sharp, 30% decline in DMN-demethylase activity, followed by a much slower, almost linear decline in activity of about 20% over the range 5–95 mM.

We then investigated the inhibition of DMN-demethylase by the secondary amines corresponding to the nitrosamines examined. Suppression of activity by the amines followed a pattern (figure 2, a and b) very similar to that of the inhibition by the nitrosamines and was quantitatively in the same range. Proline gave a biphasic inhibition profile in a manner exactly similar to nitrosoproline. The similarity of the inhibition with the nitrosamines and the amines suggests that interaction of nitrosamines with DMN-demethylase is determined in large part by structural characteristics of the amine moiety.

1 Acknowledgments. This work was supported by Public Health Service Grant 5-PO1-ES00597, Training Grant 1-732-ES07020, and Research Career Development Award 1-KO4-ES00033 (to M.C.A.) from the National Institute of Environmental Health Sciences. We are also grateful for support from Hoffmann-LaRoche Inc., Nutley, NJ.

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Attraction of primordial germ cells by notochord in seven somites chick embryo

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Summary. Chemical studies in chick embryo have indicated the existence of proteoglycan in notochordal sheath. Primordial germ cells were observed with scanning electron microscope on the notochord dorsal face, surrounded with perichordal material. We postulate the identification of such a material with proteoglycan which could attract primordial germ cells to the notochord.

In amphibians, primordial germ cells seems to be attracted by somitic mesodermical material. The extirpation of dorsal axial organs impedes gonocytes's migration and differentiation. Even a very small amount of somitical material is able to induce the migration of a great number of germ cells[1,2]. These results have not been confirmed in birds. This report describes our first observations on close relationship between primordial germ cells and notochord of the young chick embryo which can be observed with SEM.

Materials and methods. Chick embryos (White Leghorn strain) at stage 9 of Hamburger-Hamilton[3] were fixed in 4% glutaraldehyde in 0.1 phosphate buffer at pH 7.4; with the help of a tungsten needle set up on a stem, we extirpated the neural tube and the surrounding epiblast, including a little paramedian left fringe, taking care not to injure underlying somites. Notochord remained in its place. Later, embryos were postfixed in 1% osmium tetroxide in the same buffer, dehydrated in graded ethanols (50, 70, 96, 100), and immersed in isoamyle acetate. Embryos were dried with CO_2 at its critical point[4]. Following gold coating, embryos were viewed through a Jeol Jsm-50A flanked by 7 pairs of somites (figure 2). As a control, some embryos at stage 9 were fixed in Carnoy, dehydrated, and embedded in paraffin and sectioned serially at 7 µm. The sections were stained with the periodic acid-Schiff technique (PAS)[5] to facilitate identification of the germ cells[6,7], and counterstained with alcian blue and give a characteristic blue reaction in the presence of acid mucopolysaccharides[8,9] of perinotochordal material (figure 1).

Results and discussion. On notochord dorsal face, primordial germ cells appeared on level with the 2 1st somites

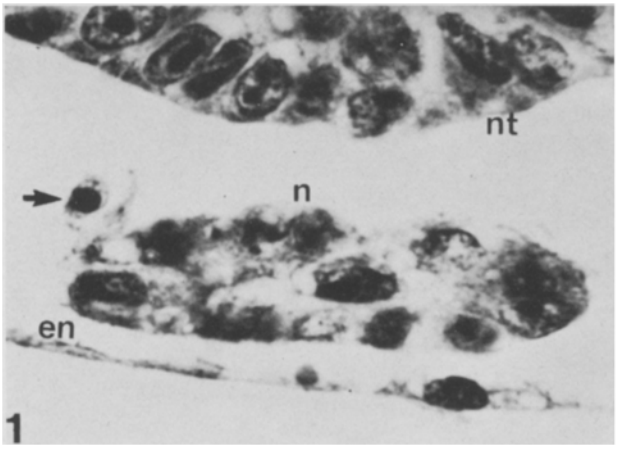

Fig. 1. Chick embryo at stage 9. PAS-alcian blue; n, notochord; nt, neural tube; en, endoblast; arrow, primordial germ cell surrounded with perinotochordal material blue coloured with alcian blue. × 1250.

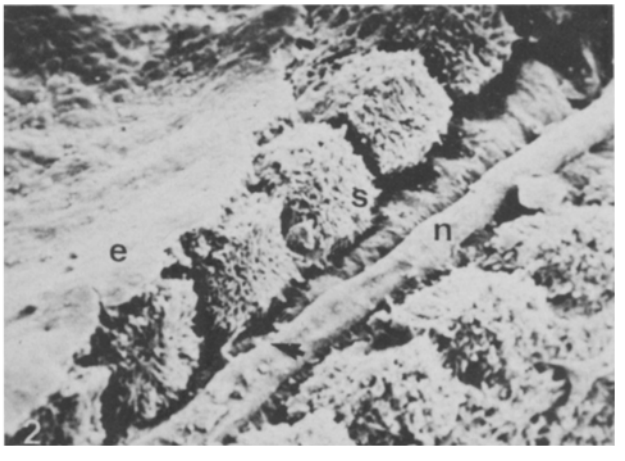

Fig. 2. Scanning electron micrograph of a chick embro of 7 somites in which the nervous system and the surrounding epiblast have been extirpated; e, epiblast; s, somite; n, notochord. The arrows show various primordial germ cells. × 300.

(figures 2 and 3). At level with the 3rd somite, we observed in notochord lateral face, 2 primordial germ cells, covered with a net which continued with perichordal material (figure 3). Distal to 4th somite, we did not observe primordial germ cells on notochord.

Clawson et al.[10] described the primordial germ cells at stage 8 close to the notochord. At stage 9, these cells can be observed in the mesenchyme surrounding the notochord and neural tube (notochord-neural tube complex)[11] (figure 1).

In chick embryo it has been described that 2 perichordal microfibrils types unite the notochord with the neural tube: the smaller fibrils are specially concentrated near the notochordal boundary membrane. These fibrils have a diameter of about 10 nm and are digestible with hyaluronidase and alpha amylase; they may be precursors of the larger type, which is about 20 nm in diameter and is digestible with collagenase[12-17]. The collagen and proteoglycan secretion by chick axial organs, has recently been made obvious[18-23]. The immunological role of proteoglycan in promoting cell recognition is now well established[24]. It seems possible, therefore, that, when the proteoglycan component of the notochordal comes into contact with the primordial germ cells, it directs their movement toward the notochord, thus promoting a chemostatic response, permitting primordial germ cells migration. According to our 1st observations, we can postulate that, as in amphibians, the notochord exhibits attraction on primordial germ cell. Our projected experiments will permit us to confirm such a postulate.

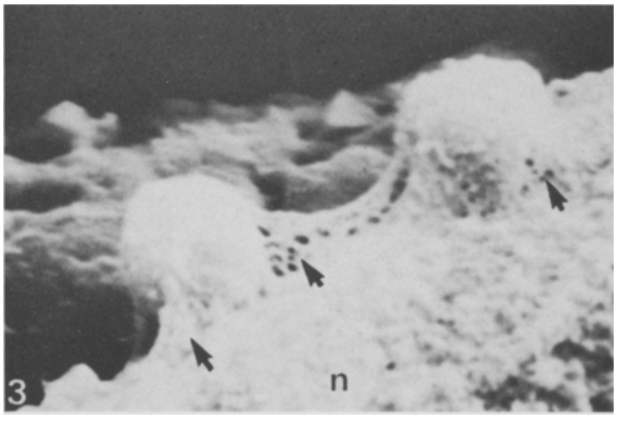

Fig. 3. Scanning electron micrograph of a chick embryo of 7 somites. The arrows show the perichordal substance surrounding 2 primordial germ cells. × 6000.

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Acetic acid pretreatment of initiated epidermis inhibits tumour promotion by a phorbol ester

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Summary. Mouse skin initiated with 7,12-dimethylbenz(a)anthracene and then exposed to multiple treatments of acetic acid, shows a decreased papilloma yield on subsequent promotion with croton oil.

The role of hyperplasia in promotion during 2-stage tumorigenesis[3] is not understood. It has been generally observed that although all promoters induce epidermal hyperplasia, not all hyperplastic agents are promoters[4,5]. For example, a recent paper has reported that acetic acid is only a weak promoting agent despite its ability to induce intensive epidermal hyperplasia[4]. This type of result has been assumed to imply that promoters must induce biochemical changes in epidermis which are unrelated to hyperplasia[4-6]. It was the purpose of the present study to determine whether cytotoxicity of compounds such as acetic acid could be an alternative explanation for their inability to act as tumour promoters.

Materials and methods. Female Swiss albino mice were used and were maintained as described before[7]. Each treatment group consisted of 25 animals. All animals were initiated with 25 µg 7,12-dimethylbenz(a)anthracene (DMBA). 1 week after initiation, 1 group was treated with 167 µmoles of acetic acid, another with 500 µmoles of acetic acid and a 3rd control group with acetone (all treatments were applied to the dorsal skin as solutions in 0.2 ml acetone). The treatments were repeated 4 times at a rate of 2 applications per week. 4 days after the final acetic acid treatment, all groups were promoted with twice-weekly applications of croton oil (0.2 ml of a 0.5% solution in acetone). The mice were examined twice-weekly for papilloma formation.

Results and discussion. As shown in the figure, pretreatment of initiated animals' skin with 5 applications of 500 µmoles acetic acid resulted in a tumour yield during subsequent promotion only about 50% of that in the control group. This decrease is unlikely to be a consequence of interference with DMBA-initiation as acetic acid treatment was not begun until 1 week after DMBA application. Consequently,