The stage-dependent resistance of the chorion to external chemical damage and its relationship to embryonic diapause in the annual fish, *Nothobranchius guentheri*

J. R. Matias

Orentreich Foundation for the Advancement of Science, 910 Fifth Avenue, New York (NY 10021, USA), 7 July 1983

Summary. The resistance of the embryonic chorion of the annual fish *Nothobranchius guentheri* to chemical damage in vivo was investigated by the exposure of the embryos to protease. Embryos at stages 20, 33, and 43 were the most resistant to enzymatic action. These stages of development correspond respectively to the stages at which diapause I, diapause II, and 'delayed hatching' may occur. The magnitude of the resistance was further enhanced when diapause was induced prior to the treatment.

The ontogeny of the East African annual fish *Nothobranchius guentheri* deviates from the typical teleostean developmental pattern because of the occurrence of developmental arrest or diapause at specific stages[1,2]. Similar to insects, annual fish embryos in diapause are able to survive extreme environmental conditions[3,4]. The embryo is enclosed by a tough egg envelope or chorion which provides protection against chemical or mechanical damage. It may be possible that the increased resistance of *N. guentheri* embryos during diapause is due to the increased effectiveness of the chorion in shielding the embryo against external stress. This hypothesis was tested in the present study by utilizing the technique of external proteolytic digestion of the chorion in vivo as a means of measuring the degree of chorion resistance to chemical insults.

Materials and methods. The source of fish, husbandry conditions, and the method of embryo collection have already been reported for *N. guentheri*[2]. The embryos were derived from 30 breeding pairs maintained at a constant photoperiod (16 h light, 8 h darkness) and temperature (23 ± 0.5 °C). The induction of diapause II was accomplished by prior incubation of the embryos at 17 °C[2]. The nomenclature established by Wourms[5] to describe the stages of the annual fish *Austrofundulus myersi* was used.

The procedure reported by Lesseps and Gast[6] for the preparation and sterilization of the dechorionating solution was followed as described. The enzyme used in this study was pronase (Type IV, Sigma Chemical Co., USA), a bacterial protease with broad substrate specificity. The temperature was maintained at 23 °C throughout the incubation period. The gross morphological changes seen during the dechorionation process was similar to that observed by Smithberg[7] for the medaka, *Oryzias latipes* and was therefore used as a guide. The length of time required for each embryo to escape through the ruptured chorion was determined[6].

Results and discussion. The annual fish chorion differs structurally from that of most teleosts because of its greater thickness and the more developed surface ornamentation of the zona pellucida[8,9]. The exposure to pronase resulted in the rapid destruction of the surface ornamentation and followed by the time-dependent softening of the inner layers of the chorin. The end result was the formation of a hole through the chorion which permits the escape of the embryo[6]. The greater the time for the rupture of the chorion, the greater the resistance to enzymatic action.

The data in figure 1 clearly illustrate that the susceptibility of the annual fish chorion to enzymatic digestion was stage-specific. The chorion of embryos at the 16-cell stage (stage 7) and those at the blastula stage (stage 11) were digested by pronase within 5.3 h. A slight increase in the time for proteolytic digestion occurred during epiboly (stage 15). The chorion of stage 20 embryos demonstrated the greatest resistance to enzymatic action (peak I) in comparison to all other stages of development. At this stage the mass of deep blastomeres have completely and randomly dispersed throughout the surface of the yolk[10,11]. Diapause I may occur at this stage if the embryos are exposed to low oxygen tension or to inhibitory substance(s) produced by adult fishes[1,12]. The complete reaggregation of the previously dispersed blastomeres signals the end of the arrest period.

Axial formation corresponded to a drastic decline in chorion resistance to pronase. The mean duration of proteolytic digestion remained at approximately 7 h throughout the early part of somite formation. The 2nd peak of resistance occurred at stage 33 (peak II). At this stage the optic cups are already distinct and 38–42 somites are present. The contraction of the rudimentary heart is erratic. Diapause II may occur at the early part of this stage[5]. Its induction is triggered by low temperature[2], low oxygen tension[12], or short photoperiod[2,13]. The commencement of regular heart contractions marks the termination of this arrest period.

The 3rd stage of high resistance occurred at stage 43 (peak III). By this time all the embryonic organs have already formed. Although active circulation and occasional movement of the embryo were evident, hatching may be delayed for many months. While diapause III has been reported for a number of annual fishes[14], this arrest period has never been observed in *N. guentheri*. These fishes appear to exhibit the delayed hatching phenomenon commonly observed in the killifish *Fundulus confluentus*[15].

To determine whether diapause caused changes in the suscepti-

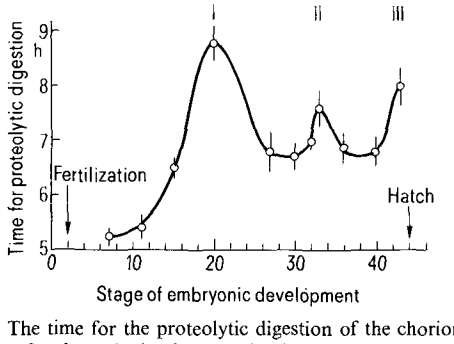

Figure 1. The time for the proteolytic digestion of the chorion at various stages of embryonic development in *N. guentheri*. Each point represents the mean ± SEM of 40–60 embryos.

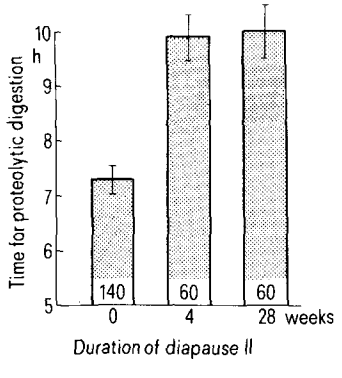

Figure 2. The relationship between the duration of proteolytic digestion and the duration of diapause II in the embryos of *N. guentheri*. The values represent the mean ± SEM. The circled numbers at the bottom of the graph represent the number of embryos in each group.

bility of the chorion to enzymatic action, a comparison was made between non-diapause embryos at stage 33 and embryos at this stage which had been induced to undergo diapause. The data in figure 2 illustrate that the chorion of diapause II embryos were more resistant to pronase digestion. The values determined for embryos at diapause II for 4 and 28 weeks were virtually identical, indicating that longer duration in diapause does not enhance the resistance of the chorion to pronase.

The use of pronase was first demonstrated by Smithberg[7] in the chorion of medaka. In this species, which does not exhibit any instance of diapause, differences in the duration of chorion digestion were not observed between embryos of different stages. The stage specific variation in chorion susceptibility to pronase in annual fish embryos may represent an evolutionary response to the erratic climatic conditions to which these fishes are normally exposed. *N. guentheri* is native to the isolated ponds and mudholes along the coastal lowlands of Kenya and Tanzania[16]. During the dry season all the adults die due to evaporative waterloss[17]. The survival of the population becomes dependent entirely upon embryos which undergo diapause at specific stages of their normal development. The evolution of a resistant egg envelope is an essential requirement for survival since these embryos are normally exposed to extremes in temperature, water chemistry, and desiccation.

The role of the chorion in the survival of annual species and the mechanism by which the embryo modifies its chorion are speculative at this time. The present study demonstrates that the susceptibility of the annual fish chorion to external chemical damage changes with the stage of development and upon entry into diapause.

1 Peters, N., Int. Revue ges. Hydrobiol. *18* (1963) 257.
2 Markofsky, J., and Matias J.R., J. exp. Zool. *202* (1977) 49.
3 Matias, J.R., and Markofsky, J., J. exp. Zool. *204* (1978) 219.
4 Matias, J.R., Experientia *38* (1982) 1315.
5 Wourms, J.P., J. exp. Zool. *182* (1972) 169.
6 Lesseps, R.J., and Gast, E.A., Anat. Rec. *187* (1977) 125.
7 Smithberg, M., Anat. Rec. *154* (1966) 823.
8 Wourms, J.P., and Sheldon, H., Devl Biol. *50* (1976) 355.
9 Schoots, A., Stikkelbroeck, J., Bekhius, J., and Denuce, J.J., Ultrastruct. Res. *80* (1982) 185.
10 Wourms, J.P., J. exp. Zool. *182* (1972) 169.
11 Lesseps, R.J., Geurtz van Kessel, A.H.M., and Denuce, J.M., J. exp. Zool. *193* (1975) 137.
12 Inglima, K., Perlmutter, A., and Markofsky, J., J. exp. Zool. *215* (1981) 23.
13 Markofsky, J., Matias, J.R., Inglima, K., Vogelman, J.H., and Orentreich, N., J. exp. Biol. *83* (1979) 203.
14 Wourms, J.P., J. exp. Zool. *182* (1972) 389.
15 Harrington, R.W., Ecology *40* (1959) 430.
16 Jubb, R.A., Ann. Capl Prov. Mus. nat. His. *8* (1969) 1.

0014-4754/84/070753-02$1.50 + 0.20/0

Differential lethality following treatment with ionizing radiations of various energies in *Drosophila*

S. Grazia, D. Guerra, R. Alicchio and M.L. Vanelli

Istituto di Genetica, Università di Bologna, Via F. Selmi 1, I-40126 Bologna (Italy), 27 July 1983

Summary. Male and female gametes of *Drosophila* were treated with various doses of ionizing radiations: X-rays at different energy, and gamma-rays from 2 sources given singly and in 2 temporal sequences. The induced lethality was assessed in successive developmental stages by scoring the number of eggs, larvae and adults. The results clearly show that the effects of various radiations appear in terms of difference among developmental stages and/or between treated sexes/genotypes. It is suggested that the various energies affect different gene functions which are not completely independent, as supported by the non-additive effects of the two temporal sequences.

The results obtained using UV in yeast[2-4] suggest that mutagenic repair is not a single process, but rather comprises a number of partially independent processes, each of which gives rise to a different category of mutation.

The comparison between UV, gamma and chemicals mutagenesis[5,6] supports the model of mutagenic repair in which partially independent sets of gene functions are required for the production of mutations of different kinds, for their formation at different sites and for their induction by different mutagens. Further results[7] indicated that both the genetic and the developmental state of the organism influence the induction and the repair of the X-irradiation damage and that many of the same processes which are utilized in normal cell growth and division are involved in the repair mechanism.

In previous work[8] we found different mutagenic responses by irradiation of selection lines genetically related and we suggested that the different mutation frequencies observed could result from some kind of gene activity control; this view was supported by further results[9] showing that when the two sexes are separately irradiated there is a different relationship between developmental stage and response to the treatment.

Since the various mutagens may involve distinctive patterns of genetic control and, on the other hand, the developmental patterns may affect the expression of induced mutations, it seemed interesting to study the interaction between mutagens and developmental stages. For this purpose we used the biological system tested in our previous work[9], on which we checked whether lethality expression is modified during development by ionizing radiations of various energies.

Table 1. Regression coefficients (b ± SE) estimating the relationship between the dose applied and the survival at different developmental stages after treatment with X-rays at 30 and 97 KeV

		X-rays 30 KeV b ± SE	d.f.	X-rays 97 KeV b ± SE	d.f.
L/E	Cross A	−1.82 ± 0.545***	12	−0.30 ± 0.494	12
	Cross B	−2.80 ± 0.280***	12	−2.12 ± 0.850**	12
F/L	Cross A	−1.31 ± 0.722*	12	−1.37 ± 0.697*	12
	Cross B	−1.67 ± 0.510***	12	−0.79 ± 0.772	12
1-(L-F)/E	Cross A	−0.16 ± 0.417	12	−0.71 ± 0.499	12
	Cross B	0.42 ± 0.276	12	0.59 ± 0.448	12
F/E	Cross A	−1.97 ± 0.698***	12	−1.29 ± 0.514**	12
	Cross B	−2.70 ± 0.329***	12	−1.80 ± 0.800**	12

* $p < 0.10$; ** $p < 0.05$; *** $p < 0.01$.