Histology. — "*On the Determination of Polarity in the Epidermal Ciliated cell. (After experiments on Amphibian Larvae)*". By Dr. M. W. WOERDEMAN. (Communicated by Prof. L. BOLK).

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It is a well-known fact that in the early stages of their life the larvae of amphibians have an epidermis, provided with ciliated cells. This cannot be observed distinctly in all species, for they differ largely as to the number of ciliated cells. Nor are these cells evenly distributed over the epidermis of one and the same larva; there are spots where they are scattered thickly, while they occur more sparsely in other spots.

The ciliary movement causes a slow rotation of the larvae while the latter are still inclosed in their jelly-like envelope. When this envelope is removed, the exposed larvae will be seen to keep up their rotatory motion owing to the ciliary movement, just as the larvae that have already left their envelope. At the same time a rather violent current may be observed in the water encircling the larva. It is self-evident that this current is strongest where most ciliated cells are collected. Strong currents are, therefore, distinguishable along certain parts of the larval body, weaker streams along other parts, which e.g. have been minutely examined by ASSHETON [1]) for Rana temporaria and Triton cristatus and have been represented in plates for larvae of various age-periods.

It appears that in these animals the first action of cilia is noticeable in larvae where the neural folds are still open, shortly before their closure. There is a strong current in the water round about the larva from head to tail along the neural walls. My own researches were made on Rana esculenta and Triton alpestris larvae. I found that in these amphibians the ciliary movement begins when the neural walls are in part united. The direction of the fluid-streams along the larval body I found to agree in the main with ASSHETON's schemata, although there were also some differences. This, however, is not to the purpose. The direction of the ciliary movement in normal larvae of Rana esculenta and Triton alpestris was,

[1]) R. ASSHETON. Quarterly Journ. of micr. Science New Series. Vol. 38. 1896, p. 465.

therefore, closely examined and represented in diagrams. It was further established that the fluid-streams flow invariably in the same direction. A reversed direction of the ciliary movement seems to have rarely been observed in metazoa. (ERHARD)[1]).

This implies such a structure of the ciliated cells that a ciliary movement is only possible in one direction, the cells present a certain asymmetry in their structure; besides their polarity (by which base and ciliated free surface are distinguished) there is an "accessory polarity" (vide ROUX[2]) for these ideas). The question has been considered whether this accessory polarity could be reversed artificially, in other words, whether the ciliated cell could by some artificial method be made to move in the opposite direction. This question is connected with another, viz. in how far the ciliary movement depends on the position of the ciliated cells relative to the axis of the body.

Experiments performed by v. BRÜCKE[3]) and those made this very year by MERTON[4]) bear on this question. They did not succeed in bringing about a reversion of the polarity. Now it has been evidenced by numerous experiments that in the embryonic development there is a period in which the ectoderm, from which the larval epidermis is derived, is still indifferent. SPEMANN[5]) e.g. found that at the beginning of the gastrulation ectoderm, destined to build up the medullary plate (so-called presumptive medullary plate), could be replaced by presumptive epidermis. Larvae developed with normal medullary plate and normal epidermis. The fate of the ectoderm-cells in that stage of development has not been, or has not yet been determined. The ectoderm is still in a high degree liable to change („umbildungsfähig"). Whether in that phase it is still completely indifferent cannot be decided without a detailed inquiry. It occurred to me that an inquiry into the polarity of the cell might afford some indication, as the polarity of the cell may already be determined before its organogenetic function. SPEMANN's experiments regard the organ-determination. Now, how about the polarity of the cell? When is it determined? The experiments in which I tried to solve these questions, I performed on larvae of Rana esculenta and of Triton alpestris in the Zoological Institute of the Freiburg University (Director Geheimrat Prof. Dr. H. SPEMANN).

[1]) ERHARD in ABDERHALDEN's Handbuch der biologischen Arbeitsmethoden.

[2]) W. ROUX. Terminologie der Entwicklungsmechanik der Tiere und Pflanzen. Leipzig. Engelmann. 1912.

[3]) E. TH. v. BRÜCKE. Pflüger's Archiv. f. d. ges. Physiol. Bnd. 166. 1917.

[4]) H. MERTON. Pflüger's Archiv. f. d. ges. Physiol. Bnd. 198. 1923.

[5]) H. SPEMANN. Sitzungsber. d. Gesellsch. naturf. Freunde. Berlin. 1916. N°. 9.

I started by ascertaining whether there were developmental stages in which the polarity of the ciliated cell is reversible, that is stages in which the ciliated cells can be forced to move in a direction other than the normal.

After circumcision with fine glass-needles patches of ectoderm were detached from their sublayer and after a rotation of 180°—90° brought again to coalescence. After the wounds thus made were healed, which occurred in a marvellously short time, the direction of the ciliary movement was determined by examining the larvae in water in which granules of carmine had been suspended. A disadvantage of this procedure appeared to be that the borders of the wound are soon altogether invisible, so that the extent of the reversed regions cannot be traced out. For this reason I used the method adopted by W. VOGT [1]), who interchanged ectoderm patches of larvae stained vitally and those of nonstained larvae. After it had first been ascertained that vital staining with Nile-blue sulphate did not affect the ciliary action, I stained one of two larvae of the same age-period, and the other I did not. Of these two larvae fragments of ectoderm of a very well-defined shape and of the same size were excised and interchanged. In the transplantates the colour remains very well localized, it does not diffuse and enables us to recognize the contour of the implantate for many days still. Moreover, the shape of the implantate is indicative of its original position, consequently of the direction of the currents produced by the ciliary movement under normal circumstances. I shall not give an account of the various experiments, but I will describe briefly the final result of all of them.

It became evident that when a ciliated cell has once begun to vibrate it cannot be made to move in another direction. Patches of ectoderm being implanted in the wrong direction persisted to move in their original direction for days, nay, even till the ciliated cells had disappeared from the epidermis. Even before the ciliary movement has begun, its direction has already been established. When ectoderm fragments are reversed 180° before the ciliary movement begins, the ciliated cells will afterwards reveal a vibration opposite to that under normal circumstances. The youngest stages of development, however, are excepted in this respect, as it appeared that in blastulae and in incipient gastrula-stages the blastula-roof resp. ectoderm-patches can be reversed, without affecting the direction of the movement, when afterwards the larvae begin their ciliary action.

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1) W. VOGT. Verhandl. deutsch. zoolog. Gesellsch. Bnd. 27. Sept. 1922. p. 49.

It is evident, then, that in the young stages, just referred to, the polarity of the cell has not yet been determined. It also appeared from the experiments that the determination takes place during the gastrulation. If the blastopore is still like a straight or slightly crescent-shaped slit, the future ectodermal ciliated cell is still indifferent. But as soon as the blastopore has become horseshoe-shaped and still later circular, reversion of ectoderm without reversion of the future direction of the ciliary movement is not possible.

It follows, then, that the period of determination of the polarity of the epidermal ciliated cell falls in an early stage of gastrulation.

Now we had to ask if the determination of the polarity of the cell coincided with the organ-determination.

To ascertain this we interchanged patches of presumptive epidermis and presumptive medullary plate in very young larvae, and we watched the subsequently developing ciliary movement while giving due attention to the original position (vital staining). Stated briefly the results were to the following effect: When after the operation larvae appeared with a normally developed medullary plate (part of which was consequently generated by presumptive epidermis) and with a normally developed epidermis (part of which was consequently formed by presumptive medullary plate), the larvae exhibited normal direction of ciliary movement i.e. the ciliated cells have not developed as they would have done originally, but have adapted themselves to their new environment. If the organ-determination has not yet been effected, the direction of the ciliary movement can still be influenced by the environment. But if abnormal larvae developed with a deficient medullary plate or with pieces of the medullary plate in their epidermis, then the direction of the movement appeared to have developed on the implantates according to the origin of the implantates and appeared not to have been influenced by the new environment.

Our experiments, therefore, seem to imply that the determination of the polarity of the cells and of the organogenetic function either occur synchronously or at all events with a very brief interval of time.

It should be borne in mind, however, that the organ-determination in the ectoderm does not occur everywhere at the same time. SPEMANN'S and Mrs. MANGOLD-PRÖSCHHOLD'S [1]) experiments have shown that this determination starts from what they have termed an „organisation centre", which is located in the dorsal lip of the blastopore.

[1]) H. SPEMANN. Arch. f. Entw. mech. der Organismen. Bnd. 48. 1921.

Furthermore, experiments by O. MANGOLD[1]) tend to show that after the conclusion of the gastrulation, i.e. when the region of the medullary plate has already been determined, ectoderm of the ventral half of the larva can still form mesoderm or entoderm. From this we see that this ectoderm has not yet been determined.

My experiments to find an answer to the question if there is any relation between the determination of the polarity of the cell and of its organogenetic function, were carried out in the region of the future medullary plate. A more extensive investigation is required for the purpose of ascertaining whether the phenomenon that the determination of the polarity of the cell almost coincides with that of the organogenetic function of the cells holds generally or only for the region of the medullary plate.

In a subsequent communication I intend to discuss the histophysiological data regarding the ciliary movement obtained in the experiments reported in this paper.

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[1]) O. MANGOLD. Verhandl. deutsch. zoolog. Gesellsch. Bnd. 27. Sept. 1922. p. 51.