Technique

A modification of charcoal adsorption immunoassay of gastric intrinsic factor

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Gastric intrinsic factor by immunoassay is measured as the reduction in vitamin B₁₂-binding capacity of gastric juice after the addition of pernicious anaemia serum containing intrinsic factor antibody. The antibody serum itself binds a small amount of vitamin B₁₂. This is ignored by Gottlieb, Lau, Wasserman, and Herbert (1956). Rødbro, Christiansen, and Schwartz (1965) and Irvine, Cullen, Scarth, and Simpson (1968) use normal serum during the determination of total vitamin B₁₂-binding capacity of the gastric juice to 'balance' the antibody serum. However, unless great care is taken in choosing antibody and normal sera, it is unlikely that the vitamin B₁₂-binding capacity of the two would balance in the assay. In the method of Ardeman and Chanarin (1963) additional determinations of vitamin B₁₂-binding capacity of normal and antibody serum are carried out. These are subtracted from the binding capacity of gastric juice + normal serum and gastric juice + antibody serum, respectively. In our opinion these steps involving additional radioactivity counts add to the error of the method. To circumvent these difficulties we have devised a modified assay in which the contribution to vitamin B₁₂-binding by the antibody serum is 'balanced' by the same serum.

Method

The assay is carried out in two parts. In the first part, 0.1 ml of gastric juice is pipetted into a test tube containing 0.7 ml of physiological saline. One ml of an aqueous solution containing 15 ng of ⁵⁷Co-labelled vitamin B₁₂ is added and thoroughly mixed. Then, 0.2 ml of a pernicious anaemia serum containing a high titre of intrinsic factor antibody is added and mixed well followed by 2 ml of a 5.25% suspension of charcoal in 1% bovine albumin. The tube

is then centrifuged at 3,500 rpm for 20 minutes. Charcoal with the adsorbed free vitamin B₁₂ sediments. The supernatant is decanted and its radioactivity counted in a well-type scintillation counter. In the above procedure antibody serum is added at a stage when it cannot interfere with binding of vitamin B₁₂ to the gastric juice. The radioactivity of the supernatant measures ⁵⁷Co-labelled vitamin B₁₂ bound to gastric juice and to the antibody serum.

The second part of the assay is carried out exactly as the first part except that antibody serum is added to the gastric juice *before* vitamin B₁₂ solution thus preventing the uptake of vitamin B₁₂ by the intrinsic factor component of the gastric juice. The radioactivity of the supernatant here measures the vitamin B₁₂ bound to non-intrinsic factor binders of the gastric juice and to the antibody serum.

The two parts of the assay are carried out in parallel and in duplicate. From the mean difference in the radioactivity counts of the two supernatants compared with an isotope standard (prepared with 1 ml of ⁵⁷Co-labelled vitamin B₁₂ solution and physiological saline), gastric intrinsic factor content is calculated in terms of vitamin B₁₂-binding capacity in nanograms.

Results and Comments

In a series of intrinsic factor assays on two gastric juices, one from a patient with pernicious anaemia and the other from a normal volunteer, using five different randomly selected antibody sera, the standard error of the results (ng/ml) by our modification was 0.670 for the normal gastric juice and 0.199 for the pernicious anaemia juice. Using the same five antibody sera the standard error by the Gottlieb type assay was 4.518 for the normal gastric juice and 2.081 for the pernicious anaemia juice. When the antibody sera were paired with five randomly chosen normal sera and the latter used in the first part of the assay, as by Irvine *et al* (1968) and Rødbro *et al* (1965), the standard error for the normal gastric juice was 4.278 and 1.555 for the pernicious anaemia juice. The consistency in the results by our assay using different antibody sera obviates the need for large fixed pools of serum. Normal serum is not required. The number of radioactivity measurements and hence counting errors are kept to a minimum. We feel that this simple modification offers a considerable advantage over the existing methods.

This work has been carried out whilst in receipt of an Endowment Fund Research Grant for which we are grateful to the Board of Governors of United Sheffield Hospitals.

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