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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been found in all guinea pigs between the 6th and the 11th day after transplantation to the anterior chamber(1). Regression of tumor tissue invariably takes place, when, in the great majority of animals, only a small portion of the anterior chamber contains tumor tissue. Regression begins at the end of the 2nd week, and is completed within the 3rd week after transplantation. Growth of this tumor has not been observed following subcutaneous transplantation to guinea pigs. The subdural space was selected because it resembles the anterior chamber in the following respects: (1) it contains clear fluid (similar to the cerebrospinal fluid), which drains directly into the blood stream (*cf.* canal of Schlemm); (2) the normal fluid in both spaces is apparently low in protein; (3) both spaces are limited on one surface by a relatively avascular fibrous membrane, and on the other, by a highly vascular reticular tissue; (4) both the iris and the arachnoid develop from mesenchyme in the immediate vicinity of the brain.

Material and methods. Mouse Sarcoma 37, obtained from the National Cancer Institute, has been carried in this laboratory for 58 transplant generations by subcutaneous inoculation of mice of the ABC strain. Adult guinea pigs of either sex were obtained locally, and fed a commercial diet regularly supplemented with ascorbic acid. The transplantations to the subdural space were done as follows: The fronto-parieto-occipital area was depilated (barium sulfide). A curved incision was made extending from behind the right ear to the midline and down to the right eye. A rectangular bone flap (about 1.0 x 0.8 cm) was cut from the right parietal region, (using a portable, electric disc saw), leaving the dura intact. The bone flap was lifted medially, leaving it hinged laterally. A 2 mm slit was made in the inferomedial corner of the dura, and a tumor-laden trocar (No. 17 spinal needle) inserted into the subdural space. The tumor was deposited under the semi-transparent dura in the diagonally opposite corner. The hinged bone flap was closed, and the skin approximated with continuous No. 60 cotton suture. The animals were anesthetized with ether. Cotton applicators moistened with penicillin solution (approximately 25,000 units/cc) were used for sponging. The procedure presented little difficulty when care was applied to avoid hemorrhage on approaching the dura. It lasted less than five minutes and was withstood well by all animals. There were no post-operative deaths. The animals were sacrificed with ether and by exsanguination. The area beneath the bone flap, consisting of dura and subjacent tissue (tumor, arachnoid and brain) was removed *en bloc* and fixed in 10% formalin.

Experiment. Mouse Sarcoma 37 was transplanted to the subdural space of 14 guinea pigs. The animals were sacrificed after 10 hours, and on the 1st, 2nd, 3rd, 5th, 7th, 9th, 11th, 14th, and 18th day after transplantation. One animal died on the 21st day.

The subdural transplantation sites were readily identified during the first 5 days as a hemorrhagic area in the region of the original graft. Microscopically the bulk of the tumor had become necrotic by the first day and free tumor cells were found to penetrate the arachnoid and spread through the subarachnoid space. Growing tumor was found in all animals sacrificed thereafter.

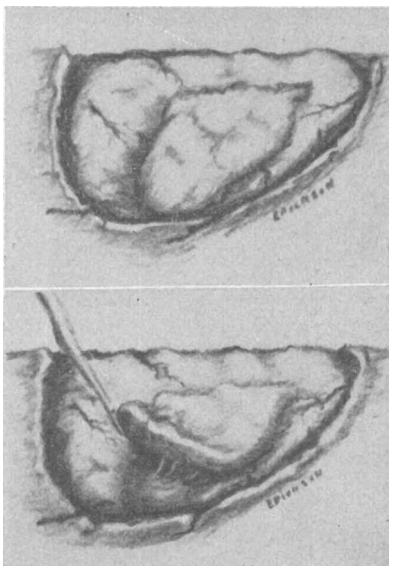

FIG. 1. Mouse Sarcoma 37, 11 days after transplantation to subdural space of guinea pig.

1. Eichwald, E. J., and Chang, H. Y., *Am. J. Path.*, in press.

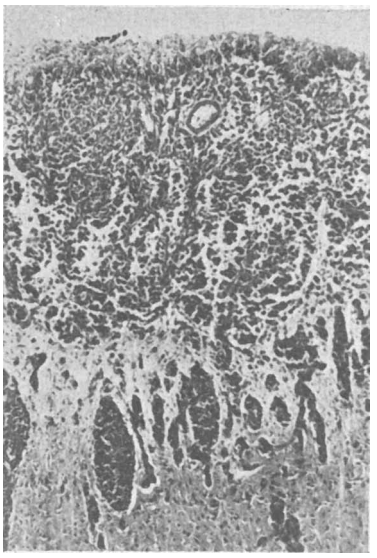

FIG. 2. Mouse Sarcoma 37, 14 days after transplantation to subdural space of guinea pig.

By the end of the 1st week a solid layer of tumor tissue was present in the subarachnoid space, and rows of tumor cells infiltrated the brain along the perivascular spaces. Areas of necrosis and fibrosis were present in the upper layers of the tumor. The animal sacrificed on the 11th day showed an ovoid polypoid meningeal mass measuring 1.1 x 0.8 x 0.4 cm, lying upon and depressing the brain surface (Fig. 1). On the 14th and the 18th day there was deep invasion of the brain with gross extension to the proximity of the ventricle and thick cuffs of tumor cells around the cerebral vessels (Fig. 2). The tumor of the animal sacrificed on the 18th day measured 1.5 x 1.0 cm over its surface and extended 0.4 cm into the brain tissue. This is 3 to 4 times larger than the largest size obtained in this laboratory following transplantation of this tumor to the anterior chamber and several hundred times larger than the average size. The last surviving animal with subdurally transplanted tumor developed a gait disturbance during the 3rd week and died 21 days after transplantation. The autopsy showed hemorrhage and necrosis of the brain and abundant well preserved tumor tissue. No lesions of note were seen in the other viscera.

Summary. Mouse Sarcoma 37 was transplanted to the subdural space of 14 guinea pigs. Evidence of growth was present in all animals sacrificed between the 2nd and 18th day after transplantation. One animal died at the end of the third week, apparently as the result of invasion of the brain by tumor tissue. The pattern of growth in the meninges and brain was similar to that previously observed in the anterior chamber of the eye of guinea pigs, but, after the 9th day the mass of tumor tissue in the brain was greater than that in the eye for comparable periods. There was no evidence of regression of tumor in the brain in contrast with tumor transplanted to the anterior chamber, which usually begins to regress by the end of the 2nd week.

Since this paper was submitted for publication, H. S. N. Greene published an excellent paper on "The Transplantation of Tumors to the Brain of Heterologous Species," *Cancer Research,* 1951, v11, 529.

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Depressor Effect of Potassium Restriction on Blood Pressure of the Rat. (18978)

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Although there is ample evidence(1,2) that an adequate intake of potassium is necessary for the structural integrity of the cardiovascular system, almost no hemodynamic studies

1. Schrader, G. H., Prickett, C. O., and Salmon, W. D., *J. Nutrition,* 1937, v14, 85.

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