the somatotrops presented a marked hypertrophy of the Golgi complex, extended over a large area of the cytoplasm. The vesicles were very numerous and the vacuoles considerably dilated. Many microvesicles were scattered in the Golgi zone and the occurrence was frequent of granules of secretion in different stages of formation, ranging from condensing secretory material to dense and well-defined granules enveloped by Golgi membranes (Figure 2). Multivesicular bodies were also found in some cells.

Some authors report that the Golgi complex of STH cells is well defined but small[3,6], as we have described here in the animals killed at midnight. Other authors report pictures coincident with the one that we have described in the animals killed at 18.00 h[7]. Perhaps the differences that we have found might help to explain these apparent contradictions in the literature.

More times of a 24-h period must be explored before attempting to correlate the changes in the Golgi complex with the circadian changes existing in growth processes.

Resumen. El aparato de Golgi de las células somatotropas de la pars distalis de la hipofisis del ratón, presenta importantes diferencias ultraestructurales en distintos momentos de un período de 24 horas. Es pequeño a mediodia y marcadamente hipertrófico a mediodía y 6 de la tarde.

C. L. GOMEZ DUMM and J. M. ECHAVE LLANOS

Instituto de Embriologia, Biología e Histología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata (Argentina), 12 August 1969.

The Composition of Extracted Nuclei of Developing Frog Embryos used as Template Material for RNA synthesis in vitro

After neurulation, *Xenopus laevis* and *Rana pipiens* embryos synthesize less DNA-like RNA (D-RNA) per cell[1,2] and fewer kinds of D-RNA per cell[3,4] as development proceeds to the larval stage. In addition, the ability of isolated chromatin of *Rana pipiens* embryos to act as template for RNA synthesis in vitro without the addition of exogenous microbial RNA polymerase decreases over this same period[4–6]. However, if microbial RNA polymerase is added to the in vitro system, there is a progressive increase in RNA synthesis from chromatin of the later stage embryos[5,7]. It seemed possible that a greater amount of RNA polymerase in the non-basic residual protein fraction might account for the higher RNA synthesis in vitro without added RNA polymerase, but that increased masking of the DNA template by total protein, histones, or D-RNA might account for the lower levels of RNA synthesis with the addition of exogenous RNA polymerase at the early stages. The present study examines this problem by determining the amounts of basic and residual protein, phosphoprotein, and D-RNA of extracted nuclei of gastrulae, tailbuds and larvae (stages 10, 19, 25 of SHUMWAY[8]).

The problem involved in measuring such constituents in isolated nuclei or chromatin is that significant contamination by yolk and pigment granules cannot be avoided. However, it is possible to remove the pigment granules and yolk protein by partially deproteinizing isolated nuclei with one chloroform-isoamylalcohol (24:1) extraction in the presence of 1 *M* NaCl[9]. We feel that these preparations can be used for the composition study since they show the same increase in templating activity with development as did isolated chromatin in the in vitro system containing added RNA polymerase[5]. The preparations are devoid all pigment and are optically clear after centrifugation at 65,000 *g* for 1 h, as compared to isolated chromatin or nuclear preparations which are contaminated by small amounts of pigment. It was impossible to remove protein and D-RNA from these DNA preparations by repeated precipitation with cold ethanol or isopropyl alcohol, or by wrapping them around a rod and washing with 0.01 *M* *Tris*-HCl (pH 7.5)[7].

Materials and methods. Nuclei were isolated from gastrulae, tailbuds and larvae, and were partially deproteinized as described above. The method of MARUSHIGE and OZAKI[10] was used to determine the amounts of basic and residual protein in the extracted nuclear preparations. Aliquots of the nuclear extracts (1–4 mg) in 0.1 *M* *Tris*-0.1 *M* NaCl (pH 7.5) were precipitated and washed 3 times with cold 10% trichloroacetic acid (TCA). Basic proteins were extracted twice from the pellets by shaking at 4 °C for 1 h with 0.4 ml of 0.4 *N* H_2SO_4. The combined extracts were adjusted to pH 10 with 4 *N* NaOH and brought to a final volume of 1 ml. Protein concentration was determined by the method of LOWRY et al[11] using calf thymus histone as a standard.

Nucleic acids were removed from the residues of the acid extractions by heating at 95 °C for 20 min in 5% TCA. The residual protein was dissolved in 0.1 *N* NaOH and protein was estimated using the LOWRY[11] method with a bovine seum albumin standard. The DNA content of the TCA hydrolysate was determined by the Dische diphenylamine reaction[12], and the protein:DNA ratios were calculated.

In assaying for phosphoprotein, the nuclear extract was treated with 10% TCA, centrifuged and washed.

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Constituents of extracted nuclei of developing frog embryos

Stage	% Total basic protein	% Residual protein	% P of phosphoprotein	% D-RNA
Gastrula Stage	28.7 ± 3.3	515.0 ± 29.9	11.4 ± 0.51	3.4 ± 1.1
Tailbud Stage 19	2.77 ± 0.187	35.8 ± 6.0	1.11 ± 0.071	0.51 ± 0.09
Larvae Stage 25	0.73 ± 0.162	3.27 ± 0.84	0.34 ± 0.22	0.09 ± 0.0035

Values are given in terms of % of DNA with standard error calculated.

Phospholipids were removed by boiling 3 times in ethanol-ether (3:1) for 4 min. The washed residue was suspended in 5% TCA, and nucleic acids were hydrolyzed by incubating for 20 min at 95 °C. The protein residue was washed, dissolved in 1 *N* NaOH, and heated at 100 °C for 15 min to selectively hydrolyze phosphoprotein phosphate which was estimated according to BERENBLUM and CHAIN[13].

In order to determine the amounts of nuclear D-RNA, isolated nuclei were extracted 3–4 times with phenol at 45 °C until no more RNA could be removed. Base composition studies have shown that the remaining RNA bound to the DNA is mostly D-RNA[14]. This RNA, not extracted with phenol at 45 °C, was hydrolyzed with 0.3 *N* KOH at 37 °C for 18 h and was determined quantitatively by the orcinol reaction[12].

Results and discussion. The amounts of basic and residual protein, phosphoprotein, and D-RNA associated with the DNA of the extracted nuclear preparations decrease from the gastrula to the larval stage (Table). The results show that the non-basic residual protein is at a high level at the gastrula stage when RNA synthesis is more active in vivo and in the in vitro system without added RNA polymerase, and that this fraction decreases proportionately more than other constitutents with development. It is known that more functional endogenous RNA polymerase is associated with DNA in earlier stages of *Rana pipiens* embryos[1,6]. A greater amount of the residual protein fraction in the younger *Rana pipiens* embryos could account for the higher levels of RNA synthesis per cell in vivo and in the in vitro system without added microbial RNA polymerase if RNA polymerase is located in this fraction. An excess of non-basic nuclear proteins[10,15] and RNA polymerase[16] is also associated with chromatin actively synthesizing RNA in other systems.

The results also suggest that isolated chromatin and extracted nuclei of progressively later stages are more active in vitro as templates for RNA synthesis in the presence of added microbial RNA polymerase because reduced levels of total protein and D-RNA make the DNA more accessible to the added RNA polymerase[17].

Résumé. Les quantités de protéines basiques et résiduelles, de phosphoprotéine et de D-RNA se trouvant dans les noyaux extraits d'embryons de *Rana pipiens* diminuent en passant du stade de blastula à celui de la larve. Cette évidence suggère que l'accumulation progressive des protéines ou du D-RNA, en masquant le DNA n'offre pas le contrôle principal de la synthèse de l'RNA in vivo. Une réduction de la quantité de l'endogène RNA polymerase fonctionelle incluse dans la protéine résiduelle explique mieux la réduction de la synthèse du D-RNA dans les stades plus avancés du développement embryonnaire.

J. A. REMINGTON and R. A. FLICKINGER

Department of Biology,
State University of New York,
Buffalo (New York 14214, USA), 30 May 1969.

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Effects of Ionizing Radiation on Myelinogenesis in the Chicken

Numerous studies in the rat have demonstrated that ionizing radiation has profound effects on the functional, anatomical, and biochemical development of the central nervous system (CNS), effects that depend upon the dose of irradiation, the developmental period during which the animal is exposed, and the specific structure or characteristic under study. For example, prenatal irradiation accelerates the proliferation of oligodendroglia[1,2], the cells that are believed to form myelin in the CNS[3], as well as the accumulation of cerebrosides[4] which, together with spingomyelin and cholesterol, are the lipid components characteristic of myelin[5].

The present study adds the chicken to the few species in which such studies have been carried out, and focuses attention on the fact that the effects of radiation may be exerted upon individual aspects of myelin formation rather than upon myelinogenesis as a unitary process.

White Leghorn cockerels were exposed to 1600 R of whole-body gamma irradiation (Co^{60} source, 10.4 R/min) at 24 days after hatching. This dose and dose rate was selected in order to compare its known effects on extraneural structures with potential effects on neural components. As suggested in previous studies[6], the cockerels

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