LETTER TO THE EDITOR

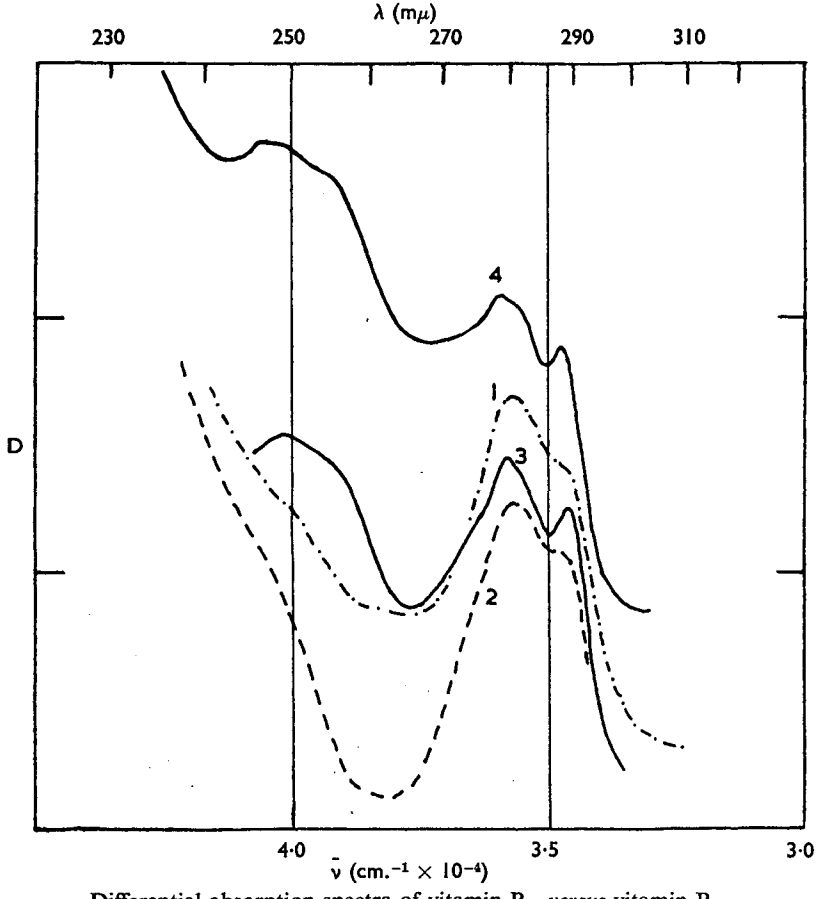
Spectroscopic Evidence for the Presence of the Benziminazole Chromophore in Intact Vitamin B_{12}.

Pfiffner[1] has reported the occurrence of pseudo vitamin B_{12} (ψB_{12}) in the harvest broth of a rumen anærobe. Vitamin B_{12} from liver and from *S. griseus* cultures yields, on acid hydrolysis, 1-aminopropanol-2, a benziminole glycoside and a red cobalt-containing fragment.[2] Pfiffner has shown, however, that ψB_{12} does not yield a benziminzole-containing fragment but an adenine glycoside. In view of a consequent necessary subclassification of vitamin B_{12}-like factors it seems to us important to establish whether the benziminazole exists intact in the B_{12} molecule or is produced under the conditions of acid hydrolysis which, so far as reported in the literature, provides the only means available for obtaining substituted benziminazoles from B_{12}. In our original communication[3] on the spectroscopic identification of 5:6-dimethylbenziminazoles in the hydrolysis products of B_{12} we gave evidence for the pre-existence of the benziminazole in the B_{12} molecule, based on the presence of some of the fine-structure features of the benziminazole chromophore in a moving-plate spectrogram[4] of an aqueous solution of B_{12}. Further more definite confirmation of this has now been obtained by examination of the difference between the spectrum of intact B_{12} and that of a specimen of the red cobalt-containing fragment obtained by acid hydrolysis of B_{12}; the preparation and properties of this fragment will be reported in a future paper.

Since the molecular weight of the red fragment (R.F.) is not yet known, it is not possible to make a simple subtraction of the molar absorption curve of R.F. from that of B_{12} and thus obtain a difference curve representing the absorption of that portion of the B_{12} molecule which is absent in R.F. It is possible to show, however, that a solution of red fragment can be found by trial whose concentration and thickness is such that the difference between its spectrum and that of a given solution of B_{12} is a "most probable" spectrum for the chromophore absent in R.F. This type of analysis has been treated theoretically by Hardy and Young.[5] The method is very laborious when using a manual spectrophotometer, since it involves the calculation or measurement of several absorption curves for different concentrations of R.F. and subtraction of these from that of the solution of B_{12}. However, with a twin-beam automatic recording spectrophotometer, a whole series of difference curves can be directly obtained by placing a solution of R.F. in a variable-length cell in the "control" beam of the spectrophotometer and the solution of B_{12} in the "sample" beam, and altering the thickness of the variable-length cell for each run. The spectrophotometer is, in effect, used as a computor by means of which, by the method of Hardy and Young, a "most probable" difference curve may be selected in a favourable case such as this turns out to be. The principles of selection will be given in a later communication in a more general context. Suffice it to say here that the absorption spectra of vitamin B_{12} and of our red fragment R.F. are, under certain conditions, very similar in the wavelength region 310 to 650 mμ and their "dicyanide" derivatives are indistinguishable in this range. The differences in their absorption characteristics are found at wavelengths shorter than 310 mμ.

The figure shows differential absorption curves between a solution of vitamin B_{12} and one of R.F. under three different conditions: (1) at *p*H 4·0, (2) at *p*H 10·0, (3) after adding a small amount of potassium cyanide to each solution at

*p*H 10. For comparison, curve (4) gives the absorption spectrum of the benziminazole fragment obtained by hydrolysis of vitamin B_{12} (component α of Beaven *et al.*[3]) in neutral or alkaline solution. From inspection of the curves the following points may be noted: (*a*) the great similarity between curve 3 and curve 4; (*b*) the lack of resolution of the 288 mμ band in curves 1 and 2; (*c*) the presence of a band at 250 mμ in curves 3 and 4; (*d*) the general similarity of all four curves in the 270 to 290 mμ region.



Differential absorption spectra of vitamin B_{12} *versus* vitamin B_{12} "red fragment"; solvent, water

No. 1 –·–·– *p*H 4
No. 2 – – – – *p*H 10
No. 3 ——— *p*H 10 plus potassium cyanide
Refer to text for conditions used to obtain spectra Nos. 1, 2 and 3.
No. 4 ——— "α Fragment" at *p*H 10 with optical zero displaced vertically for clarity.

These facts, in our opinion, support the hypothesis that the benziminazole chromophore is present in the intact vitamin B_{12} molecule, otherwise one would have to postulate a benziminazole precursor with the same absorption spectrum but with a different cyclic structure, which, in the case of this type of compound is most improbable.

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The lack of resolved fine-structure and of acid-alkali shift in curves (1) and (2), the absence of a band at *ca.* 250 mμ in curve (2) and the presence of resolved fine-structure and the 250 mμ band in (3) are fully consistent with our previous suggestion[6] that in vitamin B_{12} the benziminazole chromophore is co-ordinated to the cobalt atom.

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ABSTRACTS (*Continued from page* 340.)

about 200 million organisms per ml., as determined by opacity and autoclaving at 115° C. for half-an-hour. Organisms were also suspended in sterile water, incubated for 16 days at 37° C. and filtered or centrifuged. These preparations were injected into rabbits at the rate of 0·5 ml./kg. Emulsions, filtrates and supernatant liquids from the centrifuge were almost equally pyrogenic. The bacteria tested included 10 known pyrogenic species and some species of *Bacillus*, *Chromobacterium*, *Flavobacterium*, *Pseudomonas*, *Sarcina* and *Staphylococcus* which were isolated from pyrogenic samples of distilled water and classified. All proved to be pyrogenic in rabbits. For some species (for example *Ps. æruginosa*) there was a constant pyrogenic power, while for others (for example *B. subtilis*) it varied according to origin. No correlation was observed between pathogenic and pyrogenic power. G. B.

Pyrogens from Various Bacterial Species. L. G. Ginger, N. M. Nesset, B. Riegel and E. J. Fitzsimons. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 421.) Pyrogenic concentrates have been prepared from *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Serratia marcessens* and *Proteus vulgaris* by subjecting the cellular materials to tryptic digestion. Analysis of these concentrates has yielded information which indicates that there are essential differences in their chemical composition and biological activity; in particular, the concentrate prepared from *B. subtilis* differs distinctly from the other concentrates. At least three possible contaminants are associated with the pyrogenic polysaccharides, namely, nucleic acids, other nitrogenous material, and free lipid. The amount of contaminating nucleic acids in each concentrate is related to the initial nucleic acid content of the cellular material, while the amount of nitrogenous residues varies with the cellular species under consideration. The pyrogenic polysaccharides have been shown to consist of hexosamine, an unclassified reducing sugar, and a non-reducing fraction, but there seems to be no direct correlation between the amount of any of these constituents present in the concentrates and the pyrogenicity of the latter. It is possible that some yet undetermined component is responsible for the observed differences in biological activity. S. L. W.