

ascorbic acid is the error which will result from accepting a 'folate serum', preserved by ascorbic acid, for vitamin B<sub>12</sub> assay. Using radioisotopic dilution techniques dependent on intrinsic factor binding such a serum will exhibit a misleadingly low vitamin B<sub>12</sub> activity in a clinical situation where a low level might be anticipated.

Having noted the effect of ascorbic acid *in vitro* we considered the possibility of an effect *in vivo* and now have evidence that very high oral doses of ascorbic acid can produce a lowering of vitamin B<sub>12</sub> activity as measured by this technique.

We hope to publish the full details of our modifications to Lau's original technique and the results of our further studies.

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## NORMAL VALUES FOR INDIVIDUAL PLASMA COBALAMINS

In this letter we wish to make a preliminary communication of normal values for individual plasma cobalamins, and to report values obtained in two cases of untreated pernicious anaemia. It has been known for some years that the total serum (or plasma) vitamin B<sub>12</sub> is made up of several compounds, of which methylcobalamin is usually predominant (Lindstrand and Ståhlberg, 1963). Quantitative values for the individual cobalamins have not, however, yet been published.

Heparinized blood samples were taken from 20 healthy people, 17 of whom were smokers. Plasma cobalamins were separated and located by thin-layer chromatography and bioautography as described by Linnell, MacKenzie, Wilson, and Matthews (1969). The proportion of each fraction was estimated by photo-

metric scanning of stained growth areas and by comparison with standard cobalamin solutions treated similarly to the plasma extracts (Linnell, MacKenzie, and Matthews, 1969). Total plasma B<sub>12</sub> was estimated by radioisotopic assay (Matthews, Gunasegaram, and Linnell, 1967).

In all normal samples, the predominant component was methylcobalamin. The second major component (recorded as hydroxocobalamin but probably also containing some deoxyadenosyl coenzyme B<sub>12</sub>, averaged about one quarter of the total B<sub>12</sub>; the range for this component was extremely wide. Six samples showed a small proportion of cyanocobalamin; in most of these it was negligible, but in one individual it amounted to about 10% of the total B<sub>12</sub>. The results suggest a tendency to slight overestimation of the hydroxocobalamin and cyanocobalamin growth areas when chromatograms are assessed visually (Linnell *et al.*, 1969).

In the cases of pernicious anaemia, the pattern of distribution of the two major components was abnormal, the ratio of methylcobalamin to hydroxocobalamin, normally greater than 1·1, being very much reduced. In both cases, hydroxocobalamin, though low, was still within normal limits. The significance of this abnormality is not yet clear.

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## VALUES FOR INDIVIDUAL PLASMA COBALAMINS IN NORMAL SUBJECTS AND CASES OF UNTREATED PERNICIOUS ANAEMIA

	Total B <sub>12</sub> (μg/ml)	Me-B <sub>12</sub> (μg/ml)	CN-B <sub>12</sub> (μg/ml)	OH-B <sub>12</sub> (μg/ml)
Twenty normal subjects (mean, SE, range)	544 ± 45 (250-1085)	398 ± 33 (158-635)	5·5 ± 2·8 (0-45)	141 ± 23 (22-488)
Individual values for two cases of pernicious anaemia	30 65	7 <5	0 20	23 45