

phages. Control cultures were made of algae alone and others of chick tissue alone. Other cultures were made with amphibian cells instead of chick cells. The medium consisted of embryonic chick extract, chicken plasma and Tyrode's solution.

There was a very marked effect on the alga. In cultures containing algae alone, there was slow growth; the individual cells gradually became pale and the chloroplast appeared greatly shrunken. In the cultures of algae with tissue, the plant cells grew abundantly until there were numerous colonies. There was a marked difference, even here, between the algae immediately around the tissue and those a considerable distance away from the tissue. This was particularly true in the slow-growing amphibian cultures. Thus in cultures of adult amphibian heart, the algae became very dense about the beating heart, but showed little growth over the control in the periphery of the drop of medium. Not only did the algae multiply more rapidly in the mixed cultures, but they were larger, greener and the chloroplast did not shrivel up as in cultures of algae alone. The tissue cells grew around many of the algae colonies and individual cells. Many colonies were thus embedded in the tissue, and these remained green and increased in size, even through subcultures. Macrophages and fibroblasts took up algal cells in great numbers. Some of the tissue cells could be seen with included algal cells in all stages of disintegration, indicating digestion. It appeared that in some cases, after a macrophage accumulated a number of algae, it died, and the algae became a free colony, traces of the animal cell still adhering.

Macrophages engulfed algae in great numbers, and digestion probably occurred. These algae-filled macrophages seldom became fatty, as is characteristic of macrophages after a few days in cultures of tissues alone. The fibroblasts were also unusually devoid of fat. At the same time that the cultures of tissues alone showed almost every cell gorged with fat droplets, the cells of the cultures with algae rarely had fat droplets. In a few cultures in which there were algae on only one side of the explant, only the cells farthest from the algae produced fat. In rate of growth, as determined by increase in surface area, the mixed cultures were far superior to the cultures of tissue alone. Further, mixed cultures of algae and tissues grew and remained in a healthy condition for at least twice as long as cultures of tissue or algae alone.

Although it has not yet been demonstrated that the plant cells really do use the nitrogenous wastes and carbon dioxide of tissue respiration in vitro, it is true that they live and grow better under these conditions in the presence of animal cells. Similarly, although we have not shown that the animal cells really use

the oxygen and carbohydrates produced by photosynthesis of the algae, it is true that they appear to be much healthier as evidenced by marked absence of fat and increased rate of growth (as increase in surface area). It is possible that the mutual benefit may be due to a decrease in toxicity of the medium, such as a mutual hydrogen-ion adjustment or a change in the physical structure of the plasma clot. However, we believe that whatever the mechanism, this is a case of an artificial symbiosis.

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PURE CULTURES OF PARAMECIUM

IN September, 1933, Glaser and Coria¹ described a method for the culture of *Paramecium caudatum* free from living microorganisms. The medium employed was a liver extract broth, into each tube of which was placed aseptically a bit of fresh unheated rabbit kidney and a suspension of heat-killed yeast cells. The medium was quite simple in composition and, for any one accustomed to working aseptically, easy to prepare. In May, 1934, Hetherington² reported that he had been unable to cultivate *Paramecium caudatum* free from bacteria in the medium of Glaser and Coria. In fact he found the medium as prepared by him toxic for *Paramecium*. He concluded that "Glaser and Coria probably did not have sterile paramecia."

Glaser has supplied me with three presumably pure strains of *Paramecium caudatum* and three of *Paramecium multimicronucleatum* growing in his medium. He also supplied some of the sterile medium which I used for transplants of the cultures. The cultures were examined microscopically, unstained and stained by various methods, for the presence of bacteria, but none were found. Detached cilia and certain needle-like crystals were easily distinguished from bacterial cells. All the cultures were inoculated onto the surface of slants of meat infusion agar, infusion agar plus ascitic fluid and Sabouraud agar, which were incubated at 22°C. and 37° C. aerobically and anaerobically. They were also inoculated into the depths of deep tubes of infusion agar and infusion agar plus glucose and ascitic fluid incubated at 22° C. and 37° C. These media were examined grossly and microscopically at intervals for two weeks and showed no evidence of growth of any kind. In transplants of the *Paramecium* cultures into the medium supplied by Glaser no evidence of toxicity was noted. All the cultures grew, and actively motile paramecia were seen for as long as two months after inoculation of the media.

¹ *Jour. Parasit.*, 20: 33, 1933.

² *SCIENCE*, 79: 413, 1934.

The cultures of *Paramecium* supplied by Glaser appeared to be pure and, in so far as could be determined, were free from living yeasts or bacteria. They

multiplied and remained actively motile for two months in the medium of Glaser and Coria.

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SPECIAL ARTICLES

DETAILED SURVEYS OF SUBMARINE CANYONS

IN recent years the United States Coast and Geodetic Survey have constructed charts which make it possible to draw contours of submarine canyons off both the east and west coasts with a considerable degree of accuracy.¹ These charts, however, fail to give many of the fine points which are desirable in obtaining an accurate appreciation of the form of these canyons. For example, they do not give the data necessary to determine the exact slope of the walls and gradients of the floors, nor do they show whether the floors contain small basin depressions and whether the tributaries come in at grade as do the tributaries of land river valleys. In order to obtain this additional information the writer has made surveys in canyons near La Jolla and Carmel, California, which are from 10 to 20 times as detailed as any ever constructed by a coast survey party. Also, because of the methods used, somewhat greater accuracy of positions should have been attained than is possible in ordinary surveys.

This work involved no complicated mechanism and required only a rowboat, so that any one with a rudimentary knowledge of surveying and some simple equipment may make the same type of chart of any submarine or sub-lacustrine topographic feature which might interest them. In this machine age it may cause some amazement to state that neither were any motors used in the work nor would they have been of any material assistance. The modern method of echo sounding could not have been used, since it does not give accurate depths where steep slopes are involved, nor does the method allow a high degree of accuracy of location. Using a boat with an engine would not have given the maneuvering ability which is possible with oars and was vital to this type of survey. Also, since it was only necessary to pull in the sounding lead once or twice in an hour and depths did not exceed 1,000 feet, a hand reel proved quite satisfactory.

A range system of surveying was used (Fig. 1). Sextant angles give accurate locations at sea if angles between three or more land objects can be obtained. Where two of the objects are in range and thus only one angle is necessary a greater accuracy is obtainable than where two angles between the three objects are necessary. Also with a range it is possible through

¹ The original survey sheets should always be used for this purpose, since they contain many more soundings than the published charts.

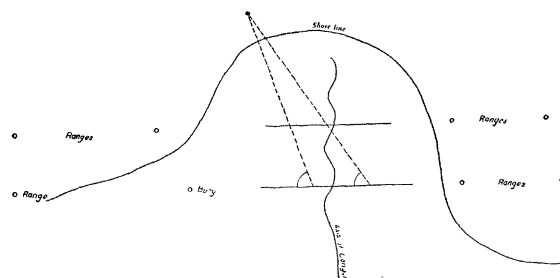


FIG. 1. Illustrating the method used in surveying the submarine canyons. Soundings were made at close intervals along these range lines and the exact positions obtained by taking angles to some shore object. In many cases it was not practical or possible to use ranges on both sides as are here indicated.

previous determinations to tell how much of a change of angle is necessary in order to move the desired number of feet for the successive soundings in running a cross section and furthermore the range makes the section a straight line. These ranges were developed before running the lines by using shore objects located at the proper positions or if necessary planting flags or even buoys.² While running each of the lines developed by these ranges it proved desirable to make many checks on the position of the line by taking two angles.

The soundings were made by running a wire with lead attached over the stern from the sounding machine and through a sheave hung from a small davit. The sheave had a depth indicator which operated like a speedometer. In making transverse sections of the submarine canyons the soundings were started on the shelf adjacent to the depression. After letting out the wire for the first sounding, it was not pulled in again, but the boat was rowed ahead trailing the lead and wire behind. Then before taking the next sounding the boat was backed till the wire became vertical and then the sounding made. Since most soundings showed an increased depth going down the side of the canyon the method was quite satisfactory and saved much time. Also in places where there were pinnacles or ridges on the canyon sides dragging the line served to detect their presence, where successive soundings might have straddled them. In a few places the lead caught on bottom and it was not always possible to free it, but this trouble was unusual, and out of almost 2,000 soundings off La Jolla not one lead was lost.

² A network of buoys may be used for this type of survey where it is run out of sight of land.