

were found to contain 10, 11.6 and 4.3 per cent, respectively, of the amino-sugar.

Work on the identification of the substance is proceeding in this laboratory.

R. E. STRANGE  
F. A. DARK

Microbiological Research Department,  
Porton, Nr. Salisbury,  
Wilts.  
Sept. 26.

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### Vitamin B<sub>12</sub> Activity of *Chlorella vulgaris* Beij and *Anabaena cylindrica* Lemm

MICROBIOLOGICAL tests by means of vitamin B<sub>12</sub>-dependent bacteria and *Euglena gracilis* have shown that higher plants contain only traces (less than 1 µgm. per 100 gm. dry weight) of vitamin B<sub>12</sub>, whereas microbiological activity corresponding to 10–50 µgm. vitamin B<sub>12</sub> per 100 gm. dry weight has been reported in a number of algae.

Several substances, such as factor A and pseudo-vitamin B<sub>12</sub>, are closely related chemically to cobalamin and capable of supporting growth of the bacteria and *Euglena* used for microbiological assay of the vitamin<sup>1,2</sup>; but they cannot replace cobalamin in the nutrition of higher animals<sup>3</sup>. Some micro-organisms are known to synthesize these substances as well as vitamin B<sub>12</sub> itself<sup>2,4</sup>. The vitamin B<sub>12</sub> activity of two algae was therefore investigated to determine whether this was due to cobalamin or to one or more of the related factors. Pure cultures of algae, which had been maintained free from bacteria during many transfers on inorganic media, were employed to ensure that any vitamin B<sub>12</sub> activity detected must have originated from the synthetic activity of the organisms studied.

Vitamin B<sub>12</sub> assays were carried out by a modification of the *E. coli* mutant plate-assay method<sup>5</sup>, the solution for assay being obtained by extracting the algae with hot aqueous methanol in the presence of ammonia and cyanide. The nature of the substances present was determined by chromatography, ionophoresis and bio-autography<sup>6</sup>. By these tests the sample of *Chlorella vulgaris* was shown to contain 6.3 µgm. cobalamin per 100 gm. dry weight; no other compounds showing vitamin B<sub>12</sub> activity could be detected. The results obtained with *Anabaena cylindrica* are shown in Table 1.

About 65–70 per cent of the activity of the *Anabaena* was due to vitamin B<sub>12</sub>, the remaining effects being

Table 1

Added cobalt	Total vit. B <sub>12</sub> activity ( <i>E. coli</i> ) per gm. dry wt.	Activity of B <sub>12</sub> and related substances (in terms of B <sub>12</sub> ) per gm. dry weight			
		Vit. B <sub>12</sub>	Pseudo-vit. B <sub>12</sub>	Factor A	Factor B
Nil	0.63 µgm.	0.44 µgm.	0.19 µgm.	trace	trace
5 p.p.m.	1.1 µgm.	0.70 µgm.	0.40 µgm.	trace	trace

caused by the presence of pseudo-vitamin B<sub>12</sub> and traces of factors A and B. In one experiment, cobalt (5 p.p.m.) was added to the medium to determine whether additional cobalt would allow synthesis of more vitamin B<sub>12</sub>. Although the concentration of vitamin B<sub>12</sub> was thereby almost doubled, scarcely any more total vitamin B<sub>12</sub> activity was detected, because the dry-weight yield of alga was simultaneously depressed.

The cobalamin content of *Chlorella* used in these studies is of the same order as the microbiological activity reported by Combs<sup>7</sup> for this organism: it indicates that the algae, grown under controlled conditions, may synthesize sufficient cobalamin to contribute to the dietary needs (up to about 5 µgm./100 gm. food) of higher animals and man.

The higher animals and many Protista, for which vitamin B<sub>12</sub> is an essential nutrient, have been thought to be dependent on bacterial and fungal synthesis of the vitamin. The presence of cobalamin in at least two different algae may point to an alternative source of this nutrient, particularly for B<sub>12</sub>-dependent organisms living in soil or water and for the food chains of animals living on these organisms.

F. BROWN  
W. F. J. CUTHBERTSON

Glaxo Laboratories, Ltd.,  
Greenford, Middlesex.

G. E. FOGG

Department of Botany,  
University College,  
London.

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### Influence of the Adrenal Cortex on Wool Growth and its Relation to 'Break' and 'Tenderness' of the Fleece

WE were interested to know whether the adrenal cortex exerted a depressant action on wool growth in sheep. Such a mechanism might underlie the decline in wool growth observed in winter<sup>1</sup> and during pregnancy<sup>2</sup>, since cold and gestation are known to increase adrenocortical activity in other species<sup>3</sup>. The marked increase in the number of active follicles which begins about ten days after birth in the lamb (Ferguson, K. A., Schinckel, P. G., Carter, H. B., and Clarke, W. H., in preparation) may possibly be due to release from adrenocortical inhibition. Field-observations link the occurrence of 'break' or 'tenderness' of the fleece<sup>4</sup> with disease, advanced pregnancy or environmental stresses which may be expected to stimulate secretion of adrenocorticotrophic hormone.

We have observed the effects on wool growth of treating sheep with adrenocorticotrophic hormone and with cortical steroids. The sheep were main-