N'ayant pas étudié de Chéloniens, il nous est évidemment impossible de nier *a priori* les affirmations des cytologistes japonais. Cependant, ceux-ci ayant commis une erreur dans le cas du Lézard vivipare, matériel relativement facile, il n'est pas exclu que chez les Tortues, dont le type cinétique est beaucoup plus complexe, ils se soient trompés dans le compte des microchromosomes. Une discussion à ce sujet trouvera place dans la seconde partie de nos recherches, consacrée aux Oiseaux.

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Laboratoire de zoologie, Université de Lausanne et Institut de génétique, Université d'Utrecht, le 27 novembre 1955.

Summary

In contradiction to some Japanese authors (Oguma, Makino, Nakamura), we are unable to find any difference in the chromosome sets of the male and female of *Lacerta vivipara* ($2N = 36$). In a saurian, where the analysis is very easy (*Chamaeleon bitaeniatus*), there are, without the smallest doubt, 24 chromosomes in both sexes ($20M + 4\ m$). The evidence of a female heterogamety in turtles is by no means satisfying.

The Mitochondrial Pattern in the Development of the Ascidian Egg

(1)– Janus green has been, for a long time, considered a vital stain specific for the mitochondria. Recent investigations seem to have produced plausible evidence to explain such a specificity: the mitochondria are indeed supposed to be the seat of many enzymes, particularly the cytochrome-oxidase, with which Janus green chemically reacts.

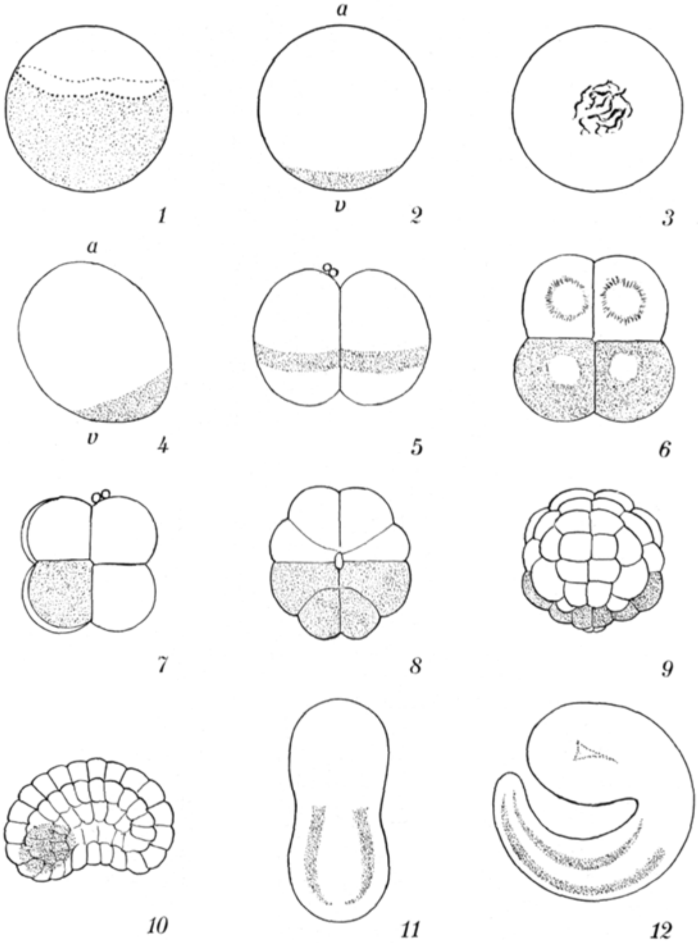
As Janus green in very diluted solutions is not toxic for the cell, it can be very profitably used to follow the modification and distribution of the mitochondria in the developing egg.

In the Ascidian egg, the distribution of mitochondria in specific cells during the embryonic development has been described by Duesberg[1]. Duesberg showed that the mitochondria of *Ciona* follow, in their distribution and segregation, the same pattern as the yellow pigment described by Conklin[2] in *Styela.*

Janus green has been used very little to study the differential distribution of mitochondria in different kinds of eggs or in the Ascidian egg itself. Only Child[3] recently used it in *Clavelina* egg, in the leucobase form after the reduction with sodium hydrosulfite, showing the existence of oxido-reduction gradients or peculiar oxido-reduction patterns.

The present work will be an introduction to more extensive research on the segregation of the mitochondria in the different cells of a developing embryo, and on the rôle of mitochondria in the processes of embryonic differentiation and induction.

(2)– *Phallusia mamillata* eggs were used as material for the present research. They are glass-clear, but an accurate observation reveals in the fertilized egg the yellow crescent, which was described by Conklin[2] in *Styela*. The *Phallusia* egg is therefore very suitable for treatment with vital dyes. The observation is greatly facilitated in eggs which have been deprived, mechanically or chemically, of their two membranes: the chorial one, with follicular cells, and the inner one, which is very thin and closely adhering to the egg; in the space between the two membranes there are numerous testal cells. Janus green colours the follicular cells purple, the testal cells blue and the egg green. Important differences are observed in the egg before and after fertilization.



Figs. 1–12.—Developing egg of *Phallusia*, after staining with Janus green (see the text). *a* = animal pole; *v* = vegetal pole.

The unfertilized egg possesses at the animal pole—which can thus be very easily determined—a small refringent spot, corresponding to the first metaphasic figure: the nuclear sap of the broken germinal vesicle is spread all over this animal zone. This zone does not take up any colour with Janus green: the rest of the egg, which contains vitelline granules and pigment granules is on the contrary coloured green. The two zones are very sharply separated by a circular line of intensively coloured granules or dots, which are situated subcortically (Fig. 1). The circular line is not even: it is rather sinuous, with slight differences in different eggs. An accurate search for evidence of the existence of a bilateral organization of the egg from the sinousity of the line did not give positive results. Child[3] was also unable to find evidence of such an organization with oxido-reduction indicators in the unfertilized *Clavelina* egg.

The aspect of the egg changes completely after the fertilization: the egg gradually becomes clearer, owing to a migration of the green particles towards the vegetal pole: the migration is very perceptible to the eye; it

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[1] J. Duesberg, Bull. Acad. roy. Belgique *5*, 463 (1913).

[2] E. G. Conklin, J. Acad. nat. Sci. Philadelphia *13*, 1 (1905).

[3] C. M. Child, Physiol. Zool. *24*, 353 (1951).

would be very useful to analyse it by cinematography. As a consequence of such a migration, the vegetal pole becomes intensively green (Fig. 2). Observing the egg from the vegetal pole, one can distinguish a mass of intersecting green filaments (Fig. 3) which seem to be constituted by a peculiar aggregation of mitochondria.

Before the expulsion of the first polar body the spheric shape of the egg changes; the morphological modifications are continuous, especially at the vegetal pole, where a lobe appears, which makes the egg pyriform or oblongated. It reabsorbs rapidly after its appearance. It is difficult to say what is the meaning of such a lobe; it recalls the vitelline lobe of an egg of *Chaetopterus* or *Myzostoma*. What is important is that the lobe contains the whole green mass of mitochondria (Fig. 4). After the reabsorption of the lobe, the mass of mitochondria becomes located, no longer in the vegetal pole, but subequatorially. It looks like transverse green stripes, which are the equivalent of the yellow crescent of CONKLIN. The localization of the green mass is sharply subcortical.

The first plan of segmentation divides the transverse mitochondrial stripes into two parts and distributes each of them in a cell (Fig. 5); the second plan of segmentation segregates the green mass of mitochondria into the two posterior cells (Fig. 6); and the third one in the two vegetal posterior blastomeres (Fig. 7). It is well known that these blastomeres contain all the mesoplasm and a part of the endoplasm.

It would be deceptive to imagine that all the mitochondria of the egg are segregated only in the two posterior vegetal blastomeres of the 8-cell stage; in fact, sparse green granules can be perceived in the plasm of the other blastomeres. That is very evident in the stages which precede the segmentations, because at that time the mitochondria "orient" themselves in the form of astral rays around the clear area of the broken nuclei (*cf*. Fig. 6).

The segregation of the mitochondria in particular cells becomes stricter with the following segmentations (Fig. 8); gradually the mitochondria become segregated in the prospective muscle cells. The 64-cell stage shows an intense green coloration in the exterior ring of cells of the vegetal posterior part (Fig. 9), which is demonstrated by ORTOLANI[4] to give rise only to the muscle cells; the A7·6 cells, whose fate is not yet ascertained, do not stain. At the gastrula stage the vital coloration is limited to the lateral lips of the blastopore (Fig. 10); at the early tail-bud stage two lateral stripes in the posterior part of the embryo are coloured (Fig. 11), corresponding to the muscle cells. In the tadpole only the muscles result differentially coloured (Fig. 12).

It is of course possible to follow all these aspects described in the same egg, since diluted solutions of Janus green are not toxic for its development.

(3)—As indicated above, CHILD[3], using reduced Janus green, came to the conclusion that in the *Clavelina* egg there is an oxido-reduction pattern and a gradient whose maximum should be at the animal pole. CHILD did not seem to realize the meaning of such a pattern in the egg of Ascidians, because he did not relate it to the segregation of mitochondria and of the oxidative enzymes contained in them. In any case it must be said that there is no gradient in the uncleaved egg; the Figure 9 in CHILD's paper represents an egg before the fertilization, which was treated with oxido-reduction agents: CHILD was not able to give the orientation of the egg, but my observations show that the more densely dotted part of the egg corresponds to the vegetal pole, not to the animal one. On the other hand there is a perfect agreement between my observations and the Figures reported by CHILD: only they are not in favour of the existence of a gradient. That can be deduced also from Figure 10 of CHILD's paper, where a fertilized egg is represented, after treatment with the oxido-reduction agents: in the Figure, by my interpretation, the mitochondria are still migrating towards the vegetal pole, where they have already formed a mass. Here no question of the existence of a gradient can be raised.

(4)—What I have described above is the pattern of mitochondria along the development of *Phallusia*. This pattern is achieved by their gradual segregation along the lineage of the muscle cells. Muscle cells are related to the movement of the tail of the tadpoles, and every movement requires energy. The problem of energy is connected, although not exclusively, with some mitochondrial enzymes. In relation to the existence of oxidative enzymes in the mitochondria of the Ascidian egg, there are at present not many data. My research using some specific substances which block the activity of certain enzymes shows that in some mitochondria at least the cytochrome-oxidase, the succino-dehydrogenase and probably the benzidine-peroxidase are localized. The question of the existence of qualitatively different mitochondria; and the question of their qualitative segregation in different blastomeres, whose determination they possibly influence, is again raised by this research.

This idea was present in the minds of the early cytologists such as MEVES[5], REGAUD,[6] and DUESBERG[1]. Let us recall what DUESBERG wrote:

> «Je suis tenté d'admettre qu'il y a une répartition qualitativement inégale des plastosomes, et cette répartition qualitativement inégale me paraît devoir être le résultat d'une différentiation plus ou moins précoce des plastosomes... On peut dès lors se figurer avec MEVES que dans les œufs doués d'un pouvoir régulateur, les plastosomes persistent pendant une partie du développement à l'état indifférent et donnent aux blastomères une potentialité totale, tandis que dans les œufs à mosaïque la différentiation se produit immédiatement après la fécondation, de telle sorte que chaque blastomère reçoive des matériaux différents.»

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Zoological Institute, University of Palermo, November 6, 1955.

Riassunto

Con il verde Janus è stata seguita la distribuzione dei mitocondri lungo lo sviluppo dell'uovo di Ascidie. I mitocondri hanno una distribuzione diffusa nell'uovo vergine: ma dopo la fecondazione sono trasportati al polo vegetativo dell'uovo e successivamente nella porzione subequatoriale dorsale. Vengono poi gradualmente segregati nelle cellule della linea muscolare. Sono queste anche le cellule più ricche in citocromoossidasi, e che presentano un'attività ossido-riduttiva più intensa.

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[4] G. ORTOLANI, Exper. *11*, 445 (1955).

[5] F. MEVES, Arch. mikr. Anat. *82*, 215 (1913).

[6] CL. REGAUD (quoted by MEVES).