REGULATION OF AMPHIBIAN GASTRULAE WITH ADDED ECTODERM[1]

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(With Four Text-figures)

If a young Urodele gastrula is cut or constricted into two along the frontal plane, so that the dorsal and ventral halves are separated, the dorsal part contains the whole organization centre but an abnormally small amount of presumptive ectoderm; nevertheless, as Spemann (1903) and Ruud & Spemann (1922) showed, it regulates to form a complete embryo of normal proportions but of small absolute size. Schmidt (1933) demonstrated the same thing for Anuran gastrulae. Thus an absence of ectoderm causes modification of the normal development of the organization centre. We have much less information of the effect of increase in the proportion of presumptive ectoderm. Bruns (1931) added fairly small pieces of ectoderm to entire gastrulae, and obtained embryos apparently indistinguishable from normal; but the amounts added were so small that one could not expect easily visible effects. In the experiments to be reported here, very much larger quantities of ectoderm were added to normal gastrulae, and, as will be shown, it was found that the presence of this added material caused an increase in size of the invaginated roof of the gut, so that an embryo of normal proportions but increased size was developed.

METHODS

The experiments were performed on the eggs of the Anuran *Discoglossus pictus*, the animals having been originally obtained from Algiers through the good offices of Prof. Courrier. Some preliminary experiments were made in which the regulation of half gastrulae was tested. The young gastrulae, with a sickle-shaped blastopore, were cut in half with a glass needle, along the frontal plane, that is to say, along the plane passing through the animal and vegetative poles and at right angles to the dorso-ventral plane. In the main series of experiments, a gastrula was first cut in half in this way and then a large part of a second gastrula was added, sandwiched in between the dorsal and ventral halves. This added piece was obtained by removing, from the second gastrula, the dorsal region containing the organization centre, and the corresponding ventral region. The piece therefore contained a large part of the

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presumptive ectoderm, with part of the presumptive mesoderm, which would normally form part of the lateral lips of the blastopore, and also a small amount of endoderm. The added pieces amounted to about two-thirds of the total material of the gastrula from which they were taken. The actual grafting together of the pieces was carried out in Holtfreter solution, the pieces being placed in a groove in a wax operating dish which held them together until they were healed.

In the scheme of the operation (Fig. 1) the presumptive areas have been drawn according to the map given by Pasteels (1936). It will be realized that there are two possible orientations of the added middle piece; it may be arranged so that its own gastrulation movements would be in the same direction as those of the main host egg (concordant orientation), or it may be arranged so that its original dorsal region is now joined to the ventral part of the host (discordant orientation).

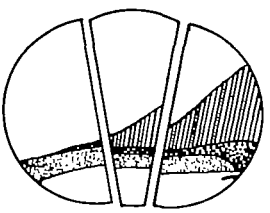


Fig. 1. Diagram of operation. The wedge-shaped piece of a second gastrula, which is being inserted into the first gastrula, is here drawn smaller than it was in most of the experiments. In the figure it is being inserted with its anterior-posterior orientation concordant with that of the host. *Vertical shading*, presumptive neural plate; *coarse spotting*, presumptive notochord; *fine spotting*, presumptive mesoderm.

EXPERIMENTAL RESULTS

(*a*) *The regulation of half-gastrulae*

As might be expected, the dorsal and ventral half-gastrulae obtained by cutting along the frontal plane as described above, developed in quite different ways. The dorsal halves contained nearly the whole presumptive neural plate and notochord, and the greater part of the presumptive axial mesoderm. In spite of this, they did not regulate to harmonious, half-sized embryos, but developed into the anterior part of an embryo of normal size. This lack of regulation is probably connected with the fact that the cut surface did not heal and the half-gastrula did not round up into a spherical, or approximately spherical ball. Instead the cut surface remained open as an enormously enlarged blastopore, and persisted in the larva as a region, uncovered by ectoderm, stretching forwards from the posterior end of the ventral surface. The posterior region of the half-embryo is always considerably distorted, so that it is difficult to determine just how much of the posterior end of the body is present. There is however never any sign of a tail, so the embryo must be truncated somewhere in the trunk region. From what we know of the location of the presumptive areas, this is rather surprising. It seems certain that some at any rate of the tail mesoderm must have been included in the dorsal halves. The reason why we find, not regulation, but the production of even less of the embryo than corresponds to the presumptive fate, must presumably be sought in the mechanical conditions

produced by the unhealed wound, which must in some way hinder the invagination and elongation of the posterior mesoderm.

The ventral half-gastrulae develop as "Bauchstucks", which consist mainly of a ball of endoderm enclosed in a skin of ectoderm. The wound is in some cases not completely healed, so that part of the endoderm may be uncovered. Between the endoderm and the ectoderm, a certain amount of mesoderm is present. It has usually developed as mesenchyme or blood, and often these are the only two mesodermal tissues recognizable. The ventral halves should, however, contain a fair quantity of presumptive somite material, in particular of material for the tail somites. This never develops into anything at all like a tail, and there is never any apparent expression of the normal elongation of the tail material. In a few cases, small patches of differentiated somites can be found. They are not accompanied by any nervous tissue, and, perhaps for this reason, the segmentation is rather irregular, though it is not altogether absent. In one specimen some pronephros is also present. On the whole, however, the Bauchstucks contain fewer differentiated mesoderm derivatives than might be expected. In fact, since the lateral-plate mesoderm also probably possesses organizing power at this stage (Waddington, 1936) one might expect that a certain amount of regulation of mesoderm would occur and that neural tissue might be induced. But, except for a doubtful case reported by Mangold & Seidel (1927), neural tissue has never been found in ventral half-gastrulae. It seems rather as though the weak inducing power of the mesoderm included in the ventral half-gastrulae becomes entirely swamped by the large amount of ectoderm with which it is in contact.

(*b*) *The regulation of gastrulae with added material*

The regulation which is achieved in the gastrulae to which material has been added in the way described above is considerable; in some cases completely normal embryos were produced. There are two main respects in which regulation is called for. Firstly, the added ectoderm must be incorporated, and secondly, the invagination of the added mesoderm must be controlled. The second of these seems to be the more easily attained, since in only 20% of the operated embryos is there a development of a secondary embryonic axis. In all the other specimens, the added mesoderm is either completely incorporated, or in some cases perhaps takes part in the formation of excess blood collected in the space caused by the excess of ectoderm.

When the regulation of the added ectoderm is incomplete, the excess material is left as a bulge, which later contracts into a wrinkled excrescence, near the anus (Figs. 2 and 3). This excrescence, then, is formed from ectoderm which lay near the ventral lip of the blastopore, and is therefore mostly composed of ectoderm derived from the original gastrula, while part of the smooth surface of the anterior belly is derived from the added material. Thus even in incomplete regulation, the added ectoderm is incorporated and it is what may be referred to as host ectoderm which is left in excess and therefore forms a disordered mass. The ectoderm of the excrescence is often considerably thicker than normal and this may also be true of part of the

smooth, regulated, ectoderm. Inside the excrescence there is usually little mesoderm other than blood.

In cases of complete regulation, the added ectoderm has not been disposed of in some way within the body of a tadpole of normal size. In particular, it cannot all be accounted for by the slight thickening of the skin which is sometimes to be noted. On the other hand, the size of the embryonic axis has been increased so as to balance the added material (Fig. 4).

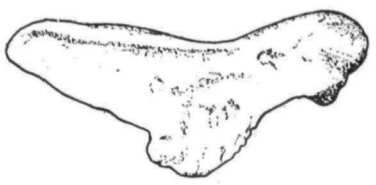


Fig. 2.

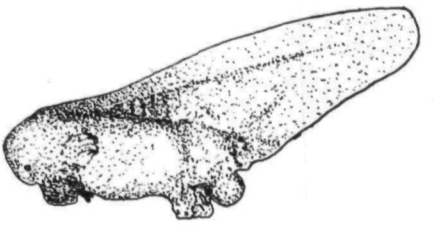
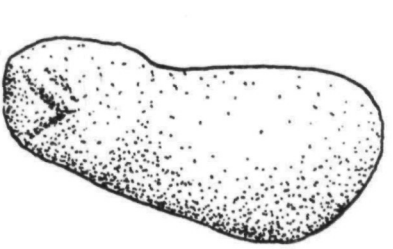


Fig. 3.

Fig. 2. No. E124*c*–5. Young tadpole showing considerable regulation; orientation of added material was concordant.
Fig. 3. No. E125*c*–1. Regulation complete except for a small excrescence near the anus. Orientation discordant.



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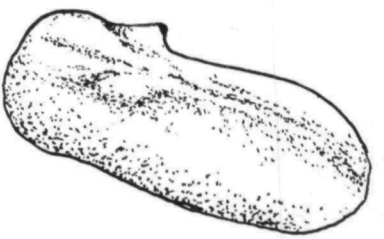

b

Fig. 4. No. E124*c*–1. Ventral and latero-dorsal views of neurula showing complete regulation. Orientation concordant.

DISCUSSION

The increase in size of the embryonic axis in the gastrulae with added material must be dependent on a more extensive invagination of mesoderm. The amount of mesoderm invaginated, and the distance it migrates forward from the blastopore, must therefore result from some kind of interaction between the presumptive mesoderm and the presumptive ectoderm. The same conclusion follows, of course, from the well-known regulation of half-gastrulae, which occurs when healing has restored the spherical shape and brought the presumptive mesoderm and ectoderm into a single system (Spemann, 1903; Ruud & Spemann, 1922). The normal proportions between invaginated mesoderm and ectoderm must represent the equilibrium state of this interaction, which any system of ectoderm and mesoderm, if they are allowed to interact in this way, tends to attain.

The incorporation of the added mesoderm involves a suppression, or at least control, of its tendencies of invagination; this is most obvious for mesoderm added in the discordant orientation. This control seems to be exerted with considerable ease. Bautzmann (1933) has already described a similar phenomenon when discussing the implantation of lateral germ-ring material into various parts of the presumptive mesoderm. In other experiments on *Discoglossus*, which will be described in the near future, it has been found that the dorsal regions of the presumptive mesoderm show very much stronger tendencies for autonomous movement, the direction of movement being hardly affected when the grafts are made in abnormal orientations. The capacity for gastrulation movements therefore seems to be much more rigidly fixed in the dorsal region than in the lateral lips of the blastopore. Even in the dorsal region, however, the regulation in size mentioned above shows that the extent of the movement may be subject to alteration in accordance with the amount of ectoderm present.

SUMMARY

1. If a young gastrula of *Discoglossus pictus* is cut in half along the frontal plane the dorsal half develops into the anterior part of an embryo of normal size, there being no regulation; this is probably dependent on the failure of the wound to heal. The ventral half develops as a "Bauchstuck" of the usual kind, containing endoderm, mesenchyme, with occasionally some somitic mesoderm, enclosed in a bag of ectoderm.

2. Gastrulae were cut in half along the frontal plane, and the greater part of a second gastrula, from which the dorsal and ventral regions had been removed, was inserted in the plane of the cut (Fig. 1). Complete regulation occurred in many cases, leading to the production of abnormally large embryos. In most cases, some regulation occurred, but some ectoderm was left in excess and formed an excrescence near the anus.

3. The extent of the invagination of mesoderm is determined by an interaction between the presumptive mesoderm and presumptive ectoderm.

REFERENCES

BAUTZMANN, H. (1933). *Arch. EntwMech. Org.* **128**, 665.
BRUNS, E. (1931). *Arch. EntwMech. Org.* **123**.
MANGOLD, O. & SEIDEL, F. (1927). *Arch. EntwMech. Org.* **111**.
PASTEELS, J. (1936). *Arch. Biol., Paris*, **47**, 631.
RUUD, G. & SPEMANN, H. (1922). *Arch. EntwMech. Org.* **52**, 95.
SCHMIDT, G. A. (1933). *Arch. EntwMech. Org.* **129**, 1.
SPEMANN, H. (1903). *Arch. EntwMech. Org.* **16**, 552.
SPEMANN, H. & BAUTZMANN, H. (1927). *Arch. EntwMech. Org.* **110**.
WADDINGTON, C. H. (1936). *J. exp. Biol.* **13**, 75.