Table 2. Effect of continuous light on the urine volume and urinary glucose of alloxan diabetic rats

Treatment	Urine volume ml/24 h after 3 days	Urinary glucose g/100 ml/24 h after 3 days	Urine volume ml/24 h after 17 days	Urinary glucose g/100 ml/24 h after 17 days
Control	14.36 ± 0.38*		14.98 ± 0.44	
Diabetic rats	46.38 ± 1.09	3.53 ± 0.09	145.01 ± 0.88	8.60 ± 0.11
Diabetic + light	47.17 ± 1.11	3.68 ± 0.08	105.3 ± 1.36	5.82 ± 0.09
p-value:				
Control vs diabetes	< 0.001	-	< 0.001	-
Control vs diabetes + light	< 0.001	-	< 0.001	-
Diabetes vs diabetes + light	NS	NS	< 0.001	< 0.001

* Mean ± SE; NS, indicates statistically not significant.

days of light exposure urine samples were collected and the blood was obtained by cardiac puncture. Finally both the blood and urinary glucose content were estimated. All the animals were killed by decapitation. The adrenal glands were dissected out free of fat and weighed.

Results. Alloxan treatment in the female rats resulted in a significant rise of blood glucose and its excretion in the urine. The urinary volume and the weight of the adrenal glands were also increased markedly in alloxan diabetic rats as compared with controls (LD 10:14 h). The alloxanized rats showed a significant fall in their body weights compared with the controls. Continuous light exposure of diabetic animals appeared to decrease the rise of blood and urinary glucose along with the urinary volume. The weight of the adrenal glands showed a return to control levels in the light-exposed diabetic rats. Body-weight gain was found in diabetic rats shifted to continuous light (tables 1 and 2).

Discussion. The data reported here show that continuous illumination results in a considerable fall of blood and urinary glucose content as well as urine volume in diabetic rats. Similar depression of blood glucose level has been noted in the normal grass snake exposed to lighting for a prolonged period[12]. The mechanism through which light affects blood glucose level is yet to be determined. It has been reported previously that continuous illumination depresses the adrenocortical activity in alloxan diabetic rats[8,9]. Plasma corticosterone values appear to be higher in the female rats during the evening than in the day time[13]. On the other hand Cheiftz et al.[7] have reported a fall of plasma corticosterone levels due to reduced corticotrophin secretion in intact female rats exposed to constant light. A decrease in adrenal weight, as observed in alloxan diabetic rats shifted to continuous illumination, might be due to a similar cause.

Since the adrenocortical hypersecretion increases the severity of diabetes[2,3] and the removal of both the adrenal glands reduces the glucose output in alloxan diabetic rats[6], the present investigation suggests that the fall of blood and urinary glucose content in alloxan diabetic rats exposed to light is possibly the result of suppressed adrenocortical activity.

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Stimulating effect of the divalent cation ionophore A 23187 on in vitro neuroblast differentiation; comparative studies with myoblasts

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Summary. The effects of the divalent cation ionophore A 23187 and papaverine on the in vitro differentiation of isolated embryonic cells from young neurulae *(Pleurodeles waltlii)* were observed. These experiments suggest that an intracellular increase or decrease of the divalent cation concentration (Ca^{++}) stimulates or disturbs the morphological differentiation of already determined embryonic cells from neurulae but does not change their developmental pathway.

The involvement of Ca^{++}, Mg^{++}, Na^{+} and K^{+} in fundamental biological processes is now well established[2-5]. The important role of these cations in, for example, the early steps of embryonic development[6-8], in the regulation of embryonic induction[9-13] and in morphogenetic movements such as neurulation[14], is also now evident.

The primary effect of the ionophore A 23187 is to promote the transport of divalent cations, mainly Ca^{++}, either across an artificial lipid bilayer[15,16] or through the plasma membrane of cells[17,18] and also to release Ca^{++} ion from the intracellular organelles[8,13,19]. It is because of this that ionophore A 23187 is a very good tool for the in vivo study

of intracellular cation changes. Studies in vitro carried out by Barth and Barth with ectoderm of *R. pipiens* gastrulae suggest the ion dependence of embryonic induction. In this light, the aim of our experiments was:

- to find out whether neural plate cell determination (ontogenic step just following gastrulation) may still be changed by ionic intracellular modifications brought about by ionophore A 23187.
- to observe the comparative effects of this ionophore on the in vitro cytodifferentiation of various other embryonic cells (melanoblasts, myoblasts).

Material and methods. Isolated embryonic cells were prepared from neural plate, neural ridges and chordamesoblast of stage 13 neurulae[20] of *Pleurodeles waltlii* according to the technique already described[21]. The dissociated cells were cultured in the standard medium of Barth at 18 °C for approximately 3 weeks.

Treatment with ionophore A 23187 took place for 1 h (or 5 h): - at the beginning of the culture; - after the spreading of cells on the bottom of the culture-chamber, before the appearance of morphological differentiation.

The treating medium was removed and replaced, after several washings, by standard medium. The concentrations used here were determined in preliminary experiments. The active concentration of ionophore A 23187 (Lilly, France) which did not involve marked cytotoxicity (treated cells living as long as the control ones) was 1 $\mu g \cdot ml^{-1}$ ($5 \cdot 10^{-5}$ M). The concentration of methanol in which the ionophore was dissolved was also tested; it had no effect.

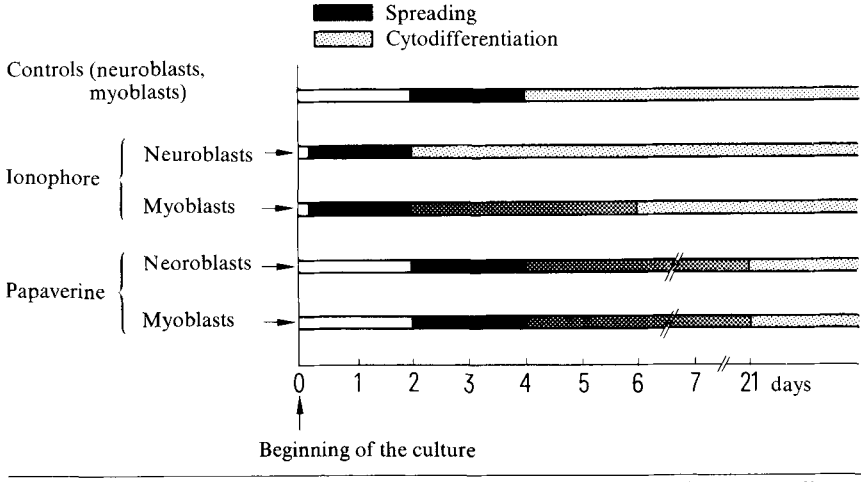
In the present investigation, experiments were also performed with papaverine which disturbs the Ca^{++} flux and induces Ca^{++} efflux[22-25]. Papaverine 100 $\mu g \cdot ml^{-1}$ ($4 \cdot 10^{-3}$ M) was directly dissolved in Barth's medium; treatment took place for 1 h at the beginning of the culture period. The observations involved 104 control cultures, 158 treated with ionophore A 23187 and 120 treated by papaverine.

Quantitative assessment of differentiation. A microscope field was selected. A count was made of the total number of cells in the field, followed by a count of the nerve cells. The fields contained between 50 and 250 cells. The number of nerve cells was expressed as a percentage of the total for each field. 1 culture-chamber yielded about 5 fields. 20 fields were taken for each condition tested.

Results and discussion. I. Effect of the ionophore on attachment and spreading of cells. The control cells, when put in the culture-chamber, with standard medium, settled at the bottom. They were round and filled with yolk platelets. Attachment of these cells to the glass required 2 or 3 days. Spreading took place only during the 3rd or 4th day (70% of cells attached and spread). Cytodifferentiation appeared after that (5 or 6 days of culture).

Based on in vivo morphological criteria the following cell types were clearly distinguished in normal cultures: myoblasts (approximately 20% of the total population of differentiated cells), neuroblasts (10-15%), melanoblasts (20%) occasionally chordal cells, and other unidentifiable cell types (glial cells, epithelial cells, fibroblasts). Similar observations were recently described for *Xenopus* neurulae

Effects of ionophore A 23187 and papaverine on the spreading and differentiation of embryonic cells of neurulae *(P. waltlii)*



	Attachment spreading	Differentiation
Control	+ + + (70% of cells)	+ + + { Myoblasts 20%, Neuroblasts 10%, Melanoblasts 20%
Ionophore A 23187	+ + + +	Myoblasts + + Neuroblasts + + + + Melanoblasts + + + +
Medium without Ca^{++}	+ (10%)	Myoblasts + Neuroblasts − Melanoblasts −
Medium without Ca^{++} + ionophore	+ + (30%)	Myoblasts + Neuroblasts + Melanoblasts +
Papaverine	+ + +	Myoblasts + Neuroblasts + Melanoblasts +

+ + +: Normal processes; + + + +: stimulated; + +: reduced or disturbed; +: very poor.

cells[26]. These cells were differentiated after 10–12 days[27]. They lived for about 3 weeks, as long as they had yolk and lipid in their cytoplasm.

Treatment with ionophore A 23187 (1 or 5 h), hastened attachment and spreading of cells (figures 1 and 2; table) which was almost total after only 2 h (3 or 4 days for the control cells).

II. Effects of the ionophore on cell differentiation. This substance strongly stimulated the differentiation of neuroblasts. The presence of axons was noticed very early in the treated cultures (2nd day instead of 5 or 6 days for controls). They were more numerous and better developed than in control cultures (figure 4, table). Quantitative analysis showed that in the controls, nerve cells formed between 4 and 16% of the total population of differentiated cells (per field): 11.2% mean. In treated cells, the range was 10–31% : 23.6% mean. This stimulatory effect was very similar to that we described for the nerve growth factor[28].

Melanoblasts were also seen to be stimulated (table). Muscle cell behaviour was different. Although myoblasts spread earlier, a delay of 1 or 2 days was observed in the appearance of striated myofibrils as compared to the controls (table). Moreover, in treated cultures, myofibrils did not show the normal arrangement along the main axis of the cell, but instead were irregularly arranged in thin and very long myoblasts (figure 5). This indicates that differentiation of myoblasts was disturbed, but not totally inhibited. These experiments suggest that, at the neurula stage, cell determination did not change after treatment with ionophore A 23187. All the cell-types which were observed in the control cultures were also differentiated in the treated ones. Contrary to what happens immediately after the

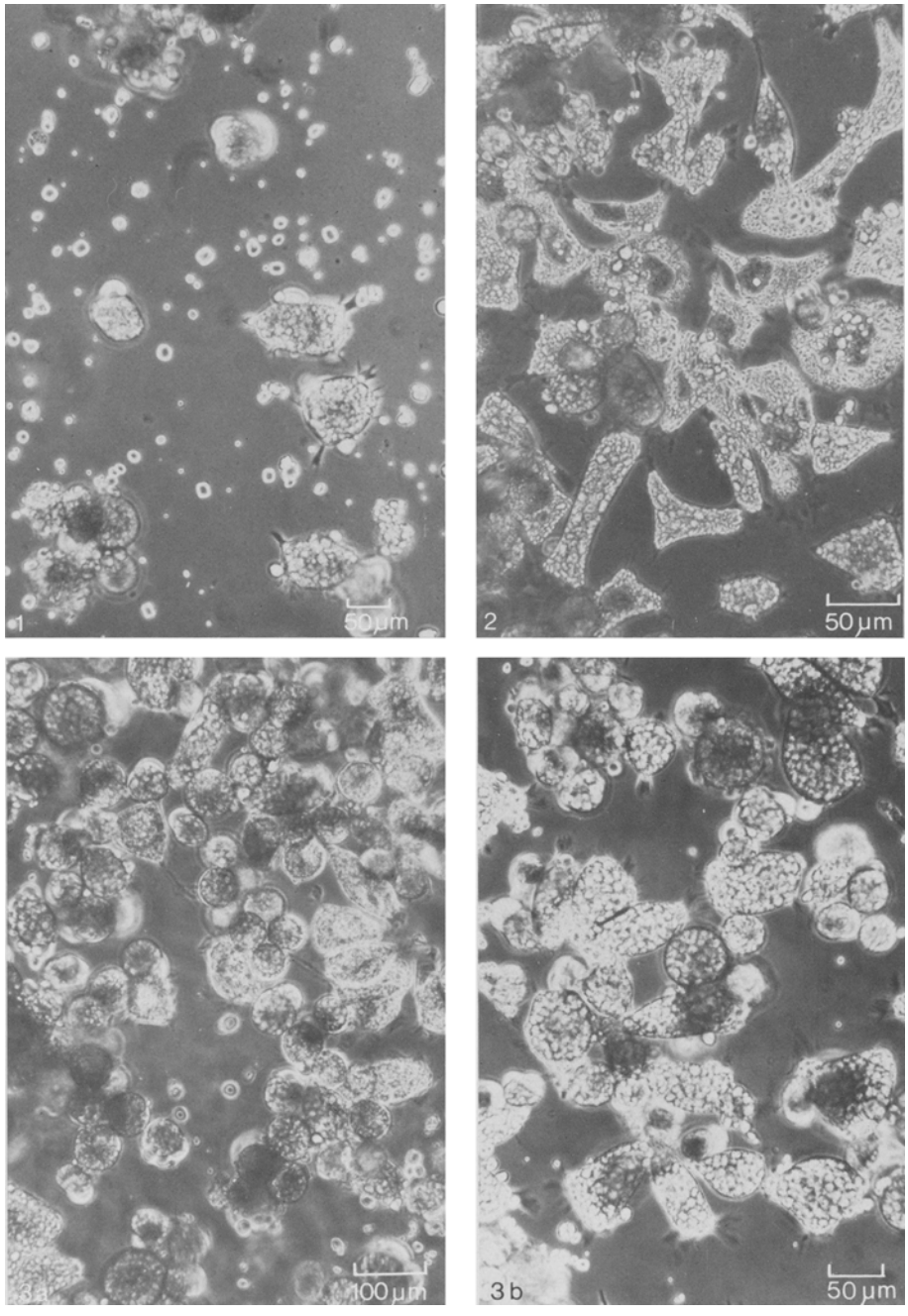


Fig. 1. Control embryonic cells from neural plate, neural ridges and chordamesoblast of a young neurula *(P. waltlii)*. Onset of spreading, 24 h of culture. Fig. 2. Large spreading of cells treated with ionophore A 23187 (1 $\mu g \cdot ml^{-1}$, 1 h), 24 h of culture. Fig. 3. *a* Control cells cultured in medium without Ca^{2+}, 6 h of culture. Poor adhesiom (10%). *b* Ionophore increases spreading of cells even in a medium without Ca^{2+} (30%).

determination of the gastrula ectoderm[13], a change in intracellular ionic concentration did not modify this determination at the young neurula stage. It should be underlined that the response of the various embryonic cell-types to the ionophore A 23187 was different. For all the cultures, identical experimental conditions were observed. The different cell-types were all taken from the same neurula and cultured in the same culture-chamber.

Treatment without Ca^{++} in the culture medium. Only 10% of the cells cultured without Ca^{++} (90 mM Na^{+}, 1 mM

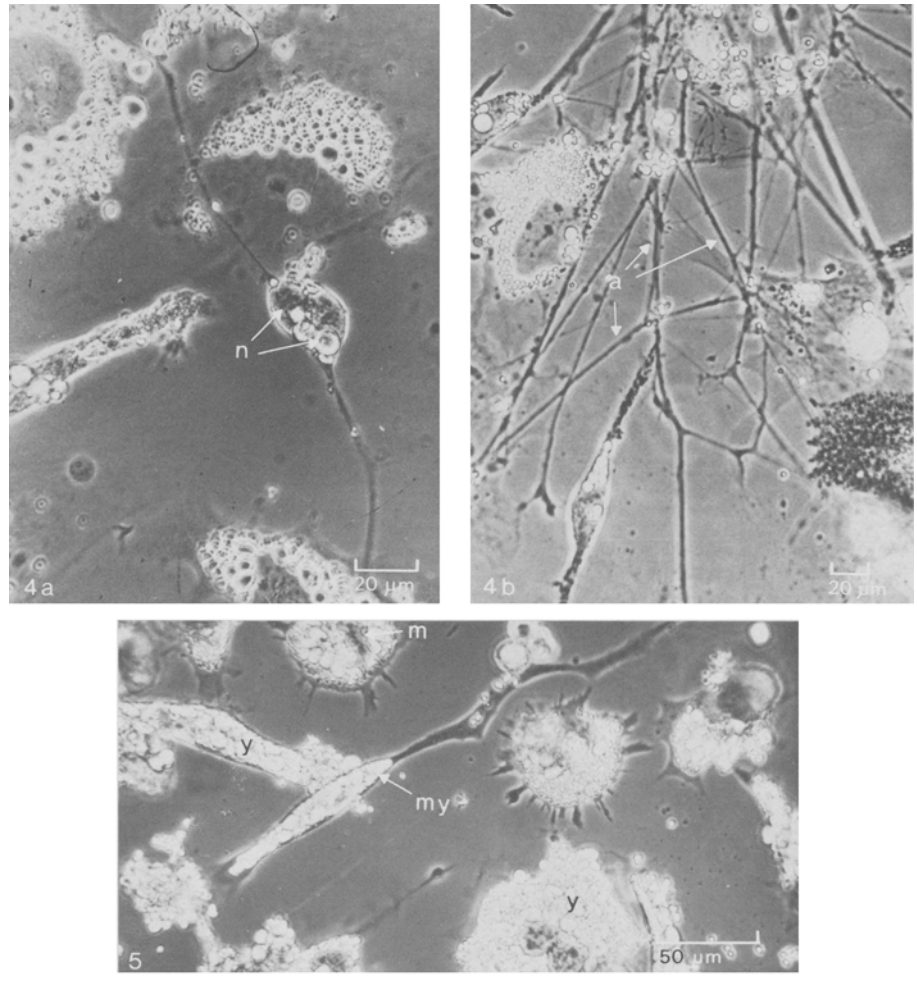


Fig. 4. *a* Control neural cells from a young neurula *(P. waltlii)*; n: neural cells. *b* Promoting action of ionophore A 23187 on neurites, 17 days of culture; a: axons. Fig. 5. Myoblasts treated by ionophore: Streching and narrowness of the cytoplasmic layer. 2 days of culture; m: mitosis, my: muscle cell, y: yolk.

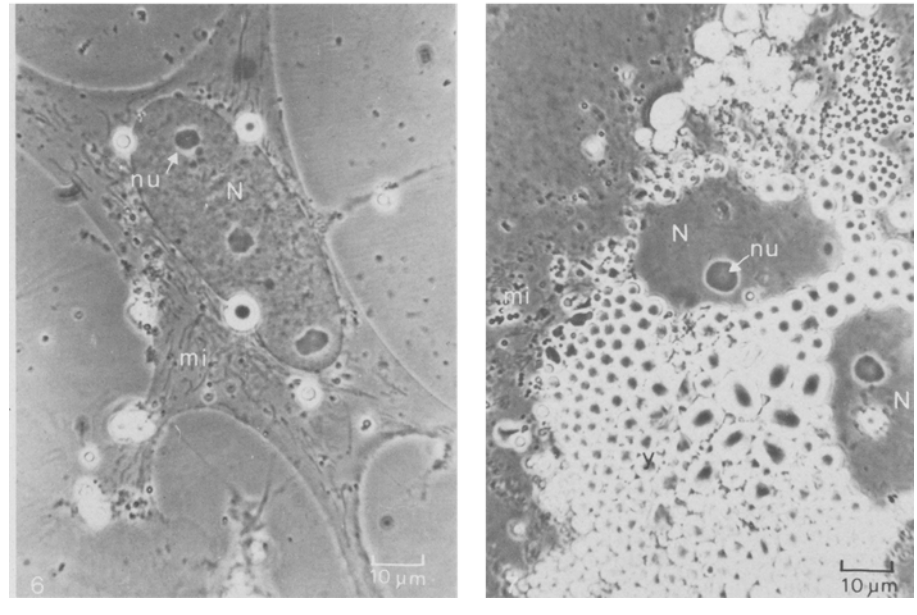


Fig. 6. Control cell from a young neurula *(P. waltlii)*. Easy observation of the cellular constituents. 19 days of culture; mi: mitochondria, N: nucleus, nu: nucleolus. Fig. 7. Papaverine (100 $\mu g \cdot ml^{-1}$, 1 h) blocks the utilization of the yolk. 24 days of culture; mi: mitochondria, N: nucleus, nu: nucleolus, y: yolk. Nuclear and cytoplasmic alterations (nucleolar fusion, round mitochondria).

K^+, 0.82 mM Mg^{++}) spread; the majority of them degenerated quickly, and the few surviving cells were poorly-differentiated myoblasts. Treatment with ionophore A 23187 facilitated attachment and spreading of cells cultured in Ca^{++}-free medium. About 30% of the treated cells spread (figure 3, table). A few neurites appeared after 5 days of culture. In myoblasts cross-striation was clearly seen on the 5th day, preceding formation of striated myofibrils in control cells cultured without Ca^{++}.

These stimulating effects of the ionophore on spreading and differentiation of cells cultured without Ca^{++} in the extracellular medium, could result from the release of intracellularly bound Ca^{++} from yolk platelets[13,19] and/or mitochondria[8] promoted by the ionophore A 23187. These experiments show that at the neurula stage there is a necessity for Ca^{++}, however there is already a different dependence of the various as yet morphologically undifferentiated cell-types, towards cations and principally Ca^{++}.

III. Effects of papaverine on this embryonic system. To complete these observations on the action of the ionophore, experiments were carried out with papaverine, which promotes Ca^{++} efflux.

The treatment by papaverine had no marked inhibitory effect on the attachment and spreading of cells. This suggests that papaverine does not alter the sites on the plasmalemma involved in cellular adhesion and spreading. Later on, papaverine treatment (only 1 h at the beginning of the culture) caused morphological modifications which were maintained during the whole life of the treated cells: yolk utilization was delayed (figures 6 and 7; table) and there were various nuclear and cytoplasmic alterations (figure 7). These modified cells did not degenerate and remained attached to the support when the culture-chamber was turned over, maintaining this new position for several weeks. Such cells survived for more than 1 month.

After about 3 weeks, there was a slight progress in differentiation but yolk utilization was still considerably slowed down. In neural cells, short and dumpy-looking axons appeared; myoblasts were fusiform and showed delayed and imprecise striation, masked by abundant yolk platelets; there were few melanophores (table). The cells degenerated after only 4 or 5 weeks.

Here again, papaverine disturbed, but did not change, the cell differentiation pathway. All the cell-types seen in the controls were also present in the treated cultures, indicating no differential inhibition of differentiation.

The effects of ionophore A 23187 and papaverine were quite irreversible since the duration of treatment was only 1 h (or 5 h) and the cells were then passed through 3 washes and maintained in standard Barth-medium for several weeks.

The experimental studies reported here provide evidence for differential cation (Ca^{++}) dependence of different types of cells in the amphibian neurula. Furthermore, they demonstrate that Ca^{++} does not alter cell determination at this stage.

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α-L-Aspartyl-L-alanine (a neural dipeptide) enhances synaptic transmission

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Summary. α-L-aspartyl-L-alanine, a dipeptide found in the brain, increases the amplitude of the miniature endplate potentials (MEPPs) in phrenic nerve-diaphragm preparations from rats. The peptide also stimulates the appearance of a population of 'giant' MEPPs.

The peptide, α-L-aspartyl-L-alanine, was recently identified by us[2] in the pig brain. As an initial step in determining whether this substance may have physiological activity in the nervous system, we studied its effect on spontaneous quantal release of acetylcholine at the neuromuscular junction in phrenic nerve-diaphragm preparations from rats. α-L-aspartyl-L-alanine increased the amplitude of the miniature endplate potentials (MEPPs) when used at 10^{-5} M concentration (figure 1). There was a shift in the mean amplitude of the regular MEPPs from 0.45 to