XCV. OBSERVATIONS ON A DIATOM (*NITZSCHIA CLOSTERIUM* W.SM.) AS A SOURCE OF VITAMIN A.

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INTRODUCTION.

IN 1922 Jameson, Drummond and Coward [1922] studied *Nitzschia closterium*, a typical unicellular marine diatom, and showed that it possessed considerable growth-promoting activity. At that time the fat-soluble vitamins A and D were not differentiated, and these tests, therefore, did not preclude the possibility of the observed growth being due to a factor other than vitamin A. This possibility was indirectly supported by the observation of Drummond and Watson [1922] that the oil from this organism failed to give a positive colour reaction with strong sulphuric acid which Rosenheim and Drummond [1920] had previously shown to be associated with vitamin A. On the other hand, in view of the experiments of Leigh-Clare [1927] it seemed improbable that the growth-promoting activity could be ascribed to vitamin D.

Recently the specificity of the colour reactions of vitamin A with AsCl_3 and SbCl_3 , described by Rosenheim and Drummond [1925] and by Carr and Price [1926], in support of which a weight of evidence has accumulated during the last few years, has been questioned by various investigators. The discrepancies have been satisfactorily explained by Drummond and Morton [1929] and by Ahmad and Drummond [1930], but the controversy recalled the earlier observations of Drummond and Watson [1922] regarding the oil from *Nitzschia closterium* which, though apparently a potent source of vitamin A, did not give the colour reaction. It was decided, therefore, to repeat the experiments, and also get a more quantitative estimate of the activity of this organism in the light of our present knowledge of the subject.

EXPERIMENTAL.

Following mainly the technique of Allen and Nelson [1910], large quantities of the organism were grown from a pure culture obtained by the courtesy of Dr E. J. Allen, F.R.S., Director of the Marine Biological Laboratory, Plymouth. 100 cc. of sea water, to which Miquel's nutrient solutions, as modified by Allen, had been added, were placed into each of a large number of conical

Pyrex flasks of 250 cc. capacity, plugged with cotton-wool and sterilised in steam at 95° for 20 minutes on three successive days. Each flask was then inoculated with 0.01 cc. of the pure culture and placed in front of a window in diffused daylight. The bottoms of the flasks were soon covered with a brown growth of the organism.

A large quantity of the organism being required, cultures and subcultures were continued for a period extending over a year. Cultures usually stopped growing after 4–6 weeks. During the winter months when the daylight was poor the growth was scanty.

Four to six weeks-old cultures were filtered on a tared filter, dried in a vacuum desiccator and weighed. They were stored in a desiccator, which had been re-evacuated after the air had been replaced by nitrogen, in a cool, dark cupboard. The oil was extracted with redistilled ether by continuous extraction in Soxhlet's apparatus. Nitrogen was passed in during the extraction and other precautions were taken to prevent oxidation.

The results of culture experiments are summarised in Table I.

Table I.

Crop	Time of year	Vol. of the culture sol. (litres)	Dry wt. of organism (g.)	Quantity of oil obtained (g.)
1	Apr. and May	3.7	3.55	0.103
2	June, July and Aug.	12.5	11.74	—
3 (a)	Sept. and Oct.	5.5	5.53	0.156
(b)	Sept. and Oct.	3.0	1.10	—
4	Oct., Nov. and Dec.	15.5	9.075]	0.374
5	Dec., Jan. and Feb.	15.0	2.765]	

Under satisfactory conditions of growth approximately 1 g. of the dry organism was obtained from each litre of a month-old culture. During the dark winter days the yield fell even below 0.2 g. per litre. The amount of the oil in the dry organism was, however, fairly constant and varied between 2.8 and 3.1 %.

The oil is extremely rich in natural plant pigments, chlorophyll, xanthophyll, carotene, and fucoxanthin, the characteristic pigment of the brown algae. It gave a strong blue colour reaction with SbCl_3 . Drummond and Watson failed to get the colour reaction with H_2SO_4 , probably because they had rather a small amount of the material available, and because with this reagent the test is not very delicate.

Table II shows the yellow and blue colour units given by 10 % solutions in a 1 cm. cell and examined in the Rosenheim-Schuster colorimeter. In the case of blue units the figures refer to 0.2 cc. of 10 % oil mixed with 2 cc. of SbCl_3 reagent, and examined after 30 seconds. The technique of Carr and Price [1926] was mainly followed, though 10 % values were obtained from an intensity-dilution curve as explained in our previous communication [Ahmad and Drummond, 1930].

The pigment of *Nitzschia closterium* interferes with the application of the SbCl_3 reaction. Attempts have been made to obtain the non-saponifiable fraction of the oil in order to remove chlorophyll, but sufficient material was not available. For this reason the measurement of the blue colour must be regarded as approximate.

Table II.

Crop	Dilution %	Colour units 1 cm. cell	Blue units with SbCl_3 reagent
1	10	3,000 yellow 429 red	57.1 blue 52.4 yellow
3 (a)	10	10,000 yellow 830 red	100-150 blue 100-120 yellow
4, 5	10	10,000 yellow 800 red	100-150 blue 100-120 yellow

The technique of the biological test was that usually employed in this laboratory [see Drummond and Morton, 1929]. The basal diet consisted of purified caseinogen, rice starch and salt mixture without the addition of any extraneous fat. Vitamin D was supplied as irradiated ergosterol dissolved in medicinal liquid paraffin, and 0.01 mg. was given twice a week to each rat. Ethyl laurate was used as a diluent for the oil. Its satisfactory nature has been shown by Drummond, Ahmad and Morton [1930].

For feeding, the filter-paper with the organism was broken up into small pieces, mixed with a small quantity of water and the basal diet, and worked up into a dough. This was given to the rats in aliquot portions on small porcelain dishes. They devoured it readily.

The results of the biological tests are shown in Table III, and Figs. 1-3 show some representative curves.

Table III.

No. and sex of rat	Dose and its nature	Total days of test	Total difference in wt. (g.)	Rate of growth per week (g.)
27 ♂	0.2 mg. oil	16	- 3	- 1.3
26 ♀	0.4 mg. oil	16	0	0
28 ♂	1.0 mg. oil	16	- 11	- 4.8
69 ♂	0.35 g. organism	21	+ 45	+ 15.0
70 ♀	0.35 g. organism	21	+ 31	+ 10.0
72 ♀	0.1 g. organism	21	+ 27	+ 9.0
95 ♀	5.0 mg. oil	14	+ 13	+ 6.5
362 ♀	1 mg. oil	7	0	0
	5 mg. oil	14	+ 15	+ 7.5
385 ♂	2 mg. oil	11	- 5	- 3.2
	0.15 g. extracted organism	7	- 3	- 3.0
	0.15 g. extracted organism plus 5 mg. oil	14	+ 30	+ 15.0
388 ♀	3 mg. oil	11	+ 2	+ 1.2
	5 mg. oil	14	+ 15	+ 7.5

Fig. 1. Administration of dried organism.

Fig. 2. Administration of oil.

Fig. 3. Administration of extracted organism plus oil.

DISCUSSION.

The feeding experiments conclusively indicate that *Nitzschia* and the oil obtained from it are good sources of vitamin A. The extracted oil gives a positive reaction with SbCl_3 and the apparent discrepancy observed by Drummond and Watson does not exist.

The extreme richness of the oil in the natural plant pigments will be clear from a study of Table II. The greater part of the colour is due to chlorophyll and xanthophyll, both of which are inactive as a source of vitamin A [Willimott and Moore, 1927]. Carotene, whose relation to vitamin A is supported by increasing evidence, is also present, and it is possible that the greater part of the physiological activity of the oil is due to this pigment. A spectroscopic examination would have indicated the part of the activity due to carotene and the part due to vitamin A, but unfortunately enough material was not available for this to be made. If we compare the biological activities of this pigmented oil and carotene (rat dose 5 mg. and 0.005 mg. respectively) and their respective yellow colours (10,000 and 300,000 Lovibond units in 10 % dilution in 1 cm. cell), we find that the whole of the biological activity of the oil can be accounted for if 3 % of the yellow pigment present be carotene. This is interesting when considered with the earlier observations of Steenbock and his colleagues pointing to the association of the lipochrome pigments and vitamin A activity of vegetable sources; of Coward [1923] that the lipochrome chiefly concerned in this association was carotene; the more recent work on the activity of carotene; and our own observations and that of Moore [1929, 1930] that from the livers of rats fed on large quantities of carotene (0.02–0.05 mg. per day) a colourless or a lightly coloured fat is obtained which gives an intense colour reaction with SbCl_3 . It will probably not be far from the truth if it is suggested that these diatoms are the ultimate source of vitamin A of the fish-liver oils, and that a similar change takes place in the cod or the intermediate fauna giving rise to the stores of the colourless vitamin A present in the liver oils. It also explains why liver oils of fish may be very pale in colour and at the same time very rich in vitamin A, a fact which had rather perplexed the earlier supporters of the view that vitamin A was related to a lipochrome pigment.

SUMMARY.

1. The oil obtained from a marine diatom (*Nitzschia closterium*) growing in pure culture, has been studied both colorimetrically and biologically as a source of vitamin A.
2. It has been found that the oil is very potent in this respect and gives a strong colour reaction with SbCl_3 .
3. The greater part of the biological activity of the oil is probably due to carotene, synthesised by the organism, and extracted with the oil.

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