A Biological Assay of the Vitamin A Value of Algae Grown on Domestic Sewage[1]

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Because there are so many starving people in the world today, there are many investigations of possible additional sources of food. Not only is there insufficient tillable land to feed all people (Sure, 1956; Weaver, 1956), but in many countries much of the land is unsatisfactory for producing food. Also, these countries usually are thickly populated. For some time seaweeds have been used in some lands as food. Later other algae were considered and, when tested, were found to have nutritive value.

Algae contain protein, carbohydrate, fat, mineral matter and vitamins (Hardy, 1941), with considerable variation in the amounts. In some species the proteins lack some of the essential amino acids (Mazur and Clarke, 1938), although some produce satisfactory growth in rats when used as the only protein source (Gaffron, 1953). Algae are believed to contain all the mineral elements essential to man (Beharrel, 1942; Ericson, 1952). The most important nutritive value is due to the vitamin content. In some varieties the B vitamins are abundant (Norris, et al, 1937), and the carotene content in certain types indicates considerable vitamin A value (Braarud and Sorensen, 1956).

[1] The Material for this report was taken from a thesis submitted by Joyce G. Sibley in partial fulfillment of the requirements for the Master of Science Degree at the University of Oklahoma.

The methods used to determine the composition of algae are the spectrographic, which gives the chemical composition but not the behavior in the body, and the biological assay which determines the digestibility, palatability and utilization of the product by the animal.

In an early biological test, cows were fed seaweed, and the milk production, fat percentage and vitamin content of the butter were all increased (Freudenthal, 1949). Another test showed that algae fed in place of an equal amount of soybean meal resulted in increased growth in chicks and that further increase depressed the growth (Combs, 1952). Probably the riboflavin and carotene content was responsible for the increased growth. Using rats, certain forms of algae were found to be good sources of carotene.

There have been some studies on the use of algae by humans. One type dried, tastes like green tea. Algae have been used in soups, noodles, French bread, cookies and ice cream. When it was realized that this form of life had nutritional value, its use in space and submarine travel became of interest. In this connection, a recent leaflet from the National Live Stock and Meat Board carries this information. "After three days of seaweed fare (the proposed space traveler's diet) at the United States Army Medical Research and Nutritional Laboratory, Denver, Colorado, four volunteers agreed it was edible, but not tasty. They complained of nausea, abdominal fullness, and headaches. Algae were added to gingerbread, chocolate cake, milkshakes and other foods. Acceptability problems are a strong spinach-like flavor, a greenish color and incomplete digestibility. When processing techniques are improved, seaweed may be a possibility as a nutritional source of food." Earlier studies show algae may cause gagging, dysentery, allergic reactions (Schwimmer and Schwimmer, 1955). However, some nations have eaten seaweed for centuries.

In the present study the biological assay method, using albino rats, a procedure recommended by the U.S. Pharmacopoeia, was followed (Rosenberg, 1945). Eighteen male weanling rats were used. When received they were put in separate cages and fed a diet adequate in every way except lacking vitamin A, a procedure necessary to deplete the body stores of this vitamin. The diet, obtained from the Biochemical Corporation, was composed of U.S.P. Salt Mixture No. 2—4%; Irradiated Yeast for Vitamin D—8%; Starch—65%; Vegetable Oil—5%; Vitamin Test Casein—18%. The diet in powder form was mixed with water and made into dough balls which were easy for the rats to manage. At first each pellet contained seven grams (wet weight basis) but as this was insufficient, the amount was increased to 11.5 grams (wet weight) or about 10 grams on a dry basis.

The cages were wire mesh and sheet metal with a removable tray under each cage. The usual cleaning procedures were followed. The rats were allowed to drink tap water ad libitum. The food was placed in shallow metal dishes with glass bottoms.

The rats were purchased in two lots, but all were born on the same date. Due to unexpected complications, many rats of the first set died and more had to be ordered. After three to four weeks on the depletion diet all rats showed loss of weight or stationary weight for two to three days. They were then divided into four groups, three rats in the positive control group and five animals in each of the three experimental groups. All groups were continued on the depletion diet. The positive controls had in addition 10.32 mcg. of vitamin A per rat per day, experimental group I, 0.15 gram algae, experimental group II, 0.35 gram algae and experimental group III, 0.55 gram algae per rat per day. To determine, if possible, the minimal amount of algae needed for normal growth, the amount of algae fed group I was reduced to 0.075 gram per day after the third week of the experimental period.

One hundred milligrams of the vitamin A preparation, crystalline acetate, obtained from the Nutritional Biochemical Corporation, were dissolved in vegetable oil. A portion was weighed on a Becker Chainomatic Balance to give 2.89 mg of the acetate, enough for four rats daily for 10 weeks. This amount was carefully mixed with enough basal diet to allow each rat five grams of the mixture per day for 10 weeks. Besides this five-gram pellet containing the vitamin A, each animal received a five-gram pellet of the unadulterated basal diet. Both dough balls were given at the same time as it was noted that the rats did not object to the vitamin-treated pellet.

The algae, mainly, *Microspora, Syndera, Chlorella, Oscillatoria, Urenema* and *Anabaena* (Reid and Assenzo, 1960), were obtained at the Norman Sewage Treatment Plant. They were collected in April between thunder-storm activity that washed off considerable slime. Also, the material was obtained in the late afternoon when the density of the algae was greatest and of the slime the least. The algae were dried in a drying oven at 55° C., a temperature satisfactory to protect the nutritive value, and ground to a fine powder in a mortar. Portions were weighed to give the three different intakes previously mentioned, and each added to five grams of the basal diet. The animals were fed daily this five-gram portion plus another five-gram portion of the basal diet. The basal diet-algae mixtures were prepared in dried form for the whole experiment. All food was kept in dark containers in the refrigerator except when weighing amounts for the day.

The food intake and condition of the rats were checked daily. During the depletion period they were weighed every four to seven days and during the experimental period, which lasted a little over six weeks, every three to seven days. Towards the end of the depletion period the rats were irritable and restless. There were no signs of extreme A deficiency, but all animals showed loss of weight or stationary weight.

Throughout the experimental period experimental group I on the least amount of algae gained at the same rate as the positive controls. Even when the intake of algae was reduced one-half, there was no decline in the weight gain for this group (Table 1).

TABLE 1 SUMMARY OF AVERAGE GROUP GAINS IN WEIGHT DURING THE EXPERIMENTAL PERIOD

Group	Avg. gain During the 1st 3 weeks	Daily gain	Avg. gain during the 2nd 3 weeks	Daily gain	Avg. gain during the total exp.	Daily gain
	gms.	gms.	gms.	gms.	gms.	gms.
E.G. I	27.2	1.3	35.1	1.6	62.3	1.5
E.G. II	26.8	1.3	32.8	1.5	59.6	1.4
E.G. III	29.5	1.4	30.0	1.4	59.5	1.4
P. C.	27.2	1.3	36.1	1.6	63.3	1.5

Four animals in the experimental groups have been omitted from the calculations as three died during the experiment and one was in such poor condition that he doubtless would have died if the experiment had been continued longer. All began by leaving a portion of their food containing the algae. Thus death might have been due to a deficiency of vitamin A, but the exact cause of death is unknown. Some of the unsatisfactory conditions, noted only in the experimental groups, were asthmatic type of breathing, hair patched and rough, uncoordinated movements, depigmented appearance of the teeth, scaly-looking tail and feet, diarrhea, crouched posture and decline in weight. Some of the experimental animals developed diarrhea or anorexia, but recovered and grew fairly normally. The rats in experimental group I (least amount of algae) were in the best condition and nearest like the control group. The growth patterns of all the experimental animals were rather irregular while in the positive controls the growth was more regular.

The average weight gains were similar for both the experimental groups and the positive controls. (Table 1). In general, there was not much difference between the experimental and positive control animals. Apparently, all experimental groups had more vitamin A than the minimal amount. Considering factors other than weight gain, experimental group III possibly suffered from hypervitaminosis A.

Experiments with non-sewage grown algae indicated positive growth with doses above 0.5 gram per animal per day on some types of plants and no positive growth on amounts less than 0.2 gram. In the present study, using sewage-grown algae, normal growth resulted even on 0.075 gram per rat per day. Doubtless, the sewage used to nurture the algae contained nutrients in addition to vitamin A. These nutrients, therefore, may have been at least partly responsible for the growth response, as well as the carotene in the algae.

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