A CULTURE MEDIUM FOR EUGLENA WITH NOTES ON THE BEHAVIOR OF EUGLENA

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I. INTRODUCTION

Quince seed jelly has been in common use as an agent for retarding the movements of Protozoans but as far as the writer can determine it has not been suggested as a culture medium.

While working with quince seed jelly to retard the action of the flagellum of *Euglena* it was discovered that *Euglena* would live for several days in the medium. Paramoecium also existed for several days in depression slides surrounded by the medium. It was conceived that Protozoans might be kept alive for a considerable length of time and an attempt to test this possibility suggested the value of further experimental work.

It was found that not only would *Euglena* live in the medium but it seemed to obtain nourishment from the medium and increased in great numbers. Cultures have been kept for fourteen months. Two hundred successful transplants have been made from a single culture and *Euglena* has been available for class work at all times throughout the year. The following experiments have been carried out to determine the best conditions for raising *Euglena*.

II. EXPERIMENTS TO DETERMINE THE OPTIMUM CONDITIONS FOR THE DEVELOPMENT OF EUGLENA

1. *Acidity and alkalinity*

Experiment a. Quince seed jelly was used which had been exposed to the air for several weeks and had acquired a strong acidity evidently through fermentation. Twenty-five tubes of the medium were prepared and inoculated with cultures of *Euglena* which had been grown on a neutral medium.

Six of the cultures contained live *Euglena* at the ends of three weeks. Two cultures contained *Euglena* at the end of six weeks and all were dead at the end of twelve weeks. Moulds were abundant in the cultures after five days. In three of the cultures a mould developed which contained a brilliant orange pigment.

Experiment b. Acid medium from the same stock as that used in the preceding experiment was neutralized with sodium carbonate.

Twenty tubes of the medium were inoculated with *Euglena* from the same culture as in the preceding experiment.

At the end of three weeks all the cultures were living and only three were dead at the end of six weeks. Sixteen of the twenty cultures were alive at the end of twelve and all had shown a great increase in the number of *Euglena*.

Experiment c. Twenty test tubes were prepared each containing 50 cc. of neutral medium. Five were kept as controls while amounts varying from one to seven drops of $\frac{N}{10}$ sodium carbonate were placed in the others. They were inoculated with *Euglena* from a neutral culture,

All cultures were alive at the end of two weeks but no marked difference in their progress could be distinguished. At the end of four weeks there was a slight difference in favor of the more alkaline cultures. After a period of seven weeks all the cultures were flourishing. There was no perceptible difference in cultures differing only slightly in their alkaline content but the most alkaline had progressed somewhat more than the neutral and the slightly alkaline ones.¹

Experiment d. The acid medium used in experiments a and b was used here. Fifty lots of media of 50 cc. each were prepared and arranged in ten equal groups. (The medium at this time had a total acidity which required 8 drops of $\frac{N}{10}$ sodium carbonate to neutralize 50 cc.) Five cultures were kept on the acid medium as controls. Amounts of sodium carbonate varying from 3 to 17 drops were placed in the other lots. Group number one (the controls) were thus rather acid while group number ten was equally alkaline. Group number five was about neutral. *Euglena* from a 'Wild' culture were used to inoculate the tubes.

After six weeks active *Euglena* were found in the slightly acid, the neutral and all the alkaline cultures. In the alkaline cultures there had been a substantial increase in the numbers of individuals. No *Euglena* were found in the strongly acid cultures but a few encysted individuals were found in the cultures having a moderate acidity.

Experiment e. In cultures kept for a year in a thick jelly it was noticed that the medium was reduced to a thin liquid having the consistency of water wherever the animals had remained in one place for some time. This liquid was tested from time to time and gave a strong alkaline indication while the medium at the center of the tubes was still neutral. The liquid part of the cultures increased in its alkalinity with the age of the cultures. It is not necessary to postulate a specific substance in the decomposition products of *Euglena* to account for this liquefaction. A thick jelly may be reduced to a smooth liquid by ammonium hydrate or sodium carbonate and conversely a thin medium may be set to a jelly by a strong acid.¹

¹ In tests for alkalinity and acidity $\frac{N}{10}$ and $\frac{N}{50}$ solutions of sodium carbonate and of hydrochloric acid were used with alizarin as an indicator.

2. *Light*

Cultures kept in a moderate light were successful when other factors were equal. Cultures kept in the sunlight died in a short time. *Euglena* will not live in the dark.

3. *Density*

Experiment a. Two cultures were started in the fall of 1915 in very thick jelly. No effort was made beyond a preliminary boiling to keep the tube sterile. About five hundred individuals were placed in each culture. At the end of three months each culture showed a conspicuous green color at the top. After six months the green color was present at the tops, sides and bottoms. The medium at the top, sides and bottom was thin while that at the center of the tubes was unchanged. At the end of nine months about a third of the entire volume of the medium had been liquefied and this zone was occupied by enormous numbers of *Euglena*. Over two hundred transplants were made from one of the cultures at this time. At the end of the thirteen months both cultures had shown a still greater increase in numbers. The liquid zone had increased and as previously stated gave a strong alkaline reaction, whereas the original medium had been neutral.

Experiment b. Forty cultures were started under the same conditions as in experiment a.

After five weeks twenty-eight of the cultures had started through the course just described in experiment a. Twelve were dead and a thick growth of mould had taken possession of them. Most of the dead cultures displayed a slight acid reaction.

Experiment c. Media was prepared by evaporating the jelly to dryness and making up solutions to the desired consistency with distilled water. The following dilutions were made up: 0.75 per cent, 0.5 per cent, 0.4 per cent, 0.3 per cent, 0.25 per cent, 0.2 per cent, 0.1 per cent, 0.05 per cent, 0.025 per cent. Forty cultures were started with *Euglena* from a 'Wild' culture, five cultures upon each of the above named dilutions. Test tubes having a bore of 8.25 mm. were used.

At the end of a few weeks a few inactive *Euglena* were found in the cultures presenting a 1 and a 0.75 per cent dilution. A growth of mould had started at the tops of some of the cultures. In all the other cultures there was a downward migration, all the *Euglena* being arranged in a horizontal plane not more than 0.5 mm. in width. The extent of this downward migration varied directly in proportion to the density of the medium.

Dilution per cent	Vertical distance of migration mm.
0.5	2.8
0.4	3.25
0.3	3.6
0.2	4.25
0.1	8.0
0.05	11.0
0.025	17.0

At the end of two months only a few slowly moving and encysted individuals were found in the cultures representing a 1 per cent, a 0.75 per cent and a 0.5 per cent solution. In the others the migration had reached the bottoms of the tubes and there had been a great increase in the actual number of *Euglena*.

Heat and cold

Sixty cultures were prepared, twenty being in an incubator at 37°C., another twenty kept at room temperature and the rest in a temperature of about 7°C. Those kept at room temperature produced flourishing cultures. The increased heat of the incubator tended to promote bacterial growth and to dry up the medium. Those kept at 7°C. were slow to develop but produced good cultures when returned to room temperature. The lower temperature was favorable for the growth of mould.

5. Shape of receptacle

Cultures made the most rapid progress in test tubes, vials and jars where the *Euglena* had a considerable vertical range. Cultures were also tried in flat bacteria dishes where there was a large surface exposed.

These cultures were generally failures.

6. Sterile cultures

About 70 per cent of all cultures attempted with no precautions as to their being sterile were successful. Sterile media can be easily prepared, however, by boiling and cultures planted in sterile media give more uniform results. It is very difficult to keep cultures sterile as the introduction of *Euglena* can hardly be accomplished without giving access also to some bacteria.

7. Bacteria and moulds

The presence of a small quantity of bacteria does not seem to materially affect the progress of a culture except when the cultures are kept at a temperature of more than 21°C. The presence of moulds not only retards the progress of the culture, but the mycelium makes it difficult to follow the progress of *Euglena*.

Cultures containing a dense medium were found to contain a thicker growth of mould than those grown on a thinner medium. Experiments proved that a medium representing a 0.25 per cent solution of dried jelly in distilled water will not support a growth of mould. Neutral and slightly acid cultures usually contained growths of mould but the alkaline cultures, whether weak or strongly alkaline were free from it. Old cultures of *Euglena*, grown on a thick medium, will automatically clear themselves of mould by becoming alkaline.

8. *Effects of transplanting*

No weakening effects have been observed in transplanting cultures and frequently a slowly developing culture in which there is a tendency for the *Euglena* to encyst will become rejuvenated by being transplanted.

9. *Media prepared from ground seed*

A tendency to group about pieces of seed was exhibited by *Euglena* in some early cultures. An effort was made to test this tendency to see whether it might be of practical use. Seed was ground to a fine meal and a medium prepared by adding water in the proportion of 20 grams of ground seed to 2.5 liters of water. This medium produced the most favorable results for the rapid development of a culture. Fifty cultures of 50 cc, each were started, using this medium. About a thousand *Euglena* were placed in each culture. At the end of six weeks the numbers had increased till the entire volume of the cultures had assumed a bright green color and it was estimated that each culture contained 15,000 individuals per cubic centimeter.²

III. NOTES ON THE BEHAVIOR OF EUGLENA

The usual positive phototropism in exhibited *Euglena* shows a marked difference in its behavior in thick and thin media. Whatever the density of the medium there is a persistent effort to migrate downward. In thin jelly locomotion by means of the flagellum is possible and the downward migration is accomplished by this means. During this migration all the animals are to be found in a narrow horizontal zone as described under 'density', experiment three. In a very dense medium locomotion can take place only by means of 'euglenoid' motion and this is relatively ineffectual for progress unless the animal is in contact with a solid surface. The behavior described under 'density', experiment one can be explained according to this observation. Here, only the tops, sides and bottoms of the tubes are occupied or only those regions which can be penetrated by euglenoid locomotion.

² In estimating numbers a known quantity of media with the *Euglena* was diluted to a known volume. The tube was thoroughly shaken to insure an even distribution of the animals. A small measured amount of the medium was then placed under a binocular and the *Euglena* counted.

The density of the medium thus plays an important rôle in the behavior of *Euglena*, both in determining the mode of locomotion (whether euglenoid or flagellate) and second, in limiting the distance of migration within a given time.

IV. CULTURES OF OTHER ANIMALS IN QUINCE SEED JELLY

A minute green flagellate appeared in some of the *Euglena* cultures which had been inoculated from 'Wild' cultures. Transplants have been made and pure cultures obtained. So far these cultures have lived for four months and a half and are reproducing rapidly.

A large spiral shaped Bacterium was found to reproduce in great numbers in a slightly acid medium that had been kept at room temperature.

Paramoecium and a large Stylonychian have been kept for three months in mixed cultures. They feed upon *Euglena* and the bacteria.

Two small species of Rhizopods appeared in some mixed cultures and lived for about four weeks. An attempt was made to raise *Amoeba Proteus* in the medium but without success.

Nematode worms have been kept alive for a period of six weeks in the medium.

Mosquito larvae lived for three weeks in a rather dormant condition.

The medium seems to serve as food for the chlorophyll-bearing Protozoans and for some bacteria. The other animals live only as long as food is furnished in the form of *Euglena* and bacteria.

V. FLAX SEED JELLY AS A SUBSTITUTE FOR QUINCE SEED JELLY

A jelly may be obtained from flax seed by boiling the whole seed in water for a short time. At the date of this writing cultures of *Euglena* have been kept for five weeks in this medium and seem to be thriving quite as well as those in the quince seed jelly.

VI. PREPARATION

1. Quince seed jelly may be prepared quickly for laboratory use in the following manner:

Boil 20 grams of dry quince seed in 1.5 liters of distilled water for about half an hour, stirring occasionally. A gelatinous exudate will be obtained from the seeds. Pass the water containing the exudate through a wire sieve to remove particles of seed. (An Eimer and Amend sieve number 80 is fine enough to clarify the jelly and still permit it to pass through freely). Make up the volume to two and a half liters with distilled water. The medium will keep for several months if sterilized.

2. It is possible to obtain for experimental purposes a medium having a constant as regards density and chemical content by evaporating the jelly to dryness and making up standard dilutions with distilled water.

3. *Euglena* have been found to develop most rapidly on the following medium: ground quince seed, 20 grams; distilled water, 3 liters.

The above methods are practical for the laboratory because they require no special technique for their preparation and the cultures need but little attention when once started.

VII. SUMMARY

1. *Euglena* have been kept for over a year on a medium composed of quince seed jelly and water.

2. For cultures expected to last for a long time a thick jelly which has been rendered slightly alkaline should be used. Cultures planted on such a medium develop slowly.

3. Cultures develop more rapidly on a thinner medium.

4. Cultures develop most rapidly on a medium composed of ground quince seed and water. This medium has the disadvantage of being opaque.

5. Cultures should be kept at room temperature, in an moderate light and in glass containers having a considerable vertical range.

6. Other Protozoans have been kept for several months in the medium.

7. The behavior of *Euglena* is modified by the medium.

8. Transplants can be made successfully.