LETTERS TO THE EDITOR

The Inactivation of Penicillin by Oil of Theobroma

SIR,—During the development of penicillin formulations for human and veterinary use we have had occasion to investigate the effect of incorporating various penicillin salts in bases containing oil of theobroma. The rapid fall of potency in these preparations led us to suspect that the latter was exerting a definite inactivating effect. A series of dispersions of penicillin in oil of theobroma was accordingly prepared, using separately calcium penicillin and crystalline potassium penicillin at concentrations of approximately 10,000 I.U./g. These were assayed at time intervals after storage at room temperature and a progressive potency fall was observed. For calcium penicillin a fall of 27 per cent. was recorded after 3 months and for crystalline potassium penicillin a fall of 90 per cent. after 12 months. Further work is in progress to investigate this change in more detail but meanwhile we think it advisable to draw attention to what may be a serious incompatibility.

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August 16th, 1949.

Vitamin B_{12} as a 5:6-Dimethylbenziminazole Derivative

SIR,—A paper chromatogram of an acid hydrolysate of vitamin B_{12} (*n*-butyl alcohol-acetic acid being employed as the irrigation solvent) was exposed to the light of a low-pressure mercury resonance lamp fitted with a Corning 9863 glass filter, when three blue fluorescent spots were revealed. Spectroscopic examination of eluates from these areas indicated their close chemical similarity. The compounds responsible for the fluorescent zones have accordingly been termed by us *components* α-, β-, and γ. Comparison of their ultra-violet absorption spectra with those of known heterocyclic ring systems led to their identification as derivatives of benziminazole. Spectroscopic comparison with 22 methylated benziminazoles synthesised for this purpose led to the identification of *component* γ with 5:6-*dimethylbenziminazole*, and of components α- and β- with 1-substituted 5:6-dimethylbenziminazoles. Moreover, both spectroscopic and chemical work has led us to the conclusion that vitamin B_{12} itself contains one preformed benziminazole residue in the molecule. It may therefore be inferred that *components* α-, β-, and γ represent different stages of degradation of a common precursor. It is interesting to note that vitamin B_{12} and riboflavine may thus both be regarded as derived chemically from 4:5-dimethyl-*o*-phenylenediamine, and speculation on the biogenesis of vitamin B_{12} thus becomes possible.

Both Dr. K. Folkers and one of us (V.P.) have to-day simultaneously reported at the 1st International Congress of Biochemistry held at Cambridge the identification of hydrolytic fragments of vitamin B_{12} with 5:6 dimethylbenziminazoles. It therefore seems desirable to place these observations on

record at this stage. Our detailed results will be submitted shortly for publication in your Journal.*

It is a pleasure to acknowledge the encouragement of the Directors of The British Drug Houses, Ltd., in this work.

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The "Ninhydrin-Reacting" Hydrolytic Fragment of Vitamin B_{12}

SIR,—We have previously reported[1] that hydrolysis of vitamin B_{12} with 20 per cent. hydrochloric acid at 100°C. for 6 hours followed by examination of the hydrolysate by unidimensional paper-strip chromatography, reveals the presence of *one* "ninhydrin-reacting" substance which could not be identified with any of the known amino-acids. Our studies have hitherto been handicapped by incomplete separation on paper chromatograms of the "ninhydrin-reacting" fragment from other products of vitamin B_{12} hydrolysis. By using *n*-butyl alcohol-acetic acid as the irrigation solvent, however, complete separation has now been obtained. The "ninhydrin-reacting" area occupies a position well removed from zones which fluoresce under the light of a low-pressure mercury resonance lamp fitted with a Corning 9863 glass filter[2], and which form the subject of a separate communication (*vide infra*). Elution of the "ninhydrin-reacting" area with dilute hydrochloric acid gives a solution transparent to ultra-violet light. From this and other observations we concluded that the "ninhydrin-reacting" substance was probably an aliphatic base.

We now find that the "ninhydrin-reacting" substance and 2-aminopropanol show identical behaviour on paper chromatograms irrigated with four different solvent systems. The two substances thus have the same partition coefficients in each of these solvent systems, and it is therefore reasonable to conclude that they are identical. A final decision, however, must rest on a direct chemical comparison. Full details of this work have already been submitted for publication[3].

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August 22, 1949.

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1. Ellis, Petrow and Snook, *J. Pharm., Pharmacol.*, 1949, **1**, 60.
2. Holiday and Johnson, *Nature*, 1949, **163**, 216.
3. Ellis, Petrow and Snook, *J. Pharm. Pharmacol.*, in the press.