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# AN ANALYSIS, BY MEANS OF THE MITOTIC INDEX, OF THE PROCESSES OF THE EARLY DIFFERENTIATION OF ORGANS IN FUNDULUS HETEROCLITUS

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At the end of the last century, the study of embryology assumed a decided cytological cast. As a result of this emphasis, experimental embryologists today are giving considerable attention to the relationships between cellular proliferation, differentiation and growth. Various mathematical techniques have been used to express these relationships, among which has been that of the "Mitotic Index."

This method consists of determining, by actual count, the frequency of mitotic figures among the cells of a tissue at various developmental stages. Those regions, where the greatest percentage of the cells show mitotic figures, would be, supposedly, the places of greatest mitotic activity

at the time of fixation. The term "Mitotic Index" was first used by Minot in 1908 and was applied to the number of dividing cells per thousand. Later usage has extended the meaning of the term to be "the percentage of cells in division." The relationships of the mitotic rate to the differentiation and growth of the embryo are of great importance in solving the problems confronting the embryologist and cytologist today.

The study, herein reported, is the fifth of a series in which the early processes of differentiation in fish embryos have been investigated in this cytological fashion. Because of the ease with which embryos of known age and degree of development may be secured, *Fundulus heteroclitus* was used. The specimens were obtained at the Marine Biological Laboratory, Woods Hole, Massachusetts, during the summers of 1934 and 1936. Care was taken to see that the cultures were not overcrowded and that the temperature remained approximately the same as that of the ocean nearby, thus insuring normal development.

Observations were made of the entire period of development from fertilization until the time of hatching and critical periods in the early morphosis were selected for analysis. A total of 1,500 embryos were fixed, 480 imbedded and 147 sectioned in preparing material for this study. Only embryos that appeared normal in development were sectioned and the specimens selected for counting were as nearly representative of an embryonic stage as it was possible to secure.

Three important degrees of morphogenesis were recognized. The first follows gastrulation and is the period during which the embryonic axis is formed and the blastopore closed. The second stage is one in which the brain, sensory structures and other organs are developed. The third can be described best as the functional period of differentiation.

From these periods of early organ formation eight embryonic stages were selected as follows: the embryonic shield before the formation of the axis of the embryo, immediately after the appearance of the neural cord and the embryonic axis, during the closure of the blastopore, before the formation of the mesodermal somites, after the appearance of the first seven somites, the early development of the optic vesicles, the early differentiation of the optic cup and lens, the completion of the optic cup and the lens. Nine embryos were chosen from these groups and analyzed by means of the mitotic index. As the exact ages of the *Fundulus* embryos were known, calculations were made to determine whether it is necessary to postulate methods of cell multiplication other than ordinary mitosis to account for the number of cells found. It was demonstrated that the mitotic rates observed were fully adequate to account for the increase in cell number from one embryonic stage to the next.

During the early cleavages it was noted that the new cell generations were produced approximately every hour. With the onset of gastrulation the interkinetic period lengthened and as differentiation progressed the rest periods became correspondingly longer. In the fifty-six hours between the embryos twenty-four and eighty hours of age, only two cell generations were produced, although during the first twenty-four hours fifteen cleavage cycles had occurred. This great increase in the length of the interkinetic period is interesting when one remembers that it is correlated with the development of the embryonic axis and the formation of the early organs. It would seem highly probable that the energy relationships necessary in the early processes of morphosis might account for the increased length of the resting stage in cell division.

The mitotic data were tabulated and correlated with the formative changes occurring in the embryos. The results were then compared with the conclusions concerning the ontogeny of *Fundulus* derived by other methods of study. The data determined by means of the mitotic index were found to agree in most details with the observations of workers using

other techniques. Comparisons also were made with the previous studies of the mitotic index of this and other animals. The conclusions of Richards, Porter, Derrick and Self were, in general, substantiated.

Throughout all of the embryonic series studied, it was noted that, with few exceptions, the nuclei of the cells undergoing mitosis were located near the free epithelial surface of the lumen of the neural tube. This is in agreement with Sauer's theory concerning the migration of the epithelial nucleus during mitosis.

The observations on cell migration and localization in *Fundulus* and other teleosts, made by means of the vital staining technique of Vogt, were confirmed. The origin of the endodern by invagination in the region of the blastopore is indicated.

Variations in the rate of cell division appear to be correlated with the changes in the structure and shape of the embryonic organ. Periods of differentiation or reorganization alternate with periods of cellular proliferation. During stages of differentiation the mitotic index decreases but it increases during intervals of growth.

Mitosis and differentiation are to be considered alternative processes. Growth (increase in protoplasmic volume) of an organ as a whole may occur simultaneously with increased cell division in that organ. The degree of morphological development in an embryo was not found to correlate necessarily with the cell number or the age of the embryo.

Centers of mitotic activity are frequently asymmetrical, probably due to the disturbance of the mitotic rhythms of corresponding regions. The mitotic indices of the organs and embryos that were studied vary, but as most of the processes of the period of early organ formation studied here involve an upward swing of the growth curve, the progressive decrease in the mitotic rate due to advancing age and differentiation is not evident.