late in the infection of amino acid depleted cells only with relatively high multiplicities of infection which would produce a maximal primary effect (Exp. 2, Fig. 1).

Since the stimulatory effect can be observed in the first hour of infection, is multiplicity dependent, and occurs in the presence of guanidine (an inhibitor purported(13) to interfere with synthesis of virus-induced early proteins), the primary viral effect would appear to be an interaction between the parental virion and some cellular structure.

It would seem unlikely that the teleological purpose of such an effect would be to induce a maximal rate of cellular DNA synthesis. Rather the synthesis may be incidental to some other activity essential to viral replication. By the same token, it cannot be assumed that virus-induced inhibition of DNA synthesis is a process directed primarily and exclusively to the prevention of DNA replication, particularly *in situ* in motor neurons.

Summary. The rate of DNA synthesis in HeLa cells cultivated in medium without histidine was stimulated in the early stages of infection with poliovirus and could be inhibited in the late stages. The degree of each effect increased with increasing multiplicities of infection. The inhibition, but not the stimulation, was prevented with guanidine. The effect of guanidine was blocked by dimethylaminoethanol. It is proposed that both viral effects upon DNA synthesis which are dependent upon the multiplicity of infection are indicators of one primary interaction of the parental virion with some cell structure.

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Toxic Effects of Various Penicillins and Cephalosporin Derivatives On Human Amnion Cell Cultures.* (31902)

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The penicillins and available cephalosporin derivatives are, with rare exception, virtually non-toxic for man and animals, even when administered in large doses. However, high concentrations of benzylpenicillin and low ones of cephalothin damage cultured mouse embryo and human amnion cells(1). The purposes of the present study were (a) to study a number of penicillin and cephalosporin C congeners that have not been examined before, (b) to determine the effect of degradation products of these compounds, and (c) to ascertain the activity of combinations of chlortetracycline with the other drugs on cultures of human amnion cells.

Methods. The penicillin compounds studied were 6-aminopenicillanic acid, potassium phenethicillin, sodium methicillin, sodium

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TABLE I. Concentrations of Penicillins Producing CPE_{50} in Human Amnion Cells.

Drug	mg/ml
6-Amino-penicillanic acid	17.6
Phenecillin (K salt)	9.1
Methicillin (Na salt)	9.0
Oxacillin (Na salt)	4.4
Penicillin G (Na and K salt)	2.7
Penicillin V (K salt)	2.2
Nafcillin (Na salt)	2.2
Ampicillin	1.2
3,4 Dichloro-a-methoxybenzyl penicillin (K salt)	1.2
2-Biphenyl penicillin (Na salt)	2.1
O-Cyclohexylphenyl penicillin (Na salt)	.8
2-Phenyl-3-thianaphthenyl penicillin (K salt)	.16
2-Phenyl-3-benzofuranyl penicillin (Na salt)	1.1

CPE_{50} = Dose of the drug causing cytopathic effect in 50% of tissue cultures.

oxacillin, sodium and potassium salts of benzylpenicillin, potassium phenoxymethylpenicillin, sodium nafcillin, alpha-aminobenzylpenicillin, 3,4 dichloro-a-methoxybenzylpenicillin (K salt), 2-biphenylpenicillin (Na salt), O-cyclohexylphenyl penicillin (Na salt), 2-phenyl-3-thianaphthenyl penicillin (K salt) and 2-phenyl-3-benzofuranyl penicillin (Na salt). The cephalosporin C derivatives examined included 7-aminocephalosporanic acid, cephalothin, cephaloridine, cephaloglycin and cephalosporin C. Penicillamine and penicilloic acid were the only metabolic products of penicillin investigated. Chlortetracycline was combined with some of the penicillins and cephalosporin derivatives. All of the drugs were dissolved in Eagle's basal medium plus 10% calf serum; 2-fold dilutions carried out in the same medium.

Human amnion cells were obtained by trypsinization of human placentas delivered by Caesarean section. The cells were cultured in Eagle's basal medium to which 10% calf serum and 100 units of penicillin and 100 γ of streptomycin per ml were added. After adequate growth of the tissues had taken place, the fluid was replaced with medium containing varying concentrations of the compounds being studied. Three to five cultures were treated with each dilution of the various drugs, and then incubated in the stationary position at 36°C. The cultures were examined for cytopathic changes in stained (Giemsa) and unstained preparations daily for 10 days at the end of which time the CPE_{50} was calculated.

Results. Penicillins. The least toxic of the penicillin preparations proved to be 6-aminopenicillanic acid, 17.6 mg per ml of tissue culture fluid being required to produce the CPE_{50}. Phenethicillin and methicillin, although less damaging than other penicillin congeners, were more harmful to the tissues than 6-aminopenicillanic acid. Benzylpenicillin, phenoxymethylpenicillin, nafcillin and 2-biphenylpenicillin exhibited approximately the same degree of toxic effect, concentrations of these agents associated with the development of CPE_{50} ranging from 2.1 to 2.7 mg per ml. Ampicillin and the remaining penicillins produced CPE_{50} at levels of 0.8 to 1.2 mg per ml (Table I).

Two of the degradation products of penicillin, penicillamine and penicilloic acid were found to damage human amnion cells in concentrations much lower than those required for benzylpenicillin or 6-aminopenicillanic acid. The doses required to produce CPE_{50} were 0.25 mg per ml of penicillamine and 0.5 mg per ml of penicilloic acid.

Cephalosporin derivatives. With the exception of 7-aminocephalosporanic acid, the toxic concentration of which (2.7 mg per ml) was approximately the same as that of benzylpenicillin, the other cephalosporin compounds damaged human cells at levels significantly lower than any of the penicillin congeners except 2-phenyl-3-thianaphthenyl and 2-phenyl-3-benzofuranyl penicillins. The concentrations of these antibiotics required to induce CPE_{50} were as follows, in order of increasing toxicity: cephaloglycin - 0.4, cephaloridine - 0.27, cephalosporin C - 0.1 and cephalothin - 0.02 mg per ml. Treatment of cephaloridine with cephalosporinase failed to alter their toxic effects to a significant degree.

Penicillin and cephalothin combined with tetracycline. Since there is evidence that the antimicrobial activity of penicillin is reduced by addition of tetracycline, a study was carried out to ascertain whether this combination of drugs also interfered with toxicity. The

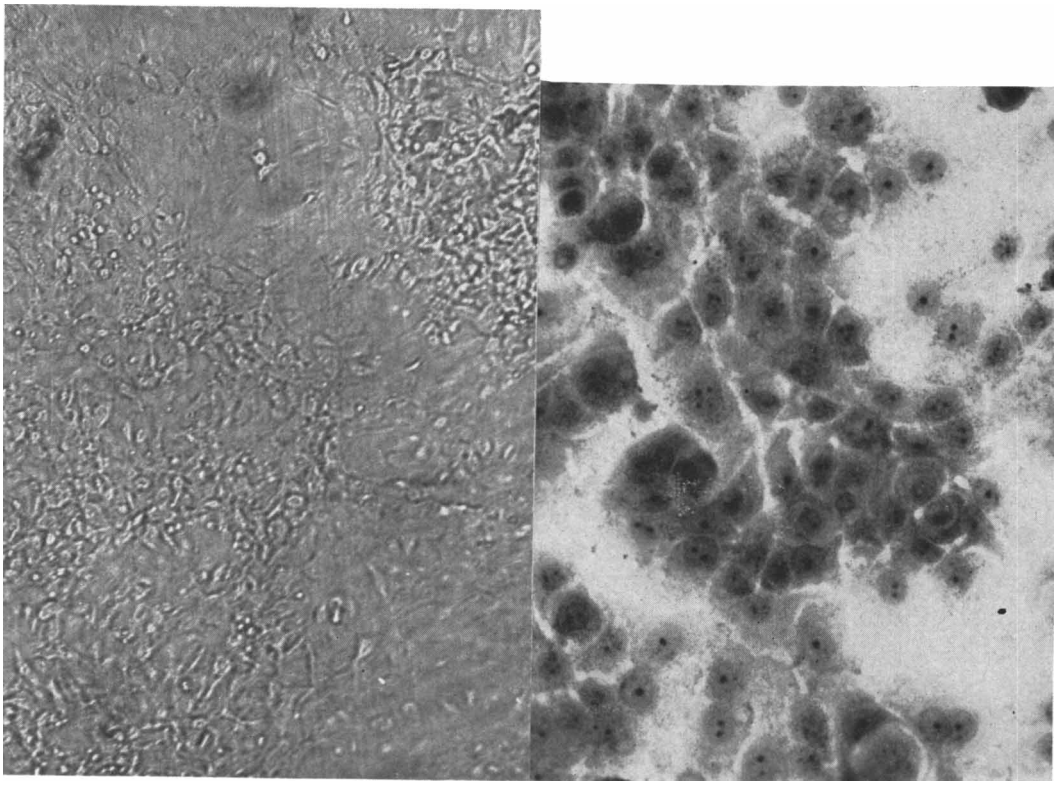

Fig. 1. Normal human amnion cell culture, unstained X 100.
Fig. 2. Granularity and accentuation of cell outlines and nuclear membranes of cells after exposure to high concentration of penicillin, unstained X 450.

same type of experiment was carried out with a mixture of cephalothin and tetracycline. Addition of subtoxic concentration of tetracycline to penicillin reduced the toxicity by about 100% (Table II). Mixture of tetracycline with cephalothin produced a decrease in the damaging effect of the cephalosporin derivative. No significant effect was produced by combining cephalothin with benzylpenicillin.

Cytologic changes induced by penicillins.

TABLE II. Effect of Chlortetracycline Toxicity of Penicillin and Cephalothin.

Drugs	Concentrations required to produce CPE_{50} (mg per ml)
CT	.04
PN	2.7
CP	.02
PN + CT	5.3 + .012
CP + CT	.05 + .05
PN + CP	3.5 + .012

CT = chlortetracycline; PN = benzylpenicillin; CP = cephalothin.

Examination of unstained cultures for cytologic changes after contact with the various penicillins revealed no differences in the effects of any of the types of this agent. Twenty-four hours after addition to the tissue cultures, all of the drugs produced granularity, retractions of the cell processes, and accentuation of the outlines and nuclear membranes of the cells. Granularity was most marked with concentrations of the drugs approximately 10 or more times greater than those required to produce CPE_{50}. At these levels of antibiotic, retraction of cell processes or rounding of cells did not develop and the cells remained attached to the glass (Fig. 1,2). With lower concentrations of the penicillins, less intense granularity but more marked rounding of cells was apparent after 24 hours (Fig. 3). All of the changes were intensified after 48 to 72 hours of incubation; fibroblast-like cells became completely round and detached from the glass. Tissue damage was

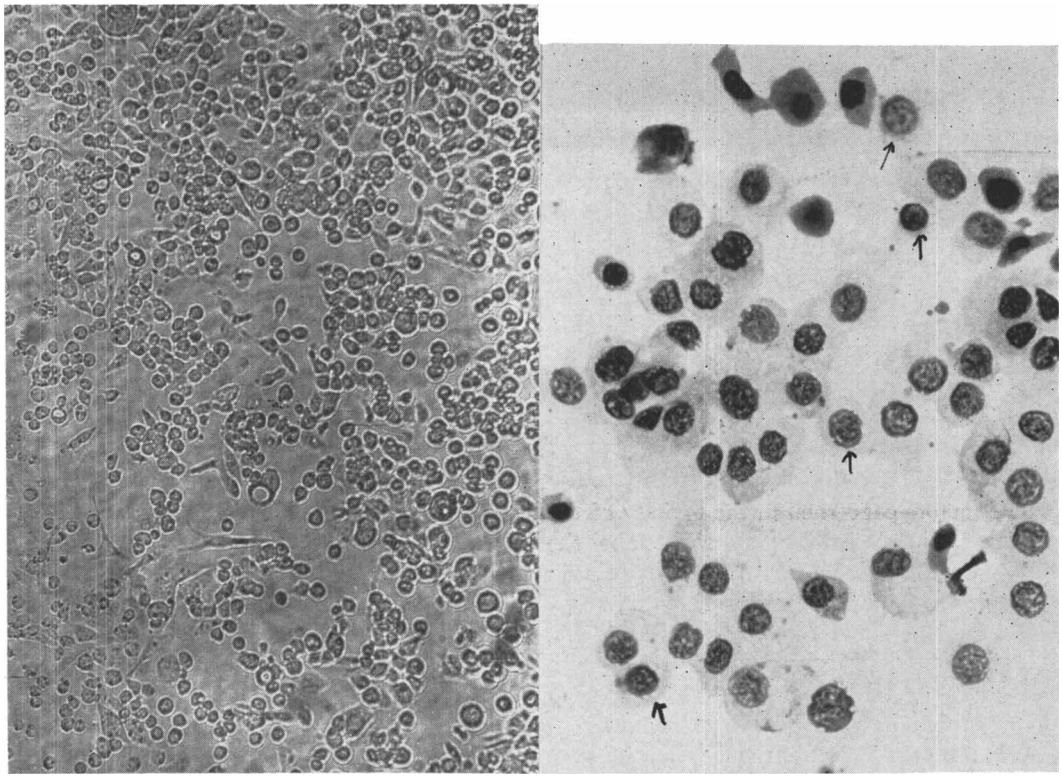

Fig. 3. Rounding and detachment of cells after exposure to lower concentration of penicillin, unstained X 100.

Fig. 4. Giemsa stained cells showing inclusion-like structures in the nuclei (Arrows) X 450

maximal by the third day.

Examination of Giemsa stained preparations of penicillin-treated tissues revealed the nuclei to be the site of the most striking changes. The chromatin became coarse and clumped, and tended to concentrate at the periphery. An inclusion-like structure surrounded by a halo, and margination of chromatin were observed in the nuclei of some of the cells (Fig. 4); these changes appeared to resemble those produced by the herpes-varicella viruses. Pyknosis of some cells was observed when high concentrations of the penicillins were used. Scattered cytoplasmic vacuoles, granularity and loss of cell processes were also noted.

Cytological changes induced by cephalosporin derivatives. The cytological changes induced in human amnion cells were strikingly similar to those developing after exposure to the penicillins. The degree of cell rounding was greater over a wider range of concentrations of the cephalosporins than with the penicillins. Thus, while this change was produced by 1 to 10 times the CPE_{50} dose of the penicillins, it was also observed with quantities 100 times greater than the CPE_{50} producing dose. Development of tissue damage was often not evident for as long as 5 days after addition of the cephalosporin compounds to the cultures.

Discussion. The varied capacity of the penicillins to produce cytotoxicity may be related to the same factors that determine differences in their antimicrobial activity. The results of the present study suggest that addition or modification of a side chain at position 6 in 6-aminopenicillanic acid, known to influence the degree of antibacterial effectiveness, may also play a role in conditioning the toxicity of penicillin. This is suggested by the fact that 6-aminopenicillanic acid was found to be the least harmful to cultured cells. These studies also indicate a pos-

sible direct relation between potential cytotoxicity and stability of a penicillin congener in aqueous solution at neutral pH(2,3). Thus, ampicillin, the most stable of the compounds examined, proved to be the most toxic. On the other hand, methicillin, the least stable penicillin, was noted to be the least damaging to cultured cells. An exception to this generalization is phenethicillin; this is more stable but less toxic than benzylpenicillin. That antimicrobial activity and the capacity to produce cellular injury are not related is indicated by the demonstration that while treatment of benzylpenicillin with penicillinase destroys the antibacterial effects of this agent it increases its cytotoxicity.

The factors concerned in the toxicity of the cephalosporin derivatives appear to be the same as those which may play a role in determining the tissue-damaging potential of the penicillins. Thus, 7-aminocephalosporanic acid was found to be the least toxic of these agents. The presence of a side chain at position 3 or 7 in this compound appeared to increase its capacity to injure cells. In contrast to the effects of penicillinase on penicillin, treatment of cephalothin with cephalosporinase decreased the cytotoxicity of this drug.

It is interesting that inhibition of the antimicrobial activity of penicillin by addtion of tetracycline appears to be associated with a similar effect on toxicity of the former(4-6). This is especially striking in view of the observation that complete destruction of antibacterial effect of penicillin by exposure to penicillinase results in an increase in its cytotoxicity.

It should be pointed out that the concentrations of the antibiotics employed in these studies were far in excess of those developing in human tissues and fluids during treatment of infections with even the highest safe doses. The implications of these data for clinical situations have not been determined.

Summary. The cytotoxicity of a number of penicillin and cephalosporin congeners for cultures of human amnion was determined. The least injurious compounds were found to be 7-aminocephalosporanic and 6-aminopenicillanic acids. Addition of a side chain to position 6 of the basic nucleus of penicillin or to position 3 or 7 of cephalosporanic acid appeared to be associated with an increase in capacity to injure cultured cells. Destruction of the antimicrobial activity of penicillin by treatment with penicillinase resulted in an increase in toxicity, while inactivation of cephalothin by exposure to cephalosporinase decreased the tissue damaging effect. These observations suggest a lack of relation between antibacterial effects and the potential for producing cellular injury. Addition of tetracycline to penicillin or cephalothin reduced the cytotoxicity of these agents.

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