stained with uranyl acetate and lead citrate, were examined in a Hitachi HS-8 electron microscope. - Almost all of the dissociated cells formed smaller and larger aggregates after 24 h of culture.

Immediately after dissociation, the striated duct cells showed only a few short microvilli on their surfaces (figure 1), while after 24 h of culture they formed deep tubular invaginations of the cell membrane (figure 2). Such invaginations were not seen in the original tissue. The tubular invaginations consisted of canals with numerous microvilli and were similar to the intracellular canaliculi in gastric parietal cells. - Similar structures were not seen in acinar cells and intercalated duct cells.

The characteristic structural feature of the surface of the striated duct cells is the presence of basal infoldings, short microvilli on the apical surface and folds and interdigitations of the lateral surface[6]. It is likely that the exposure to enzymes, such as collagenase and hyaluronidase in the present study, produced profound alterations of the cell surface. In fact, freshly dissociated striated duct cells do not show basal infoldings but only a few short microvilli. During 24 h of culture, a restoration of surface components lost by the enzymic digestion occurs and produces the intracellular canaliculi.

The short-term stationary culture system of dissociated striated duct cells of the submandibular gland may be useful as a model for analyzing mechanisms of the specialization and modification of the cell membrane.

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Inhibition of neural tube closure by ionophore A23187 in chick embryos[1]

H. Lee, R. Nagele and N. Karasanyi

Department of Biology, Rutgers University, Camden (New Jersey 08102, USA), 15 August 1977

Summary. Ionophore A23187 inhibited closure of the chick neural tube through its effects on cytoskeletal components.

Ionophore A23187, a carboxylic antibiotic isolated from *Streptomyces chartreusensis,* promotes transport of divalent cations, especially Ca^{++}, across biologic membranes[2]. This antibiotic has been shown to activate several calcium-dependent biologic processes. Some of these include: DNA synthesis in sea urchin eggs[3], secretion in mast cells[4,5], and mitogenesis in lymphocytes[6]. More recently, Moran[7] and Moran and Rice[8] found that ionophore counteracted the inhibitory effect of papaverine, a smooth muscle relaxant, on amphibian neurulation and proposed that the availability of free Ca^{++} is crucial in controlling closure of the neural tube. The present study was undertaken to investigate effects of ionophore on closure of the neural tube in chick embryos.

Materials and methods. Fertile White Leghorn eggs were incubated at 37.5 °C to obtain embryos at stage 8_+ of development[9]. A total of 96 embryos were explanted by New's[10] technique and grown on thin albumen (= nutrient medium) with or without 25 µg/ml ionophore A23187, a concentration found to selectively inhibit closure of the neural tube. Embryos were examined at intervals during incubation to determine the degree of neural fold fusion. Some were fixed in Bouin's fluid, stained with Delafield's hematoxylin and kept as whole mounts. Others were processed for microscopic studies as described below.

For histological studies, embryos were fixed in Bouin's fluid, embedded in paraffin, serially sectioned at 6 µm, and stained with Delafield's hematoxylin and eosin. For electron microscopy, embryos were fixed in half strength Karnovsky's[11] fixative for 2 h at room temperature. After a thorough washing in 0.08 M cacodylate buffer (pH 7.2), embryos were postfixed for 1 h in 1% osmium tetroxide. After an additional brief rinse in buffer, embryos were stained 'en bloc' with 1% uranyl acetate, dehydrated in graded ethanol series, embedded in Spurr's[12] resin, and sectioned. Sections (silver/pale gold) were contrasted with aqueous uranyl acetate and lead citrate and examined with a RCA EMU-4 electron microscope.

Results and discussion. In stage 8_+ embryos, apposing neural folds have not yet made contact except in the future hindbrain region[13]. However, a brief exposure (1-2 h) of these embryos to 25 µg/ml ionophore often resulted in prematurely fused neural folds even in the somite region. In this region, affected neuroepithelial cells were highly elongated, wedge-shaped, and exhibited numerous apical foldings (figure 1), features which are usually found only in the brain region at this stage of development (figure 2). In addition, the terminal web, a band of contractile apical microfilaments, was densely organized (figure 1). Ionophore is known to promote both Ca^{++} influx and release of bound Ca^{++}[2]. High concentrations of intracellular free Ca^{++} can augment the contraction of microfilaments[14], cytoskeletal components known to be responsible for apical constriction and the wedge-shaped appearance of neural tube cells[13,15]. Therefore, it seems likely that the observed premature changes in cell shape and uplifting of neural folds in ionophore-treated embryos are consequences of enhanced microfilament contraction.

The effect of ionophore on the developing neuroepithelium was found to be time dependent. After 16 h of incubation, affected cells usually lost their highly elongated shape and the neuroepithelium as a whole became thinner than that of corresponding controls (compare figures 3 and 4). This effect was most pronounced in the portion of the neuroepithelium adjacent to the notochord (figure 3). Revel and Brown[16] have observed that fine fibrils firmly anchor the neural tube to the notochord. These 2 structures were often widely separated in ionophore-treated embryos (figure 3). Also, the cells forming the floor of the neural tube were less elongated than usual, suggesting that anchorage to the notochord may account in part for their high columnar, 'pulled down' appearance during the uplifting of neural

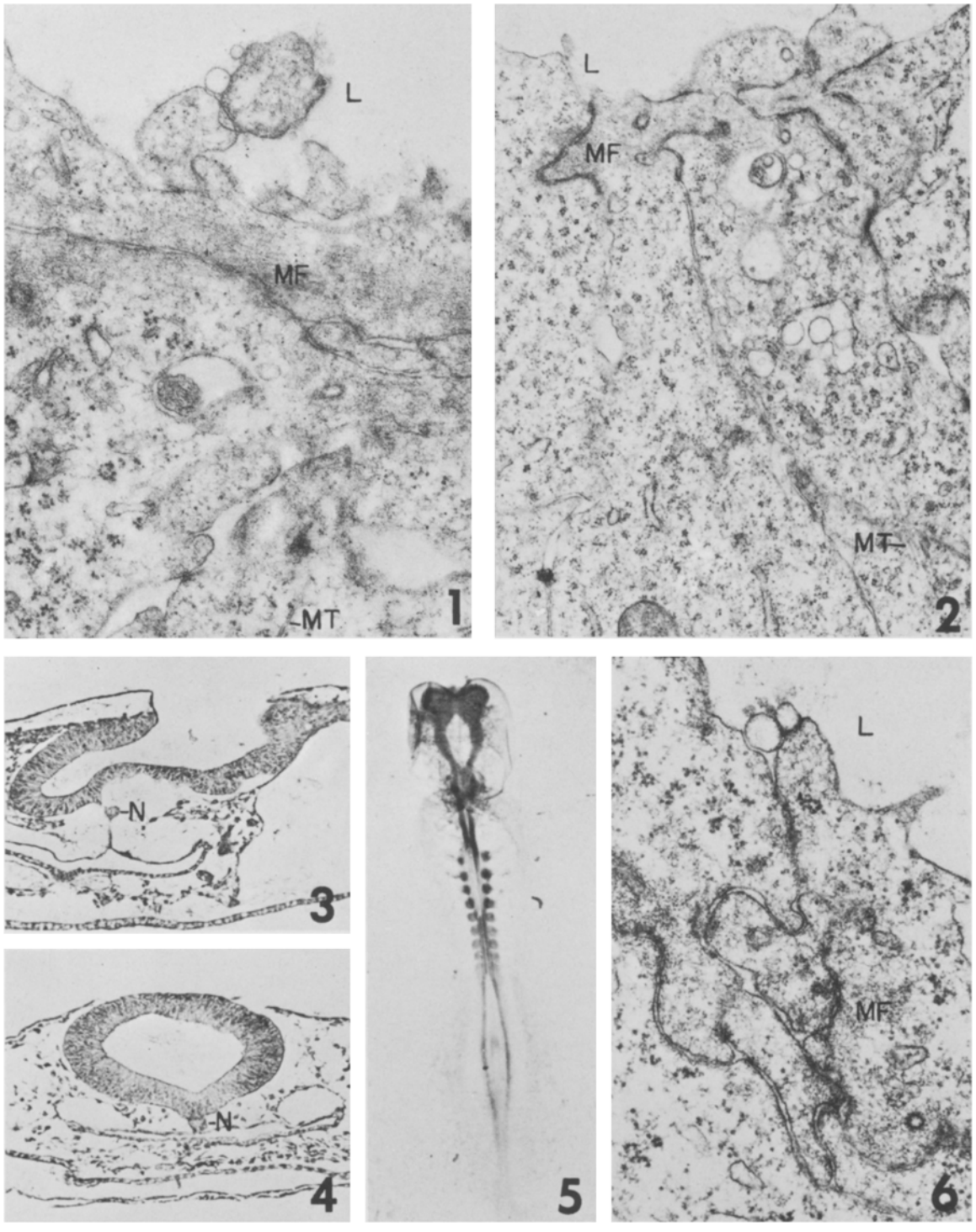

Fig. 1. Cells from the prematurely closed neural tube (first somite region) of an embryo explanted at stage 8_+ and exposed to 25 µg/ml ionophore at 37.5 °C for 1 h. The luminal surface exhibits various membrane protrusions and microfilaments (MF) are densely organized, both features usually seen in neuroepithelial cells of the brain region at this stage of development (cf. figure 2). L, lumen; MT, microtubules; MF, microfilaments. × 32,000. Fig. 2. Cells from the future midbrain region of an embryo explanted at stage 8_+ and cultured for 1 h on plain nutrient medium. × 17,000. Fig. 3. Transverse section through malformed midbrain region of an embryo explanted at stage 8_+ and cultured for 16 h on medium containing 25 µg/ml ionophore. Note the floor of the midbrain is flattened, thinner than corresponding controls (figure 4), and widely separated from the notochord (N). × 90. Fig. 4. Transverse section through the midbrain region of a control embryo explanted at stage 8_+ and cultured for 16 h on plain nutrient medium. × 90. Fig. 5. An embryo explanted at stage 8_+ and cultured for 16 h on medium containing 25 µg/ml ionophore. Note midbrain is widely open and neural tube shows varying degrees of openness. Somite formation and heart development are not apparently affected by ionophore treatment. × 28. Fig. 6. Cells from the collapsed midbrain region of an embryo explanted at stage 8_+ and cultured for 16 h on medium containing 25 µg/ml ionophore. The integrity of cytoskeletal components is not apparently affected by ionophore treatment. × 33,000.

folds. This ionophore-mediated change in cell shape apparently weakened the neuroepithelium as shown by the flattened neural groove and collapsed neural folds (figure 3). This was most apparent in the region where the neuroepithelium was expanding into the midbrain (figure 5). Electron microscopy revealed that the integrity of cytoskeletal components was not visibly altered by ionophore treatment (figure 6). Burnside[17] has suggested that the elongated shape of neuroepithelial cells is not maintained simply by the presence of microtubules, but rather by the directed flow of cytoplasm along the length of these cytoskeletal components toward the cell base. Microfilaments have been shown to be involved in cytoplasmic flow[18,19] and in neuroepithelial cells their coordinated contraction may provide the driving forces for the basally directed cytoplasmic flow. Since the contractile state of microfilaments is regulated by the availability of Ca^{++}[14,20], a possibility exists that ionophore inhibits cell elongation by raising the intracellular concentration of free Ca^{++}, thus disrupting the coordination of microfilament contraction and directed cytoplasmic flow.

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The ontogeny of α-foetoprotein in the chicken

B. Slade and J. Milne

Department of Zoology, University College of Wales, Aberystwyth SY23 3DA (Great Britain), 25 August 1977

Summary. The ontogeny of α-foetoprotein (AFP) has been studied in the chicken using polyacrylamide gel gradient electrophoresis and electroimmunoassay from 7 days of incubation until after hatching. The results are discussed in the light of previous on the ontogeny of AFP in mammals.

An embryo-specific serum glycoprotein homologous with mammalian α-foetoprotein (AFP) has been described in the chicken[1]. Studies on the localisation and synthesis of this protein[2] have shown it to be a product of the yolk sac and to a lesser extent the embryonic liver. This is very similar to the situation in mammals, although here the liver appears to be the major site of synthesis[3,4].

The ontogeny of AFP has been studied in man, rodents and rabbits, but relatively little work has yet been done on its avian counterpart. A qualitative study of the changes in the protein composition of embryonic chicken serum (ECS) was made by Weller[5] using immunoelectrophoretic analysis, but no quantitative data on AFP levels during development have been published. This investigation was undertaken to study the ontogeny of AFP in the chick embryo and to compare this with the mammalian situation.

Materials and methods. Pooled 11–15-day embryonic chicken serum was fractionated on Sephadex G200, and the AFP-rich 3rd peak concentrated by pressure filtration. After suitable dialysis this fraction was applied to a column of ConA-Sepharose, the bound glycoproteins eluted with 2% α-D-methylglucoside and concentrated. Final purification was achieved by electrophoresis on a slab on polyacrylamide gel with a monomer concentration gradient of 4–30% (Pharmacia), the pure AFP being eluted from the sliced gel. Sera were analyzed using this same electrophoretic technique.

Electroimmunoassay as originally described by Laurell[6] was used for quantitative AFP determination employing the pure protein preparation as a standard. Total serum protein concentrations were determined by the Lowry technique[7].

Results. Quantitative analyses were not undertaken before 7 days of incubation because of the difficulty in obtaining blood samples free from contamination at earlier stages. However, from 7 days there was a linear rise in total serum protein from just over 10 mg/ml to adult values of around 50 mg/ml 1 week after hatching. During the same period

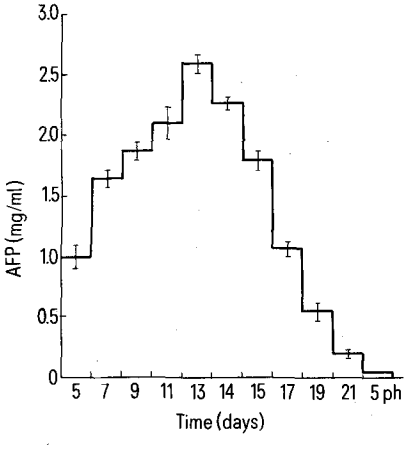

Fig. 1. AFP concentrations in embryonic chicken serum. Values represent the arithmetic mean of 6 individual samples±SE. PH, post hatch.