acid in its crystalline form, obtained from Sigma Chemical Corporation, St. Louis, Mo., USA, served as standard. The supernatant fluids of neuraminidase- and TBS-treated leukemia cells were analyzed, and the sialic acid content related to the number of cells incubated. Cellular electrophoretic mobility (epm) of cells suspended in 0.145 *M* NaCl, pH 7.2 ± 0.2, was measured with a Zeiss cytopherometer as described before[16].

50×10^6 neuraminidase-treated cells were i.p. implanted in 35 BDIX rats. 5 animals were sacrificed every 4 h up to 24 h, also at 48 h after implantation, and the tumor cells harvested as stated above. Part of the cells were again standard washed, part were treated with neuraminidase and TBS, respectively, and their epm measured. The sialic acid content was determined from the supernatants of the 2 groups of treated cells.

Results. The sialic acid content in the supernatant of L 5222 leukemia cells after treatment with neuraminidase was in the order of 27 $\mu g/10^9$ cells. A second enzyme incubation yielded negligible amounts of sialic acid only, while the supernatants of TBS-treated controls were free of sialic acid. Measurements of the electrophoretic mobility showed that standard washed leukemia cells had values of -0.84 ± 0.05 SD $\mu m\ sec^{-1}V^{-1}\ cm$ (mean of 17 different experiments). Incubation with neuraminidase lowered the mobility by 38% to -0.52 ± 0.05 SD $\mu m\ sec^{-1}V^{-1}\ cm$ (mean of 11 different experiments) if compared to TBS-incubated controls (0.84 ± 0.04 SD $\mu m\ sec^{-1}V^{-1}\ cm$ as mean of 9 different experiments). The gradual reappearance of sialic acid moieties on leukemia cells treated with neuraminidase, reimplanted, and then harvested at the time intervals indicated, is presented in the Figure. Sialic acid and surface charge start to be restituted immediately after cessation of the enzyme treatment. The original sialic acid content is regained after 20 h, while the electrophoretic mobility values are normalized after 16 h.

Discussion. The results presented in this study correspond roughly to published data on in vitro-models and show that cellular alterations induced by removal of neuraminidase-sensitive sialic acid residues are of short duration. This fact is particularly important for attempts to expose cell surface antigens masked by sialic acid moieties. It should be realized that the latter, after cessation of neuraminidase treatment, begin to reappear immediately. Although this may still be compatible with detection of the unmasked antigens by surveillance mechanisms, the situation is uncertain with regard to the ensuing immune reactions. We have to assume that classical antibodies and sensitized lymphocytes will only be available after the target cells have reconstituted their original surface configuration. However, in view of the conflicting results in the literature on the fate of neuraminidase-treated tumor cells, the operation of natural antibodies to 'cryptic' membrane antigens unmasked by neuraminidase treatment[17,18] should be taken into account.

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Further Studies on Neural Tube Defects Caused by Concanavalin A in Early Chick Embryos[1]

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Summary. Neural tube defects caused by concanavalin A in chick embryos are consequences of change in the cell surface of developing neuroepithelium.

Many physicochemical agents have been found to interfere with neurulation of the chick embryo when applied during the early stages of development. Despite new information concerning the etiology of central nervous system abnormalities, much is yet to be learned about the underlying mechanisms responsible for neural tube closure. Recent evidence has suggested that driving forces for closure of the neural tube arize, at least in part, from the constriction of microfilaments and elongation of cells under the influence of microtubules[2-4]. Our previous study[5] showed that concanavalin A (Con A), a plant agglutinin that binds to cell surface terminal glycosyl and mannosyl residues of glycolipids and glycoproteins[6,7], selectively inhibited neurulation and interkinetic nuclear migration in explanted early chick embryos. These findings suggest that closure of the neural tube is strongly influenced by the cell surface. The objective of this study was to elaborate further causative mechanisms of Con A action in producing neural tube defects in chick embryos.

Materials and methods. Two experimental series were carried out. In the first series, fertile White Leghorn eggs were incubated at 37.5 °C to obtain embryos at stage 4 of development[8]. Embryos were explanted by NEW's[9] technique. Thin albumen (nutrient medium) with or without 16 μg/ml Con A, a concentration which strongly inhibits neurulation of chick embryos[5], was added outside the glass ring. After 24 h of incubation, neural tissue was isolated from randomly selected control and experimental embryos, fixed for 1 h in 3% phosphate-buffered glutaraldehyde, rinsed several times in buffered sucrose solution, and postfixed for 1 h in 1% phosphate-buffered osmium tetroxide. After fixation, specimens were dehydrated in graded ethanol series and embedded in a mixture of Epon and Araldite. Thick sections stained with toluidine blue were prepared for light microscopy.

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Thin sections were contrasted with 3% aqueous uranyl acetate and lead citrate and examined under an A.E.I. electron microscope. In the second series, explanted stage 4 embryos were exposed to 0.01 μCi/ml Con A-H^3 (Elscint INC., Palisades Park, N.J.) at 37.5 °C for 6 h. After incubation, embryos were fixed in absolute ethanol-glacial acetic acid (3:1), embedded in paraffin, sectioned at 4 μm, and mounted on slides. Sections were deparaffinized, rehydrated, and stained with Delafield's hematoxylin. Slides were coated with Kodak NTB-2 photographic emulsion and exposed for 7–14 days at 4 °C. No obvious change in the general distribution of labeled materials was noted by increasing the exposure time. The emulsion was developed for 6 min in Kodak D-19 developer at 20 °C and fixed for 10 min in Kodak acid-fixer. The sections were dehydrated through a graded

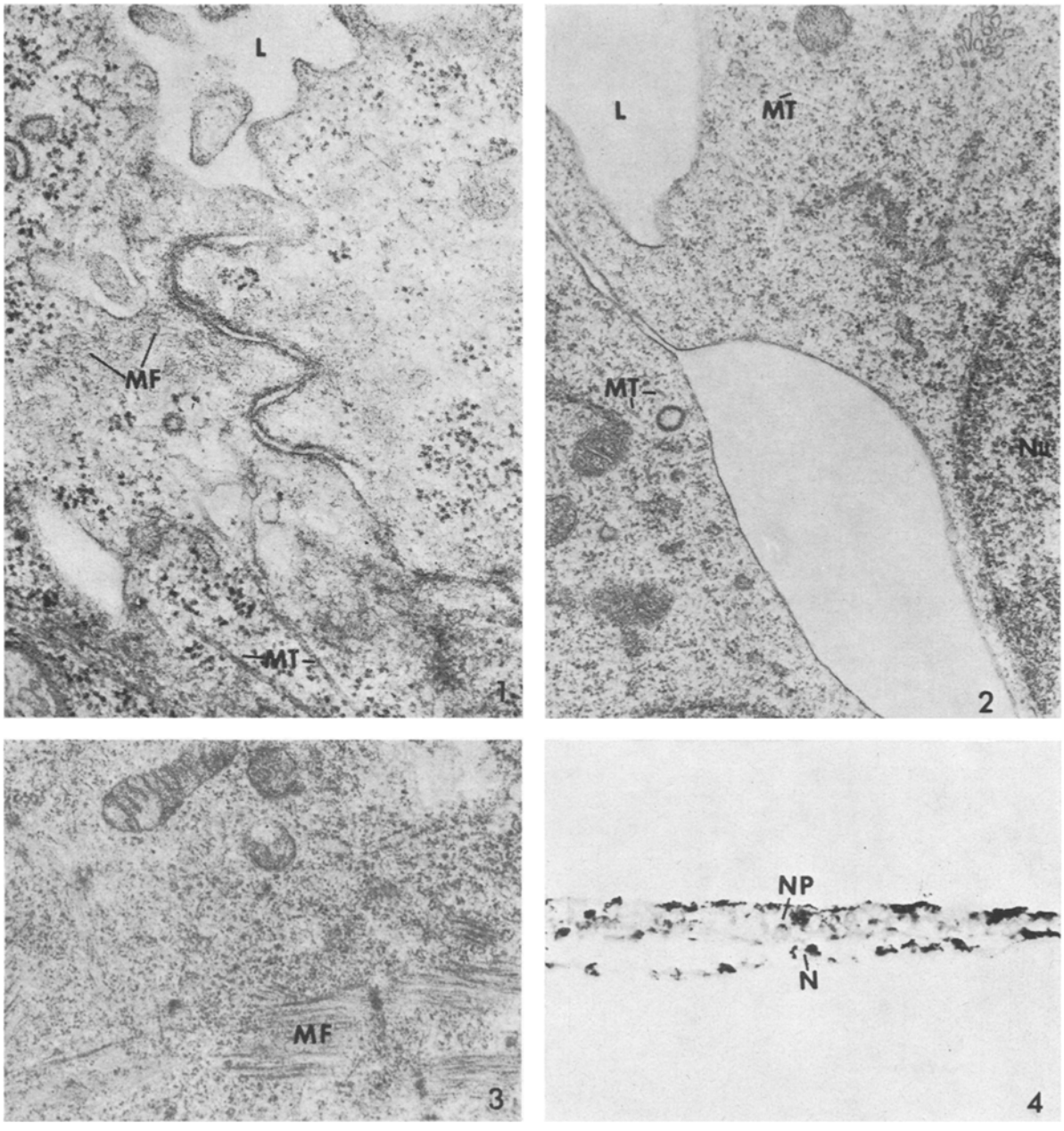

Fig. 1. Neuroepithelial cells of a control embryo explanted at stage 4 and cultured for 24 h on thin albumen. Note the luminal surface is convoluted with many cytoplasmic processes; the membranes of adjacent cells are interdigitated; microtubules (MT) are abundant and usually extend in an apical-basal plane; microfilaments (MF) are associated into large bundles. L, lumen. ×45,000.

Fig. 2. Neuroepithelial cells of an embryo explanted at stage 4 and cultured for 24 h on thin albumen containing 16 μg/ml of Con A. Note cells are not closely apposed except at the apical corner; the luminal surface is smoother than in controls. L, lumen; Nu, nucleus; MT, microtubules. ×26,000.

Fig. 3. Neuroepithelial cells of an embryo explanted at stage 4 and cultured for 24 h on thin albumen containing 16 μg/ml of Con A. Note microfilaments (MF) are associated into prominent bundles. ×20,000.

Fig. 4. Transverse section through the hindbrain region of an embryo explanted at stage 4 and cultured for 6 h on thin albumen containing 0.01 μCi/ml of Con A-H^3. Note labeled materials are found mostly on the cell surface. NP, neural plate; N, notochord. ×90.

ethanol series, cleared in xylol, and mounted in Permount.

Results. Of the 94 Con A-treated embryos, 86 had a malformed brain, which was shortened and distorted by irregular foldings. The neural tube showed varying degrees of openness. Somites, although less numerous than controls, were almost normal in appearance. Other structures were usually unaffected. Electron microscopic observations on the Con A-treated neuroepithelium revealed that cells were more rounded than those of controls, were not closely apposed, and showed loss of lateral cytoplasmic extensions (Figures 1 and 2). The apical surface was smoother than in the untreated cells and had no apical folds. Con A, at the concentration used, had no apparent effect on the integrity of microtubules (Figure 2) and microfilaments (Figure 3) except in severely affected neuroepithelium. Radioautographic studies showed that labeled materials were found mostly on the cell surface (Figure 4).

Discussion. The present study showed that 1. cellular processes associated with closure of the neural tube were more sensitive to Con A than were those essential to the formation of microfilaments and microtubules; 2. the initial site of Con A effect on neuroepithelium was the cell surface. These findings along with the known biological property of Con A[6,7] indicated that the observed neural tube defects were consequences of alterations in the cell surface of developing neuroepithelium. Indeed, the presence of glycoproteins and glycosyltransferases on the surface of cells which are in contact with a lumen has been implicated in cell adhesion and recognition during embryonic development[10–15]. Further experiments are presently underway in our laboratory to investigate the possible role of cell surface coat material in neurulation of chick embryos.

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Extravesicular Noradrenaline in Developing Peripheral Adrenergic Nerves

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Summary. Using a fluorescence technique numerous developing noradrenergic nerve terminals were observed in the muscle coat of the rat ductus deferens between 2 and 6 days postpartum. In the electron microscope similar developing nerve terminals possessed an extensive system of tubular endoplasmic reticulum but did not contain the small dense cored vesicles characteristic of mature noradrenergic nerve terminals. Thus the tubular reticulum is proposed as an alternative storage site for noradrenaline in developing adrenergic nerves.

Noradrenaline (NA) is believed to occupy membrane bound stores within peripheral adrenergic (sympathetic) nerve terminals and both pharmacological and electron microscopic studies[1–3] have shown that intra-axonal dense cored vesicles – both small (50 nm diameter) and large (90 nm diameter) – are the structures concerned with this amine storage. However, these and many other studies apply to adult material and relatively little information is available on NA in immature peripheral adrenergic nerves. Consequently, the distribution of NA in developing axons was examined using a microfluorescence technique and these results have been correlated with the fine structure of similar immature adrenergic nerves. The rat ductus deferens was chosen for examination since this tissue has been used extensively in studies on adult autonomic innervation and it is known to possess a rich supply of NA containing nerves[4,5].

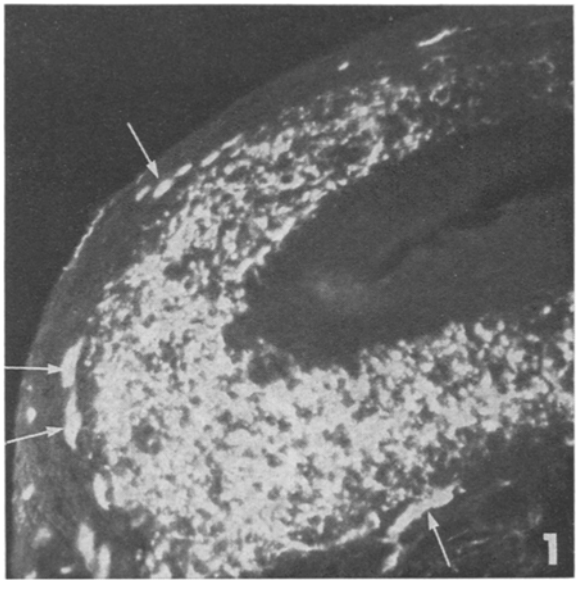

Fig. 1. An oblique section through the ductus deferens of a 6-day-old rat showing a dense plexus of fluorescent nerves throughout the muscle coat. Note the large fluorescent adventitial nerves (arrows). × 200.

Materials and methods. Male Sprague Dawley rats were killed at birth and on alternate days thereafter to provide a series of animals in age from 0–24 days. For each group, a minimum of 2 animals was examined and from every rat one ductus was processed histochemically for tissue NA using the technique described by FALCK and OWMAN[6]. The other ductus deferens from each animal was examined electron microscopically following fixation in either buffered potassium permanganate[7] or osmium tetroxide. Particular attention was focused on the urethral end of

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