chronic spinal rats. These tremors have been blocked by the anticholinergic, anti-Parkinson agent, procyclidine, as well as by dorsal root section. In contrast, prostigmine methylsulfate produced only muscle fasciculations which were not blocked by procyclidine or acute limb denervation. The implications of these findings are briefly discussed.

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Trachoma Viruses Isolated in the United States. V. Growth Rates and Drug Inhibition Patterns in Embryonated Eggs.* (27153)

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It has been known for some years that clinical trachoma and inclusion conjunctivitis respond to several antimicrobial drugs. In fact it is likely that trachoma could be controlled throughout the world, and perhaps eliminated altogether, if infected persons would undergo adequate treatment with tetracyclines or other effective drugs. When trachoma viruses became available for laboratory study it was found, as expected, that they were strongly inhibited by tetracyclines, less markedly by penicillin or chloramphenicol and virtually unaffected by streptomycin (1,2,3). After isolation of the first trachoma viruses from the United States in our laboratory(4) pronounced strain-specific behavior was encountered with tetracycline and penicillin. Each strain tended to be inhibited in its lethality for eggs by a constant and distinct quantity of a given antimicrobial drug. These initial results suggested the possibility that the reaction patterns to antimicrobial drugs might be genetically stable markers of trachoma strains(3). Consequently it was desirable to submit a larger number of isolates of trachoma and inclusion conjunctivitis (IC) viruses to tests with antimicrobial drugs. This report summarizes quantitative experiments on the action of penicillin, tetracycline and tylosin tartrate on 5 strains of trachoma and 2 strains of IC viruses. The action of antimicrobial drugs on viruses on the psittacosis-LGV-trachoma group is remarkably similar to their action on bacteria. The latter is greatly influenced by metabolic activity and growth rate of microorganisms. Therefore it was of interest to compare the growth rates of different trachoma and IC viruses in embryonated eggs and attempt to correlate them with susceptibility to drugs. Results of such studies are presented here.

Materials and methods. Viruses. Isolates from the United States were employed at 2 or more passage levels with the following egg LD_{50} titers (ELD_{50}/ml): Trachoma strains BOUR YS 9-11 ($10^{4.7}$ to $10^{7.0}$), ASGH YS 7-9($10^{5.1}$ to $10^{7.4}$), APACHE #1 YS 7-9 ($10^{6.5}$ to $10^{6.8}$), APACHE #2 YS 8-10 ($10^{5.6}$ to $10^{5.9}$); and inclusion conjunctivitis strains IC Cal 1 YS 6-9($10^{5.0}$ to $10^{6.8}$) and IC Cal 3 YS 7-8 ($10^{6.3}$ to $10^{6.8}$). In addition one of

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the trachoma virus strains originally isolated by T'ang *et al.*(1) in Peiping and passed in eggs for several years was kindly supplied by Dr. J. Snyder. Its passage level is unknown and it was employed as our TANG-55 YS 2-4 ($10^{7.3}$ to $10^{8.3}$). Stock virus of each strain was prepared, stored and titrated as described(5).

Assay of antibiotic activity. Crystalline potassium benzylpencillin and tetracycline hydrochloride were prepared as sterile solutions as described(3). Tylosin tartrate made available as a powder by Dr. R. S. Griffith was weighed out, dissolved in 0.85% salt solution and sterilized by passage through Seitz filter. Stock solutions of all antibiotics were freshly prepared for each test or kept at −20°C until diluted and mixed with virus inoculum. Stock virus was rapidly thawed, serial 10-fold dilutions were prepared in chilled broth and promptly added to antibiotic dilutions. The mixture was kept for 30 minutes in an ice bath, then 0.5 ml was inoculated into the yolk sac of groups of 7-day embryonated eggs. Six to 8 eggs were used per drug-virus mixture. Eggs were incubated at 35°C and candled twice daily. Deaths occurring within 48 hours of inoculation were discarded as non-specific. All deaths from the 3rd to the 12th 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 or absence of virus. Such virus was regularly seen in eggs dying on or after the 6th day of incubation. Fifty per cent endpoints were calculated by accepted methods(6) and expressed as egg lethal titer (ELD_{50})/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" (*i.e.*, difference between ELD_{50} of antibiotic-free and antibiotic-containing virus mixture) by a given concentration of antibiotic.

Growth rate in eggs. Seven-day embryonated eggs were inoculated *via* the yolk sac with either 10^1 to 10^2 or 10^4 to 10^5 ELD_{50} of a given virus strain, and incubated at 35°C. At daily intervals 3 eggs were removed from each group at random, the yolk sacs harvested, pooled, and kept frozen at −50°C. Subsequently (within a few days to 6 weeks) the pools were thawed, homogenized, serially diluted and titrated for infective virus content as described(5).

Results. The rise of infective virus in the yolk sacs of inoculated eggs is shown in Fig. 1. In view of the thermal instability of this group of viruses it is obvious that each point represents the total amount of infective virus that accumulated during incubation and withstood inactivation during this temperature exposure. With the smaller viral inocula infective virus appeared in 48 to 72 hours and increased logarithmically until the 6th day. There was no difference in the growth rates of the 3 viruses isolated in our laboratory and of TANG 55 virus which has undergone years of egg passage.

With the larger infective dose, virus was demonstrable during the first day of incubation, perhaps representing residual inoculum. On the second day of incubation there was a hundred- to thousand-fold increase in virus, evidently because of rapid multiplication. From then on to day 5 the apparent rate of growth was the same as observed with the smaller inoculum. TANG 55 virus often reached somewhat higher titers than the strains which were in early egg passages, but the slope of the logarithmic increase appeared to be similar for all strains.

The activity of benzylpenicillin against 5 strains of trachoma and one of inclusion conjunctivitis virus is shown in Fig. 2. The results of individual experiments with any one strain were grouped rather closely together and at a given drug concentration the difference in amount of virus "inactivated" varied up to 10.000-fold between strains. Thus strain IC Cal 1 lost 99.9% of its lethality upon exposure to 7.5 μg penicillin/ml whereas trachoma strain APACHE #1 appeared unaffected by that amount of drug. However, the pattern of antiviral effect fell into a broad band with some overlapping of strains.

Results obtained with the same group of strains exposed to tetracycline hydrochloride are shown in Fig. 3. Again at some critical drug concentrations there were large differences in strain behavior: Eight μg tetracy-

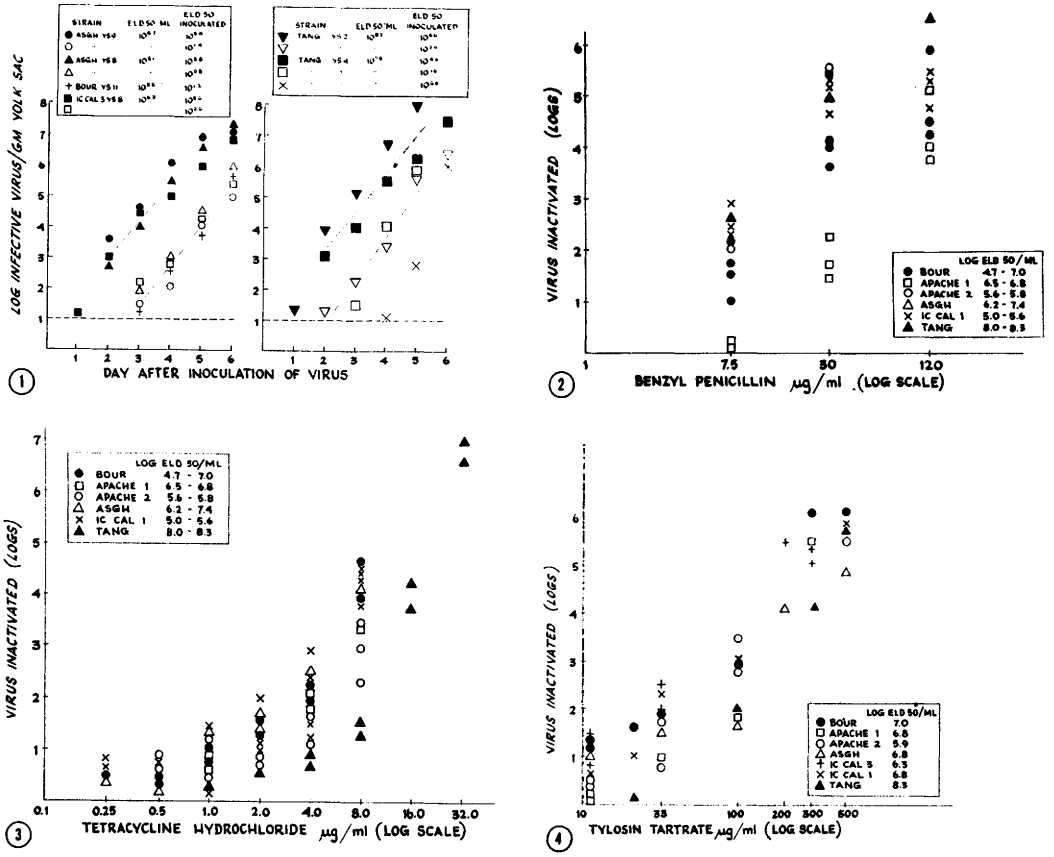

FIG. 1. Growth curves of low egg passage United States strains of trachoma and inclusion conjunctivitis viruses compared with the high egg passage Chinese strain of trachoma virus.

FIG. 2. Effect of benzylpenicillin on 5 strains of trachoma and 1 strain of inclusion conjunctivitis virus. Amount of virus "inactivated" by various concentrations of potassium penicillin G.

FIG. 3. Effect of tetracycline hydrochloride on 5 strains of trachoma and 1 strain of inclusion conjunctivitis virus. Amount of virus "inactivated" by various concentrations of tetracycline.

FIG. 4. Effect of tylosin tartrate on 5 strains of trachoma and 2 strains of inclusion conjunctivitis virus. Amount of virus "inactivated" by various concentrations of the erythromycin-like drug tylosin.

cline/ml had little effect on TANG 55 but "inactivated" 99.99% of several US strains. However, *all* strains were suppressed by amounts ranging from 2 to 32 μg tetracycline/ml and the reaction pattern represented a band with much overlapping of strains.

With tylosin tartrate (Fig. 4) the differences between strains were even less marked than they were with the other 2 drugs. Whereas less than 30 μg/ml of that drug had little effect on any strain, all viruses were inactivated by 100 to 500 μg/ml.

Discussion. After inoculation of trachoma or IC viruses into the yolk sac there was a latent period during which virus could not be detected. Subsequently a period of logarithmic growth took place for 4 days with an average daily increase of 1.2 to 1.5 logs of infective virus. This rate appeared to be independent of the size of the original inoculum and was similar for all strains examined. Trachoma-IC viruses lose infectivity fairly rapidly if infective yolk sac suspensions are exposed to 35°C *in vitro*. The degree of thermal inactivation of these agents *in ovo* is uncertain but the amount of virus titrated in our experiments probably represents only a fraction of the total amount synthesized.

TANG 55 virus, a strain which had been in continuous egg passage for 5 years in various laboratories, regularly exhibited an egg infective titer 1 log higher than any of our isolates. This might be explained by faster growth rate, lower toxicity for the egg, or greater temperature stability permitting accumulation of infective TANG 55 virus. The first of these possibilities appears to be excluded by the similar slopes of growth curves observed for all strains. The other possibilities are currently under study.

It is of interest that to date TANG 55 is the only strain of trachoma virus successfully propagated in cell culture with large yields of infective virus(7). Perhaps the features which enhance growth in embryonated eggs also favor growth in tissue culture systems. Conversely it must be noted that TANG 55 and several other strains have lost infectivity for primates in the course of prolonged egg passage(8) whereas at low egg passage levels our strains maintain this pathogenicity. It will be important to determine whether prolonged egg passage regularly leads to loss of one set of characteristics and gain of another set. It appears that TANG 55 is a highly modified laboratory strain and conclusions regarding its growth, development or drug sensitivity(9,10) need not apply to trachoma strains occurring in nature.

The results of drug action on different strains do not bear out earlier hopes that drug susceptibility patterns might be sufficiently specific and stable to serve as strain markers(3). The inhibition pattern was fairly constant for each strain but there was much overlapping in the behavior of different strains. At certain critical drug concentrations one strain was often much more susceptible than another but it is uncertain whether these strain relationships would remain constant with prolonged egg passage. The high egg passage level TANG 55 strain was more resistant to tetracycline than any other isolate tested, but was among the most susceptible to penicillin. However, this behavior was not a function of growth rate. It is remarkable that the strain was originally isolated in the presence of penicillin(1).

By weight tetracycline hydrochloride was the most active of the 3 drugs tested in abolishing lethality of these viruses for embryonated eggs. The clinical advantage of tetracycline over penicillin is established. Pollard(10) recently reported that tetracycline and tylosin "interrupted the cytochemical sequence which reflected maturation" of TANG virus in tissue culture. It remains to be seen whether this applies to the more complex situation *in vivo*.

Summary. All isolates of trachoma and inclusion conjunctivitis viruses from the United States in early egg passages grew in embryonated eggs at a rate similar to that of a Chinese isolate after many egg passages. During the phase of logarithmic growth there was an average increment of 1.3 logs of infective virus per day per gram of yolk sac. The size of inoculum determined the delay until the onset of logarithmic growth, but had no influence on rate of growth. The action of penicillin, tetracycline, or tylosin was fairly constant on each virus strain. However, there was much overlapping in drug-virus patterns so that the over-all effect of each drug could be depicted as a broad band. It now appears doubtful that the drug-susceptibility of virus strains is sufficiently fixed to serve as a genetic marker.

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