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Short Report

Organelle distribution in chick neuroepithelial cells: effects of colchicine and cytochalasin B

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ABSTRACT. The intracellular distribution of mitochondria, cytoplasmic inclusions and rough endoplasmic reticulum cisternae of chick neuroepithelial cells was investigated at neurulation stages 6,8,10 and 12. These neuroepithelial cells were subdivided into three zones: apical, median and basal and the distribution percentages of distribution of these organelles were obtained. Mitochondrial distribution was related to the energy supply that mitochondria provide for apical microfilament contraction. Cytoplasmic inclusions were distributed preferentially in the apical zone of the neuroepithelial cells during the four stages. Rough endoplasmic reticulum cisternae were homogeneously distributed in the three zones at stages 10 and 12, but at stages 6 and 8 there are more elevated percentages of rough endoplasmic reticulum in the apical zones than in the other zones. Experimental treatments with colchicine and cytochalasin B does not modify the patterns of mitochondria and rough endoplasmic reticulum cisternae but alters the distribution of cytoplasmic inclusions. Finally, there is a correlation in the normal neurulating neuroepithelial cells between the distributions of mitochondria and rough endoplasmic reticulum distribution and between the distributions of mitochondria and cytoplasmic inclusions distribution. This relationship is retained in the treated neuroepithelial cells.

KEY WORDS: *neuroepithelial cells, colchicine, cytochalasin B, chick embryo*

The intracellular distribution of cytoplasmic organelles present in neuroectoderm cells is undoubtedly related to cell shape and other specific changes of neurulation. To date the evolution of this distribution and its relationship to the subcellular components has not been documented. Studies on the subcellular topographic organization of cells undergoing morphogenetic processes become necessary since Burgess and Schroeder (1979) have clearly shown that subcellular events alter the shapes of cells participating in morphogenetic changes.

Mitochondria are ubiquous organelles whose intracellular distribution varies according to the specific functions of each cell type (Bereiter-Hahn and Voth, 1983; Paz *et al.*, 1986).The intracellular localization of mitochondria can be conditioned by the presence of certain cytoplasmic elements such as lipidic inclusions (see Stemberger *et al.*, 1984), myofibrils (Fawcett, 1981), etc. In the neurulating amphibian embryo, Schroeder (1970) pointed out that mitochondria occupy a significant volume within neuroepithelial cells and are sometimes closely associated with lipid bodies (Burnside, 1971). In the chick embryo there is a great variability in mitochondrial shape and size (Paz *et al.*, 1986) although in the neuroepithelial cells a longitudinal arrangement of mitochondria is characteristic (Bancroft and Bellairs, 1975).

During neurulation the neuroepithelial cells have a rough endoplasmic reticulum which is poorly developed in rat (Takeuchi and Takeuchi, 1980), amphibian (Schroeder, 1970) and chick (Ruggeri, 1967; Rovasio and Monis, 1981) embryos. Miki (1981) has described the existence of an increase in the rough endoplasmic reticulum cisternae of neuroepithelial cells through neurulation.

In this study the intracellular distribution of mitochondria, cytoplasmic inclusions and rough endoplasmic reticulum is investigated in the chick neuroepithelial cells during neurulation. The interrelations between these organelles are also analysed in order to elucidate their possible relevance in the neuroepithelial cells participating in neurulation.

Cytoskeletal elements (microtubules and microfilaments) play an important role both in the intracellular distribution of organelles and in the neuroepithelial cell shape changes during chick neurulation (for review see Burnside, 1973; Karfunkel, 1974; Schoenwolf *et al.*, 1988). In the present study their first role is tested by experimentally treating the embryos with colchicine and cytochalasin B and then analyzing the distribution patterns found in the treated embryos.

Distribution of mitochondria

Mitochondria of the control neuroepithelial cells are found concentrated in the basal zone rather than in the other zones (see Table 1), and go from 39.0% at stage 6 to 48.4% at stage 10. The basal zone percentage differs significantly with respect to the median zone in all the stages studied, as the mitochondrial percentages in this zone are about 20-30%. The apical zone contains mitochondria in a

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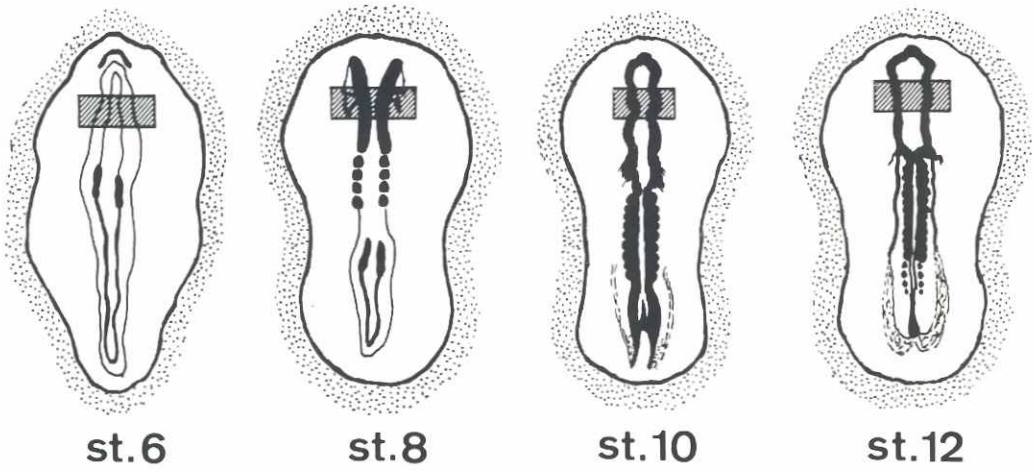

Fig. 1. *Drawings of the four stages used in this study (6,8,10 and 12) showing the midbrain region (striped area) collected at each stage.*

percentage that varies significantly with respect to basal zone at stages 6 (32.0%), 10 (24.7%- the lowest percentage of mitochondria) and 12 (30.2%), whereas the highest apical percentage of mitochondria is found at stage 8 (36.9%).

Neuroepithelial cells from embryos treated with colchicine *in ovo* show a mitochondrial distribution (Table 1) that does not change significantly with respect to untreated embryos. Treatment with cytochalasin B, however, produces a statistically significant increase ($P<0.05$) in the apical percentage of mitochondria at stage 10 (rising from 24.7% to 36.1%) in treated embryos.

Distribution of cytoplasmic inclusions

The cytoplasmic inclusions present in the control neuroepithelial cells are more frequently located in the apical zone as shown in Table 1. Thus the higher percentages of cytoplasmic inclusions are found in this cell zone (from 40.5% at stage 6 to 83.3% at stage 12).

The effect of colchicine on the intracellular distribution of cytoplasmic inclusions is observed in the percentages of the distribution from the apical and the basal zones of stages 8,10 and 12. We can detect a significant decrease in the apical percentages, as well as an increase in the basal percentages of the inclusion distribution patterns at stages 6,10 and 12 (see Table 1).

Distribution of rough endoplasmic reticulum cisternae

The distribution of the rough endoplasmic reticulum cisternae within the control neuroepithelial cells makes it possible to distinguish two different situations (see Table 1). At stages 6 and 8 there are higher proportions of rough endoplasmic reticulum cisternae in the apical zone than in the basal and median zones of neuroepithelial cells. Thus, about 40% of rough endoplasmic reticulum cisternae are in the apical zone at these stages, whereas median and basal zones contain a proportion nearer to 25% and 30% respectively. On the other hand, it can be observed that the distribution of the rough endoplasmic reticulum cisternae at stages 10 and 12 is so similar in the three zones of the cell that significant differences between cellular zones are not observed.

The distribution of rough endoplasmic reticulum in the neuroepithelial cells treated with colchicine varies significantly from controls only at stage 8 in which there is a decrease in the percentage of rough endoplasmic reticulum cisternae in the apical zone with respect to untreated embryos. Treatment with cytochalasin B does not modify the distribution patterns of rough endoplasmic reticulum cisternae as shown in Table 1.

Correlation analysis

The study of the correlations between the percentages of mitochondria and rough endoplasmic reticulum cisternae in the three cellular zones of the control, colchicine- and cytochalasin B-treated embryos of stages 6,8,10 and 12 reveals the existence of a positive correlation between the two organelle types in most of the cases considered (see Table 1).

The correlations between mitochondria/cytoplasmic inclusions can also be observed in the control, colchicine- and cytochalasin B-treated embryos but they appear more frequently in the apical zone of the cell.

The correlation data concerning rough endoplasmic reticulum/cytoplasmic inclusions are not statistically significant.

Chick embryo neuroepithelial cells during neurulation show a typical ultrastructure that has already been de-

scribed (Ruggeri, 1967; Paz *et al.*, 1985). Factors affecting their ultrastructure may be the epithelial nature of the neuroectoderm and the characteristic evolution of the neuroepithelial cell shape during neurulation. An analysis of the intracellular arrangement of mitochondria, cytoplasmic inclusions and rough endoplasmic reticulum in neuroepithelial cells can reveal specific changes during the successive stages of neurulation, as described below.

From the successive stages of neurulation (Fig. 2) it can be established that there is a close relationship between mitochondrial distribution and the different stages of development. Thus at stages 6 and 8 (neural tube formation is taking place) we observe an elevated proportion of mitochondria in the apical zone, while at stage 10 (neural tube formation has been completed) the percentage of mitochondria in the apical zone has decreased (see Fig. 2). This pattern of mitochondrial distribution could be related to the apical constriction of neuroepithelial cells by means of microfilaments (see Fig. 4).

For the active contraction of microfilament bundles, a local energy supply provided by the mitochondria is necessary. Thus in the cellular types that have high local energy requirements (muscular cells, spermatozoids, etc; Fawcett, 1981), mitochondria tend to accumulate in these locations with a high energy demand. Moreover, Burnside (1971) has pointed out that mitochondria of amphibian embryos accumulate within the apical pole of neuroectoderm cells providing energy which is sufficient for microfilament bundle contraction, while Nagele and Lee (1979) have reported that mitochondria are preferably located in the apical zone of chick neuroepithelial cells.

On the other hand, some conformational changes of mitochondria seem to exist in the chick neuroepithelial cells according to the results obtained by Mathieu and Messier (1976) and Paz *et al.* (1986). These authors have observed that the mitochondrial volume within the cytoplasm remains unchanged during neurulation, while the number of mitochondria increases. These facts lead us to think that there is mitochondrial division in neuroepithelial cells during neurulation.

Cytoplasmic inclusions of chick neuroepithelial cells also tend to be distributed within the apical zone during the four stages of neurulation considered. This fact is undoubtedly related to the mitochondrial distribution of mitochondria since the correlation analysis carried out demonstrates the existence of a positive correlation between the distribution percentages of mitochondria and cytoplasmic inclusions mainly in the apical zone. Thus one can assume there is a close relationship between the cytoplasmic inclusions and mitochondria in the neuroepithelial cells as described by Burnside (1971). This author reported that lipid bodies in amphibian neuroectoderm cells are often found in the mitochondria.

Rough endoplasmic reticulum distribution in the chick neuroepithelial cells differs at stages 10 and 12, when the three cellular zones have similar percentages of rough endoplasmic reticulum with respect to stages 6 and 8, in

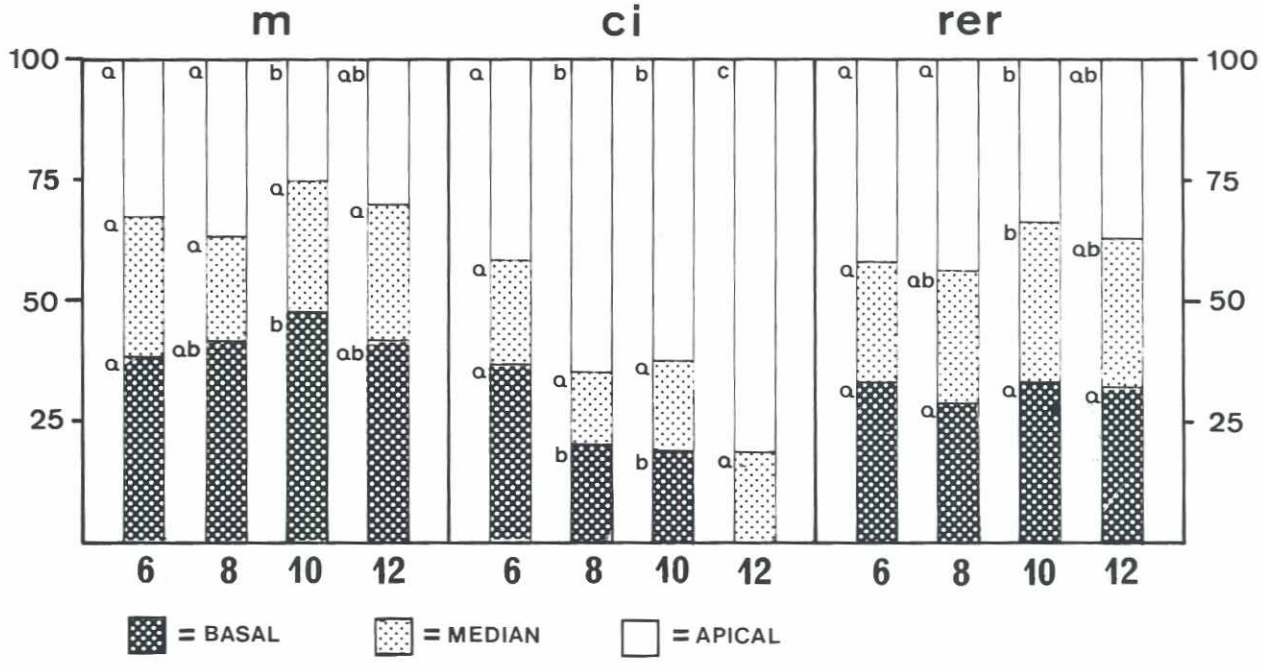

Fig. 2. *Distribution percentages of mitochondria (m), cytoplasmic inclusions (ci) and rough endoplasmic reticulum (rer) in the apical, median and basal zones of the chick neuroepithelial cells at stages 6,8,10 and 12. For each organelle and cellular zone, the significant differences (P<0.05) between stages are shown by letters a,b,c. Distinct letters indicate significant differences between stages.*

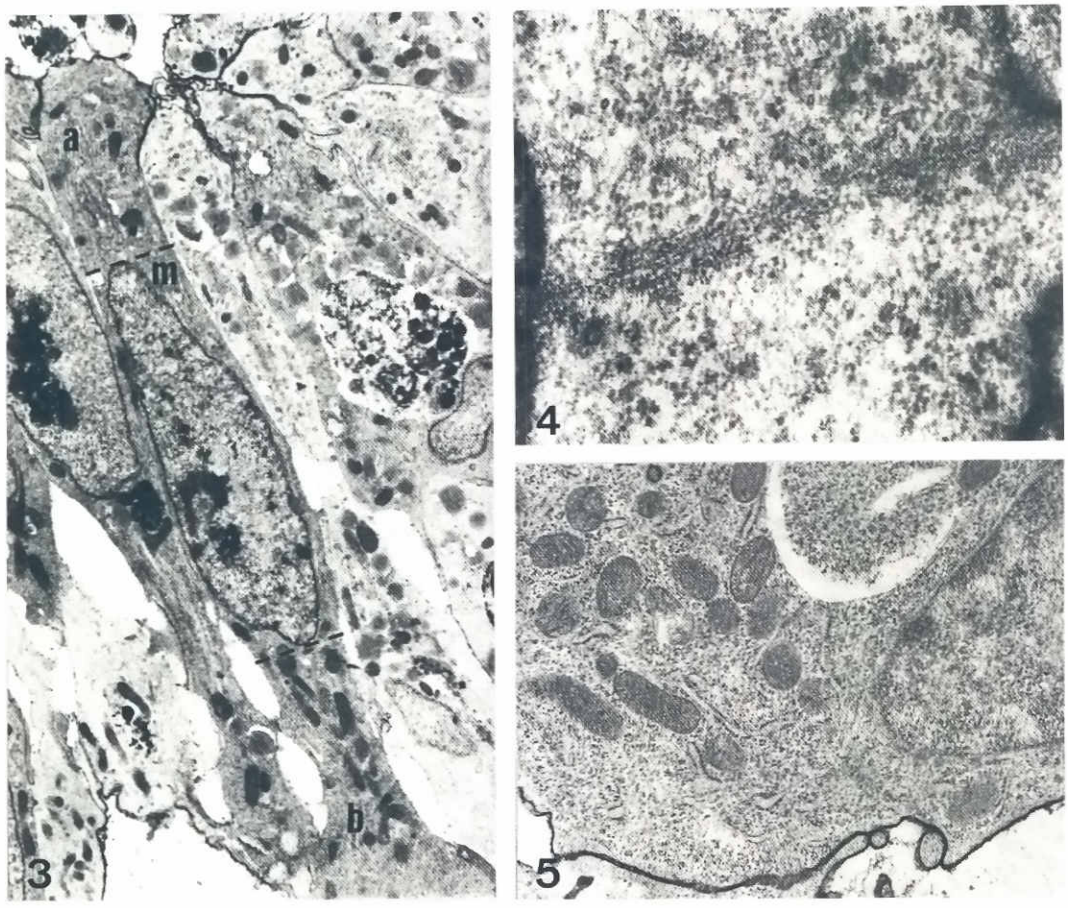

Fig. 3. *Neuroepithelial cell from stage 8-chick embryos divided into basal (b), median (m) and apical (a) zones for the organelle distribution analysis. Two lines passing through apical and basal poles of nucleus were drawn perpendicularly to apico-basal axis of cell. x 3,200.*

Fig. 4. *Microfilament bundle in the apical zone of a neuroepithelial cell from stage 8. x 43,000.*

Fig. 5. *Transmission electron micrograph cell (stage 10) showing a close spatial relationship between mitochondria and rough endoplasmic reticulum cisternae. Cell coat is stained with ruthenium red. x 27,000.*

which there is a higher percentage in the apical zone than in the median and basal zones (see Fig. 2). These distribution patterns do not apparently relate with the succesive events of neurulation. Paz *et al.* (1985) have found in the chick neuroepithelial cells that there is an increase in the surface density of rough endoplasmic reticulum cisternae which relates with the production of extracellular matrix production. The acetylcholinesterase activity present in the endoplasmic reticulum cisternae also reveals an increase in the number of the cisternae (Miki, 1981). Our results agree with this increase since the rough endoplasmic reticulum cisternae profiles pass from a non-homogeneous distribution (stages 6 and 8) to a homogeneous distribution in the three zones of neuroepithelial cells (stages 10 and 12) (see Fig. 2).

Distribution percentages of mitochondria, cytoplasmic inclusions and rough endoplasmic reticulum cisternae found in the normally developed embryos can be compared with the corresponding distribution patterns obtained in the embryos treated with colchicine and cytochalasin B (see Table 1). Both drugs markedly modify the distribution patterns of cytoplasmic inclusions (Table 2). Colchicine decreases the apical percentages of inclusions while cytochalasin B changes the distribution percentages of stages 6,8 and 12. These facts lead us to think there is a possible involvement of the cytoskeletal elements in the maintenance of the distribution patterns of cytoplasmic inclusions. Both experimental treatments seem to have minimal effects on the distribution patterns of mitochondria and rough endoplasmic reticulum cisternae. These observations suggest that microtubular and microfilamentous cytoskeleton does not play an important role in the maintenance of distribution patterns of the mitochondria and rough endoplasmic reticulum cisternae in chick neuroepithelial cells.

The correlation analysis carried out in this study in the three neuroepithelial cell zones during the four neurulating stages clearly shows a positive correlation between

TABLE 1

PERCENTAGES OF DISTRIBUTION OF MITOCHONDRIA (M), CYTOPLASMIC INCLUSIONS (CI) AND ROUGH ENDOPLASMIC RETICULUM CISTERNAE (RER) IN BASAL (B), MEDIAN (M) AND APICAL (A) ZONES OF CHICK NEUROEPITHELIAL CELLS AT STAGES 6 ,8, 10 AND 12 FROM CONTROL (C), COLCHICINE-TREATED (CO) AND CYTOCHALASIN B-TREATED (CY) EMBRYOS

		stage 6			stage 8			stage 10			stage 12		
		m	ci	rer	m	ci	rer	m	ci	rer	m	ci	rer
C	B	39.0a	37.2a	33.5ab+	42.1a	19.7a	29.2a	48.4a	20.7a	33.6	41.4a	-	32.2+
	M	29.0b	22.3b+	25.0a	21.0b	16.7a	27.1a	26.9b	18.2a	34.0	28.4b	16.7a	30.7
	A	32.0b	40.5a+	41.5b+	36.9a	63.6b	43.7b+	24.7b	61.1b+	32.4+	30.2b	83.3b	37.1+
Co	B	37.3	41.8+	32.1+	36.8	44.2*	32.3+	44.9	35.9*	34.2+	38.5	16.3*	32.9+
	M	32.3	23.3	32.1+	28.4	9.9	33.2	23.7	11.5	31.4	25.5	12.7	29.1+
	A	30.4	34.9+	35.8+	34.8	45.9*	34.5*+	31.4	52.6*	34.4+	36.0	71.0*+	38.0+
Cy	B	43.1	51.3*	36.5+	36.2	18.0	24.6+	41.9	23.5	32.4+	42.1	9.1*	26.7+
	M	22.8	9.5*	25.7+	22.9	15.8	28.9	22.0	8.1*	28.4	24.2	13.9	31.4
	A	34.1	39.2	37.8+	40.9	66.2+	46.5+	36.1*	68.4	39.2+	33.7	77.0+	41.9+

a,b In the control embryos the letters a,b indicate significant differences ($P<<0.05$) between cellular zones. Distinct letters indicate significant differences between cellular zones

* In the treated embryos there are significant differences ($P<<0.05$) with respect to control embryos

\+ In ci and rer percentages there are a positive correlation coefficient m/ci or m/rer respectively which is statistically significant ($P< 0.01$)

mitochondria and rough endoplasmic reticulum cisternae (Table 1). This correlation is significant in the apical and basal zones of the cell which implies a close relationship between both organelles. This relationship was observed in transmission electron micrographs (see Fig. 5) in which the rough endoplasmic profiles are closely associated with mitochondria. Similar associations between both organelles have been shown in endothelial cells (Bereiter-Hahn and Voth, 1983), cardiac muscle cells (Segretain *et al.*, 1981), glomus cells (Gronblad and Akerman, 1984), etc. Moreover, during the primary development of various organs, for example, kidney and intestine, Thiery *et al.* (1983) and Gaffiero *et al.* (1983) have described an analogous differentiation of mitochondria and rough endoplasmic reticulum of the renal and intestinal epithelial cells. Moreover, in the embryos treated with colchicine and cytochalasin B, the correlation between mitochondria and rough endoplasmic reticulum cisternae distribution is retained in the treated neuroepithelial cells.

Experimental procedures

Midbrain regions from chick embryos at stages 6,8,10 and 12 (Hamburger and Hamilton, 1951) were used (see Fig. 1). Samples were fixed directly after removal from the yolk with 2% glutaraldehyde in 0.1 M cacodylate buffer (330-360 mOs) with 1% ruthenium red (Luft, 1971) at pH 7.4 for 1 h at 4°C and rinsed in this buffer. Postfixation was accomplished in a buffered osmium tetroxide solution (1%) with 1% ruthenium red for 3 h at room temperature. After washing in buffer, the samples were dehydrated in a graded series of ethanol solutions and finally were embedded in Epon 812 resin. Semi-thin sections were stained with toluidine blue (0.5%) and ultra-thin sections were mounted on 200-mesh grids and stained with uranyl acetate and lead citrate. The grids were examined in a Jeol 100 CX transmission electron microscope operating at 60 kV.

Colchicine and cytochalasin B treatment

For the *in ovo* experimental treatment with colchicine and cytochalasin B a small window was cut through the shell and the sublastodermic space injected with 20 µl of a colchicine solution (5×10^{-6} M in saline) or a cytochalasin B solution (5 µg/ml in saline and stabilised with dimethysulphoxide). All embryos were illuminated obliquely and staged accurately. Windows were sealed with Parafilm (American Can Co) and embryos reincubated for an additional 2 h. A series of control embryos was injected with saline solution alone in the same way that the experimental embryos were injected with the drugs. A colchicine dosage of 5×10^{-6} M has been shown to be effective in degrading microtubules in chick neuroepithelial cells (Fernández *et al.*, 1987).

Samples from treated and control embryos were processed in the same way as the untreated embryos and the organelle distribution and correlation analysis was also done following the method described below for untreated embryos.

Sampling procedures

To obtain a representative sample we used the method described by Williams (1977) and Petrzilka *et al.* (1978), considering only sections cut perpendicularly to neuroepithelium. Briefly, eight embryos were selected at each stage from a pool of 24 embryos per stage by a digital random table, and three grids from each block were obtained. On each grid ten neuroepithelial cells

with nuclear profiles were photographed following the method reported by Hirakow and Gotoh (1975) finally obtaining 240 profiles for each stage. The micrographs were printed at magnifications of 12,000-22,000 and analysed for the organelle distribution study.

Organelle distribution and correlation analysis

The organelle distribution analysis in the neuroepithelial cells was performed taking into account the apico-basal polarity of these cells. Cells were divided into three zones: apical, median and basal by using the nucleus as a reference point (see Fig 3). At each cellular zone the number of profiles of mitochondria, cytoplasmic inclusions and rough endoplasmic reticulum cisternae was quantified, obtaining the Na (number of profiles/unit area). Finally this parameter is expressed as the percentage of distribution for each organelle and cellular zone. The cytoplasmic inclusions considered in this study include three types of vitelline inclusions (Ruggeri, 1967): yolk droplets, lipid bodies and composed vitelline inclusions.

In order to determine the degree of association of the mitochondria, cytoplasmic inclusions and rough endoplasmic reticulum cisternae, we carried out a correlation analysis between data of these organelles in the three cellular zones. Thus at each zone the linear correlation coefficient was calculated as well as its significance level (see Table 1).

Statistical analysis

The percentages of organelle distribution were statistically compared by the G-independence test (Sokal and Rohf, 1969) based on chi-square distribution, using a level of significance $P<0.05$. For each stage, the percentages obtained at one cell zone were statistically compared with those of the other zones, and for each cell zone the percentages of the four stages were also compared by the G-independence test.

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