The potential significance of such similarities of molecular structure lies in the fact that LSD for example is a potent psychotomimetic drug[14], phenothiazines are in general antipsychotic[11] and dopamine, as a probable central neurotransmitter, is being increasingly implicated in normal and abnormal behaviour patterns partly since neuroleptic potency can be correlated with dopamine antagonist potency[9,10,16–18]. If it is possible to identify a common site of action at the molecular level we may be nearer to identifying the primary cause of psychotic disorders.

The possible existence of a common receptor site for dopamine, ergot alkaloids and phenothiazines may explain some of the pharmacological interactions mentioned above, and would seem to explain some structure-activity relationships. To test the validity of these ideas it would be interesting to test a phenothiazine such as that in Figure 3A with a side chain carbonyl grouping, or that in Figure 3B with a complete 'ring D equivalent' for antipsychotic and LSD-antagonistic properties. What would be the effects of lysergic acid amide with a piperazinylethanol side chain as in fluphenazine?

Résumé. On a construit des modèles moléculaires de plusieurs phénothiazines et du LSD, et observé que la structure des phénothiazines peut se superposer à celle du LSD. Cette observation explique peut-être pourquoi les phénothiazines manifestent un antagonisme envers les alkaloïdes d'ergot. La similarité de ces structures est la plus exacte pour les phénothiazines qui combattent le plus efficacement les psychoses.

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[16] T. J. Crow and G. W. Arbuthnott, Nature New Biol. *238*, 245 (1972).
[17] J. M. van Rossum, Neuropsychopharmacology *5*, 321 (1967).
[18] N.-E. Andén, S. G. Butcher, H. Corrodi, K. Fuxe and U. Ungerstedt, Eur. J. Pharmac. *11*, 303 (1970).

PRO LABORATORIO

A Simplified Method of Nerve Organ Culture

The double coverslip assembly technique introduced by Maximow[1] is one of the most frequently used methods for nerve organ culture. It provides a good system for visual observation, using bright field and is also more economical in terms of feeding solution requirement than the other organ culture methods, e.g., the flying coverslip technique[2], the flask method[3], etc. However, it also has considerable disadvantages. The concave well, acting as an optical system, interferes with the use of phase contrast. The nutrient medium cannot be rapidly changed and

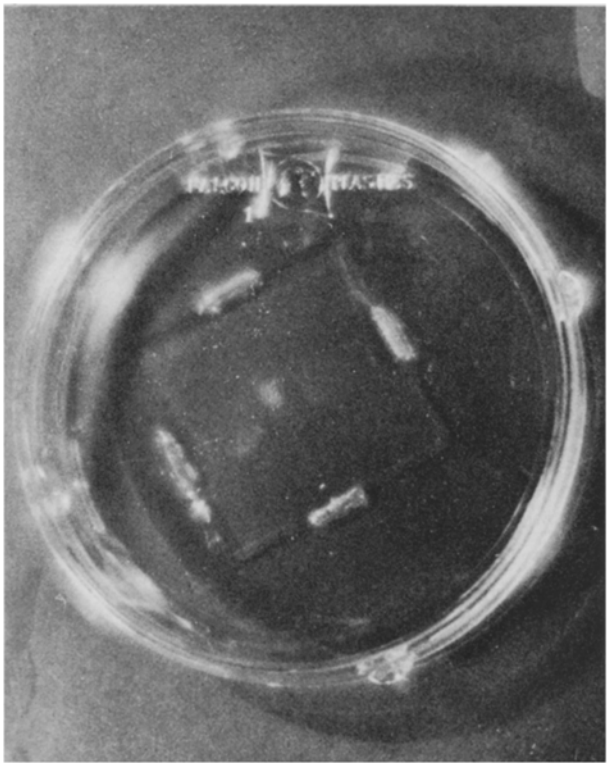

Fig. 1. Square coverslip with nerve tissue on its surface in the Falcon Plastic Petri Dish. The grooves are well seen along the side of the coverslip.

Fig. 2. Neuron and glia cells from newborn rat motor cortex, 10 days after explantation. Phase contrast. × 500.

finally, it takes a considerable time to feed the individual cultures (approximately $2^1/_2$–3 min per culture for a skilled person to remove the cultures from the double coverslip assembly, to place it on a new square coverslip, to feed it, to place it and seal it in a newchamber). Furthermore, the lengthy preparation of the glassware and the inevitable breakage of a certain percentage of the not- inexpensive glass chambers renders this method rather costly.

Some of the disadvantages mentioned, particularly the poor optical quality for phase contrast work and the difficulty with the rapid change of feeding medium, can be eliminated by the use of the ROSE multipurpose chamber[4]. The ROSE chamber, however, is relatively expensive and its use is unsuitable for a laboratory where a great number of cultures are carried at once.

A simple, inexpensive method of nerve organ culture was developed in this laboratory, essentially substituting the MAXIMOW chamber with a polystyrene plastic petri dish fitted with a tight lid (Falcon plastic petri dish No. 1006, 50×12 mm; Falcon Plastics, Division of B–D Laboratories, Inc., Los Angeles, California 90045).

Method. The collagen-coated round or square No. 1 glass coverslip with the tissue on its surface, instead of being mounted into a double coverslip[5], is placed in a No. 1006 Falcon plastic petri dish. A shallow groove is made along the edge of the coverslip, by touching the bottom of the plastic dish with the warmed prong of a flat forceps. This prevents displacement of the coverslip and also the potential drainage of the feeding medium from its surface. 0.05–0.10 ml feeding solution is placed on the coverslip and the dish is closed by pressing the tight lid to the bottom part (Figure 1). The petri dish is kept in an incubator (in an atmosphere of 95% air and 5% CO_2, with 98% relative humidity) at a temperature of 34–36 °C. The cultures are fed twice a week. The lid is pried open, the feeding solution is removed by gentle suction after the culture is slightly tilted to one side to promote collection of the fluid and is replaced by 0.05–0.10 ml new feeding solution. The coverslip, with the culture mounted on it, is never removed from the assembly, nor is the plastic dish ever changed.

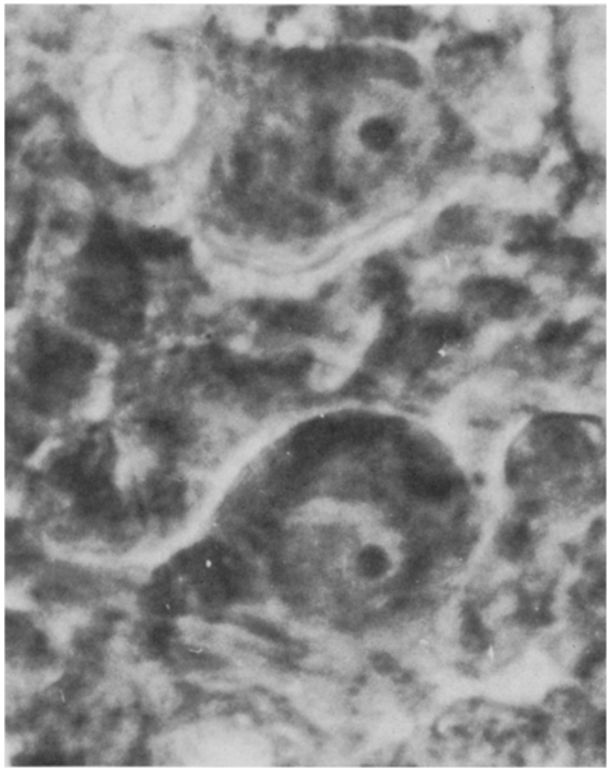

Fig. 3. Spinal ganglion cells from 14-day-old rat fetus, 30 days after explantation. Phase contrast. ×500.

With this method a 60–70% reduction in feeding time can be achieved. Evaporation of the feeding solution through the tight fitting lid is very slow even in a relatively low-humidity environment. We experienced no difficulty when this system was used on a long-term basis for time-lapse cinematography in an environment of 40% humidity, provided the culture was fed daily.

For microscopic observation a long working distance objective is used (Zeiss Planachromat 40–0.60, working distance 1.5 mm), placed in a correction mount to compensate for the relative thickness of the plastic.

The culture can be viewed either in an inverted microscope or in an ordinary microscope by 'flipping' it in an upside-down position like the MAXIMOW double coverslip system.

The morphological appearance of the tissue, its maturation, myelin formation is identical to the one observed in a nerve organ culture grown in the MAXIMOW double-coverslip assembly (Figures 2 and 3). Preparation of the collagen-coated coverslips for this system is a modification of the method described by BORNSTEIN[6]. Tendons (wet weight in the range of 0.30 g) from the tail of a 3–4-month-old rat are placed overnight in 300 ml 0.1% sterile acetic acid solution at room temperature. The non-dissolved part settles on the bottom of the flask and the supernatant is used without prior centrifugation or dialysis. Ammonia from the collagen-coated coverslips, after being exposed to ammonia fumes, is removed by vacuum suction – as described by OKUN[7]. Following this, the coverslips are stored at 4 °C in a solution of 60% horse serum, 40% Medium 199 (Grand Island Biological Co., Grand Island, N.Y. 14072) and 600 mg glucose/100 ml.

Collagen prepared from commercially available, acid soluble collagen proved to be less satisfactory in this system than the one prepared in the laboratory from rat tail tendons and could be used only in relatively short-term experiments.

The system described considerably reduces the time needed to feed the individual cultures, it provides for the relatively rapid change of the feeding medium, it is suitable for bright field and phase contrast microscopic observation, for cinematographic studies and is inexpensive.

Résumé. Une méthode simplifiée de culture d'organe nerveux est décrite. Elle réduit le temps consacré à la nourriture et permet un changement rapide du milieu nutritif. Elle convient aux études microscopique (m. à contraste de phase) et elle est relativement peu coûteuse.

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20 December 1973.

[1] A. MAXIMOW, Z. mikrosk. Anat. Forsch. *17*, 625 (1929).
[2] J. PAUL, *Cell and Tissue Culture* (E. & S. Livingstone Co., Edinburgh *1970*), p. 183.
[3] A. CARREL, J. exp. Med. *38*, 407 (1923).
[4] G. G. ROSE, Tex. Rep. Biol. Med. *12*, 1074 (1954).
[5] J. F. SCHNEIDER, *Methods of Neurochemistry* (Ed. R. FRIED; Marcel Dekker, Inc., New York 1973), vol. 4.
[6] M. B. BORNSTEIN, Lab. Invest. *7*, 134 (1958).
[7] L. M. OKUN, J. Neurobiol. *3*, 111 (1972).