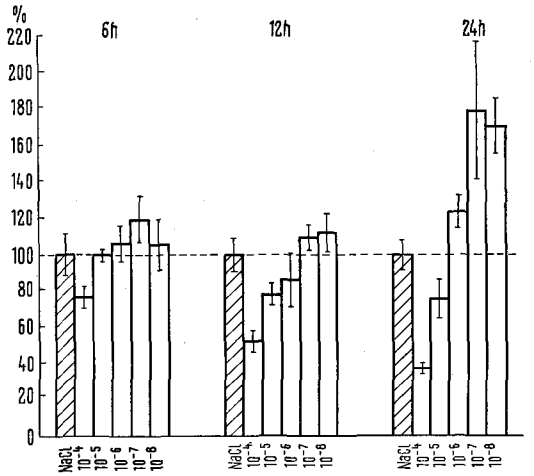
incorporation was found (down to 38% of the control value). At 10^{-7} and $10^{-8}M$, however, the incorporation was stimulated up to 180%. The degree of inhibition and of enhancement became more pronounced after 24 h. Enhanced incorporation was observed even at concentrations as low as $10^{-9}M$. Maximal enhancement occurred either after 12 or 24 h, obviously dependent on the pre-treatment of L-cells in the flask culture. The Table summarizes the incorporation data for the control cultures used in the Figure.



Thymidine incorporation into mouse fibroblasts under the influence of Prednisolone. Incubation procedures are described in the text. Given are % inhibition or increase compared to control values (saline). Each column represents the average of 5 roller tubes (3 measurements per tube), standard deviations are indicated.

Absolute thymidine incorporation into fibroblasts control cultures

	6 h	12 h	24 h
Cpm/0.1 ml culture	2971 ± 11%	7431 ± 9.9%	4465 ± 8%

Experimental details are given in the text.

Increase and decrease of thymidine incorporation appear as a function of the concentration of Prednisolone: high concentrations inhibit, low concentrations stimulate 'DNA-synthesis', no detectable effects in between. The concentrations given in the Figure are true concentrations, because no protein was present. Protein-binding, which normally reduces the concentration of free hormones, is therefore avoided[16].

Both effects, stimulation and inhibition are possibly independent of each other: enhancement of thymidine incorporation occurs without preceding inhibition and vice versa. It may be that the inhibition simply reflects toxic effects of Prednisolone. Thymidine incorporation, as measured by the method described, depends on cell number and/or the rate of actual DNA synthesis. Our experiments do not allow a separation of these 2 parameters; an enhancement of DNA synthesis, however, definitely follows from the data presented, because cell number itself is a function of DNA-synthesis, provided no polyploidization occurred. The bimodal action of Prednisolone, as found in our experiments, may give a hint how to explain the contradictory results of cortisol therapy in the treatment of tumours[17].

Zusammenfassung. Prednisolon wirkt auf L-Zellen in vitro bimodal: Der Einbau von ^{3}H-Thymidin wird in Abhängigkeit von der Hormonkonzentration gehemmt beziehungsweise gefördert.

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Embryonic Cell Surface: Electrophoretic Mobilities of Blastula Cells

In embryogenesis cells undergo ordered movements during gastrulation. If these movements are related to surface charge densities then cells from different embryonic regions might be expected to vary in this respect. Herein we report differences in electrophoretic mobilities (EPM) of presumptive ectodermal, mesodermal and endodermal cells from blastulas of *Xenopus laevis*. The EPM of early embryonic cells have to our knowledge not been reported previously.

Materials and methods. Eggs were collected in BROWN and CASTON's[1] saline with penicillin-streptomycin. Embryos (stage 8–9)[2] were dejellied in 1% cysteine:papain. Following transfer to 0.002M EDTA in $Ca^{++}Mg^{++}$ free STEARNS'[3] pH 8.0, vitelline membranes were removed manually and embryos allowed to dissociate for 1 h. A discontinuous density gradient of Ficoll ranging in density from 1.07 to 1.13 g/ml was prepared. Cells were layered on the gradient and centrifuged at 500 g, resulting in the formation of 6 bands (Figure 1). To identify the origin of these layers, 100 embryos were micro-dissected into 3 regions: animal pole, marginal zone and vegetal pole. These regions were dissociated separately and centrifuged in identical density gradients. Banding patterns for each region are shown in Table I. Ectodermal cells sedimented in the lightest regions (bands A and B), mesoderm in the intermediate bands (C, D) and endoderm in the densest regions of the tube (E, F). Some overlap, occurred presumably due to the technique of microdissection.

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Following centrifugation, cells of each band were resuspended in 0.145 *M* NaCl pH 7.2, for measurement of EPM. Measurements were made in a cylindrical cell electrophoresis apparatus[4].

To test for viability, cells collected after gradient centrifugation were cultured in Steinberg's solution[5] containing 0.5% albumin. Small aggregates were observed after 18–20 h in culture at 22 °C.

Results and discussion. Due to wide variation in the mobilities of each germ layer between different egg clutches, we measured 350 cells from each layer. Results of 15 experiments are summarized in Figure 2 and Table II. The EPM of presumptive ectodermal cells (layers A and B) is 1.38 μ/sec/volt/cm. That of presumptive mesodermal cells (Cand D) is 1.31 and 1.27 respectively, and the mobility of presumptive endodermal cells (E and F) 1.38. Differences in EPM between the mesodermal versus ectodermal and endodermal layers were statistically significant. Differences between the 2 mesodermal and between endodermal and ectodermal layers were not significant.

The EPM obtained for blastula cells is high compared to adult[6] and late embryonic cells[7]. To test whether the high EPM obtained were due to species differences or were characteristic of embryonic cells, we measured mobilities of erythrocytes from 2 species of amphibians. The mobility of *Rana pipiens* erythrocytes was 1.05 ± 0.01 and that of *Xenopus laevis* was 1.03 ± 0.07. Thus high electrophoretic mobilities are characteristic of amphibian blastula cells.

According to MAYHEW[8] dividing cells have higher EPM than resting cells. However, the high EPM obtained in blastula cells might not be due to division alone since gastrula cells have a low rate of division[9] but have higher mobilities[10]. High EPM is a consequence of cell surface charge and may reflect a high degree of cell repulsion[11]. On the other hand the hypothesis relating negative surface charge to cell adhesion through calcium binding[7] should not be overlooked.

Lower EPM of mesodermal cells compared to endodermal and ectodermal may reflect a greater tendency to deformability of the cell periphery as suggested by

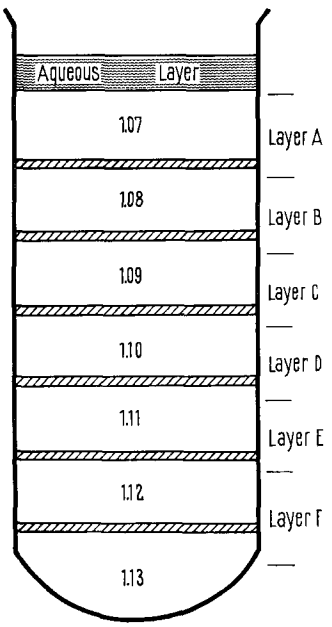

Fig. 1. Discontinuous density gradient, showing the bands obtained after centrifuging blastula cells at 500 *g* for 10 min.

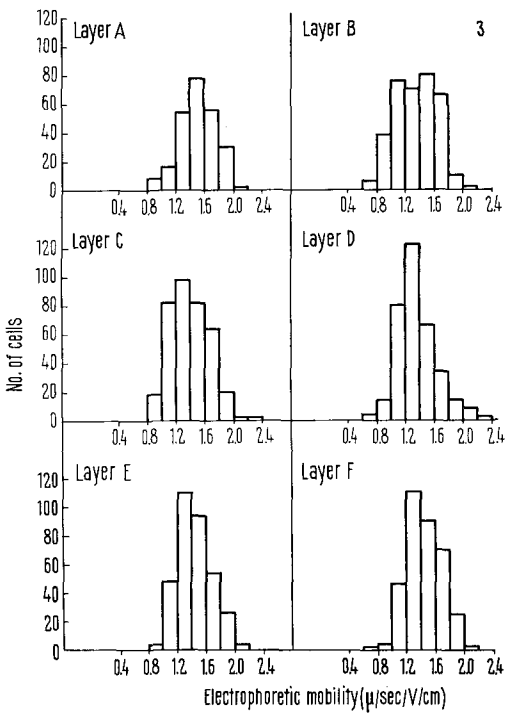

Fig. 2. Histogram indicating electrophoretic mobilities of each cell layer obtained by density gradient centrifugation. Each graph represents 350 cells derived from several clutches of eggs.

Table I. Density gradient centrifugation of germ layers at blastula stage, indicating the sedimentation of the 3 germ layers when isolated and centrifuged separately (see text)

Density	Animal pole	Marginal zone	Vegetal pole
Aqueous–1.07	+	—	—
1.07–1.08	++	—	—
1.08–1.09	++	—	—
1.09–1.10	+	++	—
1.10–1.11	—	++	+
1.11–1.12	—	—	++
1.12–1.13	—	—	++

Animal pole region contains presumptive ectoderm, marginal zone presumptive mesoderm and vegetal pole presumptive endoderm[2,5].

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From these experiments we conclude that: embryonic cells at the blastula stage have high electrophoretic mobilities, and mesodermal cells have significantly lower values than endodermal and ectodermal cells.

Table II. Electrophoretic mobilities of presumptive germ layers at the blastula stage

Layer	Density	Mobility ± S.E.
A	1.07–1.08	1.38 ± 0.015
B	1.08–1.09	1.38 ± 0.017
C	1.09–1.10	1.31 ± 0.016[a]
D	1.10–1.11	1.27 ± 0.013[a]
E	1.11–1.12	1.38 ± 0.012
F	1.12–1.13	1.38 ± 0.013

[a] Significant at the 0.01 level from ectodermal (A, B) and endodermal cells (E, F).

Résumé. La mobilité électrophorétique des couches germinales présomptives d'embryons de *Xenopus laevis* au stade blastula fut mesurée. Les valeurs obtenues ont été 1,38 μ/sec/V/cm pour les cellules ectodermiques et endodermiques et entre 1,27 et 1,31 pour les cellules mésodermiques, les différences de mobilité furent significatives.

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Localisation d'une Glycoprotéine tissulaire du rat dans les cellules de la lignée myéloblastique[1]

Nous avions isolé et caractérisé une glycoprotéine, extraite d'une tumeur primaire au 2-AAF[2,3]. Nous avions observé, par double diffusion et immunofluorescence, que cette protéine existe naturellement dans le foie embryonnaire et, chez l'adulte, dans la rate, la moëlle osseuse et le poumon[3]. La méthode de l'immunofluorescence avait montré que cette protéine se situe électivement dans les cellules de la lignée myéloblastique. Cependant, il est bien connu qu'au niveau des granulocytes et des histiocytes, il y a danger de captage non-spécifique de la fluorescéine[4]. Aussi, pour préciser davantage la localisation cellulaire de cette protéine, nous avons remplacé l'immunofluorescence par la nouvelle méthode de marquage d'anticorps avec les enzymes[5,6]. De plus, pour mieux détailler la morphologie cellulaire, nous avons utilisé la méthode des suspensions cellulaires de BIOZZI[7] qui permet de dégager d'un tissu, les cellules les unes des autres, tout en conservant intègre leur morphologie et même leur viabilité.

Matériel et méthodes. Le sang et la moëlle osseuse du rat adulte, de race Sprague-Dawley de 225 g et le foie de l'embryon de 20 jours ont été prélevés sous anesthésie au nembutal. Les suspensions cellulaires sont alors faites selon la méthode de BIOZZI[7].

Nous avons utilisé la méthode indirecte, à l'aide d'un anticorps de lapin monospécifique dirigé contre la glycoprotéine purifiée selon une méthode décrite ailleurs[3] et d'une anticorps de chèvre anti-gamma-globuline de lapin purifié par la méthode des immunoabsorbants[8]. Le marquage à la glucose oxydase des gamma-globulines de chèvre anti-gamma-globuline de lapin se fait selon la méthode d'AVRAMEAS[9] à l'aide, comme coupleur, de la glutaraldéhyde à 1%.

La vérification du marquage est faite par double diffusion, analyse immunoélectrophorétique et sur frottis de la rate du lapin hyperimmun[9]. La réaction immunohistochimique indirecte est faite sur les frottis de sang et sur les suspensions cellulaires de la moëlle osseuse et du foie embryonnaire fixés 45 min dans un mélange alcool-ether (60–40). On applique successivement sur ces frottis l'immunsérum de lapin anti-glycoprotéine dilué 1/50 et l'anticorps marqué à la glucose-oxydase (0,3 mg de protéine par ml) pour une période d'une heure. L'activité enzymatique de la glucose-oxydase est révélée selon la méthode décrite par AVRAMEAS[9].

Résultats. Cette méthodologie expérimentale permet de localiser spécifiquement la glycoprotéine dans le cytoplasme du myélocyte, du métamyélocyte, et du polynucléaire de la moëlle osseuse (Figure 1), le métamyélocyte du foie embryonnaire (Figure 2) et le polynucléaire neutrophile du sang circulant (Figure 3-B).

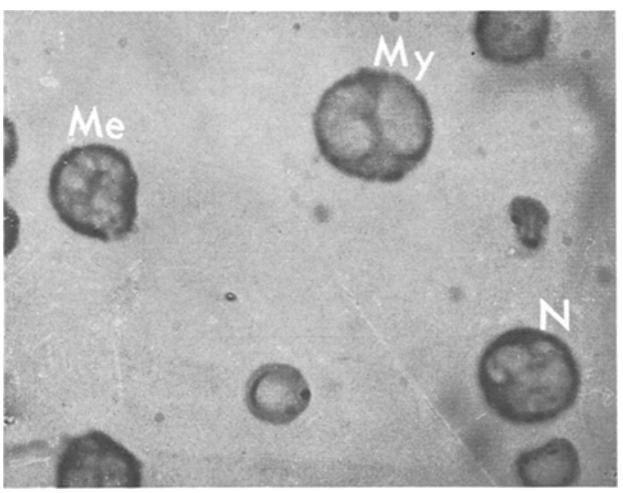

Fig. 1. Détection de la β-glycoprotéine dans les cellules de moëlle osseuse du rat adulte. Présence de la glycoprotéine dans le cytoplasme du métamyélocyte (Me) du myélocyte (My) et du polynucléaire (N).