Inhibition of rat hepatic dimethylnitrosamine demethylase by cyclic and acyclic nitrosamines and secondary amines[1]

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Summary. Inhibition of hepatic dimethylnitrosamine (DMN) metabolism by a variety of cyclic and acyclic nitrosamines was demonstrated. Nitrosoproline, a noncarcinogenic nitrosamine, behaved differently from the carcinogenic nitrosamines as an inhibitor for DMN-demethylase. Secondary amines corresponding to the nitrosamines inhibited DMN-demethylase in a manner similar to the nitrosamines.

The carcinogen dimethylnitrosamine (DMN) is metabolized to an active form by the hepatic microsomal mixed-function oxidase system in the rat[2]. In vitro experiments with liver fractions show that the methyl groups of DMN are quantitatively converted into formaldehyde and methanol[3]. Friedman and Couch[4] and Cottrell et al.[5] have shown that formaldehyde production from DMN in rat liver post-mitochondrial supernatant is inhibited by nitrososarcosine and nitrosopyrrolidine. Recently, we showed that microsomal DMN-demethylase is inhibited by diethylnitrosamine and described the detailed enzyme kinetics of this process[6].

In the present study, we have investigated the inhibition of DMN-demethylase by a number of cyclic and acyclic nitrosamines and secondary amines. We were interested in determining whether there are interactions between members of the nitrosamine series of widely differing structures, and, in addition, in determining whether a non carcinogenic nitrosamine, nitrosoproline[7], would interact with DMN-demethylase differently from the carcinogenic nitrosamines.

Male albino Sprague-Dawley rats (Charles River Breeding Labs, Wilmington, MA), aged 22–25 days, were given a standard laboratory diet ad libitum. To induce the microsomal mixed-function oxidase system, their drinking water was supplemented with 0.1% phenobarbital. After 7 days, the animals were decapitated. Microsomes were prepared from the liver as described previously[8]. Incubations were carried out under air in half-ounce amber glass bottles with air-tight stoppers maintained at 37 °C in a metabolic shaker. To ensure vigorous mixing, several glass beads were added to each incubation bottle. Each bottle also contained the following components in a final volume of 3.5 ml: 0.1 M phosphate buffer, pH 7.4; microsomal protein (4–6 mg); semicarbazide hydrochloride (9 mM); an NADPH-regenerating system consisting of glucose-6-phosphate (7.4 mM), glucose-6-phosphate dehydrogenase (3 units), NADP (0.73 mM), and $MgCl_2$ (20 mM). The mixtures were preincubated for 5 min at 37 °C prior to the addition of DMN (102 mM) and various concentrations of inhibitor (care was taken to adjust the pH of the secondary amines to pH 7.4 prior to addition). After 30 min, reactions were stopped by the addition of 1 ml 15% $ZnSO_4$ and 1 ml saturated $Ba(OH)_2$, and the bottles were rapidly cooled in an ice bath. The extent of oxidative demethylation of DMN was determined by measuring formaldehyde formation by the method of Chrastil and Wilson[9]. Quantitation of formaldehyde was based on the recovery of a known amount of formaldehyde added to a complete incubation mixture in the absence of DMN. In these control incubations, recovery of formaldehyde was always close to 100%. Formaldehyde production in the incubation mixtures was linear up to 30 min. All reactions were performed in duplicate. Enzyme activity was expressed as nmoles formaldehyde produced/mg microsomal protein/min.

Among the inhibitors examined in microsomal incubation mixtures containing no DMN, only nitrosomorpholine and dipropylnitrosamine produced low but measurable levels of formaldehyde. To determine the formaldehyde produced by DMN in the presence of these 2 nitrosamines, the small amounts of formaldehyde generated by the inhibitors alone were subtracted. In addition, we showed that neither the nitrosamine inhibitors nor the secondary amine inhibitors interfered in the quantitative determination of formaldehyde.

All of the nitrosamines examined, both acyclic and cyclic, produced a concentration-dependent inhibition of hepatic DMN metabolism (figure 1, a and b). Nitrosopiperidine and nitrosodipropylamine were the most effective inhibitors examined, with I_{50}-values (concentrations giving 50% inhibition) of 17 and 15 mM, respectively. Nitrosopyrrolidine and diethylnitrosamine were also effective as inhibitors, with I_{50}-values of 33 and 40 mM, respectively. Nitrosomorpholine was only a weak inhibitor of DMN-demethylase ($I_{25} = 42$ mM).

These results show that there is considerable interaction between nitrosamines of different structure with DMN-demethylase. The noncarcinogenic nitrosoproline inhibited DMN-demethylase, but its behavior was different from that of the carcinogenic nitrosamines investigated. Figure 1, a shows that the inhibition profile for nitrosoproline is biphasic. From 0 to about 5 mM nitrosoproline, there is a

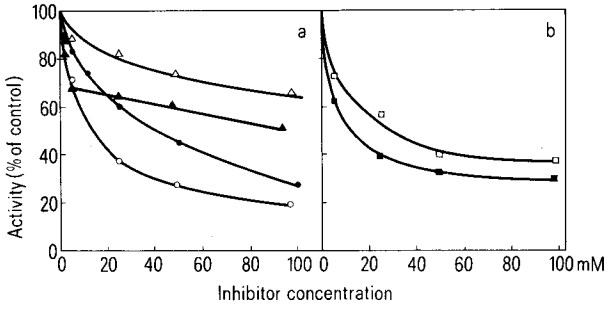

Fig. 1. Inhibition of hepatic DMN-demethylase by nitrosamines: *a* ○ nitrosopiperidine; ● nitrosopyrrolidine; △ nitrosomorpholine; ▲ nitrosoproline. *b* ■ dipropylnitrosamine; □ diethylnitrosamine.

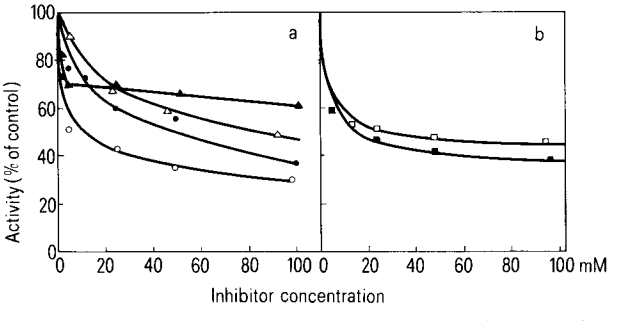

Fig. 2. Inhibition of hepatic DMN-demethylase by secondary amines: *a* ○ piperidine; ● pyrrolidine; △ morpholine; ▲ proline. *b* ■ dipropylamine; □ diethylamine.

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

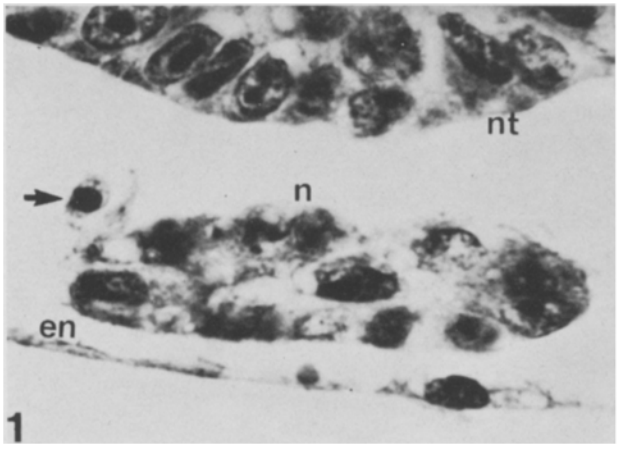

Fig. 1. Chick embryo at stage 9. PAS-alcian blue; n, notochord; nt, neural tube; en, endoblast; arrow, primordial germ cell surrounded with perinotochordal material blue coloured with alcian blue. × 1250.

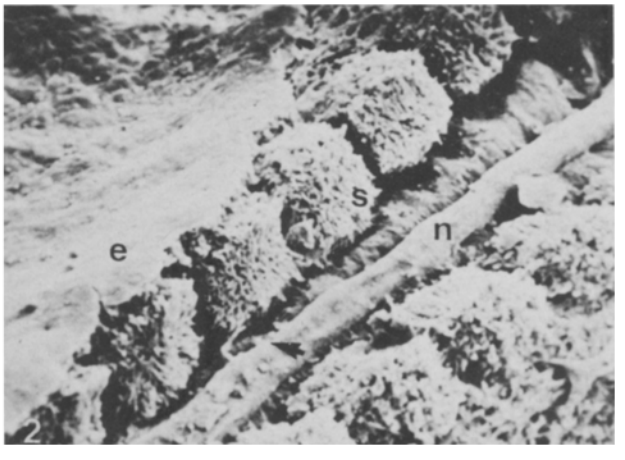

Fig. 2. Scanning electron micrograph of a chick embro of 7 somites in which the nervous system and the surrounding epiblast have been extirpated; e, epiblast; s, somite; n, notochord. The arrows show various primordial germ cells. × 300.