Mass Culture of Algae for Food and Other Organic Compounds
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media where metabolic deficiencies or upsets would not be expected to lead as rapidly to death of the cell. It is perhaps for this reason that synthetic media must be developed if stable cell populations are to be maintained successfully in vitro. Only in a chemically defined nutrient medium which permits all the normal cell processes to proceed without over-stimulation or under-nourishment will it be possible to establish and maintain stable cell populations. This is a goal toward which the present work is directed.

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MASS CULTURE OF ALGAE FOR FOOD AND OTHER ORGANIC COMPOUNDS[1 2]

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ABSTRACT

KRAUSS, ROBERT W. (U. Maryland, College Park.) Mass culture of algae for food and other organic compounds. Amer. Jour. Bot. 49(4): 425–435. Illus. 1962.—Data are being collected which appear to support the use of unicellular algae for human food. Analyses of proteins, fats, carbohydrates, and vitamins indicate that unicellular green algae, especially *Chlorella*, should be excellent sources of these nutrients. The effectiveness of the algae for the support of growth of chickens, mice, rats, and rabbits has been found to be good. However, only limited studies have been done with humans. The problem of acceptability varies with the nationality of the subjects and the preparation of the food. Serious gaps still exist both in the technology of production and in the experimentation required to establish nutritional value. Nutrition studies using algae free of bacteria are urgently needed.

INTRODUCTION—It is now 14 years since Hermon Spoehr and Harold Milner (1947–48) first suggested that unicellular algae might serve as a food source for an expanding world population. This proposal captured the imagination of scientists acquainted with the characteristics of

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the algae and also intrigued a multitude of journalists, engineers, and amateur biologists not at all familiar with the problems involved. Consequently, there has been a great deal written about the mass culture of algae for food. In view of the current attention being given to the use of algae in closed ecological systems essential for protracted space exploration, it seems appropriate to review what we know and to determine what we do not know concerning the use of unicellular algae for production of food and other useful organic products.

Although the importance of algae in synthesizing 90% of the world's organic carbon was well known and under careful study, it is important to recall that the original proposals of Spoehr and Milner were the result of observations on cultures of *Chlorella* (grown during World War II) in the hopes of obtaining antibiotics of medicinal value (Spoehr et al., 1949). Analyses of such cultures revealed that the nutrition and culture technique could result in cells with varying percentages of lipid, carbohydrate, and protein. The lipid content could be as high as 85%, the carbohydrate 37%, and the protein 88.2% (Spoehr et al., 1949; Spoehr, 1951; Burlew, 1953). The growth rate of the *Chlorella* used at that time was 3.3 $\log_2$ units/day at the maximum. This is relatively slow when compared to the rates recently obtained for *Chlorella* (Sorokin and Krauss, 1958, 1959). The photosynthetic efficiency, again at its maximum for prolonged culture, appeared to be near 25% of incident visible energy in contrast to under 1% for crop plants. Extrapolation could be readily made to show that the efficient production of protein achieved by the algae could be accomplished on a small fraction of the surface of the earth now devoted to cultivation of higher plants (Milner, 1951; Burlew, 1953; Milner, 1955). The possibility of algal culture as a method of producing fuel was also considered to be feasible (Fisher, 1955). In addition there were proponents of algal culture for production of fats—Harder and Von Witsch (1942) having performed experiments to that end in Germany during the war. Recently, considerable production for carotenoids for chick food has been achieved with *Spongiococcum* (Kathrein, 1960).

The use of algae for food, in fact, did not appear especially repugnant in view of the long history of algal consumption in numerous maritime nations —most notably Japan. There, *Porphyra tenera, Enteromorpha intestinalis, Laminaria japonica, Undaria pinnatifida,* and *Monostroma nitidum* are common components of the diet. In Scandinavia and Scotland, *Rhodymenia palmata* is frequently eaten. In the West Indies, an excellent soup is made from *Gracilaria* (Krauss and Galloway, 1960). In fact, scores of species are eaten in many parts of the world. It should be remembered that these species are large forms, high in complex polysaccharides but low in protein, fats, and digestible carbohydrates.

The central problem in dealing with the mass

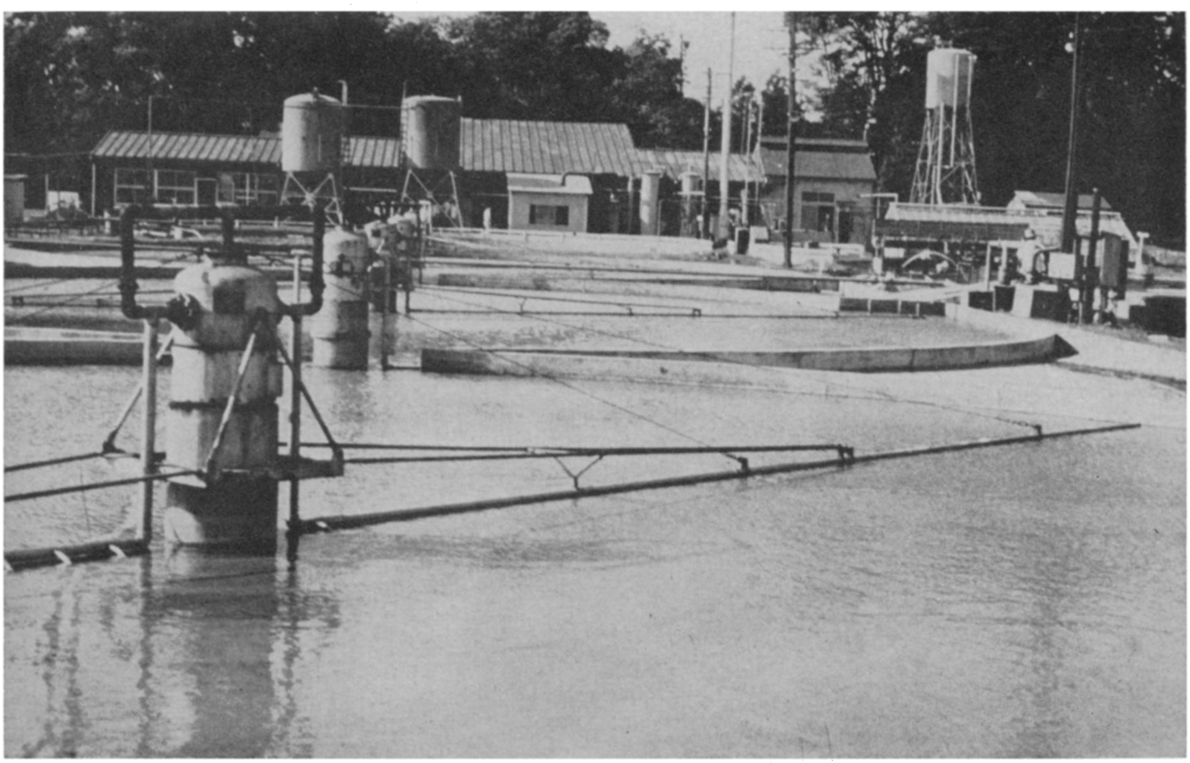

Fig. 1. A view of the Japanese Microalgae Research Institute at Kunitachi-machi, a suburb of Tokyo. The shallow vats of algae in the foreground are supplied CO_2-in-air and are stirred by revolving booms.

culture of algae appeared to be production rather than acceptability. Laboratories in the United States (Cook, 1951; Myers, Phillips, and Graham, 1951; Krauss and Thomas, 1954; Pruess et al., 1954; Krauss, 1955), Germany (Meffert, 1954), England (Geohegan, 1951), Japan (Tamiya, 1957), Sweden (Bjorkman et al., 1955), Israel (Mayer, Eisenberg, and Evenari, 1956), and Russia (Gaevskaia, 1955) constructed more or less ambitious devices for the mass culture of algae. The most notable of these early trials was that financed and supervised by the Carnegie Institution of Washington at the Arthur D. Little Company in Cambridge, Massachusetts (Burlew, 1953; Fisher, 1955). The experimental work was restricted to a few summer months in 1951, but during that time some 180 lb (dry wt) of *Chlorella* were produced. Cost analyses were considered hazardous. The A. D. Little group estimated a cost of $.17–.25/lb for production in a fairly simple apparatus. The algae were distributed to various laboratories for study.

During this same period, the Japanese, working with Dr. Tamiya at the University of Tokyo, also were experimenting with algae as a potential food source (Mituya, Nyunoya, and Tamiya, 1953; Tamiya, 1959). Only the Japanese apparatus and that of the A. D. Little Company can be considered "pilot plant" in scale. Both used natural sunlight and occupied considerable ground area. While the pilot-plant studies in the United States were terminated in 1951, those in Japan have continued and at present are centered at the Microalgae Research Institute at Kunitachi-machi, a suburb of Tokyo (Fig. 1). The Institute, under the direction of Dr. Hiroshi Nakamura, represents a commitment of $3,000,000 by the Japanese government and private industry in the mass culture of algae (Nakamura, 1961). The culture ponds at present cover one acre. The Japanese establishment is in part devoted to experimental work on culture methods and in part to production of *Chlorella* for commercial sale and experimental work. Production is maintained during summer and winter by employing species of *Scenedesmus* and *Chlorella* with appropriate temperature optima. *Chlorella ellipsoidea* is the most common species employed. Strains exist with temperature optima of 40, 25, and 15 C.

The production record of the Institute for the last 2 years is of interest (Table 1). As one might expect, the growth of the algae was more rapid during the summer months than in the winter. However, some production is apparent even in January and February when the temperature and light intensity are quite low in Tokyo.

The production cost is very high compared to previous estimates, but part of the inefficiency is due to the experimental nature of the plant. The product sells for $4.00/kg or roughly $2.00/lb. However, at this price the running cost of production is paid for by the product during months of high production. This is still a long way from the

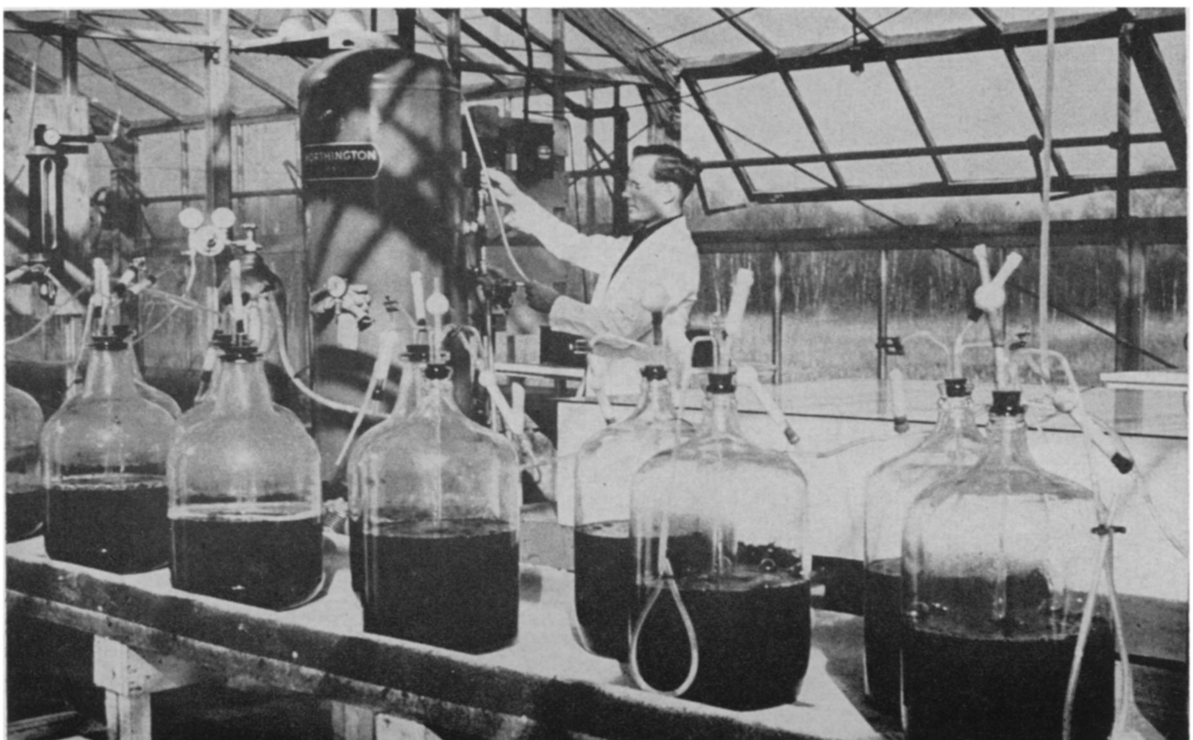

Fig. 2. Experimental bacteria-free cultures of different species of algae in the greenhouse of the University of Maryland. Such cultures may be grown using inorganic media in the light, or media supplemented with glucose in darkness.

TABLE 1. *Chlorella production at the Japanese Microalgae Institute.* (*From Nakamura, 1961*)

Month	Dry weight (kg/acre)	Average per month (kg/acre)
1959		
May	260	
June	245	
July	240	
August	500	
September	375	
October	350	
November	180	
December	185	209
1960		
January	130	
February	75	
March	70	
April	245	
May	275	
June	350	
July	450	
August	245	
September	250	
October	350	
November	185	
December	60	208

more sanguine estimates of Tamiya (1957) suggesting a goal of $.26/lb.

There are numerous inefficiencies in the production technique. Carbon dioxide is produced by burning fuel oil in a converter and forcing scrubbed CO_2 through the revolving arms in the algal ponds at about a 5%-in-air concentration. At best, only about 1/3 of the CO_2 is absorbed by the algae and the rest is lost, nor is the agitation as rapid as necessary for maximum yield. Also, the methods of CO_2 generation and of harvesting are not optimum.

The large-scale apparatus in Tokyo is undoubtedly the major effort in the world for algal mass culture. However, it should be pointed out that other culture techniques are also responsible for some production of algae for analytical purposes. These can be considered large-scale or mass cultures as well. In the United States, large-scale cultures can be found in a number of commercial, governmental, and university laboratories (Fig. 2). These are being studied often in conjunction with studies of photosynthetic gas exchangers or closed ecological systems. Also the careful scrutiny of the role of algae in the maintenance of sewage oxidation ponds by Dr. Oswald's group at the University of California has contributed to the knowledge and techniques of mass culture (Oswald et al., 1953; Gotaas, Oswald and Ludwig, 1954). The possibility of encouraging the growth of nitrogen-fixing blue-green algae in rice fields or in temporarily flooded agricultural lands may also be considered an aspect of mass culture, but in fact it would be more a case of semi-controlled ecology. Several laboratories have also been committed to studies of algal physiology and on occasion resort to mass-culture techniques (Ketchum and Redfield, 1949; Myers et al., 1951; Ryther, 1954; Krauss and Specht, 1958). This paper is not

TABLE 2. *Amino acids of algae.* (*From Fowden, 1951*)

Amino acid	*Chlorella pyrenoidosa*		*Chlorella vulgaris*		*Anabaena cylindrica*	
	Free	Comb.	Free	Comb.	Free	Comb.
Cystine	+	+	+	+	++	+
Aspartic acid	+	++	+	+	++	++
Glutamic acid	++	++	+++	++	+++	+
Glycine	++	++	++	++	++	++
Serine	++	++	++	++	++	++
Asparagine	−	−	+	−	−	−
Threonine[a]	+	++	++	++	+	++
Alanine	+++	+++	+++	+++	++	+++
Glutamine	+	−	+	−	+	−
Citrulline	+	−	++	−	−	−
β-Alanine	−	−	+	−	−	−
Tyrosine	+	++	+	++	+	++
γ-Amino-butyric acid	+	−	+++	−	−	−
Lysine[a]	+	++	+	+	+	+
Histidine[a]	−	+	−	+	−	++
Arginine[a]	+	++	+	++	+	++
Proline	+	++	++	++	−	+
Methionine[a]	+	+	+	+	+	+
Valine[a]	++	++	+	++	+	++
Phenylalanine[a]	+	+	+	+	+	+
Isoleucine[a]	+	++	+	+	−	++
Leucine[a]	++	++	++	++	+	++
Tryptophane[a]	−	+	−	+	−	+

[a] Essential amino acid.

TABLE 3. *Amino acid assay of dried Chlorella.* (*Adapted from Combs, 1952*)

Nutrient	Laboratory sample (%)
Crude protein	40.0
Arginine	2.39
Histidine	0.65
Isoleucine	1.69
Leucine	1.99
Lysine	2.43
Methionine	0.57
Phenylalanine	2.14
Threonine	1.91
Tryptophane	0.41
Valine	2.67
Glycine	2.20

designed to review these interesting aspects of mass culture but must be limited to a discussion of the economic horizons.

ANALYTICAL DATA—The analytical data that have been collected from the samples of algae produced thus far are the most convincing guides to their potential value.

Amino acids—Early qualitative analyses of the amino acid content of *Chlorella* were prepared by Fowden (1951) (Table 2). Of the 23 amino acids identified in algal protein, only the amides, asparagine and glutamine, were conspicuously low. Of the amino acids essential for mammalian nutrition, methionine, histidine, and tryptophane appeared to be lower than would seem desirable in a balanced protein. Quantitative analyses confirm the analyses for the essential amino acids for *Chlorella pyrenoidosa* (Table 3). The most recent analyses show a somewhat higher level for all amino acids but still a paucity of methionine, histidine, and tryptophane (Table 4). In practice, as will be shown later, only methionine and lysine seem to be deficient enough to require supplementation.

Lipids—The lipid levels in the algae had been determined prior to the introduction of gas-liquid chromatography (Paschke and Wheeler, 1954). Recently Schlenk et al. (1960) have presented a fairly complete analysis which can be assembled in tabular form (Tables 5, 6). The data are given in terms of per cent of total fatty acids which can vary, from 20 to 85%, depending on the method of culture. The interesting thing about the data is the presence of a number of unusual acids in the table showing minor components. Acids of short chain length are rare in nature, being unknown under 10 carbons. The odd-numbered acids C_{15}, C_{17}, and C_{19} are also rare, as are the long-chain arachidic, behenic, and lignoceric acids. It should also be noted that the majority of the fats are unsaturated. Some data on sterols are also available (Krauss and McAleer, 1953).

Carbohydrates—Carbohydrate analyses have not been as detailed as those for the amino acids and fats. A large portion of the carbohydrate fraction

TABLE 4. *Contents of amino acids in algal samples.* (*Adapted from Hayami, Matsuno, and Shino, 1960*)

Amino acid	Contents in grams per 100 grams of protein: Freeze-dried	Blanched	Methanol-treated
Isoleucine	2.88	3.43	3.42
Leucine	6.85	8.52	8.81
Lysine	5.55	5.92	6.65
Phenylalanine	4.08	4.60	4.91
Methionine	1.23	1.51	1.62
Threonine	3.88	4.38	4.76
Valine	4.97	5.26	5.09
Histidine	1.24	1.39	1.72
Arginine	5.51	6.47	6.33
Tryptophane	1.23	1.54	1.60
N(%)	12.34	9.67	10.68
Protein (%)	77.13	60.44	66.75

TABLE 5. *Composition of Chlorella pyrenoidosa fatty esters: major components* (*weight % of total esters*). (*Adapted from Schlenk et al., 1960*)

Apparent acid	Methyl ester of acid	Original GLC
Palmitic	C_{16} saturated	13.6
Palmitoleic	C_{16} monoene	3.2
	C_{16} diene	7.0
	C_{16} triene	5.1
Stearic	C_{18} saturated	3.5
Oleic	C_{18} monoene	34.7
Linoleic	C_{18} diene	17.7
Linolenic	C_{18} triene	14.6

TABLE 6. *Composition of Chlorella pyrenoidosa fatty esters: minor components* (*weight % of total esters*). (*Adapted from Schlenk et al., 1960*)

Apparent acid	Methyl ester of acid	GLC detected after distillation
	C_{12}	0.02
Lauric	C_{12}	0.01
Myristic	C_{14} saturated	0.15
Myristoleic	C_{14} monoene	0.09
	C_{14} diene	0.13
	C_{14} triene	0.03
Pentadecanoic	C_{15}	
Heptadecanoic	C_{17}	0.09
Nonadecanoic	C_{19}	0.15
Arachidic	C_{20}	0.67
		0.36
		0.20
Behenic	C_{22}	0.05
		0.06
Lignoceric	C_{24}	

TABLE 7. *Vitamin assay of dried Chlorella.* (*Adapted from Combs, 1952*)

Vitamin	Laboratory sample
Carotene, mg/lb	218.0
Thiamin, mg/lb	4.5
Riboflavin, mg/lb	16.3
Niacin, mg/lb	109.0
Pyridoxine, mg/lb	10.4
Pantothenic acid, mg/lb	9.1
Choline, mg/lb	1370.0
Biotin, μg/lb	67.0
Vitamin B_{12}, μg/lb	10.0
Lipoic acid, acetate μ/mg	—

in the cell is reserve starch with about the same amylose/amylopectin ratio as in higher plants. Bailey and Neisch (1954) report that 20% of the dry weight of *Chlorella vulgaris* is starch. They further report the absence of glucosamine components of chitin and the complete absence of cellulose. About 5% of the carbohydrate may be in the cell walls; of this, none is cellulose but rather a similar polysaccharide. Sucrose and glucose are the usual free sugars found in the green unicellular algae but only in fractions of a per cent dry weight (Brown, 1948).

Vitamins—The vitamin content of *Chlorella* has been examined. A representative analysis is the table from Combs (1952) (Table 7). In addition to this, *Chlorella* has been shown to contain 600 mg/kg of vitamin C and 6 mg/kg of vitamin K. Although vitamin B_{12} has been shown to be synthesized by green plants, the 10 μg/lb shown in the table is probably not the result of *Chlorella* synthesis. Less than ½ lb of algae would supply more than the daily requirements of the vitamins known to be essential for human nutrition, and, for most of the vitamins, a much smaller amount would be required.

Inorganic substances—The inorganic analyses of the algae have been shown to vary considerably with the concentration in the medium (Krauss, 1953; Krauss and Specht, 1958). The elements essential for human nutrition will be found in

TABLE 8. *Effect of dried Chlorella on body weight of chicks.* (*Adapted from Combs, 1952*)

Treatment	Average weight at 4 weeks (g)
Basal ration	135(13)
As 1 + 10% *Chlorella*	262(16)
As 1 + 10% *Chlorella* + 0.1% DL-methionine	298(16)
As 1 + 10% *Chlorella* + 0.1% DL-methionine + vitamine mixture	292(16)
As 1 + 0.1% DL-methionine + vitamine mixture	316(16)
Complete broiler mash	342(16)

TABLE 9. *Effect of Scenedesmus on growth of rats.* (*From Hundley et al., 1956*)

Diet	Weight gain in 27 days (g/rat)
Flour	6.4±0.6
Flour + 4% *Scenedesmus*	20.3±1.2
Flour + 0.75% lysine·HCl	25.0±1.2
Flour + 0.75% lysine + 4% *Scenedesmus*	53.2±3.1
Flour + 1.2% DL-threonine	7.3±1.0
Flour + 1.2% DL-threonine + 4% *Scenedesmus*	18.6±1.8
Flour + 1.2% DL-threonine + 0.75% lysine·HCl	46.5±2.9
Bread	22.0±1.2
Bread + 4% *Scenedesmus*	40.8±2.6

the algae in sufficient amounts with the possible exception of calcium—normally required by algae in only trace amounts—and sodium—not presently considered essential for growth of green algae.

The data are reassuring and it can be seen that there is room for optimism in viewing *Chlorella* as a food source. The analyses, however, do not reveal everything about the value of the material as food. The only satisfactory proof of the nutritional value of a food comes from carefully controlled nutritional studies on animals. These have been performed but in fewer number than one would expect in view of the apparent importance of the "new viticulture" that had been proposed.

The first nutritional studies made on animals were made by Combs (1952), at the University of Maryland, using chicks as the test animal (Table 8). The superior growth of chicks supplied with *Chlorella* in the basal ration is obvious especially in

TABLE 10. *Effect of Chlorella on growth of rats.* (*Adapted from Hundley et al., 1956*)

Diet	Weight gain in 28 days (g/rat)
Bread	14.8±1.6
Bread + 0.75% lysine	96.3±10.0
Bread + 0.75% lysine + 4% *Chlorella*	133.0±8.4
Bread + 0.75% lysine + 1.2% DL-threonine	139.3±5.7
Bread + 0.75% lysine	94.0±1.7
Bread + 0.75% lysine + 2.1% casein	116.1±4.0
Bread + 0.75% lysine + 2.1% soya protein	99.1±5.9
Bread + 0.75% lysine + 2.9% dried liver	117.6±4.3
Bread + 0.75% lysine + 5.4% dry skim milk	127.3±3.8
Bread + 0.75% lysine + 4.9% whole dried egg	123.2±8.4
Bread + 0.75% lysine + 1.2% DL-threonine	125.4±4.2

TABLE 11. *Feeding test with rabbits. (From Tamiya, 1961)*

	Chlorella diet	Soybean diet
Composition of diet (%):		
Spray-dried algal sample...	5	—
Soybean flour............	—	5
Wheat bran..............	60	60
Rice bran................	35	35
$CaCO_3$..................	0.2	0.2
NaCl....................	0.1	0.1
Average initial weight of animals (g)................	855	791
Average weight gain (g) in 49 days..................	345 (147%)	234 (100%)
Feed-efficiency (%)..........	18.4	9.0
Nitrogen-efficiency (%)......	99.2	51.2

TABLE 12. *Nitrogen balance in rabbits fed on algal diets. (From Tamiya, 1961)*

	Diet containing spray-dried alga	Diet containing methanol-treated alga
Composition of diet (%):		
Spray-dried alga...........	80	—
Methanol-treated alga.....	—	80
Wheat bran...............	20	20
$CaCO_3$..................	0.2	0.2
NaCl....................	0.1	0.1
Number of animals..........	2	3
Average weight (g)..........	1025	1250
Average quantity of —		
N consumed (mg).........	2053	1215
N in feces (mg)...........	548.6	204.7
N absorbed (mg)..........	1489	1011
Apparent quotient of absorption (%)...........	73.4	83.3
N in urine (mg)...........	1103	512.2
N assimilated (mg)........	386.3	494.2
$\frac{\text{(N assimilated)}}{\text{(N consumed)}}$ (%)	18.9	40.6
$\frac{\text{(N assimilated)}}{\text{(N absorbed)}}$ (%)	26.0	48.8

those fortified with 0.1% methionine. The heavier weights of the chicks grown on a complete broiler mash, including an antibiotic, which has been shown to be remarkably promotive of growth, could be in part attributed to the difficulty experienced by the chicks in eating the mash made hygroscopic by the particular sample of algae.

Further studies were conducted in Germany by Fink and Herold (1955) and by Hundley, Ing, and Krauss (1956) at the United States National Institutes of Health, using rats. The results of 2 experiments, with *Scenedesmus* and *Chlorella*, are shown in Tables 9 and 10. With *Scenedesmus*, the highest weight gain was in a diet of flour plus 4% *Scenedesmus* plus 0.75% lysine. Similar experiments with *Chlorella* (Table 10) show that the food value of *Chlorella*, when supplemented to a bread diet at the low level of 4%, is superior to a number of other common food additives including milk, soya protein, and liver. Soybean meal and *Chlorella* powders have been compared in rabbit feed (Tamiya, 1961) (Table 11). As with the NIH trial, the weight gain by the rabbits on algae wsa superior, being 147% of that on soybean meal.

Unfortunately, nutrition studies based solely on weight gain of mammals are insufficient for a balanced evaluation of the diet. A somewhat more searching evaluation can be made by examining the one nitrogen budget of the food consumed by rabbits (Tamiya, 1961) (Table 12). It is clear that the method of preparation of the algal sample bears heavily on the efficiency of utilization of the nitrogen contained in the sample. The extractions involved several hours of hot-alcohol extraction. There is shown a remarkably better "digestibility" or utilization of the algal protein by rabbits consuming cells that have been extracted briefly with hot methanol.

Data for human beings have been assembled in only 2 laboratories—Fitzsimmons General Hospital, USAMC, Denver (Powell, Nevels, and McDowell, 1961), and the Japanese National

TABLE 13. *Representative fecal excretion data. (Adapted from Powell et al., 1961)*

Period	Dry stool weight g/day	Ash g/day	Fat g/day	Nitrogen g/day	Carbohydrate g/day	Total calories/day
Control	49	6.3	6.2	5.6	1.8	257
50 g algae	74	8.3	7.5	7.1	13.8	401
100 g algae	85	8.6	6.7	8.8	14.6	476
200 g algae	136	12.5	15.3	15.2	22.2	806
500 g algae	217	19.6	20.8	22.4	36.4	1272

TABLE 14. *Digestibility of methanol-treated algal sample for human beings. (From Tamiya. 1961)*

Subject number	"Apparent" digestibility (%) Basal diet	Algal diet	Basal diet	Soybean diet	"True" digestibility (%) Algal protein	Soybean protein
1	86.1	87.0	86.0	86.6	89.1	85.9
2	86.2	87.3	87.9	85.9	87.7	82.1
3	86.5	86.1	87.2	87.8	83.1	90.7
4	88.7	86.2	85.2	84.7	83.4	77.8
5	87.0	86.8	85.3	87.1	88.3	90.4
Average	86.9	86.7	86.3	86.4	86.3	85.4

Institute of Nutrition, Tokyo (Tamura et al., 1958g; Hayami and Shino, 1959a,b; Hayami, Shino, and Matsuno, 1959; Hayami, Shino, and Tsuchida, 1959; Matsumoro, Morimoto, and Ito, 1959; Hayami, Matsuno, and Shino, 1960; Hayami et al., 1960). Certain recipes have been formulated and tested for taste by Morimura and Tamiya (1954). In the study by the Army Medical Corps, 5 healthy young men between the ages of 18 and 23 years consumed algae in amounts increased gradually every few days over a period of 40 days. After precautionary autoclaving, the algae were supplied in gingerbread, chocolate cookies, chocolate cake, and milk. An extensive series of metabolic analyses was maintained during the tests.

The results are in part what might have been expected. There was little difficulty with acceptability and tolerance up to the 100-g level. However, beyond this point, which is above that which would normally supply all necessary body protein, difficulties were observed in acceptability and digestive function. No abnormalities other than digestive difficulties were observed. Poor digestibility was evident. However, it can be assumed that up to 100 g of unprocessed algae may be eaten without undue discomfort for at least short periods. The poor digestibility can be seen in the data giving excretion figures for one subject on the different levels of algae (Table 13).

In Japan, Dr. Hayami, using some of the earlier recipe suggestions of Morimura and Tamiya (1954), has addressed himself to the problem of digestibility with algal samples modified in various ways. He has used 5 healthy girl students in these tests. Thirty grams of algae per day were added to normal food. The results are given in Tables 14 and 15. They show only a slightly poorer digestibility of the algal protein. The methanol-treated samples appear to be similar to soybean meal in digestibility. The tolerance to and acceptability by the Japanese of this diet appear high. Considerable work has also been done with the effects of processing on the composition of the algae (Tamura, Baba, and Tamura, 1958; Tamura et al., 1958a–f; Baba, Kobatake, and Tamura, 1958a,b, 1959; Hayami et al., 1959a–d; Hayami, Shino, and Matsuno, 1959; Hayami, Shino, and Sassa, 1959).

In concluding this review, some mention should be made of the only present commercial use of *Chlorella*. Shirota and Takechi (1961) (Table 16) have discovered that the rate of *Lactobacillus acidophilus* digestion of milk is enormously accelerated by dried *Chlorella*. In Japan, a lactic-acid-fermented milk called Yakult is sold in the amount of 3,000,000 bottles per day. The final data show the degree to which fermentation in this drink is accelerated. At present, almost all the production of the Microalgae Institute goes

TABLE 15. *Digestibility of blanched algal sample for human beings. (Adapted from Hayami et al., 1960)*

Subject number	"Apparent" digestibility during the period of Basal diet	Algal diet	"True" digestibility of algal protein
1	87.4	83.9	71.5
2	87.1	84.5	75.1
3	91.5	88.7	78.7
4	89.2	85.8	73.9
5	84.6	82.3	74.6
Average	87.9	85.1	74.8

TABLE 16. *Lactic acid fermentation by Lactobacillus acidophilus grown on milk-glucose media containing dried Chlorella. (Adapted from Shirota and Takechi, 1961)*

Per cent algae....	0%	0.25	0.50	1.0	2.0
Hours	ml N/10 NaOH to neutralize				
0	0.0	0.0	0.0	0.0	0.0
24	0.1	0.3	0.5	0.6	0.7
48	0.6	1.3	1.5	1.8	1.9
72	1.1	1.7	1.9	2.1	2.2
96	1.3	1.9	2.1	2.2	2.3
120	1.5	2.1	2.3	2.4	2.5
144	1.6	2.3	2.6	2.7	2.8

into the fermentation of milk for the production of Yakult. Active research is being directed to isolation of the factor responsible for this stimulation.

CONCLUSIONS—A review of the present status of mass culture of algae for food can be concluded with the following observations:

(1) The mass culture of algae for food is under active study. At present, the economics of the process make algal protein too expensive as a common food, in spite of yields which approach the early predictions. However, the possibilities of the closed ecological system for space exploration have made further study imperative. Furthermore, continued exploration of the algae for products of value in special situations like the milk fermentation will continue, especially by the Japanese.

(2) Further engineering experimentation is necessary to reduce the cost of production and to insure uniformity and quality of the product.

(3) Of paramount importance is the development of techniques for culturing large quantities of pure, bacteria-free algae for definitive and reliable nutrition tests. At the present, no such tests have been run and the data that we do have must be suspect because of lack of purity in the culture.

(4) Studies of processing must be accelerated. Obviously the algae are difficult to digest in the unprocessed form. However, the little experimentation that has been done argues well for success.

(5) Likely to be of even greater value to the ultimate commercial utilization of algae are the studies of fundamental physiology or biochemistry being carried out in numerous laboratories in many countries.

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