mated as cupric oxide, we are at present developing quantitative methods for these two drugs as well as sulfanilamide.

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RECEIVED SEPTEMBER 20, 1939

BIOTIN AS A GROWTH FACTOR FOR THE BUTYL ALCOHOL PRODUCING ANAEROBES

Sir:

Recently the "growth factor" requirements of the butyl alcohol-producing clostridia has excited considerable interest.[1,2,3] It has been found that several species of these organisms require a widely distributed organic acid, which cannot be replaced by any known available growth factors.

Using the test conditions of McDaniel, *et al.*,[3] and a culture of *Clostridium butylicum* (Amer. Type Culture Coll. No. 6015), we have shown that biotin[4] is the only accessory substance required by this organism for luxuriant growth even on a synthetic medium.

The results of three tests are given in Table I, the first two on the basal medium of McDaniel, *et al.*,[3] the third using a basal medium exactly the same except that an additional 0.1% asparagin was substituted for the casein hydrolysate. Cultures 2 and 3 were subcultured from the unsupplemented tube of culture 1. Turbidities at the end of the three-day incubation period were measured quantitatively with the thermoelectric turbidimeter described by Williams, *et al.*[5] The galvanometer scale was set to read zero with the uninoculated medium in the cell; a reading of 100 corresponds to complete opacity.

TABLE I

Micrograms supplement per cc. of medium	Turbidities (galvanometer readings) — Subcultures: Culture 1 (1–3 diln.) hydrolyzed casein medium	Culture 2 (1–2 diln.) hydrolyzed casein medium	Culture 3 (undiluted) asparagin medium
Biotin Supplement			
0.000000	15.0	17.0	2.2
.0000133	25.0	19.0	10.2
.0000266	32.0	24.0	29.0
.000053	46.0	34.0	54.0
.00010	60.0	49.0	75.0
.00020	68.0	66.0	88.0
.00066	81.0	67.0	94.0
Liver Concentrate Supplement			
.0133	33.0	28.0	39.0
.0333	52.0	39.0	60.0
.0666	65.0	58.0	79.0
.1332	66.0	66.0	94.0
.2664	67.0	74.0	94.0
.6660	70.0	68.0	96.0

The "liver concentrate" was prepared in accordance with work of McDaniel, *et al.*, and was kindly furnished by Dr. Woolley. Biotin proved to be approximately 500 times as active as this concentrate and it is evidently the only substance required in addition to the ordinary nutrients. Hydrolyzed casein evidently contains small amounts of some substance which is in this case physiologically equivalent to biotin. Whether the response which we have obtained is specific for biotin cannot at present be stated. In any event, these results supply further evidence for the great physiological activity of biotin.

We wish to express our thanks to Professor Kögl, who kindly furnished us with the sample of biotin which made this work possible.

(1) Weizmann, *et al.*, *Biochem. J.*, **31**, 619 (1937).
(2) Brown, Wood and Werkman, *J. Bact.*, **36**, 246 (1938).
(3) McDaniel, Woolley and Peterson, *ibid.*, **37**, 259 (1939).
(4) Kögl and Tonnis, *Z. physiol. Chem.*, **242**, 43 (1936).
(5) Williams, McAlister and Roehm, *J. Biol. Chem.*, **83**, 315 (1929).

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ON THE ABSORPTION SPECTRUM OF HYPERICIN

Sir:

The pigments of *Hypericum perforatum* causing photosensitization in animals are spectroscopically closely related to irradiated oxypenicilliopsin from *Penicilliopsis clavariaeformis* [A. E. Oxford, *Chem. and Ind.*, **57**, 975 (1938); C. Dhéré and V. Castelli, *C. R. Soc. Biol.*, **131**, 669 (1939); C. Dhéré, *ibid.*, **131**, 672 (1939)].

Extracted hypericin was found chromatographically to consist of five components, and we have designated the two most abundant as X and Y. Samples were interchanged with Professor Raistrick, and component Y and irradiated oxypenicilliopsin appear very similar but not identical in chemical behavior. We wish at this juncture to supplement spectroscopic observations by Dhéré and Castelli on these pigments. We have checked their absorption maxima in absolute alcohol within 10 Å. and are in fair agreement as to the intensities.

In Table I we show the effect of solvent composition on the absorption maxima. Values are

TABLE I

EFFECT OF SOLVENT ON ABSORPTION MAXIMA

	Solvent	Ia	Ib	Ic	IIa	IIb	IIc	IIIa	IIIb	IV
1	90% aqueous acetone	5955[a]			5510[a]			5140		4770
2	100% acetone	5990	5830		5540	5420		5130		4750
3	33% acetone, 67% ether	5990[b]	5840		5550	5420	[d]	[d]	[d]	4550
4	25% acetone, 75% ether	5990[c]	5830	5690		5420	5290	[d]	[d]	4540
5	100% ether		5815	5690		5410	5300		5060	4550
6	Ether + trace pyridine		5890	5730		5460[a]				Not detd.
7	Ether + *ca.* 20% pyridine	5990[a]			5550			5170		Not detd.
8	Pyridine	6030[a]			5580[a]			5200		4830

[a] Band slightly asymmetric. [b] Ia > Ib in intensity. [c] Ib > Ia in intensity. [d] Band not discernible.

averages of several readings made visually, and are accurate within ±10 Å. Of particular interest is the co-existence of two distinct spectral types in homogeneous solutions comprised of mixtures of acetone and ether. The case of europium salts [*J. Chem. Physics*, **7**, 824 (1939)] is the only comparable instance of which we are aware.

We have segregated the spectra into three types: A_1, A_2, and B. Hypericin was considered to have four absorption bands in the visible, and this is still true in aqueous acetone, aqueous alcohol and pyridine (type A_1). Unpublished curves for the pigment in 90% aqueous acetone show a slight but definite asymmetry for band Ia and, to an even smaller extent, for band IIa in the regions where Ib and IIb occur as maxima in the anhydrous solvent (type A_2). These curves were obtained spectrophotoelectrically with a high degree of precision and there is no doubt as to the absence of Ib and IIb in the aqueous solvent at room temperature.

The ether spectrum (type B) is essentially different as shown by the fact that Ic (5690 Å.) is absent from anhydrous acetone and alcohol. The pigment is soluble with difficulty in ether. When an ether solution is shaken with water most of the pigment separates at the interface. It has been suggested that a hydrate is formed, as one could not account otherwise for such a decrease in solubility.

Relative intensities, photometrically determined in acetone, are: Ia > Ib, IIa > IIb > IV > IIIa; in ether: Ib > Ic, IIb > IV > IIc > IIIb. In acetone the fluorescence is a vivid red, whereas in ether it is distinctly orange.

The data suggest the existence of a different molecular species in ether, possibly an addition compound because the pigment is hydroxylated. This is capable of co-existence with a second type (A_2) in anhydrous alcohol– or acetone–ether mixtures. In the presence of water or pyridine it disappears, leaving a spectrum of type A_1.

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