mierten wie auch bei Kontrolltieren je 145 unabhängige Integrationsstellen gezählt. Folgende zytoplasmatische Komponenten wurden analysiert: Mitochondrien (M), endoplasmatisches Retikulum (E), Golgi-Komplex (G), Interzellularräume (Int), Lipidgranula (L), Sekretgranula (S), Lipofuszingranula (Lf), Cilien (C) und zytoplasmatische Matrix (Mtr).

Nach beendeter Analyse verhielten sich die Relativfrequenzen der sämtlichen zytoplasmatischen Komponenten wie folgt:

	M	E	G	Int	L	S	Lf	C	Mtr
Kontrolltiere	6,4	0,7	3,0	3,9	0	3,1	0,4	0,01	82,49%
		10,1%							
Versuchstiere	10,02	4,0	4,8	7,5	0,2	1,5	0,5	0,2	71,28%
		18,82%							

Die ersten drei zytoplasmatischen Komponenten, welche als Indikatoren der Zellaktivität angenommen wurden, sind in bezug auf Trefferzahl pro Plättchenstellung am Wahrscheinlichkeitsnetz dargestellt. Aus dem Diagramm 1 ist ersichtlich, dass die Treffer in den ersten drei Komponenten Gaußsche Verteilung haben und dass bei epiphysektomierten Ratten eine grössere Zahl der Treffer diese Komponenten haben, was hoch signifikant ist ($P < 0{,}001$)[4].

Dieser Befund steht in gutem Einklang mit früheren Analysen der Epithelkörperchen nach der Pinealektomie, welche durch verschiedene Methoden gewonnen wurden[5].

Aus unseren Integrationsanalysen von zytoplasmatischen Komponenten an elektronenmikroskopischen Aufnahmen ist ersichtlich, dass eine der lichtmikroskopischen Integrationsanalyse ähnliche Treffermethode ausführbar ist und in einer früheren Arbeit[6] wurde von verlässlichen Ergebnissen dieser Methode bereits berichtet.

Schliesslich ist bei der Interpretation der gewonnenen Ergebnisse grosse Vorsicht notwendig, da diese lediglich im Zusammenhang mit allen anderen Analysen, die uns heute zur Verfügung stehen, interpretiert werden dürfen. Eine Analyse, die ausschliesslich mit dieser Methode begründet würde, wäre vollständig unzuverlässig.

Résumé. Par l'application de la méthode d'intégration aux points sur les clichés électronomicroscopiques, on a constaté l'hypertrophie des organelles cellulaires (mitochondries, le réticulum endoplasmique et l'appareil de Golgi) dans les cellules parathyroïdiennes de rats sacrifiés trois semaines après l'épiphysectomie.

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Institut für Histologie und Embryologie der Medizinischen Fakultät, Novi Sad (Jugoslawien), 5. November 1965.

[4] E. Weber, *Grundriss der biologischen Statistik* (Jena 1961).
[5] R. Krstić, Naturwissenschaften *2*, 40 (1965).
[6] R. Milin, M. Šćepović und R. Krstić, VIII. Internationaler Anatomenkongress, Zusammenfassungen der Vorträge (Wiesbaden 1965), p. 80.

A Method for Perfusing Tissue Culture with an in vivo System

Rose chambers[1–4] have been modified as follows (see Figure 1): The two stainless steel plates (A and G) are 5 mm thick, 50 mm wide, and 65 mm long; the bottom coverslip (B) lies on a gasket (C) of silicon rubber (Silastic Dow Corning Co., Midland, Mich., USA) from 1–0.1 mm thick; the top coverslip has been replaced by the 'diffusion device' (Figure 2) consisting of three components: (1) The blood-containing ring (F) is a Perspex or Makrolon plate (50 · 65 · 2.5 mm); in the centre of it is carved a 330° arc with an inner diameter of 23 mm. The carved channel. is 0.5 mm deep and 1 mm wide. The two ends of the channel are connected to the exterior of the chamber by two conical holes drilled in the thickness of the plate in which are inserted the polyethylene tubes (H) connected with the artery of the animal. Four retaining holes corresponding to those of the stainless steel plates are drilled in the corners. (2) The membrane (E) separating blood from the environmental medium, lies between the channel of the above plate and the channel of the plate described below. (3) The membrane support (D) is a plate similar to the plate (F) except that its thickness is 1.5 mm and the conical tubes connecting to the exterior are absent. Alternating parts of the carved channel are completely drilled through the plate permitting the communication between the channel and the face of the plate which is in direct contact with the medium. Thus the chamber can be assembled as any other Rose chamber by inserting the 'diffusion device' instead of the top coverslip. Sterilization of the diffusion device is performed in 70% ethanol for 24 h. After sterilization the parts are soaked for a few hours in three changes of Gey's balanced salt solution. In the described chamber, 0.6 ml of medium (or less according to the thickness of the gasket, C) are in equilibrium with the arterial circulating blood of the animal through a membrane surface of about 50 mm². The pores of the membrane can be chosen from a large series

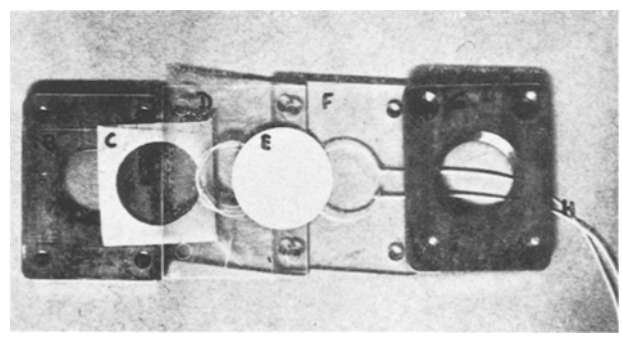

Fig. 1

[1] G. C. Rose, C. M. Pomerat, T. O. Shlinder, and J. B. Trunnel, J. biophys. biochem. Cytol. *4*, 761 (1964).
[2] G. C. Rose, Tex. Rep. Biol. Med. *12*, 1074 (1954).
[3] G. C. Rose and C. M. Pomerat, J. biophys. biochem. Cytol. *8*, 423 (1960).
[4] G. C. Rose, Cancer Res. *23*, 279 (1963).

of sizes as indicated in the Table. The cells growing in the chamber can be examined with a long-focus condenser phase-contrast microscope. Medium for chemical analysis can be drained from the chamber.

The set-up for an experiment is presented in Figure 3. 2 days before a study, rats (but other animal species can be used as well) are treated with a single oral dose of 8 mg/kg of sodium Warfarin. This anticoagulant agent, unlike heparine, is non-toxic for tissue cultured cells up to a concentration of 300 mg/ml of medium.

Because of the high toxicity of anaesthetics such as pentobarbital and ethyl-urethane, animals under light ether anaesthesia are injected the day before perfusion with 0.05 ml of ethanol into the spinal cord between the 1st and the 2nd lumbar segment. This type of spinalization permits us to keep the cannulated animal in a restraining cage in good condition for a few days when food and water are provided.

Two polyethylene cannulas of 1 mm external diameter are inserted in the proximal and distal parts of the same femoral artery. The other ends of the cannulas are connected to each of the two conical tubes of the diffusion device.

The chamber is then situated in a thermostatic box at 37 °C. Preliminary experiments have demonstrated that, with an 'm' type membrane (Table), cells of KB strain (human nasopharingeal carcinoma) cultivated in Eagle's medium[5] without serum, were able to grow normally after several hours of perfusion with rat arterial blood, when the non-perfused ones were unable to grow in such a condition. Perfusion can be carried out from a rat for 8 h or more. Anyway, 1 or 2 h of perfusion are normally enough as medium renewal. The same chamber can be perfused many times during a period of a few days.

This method is now currently used to study the cytotoxic effect of the blood of rats injected with antitumoral drugs. Besides this particular problem, the method described may have many other applications such as following the effect of hormonal endogenous secretion or the action of exogenously injected chemical agents[6].

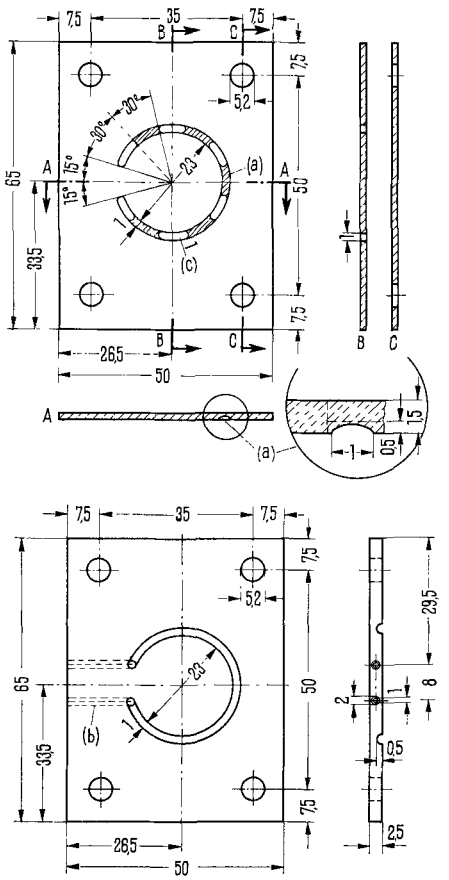

Fig. 2

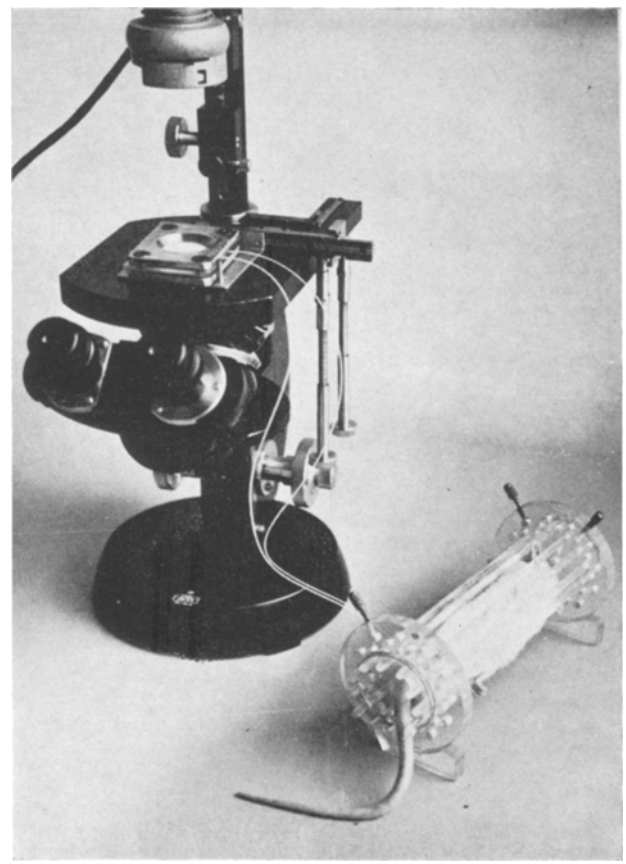

Fig. 3

Membrane filter membranes[a]

Type	Pore size
Ultracella fff	< 5 nm
Ultracella ff	5– 10 nm
Ultracella f	10– 20 nm
Ultracella m	20– 35 nm
Ultracella g	35–100 nm

[a] Membranfiltergesellschaft GmbH, 34 Goettingen (West Germany).

Riassunto. Il lavoro descrive una modificazione della 'camera' di Rose che permette la perfusione delle culture tramite una membrana semipermeabile.

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[5] P. C. PARKER, *Methods of Tissue Culture* (P. B. Hocher Inc., New York 1961).

[6] Acknowledgment: The new chamber was made by Techniplast Co., Buguggiate (Varese, Italy) according to our suggestions.