Are shape and morphogenesis independent phenomena?

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Summary. Amphibian embryos can develop inspite of dramatic deformations produced by constraints in the surrounding medium, and can even ultimately recover a normal morphology. Consequently, no sequential change of shape is necessary for normal morphogenesis and the form of the embryo appears to be determined by cell activities intrinsic to each stage.

One of the most noticeable phenomena during embryonic development is the continuous change of shape that an organism exhibits during this time of its life. It is generally accepted that the sequence which permits this development begins with the establishment of a well-defined polarity; this results in a morphogenetic field, and finally within this field pattern formation occurs. There is some evidence that the development of prepatterns in vertebrate embryos involves neither cell counting[3,4] nor the cell cycle[5], but the establishment of a 'map' of information which assigns to certain regions the development of specific differentiations by cells within them. Undoubtedly morphogeneis is the result of a great number of processes at different levels, but at the morphological level, among other things, changes in cell shape and cell contact play an important role[6,7]. From this perspective, the sequential changes in cellular activities coordinated in space and time, which the embryo exhibits during its development, seem to be connected with the sequential shape changes.

Recently normal morphogenetic shape alterations have been considered to be directly determined by mechanical stresses in cell layers in hydroids[8] as well as amphibian embryos[9]. These authors suggested that a possible causal connection between the mechanical stresses and the subsequent activation of the intracellular mechanochemical machinery could be interpreted in terms of the 'positional information' concept[10].

The main aim of this work was to obtain Bufo arenarum embryos which develop in spite of dramatic deformations produced by constraints in the surrounding medium and to show the capacity for shape regulation which those anomalous embryos exhibit later.

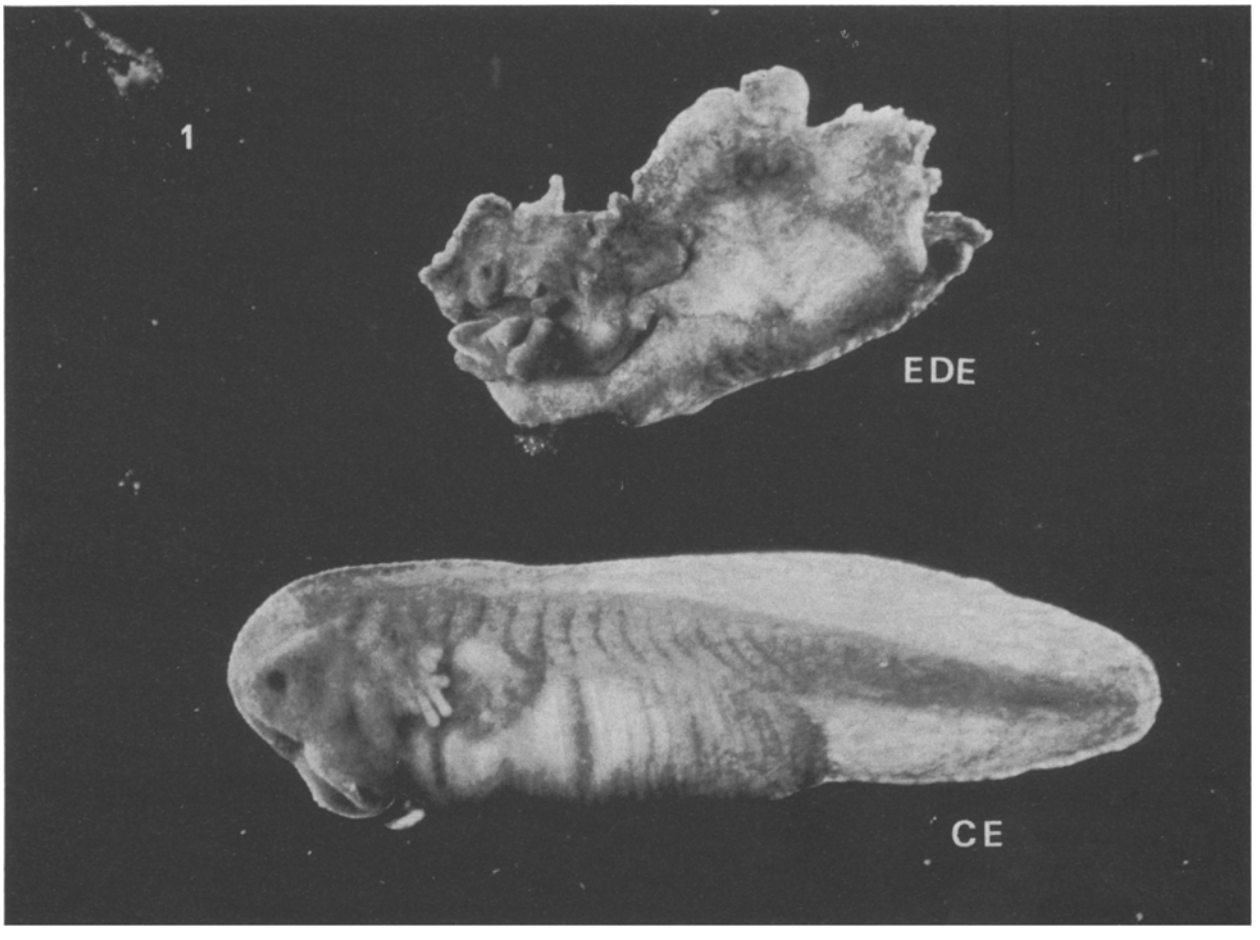

Fig. 1. Experimentally deformed embryo (EDE). Control embryo at the same stage (CE)

Material and methods. The vitelline membrane of Bufo arenarum embryos was removed at the early tail-bud stage (stage 17 according to Del Conte and Sirlin[11]) with watchmakers' forceps under a dissecting microscope. About 120 embryos were then embedded in an agar medium made up with isotonic salt solution (Holtfreter) at a concentration of 0.5% agar, and kept at room temperature till the control embryos reached stage 21 (figure 1). The gelified agar provide an isotonic and non-toxic medium allowing relatively free exchange of respiratory gases, even in higher concentrations than that used here, as was shown in growth-inhibition experiments[12]. In our experiments, it deforms the embryo and prevents expansive growth non-uniformly.

At the end of this first experimental phase, the embryos were freed from the agar and kept in Holtfreter solution. Embryos were selected for further observation, which showed no traces of damage in the ectoderm when examined under the binocular microscope. Some embryos were fixed in Ancel and Vintemberger (1948) solution when they were freed from the agar gel, and some others after they had reached stage 24. Transverse and longitudinal serial sections (5–7 μm) were stained with haematoxylin and eosin. For Scanning electron microscopy (SEM) the embryos were fixed in 2.5% glutaraldehyde in phosphate buffer, rinsed with phosphate buffer, dehydrated through an ethanol series and dried according to the critical point technique. The dried specimens were coated with evaporated gold-platinum and observed in a Jeol JSM-U3 Scanning electron microscope operating at 5–25 kW.

Results. During the first experimental period (that is, while being embedded in agar) the growth of the embryos was severely distorted, partly as a result of non-uniform restriction of expansive growth and partly because of the irregular embryo-agar interface. At the end of this period, the embryos showed different types and grades of deformation (figure 1). In spite of the severe deformations, some distinct external features, i.e. increase in the number

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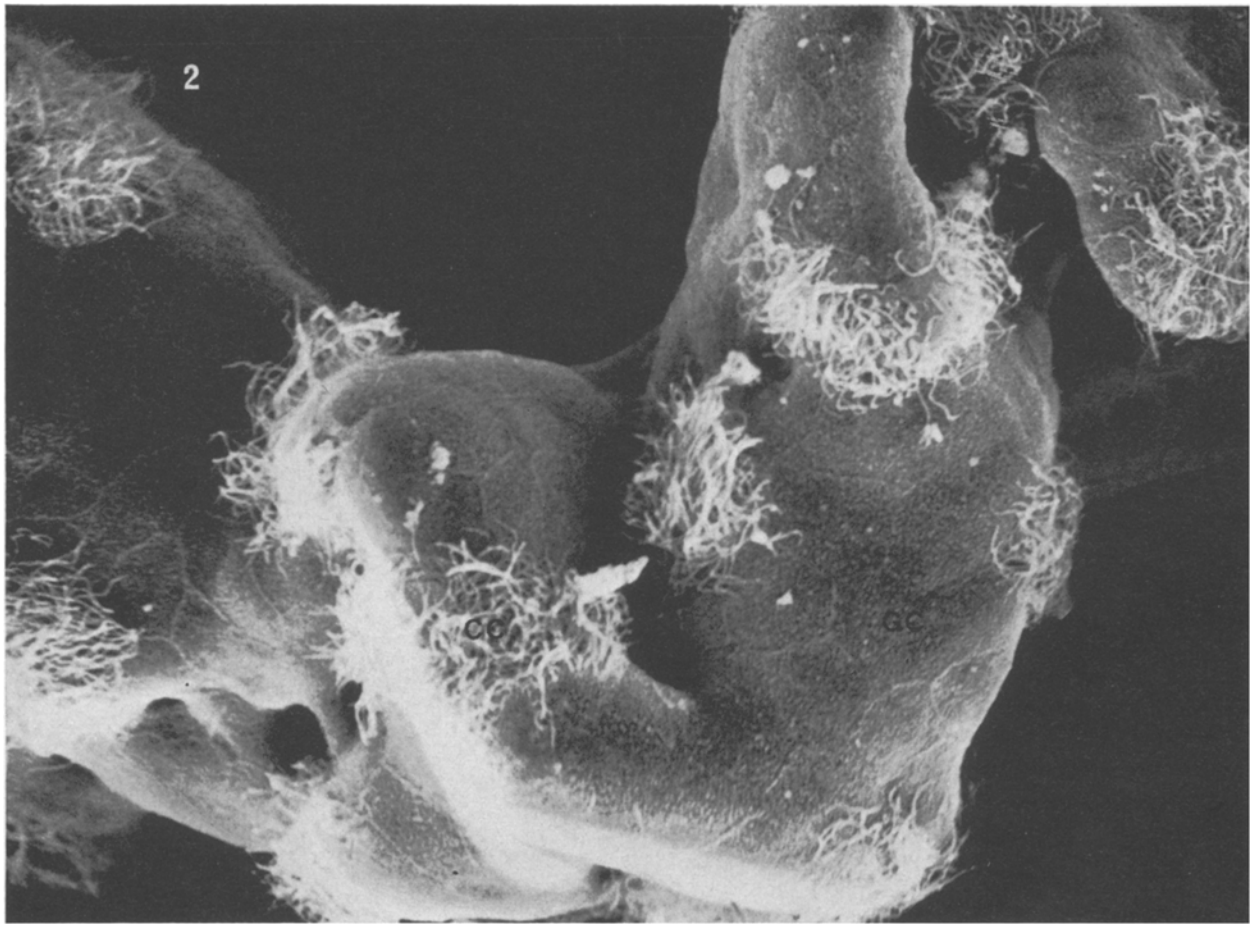

Fig. 2. Tail of an experimentally deformed embryo. Apart from alterations in cell shape, the epidermis shows normal glandular (GC) and ciliated cells (CC). ×1200.

of somites, appearance of gills, cardiovascular activity, growth of the tail, etc. clearly showed that the embryos continued developing. This was confirmed by histological analysis. In spite of the deformations, histogenesis as a whole seems normal. Even in structures deformed from the beginning, such as the tail, SEM study shows cytodifferentiation to be normal. Moreover the pattern of distribution of glandular and ciliated cells is also normal, i.e. ciliated cells occur singly (figure 2).

After the end of the first experimental phase, the embryos gradually and harmoniously tended to recover normal morphology while continuing their development. First the most conspicuous deformations disappeared. The various curvatures of the axis were corrected much more slowly and gradually (figure 3). Finally, at about stage 24, the embryos with only few exceptions became indistinguishable from the controls. The histological analysis of the embryos which had recovered normal morphology showed the internal structures also to be normal, while on the other hand SEM study showed normal development of ectoderm cells. Larval development of these embryos was normal, and metamorphosis occurred without difficulty, resulting in normal toads.

Discussion. Considering that the phenomenon described is apparently mainly one of shape-recovery, let me consider first the factors that seem to be involved in the determination of the shape of an embryo or organism at any one time: a) the pattern of association between the cells, and b) the shape of the cells.

a) The specific reassociation patterns shown by cells dissociated from different embryonic tissues, and even from different species[13–16], constitute immediate evidence: the cells form an assembly that is similar to an organism by joining in a highly specific way. There is also evidence that the transmission of morphogenetic signals between embryonic cells (that is a special form of intercellular communication) may often involve specific intercellular contacts[17–20]. Undoubtedly this specificity is maintained inspite of deformations, as the histological study and the development of the experimental embryos shows. So far, only very little evidence for alteration of intercellular junctions in the deformed embryos was found[21]. b) The shape of the cells is undoubtedly related to the specific pattern of cell association, while on the other hand cyto-

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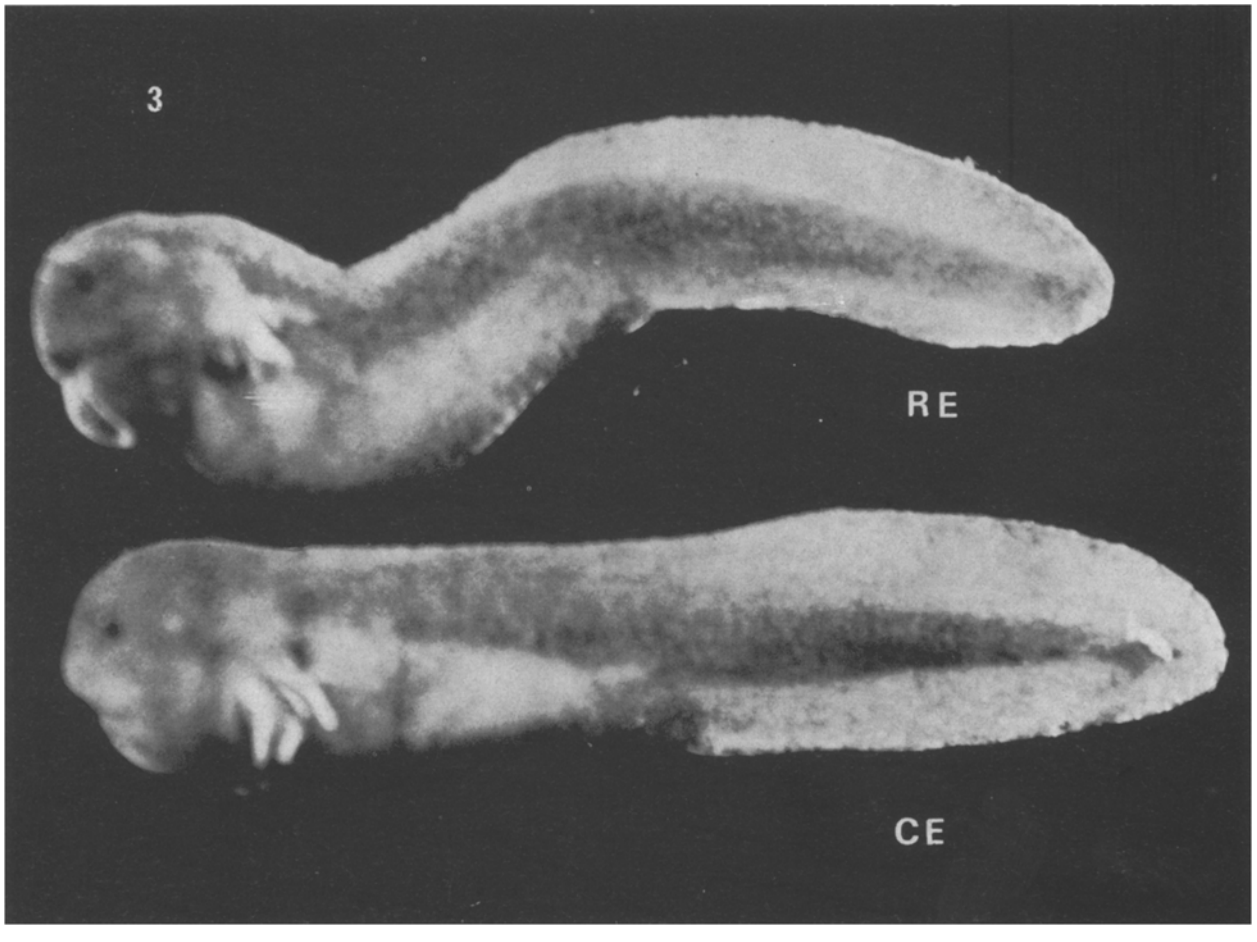

Fig. 3. Experimentally deformed embryo during recovering phase (RE). Control embryo at the same stage (CE).

plasmic organelles as microtubules are also involved[22,23]. The SEM study alone provides good evidence for cell shape alterations in the experimental embryos. However, these cell deformations do not seem to affect cell differentiation or further cell development, because the cells gradually reach their normal shape and continue to be normal as SEM analysis shows. On the other hand, at the supracellular level there is evidence that the different amphibian embryoric layers as a whole are considerably affected by the surrounding tissues[9]. These authors consider that morphogenetic shape change is directly determined by mechanical stresses in cell layers which occur as a consequence of their contraction capacity. Such stresses seem to play an important role also in the recovering phenomenon observed in experimentally deformed embryos.

The first conclusion from the results presented here is that an embryo can develop inspite of dramatic deformations (that is changes in the spatial conformation) and can even ultimately recover a normal morphology. A more general conclusion is that, within wide limits, no sequential change of overall shape is necessary for normal morphogenesis and pattern formation. Consequently, the form of the embryo appears to be determined by cell activities intrinsic to each stage.

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Chiasmate meiosis in roaches. II. Meiosis in Blatella supellectilium Serv.

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Summary. In the cockroach, Blattella supellectilium Serv., meiosis in the male is of a normal, chiasmatic type, with an average chiasma frequency of 1.3 per bivalent (2n = 22 + XO).

Until recently, the occurrence of chiasmata in cockroaches was a controversial matter. The opinion of Matthey[3], John and Lewis[4–6] and Sharma[7], that meiosis in these insects is of 'non-chiasmate' type was generally accepted. Subsequently in the light of the works of Suomalainen[8], John and Quaraishi[9], and Rajasekharsetty and Ramamurthy[10], the validity of such a view was doubted. Still the 'non-chiasmate meiosis' hypothesis first proposed by John and Lewis is retained in the literature. This was pointed out recently by the present author (Desai)[11] who described clear chiasmata in the roach Nauphoeta cinerea Oliv. In the same article it was proposed that other genera and species of roaches should be examined. This report concerns Blattella supellectilium Serv., a species in which male meiosis appears to be quite normal.

Over 100 males of this insect species were collected from different localities in and around Dharwar. The observations are based on aceto-orcein squash preparations of testes. Spermatogonial metaphases show a diploid chromosome number of 23 (22A + XO). Of the 11 pairs of autosomes, 8 pairs are metacentric and 3 submetacentric. The X-chromosome is also submetacentric. During meiosis pairing is intimate. The crossing over involves interstitial as well as distal segments. This situation is quite unlike that found in Periplaneta americana wherein chiasmata are restricted to the distal segments only (John and Lewis[4,5] and John and Quaraishi[9]). During the early diplotene stage, 3–4 chiasmata are seen in some

1 This paper is dedicated to my Professor and Principal, Dr J. C. Uttangi, who retired recently.
2 I express with pleasure sincere thanks to my colleague Dr R. M. Patil for his valuable suggestions during the course of this work.
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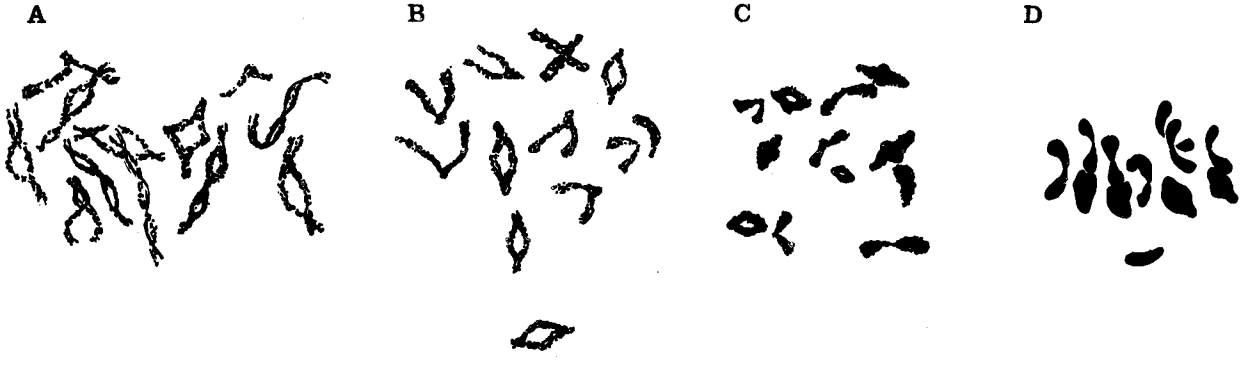

Camera lucida drawings from aceto-orcein squash preparations of testes. × 1500. *A* 11 bivalents and a univalent X at early diplotene. *B* Late diplotene with advanced terminalization of chiasmata. *C* Late diakinesis. Most chiasmata are terminalized. *D* Metaphase I.