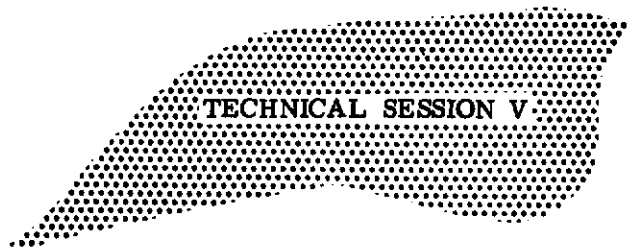

NUTRITIONAL SUPPORT FROM BIOREGENERATING SYSTEMS

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ANIMAL NUTRITION STUDIES WITH CHLORELLA 71105 *

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PART I PROTEIN QUALITY, DIGESTIBILITY, AND COMPOSITION

INTRODUCTION

For extended exploration into space, a system must be designed to provide such astronauts' needs as air revitalization, waste disposal, water recovery, and food supply. From a logistic standpoint, algae would provide an ideal gas exchange medium because of their capability to convert CO_2 into cellular constituents with the concomitant production of O_2. Furthermore, treated waste material can provide mineral nutrients and water to support algal growth.

Before algae can be accepted as food for Man, the nutritional characteristics of the product, grown by mass-culture techniques, must be evaluated. The Sorokin-Myers strain of Chlorella 71105 is currently under extensive research in connection with photosynthetic gas exchange. However, because nutritional studies with this strain have been incomplete, the following studies were undertaken:

1. Composition, i.e. proximate analysis, vitamins, minerals and amino acids.
2. Digestibility of protein, fat, carbohydrate, and crude fiber.
3. Quality of protein
4. Caloric value

*The work reported herein was carried out under Contract No. AF 33(616)-7373, Biomedical Laboratory, Aerospace Medical Laboratory, U.S. Air Force, Wright-Patterson Air Force Base, Ohio.

LITERATURE REVIEW

Fisher and Burlew 1953 (Burlew 1953) reported an average protein (crude) content of 50% in algae and compared it with fish meal at 60% protein, dried yeast at 48%, soybean meal at 44% and dried skim milk at 36%. Milner (1948) reported striking variations in the lipid content of Chlorella of from 5% to 85% of the dry weight depending on culture conditions. Values of 4 to 6% chlorophyll were reported by Fisher (Burlew 1953). Powell et al. (1961) reported 59% protein, 19% fat, 13% carbohydrate, 3% moisture, and 6% ash.

Morimura and Tamiya (1954) reported 200-500 milligrams per cent of ascorbic acid in algae while Cook (1960) reported 39.6 milligrams per cent ascorbic acid and 55.4 mg/100 gm of B-carotene on algae grown in sewage.

Fisher and Burlew (Burlew 1953) reported methionine values of 0.36 and 0.57 percent for Chlorella of somewhat lower protein content than that used in this study.

Prosky and Karinen (1960) reported a digestibility of 70% for the protein of algae supplied by the Japan Nutrition Association. Cook (1960) reported 54% digestibility for the protein of sewage-grown algae. Sixty per cent digestibility for dry Chlorella was reported by Hayami and Shino (1958).

Geoghegan (Burlew 1953) reported a protein efficiency ratio (PER) of 1.84 for Chlorella and Cook (1960, 1961) reported a PER of 1.62 for dried waste-grown algae, and 1.85 for the material after cooking. Powell et al (1961) reported 5.5 kcal/gm (bomb calorimeter) for dried algae supplied by the Japan Nutrition Association.

COMPOSITION OF CHLORELLA 71105

The composite algae powder used in these analyses was prepared from a culture of Chlorella 71105 grown in open conditions within an air conditioned facility by sanitary food plant procedures. An analysis of this dried Chlorella is found in Table 1.

It was found that Chlorella contained more crude protein than dried beef, soybean meal, dried yeast, and skim milk powder. The crude fat in Chlorella was of an unsaturated nature. The low amount of crude fiber in the algae compared favorably with the amounts found in dry legumes and expressed or extracted peanut flour.

Chlorella contained 17.5% total carbohydrate. Some peanut cake meals and flours contain as low as 23% total carbohydrate when decorticated. The amount of ash in Chlorella was about the same as that in alfalfa leaf meal and young timothy hay.

Semi-quantitative spectrographic analysis was carried out on Chlorella. Phosphorus was present in amounts of 1% to 10%. Calcium and magnesium were present in amounts of 0.1% to 1%. Iron and silicon were present in amounts of 0.01% to 0.1%. Sodium, aluminum, barium, and copper were present in amounts of 0.001% to 0.01%. Bismuth, chromium, lead, manganese, and titanium were present in quantities less than 0.001%. These results indicate that Chlorella 71105 contains significant amounts of calcium, phosphorus, and iron. Ascorbic acid, B-carotene, B_6, Thiamine, and pantothenic acid were also determined. These vitamin values are listed in Table 2. Chlorella can be considered an excellent source of B-carotene and a less significant source of ascorbic acid. Values for carotene in Chlorella were significantly greater than those reported for alfalfa leaf meals.

The determination of the amino acid content of Chlorella is given in Table 3. Protein with an amino acid pattern such as that contained in Chlorella should be suitable as supplemental food. All the essential amino acids were found to be present. The methionine value of 1.15% is substantially higher than values reported in the literature for Chlorella.

TABLE 1

ANALYSIS OF DRIED POWDERED CHLORELLA

	%
Protein (Nitrogen X6.25)	55.5
Crude Fat	7.5
Carbohydrate, Total (Anthrone method)	17.8
Ash	8.25
Moisture (Hot Air Oven at 105°C)	7.00
Crude Fiber (Cellulose and Hemicellulose)	3.1
Urea	0.08*
Chlorophyll	2.68
Total Calories	5.2 kcal/gm

*Urea was carried over from the growth medium.

TABLE 2

VITAMIN CONTENT OF CHLORELLA

Ascorbic Acid	14.6 mg/100 gm
Vitamin B_6	3.0 mcg/gm
Thiamine (B_1)	7.7 mcg/gm
Pantothenic Acid	11.2 mcg/gm
B-Carotene	50.2 mg/100 gm

TABLE 3

AMINO ACID CONTENT OF POWDERED CHLORELLA

Amino Acid	In Chlorella %	In Chlorella Protein (based on 16% nitrogen value) %
Aspartic Acid	3.74	6.58
Threonine	1.94	3.42
Serine	1.39	2.47
Proline	2.12	3.73
Glutamic Acid	5.13	9.03
Glycine	3.10	5.45
Alanine	3.87	6.81
Valine	3.27	5.76
Methionine	1.15	2.03
Isoleucine	2.02	3.55
Leucine	2.30	4.05
Tyrosine	1.67	2.94
Phenylalanine	2.70	4.77
Lysine	4.44	7.80
Histidine	0.85	1.49
Arginine	3.06	5.39
Tryptophan	0.85	1.50
(Ammonia)	1.32	2.32

FEEDING EXPERIMENTS

As a result of feeding experimentation carried out at Electric Boat Division's laboratories, the following data were obtained with regard to digestibility, protein quality, and caloric value.

Digestibility

The coefficient of digestibility was determined for the protein, fat, total carbohydrate, and crude fiber content in this strain of Chlorella as shown in Table 4. The digestibility of protein in Chlorella was 86%, a higher value than those of 54-70% found in the literature and one which compares favorably with the digestibility of proteins in beef liver, fish meal, and meat scraps.

TABLE 4

COEFFICIENTS OF DIGESTIBILITY OF CHLORELLA

	%
Protein	86
Total Carbohydrate	72
Fat	93
Crude Fiber	15

Protein Quality

Protein efficiency ratio (PER) experiments were carried out to compare the quality of algae protein with other food proteins. (PER is the ratio between the weight gained and weight of protein consumed.)

Four groups of ten littermate weanling rats were fed isocaloric rations, each containing 10% available protein. The test rations fed to the rats contained one of the following as the sole source of protein: Chlorella; Chlorella, supplemented with 2% L-methionine; purified casein; and defatted egg protein (see Table 5.) The experiment was allowed to run for 28 days. Records were kept of food consumption and food wastage. The rats were fed ad libitum and were weighed periodically. Data on PER experimentation are given in Table 6.

TABLE 5

DIETS USED FOR PROTEIN QUALITY STUDIES*

	Chlorella Diet %	Casein Diet %	Algae + L-Methionine Diet %	Egg Protein Diet %
Chlorella 71105 Lot A926-125	21	--	20.5	--
Casein +	--	12.3	--	--
L-Methionine	--	--	0.20	--
Dried Defatted ++ Egg Powder	--	--	--	11.7
Corn Oil	10	9	10	9
Starch	61	70.7	61.3	66.3
Agar	2	2	2	7
Vitamins in Dextrose+++	--	2	--	2
Vitamins in Dextrose without Vitamin A +++	2	--	2	--
Salt Mixture (USP XV)	4 /100	4 /100	4 /100	4 /100
Kcal per 100 grams	403	404	404	403

*All diets contain 10% available protein based on nitrogen x 6.25 and digestibility of protein.

+Casein (purified) was purchased from Nutritional Biochemicals Corporation, Cleveland 28, Ohio.

++Dried Defatted Whole Egg Protein Powder was purchased from Wilson Laboratories, 4221 Southwestern Boulevard, Chicago 9, Illinois.

+++Vitamin Diet Fortification Mixture in Dextrose with and without Vitamin A was purchased from Nutritional Biochemicals Corporation, Cleveland 28, Ohio.

TABLE 6

PROTEIN EFFICIENCY RATIO DATA

Diet	Average Amount of Ration Consumed gm	Average Weight Gain of Rats gm	Average Amount of Protein Consumed gm	PER*
Chlorella	386.9	84.7	38.69	2.19
Chlorella with 2% L-methionine	268.6	78.0	26.86	2.90
Casein	407.9	134.8	40.79	3.30
Defatted Egg	448.6	179.6	44.86	4.01

*Protein efficiency ratio is the amount of weight gain divided by the amount of available protein consumed.

The PER found for Chlorella is higher than that for most vegetable and cereal proteins. Too, it is slightly higher than the PER of soy protein (Table 7).

The PER of Chlorella plus methionine, although lower than our own value for casein, compares favorably with all other listed values in Table 7 for casein or milk protein. It is about the same as values listed for beef liver and hamburger.

The values obtained for egg protein and casein are higher than most values in Table 8. Campbell (1960) found PER values of 2.59, 3.18, and 3.35 for casein and 3.44, 4.00, and 4.00 for dried whole egg using three different strains of rats. Campbell concluded that the strain of the rats is the most important factor in variations of PER values among laboratories. He proposed the following correction calculation:

$$\text{PER} \times \frac{2.5}{\text{PER for casein}}$$

This calculation assumes a PER of 2.5 for reference standard casein and corrects for variation due to strain of rats. Using Campbell's correction calculation, we found the following PER values: casein 2.5, egg protein 3.03, Chlorella-methionine 2.20, Chlorella 1.66. As most investigators have not used Campbell's method of correction for strain of rats, the errors inherent in all comparisons of PER are self-evident.

More work, using higher levels of L-methionine for Chlorella supplementation to bring levels up (1) to that contained in egg and (2) to that believed to cause maximum growth in rats, should prove rewarding.

Caloric Value

The caloric value of Chlorella was determined both by calculation, using proximate analysis with corrections for digestibility, and by bomb calorimetry. The values of 5.16 kilocalories per gram by bomb calorimetry techniques and 3.29 kilocalories per gram by calculation were determined as shown in Table 8.

TABLE 7

PROTEIN EFFICIENCY RATIOS (PER) OF VARIOUS FOODS

FOOD	PER
(Chapman et al 1959, in Wrenshall, 1960)	
Whole egg dried	3.50
Fish flour	3.04
Whole milk, dried	2.70
Hamburger beef, dried	2.68
Soya flour	2.04
Beans, lima, cooked	1.72
Peas, cooked	1.57
"Protein" bread	1.29
Whole wheat bread	1.10
White bread enriched	0.77
White flour, enriched	0.59
"Protein" cereal	0.03
Gelatin	-1.25
(Hutchinson et al 1959, in Wrenshall, 1960)	
Whole egg	3.7
Bread	1.3
Bread and whole egg (50-50)	3.0
Bread and milk (50-50)	2.7
(Wrenshall, 1960)	
"Protein" bread	1.87
Nonfat milk solids	2.54
(Altschul, 1958)	
Peanut Protein average	1.5

FOOD	PER
(Albanese, 1959)	
Beef (ox muscle)	3.08
Liver (ox)	2.67
Pork	2.79
Pork liver	3.21
(Burlew, 1953)	
Chlorella	1.84
Dried skim milk	2.83
Dried brewers yeast	1.69
Peanut meal	1.34
(Cook, 1960, 1961)	
Skim milk solids	2.64
Cooked dried algae (waste grown)	1.85
Cooked oatmeal	2.35
Dried algae (waste grown)	1.62
Casein	2.39
Algae 75%, Casein 25%	1.96
Algae 50%, Casein 50%	2.05
Algae 25%, Casein 75%	2.45
(Electric Boat Div. Studies)	
Chlorella	2.19
Chlorella, L-methionine	2.90
Casein	3.30
Egg protein	4.01

TABLE 8

CALCULATION OF AVAILABLE CALORIES IN
CHLORELLA 71105

	Fraction in Food %	Heat of Combustion kcal/gm	Digestibility %	Chlorella kcal/gm
Total Carbohydrate (includes cellulose and hemicellulose)	17.8	4.15	72	0.53 +
Protein	55.5	5.65 (-1.25)*	86	2.10 +
Crude Fat	7.5	9.40	93	0.66 +
			Total kcal. per gram	3.29

*1.25 kilocalories is urinary energy loss per gram

+ kilocalories = % of Fraction in food x Heat of Combustion kcal/gm x % Digestibility per gm.

RESULTS AND DISCUSSION

Freeze dried Chlorella 71105 grown at Electric Boat Division, General Dynamics Corporation had the following composition: moisture 7.00%, crude protein 55.5%, crude fat 7.5%, ash 8.25%, total carbohydrate 17.8%, crude fiber 3.1%, chlorophyll 2.68%, and urea .08%. All essential amino acids were present.

The digestibility of the protein of the Chlorella was 86%, of the crude fat 93%, total carbohydrate 72%, and crude fiber 15% using white rats as the test animals.

Chlorella 71105 was determined to contain good quality protein. Also this algae strain was determined to be an excellent source of B-carotene and a less significant source of ascorbic acid. L-methionine value of 1.15% was higher than those assigned in the literature. Calcium, phosphorus, and iron are present as well in nutritionally significant amounts.

When the daily gas exchange requirements for one man are fulfilled, 606 grams of dried Chlorella are produced. It is interesting to note that 606 grams of freeze-dried Chlorella would furnish 2,000 kcal, 100 times the "Recommended Daily Dietary Allowance" of Vitamin A as Carotene, four times the protein allowance, slightly more than the daily requirement of ascorbic acid, about three times the allowance of thiamine, about the daily need for calcium, and at least six times the daily allowance of iron for an average adult male.

PART II GROWTH AND TOXICITY

INTRODUCTION

Research is currently being conducted on the use of algae for air revitalization and food supply in an extended exploration of space. The Sorokin-Myers strain of Chlorella 71105 is being studied extensively in connection with photosynthetic gas exchange. Since a population explosion is anticipated in the foreseeable future, it would be also behoove us to consider all possible sources of food, however exotic or impractical they may seem at the moment. Several studies on nutritional properties of algae have already been undertaken (Cook, 1960, 1961; Prosky and Karinen, 1960).

Since many foods contain substances that may be harmful to animals, it is important to conduct long term growth studies. It is not sufficient, however, to check properties of new and different foods by growth alone. Rats will grow and gain weight even though subjected to trauma, such as broken bones. Even some severe physiological abnormalities do not necessarily inhibit growth. Rats should be observed for functional or behavioral effects which might be caused by the feeding of a new material. It is prudent to perform autopsies on the subject rats to check for gross changes and to examine tissues for cellular changes associated with the feeding.

Some foods in natural form contain substances more hazardous than any of the materials that are added to foods for coloring or preserving, or as anti-oxidants. For example, anti-vitamins such as avidin, thiaminase, and dicumarol, and cyanogenetic glucosides are present in many food products, including solanine in potato sprouts, serotonin in pineapple, ricin in castor bean, and gossypol in cotton seed. It is only through the elimination of toxic portions or the development of proper culturing and processing techniques that many plants become edible.

Hayami and Shino (1958) tested decolorized (solvent extracted) and untreated, dried Chlorella as a food source for rats. While the decolorized alga was better than the untreated in promoting growth, neither was as good as a casein diet. Prosky and Karinen (1960) found the growth rate of weanling rats fed a 20% algal protein ration equaled that of the casein control animals on the same protein level.

Fink (1958) used the green alga, Scenedesmus, as the sole source of protein (except for the obligatory supplementation with brewer's yeast) in rat growth experiments. Algae, milk, and egg protein yielded nearly equal growth in weanling rats; after 100 days of growth, the algae appeared to support better growth than other protein sources. The skim milk protein diet required cereal protein supplementation. The rats showed no ill effects after eating algae for 240 days.

Powell et al (1961) fed Chlorella supplied by the Japan Nutrition Association to human volunteers. Mild to severe gastro-intestinal symptoms resulted. Physical examinations failed to reveal any abnormalities other than those associated with the gastro-intestinal tract. The gastro-intestinal symptoms were transient and disappeared soon after algae feeding was discontinued. All clinical laboratory tests remained within normal limits. The subjects were followed from one to six months after this study and no evidence of persisting toxicity was found. Preliminary tests in rats revealed no histologic evidence of toxicity when the algae was fed as the only food source for three weeks.

The program reported on in this writing explored various areas to determine the suitability of Chlorella 71105 for food:

1. Production of Chlorella under carefully controlled conditions in kilogram quantities sufficient for the investigations.

2. Growth studies with Chlorella fed to rats.

3. Determination of any toxic effect of Chlorella fed to rats both thru gross observations and histopathology.

4. Observations on acceptability of rations, and color of fur of rats fed Chlorella.

EXPERIMENTAL

Production of Chlorella 71105

The method for producing dried Chlorella consisted of growing the algae continuously at 39°C in two 600-gallon stainless steel tanks, under open nonsterile conditions. Fluorescent lamps were used as the light source and 0.7% carbon dioxide in filtered air was constantly bubbled through the medium.

The water used in the culture medium was filtered through acetate wound filters. The production facility was ventilated with filtered forced air. Food manufacturing sanitation was practiced in the production of this algae.

The algae were harvested using a Sharples super-centrifugal separator (Model #16) at 15,000 rpm. The Chlorella paste was then spread on stainless steel trays, placed in a freezer, and frozen at -10°C. The trays were then put in a vacuum dryer consisting of a Stokes vacuum chamber containing a conduction heating plate and

a refrigerated condensing coil. The algae were dried for about seven hours and then removed from the chamber. The dried material was milled to a powder in a Weber Bros. S-500 laboratory mill.

Growth Studies

Two young adult male rats were placed on a 10% algae protein diet (21% Chlorella, Table 9) and their growth observed. This work was carried out before growth studies were started in order to observe the acceptability of this diet and to ascertain any obvious toxic manifestations. The growth curve is shown in Figure 1. The amount of ration consumed by the two rats was 2426 gm. One rat gained 284 gm and the other 276 gm in 110 days, and both appeared normal and healthy.

Two male weanling rats were placed on the following ration for 37 days:

Chlorella	92%
Agar	2%
Salt mixture (USP XV)	4%
Vitamins in dextrose without vitamin A	2%

Vitamin A was omitted from Chlorella-containing rations because Chlorella is rich in carotene. The growth curve results are shown in Figure 2. The amount of ration consumed by both rats was 1120 gm for the 37 day period. One rat gained 145 gm and the other 150 gm during this period. However, it is interesting to note that one of these rats (AA1) lost 33 gm and the other gained 7 gm in the subsequent period of 8 days at which time autopsies were performed. Cook (1960, 1961) reported that the nutritional needs of rats and probably of human beings, could not be met by the use of algae alone.

Forty rats were used in a growth study. These were male littermate weanlings, caesarean-derived, weighing from 53-77 grams when received. These rats were fed laboratory ration for 24 hours. All gained weight and appeared healthy.

The 40 rats were divided into four groups of ten littermates. The rats were fed isocaloric rations, each containing 10% available protein. The test rations contained one of the following as the sole source of protein: Chlorella, purified casein, defatted egg protein, and Chlorella supplemented with 2% L-methionine as shown in Table 9. Rats were fed ad lib.

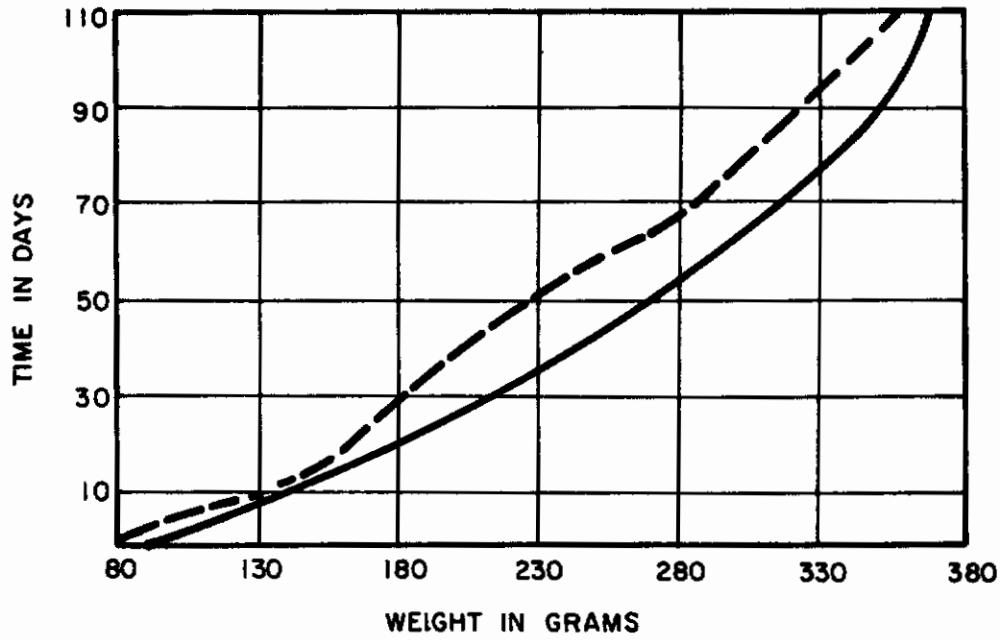

Figure 1. Growth curves of two young adult male rats fed 10% chlorella protein ration 110 days.

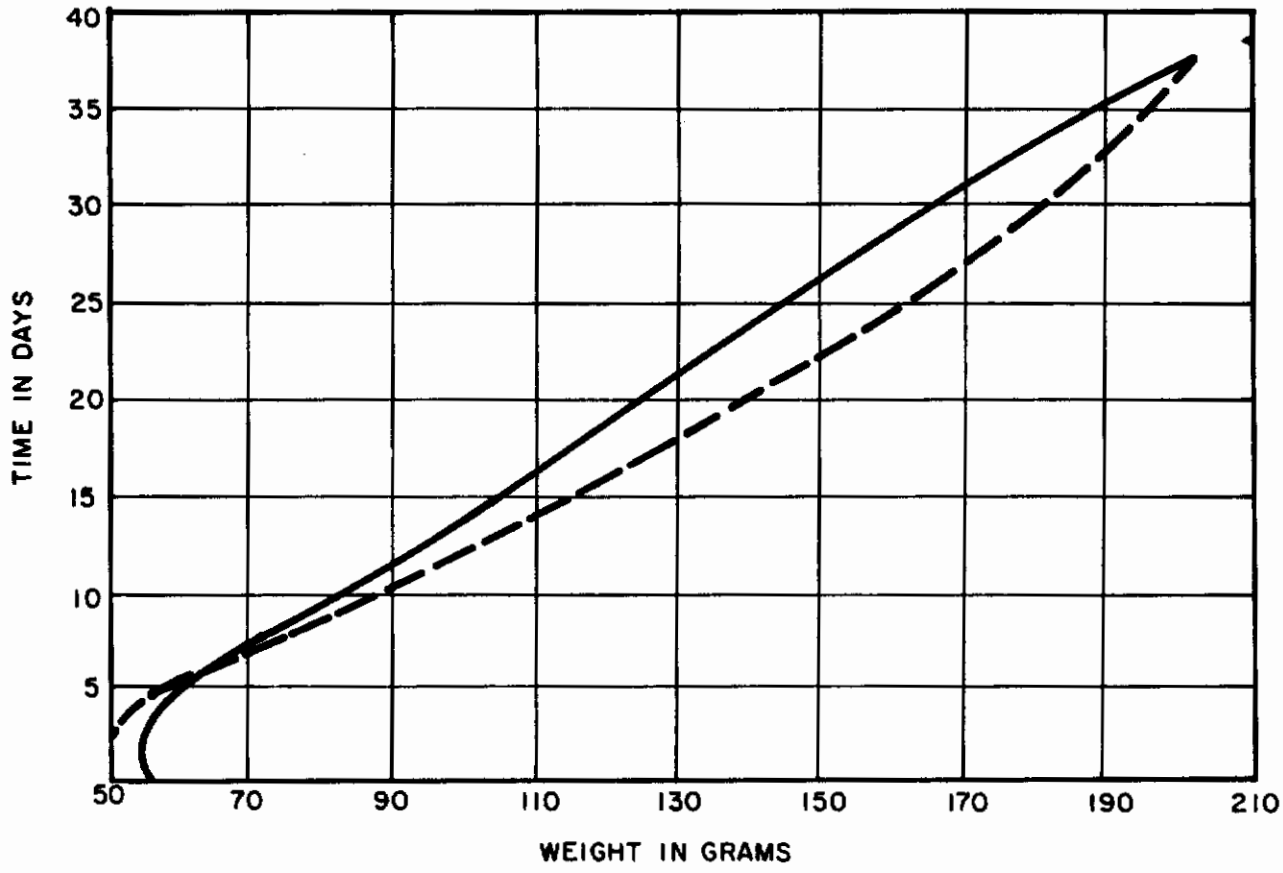

Figure 2. Growth curve of two male weanling rats fed 92% chlorella ration 37 days.

TABLE 9

DIETS USED FOR GROWTH STUDIES*

	Chlorella Diet %	Casein Diet %	Chlorella Plus L-Methionine Diet %	Egg Protein Diet %
Chlorella 71105 Lot A926-125	21	--	20.5	--
Casein+	--	12.3	--	--
L-Methionine	--	--	0.20	
Defatted Egg Protein++	--	--	--	11.
Corn Oil	10	9	10	9
Starch	61	70.7	61.3	66.3
Agar	2	2	2	7
Vitamins in Dextrose+++	--	2	--	2
Vitamins in Dextrose+++ without Vitamin A	2	--	2	--
Salt Mixture (USP XV)	4	4	4	4
Total	100	100	100	100
Calories per 100 grams	403	404	404	403

* All diets contain 10% available protein based on nitrogen x6.25 and digestibility of protein.

\+ Casein (purified) was purchased from Nutritional Biochemicals Corporation, Cleveland 28, Ohio.

++ Dried Defatted Whole Egg Protein Powder was purchased from Wilson Labs, 4221 Southwestern Boulevard, Chicago 9, Illinois.

+++ Vitamin Diet Fortification Mixtures in Dextrose, with and without Vitamin A were purchased from Nutritional Biochemicals Corporation, Cleveland 28, Ohio.

Two percent of L-methionine was added to one Chlorella protein level to bring the amount of sulfur-containing amino acids up to that of a 10% casein protein ration. The supplemented ration had twice the amount of L-methionine when compared to a 10% Chlorella protein ration.

The growth curves for the four groups of rats were compared with the growth curve furnished by the Charles River Breeding Laboratories for the C-D strain of male rat. The Charles River laboratory fed the rats "Baked D & G Research Animal Laboratory Diet for Rats and Mice," a balanced commercial type diet containing a minimum of 24% crude protein. The curves shown in Figure 3 indicate that rats grow normally when fed Chlorella rations for periods of 208 days. The rats on algae ration weighed an average of 474 gm; on algae-methionine ration, 493 gm; and on casein ration, 592 gm.

The studies show that rats can grow on rations utilizing Chlorella as the sole source of protein, fat, and carbohydrate (vitamins, minerals, and agar were added) or as the sole source of protein only.

Pathology

Pathological studies were undertaken to determine whether or not cellular changes are associated with the feeding of algal material. Dr. Charles Delahunt, veternarian in the Pharmacology Dept., Charles Pfizer and Company, performed the gross pathology and Dr. Vincent Dardin, Pathologist at Georgetown University carried out the histological examination.

Gross Pathology

Fourteen rats, (subjects in the growth studies) were sacrificed, and gross pathology of all important organs was studied. Twelve of these rats had been fed various concentrations of Chlorella in their rations for periods of 45 to 115 days. Two rats were used as control animals, one had been fed 12% casein, and the other 12% defatted dried egg.

As shown in Table 10 there were no gross adverse effects on animals fed Chlorella. The 92% Chlorella ration appears to have some possible growth retarding effect (on one out of two animals) and some possible impairment of hepatic function. The liver abnormality (a yellow or fatty liver) may be a secondary effect of the growth retardation.

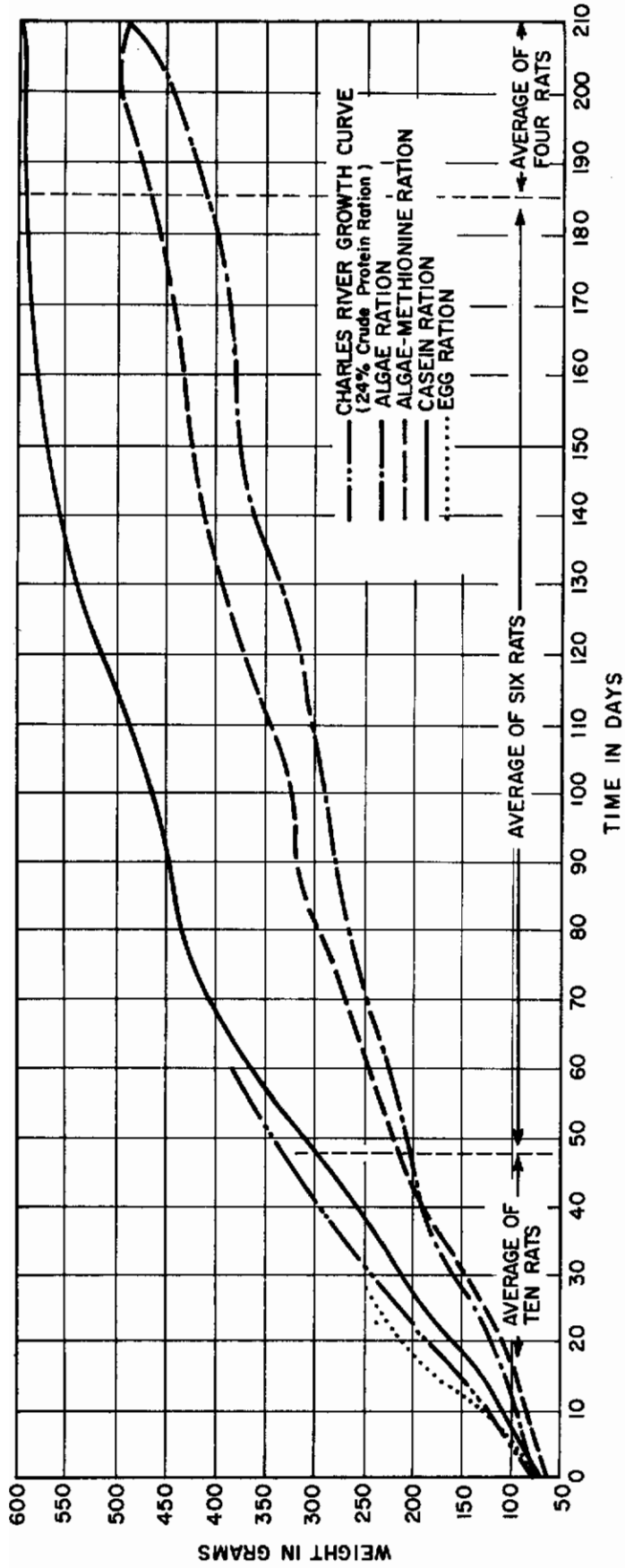

FIGURE 3 COMPARATIVE GROWTH CURVES OF SAME STRAIN RATS ON 10% PROTEIN TEST RATIONS AND CHARLES RIVER NORMAL GROWTH CURVE FOR RATS ON 24% CRUDE PROTEIN RATION

TABLE 10

SUMMARY OF GROSS PATHOLOGY OF MALE RATS FED CHLORELLA

Animal No.	Duration Days	% Chlorella Level	gm Body Weight		General Condition	Brain	Thyroid	Lung	Heart	Liver	Pancreas	Spleen	Kidney	Bladder	Eye	Adrenal	Stomach	Intestine	Prostate	Testicle	Pituitary
			Start	End																	
1. A5	45	21	58	225	N	N	N	V	N	N	N	N	N	N	N	N	N	V	N	N	N
2. A6	49	21	57	230	N	N	N	V	N	N	N	N	N	N	N	N	N	V	N	N	N
3. A7	49	21	64	190	N	N	N	V	N	N	N	N	V	N	N	N	N	N	N	N	N
4. A8	49	21	61	196	N	N	N	N	N	P	N	N	V	N	N	N	N	V	N	N	N
5. AM5	45	21	60	216	V	N	N	V	N	N	N	N	N	N	N	N	N	N	N	N	N
6. AM6	49	21	61	250	N	N	N	N	N	N	N	N	N	N	N	N	N	V	N	N	N
7. AM7	49	21	66	219	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
8. AM8	49	21	60	210	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9. A11	111	21	93	381	N	N	P	N	N	N	N	N	N	P	N	N	V	N	N	N	N
10. A12	115	21	84	360	N	N	N	N	N	V	N	N	N	N	N	N	N	N	N	N	N
11. AA1	45	92	51	168	D	P	N	N	N	P	N	N	V	N	N	N	P	P	N	N	N
12. AA2	45	92	58	210	N	N	N	N	V	P	N	N	V	N	N	N	V	N	N	N	N
..... CONTROLS																					
13. C5*	45	12	60	301	N	N	N	N	V	P	N	N	V	N	N	N	N	N	N	N	N
14. E5+	45	12	59	355	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

* Fed Casein
\+ Fed egg protein

Key
N normal
V lesion present, unrelated
P pathologic condition that is questionable
D diet induced abnormality

Histological Examination

Selected organs and tissues from the animals examined for gross effects were subjected to histological examination. Among those organs examined were the eye, heart, lung, liver, brain, kidney, parotid salivary gland, pancreas, thyroid, and testicle.

Comments on histological studies follow:

1. In preliminary studies, 30 tissues and glands from two young adult rats which had been fed a 21% Chlorella ration for 111 days were examined. One rat, whose salivary gland was not examined, showed abnormal cells in the pancreas; the other rat, whose pancreas was not examined, showed abnormal cells in the salivary gland.

2. Of two weanling rats fed a 92% Chlorella ration for 45 days, one showed histological abnormalities, the abnormalities being restricted to the pancreas and salivary glands.

3. Four weanling rats were fed a ration containing 20.5% Chlorella and 0.2% L-methionine for 45 days. Although the histopathology was incomplete on these animals, a total of 32 organs and glands were examined. Of the two salivary glands examined one showed abnormal cells. The pancreas was not examined in these animals.

4. Four weanling rats were fed a ration containing 21% Chlorella in a synthetic diet for 45 days. The histology was incomplete on these animals. A total of 43 organs and glands were examined. No abnormalities were found in these specimens which include two salivary and two pancreas glands.

5. The two control rats were histologically normal.

The preliminary histological findings can be summarized as follows:

1. Provocative changes, especially in the salivary glands and pancreas of some of the animals, were noted.

2. Both glands, especially the serous portion of the salivary gland showed marked pleomorphic acinar cells with hyperchromatic nuclei.

3. These changes were not found in all of the animals fed Chlorella, nor did there seem to be a dose response. Acinar cells in the rat have not been studied extensively.

Acinar cell change had not been observed previously by the two pathologists assisting in this study. These preliminary results clearly indicate the necessity for more detailed histopathology. The abnormalities discovered may be artifacts and it is hoped that many questions will be resolved when the remainder of the rats used in this study are autopsied and histological examinations are performed.

Miscellaneous Observations

Some observations were made concerning palatability of test diets, loss of hair in some rats, staining of hair by Chlorella, and size of feces of rats consuming various rations. These are commented upon below.

Palatability

According to Halpern et al, (1961) rats rejected dilutions of DL-methionine between 0.001 M and 0.04 M. Methionine is not unpleasant to humans in taste or odor.

There were no acceptability problems using algae rations containing 10% Chlorella protein (21% algae) in synthetic rations. However, two male weanling rats placed on a ration containing 92% Chlorella, vitamins, minerals, and agar, failed to eat the dry powdered ration and actually lost weight for the first three days. They then ate this ration satisfactorily (Figure 3). Rats ate Chlorella-methionine ration less readily than the Chlorella ration.

Alopecia (Loss of Hair)

A deficiency of inositol will cause loss of hair around the eyes of rats (Crampton & Lloyd, 1959) called "spectacle eye" syndrome (Rosenberg, 1942). Tamiya (1961) fed spray-dried algae to rabbits, chicks, and rats. He found that the rabbits began to lose hair after about four weeks of feeding.

After 12 days of 10% Chlorella protein ration, one of the ten rats developed alopecia. Two of the ten rats on the Chlorella-methionine ration developed a similar condition after 24 days.

Loss of hair started with the hindquarters and spread over part of the abdomen. The condition grew more pronounced in the two algae-methionine rats until it spread over the entire abdomen and hindquarters in a symmetrical fashion. In the case of the rat on the 10% Chlorella protein ration, the condition cleared up and all hair grew back 24 days later. This condition did not affect food consumption or weight which approached or exceeded the average gains for the group. The alopecia was not contagious. No evidence

of mites or insects was found. (Mites would be indicative of mange.) The skin was entire and no symptoms of fungus infection were present.

The loss of hair of rats fed Chlorella rations does not correspond to a loss of hair caused by inositol deficiency. All rations used in these studies contained the same vitamin supplementation; however, only some of the rats consuming Chlorella developed this condition.

Color of Rats Fed Chlorella Rations

It was interesting to note that the fur on all rats in this study which were fed Chlorella and Chlorella-methionine rations was pale green in color. This was due simply to external staining by the algae rations.

SUMMARY OF RESULTS

1. There were no acceptability (palatability) problems with rats on the 10% Chlorella protein ration. Rats ate Chlorella plus L-methionine ration less readily. Two rats on a 92% Chlorella ration lost weight for the first three days but then consumed their ration in a satisfactory manner and gained weight.

2. Three of 20 rats consuming rations containing Chlorella developed a symmetrical alopecia (loss of hair), which started with the hindquarters and spread over the entire abdomen.

3. The fur of all rats fed Chlorella in this study developed a very pale green cast. This was due to external staining of the skin and hair by the Chlorella in the rations.

4. No gross adverse effects were observed on animals fed Chlorella; however, histological examination did reveal some anomalies, particularly in the salivary gland and pancreas of some of the animals.

5. In preliminary studies, two rats were fed a 21% Chlorella diet for 110 days with Chlorella as the sole source of protein. The rats appeared normal and healthy and exhibited satisfactory growth rates. Two other rats were fed 92% Chlorella (minerals, vitamins, and agar to make 100% of ration) for 37 days. These rats also displayed normal growth and appearance. In other studies, rats grew satisfactorily when fed Chlorella and Chlorella-methionine rations as the sole source of protein at 10% protein level for almost 7 months.

CONCLUSIONS

Rats can grow on rations utilizing 92% Chlorella as the sole source of protein, fat, and carbohydrate and grow satisfactorily on rations utilizing 21% Chlorella as the sole source of protein.

No gross adverse effects were observed on rats fed Chlorella at about 21% levels. The 92% Chlorella ration appeared to have some possible growth retarding effects (on one out of two rats) and some possible impairment of hepatic function. The liver abnormality (a yellow or fatty liver) could be secondary effect of the growth retardation.

Histological examination showed provocative changes, especially in the salivary gland and pancreas.

Chlorella rations were acceptable to rats. Three rats of the 20 fed Chlorella developed loss of hair. The causative factor of this condition is unknown. The staining of fur was simply superficial.

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