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]

M. Kedinger, P.M. Simon-Assmann, B. Lacroix and K. Haffen

Unité de Recherche de Physiopathologie Digestive, INSERM UNITE 61, 3, av. Molière, F-67200 Strasbourg (France), September 20, 1982

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.

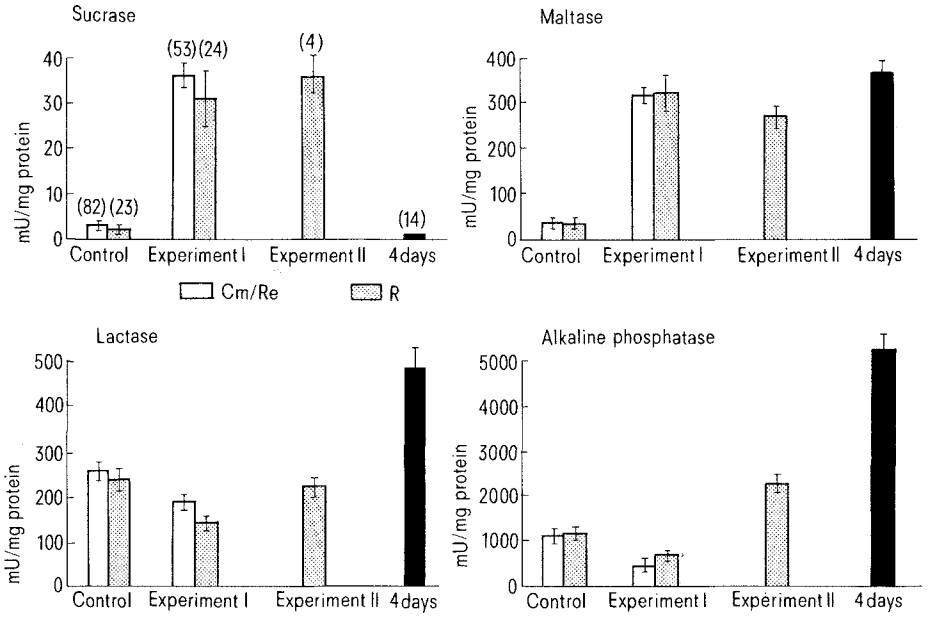
Control experiments: Cm/Re and R segments (undissociated 14-day fetal rat intestine) were grafted for 11 days into the coelomic cavity of 3-day chick embryos, providing a hormone-deficient environment[8].

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



Specific activities (mU/mg protein: mean ± SEM) of enzymes determined on the brush border fraction purified from Cm/Re (heterospecific recombinants between 5½-day chick embryonic mesenchyme and 14-day fetal rat endoderm: open columns), R segments (undissociated 14-day fetal rat intestine: hatched columns) or from 4-day rat intestine (black columns). 1 unit is defined as the activity that hydrolyzes 1 µmole of substrate/min under the experimental conditions. It is likely that maltase activity is in part (approximately 25%) due to sucrase as already mentioned in a previous paper[6]. Numbers in parentheses represent the number of experiments.

phenomenon reflecting the already-described decrease of villus height under the culture conditions used for intestinal tissue[4,5]. Explantation of the recovered grafts in the absence of DX led to an amplified loss of villus cells paralleled by a pronounced decrease of all enzyme activities, which were therefore not illustrated. The transplantion of R segments under the kidney capsule of adult rats for 11 days (experiment II) essentially resulted in the induction of sucrase and in the stimulation of maltase activities ($p < 0.001$) in comparison with the control segments developed in ovo. It must be pointed out that these 2 enzyme activities were stimulated to levels similar to those obtained in experiment I by DX added into the culture medium. It is noteworthy that, at the ultrastructural level, DX enhanced the maturation of the apical microvilli of the Cm/Re and R explants to an extent similar to that provided in R by the renal graft (not shown). Concerning lactase and alkaline phosphatase, their activities in experiment II were respectively equal to or above ($p < 0.001$) the values present in the control in ovo transplants. From the comparison with the in situ developed 4-day rat intestine, it becomes obvious that the main effects provided by both experimental conditions I and II were: the stimulation of maltase activity leading to in situ levels and the induction of sucrase activity, the latter being detectable in the rat only at weaning[2,3]. Lactase and alkaline phosphatase remained below the levels present in the 4-day rat intestine. Of interest, in the present study, are the similar responses of sucrase and maltase activities either to a well defined concentration of dexamethasone present in the culture medium or to the hormonal status supplied by the adult rat host. These results raise the possibility that the effect observed in the renal graft, also obtained by others[18-20], may be related to glucocorticoids rather than to other hormonal influences. This view is supported by experiments of our laboratory, indicating the failure of various hormones to reproduce or enhance the inductive or stimulating effect exerted by dexamethasone on sucrase and maltase activities in the neonatal[4,5] rat intestine cultured in vitro. Related to this, the failure especially of thyroxine to act directly on the maturation of disaccharidases, has been recently confirmed in vivo[21]. Moreover, taking the 4-day rat intestine as reference, it becomes apparent that neither the hormonal status of the host, nor dexamethasone was able to promote normal maturation of lactase and alkaline phosphatase, thus confirming the insensitivity of these 2 enzymes to any hormonal factors at yet tested in vitro[4,5].

In conclusion, if tissue interactions are responsible for the first expression of enzyme activities with endodermal species-specificity[8], the present data emphasize that glucocorticoids play the major role in promoting the expression of maturation of at least 2 brush border enzymes: sucrase and maltase.

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Formation of ring chromosomes by diethyl sulphate and gamma-rays

R.N. Gohil and Asha Kaul

Cytogenetics Laboratory, Department of Botany, University of Kashmir, Srinagar-190006 (India), January 4, 1982

Summary. Ring chromosomes were formed in the root tip cells of *Allium sativum* and *A. cepa* var. *viviparum* after treatment with diethyl sulphate and gamma-rays. Both centric and acentric types of ring chromosomes were observed. The behavior of these chromosomes during different stages of somatic cell division is discussed.

Ring chromosomes have been reported to occur spontaneously both in plants (maize[1-3] and barley[4]) and in animals[5]. In some organisms, they have been produced upon exposure to environmental stress. Both centric and acentric ring chromosomes have been observed. Although the mode of their origin has been hypothesized by a number of workers, the cause of breakage and reunion in nature is not fully understood. In order to induce somatic mutations, cultivated species of *Allium* were treated with some chemical and physical mutagens. We observed ring chromosomes in addition to other chromosomal anomalies in the root tip cells of the treated material.

Materials and methods. Propagules of *A. sativum* and *A. cepa* var. *viviparum* were treated with 2 chemical and 1 physical mutagen as shown in the table.

Mutagen used	Concentration/ doses	Time of treatment
1. Ethyl methane sulphonate (EMS)	100 mM 200 mM 300 mM	2, 4 and 8 h
2. Diethyl sulphate	20 mM 25 mM 30 mM	1, 2 and 3 h
3. Gamma rays	3 Gy 4 Gy 5 Gy	

Root tips from the treated propagules and the controls were fixed directly in 1:3 acetic alcohol. Root tips were hydrolyzed in 1N HCl for 10 min at 60 °C, stained in feulgen and squashed in 1% acetocarmine.

Observations and discussion. Ring chromosomes were observed in the root tip cells of *A. sativum* treated with 25 mM of diethyl sulphate and 4 and 5 Gy of gamma-rays and *A. cepa* var. *viviparum* treated with 4 Gy of gamma-rays whereas, they were absent in the controls. The frequency of cells with ring chromosomes was higher in tips exposed to gamma-rays. These chromosomes were observed at mitotic prophase (fig. 1), metaphase and anaphase (figs 2 and 3). The rings observed in *A. sativum* were of small size and in none of these could the centromere be located. At anaphase they remained as laggards either in the center of the cell or moved towards one of the poles. Their behavior during cell division was typical of acentric fragments. In gamma-ray-treated bulbils of *A. cepa* var. *viviparum*, in addition to small-sized acentric ring chromosomes, large dicentric ring

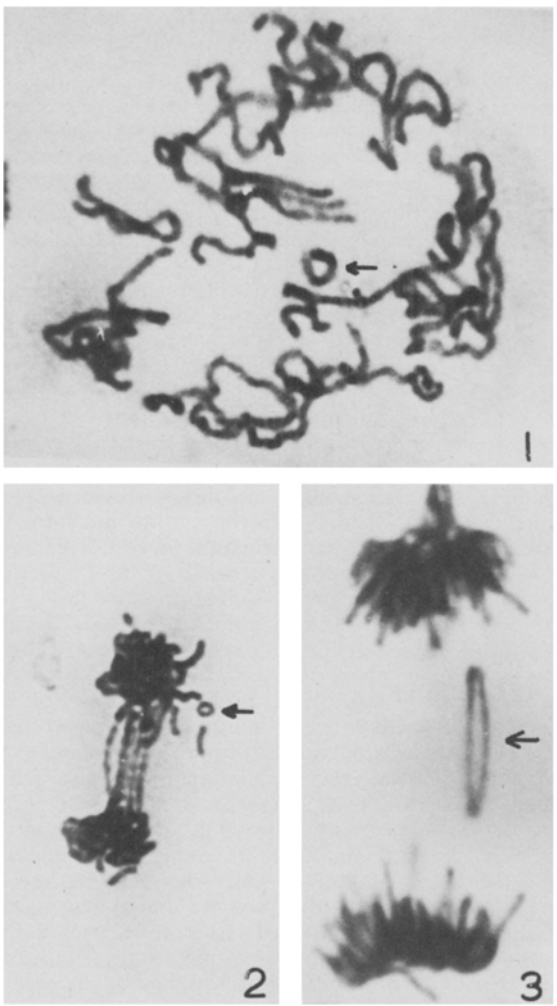

Figure 1. Root tip cell of *A. sativum* at prophase of mitosis with one acentric ring chromosome.
Figure 2. Root tip cell of *A. sativum* at anaphase of mitosis with one acentric ring chromosome which has moved towards one of the poles. Note the acentric fragments also.
Figure 3. Root tip cell of *A. cepa* var. *viviparum* at anaphase of mitosis with a dicentric ring chromosome lying in the center. (Arrows point towards ring chromosomes).