In cultures of the ventral lobe of both the dogfish and the skate were many large frothy looking cells, full of large colloidal vacuoles. These cells did not seem to multiply but migrated out among the growing cells.

Cells of the stroma usually grew out later than the epithelial cells and increased in number slowly. They formed wide-spread outgrowths of large, flat, spindle-shaped cells with large round or oval nuclei, and usually contained many irregular granules, probably composed of ingested foreign protein.

These preliminary observations have shown that the pituitary gland of the fish, particularly that of the dogfish and skate, in which the lobes are easily separated, furnishes favorable material for a study of different kinds of pituitary gland cells.

STUDIES ON THE SPONTANEOUS CARCINOMA OF THE MAMMARY GLANDS OF MICE

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The effect of ascorbic acid, dibenzanthracene and fluorescent X (reduced neutral red, Clark) was studied upon in vitro growth of mammary gland tumors of three mice of the Murray strain and ten mice of the Strong strains.

It was found that all three of these substances produced a different effect upon the cultures of carcinomatous tissue than they had exhibited in cultures of chick embryo tissue.

Ascorbic acid and dibenzanthracene had an inhibiting rather than a stimulating effect upon the growth of the cultures. Fluorescent X, instead of remaining in bright red granular form, as it does in supravitally stained cultures of normal cells, frequently formed into clusters of yellow crystals within the cell vacuoles of the growing cancerous epithelial membranes.

STUDIES IN TISSUE CULTURES

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The work of the summer consisted of growing in vitro, at laboratory temperature, various tissues of cold-blooded animals. The work was carried on in consultation with Dr. and Mrs. W. H. Lewis. The hanging drop method on a coverglass was used exclusively. The amount and character of growth in the various cases differed according to the tissue and the composition of the nutrient medium. Briefly, the results were as follows:

a) Lobster heart. The growth of the lobster heart was made up of long cells, resembling the fibroblasts of mammals and birds. These cells grew out in the blood or in centrifuged plasma of the animal itself.

b) Snake kidney. Broad, flat epithelia of dense, closely adhering cells grew out in snake plasma. These may have been, wholly or in part, a flattening out and migration of the cells from the cylindrical kidney tubules out over the flat coverglass.

c) Skate kidney. Flat cells forming a solid layer moved out from the explant, similar in form to those from the snake kidney.

d) Dogfish kidney. Again, the same type of dense, closely adhering cells grew out. All these kidney epithelia grew out slowly and only after some days. Their area greatly exceeded that of the explant in many cases and it seems probable that true growth and not simple migration took place. Several times, these epithelia in the dogfish were observed to have cilia on the lower surfaces of the cells, restricted in position to the "central body" region of the cell. These cilia covered a limited area of the cell surface, roughly corresponding to that occupied by the free dital end of the cell when in columnar form in the tubule. This may be a change in form from the columnar type to a very thin, flat type of cell in response to the environmental stimuli accompanying the opening out and flattening of the tubule into a membrane. If this is true, it is striking that the ciliated zone of the cell surface does not expand but remains localized in the midst of a much greater flat cell surface. No mitoses were observed among these cells. The beating of these cilia was not coordinated between cells or even between individual cilia in the same tuft. In the explant, however, there were still tubules with coordinated ciliary action going on in a normal manner. The ciliated membrane, spread out on the coverglass survived as long as two weeks with no change of the plasma.

e) Frog. For demonstration of dividing cells, gonads of large tadpoles grown in frog plasma or diluted chicken plasma, or in a mixture of these, gave uniformly good results. After seven to ten days of growth at laboratory temperature, large numbers of dividing cells could always be found, especially after an hour or so of slight warming of the preparations. They remained in good condition for three or more weeks without change of medium. In frog plasma, there was much more fat in the growing cells than in chicken plasma. Some of the germ cells moved out of the explant into the surrounding medium where the spireme stages of early maturation could be easily seen. One isolated oocyte moved out and exhibited active amoeboid changes of form for more than twenty-four hours. The greater part of the growing cells, however, were of the mesothelial or endothelial type, forming a sort of stroma. The very active growth of these embryonic tissues contrasts strongly with the much slower development of adult tissues.

f) *Samia cecropia.* Small bits of the ovariole wall grew out in chicken plasma, and remained alive for over a month. These cells had transparent nuclei and numerous cytoplasmic fat droplets.

g) *Bombyx mori* (Silk worm). A large number of preparations of *Bombyx* tissues were made, using various solutions as a medium, such as *Bombyx* blood, centrifuged *Bombyx* blood, chicken plasma, Locke-Lewis solution, modified White's solution and mixtures of these. The *Bombyx* blood presents the difficulty of becoming easily

oxidized in air, turning black with a granular precipitate. The best means of preventing this was to draw off the blood at once into fine capillary tubes. When not exposed to the air, the blood retained, for several hours, its clear yellow appearance. The blood from the pupa was very much more rapidly oxidized than that from the larva. Tissues cut up in blood and mounted in the same were less satisfactory than those cut in salt solution. Ringer concentrated to 1.8 per cent NaCl, 2 per cent NaCl, and Locke-Lewis all gave good results. Agglutinated clumps of amoeboid cells appeared very early after making the preparation and from these grew out long fibers and cells, resembling macrophages of chick tissues. These long, slender, sometimes branched cells migrated over the whole slide. Their nuclei characteristically are extremely granular—fine, uniform granules which stain like chromatin after fixation. Varying amounts of fat droplets are found in the cytoplasm, as well as neutral red granules and chondriosomes. In blood from the pupa or badly oxidized blood from the larva, these cells become filled with brown granules similar to the precipitate in the medium. They often become so full of these as to have a brown color. Other "fibroblasts" appeared after several days from bits of ovariole, from fat masses and other tissues, growing out as delicate spears and finally detaching themselves from the explant. Many of these cells remained alive and active after sixteen days of cultivation. In fragments of testis, clumps of spermatocytes sometimes became disorganized and the cells of the protective wall of the cyst assumed an amoeboid shape and wandered off, leaving the germ cells. These wandering cells were always richly filled with fat droplets. The spermatocytes left behind had a tendency to spread out in a thin layer against the coverglass.

These observations on the cultivation of Lepidopteran tissues represent very preliminary results and continued experiment in this direction is planned. The fact that the wandering cells, "fibroblasts" or "macrophages," grow successfully indicates an appropriate osmotic concentration and an attempt will be made to produce a more extended growth of other types of cells by varying the medium.

ROLLER TUBE CULTURES OF RAT TUMOR CELLS AND SOME RESULTS

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During the summer an analysis was made on the data previously obtained on the cultivation of the various malignant cells in test-tubes. Pure colonies of such cells were obtained by this method from a number of different rat tumors (Crocker 10 and 92 and Walker 315, 319 and 338). The cells multiply in the test-tubes and form colonies that are characteristic for each tumor. By dividing and transferring colonies from tube to tube their number can be increased indefinitely. The malignant cells from the different tumors which composed the colonies differ from one another and from normal cells.