compounds passing through membranes more easily. Our tests in this regard were negative.

DMSO is, however, a very effective solvent for a number of agents which are almost insoluble in polar solvents. For instance, 3,4-benzypyrene and 3-methylcholanthrene were readily solubilized in 100% DMSO. DMSO was also an effective solvent for highly insoluble antineoplastic agents and pesticides.

Our observations with DMSO confirm the fact that DMSO is a very effective solvent with low toxicity and few pharmacological effects(11). These data do not suggest, however, that systemically administered DMSO alters drug penetration. No evidence was seen to indicate an increased oral absorption or increased penetration to the central nervous system in the cases studied. However, it would not be unreasonable to assume that a drug dissolved in 100% DMSO and applied topically might result in some increase in absorption.

Summary. The lethality of a number of drugs dissolved in DMSO or saline has been compared. In no case was there a significant increase in drug lethality when administered with DMSO. Preliminary tests failed to indicate that DMSO possesses local anesthetic or analgetic action. DMSO also failed to alter the CSF or tissue penetration of PAH. DMSO appears to be a very good solvent with little or no effect on pharmacological action.

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Continuous Cell Strains Derived from Human Atheromatous Lesions and their Viral Susceptibility.* (29962)

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This report is concerned with a study of human atheromatous lesions, carried out by growing tissue fragments in culture. From cells which grow out from such fragments, a number of continuous cell lines were established and they have been characterized by determining their susceptibility to a variety of viruses.

Materials and methods. A total of 60 atheromatous lesion specimens obtained during surgical operations for either abdominal or thoracic aneurysms were processed for growth in tissue culture. The specimens were received in sterile petri dishes and were processed within a few hours after surgical removal. For the primary cultivation of all specimens, the following procedure was used (1,2). The specimens were washed 3 times with Eagle's medium and then minced with scissors into pieces ranging from 1-2 mm in size. About 5-10 minced pieces suspended in a small amount of growth medium were squirted with a large-bore pipette onto the flat surface of a 1-oz bottle previously heated to about 45°C. Six 1-oz bottles of explants were made from each specimen. About 3 ml

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of growth medium was then added to each bottle and the bottles incubated at 37°C with the sides containing minced tissues on top. Over a period of one week the tissues were bathed in the growth medium by turning over the bottles twice a day for a few minutes. At the end of the week the medium was changed and the bottles reincubated but now with the explants submerged in the medium. When sufficient outgrowth (covering at least ½ of surface area) was observed, the cultures were trypsinized and serial passages were initiated.

Growth medium. The initial growth medium consisted of Eagle's basal medium containing 20% bovine fetal serum. After the first trypsinization the cells were grown in Eagle's medium containing 20% calf serum. Although 10% calf serum supported the growth of these cell strains, 20% serum resulted in faster growth.

Trypsinization process. The cell sheets were washed once with warm 0.2% trypsin (pH 7.5); then fresh trypsin was added and the cultures were left at room temperature for 1-2 minutes. The trypsin was then decanted and the bottles incubated at 37°C for 15-30 minutes. Usually within this period of time, the cells were loosened from the glass surface. Warm growth medium was then added, and the cells were thoroughly dispersed by repeated aspiration with a pipette and planted in new bottles. For each serial passage the cell sheet surface was doubled (e.g., a full sheet in an 8-oz bottle was passed into one 16-oz bottle or into two 8-oz bottles). Fully grown cell sheets were normally obtained within 5-7 days after trypsinization.

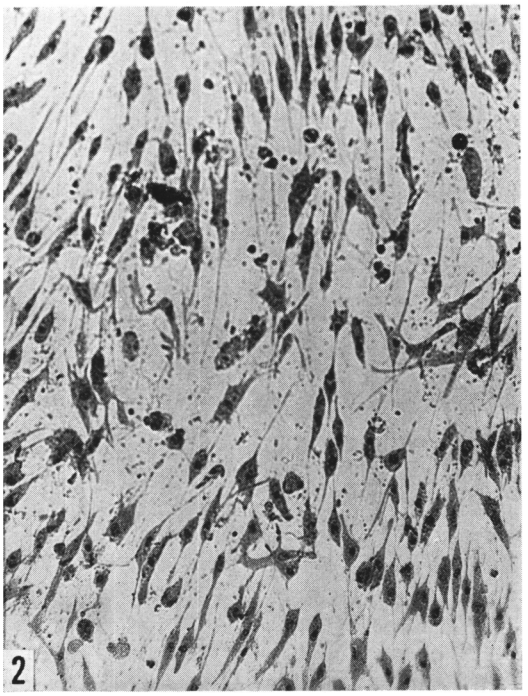
Preservation of cells by freezing. Fully grown cell sheets in 16-oz bottles were trypsinized as in serial passage; the cells were suspended in 5 ml ($1\text{-}2 \times 10^6$ cells/ml) growth medium containing 10% sterile (autoclaved) glycerol and dispensed in 2.5 ml amounts into 2 (15×45 mm) screw-capped vials. After overnight storage at 4°C, the vials were frozen slowly to —25°C and then stored at —70°C or in a liquid nitrogen tank (vapor phase). To reconstitute frozen cells, a vial was removed from the N_2 tank, immediately placed in a 37°C water bath, and rapidly thawed with agitation. The contents were then emptied into a 16-oz bottle and 20 ml of warm growth medium was added. The screw cap of the bottle was fitted loosely but covered with sterile aluminum foil and the bottle was placed in a 5% CO_2 incubator at 37°C. The medium was changed after 24 hours and the bottle reincubated as before until at least ⅓ of the surface area was covered by cells at which time the cap was tightened and a regular 37°C incubator was used. Normally a full cell sheet was obtained within 7-9 days.

Results. Establishment of cell strains from atheromatous lesions of the aorta (A cells). A total of 40 of the 60 specimens processed for explantation in tissue culture showed active outgrowths within 6-21 days. Of these, 22 cultures could not be serially passed and degenerated within 3 transfers. Eleven cell strains were serially passed beyond the sixth passage and samples of each strain were frozen for future studies. The cell strains so far tested for virus susceptibility are A-1, A-14, A-19, A-23, A-24, and A-32 and A-39. Four of the strains were selected for extended serial passage and were tested again for their viral susceptibility at higher passages. Some of the cell strains have undergone more than 30 serial passages extending over a period of about 6 months. After approximately 30 passages, the cells approach senescence and grow very slowly, similar to the Wistar diploid lines(3). At this point an early passage may be reconstituted from the frozen stock.

Morphology of A cell strains. In the original outgrowths, as well as in subsequent serial passages, cell monolayers appeared to be a mixture of polygonal and fibroblastic cells. Fig. 1 shows the A-23 cell strain after 10 subcultivations extending over 3 months. The presence of both spindle-shaped, fibroblastic cells and flattened, endothelioid, polygonal cells is illustrated. The nuclei of both cell types are relatively small and round in configuration and only one nucleus per cell is seen.

Susceptibility to viral agents. Special interest in the A cell strains developed when it was found that these cells were highly susceptible to a variety of viral agents, and the viral cytopathic effects appeared early. From 40-50 tube cultures were prepared from a fully grown cell sheet of a 16-oz bottle culture. The maintenance medium consisted of Eagle's basal medium containing 5% calf serum and

0.22% sodium bicarbonate. Under these conditions the cells could be readily maintained for about 2 weeks without the appearance of non-specific degeneration. For growth and titration of rhinoviruses (echo 28 and 16/60) 2% calf serum and 0.074% sodium bicarbonate were used and the inoculated tubes were rolled at 33°C. The range of viral susceptibility is presented in Table I. No differences have been noted in the susceptibility of dif-



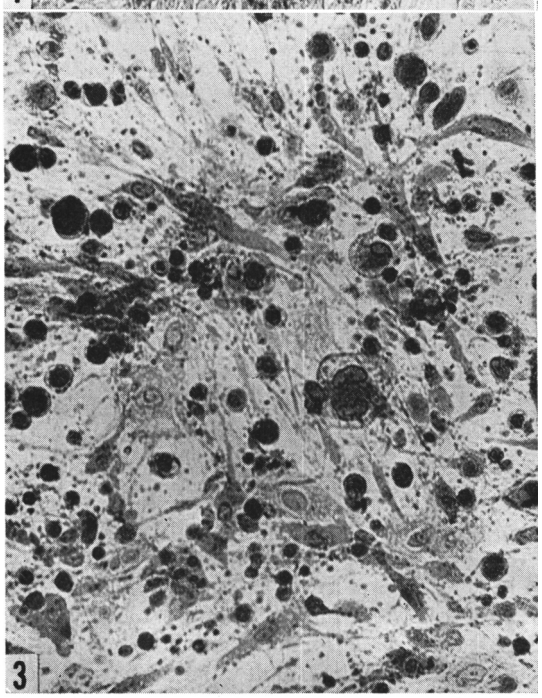


FIG. 1. A cells, strain 23, passage 10. Stained with methylene blue. × 70.

FIG. 2. A cells (strain 23, passage 10) showing cytopathic changes 3 days after inoculation with rhinovirus 16/60. Stained with methylene blue. × 70.

FIG. 3. A cells (strain 23, passage 10) showing cytopathic changes 40 hr after inoculation with herpes simplex virus. Stained with methylene blue. × 70.

TABLE I. Susceptibility of A Cell Strains to Viral Agents.

Virus*	Cell strain and passage No.	Cytopathic effects	Time of appearance of CPE following inoculation (days)
Adenovirus type 2	A-39, P13	+	2
" " 7	A-14, P6	+	3
" " 12	A-39, P13	+	4
Coxsackievirus A type 3	A-14, P6	—	0
" " " 5	A-14, P6	—	0
" " " 7	A-14, P6	+	1
" " " 9	A-1, P8	+	1
" " " 18	A-14, P6	+	1
" " " 19	A-14, P6	—	0
" " " 24	A-14, P6; A-32, P18	+	2
" " " "	A-39, P13	+	2
Coxsackievirus B type 1	A-1, P8	+	1
" " " 6	A-39, P13; A-32, P18	+	3
Cytomegalovirus	A-24, P7	+	4
Echovirus type 3	A-1, P8	+	1
" " 11	A-14, P6	+	1
" " 21	A-23, P10	+	3
" " 28	A-14, P6; A-19, P7	+	1
" " "	A-23, P10; A-32, P18	+	1
Equine abortion virus (equine herpesvirus type 1)	A-39, P13	+	2
Herpes simplex	A-14, P6	+	1
Measles	A-14, P6	+	5
Poliovirus type 1	A-1, P8; A-24, P6	+	1
" " 3	A-14, P6	+	1
Pseudorabies	A-39, P13	+	2
Rhinovirus 16/60	A-23, P6 and P10; A-24, P7	+	3
" "	A-39, P8	+	3
Reovirus type 1	A-19, P7	+	1
Respiratory syncytial virus	A-32, P18; A-39, P13	+	2
Vaccinia virus	A-14, P6	+	1

* Inoculum was 0.1 ml of undiluted tissue culture fluid per tube except in inocula of coxsackievirus A types 5 and 19, which consisted of 0.1 ml of 20% mouse brain and torso material.

ferent strains of A cells. Occasionally one blind passage was necessary for development of marked cytopathic effects. As examples the cytopathic effects produced by rhinovirus 16/60 and by herpes simplex virus are illustrated in Figs. 2 and 3.

Discussion. The growth of human and rabbit aortic cells has been reported but only for short-term cultivation(4-6). In the present study the establishment of several strains from human aortas is described. The monolayers in culture appeared to be a mixture of polygonal (endothelioid) and fibroblastic cells. Of the 11 cell strains (A cells) which were subcultured for more than 6 passages, 4 were selected for long-term subcultivation. Eventual cessation of mitosis and cellular degeneration were observed between the 32nd and the 35th passages, about 6 months after initiation of the culture. These cell strains showed a remarkable susceptibility to a variety of viral agents. With many viruses, distinctive cytopathic effects appeared earlier than in other susceptible cell cultures. The high degree of susceptibility of A cells to certain rhinoviruses which grow slowly and produce irregular cytopathic effects in other cell cultures may be of considerable practical importance. No attempt was made to test the suitability of these cell strains derived from human aortas for experiments on lipid deposition(4-6).

Summary. Of 60 biopsies of atheromatous lesions, 40 were grown in tissue culture, and 11 were successfully transferred for 6 or more passages. Four of the aorta or A cell strains were carried for about 30 passages over a

period of about 6 months. After this they become senescent and degenerate like the Wistar fibroblast strains. However, the A cell strains may be revived from the large stocks of cells being maintained in a liquid nitrogen depository. The cultures appear to consist of a mixture of fibroblastic and endothelioid cells. The cells have a high susceptibility to DNA viruses (pox-, herpes- and adenovirus groups) and to RNA viruses (myxo-, reo-, and picornavirus groups). They seem to be particularly susceptible to some of the rhinoviruses which have a limited host cell range.

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Use of Writhing Test for Evaluating Analgesic Activity of Narcotic Antagonists. (29963)

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The field of narcotic antagonist analgesics was opened by the discovery of Lasagna and and Beecher(1) that nalorphine (N-allylnormorphine), a narcotic antagonist, was by itself a potent analgesic in man. Although possessing the additional virtue of being non-addicting(2), nalorphine was nevertheless unsuitable for clinical use because of its high incidence of psychotomimetic and other side effects. Its analgesic activity in man was of particular interest because in animals nalorphine was an effective narcotic antagonist but showed no analgesic activity on the usual tests for potent analgesics, such as the mouse hot-plate and rat tail-flick tests. Other narcotic antagonist analgesics active in man, and apparently non-addicting, such as pentazocine and cyclazocine, were also found to be essentially inactive in animals by the usual analgesic tests(3,4). It has been suggested, in fact, that in the search for potent non-addicting analgesics, compounds showing analgesia on the hot-plate and tail-flick tests might be ruled out because they would almost certainly be addicting.

Siegmund, Cadmus and Lu(5) described a phenylquinone "writhing" test in mice which detected both narcotic analgesics and the non-narcotic antipyretic analgesics, although it was probably not entirely specific for analgesics. This test has been used in our laboratory since that time as one of our analgesic testing methods because it appeared to represent a different type of pain from that of the hot-plate or tail-flick, and a type which might more closely resemble surgical pain. Both phenylquinone and acetic acid were employed as the intraperitoneally injected writhing agent in mice and rats.

During the investigation of some narcotic antagonists about 2 years ago, we observed that one of them showed analgesic activity on the writhing test. This prompted further studies which suggested that some interesting correlations existed between the mouse and rat writhing tests and analgesic activity reported in man. In this connection, Bentley (6) has recently referred to unpublished work of R. E. Lister demonstrating that many morphine antagonists abolished the phenylquinone writhing response. We present here the results of writhing test analgesic studies on 6 narcotic antagonists: cyclazocine, levallorphan, nalorphine, naloxone, pentazocine, and (-)-3-hydroxy-N-cyclopropylmethlymorphinan*(7), designated for convenience as

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