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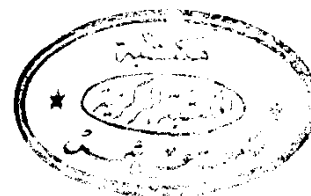
ENZYMATIC STUDIES ON SCHISTOSOMA MANSONI

A Thesis Presented to The Faculty of Medicine

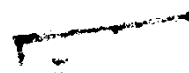
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INTRODUCTION

In recent years the possibility of utilization of unicellular fresh water algae as new source of food supply has been investigated in many institutions in America, Germany, France and Britain. These investigations include the development of mass culturing of unicellular algae, the study of the growth and chemical composition of these organisms as influenced by the variation of the different physiological and environmental conditions as well as the nutritive value of the algal products.

These studies have shown, however, that unicellular algae are remarkably flexible in the sense that their composition can be altered at will and thus by careful control of the culturing conditions a wide variation in the relative amounts of the chemical constituents especially proteins and lipids, can be attained.

Laboratory experiments on the mass culturing of autotrophic micro-organisms were carried out in the botanical Institute of Göttingen university (professor A.K. Harker).

One aim of such experiments was mainly the study of the nutritive value of these algal organisms for the production of food as well as vitamins. These organisms were cultured in a nutritive solution in glass tubes 5 x 25 - 50 cm. long.

The tubes were provided with an air stream fortified with carbon

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with CO_2 and illuminated with water - cooled - 300 watt -
lamps yielding up to 10,000 lux.

Aach, 1955; however used culture vessels illuminated
from inside. He used 5 - 15 cm. wide cylinders. Open
glass tubes were put inside and the culture solutions were
introduced in the space between the glass cylinders.

In Essen the carbon biological research station, 1953;
(Dr. Gummert, Dr. Meffert and Dr. Strattman) have been highly
interested in the mass culturing of algae which may provide a
possibility of the biological utilization of the large
quantities of CO_2 of the waste gases available in the
Industrial Ruhr district.

Since 1950, they have studied the unsterile large scale
culturing of the green algae, Chlorella and Scenedesmus.
The methods of culturing were applied in Essen. The open
air cultures and the green house cultures. The open air
cultures were carried out in culture trenches nine meters long,
10 cm. wide and 20 - 24 cm. deep at the low ends. They were
lined with plastic material. Gas mixture for aeration was
led to the cultures in iron tubes having many small holes and
protected with inert coating.

In America the mass culturing of algae was
intensively studied and pilot plants for culturing of these

organisms were installed . For the preparation of the cultures for the large scale experiments one litre Erlenmeyer flasks containing 500 ml. of the standard medium were inoculated with small quantity of the organism from the slant cultures. These flasks were set in an incubating rack illuminated from below by 8 fluorescent tubes and 5% CO_2 in air mixture was bubbled through the culture.

To produce enough cultures for inoculation of the large growth units a second intermediate step was used. The cultures in vertical cylinders, 4 inches in diameter and 4 feet long, were made.

The large scale operations were carried out in large scale units. A unit was composed mainly of large thin-walled plastic tubes (polyethylene) to form channels with simultaneous flow of cultures.

The polyethylene tubing seemed to provide a suitable combination of light transmission, chemical and physical stability under exposed conditions, freedom from toxicity and physical strength.

Taniya, 1954; compared closed circulation system similar to that run by Arthur D. Little in America and the

open trench system similar in principle to that run in Essen, Germany, and gave a detailed report on the advantages and disadvantages of both systems and concluded that the open trench cultures appeared to be superior to the closed circulation cultures in its apparently lower cost and simplicity of equipments and operations although it is rather inefficient in the CO₂ utilization. He finally constructed a new type of the open trench system which proved to give a better yield.

Tamiya and his co-workers of the Tokugawa institute for biological research were also highly interested in the physiology and biochemistry of unicellular algae and their mass culture. In his laboratory experiments Tamiya used either culture flasks or special culture chambers. The culture chamber described by Tamiya was an oblong flat flask with uniform inner thickness and total capacity of 600 ml.

In England M.J. Geoghegan, 1951; used cylinders and aspirator bottles for the culturing of unicellular algae in the laboratory, while in his outdoor experiments he used plastic tanks.

The green algae, especially Chlorella and Scenedesmus had been used for a long time by many investigators as experimental tools in the study of physiological processes. More recently, the chemical constitution of these organisms became of interest to many authors especially after the

of the mass culturing of these organisms.

From the results of more detailed investigations performed on few algal species especially Chlorella and Spirulina it has been established that the chemical composition of algae depends to a large extent upon the biological and environmental conditions under which the organism is cultured. Thus Spoehr and Milner, 1948; have studied the influence of various environmental factors on the chemical composition of Chlorella. These factors include CO₂ concentration, aerobic and anaerobic state, light, and nutrients, more particularly, Nitrogen, Phosphorus and temperature. They have demonstrated that the chemical composition of the cells varied greatly according to the conditions under which they are grown.

Spoehr and Milner, 1948; have argued that the observed variations in chemical composition are due either to environmental changes or to the growth of different populations of a strain of the organism which is subjected to different special conditions and when their growth is compared. They have concluded that the chemical composition of Chlorella - their experimental organism - is determined by its environmental conditions during its growth. They also observed selective growth of different strains.

Milner, 1959; giving data on the chemical composition of different algal species including Chlorella and Spirulina.

Veris, has demonstrated that this composition varies in response to change in environmental culture conditions. This variation is much more significant in the case of Chlorella pyrenoidosa.

Similar results were, however, obtained by Ketchum and Redfield, 1949; on growing different algae under different environmental conditions.

Powden, 1952; has also isolated bulk protein samples representative of the whole cell protein from Chlorella cultures of different ages and the amino acid composition of these protein samples has been determined chromatographically. It has demonstrated that the amino acid composition of the whole proteins remains virtually constant with increase in culture age, although the percentage of proteins within the cells gradually decreased.

Meffert, 1952 - 1955; has found that the chemical composition of her experimental organisms, namely Scenedesmus obliquus and Chlorella pyrenoidosa, when grown in large scale unsterile cultures varies but very slightly in response to change of climatic conditions during the different months of the year and also from year to year. As it has been recorded by Meffert the protein content also varies between 50 and 60% of the dry weight, the lipids between 15 and 20% while the carbohydrate content remained

more or less constant.

Though many authors have shown that the composition of the chemical composition of the algae varies within extremely wide limits depending on the physiologic and environmental conditions under which the algae grow, rather than on genetically determined characteristics of metabolism, (Heteman & Redfield, 1947; Speer & Millard, 1951; Peters, 1951) yet the results reported by Speer & Millard (1951) concerning the lipid accumulation in algae indicate that nevertheless there are considerable inherent differences in the capacity of algae to accumulate lipid.

Proteins seem to be the most abundant organic constituent of the unicellular green algae with the exception of the high lipid forming organisms (Green & Allen, 1942; Hitch, 1948) and the protein content of most of the algal species investigated was appreciably higher than that of the best vegetable sources of proteins used in animal and human feeding (Fischer, 1955).

Geoghegan, 1951; studied also the physiological behaviour and chemical constitution of Chlorella vulgaris var. viridis, and demonstrated that the true protein content of the dried Chlorella material was about 40%. He also investigated the amino acid content of this protein.

Fowden, 1951, 1952; has also studied the composition of the algal proteins in 3 different organisms namely Chlorella pyrenoidosa, Ch. vulgaris and the blue green alga Anabaena. He has shown that all the essential amino acids occurred in his 3 organisms. He also investigated and determined the relative quantities of 20 amino acids in the free (in the alcoholic extract) or in the combined form (in the protein hydrolysate). He applied paper chromatography methods.

Fowden also demonstrated that the amino acid composition of the algal proteins remains virtually constant with increasing culture age, although the percentage of protein within the cells steadily decreased. Fowden continued his studies and in a latter work, 1954; he made analysis of 4 different algae to investigate the causes of any differences in composition between algae of different strains and also to compare the composition of the algal proteins with those of other plants. These investigations revealed that the total nitrogen and protein contents of the 4 examined algae varied widely. On the other hand the proportions of the total nitrogen present as soluble and as protein nitrogen remained relatively constant for all the 4 species. The results presented by Fowden also showed that the proportions of most of the individual amino acids present in the algal protein fractions of the 4 different species examined were similar.

Some of the amino acids (glycine, phenylalanine, leucine, methionine and proline) are present in each of the 4 proteins in approximately equal amounts, if allowance is made for normal experimental error. Significant differences between the percentage of certain amino acids (arginine, histidine, lysine, threonine, serine and aspartic acid) are apparent. It is considered that such differences exist in the amino acids threonine, lysine and glutamic acid.

Powden finally concluded that the composition of the bulk of proteins of algae is similar to that of higher plants and other micro-organisms and with regard to their possible nutritional value the algal proteins are like other vegetable proteins in being deficient in the sulfur containing amino acids.

The studies of the composition of proteins of unicellular algae proceeded also in U.S.A. Thus the food research Lab. (Washington) ¹⁹⁵³ found that Chlorella pyrenoidosa contained about 44% protein. The different amino acid composition of this protein were also estimated and 11 different essential amino acids were proved to be present.

In Japan Taniya and his co-workers, 1951 - 1954; completed intensive studies in physiology and biochemistry of

unicellular algae. They have shown that the protein content of their different experimental organisms lies between 40% and 50% of the dry weight and all the essential amino acids were found present in Chlorella ellipsoidae proteins.

In Germany Meffert, 1955; giving a review of the work done in Essen station on large scale production of algae, 1951 - 1955; had shown that the protein content of Scenedesmus and Chlorella varies within limits very slightly from month to month and from year to year. The protein content lies between 50 - 60%. Also Meffert demonstrated that all the essential amino acids are present in Chlorella and Scenedesmus proteins.

Edible seaweeds have been used for centuries. A full account of the use of a number of microscopic marine algae as food is given, however, by F. Chase, 1942; along the coast of Europe some forms, are regularly collected and dried for use as cattle fodder and on the coast of Ireland and Scotland there is a variety called ulse which is often sold like peanuts or popcorn.

The Japanese also have used certain seaweeds in cooking. In America Chondrus crispus or Irish moss is harvested commercially along the New England coast for use in food products.

Recently more emphasis had been imposed on the investigations concerned with the possible utilization of fresh water algae, especially the unicellular forms, as food resources.

The primitive character of these microscopic organisms gives them a number of advantages over higher plants as a source of food. These advantages can be generally summarized in the following

- I. The whole algal plant is nutritional as little of it is devoted to indigestible structures.
- II. The algal material contains a high protein content which amounts sometime to more than 50% ^{of the dry weight} and this is much higher than that found in the edible part of any of the higher plants.
- III. The preliminary investigations of many authors had shown that the unicellular algal proteins contain the essential amino acids needed for human nutrition.
- IV. The chemical composition of the unicellular algae is more or less flexible and could be changed at will by variation of the culture conditions.
- V. The area needed for large scale cultivation of algae is limited and it has been assumed that the total area required for algal culture needed to provide the half of the nutritional requirements of proteins is less than a million acres for the present world population. In countries