Chromatid aberrations and centromeric elongation induced by different concentrations of netropsin sulfate (NtS) and netropsin hydrochloride (NtHy) in Ehrlich ascites tumour cells of the mouse in vitro

Concentration tested (Mol/l) (treatment time 21 h)		Number of metaphases analyzed	Metaphases with aberrations (%)	Types of aberrations per 100 cells				Cells with centromeric elongation (%)	Percentage of metacentric B-chromosomes with centromeric elongation (%)	Percentage of telocentric chromosomes with centromeric elongation (%)
				g	b	t	m.a.			
NtS	$1 \cdot 10^{-4}$	250	9.6	0.8	2.4	2.8	0.4	90.0	100	18.5
	$5 \cdot 10^{-5}$	100	8.0	2.0	6.0	3.0	0	20.0	85.0	2.6
	$2.5 \cdot 10^{-5}$	100	2.0	1.0	2.0	0	0	5.0	60.0	2.1
	$1 \cdot 10^{-5}$	250	2.4	1.2	2.0	0.8	0	2.0	0	2.3
NtHy	$1 \cdot 10^{-3}$	No mitosis								
	$5 \cdot 10^{-4}$	100	4.0	0	0	4.0	0	91.0	100	18.7
	$1 \cdot 10^{-4}$	300	4.0	2.0	2.7	2.3	0	33.0	87.9	2.3
	$7.5 \cdot 10^{-5}$	100	2.0	4.0	2.0	0	0	18.5	67.6	2.6
	$5 \cdot 10^{-5}$	100	2.0	2.0	2.0	0	0	13.0	69.2	2.1
	$1 \cdot 10^{-5}$	200	2.5	0.5	2.5	1.0	0	Not determined		
Control		300	0	0.7	0	0	0	0	0	0

binding constant, netropsin should be able to remove proteins thought to be responsible for chromatin compactness, especially histon H1. This effect was indeed demonstrated in experiments with calf chromatin[14] and could be a molecular reason for the heterochromatin decondensation observed. Netropsin binding to other than satellite DNA can be inferred from the observation that netropsin-treated chromosomes stain much less than untreated chromosomes with aceto-orcein. In this respect it is important to note that the bibenzimidazol derivative 'Hoechst 33258', another highly AT-specific compound which causes centromer uncoiling in some mouse cell lines[15,16] but not in human chromosomes[16], is without detectable effect on ascites chromosomes of cells grown in a permanent or the short-term suspension culture described here under the conditions used by Kim and Grzeschik[16] (unpublished results).

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Neural inducing capacity of cyclic AMP on post-nodal pieces of early chick blastoderms[1]

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Summary. Cyclic AMP (0.5 mM) induced neural differentiation in post-nodal pieces of early chick blastoderms, at least in part, through promoting cell movement, tissue condensation, and assembly of microtubules.

Hensen's node has long been recognized as the center for the primary organization of the chick embryo. Pieces isolated from regions more than 0.5 mm posterior to the node, regardless of culture techniques and duration of cultivation, do not develop well-defined axial structures[2-10]. Thus post-nodal peices (PNPs) have frequently been used for testing the inducing capacity of various agents.

Experiments previously conducted in our laboratory[11] showed that cyclic AMP and several related nucleotides could induce differentiation in PNPs. The inducing capacity of these nucleotides varied and was concentration dependent. Differentiation was usually manifest by the formation of neural tissue, but concentrations used were unphysiologically high (3–18 mM) and many PNP cells showed signs of mild cytolysis. Thus a question was raised whether the observed differentiation was a consequence of cyclic AMP treatment or sublethal damage. Somewhat different results were recently reported by Deshpande and Siddiqui[10] who found that cyclic AMP, at a lower concentration (0.5 mM), induced the formation of heart-like pulsatile tissues in 74% of the PNPs. The present study was undertaken to reinvestigate the inducing capacity of cyclic AMP on PNPs.

Materials and methods. Unincubated fertile White Leghorn eggs were obtained from the Shamrock Poultry and

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Breeding Farm, North Brunswick, N. J. They were incubated for 17–19 h at 37.5 °C to obtain embryos at stage 4 of development[12]. Blastoderms were isolated from the yolk and vitelline membrane, excess yolk was removed, and the area opaca was trimmed off. The remaining area pellucida was then transected at 0.6 mm posterior to Hensen's node as previously described[8,9]. The posterior portion (PNP) thus obtained was used in this study.

2 PNPs were placed, ventral side upward, on Ringer-agar plus yolk-albumen extract medium (= basic medium) using Spratt's[13] explantation technique. A drop (about 0.03 ml) of chick Ringer's solution (adjusted to pH 7.2 using 10% $NaHCO_3$) with or without 0.5 mM cyclic AMP (Sigma Chem. Co., St. Louis, Mo.) was added directly onto each PNP. Culture vessels were kept at room temperature (22–24 °C) for 3–4 h, followed by incubating at 37.5 °C for 2 days. PNPs were rinsed several times in chick Ringer's solution and subcultured for 2 days on fresh basic medium. After incubation, PNPs were examined under a dissecting microscope to determine the presence of pulsatile tissue. Some were fixed in Bouin's fluid, serially sectioned at 4 μm, and stained with Delafield's hematoxylin and

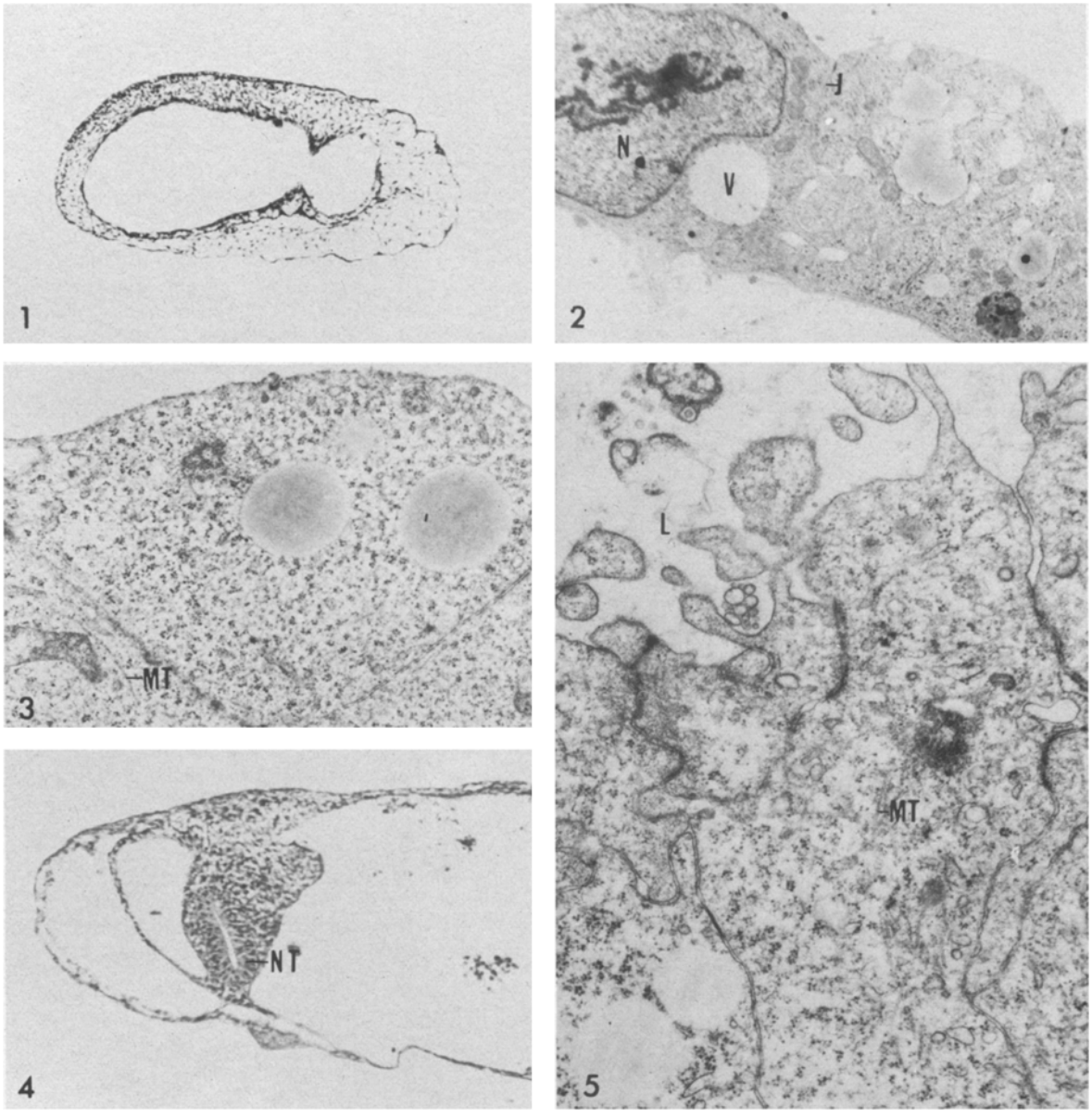

Fig. 1. Transverse section of untreated control PNP cultured for 4 days. All germ layers remained undifferentiated. ×30.

Fig. 2. Low magnification electron micrograph showing general features of control ectodermal cells. J, cell junction; N, nucleus; V, vesicle. ×6000.

Fig. 3. Electron micrograph of control ectodermal cells showing relatively smooth adjacent cell membranes and a few randomly arranged microtubules (MT). ×17,600.

Fig. 4. Transverse section of PNP treated for 2 days with 0.5 mM cyclic AMP, followed by subculturing for 2 days on basic medium. Note well-defined neural tube (NT). ×60.

Fig. 5. Electron micrograph of induced neural tube. Note luminal surface is convoluted with cytoplasmic extensions; adjacent cell membranes are interdigitated; microtubules (MT) are abundant and usually oriented in cell's long axis. L, lumen. ×21,900.

eosin. Others were fixed for 1 h in half strength Karnovsky's[14] fixative, thoroughly rinsed in 0.08 M cacodylate buffer, and postfixed for 1 h in 1% osmium tetroxide. After fixation, specimens were stained en bloc with 1% uranyl acetate for 1 h, dehydrated in graded ethanol series, and embedded in Spurr's[15] resin. Sections (silver/pale gold) were contrasted with uranyl acetate and lead citrate and examined with a JEM 100B electron microscope.

Results. Our previous studies[8,9,11] showed that PNPs treated with various inducing agents always had the most complicated and well-defined structures (neural tube, notochord, nephric tubules, somite mesoderm, and pulsatile tissue) after 4 days of incubation. Thereafter, many structures apparently underwent dedifferentiation and/or disintegration. By the 10th day only neural tissue and nephric tubules were histologically identifiable. Thus the results described below are based on the examination of PNPs cultured for 4 days.

Of the 42 control PNPs, 41 remained undifferentiated (figure 1). Here, ectodermal cells were more or less rounded with highly vacuolated cytoplasm and a large nucleus compared to the overall cell size (figure 2). The cell surface was relatively smooth; microtubules were few and randomly arranged; microfilaments were not well developed (figure 3). By contrast, 27 out of 58 experimental PNPs acquired the capacity to undergo differentiation in the form of neural tissue (figures 4 and 5). Pulsatile tissue and notochord were observed in 4 of these PNPs. The induced neuroepithelium exhibited morphological characteristics identical to that of stage 10 chick embryos[12]. Briefly, the wall of the neural tube consisted of a pseudostratified columnar epithelium. Adjacent cell membranes were interdigitated; microtubules were numerous and were usually arranged parallel to the cell's long axis; microfilaments were associated into dense bundles (figure 5).

Discussion. The present study showed that cyclic AMP (0.5 mM) could induce differentiation in PNPs which was always manifest by the formation of neural tissue. Deshpande and Siddiqui[10] reported that cyclic AMP, at the same concentration, induced heart-like pulsatile tissues in 74% of the PNPs. Identical structures were also noted in this study, but the frequency of appearance was much lower (about 7%). This discrepancy may be due to difference in culture techniques and/or methods of treatment. Our results, however, were not surprising and agreed with many investigators who reported that cells of the presumptive epidermis or of neuroectodermal origin were relatively sensitive to cyclic AMP as an inducer of cell differentiation[16–18]. Furthermore, PNPs with recognizable structures were usually more compact than those showing no differentiation, suggesting that cyclic AMP induced differentiation in PNPs, at least in part, by promoting cell movements and tissue condensation, events that are considered essential to differentiation in early chick blastoderms[5,19]. Since microtubules are known to be responsible for cell elongation during neurulation[20], the possibility exists that cyclic AMP induces neural differentiation in the presumptive chick epidermis also by stimulating the assembly of microtubules.

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Effect of cholinesterase inhibitors on differentiation of cultured sympathicoblasts

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Summary. The 2 cholinesterase inhibitors, eserine and BW 284 C 51, inhibited the nerve fibre growth and differentiation of immature sympathicoblasts at low concentrations. The effect was nerve cell-specific and appeared in the course of the in vitro development, indicating that cholinesterases might play an important role in the early differentiation of sympathicoblasts.

In addition to acetylcholinesterase activity in the plasmalemma, probably directly connected with nervous transmission, sympathetic neurons also show intracellular acetylcholinesterase, the role of which is not fully understood[1]. The appearance of cholinesterases in the sympathicoblasts early during the differentiation[2–4] suggests that cholinesterases might play a role in the maturation of the sympathetic neurons.

Tissue culture of immature sympathetic ganglia[5] provides an opportunity to evaluate the significance of the cholinesterases by studying the effect of cholinesterase inhibition on the differentiation of sympathicoblasts.

Materials and methods. Lumbar sympathetic ganglia of 7-day-old chick embryos were cultured for 2–7 days in a tricine-buffered medium containing 1 IU/ml nerve growth factor (Burroughs and Wellcome)[5] with and without cholinesterase inhibitors. Eserine (1′-methylpyrrolidino(2′:3′:2:3)1,3-dimethylindolin-5-yl N-methylcarbamate, Merck) or BW 284 C 51 (1:5-bis(4-allyldimethyl ammoniumphenyl)pentan-3-one dibromide, Wellcome Research Laboratories) were added to the cultures in 10^{-6}–10^{-3} M concentrations for the whole culture period beginning at the time of explantation. The acute effect of the inhibitors was tested by 24-h-treatment. Phase-

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