

Cell Types Found in the Harding and Passey Mouse Melanoma Grown *in vitro*.

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The growing of melanoma tissue *in vitro* has presented a good method for identifying the various cells found in this tumor. In sections of the tumor the study of the cells is more difficult because of the heavy pigmentation. In tissue cultures cells migrate from the margin of the explant, retain their specific morphology and are easily identified.

Previously, attempts had been made to grow this tumor *in vitro* but without success. At the beginning of our experiments we found that the fragments of explants rendered the medium alkaline and as long as this alkalinity was maintained no growth occurred. We found that we could best counteract the alkalinity by implanting pieces of normal tissues in the same medium. The growth of the normal tissues produces an acidity sufficient to counteract the alkalinity. Under these conditions good healthy growths were obtained. It was also found that fragments of the tumor will grow by themselves, provided that the margins of the fragments contain relatively non-pigmented macrophages because these cells begin to migrate and their continuous growth causes the medium to become less alkaline.

Three types of cells appear in the outgrowths—macrophages, fibroblasts and melanoblasts. The macrophages can be differentiated into actively moving, sparsely laden cells, and swollen, sluggish cells densely filled with melanin granules. The fibroblasts have oval nuclei and are frequently spindle-shaped. The melanoblasts have spherical nuclei and are highly dendritic and are of 2 kinds morphologically: small cells with slender, uniform dendrites, and large cells with stouter dendrites possessing knobbed swellings along their lengths.

The melanin granules in the various types of cells are distributed as follows: In the macrophages they are usually packed in irregular clumps throughout the entire protoplasm; in the fibroblasts they are massed about the nucleus and are sparse or absent in the pseudopodia; in the melanoblasts they occur in the dendritic processes and in the periphery of the main cell body. The granules are fine and

sparse in the dendrites of the small melanoblasts while in the large melanoblasts they are more numerous and tend to be collected into clumps, especially in the knobbed swellings.

The melanoblasts gave a positive reaction to the dioxyphenyl-alanine (Dopa reaction). Mitoses of the small type melanoblast were observed. No epithelial outgrowth has ever been found in the cultures we have studied.

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Permeability of the Nuclear Membrane to Vital Stains.

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The staining of the nucleus has been considered in general as a sign of decreased vitality of the cell leading to its death. In spite of that we find in the literature a few reliable data indicating that vital staining of the nucleus may occur. This has been accomplished by immersing living cells in solutions of various dyes, both of plants and of animals. It has also been accomplished by micro-injecting various dyes, especially the sulphonated pH indicators of Clark and Lubs into amoebae and tissue culture cells. The stain blown against the nuclear membrane is taken up by the nucleus, but after a short time the color disappears. The possibility at least of a transitory vital staining of the nucleus seems to be fairly well established.

It was of interest to note whether the nucleus is stainable by any dye or only by certain groups of dyestuffs, that is to say, the question was: whether the nuclear membrane is selective or freely permeable. In order to test this it was necessary to micro-inject directly into a cell, since it is well known that many dyestuffs do not penetrate living cells from the environment.

Amoeba dubia and *A. proteus* were selected for the experiments. Aqueous solutions of the stain were blown by means of a micro-pipette into the cytoplasm directly against the nucleus without injuring its wall. The staining of the nucleus was considered as vital only when the amoeba recovered completely. When the amount injected was very small it was possible to get the staining of the nucleus only, but when the amount was larger the surrounding cytoplasm was also stained. About 40 different dyes were