LIII. A SEARCH FOR VITAMIN D IN THE DIATOM *NITZSCHIA CLOSTERIUM* (W. Sm.).

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(Received March 1st, 1927.)

JAMESON, DRUMMOND and COWARD [1922] showed that the marine diatom *Nitzschia closterium* (W. Sm.) *forma minutissima*, grown in sterilised sea-water with the addition of Miquel's nutrient solutions, can synthesise large amounts of fat-soluble vitamin, and they came to the conclusion that the ultimate though indirect source of the fat-soluble vitamin was this and other marine unicellular organisms.

In view of the recent differentiation of the fat-soluble vitamins, it was suggested to me by Dr H. Chick that it would be interesting to see whether *Nitzschia closterium* also contained vitamin D.

It has been found that the green land plants examined up to the present contain very little, if any, vitamin D [Goldblatt and Zilva, 1923; McClendon and Shuck, 1923; Zucker and Barnett, 1923; Hess and Weinstock, 1924; Bethke, Kennard and Kik, 1925; Chick and Roscoe, 1926; Boas, 1926; Roscoe, 1927] and it was thought that it would be of interest to ascertain whether the same held for marine unicellular organisms containing chlorophyll. There is good reason to believe that these are the ultimate source, via plankton, copepods and small fish, whence deep-sea fish, such as the cod, indirectly obtain their supplies of vitamin A; but it is not known whether the store of vitamin D, found in the liver oils of the codfish, is obtained in this way.

TECHNIQUE.

The experiments described below were carried out on four different litters of rats. The basal diet given was McCollum's No. 3143 low phosphorus rickets-producing diet [1921].

A culture of *Nitzschia closterium* was kindly supplied to me by Professor Drummond, of University College, London, and from this further cultures were grown as described by Jameson, Drummond and Coward [1922]. At first the cultures were kept in Erlenmeyer flasks which were placed in a window facing north. They were never exposed to direct sunlight and all the light which reached them had to pass through a pane of plate-glass and the glass

wall of the flask. Very little ultra-violet light of wave-length less than $340\text{ }\mu\mu$ succeeded in reaching the *Nitzschia* in the flasks. It was therefore decided to try to grow the diatom under conditions more comparable to those to which it is exposed when floating in the superficial layers of the sea, and to grow it in shallow glass dishes on a flat roof to catch all the sunlight available, shielding it from bacterial contamination by a sheet of "windowlite."

A spectrogram of the windowlite used was kindly made for me by Dr Lucas. This showed that windowlite has a fair transparency for ultra-violet light of wave-length longer than $290\text{ }\mu\mu$ or nearly to the extreme range of the sun's spectrum. A padding of sterilised cotton-wool was introduced between the edge of the loose-fitting lid and the rim of the glass vessel in order to preserve the culture from contamination. The *Nitzschia* grew well under these conditions throughout the months of September and October.

Each litter of young rats received the dose of *Nitzschia* after somewhat different treatment. At first, the culture was filtered to dryness on a layer of starch in Gooch crucibles or Büchner funnels, and then further dried for two hours before administration, being mixed with starch in the proportions of 1 to 3. Later the drying at 37° was omitted, and in the final experiment the *Nitzschia* was given without any starch. In this last case, the diatom was filtered freshly every day from the glass dishes on the roof.

The amount of water lost, when *Nitzschia* was collected on a Büchner funnel, and dried at 100° to constant weight, was from 70–75%. The actual weight of dried material administered would therefore be one-quarter of that of the fresh diatom. The doses specified below refer to the fresh weight of the diatom, after suction on the Büchner funnel.

The *Nitzschia* was always given before the basal diet. It was eaten readily after the first few days. Each batch of the diatom was examined microscopically before filtering to make sure both that the culture was free from other diatoms and also that the diatom was alive and in a healthy condition.

To test the condition of the skeletons of the rats the junctions of the 6th and 7th ribs on the right side were examined histologically, and chemical analyses were made on the leg bones (femora, tibiae and fibulae). The methods used are described in full in the paper by Chick, Korenchevsky and Roscoe [1926].

RESULTS.

Exp. 1. The *Nitzschia* was grown in Erlenmeyer flasks in diffused light passing through plate-glass windows. The culture was filtered on starch, dried at 37° and administered with starch as described above.

A litter of five black-and-white rats was put on experiment on June 26th, 1926. The experiment lasted four weeks. Two of the rats, one male and one female, were used as controls; two others, one male and one female, received 0.1 g. (fresh weight) of *Nitzschia* in addition to the basal diet. One rat received 60 mg. of cod-liver oil.

The histological and chemical analyses of their bones, together with the initial weight and growth of the rats, will be found in the table.

Experi- ment	No. of rat	Sex	Dose of <i>Nitzschia</i> g. fresh weight	Initial weight g.	In- crease g.	Duration of exp. (days)	Degree of rickets from histology of rib junctions	Ca % in leg- bones on fat- extracted dry weight
I	7	♂	Control	45	16	26	++++	9.7
	5	♀	Control	38	27	26	++++	9.4
	10	♂	0.1	41	30	26	++++	7.9
	4	♀	0.1	37	23	26	++++	7.3
	3	♂	60 mg. C.L.O.	40	25	26		13.1
II	16	♂	Control	46	17	19	+++	8.0
	17	♀	Control	42	11	20	++	8.4
	12	♂	0.2	42	7	19	++	7.6
	13	♀	0.2	41	14	20	++	8.3
	14	♂	0.1	41	12	19	+++	7.1
	15	♀	0.1	39	20	20	++++	8.0
III	18	♂	Control 16 days	41	12	16	++	9.8
	21	♀	0.21 after 16 days	41	18	23	++++	8.3
	22	♂	Control 18 days	42	10	18	+++	10.0
	25	♀	0.21 after 18 days	41	18	25	++++	9.3
	20	♂	Control 21 days	44	12	21	+++	9.4
	23	♀	0.21 after 21 days	41	25	28	++++	9.5
	24	♂	Control 23 days	39	19	23	+++	8.9
	19	♀	0.21 after 23 days	39	12	30	++	10.9
IV	26	♀	Control	41	35	21	+	13.0
	27	♂	Control	50	24	21	++	10.6
	29	♀	An average of .378 }	39	34	21	+++	10.3
	30	♂	filtered fresh daily }	39	28	21	+++	10.1

It will be seen that all the rats except No. 3, which was receiving cod-liver oil, were intensely rachitic; it was impossible to find any significant difference between them histologically. In the chemical analysis it will be seen that, disregarding the animal receiving cod-liver oil (No. 3), the bones of the control animals (Nos. 7 and 5) had a higher percentage of calcium than those of the animals receiving the supplementary rations of *Nitzschia*.

Exp. 2. The *Nitzschia* given had been grown in Erlenmeyer flasks for six weeks in the same manner as in *Exp. 1*. At the end of this period it was put into the shallow glass dishes and exposed on the roof, as described at the beginning of this paper. The diatom was filtered off on to starch, dried and administered as in *Exp. 1*.

A litter of six white rats was used. The experiment was started on Sept. 17th, 1926. Two of the rats, a male and a female, were used as controls. These received only the basal diet. Two were given 0.2 g. of *Nitzschia* and two 0.1 g. in addition to their basal ration.

The experiment lasted 19–20 days. There was no striking difference in the calcium content of the bones of the animals, though in each case the bones of the control rats had higher calcium-content than those of the rats of the same sex receiving *Nitzschia*. The histological examination showed little difference between the various animals. They were all rachitic.

Exp. 3. The *Nitzschia* was propagated at first indoors and transferred to the roof after three weeks. It remained on the roof for a week or more

before being filtered off. It was given as before, mixed with starch in the proportion of 1 in 4.

A litter of eight black-and-white rats was used. The experiment was commenced on Sept. 27th, 1926. Four rats were taken as controls and were killed after 16, 18, 21 and 23 days respectively. Four other rats of corresponding sex received doses of *Nitzschia* for one week after being on basal diet alone for 16, 18, 21 and 23 days respectively. These rats were then killed. There was very little difference in the calcium content of the bones of two groups of animals. Two controls showed a higher calcium content in their bones than the corresponding animals fed with *Nitzschia* for a further week, and two other controls showed a lower content. Histologically, also, there was little to choose. They all had rickets in a marked degree.

Exp. 4. The culture of *Nitzschia* was started indoors for a short time and subsequently grown on the roof and filtered fresh daily for administration to the rats.

A litter of 4 black-and-white female rats was put on experiment on October 7th, 1926. Two of these rats were used as controls and two received *Nitzschia* filtered fresh daily. Their daily dose averaged 0.40 g. (fresh weight) during the first, 0.46 g. during the second and 0.27 g. during the third week.

Chemical analysis of the bones showed that the calcium content of the bones of the control animals was not lower than that of the rats receiving freshly filtered *Nitzschia*.

DISCUSSION.

From a paper by Chick and Roscoe [1926], it appears that the anti-rachitic potency of cow's milk is very little increased by feeding the animal on green pasture food while the cow itself is kept in a dark stall. It is only when the cow is itself exposed to the rays of the sun that the anti-rachitic value of its milk increases appreciably. Cows fed on cod-liver oil in a dark stall, however, yield a milk of increased anti-rachitic potency [Golding, Soames and Zilva, 1926]. It seems as if it may be possible to trace a parallel in this respect between land and marine animals.

There are at present only two known sources from which the cod could obtain its supplies of vitamin D:

(1) The activation of the precursor of vitamin D by direct action of the sun's rays on the cod.

(2) The food it consumes.

It is difficult to suppose that the cod, a deep-sea fish, is ever exposed to enough sunshine to activate a precursor of vitamin D, so presumably it must obtain its supplies from the food which it consumes. The ultimate source of vitamin A in cod-liver oil has been traced by Zilva and Drummond [1922] to unicellular marine organisms. They say: "It is not directly from these organisms that the cod receives its vitamin but through several intermediaries such as the copepods and larval decapods and mollusca which are present in

plankton and which feed on these unicellular plants. These in turn are consumed by the capelan and other forms of food of the cod."

From the experiments on *Nitzschia closterium* described in this paper it does not appear probable that unicellular marine organisms are the ultimate source of vitamin D in cod-liver oil, and if this is correct there remain to be examined the plankton and the smaller fish, both of which might conceivably be exposed to sufficient radiant energy of the necessary wave-length for synthesis of vitamin D.

My thanks are due to Professor C. J. Martin, Dr H. Chick and Dr Zilva for kind criticism and help, to Professor Drummond for the culture of *Nitzschia*, to the Medical Research Council for a whole time grant, and to the Lister Institute for the hospitality of its laboratories.

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