THEORIA

Ligand-Leakage in Affinity Chromatography: a Note on the Mathematical Approach

In a recent paper, GRIBNAU and TESSER[1] derive mathematical expressions for the concentration of free ligands and the half-time of leakage. The authors use graphical solutions and the Newton-Raphson method to solve their equation (3)

$$e^{-k\tau}\sum_{p=0}^{n-1}\frac{(k\tau)^p}{p!}=\frac{1}{2} \qquad (1)$$

This equation can easily be solved using a wellknown relation in mathematical statistics. Equation (1) is the cumulative probability function of the POISSON-distribution with parameter $\lambda = k\tau$ and is related to the χ^2-distribution with ν degrees of freedom by

$$Q(\chi^2|\nu) = \sum_{p=0}^{n-1} e^{-kt}\frac{(kt)^p}{p!} \qquad (2)$$

Table I. Computation of $k\tau_n$, using equation (2) and tabulated values of the χ^2-distribution

n	ν	$\chi^2_{Q;\nu}$	$k\tau_n$
1	2	1.38629	0.69315
2	4	3.35670	1.67835
3	6	5.34812	2.67406
4	8	7.34412	3.67208
5	10	9.34182	4.67091
6	12	11.3403	5.67015

Table II. Values of kt for varying values of n and C_N/a, using tabulated values of the χ^2-distribution[3].

C_N/a		10^{-2}		10^{-4}	
n	ν	χ^2	kt	χ^2	kt
1	2	0.02010	0.01005	0.00020	0.00010
2	4	0.29711	0.14856	0.02842	0.01421
5	10	2.55821	1.27911	0.88892	0.44446
10	20	8.26040	4.13020	4.39516	2.19758

where $\chi^2|\nu$ is the Q-quantile of the χ^2-distribution with $\nu = 2n$ degrees of freedom and $kt = (\chi^2)/2$. Hence the value of $k\tau_n$ can be found from any table of the χ^2-distribution[2,3] by taking half of the tabulated χ^2-value for $\nu = 2n$ degrees of freedom and $Q = 0.5$ (Table I). A very accurate approximation for τ_n can be derived from the WILSON-HILFERTY-approximation of the χ^2-distribution[2] for large ν ($\nu > 30$).

$$\chi^2_{Q;\nu} \simeq \nu\left\{1-\frac{2}{9\nu}+u_Q\sqrt{\frac{2}{9\nu}}\right\}^3 \qquad (3)$$

where u_Q is the Q-quantile of the standard normal distribution. Setting $Q = 0.5$, $u_Q = 0$, the approximation yields

$$\tau_n \simeq \frac{n}{k}\left(1-\frac{1}{9n}\right)^3 \simeq \frac{n}{k}\left(1-\frac{1}{3n}\right) \qquad (4)$$

This approximation is even accurate enough for n as small as 5 (it should be noted that for $n = 1$, $\tau = (ln\ 2)/k$ as given in[1] is exact!). Using relation (2), solutions to kt for varying values of C_N/a can be found using appropriate tables of the χ^2-distribution[3] and setting $P = 1 - Q = C_N/a$ (Table II).

Zusammenfassung. Für die von GRIBNAU and TESSER angegebene Auswaschfunktion wird eine Lösungsmöglichkeit mit Hilfe der χ^2-Verteilung angegeben.

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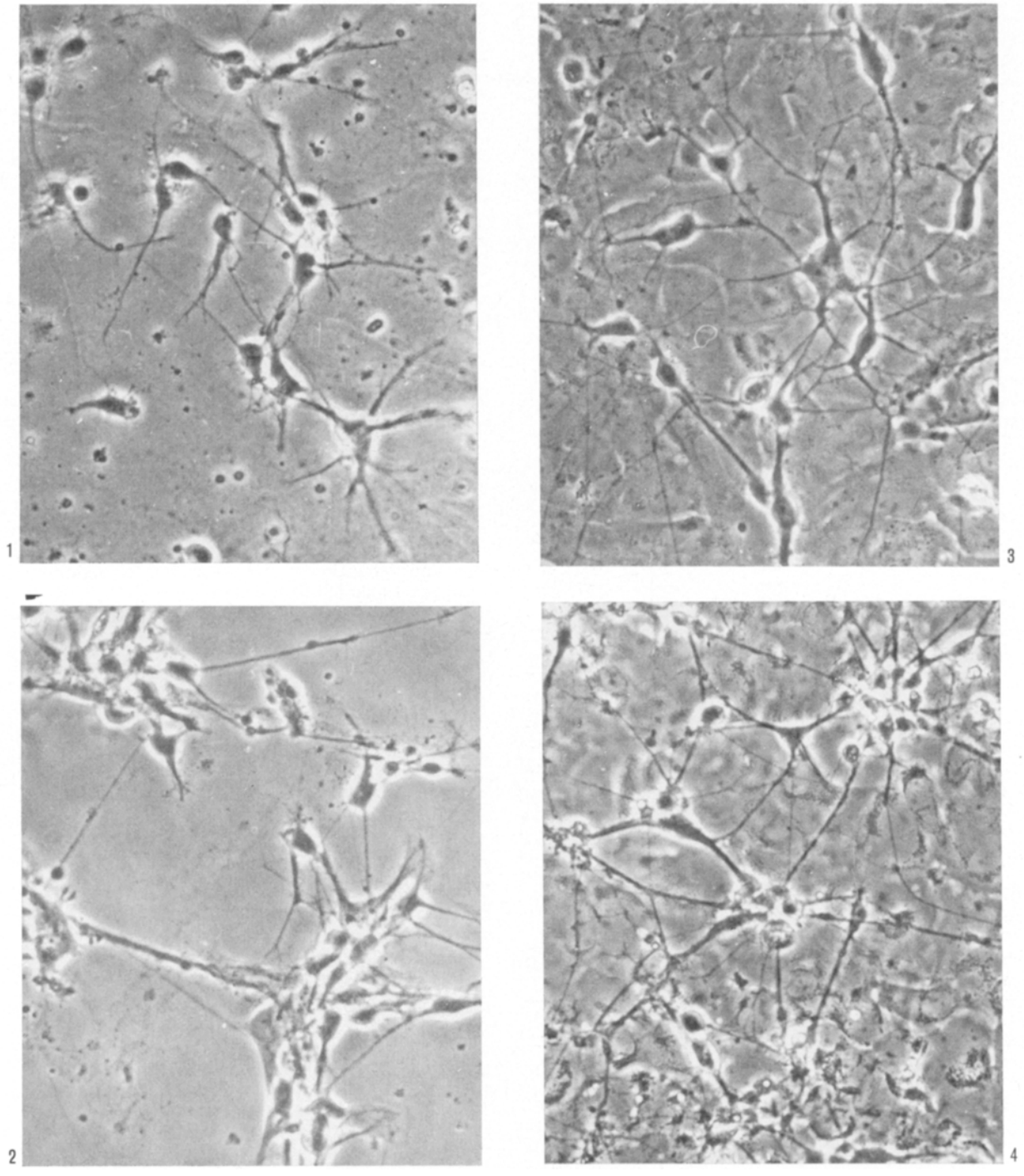
[1] T. C. J. GRIBNAU and G. I. TESSER, Experientia *30*, 1228 (1974).
[2] M. ABRAMOWITZ and I. A. STEGUN, *Handbook of Mathematical Functions* (Dover Publications, New York 1965).
[3] E. S. PEARSON and H. O. HARTLEY, *Biometrika Tables for Statisticians*, vol. 1 3rd edn. (Cambridge University Press 1970), vol. 2 (1972).

PRO EXPERIMENTIS

Simplifications to Substrate Preparation for the Cultivation of Dissociated Nerve Cells

Reconstituted collagen[1] has been used with success as substrate for the cultivation of nerve tissue explants[2] and of dissociated nerve cells[3,4]. However, the presence of toxic ammonia vapours used for the reconstitution process necessitates a time-consuming rinsing procedure, making the method inconveniently long, especially in laboratories where a great number of experiments have to be performed. Plastic substrate has been used without collagen, but in this case the settlement and the onset of differentiation of the nerve cells were retarded[5]. A method of photo-reconstitution for the collagen, that makes the preparation of the substrate more rapid, was



Figs. 1–4. Dissociated nerve cells from chick embryo cerebral hemispheres in culture. Phase contrast. ×250. 1 and 3. On collagen prepared by method 1. 1: 4 days in culture; 3: 14 days in culture. 2 and 4. On collagen prepared by method 2. 2: 4 days in culture; 4: 14 days in culture.

described recently[6]. In the present communication we report our experience in the use of non-reconstituted collagen as substrate for nerve cell cultures.

Materials and methods. Dissociated cerebral hemisphere cells from 7-day-old chick embryos were obtained by the method described elsewhere[4]. The nerve cells were cultivated in ROSE chambers[7] as well as in plastic Petri dishes and plastic flasks. The medium used was Eagle's minimal medium supplemented with 20% fetal calf serum and 500 mg/100 ml glucose. The denaturated collagen was obtained from the tail of the rat by a slightly modified version of the original method[1,2]. At this stage, instead of reconstituting the collagen, we prepared the substrate by one of the following two methods.

Method 1: a few drops of denaturated collagen (depending on the size of the surface) were spread out in a thin film

over the culturing area. The preparation was left standing at room temperature for 20 min, after which the cellular suspension was introduced into the culturing chamber.

Method 2: the denaturated collagen was mixed with balanced salt solution (BSS) in various proportions (1 to 50%). The mixture was introduced into the culturing chamber and left standing for at least 12 h, after which it was replaced by the cellular suspension. The presence of a thin layer of collagen was established in some control experiments by staining with anilin blue-orange G-acetic acid according to the method of HEIDENHAIN[8].

Results. The results described in the present communication have first been indicated to us by a previous series of experiments in which we prepared over 100 cultures of dissociated cerebral hemisphere cells in ROSE chambers. For the purpose of the experiments, only a small portion of each coverslip was prepared with reconstituted collagen according to the method of BORNSTEIN[2]. It was noticed, however, that in all the cultures cellular differentiation proceeded on the entire surface of the coverslip. We postulated that some collagen remaining in the denaturated state must have been transferred to the uncovered surface during the washing procedure. Consequently we reasoned that the reconstitution process was not necessary at all, and we performed over 200 experiments to prove it.

We found that both methods of substrate preparation, described above, gave rise to good cellular differentiation, but that the appearance of the cultures during the first 4 days was different and depended on the concentration of the denaturated collagen. With a 100% collagen (method 1) mainly isolated neurons differentiated during the first 4 days (Figure 1) and the glia only started to multiply and to form a monolayer after the 4th day. With a lower percentage of denaturated collagen (1 to 50%, method 2), the flattening of the glia started already after 24 h and the neurons differentiated on the glial layer (Figure 2). This was an improvement in comparison with cultures on plastic alone where the above-mentioned process started only after 3 days. After about 1 week, when the complete glial layer has been formed, there were no discernible differences between the cultures prepared by the 2 methods using the denaturated collagen (Figures 3 and 4).

Discussion. Apart from the simplified procedure for both of the methods described, the second of them (viz, using BBS-denaturated collagen mixture) has a further advantage. As the result of only a thin film of denaturated collagen, the cultures can be used for biochemical investigations equally well as those on plastic substrates. With normal collagen substrate preparations, certain of these investigations have proved to be difficult because of the interference of the excessive amount of collagen with the protein determinations[9].

The method incorporating the simplifications has been equally successful on glass and on plastic surfaces and is therefore applicable to cultures in ROSE chambers, in plastic Petri dishes and in plastic flasks. LUDUENA[10] has cultivated dissociated ganglionic neurons on gelatin substrate with success. It is probable that the denaturated rat tail collagen used in our experiments is structurally very similar to the gelatin used by the above-mentioned author.

Résumé. Nous avons simplifié la méthode de préparation du substrat «collagène» pour la culture de cellules nerveuses dissociées. On obtient une fixation et une différenciation rapide des cellules sur ce substrat. Les cultures conviennent pour des études morphologiques, biochimiques et électrophysiologiques.

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[1] R. L. EHRMANN and G. O. GEY, J. natn. Cancer Inst. *16*, 1375 (1956).
[2] M. B. BORNSTEIN, Lab. Invest. *7*, 134 (1958).
[3] Z. LODIN, J. BOOHER and F. H. KASTEN, Expl Cell Res. *59*, 291 (1970).
[4] M. SENSENBRENNER, J. BOOHER and P. MANDEL, Z. Zellforsch. *117*, 559 (1971).
[5] J. BOOHER and M. SENSENBRENNER, Neurobiology *2*, 97 (1972).
[6] E. B. MASUROVSKY and E. R. PETERSON, Expl Cell Res. *76*, 447 (1973).
[7] G. ROSE, Tex. Rep. Biol. Med. *12*, 1074 (1954).
[8] M. HEIDENHAIN, Z. wiss. Microsk. *30*, 161 (1913).
[9] A. EBEL, R. MASSARELLI, M. SENSENBRENNER and P. MANDEL, Brain Res., in press.
[10] M. A. LUDUENA, Devel. Biol. *33*, 268 (1973).
[11] Permanent address: Department of Physiology, University of Pretoria, Pretoria, South Africa.
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A Simple Method of Studying the Effects of Drugs and Cytotoxic Agents at the Ultrastructural Level

A variety of ways have been described for culturing cells in vitro[1,2]. Before techniques for thin sectioning were developed, several investigators grew cells on formvar-coated cover-slips and examined them after fixation under the electron microscope[3]. A double-embedding procedure was developed by GAY and modified by HOWATSON and ALMEIDA[4]. Many workers now employ standard methods to culture cells and then treat the culture with a chelating agent or with an enzyme in order to disaggregate the cells so that they can be centrifuged down into a pellet for embedding in epoxy resin[1,5]. Such procedures, however, may adversely alter the morphology of the cell. We have found it convenient to grow HeLa cells in 'BEEM' capsules (Ernest F. Fullam, Inc., Schenec-

[1] J. PAUL, *Cell and Tissue Culture*, 4th edn. (E. and S. Livingstone, Edinburgh 1970).
[2] R. C. PARKER, *Methods of Tissue Culture*, 3rd edn. (Pitman Medical Publishing Co. Ltd., London 1961).
[3] K. R. PORTER, A. CLAUDE and E. M. FULLAM, J. exp. Med. *81*, 233 (1945).
[4] A. F. HOWATSON and J. D. ALMEIDA, J. biophs. biochem. Cytol. *4*, 115 (1958).
[5] J. H. T. CHANG, Tissue Cell *4*, 561 (1972).