discarded, and the plates carefully rinsed once or twice with medium before being returned with fresh medium, to the incubator. Large plates used in the selection procedure for myoblast cultures also constituted a valuable source of pure cultures of mesenchymal cells, if care was taken to rinse them before returning them to the incubator, in order to remove all floating myoblasts[10].

Résumé. La plupart des cellules mésenchymateuses présentes dans une suspension cellulaire obtenue par trypsination de cœurs de rats nouveau-nés s'attachent au récipient de culture avant les myoblastes. Cette propriété est utilisée pour obtenir des cultures confluentes contenant une proportion élevée de myoblastes actifs.

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Quantitation of Fibrinolytic Agents Released in Tissue Culture

The endothelium of certain vessels contains a potent fibrinolytic agent[1,2] that is believed to be continuously presented to the bloodstream to break down fibrin deposits and thereby prevent thrombosis[3,4].

Tissue cultures have been used to investigate the mechanism of this release. Supernatants of cultures of renal and of some other tissues[5,6] show fibrinolytic activity on fibrin plates, according to ASTRUP and MÜLLERTZ[7]. But we have found the results of such assays during tissue culture to be less uniform. This may perhaps be explained by variation of the rate of release of the fibrinolytic agent and by the unknown amount of the agent inevitably inactivated at the temperature (37 °C) prevailing during culture.

The method described below was first developed to avoid the error due to possible fluctuation in the rate of release of this activity; and secondly, to avoid the loss due to denaturation and thereby to get a better idea of the amount of the agent released during a certain period. We cultured human tissues in the presence of, but not in direct contact with, a standard fibrin clot. The amount of fibrin digested was then calculated directly from the degradation products accumulated in the culture medium.

Lung, liver, bone marrow and kidneys from normally developed 16–20-week-old foetuses were obtained at legal abortion by hysterotomy. We used foetal tissue so that we could culture it in a defined synthetic medium. Explants from the organs were cultured as organ cultures on gelatine-sponge (Spongostan, Ferrosan, Malmö) in Leighton tubes containing 1 ml of Parker 199 (Statens Bakteriologiska Laboratorium, Stockholm) synthetic medium and a preformed clot obtained by adding 1 ml of human fibrinogen (Fibrinogen Kabi, 1% in distilled water) to 0.02 ml of thrombin (Topostasin Roche, 75 NIH U/ml saline). The explants were not in contact with the standard clot (Figure). Every 24 h after the beginning of culture, a small volume (0.06 ml) of medium was aspirated and assayed quantitatively for fibrin degradation products, according to an immunochemical method[8]. At the end of the culture period (4 days), the explants were examined histologically as well as histochemically for activators of fibrinolysis by a modified[9] fibrin slide technique[2]. We also determined the inhibitors of urokinase, the α_2-macroglobulin and the total antitrypsin activity[10].

Fibrin degradation products regularly appeared in increasing amounts in the medium of lungs, kidneys and bone marrow (Table). They were practically absent in the culture medium of liver and controls, i.e. gelatin sponge alone. Conventional histological sections showed good survival of the explants. The fibrin slide technique revealed fibrinolytic activity located around small blood vessels in the explants from all the organs except those of the liver. Such activity was also demonstrable in explants cultured for up to 2 weeks. No inhibitors of

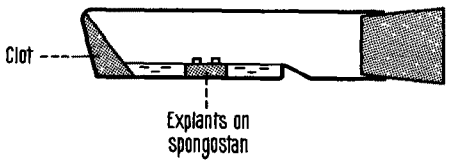

Diagram of culture system.

Illustrative example of fibrinolytic split products in the culture medium (mg/100 ml)

Day	1	2	3	4
Control 1	0	0	0	traces
Control 2	0	0	0	1
Liver	0	0	0	1.5
Lung	3.5	5	100	145
Bone marrow	2.5	9.5	24	67
Kidney	3.5	40	350	450

[1] T. ASTRUP and O. K. ALBRECHTSEN, Scand. J. clin. Lab. Invest. *9*, 233 (1957).
[2] A. S. TODD, J. Path. Bact. *78*, 281 (1959).
[3] G. R. FEARNLEY, *Fibrinolysis* (Williams and Wilkins, Baltimore 1965), p. 56.
[4] I. M. NILSSON and M. PANDOLFI, XV Annual Meeting International Committee on Haemostasis and Thrombosis. Conference on Vascular Factors and Thrombosis, Bath, England (1969).
[5] R. H. PAINTER and A. F. CHARLES, Am. J. Physiol. *202*, 1125 (1962).
[6] M. B. BERNIK and H. C. KWAAN, J. clin. Invest. *48*, 1740 (1969).
[7] T. ASTRUP and S. MÜLLERTZ, Arch. Biochem. *40*, 346 (1952).
[8] J.-E. NILÉHN, Thromb. Diath. haemorrh. *18*, 3 (1967).
[9] M. PANDOLFI, I. M. NILSSON, B. ROBERTSON and S. ISACSON Lancet *ii*, 127 (1967).
[10] U. HEDNER, I. M. NILSSON and C. D. JACOBSEN, Scand. J. clin. Lab. Invest. *25*, 329 (1970).

urokinase or total antitrypsin activity were demonstrable in any of the tissue cultures but α_2-macroglobulin was found in small amounts (4–8% of that in normal plasma) in the medium of all the cultures.

The results are in good agreement with the known high fibrinolytic activity of lung tissue, bone marrow and renal tissue and with the absence of such activity in the liver of the adult[11] and in the foetus[12,13]. The minute amounts of degradation products in liver cultures and in the controls on the last day of culture may presumably be explained by spontaneous formation of plasmin from plasminogen contaminating fibrinogen and thrombin. The persistence of activity, even in the explants cultured for 2 weeks, suggests that the fibrinolytic agent is not only released from, but also synthetized, in the explants.

Denaturation of the plasminogen content of the fibrin by heating the clot at 85°C[14] and the use of medium containing a specific inhibitor of plasminogen activator, such as Cyklocapron® (0.01 ml per ml Parker medium), prevented the digestion of the clot and thereby suggested that the agent liberated by the cells is an activator of plasminogen[15].

Zusammenfassung. Es wird eine neue Methode zur Bestimmung der freigesetzten Menge fibrinolytisch wirksamer Stoffe in Gewebekulturen beschrieben. Das Gewebe wird in Leightonröhrchen mit einem standardisierten Fibringerinnsel gezüchtet. Die abgebaute Fibrinmenge – als Mass der fibrinolytischen Aktivität – wird durch immunologische Bestimmung der Spaltprodukte gemessen.

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University of Lund (Sweden), 29 October 1970.

[11] T. Astrup, Fedn Proc. *25*, 42 (1966).
[12] Aa. Ladehoff, Acta paediat., Stockh. *57*, 500 (1968).
[13] M. Pandolfi, B. Åstedt and I. M. Nilsson, XIII International Congress of Hematology, Munich, Germany (1970).
[14] M. Lassen, Acta physiol. scand. *27*, 371 (1952).
[15] This research was supported by grants from the Swedish Medical Research Council No. B71-19X-87-07A and the Faculty of Medicine, University of Lund (Sweden).
[16] Koagulationslaboratoriet, Allmänna Sjukhuset, S-21401 Malmö 8 (Sweden).

The Chorio-Allantoic Membrane. A New Site for Development of Mouse Embryos

Mouse embryos have been successfully grown in various extra uterine sites[1–5]. Their ability to survive even in inter-strain and inter-specific transplantation[6,7] emphasizes the absence of an immune response in early embryos and shows they can be used under various experimental conditions.

The chorio-allantoic membrane (CAM) of the chick has been widely used for the maintenance of a variety of explants and micro-organisms. Different types of cells have been grown on the membrane successfully. The present method was developed to study the interaction of different cell types grown in contact with pre-implantation embryos at the onset of differentiation of embryonic cells. A millipore filter was used to locate the embryos on the CAM.

White leghorn eggs incubated for 9 days were used as hosts for the embryos. A window was made in the shell, the shell membrane removed and the CAM exposed. Mouse embryos from animals autopsied 3 days after the

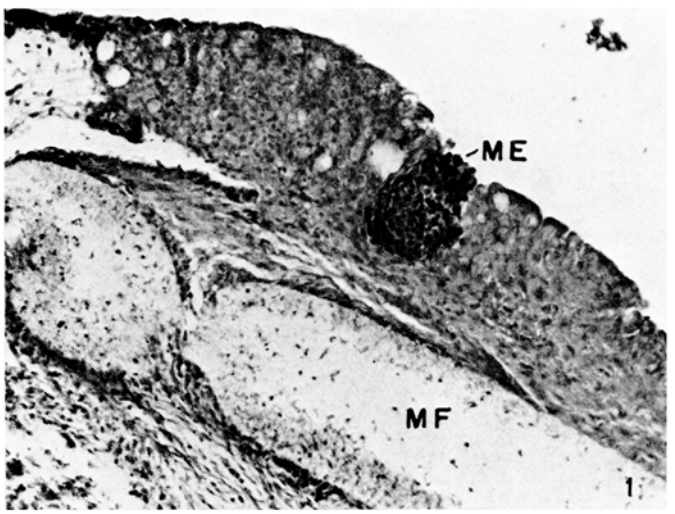

Fig. 1. Surface epithelium of CAM.

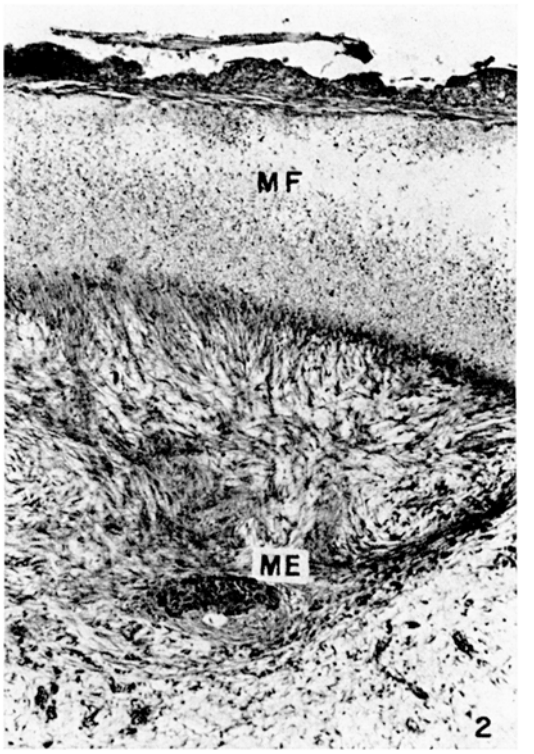

Fig. 2. Surface epithelium below the CAM epithelium and deep in the stroma.