# A RADIOAUTOGRAPHIC STUDY OF RNA SYNTHESIS IN THE RETINA OF CHICK EMBRYO[1)]

## (Studi Mengenai Sintesis RNA pada Retina Mata Embrio Ayam dengan Menggunakan Radioautografi[1)])

Wisnu Gunarso[2)], Nagata, Shimizu dan Segawa

## ABSTRAK

Penelitian dilakukan untuk mengetahui penggabungan $^3$H-uridine pada RNA dari retina mata embrio ayam dengan menggunakan radioautografi baik pada tingkat mikroskop cahaya maupun mikroskop elektron. Penghitungan noktah perak yang terjadi dilakukan terhadap tiga komponen sel (nukleus, nukleolus dan sitoplasma) dari tiga bagian retina mata embrio ayam (anterior, ekuator dan posterior) untuk masing-masing kelompok embrio berumur 2, 3, 4 dan 7 hari.

Hasil penelitian ini menunjukkan terjadinya peningkatan jumlah noktah perak yang terjadi sejalan dengan peningkatan umur embrio yang diteliti. Pada masing-masing tahap perkembangan embrio tersebut terlihat bahwa jumlah noktah terbanyak selalu pada bagian anterior. Untuk masing-masing kelompok embrio yang diamati, dari ketiga komponen sel yang diteliti terdapat kecenderungan bahwa noktah perak terbanyak adalah pada nukleus. Noktah perak yang jumlahnya masih sedikit pada sitoplasma dari kelompok embrio berumur 2 hari, terlihat semakin meningkat pada kelompok embrio yang berumur lebih tinggi, terutama pada kelompok embrio berumur 7 hari.

## INTRODUCTION

It has been shown in a variety of systems that, following the administration of labeled RNA precursors, the labeled RNA was located in the nucleus, nucleolus and cytoplasm of cells. The RNA synthesis initially occurs in the nucleus and nucleolus, then it moves to the cytoplasm (Fakan and Bernhard, 1971). The extra-nuclear RNA synthesis, however, may take place in various cell organelles, such as ribosomes, endoplasmic reticulum, and mitochondria (Nagata, 1972; Nagata *et al.*, 1967a, 1975, 1982).

This paper deals with the radioautographic study on the RNA synthesis in three different compartments of the cell (nucleus, nucleolus, and cytoplasm) in three different regions of the same retina of 2, 3, 4 and 7 days old chick embryo groups after an in vitro incubation in media containing $^3$H-uridine.

---

[1)] Sebagian dari tesis penulis pertama untuk memperoleh gelar Ph.D Med. Sci dari Shinshu University School of Medicine, Jepang, yang ditulis dalam bahasa Jepang. Penelitian dibiayai oleh Ministry of Education, Science and Culture, Jepang.

[2)] Berturut-turut Staf Pengajar pada Jurusan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Pertanian Bogor, Profesor-profesor pada Departemen Anatomi dan Opthalmologi, Fakultas Kedokteran, Universitas Shinshu.

## METODOLOGY

Fresh fertilized White Leghorn chick (*Gallus domesticus*) eggs from the local hatchery were incubated in a moist incubator at 38.5°C. The retinas of 2, 3, 4 and 7 days embryonic age (stage 9-12, 14-20, 23-24, and 30-32 of Hamburger and Hamilton, 1951) were dissected in Hanks' balanced salt solution (B.B.S.). The tissues were cultured *in vitro* in Eagle's MEM suplemented with 10% calf serum containing 20 $\mu$Ci/ml $^3$H-uridine (Amersham, England, specific activity 30 Ci/mM). Cultures were maintained in a $CO_2$ incubator (Te-Her, Tokyo, Japan) at 37.5°C, 5% in air, 90% relative humidity and pH 7.5 ± 0.1. Cultures were exposed to labeled precursor for 1, 2, and 4 hrs and then prefixed in ice-cold 2.5% glutaraldehyde buffered with Millonig's phosphate buffer for 1 hr at pH 7.4, postfixed for 1 hr at 4°C in 1% osmium tetraxide buffered with Millonig's phosphate buffer at pH 7.4. Tissues were repeatedly washed in the same buffer, and dehydrated in ethanol series. Some of them were embedded in paraplast (Lancer, Sherwood Med., Brunswick Co., St. Louis, USA) and some in Luveak 812 (Nakarai Chemicals Ltd., Osaka, Japan). For light microscopic radioautography, 4-8 µm thick sections of paraplast blocks were cut with a sliding microtome (Yamato Koki Co., Tokyo, Japan) and 1.5 µm thick sections of Luveak blocks were cut with a Porter Blum MT2-B ultratome (DuPont-Sorvall, Biomedical Co., Newton, Conn., USA). They were mounted on glass slides and dipped (Nagata *et al.*, 1967b) in Sakura NR-M2 emulsion (Konishiroku Photo Co., Tokyo, Japan), exposed for 8 weeks and developed by SDX-1 developer (Nagata *et al.*, 1967b). After the development, sections of paraplast blocks were stained with haematoxylene-eosin, while the sections of Luveak blocks were stained with 0.5% toluidine blue (pH 7.6) and observed with an Olympus Vanox microscope (Olympus Opt. Co., Tokyo, Japan). For electron microscopic radioautography, ultrathin sections of Luveak blocks were cut at 500 Å thick with an LKB Ultrotome (LKB, Bromma, Sweden) and coated with Sakura NR-H2 emulsion (Konishiro Photo Co., Tokyo, Japan) by a wire loop method (Yoshida *et al.*, 1978), exposed for 8 weeks, developed by GL-phenidon developer (Murata *et al.*, 1979), stained singly with lead citrate (Reynolds, 1963) and observed in a Hitachi HU-11A electron microscope (Hitachi, Tokyo, Japan).

For the purpose of the qualitative and quantitative observations, the appearance and the distribution pattern of grains within the labeled cells from each of the given embryo group were observed, and the average grain count was calculated. The number of grains in three different cell compartments (the nucleus, the nucleolus, and the cytoplasm) of three different regions (the anterior, the equatorial, and the posterior region) of the retina (Fig. 1) of 2, 3, 4, and 7 day old embryo groups were counted. As replication, 10 labeled cells per retinal region were taken at random, and each of the embryo group was represented by 3 embryonic retinas.

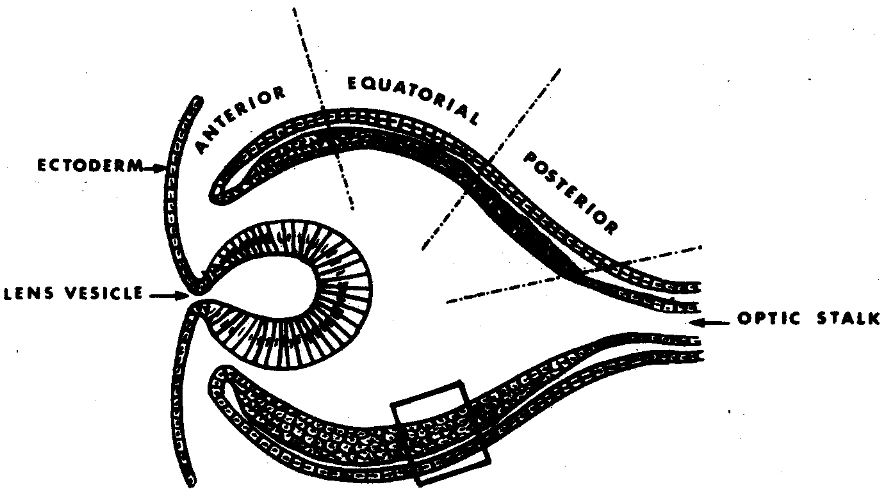


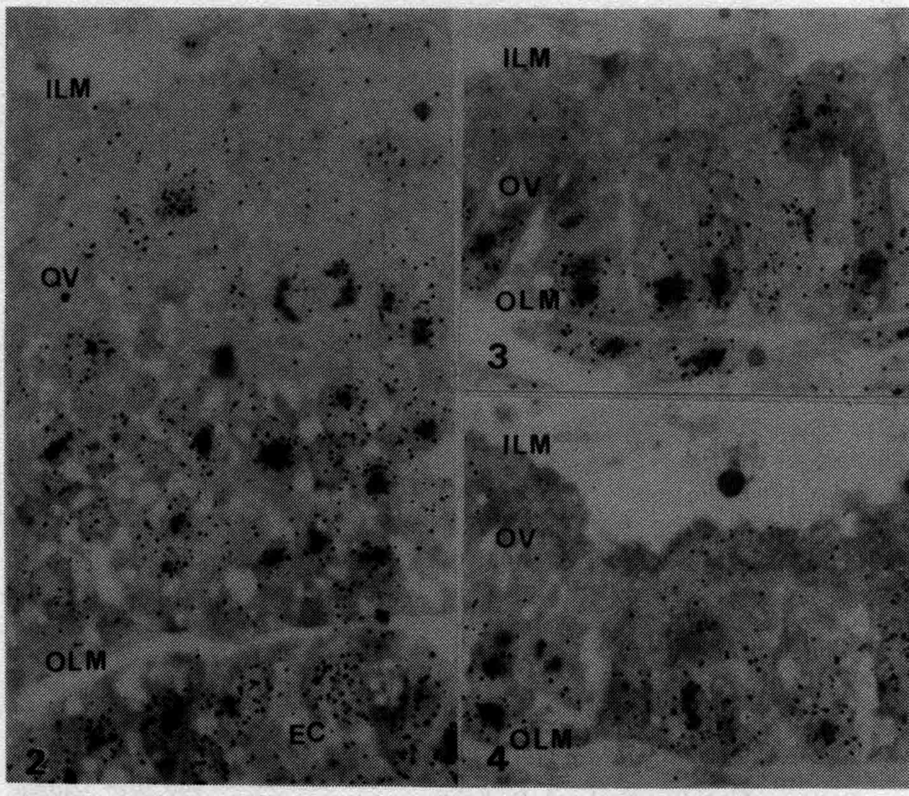

Figure 1. Diagram of regions of the chick embryo retina from which the distribution of labeled cells are observed and calculated.

## RESULTS

### Qualitative observations

Pattern of labeling on day 2. By light microscopic radioautography, all cells of all three regions of the optic vesicle were found labeled, where the optic cup was not yet formed. Slightly dense labeled cells were noticed in the anterior region as compared with the other two regions (Figs. 2-4). By electron microscopic autoradiography, it was noticed that most of the grains occured in the nuclei over the euchromatin, nucleoli, and the cytoplasms, which were still poor in the ribosomes and mitochondria (Fig. 5).

Pattern of labeling on day 3. By light microscopic radioautography, all cells in all three regions of the retina were labeled, where the optic vesicle was obliterated and the optic cup was formed. As found on day 2, more densely labeled cells were noticed in the anterior region as compared with the other two regions of the retina. Moreover, in comparison with the day 2 group, the density of the grains within cells of the day 3 embryo group increased (Figs. 6-8). By electron microscopic radioautography, it was found that the grains could be noticed over the euchromatin, the nucleolus, and the nuclear membranes. In the cytoplasm, which were still found to possess few cell organelles, grains were noticed to occur over the ribosomes, endoplasmic reticulum, and mitochondria (Fig. 9). The differentiating ganglionic cells possessing growth cones were




Figures 2-4. Light microscopic radioautograms of the optic vesicle of day 2 chick embryo after 1 hr incubation in vitro with $^3$H-uridine. Note that all of the cells are labeled and most of the silver grains are localized in the euchromatin of the nuclei. 2. Anterior region. 3. Equatorial region. 4. Posterior region. Abbreviations: EC, ectoderm. ILM, inner limiting membrane. OLM, outer limiting membrane. OV, optic vesicle. $\times 700$.

noticed in the marginal zone of the retina (Fig. 10). In the apical zone of the retina, the presence of the differentiating photoreceptor cells with only few grains but possessing the initial formation of the cilia of centrioles, indicating the outer segments formation, were observed (Fig. 11).

Pattern of labeling on day 4. By light microscopic radioautography, cells of all three regions of the retina were found labeled. The density of grains within each cell of this embryo group increased as compared with those younger embryo groups. The densely labeled cells were still found to dominate in the anterior region in comparison with the equatorial and posterior regions (Figs. 12-14). By electron microscopic radioautography, it was found that an increase in number of grains were noticed in the nuclei, where the grains were found to

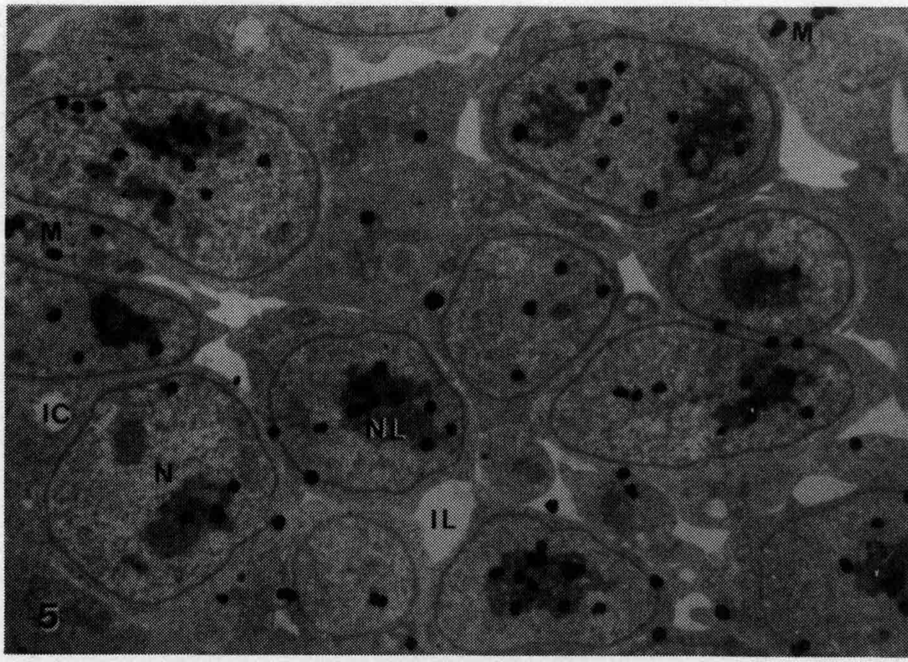
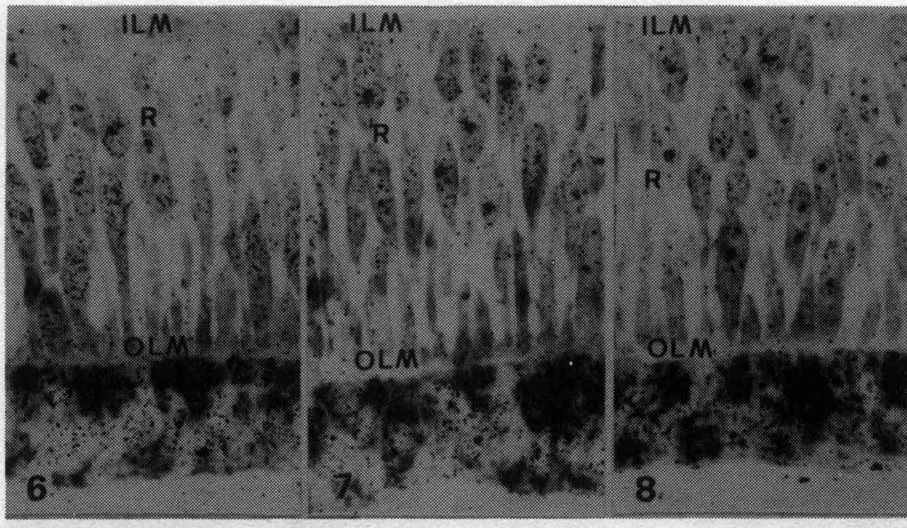



Figure 5. An electron microscopic radioautogram showing some labeled cells of the day 2 chick embryo optic vesicle after 1 hr incubation in vitro with $^3$H-uridine. Note that the grains are found over the euchromatin, nuclear membranes, nucleoli and cytoplasms. Some of the mitochondria are labeled. Abbreviations: M, mitochondria. N, nucleus. NL, nucleolus. IL, intercellular lacunae. IC, innercellular cisternae. ×5.250.

disperse over the chromatin, nucleoli and cell membranes. The cytoplasms were observed to possess more cell organelles as compared with those of the earlier embryo groups, and the occurrence of the grains was noticed over the ribosomes, endoplasmic reticulum, and the mitochonria (Fig. 15). In the marginal zone of the retinal layer, the differentiating ganglionic cells possessing growth cones were found to increase in number (Fig. 16). In the apical zone of the retinal layer, the appearance of the ciliary stalks of future outer segments were more developed. They were sticking out from the differentiating ellipsoid photoreceptor cell inner segments, elongated and reached the apex of the pigment apithelium layer with a few grains (Fig. 17).

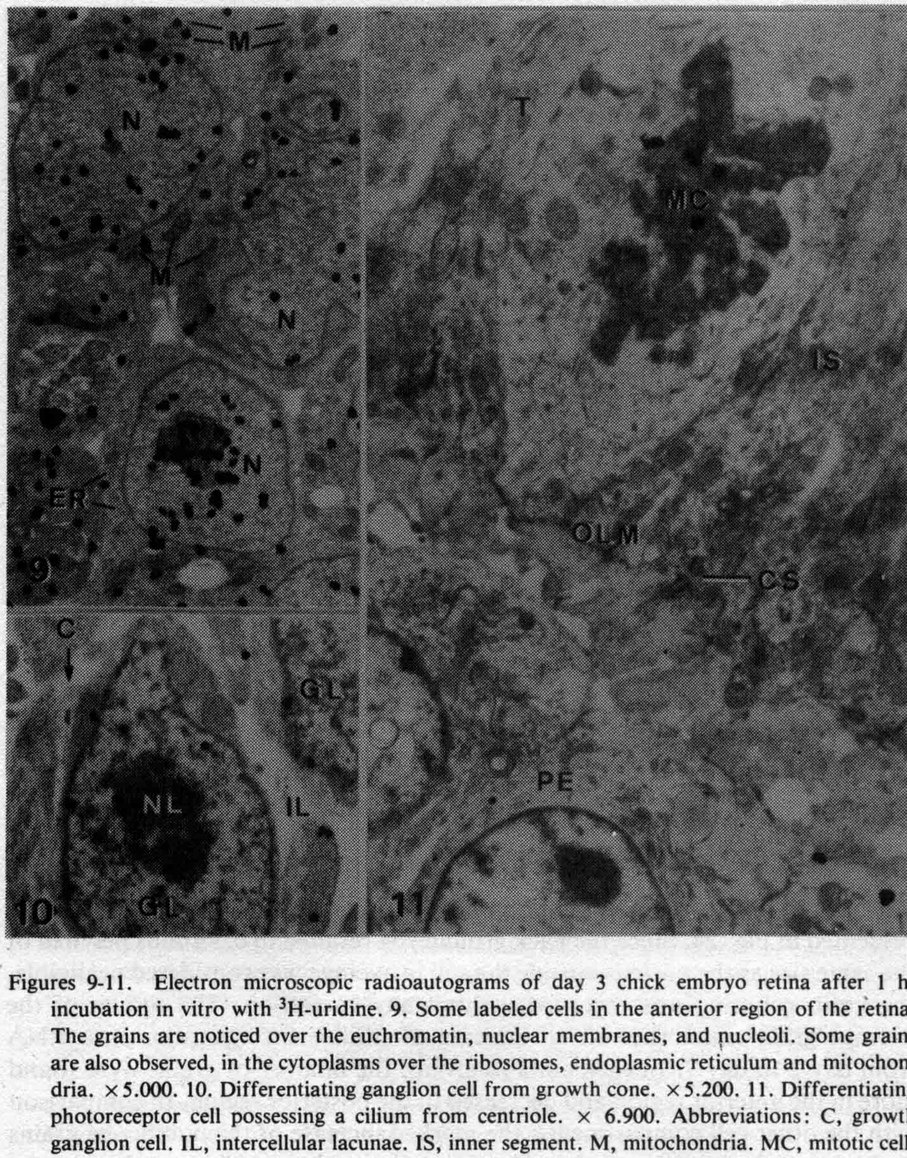
Pattern of labeling on day 7. By light microscopic radioautography, the number of grains within each cell of all three regions of the retina continued to increase, since the cells of those three regions were found densely labeled in comparison with the younger embryo groups. More dense labeled cells were still found to occupy the anterior region. More grains were noticed in the nuclei,



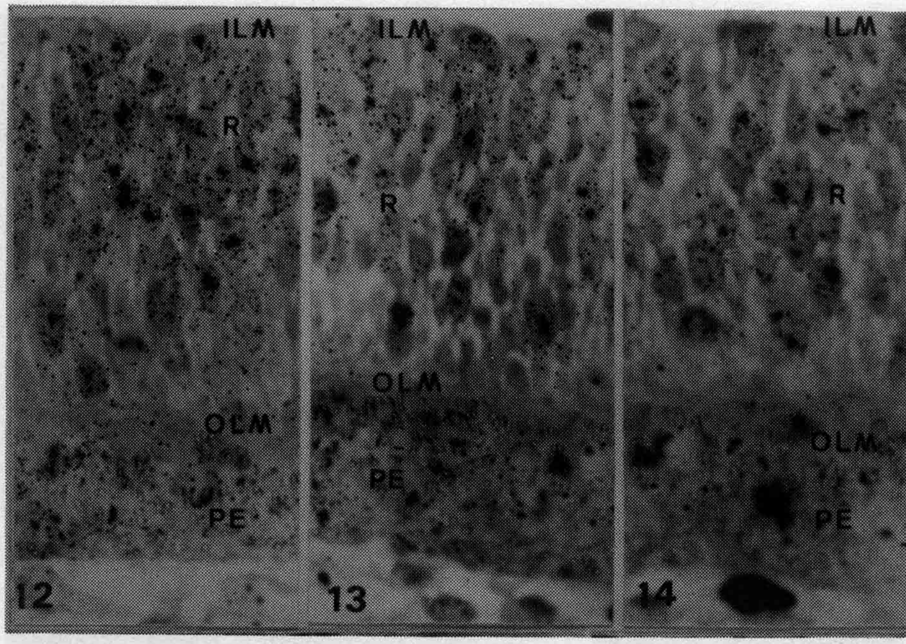

Figures 6-8. Light microscopic radioautograms of day 3 chick embryo retina after 1 hr incubation in vitro with $^{3}H$-uridine. 6. Anterior region. 7. Equatorial region. 8. Posterior region. Abbreviations: ILM, inner limiting membrane. OLM, outer limiting membrane. PE, pigment epithelium, R. retina. ×750.

however, an increase in number of grains were also noticed in the cytoplasms (Figs. 18-20). By electron microscopic radioautography, the distribution of grains within the cells could be noticed to disperse over the chromatin, the nuclear membranes, the nucleoli, and the cytoplasms. Most of the cytoplasms of day 7 retinal cells possessed more cell organelles such as Golgi apparatus, endoplasmic reticulum, ribosomes, and mitochondria. The silver grains in the cytoplasm were found to occur in the ribosomes and mitochondria (Fig. 21). The differentiating ganglionic cells in the marginal zone of the retinal layer were found to possess developed axons (Fig. 22). In the apical zone, the developing photoreceptor cells were noticed with outer segments possessing vacuoles or tubular structures or even lamellar discs but few grains (Fig. 13).

The obseıvations described above were almost the same in the same embryo groups eventhough the incubation time was changed from 1 hr to 2 and 4 hrs. However, the number of grains was found to increase according to the increase in the duration of incubation time.



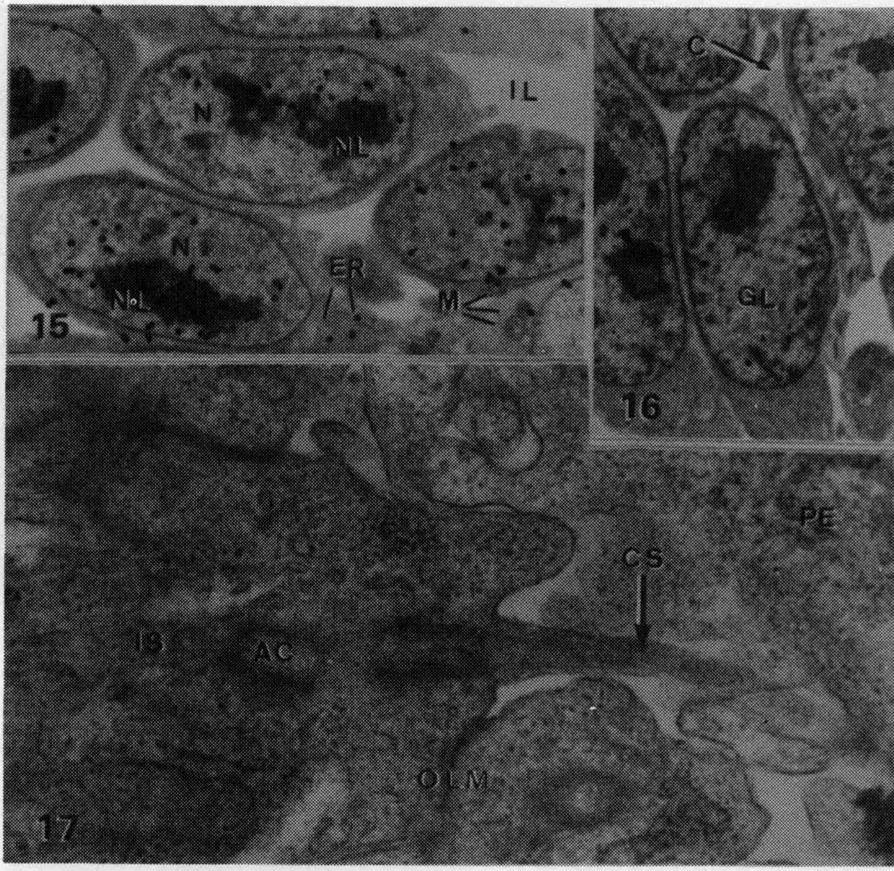

Figures 9-11. Electron microscopic radioautograms of day 3 chick embryo retina after 1 hr incubation in vitro with $^3$H-uridine. 9. Some labeled cells in the anterior region of the retina. The grains are noticed over the euchromatin, nuclear membranes, and nucleoli. Some grains are also observed, in the cytoplasms over the ribosomes, endoplasmic reticulum and mitochondria. ×5.000. 10. Differentiating ganglion cell from growth cone. ×5.200. 11. Differentiating photoreceptor cell possessing a cilium from centriole. × 6.900. Abbreviations: C, growth ganglion cell. IL, intercellular lacunae. IS, inner segment. M, mitochondria. MC, mitotic cell. N, nucleus. NL, Nucleolus. OLM, outer limiting membrane. PE, pigment epithelium.



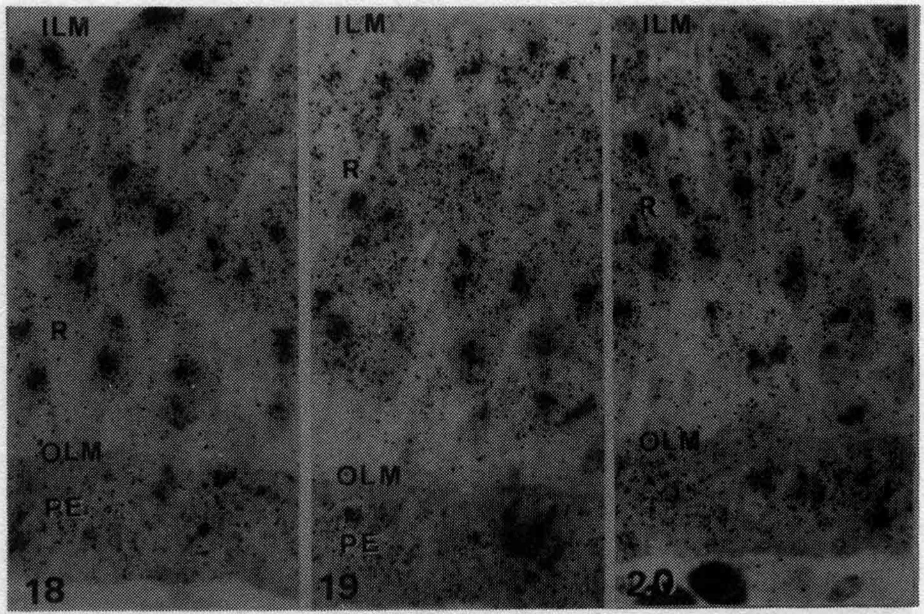

Figures 12-14. Light microscopic radioautograms of day 4 chick embryo retina after 1 hr incubation in vitro with $^{3}$H-uridine. 12. Anterior region. 13. Equatorial region. 14. Posterior region. Abbreviations: ILM, inner limiting membrane. OLM, outer limiting membrane. PE, pigment epithelium. × 675.

**Quantitative observations**

The results of the average grain count from 1 hr incubation groups were presented in Fig. 24. Since the back ground fog resulted in 0.3 grains per area of the same size as the nucleus outside the cell in average was considered negligible, the grain counting was recorded without any correction. The results of the average grain count showed an increase of uridine incorporation into RNA from day 2 to day 7. In each embryo group, the number of grains were found more in the anterior region and decreased in the posterior region. In comparison with the other cell compartments, the marked increase of the number of grains could be noticed in the nuclei. However, the grains which were less in the cytoplasms of the second day of development, were found to increase in number as the development proceeded, especially on the seventh day of development. The results of the differences in number of grains between the given embryo groups, the retinal regions, and the cell organelles, were analyzed stochastically (Table 1). From the analysis it was clarified that for those of blocks (between embryo groups), factor A (between retinal regions), and factor B (between cell




Figures 15-17. Electron microscopic radioautograms of day 4 chick embryo retina after 1 hr incubation in vitro with $^{3}$H-uridine. 15. Some labeled cells of an area close to the inner limiting membrane of the posterior region of the retina. The grains are noticed over the euchromatin, nuclear membranes, nucleoli, and over the ribosomes, endoplasmic reticulum, and mitochondria of the cytoplasm. ×4.800. 16. The differentiating ganglion cell possessing growth cone. ×3.200. 17. The developing ciliary stalk of the photoreceptor cell. ×17.500. Abbreviations: AC, assesory centriole. C, growth cone. ER, endoplasmic reticulum. GL, ganglion cell. ILM, inner limiting membrane. IL, intercellular lacunae. IS, inner segment. M, mitochondria. N, nucleus. NL, nucleolus. OLM, outer limiting membrane.



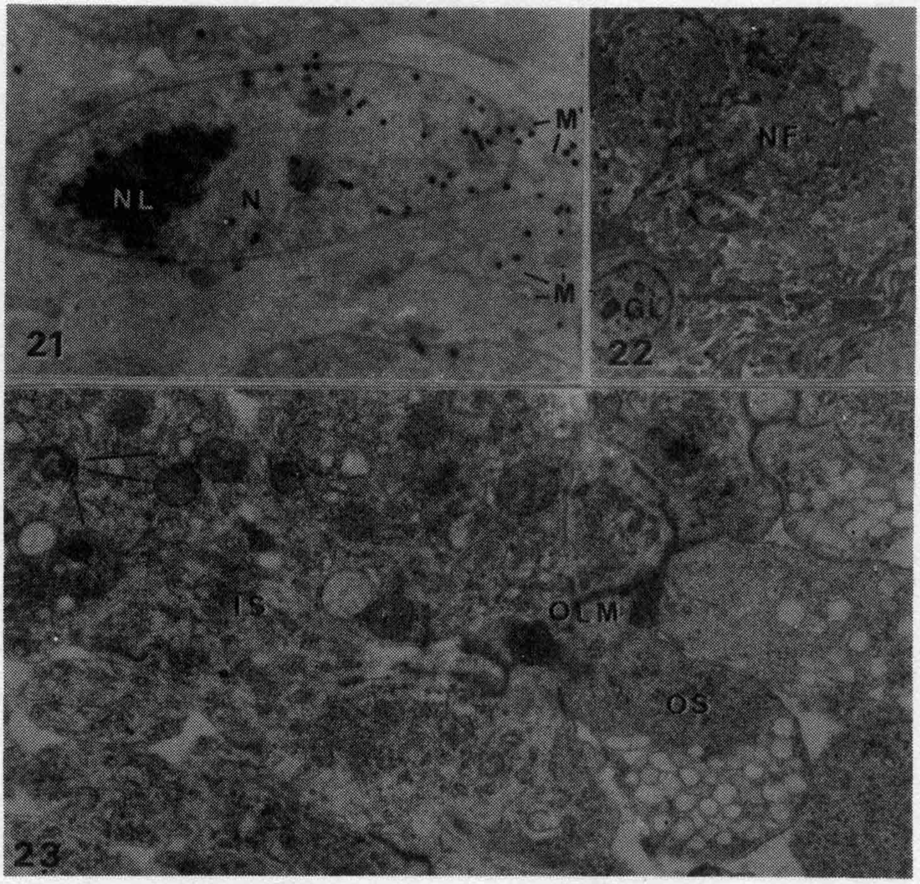

Figures 18-20. Light microscopic radioautograms of day 7 chick embryo retina after 1 hr incubation in vitro with $^3$H-uridine. 18. Anterior region. 19. Equatorial region. 20. Posterior region. Abbreviations: ILM, inner limiting membrane. OLM, outer limiting membrane. PE, pigment epithelium. ×675.

Table 1. Factorial design on the grain counts.

| Source of variation | Degree of freedom | Sum of square | Mean square |
|---|---|---|---|
| Blocks (between embryo groups) | 3 | 16508.47 | 5502.82** |
| Factor A (between retinal regions) | 2 | 5654.66 | 2827.33** |
| Replications (R) | 9 | 701.51 | 77.94 |
| Main-plot error | 18 | 176.86 | 9.83 |
| Factor B (between cell compartments) | 2 | 33053.55 | 16526.77** |
| Interaction AB | 4 | 3733.62 | 933.40** |
| Sub-plot error | 321 | 13400.80 | 41.75 |

** Highly significant at 1% level of significance.

The analysis of variance of the split plot design was carried out to analize the difference of the number of grains among the three different cell compartments of three different regions of the retinal cells of respective embryo groups.




Figures 21-23. Electron microscopic radioautograms of day 7 chick embryo retina after 1 hr incubation in vitro with $^3$H-uridine. 21. A labeled cell as observed from the equatorial region of the retina. The grains are observed over the euchromatin, nuclear membrane, nucleolus, and over the ribosomes and mitochondria of the cytoplasm. ×5.500. 22. A ganglion cell possessing developed axon. ×2.000. 23. A developing photoreceptor cell outer segment possessing vacuoles or tubular structures at the apical end of its flagelar process. ×11.900. Abbreviations: GL, ganglion cell. ILM, inner limiting membrane. IS, inner segment. M, mitochondria. N, nucleus. NF, nerve fibre. NL, nucleolus. OLM, outer limiting membrane. OS, outer segment. PE, pigment epithelium.

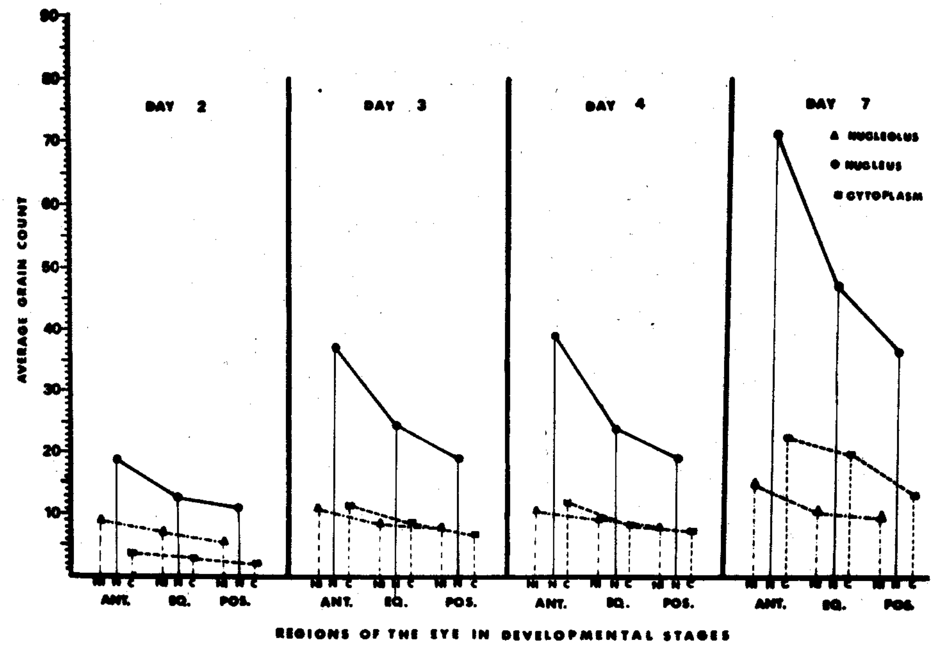



Figure 24. Histogram of average grain counts in respective three different cell compartments of three different regions of chick embryo retinas labeled with $^{3}H$-uridine for 1 hr during 2, 3, 4, and 7 days of development. Abbreviations: ANT, anterior region. C, cytoplasm. EQ, equatorial region. N, nucleus. NL, nucleolus. POS, posterior region. The analysis of variance of the split-plot design (Snedecor and Cohran, 1956) was carried out to analize the difference of the number of grains among the three different cell compartments of three different regions of the retinal cells of respective embryo groups.

compartments), the differences were stochastically significant at the 1 per cent level. The interaction of factors A and B was also found significant at 1 per cent level, which means that the increase and the difference in number of grains within the cell compartments tend to be significantly higher than the increase and the difference in number of grains within retinal regions.

## DISCUSSION

From the results obtained in the present study concerning the morphological development, it was shown that the optic vesicle was formed on day 2, while the optic cup was formed on day 3. However, the early differentiation of the ganglionic cells and the photoreceptor cells seemed somewhat earlier in comparison with the investigations of some previous investigators, and the detailed description of this findings has been presented in the other communication concerning the DNA synthesis in early development of chick embryo retina. The first appearance of the differentiating ganglionic cells was noticed on day 3 of the present study, however, several investigators noticed it to rise around day 4 and 5 of embryonic age (Fujita and Horii, 1963; Coulombre, 1965; Mishima, 1974; Mishima and Fujita, 1978; Hasebe, 1979), while a number of authors (Cajal, 1911; Rogers, 1957; Golberg and Coulombre, 1972; Khan, 1974) suggested that the first appearance of juvenile post-mitotic ganglionic cells occurred on day 3. In the present study, the first differentiating photoreceptor cells possessing the primitive cilium of centrioles, indicating the first development of the presumptive outer segments, were noticed to appear on day 3. On day 4, the elongation and enlargement of balooning of the apical ends of the ciliary stalks were observed, and by day 7 the appearance of the outer segments with the flagelar processess containing vacuoles or tubulat structures, or even the lamellar discs, was noticed. The first appearance and the development of the photoreceptor cells of the present study were also found to rise earlier than those observed by several previous investigators (Fujita, 1963; Coulombre, 1965; Hanawa *et al.*, 1979). The main reason for these differences should be due to various factors influencing on the condition of incubation such as the species of the eggs, temperature, humidity of the incubator and so on.

The distribution and the labeling pattern of grains, indicating RNA synthesis, as well as the grain count, in different cell compartments of different regions of the developing chick embryo retina from day 2 to day 7 were obtained in the present study by $^{3}$H-uridine radioautography which revealed the changes of labeling patterns to both the regional and compartmental differences. Observations on RNA synthesis had been reported by many investigators in various animals including the invertebrates (Wilt, 1970; Firtel and Monroy, 1970; Hartmann *et al.*, 1971; Prescott *et al.*, 1971; Davis and Wilt, 1972; Eckert *et al.*, 1975; Choi and Nagl, 1977) and vertebrates (Nagata *et al.*, 1967a; Landesman and Gross, 1968; Ellem and Gwatkin, 1968; Piko, 1970; Daentl and Epstein, 1971; Fakan and Bernhard, 1971; Landesman, 1972; Karp *et al.*, 1973; Nagata *et al.*, 1975; Dziadek and Dixon, 1977; Wise *et al.*, 1978; Remington and Flickingers, 1978; Yew, 1979; Counis *et al.*, 1982). Most of those observations dealt with the early development of embryos, but not with the analytical procedure on counting the number of grains in cell compartments.

From the results of the average grain count obtained in the present study as shown in Fig. 24, as well as the results through the analytical procedures (Table 1), it appeared that in significant increase the number of grains was due to the developmental progress. Similar results of $^{3}$H-uridine incorporation into RNA were also noticed by some of the previous authors mentioned above. In fact, several experimental conditions, such as the use of certain inducers (Nagata, 1974; Prasad *et al.*, 1975), the changes of certain conditions of incubation (Ward and Plageman, 1969; Ohtsuki and Amano, 1972), an increase in the dose of labeled precursor (Daentl and Epstein, 1973), or an increase of incubation time (Davis and Wilt, 1972; Nagata, 1972, 1974; Remington and Flickingers, 1978), could influence $^{3}$H-uridine incorporation into RNA. In the present study, the incorporation of $^{3}$H-uridine into RNA was examined in early development of chick embryo retina (from day 2 to day 7) after 1, 2, and 4 hr cultured *in vitro*. The increase of $^{3}$H-uridine incorporation into RNA, as indicated by an increase in the number of grains, also occurred. However, since particular attention was paid only to the distribution of labeled cells of each embryo group of 1 hr incubation in a medium containing $^{3}$H-uridine, it was assumed that the pattern of RNA synthesis in the chick embryo retinas observed in the present study was due to the progressive initiation of RNA synthesis as the development proceeded, since the amount of label precursor used during the incubation (20 uCi/ml of $^{3}$H-uridine) and the incubation time (1 hr) remained constant on all of the embryo groups observed.

Based on the increase in incorporation of $^{3}$H-uridine into RNA observed in the invertebrates (Firtel and Monroy, 1970: Wilt, 1970; Prescott *et al.*, 1971; Hartmann *et al.*, 1971; Davis and Wilt, 1972; Eckert *et al.*, 1975; Choi and Nagl, 1977), as well as vertebrates such as amphibian embryos (Landesman and Gross, 1968; Landesman, 1972; Dziadek and Dixon, 1977), mouse embryos (Landesman and Gross, 1968; Landesman, 1972; Daentl and Epstein, 1971, 1973), and rabbit embryos (Karp *et al.*, 1973), most of the investigators concluded that this was due to the increase in the formation of rRNA and mRNA. From results of the present study, it is impossible to determine which particular type of RNA was synthesized due to the developmental progress in early chick embryo retina. It is possible that the increase in RNA synthesis in early development of the chick embryo retina, as the development proceeded, is due to an increase of rRNA and/or mRNA, which is translated during the synthesis of the neural retina factor(s) or any other proteins playing a role in the differentiation throughout the development. These two processes might be related to each other and would be of interest for further investigation.

On the other hand, regional differences in RNA synthesis were found to occur during the early development of chick embryo retina. The RNA synthesis was examined in three different regions of the retina; the anterior, the equatorial, and the posterior region. The results showed that within each of the given embryo group, the grains were found more in the anterior region and descreased in the posterior region. The differences between those three regions were analysed stochastically. Such regional differences were also noticed by Yew (1978) in neonatal albino rats. Glücksman (1940) reported that the continuous dividing cells during the development and differentiation were found in the germinative zone of the anterior region. However, it is well known that the differentiation of the retinal layers begins in the fundus (central or posterior region) of the retina (Sidman, 1961; Fujita and Horii, 1968; Khan, 1974). From the present study, it was found that as early as the third day of development, the differentiation from retinal neuroblasts into presumptive ganglion and photoreceptor cells was noticed especially in the posterior region of the retina. Thus, it could be expected that as the development proceeded, the RNA synthesis would occur more in the posterior region in comparison to the other two regions of the retina. The present study however showed that the RNA synthesis, preceding the protein synthesis, was found more in the anterior region. It seems probable that the anterior region, as the developmental progress went on, performed an active continuation of protein synthesis more than the other two regions of the retina.

As for the distribution of silver grains in three cell compartments (the nucleus, the nucleolus, and the cytoplasm) of different regions of the retinal cells in the present study, the number of grains were found more in the nuclei than the other two regions of the retina. The differences between those three cell compartments were stochastically significant. The difference of grain distribution in various cell compartments in the present study, was also observed in many other animal materials such as; in sea urchin embryos (Hartmann *et al.*, 1971), in fibroblast-like cells of Chinese hamster (Abramova and Neyfakh, 1977), in preimplantation of rabbit embryos (Karp *et al.*, 1973) or in young albino rabbits (Bracher, 1967), in early mouse embryos (Ellem and Gwatkin, 1968), and also in rat hepatoma cells (Dziadek and Dixon, 1977). Moreover, intensive work to demonstrate the presence of soluble uridine in those three cell compartments, before uridine is incorporated into RNA, was done by Nagata *et al.* (1969, 1977b) in the livers and kidneys of chick embryos and new born mice, and also in liver and pancreas cells of adult mice and HeLa cells. On the other hand, Nagata *et al.* (1977a, 1977c, 1982) demonstrated the incorporation of $^3$H-uridine into RNA in three cell compartments of HeLa S3 cells, YS cells, and rat liver cells, while Murata *et al.* (1977, 1978) demonstrated it in mastocytoma and mast cells.

The principle function of nucleolus is as the source of ribosomal RNA synthesis, which is needed and essential for the protein synthesis during the development and differentiation (Ellem and Gwatkin, 1968). Therefore, the dominant localization of label, indicating ribosomal RNA synthesis, is supposed to occur in the nucleoli of retinal cells throughout the developmental progress. However, the results of the distribution pattern obtained from the average grain counts of three different cell compartments of three different regions of retinal cells showed that more grains were noticed in the nuclei than in the nucleoli and the cytoplasms. It is therefore difficult to take a conclusion from these two contradiction of facts.

Concerning the distribution of grains observed in the cytoplasms, they were found less in the cytoplasms of day 2 embryo group, but found more in the day 7 embryo group. The distribution of grains in the cytoplasm, indicating the occurance of the extra-nuclear RNA synthesis, could be found in the ribosomes, endoplasmic reticulum, and also in the mitochondria (Ellem and Gwatkin, 1968; Nagata *et al.,* 1967a, 1975, 1977a, 1982; Murata *et al.,* 1977, 1978). The occurrence of grains in the ribosomes, polysomes, and endoplasmic reticulum would indicate that the label precursor first localized in the nucleolus and nucleus of a cell was later transfered to the ribosomes of the cytoplasm (Ellem and Gwatkin, 1968; Fakan and Bernhard, 1971). As for the occurrence of grains in the polysomes, Firtel and Monroy (1970) noted that as the development proceeded, the amount of labeled RNA presented in the polysomes was increased. Furthermore, concerning the synthesis of RNA on mitochondrial genes during development, it had been largely ignored or unrecognized in the past. But later, from the intensive works of Nagata (1972, 1974), Nagata *et al.* (1977a, 1977c), Hartmann *et al.* (1968), Karp *et al.* (1973), and Murata *et al.* (1977, 1978), it was demonstrated that RNA synthesis was performed by the mitochondria, and the transcription products of mitochondrial genes represented a significant contribution to the total newly synthesized cytoplasmic RNA. The labeled mitochondria, indicating RNA synthesis, were also observed in the present study. It requires further investigation to quantify this phenomenon.

From the results obtained in the present study, it is concluded that the labeling pattern in the chick embryo retinas labeled with $^3$H-uridine showed an increase of uridine incorporation due to the developmental progress and the average grain count was different at three regions of the retina.

## REFERENCES


Abramova, N.B., A.A. Neyfakh. 1973. Migration of newly synthesized RNA during mitosis. III. Nuclear RNA in the cytoplasm of metaphase cells. Exp. Cell. Res. 77: 136-142.

Bracher, R. 1967. Radioautographic analysis of the synthesis of protein, RNA, DNA, and sulfated mucopolysacharides in the early stages of corneal wound healing. Invest. Opthalmol. 6: 565-573.

Cajal, S.R. 1911. The development of retinal cells. *In* The structure of the retina. Translated by Thorpe S.A. and Glickstein M. Charles C. Thomas, Springfield, Illinois, pp. 140-152.

Choi, W.C. and W. Nagl. 1977. Pattern of DNA and RNA synthesis during development of ovarian nurse cells in *Gerris najas* (Heteroptera). Develop. Biol. 61: 262-272.

Coulombre, A.J. 1965. The eye. *In* De Haan RL, Ursprung H. eds. Holt, New York, pp. 219-251.

Counis, M.F., E. Chaudun, Y. Courtis, J.P. Carreau, J. Jack, R. Clayton. 1982. Differential protein synthesis in chick embryonic lenses during in vitro differentiation. Exp. Eye Res. 34: 861-876.

Daentl, D.L. and C.J. Epstein. 1973. Uridine transport by mouse blastocysts. Develop. Biol. 31: 316-322.

Davis, F.C., and K.E. Dixon. 1977. An autoradiographic analysis of nucleic acid synthesis in the presumptive primordial germ of *Xenopus laevis*. J. Embryol. Exp. Morph. 37: 13-31.

Eckert, W.A., W.W. Franke, U. Scheer. 1975. Nucleocytoplasmic translocation of RNA in *Tetrahymena pyriformis* and its inhibition by actinomycin D and cycloheximidine. Exp. Cell Res. 94: 31-46.

Ellem, K.A.O. and R.B.I. Gwatkin. 1968. Pattern of nucleic acid synthesis in the early mouse embryo. Develop. Biol. 18: 311-330.

Fakan, S. and W. Bernhard. 1971. Localization of rapidly and slowly labeled nuclear RNA as visualized by high resolution autoradiography. Exp. Cell Res. 67: 129-141.

Firtel, R.A., and A. Monroy. 1970. Polysomes and RNA synthesis during early development of the surf clam *Spisula sallidisum*. Develop. Biol. 21: 87-104.

Fujita, S. 1963. The matrix cell and cytogenesis in the developing central nervous system. J. Comp. Neurol. 120: 37-42.

Fujita, S. and M. Horii. 1963. Analysis of cytogenesis in the chick retina by tritiated thymidine radioautography. Arch. Histol. Jap. 23: 359-366.

Glücksman, A. 1940. Development and differentiation of the tadpole eye. Brit. J. Ophthalmol. 24: 153-178.

Goldberg, S. and A. Coulombre. 1972. Topographical development of the ganglion cell fibre layer in the chick retina. A whole mount study. J. Comp. Neurol. 146: 507-518.

Hamburger, V. and M.I. Hamilton. 1951. A series of normal stages in the development of chick embryo. J. Morphol. 88: 49-92.

Hanawa, I., K. Takahashi, N. Kawamoto. 1979. A correlation of embryogenesis of visual cells and early receptor potential in the developing retina. Exp. Eye. Res. 23: 587-594.

Hartmann, J.F., M.M. Ziegler, D.G. Comb. 1971. Sea urchin embryogenesis. I. RNA synthesis by cytoplasmic and nuclear genes during development. Develop. Biol. 25: 209-231.

Hasebe, H. 1979. Histogenesis of the chick embryo retinal pigment epithelium. II. $^3$H-thymidine autoradiography (in Japanese). Acta Soc. Ophthal. Jap. 83: 598-607.

Karp, G., C. Manes, W.E. Hahn. 1973. RNA synthesis in preimplantation rabbit embryo: radioautographic analysis. Develop. Biol. 31: 404-408.

Landesman, R. 1972. Ribosomal RNA synthesis in pre-and postgastrula embryo of *Xenopus laevis*. Cell. Differ. 1: 209-213.

Landesman, R. and P.R. Gross. 1968. Pattern of macromolecule synthesis during development of *Xenopus laevis*. I. Incorporation of radioactive precursors into dissociated embryo. Develop. Biol. 18: 571-589.

Mishima, H. and H. Fujita. 1978. Studies on the cytodifferentiation of the matrix cell to neuroblast (in Japanese). Acta Soc. Ophthal. Jap. 78: 1410-1417.

Murata, F., Y. Momose, K. Yoshida, S. Ohno, T. Nagata. 1977. Nucleic acid and mucosubstance metabolism of the mastocytoma cells by means of electron microscopic radioautography. Acta Pharmacol. Toxicol. 41: 58-59.

Murata, F., K. Yoshida, S. Ohno, T. Nagata. 1978. Ultrastructural and electron microscopic radioautographic studies on the mastocytoma and mast cells. J. Clin. Electron Microscopy 11: 5-6.

Murata, F., K. Yoshida, S. Ohno, T. Nagata. 1979. Electron-microscopic radioautography using a combination of phenidon developer and domestic emulsion. Acta Histochem. Cytochem. 12: 443-450.

Nagata, T. 1972. Electron microscopic radioautography of intramitochondrial RNA synthesis in HeLa cells in culture. Histochemie 32: 163-170.

Nagata, T. 1974. Electron microscopic radioautography of intramitochondrial nucleic acid synthesis in mammalian cells in vitro. Proc. 8th Internat. Cong. Elect. Microsc. II: 346-347.

Nagata, T., N. Iwadare, F. Murata. 1975. Relationship of intramitrochondrial nucleic acid synthesis to the nucleoli in cultivated cells as revealed by electron microscopic radioautography. Proc. 10th Internat. Cong. Anat.: 474.

Nagata, T., N. Iwadare, F. Murata. 1976. Mitochondrial and nucleolar RNA synthesis as revealed by electron microscopic radioautography. Proc. 5th Internat. Cong. Histochem. Cytochem.: 242-244.

Nagata, T., N. Iwadare, F. Murata. 1977a. Electron microscopic radioautography of nucleic acid synthesis in cultured cells treated with several carcinogens. Acta Pharmacol. Toxicol. 41: 64-65.

Nagata, T. and F. Murata. 1977b. Electron microscopic dry-mounting radioautography for diffusible compounds by means of ultracryotomy. Histochemistry 54: 75-82.

Nagata, T., F. Murata, K. Yoshida, S. Ohno, N. Iwadare. 1977c. Whole mount radioautography of cultured cells as observed by high voltage electron microscopy. Proc. 5th Internat. Conf. on High Voltage Elect. Microsc.: 347-349.

Nagata, T., T. Nawa, S. Yokota. 1969. A new technique for electron microscopic dry-mounting radioautography of soluble-compounds. Histochemie 18: 241-249.

Nagata, T., S. Ohno, F. Murata. 1977d. Electron microscopic dry-mounting radioautography for soluble compounds. Acta Pharmacol. Toxicol. 41: 62-63.

Nagata, T., S. Ohno, K. Yoshida, F. Murata. 1982. Nucleic acid synthesis in proliferating peroxisomes of rat liver as revealed by electron microscopical radioautography. Histochem. J. 14: 197-204.

Nagata, T., O. Shibata, T. Nawa. 1967a. Electron microscopic radioautographic studies of nucleic acid and protein synthesis in the liver and kidney cells of chickens and mice in tissue culture. Arch. Histol. Jap. 28: 540-541.

Nagata, T., O. Shibata, T. Nawa. 1967b. Simplified methods for mass production of radioautographs. Acta. Anat. Nippon 42: 162-166.

Ohtsuki, H. and M. Amano. 1972. RNA synthesis in the different phases of cell cycle of chick embryo cells. Dev. Growth Differ. 14: 197-204.

Prasad. K.N., S.C. Brandy, J.L. Purdy. 1975. Polyadenylic containing cytoplasmic RNA increase in adenosin 3′, 5′ cyclic monophosphate-induced differentiated neuroblastoma cells in culture. Exp. Cell Res. 94: 88-94.

Prescott, D.M., C. Bostoct, E. Gamow, M. Lauth. 1971. Characterization of rapidly labeled RNA in *Tetrahymena pyriformis.* Exp. Eye Res. 67: 124-128.

Piko, L. 1970. Synthesis of macromolecules in early mouse embryo cultures in vitro: RNA, DNA, and a polysacharide component. Develop. Biol. 21: 257-279.

Remington, J.A. and R.A. Flickingers. 1978. Timing of incorporation of tritiated nucleoside into DNA and RNA of embryonic cells of *Rana pipiens.* Dev. Growth Differ. 20: 11-15.

Reynolds, E.S. 1963. The use of lead citrate of high pH as an electron opaque stain in electron microscopy. J. Cell. Biol. 17: 209-212.

Rogers, K.T. 1957. Early development of the optic nerve in chick. Anat. Rec. 127: 97-107.

Sidman, R. 1961. Histogenesis of mouse retina studied with thymidine-$H^3$. In: The structure of the eye. Smelser GK ed. Academic Press, New York, pp: 487-505.

Snedecor, G.W. and W.G. Cohran. 1956. Factorial arrangements of treatments. In: Statistical methods. Fifth ed. The Iowa State Coll. Press, Ames, Iowa, pp: 329-393.

Ward, G.A. and G.W. Plagemann. 1969. Fluctuations of DNA-dependent RNA polymerase and synthesis of macromolecules during growth cycle of Novikoff rat hepatoma cell in suspension culture. J. Cell Physiol. 73: 213-231.

Wilt, F.H. 1970. The acceleration of ribonucleic synthesis in cleaving sea urchin embryo. Develop. Biol. 23: 444-455.

Wise, S.P., E.G. Jones, N. Bermen. 1978. Direction and specifisity of the axonal and transcellular transport of nucleosides. Brain Res. 139: 197-217.

Yew, D.W. 1979. Autoradiographic study of neonatal rat retina after labelled uridine uptake. Acta Histochem. 64: 1-4.

Yoshida, K., F. Murata, S. Ohno, T. Nagata. 1978. A modified wire-loop method for quantitative electron microscopic radioautography. Histochemistry 57: 93-96.