XL. SYNTHESIS OF VITAMIN *A* BY A MARINE DIATOM (*NITZSCHIA CLOSTERIUM* W.Sm.) GROWING IN PURE CULTURE.

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DURING the early stages of an investigation of the nutrition of certain species of marine molluscs it became necessary to gain some information concerning the food value of a number of marine plants upon which the shell-fish directly or indirectly feed[1].

Amongst other dietary factors to be studied that known as vitamin *A* claimed our early attention. By now it is generally understood that the higher land animals draw their supplies of this indispensable factor directly or otherwise from green plants, since their own tissues do not appear to possess the power to synthesise this organic complex. That a parallel condition would exist in the sea appeared to us to be highly probable from the fact that certain marine algae had been found to share with land plants the power to synthesise vitamin *A* [Coward and Drummond, 1921]. The algae examined in the former work were Fucus, Ulva, Cladophora, Polysiphonia and Enteromorpha, but these are not truly representative of the ultimate food supply of the species of molluscs with which we were working. We therefore decided to test whether a typical unicellular marine alga can synthesise vitamin *A*.

To obtain the necessary material it was decided to grow the organisms in pure culture following the technique described by Allen and Nelson [1910]. Pure cultures of three common marine diatoms, *Nitzschia closterium* W. Sm. *forma minutissima*, *Thalassiosira gravida*, and *Skeletonema* were very kindly placed at our disposal by Dr E. J. Allen, F.R.S., Director of the Marine Biological Laboratory, Plymouth.

To grow the relatively large quantities of the organisms required for the feeding tests was not a simple matter, and in the case of two of the three species named our attempts failed owing to their slow rate of growth. With Nitzschia, however, we found it a relatively simple matter to prepare ample

[1] Unfortunately owing to the sudden and untimely death of our colleague Dr H. Lyster Jameson the main line of this work has been discontinued. The present communication records the chief result which we had obtained up to the time of his death, by which science and particularly marine biology suffers a grievous loss. J. C. D.

quantities of the diatom. The cultures were prepared by carefully isolating a few of the organisms from the original Plymouth sample and allowing them to grow in small flasks containing Miquel's culture fluid or sterilised sea water (see reference to Allen and Nelson's work). At the end of a month the bottom of the flask was covered with a thick brown growth of the diatom, but, as Allen and Nelson also observed, the cultures do not thrive well after more than a month so that at that stage the cultures were transferred to a number of large Kilner jars of 4-litre capacity containing the sterile culture fluid. The covered jars were allowed to stand in diffuse daylight in front of a window facing north, and the growth was accompanied by quite an appreciable evolution of oxygen on the brighter days. Frequent microscopical examination of the cultures demonstrated their freedom from contamination. By repeated sub-culturing in this manner large amounts of the organism were prepared without much trouble.

We were at first doubtful whether the administration of the fresh diatoms to rats in the usual manner of testing for the presence of vitamin *A* would be of any value but decided to test this method before going to the trouble of preparing by chemical means a special fraction. Somewhat to our surprise the organisms were digested during their passage through the alimentary canal of the rats as was indicated by the recovery of growth and by the presence of empty frustules in the faeces. The usual method of testing was followed [Drummond and Coward, 1920], the supplement being administered before the daily ration of basal diet was given. To prepare the organisms for administration the fluid was decanted off from a month-old culture and the thick flocculent growth of diatoms on the bottom filtered on to a thin layer of starch at the bottom of a Gooch crucible. After washing with a very small quantity of distilled water the almost dry mixture of starch and organisms was thoroughly mixed and an aliquot part given to the animals who invariably consumed it with readiness.

The organisms although possessing a deep brown colour whilst in culture assumed a dark green tint on drying. The total fat content (ether extract) was 5·25 % of the dry weight, and in addition to the characteristic pigment of the brown algae, fucoxanthin, there were also present chlorophyll and relatively large amounts of the lipochrome pigments carotene and xanthophyll.

Although it was impossible accurately to measure the dose of Nitzschia given daily an approximation was made on the basis of the average dry weight of a month-old culture which was 0·32 g.

The curves shown in Fig. 1 show that this diatom may serve as a source of vitamin *A*, the resumption of growth being especially marked at the higher dosage of approximately 0·08 g. (dry weight) per day.

The extraordinary potency of these diatoms as a source of the vitamin *A* may be judged from the fact that this ration of diatom represents approximately 4 mg. of fat; which recalls the observations of Zilva and Miura on the potency of cod liver oils [1921].

It is significant that this organism promoted growth very much more effectively than did the common seaweeds we previously tested. This difference may possibly be accounted for by taking into account the much greater relative surface of the diatoms.

The enormous stores, relatively speaking, of vitamin *A* which are found in the tissues of certain marine animals lead one to conclude that they are derived from some highly potent source of that factor such as is represented by the diatom we have been studying.

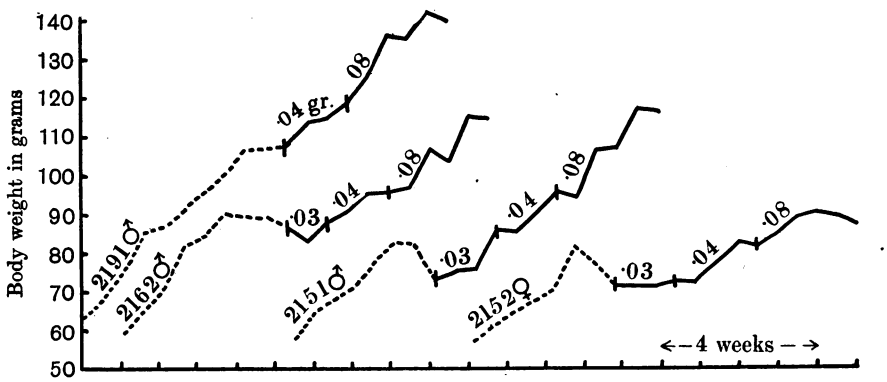

Fig. 1. Daily ration of Nitzschia (dry weight) shown in each period. Preparatory period shown by dotted line.

We essayed to prove the transference of the vitamin from the diatoms to the molluscs we were investigating but owing to the sad death of our colleague the experiments were carried no further than the demonstration that many species of molluscs are valuable sources of the *A* factor. The production of vitamin *A* in green land plants capable of carbon assimilation [Coward and Drummond, 1921] is therefore paralleled by a similar synthesis in marine plants containing photocatalytic pigments. Further, the fundamental dependence of terrestrial animal life on the fresh green leaf for its supplies of vitamin *A* would appear to be paralleled by a similar dependence of marine animals on the marine flora, particularly the microscopic plants.

Summary.

1. Pure cultures of a common marine diatom, *Nitzschia closterium*, grown in Miquel's solution or sterilised sea water synthesise large amounts of vitamin *A*.

2. A parallel is drawn between the dependence of land animals on fresh green leaves and that of marine animals on the synthetic activity of the marine flora for their supplies of vitamin *A*.

3. A number of molluscs were found to contain considerable amounts of vitamin *A*.

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