Intrinsic factor antibodies and intrinsic factor mediated vitamin B₁₂ absorption in pernicious anaemia

S. ARDEMAN AND I. CHANARIN

*From the M.R.C. Experimental Haematology Research Unit and
Department of Haematology, St. Mary's Hospital, London*

EDITORIAL SYNOPSIS These studies indicate the possibility of an antibody to human intrinsic factor operating at cellular level in the small intestine in certain patients suffering from Addisonian pernicious anaemia.

There is considerable evidence to suggest that Addisonian pernicious anaemia is an autoimmune disease and that the transition from simple atrophic gastritis to pernicious anaemia is brought about by the development of an antibody to intrinsic factor. Briefly, intrinsic factor antibody is absent in patients with simple gastric atrophy but present in the majority of patients with Addisonian pernicious anaemia (Coghill, Doniach, Roitt, Mollin, and Williams, 1965; Ardeman, and Chanarin, 1963). The development of pernicious anaemia at a very young age (so-called 'juvenile autoimmune group') seems invariably to be associated with the presence of an intrinsic factor antibody (Doniach and Roitt, 1964). The association of pernicious anaemia and autoimmune thyroid disease (Doniach, Roitt, and Taylor, 1963) is such as to suggest that in both we are dealing with patients who have a genetically determined liability to develop an autoimmune type of disorder. On the other hand pernicious anaemia appearing in the first year of life with normal gastric histology is probably due to an inborn error of metabolism and is unrelated to the type presenting in later life.

The fact that intrinsic factor antibody is demonstrable in the serum of only 55% of the patients with Addisonian pernicious anaemia suggests that in the remaining 45%, either (1) the antibody is present at cellular level while absent from the serum, or, (2) in these the final failure of intrinsic factor mediated-vitamin B₁₂ absorption occurs by a mechanism not dependent on intrinsic factor antibody.

If an antibody against human intrinsic factor is present in all patients with pernicious anaemia, it seemed pertinent to enquire into the relative efficacy of hog intrinsic factor as opposed to human

intrinsic factor in promoting vitamin B₁₂ absorption in pernicious anaemia. If antibodies to human intrinsic factor are present, human intrinsic factor might prove less efficacious than hog intrinsic factor in potentiating vitamin B₁₂ absorption; further if human intrinsic factor antibodies are present only in those patients in whom it can be demonstrated in the serum, then only these patients should show a poorer result in vitamin B₁₂ absorption tests with human intrinsic factor as compared to hog.

Despite the vast number of publications dealing with vitamin B₁₂ absorption we are aware of only two studies comparing the potentiating effect of human and hog intrinsic factor on vitamin B₁₂ absorption in the same pernicious anaemia patients. Schwartz, Lous, and Meulengracht (1958), using an 0.5 µg. dose of oral vitamin B₁₂, found that in three previously untreated patients the mean urinary excretion in the Schilling test was 22% of the dose with hog intrinsic factor and 12% with human intrinsic factor.

Abels (1959), using a 1.0 µg. oral dose of vitamin B₁₂, found that the mean urinary excretion in 10 patients was 18.1% with 25 ml. human gastric juice and 17.9% with a hog intrinsic factor preparation. The volume of gastric juice used would probably have provided an excess of human intrinsic factor but there is some doubt whether the hog intrinsic factor was present in excess. Reduction in the amount of hog intrinsic factor produced a fall in the urinary excretion of vitamin B₁₂.

The purpose of the observations described in this paper was simply to compare the efficacy of a hog intrinsic factor concentrate with that of normal human gastric juice in promoting vitamin B₁₂ absorption in patients with Addisonian pernicious

anaemia; and secondly, to see if the results bore any relationship to the presence or absence of antibodies to intrinsic factor in the sera of these patients.

MATERIALS AND METHODS

Observations were made on 35 hospital patients not suffering from any gastrointestinal disorder and with normal renal function, and on 27 patients with megaloblastic anaemia due to vitamin B₁₂ deficiency. In 19 patients the megaloblastic anaemia was due to Addisonian pernicious anaemia and in eight it followed partial gastrectomy. None of these patients had received oral hog preparations.

Vitamin B₁₂ absorption was assessed by the urinary excretion method (Schilling, 1953) using a 1.0 µg. dose of ⁵⁷Co-vitamin B₁₂. One mg. of non-radioactive vitamin B₁₂ was given intramuscularly immediately after the oral dose and urine collected for 24 hours. The radioactivity in urine and standards was counted in a Packard auto-gamma spectrometer.

The source of human intrinsic factor was gastric juice collected after histamine stimulation. The juice was brought to pH 7.0 and stored at -20°C. One unit of intrinsic factor was the amount that took up 1.0 µmg. of vitamin B₁₂ (Ardeman and Chanarin, 1963). Five hundred units are required to produce a maximum urinary excretion of vitamin B₁₂ with a 1.0 µg. oral dose in the Schilling test (Ardeman and Chanarin, 1965a). To ensure an excess of intrinsic factor 1,500 units (20 to 25 ml. gastric juice) was given.

The hog intrinsic factor preparation was Lederle (WES 818). Ten mg. was used in the tests. This quantity bound over 3,000 µmg. of vitamin B₁₂ but the proportion of this binding due to intrinsic factor is uncertain. Tests in one patient with pernicious anaemia showed that 5 mg. of this preparation gave maximum excretion of labelled vitamin B₁₂ in the Schilling test.

Antibody to human intrinsic factor was detected by the method of Ardeman and Chanarin (1963).

RESULTS

Antibody to human intrinsic factor was present in the sera of seven of the 19 patients with Addisonian pernicious anaemia, and was not detected in the sera of the remaining 12 patients.

TABLE
URINARY EXCRETION TEST
(% of 1.0 µg. oral dose of ⁵⁷Co-B₁₂)

	Vitamin B ₁₂ Alone	With Human Intrinsic Factor	With Hog Intrinsic Factor
Control subjects (35)	19.3 ± S.E. 0.95 (11.2-32.0)		
Pernicious anaemia (19)	1.8 ± 0.1 (0.0-5.4)	11.7 ± 0.98 (5.8-26.3)	14.2 ± 1.06 (6.0-22.3)
Post-gastrectomy (8)	4.3 ± 0.34 (0.7-9.4)	14.4 ± 1.9 (8.2-24.9)	13.2 ± 1.8 (6.6-21.0)

FIG. 1. A comparison of the urinary excretion of ⁵⁷Co-vitamin B₁₂ in the Schilling test with hog intrinsic factor and human intrinsic factor respectively in 19 patients with Addisonian pernicious anaemia. The closed circles denote those patients who had an antibody against human intrinsic factor in their serum.

Human intrinsic factor was less effective than hog intrinsic factor in potentiating vitamin B₁₂ absorption in patients with pernicious anaemia (Table) and this applied equally to those patients with demonstrable intrinsic factor antibodies and to those without. Human gastric juice gave a higher urinary excretion of labelled vitamin B₁₂ in only three of the 19 cases (Figure). The difference between the hog and human intrinsic factor results in the pernicious anaemia group was statistically significant (p = 0.05).

On the other hand, in post-gastrectomy subjects a slightly higher value in the Schilling test was obtained when the test was carried out with human intrinsic factor as opposed to a hog preparation.

COMMENT

The use of a heterologous (hog) intrinsic factor preparation resulted in the excretion and presumable absorption of a greater amount of vitamin B₁₂ than was the case when the homologous intrinsic factor was used. The probable explanation is the presence of an antibody to human intrinsic factor acting at small gut level. There is some degree of cross reaction between the human intrinsic factor antibody and hog intrinsic factor and this may be the expla-

ation for the urinary excretion values in pernicious anaemia even with the hog preparation remaining below that found in control subjects. On the other hand this can hardly be the explanation for the lower urinary excretion in post-gastrectomy subjects. It is possible that after gastric resection intestinal malabsorption may be of some importance in producing a lower vitamin B₁₂ absorption. Alternatively, Callender and Evans (1955) have shown in a different context that a large excess of intrinsic factor may improve vitamin B₁₂ absorption. It is possible that a large excess of intrinsic factor may restore vitamin B₁₂ absorption in pernicious anaemia to the values found in control subjects. Since the amount of intrinsic factor used in this study was sufficient to bind fully all the orally-administered vitamin B₁₂, a large excess of intrinsic factor might function by protecting vitamin B₁₂ intrinsic factor complex from the action of proteolytic enzymes.

There appeared to be no difference in the manner of vitamin B₁₂ excretion in patients with demonstrable intrinsic factor antibodies and in those without. Thus if the reduced excretion with human intrinsic factor is determined by an antibody, then this antibody is present in both groups of patients. It is of some interest that when patients with Addisonian pernicious anaemia are treated with steroids an improvement in vitamin B₁₂ absorption and return of intrinsic factor and hydrochloric acid to the gastric secretion occurs both in patients with a demonstrable intrinsic factor antibody and also in those without. Patients after gastrectomy, however, do not improve their vitamin B₁₂ absorption with steroid therapy (Ardeman and Chanarin, 1965b).

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SUMMARY

A comparison was made of the ability of hog intrinsic factor and human intrinsic factor given in excess to potentiate the absorption of ⁵⁷Co-vitamin B₁₂ in Addisonian pernicious anaemia. The mean urinary excretion of ⁵⁷Co-vitamin B₁₂ with the hog preparation was 14.2% and with human intrinsic factor 11.7%. The difference between the values was statistically significant ($p = 0.05$). This difference was not found in post-gastrectomy patients. The lower value with human intrinsic factor was attributed to the presence in cells of the villus of an antibody to human intrinsic factor.

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