Effects of Antibiotics on Trachoma Virus in Tissue Cultures.* (26670)

MORRIS POLLARD AND YOH TANAMI
University of Texas, Medical Branch, Galveston

The propagation of trachoma virus in the yolk sac of the chick embryo by T'ang *et al.* (1) was followed by confirmatory reports (2-7) and by reports of propagation of the virus in explanted tissues from chick embryos(8), and in tissue cells of human origin (9-11). Trachoma virus was identified serologically and morphologically with the psittacosis-LGV family of agents(7,12,13). Propagation of the inclusion blenorrhea agent in the yolk sac of chick embryos substantiated its identification with the same family(14,15).

Propagation of trachoma virus by laboratory procedures provided for screening the sensitivity of virus to drugs(16,17). The screening of drug effect in chick embryos has involved inoculation of drug-virus mixtures simultaneously into the yolk sac of the embryonated egg. Trachoma is an intracellular infection. A response of extracellular virus to drug action may not reflect accurately the response of the intracellular infection in man to such treatment. Jawetz *et al.* (17) attempted to affirm the value of the WHO-recommended treatment with CTC†; however, tetracycline was substituted for CTC and the drug was mixed with the virus prior to inoculation into the yolk sac.

As psittacosis and trachoma viruses replicate and mature in human cells they induce a cytochemical sequence which involves viral DNA, an intermediate RNA matrix around the DNA virus, and emergence of antigenically mature particulate DNA-staining virus (18,19). The cytochemical changes were demonstrable in infected tissue cells stained with acridine orange and observed by ultraviolet microscopy. Antibiotics for psittacosis virus have an ability to interrupt the cytochemically defined maturation of intracellularly established virus(20,21). The technic has now been applied to trachoma-infected cells. This paper is a report of a drug, other than CTC, which has shown therapeutic effect on tissue cells in which trachoma virus was propagating.

Methods. The technics concerned with propagation and analysis of psittacosis virus replication have been described(19); the same procedures are applicable to trachoma virus.

Cells. Three human cell-lines have been used. (A) The McCoy cell(22) which was originally derived from human synovial tissue. McCoy cells were propagated and maintained in a medium consisting of Mixture 199 plus 0.5% LAH, 5% heat-inactivated calf serum and streptomycin 0.1 mg per ml medium. (B) S 3 clonal strain of HeLa cell (23) obtained from Dr. Harry Eagle, Bethesda, Md. was propagated and maintained in the same medium as for (A) except that 10% heat-inactivated calf serum was used. (C) FL line of human amnion cell(24) obtained through the courtesy of Dr. W. F. McLimons, Atlanta, Ga. The medium for FL cell line was the same as for (A) except that 20% heat-inactivated calf serum was used.

The cells were propagated as monolayer cell-sheets on cover slips in Leighton tubes. The cell sheets were washed twice with Mixture 199 and inoculated with 0.1 ml of trachoma virus in yolk-sac extract or in chorioallantoic fluid. One ml of nutrient fluid was added to each tube and cultures were then incubated at 35°C. At intervals thereafter, the infected cells were stained with acridine orange and examined by ultraviolet microscopy. At intervals after infection, the cell cultures were washed with Mixture 199 and replacement nutrient medium containing antibiotic drugs, in graded dosages, were each added to groups of cultures. The cultures were thereafter examined by fluorochrome microscopy and assayed for virus content by inoculation into fresh tissue cultures(25,11) or into the yolk sacs of 7-day embryonated eggs.

Virus. The early trials in tissue cultures were performed with a slow-growing strain

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† Chlortetracycline HCl, Lederle Laboratories, Pearl River, N. Y.

of trachoma virus (SA-2) transferred to this laboratory through the generous assistance of Dr. John Snyder of Boston, Mass.(6). More recently, T'ang strain of virus(1) was transferred here through the kind interest of Dr. L. H. Collier of London, England. Both strains of virus were propagated by inoculation of virus in yolk sac extracts into the yolk sacs of 7-day embryonated eggs. Infected eggs usually died within 4 to 7 days and Giemsa-stained smears of their yolk-sacs contained numerous microscopically detected elementary bodies. Stock virus was stored at −50°C until used.

In the course of passaging T'ang virus through alternate yolk sac and tissue cultures (FL cells) a deviant appeared which grew rapidly in the allantoic cavity of embryonated eggs and killed mice rapidly by the intracerebral route. The effects of drugs on both fast- and slow-growing lines of virus were determined.

Antibiotics. Six drugs have been tested for effect on trachoma virus propagation in cells: Dihydrostreptomycin SO_4; sulfanilamide; penicillin G; chlortetracycline HCl; tylosin tartrate‡(26,27); and tylosin SO_4.* Each drug was diluted in tissue culture medium and added to groups of cell cultures on cover slips. Drug dosages ranged from 200 μg/ml to 0.5 μg/ml. The cells were examined at daily intervals thereafter by fluorescence microscopy(19) for evidence of cytotoxicity. Cell cultures on cover slips were inoculated with trachoma virus (as described above) and at intervals thereafter the nutrient fluid was exchanged for nutrient fluid containing a graded dose of one antibiotic. The cells were thereafter examined for cytochemical evidence of virus maturation and washed cell cultures were assayed for infective virus.

Staining procedures. Cell cultures with virus were fixed in Carnoy's solution, stained with acridine orange, mounted in buffer pH 3.8 and examined microscopically with ultraviolet light. The technic has been described (19).

Results. By the fluorochrome procedure, a pattern of cytochemical changes associated with replicating trachoma virus was visualized which resembled that described for psittacosis virus, except that: (a) the reproductive cycle of SA-2 virus required from 5 to 7 days and the mass of mature virus per cell was relatively small(9); (b) T'ang virus occupied large vacuoles which were 4 to 5 times as large as the viral inclusion; and (c) the fast-growing T'ang virus usually filled the cell with mature virus particles within 48 hours after inoculation. The latter virus killed chick embryos within 3 to 4 days following inoculation into the allantoic cavity and the allantoic fluid from such embryos contained virus. T'ang virus and its enhanced form replicated readily in FL and in McCoy cells but showed less activity in HeLa cells.

Within 24 hours after inoculation of T'ang virus into McCoy and into FL cells, small spheres of RNA-staining material ("red balls") appeared. This represented a matrix of RNA around the initial virus DNA particle (Fig. 1). During the next 48 hours the "red balls" enlarged, multiplied and changed color from red, to orange, to yellow, to green. At the later stage, yellow-green pin-point virus particles appeared (Fig. 2). When various antibiotics were each added to T'ang virus represented by the "red ball" stage a gradient of virus inhibition was observed (Table I). 1. In the presence of 200 μg of streptomycin per ml nutrient medium the virus attained cytochemical maturity and was infective. 2. Penicillin and sulfanilamide each delayed maturation of virus; however, cultures with each drug contained a significant number of cells with mature virus, and infective virus was recovered from both in fresh cells. 3. CTC interrupted the virus-associated cytochemical sequence at the stage at which it was exposed to the drug. The treated "red ball" appeared solid, homogeneous and intensely red. In a significant number of cells there was no visible evidence of virus by the fifth day of treatment and infective virus could not be recovered from the CTC-treated cultures. 4. Tylosin tartrate, like CTC, interrupted the trachoma virus-induced cytochemical sequence at the stage at which it was added. The "red balls" developed a target-like appearance; the red sphere developed distinctive concentric rings of rarefaction and appeared to be disintegrating. Infective virus

‡ Eli Lilly and Co., Indianapolis, Ind.

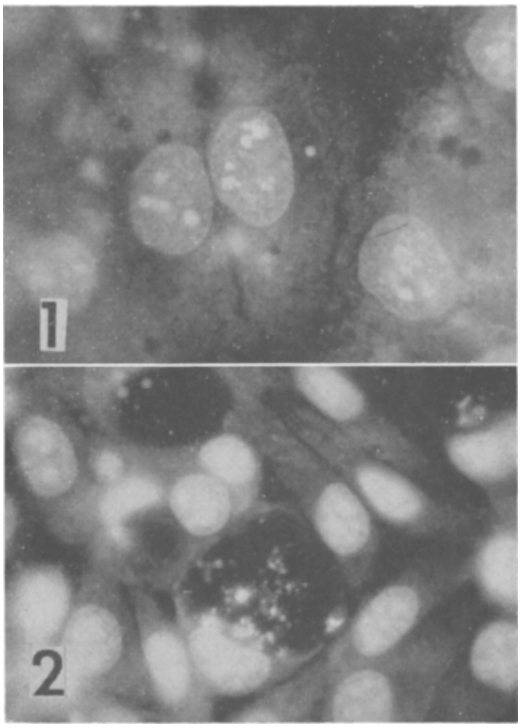

FIG. 1. FL cell infected with T'ang trachoma virus. At 24 hr after inoculation this culture was treated with tylosin tartrate 25 μg/cc for 2 additional days. In the original color transparency, the white sphere in the cytoplasm is stained red. Acridine orange stain and U.V. light × 640.

FIG. 2. FL cell infected with T'ang trachoma virus 3 days previously. No treatment. Viral material occupies a large cytoplasmic vacuole. In the original color transparency the colors induced in developing virus range from red to orange to mature green, pin-point elementary bodies. Acridine orange stain and U.V. light × 640.

could not be recovered from such cultures. Tylosin tartrate caused visible shrinkage of nucleoli, as did CTC, but was otherwise nontoxic at the doses used. 5. Addition of Tylosin SO_4 to infected cultures delayed maturation of virus for one day and thereafter the virus was indistinguishable from that in drug-free cultures.

Discussion. Except for streptomycin, antibiotics inactivated extracellular psittacosis virus(21) thus yielding results which were not representative of their clinical value. The same effect can be ascribed to trachoma agent. It is likely that drugs may be ineffective therapeutic agents because they fail to penetrate the wall of the infected cell, deteriorate rapidly, or are excluded from the biosynthetic mechanism of replication. Tests on psittacosis virus have shown a gradient of inhibitory effect among tylosin derivatives in which tartrate and gluconate were most effective, lactate was intermediate and sulfate was least effective(21).

The virus-inhibiting effects of CTC and of tylosin tartrate may be attributed to interference with the process by which the virus-oriented RNA matrix is mobilized or, at a preceding stage, with elaboration of viral DNA in the cell. This contrasts with the inhibitory effects of fluorinated pyrimidines after being applied to the "red ball" stage of psittacosis virus; incorporation of the drug into the synthetic sequence resulted in accumulations of visible masses of "fraudulent" virus-associated RNA or of incomplete viral DNA(28). Trachoma virus responds similarly to treatment with fluorinated pyrimidines(29). Thus, in addition to morphologic and serologic similarities among members of the psittacosis-LGV family of agents, the characteristic pattern of biosynthetic replication adds another bond to this relationship. This cytochemical sequence is unique and has been observed neither with rickettsial agents nor with vaccinia virus; therefore, it

TABLE I. Effects of Antibiotics on Replication of Trachoma Virus in McCoy Cells.

Drug	Units/ml medium	Microscopic effect*	Virus recovery after treatment, particles/ml†
Streptomycin	200 μg	Mature	5.85×10^6
Sulfanilamide	200 "	"	5.59×10^6
Penicillin	100 units	Partial inhibition	1.04×10^5
Chlortetracycline	25 μg	Inhibition	Negative
Tylosin tartrate	25 "	"	"
Tylosin SO_4	25 "	Mature	9.1×10^5

* As observed with acridine orange stain and U.V. microscopy on day 3 after inoculation.
† As determined in tissue cultures(28).

may become another basis for identification of psittacosis and related agents.

It would be hazardous to transpose arbitrarily information from a laboratory procedure to clinical application; however, the experimental procedure has yielded results with psittacosis virus which reflected accurately the clinical value of drugs. The evidence presented here substantiates the ineffective role of streptomycin, penicillin, and sulfonamide in specific treatment of trachoma, confirms the therapeutic value of CTC, and endorses tylosin tartrate as a drug with sufficient merit to warrant clinical trial.

Summary. Six antibiotic drugs were tested for inhibitory effect on trachoma virus in human tissue cells. Chlortetracycline hydrochloride and tylosin tartrate interrupted the cytochemical sequence which reflected maturation of the virus. Penicillin and sulfanilamide only delayed maturation and streptomycin had no effect on the virus. A cytochemical procedure for detection of trachomastatic drugs is described.

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