

Food Value of Red Tide (*Gonyaulax polyedra*)

Abstract. Two harvests of ocean-growing red tide, comprised mainly of *Gonyaulax polyedra*, were evaluated in limited trials of rat feeding. The protein of red tide (25 to 30 percent, dry basis) supported growth satisfactorily. The essential amino acid composition of the protein closely resembles that of casein, the major protein of milk. As a marine resource, plankton represents a challenge for research.

Plankton is one of the potential sources of food for expanding world needs. We studied this possibility after observing the so-called red tides which commonly occur during early summer in the coastal waters of southern California. Although economic harvesting of such minute organisms poses difficult technological problems, the hundreds of square miles of ocean area often covered by plankton blooms suggest the substantial quantities of material involved. In addition to problems of harvesting, the dominant organism in southern California red tide, *Gonyaulax polyedra*, is reputed to be toxic (1).

Chapman (2) states that anchovy and larger fish represent a satisfactory size for harvesting from the ocean but that lesser fish and plankton are too small to be recovered economically. Hence, the principle at present is to catch fish after the fish have harvested the plankton. However, because there is always a loss of efficiency during passage of nutrients along a food chain, it is important to determine the nutritive value of plankton to nonaquatic species of animals. We now describe tests of this value (particularly protein quality) in red-tide samples collected at La Jolla, California, in July 1965 and December 1966. We have reported on fatty acid composition of lipids from red tide (3).

Gonyaulax polyedra (4) was the predominant organism in our two harvests of red tide. Cells were recovered from the ocean by filtration, packed by sedimentation, and held in deep-frozen blocks for later drying to a free-flowing powder. This was done either by lyophilization or by drying of the melted slurry of cells under vacuum with a rotating evaporator.

To gain some impression of the nutritional qualities of the material, we made a proximate analysis of the sample taken in July 1965. As dry matter, the results (in percentage) were: pro-

tein, 27.5; ether extract, 4.7; fiber, 19.5; ash, 17.6; and nitrogen-free extract, 30.6. The high ash content could also be derived by assuming that the 82-percent moisture in the wet-packed cells has about the 3-percent salinity of seawater; this salinity is increased roughly fivefold by drying of the cells. Although not submitted to the complete analysis, the sample taken in December 1966 exhibited a comparable quantity of protein (26 percent as dry matter) and an amino acid composition very similar to the earlier sample.

Amino acid analyses were made in triplicate for the two plankton samples and for a culture of *G. polyedra* grown (5) in the laboratory. Each sample (1 to 3 mg) was hydrolyzed in a vacuum for 24 hours at 110°C in 6N HCl. The digested samples were analyzed by the Piez-Morris system with triangulation of the peak areas (6). The results (Table 1) are averages of the triplicates. For comparison, data on casein (7) are included. Tryptophan was determined in duplicate for two of the samples (8).

The data on amino acid composition indicate that the protein of *G. polyedra* is of good nutritional quality. This composition is quite similar to that of casein, a protein well known for high nutritional value. The three samples of *G. polyedra* (from the ocean in summer or in winter or grown in the laboratory) have very similar patterns of amino acid composition. We attribute this to strong genetic control of protein composition.

Table 1. Percentage of amino acids in whole casein and in three cultures of *Gonyaulax polyedra*. (A) Sample from the ocean in July 1965; (B) sample from the ocean in December 1966; (C) sample from cultures in the laboratory.

Amino acid	Whole casein	Cultures of <i>G. polyedra</i>		
		A	B	C
Asp	7.1	10.0	10.0	9.9
Thr*	4.9	4.8	4.8	4.9
Ser	6.3	4.7	4.9	4.8
Glu	22.4	12.4	12.7	13.7
Pro	10.6	5.2	5.2	5.2
Gly	2.0	5.7	6.1	7.3
Ala	3.2	7.4	7.9	8.0
½ Cys	0.3	1.0	0.7	0.9
Val*	7.2	6.5	6.4	6.4
Met*	2.8	2.9	2.9	2.7
Ileu*	6.1	4.7	4.5	4.2
Leu*	9.2	9.5	9.0	8.9
Tyr	6.3	3.9	3.6	3.3
Phe*	5.0	6.0	5.5	5.1
Lys*	8.2	6.7	7.5	6.4
His*	3.1	2.5	2.4	2.2
Try*	1.7		1.5	1.5
Arg	4.1	6.1	6.0	6.0

*Essential amino acids for the rat.

Red tide was fed to rats in two trials. The first was a simple evaluation of toxicity; when the plankton-fed rat grew about as well as its controls, we made a second trial to study nutritive quality of the material. Unfortunately, the available amount of processed red tide was of the order of only a few hundred grams; trials, therefore, were largely exploratory.

In the first trial, dried red tide (July 1965) was substituted in a purified diet for casein and cornstarch, so that the protein from red tide replaced approximately 20 to 25 percent of the protein supplied by casein in the purified diet (the control diet of the second trial with minor quantitative variations). The non-protein fraction of red tide replaced cornstarch; this caused the experimental ration of red tide to be lower in energy than the control because of the lower energy value of red tide with respect to cornstarch. Because of the small amount of red tide, we were able to test this material with only one animal. One female rat (74 g) was fed this diet for 10 days. During this period, the animal gained 3.0 g per day. This rate of gain was slightly lower than that (3.5 g per day) exhibited by comparable females on the casein control diet. The animal consumed the red tide in an amount similar to that of the control diet.

In the second trial, control and red-tide diets were formulated (9) to be equal to both protein and energy. Protein and energy values were obtained for all ingredients except minerals and vitamins. Red tide (an equal mixture of samples from July 1965 and December 1966) was added until it supplied 20 percent of the protein; the remainder was supplied by casein. Cornstarch and corn oil were adjusted so that the energy value was equal to that of the control diet.

Four male littermate rats, about 4 weeks of age, were paired and assigned to the two diets. The two animals receiving red tide grew at the same rate as those receiving the control diet. The feed intakes were about the same for both diets, and the gain for each animal during the 30-day test period was approximately 160 g. All the animals were placed on a diet of commercial rat chow for 7 days beginning on day 23. Evidently, the animals on the diet of red tide had deposited body protein rather than increased weight through large intakes of water. At the end of the trial, when the rats were killed in order to determine body fat and abnor-

malities, all animals seemed to have been normal, and no difference in the amount of internal fat was apparent.

Two trials for nitrogen balance were conducted during days 5 to 10 and days 16 to 23. Urine and feces, collected separately for each trial, were blended, sampled, and analyzed. The animals were in positive nitrogen balance at all times; the amount of nitrogen retained was higher during period I (60 percent) than during period II (50 percent). The digestion coefficient for nitrogen was 90 percent in period I, but it was slightly lower in period II. The animals on the diet containing plankton, as well as the control animals, seemed to utilize nitrogen during both periods. Palatability of the red tide was not a problem at any time despite marine odor and high salinity.

Our analyses of the nutrient composition of *G. polyedra* indicate its potential as a source of food. Exploratory feeding trials showed that the rats had good early growth and no toxicity during that period. Factors determining the occurrence, size, and variety of organisms of plankton blooms in the open ocean are not well known, and *G. polyedra* is not one of the more common varieties. However, controlled farming of plankton could become an integral part of the recovery (by atomic energy) of potable water and chemicals from seawater. In many arid coastal regions of the world, drying of plankton may be practical because of climates of high heat and low humidity.

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2. W. M. Chapman, *Food Technol.* **20**, 45 (1966).
3. S. Patton, G. Fuller, A. R. Loeblich III, A. A. Benson, *Biochim. Biophys. Acta* **116**, 577 (1966).
4. *G. polyedra* is a unicellular, photosynthetic, marine dinoflagellate averaging about 40 μ in diameter [see F. T. Haxo, in *Comparative Biochemistry of Photoreactive Systems*, M. B. Allen, Ed. (Academic Press, New York, 1960), p. 345].
5. Minor modifications of the culturing method of Schradie and Bliss (1) were used.
6. K. A. Piez and L. Morris, *Anal. Biochem.* **1**, 187 (1960).

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8. J. R. Spies, *Anal. Chem.* **39**, 1412 (1967). We thank him for the tryptophan analyses.
9. Composition (in percentage) of the control and red-tide diets, respectively, for each ingredient was: casein, 20 and 16; red tide, 0 and 17.89; cornstarch, 64 and 47.61; corn oil, 10 and 12.5; minerals, 4 and 4; and vitamins, 2 and 2.
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5-Hydroxytryptamine in the Carotid Body of the Cat

Abstract. *Glomus cells, at least in the carotid body of cats, contain catechol and indolamines. Cells containing adrenaline, noradrenaline, and 5-hydroxytryptamine were identified.*

Catecholamines are present in the carotid body of some species. Using histochemical methods, Muratori and Battaglia (1) demonstrated the presence of catecholamines in the carotid body of the pig; Muscholl *et al.* (2) obtained similar results in calves. The presence of noradrenaline in the carotid body of calves and pigs was confirmed by Rahn (3). With Falck's (4) technique, Niemi and Ojala (5) found that the human carotid body fluoresces, and they attribute this to catecholamines. Using biochemical and histochemical methods, we found various amounts of dopamine, noradrenaline, and adrenaline, with dopamine comprising more than half of the total catecholamine content of the carotid body. The glutaraldehyde-silver technique enabled us to identify cells containing adrenaline and noradrenaline (6). At the same time, Filenz and Woods (7) reported the presence of dopamine in the carotid body of the rabbit.

The only available information on the presence of indolamines in normal carotid bodies was reported by Hamberger, Ritzén, and Wersäll (8) who examined human carotid bodies with a fluorescence method for histochemical visualization of certain monoamines. They found cells with a green to yellow-green fluorescence, presumably norepinephrine, and yellow fluorescent cells containing 5-hydroxytryptamine (5-HT). Using a fluorescence microspectrographic method, they established the presence of both monoamines in human carotid bodies.

During our study of the carotid body with the glutaraldehyde-silver technique, we noticed that there was more than one type of silver-reducing cell. Cannata *et al.* showed that this technique permits the differentiation of noradrenaline from cells containing 5-HT. After glutaraldehyde fixation, the cells containing noradrenaline reduce the silver immediately, whereas the cells containing 5-HT need a longer period of silver treatment (9). Therefore, the study of the 5-HT content in the cat's carotid bodies was undertaken. Once we had proved the presence of this indolamine, we identified the cells containing 5-HT. The carotid bodies were removed from adult cats anesthetized with Nembutal (40 mg/kg), dissected, immediately immersed in 5 percent trichloroacetic acid, and assayed for 5-HT by the technique of Bogdansky *et al.* (10). In each of ten experiments, four carotid bodies were assayed. For the assay of catecholamines, the same number of carotid bodies was dissected and distributed in ten experiments. The carotid bodies were weighed and immersed in 0.4M perchloric acid. The extraction was performed by the method of Bertler *et al.* (11). Adrenaline and noradrenaline were assayed with the technique of Donoso *et al.* (12), whereas for the assay of dopamine the method of Carlsson and Waldeck was used (13). For histochemical studies by electron microscopy, the carotid bodies were fixed in 6.5 percent glutaraldehyde solution in Millonig buffer at pH 7.2, 4°C, for 6 hours. The glutaraldehyde-silver technique (14) was used after a careful wash in distilled water. Silver treatment was applied at various times (2, 5, 15, and 30 minutes).

The biochemical assays showed that 5-HT was present in a large amount ($6.9 \pm 0.27 \mu\text{g/g}$); the amount of noradrenaline was $2.4 \pm 0.37 \mu\text{g/g}$, that of adrenaline was $0.4 \pm 0.08 \mu\text{g/g}$, that of dopamine was $4.4 \pm 0.41 \mu\text{g/g}$. Extracts of carotid bodies prepared for 5-HT assays excited at 295 m μ showed that the fluorescence spectrum obtained with an Aminco Bowman spectrofluorometer was the same as that obtained with pure 5-HT. Both showed a fluorescence maximum at 575 m μ (uncorrected).

Electron microscopy with the glutaraldehyde-silver technique revealed three different types of cells. The first type, distributed irregularly, contains large, polymorphic granules; with 1 or 2 minutes of treatment with silver, a positive glutaraldehyde-silver reaction