such as clouding of the cornea, depilation or change in hair color was ever observed.

The subsequent development and status of the calves which survived after treatment with a lethal dose of gamma-radiation and autologous bone marrow is of interest. After 60 days they were fed a ration of alfalfa hay supplemented with a grain mixture and kept outdoors most of the time. Since spring of 1960 they have been on pasture. One animal ingested a foreign body and was slaughtered because of ensuing traumatic pericarditis and reticulitis, 11 months after irradiation. The 8 other animals developed normally and had estrous cycles with regular intervals. They have been bred by artificial insemination and seven are now (19 to 27 months after irradiation) pregnant. At various times their blood counts have reached levels which are considered normal for heifers of this age. There has been a tendency toward thrombocytopenia in 4 of the animals with counts of 200,000 to 300,000 per cmm being recorded several times. No evidence of leukemia has been obtained. With the exception of 1 heifer which is somewhat gaunt, the animals appear to be normal.

Summary. Intravenous injection of homologous bone marrow or of a mixture of fetal liver, spleen and thymus after exposure to a lethal dose of whole-body gamma-radiation failed to protect calves against death from bone marrow damage. Nine of 15 calves treated with autologous bone marrow recovered after a severe hematologic crisis; 8 of these developed normally, showed no apparent signs of late radiation damage, had regular estrous cycles and 7 are now pregnant.

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Trachoma Viruses Isolated in United States. 3. Strain specific effects of tetracycline and penicillin.* (26097)

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Members of the psittacosis—LGV—trachoma group of viruses differ from "true" viruses in being susceptible to the action of many antimicrobial drugs both experimentally and clinically. Long before trachoma viruses were first successfully cultivated(1) millions of persons in trachomatous areas were being treated with topical tetracycline or sulfonamide applied to the eye. Under the auspices of the World Health Organization extensive trials of various time-dose regimens

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in trachoma treatment are being conducted (2). In laboratory studies(1,3) it has been observed that virus proliferation was completely inhibited by tetracyclines and penicillin in high concentrations, partially inhibited by chloramphenicol and unaffected by streptomycin. These findings formed the basis for the now uniform usage of streptomycin in isolation of trachoma viruses from clinical specimens(4,5). In view of reports of striking failures of penicillin to eradicate trachoma virus from experimentally induced infection in man(6) we undertook quantitative studies of antimicrobial drug action on trachoma viruses. This report deals with the effect of tetracycline and penicillin on 3 representative strains isolated in United States.

Materials and methods. Viruses: Three representative strains of trachoma virus isolated in United States(5) were employed. To evaluate possible strain-specific behavior, pools from 2 yolk sac passage levels (YS) of each isolate were used with the following egg LD_{50} titers: Strain BOUR, YS 10 ($10^{-6.4}$), YS 12 ($10^{-4.4}$), strain ASGH, YS 7 ($10^{-7.4}$), YS 8 ($10^{-6.7}$), strain APACHE #1, YS 7 ($10^{-6.7}$), YS 8 ($10^{-5.8}$). Stock virus pools were prepared as described(7) and stored at –40°C. Bacterial sterility was established by suitable cultures.

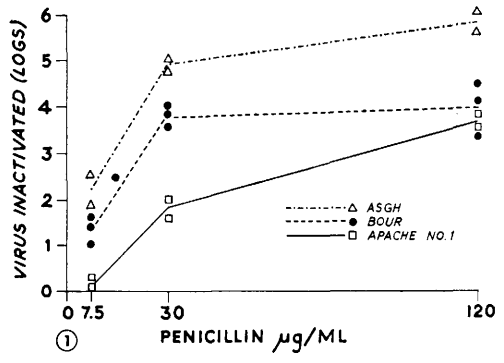
Antibiotics. Crystalline potassium penicillin G was obtained commercially in sterile vials containing 1 million units. Stock solutions of 50,000 units/ml (30 mg/ml) were prepared in saline freshly for each test. Tetracycline hydrochloride in sterile vials containing 20 mg was supplied by Dr. F. Fink. Stock solutions of 1 mg/ml were prepared in saline freshly for each test. Further dilutions of antibiotics were made in broth and kept refrigerated until mixed with virus dilutions. Drug concentration is expressed as μg/ml in the final drug-mixture that was inoculated into eggs. No additional doses of drug were administered.

Assay of antibiotic activity. A vial of stock virus was rapidly thawed, 10-fold dilutions were prepared in chilled broth and promptly added to the antibiotic dilutions. The mixture was kept for 30 minutes in an ice bath, then inoculated in 0.5 ml volume into the yolk sac of groups of 7-day embryonated eggs. Five or 6 eggs were used per drug-virus mixture. Eggs were incubated at 35°C and candled twice daily. Deaths occurring during the first 48 hours were discarded as non-specific. All deaths from the third to the twelfth days of incubation were recorded. Random yolk sacs from dead eggs in each group were smeared and stained by Macchiavello's method to confirm microscopically presence of virus. Such virus was seen regularly in eggs dying on or after the 6th day of incubation. Deaths between day 3 and 5 of incubation were exceedingly rare, and in them virus was seen infrequently. Fifty per cent death endpoints were calculated by accepted methods(8) and expressed as egg lethal titer (ELD50)/g of yolk sac. Each test included a series of virus dilutions mixed with antibiotic-free broth. Final results are presented as the amount of "virus inactivated" by a given concentration of antibiotic (*i.e.*, difference between ELD50 of antibiotic-free and antibiotic-containing virus mixture).

Results. For simplicity of presentation amounts of "virus inactivated" (logs) were plotted against concentration of drug (arithmetic scale). This presentation seemed preferable as it yielded a number of linear or parallel relationships whereas no simple relationships were apparent on log-log plots. At least 2 different stock virus pools with different egg LD_{50} titers were titrated in the presence of varying amounts of each drug. In this fashion it was hoped that the behavior of a given single virus pool might not be mistaken for a strain characteristic.

The results with penicillin are shown in Fig. 1, those with tetracycline in Fig. 2. For each strain the results appeared remarkably constant, as evidenced by the proximity of individual points in the figures. Each strain appeared to behave in an individual and reproducible manner.

Penicillin (Fig. 1): At each of 3 concentrations strain APACHE #1 was markedly more resistant than strain ASGH, by 2-3 logs, with strain BOUR in an intermediate position. Thus penicillin 7.5 μg/ml had no effect on APACHE #1, but "inactivated" 99%



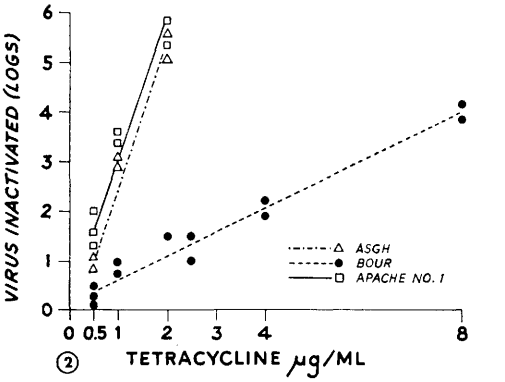


FIG. 1. Effect of penicillin on 3 strains of trachoma virus. Amount of virus "inactivated" (Log ELD_{50} without antibiotic − log ELD_{50} in presence of antibiotic) by various concentrations of penicillin G potassium.

FIG. 2. Effect of tetracycline on 3 strains of trachoma virus. Amount of virus "inactivated" (log ELD_{50} without antibiotic − log ELD_{50} in presence of antibiotic) by various concentrations of tetracycline hydrochloride.

of ASGH. The 4-fold increase in penicillin concentration from 7.5 to 30 μg/ml reduced viable virus of any strain by 99%, but the next 4-fold step had much less effect. At a penicillin concentration of 800 μg/ml (not shown in chart) no virus-specific deaths were observed. Thus 800 μg/ml penicillin completely blocked viral proliferation to lethal levels, in spite of drug inactivation during incubation at 35°C. No blind passage of surviving eggs was done and thus the persistence of sublethal amounts of infective trachoma virus cannot be ruled out. However, smears of surviving eggs showed no microscopic evidence of virus. Fig. 1 suggests that different strains of trachoma virus have basically different susceptibility to penicillin and that in each strain members of the population vary in penicillin-susceptibility over a fairly wide (more than 16 fold) range.

Tetracycline (Fig. 2): The response of the 3 strains of trachoma virus to tetracycline appears to differ markedly from their response to penicillin. For any one strain the relationship of drug dose (arithmetic) to amount of "virus inactivated" (logs) appears to be linear, and the slopes of the lines are distinct for a strain. Whereas 0.5 μg/ml tetracycline affects strains BOUR and ASGH only to a negligible extent, it reduces the infective titer of APACHE #1 by from 20 to 99%. A concentration of 2 μg/ml tetracycline completely prevents growth of strains ASGH and APACHE #1 but affects BOUR little. Even 8 μg/ml does not completely "inactivate" BOUR. Thus it appears that 2 strains are completely "inactivated" over a very narrow (4-fold) range of tetracycline, apparently all infective units being uniformly susceptible to that drug. Strain BOUR, on the other hand, is "inactivated" over a very wide range of drug concentrations, suggesting that some infective virus units are markedly more resistant to tetracycline than others.

Discussion. The results reported here do not at present have any direct practical applicability to treatment of human trachoma. Ophthalmic ointments or solutions usually contain tetracycline 10 mg/g. Thus the concentration of drug placed on trachoma-infected epithelial cells is many times higher than the concentration necessary for *in ovo* inhibition of growth of the more resistant virus strains. However, rapid removal of topically applied preparations from the conjunctival surfaces by lid action and uncertainty of diffusion and penetration might result in barely effective concentrations of tetracycline within infected epithelial cells. Under such circumstances differences in strain susceptibility might make themselves felt in the clinical response of the patient. A much more important aspect of these strain differences might pertain to the suppression versus eradication of trachoma infection in the eye. Intracellular drug concentrations might be sufficient to interfere completely with the multiplication of the more susceptible strains,

yet might permit the survival and slow proliferation of the more resistant strains. While it has been reported that penicillin is effective in trachoma(9), controlled experimental evidence(6) suggests that it often fails to eradicate the infection. This result might be anticipated from our laboratory observations that even 120 μg/ml penicillin failed to completely destroy infectivity of some strains while as little as 7.5 μg/ml reduced infectivity of other strains by 99% or more. The shape of the lines in Fig. 1 suggests that the curves might become asymptotic when complete block of virus proliferation is approached at high drug levels. Thus it might be expected that very high concentrations of penicillin would largely but not completely suppress virus multiplication. Expressed clinically this would mean amelioration of the disease without cure, and a high probability toward relapse—as was indeed observed in patients(6). It may be remembered in this connection that T'ang(1) isolated some trachoma viruses from clinical specimens containing 30-120 μg/ml penicillin, perhaps because in conjunctival scrapings rich in trachoma virus some particles might retain infectivity even after exposure to penicillin.

Apart from possible practical implications, the demonstration of marked strain-specific effects of tetracycline and penicillin has several points of interest. The selection of very highly drug-resistant mutants may be possible and their use as "markers" for genetic studies suggests itself(10). Antibiotic action should be related to growth cycles of trachoma virus in eggs to compare the inhibition dynamics of these large viruses to those observed with bacteria. For example it is possible that the high susceptibility of strain ASGH to penicillin is a function of its rapid growth rate, as an analogy of the dependence of penicillin effect upon growth rate of bacteria. Evidence has been presented(7) that ASGH indeed kills eggs faster than other strains of trachoma virus, perhaps because of faster growth rate.

All virus inocula employed in the present work contained streptomycin. The effects described may therefore represent combined antibiotic action rather than the effect of the single drug under study. While 20 mg/ml streptomycin has no demonstrable effect on trachoma virus infectivity *in ovo*(3,4,5) and while Gordon *et al.*(10) failed to demonstrate any combined antibiotic effect on psittacosis viruses, the possibility exists that biologically inactive drug may enter into combined action with another drug(11). Therefore future drug assays must include truly antibiotic-free preparations of trachoma viruses.

Summary. The ability of varying amounts of penicillin or tetracycline to interfere with the growth of trachoma virus in eggs was studied. Three strains of this agent isolated in the United States behaved in a reproducible and individual manner toward these drugs. They exhibited a very large range of differences in susceptibility to these antibiotics. The theoretical and practical implications of these findings are discussed.

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