activated cells, particularly during initation of cell growth and proliferation[8,9]. Our previous results showed that during compensatory renal growth stimulation of inositide metabolism occurs[2]. During postnatal renal growth the incorporation rate of ^{32}P into phosphatidic acid and phosphatidylinositol showed an identical pattern with other phospholipids, and did not show a phosphatidylinositol effect (fig. 2). These findings are in accord with the hypothesis that there must be a significant difference in the factor(s) stimulating and maintaining normal and compensatory renal growth[10] and that the involvement of cellular membranes in the initiation of compensatory kidney growth is different from that in normal kidney growth.

1 Bean, G.H., and Lowenstein, L.M., J. clin. Invest. *61* (1978) 1551.
2 Banfić, H., Pokrajac, N., and Irvine, R.F., Biochim. biophys. Acta *833* (1985) 223.
3 Folch, J., Lees, M., and Sloane-Stainly, G.H., J. biol. Chem. *226* (1957) 497.
4 Bartlett, G.R., J. biol. Chem. *234* (1959) 446.
5 Armitrage, P., Statistical Methods in Medical Research, p. 118. Blackwell, Oxford and Edinburgh 1971.
6 Priestley, G.C., and Malt, R.A., J. Cell Biol. *37* (1968) 703.
7 Pushpendran, C.K., and Eapen, J., Biol. Neonate *23* (1973) 303.
8 Michell, R.H., Cell Calcium *3* (1982) 429.
9 Michell, R.H., Trends biochem. Sci. *4* (1979) 128.
10 Silber, S., and Malvin, R.L., Am. J. Physiol. *226* (1974) 114.

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Possible involvement of calmodulin in apical constriction of neuroepithelial cells and elevation of neural folds in the chick[1]

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Summary. Chlorpromazine and trifluoperazine HCl, antipsychotic drugs known to bind to calmodulin, reversibly inhibited elevation of neural folds by interfering with the contractile activity of apical microfilament bundles in developing chick neuroepithelial cells.
Key words. Chick embryo; neurulation; microfilaments; calmodulin; phenothiazine antipsychotic drugs.

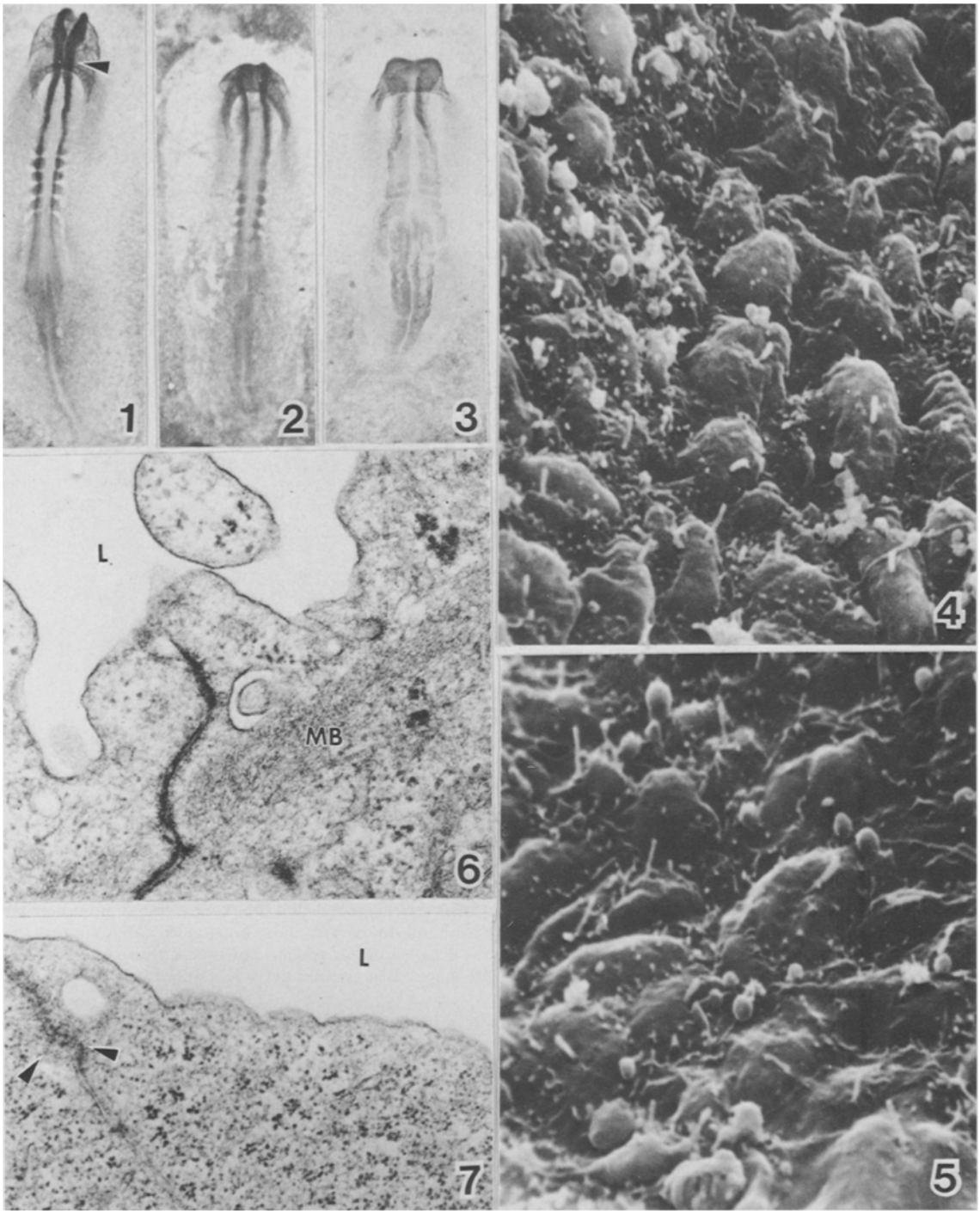
Schroeder[2] originally suggested that microfilaments in developing neuroepithelial cells are structurally and functionally comparable to muscle actin. This was later confirmed by the observations that 1) these microfilaments have the ability to bind heavy meromyosin to form characteristic arrowhead configurations[3] and 2) following indirect immunofluorescence staining with anti-actin antibodies, intense fluorescence is always found in apical regions of neuroepithelial cells where microfilaments are organized into prominent bundles[4,5]. It is well known that muscle contraction is regulated by local modulation of intracellular free calcium ion (Ca^{++}) levels. Thus a question arises as to whether or not Ca^{++} also serves as a regulator of microfilament contraction in the developing neuroepithelium in a manner similar to that of skeletal muscle. This view is supported, at least in part, by the finding that chemical agents (e.g., papaverine and ionophore A23187), which alter intracellular free Ca^{++} levels, disrupt elevation of neural folds through their action on the contractile activity of microfilaments[6-8]. Although troponin C, the skeletal muscle calcium-binding protein, has not yet been identified with certainty in non-muscle cells, studies have shown that Ca^{++} exerts control over a variety of cellular functions through a similar protein called calmodulin[9]. The recent demonstration that chlorpromazine (CPZ) and trifluoperazine HCl (TFP) bind specifically, in a calcium-dependent fashion, to calmodulin has raised the possibility that these antipsychotic drugs can be used to assess the contributory role of calmodulin in morphogenetic movements[10-13]. As a first part of our interest in this problem, we investigated the effects of CPZ and TFP on apical constriction of neuroepithelial cells and elevation of neural folds in the chick.

Materials and methods. Calmodulin antagonists (CPZ and TFP) were obtained from Smith Kline and French, Co. Fertile White Leghorn eggs were incubated at 37.5 °C to obtain embryos at stage 8 of development[14]. These embryos were chosen for investigation because the forming neural tube exhibits a gradual variation in the degree of openness along its length, providing an excellent opportunity to study the effects of chemical agents on closure of the neural tube[5]. Embryos were explanted using New's[15] technique and grown on medium (thin albumen) with or without a calmodulin antagonist. They were examined at intervals under a dissecting microscope to determine the degree of folding of the neuroepithelium. Unless otherwise indicated, several embryos were randomly selected from each treatment group after 6 h of incubation and processed for scanning and transmission electron microscopy (SEM and TEM)[5] to examine ultrastructural changes caused by the drugs.

Results and discussion. In a series of four experiments, 98 chick embryos were explanted at stage 8, cultured on thin albumen with or without a calmodulin antagonist, and examined at 2-h intervals (up to 6 h) under a dissecting microscope. At recovery, all (18) of the control embryos had advanced to stage 8+ or 9− of development. They had 5–6 somite pairs, and their neural folds in the future midbrain and anterior portion of the hindbrain already had made contact (fig. 1). In contrast, embryos responded to CPZ and TFP in a dose-related manner. Elevation of neural folds was inhibited in over 80% of the embryos by 40 μg/ml CPZ or 9 μg/ml TFP (fig. 2; table), whereas lower concentrations (e.g., 10 μg/ml CPZ and 3 μg/ml TFP) had no apparent effect on the morphogenesis of embryos. Higher concentrations (e.g., 80 μg/ml CPZ and 15 g/ml TFP) were deleterious and less than 10% of the embryos remained viable after 6 h

Effects of CPZ (40 μg/ml) and TFP (9 μg/ml) on elevation of neural folds in chick embryos explanted at stage 8 and cultured for 6 h

Appearance of neural folds in future midbrain region	No. and % (in parentheses) of embryos		
	Control	CPZ	TFP
Neural folds made contact (fig. 1)	18 (90.0)	2 (8.3)	0 (0.0)
Neural folds further elevated but showed no signs of contact	6 (10.1)	2 (8.3)	3 (12.5)
Neural folds appeared to be 'relaxed' (fig. 2)	0 (0.0)	20 (83.3)	21 (87.5)



Embryos were explanted at stage 8 and grown for 6 h on thin albumen with or without a calmodulin antagonist. Figure 1. Dorsal view of a control embryo. Apposing neural folds in the future midbrain region (arrow) have already made contact. × 20. Figure 2. Dorsal view of a TFP (9 μg/ml)-treated embryo. Neural folds appear to be 'relaxed' and are widely separated throughout the embryo axis. × 20. Figure 3. Dorsal view of a CPZ (60 μg/ml)-treated embryo. This embryo exhibits short embryo axis, 'relaxed' neural folds, and poorly defined somite pairs. × 20. Figure 4. Scanning electron micrograph showing apical surfaces of cells forming the floor of the future midbrain of a control embryo (cf. fig. 1). × 4200. Figure 5. Scanning electron micrograph showing apical surfaces of cells forming the floor of the midbrain of a TFP (9 μg/ml)-treated embryo (cf. fig. 2). Apical surfaces are smoother and broader than those shown in figure 4. × 4200. Figure 6. Transmission electron micrograph showing apical regions of cells forming the floor of the future midbrain of a control embryo (cf. fig. 1 and 4). L, lumen; MB, microfilament bundles. × 36,000. Figure 7. Transmission electron micrograph showing apical regions of cells forming the floor of the future midbrain of a TFP (9 μg/ml)-treated embryo (cf. fig. 2 and 5). Microfilament bundles (arrows) are thinner and much less conspicuous than those shown in figure 6. L, lumen. × 36,000.

of incubation. Viable embryos were characterized by reduced blastodermal area, 'relaxed' neural folds, and poorly defined somite pairs (fig. 3). Furthermore, TFP (9 µg/ml)-treated embryos showed the first signs of neural tube closure defects within 3 h of incubation compared to 4.5 h of incubation in CPZ (20 µg/ml)-treated embryos, a finding consistent with that of Levin and Weiss[10] who reported that TFP was approximately four times more effective than CPZ in inhibiting calmodulin binding activity in vitro. When embryos showing 'relaxed' neural folds (cf. fig. 2) were subcultured for 6–8 h on plain thin albumen, they underwent morpohogenesis comparable to corresponding (untreated) controls, indicating that 1) the inhibitory action of CPZ and TFP was readily reversible and 2) the observed relaxation of neural folds was not a consequence of general cytotoxicity[11].

SEM revealed that apical (luminal) surfaces of treated neuroepithelial cells were often smoother and broader than those of controls (figs 4 and 5), suggesting that the contractile activity of apical microfilament bundles responsible for apical constriction of neuroepithelial cells during elevation of neural folds had been impaired[3]. This view is supported by the observation that apical microfilament bundles in cells showing poor apical constriction were thinner and much less conspicuous than those in controls where they are known to progressively increase in thickness during apical constriction[16].

While we have shown that calmodulin antagonists reversibly inhibit elevation of neural folds through their disruptive action on the contractile activity of apical microfilament bundles in neuroepithelial cells, further work is needed to 1) elucidate the molecular basis of this inhibition and 2) determine if calmodulin indeed participates in closure of the neural tube. Experiments along these lines are presently underway in our laboratory.

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2 Schroeder, T. E., Am. Zool. *13* (1973) 949.
3 Nagele, R. G., and Lee, H., J. exp. Zool. *213* (1980) 391.
4 Sadler, T. W., Greenberg, D., Coughlin, P., and Lessard, J. L., Science *215* (1982) 172.
5 Lee, H., Kosciuk, M. C., Nagele, R. G., and Roisen, F. J., J. exp. Zool. *225* (1983) 449.
6 Moran, D., and Rice, R. W., Nature *261* (1976) 497.
7 Lee, H., Nagele, R. G., and Karasanyi, N., Experientia *34* (1978) 518.
8 Lee, H., and Nagele, R. G., Teratology *20* (1979) 321.
9 Cheung, W. Y., Science *207* (1980) 19.
10 Levin, R. M., and Weiss, B., Molec. Pharmac. *13* (1977) 690.
11 Forsén, S., Thulin, E., Drakenberg, T., Kreb, J., and Seamon, K., FEBS Lett. *117* (1980) 184.
12 Chernoff, E. A. G., and Hilfer, S. R., Tiss. Cell *14* (1982) 435.
13 Meijer, L., and Guerrier, P., Devl Biol. *101* (1984) 257.
14 Hamburger, V., and Hamilton, H. L., J. Morph. *88* (1951) 49.
15 New, D. A. T., J. Embryol. exp. Morph. *3* (1955) 326.
16 Burnside, B., Am. Zool. *13* (1973) 989.

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Alternariol, a dibenzopyrone mycotoxin of *Alternaria* spp., is a new photosensitizing and DNA cross-linking agent

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Summary. The mycotoxin alternariol (3,4',5-trihydroxy-6'-methyldibenzo [a] pyrone) but not alternariol monomethyl ether (3,4'-dihydroxy-5-methoxy-6'-methyldibenzo [a] pyrone) is phototoxic to *Escherichia coli* in the presence of near UV light (320–400 nm). The phototoxicity bioassays with a DNA repair-deficient mutant of *E. coli* suggested that DNA may be the molecular target for photo-induced toxicity of alternariol. Interactions between alternariol and double-stranded, supercoiled DNA suggest that alternariol interacts with DNA by intercalation. No DNA breakage was detected in this system; however, alternariol forms a complex and cross-links double-stranded DNA in near UV light. These results suggest that alternariol is a new phototoxic, DNA-intercalating agent and is a DNA cross-linking mycotoxin in near UV light.

Key words. Alternaria spp.; mycotoxin; alternariol; alternariol methylether; photosensitizer; DNA cross-linking.

Light modifies the biological activity of naturally occurring photosensitizers[1–10]. The cellular targets of these molecules have been identified as membrane components and the nucleus[3]. Indeed, DNA is the molecular target in the presence of near UV light (n-UV, 320–380 nm) for some of these photosensitizers[1–6]. Compounds that react with DNA in the presence of n-UV can cause chromosomal aberrations and are powerful mutagens[4, 11]. The possibility that the photochemical properties of such natural products can be exploited in the treatment of neoplastic disorders has been considered (see Elespuru and Gonda[1]). However, the photosensitizing nature of mycotoxins is relatively unexplored.

Mycotoxins are toxic metabolites produced by various microfungi[12]. The compounds are commonly encountered in mold-contaminated food-stuffs and pose serious threats to the health of humans and livestock. At least three classes of mycotoxins are known to be phototoxic. These are the aflatoxins[6], the extended quinone cercosporin (see Towers[3]) and the dimeric anthraquinone rugulosin[12]. Sporodesmin, an amino acid-derived fungal metabolite is a known photosensitizer[12]. Alternariol, however, a well-known myco- and phytotoxin produced by *Alternaria* spp., has not been previously examined for any phototoxic effects, although the compound is known to be cytotoxic[12]. It is therefore relevant that alternariol, as well as some other *Alternaria* spp. metabolites, were recently found to be mutagenic[13].

We have employed the relatively simple and sensitive bioassay promulgated by Ashwood-Smith et al.[9] in our study of alternariol and alternariol monomethyl ether (fig. 1). For this study we developed a rapid method for detecting DNA-cross-linkage using agarose gel electrophoresis. Alternariol was found to be a new photosensitizer, and the results suggest that DNA is the

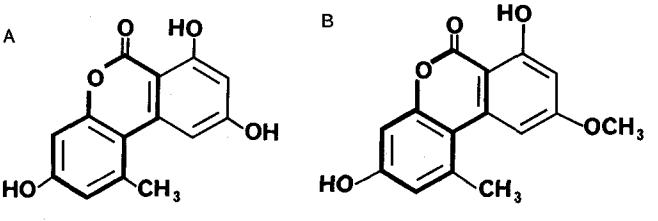

Figure 1. The chemical structures of alternariol (A), and alternariol monomethylether (B). The coumarin moiety is outlined.