the fundus and body of the stomach. This results in decreased absorption of dietary vitamin B₁₂ to an amount less than the body requirement. Body stores are utilized and eventually depleted. The serum vitamin B₁₂ concentration then falls below normal and megaloblastic erythropoiesis with an associated macrocytic anaemia results.

The certain diagnosis of pernicious anaemia can be made only by demonstrating reduced secretion of intrinsic factor of sufficient degree to impair vitamin B₁₂ absorption.

When a small oral dose of radioactive vitamin B₁₂ is given to a patient with pernicious anaemia impaired absorption can be demonstrated by several methods. If the radioactive dose is given with a source of intrinsic factor normal absorption occurs. For the purpose of such tests either human gastric juice or partially purified preparations from hog stomach or duodenal mucosa have been used successfully as sources of intrinsic factor. Radioactive vitamin B₁₂ absorption tests make it possible to establish the diagnosis of pernicious anaemia even in patients who are in haematological remission, and for this reason have a wide application in the investigation of patients with megaloblastic anaemia.

It is more convenient to use animal intrinsic factor preparations than human gastric juice in performing radioactive vitamin B₁₂ absorption tests. In this communication we present the findings in a patient with pernicious anaemia previously treated by an oral vitamin B₁₂-intrinsic factor preparation who showed impaired absorption when the radioactive vitamin B₁₂ was administered orally with hog intrinsic factor. This intrinsic factor preparation regularly promotes normal absorption of radioactive vitamin B₁₂ in patients with pernicious anaemia. Our patient absorbed radioactive vitamin B₁₂ normally when the dose was given with human gastric juice. The phenomenon of refractoriness to animal intrinsic factor following oral therapy has been observed recently by Schwartz *et al.* (1957).

Materials and Method of Investigation

We have employed a modification of the urinary excretion test (Schilling, 1953) to demonstrate absorption of radioactive vitamin B₁₂. At present we believe the urine test to be the most readily applicable to clinical problems. Mollin and Baker (1955) showed that in some subjects an injection of carbachol (carbamylcholine chloride) increases the secretion of gastric intrinsic factor, and we use carbachol routinely in our modification of the test. We collect urine for 48 hours after the oral dose of radioactive vitamin B₁₂, since Ellenbogen *et al.* (1955) have shown that considerable

amounts of radioactivity may be excreted on the second day. The test is carried out in the following manner:

The patient fasts for at least four hours prior to the test and for one hour after the oral dose of radioactive vitamin B₁₂. Fifteen minutes after an intramuscular injection of 0.25 mg. of carbachol the patient drinks 0.6 µg. ⁵⁷Co vitamin B₁₂ (specific activity 0.24 µC./µg.) in 10 ml. of water. Then 1 mg. of non-radioactive vitamin B₁₂ is immediately injected intramuscularly and urine collected for 24 hours. At the end of 24 hours a further 1 mg. of non-radioactive vitamin B₁₂ is injected intramuscularly and urine collected for a further 24 hours.

Radioactivity of 100-ml. samples of urine is measured in an annular type scintillation counter and urinary radioactivity expressed as a percentage of the administered dose. If this test shows impaired absorption of radioactive vitamin B₁₂, the dose of ⁵⁷Co vitamin B₁₂ is mixed with 50 mg. of a preparation of hog-stomach intrinsic factor immediately before administration and the whole test repeated. This intrinsic factor (Batch 186-1) is from the same batch as that used by Ellenbogen *et al.* (1955) and Baker and Mollin (1955).

Our experiences with this test are shown in Table I. In 13 of 14 proved cases of pernicious anaemia in relapse, 50 mg. of the intrinsic factor preparation was found sufficient to increase urinary excretion of radioactivity into the range shown by 22 normal subjects.

TABLE I.—*Urinary Excretion Test Following an Oral Dose of 0.6 µg. ⁵⁷Co Vitamin B₁₂ in 22 Normal Controls and 14 Proved Pernicious Anaemia Patients in Relapse*

	No. of Subjects	Percentage Oral Dose Excreted			
		0-24 Hours		0-48 Hours	
		Range	Mean	Range	Mean
Normal controls	22	9-29	16.9	16-36	23.7
Pernicious anaemia patients in relapse:					
Without intrinsic factor	14	0-4	1.4	0-7	2.2
With 50 mg. intrinsic factor	14*	7-20	14.6	12-33	21.3

* 13 of the 14 patients were within the range shown by the normal controls.

The pooled human gastric juice used in this investigation was prepared by continuously aspirating fasting juice from two patients with a duodenal ulcer into a flask immersed in melting ice. N NaOH was added at frequent intervals to maintain the pH at 7 during collection. The gastric juice was then strained and stored at -20° C. until required.

Case History

On January 8, 1957, a 52-year-old woman was referred for the investigation of an anaemia for which she had received various forms of treatment for six years. Treatment included oral and parenteral iron, injections of liver extract, and recently vitamin B₁₂ intramuscularly. Five months before referral parenteral therapy was stopped temporarily while she was treated with "bifacton" tablets,* one three times a day for a period of eight weeks. Thereafter intramuscular vitamin B₁₂ was recommenced.

A complete clinical examination failed to reveal any abnormality. Haemoglobin was 12.8 g. per 100 ml.; P.C.V., 43%; leucocytes, 3,900 per c.mm. (55% segmented polymorphs, 38% lymphocytes, 6% monocytes, and 1% basophils). A moderate degree of poikilocytosis and slight anisocytosis of the erythrocytes were found. A fractional test meal revealed histamine-fast achlorhydria.

Urinary Excretion Tests.—The results of these tests are set out in Table II. Vitamin B₁₂ absorption was considerably impaired, and doses of 50 mg. and 200 mg. of the hog intrinsic factor preparation (Batch 186-1) were ineffective in significantly increasing vitamin B₁₂ absorption. The test was repeated using two volumes of pooled human gastric juice. Urinary radioactivity increased significantly with 50 ml. and fell within the normal range with 100 ml. Thus the diagnosis of pernicious anaemia was established.

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*Each tablet contains not less than 7.5 µg. of vitamin B₁₂ and not less than 18 mg. of hog-stomach intrinsic factor concentrate.

TABLE II.—*Urinary Excretion Test Following an Oral Dose of ^{60}Co Vitamin B₁₂ With Varying Amounts of Animal Intrinsic Factor and Human Gastric Juice*

	Percentage Oral Dose Excreted	
	0-24 Hours	0-48 Hours
Animal intrinsic factor:		
Nil	3	3
50 mg.	1	2
50 mg.	Nil	1
200 mg.	3	—
Human gastric juice:		
50 ml.	9	12
100 ml.	12	31

Discussion

The investigation of this patient confirms the observation of Schwartz *et al.* that the effectiveness of animal intrinsic factor may be reduced in pernicious anaemia patients who have been treated orally with preparations containing intrinsic factor and vitamin B₁₂. This phenomenon presents certain difficulties in the interpretation of vitamin B₁₂ absorption tests in the diagnosis of pernicious anaemia. If failure to absorb radioactive vitamin B₁₂ when given with animal intrinsic factor occurs in a patient previously treated orally, it would be advisable to repeat the test, using human gastric juice. It seems possible that other patients reported in the literature as having a selective malabsorption to the vitamin B₁₂-intrinsic factor complex may also be identified as having pernicious anaemia when reinvestigated using pooled human gastric juice.

It is not yet known how often or how rapidly this phenomenon occurs in such patients. Mollin and Ross (1954) noted in a pernicious anaemia patient on continuous oral therapy with bifacton tablets that the serum vitamin B₁₂ level fell after approximately six months. In a patient of Schwartz *et al.* the values for the haemoglobin and red-cell count, while never normal during the successful period of oral therapy, began to fall after about eight months. In our patient, bifacton tablets were taken for a period of eight weeks.

In the treatment of pernicious anaemia patients, whether in a state of relapse or remission, it appears that parenteral therapy with vitamin B₁₂ is undoubtedly the more certain method and is therefore to be preferred.

Summary

The results of radioactive vitamin B₁₂ absorption tests using hog stomach and pooled human gastric juice as sources of intrinsic factor are presented in a pernicious anaemia patient previously treated with bifacton tablets. It was shown that potent animal intrinsic factor did not increase vitamin B₁₂ absorption in this patient. However, normal absorption occurred when human gastric juice was used as the source of intrinsic factor.

The implication of this phenomenon in the future investigation and management of pernicious anaemia patients is discussed.

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Addendum

Dr. Ellenbogen supplied a more highly purified hog intrinsic factor preparation for trial on the patient. 5 mg. of this material is at least as potent as 50 mg. of Batch 186-1 in potentiating the absorption of radioactive vitamin B₁₂ in patients with pernicious anaemia. The urinary excretion test was repeated on the patient, using the purified intrinsic factor, and was still abnormal. After an oral dose of 0.6 $\mu\text{g.}$ of ^{60}Co vitamin B₁₂ and 5 mg. of the purified material, 6% of administered radioactivity appeared in the urine in 48 hours. Three patients with pernicious anaemia in relapse excreted between 23% and 30% of administered

radioactivity when tested with the same dose of purified intrinsic factor.

To investigate the possibility that the patient's gastric juice contained a substance inhibitory to hog intrinsic factor, 75 ml. of gastric juice was mixed with 5 mg. of purified intrinsic factor. This was administered to a patient with known pernicious anaemia in remission, together with 0.6 $\mu\text{g.}$ ^{60}Co vitamin B₁₂. The pernicious anaemia patient excreted 15% of the radioactivity in the urinary excretion test. When the radioactive vitamin B₁₂ was given without intrinsic factor the urinary radioactivity in this patient was 2%.

Immunological studies using the patient's serum and hog intrinsic factor have not demonstrated the presence of circulating antibody.

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Medical Memoranda

Infected Facial "Cysts" and Sinuses of Dental Origin

An acute pulp infection of a tooth or root fragment is usually a painful event that demands urgent relief. Occasionally, however, the pulp undergoes a slow and silent degeneration without symptoms and without producing any visible change in the mouth. There is only an apparently sound and filled tooth, or a gap where a tooth was extracted months or even years ago, and a forgotten root fragment has become infected. In either case it would pass unnoticed at a routine dental examination unless a dental x-ray film was taken, where it would be clearly revealed.

After a variable period of chronic infection pus discharges through the thin outer alveolar plate into the mouth, or through into the soft tissues of the cheek. There is then left an established sinus from the infected tooth or retained root, either into the mouth or into the soft tissues. In the latter case a cystic swelling appears in the cheek, which can persist for weeks or months and is similar in appearance to a low-grade infection in a sebaceous cyst. Often it is treated as a simple cyst, by excision, and is followed by a chronic discharging sinus. The communicating track between the cystic swelling and the primary focus of infection is not necessarily the shortest distance between the two, and an oblique or variable course of the communicating track can be a misleading feature.

These cases generally come to us because we are expected to deal with the presenting facial lesion with the minimum of scarring, not because of the dental condition, as this has been overlooked. Obviously, any attempt to treat the facial lesion without first removing the dental focus is a waste of time. Sometimes we are able to deal with both the dental and the facial conditions simultaneously, although occasionally it is advisable to clear the dental focus before tidying the face.

FIG. 1.—Chronic infected facial cyst.