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.

differences in the free amino acid content of various species of female mosquitoes might have some bearing upon their differential susceptibility or resistance to malarial parasites. It was therefore desirable to ascertain whether such differences actually existed.

Materials and methods. Laboratory-reared mosquitoes (*Culex quinquefasciatus, Culex pipiens, Aedes aegypti* and *Anopheles quadrimaculatus*) were used as well as *Culex salinarius, Aedes sollicitans* and *Culiseta inornata* which were collected in this vicinity as fourth stage larvae and pupae and reared to adulthood in water from their respective breeding habitats. Those species colonized in the laboratory were fed a standard diet consisting entirely of finely powdered dog chow. Unfed, adult female mosquitoes were collected 2 to 3 days after emergence and lightly anesthetized with chloroform. These were immediately weighed and homogenized in a tissue grinder with 1 ml of water. The material was centrifuged and the supernatant retained. Protein precipitation was carried out by adding one volume of 95% ethanol to about 5 ml of sample, and centrifuging. The supernatant was shaken with 3 volumes of chloroform (15 ml) with subsequent centrifugation at a low speed, and the aqueous fraction was removed for amino acid determinations. The paper chromatographic method was employed for qualitative and semi-quantitative determinations of free amino acids in the mosquito extracts. Whatman filter paper No. 4 was used with water-saturated phenol as the first solvent and 2,4-lutidine (3 parts to 1 part water) as the second. The quantity of extract usually delivered to the filter paper represented 12.5 mg (approximately 6 mosquitoes) for the one-dimensional chromatograms and 40 mg for the 2-dimensional runs. Amino acids and peptides were revealed by spraying the filter paper with a solution of 0.2% ninhydrin in water-saturated butanol and heating it for approximately 15 minutes at 80°C. Amino acids in pure form were added to various samples of unknowns for verification of preliminary identification based upon position and color of the spots appearing on the 2-dimensional chromatogram.

Results. The following amino acids were demonstrated in the extracts of all species of mosquitoes included in the present study: Alanine, glutamic acid, beta-alanine, isoleucine, leucine, taurine, proline, histidine, serine, valine, methionine, threonine, lysine, arginine, aspartic acid, glycine, tyrosine and tryptophan. Alanine and beta-alanine were present in relatively large amounts as indicated by the size and color of the spots. Glutamic acid and the leucines were less concentrated and the remaining amino acids appeared as much lighter spots indicating a comparatively low concentration. Although isoleucine and leucine were not usually completely separated on the typical chromatogram, this was accomplished by modification of the pH of various extracts. Tryptophan was represented by a very faint spot which could be seen immediately upon heating the ninhydrin-sprayed paper but disappeared soon thereafter. Although it was expected that cystine might be present, a small amount of hydrogen peroxide added to a portion of the various mosquito extracts revealed in only one instance a spot in the position of cysteic acid in the oxidized sample which was absent in the non-treated sample. A spot appearing immediately above beta-alanine, possibly a peptide, has not been identified due to considerable overlapping. This spot is blue when first seen, but it becomes purple in a matter of a few minutes.

Inasmuch as there were no apparent qualitative differences in free amino acids of various mosquito species, it was desirable to determine whether there were any consistent differences in the concentration of amino acids in the various genera and species as judged by parallel, one-dimensional chromatograms in phenol using standard amounts of extracts. A typical chromatogram of three genera of mosquitoes is seen in Fig. 1 (A-C). It will be noted that although there is only a slightly greater intensity of amino acid spots in *C. quinquefasciatus* than in *Aedes aegypti*, both of these culicine mosquitoes exhibit a considerably higher concentration of amino acids than *A. quadrimaculatus*. In contrast to these

3. Geiman, Q. M., Proc. 4th Int. Cong. Trop. Med. Mal., 1948, v1, 618.

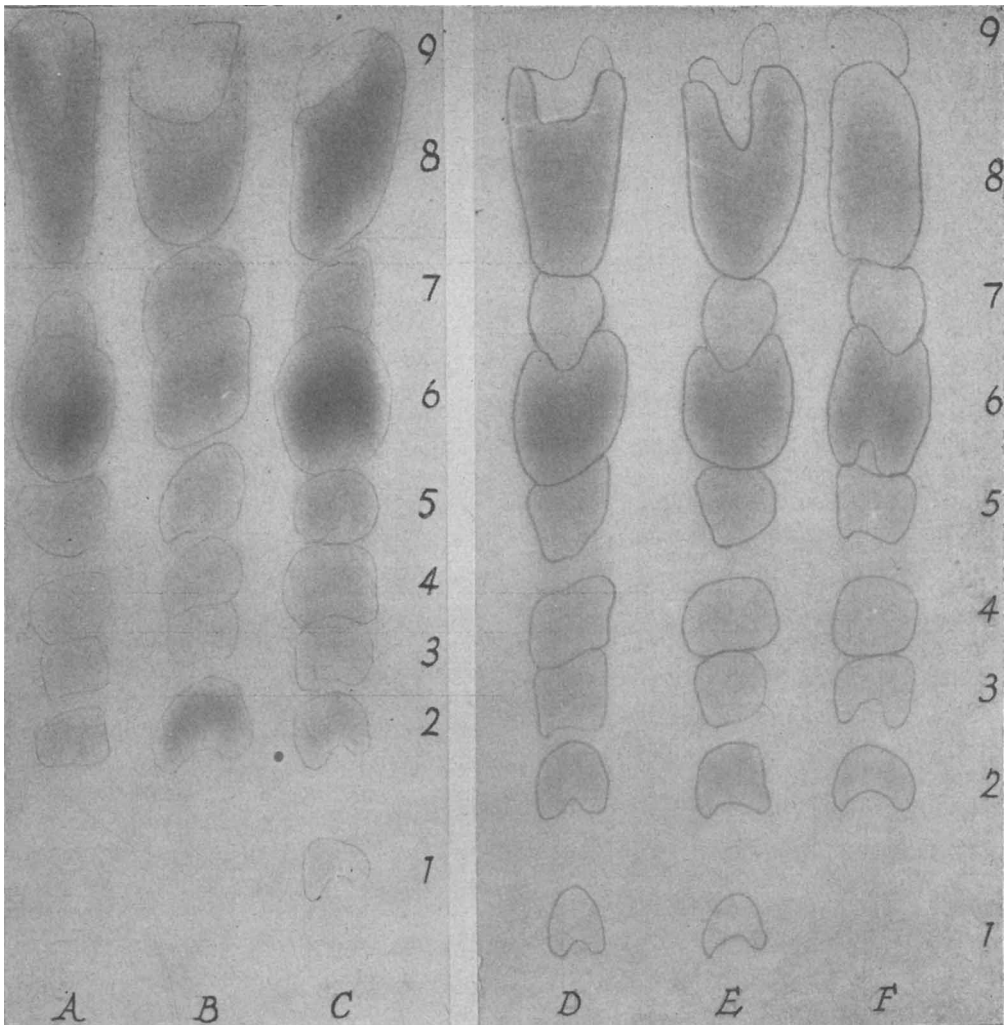

FIG. 1. Chromatograms of ethanol extracts. A, *Aedes aegypti*, B, *A. quadrimaculatus* and C, *C. quinquefasciatus*. (1, aspartic acid; 2, glutamic acid; 3, taurine; 4, serine and glycine; 5, threonine and lysine; 6, tyrosine, alanine and beta-alanine; 7, valine and arginine; 8, tryptophane, leucines, methionine and histidine; 9, proline.) D, *C. pipiens*, E, *C. quinquefasciatus* and F, *C. salinarius*. (1-9 as above).

intergeneric variations semi-quantitative differences between species belonging to a single genus were much less marked. The consistency of the amino acid pattern in 3 species of *Culex* mosquitoes is indicated in Fig. 1 (D-F). Except for the absence of a spot in the position of aspartic acid for *C. salinarius*, intraspecific differences are practically negligible. It is interesting, however, to note the striking similarity between the patterns of *C. pipiens* and *C. quinquefasciatus* since these mosquitoes though differing in their geographic distribution and in certain morphological characters are thought to be subspecies(4).

Extracts of *A. aegypti* and *A. sollicitans* were compared in a similar fashion and again, only slight differences in the intensity of certain spots were noted.

Discussion. It is apparent from the foregoing results that adult, female mosquitoes, like other insects studied, have a relatively high free amino acid content in their body

4. Farid, A. F., *Amer. J. Hyg.*, 1949, v49, 83.

fluids. Other members of the order Diptera have been investigated; however, immature stages were used in order that the hemolymph *per se* could be extracted in sufficiently large quantities for analysis. Agrell(1), working with *Calliphora erythrocephala* pupae, demonstrated the presence of all the amino acids found in the present study with the exception of histidine and tryptophan. He also reported beta-alanine, taurine and methionine for the first time, all of which are present in adult mosquitoes. It is interesting to note that alanine, which is present in high concentrations in adult mosquitoes, likewise was predominate in both *Calliphora erythrocephala* larvae(5) and pupae(1). Although Golberg and DeMeillon(6) found that cystine was a necessary constituent of the diet of *Aedes aegypti* larvae, the present study suggests that it does not occur in free form in the body fluids of the adult. Tryptophan has not previously been reported for any of the Diptera studied. Phenylalanine also appears to be an uncommon constituent of insect hemolymph and other body fluids, having been reported only from larvae of the order Diptera by Finlayson and Hamer(5) in *Calliphora erythrocephala* and by Levenbook (2) in *Gastrophilus intestinalis*. According to Golberg and DeMeillon(6) it is an essential dietary constituent for *Aedes aegypti* larvae. It is the only amino acid of those found by them to be essential for these mosquito larvae that was not detected in the extracts of adult mosquitoes.

Summary. The free amino acids of adult, female mosquitoes (*Culex pipiens, Culex quinquefasciatus, Culex salinarius, Aedes aegypti, Aedes sollicitans, Culiseta inornata* and *Anopheles quadrimaculatus*) were extracted with ethanol and identified by paper chromatography. Extracts of all species of mosquitoes contained 18 amino acids: Alanine, glutamic acid, beta-alanine, isoleucine, leucine, taurine, proline, histidine, serine, valine, methionine, threonine, lysine, arginine, aspartic acid, glycine, tyrosine and tryptophan.

Extracts of *C. quinquefasciatus* and *A. aegypti* exhibited relatively larger amounts of most amino acids than those of *A. quadrimaculatus* as indicated by the size and intensity of spots on the chromatograms. Such differences between 3 species of *Culex* mosquitoes and 2 species of *Aedes* were practically negligible.

5. Finlayson, L. H., Hamer, D., *Nature,* 1949, v163, 843.

6. Golberg, L., DeMeillon, B., *Biochem. J.,* 1948, v43, 379.

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Tumor Transplantation to the Subdural Space of Heterologous Hosts.* (18977)

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It is not known why the anterior chamber of the eye of heterologous hosts is a suitable site for tumor transplantation. The experiment to be reported was devised to determine whether or not the subdural space of guinea pigs is as favorable a transplantation site for Mouse Sarcoma 37 as the anterior chamber of the eye. Following transplantation to the anterior chamber, grafts of this tumor have been found to become subtotally necrotic within 24 hours. Cells from the periphery of the graft invade the iris and the pectinate ligament. Here they multiply and become vascularized by the host. Growing tumor has

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