Letter to the Editor

RADIOISOTOPIC ASSAY OF VITAMIN B12

We heartily endorse the favourable views of Raven, Walker, and Barkhan (1966) and Matthews, Gunasegaram, and Linnell (1967) on methods for assay of vitamin B12 in serum by radioisotope dilution. However, we feel that for simplicity and speed the original method of Lau, Gottlieb, Wassermann, and Herbert (1965) has considerable advantages, which have not as yet achieved the recognition in this country which they deserve.

In the method of Raven et al. the principle is that of Lau et al., but because the modifications introduced necessitate the use of larger volumes of saline and reagents with more dilution steps and several additional rows of tubes, labour and costs are greater than for the bio-assay, while the method of Matthews et al., using the non-specific protein binders in normal serum as described by Barakat and Ekins (1961), requires the preparation of a standard curve for each run of tests and two hours for binding to take place.

In our hands the technique of Lau et al. has proved sufficiently reliable for clinical purposes with results comparable to bio-assay (see Figure) and has been in routine use here for 18 months. It will be seen that, like other workers, our results in the low range are lower by radioisotope dilution than by bio-assay. Our results in 40 untreated cases of pernicious anaemia ranged from 5 to 135 μg/ml (mean 69 SD±44.5, SE 7.05).

However, we would like to stress certain points in technique which may have led to difficulty with the method:--

(a) It is most important to use only the grades of reagent recommended by Lau et al. The National formulary intrinsic factor supplied by Armour and the Norit A charcoal of Hopkin and Williams Ltd. have proved satisfactory. We use the latter coated with haemoglobin.

(b) Both the time for binding of intrinsic factor and absorption by charcoal should be standardized. We have found a period of 15 minutes for each satisfactory.

(c) For greater accuracy in the critical range we add only 200 to 250 pg. of Co57B12 to each tube and for ease of measurement the solution is so standardized that this amount is contained in 1 ml.

(d) Unlike Matthews et al., we have not found the addition of cyanide to increase reproducibility. A low serum control is put through with each batch of tests. Occasionally we have found, like Raven et al., that the net counts of the low control serum (if this contains less than 25 μg/ml) are higher than the net counts of the intrinsic factor control tubes. The serum value is then the one used in the calculation of serum B12 concentration. This seems to occur through the action of acid on protein-coated charcoal that has deroxidated during storage, rather than from its action on intrinsic factor as postulated by Raven, since we have found the effect disappears in the control tubes either if acid is omitted in them or if freshly prepared protein-coated charcoal is used.

By the method of Lau et al., to put up and read a batch of 12 to 18 tests is a half-day's work for one technician, only the simplest of counting equipment being required. This puts this most important investigation within the scope of even the smallest laboratory.

Group Laboratory, S. J. DOWNER
Mayday Hospital, R. A. M. OLIVER
Croydon

REFERENCES


FIG. 1. Comparison of the B12 levels of 44 sera assayed by the Lactobacillus leichmanii and isotope dilution methods. Results enclosed by the dotted lines are below 150 pg/ml; with perfect agreement the results would lie along the continuous line.
Radioisotopic assay of vitamin B12.

S J Downer and R A Oliver

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