than obtained with deuterium(8-10). The different rates of passage of various elements into cerebrospinal fluid point to the process of secretion rather than ultrafiltration.

Summary. Comparative rates of passage of strontium and calcium from plasma into cerebrospinal fluid have been studied in man following intravenous injection of Sr^{85} and Ca^{45}. Cerebrospinal fluid to plasma ratios of Sr^{85} and Ca^{45} were approximately the same except in one patient who showed a greater ratio for Ca^{45}. As a greater proportion of Sr^{85} than of Ca^{45} is unbound to protein, differential passage into the cerebrospinal fluid in favor of Ca^{45} appears to take place. The data indicate that cerebrospinal fluid may be formed by secretion rather than ultrafiltration. Rate of formation of fluid has been calculated to be approximately 0.03 to 0.04 ml/hr/kg of body weight.

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Effects of Antibiotics on Multiplication of L Cells in Suspension Culture. (25223)

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A number of agents known to have an antitumor effect in animals or man were recently shown by Eagle and Foley(1,2) to be highly toxic against a number of cultures of human and animal cells. They suggested that determination of cytotoxicity in cell cultures might prove to be of value as a primary screen for detection of new carcinolytic agents. Their technic depends on the cytotoxic agent affecting ability of the cells to adhere to glass and to multiply under these conditions. During the past few years the method of Owens *et al.* (3) for growing mammalian cells in suspension culture has been reproduced (with modifications) in several laboratories(4,5), and it seemed to us that cells growing in suspension would be more suitable for study of the effects of cytotoxic agents than cells growing on glass surfaces. We have examined the effects of many substances on the L cell line of mouse fibroblasts growing in suspension culture and wish to report here on the effects of certain antibiotics on this cell line.

Methods. Stock cultures of the L cell line were grown in suspension in the modification of Eagle's medium(6) summarized in Table I. Approximately 200 ml of sterile medium and 20 million cells (taken from suspension cultures) were added to a sterile 500 ml flat-bottomed centrifuge bottle containing a sterile teflon-covered 2-inch magnet and capped with a black rubber stopper. The bottle was then placed on a magnetic induction stirrer located in a 37° incubator and the power adjusted so that the magnetized bar turned at 225 rpm. At the end of a 3-or 4 day incubation period

TABLE I. Composition of Medium Used for Growth of L Cells in Suspension Culture.*

Glucose	2.5 g	L-arginine • HCl	.021 g
NaCl	7.0	cystine	.012
KCl	.4	histidine • HCl	.008
$MgSO_4 \cdot 7H_2O$	.2	isoleucine	.026
Na_2HPO_4	1.44	leucine	"
D-biotin	.001	lysine • HCl	"
Ca pantothenate	"	methionine	.008
Choline chloride	"	phenylalanine	.016
Folic acid	"	threonine	.024
Nicotinamide	"	tryptophane	.004
Pyridoxal • HCl	"	tyrosine	.018
Thiamine • HCl	"	valine	.024
Riboflavin	.0001	glutamine	.3
Phenol red	.01	Potassium benzylpenicillin	.06
Methocel (4000 cp)	.3	Dihydrostreptomycin sulfate	.1
Calf serum	100 ml	Nystatin	50,000 units
Pyrogen-free water q.s.	1 l.		

* This medium is a modification of that described by Eagle(6).

the population (as determined by direct count using a hemocytometer) was usually between 120 million and 180 million cells. Aliquots were removed for inoculation of secondary bottles and for use in cytotoxicity tests. The cytotoxicity tests here described were carried out by growing the L cells in 1 x 6 inch test tubes capped with teflon-lined screw caps. Aliquots of sterile medium (Table I) were added to sterile flat-bottomed centrifuge bottles together with sufficient inoculum to give a population of about 100,000 cells per ml. The suspension was stirred with a magnetic stirrer for 1 hour to disperse the cells, then 25 ml aliquots were added to the sterile 1 x 6 inch screw-capped test tubes. Appropriate dilutions of sterile solutions of the antibiotics under study were added to the inoculated tubes, and the tubes were placed on a roller tube apparatus (New Brunswick Scientific Co., model TC-3) located in a 37° incubator. After 1 to 3 hours 2 ml aliquots were removed from each tube and cell population determined by direct count in the hemocytometer. These counts were taken as the "zero" time count, and after 3 days incubation a second series of counts was made. The multiplication factor shown on the ordinate of Fig. 1 was calculated from the increase in cell population over this interval. The multiplication factors in unsupplemented control tubes have varied from 6 to 9 over a series of 45 experiments. The amount of antibiotic needed to give 50% inhibition of growth was determined graphically from dose-response curves similar to those shown in Fig. 1. More consistent results were apparently obtained by direct count of the cell population than when increase in cell numbers was determined by a chemical method based on increase in protein nitrogen(7).

Results. Addition of solutions of various antibiotics to L cells growing in the modified Eagle's medium resulted in inhibition of growth with 10 of the 38 antibiotics studied. Those inhibiting growth and the concentrations of each needed to reduce growth to approximately 50% of that obtained in the unsupplemented control culture are listed in Table II. Those antibiotics which did not affect growth when added to L cultures at levels of 1 μg per ml are listed in Table III. Typical dose-response curves are shown in Fig. 1. Repeated experiments showed that the concentrations required for 50% inhibition of growth varied as much as 30% from one ex-

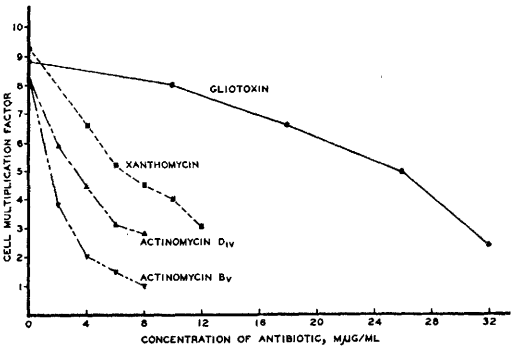

FIG. 1. Inhibition of multiplication of L cells by antibiotics.

TABLE II. Antibiotics Inhibiting Multiplication of L Cells in Suspension Culture.

Antibiotic	Approx. conc. giving 50% inhibition of multiplication of cells, mμg/ml
Actinomycin B_V	1.3
" D_{IV}	3
" $B_{(mixture)}$	4.6
Xanthomycin	7
Cycloheximide	33
Gliotoxin	36
Mitomycin C	41
Thiostrepton	70
Patulin	154
Hygromycin	1300

TABLE III. Antibiotics Not Affecting Multiplication of L Cells in Suspension Culture.*

Amphotericin A	Fumagillin
" B	Gramicidin
Aspergillic acid	Griseofulvin
Bacitracin	Methymycin
Benzylpenicillin potassium	Neomycin B sulfate
Carbomycin	Nystatin
Chartreusin	5-Oxytetracycline•HCl
Chloramphenicol	Polymyxin B sulfate
7-Chlortetracycline • HCl	Subtilin
Citrinin	Tetracycline • HCl
Colistin	Tyrothricin
Cycloserine	Vancomycin
Dihydrostreptomycin sulfate	Vernamycin
Erythromycin	Viomycin sulfate

* Non-inhibitory when added to medium to give a concentration of 1 μg/ml.

periment to the next; this variation was reduced when several replicate tubes were started at each antibiotic concentration. Of the substances tested that inhibited growth the actinomycins and xanthomycin were significantly more cytotoxic than any of the others. Experiments with concentrates obtained from fermented media containing cytotoxic substances showed that the dose-response curves could be used in estimating the potency of the cytotoxic factors present.

Examination of the literature shows that the cytotoxicity of only a few antibiotics have been examined in tissue culture. Eagle and Foley(1) found that approximately 60 mμg per ml of actinomycin D were required to inhibit growth of 6 different cell lines of human and mouse origin. Whether the differences in activity of actinomycin D and the actinomycins listed in Table II is due to the chemistry or the sensitivity of the cell lines is unknown. Smith(8) has reported that approximately 100 mμg per ml of cycloheximide is required to inhibit growth of the KB cell line. This is somewhat more than the 33 mμg per ml that we need to inhibit the L cell line.

Summary. In a study of effects of antibiotics on multiplication of L cells in suspension culture, positive inhibition of multiplication was found when less than 1 μg/ml of any one of the following were added to the media: actinomycins B, B_V, or D_{IV}; cycloheximide; gliotoxin; hygromycin; mitomycin C; patulin; thiostrepton; and xanthomycin. Actinomycin B_V was the most active of the group tested as 1.5 mμg/ml was sufficient to cause a 50% reduction in growth of culture. While the shape of dose-response curves varied with different inhibitors, it was possible to use the linear portion as the basis of a method for quantitative estimation of concentration of inhibitor present. This method may be useful in determining the presence of cytotoxic metabolites formed in microbial fermentations.

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Influence of Fighting on Leukemia in Mice.* (25224)

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It has been observed, in leukemic strains of mice, that lymphoid leukemia occurs less frequently and later in males(1,2). This observation has been confirmed in our subline of inbred AK mice, in which: 1) incidence of leukemia is 15% less in males; 2) average age at death from leukemia is 315 days in males and 281 in females, a highly significant difference. Such a difference of susceptibility is commonly attributed to sex hormones. Estro-

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