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.

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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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Table I. Effects of γ-iradiation on body, brain, and endocrine organ weights in immature cockerels

Group	Weight of organ or body						
	Body (g)	Brain (g)	(g/100g)	Left testis (mg)	(mg/100g)	Adrenals (mg)	(mg/100g)
Controls	350 ± 8	2.18 ± 0.03	0.623 ± 0.005	49.9 ± 5.1	14.3 ± 1.6	46.1 ± 2.5	13.2 ± 2.6
Irradiated[a]	221 ± 15	1.81 ± 0.03	0.869 ± 0.049	11.4 ± 1.2	5.4 ± 0.5	35.7 ± 3.0	17.4 ± 2.2
	(< 0.001)[b]	(< 0.001)	(< 0.001)	(< 0.001)	(< 0.001)	(< 0.002)	–

[a] Dose 1600 R at 10.4 R/min (Co 60 source) at 24 days of age; sacrifice at 35 days of age. [b] Significance of difference from mean of controls (t-test).

Table II. Effects of γ-irradiation on total lipid and total sphingolipid, cerebroside, and cholesterol in brain of immature cockerels

Group	Weight of Lipid							
	Total lipid (mg)	(mg/g)[a]	Total sphingolipid (mg)	(%)[b]	Cerebroside (mg)	(%)[b]	Cholesterol (mg)	(%)[b]
Controls	151 ± 3	69.5 ± 0.9	26.1 ± 0.7	17.4 ± 0.2	16.1 ± 0.4	10.7 ± 0.1	30.1 ± 0.6	20.0 ± 0.1
Irradiated[c]	130 ± 3	71.8 ± 0.7	21.6 ± 0.4	15.3 ± 0.5	13.8 ± 0.5	10.6 ± 0.2	25.9 ± 0.7	19.9 ± 0.1
	(< 0.001)[d]	–	(< 0.001)	(< 0.001)	(< 0.001)	–	(< 0.001)	–

[a] Mg/g brain weight. [b] Percent of total lipid. [c] Dose 1600 R at 10.4 R/min (Co 60 source) at 24 days of age; sacrifice at 35 days of age. [d] Significance of difference from mean of controls (t-test).

were protected by injection of homologous bone marrow 24 h post-irradiation to assure survival. Irradiated birds and sham-irradiated controls were given, ad libitum, chick starter ration, and water containing 1 g of Terramycin (oxytetracycline hydrochloride) per liter. On the 11th day after treatment, the 9 sham-irradiated controls and the 8 survivors among the 9 irradiated birds were sacrificed by decapitation.

Extraneural effects as well as effects on brain weight are reported in Table I. Growth of body, brain, testis, and adrenals was retarded in the irradiated cockerels. The testis was most radiosensitive, and the adrenals and brain were far more resistant to the growth-retarding effect of irradiation than was the body as a whole. A similar pattern of differential effects of irradiation upon the growth of these organs has been reported previously in the chicken[7,8] as well as in the rat[9–11].

Brain lipid was extracted by homogenization of the whole brain in chloroform:methanol (2:1), and the crude extract was washed with 0.88% KCl to remove gangliosides and non-lipids[12]. Total lipid was determined gravimetrically[13]. Total sphingolipid[14] and cerebroside[15] were determined after hydrolysis of the extract with methanolic boron trifluoride, a reagent which quantitatively hydrolyzes sphingomyelin[16] as well as cerebroside.

That the total lipid content of the brain was low in the irradiated cockerels compared to controls was attributed primarily to differences in brain weight (Table II). When lipid components were expressed as percent of total lipid, however, cholesterol content was unaltered, and total sphingolipid content was significantly less ($P < 0.001$) in irradiated than in control brains. Because of the negligible decrease in percent of cerebroside, the other major sphingolipid present in the ganglioside-free purified lipid extracts, this finding may be interpreted as a selective decrease in sphingomyelin.

Whether radiation affects myelination directly, or indirectly by inducing metabolic or endocrine changes remains to be clarified. On the one hand, recent studies of nervous tissue in culture have shown that radiation can alter myelination[17]. On the other hand, it is well known that malnutrition[18], for example, and hormonal disturbances[19–21] affect the deposition of myelin. Nevertheless, the present finding of a selective effect of radiation upon sphingomyelin accumulation in chicken brain points to the value of detailed lipid analyses as an approach to clarifying the multiple aspects of myelination[22].

Résumé. Chez le poulet, l'irradiation (1600 R rayons gamma) retarde la croissance de l'organisme in toto et de plusieurs organes, et affecte sélectivement les constituants individuels de la myéline plutôt que le processus global de myélinisation, l'accumulation de la sphingomyéline étant plus retardée que celle de la cérébroside et du cholestérol.

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