Histology. — *"On the Development of the Structure of the Eye-lens in Amphibians"*, by M. W. WOERDEMAN. (Communicated by Prof. L. BOLK).

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The fibres of the crystalline lens in amphibians do not run straight, no more than in other animals but are curved and specially arranged. This has been examined in detail by C. RABL [1]).

Among the data produced by this author we only mention here that every fibre has two free endings; the one lies in the anterior, the other in the posterior part of the lens. In the anterior hemisphere of the crystalline lens the endings of the lens-fibres come into contact into a vertical plane while in the posterior hemisphere a horizontal plane is formed. The planes extend from the surface to the centre of the lens, gradually decreasing in size. The front plane, namely, is a vertical triangle, the hindmost is a horizontal one.

It struck SPEMANN that the anterior lens-ray coincided with the plane of the foetal eye-fissure. Seeing that the close co-operation between the development of the lens and the optic vesicle has been proved in many experiments with larvae, the question might be raised whether also this phenomenon resulted from a similar co-operation. Prof. SPEMANN suggested to me an experimental research on this problem, for which suggestion I wish to express my obligations to him, as well as for the free access he granted me to his laboratory during this research.

Two ways offered for solution. First of all, before a lens-anlage is visible, the epidermic region from which it is to originate may be excised, rotated and brought to coalescence again. When assuming the disposition of the lens-fibres to depend on the position of the optic vesicle, it may reasonably be expected that the normally located optic vesicle will induce from the rotated epidermic region a normally structured lens.

If, however, the structure of the lens grows through "self-differentiation" from the epidermis, a lens will be engendered of which

[1]) C. RABL, Zeitschr. f. wissensch. Zoölogie. Bnd. 63. Heft 3. 1898.

the anterior ray does not coincide with the plane of the foetal eye-fissure, but is inclined to it.

The second method to be followed was the rotation of the anlage of the eye, while the epidermic region, from which the lens is to arise, is left unchanged.

I acted in either way, but the first proved to be preferable by far from a technical point of view.

The rotation of the future lens-anlage, however, is liable to dangers that might tend to annul the validity of the results. The possible errors are the following: In the first place the larvae are operated upon long before the anlage of the lens has appeared. There are no outward signs then by which it is possible to ascertain which cells of the epidermis are destined to become lens cells. It has, therefore, first to be established where the cell-group lies that will furnish the anlage of the lens, lest the wrong area should be rotated.

The determination of the topographical relations in young larvae I performed by means of local vital staining. To this end small pieces of agar, soaked in a solution of Nile-blue sulphate, are laid for the purpose on a special part of the larval body. The ectoderm cells of this part take up the stain and thus a clearly defined region of the larva is stained vitally. As the stained region remains visible for a long time, its further destiny can easily be traced. This method, suggested by VOGT, yielded very good results. Thus it is also possible to define the area, whose cells are afterwards developed into the crystalline lens.

But even if this area is known, its rotation is yet attended with peculiar difficulties, since the extension of the area in a special larva cannot possibly be distinguished before the first stage of development of the lens. When excising a definite ectodermic region care should be taken to keep the lens-anlage in the centre of it, since only in that case the anlage will not be displaced relative to the optic vesicle.

Fig I*a* represents a larva with a diagram of the optic cup and the lens in their latter position. The anlage of the lens is dotted and divided by a line (the anterior lens ray) into an anterior and a posterior half. Fig. I*b* shows a correct rotation. The upper border of the wound *A* becomes the posterior border, the posterior border *B* becomes inferior border. The lens-anlage is also rotated 90°, but is held in its position relative to the eye-anlage. In Fig. II*a*, however, we see an epidermic region cut out, that has not the lens-anlage for its centre. Rotation of this region induces the position shown in II*b*, from which it is evident that now the anlage of the lens does not correspond anymore with the optic vesicle. Now we have to ask whether normal development of a lens is possible here.

We know from SPEMANN's[1]) researches that in Bombinator larvae

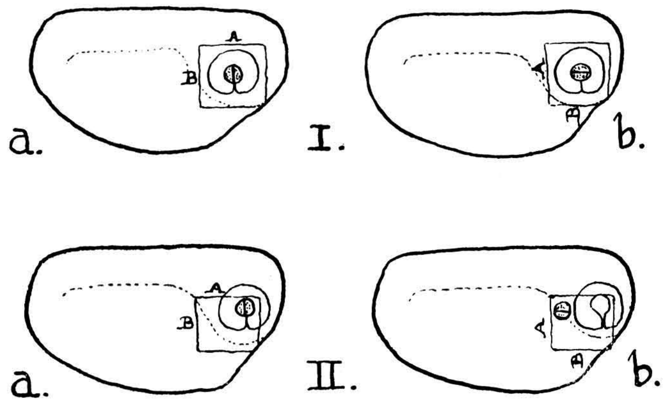

Fig. 1. Schemata (explanation in the text).

the lens-anlage is not competent to differentiate itself, i. e. that the displaced anlage cannot develop into a lens without the presence of the optic vesicle. In case II*b* therefore, it might occur that the displaced anlage of the lens does not develop further after the operation. On the other hand we also know that in Bombinator a lens can be developed from other epidermic regions than are commonly used in normal circumstances, if only they are transplanted over the optic vesicle.

It is conceivable, therefore, that in the case II*b* the ocular cup induces a lens from the epidermic region that has been transplanted over the optic cup. This will give origin to an eye with a lens that has not emanated from the rotated anlage of the lens.

In Rana esculenta the relations are different. In this amphibian the lens-anlage transplanted outside the sphere of influence of the optic cup, can develop into a lens, but it seems that the optic cup is not competent to induce a lens from other epidermic regions.

It follows then that, in Rana esculenta, in the case II*b* an eye without a lens and an ectopic lens may be expected.

I had to bear in mind, therefore, that the result of my experiment would be of value only if it could be established with certainty that after the rotation the lens-anlage would again coincide with the optic cup. Since the anlage of the lens and the optic cup were invisible in the stages operated upon, this certainty could be acquired

[1]) H. SPEMANN, Zoöl. Jahrb. Bnd. XXXII. Heft 1. 1912.

only through a special technique, by which we were also able to preclude a third error.

This third error is the following: After embryonic transplantation it will sometimes occur that the growth of the implantate is slightly slower than that of its environment. This was also possible in the experiment with rotation of the lens, and might lead to a secondary displacement of the implantate, that is pushed away by the more rapidly growing environment.

Considering that the borders of the implantate remain visible only for a short space of time, I had recourse to the following artifice to distinguish the borders for a longer interval. The operations were performed on larvae of Bombinator pachypus and Rana esculenta. Since among these larvae some specimens occur of different pigmentation, two larvae of equal age-period may be taken, the one of which being of a dark, the other of a light pigmentation. The lenticular regions of these larvae are then exchanged and rotated, so that we then have a dark larva with a light implantate and the reverse. Now the borders of the implantate will be visible for some time and we are in a position to ascertain whether the eye-anlage, on becoming visible, really lies beneath the centre of the implantate.

If the larvae, at my disposal, did not differ sufficiently as to pigmentation, I had recourse to vital staining also of some of the larvae with Nile-blue sulphate, while others were not stained. Now, in the operations the lenticular area of a vitally stained larva was substituted by that of an unstained larva of the same age-period, and was also rotated. This again yielded the advantage that the borders of the implantate remained visible for several days running.

For further investigation I discarded all those cases in which the lens-anlage appeared to have shifted from its normal location after the operation, so that I feel assured that the lenses, obtained without exception by the other experimental animals, have indeed originated from rotated lens-anlages.

The stage used for the operations was very young. The neural tube was not yet closed, so that optic vesicles had not taken origin yet. A thickening of the epidermis on the spot of the future lens-anlage is also still totally lacking. Square patches of ectoderm were excised, and subsequently rotated 90°. After the larvae had acquired distinctly visible eye-lenses, they were killed and fixed in MICHAELIS's mixture, stained in toto with borax-carmin and embedded in celloidin-paraffin. The eyes had been cut in such a way that the plane of section runs parallel with the plane of the iris. Hereby the foetal eye-fissure and the direction of the lens-rays become visible in the microscopical section.

Unfortunately the lens is a very difficult object for microscopical examination and I should have killed my experimental animals earlier, for on the whole the lenses were already too hard for suitable sections. In spite of this of the rather large number of experimental animals enough specimens were left to obtain usable preparations. It now appeared that the anterior lens-ray was disposed horizontally in the larvae operated upon, as well in those of Bombinator as of Rana, in other words, that the ray had rotated 90°. This phenomenon is more or less surprising, as it proves that the development of the structure of the lens takes place through "self-differentation", i. e. the environment plays no influence upon it. Now, since in Bombinator the development of the lens depends on the influence of stimuli emanating from the optic cup, one might rather have expected, in the experiments with Bombinator larvae described above, that the optic cup should have induced a normally structured lens from the rotated lens-anlage.

In the Rana-larvae the result comes up to our expectations. When examining the results of the experiments more narrowly it appears now that in the young stages used for operation, the future lenticular structure has already been determined, in other words, that then the direction in which the cells are to grow is established. This is a new fact contributing to our knowledge of the polarity of cells, on which I published a paper some months back [1]).

It also appears that the optic cup has no influence on the direction of the growth of the lens-fibres.

Lastly it follows from the foregoing that, although the optic cup plays a rôle in the development of a lens from a special epidermic region in Bombinator, the structure of that lens is determined by the direction of the growth of its cells, which is independent of the optic cup.

We should, however, emphasize the fact that the relations between lens and optic cup may differ much in two related amphibians. What has been brought forward in this paper holds only for Bombinator pachypus and Rana esculenta.

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[1]) M. W. WOERDEMAN, These Proceedings, Vol. XXVI, p. 702.