The embryo-forming potencies of the young chick blastoderm

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WITH ONE PLATE

INTRODUCTION AND METHODS

In an attempt to find a part of the young chick blastoderm which would not develop into an embryo when explanted and would therefore be suitable to serve as a reacting system in embryonic induction studies, a procedure was developed, the results of which are interesting in their own right.

The blastoderm (unincubated or after up to 5 hr. of incubation) was explanted with its lower surface up on an egg extract culture medium (Spratt & Haas, 1960a) and what was thought to be its posterior side marked with carmine powder. A semicircular area located centrally to the marginal zone was cut out of the anterior half and explanted in an inverted position on the culture medium (Text-fig. 1). On the basis of earlier experiments (Spratt & Haas, 1906b) it was believed that this area would probably lack any autonomous developmental tendencies. Both the operated blastoderm and isolated piece were incubated for 3–4 days before fixation. A total of seventy such pairs were fixed and the following results observed.

Text-fig. 1. Scheme of operation. P, posterior; A, anterior.

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RESULTS

Most of the experimental pairs (57) can be roughly divided into three groups.

**Group I** (14 blastoderms). This consists of isolates containing embryos or embryonic structures, usually developed along one of the edges of the explants. The complementary blastoderms also developed embryos. It seems rather peculiar that the latter embryos developed from the lateral side of the blastoderm at an angle of 90° to the prospective longitudinal axis of the blastoderm. The embryos always lie posterior to the straight cut edge but very close and parallel to it (Text-fig. 2).

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<tr>
<th>Group</th>
<th>Donor Blastoderm</th>
<th>Isolate</th>
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<tr>
<td>I</td>
<td><img src="image" alt="Group I Diagram" /></td>
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<td>II</td>
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<td>III</td>
<td><img src="image" alt="Group III Diagram" /></td>
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**Text-fig. 2.** Schematic representation of experimental results. Each arrow represents an embryonic axis. The semicircular area marked on the blastoderms represents the original position of the hole formed by cutting out the isolate.
Embryo-forming potencies of the chick blastoderm

Group II (37 blastoderms). All the isolates developed into atypical vesicles with no sign of embryonic structures in them. In all the complementary blastoderms embryos developed from the original posterior (marked) region.

Group III (6 blastoderms). Embryos developed in all the isolates and each of the complementary blastoderms developed an embryo from the original posterior region.

A check of the experimental data revealed that the above groups are clearly age groups. The blastoderms belonging to Group I were obtained from winter eggs, unincubated or incubated for 2–3 hr. None came from a group of sixteen unincubated summer eggs. All blastoderms of this group looked very young, mostly without any sign of hypoblast formation.

Most of the blastoderms comprizing Group II were obtained from 4–5-hr. incubated winter eggs or unincubated summer eggs, only one being an unincubated winter blastoderm. The blastoderms of this group were easiest to orientate since most of them showed a slight thickening of the posterior section of the marginal zone.

The third group includes summer blastoderms only, some of them unincubated and some briefly incubated (up to 5 hr.). All the six blastoderms of this group had reached the same developmental stage at the time of operation, with their hypoblast fully expanded but prior to the first appearance of a primitive streak. It was realized at the time of the operation that the position of the longitudinal axis of the blastoderm could be reliably determined, but it was impossible to distinguish between the anterior and posterior sides which looked identical. One end of this axis was therefore marked with carmine powder. The carmine was expected to mark either the anterior or the posterior side of the developing blastoderm. The results seem to indicate that only in two of the six blastoderms operated on were the isolated pieces actually cut out as intended from the anterior part. In three cases the explants were probably cut out of the posterior part, and in one other from a somewhat different location. This did not, however, affect the development of the isolates which were all double layered and formed embryos, usually from a marginal zone which developed along the circular cut edge. It is suggested that the embryos in the complementary blastoderms developed in all cases from the prospective posterior side. In the two blastoderms in which the operation was done correctly, the embryos developed in the normal position. In the other cases the embryonic axis was curved around the hole produced by the operation.

The remaining blastoderms could not be classified in any of the above three groups. In two pairs, embryos developed in the isolates, but the axial orientation of the poorly formed embryonic structures which developed in the donor blastoderm could not be determined. The remaining eleven pairs of blastoderms and isolates show very interesting intermediate stages between the groups. Three types of intermediate stages between Groups I and II were detected. In one case the embryo in the blastoderm developed at 90° to the original longitudinal axis
but the isolate failed to develop embryonic structures. In a single case the isolate again failed to develop an embryo, but in the donor blastoderm two axial systems were formed, the normal one developing from the original posterior side while the second, very rudimentary axis developed at 90° to the first (Text-fig. 3 and Plate). The third intermediate stage between the two groups is represented by eight blastoderms. Here the isolates contain some unorganized embryonic structures while the complementary blastoderms developed embryos from the originally posterior side.

![Text-fig. 3. Blastoderm No. 51, representing a stage intermediate between Groups I and II.
The better developed embryo was formed from the prospective posterior side of the blastoderm. A second rudimentary was developed from the left side at 90° to the first as result of the operation. Ventral view.](image)

Only one case is intermediate between Groups II and III. Here the hypoblast of the blastoderm was already partially extended and the posterior part of the isolate was double layered. The isolate contains poorly organized embryonic structures, whereas its complementary blastoderm formed a posterior embryo.

**DISCUSSION**

Lutz (1949), and Lutz et al. (1963) regard the unincubated duck blastoderm as a totipotential system, the posterior region acting as a dominant embryonic centre. In an undisturbed blastoderm the embryo develops from this centre. However, by dividing the blastoderm *in situ* into several pieces, anterior and lateral parts could be freed from the dominance of the embryonic centre and express their own potentiality by forming embryos. Embryos formed from posterior sections, however, always retain the original postero-anterior orientation, whereas embryos developed from anterior sections, according to Lutz, either develop in an orientation reversed to the original or retain the original axial orientation of the blastoderm. The second possibility contradicts the observations of Spratt & Haas (1960b) in the chick. They postulate that: 'In all cases, embryonic axes developing in isolates (*in vitro*) initially lie in or very close to a radius of the original blastoderm with the head pointing toward the centre and the tail close to the marginal zone. This means that the embryo forma-
PLATE

The blastoderm depicted in Text-fig. 3. PE, posterior embryo; LE, left embryo.

Fig. A. Dorsal view.
Fig. B. Ventral view.

H. EYAL-GILADI and N. T. SPRATT

(Facing page 270)
tion and polarity are initiated from a centre, derived from and subsequently located just within the marginal zone. In its embryo initiating capacity, the marginal zone of the bird blastoderm is functionally analogous with the amphibian Randzone and the teleost germ ring. Spratt & Haas (1960b) suggested that the marginal zone of the unincubated blastoderm exhibits a gradient in embryo forming potentiality the high point of which is in the prospective posterior-median position. Decline in embryo initiating capacity occurs most rapidly in the more anterior, less rapidly in lateral parts of the marginal zone. The lateral and posterolateral portions of the zone retain this capacity at least up to the intermediate streak.

Checking Lutz's figures it occurs to us that in certain cases there might have been a misinterpretation of the embryos in his divided blastoderms. The two embryos shown in Fig. 35a (Lutz, 1949) which developed from the anterior half of the blastoderm are, according to Lutz, in a cephalocaudal orientation, which is the same as the orientation of the single embryo which developed from the posterior half. We think that the two anterior embryos probably developed from the lateral marginal zone with their heads initially pointing centrally and later shifting in an anterior direction.

It is, however, clear that in some of Lutz's experiments anterior embryos undoubtedly developed with their posterior end at the original centre of the blastoderm and their heads pointing towards the anterior marginal zone. In other words, embryo formation probably did not originate in those cases from a centre derived from or located within the marginal zone.

The observations reported here are believed to form part of the missing link which serves to fit the chick and duck blastoderm into the same picture, thus shedding some light on the potencies of the early avian blastoderm. The blastoderms of Group I, which are the youngest, demonstrate a pronounced potency of central parts of the blastoderm, which are devoid of marginal zone, to form an embryo. This potency was not detected by Spratt & Haas who used a different technique and probably slightly older blastoderms.

In these almost equipotential systems (the dominance of the posterior region being expressed in undisturbed blastoderms only), the mere act of cutting is probably enough to initiate in the donor blastoderm the development of an embryo along the cut edge. It is assumed that the wound probably causes local metabolic changes which at this stage are sufficient to overcome the very labile dominance of the posterior part of the blastoderm. The ectopic axial systems were in all cases observed to be connected with the marginal zone with the head of the embryo pointing towards the centre of the blastoderm. As soon as this atypically located embryo starts to develop, the normal embryo-forming centre of the blastoderm is suppressed.

The central part of the blastoderm gradually loses the above potency which becomes confined to the marginal zone, being at first highest at and later on confined to the latter's posterior side. This condition is demonstrated in all the
blastoemrs of Group II. In them the isolates did not develop embryos, and the cut edge in the donor blastoderms is no longer able to initiate the formation of an atypical embryonic centre. The embryo will be formed preferentially from the posterior side, although it can still be formed from lateral marginal zone in isolated anterior halves (Spratt & Haas, 1960b).

The different types of intermediate stages between Groups I and II demonstrate that the rate at which the embryo-forming potency of the central part of the blastoderm is lost and its concentration at the posterior side are not equal for all blastoderms. Very rarely (one case) the central part was no longer capable of forming embryonic structures when isolated, although the operation was still sufficient to force the formation of an embryo at 90° in the donor blastoderm. In other cases the central part, as shown by the isolates, has not lost its capacity completely but the posterior marginal zone is already strong enough to develop an embryo. The most striking is the single example in which both opposing tendencies are demonstrated in the blastoderm, with the posterior winning the game (Text-fig. 3 and Plate). In this last-mentioned case the central part has already lost its embryo-forming capacity.

A further stage in the process of changing potencies of the blastoderm is related to the spreading of the hypoblast. During this process regions of the epiblast with underlying hypoblast re-acquire the capability to form an embryo.

The above studies support the view that the developmental potencies of chick and duck blastoderms are not basically different. It seems that if one can obtain very young chick blastoderms the same developmental flexibility can be demonstrated in them as was described for the duck by Lutz et al. (1963). In both bases the embryo initiating capacity is not limited initially to the marginal zone. It also exists in the central region of the young blastoderm but is normally dominated by the stronger potency of the marginal zone. In the chick the embryo-forming potency of the central region is clearly expressed in the isolates of Group I only.

SUMMARY

Very young unincubated chick blastoderms (obtained from winter eggs) are almost equipotential (or totipotential) and under experimental conditions even central fragments can form an embryo in vitro. When a central cut is made in a blastoderm of this age an embryo tends to form along the cut edge and the original embryo-forming centre at the prospective posterior side is suppressed.

In slightly older unincubated chick blastoderms (obtained from summer eggs) and in 4–5 hr. incubated winter eggs the embryo-forming potency is lost from the central part and becomes confined first to the marginal zone and, within it, gradually to its posterior region.

In still older blastoderms, with an expanded hypoblast but prior to streak formation, central fragments of the epiblast with their underlying hypoblast re-acquire the capability to form an embryo.
Les potentialités embryogenes du jeune blastoderme de poulet

De très jeunes blastodermes de Poulet, non-incubés, (obtenus à partir d’œufs d’hiver) sont presque équipotentiels (= totipotentiels), et dans certaines conditions expérimentales, en culture in vitro, même des fragments centraux peuvent former un embryon.

Si l’on pratique la section de la partie centrale dans un blastoderme de cet âge, un embryon tend à se former tout le long du bord de la section et le centre embryogène normal de la partie postérieure présomptive est supprimé.

Dans des blastodermes de Poulet plus âgés (obtenus à partir d’œufs d’été non-incubés, ou à partir d’œufs d’hiver de 4 à 5 heures d’incubation), la potentialité embryoformatrice de la partie centrale est perdue. Le pouvoir embryogène se restreint d’abord à la zone marginale entière pour ne subsister, finalement, qu’à la partie postérieure de cette même zone.

Dans des blastodermes encore plus âgés (stade précédant la formation de la ligne primitive, après l’extension de l’hypoblaste), des fragments centraux d’épiblaste, prélevés avec l’hypoblaste sous-jacent, on retrouvé leurs potentialités embryogènes.

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REFERENCES


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