

habits and parasitism of *Krameria canescens*, xeno-parasitism (experimental production of parasitism), and the origination of parasitism, the latter a most suggestive philosophical discussion.

"Some Contributions to the Life History and Cytology of the Smuts," by B. F. Lushman (*Transactions Wisconsin Academy of Sciences*, 1910), adds materially to our knowledge of the development of these plants.

"A Catalogue of the Flowering Plants and Ferns of Connecticut," by a committee of the Connecticut Botanical Society (Geological and Natural History Survey, 1910), attempts to give "an accurate and authoritative catalogue of all the plants known to grow without cultivation in Connecticut," and it appears to have accomplished this purpose so far as the flowering plants and ferns are concerned. In the summary we learn that there are included in the catalogue 74 species of pteridophytes (all native) and 1,407 native spermatophytes, with 461 introduced species.

In passing we should notice favorably Wettstein's "Handbuch der Systematischen Botanik" (Leipzig, Franz Deuticke, 1910-11), a thick volume of over nine hundred pages. Seven great phyla ("Stämme") are recognized, viz., *Myxophyta* ("conventionally" placed here), *SCHIZOPHYTA*, *ZYGOPHYTA* (including *Peridinieae*, *Bacillarieae* and *Conjugatae*), *PHAEOPHYTA*, *RHODOPHYTA*, *EUTHALOPHYTA* (including *Chlorophyceae* and *Fungi*) and *CORMOPHYTA* (including *Archegoniatae* and *Anthophyta*). The work will prove a most helpful one for the student of systematic botany, and merits translation into English.

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SPECIAL ARTICLES

ON THE STEREOTROPISM OF EMBRYONIC CELLS

IN a former paper, describing the development of nerve fibers in foreign media, the hypothesis was advanced¹ that the fibers require some form of solid support in order to carry out the growth process, which, as was

shown, is a form of protoplasmic movement. The present communication presents in brief form the results of some experiments on the movement of embryonic cells, which show beyond doubt that the hypothesis holds true for the cells of the mesoderm and the medullary tube of the frog embryo. With reference to the outgrowing nerve fibers, however, the observations are too few to warrant any more definite statement about them at present.

In the previous experiments the solid support was given in the form of a fibrin network, derived from the clotting of fresh lymph. In the present study spider web was used to support the small pieces of transplanted tissue immersed in various fluid media. The object of the investigation being to compare the behavior of embryonic cells in the same medium, with and without solid support, two sets of preparations were made; one in which the tissue was placed in a simple hanging drop in a moist chamber, the other in which the drop was supported from below by a closely woven spider web. The moist chambers were made by sticking glass rings to object slides by means of vaseline and sealing to the ring a cover slip with the culture drop. The spider webs were tightly spanned over the upper surface of the glass rings prior to fastening the latter to the slide; the pieces of tissue to be studied were then transferred in very small drops of fluid to the web, and the preparations immediately covered by cover slips, also coated with the web, so that the pieces of tissue remained supported between two layers of the fabric.

The fluid media which were employed were purposely varied considerably; physiological salt solution, Locke's solution and Ringer's solution (without sugar) of full strength and diluted, and also defibrinated frog's serum, were all used. The best results were obtained with the defibrinated serum, but some positive results were obtained with all the inorganic solutions used, showing, in agreement with the work of M. R. and W. H. Lewis,² compatibility of wide range between tissue and medium.

¹ *Journ. of Exp. Zool.*, Vol. 10, 1910.

² *Anatomical Record*, Vol. 5, No. 6, June, 1911.

The results of the work are best typified in a single group of experiments which were made on May 21. Twelve embryos of *Rana palustris* were used and from each the whole neural tube with the adjacent mesoderm was cut out in a dish of saline. In each case the piece of tissue was divided into two parts and one half mounted in a simple hanging drop of defibrinated frog's serum, the other half in a drop of the same fluid held between two layers of spider web, as described above.

Of the twelve specimens in the first group, none showed any active cell movements during the first six days, and after that only one specimen, to be referred to below, manifested anything of the kind. In these specimens many cells became loosened from the main mass of tissue, remaining inactive and rounded, though the main mass itself remained alive and in a number of cases differentiated into striated muscle which exhibited frequent twitching.

Of the twelve specimens mounted between webs, eleven showed very active movements of the embryonic cells, which began even on the same day on which the preparation was made. Only one of these preparations gave negative results, and this was one that was injured by rough handling in mounting. The behavior of the cells in these cases is fundamentally different from that of the other cells in the simple drop without support, and the general appearance of the preparation is not unlike that of specimens isolated in clotted lymph, though there are some differences. Numerous cells extend from the main mass of tissue, sometimes singly, sometimes in masses. The cells are spindle shaped, branched or polygonal, with hyaline protoplasm in the processes and at the angles of the cells, the cell body being gorged with yolk granules. Careful focusing shows that the active cells are confined to two levels, viz., the under surface of the cover slip and the lower surface of the drop which rests on the layer of web spanned across the glass ring. Cells which are partly loosened between these two levels remain rounded and inactive, just as in the hanging

drop preparations. In a very large number of cases the active cells are found to have definite relations to the web fibers. Often strands of spindle-shaped cells, resembling the cells of an embryonic tendon or the Schwann cells of a developing nerve, are found closely applied to slender bundles of web fibers. Again, spindle-shaped cells, sometimes with very long processes, lie in intimate contact with single web fibers. Where two such fibers cross, the cells may assume a tri- or quadripolar shape, with a process running along each fiber. Frequently the cells are closely attached to the cover slip and are then usually of flattened polygonal shape, forming in many cases extensive sheets. The cells may change their shape and move from place to place, or they may remain in one spot for days, practically unaltered in shape. After a few days typical pigment cells developed in a number of cases and these too assumed definite relations to the web fibers. In only two of the specimens were outgrowing nerve fibers observed. They were in all essentials like those previously found in the clotted lymph preparations, and in each case they crept along the lower surface of the cover slip, without definite relations to the web.

In this series of experiments the contrast between the preparations in the free hanging drop and those supported by web is so marked and so constant that it is impossible to escape the conclusion that the cells are able to execute their movements only when some solid framework is given them. The experiments in which saline solutions were used instead of serum bear out this same conclusion, though they are not so striking on account of a larger proportion of them giving negative results. The saline solutions are clearly not such good culture media as the serum, but notwithstanding this a number of specimens showed marked cell movement and remained alive for days.

Some of the experiments at first sight seemed to oppose the conclusion stated above, but on more careful study were found to afford a striking confirmation of it. This was brought out especially in a series cultivated in Locke's

solution, where the small hanging drop of saline frequently spread out and the tissue was left closely adherent to the cover slip. In two such cases, and in a similar one in which serum was used, cell movements took place, but the cells which exhibited movements were all in contact with the lower surface of the cover. One case showed nerve fibers. In another interesting case the drop of culture fluid was larger and touched the bottom of the chamber. The piece of tissue sank to the bottom and became adherent, sending out a number of short hyaline protoplasmic processes along the surface of the glass.

These and previous experiments show, then, that the movements of embryonic cells take place when the cells are in contact with a fibrin net, the fibers of a spider web or the surface of glass coverslips and slides, and that they occur in a considerable variety of fluid media. On the other hand when the embryonic tissue is suspended free in a drop of fluid,³ no cell movement takes place, though differentiation of tissue may result. In this movement and orientation of the cells we have before us a form of stereotropism, of which, however, the exact nature remains for the present undetermined. Whatever it may prove to be, it can scarcely be doubted that it is an important factor in normal development, influencing the movement and segregation of pigment, mesenchyme and nerve cells at least, and probably also the growth movements of nerve fibers.

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³M. R. and W. H. Lewis (*op. cit.*) state that growth apparently takes place as well in fluid media as in solid, though they admit that the outgrowing cells often creep on the cover glass or the bottom of the dish. I feel confident that careful examination of specimens with reference to this point will show that the growing cells are always in contact with the glass, except that the surface film of the free hanging drop may sometimes act as a substitute for the solid surface, as I have occasionally found to be the case in a series of experiments with tissues of the chick embryo, to be described later.

ON THE INCREASE IN OXIDATION IN THE EGG AT THE BEGINNING OF DEVELOPMENT

It was observed by O. Warburg that the oxidation in the sea-urchin's egg is increased when it is fertilized or placed in a hypertonic solution, which induces parthenogenesis. Warburg observed an increase in oxidation in the fertilized egg when placed in pure NaCl solution (which also causes parthenogenesis in the unfertilized egg), but in order to insure the life of the eggs long enough for the experiment he added a trace of NaCN to the NaCl (and to the control).

The addition of NaCN was objected to by Loeb and Wasteneys, who found no increase in oxidation if the cyanide was omitted. Apparently the cyanide, or hydroxyl ions liberated by hydrolysis, had something to do with the result. We found that NaCl increased oxidation in unfertilized eggs about one fourth.

We made similar experiments on unfertilized eggs using an isotonic NaCl solution containing the same hydroxyl ion concentration as the sea water (made by the addition of NaOH). The result was an increase in oxidation in the NaCl solution to double its rate in sea water. In other words, the NaCl solution in the presence of OH-ions causes an increase in oxidation in the unfertilized egg. This was found true also of another parthenogenetic agent.

Microscopic examination showed that the eggs formed "fertilization membranes" in the NaCl solution, and some of them that were returned to sea water after the close of the experiment segmented and produced cilia. In short, the NaCl + OH ions start development and the increased oxidation may be due to the same cause as the increased oxidation in the fertilized egg.

One of the authors has shown an increase in permeability of the egg to ions, at the beginning of development. He suggests that the NaCl solution causes an increased permeability of the egg to OH-ions, and that the latter penetrate the egg and accelerate oxidation. The morphological changes in the egg