EFFECTS OF CAUTHERYZATION OF TETRODONTOPHORA BIELANENSIS (COLLEMBOLA) EGGS FROM OVIPOSITION TILL PREMATURE BLASTODERM STAGE. INSTRUCTIVE EVENTS IN EARLY DEVELOPMENT

Czesław Jura

Sugadaira Montane Research Center, University of Tsukuba,
Sanada, Nagano, 386-22 Japan
(Permanent Address: Zoological Institute, Jagiellonian University,
ul. Karasia 6, 30-060 Kraków, Poland)

The ability of the fertilized egg or of the developing embryo to readjust, following experimental intervention, toward normal development varies from species to species even within order. But generally speaking the Pterygota may be divided into two groups.

Considerable differences exist between species in respect to the extend of the territory which is occupied by the germ anlage within the egg. The eggs of short germ anlage type have great regulative power, in opposition to those of long germ anlage type which are determined. These two main groups are characterized also at other levels, starting from type of oviposition, through egg cytoarchitecture, up to the length of time required for hatching. In general, the eggs of short germ anlage are produced in result of panoistic oogenesis, when laid, are characterized by thin peripheral cytoplasmic layer (periplasm) and developmental period of these eggs is long. The eggs of the second type are produced in result of meroistic oogenesis, they have thick periplasm (Fig. 1) and short developmental period (for review see Sander 1983).

The egg types outlines above are of course extremes with very variants in between, nevertheless, they remain in agreement with separation of Pterygota into two main taxa: Hemimetabola and Holometabola .

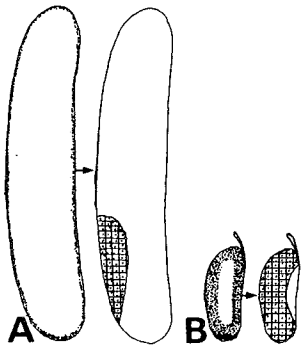

Fig. 1. Two pterygotan egg types (drawn to scale). The eggs are seen from left hand side and the region of periplasm spotted, the region of germ anlage spotted and stippled. Represented are: A- Achaeta domesticus (adapted from Mahr, 1960), Drosophila melanogaster (adapted from Turner and Mahowald, 1976).

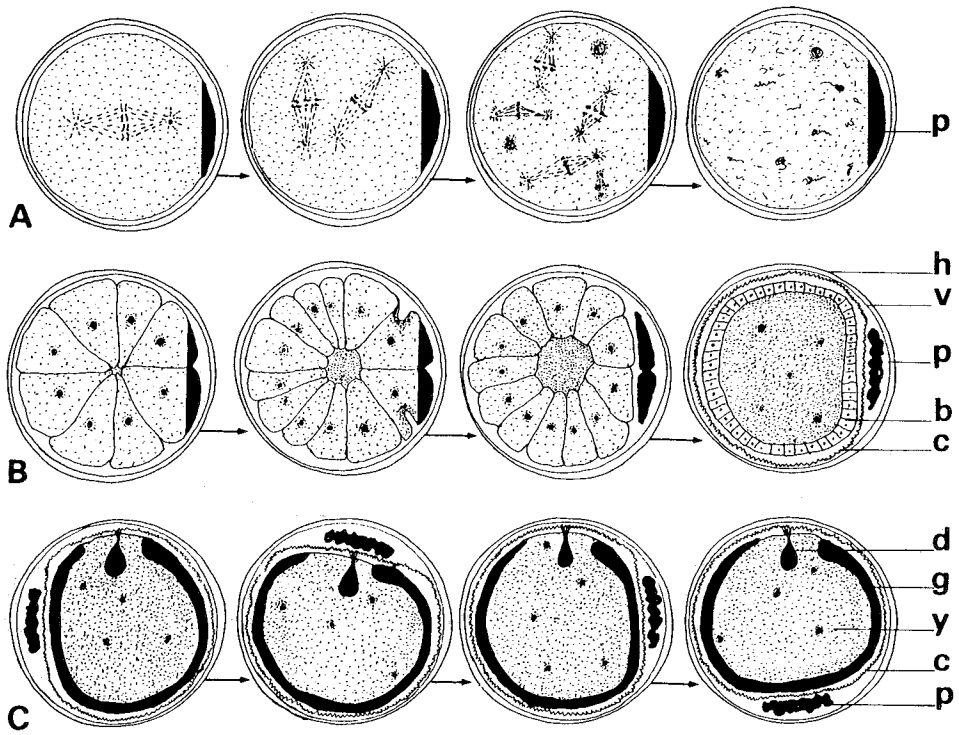

Fig. 2. Effects of cautheryzation of Tetrodontophora eggs: A- immediately after oviposition, B- at 8-blastomere stage, C- localization of coagulated egg portions in relation to germ anlage in eggs cautherized at 8-blastomere stage and succeeded development. Details in text. b- blastoderm, c-blastodermic cuticle, d- dorsal organ, g- germ anlage, h- chorion, p- coagulated egg portion, y- yolk, v- vitelline membrane.

According to the regulative power of the eggs of wingless insects (Apterygota, Ametabola) very little is known. In 1975 Jura has shown that the eggs of Tetrodontophora, when cautheryzed prior to 8-blastomere stage, are not able to repair damage. During successive years further developmental stages were tested, from 8-blastomere to premature blastoderm stage. Results of both series of experiments are summarized in this essay.

Tetrondontophora is especially intrique. The eggs of this species are produced in result of polytrophic oogenesis but they are characterized by extremely thin periplasm, long germ anlage (Jura, 1965) and very long developmental period (about 7 months).

The eggs of Tetrodontophora are very suitable for experimental manipulation because they are comparatively large (0.5 mm in diameter). The chorion is sufficiently transparent and the progress of development in living egg may be observed.

For testing the regulative capacity of the eggs of Tetrodontophora the thermocauter was used. In all cases only eggs peripheries were injured (for method see Jura 1975). The smallest lesions, which could be obtained by the method used, measured about 150 um in diameter, embraced about 8 % of egg surface and about 1.5 % of egg volume into the depth of about 20 um. The eggs were also cautheryzed above this limit, up to lesions involving about 20 % of egg volume.

Since the living eggs are spherical and nothing can be observed which would indicate their polarity, for cautheryzation the egg poles were checked haphazardly. In each particular experiment no less than 300 eggs were cautherysed, thus the possibility of injuring of the same egg pole in given series of experiment was excluded.

The eggs of Tetrodontophora exhibit a phase of total cleavage preliminary to the formation of blastoderm. The first two nuclear divisions are synchronous. These two nuclear divisions are completed within an undivided yolk mass, each quadrant of which becomes nucleated and the egg then divides directly into four equal blastomeres (Fig. 3 control). The third cleavage division, perpendicular to the previous two, is again equal and in result of this 8-blastomere stage is produced. From this last stage the furrows do not involve the whole yolk material of blastomeres, and innermost parts of pyramidal blastomeres are cut off as anucleate yolk masses, then the yolk masses fuse into a single mass filling the interior of the embryo, leaving the layer of blastomeres at the surface. At about 64-cells, radial divisions ensue in the pyramidal blastomeres, cutting of yolky cells invards which unite with central yolk mass and became yolk cells or primary germ cells. The peripheral

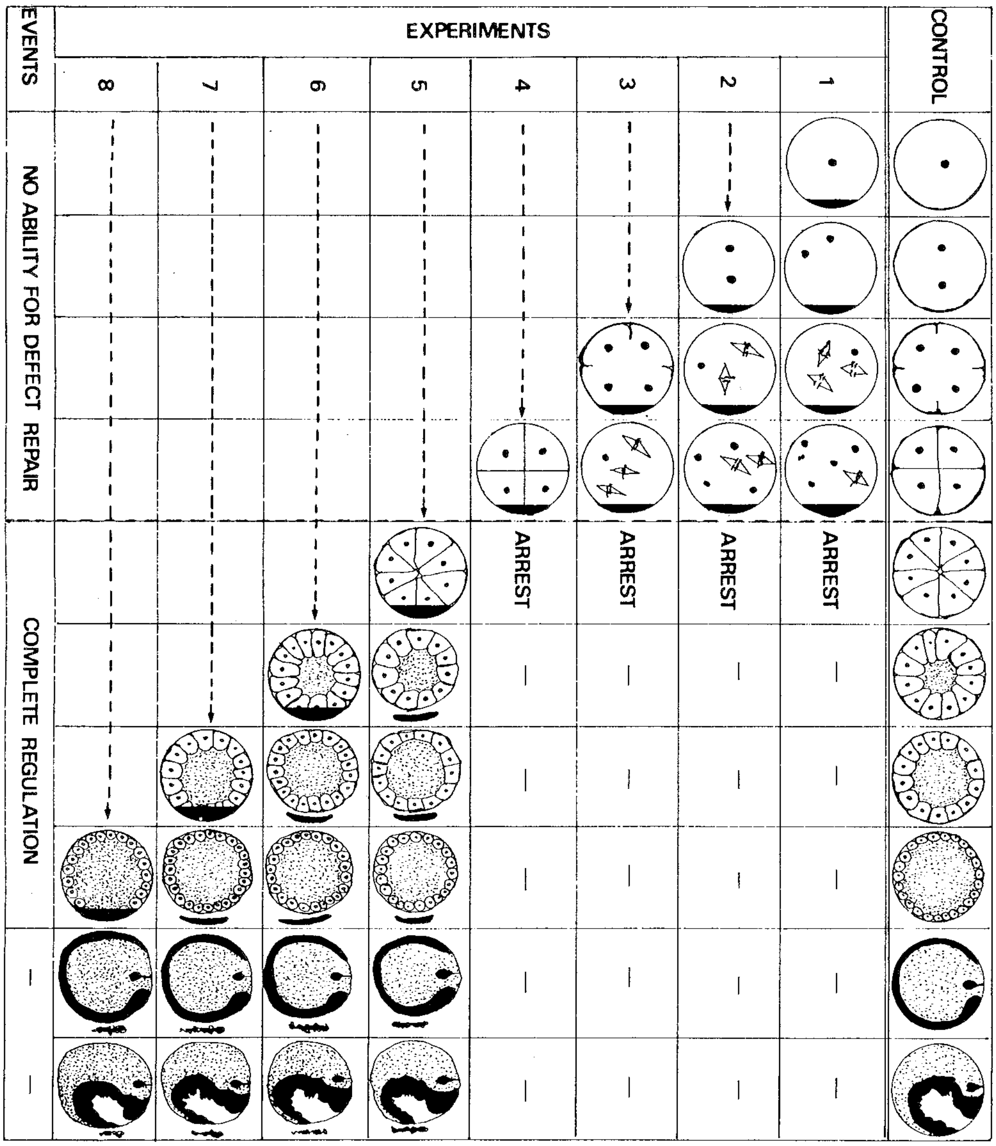

Fig. 3. Diagram summarizing results of cautheryzation of Tetrodontophora eggs from oviposition till premature blastoderm stage (experiments 1-4 after Jura, 1975). Details in text.

cells continue to divide with tangential spindles and again at every division the innermost yolky parts of blastomeres are cut off. At about 2500-cell stage the peripheral blastomeres became free of yolk. Now they are irregulary spherical in shape, loosely dispersed at the egg surface and represent premature blastoderm (Fig. 4). The blastomeres continue to divide and give rise to uniform mature blastoderm.

The eggs were cautheryzed from oviposition till premature blastoderm stage (*compare* Fig. 4). The effect of cautheryzation was not visible immediately (Fig. 5), only after some time the coagulated egg content was observable as milky spot. For observations only those eggs were selected which showed clearly a coagulation of peripheral region.

Cautheryzation of eggs immediately after oviposition, at 2- or 4-nucleus stage gave same results. About 50 % of embryos failed to develop shortly after cautheryzation. The abnormalities took unity of form. The developmental processes were limited to the nuclei mitotic divisions only. In all survived eggs depolarization of cleavage spindles, in relation to each other and in relation to egg surface, was observed (Fig. 2A; see also Jura 1975 Figs. 2 and 3). In opposition to controls, the mitotic divisions did not show synchronization. Within no one of these eggs cytokinesis occurred. In result of described abnormalities all eggs of this series of experiments died after several nuclei divisions (Fig. 3 experiments 1, 2 and 3). Analysis of sections revealed that the embryos represented many nuclear masses with no signs of morphological differentiations.

The eggs cautheryzed at 4-blastomere stage (Fig. 3 experiment 4) never continued development, all died shortly after operation.

Beginning with 8-blastomere stage the results were completely different from those described above (Fig. 3 experiment 5, 6, 7 and 8). We have also to stress out that beginning with 8-blastomere stage the analysis of experimental eggs was much more easier then in the previous case. Amongst both living as well as fixed eggs the borderlines between damaged and unaffected parts were well observable. When eggs were cautherized at more advanced stages, then the 8-blastomere stage, the moment of operation was especially easy to determine since the killed blastomeres kept unchanged shapes during long time.

The eggs cautheryzed at 8-blastomere stage, as the former ones, did not show visible effects immediately after operation (Fig. 5). Only after prolonged period of time a milky spots were observable marking the coagulate areas. The mortality of the eggs operated at this developmental stage was very high, reached about 97 %, of these 91 % died immediately after operation or somewhat latter, about 3 % developed into germ anlage but did not pass

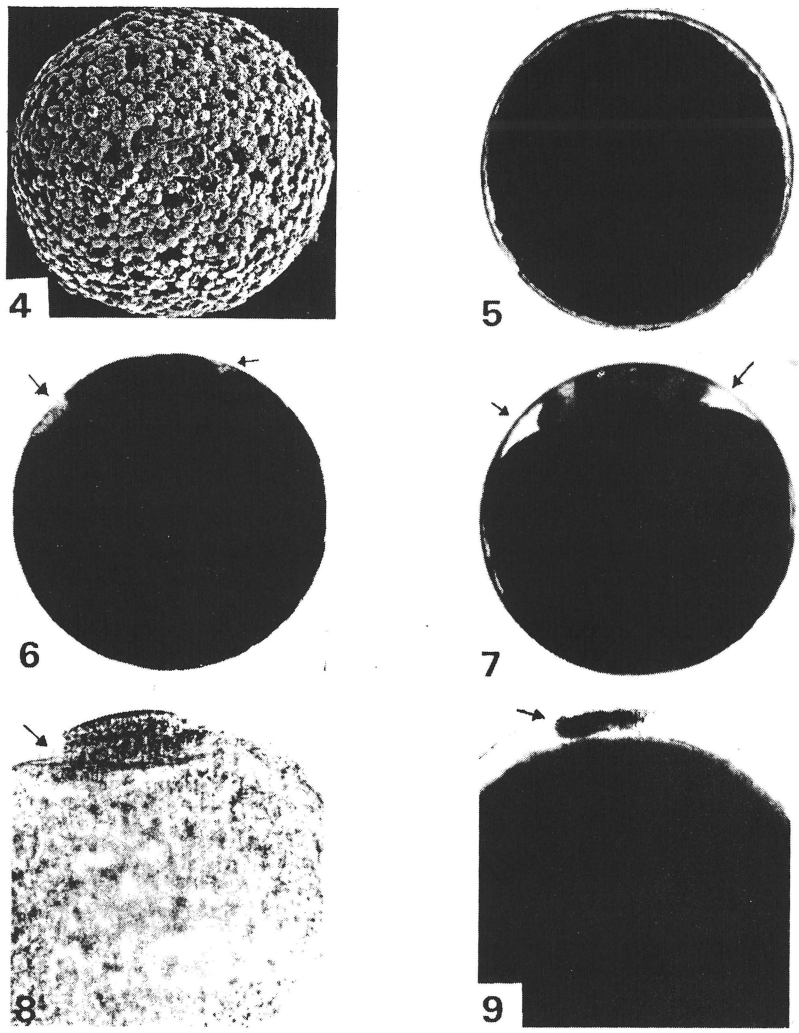

Fig. 4. Embryo of Tetrodontophora at premature blastoderm stage as seen under scanning electron microscope.

Fig. 5. Living cautheryzed embryo at 8-blastomere stage. The effect of cautheryzation is not visible immediately after operation (compare in this respect Figs. 6-9).

Fig. 6. Same embryo as shown in Fig. 5 but two days older. Arrows show incissions forming between cautheryzed and uninjured egg portions.

Fig. 7. Same embryo as shown in Fig. 5 but four days older. Beginning of separation of coagulated portion from the rest of the embryo body (arrows).

Fig. 8. Whole mounted experimental embryo of the same age as show in Fig. 7. Note separation of coagulated region (arrow).

Fig. 9. Same embryo as shown in Fig. 5 but one month older. The coagulated egg portion already separated (arrow).

beyond invagination stage. Only about 3% succeeded invagination (Fig. 3 experiment 5), developed further and before hatching did not show externally morphological abnormalities. However, the retardation of development of these embryos, when compared with controls, ranged about two months.

Analysis of living eggs cautheryzed at 8-blastomere stage, which survived operation, revealed that the cleavage of injured blastomeres was temporalily arrested, while uninjured ones cleaved further. Meantime, between uninjured and injured embryo portions incisions appeared, especially well observable on living material (Fig. 6). As the development proceeded, the incisions deepened and in result of this the uninjured and injured portions became separated from each other (Fig. 6-9). After separation the blastomeres being temporarily arrested in development recovered lost time and the embryos passed through successive developmental stages, comparable to those of controls (Figs. 2B, 3 experiment 5).

Survived eggs of this series of experiments, analysed at germ anlage stage, showed different localization of coagulated separated egg portions in relation to embryo (Fig. 2C).

Cautheryzation of the eggs at 16-blastomere stage or at more advanced stages, up to premature blastoderm stage, born same effects as in case of cautheryzation of 8-blastomere stage (Fig. 3 experiments 6, 7 and 8). Also the retardation of developmental processes was similar. Marked difference concerned the percentage of operated eggs which succeeded in passing beyond the germ anlage stage. The percentage strikingly increased accordingly to the age of the operated eggs (Tab. 1). Moreover, beginning with 400-blastomere stage some eggs in which about 12 % of their volume was coagulated could regulate further development and at prehatching stage did not show externally abnormalities. Thus the proportions of fragments, which could be killed raised accordingly to more advanced age of embryo.

Table 1. Capacity of Tetrodontophora eggs for complete recovery following cautheryzation of about 1.5% of ooplasmic content.

Stage treated (blastomere no.)	8	18	36	100	400	2500
% of embryos (of total 300) which succeeded development	3	3	5	9	18	21

The first impression which one may get when analysing figure 3, being summary of obtained results, is that the egg of Tetrodontophora up to 4-blastomere stage is not able to repair damage, but beginning with 8-blastomere stage shows great regulative power. Shortly speaking, the egg is first determinative then regulative in type. However, these results are open for certain criticism.

Ksiazkiewicz-Ilijeva (1974) has centrifuged Tetrodontophora egg prior cleavage using low speed. This procedure stratified the egg cytoplasm to gave band of lipid droplets, soluble cytoplasm and yolk granules, arranged perpendicular to the centrifugal direction. The treatment has been shown to have no effect on the subsequent development of fertilized egg, the embryo before hatching was normal. This implies that the regulative response may reflect the experimental technique utilized, but very striking difference in capacity for regulation exhibited by the eggs of Tetrodontophora following cautheryzation and centrifugation must be explained.

Eggs cautherized prior to cleavage did not succeeded development because of:

- desynchronization of nuclei mitoses,
- depolarization of mitotic spindles orientation,
- formation of supernumerous nuclei descendants in result of arrest of cytokinesis.

Since by the method used only egg periphery was injured we may conclude that syngamy initiates development but in egg periphery there exists extracaryotic information, which regulates the behaviour of nuclei undergoing mitoses. Extracaryotic information controls, or most probably, also initiates early sequestration of egg ooplasm into first four individual cells. Such conclusion does not contradict with the results obtained by means of centrifugation. It is well known that low speed centrifugation does not destroy egg cortex which is much more stiff than the inner ooplasm. on the other hand, the conclusion is also in agreement with recent findings that in most insects species the genome of zygote nucleus is not only store of information for early embryogenesis (see Sander,1983). Unfortunately the method used is not possible to resolve the problem in which component of egg periphery the extracryotic information is situated. Within the plasma membrane or under- lying periplasm? Since both components were injured by the method used.

In case of Tetrodontophora we must pay special attention to 4- and 8-blastomere stages. The 4-blastomere stage is the last being not able to readjust after cautheryzation, while 8-blastomere stage shows surprising

capacity for complete regulation, though both stages are morphologically similar. To give answer for this question we must turn again to normal development.

From 8-blastomere stage begins transition from total to superficial cleavage and thus this stage must be already equiped with information for decissive developmental events. It is reasonable to postulate that at 4-blastomere stage, or during the transition from 4- to 8-blastomere stage, new information is elaborated because beginning with 8-blastomere stage the process of yolk system formation must be controled.

Jura (1966), Jura and Krzysztofowicz (1977) have documented that in Tetrodontophora midgut origins exclusively from yolk cells. Basing on this finding we may postulate that the transition from total to superficial cleavage may be homologized with gastrulation. It is well known fact that in every animal group the pregastrula is a critical stage for further development. This stage is extremely sensitive for any intervention. In case of Tetrodontophora most probably 4-blastomere stage is such a sensitive phase in which important events occur leading to equip 8-blastomere stage with necessary information for gastrulation. Question is what is the nature of this information?

It is reasonable to postulate that new information is connected with rapid increase of gene expression. The gene products start at this time to interplay with extracaryotic information, as it occurs in other animal groups at the onset of gastrulation. This postulation finds also support in results obtained by Gancarzewicz (1975). The last author, by means of histochemical reactions, has shown at 8-blastomere stage a particularly high RNA concentration in the central area of egg, including the innermost parts of blastomeres destined for future yolk system (compare Gancarzewicz, 1975; Fig. 8). It has been clearly demonstrated in diverse animal groups that synthesis of various molecular species of RNA, by embryonic cells is not initiated immediately after fertilization, for insects in particular. There are evidences that in insect embryos mid-cleavage stages may represent an earlier synthesis of rRNA. We may assume same for Tetrodontophora, and because from 8-blastomere stage the nuclei are active and interplay between caryotic and extracaryotic information occurs,from this stage the embryo is able for reprograming its development in case of damage.

We may postulate such events because the egg of Tetrodontophora shows another properties being similar between many groups of animals. This is the common ability of early embryos to regulate their proportions following an alternation in normal size. As we have seen in these studies the normally

proportioned embryos can develop from 82 % sized fragments of cautheryzed eggs at 400-blastomere stage. In this last case not only ooplasm was killed but also blastomere nuclei. Thus the embryo of Tetrodontophora exhibits the universality concerning the totipotentiality of blastomere nuclei.

Another fact is of interest more from the evolutionary than from developmental point of view. In Pterygota localized cautheryzation of the surface ooplasm, prior to cleavage or at intralecital nuclei multiplication phase, does not prevent formation of cellular blastoderm (See Counce, 1973). Tetrodontophora also shows transitional intralecital nuclei multiplication phase (first and second nuclei division) but descendants of zygote nucleus are not able, in case of cortex damage, to form cellular blastoderm. Apparently the egg of Tetrodontophora at early developmental period represents different dynamic system from that of pterygote egg cleaving purely superficially. The egg of Tetrodontophora is at early period comparable rather to those of amphibian or molluscan eggs, in which partial injury of cortex after fertilization causes arrest of cytokinesis (Brachet and Hubert, 1972).

The difference between Tetrodontophora and Pterygota eggs is probably connected with different mechanism which regulates formation of cellular blastoderm. As we remember, in Tetrodontophora beginning with 8-blastomere stage at every following cleavage the innermost parts of blastomere are cut of as yolk masses. As the blastomeres became narrow, the nucleus and associated cytoplasmic halo within each blastomere moves outwards towards the exposed surface of the cell. Such process of formation of blastoderm is connected with repetitious synthesis and lossing of cell plasma membranes. This type of blastoderm formation is energy-absorptive and in consequence of it the development is relatively slowed and may be estimated as primitive. In Pterygota the cleaving nuclei, with their cytoplasmic halo, wander directly to periplasm. Synthesis of cell plasma membranes is completely ommited until the nuclei invade the periplasm. Thus the eggs of Tetrodontophora make link between the eggs cleaving purely totally and those cleaving purely superficially ones.

The eggs of Tetrodontophora were haphazardly injured. The killed egg portions were localized in different positions in relation to future germ anlage and the effects were always same, the localization of injury was not correlated with regulatory capacity of the embryos. Thus the eggs did not show potentiality connected with polarity. Such egg organization is different from that of Pterygota. Typical pterygotan egg is bilaterally symmetrical. But we must point out that only external morphology of experimental embryos was analysed.

Gancarzewicz (1975) has shown by means of histochemical methods that one pole of freshly laid egg of Tetrodontophora is especially rich in RNA. According to Klag (1982) this egg ooplasmic heterogenity bears for formation of primary germ cells. The regional heterogenity may refer to internal morphology, this is to formation of germ cells only, but for final conclusion further studies are necessary.

The general findings are that Tetrodontophora does not exhibit correlations established for pterygotan egg types. The oogenesis in case of Tetrodontophora is polytrophic but eggs are characterized by extremely thin periplasm, long germ anlage and very long period of development. Injuring of outer egg portion, prior to zygote nucleus division or during first cleavage divisions, is critical for subsequent developmental events. From 8-blastomere stage the same experimental treatment gives different effect, the embryo is able to realize consequently further developmental program. We have postulated that early developmental program is under influence of ooplasmic information, situated in outermost part of the egg. Beginning with 8-blastomere stage genetic information comes into interplay with ooplasmic information. However, nothing is known at this moment what is the nature of signals and responses which regulate developmental decisions.

In case of Tetrodontophora experiments with cautheryzation have showed failure to understand exactly what is meant by determination or regulation. These terms imply radically different mechanisms regulating developmental events. We have seen that the arrest of developmental processes following experimental intervention does not necessary mean determination. Distinction between determinative and regulative insect embryos, established in early part of this century, is probably not as valid as being used.

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References

Brachet, J. and Hubert, E. (1972) Studies on nucleocytoplasmic interactions during early amphibian development. J. Embryol. exp. Morph., 27:121-145.

Counce, S. J.(1973) The causal analysis of insect embryogenesis. In "Developmental Systems: Insects". (S.J. Counce and C. H. Waddington, Eds.), Vol. 2, pp.2-145, Academic Press, London and New York.

Gancarzewicz, B. (1975) Histochemical analysis of RNA in early stages of embryonic development of Tetrodontophora bielanensis (Waga)(Collembola). Zeszyty Naukowe UJ Prace Zool., 21:85-94.

Jura, Cz. (1965) Embryonic development of Tetrodontophora bielanensis (Waga) (Collembola) from oviposition till germ band stage. Acta Biol. crac. Zool., 8:143-159.

Jura, Cz. (1966) Origin of the endoderm and embryogenesis of the alimentary system in Tetrodontophora bielanensis (Waga)(Collembola). Acta. Biol. crac. Zool., 9:94-102.

Jura, Cz.(1975) Cleavage without cytokinesis of the egg of Tetrodontophora bielanensis (Waga) (Collembola) after periplasm damage with thermocauter. Acta Biol. crac. Zool. 18:97-102.

Jura, Cz. (1977) Ultrastructural changes in embryonic midgut cells developing into larval midgut epithelium of Tetrodontophora bielanensis (Waga) (Collembola). Rev. Ecol. Biol. Sol., 14:103-115.

Klag, J.(1982) Germ line of Tetrondontophora bielanensis (Insecta, Collembola). Ultrastructural study on the origin of primordial germ cells. J. Embryol. exp. Morph., 72:183-195.

Ksiazkiewicz-Ilijeva, M. (1974) Development of the eggs of Tetrodontophora bielanensis (Waga) (Collembola) when centrifuged before cleavage. Acta. Biol. crac. Zool., 17:69-75.

Mahr, E. (1960) Normale Entwicklung, Pseudofurchung und die Bedeutung des Fruchungszentrum im Ei des Heimchens (Gryllus domesticus). Z. Morph. Okol. Tiere, 49:263-311.

Sander, K. (1983) Extracaryotic determinants, a link between oogenesis and embryonic pattern formation in insects. Proc. of 19th An. Meeting of Arth. Embryol. Soc. of Japan :1-12.

Turner, R. F. and Mahowald, A. P. (1976) Scanning electron microscopy of Drosophila embryogenesis. I. The structure of the egg envelopes and the formation of cellular blastoderm. Develop. Biol., 50:95-108.