BAPN has been found to exert a toxic effect on cultured smooth muscle cells[6]. Since BAPN represents a potentially useful agent in experiments on elastin formation in vitro, its influence on cell growth and elastic fiber formation in cultures of auricular chondrocytes was examined in more detail.

Materials and methods. Chondrocytes were isolated from auricular cartilage of 4-day-old rabbits, as described elsewhere[7,8]. The chondrocytes were cultured in Medium 199 containing Earle's salts and supplemented with 10% newborn calf serum as well as 10 mg penicillin, 10 mg streptomycin, and 1 mg mycostatin per 100 ml medium. Cultures were started at a density of 1×10^6 cells per 35 mm plastic dish (Falcon) and kept in an atmosphere of 5% CO_2 in air at 37 °C, usually for 14 days. The medium was changed every 2–3 days. Before the addition of BAPN (BAPN-fumarate, Sigma) the cells were cultured overnight to permit adherence to the plastic surface of the dishes. The DNA content of the cultures was determined according to Karsten and Wollenberger[9]. After fixation of the cultures in 70% ethanol, elastic fibers were stained with orcein.

Results and discussion. As can be seen in the table, the DNA content of the cultures increased considerably during the 14-day period, thus indicating intensive proliferation of chondrocytes. BAPN exerted only a slight, although statistically significant inhibitory effect. Control cultures contained numerous elastic fibers (fig. 1). As in previous studies[4,7,8], some fibers had a straight course, whereas others formed loops around the chondrocytes. These loops were usually provided with patches of elastin. The number of fibers was higher than in previously-studied cultures of chondrocytes from 1-week-old rabbits[4], which is consistent with recent data[10] suggesting that the amount of elastin produced by auricular chondrocytes declines sharply during the first few weeks of post-natal life. BAPN used at a concentration of 40 µg/ml completely prevented the formation of elastic fibers (fig. 2). This indicates that the drug effectively inhibited cross-linking of elastin and thus conversion of the soluble into the insoluble form[11]. At a concentration of 10–20 µg/ml the formation of elastic fibers was depressed and only thin fibers were formed. At a still lower concentration (5 µg/ml), BAPN only prevented the deposition of the patches of elastin, whereas the fiber formation appeared unaffected (fig. 3). This suggests that the patches constitute an addition to the fibers and not a stage in their formation.

The concentration of BAPN required to inhibit the formation of elastic fibers completely, was higher than that reported previously[4], but this discrepancy is probably attributable to the more vigorous formation of elastic fibers by the younger chondrocytes in the present study.

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The effect of exposure to gaseous ammonia on the duration of diapause II in the embryos of the annual fish, *Nothobranchius guentheri*

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Summary. The exposure of the embryos of *N. guentheri* to gaseous ammonia at stages prior to the onset of diapause II caused a reduction in the frequency of diapausing embryos and the shortening of the duration of diapause. The optimum exposure time was approximately 30 min. There was a prolongation in the duration of diapause II when the ammonia treatments were conducted using embryos which were undergoing diapause II.

Instances of diapause or developmental arrests have been described in the embryos of the killifish, *Nothobranchius guentheri* (Pisces: Cyprinodontidae)[1,2]. This species belongs to a unique group of freshwater teleosts known as annual fishes[3]. *N. guentheri* is native to the seasonal ponds and stagnant pools along the coastal lowlands of East Africa[4]. The evaporative waterloss during periods of drought results in the death of the adult and juvenile fishes. The survival of the species during the dry season becomes dependent entirely upon the embryonic population deposited in the muddy substrate. It has been postulated that annual fishes escape extinction by undergoing diapause at specific stages of their normal embryonic development[1,5–7].

Many of the factors that may induce the onset of diapause in *N. guentheri* are known[2,8,9]. However, the conditions which regulate the termination of diapause remain unclear. Since the physiology of the annual fish diapause bears a striking resemblance to the well studied diapause in insects, the phenomena which influence diapause termination in insects might provide valuable insights on the mechanism that may control diapause termination in fishes. This report demonstrates that, like in insects[10,11], the arrest periods in this fish may be influenced by the exposure of the embryos to ammonia.

Materials and methods. All embryos were collected from a randomly bred laboratory population of the annual fish, *N. guentheri.* The source of fishes, husbandry conditions, and method of embryo collection have been reported previously[2]. The fishes were kept at a photoperiod of 9 h light and 15 h darkness. This photoperiod has been report-

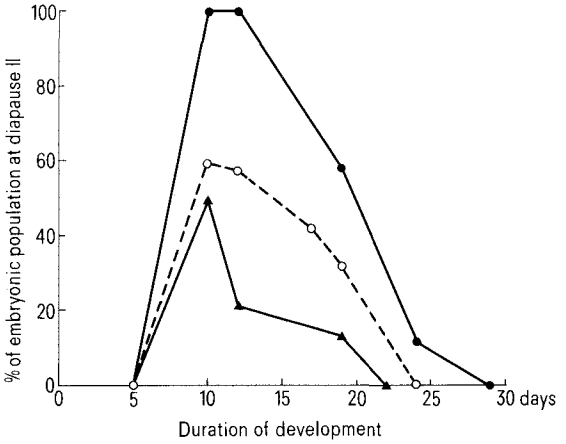

Figure 1. The exposure of early embryos to gaseous ammonia for 30 min and the subsequent effects on the frequency of diapause II. ●, Control (n=25); ○, 0.5% ammonium hydroxide (n=12); △, 1.0% ammonium hydroxide (n=14).

ed to induce the females to produce embryos that undergo diapause II[8]. The numerical stages of development established for the annual fish *Austrofundulus myersi* Dahl was used in describing the stages of development in *N. guentheri*[12].

Embryos at the early stages (stage 19–20) or at Diapause II (stage 32) were placed on a moist 35×7 mm strip of Whatman filter paper. The strip, containing up to 10 embryos, was placed inside a shell vial with open-bottom polyethylene stopper (15 mm o.d.×45 mm high; Kimble Glass). Ammonium hydroxide (28–30% NH_3, Aldrich Chemical Co.) was diluted with distilled water to obtain the desired concentrations. The embryos were exposed to gaseous ammonia by aliquoting 15 µl of the solution in a 25 mm^2 filter paper affixed inside the hollow of the stopper. The vials were capped immediately with the stoppers. At the end of the exposure period, the embryos were removed and washed twice with 25 ml of aquarium water. The embryos were incubated at 25±0.5 °C in 125-ml flasks containing 50 ml of aquarium water. Control treatments were similar to the above procedure except that distilled water was used instead of the ammonium hydroxide solution. The mortality rate and the number of the embryos which entered diapause II were determined under a dissecting microscope.

Results. Figure 1 illustrates the effect of a 30-min exposure of early stage embryos to gaseous ammonia on the subsequent embryonic development. The ammonia generated by aliquots of 0.5 and 1.0% ammonium hydroxide caused a marked decline in the frequency of embryos which entered diapause II. The development of the embryos at higher temperatures was not followed due to the high mortality rate (table 1). The majority of the deaths were observed within 24 h after the exposure. Those embryos which survived the treatments developed without showing any visible signs of gross deformities. The mean duration of diapause II and the total duration of embryonic development was significantly reduced by the experimental treatment.

Table 1. The effect of 30 min exposure of early stage embryos (stage 19–20) to gaseous ammonia on the mortality rate, duration of diapause II, and the total duration of embryonic development in the annual fish, *N. guentheri*

Ammonium hydroxide (% concentration)	n	Mortality rate (%)	Duration of diapause II (days)	Total duration of development (days)
Control	27	7.4	11.7 ± 0.9*	28.7 ± 0.9*
0.5	14	14.3	10.4 ± 1.7	22.8 ± 1.8
1.0	16	12.5	6.1 ± 2.2	19.5 ± 1.0
5.0	25	52.0	– –	– –
10.0	30	83.3	– –	– –

* Mean ± S.E.M.

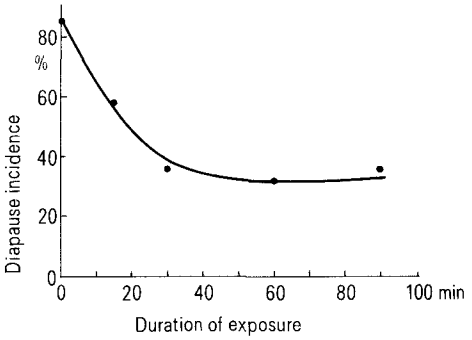

Figure 2. The effect of various durations of exposure to 1.0% ammonium hydroxide on the incidence of diapause in the embryos of *N. guentheri*. Each point represents 20–25 embryos.

To determine the optimum period of exposure, the early stage embryos were exposed to gaseous ammonia for various durations of time. Figure 2 demonstrates that a 15-min exposure was sufficient to demonstrate a decrease in the incidence of diapause II. The optimum exposure time was 30 min. Further increase in the duration did not elicit a further decline in the incidence of diapause. Embryos already undergoing diapause II were also exposed to various concentrations of ammonium hydroxide for 30 min (table 2). The data demonstrate that exposure at this stage delayed the termination of diapause II. The ammonia treatments conducted at this stage of development produced a lower mortality rate than at earlier stages of development (table 1).

Discussion. The acceleration of the rate of diapause termination after exposure to gaseous ammonia was first described by Hogan[10,11] in diapausing embryos of the field cricket, *Teleogryllus commodus*. The experiments by Beck and Alexander[13] showed that the termination of larval diapause in the European corn borer, *Ostrinia nubilalis* can be accelerated by the injecton of ammonium acetate into diapausing larvae.

It is generally accepted that injurious conditions may accelerate the termination of insect diapause. The action of ammonia in *N. guentheri* diapause may follow a mechanism akin to what occurs in insects during injury. To date the precise biochemical or biophysical events which lead to the resumption of normal development still remain unclear.

Table 2. The effect of exposure of embryos in diapause II to various concentrations of ammonium hydroxide for 30 min. The frequency of the surviving embryos which remained at diapause II was recorded 10 days after the exposure

Ammonium hydroxide (% concentration)	n	Mortality rate (%)	% at diapause II
Control	33	0.0	27.3
0.5	25	4.0	41.7
1.0	27	3.7	46.2
5.0	27	3.7	50.2
10.0	23	21.7	61.1

Our observations on the embryos of *N. guentheri* differ from that observed in insect diapause in one respect. Ammonia shortened the duration of diapause in *N. guentheri* only when the treatments were carried out prior to the onset of diapause II. The duration of diapause II was actually prolonged when similar treatments were conducted using embryos in diapause. This is in agreement with our recent findings which show that environmental insults, such as desiccation, during periods of diapause II may stimulate a longer sojurn in the diapause state[14].

These fishes can survive in areas that normally experience erratic climactic cycles. It is a current concept that the majority of the embryonic population exists as embryos in diapause II. To maintain permanent populations under such conditions, these fishes must develop a mechanism which enhances the duration of diapause during prolonged periods of unfavorable conditions. The fact that stressful or injurious treatments at this stage of development prolonged the duration of diapause II supports the idea that such a mechanism may exist in nature as a normal part of the vast array of survival adaptations in the annual fish.

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Role of glucocorticoids on the maturation of brush border enzymes in fetal rat gut endoderm[1]

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Summary. Heterospecific recombinants between fetal rat intestinal endoderm and chick mesenchyme, and also undissociated fetal rat intestine, were submitted to different hormonal environments. The present study shows that exogenously-supplied dexamethasone in organ culture, like endogenous hormones provided by the adult rat (grafting experiments) led to similar qualitative and quantitative results, i.e., a 9-fold stimulation of maltase and a precocious induction of sucrase activity in comparison with anhormonal conditions.

The elucidation of the mechanisms which may regulate the development of intestinal brush border enzymes centers on the roles of hormonal factors, mainly glucocorticoids and thyroxine, studied in vivo (for review see Moog[2] and Henning[3]) or in vitro[4-6]; of nutritional substrates[3,7], and of tissue interactions[8]. The role of epithelial-mesenchymal interactions in the morphogenesis of the gut is well established[9-12]. Concerning the biochemical maturation of the gut we have already shown, by the use of heterospecific recombinants between endoderm and mesenchyme of chick and rat intestines, that the enzymatic pattern of the specimens corresponded to that of the species from which the endoderm originated[8]. This study also showed that only minor influences are exerted by the mesenchyme and that rat endoderm did not fully mature in a hormone-deficient environment.

The purpose of the present study is to define further how the effect of tissue interactions on the enzymatic differentiation of the endoderm might be modulated by hormones.

Materials and methods. Heterospecific recombinants between 5½-day chick embryonic intestinal mesenchyme and 14-day fetal Wistar rat intestinal endoderm (Cm/Re) were performed as described earlier[8] and submitted to the following hormonal conditions.

Control experiments: Cm/Re and R segments (undissociated 14-day fetal rat intestine) were grafted for 11 days into the coelomic cavity of 3-day chick embryos, providing a hormone-deficient environment[8].

Experiment I: Cm/Re and R segments were first grafted in ovo for 9 days and were thereafter maintained in organ culture for 2 days in the absence or presence of an optimal concentration of dexamethasone (DX; Roussel Uclaf: 30 ng/ml dissolved in ethanol), according to the method described previously[4].

Experiment II: Cm/Re and R segments were grafted for 11 days under the kidney capsule of adult Wistar rats, according to the technique of Ferguson and Parrott[13]. In addition, 4-day newborn rat small intestine (stage corresponding to that presumably attained by the rat endoderm after the experimental periods) was used as reference. Sucrase, maltase[14], lactase[15] and alkaline phosphatase[16] activities of grafted and cultured specimens were determined in the purified brush border membranes[17]. Results were expressed as milliunits (mU) per mg of brush border proteins and were evaluated statistically by the unpaired Student's t-test. Differences with a p value of less than 0.05 were considered significant.

Results and discussion. In the experimental conditions used, Cm/Re and R segments grew and were able to differentiate, except that the former were rejected when grafted into the adult rat.

Results of the enzymatic analysis are given in the figure. After 11 days of in ovo transplantation (control experiment), Cm/Re exhibited enzymatic activities which are characteristic of the fetal rat intestine; traces of sucrase and high lactase activity[8]. No significant differences could be noted between the enzymatic activities present in the grafted Cm/Re and R segments. When Cm/Re or R segments were first implanted in ovo for 9 days and subsequently cultured in the presence of DX (experiment I), an induction of sucrase and a 9-fold stimulation of maltase activities ($p < 0.001$) were obvious when compared to the control Cm/Re and R grafts. In contrast, lactase and alkaline phosphatase activities dropped ($p < 0.001$), this