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The Nutritional Requirements of Two Species of *Euglena.*

By

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Although *Euglena gracilis* KLEBS is photosynthetic, it grows so well in certain complex media such as commercial "peptone" to which sodium acetate has been added, that it can be maintained indefinitely in the dark. As *Euglena* is one of the most plant-like in a series of flagellates that ranges from organisms with typically algal-flagellate morphology to forms with an animal mode of nutrition, i. e. organisms which ingest solid food, ignorance of its nutritional requirements represents a serious obstacle to the advancement of our knowledge of the nutritional factors concerned in the loss of photosynthetic ability and evolution towards saprophytism and animal mode of nutrition. The object of this study was narrowing down the unknown food requirements of *E. gracilis* and also of *E. anabaena* var. *minor* MAINX which has not been grown in the dark and grows far less vigorously than *E. gracilis* in any of the media tried thus far.

Materials and methods.

The euglenas were secured through the kindness of Dr. FELIX MAINX of the Institute of Plant Physiology of the German University in Prag. The other workers mentioned in this paper used the same strains. Cultures were maintained in 125 cc. Pyrex ERLENMEYER flasks at room temperature (24—30° C.) under continuous illumination from overhead incandescent electric lamps. Where dark-

colored media were used the light intensity was increased so that light was never the limiting factor for growth. Each flask contained 35 cc. of culture medium: 25 cc. of "basic" nutrient solution (henceforth called "solution B") made up to 35 cc. with the material to be tested dissolved in water. Media were sterilized by autoclaving for 15 minutes at 120° C. Crop yields were determined when growth attained nearly the maximum, which was from two weeks to a month after seeding.

The composition of solution B is as follows:

NH_4NO_3	0.5 gm.	Na acetate·$3H_2O$	5.0 gm.
$MgSO_4 \cdot 7H_2O$	0.5 „	K_2HPO_4	0.35 „
$FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$	0.014 „	KH_2PO_4	0.15 „
Na citrate·$11/2H_2O$	0.5 „	H_2O	to make one liter.

This solution has a p_H of 7.0 (all hydrogen-ion determinations were carried out with the quinhydrone electrode). Variations in the p_H of the medium resulting from the addition of other materials were kept within the limits 6.7—7.2. The sodium citrate, thrice recrystallized, was added to keep iron and manganese available. The acetate served as a source of energy and was prepared by neutralizing with $NaHCO_3$ acetic acid redistilled in an all-Pyrex apparatus. Water for the early experiments was obtained from a still in which the water vapor came in contact only with Pyrex and cotton. Later it was found that the distillate from a block-tin apparatus served equally well. Inorganic chemicals were SCHERING-KAHLBAUM'S analytical grade with guarentee of purity; organic chemicals were nearly all obtained from the Eastman Kodak Company and used without further purification. The concentration in the medium of added materials was calculated on a basis of 25 cc. of medium, for evaporation in the experiments of longer duration frequently reduced the original 35 cc. of solution to 25 cc.

The experimental *Euglena* cultures were inoculated with one drop from a culture in stock-medium consisting of solution B + 0.5 % DIFCO "tryptone" (an enzymatic protein digest). All media were tested for gross toxicity by parallel inoculation with *Chlorella* sp. This alga grew freely in every solution described here. As a check on bacterial contamination and viability the euglenas used in every test were concurrently introduced into new flasks of the stock medium. When a quantitative measure of growth was desired cultures were filtered on asbestos mats in Gooch crucibles and dried to constant weight at 98° C. The difficulty in filtration caused by the slime of the euglenas made it advisable to use more than one crucible

for the same culture when much growth was to be measured. All trials were run in duplicate, and when subculture from the experimental medium was contemplated, in triplicate.

Previous work on synthetic media for *Euglena*.

Several workers have attempted to develop synthetic media permitting growth equal to that in commercial "peptone". MAINX (1928) found the best diet for *E. gracilis* to be peptone digested enzymatically until biuret-negative; gelatin, intact or digested, also allowed of fair growth. He did not succeed in growing the organism on a mixture of some of the principal amino-acid components of gelatin. LWOFF (1932) and his co-worker DUSI (1933) tested without success many combinations of amino-acids and attributed the vigorous growth of *E. gracilis* in enzymatic digests of muscle and other protein-rich substances to the polypeptide content.

Growth in mineral solutions.

It would be highly desirable from the outset to have a simple medium of known chemical composition in which the organisms would grow through any number of subcultures. Then by the addition of suitable known chemicals growth could gradually be brought up to the peptone level, each such stepwise improvement representing a recognition of another factor responsible for the good qualities of peptone. DUSI (1933) reported having carried *E. gracilis* through four transfers of an all-mineral solution, and *E. anabaena* var. *minor*, though with difficulty, through eight. It therefore came as a surprise when both euglenas died out at the second transfer in solution B. Neither omission of citrate or acetate, or both, nor inoculation into a duplicate of DUSI's all-salt medium, nor complete substitution of KAHLBAUM by J. T. BAKER chemicals, nor wide variations in the proportions and absolute amounts of the mineral constituents nor employment of "tryptone" ash allowed of any growth beyond the second transfer. *Chlorella* grew in all these solutions [1]).

[1]) Dr. LWOFF has kindly informed me by letter that DUSI will publish data modifying his earlier conclusions as to continued growth in inorganic solutions. There remains to be explained a lately noted isolated instance of growth of *E. gracilis* in solution B minus acetate. DUSI used a smaller volume of solution (10 cc.) in contrast to the 35 cc. used here: consequently carry-over from the peptone stock culture may have been relatively greater. Also his cultures were not subjected to continuous light. It is conceivable that under those conditions the death of a number of cells may have occurred and subsequent autolysis enabled the release from the dead euglenas of substances essential for their continued growth.

Effect of proteins and protein hydrolysates.

The basis for a renewed attack on the problem was furnished by the already-cited observation of MAINX's to the effect that *E. gracilis* grew not only in enzymatically hydrolyzed gelatin but in untreated gelatin too. This was easy to verify: gelatin (DIFCO or BAKER) added in 1 % concentration to solution B permitted uniform good growth through three subcultures, though far inferior to that in peptone solutions. Similar results were attained with a sample of grain-curd casein [1]) and PFANSTIEHL's "nutrose" (sodium caseinate). The crop from the third consecutive transfer in each of the protein media are shown in Table 1. *E. anabaena* var. *minor* did not multiply at all, as checked by direct microscopic observation, in any of the solutions other than the peptone, in which a barely transferable growth took place. The figures for *E. gracilis* in tryptone illustrates the superiority of the peptone type of medium over other kinds. The yield in solution B alone indicates the extent of growth rendered possible, as will be shown later, by carry-over of material from a previous favorable medium. Growth in solution B initiated by inoculation from a very good medium, e. g. stock solution, was generally of the order of magnitude of one milligram.

Table 1.
Growth of *E. gracilis* in solution B + 1 % protein.

Substance	Cell Yield, Mgs.	
Grain-curd casein . . .	4.3	3.9
Nutrose	4.4	4.3
Gelatin (DIFCO)	2.9	4.3
„ (BAKER)	4.5	4.0
Tryptone	18.2	18.6
Solution B (inoc. from gelatin medium)	0.2	0.5

The failure of *E. anabaena* var. *minor* to grow in the protein solutions might be ascribed to its lack of a proteolytic enzyme, as witnessed by its inability to liquefy gelatin. Thus the amino-acids which it might require would be unavailable when combined into proteins. Indeed the possession of a proteinase by *E. gracilis* (MAINX, 1928, JAHN, 1931) encouraged the belief that the growth-promoting power of peptone might reside in its amino-acid content. The next step was to test the growth of the two euglenas in proteins hydro-

[1]) Received as a gift from Dr. J. A. DE TOMASI.

lyzed by acid or alkali to eliminate the uncertainties introduced by the incompleteness of the usual enzymatic hydrolysis and by the presence of the enzyme preparation itself.

Fifty grams of BAKER's gelatin or grain-curd casein were autoclaved at 120° C. in one liter of 5 % H_2SO_4 until filtrates rendered colorless with beechwood charcoal were biuret-negative (approximately 72 hours for gelatin, 96 for casein), then autoclaved an additional 12 hours. The acid was removed quantitatively with $Ba(OH)_2$ in the usual manner, special pains being taken to boil up the $BaSO_4$ precipitate to dissolve any aspartic or glutamic acid that might have been precipitated as the barium salt. The filtrate was evaporated nearly to dryness under diminished pressure and the residue taken up in water to which a few drops of chloroform had been added as a preservative. This was then added to solution B to furnish a 1 % solution of the hydrolysate calculated on a basis of dry weight of hydrolysate.

The *E. gracilis* was introduced from unhydrolyzed gelatin medium and *E. anabaena* var. *minor* from peptone. Neither *Euglena* made any growth in these hydrolysates even when tryptophane, which was destroyed largely if not entirely in the course of hydrolysis, was added. Hydrolysis with alkali was tried next.

Fifty grams of BAKER's gelatin or grain-curd casein were autoclaved in one liter of 5 % $Ba(OH)_2$. As before, heating was continued for 12 hours after the solution had become biuret-negative. The alkali was removed quantitatively with H_2SO_4, and the precipitate worked up as before. The rest of the procedure was the same as that described for the acid hydrolysis.

Again there was no growth even when cystine or cysteine hydrochloride was added. The various hydrolysates were not toxic for *Euglena* if the lack of inhibition of growth when the hydrolysates were added to stock cultures in concentrations as high as 5 % be taken for a criterion.

In one striking experiment the $BaSO_4$ precipitate from one of the hydrolysates was added directly to a stock culture of *E. gracilis* to form a fine suspension. The euglenas swam freely among the particles and multiplied at their usual rate.

For a conclusive check on the failure of hydrolysates to support growth, a quantity of DIFCO "tryptone" was prepared with H_2SO_4 in the manner described for gelatin and casein, and again neither *Euglena* grew. Mixtures of acid and alkali hydrolysates were also unsuccessful.

Up to this point the possibility that the euglenas might need something other than, or in addition, to amino-acids hat not been seriously considered and several experiments were performed with the protein hydrolysates by adding various amino-acids, singly or in combination, to them. Among these experiments the following

was the most elaborate: To 50 cc. of 0.5 % acid-hydrolyzed gelatine or acid-hydrolyzed casein in solution B the following amino-acids were added (quantities are in milligrams):

Tryptophane	10	dl-Phenylalanine	10
dl-Methionine	10	Histidine hydrochloride	5
Glutamic	20	Arginine carbonate	5
Tyrosine	5	dl-Valine	10
Glycocoll	35	Cysteine hydrochloride	20
dl-Alanine	35	Asparagine	10
dl-Isoleucine	10		

There was never any growth beyond that accounted for by carry-over from the stock medium.

Two alternative theories could be used to explain the failure of hydrolyzed proteins and amino-acids to support growth while untreated proteins did, namely, that the growth factor (or factors) was destroyed by hydrolysis and was present in proteins either as

a) A hitherto unrecognized integral constituent, or

b) An impurity.

To decide between the two it was necessary to prepare highly purified proteins. Casein may be easily purified by means of its complete precipitation at a sharply defined isoelectric point. The precipitate may be dissolved with alkali and re-precipitated with acid as many times as needful.

Both samples of casein were precipitated with dilute HCl and redissolved with just enough weak NaOH for the resulting solution to barely redden phenolphthalein. This process was repeated four times. Each successive precipitate was washed in several changes of distilled water in the centrifuge and the fourth precipitate was defatted with alcohol and ether.

Gelatin (Baker) was precipitated from aqueous solution with 95 % alcohol, the precipitate washed in the centrifuge as before, redissolved in water and reprecipitated with alcohol. This was repeated four times, and the last precipitate also had its fat removed with alcohol and ether.

A sample from each successive casein precipitate was adjusted to p_H 7.0 with $NaHCO_3$ and added in 1 % concentration to solution B. Each gelatin precipitate was tested separately also. The yields are shown in Table 2.

The second, third and fourth casein precipitate cultures were left unweighed as the amount of growth was very slight and no more than could be accounted for by carry-over. There was no growth on consecutive subculture from the third and fourth casein precipitate media into more of the same. The extinction of growth in the purer caseins was striking. When washing of the casein curds was not as long-continued the effect was much less evident.

Table 2.
Effect of purified proteins on growth of *E. gracilis*.

	Cell yield in milligrams				
	Untreated	Successive precipitates			
		I	II	III	IV
Grain-curd casein	3.3	1.4	trace	trace	trace
	3.5	2.0	„	„	„
Gelatin	4.5	2.2	2.0	1.6	1.6
	5.1	2.6	2.4	1.7	lost

A likely explanation for the growth in the gelatin precipitates was that the skein-like texture of the precipitate let much of the mother liquor be mechanically entrapped and thereby rendered the washing incomplete. There was no growth in edestin and concanavallin A (a protein from the jack-bean) obtained from Prof. J. B. SUMNER.

Effect of various carbon sources on *E. anabaena* var. *minor*.

Before undertaking a search for the growth factors for *E. gracilis*, an attempt was made to determine whether the growth of *E. anabaena* var. *minor* in the peptone medium employed for its maintainance was limited by lack of a suitable energy or carbon source. HALL (1934) had reported a favorable influence exercised by some carbohydrates on the growth of this euglena in peptone medium (mainly DIFCO "tryptone"). Tests were carried out on each of the following substances, added in 0.3 % concentration to solution B with the acetate omitted, + 1.0 % tryptone. The acids were supplied in the form of their sodium salts.

glucose	d-arabinose	glycocoll
mannose	xylose	dl-alanine
galactose	mannitol	cysteine hydrochloride
fructose	glycerol	thioglycollate
soluble starch	acetate	tryptone
maltose	malate	
lactose	lactate	

The only appreciable stimulation of growth was in the flasks containing extra tryptone. *E. gracilis* on the other hand, as was to be expected from the data of previous workers, showed a great increase in growth in the acetate medium and no significant increase in any of the others except the tryptone. One may conclude from this that in the ordinary acetate-less peptone medium lack of an energy source limits the growth of *E. gracilis* and lack of a probably none-energy-furnishing material checks the multiplication of *E. anabaena* var. *minor*.

Growth of the two euglenas in amines.

Amines are common products of anaerobic protein breakdown by bacteria, and euglenas are most often found in waters with a high bacterial activity. The possibility that simple aliphatic amines might contribute to the peptone effect was investigated in the same manner as for the carbon sources, acetate being omitted from the medium as before in one set of experiments and added in another. Neither of the two euglenas showed any increase in growth when the following amines were added (0.3 %) as their hydrochlorides to solution B + 1 % tryptone.

monomethylamine	ethylamine
dimethylamine	diethylamine
trimethylamine	triethylamine

Sources of the growth factor.

As commercial peptone preparations are very costly for the large-scale work necessary for isolating the growth factor (or factors), search was initiated for a more convenient source of euglena-vitamins. *E. gracilis*, though not *E. anabaena* var. *minor*, grew well in DIFCO, LOEFFLER'S Blood Serum. If the factors in serum were soluble and of low molecular weight they should traverse the kidney and appear in the urine, which was therefore tested. A toluol-preserved pooled sample of male urine was secured for this purpose. An independent plant source was also desired and yeast appeared to be a good place to look for it.

Ten grams of FLEISCHMANN'S autolyzed yeast was autoclaved in 50 cc. of distilled water at 120° for one half hour. Most of the cell debris was removed by centrifugalization. The turbid liquid remaining was clarified by repeated filtration by suction through a thick fine-fibered asbestos mat in a Gooch crucible. To ensure the presence of adequate amount of amino-acids, should they be required at all, 1 % acid-hydrolyzed gelatin was incorporated into solution B. Materials to be tested as sources of the growth factor where added in 0.05 % concentration (by dry weight) except for the urine. The yields for *E. gracilis* are given in Table 4.

The luxuriance of growth in the yeast-containing medium was comparable only to that seen in solutions with a high concentration (e. g. 2.5 %) of peptone along with the optimal acetate concentration for that level. The increased growth in the presence of urine, while scarcely of the order of that occasioned by yeast, was most en-

Table 4.
Efficacy of various materials as growth-factor-source for *E. gracilis*.
Solution B + 1 % acid-hydrolyzed gelatin + 0.05 % yeast-extract.

Substance	Cell yield, mgs.	
Difco tryptone	7.1	8.0
„ beef extract	6.5	7.5
yeast-extract	39.5	37.5
urine (0.5 %)	4.4	4.4
unhydrolyzed gelatin	1.3	2.0
nothing added	1.3	1.1

couraging, for the bulk of the solids in urine consisted of inactive inorganic compounds easy to remove by concentration and alkalinization, etc., of the urine. Cells in the yeast cultures remained motile long after those in the others had become palmelloid. *E. anabaena* var. *minor* showed no significant improvement of growth in the yeast-extract media even when tryptone was added in concentrations below 0.1 %. Work on this euglena was discontinued at this point.

The problem of developing an appropriate growth-factor-free medium for the assay of *E. gracilis*-vitamin preparations could now be attacked by making use of a 0.05 % solution of yeast-extract in solution B while investigating the need, if any, for the acid-hydrolyzed gelatin employed in the experiment just described. Preliminary tests indicated that 0.05 % was a suitable concentration of yeast-extract.

Role of acid-hydrolyzed gelatin.

When the gelatin hydrolysate was omitted growth fell to a very low level. Growth in the other hydrolysates was about the same; nevertheless the gelatin hydrolysate had a number of advantages over the others:

1. Alkali hydrolysis introduced the complication of racemization.
2. Gelatin contains fewer amino-acids than casein, thereby simplifying identification of the effective constituents.
3. The acid hydrolysate was less colored than the corresponding casein preparation.

The acid-hydrolyzed gelatin presumably lacked, among others, the amino-acids tryptophane, cysteine (or cystine) and tyrosine. The addition of 0.05 % of each of these, singly or together, to the yeast-extract + hydrolysate medium did not improve the already very good growth.

As a beginning for the identification of the effective constituents of the hydrolysate, growth was tested in the three fractions

of the hydrolysate prepared by Dakins (1920) butyl alcohol solubility method. Several amino-acids were tried simultaneously. The basal medium was solution B + 0.05 % yeast-extract. Yields are shown in Table 5.

Table 5.
Effects of components of acid-gelatin hydrolysate on *E. gracilis* in sol. B + 0.05 % yeast-extract.

Substance	Cell yield, mgs.	
Control (neither hydrolysate nor amino-acids added)	1.6	0.6
Acid-hydrolyzed gelatin 1 %	21.7	22.3
Butyl alcohol fractions		
a) H_2O-soluble fraction 1 % .	32.7	29.0
b) „monoamino" „ 1 % .	31.9	lost
c) „proline" „ 1 % .	8.0	7.2
Glycocoll 0.5 %	3.3	3.0
„ 0.5 % + 0.05 % each of arginine carbonate, glutamic acid, histidine hydrochloride and asparagine . . .	14.1	18.4

The not very uneven distribution of effectiveness among the three fractions suggested that the amino-acid requirement might be fulfilled by more than one amino-acid. Glycocoll alone had no apparent effect. Growth in the mixture of single amino acids was so good that the identification of the utilisable amino-acids of gelatin may prove an easy task. But decision upon the absolute and relative worth of any amino-acid must be deferred until the growth factor in yeast and urine is identified, or concentrated to a degree such that there can be no question that the amount of amino-acids likely to be found in the vitamin preparation is far inadequate for any direct participation in syntheses of substances found in the cell in appreciable quantity. For this reason work on amino-acid nutrition was temporarily discontinued and attention directed once more to the concentration of the growth factor in yeast and urine. The standard vitamin-free medium for the assay of growth factor concentrates now consisted of solution B + 1 % acid-hydrolyzed gelatin.

Properties of the growth factor.

Through the courtesy of the Vitamin Food Company of New-York a large quantity of dried brewers' yeast was secured for extraction of the growth factor. It was found that the *E. gracilis*-vitamin was present in negligible available amount in unautolyzed

yeast and abundantly in autolyzed yeast; that fat solvents with the exception of aqueous methyl and ethyl alcohol did not extract it; and that it would not pass into butyl alcohol from aqueous solutions at different hydrogen-ion concentrations. The factor in urine behaved similarly. The details of the concentration of the vitamin are reserved for a later paper but it nevertheless appears safe to say that it is clearly of the water-soluble type.

Growth of *Chlorella* sp. in peptone and protein hydrolysates.

An illustration of the fact that a marked stimulation of growth in "peptone" does not necessarily imply that the peptone is supplying needed amino-acids or vitamins is afforded by the behaviour of the *Chlorella* used for a control in this work. SKINNER and GARDNER (1930) noted that their *Chlorella* isolated from soil, as was also the writer's, made better growth when peptone was added to an inorganic nutrient solution than when glucose was added. As their medium contained much calcium and no soluble-metal-complex-former, e. g. citrate ion, the favorable action of peptone may have resulted from the readier availability of metals such as iron and manganese in peptone. An experiment was performed to test this theory: to the mineral constituents of solution B (magnesium sulphate, ammonium nitrate, mono- and di-basic potassium phosphate, ferrous ammonium sulphate) glucose, citrate and peptone (in this instance DIFCO "tryptone" was used as the "peptone") were added separately and in combination and inoculated with *Chlorella* and *E. gracilis*. Table 7 shows the yields.

Table 7.

Effect of citrate, glucose and tryptone on growth of *Chlorella* and *E. gracilis*.

Medium	Cell Yield, Mgs.			
	Chlorella		*E. gracilis*	
Glucose 0.5 % + citrate 0.05 % . .	99.9	104.6		
„ 0.5 % — „ 0.05 % . .	15.6	37.0		
Tryptone 0.5 % + citrate 0,05 % .	8.9	6.6	10.2	10.1
„ 0.5 % — „ 0.05 % .	5.7	7.0	9.2	11.3

These figures show plainly that while growth in mineral solutions with glucose was more than doubled by the addition of citrate, which is not at all utilized as such by *Chlorella*, this was not so for the tryptone solutions. Evidently the heavy metals in tryptone are so available that the addition of citrate is unnecessary. The

growth of *Chlorella* used as a control during the gelatin-hydrolysate fractions experiments was best in the "monoamino" fraction, most probably because of its alanine content (Genevois, 1927), and was always superior to the corresponding growth in peptone.

Discussion.

Perhaps the salient feature emerging from this study is that the possession of chlorophyll is no guarantee that an organism's nutritional requirements will closely resemble those of its presumably autotrophic ancestors: the organism's needs may transcend the readily surmised dependence upon an auxiliary energy source and pre-existing building blocks, e. g. amino-acids, for synthesis of conspicuous cellular materials, e. g. proteins, as photosynthesis becomes progressively less important for the life of the organism. Indeed to the best of the writer's knowledge, *E. gracilis* is the first photosynthetic plant proven to have a vitamin-like food requirement, i. e. requiring some organic material in trace amount. A strong likelihood exists that the same condition holds for *E. anabaena* var. *minor*. Some of the implications of these findings may be briefly mentioned:

1. It is likely that great difficulty will be encountered in discovering obligately autotrophic *Euglenophyceae* to be used as the basis of a comprehensive theory to account for the varying food requirements of the members of this group. The problem will be no doubt further complicated by uncertainty whether a pattern of unitary increments in the number of dietary essentials corresponds to a true linear evolutionary series with stabilizations at different levels of nutritional complexity, or merely represents convergences and artificial juxtapositions. A thorough study of the morphologically indistinguishable but physiologically different strains of *E. gracilis* recently reported by Miss Sauer (1935) would prove of great value in defining the position of *E. gracilis*.

2. One may expect that the requirements of the animal euglenoid flagellates will be very complex.

3. How does *E. gracilis*, with its apparently complex needs, and *E. anabaena* var. *minor*, with its exacting requirements, manage to survive in nature?

One other feature of these anomalous organisms may be pointed out. Old analytical data (Gottlieb, 1850; Habermann, 1874) point strongly to the paramylon or starch-like granules of *Euglena* being composed of a hexose sugar. Kutscher (1898), without furnishing

details, stated that the hydrolysis product was fermented by yeast. Yet *E. gracilis*, which may accumulate large amounts of this substance in its body, responds only to the acetate, butyrate and caproate radicals for energy sources in addition to light, and *E. anabaena* var. *minor* poorly, if at all, to carbohydrates alone [LWOFF (1932), DUSI (1933), HALL (1934), LWOFF and DUSI (1934) and JAHN (1935)]. Contrary to the findings of these authors, soluble starch (BAKER'S) was inert for *E. gracilis*. Should the sugar of paramylon turn out to be one of the carbohydrates employed in the experiments mentioned, the problem of the failure of *Euglena* to utilize added carbohydrate would take on a deeper significance. It is also evident that the matter of utilization of soluble starch calls for additional work.

It is clear, at any rate, that a comparison of the vitamin and other food necessities among the algae and protozoa may provide a useful tool for the solution of phylogenetic problems aside from its interest from the standpoint of cell physiology.

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Summary.

1. *E. gracilis*, although photosynthetic, requires in addition to one or more amino acids, a water-soluble, thermostable material widely distributed in nature and present in relatively high concentration in autolyzed yeast and urine.

2. *E. anabaena* var. *minor* could not be grown in any of the simplified media effective for *E. gracilis* and barely survived in complex media, such as peptone.

3. No evidence for the assimilation of a number of simple aliphatic amines was found.

4. Nothing was found that would take the place of the lower even-carbon saturated fatty acids as an energy source for *E. gracilis* and no compound definitely assimilated by *E. anabaena* var. *minor* could be detected.

5. The growth of *Chlorella* is increased by peptone because of the available heavy metals and amino acids in peptone and not by a vitamin-like substance in peptone.

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