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 $\mu\text{g}/\text{ml}$ (mean 69 SD44.5, 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 $\mu\text{g}/\text{ml}$) are higher than the net counts of the

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.

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 deteriorated 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,
Mayday Hospital,
Croydon

S. J. DOWNER
R. A. M. OLIVER

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