DISTRIBUTION OF CELL DIVISIONS IN THE OPTIC VESICLE AND PRESUMPTIVE ECTODERM OF CHICK EMBRYOS

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Foci of cell division with the character of "nests" of varied extent, or of small clusters, have been described in the nodules of Ehrlich's adenocarcinoma of mice [1, 2]. In the overwhelming majority of cases the paired mitoses are out of step, i.e., their phases of mitosis are different. A similar pattern has been found in the corneal epithelium of the mouse [5, 6]. This focal character of mitosis has been found also in the myocardium of chick embryos treated with serum [3].

The object of the present investigation was to study the distribution of mitoses in embryonic material: in the optic vesicle and the presumptive ectoderm in the period during which they are in contact in the chick embryo (48 h of development) at different times of day.

EXPERIMENTAL METHOD

Experiments were carried out on 48-hour embryos. Standard hen's eggs were placed in the incubator every 2 h for 24 h, starting at 12 noon. The same eggs were opened 48 h later at the same times of day, the embryos were taken out, and histological preparations were obtained and stained with Carazzi's hematoxylin and eosin.

The mitoses were counted in five embryos at each time corresponding to the beginning of incubation all round the clock (60 embryos altogether). The embryos in which the counts were made were chosen from many specimens by the uniformity of the morphological picture of the topography of the eye and the presumptive ectoderm. Mitoses were counted in central sections through the eye anlage at the place where the ectoderm, somewhat thickened in its central part, touches the optic vesicle. They were counted in three successive sections (7 μ thick) at intervals of one, or sometimes two, sections. The area to be studied was situated in the optic vesicle, from the center of the zone of contact with the ectoderm to the midline of the optic vesicle, and in the ectoderm on both sides of the central thickening as far as the point where the ectoderm became thin. By means of a drawing apparatus the outlines of the sections of the optic vesicle and presumptive ectoderm were transferred to paper. The number of cells and of mitoses in the various phases was then counted in the sections. The localization of the mitoses in the optic vesicle and ectoderm was noted.

EXPERIMENTAL RESULTS

Considerable individual differences in the development of chick embryos are recognized. These can be substantially reduced if the morphological picture is standardized in accordance with a particular sign, in this case the degree of contact between the optic vesicle and the ectoderm.

No strict rules could be discovered governing the distribution of the paired mitoses and its relationship to the time of day. So far as the single mitoses were concerned, they increased in number with an increase in mitotic activity, and by 8 P.M. (at the maximum of mitotic activity [4]) they were most numerous. This applied equally to the optic vesicle and to the ectoderm.

The following conclusions were drawn from the counts of the dividing cells. The total number of mitoses in the optic vesicle of all the embryos was 2968, and of these 874, i.e., 29.4%, were single mitoses. The number of grouped mitoses was 740 (25.9%), including 197 (13.113%) in step and 185 (12.406%) out of step. Of the total number of mitoses 582 (19.6%) were in clusters of three, 268 (9.03%) in clusters of four, 180 (6.06%) of five, 84 (6.06%) of six, and 63 (2.12%) in clusters of seven mitoses. All other combinations accounted for 46 mitoses (1.54%).

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The pattern of distribution of the mitoses in the ectoderm was as follows. The total number of mitoses was 2650, of which 1926 (72.68%) were single. The distribution of the grouped mitoses was as follows: 484 (18.26%) were paired, 127 (4.79%) were in clusters of three, 60 (2.3%) of four, 5 (0.18%) of five, 7 (0.26%) in clusters of six mitoses, and so on.

In the overwhelming majority of combinations, in both the optic vesicle and the ectoderm, the prophase was an essential component.

Despite the fact that in the optic vesicle and the ectoderm the total number of mitoses (single and grouped) was almost the same (2969 and 2650 respectively), the number of single mitoses in the optic vesicle was 29.45% and in the ectoderm 72.68%. The degree of "nesting," i.e., the number of cells per nest, was interesting. Some nests in the optic vesicle contained from 2 or 3 to 7 clustered cells. In the ectoderm it was very rare to find more than 4 cells to a nest. The percentage of mitoses out of step also differed: 24.84% for the optic vesicle and 13.44% for the ectoderm.

To discover whether the distribution of the dividing cells per nest was random or regular, the material was analyzed by the statistical method proposed by L. Ya. Blyakher and V. N. Dobrokhotov [1].

Comparison of the true incidence of paired mitoses with that expected theoretically showed that the coefficient of excess of the true over the theoretically expected in the optic vesicle was 1.6, and in the presumptive ectoderm 1.1. Since the true incidence of 3, 4, or more mitoses per nest is of the same order of magnitude, whereas the theoretical probability of the incidence of 3 mitoses per nest is lower by two orders of magnitude than the probability of finding 2 mitoses, the authenticity of the existence of groups of 3, 4, or more mitoses per nest in more than the calculated number is obvious.

These results show that the distribution of the dividing cells in the optic vesicle and ectoderm differs essentially both in the number of nests of cells and the number of cells to a nest, and also in the number of asynchronous mitoses. At the same time, no difference could be detected in the number of nests of mitoses at different times of day, apart from an increase in the number of single mitoses at 8 P.M., the time of maximal mitotic activity.

The experimental results thus demonstrate that the course of mitosis in embryonic tissues obeys definite topographical principles.

Only further investigations will reveal whether the nesting is the only one, or whether other principles governing cell division may be observed in embryonic tissues; the character of these principles, and whether the nesting is the result of the regulatory mechanisms of the growing organism or whether intracellular factors or a combination of both play the leading role in this process; and lastly, the changes in the character of nesting at different stages of development of the organism.

SUMMARY

It has been shown that dividing cells are arranged in nests in the optic vesicle and the presumptive ectoderm in the period of contact between the two in a 48-hour-old chick embryo. The degree of this arrangement in the optic vesicle and ectoderm is variable: in the optic vesicle there are 29.4% of isolated mitoses and 25.9% of paired mitoses. The number of dividing cells per nest in the ocular rudiment is up to seven. In the ectoderm isolated mitoses account for 72.7%, paired ones – 18.3%. The number of mitoses per nest does not exceed four. The number of isolated mitoses and various combinations of conjugate mitoses varies during a day.

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