LETTERS TO THE EDITOR

Some Observations on the Relationship between Vitamins B_{12a} and B_{12b}.

SIR,—A certain amount of confusion is apparent in the literature regarding the relationship between vitamins B_{12a} and B_{12b} The designation vitamin B_{12a} was given by Kaczka, Wolf and Folkers[1] to the compound resulting from catalytic hydrogenation of vitamin B_{12a} followed by atmospheric oxidation of the product, whilst the designation vitamin B_{12b} was subsequently given by Pierce, Page, Stokstad, and Jukes[2] to a compound isolated from cultures of *Streptomyces aureofaciens* and from liver.

Early biological studies on vitamins B_{12a} and B_{12b} appeared to indicate their dissimilarity, a conclusion supported by ultra-violet absorption data (see Table).

ABSORPTION SPECTRA OF VITAMINS B_{12a} AND B_{12b}

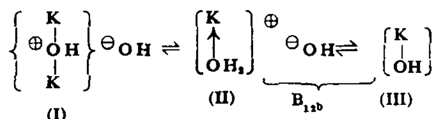
		Maxima					Minima
B_{12a}	λ =	2700	3525	4150	5300	Å	3150 Å
	$E^{1\ \text{per cent.}}_{1\text{cm.}}$ =	137	150	29	58		80
B_{12b}	λ =	2740	3510		5250	Å	3150 Å
	$E^{1\ \text{per cent.}}_{1\text{cm.}}$ =	119	146		52		40

Thus, although the two compounds showed nearly identical maxima, a significant difference could be observed in the minima in the region of 3150Å[3].

Further work failed to supply unequivocal evidence that vitamins B_{12a} and B_{12b} are dissimilar. Thus Brockman, Pierce, Stokstad, Broquist and Jukes[4] repeated the catalytic hydrogenation procedure of Kaczka *et al.*[1] but obtained only the compound designated B_{12b}. Wijmenga, Veer and Lens[5] found that vitamins B_{12a} and B_{12b} show identical absorption spectra in the visible and ultra-violet regions and that the two compounds cannot be distinguished by paper chromatography using *n*-butanol as a solvent. More recently, Kaczka, Denkewalter, Holland and Folkers[6] have reported that vitamins B_{12a} and B_{12b} show identical biological properties and identical behaviour on paper-strip chromatography, but differ slightly in their behaviour during ultra-violet absorption measurements. Thus, whereas vitamin B_{12a} gives the recorded " 12a " spectrum immediately on solution, changes occur on standing so that after 24 hours at room temperature the spectrum becomes indistinguishable from that of B_{12b}. Conversely, intensive drying of B_{12b} gives a material spectroscopically characteristic of B_{12a}, providing the measurements are made immediately after solution of the material in water. Pierce *et al.*[3], it should be noted, specifically state that their vitamin B_{12b} was dried at room temperature in a vacuum desiccator over phosphorus pentoxide, a procedure which left about 6 per cent. of

residual moisture which could be removed by heating at 100°C., but that the spectroscopic data reported were obtained with unheated preparations.

The foregoing evidence, in our view, does not provide conclusive proof that vitamins B_{12a} and B_{12b} are identical in the solid state. The recorded data can, in fact, be equally well interpreted on the assumption that the two compounds are separate entities, and that the conversion of 12b into 12a during dehydration involves structural changes of a more profound character than those usually associated with simple removal of water molecules.



We have already advanced[7] the hypothesis that B_{12b} exists normally in aqueous solution in the aquocobalamin form (II) (*see* reference 7 for nomenclature employed), and that, in alkaline solution, elimination of water occurs with redistribution of charge to give hydroxocobalamin (III). The changes undergone by B_{12b} during dehydration to B_{12a} may well be of a similar character. In this instance a structure such as (I) for B_{12a}, in which a redistribution of charge has occurred between two molecules, may be worthy of consideration.

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