ciently to reduce this to 15 μ and still allow free circulation of fluid (J). Deeper chambers (K) can be obtained by inserting thin metal washers (D) under the O-ring; chambers of all depths between approximately 15 and 1500 μ can thus be provided.

Nutrient can be circulated through such chambers by any means desired. We first tried a "Murphy-drip," but the total volume of a 20-mm chamber, even as deep as 500 μ, is only about 0.16 ml, which means that a single "drop" of fluid would represent a complete change of nutrient, and even the slowest feasible drip would be equivalent to changing the nutrient several times an hour. Most cultures do best if the nutrient is somewhat "conditioned" by exchange of metabolic products with the tissue. This is particularly important for single-cell cultures, and it is more satisfactory to renew the nutrient for these cultures at fixed intervals, by injection with a hypodermic needle. The chamber can be closed between injections by carrying the inlet end of the tube around and attaching it to the exit tube.

Perfusion chambers have in general been handled as single units. There are, however, certain advantages in connecting them in pairs, which can be done easily with these small chambers, and in having the two chambers resting on a metal carrier (38 × 76 mm), the dimensions of a standard, wide microscope slide (L). Since the two chambers may have different volumes (depths), it is possible to place a large culture—up to a gram of tissue—in one chamber, to use the large culture to "condition" the nutrient, and to connect this chamber to a second shallow chamber that has been charged with single cells; or the two chambers may contain different types of cells. With animal cultures, for example, it should be possible to place pituitary tissue in one chamber and ovarian tissue in a second, to flow nutrient from one chamber to the other, and to follow directly the effects of specific metabolites. Or a plant stem tip could be placed in a deep chamber, a massive undifferentiated callus culture of the same or a different species placed in the second, and their reciprocal effects could be studied. The possible permutations are countless.

Culture plates, O-rings, and cover glasses can be sterilized by autoclaving. The chamber can be of any metal, including brass, since the nutrient does not come in contact with it. It is best to discard supply tubes after use, even though they are made of nontoxic, surgical vinyl material.

One objection to the use of these chambers in pairs, in the study of animal materials, is the tendency of animal cells to migrate in any circulated fluid. This can be obviated by placing a disk of viscose membrane over the culture and under the O-ring, and thus separate the culture from the flowing nutrient. Diffusion through such a membrane will usually be adequate for metabolic needs of the culture. If, however, there are objections to the membrane, migration can be prevented by filling the quadrant grooves with sintered nylon sponge or by introducing a Swinney-type filter into the tube connecting two chambers.

Figure 2 gives some idea of the results that can be obtained. The two upper pictures are of plant cells (Picea glauca) grown in shallow chambers; the lower ones are of animal materials (mouse). Equally sharp images can be obtained under oil immersion (not shown), and, if the preparations are thin and in unencumbered monolayers, phase contrast can be used. The versatility of these chambers should widely commend them for use with both plant and animal materials.

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1-Adamantanamine Hydrochloride: Inhibition of Rous and Esh Sarcoma Viruses in Cell Culture

Abstract. 1-Adamantanamine hydrochloride, added to chick embryo cells, inhibited focus production upon subsequent inoculation of the cells with Rous and Esh sarcoma viruses. Addition of the chemical to suspensions of Rous and Esh viruses before they were added to chick cell cultures did not inactivate the viruses; and the addition to chick cells did not prevent plaque formation following their inoculation with Newcastle disease virus. This indicates that cells treated with 1-adamantanamine hydrochloride are capable of supporting viral replication, and these observations suggest that the drug inhibits growth of two oncogenic viruses by prevention of virus penetration, but not by a virucidal effect or cell intoxication.

1-Adamantanamine hydrochloride (amantadine HCl) inhibits certain myxoviruses; that is, several strains of human influenza and parainfluenza viruses in addition to their animal moieties. Other viruses, rubella and pseudorabies, are also sensitive in one or more systems (1, 2, 3).

Avian oncogenic viruses share many characteristics with myxoviruses: they are ether labile, sensitive to pH 3.0, within the size limits of 80 to 200 mμ, and contain pentose nucleic acid (4). Hence, it appeared pertinent to investigate the action of amantadine on these viruses in a quantitative cell system.

In the assay, patterned after that of Rubin (5), secondary chick embryo fibroblasts were used for focus production by Rous sarcoma virus (RSV) (Bry-an strain) and Esh sarcoma virus (ESV), a recent field isolate (6). Growth medium (5) was modified to include Eagle's basal medium as well as medium 199; calf serum was substituted for chicken serum.

Immediately after adding amantadine HCl, tubes containing the chick cell suspension were placed in a water bath (37°C) for 15 minutes. After incubation, the drug-cell suspension was pipetted into small plastic petri dishes and virus was added to all plates within 5 minutes. Next day, cultures were overlayed with agar that contained amantadine. After incubation for 7 days in a CO_2 incubator (5 to 7 percent CO_2 and 90 to 98 percent relative humidity) virus-induced foci were counted to quantitate inhibitory activity.

Data presented in Fig. 1 indicate that the drug inhibited production of RSV foci. A concentration of 18.4 μg/ml of amantadine HCl elicited 50 percent viral inhibition. Although the results were less consistent than with RSV, regression curves obtained for ESV also show activity against the virus (Fig. 1). Approximately 11.3 μg/ml (range 4.8 to 17.7) of the compound caused 50 percent viral inhibition. The slopes of the regression

Table 1. Effect of amantadine HCl on suspensions of Rous sarcoma (RSV) and Esh sarcoma (ESV) viruses at 37°C for 2½ hours.

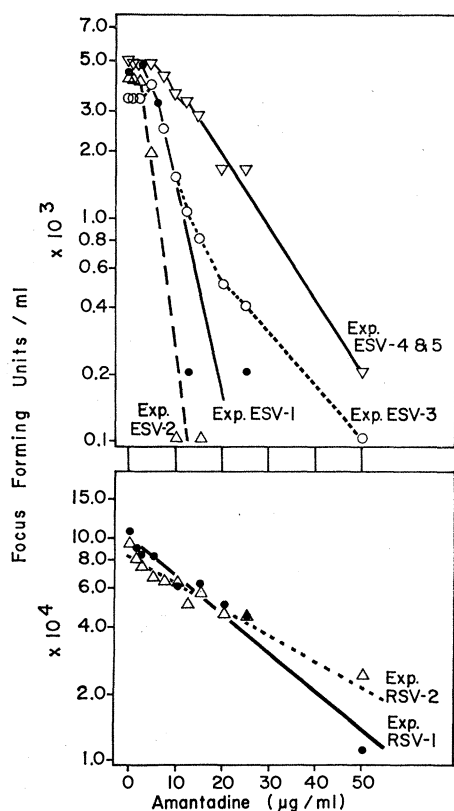| Virus	Amantadine HCl (μg/ml)	Focus forming units (per ml)
RSV	6.25	6.2×10^4
	12.50	6.5×10^4
	25.00	7.1×10^4
	50.00	6.1×10^4
	0	6.4×10^4
	0*	7.6×10^4
ESV	6.25	2.6×10^3
	12.50	2.8×10^3
	25.00	2.5×10^3
	50.00	2.3×10^3
	0	2.9×10^3
	0*	2.2×10^3

* 2°C for 2½ hours.

Fig. 1. Number of foci per milliliter produced by Rous sarcoma virus (RSV) and Esh sarcoma virus (ESV) in chick embryo fibroblasts in the presence of amantadine HCl. The points were identical for experiments ESV 4 and 5.

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curves were different in experiments ESV 3, 4, and 5 as compared with ESV 1 and 2 (Fig. 1), but in every instance the influence of amantadine was evident.

In each experiment with RSV and ESV, cells grown in the various concentrations of amantadine HCl were compared with both normal and virus-infected cells. Occasionally at drug concentrations of 25 μg/ml, and more often at 50 μg/ml, it was difficult to count the foci. This difference from the usual appearance of foci suggests that chick cells were affected in some manner by the compound without being rendered completely impregnable to virus invasion.

To eliminate the possibility of preventing viral replication by direct toxic effect of amantadine on chick cells, Newcastle disease virus (NDV), GB strain, a member of the myxoviruses known to be resistant to amantadine (2), was employed to infect secondary chick embryo cells treated with this compound at concentrations of 25, 50, and 100 μg/ml. The agar overlay also contained the amantadine. Cells and media used in this test were similar to those used in tests with tumor viruses. Control cells and amantadine-treated cells gave the same titer with NDV.

A virucidal effect was ruled out by adding amantadine to a viral suspension. Data in Table 1 show that amantidine HCl, at the concentrations used in these studies, was not virucidal to either RSV or ESV in suspension. There is good evidence to show that amantadine acts by blocking the penetration of virus into the cell (3).

Previous work indicates that amantadine elicits no effect on RSV pock-production on chorioallantoic membranes (2) or in a cell culture–drug screening system (7) and is ineffective against Friend virus in mice (8). The special significance of our results is that this is the first evidence that amantadine inhibits oncogenic viruses.

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Cytochemical Localization of Lactate Dehydrogenase in Muscular Dystrophy of the Mouse

Abstract. By use of phenazine methosulfate and the "incubation mixture film method," lactate dehydrogenase activity has been demonstrated in the dystrophic muscle fibers of strain 129 mice. The results indicate that for demonstration of lactate dehydrogenase activity in dystrophic muscle fibers phenazine methosulfate is necessary. This finding is typical for the "white" muscle fibers in the normal muscle and suggests that the dystrophy affects primarily the "white" muscle fibers.

The biochemical lesion in the striated muscles of dystrophic mice has been the subject of histochemical and biochemical study. The contents of lactate dehydrogenase (LDH) and of α-glycerophosphate dehydrogenase (linked to nicotinamide adenine dinucleotide, NAD) in the muscles of such strain 129 mice have been reported to decrease to about 75 and 50 percent of the control values, respectively (1). Subsequent histochemical studies, however, have not confirmed these biochemical findings (2).

Investigation of cytochemical localization of LDH in skeletal muscle (3) is complicated by the diffusion of LDH from tissue sections into the aqueous incubation media and also by the dependence on diaphorase activity of the final electron transfer from NADH to the tetrazolium salts. An "incubation mixture film" technique (3) has overcome the problem of enzyme diffusion, and the use of phenazine methosulfate

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