The Behavior of Clava leptostyla Agassiz In Vitro*

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Invertebrates, especially those from the lower phyla with a high power of regeneration, form an interesting group which may be used for experiments on redifferentiation of tissues and cells. Encouraged by the simplicity of tissue culture methods employed by Philip R. White and by the abundance of hydroids at the shore of the Mt. Desert Island Biological Laboratory the author decided to use Clava for experiments on the behavior of the cells in vitro.

Clava leptostyla Agassiz is a common hydroid on the Atlantic coast from Florida to Labrador. It is a hardy form which is able to survive many hours outside the sea during the low tide. The moisture of Fucus, to which it is attached, seems to be sufficient to keep it alive. There is no difficulty in culturing Clava in an aquarium with running sea water and even in dishes provided that the water is changed every 48 hours.

The stalk of Clava is smooth and, contrary to other marine hydroids, not inhabited by commensals such as sessile protozoa and other organisms. For this reason and because of its high regenerating ability, it was considered to be good material for studies on the behavior of its cells in tissue culture. Healthy looking organisms with long stalks were washed about 10 times by transfer to small petri dishes (2 inches diameter) filled with autoclaved sea water. After this preliminary washing the upper part of the body containing the tentacles and gonophores and also the base of the stalk were cut off with a sterile knife or scissors. The stalk was transferred again several times into dishes containing autoclaved sea water and finally cut into small pieces in a sterile dish without water. By means of a sterile pipette 2 - 3 fragments were mounted in a hanging drop on a sterile coverslip and placed in a moist chamber.

The medium in the first experiments was autoclaved sea water with penicillin (100 units per 1 cc.), autoclaved sea water without penicillin, and filtered sterile sea water without

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penicillin. No contamination was found in these three series of experiments. This was an indication that there was no need for using penicillin for bacteria-free cultures of Clava. In media rich in organic materials such as plasma, serum, tissue extract, or White's synthetic medium, only one contamination was found among 50 cultures (a fungus, probably Saprolegnia).

In the first experiments it was found that all fragments did not behave in the same way in the same medium. It was surmised that the differences were due to the size of the fragments. To test this hypothesis, three series of experiments were performed. In the first series the stalk of Clava was cut into thin cross sections (less than 1/2 mm.) by means of a fine knife made from a ground sewing needle. As the culture medium autoclaved sea water, filtered sea water, modification of White's synthetic medium* was used. Fragments were placed on coverslips, in roller tubes, or in Carrel flasks and covered with perforated cellophane. In all experiments the cells rounded up after a few hours and underwent dissociation. After 48 hours disintegration took place.

In the second series of experiments fragments about 2 mm. long were used. It was found that almost all the pieces healed on the cut edges without further changes in the next 24 to 48 hours.

The most interesting results were obtained with fragments 1/2 mm. to 1 mm. long which were used in the third series. In autoclaved sea water in hanging drops, on both cut edges of the fragment, migration of endodermal cells started after 16 hours. The form of the migrating cells became oblong, the most characteristic feature being the formation of long projections extending along their lengths. The slow progressive migration changed the endodermal layer after four days into dissociated cells with ameboid projections. The ectodermal cells showed a tendency for migration in sheets composed of cells with small projections along the free borders of the sheets. Such cultures were maintained in hanging drops for over two weeks. Subcultures of small groups of cells or single cells were made for observations on growth. Indications of growth were slight probably due to the poor nutritional values of the medium (autoclaved sea water).

It should be noted that the cell migration occurred only when the medium consisted of autoclaved sea water. It never

* White's synthetic medium was modified for Clava in that the salts (NaCl and KCl) were concentrated 1.5 times. This medium approximated the osmotic values of sea water.

occurred if, as culture medium, sterile filtered sea water was used.

Cultures in Carrel flasks and roller tubes behaved quite differently. First of all the cells on the cut edges rounded up. Following this all the cells of the fragment dissociated, rounded up, increased in size, underwent vacuolization and disintegrated after 48 hours. The same results were obtained in all kinds of media used.

In filtered sterile sea water or in White's modified synthetic medium in hanging drops, the cells at the cut edges became spherical. These cells underwent vacuolization, dissociation and disintegration. The rest of the fragment remained unchanged for as long as 4 - 5 days.

In Vitro Studies of Tissues from Sensitized Animals

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More than half of the summer period was spent in learning tissue culture technique. In the remaining time two projects were undertaken.

1. An attempt to induce skin sensitivity to 2, 4-dinitrochlorobenzene in rabbits by means of repeated applications to the shaved skin of a 10% alcoholic solution of the allergen, could not be carried to the tissue culture stage since no sensitivity appeared in the experimental animals. This finding agrees with results reported by other investigators, namely that rabbits are very difficult to sensitize in this manner.

2. A rabbit was inoculated in each footpad with 0.1 ml. of heat killed tubercle bacilli in mineral oil, 4 mg/ml.; the inoculation was repeated one week later. A skin test on the 13th day with undiluted old tuberculin (OT) gave a typical severe reaction with necrosis. On the 38th day, fragments of popliteal lymph node and cerebral cortex from this animal and from a control uninoculated rabbit were planted in roller tubes in a thin plasma clot with 2 ml. of nutrient medium (embryo juice, horse serum, and Gey's solution in a ratio of 1:4:5) to which in some tubes OT was added in a final dilution of 50 or 100. Observation for several days suggested that the OT caused slight inhibition of the growth and migration of macrophages and fibroblasts in the lymph node cultures from the tuberculin sensitive rabbit and of ependymal(?) cells and macrophages in the brain cultures from this animal. Later similar experiments (in the winter) confirmed this result or