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ON THE CELL PROLIFERATIVE ACTIVITY OF THE CHICK EMBRYO OPTIC CUP AFTER DIVIDING IT INTO NINE SECTORS

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ABSTRACT

In this paper we describe the results of the analysis of cell reproduction in the developing avian neural retina after dividing it into nine sectors. The distribution and frequency of different mitotic phases in the retina of the optic cup was studied in chick embryos in Hamburger-Hamilton's stages 13-14 and 17-18. A stereometric method was used to locate the figures for the mitotic index and their corresponding prophase, metaphase and anaphase-telophase indices throughout the optic cup.

Keywords: Cell division, chick embryo, optic cup.

INTRODUCTION

In observing the component cells of the optic cup in early stages of development, it is clear that the entire population possesses the potential to undergo mitosis. As has been pointed out (Langman et al., 1966), the nucleus of cells preparing to divide moves toward the ventricular margin, hence it is in this region where mitotic figures can be observed. Although studies have been carried out about the developing optic cup (Fujita and Horii, 1963; Kahn, 1973; Prada et al., 1981), few studies have been published to date on the possible differences in mitotic indices among the early stages of development in the chick embryo optic cup. In this paper a study was carried out on the frequency and distribution of different mitotic figures by dividing the area and volume of the structure to be studied into nine sectors.

MATERIAL AND METHODS

The material examined was taken from a total of 20 chick embryos, 10 in Hamburger-Hamilton's stages 13-14 and 10 in HH stages 17-18. The developmental stage was determined according to the parameters described by Hamburger and Hamilton (1951) and O'Rahilly and Meyer (1959).

Tissues were fixed in a mixture of glutaraldehyde and formaldehyde embedded in Spurr's resin and cut into 4 μ thick sections. After staining with toluidine blue, for interphase cell counts the nucleolus was taken as the unit of measurement, and the remaining mitotic phases were identified and counted as prophase, metaphase, anaphase or telophase according to the size and distribution of chromosomic material.

Two different procedures were used to determine precise values for

this work: a) an appropriate correction of the raw mitotic value counts was performed according to the formulas proposed by Calvente et al. (IV ESS, Göt. - 1985 -) and b) the exact location of each figure was determined by the method of Valderrama et al. (IV ESS, Göt.- 1985 -). For the values obtained the averages were calculated from 20 animals from which slices between 15% and 50% of the total were taken as a sample.

OBSERVATIONS

The first problem to be solved when studying the developing optic cup consists in a correct orientation of the material. The histological sections obtained can show two basic orientations, frontal and horizontal, with respect to the optic cup. Figure 1a illustrates a frontal section from a stage 13 chick embryo in which the lens has barely begun its invagination and the optic cup is still in the primitive stages of formation; in a corresponding section from a stage 17 embryo (Figure 1b) the lens is seen in the final moments of invagination. On the other hand, as can be seen in Figure 2, the horizontal sections show the retina in a "C" form, distinguishing the anterior part ("Ant.") from the posterior ("Post.")

Figure 3 shows the distribution of various mitotic phases in the ventricular surface of the developing retina. The possible variations in the figures may represent differences in the frequency and distribution of the different mitotic phases. These differences are even more apparent when the optic cup is divided into sectors.

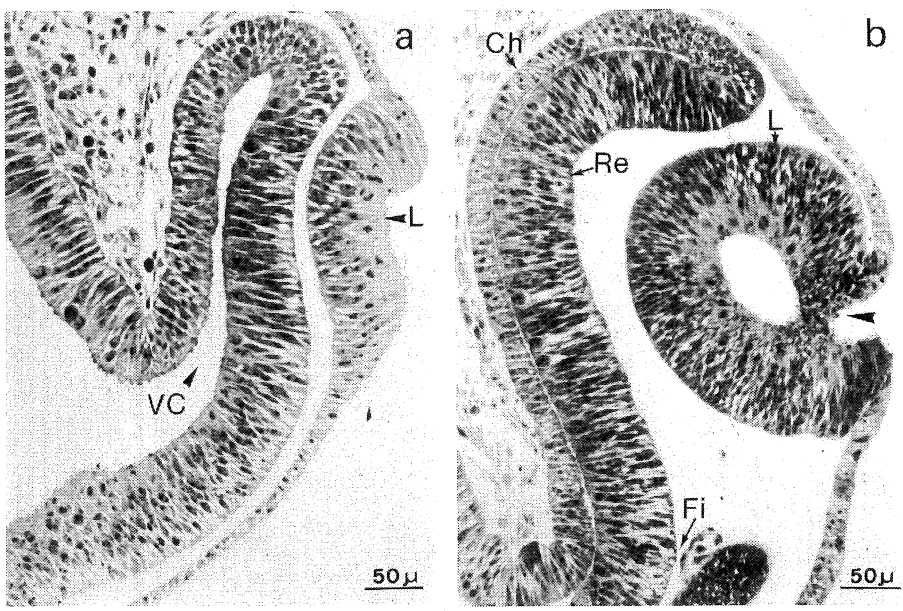

Fig. 1. (a) Frontal section of the optic cup in a HH stage 13 chick embryo. (b) Frontal section taken at the choroid fissure in a HH stage 17; the arrow shows the lens, which has not yet completed invagination; the upper and lower regions of the optic cup are unequally developed. L = lens, Fi = choroid fissure, Re = retina, Ch = choroid, VC = ventricular cavity.

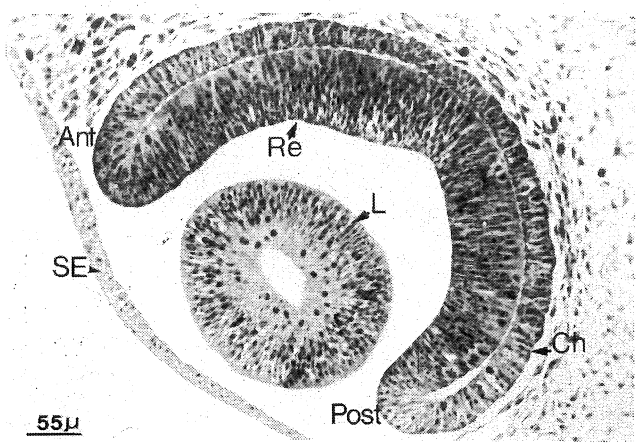

Fig. 2. Horizontal section of the optic cup in a HH stage 17 chick embryo. Post. = posterior region, Ant. = anterior region, L = lens, Ch = choroid, Re = retina, SE = surface epithelium.

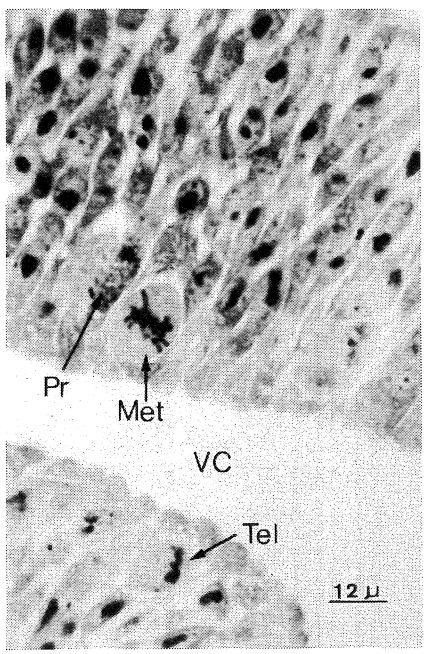

Fig. 3. Detail from a section of the optic vesicle in a HH stage 13 chick embryo. A prophase and a parallel oriented metaphase can be seen next to the ventricular surface; a telophase is also visible.Pr= prophase, Met = metaphase, Tel = telophase, VC = ventricular cavity.

Table 1. Values of the total indices for mitosis (MI), prophase (Pr), metaphase (Met) and anaphase-telophase (A-T). Comparisons between the values for stages 13-14 and 17-18.

T O T A L

INDICES (mean values)

STAGE	MI	Pr	Met	A-T
13-14	4.11	1.77	1.56	0.78
variation	-24.33%	-50.85%	-3.85%	-6.41%
17-18	3.11	0.87	1.50	0.73

% TIME (relationship with the total mitotic index)

				Pr + M	
13-14	100.0%	44.04%	35.70%	79.74%	20.26%
17-18		27.80%	48.46%	76.26%	23.74%

Tables 1-3 present the values for the frequency and distribution of the different mitotic phases. Table 1 includes total values for the entire optic cup in stages 13-14 and 17-18. Mean values are shown for mitotic index (MI), prophase (Pr), metaphase (Met) and anaphase-telophase (A-T). Because of the scarcity of anaphases observed in the sections, owing most likely to the brief duration of this phase, values for anaphase and telophase were lumped together. As the table shows, mitosis is more frequent in stages 13-14 than 17-18. Differences are indicated in the corresponding coefficients of variation. A negative sign (-) reflects a decrease in frequency from stage 13 to 18. Significant differences appeared in the mitotic indices and in prophase frequency and distribution (Pr), however, differences in metaphase (Met) and anaphase-telophase (A-T) were not significant. The lower portion of Table 1 presents values for prophase and metaphase in percentage of MI. As can be seen, the percentage of prophase participation decreased considerably during the mitotic period. Changes in metaphase, however, were not significant, as this percentage varied only in relative terms from stage 13-14 to 17-18 at -3.85%.

Table 2 is a schematic diagram of the retina volume divided into 9 sectors. In each sector the mitotic index was calculated for stages 13-14 and 17-18. The data show that mitosis is more common in stages 13-14 with exception two opposite sectors, P-I and A-S.

Table 3 shows the percentage values of prophase and metaphase duration related to the mitotic indices and based on the data in Table 2. For example, in sector P-S in stages 13-14 the mitotic index is 4.88; of this value and in view of the data for sector P-S in Table 2, 37.92% corresponds to prophase and 40.75% to metaphase. At the botton in each sector of Table 3, totals for prophase and metaphase are shown with "+" signs. It should be noted that

Table 2. Mitotic index in the nine sectors into which the retina was divided

STAGE	SECTORS		
	P-S	M-S	A-S
13-14	4.88+0.58	4.11+0.45	3.68+0.50
Variation	-25.21%	-33.10%	-6.30%
17-18	3.65±0.24	2.75± 0.21	3.45±0.35
	P-M	M-M	A-M
13-14	4.17+0.47	3.40+0.55	3.97+0.41
Variation	-25.66%	-12.65%	-26.60%
17-18	3.10±0.26	2.97±0.23	2.91±0.25
	P-I	M-I	A-I
13-14	4.90+0.86	4.76+0.79	4.51+0.55
Variation	+7.55%	-37.00%	-20.62%
17-18	5.28±0.61	3.01±0.32	3.58±0.70

Table 3. Percentages of values of prophase and metaphase based on the data of Table 2.

PHASES	SECTORS					
	P-S		M-S		A-S	
STAGE	13-14	17-18	13-14	17-18	13-14	17-18
PROPHASES	37.92%	26.87%	49.81%	27.60%	42.56%	29.79%
METAPHASES	40.75%	48.92%	28.63%	49.37%	37.92%	45.87%
+	78.67%	75.79%	78.44%	76.97%	80.48%	75.66%
	P-M		M-M		A-M	
PROPHASES	50.06%	28.25%	35.07%	22.20%	37.06%	26.84%
METAPHASES	29.91%	49.29%	30.18%	54.05%	43.93%	48.44%
+	79.97%	77.54%	65.25%	76.25%	80.99%	75.28%
	P-I		M-I		A-I	
PROPHASES	54.38%	24.62%	37.61%	33.65%	51.96%	30.49%
METAPHASES	28.75%	51.33%	49.22%	45.37%	31.97%	43.62%
+	83.13%	75.95%	86.83%	79.02%	83.93%	74.11%

these totals usually amount to about 80% of the total mitotic value in all sectors with the exception of M-M and M-I.

COMMENTS

When comparing in each of the two stages of development studied (13-14 and 17-18) the values found in these data can be expressed in terms of total mitotic density or index as well as by values of each subphase found.

In a homogenously developing region undergoing cell division, the cells are known to follow a cycle such that at any given moment most of them are in interphase while a relatively low percentage of them is in mitosis. As the entire population has the same possibility to multiply, the number and kind of mitotic figures counted are related to the duration of each

phase.

A noteworthy observation is that the sums of prophase and metaphase values respectively for all sectors is quite similar. It is possible that as development advances and differentiation continues, prohase is temporarily shortened, followed by or compesated by a real or apparent lengthening in the mitotic indices in stage 17-18. However, these figures are in fact lower in the later stage, apparently as a result of the lengthening of interphase during development, which leads to a proportionate decrease in the mitotic index. This notwithstanding, a comparison of the percentages of variation for prophase and metaphase reveals that while the former indeed becomes really shorter, metaphase duration in absolute terms remains the same and only seems to lengthen in relation to mitosis.

The pattern of differentiation in the entire retina is not uniform. The highest numbers are seen in the posterior sectors in which it may supposed that the rate of mitosis lags behind that in other sectors, e.g. in P-S and P-I, whereas the most advanced sectors in terms of cell differentiation are those located anteriorly.

A wothwhile feature of the present study that we would like to point out is the relationship between the differences observed with these stereological methods and their availability in the study of the corresponding stochastic processes, as long as can be assumed that the changes noted are time related and occur along precisely predetermined patterns.

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