

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 dioxyphenylalanine (Dopa reaction). Mitoses of the small type melanoblast were observed. No epithelial outgrowth has ever been found in the cultures we have studied.

## 8027 P

### 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 micropipette 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

used and in all cases in which a vital staining of the nucleus occurred the color was transitory, fading away sooner or later.

Vital staining of the nucleus was obtained with dyes falling into the following groups\*: 3 nitro dyes, 7 azo-dyes, 5 thiazins, 4 oxazins, 1 amido-azin, 1 safranin, 1 diamino-triphenylmethane, 4 tri-amino-triphenyl-methanes, 2 amino-hydroxy-triphenylmethanes, 1 pyronin, 1 rhodamin, 3 fluoran derivatives, 4 sulphonphthaleins, 1 acridin. No dye was found that would not penetrate the nuclear membrane by injecting the amoeba close to the nucleus. The following dyes, not included in the above list, in the most diluted solution, also penetrated the nuclear membrane, but recovery occurred only when a small part of the nucleus was stained: Water blue, Azo blue, Methyl blue, Alizarin red, and Ruthenium red. Such dyes as Sodium carminate, Trypan red, Nigrosin, Vital red HR, Rosindulin GG and Congo red always kill when they enter the nucleus, even in the most diluted solution.

The listed dyes are basic or acid, used in neutral, basic or acid solutions, lipid soluble or lipid insoluble, crystalloidal or colloidal, organic or inorganic compounds. It is of interest to note that these dyes gave vital staining of the nucleus only when injected. By immersing amoebae into the dye solutions no staining of the nucleus occurred even when the dye was able to penetrate the cell. The stain fades within a few seconds (*e. g.*, some acid dyes) to at least 15 minutes (*e. g.*, Toluidine Blue). The length of time depended largely upon the concentration, the amount injected and the acidity or basicity of dyes. The coloration with acid dyes progresses and disappears quickly. In general, basic dyes penetrate the nucleus in an advancing wave and the color disappears slowly. The decoloration of a nucleus stained with basic dyes inside the cytoplasm proceeds faster than that of an isolated nucleus stained outside the cell in the culture medium. Dyestuffs having a complicated chemical structure are more toxic than those having a simple one. We may roughly say that dye molecules having many benzene rings are more toxic than those having few. But besides that we must take other factors of toxicity into account.

An irreversibly injured nucleus is always eliminated or pinched off. When, during the pinching off process, the irreversibly injured nucleus was prevented from passing out by holding it back with a microneedle, first, only the injured cytoplasmic part was thrown out, but afterwards the nucleus was also pinched off.

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\* Classification of the dyes following Conn, Biological Stains 1929.

We conclude that the nuclear membrane is freely permeable to a great variety of substances both crystalloidal and, at least, fine colloidal. How far this may be generalized further researches will show.

## 8028 P

**Disposal of Dyes by Proximal Tubule Cells of Chick Mesonephros in Tissue Culture.**

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Dyestuffs have been used extensively for studying renal activity. However, from evidence in the literature there seems to be no rule governing intake of the various kinds of dyes by the tubule cells. For example, some lipid-soluble dyes, to which cells are known to be permeable, are not passed into the tubular urine while, of the lipid-insoluble dyes, some are known to be passed into the urine and others are not, with no apparent reference to their chemical constitution.

By means of the tissue culture method we have been engaged in testing the behavior of the proximal tubules to a series of dyes. Aqueous solutions of the dyes in various concentrations are mixed with the usual tissue culture medium in which are planted fragments of the functioning mesonephros of a 9-11-day chick. This method affords a means of studying the problem in a more direct manner than hitherto possible. Moreover, it enables one to restrict the problem to the proximal tubules which remain alive and functional for days in the explant. Isolated segments of the tubules regenerate their cut ends and become converted into closed sacs into which the progressive accumulation of color can be observed microscopically.

In this report the results of experiments are given on the use of the following dyes. They are the lipid-soluble basic dyes, Neutral red, No. 825; and Nile blue sulfate, No. 913; which are in general use as vital stains, and the lipid-insoluble acid dyes, Xylene cyanol FF, No. 715\*; Amaranth, No. 184; Acid fuchsin, No. 692; and Orange G No. 27. These acid dyes resemble the sulphonephthaleins in forming highly dissociated, sulfonated compounds in

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\* The numbers appended to the names of the dyes are those given in Rowe's Colour Index, 1st edition (Society of Dyes and Colourists, Bradford, Yorkshire).