STUDIES ON THE DEVELOPMENT OF THE GONAD IN THE SILKWORM, *BOMBYX MORI* L.

7. DIFFERENTIATION OF GERM CELLS IN EGGS CAUTERIZED AT THE CLEAVAGE STAGE.

By Keiichiro MIYA

Institute of Applied Entomology, Iwate University

Received Nov. 12, 1956.

UDC : 535.787 : 638.22 : 591.39

In one of the previous papers, the author reported on a morphological study of the presumptive genital region in the blastoderm stage of the silkworm eggs (Miya 1953). It has been shown that the differentiation of germ cells in the silkworm may take place just after certain cleavage nuclei enter into the definite region of the periplasm, as was observed in the Diptera, some species of the Coleoptera and Hymenoptera. The definite region, called the "pole plasm", "germinal cytoplasm", "germ-track determinant", "oösome" and so on, in the Diptera and some others, lies in the posterior pole of the egg (Johannsen and Butt 1941), whereas the corresponding region of the silkworm seems to be located at the ventral side of the egg (Miya 1950). Unlike the Diptera and some others, the region is morphologically indistinguishable in the silkworm from the other parts of the periplasm. In order to distinguish the region from others in the silkworm, the author cauterized the ventral area of the egg at the cleavage stage and examined the relational configuration between the degree of injury and the differentiation of germ cells. This paper is to record the results of the study so undertaken.

Before going further, the author wishes to acknowledge Prof. Saji Makino for his direction and reading through the manuscript.

Material and Method

Non-hibernating eggs of the strain "p 22" (normal, white cocoon, bivoltine) were used as material for this study. At the early cleavage stage (4 hours after egg-laying) and the late stage (7 hours after), the eggs were cauterized with a hot needle in the several parts of the ventral side. Eighty-four hours after egg-laying they were fixed with Carnoy's fluid and sectioned into 8 micra thick. Delafield's hematoxylin was used for staining. Drawing and measurements of the cauterized parts were made by the aid of a camera-lucida.

Results and Discussion

The operation of the egg at early and late cleavage stages resulted in a coagulation of the periplasm and the yolk in situ: some cleavage nuclei were disturbed from migrating into the peripheral layer. As shown in the former experiments (Takami 1946, Miya 1953), the cauterization experiments performed at the blastoderm stage brought about the splitted embryos only, while the operation at the cleavage stage produced not only the splitted embryos, but also the non-splitted ones (Takami 1946). Fig. 1 shows the relation between the degree of injury and the formation of splitted embryos. Both the eggs operated 4 hours after laying and those treated 7 hours after showed similar results; there was a limitation near 10% on abscissa. When the degree of injury was below 10%, the non-splitted embryos were brought about. When it was over 10%, the splitted embryos were produced. No such relationship was observable on ordinate; when the degree of injury in the cross section was 10% and the percentage for the longitudinal axis was 32%, the embryos produced were not splitted. On the other hand, when the former value was 18.8% and the latter 18%, the formation of splitted embryos resulted.

Fig. 1. Relation between the degree of injury and the splitting of embryo. Abscissa: degree of injury (percentage for the entire circuit of the cross section of the egg); ordinate position of the cauterized part (percentage for the major axis of the egg from the posterior pole); ○: non-splitted embryo in the egg cauterized 4 hours after egg-laying; ●: non-splitted one in the egg treated 7 hours after; ×: splitted one in the egg treated 4 hours after; ⊗: splitted one in the egg treated 7 hours after.

The cleavage nuclei lying along the cauterized portion usually remained their original configuration; they mostly degenerate in future, while the nuclei which had migrated into the peripheral layer showed proliferation with the subsequent formation of cell-wall. The relationship is thus; the narrower the destroyed part was, the fewer the degenerated cells occurred. Then the closure of the separated part occurred rather fairly due to proliferation of the cells which had migrated into the sound periplasm. Figs. 2 and 3 are the cross sections of certain cauterized eggs at the germ-band stage. In Fig. 2, the germ band was completely contiguous as seen in the normal embryo, with the normal differentiation of germ cells. In fig. 3, the

Fig. 2. Cross sections of cauterized eggs showing the non-split embryo.
cr: coagulated region; gb: germ-band; gc: germ cell.

Fig. 3. Cross section of a cauterized egg showing the splitted embryo.
db: degenerating cells.

germ-band was discontinuous, the damaged part being larger than that in the former. Moreover, there existed many cells without forming cell-wall in a form of syncytium. Takami (1948) reported in the cauterization experiment the abnormal proliferation of the cleavage nuclei lying along the artificial surface. Probably the cells showing the syncytium may be caused by a similar mechanism.

Fig. 4 shows the relation

between the degree of injury and the existence or absence of both mesoderm and germ cells in the splitted embryos. As seen in the figure, the limitation existed in a manner similar to that reported in the previous paper (Miya 1953). The one limitation was shown at ca. 9% on abscissa: when it was over 9% the mesoderm was absent, while the reverse relation occurred when it was below. The other limitation was shown at ca. 5% on abscissa: when it was below 5% the germ cells made their appearance. But the latter limitation was applicable only when it was nearly 30% on ordinate; this suggests the existence of "germinal cytoplasm". Different from the experiment performed at the blastoderm stage, there was no correlation between the degree of injury and the number of germ cells

(correlation coefficient=0.196); this result suggests the first proliferation of primordial germ cells.

Diptera, a certain forms of Coleoptera and some Hymenoptera, generally show at the posterior pole of the egg a particular region in which the cleavage nuclei differentiate into germ cells. This region is clearly distinguishable by the existence of peculiar chromophile granules or the special affinity for basic dyes; it is designated as "pole plasm", "germinal cytoplasm", "germ-track determinant", "oösome" and so on. When the germinal cytoplasm was destroyed by means of experimental ways such as cauterization or irradiation with ultraviolet light prior to the migra-

Fig. 4. Relation between the degree of injury and the structure of embryo. Abscissa: degree of injury (percentage for the semi-circuit of the cross section of the egg); ordinate position of the cauterized part (percentage for the major axis of the egg from the posterior pole); ○: embryo with the mesoderm and germ cells; ●: embryo with the mesoderm and without germ cells; ×: embryo without the mesoderm and germ cells.

tion of the cleavage nuclei, the germ cells did not make their appearance resulting in the formation of the gonad without germ cells in some cases (Hegner 1908, Reith 1925, Geigy 1931). In the silkworm, however, no particular region of cell differentiation was distinguishable in any part of the egg. Miya (1950) found that the posterior pole was not the place of germ-cell differentiation. The results of the present observations made clear the existence of the germinal cytoplasm and its locality; this seems to correspond to the presumptive genital region found at the blastoderm stage. It is most probable that at the blastoderm stage the number of germ cells may be predetermined and that they may not be complemented even if they are partly damaged, while at the cleavage stage they may be complemented to some extent due to proliferation of cells when a small part of the germinal cytoplasm is free from breaking down. This can be explicable in connection with the formation of the non-splitted embryos.

An important study has been made by Takami (1946) on the formation of the non-splitted embryo. In the non-splitted embryos, when the degree of injury at one side is smaller than the presumptive mesodermal region, the mesoderm develops at both sides, even if the injury at the other side is larger. Similar fact was observable from the present study in the differentiation of germ cells. It is then apparent that when the degree of injury at one side is smaller than the germinal cytoplasm the formation of non-splitted embryo is resulted having germ cells on the both sides.

Summary

1. The non-hibernating eggs of the silkworm were cauterized at the cleavage stages, 4 and 7 hours after egg-laying; they were fixed 84 hours after laying to examine the relational feature between the differentiation of germ cells and the degree of injury.

2. When the degree of injury falls within about 10% for the entire circuit of the cross section of the egg, the non-splitted embryos are formed, while it is over 10% the formation of the splitted ones is resulted.

3. The germinal cytoplasm was found to exist in the silkworm; it corresponded to the presumptive genital region observed at the blastoderm stage. There was no correlational evidence between the extent of the germinal cytoplasm and the number of germ cells. From this result the proliferation of the primordial germ cells may probably go ahead the completion of the blastoderm.

References

- Johannsen, O. A. and F. H. Butt (1941). Embryology of insects and myriapods. New York.
Miya, K. (1950). Trans. Sapporo Nat. Hist. Soc., 19: 36-39.
——— (1953). J. Fac. Agr. Iwate Univ., 1: 223-227.
Takami, T. (1946). Seibutsu, 1: 208-211.
——— (1948). Zool. Mag. Tokyo, 58: 51-52.