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A note on the mechanism of action of UV-irradiation of amphibian embryos

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Summary. The actions on amphibian embryos of UV-irradiation, exposure to Li^+ or exposure to ouabain show interesting parallels with their effects on spontaneous release at the presynaptic terminals of the neuromuscular junction. It is suggested that these treatments serve to raise intracellular Ca^{2+} ($[Ca^{2+}]_i$) in these examples, and that UV-promoted abnormalities in embryogenesis are a consequence of changes in $[Ca^{2+}]_i$ at critical stages in development.

Two treatments that are known to produce abnormal development in amphibian embryos are exposure to UV-irradiation or to salines containing high concentrations of Li^+.

Li^+ has a typical 'vegetalising' action and produces characteristic abnormalities in which the normal balance of cell populations and the control of morphogenesis are disturbed[1]. Li^+ presumably accumulates intracellularly and is not readily removed by the cation pump[2]. The way in which Li^+ produces these effects is unknown, but biochemical comparisons between experimental and control embryos have shown several differences at the transcriptional and translational level[1] and it is suggested that the delay in cell differentiation of ectoderm is related to a lesion in the mechanisms of gene expression[3]. Li^+ has also been implicated in modifying cAMP levels via its inhibitory action on adenylate cyclase[4]. It may decrease the rate of cell division[5] and it has been suggested that it causes sub-lethal cytolysis[6]. We have now shown that intracellular accumulation of Na^+, produced by treating *Xenopus* embryos with ouabain, the inhibitor of the cation pump, also causes abnormal development[2].

UV-irradiation of the vegetal hemisphere leads to abnormalities in neural morphogenesis[7] and also to a complete subsequent absence of primordial germ cells, although primordial germ cells can be re-established if vegetal pole cytoplasm is injected into the vegetal hemisphere of irradiated eggs[8-10]. This action of UV-irradiation on the primordial germ cells has been of particular interest to embryologists; it has been suggested that the target for UV-irradiation is nucleic acid[8] or nucleoprotein[11]. The initial effect of UV-irradiation is an inhibition of cleavage, although nuclear division continues so that a syncytium is formed[11,12]. This inhibition of cytokinesis by UV-irradiation has also been shown in sea urchin eggs, cultured mammalian cells, protozoans and *Drosophila* eggs (see Beal and Dixon[11]).

There is an interesting parallel with the effects of these treatments on the rate of spontaneous release of transmitter at the amphibian neuromuscular junction, as measured by the frequency of the miniature endplate potentials (MEPPs). Exposure to Li^+ saline[13-15], or treatment with ouabain[16-18] or UV-irradiation[19] all produce a marked rise in MEPP frequency. There is good evidence that MEPP frequency is determined primarily by $[Ca^{2+}]_i$ at the presynaptic terminals[20]. Thus, Li^+-saline and ouabain both promote a rise in intracellular alkali metal cations which, in turn, causes a rise in $[Ca^{2+}]_i$[21,22] probably by release of Ca^{2+} from storage sites; both Na^+ and Li^+ have been shown to cause a dramatic release of Ca^{2+} from isolated mitochondria[23,24]. UV-irradiation at 255 nm (a wavelength close to that used in many of the studies with amphibian embryos) has been shown to act at a protein site essential for Ca^{2+}-binding on the inner face of crab axon[25]. It is concluded that these treatments all serve to increase MEPP frequency by raising $[Ca^{2+}]_i$ at the presynaptic terminals.

UV-irradiation of sea urchin eggs extends the time of the increased Ca^{2+}-ATPase activity during the 1st part of the cell cycle if the eggs are irradiated before fertilization. Irradiation at 50 min after fertilization causes delay of the 2nd cell cycle and also extends the increased Ca^{2+}-ATPase activity of the 1st part of the 2nd cycle. The Ca^{2+}-ATPase itself, however, is not directly sensitive to UV-irradiation[26].

It is therefore suggested that Li^+-saline[2] and UV-irradiation could both produce abnormalities in amphibian development by causing, initially, a rise in $[Ca^{2+}]_i$ in the embryos. Thus, the level of $[Ca^{2+}]_i$ produces a graded control of junctional permeability in *Chironomus* salivary gland cells, thereby providing a means of selective transmission of intercellular molecular signals[27]. Such an explanation would be in accord with recent studies with the divalent cation ionophore A23187 which suggest that an experimentally-induced rise in $[Ca^{2+}]_i$ in *Xenopus* embryos interferes with normal, integrated cell division[2].

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Release of plasminogen activator from normal and neoplastic endometrium[1]

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Summary. In the medium of endometrial carcinoma cultures, anti-urokinase-reacting plasminogen activator was released in contrast to cultures of normal or hyperplastic endometrium.

It has long been known that neoplastic tissue in culture releases fibrinolytic enzymes. Certain transformed mammalian cells in culture release a plasminogen activator not released by their untransformed counterparts[2,3]. In human ovarian carcinoma, a stable plasminogen activator is released which has recently been shown to cross-react with the plasminogen activator present in urine, i.e. urokinase[4]. A radioimmunoassay (RIA) has been designed for urokinase and used for the detection of neoplastic anti-urokinase-reacting plasminogen activators[5].

We report here the release of a plasminogen activator from normal and neoplastic endometrium in culture determined by degradation of a fibrin clot preformed in the culture tube and by RIA.

Material and methods. Endometrial specimens were obtained at hysterectomy. Histological examination of the specimens was performed and the diagnoses were found to agree with those from previous curettage specimens.

The specimens were divided into pieces of about 1 mm^3 and cultured as organ cultures with 4 cultures per specimen on gelatin foam (Spongostan, Ferrosan) in Leighton tubes. A purely synthetic medium (Parker 199, SBL) was used supplemented with the tripeptide Gly-Lys-His[6]. The culture tube contained a preformed fibrin clot formed by adding 1 ml of human plasminogen containing fibrinogen (Fibrinogen Kabi, 1% in distilled water) to 0.02 ml of thrombin (Topostasin, Roche, 75 NIH U/ml saline)[7]. To some of the cultures, the inhibitor of plasminogen activation, tranexamic acid (AMCHA) was added to the medium in a concentration of 1-2 mg/ml. At 24-h-intervals after beginning of the culture, a small volume (0.06 ml) of medium was aspirated and assayed quantitatively for fibrin degradation products by immunoelectrophoresis[8] and for anti-urokinase-reacting material in the RIA. Survival of the explants was checked by histological examination at the termination of the culture period. Control tubes contained preformed clot, gelatin foam and medium.

For radioimmunoassay, an antiserum against human urokinase was produced in rabbits[9]. J^{125}-urokinase was prepared

Mean concentration of FDP and levels of anti-urokinase-reacting material in the culture medium of normal endometrium, adenomatous hyperplasia and endometrial carcinoma (n = number of patients)

	Mean value of FDP (μg/ml)			Mean levels of anti-urokinase-reacting material (ng/ml)
	Day I	Day II	Day III	Day III
Normal endometrium (n = 5)	466	2473	6121	4
Normal endometrium with AMCHA 0.01 mg/ml	0	10	1172	
Normal endometrium with AMCHA 0.02 mg/ml	0	10	1215	
Control	0	0	0	
Adenomatous hyperplasia (n = 3)	1757	4045	5481	4.2
Adenomatous hyperplasia with AMCHA 0.01 mg/ml	0	572	4750	
Adenomatous hyperplasia with AMCHA 0.02 mg/ml	0	584	4030	
Control	0	7	62	
Endometrial carcinoma (n = 7)	1725	5773	8203	53.3
Endometrial carcinoma with AMCHA 0.01 mg/ml	14	2318	4856	
Endometrial carcinoma with AMCHA 0.02 mg/ml	17	2269	4635	
Control	0	18	46	