transducer through a cannula implanted in the femoral artery and recorded on a Grass polygraph. Drugs (dopamine hydrochloride and amantadine hydrochloride) were rapidly injected through a polyethylene cannula in the femoral vein in aqueous solutions (0.1 ml/kg). Body temperature was maintained at 37° ± 1°C.

One group of six dogs (Fig. 1A) received five injections of dopamine hydrochloride (0.1 mg/kg, base weight; intravenous) spaced 30 minutes apart. Each of the first four injections of dopamine was followed generally 6, but as much as 8, minutes later by a dose of amantadine hydrochloride [0.016 to 2.0 mg/kg, base weight (cumulative); intravenous]. Two control groups of six dogs each were treated similarly except that one group (Fig. 1B) received saline (0.1 ml/kg; intravenous) instead of dopamine, and the other group (Fig. 1C) received saline in place of amantadine.

Amantadine caused a dose-related pressor response in dogs that were given amantadine 6 minutes after an injection of dopamine (Fig. 1A). The amantadine pressor effect in these dogs primed with dopamine was significant ($P < .05$ by two-tailed t-test) at a dose of 0.08 mg/kg or more when compared with the corresponding response to amantadine in a group of saline control dogs (Fig. 1B). Priming with dopamine had an obvious effect on the pressor response to amantadine, but amantadine did not seem to affect the amplitude of the pressor response to dopamine given 24 minutes later. Although the pressor response to dopamine increased in amplitude over the five successive doses of dopamine (Fig. 1A), the change in amplitude over the five doses was not significantly different from that of the dogs receiving dopamine and saline (Fig. 1C). Thus, we have no convincing evidence that amantadine blocked the uptake of dopamine into peripheral nerve storage sites; at least not under the present experimental conditions. If amantadine had blocked dopamine uptake we should have seen a significant increase in the amplitude of the pressor response to dopamine as a consequence of prior treatment with amantadine. Amantadine blocks uptake of norepinephrine at relatively high intravenous doses (*5*).

In contrast to the pronounced pressor response to amantadine in the dogs primed with dopamine (Fig. 1A), the control dogs which received amantadine after saline (Fig. 1B) showed only a small transient pressor effect which was statistically significant only at the highest dose of amantadine [when compared with the corresponding pressor response to saline (Fig. 1C)]. The pressor response to repeated injections of dopamine increased somewhat, and an injection of saline after dopamine had no significant effect on blood pressure (Fig. 1C). Some dogs receiving saline and amantadine showed a biphasic blood pressure response (a rise followed by a fall in pressure) after the dose of 2.0 mg of amantadine per kilogram (Fig. 1B).

The data in Fig. 1 suggest that amantadine releases catecholamines from peripheral nerve storage sites. We think that amantadine may have the same action within the central nervous system. The slight vasopressor response to amantadine alone (Fig. 1B) was probably not due to a direct action upon receptors, since Vernier *et al.* (*5*) reported that a small positive inotropic effect of amantadine in dogs was abolished by prior treatment with reserpine and was restored by infusion with norepinephrine. The catecholamine-releasing action of amantadine was considerably enhanced in the present study by priming the dogs with dopamine shortly before each injection of amantadine (Fig. 1A). Priming with dopamine did not create but simply amplified an inherent pharmacological action of amantadine, thus making it more readily measurable. The lowest effective intravenous dose of amantadine in dogs primed with dopamine (0.08 mg/kg; intravenous) was well below the oral doses of amantadine used in the treatment of parkinson patients (2 to 3 mg/kg).

R. P. GRELAK
R. CLARK
J. M. STUMP
V. G. VERNIER

E. I. du Pont de Nemours and Co., Inc., Pharmaceuticals Division, Industrial and Biochemicals Department, Wilmington, Delaware

References and Notes

1. W. L. Davies, R. R. Grunert, R. F. Haff, J. W. McGahen, E. M. Neumayer, M. Paulshock, J. C. Watts, T. R. Wood, E. C. Hermann, C. E. Hoffmann, *Science* **144**, 862 (1964); J. W. McGahen, C. E. Hoffmann, *Proc. Soc. Exp. Biol. Med.* **129**, 678 (1968).
2. G. G. Jackson, R. L. Muldoon, L. W. Akers, *Antimicrobial Agents and Chemotherapy*, J. D. Sylvestor, Ed. (American Society for Microbiology, Ann Arbor, Michigan, 1964), p. 703; W. L. Wingfield, D. Pollack, R. R. Grunert, *N. Engl. J. Med.* **281**, 579 (1969).
3. R. S. Schwab, A. C. England, D. C. Poskanzer, R. R. Young, *J. Amer. Med. Ass.* **208**, 1168 (1969); J. D. Parkes, D. M. Calver, K. T. Zilkha, R. P. Knill-Jones, *Lancet* **1970-I**, 259 (1970); J. B. Weeth, C. N. Shealy, D. A. Mercier, *Wis. Med. J.* **68**, 325 (1969); P. Millac, I. Hason, M. L. E. Espir, D. G. Slyfield, *Lancet* **1970-I**, 464 (1970).
4. H. O. Schild, *Brit. J. Pharmacol.* **2**, 189 (1947).
5. V. G. Vernier, J. B. Harmon, J. M. Stump, T. E. Lynes, J. P. Marvel, D. H. Smith, *Toxicol. Appl. Pharmacol.* **15**, 642 (1969).
6. W. A. Price, personal communication.
7. H. Ehringer, O. Hornykiewicz, *Klin. Wochenschr.* **38**, 1236 (1960); A. Barbeau, C. F. Murphy, T. L. Sourkes, *Science* **133**, 1706 (1961); H. L. Klawans, *Dis. Nerv. Syst.* **29**, 807 (1968).
8. We thank B. K. Robertson, R. J. Millelot, M. M. Ruckman, and R. R. Rettig for technical assistance.

10 April 1970

Automated Continuous Culture of Mammalian Cells in Suspension

Abstract. *A system has been developed for the continuous culture of mammalian cells in suspension. The system maintains constant cell concentrations (monitored as the degree of light scattering) over a wide range of previously selected values by automatic additions of known amounts of medium and simultaneous withdrawals of equal volumes of cell suspension.*

We have devised an automated system for the continuous culture of mammalian cells and have used it to maintain a suspension culture of HeLa cells for several months. Our objective was the development of a small bench-top facility which could be used to maintain growing stock cultures of mammalian cells indefinitely in a laboratory not equipped with standard facilities for cell culture, and to grow cells in sufficiently large quantities for biochemical studies. Both criteria have been satisfied. The flexibility of the system allows broad adjustments in performance to accommodate differences in the behavior of various types of cultures; its capacity can be readily varied to suit the requirements of the investigator.

The system incorporates a Nephelostat, controlled by a photocell and originally designed for the continuous culture of microorganisms (Fig. 1) (*1*).

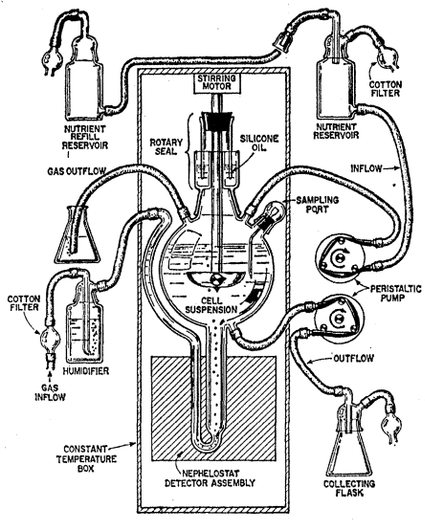

Fig. 1. Diagrammatic representation of the culture apparatus. All flexible tubing is medical grade silicone rubber (Silastic, Dow-Corning, Midlands, Michigan). The sampling port holds a silicone rubber stopper and a stainless steel needle. The stirring paddle is Teflon, and the stirring shaft is Teflon-coated glass. The interior surface of the culture vessel is coated with silicon.

The cell concentration, measured as the degree of light scattering, is held constant at a previously selected value by the automatic addition of known volumes of fresh medium (*2*) coupled with the withdrawal of equal volumes of cell suspension. This is accomplished by means of a peristaltic pump (*3*) controlled by the Nephelostat control console (not shown). The same pump controls both inflow and outflow; the pumping chambers are separated in Fig. 1 for clarity. The frequency with which medium is added is recorded by the Nephelostat control console and gives an indication of the growth rate of the cells at any given concentration

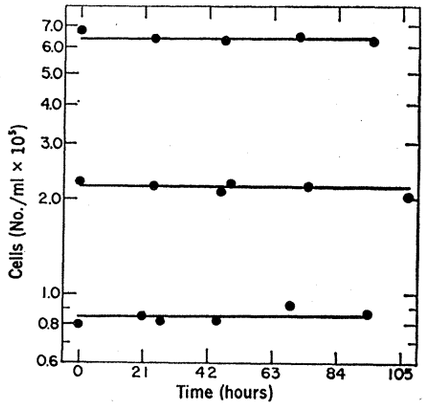

Fig. 2. Maintenance of a constant cell concentration at previously selected levels.

(*1*, *4*). Adjustable instrument settings include: (i) the frequency with which the light scattering is monitored; (ii) the threshold control for the addition of medium; (iii) the duration and rate of pumping; and (iv) the culture temperature. The culture volume can be varied between 50 and 1200 ml. The smaller volume, used for long-term maintenance between experiments, requires a minimum expenditure of culture medium. To obtain a larger working volume, the outflow from the culture vessel is occluded by means of a pinch clamp so that the volume increases to the desired level while the concentration of cells remains constant. The volume is decreased at constant cell concentration by clamping the inflow from the nutrient reservoir. The nutrient reservoir is kept at 4°C and is filled periodically by attaching the nutrient refill reservoir. The culture vessel, stirring motor, and Nephelostat detector assembly are kept at 37°C in a controlled temperature chamber. Direct access to the culture is provided through the sampling port. The culture is gassed with a mixture of 95 percent air and 5 percent CO_2 at a flow rate of 75 cm^3/min and stirred continuously at 150 rev/min. The complex of glassware and tubing shown in Fig. 1 is autoclaved as a unit (nutrient refill reservoir not attached) with the well of the rotary seal filled with silicone oil.

The ability of the system to maintain a constant cell concentration at different preselected levels is demonstrated in Fig. 2. Samples were taken from the sampling port at the intervals shown, and the cells were counted in a Coulter counter. The cell concentration was initially decreased by adjusting the nutrient feed threshold control on the Nephelostat console to the appropriate low value; the cells were thus automatically diluted to the corresponding concentration which was then kept constant. The cell concentration was raised in stages by periodically adjusting the control to progressively higher levels. This procedure ensured frequent replenishment of the medium and, under these conditions, the highest concentration obtained for HeLa cells was 6.4 $\times$ 10^5 cell/ml (Fig. 2). Beyond this level, the feeding frequency decreased sharply, indicating a pronounced drop in the growth rate.

Growth rates of HeLa cells after 6 and 12 weeks in culture were obtained by initially bringing the cell concentration to the levels indicated at time zero

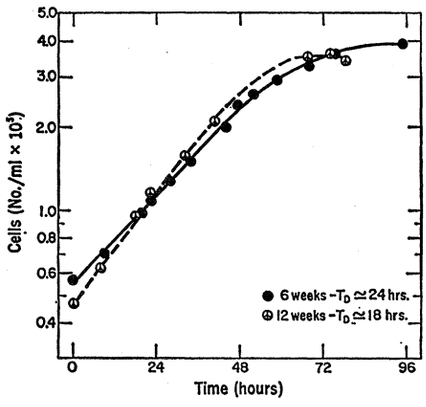

Fig. 3. Growth of HeLa cells when the threshold control for addition of medium is raised to the maximum so that no medium is added.

in Fig. 3, and then turning the control setting to maximum so that no further additions of medium would be made. The trend toward an increase in the growth rate suggests that the culture conditions may favor the selection of more rapidly growing cells. The plateau in the growth curves at a concentration of 3.5×10^5 cell/ml, approximately half that attained in Fig. 2, reflects the depletion of essential nutrients.

We have used this system primarily to grow cells in quantity for biochemical studies, and are presently able to harvest approximately 10^9 cells at 5-day intervals. This number can be increased easily by enlarging the culture vessel.

CARL PERAINO
SILVIA BACCHETTI
WILLIAM J. EISLER
Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439

References and Notes

1. W. J. Eisler and R. B. Webb, *Appl. Microbiol.* **16**, 1375 (1968). The Nephelostat is now being manufactured by Lab-Line Bio-Medical Products Inc., Melrose Park, Illinois.
2. Minimal essential medium for suspension cultures (Eagle) containing, in addition, fetal calf serum (10 percent), glutamine (2.0 mmole/liter), penicillin and streptomycin (200 units of each per milliliter). All ingredients were obtained from Grand Island Biological Co., Grand Island, New York.
3. RL 1975 Holter bilateral roller pump, with silicone pumping chambers and nylon connectors; Extracorporeal Medical Specialties, Inc., Mt. Laurel Township, New Jersey.
4. H. E. Kubitschek, *Introduction to Research with Continuous Cultures* (Prentice-Hall, Englewood Cliffs, New Jersey, in press).
5. Supported by the AEC. We thank T. Doody and J. Tornatore of the Division of Biological and Medical Research for making the glassware, and Drs. K. Kajiwara and G. Mueller, McArdle Laboratory, University of Wisconsin, for providing the HeLa cells.

10 April 1970