Electrodiffusion simple en plaque

Dilution de	Quantités de l'ASH dans 0,5 ml de solution (μg)						
l'immunsérum	10000	1000	100	10	1	0,1	0,75
1: 6	+7,2	+3,6	+4,2	−1,0	−1,8	−2,4	
1:12	+6,8	+5,1	+1,0	−1,0	−1,8	−2,4	
1:24	+6,6	+5,6	+2,5	−0,4	−0,8	−1,8	
1:48	+6,4	+5,8	+3,5	+0,5	−0,4	−1,2	−1,5

Les chiffres représentent en mm les distances entre les fronts de précipités et les points de contact Ag/Ac.

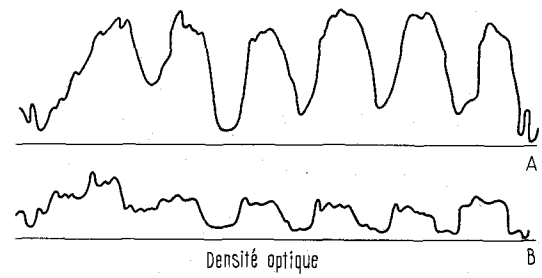

Fig. 4. Graphiques d'enregistrement de densités optiques (voir Figure 2): A) immunsérum dilué 1:6, B) immunsérum dilué 1:48.

immunoglobulines-anticorps ont été incorporés dans l'agar à pH 8,2.

Comme résultat, il a été constaté qu'au pH appliqué, la vitesse de migration d'IgG accuse de petites différences mais elles sont si peu marquées, qu'on ne peut les prendre en considération; au pH 5,5 l'IgG précipitait par dénaturation acide.

Dans une autre expérience, nous avons tenté de réaliser l'EDS en plaque d'agar à pH 8,2 en utilisant l'IgG humaine et l'immunsérum anti-protéines humaines, bien que les deux réactifs se caractérisant par la même direction de migration électrophorétique. Il a été observé que dans l'EDS en plaques des immunoglobulines (Ag), les précipités sont toujours situés dans des réservoirs contenant les mélanges l'IgG-antigène/agar. Avec dilution de l'immunsérum utilisé, on obtient des précipités plus faibles.

Les plaques peuvent être soumises à la lecture au densitomètre enregistreur qui traçe des graphiques de pics constitués par des précipité. En fonction d'un immunsérum dilué, les pics densitométriques sont plus hauts ou plus bas.

Les investigations entreprises, ont permi de constater que l'électrodiffusion simple en plaque s'adapte bien aux analyses qualitatives et quantitatives d'albumine, qui est une protéine anodique à pH 8,2. L'application du courant électrique augmente le rendement des analyses, étant donné que sous son action les Ag et Ac se déplacent plus énergiquement les uns vers les autres dans le gel, grâce à leur charge électrique opposée[6–11].

Les immunoglobulines – antigènes ou anticorps – se prêtent aussi aux analyses d'EDS donnant des résultats réguliers bien que dans ce cas la précision des réactions puisse être influencée par le fait que les immunoglobulines (antigènes) et les immunoglobulines (anticorps) se déplacent dans le champ électrique vers la cathode.

Appliquant la méthode EDS à l'étude d'un système donné Ag/Ac, on peut déterminer quantitativement les antigènes recherchés s'il a y excès d'antigènes par rapport aux anticorps ou inversement, ou s'ils se trouvent au point d'équivalence.

Summary. A simple electrodiffusion method on plates at pH 8.2 is described. Migration of proteins is accelerated by combination of diffusion and electrostatic forces. This procedure has several advantages: the results are obtained in much shorter time, antigens and antibodies are concentrated and for this reason the precipitation bands are visible at very low concentration of proteins. The results of this method can be presented graphically.

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18 mars 1971.

A Method for Time-Lapse Cinematography of Primitive Streak Stage Rat Embryos in Culture

The study of morphogenetic movements in living mammalian embryos presents many difficulties. With membranes intact, the embryos are too opaque and three-dimensional for clear high-power photographs, but an isolated, flattened embryonic disc may not survive long at early stages and undergoes at best a stunted development. Some limited success was achieved by earlier workers with rabbit and mouse embryos of primitive streak stages grown on plasma clots[1–4], and more recently DANIEL and OLSON[5] were able to follow some of the cel

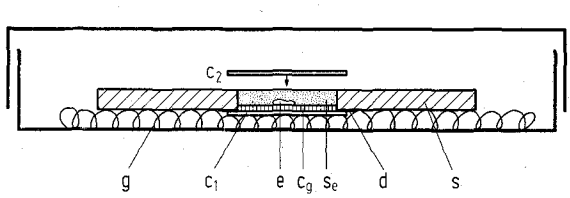

Fig. 1a. Culture slide, enclosed in Petri dish. C_1, C_2, coverslips; C_g, collagen; d, DPX seal; e, embryo; g, gauze; s, slide; Se, serum.

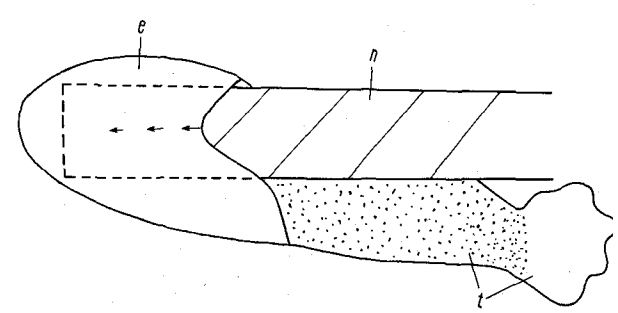

Fig. 1b. Diagram of operation. Arrows show direction of cut. e, embryonic vesicle; n, glass needle; t, trophoblast region. ---, part of needle inserted into vesicle.

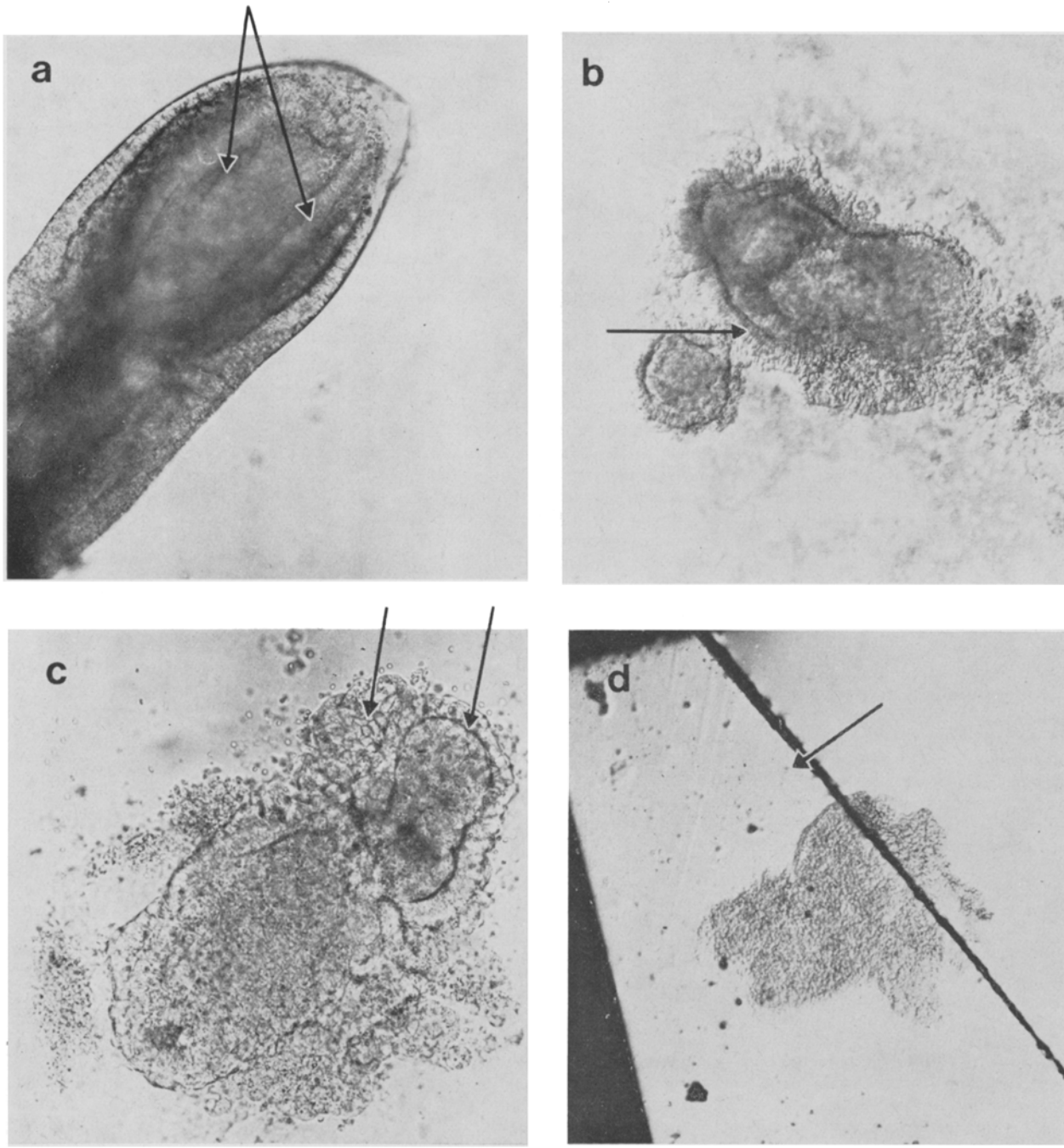

Fig. 2. a) Whole embryo (developing axis arrowed). ×400. b) Flattened embryonic disc, showing columnar neural fold cells (arrowed) at edge. ×400. c) Higher power view of flattened preparation showing some cell detail (arrowed). ×700. d) Preparation held flat by strip of cellophane (arrowed). ×300.

movements during axis-formation in the rabbit by vital dyeing techniques. But we still lack any detailed and continuous observations of the formation of the primitive streak and early embryonic axis in the mammal, comparable to those carried out on avian embryos.

Considerably improved development of neurula and somite-stage mouse and rat embryos was obtained by NEW and STEIN[6] and NEW[7] using liquid serum in watch-glass cultures and divesting the embryo only of its outer, Reichert's membrane. This method was found suitable for experimental work on 9–11-day rat embryos[8,9] and has now been modified for the culture of 8-day embryos for cinematography, in work to be described here. The culture chamber (Figure 1a) consists of a glass square, 2 mm thick with a hole 1 cm diameter bored through it, to the base of which a coverslip is sealed with DPX mount-

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ing medium (baked at 140° for 2 h after drying, to harden it and also for sterilisation purposes). The coverslip is coated with rat-tail collagen[10], equilibrated with Tyrode saline before use. The chamber is filled with rat serum (obtained by heart puncture from the pregnant female before sacrificing her for removal of embryos) and is enclosed in a Petri dish with damped gauze (cf. NEW[7]).

Eight-day rat embryonic vesicles are dissected out of the uterus in Tyrode saline, washed in 2 changes of serum, then placed in the culture dishes. If they are to be photographed entire, they are placed centrally and left undisturbed so that the ectoplacental cone can attach to the collagen (cf. HSU[11]). Flat preparations of the embryonic disc are made by first splitting and removing the thin, tough outer Reichert's membrane with jagged glass needles, then with one glass needle inserted into the central cavity, making a longitudinal cut with a second needle (see Figure 1b) after which the embryonic vesicle can be opened and the apical, disc region of it flattened onto the collagen surface, to which it sticks. Either the outer, endoderm surface or the inner, ectoderm surface can be made to adhere to the collagen. In our experience, preparations stay flat longest with the ectoderm surface next to the collagen. In some cases, the ectoplacental cone was left attached to the flattened disc by a thin strand of tissue, but although this may have promoted survival it enhanced the tendency of the disc to round up into a vesicle after 6–8 h. Such vesicles contained disorientated neural fold tissue. A few of our earlier preparations stayed flat for at least 12 h however, so that a time-lapse cinematographic record could be taken of their development.

Before filming is started, the culture dishes are gassed with 95% O_2/5% CO_2 as described previously[7,8] and left for at least 2 h in the incubator undisturbed at 37°C. Cultures which had not been pre-gassed in this way were not able to survive during the filming, which was carried out in a Prior filming hot-box with a Wild inverted microscope, so that the culture was viewed from the bottom surface next the collagen. A coverslip was placed on top to prevent evaporation, and in these conditions both whole embryos and flattened pieces remained healthy and continued development for at least 24 h. Figure 2a shows a whole embryo with neural axis forming and 2b shows a blastodisc with neural fold tissue along its borders, both photographed after a 24-h filming period. A number of time-lapse films of both types of preparation are being obtained and will be analyzed for details of morphogenetic movements. Whole embryos are still sufficiently transparent at this stage to be viewed remarkably clearly under a low-power objective, and details of the shape changes and movements of surface cells can be followed under high power in the flattened preparations which show cell detail quite well (Figure 2c). It has been found possible to invert the chamber during the culture period and so to obtain views of the opposite surface as well as of that next the collagen. To prevent the disc from rolling up too rapidly a small piece of cellophane may be laid on it (Figure 2d) which keeps it fully flattened for 18–24 h.

Zusammenfassung. Eine Kammer für die Kultur von Embryonen wird beschrieben, die es erlaubt, Zeitrafferfilme der Entwicklungsvorgänge über sehr lange Zeitspannen herzustellen.

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[10] The authors are grateful to Dr. L. W. GREENHAM and Mrs. J. TOWNSEND for supplies of collagen and for advice on its preparation. The expenses of this work are partly covered by a grant from the Agricultural Research Council.

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ACTUALITAS

Roussel Prize 1972

The prize is given every two years to a chemist or biochemist whose work has been chosen as the best by an international Committee of outstanding scientists in the field.

The first Roussel Prize was awarded to Professor W. S. Johnson for his work on non-enzymatic biogenic-like steroid synthesis. The appropriate lecture was delivered on June 17th 1970 in Paris at the celebration of the 50th anniversary of the Roussel group foundation.

The Award Committee for the year 1972 is as follows: Prof. D. H. R. Barton, Prof. D. Arigoni, Prof. K. Bloch, Prof. L. Canonica, Prof. M. Fetizon, Prof. J. Jacques, Prof. G. Storck.

The Prize is not intended to reward a scientific career but to honour original and pioneering work in the steroid field. Nomination should be submitted before March 1972. Any supplementary information from the Secretary: Prof. J. Mathieu, Centre de Recherches, Roussel-UCLAF, F-93 Romainville (France).

CONGRESSUS

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Further information by Doz. Dr. K. Moser, Wiener Medizinische Akademie, Alserstrasse 4, A-1090 Wien (Austria).