rabbit retina in vitro[23]. It is worthy of note, however, that dobutamine, like adrenaline, noradrenaline or an isomer of ADTN (i.e. 5,6-dihydroxy-tetrahydronaphtalen)[9,10] was able to increase cyclic AMP, when it was applied at 10^{-4} M. This concentration of dobutamine is hardly obtained in plasma in vivo, when applied in the range of therapeutic doses advised for man[15]. Nevertheless, in case of accidental overdose, one has to keep in mind that besides its specific β_1-stimulating properties, dobutamine may possibly display some dopamine-mimetic activity which can modify the symptoms of drug intoxication.

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Effects of colchicine, cytochalasin-B and papaverine on wound healing in *Xenopus* early embryos

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Summary. The effects of colchicine, cytochalasin-B and papaverine on wound healing in *Xenopus* early embryos have been studied. Colchicine does not prevent wound healing, whereas cytochalasin-B does. Papaverine, under conditions which prevent the completion of neurulation, does not prevent wound healing. A model is given which might explain these observations.

Amphibian embryos have been used extensively for studies in experimental embryology, partly because they show a remarkable ability to heal following manipulation. However, until recently there has been little systematic study of wound healing in amphibian early embryos, although a number of observations have been made en passant. For example, gaping of a wound made in amphibian neurulae has shown that the ectoderm is under lateral tension[2]. Also, it has been noted that healing of the wound following micro-injection of amphibian gastrulae was inhibited by cytochalasin-B[3], although it is difficult to determine the effective concentration of cytochalasin-B in this case. Recently, a scanning electron microscopical study of wound healing in *Xenopus* neurulae has shown that following initial gaping of the wound, the cells surrounding the wound become tapered towards the cut edge[4]. This observation is compatible with the idea that wound closure is effected, at least in part, by coordinated changes in cell shape. The aim of the present work has been to attempt to determine the mechanisms by which such changes in cell shape are effected, by studying the effect of inhibitors on wound healing. Colchicine is thought to disrupt microtubules[5], which have been observed in a number of developing systems where morphogenesis is occurring[6]. Cytochalasin-B is thought to reversibly disrupt microfilaments[7], which have been observed in amphibian gastrulae[8] and neurulae[9], and has been shown to inhibit gastrulation in *Xenopus*[3]. Papaverine has been shown to prevent neurulation in amphibian embryos[10], which is accomplished by changes in cell shape similar to those described in wound healing[6], and it has been suggested that papaverine acts by inhibiting calcium fluxes.

Methods. Xenopus embryos were obtained by injecting pairs of adults with chorionic gonadotrophin (Chorulon, Intervet Ltd). The jelly coats were removed by placing the embryos in 2% cysteine hydrochloride in 10% Steinberg saline, brought to pH 7.8 with 2 M NaOH. The embryos were washed and subsequently cultured in 10% Steinberg saline, pH 7.3. When the embryos had reached the late blastula stage (stage 9[11]), the vitelline membranes were removed using Watchmakers' forceps, and the embryos were cultured overnight and subsequently handled over 1% agar. When the embryos had reached the neurula stage (stage 17-18[11]), they were wounded by making a longitudinal incision approximately 0.5 mm long in the lateral ectoderm using an electrolytically-sharpened tungsten needle. The process of wound healing was followed over the next 60 min.

For colchicine treatment, embryos were exposed either to 10^{-3}-10^{-5} M colchicine for 2 h before wounding and during recovery, or to 1.25×10^{-3} M colchicine overnight from the late gastrula stage. In this latter case, a rent was made in the roof of the blastocoel of the gastrula to facilitate penetration of the drug[12]. For cytochalasin-B treatment, a different protocol was used since prolonged exposure to cytochalasin-B causes disaggregation of amphibian embryos. Neurulae were exposed to 0.3-5.0 µg/ml cytochalasin-B in dimethylsulphoxide (DMSO) for 5 min before wounding and during recovery. Controls included embryos wounded in DMSO alone, and intact embryos in cytochalasin-B. For

papaverine treatment, embryos were exposed to 300 µg/ml papaverine hydrochloride for 2 h before wounding and during recovery, or to 300–1000 µg/ml papaverine overnight from the late blastula stage. Papaverine prepared in 10% Steinberg saline buffered to pH 7.3 was found to precipitate overnight, and so papaverine was prepared in unbuffered saline, when it caused the pH to drop to 5.0. Thus controls included embryos cultured and wounded in unbuffered saline brought to pH 5.0 with HCl.

Some embryos were fixed in paraformaldehyde/glutaraldehyde[13] 15 min after wounding for semi-thin sections. Fixed embryos were washed in cacodylate buffer, dehydrated in an ethanol series and embedded in Spurr's resin. The resin was polymerized at 70 °C for 24 h and sections were cut at 2 µm. The sections were placed on glass slides on a drop of water, flattened by heating to 80–90 °C and air dried. The resin was removed by treating the slides for 4 min with a 1:1 mixture of 100% ethanol and propylene oxide brought to a high pH with solid NaOH[14]. The slides were then washed in 100% ethanol, rinsed in water, and stained for 4 min in a modified Cason's stain[15]. Finally, the slides were rinsed briefly in water, air dried, and coverslips were mounted on the slides.

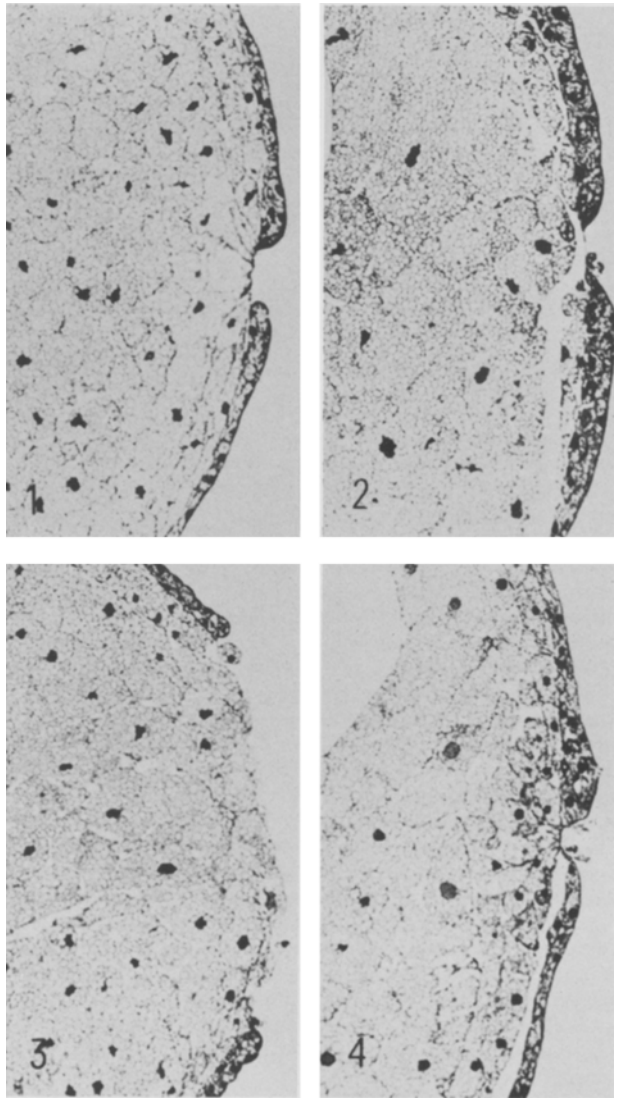
Results and discussion. Each experimental series was accompanied by control embryos as described in the materials and methods section. Results for the control embryos of different experiments were consistent and so are described first.

Control embryos: In control embryos the wound healed rapidly. Immediately after incision the wound gaped, but within 5 min the wound had started to close. 15 min after incision the wound had closed further (figure 1), and after 45 min it appeared almost completely healed. The wound usually became filled with extruded cells and cell debris, and this material was gently removed with a hair loop before fixation at 15 min.

Colchicine-treated embryos: In initial experiments it was found that treatment of embryos for 2 h before wounding and during recovery with 10^{-5}, 10^{-4} or 10^{-3} M colchicine did not inhibit wound closure. Embryos were then kept overnight in 1.25×10^{-3} M colchicine from the early gastrula stage, the earliest stage from which embryos can be maintained under mitotic block and develop normally[12]. Making a rent in the blastocoel roof of the gastrula to facilitate drug penetration produced some abnormal embryos with double axes, but only normal embryos were used for wounding experiments. Prolonged exposure to colchicine in this manner did not prevent wound closure (figure 2), suggesting that microtubules are not required for wound healing in *Xenopus* early embryos.

Cytochalasin-B-treated embryos: Cytochalasin-B at 0.3 µg/ml appeared to have little effect on wound closure. As in the controls, immediately after incision the wound gaped, but within 5 min the wound was reclosing, and during the following 40 min wound closure proceeded as normal. With 0.6 µg/ml cytochalasin-B the results were variable: in some embryos the wound healed normally; in others it closed partially; in others the wound failed to close. At the higher concentrations, 1.25, 2.5 and 5 µg/ml, cytochalasin-B completely prevented wound closure (figure 3). The wound gaped immediately after incision and continued to gape even more widely during the next 40 min. At the highest concentration used, 5 µg/ml, disaggregation of the endoderm cells within the wound became evident as the experiment proceeded. At the lowest concentration used that still prevented wound closure, 1.25 µg/ml, the ectoderm showed some tendency to curl under at the cut edge at later times as previously described[4]. These results suggest that microfilaments are essential to wound healing in *Xenopus* early embryos.

Papaverine-treated embryos: In initial experiments it was found that treatment of embryos for 2 h before wounding and during recovery with 300 µg/ml papaverine did not inhibit wound healing. Subsequently, embryos were treated overnight with 300, 600 and 1000 µg/ml papaverine. At 1000 µg/ml papaverine many of the embryos had flattened neural structures, and in some embryos development was delayed. At 600 and 300 µg/ml some of the embryos had flattened neural structures, and some of the embryos appeared normal. Those embryos in which the neural folds had failed to elevate fully and fuse were selected for wounding. When these embryos were wounded, the ectoderm appeared less rigid than normal. However, the



Semi-thin sections of *Xenopus* neurulae fixed 15 min after wounding. × 115.

Fig. 1. Control embryo. The wound is closing.

Fig. 2. Embryo treated from the early gastrula stage with 1.25×10^{-3} M colchicine. Wound is closing normally.

Fig. 3. Embryo treated with 1.25 µg/ml cytochalasin-B. Wound is gaping.

Fig. 4. Embryo treated from the late blastula stage with 600 µg/ml papaverine. Neural folds in this specimen had failed to erupt fully, but the wound is closing normally.

process of wound healing appeared to proceed normally in embryos in 300, 600 or 1000 µg/ml papaverine (figure 4). Thus, although papaverine had already inhibited 1 morphogenetic movement in these embryos, it did not prevent wound healing, and thus the control of wound closure is not identical to the control of neurulation.

Embryos in which neurulation has been blocked by papaverine can be induced to neurulate rapidly by treatment with the divalent cation ionophore A23187[16]. Thus papaverine might block the calcium fluxes which normally trigger neurulation, and the ionophore might overcome this block and allow neurulation to proceed. Calcium ions could also act as the trigger for the changes in cell shape which effect wound healing in *Xenopus* embroys[4]. One model for wound healing in this system consistent with the present results would be that the newly exposed lateral cell membranes at the cut edge of the wound have a greater ionic permeability than the normally exterior membranes. An influx of calcium through this newly-exposed membrane, resulting in a calcium-activated contraction of microfilaments localized in the margins of cells at the wound periphery could lead to the cell shape changes observed by scanning electron microscopy[4]. Further experiments are in progress to test this model.

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Regression of granular pericytes in cerebral fine vessels of rats after administration of a vitamin E deficient diet[1]

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Summary. Prolonged administration of a vitamin E deficient diet to Wistar rats resulted in regressive changes in granular pericytes distributed around fine vessels of the cerebral cortex. The regressive signs included expansion of endoplasmic reticula, swelling of mitochondria and increase of vesicular structures. Rupture of the limiting membranes of the intracellular granules often accompanied these changes. The finding seems important for an understanding of the physiology and nutriology of granular pericytes in the brain.

Granular pericytes localized in cerebral fine vessels were characterized by the presence of a lot of intracellular electron-opaque granules of various sizes. According to a previous study by the authors, the granular pericytes change in shape and content as the rats age. They possess a high concentration of acid phosphatase in intracellular granules[2].

Recently, the authors had a chance to observe granular pericytes in the cerebral cortex after prolonged administration of a vitamin E deficient diet, and found regressive signs in them. These findings seemed to be important for an understanding of the physiology and nutriology of granular pericytes. This paper deals concisely with an ultrastructural change in granular pericytes under the conditions described below.

Material and methods. The vitamin E deficient diet contained 0.3 IU of vitamin E per 100 g of food and Kodak corn oil prepared with Oriental Co. (Tokyo), and supplied by courtesy of Eisai Co. (Tokyo). 10 Wistar male rats were fed with this diet for 10 months. The hair on their bodies became rough and they looked somewhat yellowish. The animals were killed by decapitation, and the cerebral cortex was sliced with a blade after removing pial tissue carefully in cold physiological saline, and then immersed in a mixture containing 2% paraformaldehyde and 2.5% glutaraldehyde buffered with 0.1 M phosphate solution (pH: 7.4) for 2 h. The specimens were then postfixed with 1% osmium tetroxide buffered with the same phosphate solution for 2 h. The other procedures for embedding and cutting were the same as in a routine method.

Observations. Figures 1 and 2 were obtained from controls and figures 3–6 from rats fed with the vitamin E deficient diet for 10 months. As seen in figures 1 and 2, the cytoplasmic organelles of the granular pericytes in control specimens were clearly defined, and electron-dense bodies with round or irregular shapes were distributed throughout the cytoplasm. Golgi lamellae and vesicles were evident in figure 2 and condensation of the perinuclear chromatin was obvious. Granular pericytes were delimited with a basal lamina. Surrounding them, smooth muscle cells and neuronal processes were depicted. However, the granular pericytes shown in figures 3–6 revealed dense matrices, and a lot of vesicles and vacuoles. Occasionally, a basal lamina became obscure and the interstices between the granular pericyte and the surroundings became wide (figures 3 and 4). In some parts, collagen fibers appeared (figure 4). Electron-dense bodies vacuolized and lost their limiting membrane (figure 5). Often mitochondria were swollen and the arrangement of their cristae became irregular (figures 4 and 6). As shown in figures 5 and 6, no signs of regression were observed in endothelium and smooth muscle cells adjacent to granular pericytes were observed in samples from the experimental group.

To summarize the findings mentioned above; a morphological difference in cytoplasmic organelles between control and experimental groups was marked in mitochondria,