

## BIOLOGY OF THE TWINNING PHENOMENON: EMBRYOGENESIS AND TERATOGENESIS

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*The twinning phenomenon has always interested the great public, the artists, and naturally the scientists. Isidore Geoffroy St. Hilaire has established a classification still now valid. This classification considers the different types of double monsters which are found in nature in all classes of vertebrates, including man. To explain the twinning phenomenon, the experimental realizations have progressed by successive bounds after the preliminary attempts of different authors. Now, when it is question of experimental duplication, anybody thinks of Speman for the amphibians, of Lutz for the birds, of Seidel for the rabbit, and of Tarkowsky for the mouse. Now, it is possible to conceive a twinning resulting from the separation of the first blastomeres (amphibian, rabbit, mouse) and a twinning originating from the fissuration of the blastoderm (bird, mammal). All these experiments confirm the unicist-theory. If a total or partial regulation of the excedents may be experimentally realized, no argument can however support this theory in the realization of the double monstrosity.*

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### INTRODUCTION

At all times, the twinning phenomenon has interested the general public; the drawings of Sebastian Brant (1495) (Fig. 1). and Albrecht Dürer (1512) (Fig. 2) supply proof of it.

MZ twins always have the same sex because of their monovulatory origin, whereas DZ twins often have different sexes for they originate from a polyovulation as is obvious in the case of triplets, quadruplets, quintuplets or other multiple births (e.g., the birth of seven children in Hameln on the Weser in the year 1600: Lotze 1937) and this cannot be the result of an hormonal intervention.

The case of DZ twins, although of interest for the physiologist, is considered by the embryologist and the teratologist as a mere curiosity, but they both give more attention to MZ twins which raise the problem of mechanism of this monstrosity. According to the definition, a single egg gives birth to two or more individuals; this phenomenon is called the phenomenon of regulation or physiological regeneration. We must at once notice that the separation between two individuals can be complete or partial; in the last case, the result is the formation of numerous varieties of conjoined or double monsters: teratopages, teratodymes, teratodelphes (Fig. 3) according to Geoffroy St. Hilaire's classification (1836) reviewed by Lesbre (1927). The majority of these monsters are not viable; others (thoracopages or pygopages) remain alive for a long time (e.g., Chang and Eng, Josepha and Rosa Blazek).

In another kind of double monsters, one of the components can be more developed than the other; the first one is considered as an autosite and the second as a parasite; but these are only variations which can be included in Geoffroy St. Hilaire's classification. All these monstrosities are to be found in all groups of Vertebrates. Some are better known than others; this is the case for Salmonidae which are found in great numbers in fish hatcheries, for domestic mammals, for chick embryos whose

spontaneous malformations are collected by research workers who are interested in this material. But for many species only the viable malformations come into the hands of specialists (Fig. 4). For a long time, the question concerning conjoined monsters has interested scientists. As far back as 1724, Lemery (1738, 1749*a, b*) presented the French Académie des Sciences an embryo with two heads on one body; he expressed then the idea that this fetus was the result of an accidental conjoining of two individuals; a discussion which has remained famous, followed and ended only in 1743 with Lemery's death. This discussion was due to the fact that beside this dualistic notion, another one, the monoovulatory theory, assumed that duplication resulted from a secondary scission; this point of view was first supported by Duvernay and Winslow (1724), then by von Baer (1827); in fact, such a duplication is very harmonious. Dareste (1891) remained an adept of the dualistic theory, but his very numerous observations soon led him to vary his point of view slightly. He says (p. 456): "Tantôt elle (la gémellité) consiste dans l'existence de deux embryons produits dans deux blastodermes distincts, au moins au début, tantôt elle consiste dans l'existence de deux embryons produits dans un blastoderme unique." Since then, a great number of investigations have been undertaken in the field of diplogenesis. Many authors have proved experimentally that the unicist theory was true. Two kinds of methods are used: indirect methods and microsurgical interventions.

#### INDIRECT METHODS

Indirect methods were used by Lereboullet (1864) on pikes' eggs, by Stockard (1921) on *Fundulus heteroclitus* or trout's eggs by exposing them alternately to heat or cold, or to cold or hypoxia. Bataillon (1901) had recourse to lampreys' eggs and submitted them to hypertonic solutions of cane sugar or sea salt. Schultze (1894), Schleip and Penner (1926) compressed Batrachia's eggs between object-slides; Pasteels (1948) applied the centrifugal techniques to the same type of eggs. Dareste (1874) and later Gerlach (1882) varied the conditions of incubation of hens' eggs or coated them partially with varnish. After these interventions, all the authors observed more or less important and numerous duplications, but as their techniques lacked accuracy, the results they obtained were not constant; furthermore, in many cases, it was difficult to establish an intervention-duplication relationship.

#### MICROSURGICAL INTERVENTIONS

These interventions can be carried out either at the first stages of segmentation or at a more advanced stage corresponding to blastula. The first experiments in this field were made by Spemann (1901, 1902, 1903) on newts' eggs; with a fine hair, he ligatured the isthmus between the first two blastomeres; when the first segmentation plane was perpendicular to the assumed bilateral symmetry plane of the egg, two well constituted embryos were formed (Fig. 5). When the plane of segmentation was parallel with the bilateral symmetry plane, only the dorsal blastomere would give rise to an embryo, whereas the other remained very abnormal and formed a hyperblastula. When the ligature was loose, a double monster was formed. These experiments were completed by Ruud (1925), Dalcq and Huang (1948), Dalcq and Dollander (1948) and supplied the first explanations about twinning formation and double monstrosity. Experiments of the same type were carried out by Wilson (1893) who succeeded in separating the first two blastomeres by shaking *Amphioxus*' eggs in a glass tube and obtained two embryos from one egg. Conklin (1933) completed this experiment by pointing out that this effect can be obtained only when the first segmentation is perpendicular to the bilateral symmetry plane. This result was amply confirmed by Tung et al. (1958) who succeeded in separating the first blastomeres with a glass needle. On the other hand, mammals' eggs seemed impossible to be dealt with a surgical intervention, but the perfecting of the techniques of reimplantation of eggs, previously cultivated *in vitro*, in the uterus of pregnant female (or females prepared for pregnancy) permitted such experimentation. After the first data given by Nicholas and Hall (1942), Seidel (1952) on rabbits' eggs and Tarkowsky (1959) on mice's eggs obtained very striking results. Seidel (1960) extracted rabbits' tubal eggs preferentially at the two blastomere stage; one of the blastomeres was killed with UV rays or mechanically by pricking it with a fine glass needle. The egg was then cultivated *in vitro* for 24 hours in a medium containing serum and physiological liquid. It was therefore possible to observe the development process of the intact blastomere and, at the same time, to make sure that the irradiated blas-

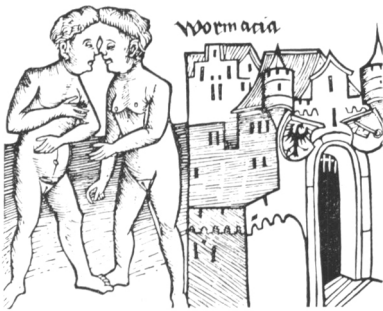


Fig. 1. The twins of Worms: Frontispiece after Sebastian Brant (1495).

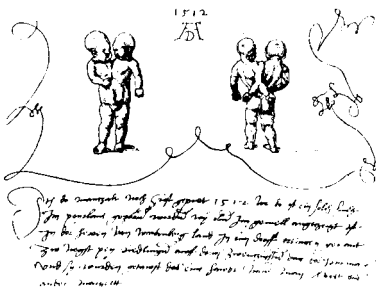


Fig. 2. Conjoined monsters: Frontispiece after Albrecht Dürer (1512).

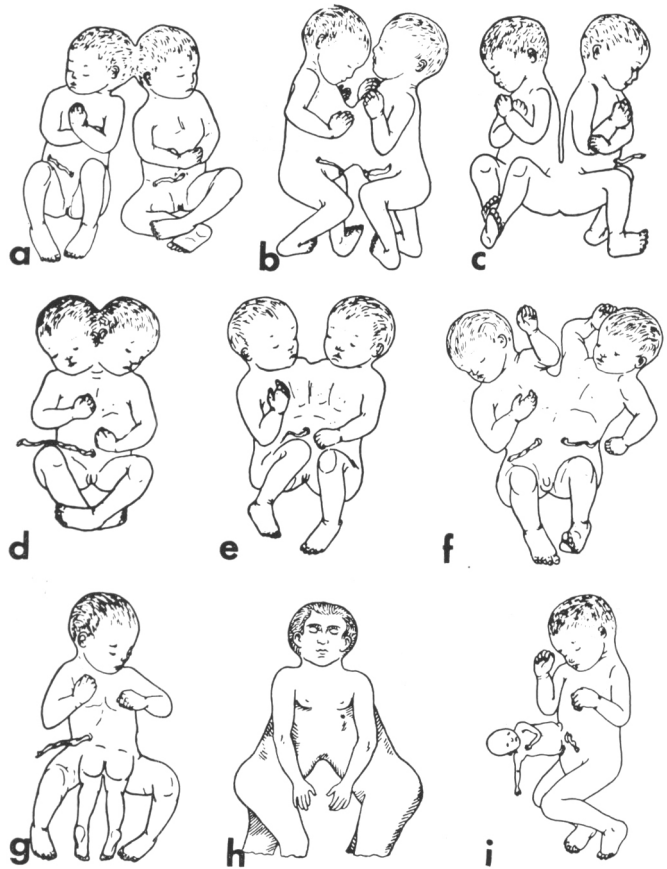


Fig. 3. Different double-monster types. I. *Teratopage series*: (A) Occipital Cephalopages; (B) Thoracopages; (C) Pygopages. II. *Teratodyme series*: (D) Iniodymes; (E) Sternodymes; (F) Thoracodymes. III. *Teratodelphe series*: (G) Pelvadelphes; (H) Iniotes. IV. *Parasitic monsters*: (I) Parasitic thoracopages.

The three series: teratopages, teratodymes and teratodelphes, lead to the complete separation of the two individuals; that's why the twins can be considered as perfect monsters. Likewise, it can be admitted that the double monstruosity begins with anterior duplication: rhinodyme monster (duplication of the nose and the mouth) or even stomatodyme (duplication of the mouth), or by posterior duplication: pelvadelphe or pygomele monsters. (According to different authors).

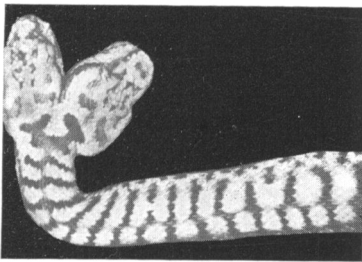


Fig. 4. Two-headed viper aged several months. (H. Lutz).

tomere did not develop. The egg was then introduced into the uterus of a female of a different strain and color, which, in case of birth, permitted to be quite certain of the obtained result (Fig. 6). When the egg was operated at the two blastomere stage, the intact blastomere could develop into a perfectly constituted embryo (Fig. 7); but, as shown by the experiments carried out on the four blastomere stage, the effect depends on the establishment of the first plane of segmentation which has any orientation in relation to the bilateral symmetry plane. Only one or the other, or the first two blastomeres are able to produce regulation, therefore to form a complete embryo; as a matter of fact, a *Bildungszentrum* or formation centre appears very soon at the posterior pole of the egg; any blastomere containing this centre or a part of it is able of regulation (Fig. 8). From Seidel's principle, we could deduce that the parasitic monstruosity is explained by the fact that a blastomere contains only a very small part of the formation centre.

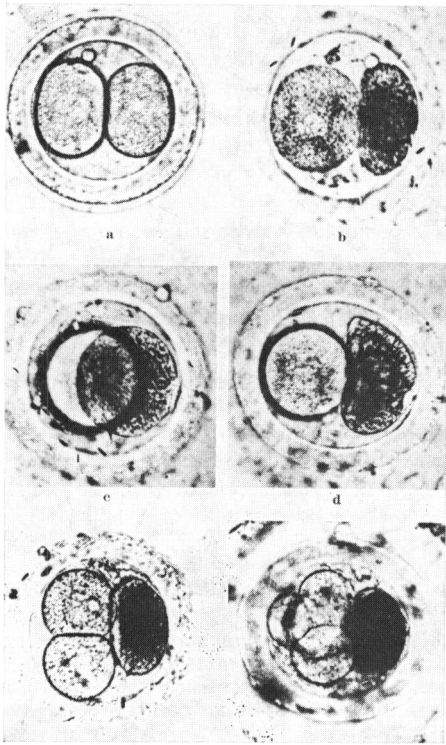


Fig. 6. Intervention at the two-blastomere stage on the rabbit's egg. One of the blastomeres is killed by UV rays. After *in vitro* cultivation for 24 or 48 hours, the whole egg is reimplanted in the uterus of a pregnant rabbit of different breed and colour. (According to Seidel).

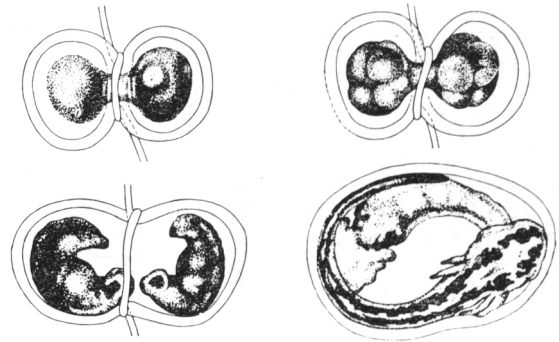
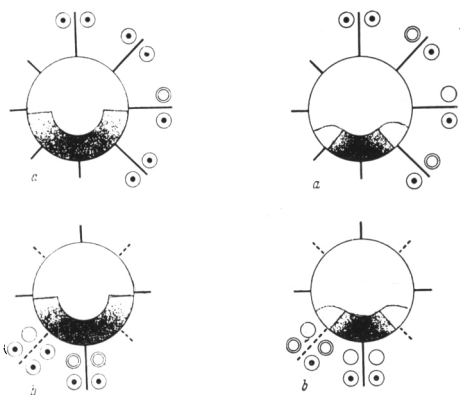
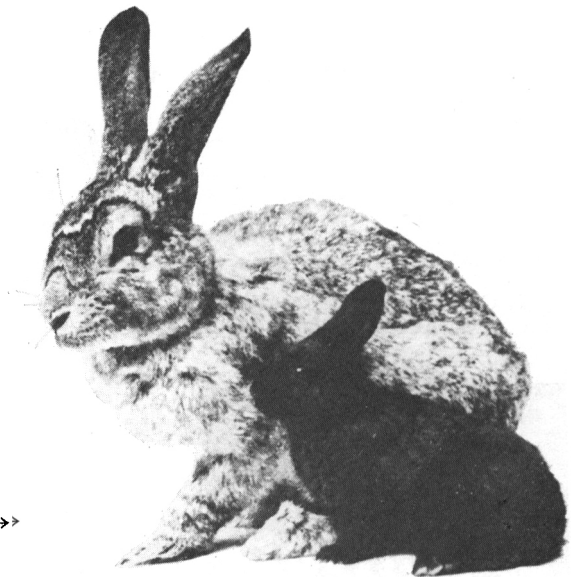


Fig. 5. Obtaining two embryos by constriction, at the two-blastomere stage, of the newt's egg. (According to Spemann).

Fig. 7. Result of Seidel's experiment: birth of an Alaska young rabbit from a Chinchilla host-mother. →



← Fig. 8. Hypothesis on the embryonic formation center (*Bildungszentrum*). In the two left figures, this center fills the subcortical half of the egg, and in the right figures, the quarter of this zone. At the top (a), results of the isolation, at stage II, of one of the blastomeres. Three types of position of the division plane in relation to the bilateral symmetry plane indicated by the *Bildungszentrum* crescent are represented by full lines (—). At the bottom (b), results of the isolation, at stage IV, of one of the blastomeres. The typical positions of the blastomeres' quartet are indicated by full (—) and dotted lines (- - - -). Symbols of the result of the separation: circle with a point = complete blastomere able to give rise to an embryo; empty circle = trophoblastic vesicle; two concentric circles = didermic blastocyst only reduced to the trophoblast and the endophyllus. (According to Seidel).

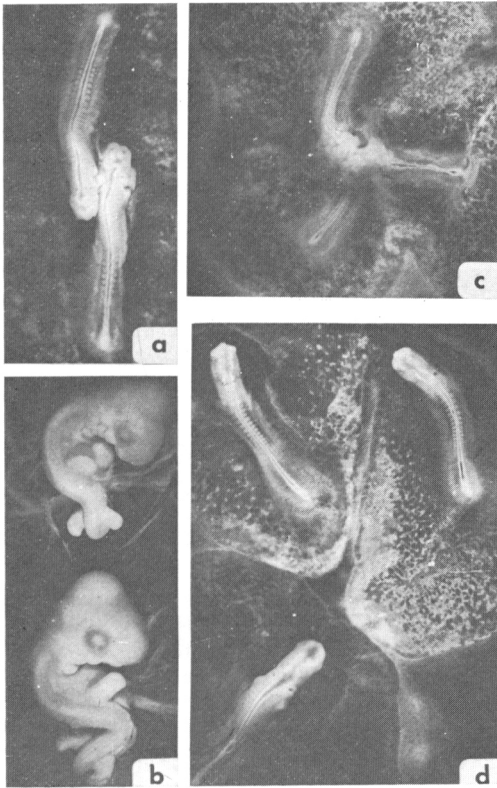


Fig. 10. Fissurations of the duck's egg. (H. Lutz). (a) Twins in head-to-head position; (b) Twins one behind the other; (c) Triplets with associated heads; (d) Triplets, two anterior and one posterior, with parallel axes.

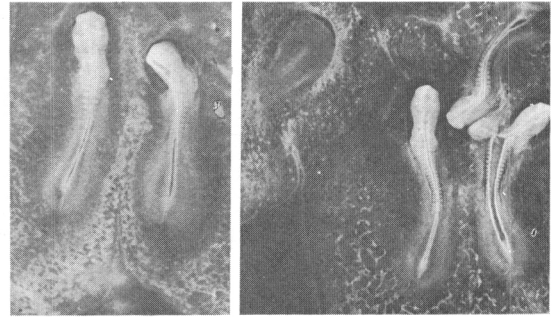


Fig. 9. Results of the fissuration experiments on the duck's or quail's egg: twins and quintuplets with parallel axes. (H. Lutz).

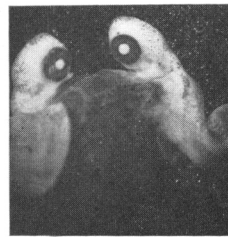


Fig. 11. Fissuration of the *Salmo umbla*'s egg. (H. Lutz and Y. Lutz-Ostertag).

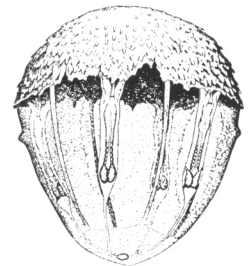


Fig. 12. Blastocyst of *Dasyypus* with four embryos. (According to Newmann).

In the case of birds, the experimental duplication is obtained at a later stage, the egg being laid at a blastula stage; the blastomeres — hundreds in number — form a blastoderm spreading on the yolk surface. S. Morita (1936), using the grafting technique, introduced dead organs under the blastoderm; Twiesselmann (1938) applied an electrolytic marking method or UV irradiation to the blastoderm itself; both authors obtained double or triple formations sporadically, therefore in a low percentage of cases.

We have studied the same problem again, not on domestic hens', but on ducks', quails' or Guinea hens' eggs (Lutz 1949, Lutz and Lutz-Ostertag, 1963). The technique we used consists in cutting the blastoderm with a fine glass needle; this fissuration was performed so that the vitelline membrane lying over the germ should not be damaged. In these conditions, it is possible to obtain two, three, four or even more embryos with a disposition which can be foreseen beforehand (Figs. 9 and 10). This arrangement depends on the orientation of one or more fissures in relation to the blastoderm symmetry plane, which is given by von Baer's rule; according to this rule, when the obtuse pole is on the left of the experimentalist, the axis of the future embryo is perpendicular to the main axis of the egg and head is directed forwards in about 70% of cases. The same technique also leads to the double formations described by Geoffroy St. Hilaire (1836).

The same duplication can also be obtained from a Salmonidae blastoderm: the char (*Salmo umbla*) (Fig. 11), as we demonstrated in collaboration with Y. Lutz-Ostertag (1952). Because of the great fragility of this egg, we used localized X-rays which destroyed a small strip of material, thus separating the blastoderm into two parts about the same size. Besides, Eismond (1910) on rays' eggs, Vivien and Hay (1954) on the blastodisc of the dog-fish (*Scyliorhinus canicula*) obtained similar results by using the fissuration method.

We could also refer to Devillers's experiments (1951), showing that the suppression of one half of the blastodisc of the trout does not prevent the other half from forming a complete embryo. In connection with this, we must

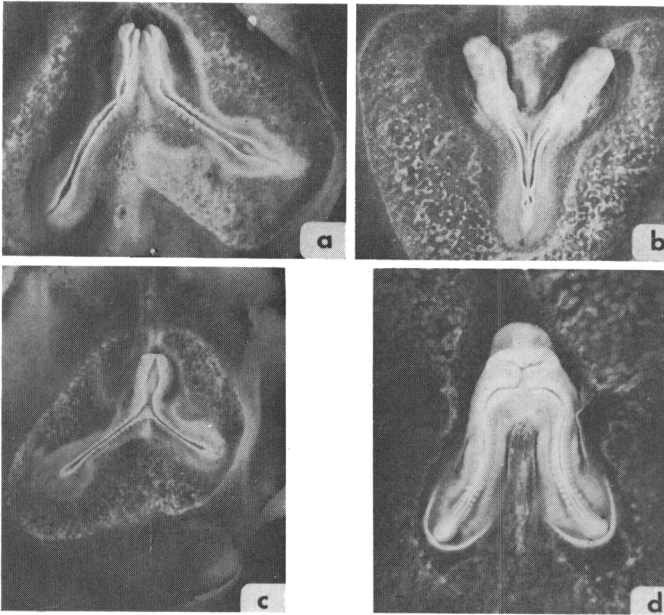


Fig. 13. Experimental conjoined monsters obtained by fissuration of the quail's blastoderm. (H. Lutz and Y. Lutz-Ostertag). (a) Teratopages; (b) Teratodymes; (c) and (d) Teratodelphes; d, besides, presents a malformation of omphalocephalic type.

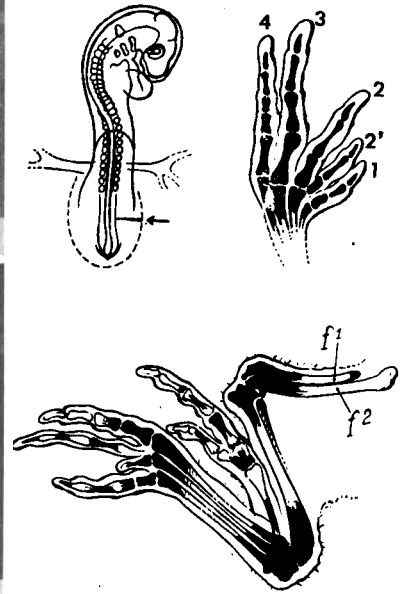


Fig. 14. Fissuration of the limb-foot *Anlage* of the chick's embryo. Formation of a foot partially divided in two, or only of a polydactyle. (According to Wolff and Kahn).

also mention the normal polyembryony in two species of armadillos (*Dasyus hybridus* and *Dasyus novemcinctus*) described by Patterson (1913) and Fernandez (1913); the embryos — four or eight in number — originate from the same blastoderm which is composed of a hollow sphere limited by the trophoblast and a clump of cells constituting the embryonic bud which will form the amniotic cavity (Fig. 12). The polyembryony itself is due to the subdivision of this cavity; this is also a case of blastodermic type of polyembryony.

#### DISCUSSION AND CONCLUSION

All these groups of experiments permit to conclude that we must distinguish blastomeric twinning on the one hand, and blastomeric twinning on the other hand. Both are directly related to the cellular differentiation which can occur at a more or less early stage.

As a matter of fact, in the case of the blastomeric twinning of Batracians' eggs, the grey crescent appears two or three hours after fecundation; this crescent indicates the future position of the dorsal lip of the blastopore and also corresponds to the dorsal region of the embryo and it marks the symmetrization of the egg. In the case of mammals' eggs, Seidel (1960) mentions the *Differenzierungszentrum* (Fig. 8). Dalcq (1962) discovered the apical cap or embryogenic dorsal cap which contains a large amount of RNA and polysaccharides. These phenomena too show the early symmetrization of the egg. Now, the phenomenon of twinning regulation is possible only when both the first blastomeres include a part of this region. Consequently, the cellular differentiation occurs very early, in the first hours after fecundation.

In the case of blastodermic twinning, any region of the blastodisc is still able to develop a complete embryo, although the bird blastoderm is already symmetrized as is shown by von Baer's rule, whose authenticity was verified in our experiments on thousands of hens', ducks' and quails' eggs; according to Clavert (1960), this rule can be established a few hours before laying-time. Does it mean that in

this case the process of cellular differentiation is slower? There is no proof of it; what is certain is that an important mass of cells, physiologically isolated from the blastoderm, organize themselves and form an embryo whose orientation, in the posterior part of the blastoderm, is determined by the underlying endoblast, whereas in the anterior region this orientation remains labile. So we can speak of cellular fields which are able to regulate to give an embryo.

An identical phenomenon probably occurs in the eggs of trout, dog-fish and ray on which the intervention is carried out at the same stage. As is shown by the natural example of the *Dasytus*, a blastodermic type of regulation can also appear at the germinative disc stage. Therefore, we can admit that in mammals blastomeric and blastodermic twinning exist. If, in the first case, all the embryonic annexes are divided into two, in the second case, there is formation of only one amnion, one chorion and one placenta.

As for double monostruosity, it results from an incomplete separation of the first blastomeres. Yet, this separation must affect blastomeres with the same potentiality. The evidence is given by Spemann's above-mentioned experiment; an imperfect ligature at the two blastomere stage, perpendicular to the grey crescent, leads to the formation of teratodome type monsters. But conjoined twinning can still occur at a more advanced stage of development; thus Spemann (1901, 1902, 1903) obtained a slow and gradual division of the lip of the blastopore by operating at a gastrula stage with a glass needle which sank into the egg owing to a counterpoise. Here, too, there is formation of a teratodome monster but, in this case, it is the "organization centre" itself which is affected; each of its halves acts to the organization of an embryonic axis. Similar results can be obtained on birds' embryos at the beginning of the gastrulation stage (Lutz 1949, Lutz and Lutz-Ostertag 1963) (Fig. 13).

A demonstration of the same type is given by the experiment of Wolff and Kahn (1947) who provoked a partial or total duplication of the foot-limb of the chick's embryo by fissuration of the limb-bud *Anlage* at an early stage of development (Fig. 14). These experiments show that the axial duplication of an embryo or even of a limb can occur at a relatively late stage of development, as long as the determination of the part of the axis, or *Anlage*, is not effective. Anyway, twinning and conjoined monostruosity are both part of the embryonic regulation, the mechanism of which is now known: it requires a cellular reorganization, a change of orientation in the fate of some cells; some day, this cellular reorganization will probably be expressed in the field of molecular phenomena.

We can conclude that all the experiments carried out till now confirm the unicist theory: twinning is due to the duplication of a single germ at a more or less early stage of development; this phenomenon corresponds to a regulation of deficiencies; but, beside this type of regulation, Wolff (1949) also mentions the regulation of excedents. It is the association and contraction of organs or even organisms of the same type which elaborate together an harmonious whole which can be compared with a normal organ or organism. Tarkowsky (1964) took two mouse eggs at the blastocyst stage and pressed them together; these eggs formed only one embryo with a perfectly normal appearance. In our own experiments, it was possible to obtain the regulation of excedents, therefore the formation of a single embryo from two blastoderms which were joined together (Lutz 1961). From these examples, we could perhaps find arguments to defend the dualistic theory for the formation of conjoined monsters such as teracopages, teratodymes or teratodelphes; two mammal eggs could perhaps develop side by side and fuse partially to form such a monster; at the same time, it must therefore be admitted that such a fusion can be realized only between embryos having the same genetic sex, because till now, as far as we know, no monster whose one component was a male and the other a female has been described.

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