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ON THE PROTEIN QUALITY AND THE LIVER NECROSIS PREVENTIVE FACTOR OF UNICELLULAR ALGAE.

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SECTION B Business Administration Building ROOM 111

DAY Monday HOUR 7:00 p.m.

For the past 15 years we have experimented on the nutritional value, especially of the biological quality, of microorganisms, yeasts, fungi and bacteria, as well as of symbioses of these. Preponderatingly such microorganisms served as experimental objects which are able to grow in masses on cheap raw materials, such as wood-sugar, waste pulp-liquors, straw hydrolysates, and which may become important for animal and human nutrition. The experiments were carried through with growing white albino rats nearly without any exception.

Now it was fascinating to extend our experiments on autotrophic microorganisms, namely, unicellular algae, instead of examining the above-mentioned heterotrophic microorganisms. In Germany the Carbon Biological Research Station, Essen, (director: Dr. Gummert) has rendered this possible, being our source of the pure algal material in the necessary quantity of many kilograms. According to our plans, a young dipl. agr. of Bonn University, Miss Elisabeth Herold, as a candidate for a doctor's degree, carried the animal experiments through in the research station for physiology of nutrition of our institute.

At the beginning of this work in 1952 there did not exist much exact knowledge about unicellular green algae as feed and food-stuffs. In the Carnegie Monograph which was published soon after (in 1953), only few experiments are reported with a number of experimental animals, a number still much too small, as the author himself wrote. Furthermore, these experiments had been carried through during a time much too short, so that it was impossible to give a final decision. Nevertheless out of these results it may be concluded, that the unicellular algae of the Chlorella group, i.e. their protein, represent a good feed-protein of about the same quality as other plant protein which undoubtedly proves to be inferior to the usual food and feed protein of animal origin.

Our examined algal material was Scenedesmus obliquus. We obtained it dried as a powder from Essen. We are informed that the drying process of the algal paste, spread in a thin layer, had been done by infrared-radiation immediately after centrifuging of the algae.

The examination of dried algal material instead of fresh, on the basis of protein value, rather means a judgment much too severe, because in general drying processes cause a change for the worse. On the other hand, it is sometimes the case that plants lose their "poisonous" nature by drying or cooking—for instance, edible turbantops or the green pods of scarlet-runners. With the green unicellular algae this question seems to be especially remarkable, because in literature hints are not lacking at the possibility of finding poisonous green unicellular algae.

If compared with our former researches, methods of our animal experiments were somewhat improved and also changed on principle. Some years ago we began to take only young albino rats of our own inbreeding species without any exception, and so a very uniform animal material is always at our disposal. Runt rats were eliminated. Liver, kidneys and lungs of one of each litter, were always sent to the Federal Research Center for Meat Management, Kulmbach, for histologic-pathologic examination in order to find anomalies especially of the inner organs. The other litter-mates, males and females as equally represented as possible, were distributed among the single experiments with the various diets. This is important, because from our several-years-old experiments a somewhat different behavior of sexes resulted on the one hand regarding gain in weight, on the other hand with respect to susceptibility to liver diseases, as well as for frequency of mortality.

The animals are put on the various diets when they have reached weights of 50 - 60 g. In general they do not drink much mother's milk any more in this stage and have just begun to eat the many-sided food-stuffs together with the old and to drink also the daily-given cow's whole milk. On purpose we sometimes took heavier mates (already weighing nearly 100 g.) for the experiments. We were able to state - moreover in agreement with other authors - that hereby nothing is changed systematically with the elaborated problems regarding the course of symptoms, neither the growing curves nor the incidence of deaths with and without specific organ-changes. Only the dimensions and the moments are somewhat dislodged in such a manner that the animals when double the weight and also older have become more resistant in general, probably because they bring greater reserves with them at the beginning of the experiments.

The diets fundamentally lean upon McCollum's well-tried rat diet which is characterized by a sufficient foodstuff ratio of 1:10 (1 part digestible protein to 10 each parts starch value (calories)) and probably contains all basic and complement nutritive substances, which rats need. They are provided with fat-soluble vitamins including the unsaturated fatty acids by medical cod liver oil DAB VI. The water-soluble vitamins are supplied by certain quantities of best brewer's yeast. The protein content of the latter is taken into consideration, of course, when the compound of diets is calculated. From experiment to experiment the used protein source varies. Complete milk protein in form of always fresh skim milk powder or, lately, of white-of-an-egg protein, both representing animal protein of highest biological quality, are assumed as the norm and put = 100. For reasons which will be mentioned later on, diets are calculated and mixed partly for equivalence of crude protein, partly for equivalence of digestible protein. Therefore digestibility or reabsorption is determined in advance in vitro as well as in vivo experiments with adult rats of our inbreeding species.

In order to determine biological quality, mates of one or several litters are put on the diets which are to be tested, and on our comparison-diets (milk or egg) according to our "Meander principle" and in consideration of a distribution of female

and male as even as possible. Food always exceeds the quantity required; consumption of food - being a sometimes important qualitative sign - is determined daily by reweighing the rest. The rats receive water (Cologne tap water) with 35 degrees of hardness (therefrom 12 degrees of permanent hardness) in special bottles; it is renewed every day. In general 6 - 20 animals, in decisive cases, many more, are put on our diet. (In zinc cages, each containing 6 boxes).

For determination of weight quick scales are used. Weighing is done every fifth day at the same hour, before feeding. Later on, the gains in weight-ranges are found out separately for all male and female rats of one and the same experiment. Out of both averages, the average is taken once more. Growth curves obtained in the above-mentioned manner - average gain in weight as function of time in experimental days - are looked upon as criterion of the biological quality of a protein. The steeper the growth curve the better the biological quality, wherein generally milk protein or protein of the white-of-an-egg may be regarded as 100.

Because of our researches on protein quality carried on for many years we regard the growing curves as <u>one</u> criterion. Not less important is the <u>mortality quotient</u>, i. e. the ratio of animals dying during an experimental time of 120 days to the total animal number of an experimental group; and the <u>necrosis quotient</u>, i. e. the ratio of animals, undoubtedly dying from dietetic liver necrosis during an experimental time of 120 days, to the total animal number. The simplest way of talking about both factors is to do so when the experiments with algae, milk protein and protein of the white of an egg are described and discussed. Also the daily average <u>food-intake</u> during the course of experiments expressing the appetite for their food is a significant sign, as we shall see later.

The experiments - described as follows - with the complete cell substance of the unicellular alga, <u>Scenedesmus obliquus</u>, were carried through during nearly 3 years with altogether 12 samples of infrared dried material given to young white rats. They are divided into 2 main groups, in which always analogously composed and thus comparable diets containing animal protein of highest value (complete cow milk protein or protein of the white-of-an-egg) were fed to mates of the same litter.

I. The algal diets still contained small supplements of bread cereal protein (bruised rye and wheat) besides a small quantity of brewer's yeast, protein which we believed necessary in order to provide the animals with the known and still unknown factors of the Vitamin B-group.

   a) The basis of calculation for these diets was equivalence of crude protein.

   b) For these diets, equivalence of digestible protein served as calculation basis.

II. The algal diets did not contain any other protein ("monochromatic" algal protein diets) except the obligatory supplement of brewer's yeast rich in vitamins.

   a) Normal experiments with young animals weighing 50 g.

   b) Experiments with rats weighing up to 100g.

   c) Normal experiments (50 g. animals) in order to compare algal protein with the protein of the "green-leaf" of higher plants (alfalfa, spinach).

Experimental Group I a

A small amount of rye and wheat protein, (equal supplements bruised grain of these cereals) besides the brewer's yeast protein, was added to the algal diets of this group. The protein of the rat food (ratio of nutritive substances 1 : 10) consisted of

77 % algal protein,

15 % rye and wheat protein (1:1),

8 % brewer's yeast protein (Cenovis first class yeast)

The basis for the calculation of all protein supplements was crude protein (N x 6.25). With the supplement of rye and wheat in the above-mentioned quantity we adhered to the purpose to be able to judge of the biological quality of algae and to observe their general behaviour as a food stuff, when at first using a diet not so very one-sided which nevertheless possessed algal protein as the main constituent.

By this diet-composition at the same time it was possible to join and compare with our former researches on biological quality of the protein of different heterotrophic microorganisms (brewer's yeasts, baker's yeasts, xylose yeasts, waste pulp-liquor yeasts, straw hydrolysate yeasts (the latter are Torula yeasts grown on wood sugar, waste sulphite lye, straw hydrolysates), Aspergillus orycae, Oospora lactis (Biosyn), Penicillium notatum, kephir etc.). Because the diet was fundamentally the same with all these investigation-objects and also contained 15% cereal protein, a comparison between autotrophic and heterotrophic unicellular organisms became possible. It ought to be remembered that the supplement of just 15% bread grain-protein originally was meant to represent the circumstance that up to the beginning of the second world war about 15% of the protein provisioning of German people was secured by eating bread.

In the following table (table 1) the analysis of feed and composition of diets is shown. It may be seen, that also in the control diet (assumed = 100) with skim milk powder 15% of crude protein consists of bread-grain protein (77 + 15 + 8).

Young albino rats, mates out of 5 litters, male and femal animals as equally as possible - 20 each - were divided among the two diets according to the Meander principle. The experimental time amounted to 120 days, in former times only 90 days. In this way the observation time is rather long; it encompasses the biggest part of the growth time of our rat species. According to this fact rather sure statements become possible. The nearly grown-up animals then reached weights of about 270 g.

During the first ten days it was striking that the daily average food intake undoubtedly was better with the animals which were fed the algal diet. It amounted to 12.2 g. with the algal animals daily, with the milk protein animals to 9.0 g. only. The relation remained similar during the whole experimental time. Undoubtedly it seems remarkable that the algal food was eaten with such pleasure by animals whose "ancestors" probably never had any opportunity to eat green algae. In this connection it must be added, we had the occasion to observe again and again during our several years old inquiries that the food intake was biggest when milk protein diets were fed. We are going to refer to this fact once more. Nevertheless the growing curves which may be regarded as the manifestation of biological protein value show the most important result. They demonstrate that the algal animals reached gains in weight as good as the milk protein animals during the whole time of 120 days. The scattering within the 2 groups with 20 male and female young rats each was unimportant (see Figs. 1a and 1b). All 40 animals survived the duration of 120 experimental days; they all made a healthy and lively impression. The hair covering looked beautifully bright and dense.

Table 1.

Composition of the diets
referred to dry weight

on basis of:	crude protein		digestible protein	
	algal diet	milk diet	algal diet	milk diet
algae	19,71	—	21,10	—
skim milk powder	—	21,05		20,52
rye	8,43	6,11	7,43	8,16
wheat	7,08	5,34	5,45	5,98
brewer's yeast	1,92	1,45	1,40	1,54
cod liver oil	3,08	2,58	1,90	2,56
salt mixture	5,46	3,43	3,64	3,65
starch	54,46	60,06	59,08	57,90

Our inquiries led to the new and unexpected result, that the body substance of the unicellular green alga, Scenedesmus obliquus, as it is cultured in the Carbon Biological Research Station, Essen, and as it was at our disposal, contains a protein, that promotes the growth of young albino rats as well as does the protein of cow's milk in the form of skim milk powder.

Now it is interesting to compare with the protein of other microorganisms. For this purpose we took results of former inquiries during war time as well as newest conclusions which we drew in our research station for physiology of nutrition primarily with heterotrophic microorganisms. In order to compare them and to put them down in one diagram a conversion was undertaken by using the former milk protein growing curve concerned. This seems to be permitted, if the corresponding ratios of fungi protein to milk protein are taken as a basis. Now we were of course, not able to take into account a few changes regarding methods and at any rate some refinements, which we have introduced during the last years. But principally they do not influence the result of this comparison.

We observe that none of the microbe proteins tested until now is equal to the algal protein as regards biological quality in our experiments -- which also contained 15% bread cereal protein and 8% yeast protein in the diet. When calculating numerical relations from the curves, specifically from the gain-in-weight values in the above-mentioned manner, the following table (table 2) of biological protein values results. This shows much more than words can show.

Experimental group I b

Against our inquiries to date the following objection might arise, which concerns the different protein digestibility of algae on the one hand and milk protein on the other, as well as of heterotrophic microorganisms.

Table 2

milk protein	100
kephir	90
brewery food yeast	84
straw hydrolysate yeast	64
waste sulphite lye yeast	34
wood sugar yeast	33
Aspergillus orycae	44
"Biosyn" (oospora lactis)	53
potatoe	54
mycelium from penicillin-production	60
algae	108

As we are able to determine by experiments in vivo and in vitro, this amounts to about 60% with the algal material we had at our disposal (besides it was the same with fungi, for instance Pencillium notatum), while the corresponding percentage rate is known to be about 90% with yeast, whereas according to literature and own inquiries species and origin of the yeast concerned seems to be of no special influence. In case the indigestible part of protein passes through the gastro-intestinal tract as an inert ballast - an opinion which absolutely seems to be allowed according to our hitherto prevailing points of view-with our hitherto used diet compositions, calculated on the basis of crude protein equivalence, there would be

not only less utilizable protein on the whole, and a corresponding quantity of unutilizable protein, but the effective relations of protein-bearing substances would be changed, too. For instance in a milk-protein or yeast diet, the crude protein composition of which amounts to 77 : 15 : 8 also, the relative part of rye and wheat protein and also of yeast-protein and at the same time the influence on the results of feeding experiments would be less important than with algae or culture Psalliota-diets. The degree of influence on the results could not be decided of course. Therefore we undertook the experiments once more with the unicellular alga Scenedesmus obliquus, as we did with the formerly determined object Penicillium notatum, whereby the diets were calculated on the basis of digestible protein equivalence.

But we made up our mind that even by this manner there could not be reached any ideal and absolute comparison of results, for with this calculation method, which seems to be exact, the share of "inert ballast of indigestible protein" became different in the several kinds of diets. This seemed to be the smaller evil, whereby we are sure, that the until-now nearly unknown influences of this kind cannot be unimportant for the whole course of digestion especially by the bacteria of the intestine. Even as things are the completion and extensions of our first series of experiments, in which diets had been calculated on the basis of crude protein, and a second series of experiments, with equivalence of digestible protein as a basis, should give us a clear judgment of the biological quality of green algae and also to compare it with the previously investigated microbe material. The algal diet and the milk protein diet for comparison were now containing 77% each of digestible protein of the chief protein source Scenedesmus obliquus on the one side and of skim milk powder on the other side. We took 8 albino rats each male and female mates out of 3 litters. This time, also, one young rat of each litter had been investigated at Kulmbach. During the experimental time of now 120 days the greater food intake of the algae animals was striking when comparing it to the intake of the milk protein animals, who were also eating much. While with the first group of experiments there was the possibility of assuming that the augmented food intake of the algae-fed animals might be explained by the above-mentioned somewhat smaller content of digestible (utilizable) protein, this explanation is beyond question this time. At any rate the dried algal material seems to stimulate the appetite of young albino rats.

The growth curves as indicators of biological protein quality indicate that the digestible algal protein is at least equal to dried skim milk. Comparing gains in weight with the algae-fed animals there was nearly no difference from the first experimental group. The very uniform course of growing also observed this time is a favorable indication for a foodstuff. But this was not the case with milk protein animals. The animals were increasing less in weight this time, at least temporarily. But during the biggest part of experimental time the differences did not prove to be significant.

Two animals died on the 40th and on the 45th day of experiment. Liver necrosis was stated without doubt as cause of death. The rest survived the experimental duration and made a healthy and lively impression. In the end of the experiment no diseases or other changes could be stated.

Group of Experiments II

Encouraged by these surprising and unexpectedly favourable results concerning the biological quality of algal protein, we chose conditions of examination still much more "severe." We passed over to much more "monochromatic" diets, which intentionally ought to be extremely one sided regarding the protein offered to the rats. We omitted the 15% supplement of rye and wheat protein in the algal diets and the other diets for comparison. Instead of the above mentioned ratio 77 + 15 + 8 = 100 now the proportion 92 + 8 = 100 was taken, 92 parts of which consisted of the digestible protein of algae and milk (or white of an egg) and 8 parts again out of brewer's yeast added in favour of vitamin provisioning. We did not wish to renounce this supplement because of provisioning as natural as possible with known and also up to date unknown factors. From the point of view of protein providing, this percentage of only 8 represents neither an important contradiction nor support in spite of a certain natural protein completion value, which cannot be denied.

New with this group of experiment was also the employment of the white of an egg* protein as a further representative of animal protein of highest quality, i. e. as some sort of second indicator for the biological protein quality. We knew from our inquiries concerning other subjects, that the aptitude of milk protein of skim milk powder would be limited with such "monochromatic" diets expecially if all experimental animals should die of liver necrosis early.

Young albino rats, 16 each of about 50 g. weight, again litter mates of both sexes out of 5 litters, were grown up on these diets, which contained everything needed for the good growth of these experimental animals. The question was only whether the absolutely one-sided protein offering by algae alone was sufficient respective to warranting a good provisioning by essential amino acids. The experiment lasted again 120 days. One animal pro litter was again sent to our coworkers at Kulmbach (Federal Research Station for Meat Management) in order to be examined regarding normal state of health. Now we come to the results concerning quality as well as quantity.

Food intake was similar to that of our experiments carried through until now. Algal food at least is eaten with the same pleasure as the diets containing skim milk powder and white of an egg. Concerning the course of growing curves there is no difference worth mentioning during the first 40 days, when the three analogously compounded diets are compared. It is remarkable that, on the one hand, protein of such a different origin enables nearly equal growth and, on the other hand, protein of Scenedesmus obliquus is able to keep pace with the animal protein in spite of the monochromatic form of diet. In the second part of the experiment from about the 55th day, the algae-fed animals continued to grow as quickly as before, while with the milk-and egg-animals growth became much slower. From about the 100th day the advantage of the 16 algal animals becomes significant. The protein of the Scenedesmus algae is not only equal according the course of growing curves, but it seems to provide the growing rats with amino acid building stones in a steady manner—especially for the later periods of growth— better than egg protein and milk protein (the latter as dried skim milk) do when used nearly as the only protein source.

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* Because the crude white of an egg is able to show certain anomalous aspects as foodstuff, we took care to pre-heat it above the coagulation-temperature for an hour (158° F). In literature we did not find any hint at the sufficiency of this treating for destroying the avidin-complex. Certain observations during the experiment led us to doubt it to a certain degree.

Table 3.

Composition of the diets
referred to dry weight

on basis of:	crude protein		digestible protein	
	algal diet	milk diet	algal diet	milk diet
algal	20,15	—	24,42	—
skim milk powder	—	24,21	—	24,67
brewer's yeast	1,88	1,45	1,40	1,56
cod liver oil	3,26	2,74	1,83	2,65
salt mixture	6,21	3,40	3,88	3,20
starch	68,46	68,23	68,47	67,87

When discussing the milk protein curves it must be taken into consideration that most of the animals had perished before the researches were finished, as will be reported afterwards. If the course of algal curves is compared with the one of our former experiments which still contained 15% of rye and wheat-protein in the diet, the characteristics prove to be principally the same. Supplement of rye or wheat protein seems to be without influence or in other words: for digestible protein of the Scenedesmus algae no supplementation by amino acids of cereals is necessary. But with the skim milk- protein the situation apparently is different. Supplementation of cereal protein brings about the "ideal" course of growth curve. This is emphasized in advanced phases of growth after the 60th day (diagram 1 and 2). Without this supplementation a distinct delay takes place.

All animals fed on the algal diets survived the duration of experiment. They made a lively and healthy impression and had shining dense coats. Surprising were the good gains in weight (diagram 3).

During the investigations of the milk diet one animal died after 20 days. The autopsy macroscopically suggested liver necrosis and the histologic examination had the same result. Soon after that many of the milk animals died of more or less massive necrosis during a relatively short time. The biggest percentage lay between the 30th and 70th day. The animals died very suddenly without having shown any sign of animal food intake or decreases of weight. The animals died even after having reached a body weight of 150 - 160 g. Only two animals survived. The course of milk animal curves shows a small incorrectness in the end (from the 80th day), because it was only calculated by taking into account two females. No one of the males had survived the experiment.

At the end of the research we again killed some animals representing the 3 groups of diets. Because some of them showed beginning necrosis with the second group of this series, with 8 animals each, we resolved to feed the animals for more than 120 days on this diet.

The observations of the first experiment principally were confirmed (diagram 3), with unimportant differences. All milk protein-animals perished after 40 days. The 8 algal animals again showed remarkable gains in weight, which proved to be much higher than in former experiments. In this case the unfavourable ratio of sexes (seven females and one male) may share the responsibility for the unusual gains in weight. Because the male animal became extraordinary heavy, but in our experiments faced seven normal females, somewhat higher value had to result for the average. With the white-of-an-egg animals, regarding gains in weight as well as food intake and whole appearance, only trifling changes could be observed.

After 120 days the main growth of rats had ceased. Regarding vitality, hair covering, and general state of health, no essential changes occurred after that time. After 240 days we had to interrupt the experiment, because we had no more algae material. Only one of the rats killed afterwards showed a necrotic liver. All algal animals had even survived this long time of experiment without any harm.

Besides these main experiments it too was desired to determine qualitatively to what degree the initial weight of rats influences the growth and the resistance of animals to liver necrosis.

Ten animals weighing 100-110 g., a body weight with which in former milk diets the first deaths occurred because of liver necrosis, were divided among algal and milk diets with any rye and wheat supplement. For these experiments only green house cultured algae were at our disposal. Because this material was cultured nearly under the same conditions as the open-air-cultured algae, we might assume that there would be no important differences when used as foodstuff.

With respect to growth-promoting qualities there were nearly no differences when compared with the animals of initial weight of 50-65 g. (diagram 4). But the influence of the high initial body-weight on the degree of liver necrosis was different. The animals all survived the first critical period of experiment, i. e. the first 20 - 30 days. But during the second critical period (60th - 70th day of experiment) 2 milk animals died of typical necrosis. All the other rats survived the duration of experiment and did not show any sign of illness like the algal animals. Also, after killing them we could find no necrosis.

Final discussion of the value of the unicellular algae, Scenedesmus obliquus, as nutriment protein and liver protection substance.

If we had known three years ago at the start of our experiments all the facts we know today about the "poisonousness" of phytoplankton, especially of Chlorella and Scenedesmus species, perhaps we would not have started the numerous experiments on animals, which we have reported in these papers. At least we would not have chosen this subject as a basis for experiments carried out for a doctoral degree. Now after having done the work the results which we believe to be very convincing, we find it contradicts 100% two recent communications (Russian and an English) published in 1954. In the following we repeat a summary (translated from German) by H.J. Elster (Naturw. Rundschau 8, 318 (1955)):

"Poisonous effects of phytoplankton."
"For some time it has been well known, that some plankton algae secrete toxins, which prove to be poisonous for other organisms, especially chlorellin, the toxin of the well known Chlorella algae. J.E. Rhyther examined the toxic effect of Chlorella vulgaris and Scenedesmus quadricauda on the zooplankton. The excreta of these algae impair filter activity and food intake of daphnes, less by toxins dissolved in water than by excretion in the intestine. Actively growing algal populations on the ascending branch of their development produce the smallest quantity and aging algae most toxins. If quickly growing algae well able to divide are fed to daphnes, qui quick growth and good augmentation of zooplanktons are the consequences, while aging algae retard growth, impair the population, and cause death of the fed daphnes after 10 - 13 days. The often observed inverse quantitative relation of phyto- and zooplankton must not be necessarily explained by the grazing of algae undertaken by zooplankton but be caused by the effect of algal toxins. In another publications Vinberg, especially discusses the effect of the toxins of phytoplankton on animals (fishes, birds, cats, dogs). Paralysis of hind legs and liver diseases were observed, among others. With man serious muscle aches and passing paralysis may follow. These effects especially were observed near eutrophic lakes, i. e. extraordinarily productive lakes when a "a water blooming", a mass growth of phytoplankton occurred and larger algal quantities gathered near the bank.
"According Vinberg toxins are especially effective as long as the producing cells are living. After death of cells the effect of toxins ceases quickly. Microcystis, Aphanizomenon, Oscillatorie as well as Gonyaulax and Prymnesium are well known toxin producers and partly (for instance) Prymnesium have killed fish. Also in this case toxins get into the body via skim immediately with the cells or after being dissolved in water, where they are accumulating within the inner organs and especially within body fat. Toxins prove to be water-, alcohol-, and acetone soluble, pH and thermo stable and penetrate cellophane and animal membranes when dialyzed."

Because we have no cause for doubting the truth of the results of these two investigators, there arises the question, how to explain the contradictions. The assumption is right at hand, that the investigators, who were working with other but closely related species of green algae, had actually used such, which proved to be notoriously "poisonous". According to a remark, which Professor Strugger, of Muenster, made in a discussion, there are 40,000 (!) species of unicellular algae. This enormous variety of nature in the field of phytoplankton is very precious especially for algal research performed for industry. It is very possible to find out the most useful species or varieties corresponding to required conditions and expected effects in the best manner, species which simultaneously possess the greatest unpretendingness, resistance against infections, adaptation and good growth. As Henneberg during the first world war, by discovering the Torula utilis (utilis useful), laid the real basis for microbiological protein synthesis, perhaps today better races must be found in the field of phytoplanktons for technical mass culture. On the other hand, it must be understood that among 40,000 species there are more or less notoriously poisonous or toxic, which are responsible for the reported poisonous effects.

Besides, it has been aquarium knowledge for a long time that one should put infected ornamental fish into a glass filled with water that has turned green, (i. e. interspersed with unicellular algae), and stand it in full light. The nascent oxygen (or in our today's opinion probably rather the antibiotica of phytoplankton organisms) is then to do its work. As we look upon it, no symptons of poisoning are known. Regarding our today's knowledge, the assumption seems to be more probable that the toxins, the presence of which must be assumed with the Essen species of Scenedesmus obliquus, which has turned out so well when cultured, are inactivated when the material is dried. Because drying is done so very carefully, as we were told, by infrared radiation without local over heating, as attested by the high, indeed scarcely diminished, protein quality, poisons would be very labile indeed. We hope to be able to examine this second possibility practically by feeding living and dried algal material to rats. In case this second assumption should be right, our algal substance would behave similarly to several other parts of plants used in nutrition -- for instance, edible scarlet runners (Phaseolus multiflorus), the uncooked green pods of which are said to be very poisonous. But any quantity of them is known to be eaten as vegetables when cooked. As another example edible turbantops may be mentioned, which are said to have a poisonous effect when eaten raw and only become innocuous by cooking or drying.

Now we are going to discuss our findings in detail. Regarding the astonishingly high biological value of Scenedesmus obliquus, which we observed in all our experimental groups, especially in the monochromatic protein diets, it must be reported that we did not find any nutriment protein of higher quality at the very least of plant origin even though we have been occupied with examining biological quality of very differently originated proteins, especially of microbe proteins. Since 1941 we have examined: yeasts (very differently originated and grown), fungi (Aspergillus orycae, Penicillium notatum, Oospora lactis), bacteria (kephir, i. e. syvbiosis of yeasts and bacteria), fish meal, mushrooms (culture Psalliota) soybeans, peas, rye, wheat, casein, gelatin, barley, malt, spent grains, malt rootlets, brewing sediment, beer and so on.

It is just possible that whole egg protein, which we have not yet examined, and perhaps total protein of the whole milk might be superior to a certain degree. But in our opinion its significance would be proved by many animals. However in these experiments, larger numbers would not have been practical. After all these experiments, it seems to be established that the dogma of the inferiority of plant protein as compared with animal protein may be regarded as undoubtedly upset. Other criteria: liver protecting effect, mortality quotient and necrosis quotient which have to be considered for final judgment,, support this fact. We will refer to it again later.

In addition to the foregoing, the unusually high biological quality of the Essen Scenedesmus algae, equal to best animal protein, also is of practical and economical importance, to which we may be allowed to refer here briefly. In contrast to most plant proteins, looked upon to be more or less inferior, which are well known to be transformed into animal protein of high quality for men (meat, milk, egg), especially if they are rich in ballast, by feeding them to farm-useful animals this conversion proceeding with a loss of 80% - 90% might be disregarded. The depressingly low conversion factor of 4 : 1 respectively 9 : 1 might be eliminated for many an economic consideration in the course of the future industrial algal culturing. In this circle there may be no necessity of referring especially to the remarkable part this loss factor is playing within many considerations regarding present and future provisioning of mankind with sufficient nutrition on the whole and especially with a suitable proportion of 2/3 plant to animal protein. To think that already many nutrition scientists believe it impossible to nourish the whole mankind by optimum proportion of plant and animal protien the unexpected result — that algal protein is equal at least to protein of white of an egg and milk (the latter in form of skim milk powder) — is gaining in importance.

One point of our working programme, which by mere accident we fortunately had not yet begun to elaborate became rather uninteresting in consequence of this result, that is to say the question, of which value this transversion factor which proves to be so important for agriculture, is for zoo- and phytoplankton that means when Scenedesmus algae are fed to Daphnia or Cyclopes and when these proceedings would be balanced in certain directions. For the consideration of whether algal masses are to be taken directly for human nutrition or by the roundabout way through animal protein involving great losses, it is an important point whether it will be possible to use the algae for human nutrition in a form which does not offend sensation of taste. Many a mushroom species of our woods, which are known to be non-poisonous, well digestible and perhaps dietetically more valuable than cultured Psalliota - and might be harvested in hundredweights, cannot be utilized, because it does not taste good in any cooking.

And also the intensive troubles during the last world war, to prepare a dish in the proper sense of the word out of brewer's yeasts of industrially particularly cultured yeasts were not at all successful. As things are now with the algae and how far matter has come in this direction, I do not know except fanciful newspaper-articles, the real basis of which I was not able to examine. Some partakers in this international congress have come here expecting to hear new and positive results about this matter - I suppose. A hint at this question may probably be the fact we observed again now, that our algae-fed animals whose "ancestors" probably never had eaten unicellular algae, daily are eating more of the diets containing Scenedesmus as protein source than litter mates of the analogously composed diets even with milk protein, of which up to date most was eaten remarkable by the somewhat paradoxical circumstances, that our young rats did not like the foodstuff containing costly culture Psalliota at all. Therefore we had trouble to make them eat the protein minimum just adequate for producing liver necrosis.

Because taste is a matter of opinion, as people are saying all over the world, just concerning this point it would be very risky to undertake far-going and too optimistic extrapolations out of our experiments on white rats regarding the situation with man and his appetite for algae.

We now refer to our observations regarding the liver-protecting effects of the Essen algal material. Because we were looking for the occurrence or non-occurrence of dietetic liver diseases in animals on algal diets as well as on control diets of the litter-mates, we are able to judge the dietetic value of the Scenedesmus algae in a deeper and more comprehensive manner. We have already reported that in our three-year old investigations, during which we fed 12 different samples of algae (one after the other, cultured at different times) to young albino rats within the described diets, not one animal perished during the 120 days growth time; while with the control diets prepared on basis of many skim milk powders a high percentage of deaths occurred--the so called nutritional or dietetic liver necrosis being the cause of death in most cases. Out of these facts we draw the conclusion that dried algal material contains one or more of these substances which singly or together are able to prevent or heat dietetic liver necrosis--at least in a quantity sufficient for complete protection. Today we are not yet able to decide which of the three substances known as belonging to very different groups of compounds, thioamino acids, Vitamin E or one or more as yet unisolated factor. This last could be the factor 3 which was determined and enriched in unpurified casein and many brewer's yeasts by Claus Schwarz, or the factor which obviously is destroyed when technically drying skim milk. We called it factor Y, because we are not yet sure whether it involved with or identical with factor 3, which seems to be more thermostable.* Because time is lacking I am sorry to have no possibility of referring to these things in detail. I just want to hint at the necessity of calling the algae extremely hepatophil nutrient substances or protein sources, in view of their extreme liver-protecting properties. Beside the algae, undried skim milk, many brewer's yeasts, beer, egg protein, and spinach proved to have this effect too. On the contrary nearly all skim milk powders,** Torula culture yeasts, especially Waldhof yeast grown on sulphite lye, and cultured Psalliota must be called hepatophobic. Out of former experiments - in cooperation with Hock - potatoes, peas, cereals, gelatin or their protein parts must be added, and according to Matet, Matet and Fridenson (1947) soy bean meal and according to Olson (1953) soy protein also.

It must be emphasized, that we got our results when using dried algal material, but to be sure, as the Essen station told us, conditions of drying were rather careful. The algal material was dried by infrared radiators* when spread in a thin layer. We mentioned already that probably in this manner toxic substances observed in many a Chlorella and Scenedesmus species may be inactivated in our algae. The liver-protecting factors of our Scenedesmus algae at any rate have survived drying well, according to all symptoms. Whether this would be the case when technically drying it differently, we dare not decide yet. Once more our discovery may be recalled that fresh skim milk used as protein source, which is able to give full liver protection in our diets, becomes highly liver necrogenous when technically dried, especially on roller driers. It must be determined by investigation whether algal substance may be subject to changes of this pernicious kind. With regard to skim milk we have already been for some time occupied with the chemistry of this change, but we are not yet

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* Of course it may be that factor 3 or Y is so easily destroyed when skim milk is dried, because in this case a Maillard reaction may take place owing to the sugar content.

** Function of technical drying process, whereby spray drying preserves more than roller drying (see "Naturwissenschaften" 42, 446 (1955).

* The thermometer showing temperatures of 104°-140° F within the algal layer.

able to decide whether an antinecrogenous substance - our presumed factor Y perhaps identical with Claus Schwarz's factor 3 or thioamino acids - is destroyed or inactivated. Because of its high content of carbohydrates, possessing in our opinion a high reacting ability, conditions for inactivation of skim milk are much more convenient that for inactivation of our Scenedesmus algae. Protein substances of Scenedesmus obliquus seem to be highly resistant to drying too, a fact which undoubtedly may be derived from the very high bioligical quality of dry algal material.

Now it is necessary to discuss an objection which nutrition scientists and also microbiologists might raise. In case of our investigated dry material we were not dealing with pure cultures obtained according to principles of absolutely pure culture. Today this is not yet technically possible, at any rate not at the Essen Research Station. We were dealing partly with "open air algae" cultured in trenches lined with plastic and partly with "Green house" cultures. Because, contrary to our expectations, our experiments on animals took such a favorable course quantitatively, as well as qualitatively, and because the biological quality of digestible protein and the antinecrogenous effect were at least equal or better than the corresponding properties of skim milk powder, we suspected that perhaps large quantities of zooplankton, for instance Protozoa, might be simultaneously cultured and harvested along with algae. Though we did not know (and probably up to date it has not been investigated) what digestibility of protein and what growth curves or biological quality Protozoa or similar organisms of infections might have in rat experiments, for some time we believed - remembering the dogma of plant protein being inferior to animal protein - that our very favorable results might be falsified, or at least influenced, by the presence of animal protein or APF factors. Dr. Meffert, of the Carbon Biological Research Station, Essen, reassured us indeed, because of the result of her current microscopical "Factory controls". Subsequently we had newly harvested substances sent to us in order to control it ourselves. Observing many dozens of fields of view we very seldom saw microorganisms which were different from green algae and had to be regarded as zooplankton.* (Besides, the finding and description of antibiotic excretions of Chlorellin and other substances in algal cultures might point in the same direction.) To some practical extent for manufacturing future algal culture it would be nearly indifferent whether cultured protein material should be a pure or mixed culture, provided only that it is of as high quality as possible, at any rate of equal value with animal protein if best quality. From a theoretical point of view the dogma of inferiority of plant protein would not be upset with Scenedesmus --the high values would be explained by admixture of animal protein, for instance of Protozoon protein, while a somewhat lower protein value equal to good plant protein must then be assigned to algae.

Furthermore, I should like to refer to the question of therapeutical use of unicellular algae as far as this seems to be successful according to our experiments. Not only these results we had when examining the Scenedesmus algae point in this direction, but also the conclusions we draw from skim milk and skim milk powder, which I mentioned before. Our discovery forecasts therapeutic possibilities. According to our data, in our opinion Scenedesmus substance might be called a therepeutic and prophylactic substance, good for protein deficiencies and for states usually occurring during long malnutrition, especially protein malnutrition. The discovery, attributed to the tropical physician Trowell, that the very wide-spread tropical disease kwashiorkor (the etiology of which only became known during the last few years) proves to be a

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* This very unimportant part of weight in percent (or better per thousand) scarcely would have had any influence on the result even if the contamination had consisted of the best "Animal protein." Therefore this objection may pass for being refuted.

complex protein malnutrition damage and that, up to date, this kwashiorkor syndrome may be successfully healed and prevented by dried skim milk to an astonishing degree, made us see our algae and skim milk results in a new light. Might the algal material be effective on kwashiorkor syndrome too because of its extremely high protein quality and its superior content of liver-necrosis-preventing factors? Tropical physicians will perhaps object, that kwashiorkor liver changes in man are not identical with acure * necrotic liver damages in rats (!). Kwashiorkor conditioned lesions rather suggest fatty infiltration and cirrhosis. As regards our postulated effect of Scenedesmus algae on kwashiorkor we are not only looking at this problem from the side of liver changes but also of results in the field of physiology of protein nutrition and the superior position of Scenedesmus protein. Moreover we do not yet know whether the skim milk powders which are successfully used by tropical physicians for treating kwashiorkor syndromes will have a necrogenous effect in our animal tests too and will behave like our German preparations. We are quite sure of their producing liver necrosis too, for nearly all differently dried skim milk powders of various German origins, thus far investigated, proved to be more or less liver necrogenous - contrary to the 12 algal samples we received. Medical experiments on kwashiorkor cases must decide. Tropical physicians ought to carry out investigations in this direction.

In the end I want to refer to two subjects. The first subject concerns the constancy of the very favorable dietetic results, which we got during nearly 3 years when investigating 12 algal samples - in other words the question whether there may not occur a diminution or at least qualify fluctuations of the cultured algae for nutrition. We believe that to be possible indeed, even without any infection by inferior or even noxious species. Perhaps one of our last results is pointing at this direction. One sample of dried Scenedesmus obliquus which had grown on waste-water, did not behave so favorably as regards animals' appetite and growth-curves. But even then liver damages could not be observed during the usual time of experiment. Why these waste-water algae proved to be of lower value, cannot yet be reported. Besides, the changed miliue conditions, perhaps the reason was a different "age" of the algal cells because of different culturing and different times of harvesting, in agreement with Vinberg's communication, or a diminishing of quality of the cultured protein.

Obviously the yield of algal culturing station must be investigated as to biological quality by experiments on animals at regular intervals.

The second subject concerns the question whether there are plant organs in the field of higher plants too, which possess high dietetic and antinecrogenous effects just like the algae. Out of our extensive results I am only able to say briefly that artificially dried alfalfa meal, as it may be bought on the market (concentrated feed for farm animals), proved to be significantly inferior for growing rats, while on the other hand fresh spinach, after preliminary cookery-like treatment (cooked in a bit of water for some minutes), when fed in a monochromatic diet nearly approaches the algae and at the same time the milk protein and the white-of-an-egg protein without reaching this group of protein sources of highest value. This is shown by Diagram 5. Our investigations are telling in favor of the fact, that beside the unicellular algae as prototype of green-leaf freshly-grown green parts of higher value than proteins of plant seeds for instance of cereals and legumes and of tubers (for instance of potatoes). Because animal protein of high quality will always remain rare for nutrition of mankind on the whole, it will be necessary to attribute greater importance than up to the present to protein of algae and young green plant parts especially in order to prevent protein deficiency diseases.

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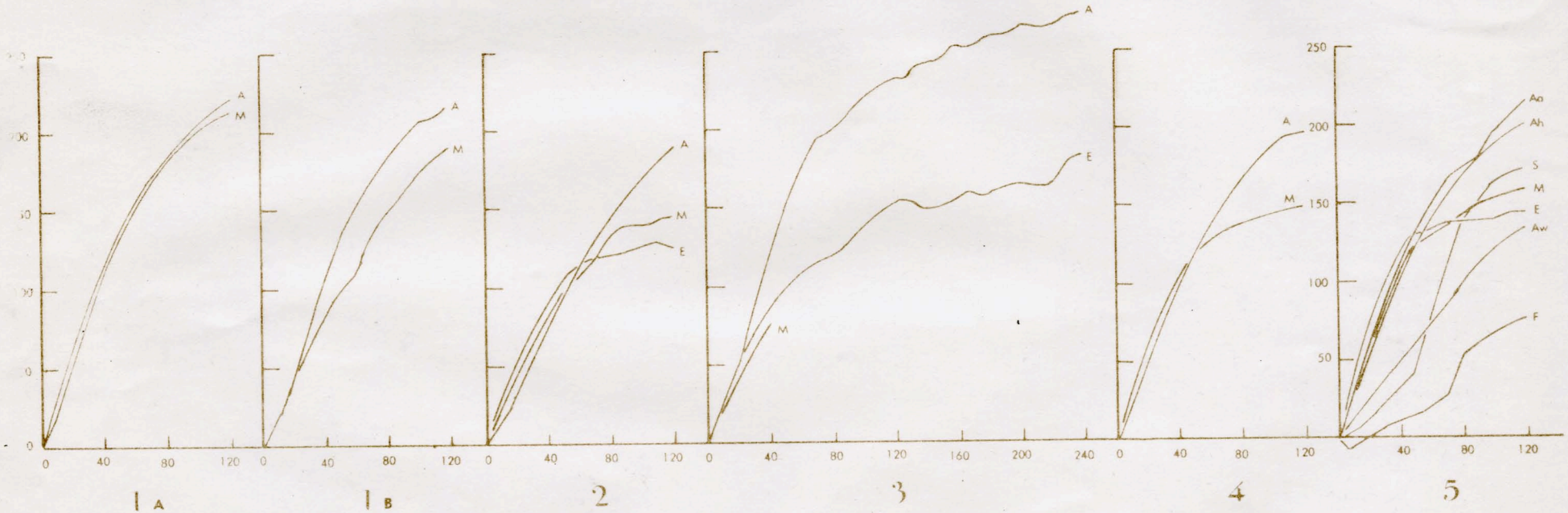
* Recently we also met incidences which did not lead to death during the well known short terminal phase, but must be regardedas chronic.

AVERAGE CURVES OF GROWTH

Abscissae: Elapsed time in days since the start of the experiment

Ordinates: Average gain in weight (grams).



DIET SUPPLEMENTED BY RYE AND WHEAT

DIET NOT SUPPLEMENTED BY RYE AND WHEAT

Identification of Source of Protein

A	Algae.	E	Egg-white.
Aa	Algae cultured in open air.	F	Alfalfa.
Aw	Algae cultured in waste water.	M	Skim milk powder.
		S	Spinach.

The symbol Ah designates heavy animals fed on Algae