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This Week's Citation Classic®

Ardeman S & Chanarin I. A method for the assay of human gastric intrinsic factor and for the detection and titration of antibodies against intrinsic factor. *Lancet* 2:1350-4, 1963.
[MRC Experimental Haematology Res. Unit and Dept Haematology, Wright-Fleming Inst Microbiology, St. Mary's Hospital Medical School, London, England]

The methods described in this publication enabled the direct measurement of human intrinsic factor in normal individuals and in patients with disorders of the stomach. Furthermore, it was established that circulating intrinsic factor antibodies were found exclusively in patients with Addisonian pernicious anaemia. [The *SCI*® indicates that this paper has been cited in over 270 publications since 1963.]

Simon Ardeman
Department of Haematology
Edgware General Hospital
Edgware, Middlesex HA8 0AD
England

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As a fledgeling haematologist in the early 1960s, I was honored to be invited by I. Chanarin to join the staff of the Medical Research Council's Experimental Haematology Research Unit, whose director was P.L. Mollison. My task was to devise a method for the measurement of human intrinsic factor (IF), the stomach secretion needed for the absorption of dietary vitamin B_{12}, which is vital for normal haemopoiesis.

It had been known for many years that gastric juice (GJ) "bound" B_{12},[1] and although IF was thought to be the major component, it was known that there were other "nonspecific" factors (R-binders) present, such as those in bile and saliva. Therefore measurement of the B_{12}-binding capacity of GJ alone was not an assessment of its IF content. It was necessary to sort out the wheat (IF) from the chaff (IF + R-binder).

The way ahead was signposted by the crucial observations of K.B. Taylor,[2] who demonstrated that a proportion of patients with Addisonian pernicious anaemia (PA) had a circulating antibody to IF. The technique used was cumbersome, involving B_{12} absorption tests in patients with PA. The patient drank PA serum together with hog IF and radioactive B_{12}. If the PA serum contained antibody, there was impaired B_{12} absorption. We devised an *in-vitro* test for detecting IF antibodies that involved pre-incubating known normal GJ with PA serum, adding radiolabelled B_{12} in excess, removing unbound B_{12} with activated charcoal, and calculating the amount of radioactive B_{12} in the supernatant following centrifugation. If antibody was present, it would react with IF and prevent it binding labelled B_{12}. A control using normal serum was also set up, and an antibody was deemed to be present in the PA serum if there was a reduction of B_{12} binding capacity relative to the control.

Using IF-antibody-containing sera of sufficiently high titre to neutralise all the IF present in normal GJ, it was now possible to evaluate the IF-content of any GJ sample. Thus the difference between total B_{12}-binding capacity (IF + R-binder) using control serum in the assay system and R-binding (using IF-antibody serum) was a measure of IF content. We defined the unit of IF as the amount that bound one ng of labelled B_{12}. A method using a B_{12} analog (cobinamide) to block R-binder has recently been described,[3] which enables IF to be measured directly and obviates the need for IF antibody in the assay system.

I think our publication has been highly cited because it was the first account of a simple, rapid, and reproducible *in-vitro* quantitative assay of a vital body secretion that had been discovered more than 30 years previously.[4] Furthermore, our work facilitated the accurate study of the physiological and pharmacological properties of IF and was also of value in the differential diagnosis of patients with megaloblastic anaemia. The detection of circulating IF antibody was of particular importance in this context, since this is virtually only found in patients with PA.

1 **Ternberg J L & Eakin R E.** Erythein and apoerythein and their relation to the antipernicious anemia principle. *J Amer Chem Soc* 71 3858, 1949 (Cited 50 times)
2 **Taylor K B.** Inhibition of intrinsic factor by pernicious anaemia sera *Lancet* 2 106-8, 1959 (Cited 160 times)
3 **Begley J A & Trachtenberg A.** An assay for intrinsic factor based on blocking of the R binder of gastric juice by cobinamide *Blood* 53 788-93, 1979
4 **Castle W B & Townsend W C.** Observations on the etiologic relationship of achylia gastrica to pernicious anemia II The effect of the administration to patients with pernicious anemia of beef muscle after incubation with normal human gastric juice *Am J Med Sci* 178 764-77, 1929

