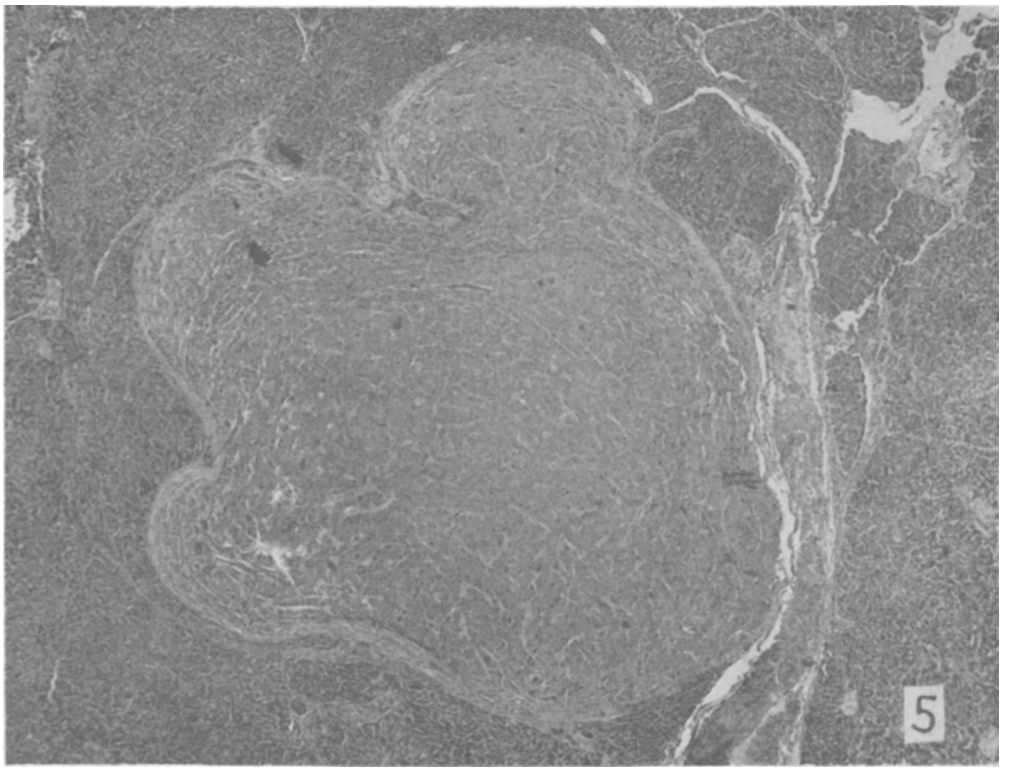

FIG. 5. Benign trabecular hepatoma. H & E. × 31.

hyperplasia, and cirrhosis. (3) Although these findings confirm the frequency of hyperplasia preceding malignant tumor formation, the actual conversion to malignancy does not seem to follow necessarily the condition of hyperplasia and/or benign neoplasia. (4) Repeated partial hepatectomies did not increase the incidence or accelerate the occurrence of hepatic tumors. (5) The percentage of liver tumors was considerably higher in males, which were found to be more susceptible than female rats to the hepatotoxic action of 2-AAF. (6) The incidence of liver tumors apparently depends on the production of hepatic damage, which has a localizing effect on the carcinogenic action of 2-AAF.

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Difference between Autoclaved and Sterile Filtered Medium in Culturing Fragments of a Marine Hydroid.* (18975)

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Invertebrates, especially those from the lower phyla which possess a high regenerative capacity, present interesting material for ex-

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periments on redifferentiation of tissues and cells. Encouraged by the simplicity of the methods for tissue culture employed by Dr. Philip R. White and by the abundance of hydroids at The Mount Desert Island Biological Laboratory, the author decided to use a hydroid for experiments on the behavior of invertebrate cells *in vitro*.

Clava leptostyla, Agassiz(1), is a hardy hydroid common on the north Atlantic coast. It is found attached to Fucus plants and is able to survive many hours in the sea water clinging to the plants exposed at low tide. Clava is best cultured in an aquarium with running sea water or in dishes in which the sea water is changed every 48 hours. The stalk of Clava is smooth and in contrast to many other marine hydroids is not inhabited by commensals such as sessile protozoa and other microorganisms. For these reasons and because of its high regenerating power the author selected Clava as material for cultivation. Healthy looking organisms with long stalks were washed by repeated transfers (at least 10 times) in small Petri dishes filled with autoclaved sea water. After this preliminary washing the base of the stalk and the upper part of the body carrying the tentacles and the gonophores, were cut off with a sterile knife or scissors. The separated stalks were then picked up and again washed several times in autoclaved sea water. Finally the stalks were lifted out of the water and cut on a sterile dish into small pieces. By means of a sterile pipette the fragments were transferred to hanging drops of a liquid medium, 2 to 3 fragments for each drop, on a sterile coverslip and placed in a moist chamber. The moist chamber consisted of a glass ring fastened to a slide with vaseline. Only the coverslip, the medium and the pipette were sterile. Cultures were also made in roller tubes and in Carrel flasks under perforated cellophane. From preliminary experiments the use of penicillin (100 units per ml) was found to be superfluous since sterile conditions could be obtained without its use.

1. Fraser, C. M., Hydroids of the Atlantic Coast of North America, 1944, Univ. of Toronto Press, Toronto, p16.

The fragments were selected in lengths of 0.5, 1.0 and 2.0 mm and were immersed in 3 types of media all at 17-22°C. The media were A, autoclaved sea water, B, sea water sterilized by filtration through a Selas porcelain filter and, C, a modification of White's synthetic medium, M & W(2,3).‡

The cells of the 0.5 mm fragments rounded up after a few hours and separated from one another. On the cut edges of the fragments most of the cells became highly vacuolated and increased in size. In Carrel flasks and roller tubes all cells underwent vacuolization. The individual cells survived 24 to 48 hours after which they disintegrated. Of the 2.0 mm fragments almost all the pieces closed over their cut ends. No further change occurred during the next 24 to 48 hours irrespective of the kinds of media and culture dishes used. The 1.0 mm fragments showed the most interesting results. In hanging drops containing autoclaved sea water a migration of endodermal cells started at both cut edges of the fragment after 16 hours. The migrating cells became oblong and narrow. The most characteristic feature was the formation of long projections. The slow progressive migration changed the endodermal layer after 4 days into dissociated cells with long projections. The ectodermal cells showed a tendency to migrate 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. Evidence of growth was very slight, possibly due to the poor nutritional quality of the medium, which consisted only of autoclaved sea water.

In hanging drops containing sea water sterilized by filtration or in modified White's

2. White, P. R., *Growth,* 1946, v10, 249.

3. White, P. R., *J. Cell. and Comp. Physiol.,* 1949, v34, 223.

‡ White's synthetic medium, M & W, used in these experiments was modified by increasing the concentration of the salts (NaCl, $MgSO_4$, $Ca(NO_3)$ HOH, KCl) threefold. The purpose of the modification was to adjust the osmotic value of White's synthetic medium to the osmotic value of sea water.

synthetic medium the 1.0 mm fragments behaved in an entirely different manner. The cells on the cut edges of the fragment rounded up and underwent vacuolization dissociation and disintegration. The rest of the fragment remained unchanged during the following few days. The results with those placed in Carrel flasks and roller tubes (in all kinds of used media) were similar to those in experiments with 0.5 mm fragments.

These experiments show that the behavior of the animal fragments depends not only on the size of the fragments but also on the medium and on the amount of culture fluid, which differed according to the kind of culture dishes used.

In comparing the different media it is of interest to note that migration of cells occurred only when the medium consisted of autoclaved sea water. This was surprising because it had been assumed that sterilization by filtration would be less damaging to the chemistry of the sea water than the prolonged heat of autoclaving. W. H. Johnson(4), working with sterile cultures of *Paramecium multimicronucleata* has also found media sterilized by autoclaving more satisfactory than those sterilized by filtration through a Seitz or a sintered glass filter.§ Possibly sterilization by filtration removes some essential components from the medium.

Summary. *Clava leptostyla,* Agassiz, a marine hydroid, is good material for obtaining separated and migrating cells in tissue culture. A thorough washing of fragments in autoclaved sea water was sufficient to provide bacteria-free conditions. The behavior of the cells depended on the size of the fragments, the kind of medium and the amount of culture fluid. Fragments about 2 mm in length tended to close over along the cut edges thus preventing migration of cells. In 0.5 mm fragments the cells rounded up, underwent vacuolization and distintegration after 24 to 48 hours. In fragments about 1 mm in length migration of endodermal cells was observed but only in hanging drops of autoclaved sea water. Cell migration was never observed in cultures containing sea water sterilized by filtration with a Selas porcelain filter.

4. Johnson, W. H., *Proc. Am. Soc. of Protozool.,* 1950, v1, 9.

§ Personal communication.

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Free Amino Acids in Adult Mosquitoes.* (18976)

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Most of our information concerning amino acids in insects has been accumulated during recent years mainly through the application of the paper chromatographic method. Attention has been centered largely upon the amino acids occurring free in the hemolymph and other body fluids of insects in different stages of development(1,2). Such data have been obtained from a relatively small number of insect species representing only a few orders. In general these studies have been confined to large, immature forms which better lend themselves to analysis of body fluids.

The present work was undertaken as part of a study to elucidate factors which govern the susceptibility of mosquitoes to malarial parasites. Inasmuch as certain amino acids are known to be essential for *in vitro* growth and multiplication of malarial parasites, and since strains are known to differ in their amino acid requirements(3), it appeared that

* This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.

1. Agrell, I., *Acta Physiol. Scand.,* 1949, v18, 247.
2. Levenbook, L., *Biochem. J.,* 1950, v47, 336.