Serial Propagation of *Toxoplasma gondii* in Roller Tube Cultures of Mouse and of Human Tissues.* (20788)

ELI CHERNIN AND THOMAS H. WELLER.

From the Research Division of Infectious Diseases and the Department of Medicine, Children's Medical Center, and the Department of Tropical Public Health, Harvard School of Public Health.

The demonstrated usefulness of roller tube cultures of mammalian tissue for the isolation of certain viruses of human origin has stimulated interest in the behavior of other types of human pathogens in tissue culture. While multiplication of *Toxoplasma gondii* has been demonstrated in cultures of chicken embryonic tissues(1-6) and on a single occasion in mammalian tissue(7), the propagation of *T. gondii* in cultures of the roller tube type has not yet been explored. Apart from the diagnostic considerations involved, a study of the capacity of *T. gondii* to propagate in roller cultures of human and of other mammalian tissues might be expected to augment our knowledge of the bionomy and the pathogenic attributes of the parasite. This paper deals in a preliminary manner with the behavior of *Toxoplasma* in roller cultures of mouse embryonic and various human tissues.

Materials and methods. A. *Preparation and maintenance of cultures.* Roller tube cultures were prepared as described by Robbins, Weller and Enders(8) in studies on cultivation of the poliomyelitis viruses. The tissues employed were either of mouse embryonic origin, derived from white mice of the Harvard Medical School strain between the 13th and 18th day of gestation, or were of human origin, including foreskin, myometrium and embryonic skin and muscle. The mouse embryos or individual human tissues were minced with scissors and fragments explanted in a thin layer of clotted chicken plasma on the walls of roller tubes or on narrow coverslips which were subsequently inserted into roller tubes. The fluid medium of Enders(9) (beef amniotic fluid, 90%; beef embryo extract, 5%; and inactivated horse serum, 5%) with penicillin, streptomycin, soybean trypsin inhibitor and phenol red(8), was employed. All roller cultures were maintained at 35°C and the nutrient fluids (2 ml per tube) were changed at 3- to 4-day intervals. Cultures were inoculated as tissue growth became well established, usually after 4 to 8 days incubation in the case of the mouse or human embryo preparations, and after 10 days for the cultures of human prepuce and myometrium. For histologic examination coverslip preparations were withdrawn at intervals, fixed in Zenker's-acetic or Bouin's, and stained *in toto* with hematoxylin-eosin or Giemsa's stain. B. *Strains of Toxoplasma employed.* The RH strain was supplied by Dr. Quentin M. Geiman in whose laboratory it had been maintained by intraperitoneal passage in mice at 4- to 5-day intervals since its receipt in February, 1953, from Dr. Leon Jacobs of the National Institutes of Health. A second strain (KM), recently isolated at the Children's Hospital by Dr. William D. Winter, Jr. from a fatal case was supplied by him in the form of material from the 24th mouse passage. C. *Inoculation and subculture procedures.* The inoculum for the first passage in roller cultures consisted of 0.1 ml of a 1:20 dilution in medium of peritoneal fluid removed from an infected mouse on the third day of infection. Subsequently, as organisms became numerous in the medium, (determined by microscopic inspection), fluids from a group of infected tubes were pooled and 0.1 ml aliquots transferred to each of a new group of 3 or 4 roller cultures. Comparable control cultures were maintained and subinoculation of fluids from such uninfected cultures was performed at the time of serial passage. Routinely, aliquots of materials used for transfer were cultured on blood agar plates and in thioglycollate broth.

Experimental observations. A. *Propagation of the RH and KM strains of Toxoplasma in mouse embryo roller cultures.* The two strains behaved similarly *in vitro* and serial passage of each was accomplished without difficulty.

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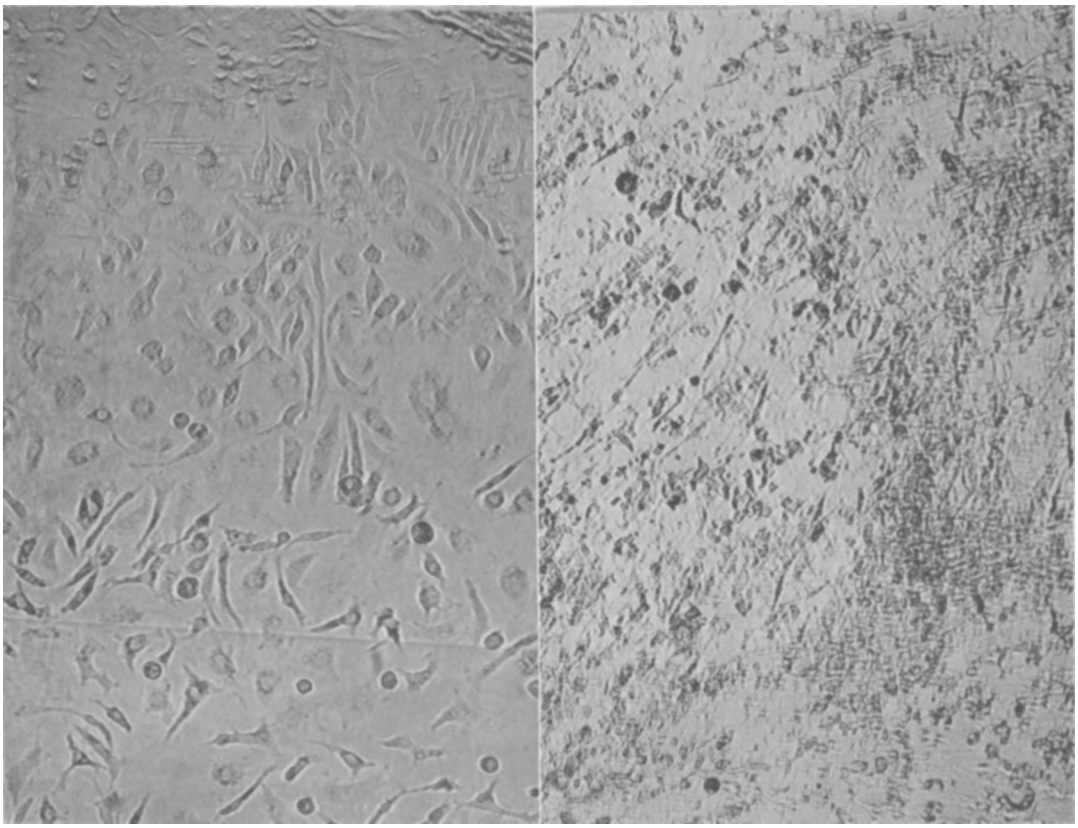

FIG. 1. Photographs (× 150) of 15-day-old mouse embryonic roller cultures. Left: control showing normal cellular growth. Right: extensive cellular destruction 11 days after inoculation with *Toxoplasma*.

The RH strain has been maintained *in vitro* for 15 consecutive passages during a cumulate period of 81 days while the KM strain has been propagated for 7 passages over a 56-day period. It is to be noted that the manipulations carried out during the maintenance of the RH strain for 15 passages resulted in a dilution of the order of 10^{-37} of the initiating inoculum; yet, the LD_{50} of infected fluids from the 15th passage as determined in mice was $10^{-5.5}$ per ml.

After inoculation cultures were usually examined daily employing a magnification of 100 X. Organisms floating free in the medium were easily recognizable and usually appeared in small numbers between the 3rd and 5th day after inoculation. Thereafter, the number of free organisms increased rapidly reaching maximum levels around the 6th or 8th day; at this time the organisms appeared as loose aggregates or floating plaques which sometimes extended across the breadth of the low-power microscopic field. Such cultures contained as many as 2.5 million organisms per ml of medium as ascertained by counts with a haemocytometer. It should be noted that infrequently, for reasons not now understood, organisms were first observed 10 to 12 days after inoculation.

Striking degenerative changes, readily visible under low magnification, occurred in the tissue phase of the cultures coincident with the appearance of large numbers of *Toxoplasma* in the medium. Typically, diffuse fragmentation and death of the cell population developed over the 48- to 96-hour period after free organisms were first observed. These specific cytopathic alterations in cultures of mouse tissue are illustrated in Fig. 1. At this stage a difference between the pH of the medi-

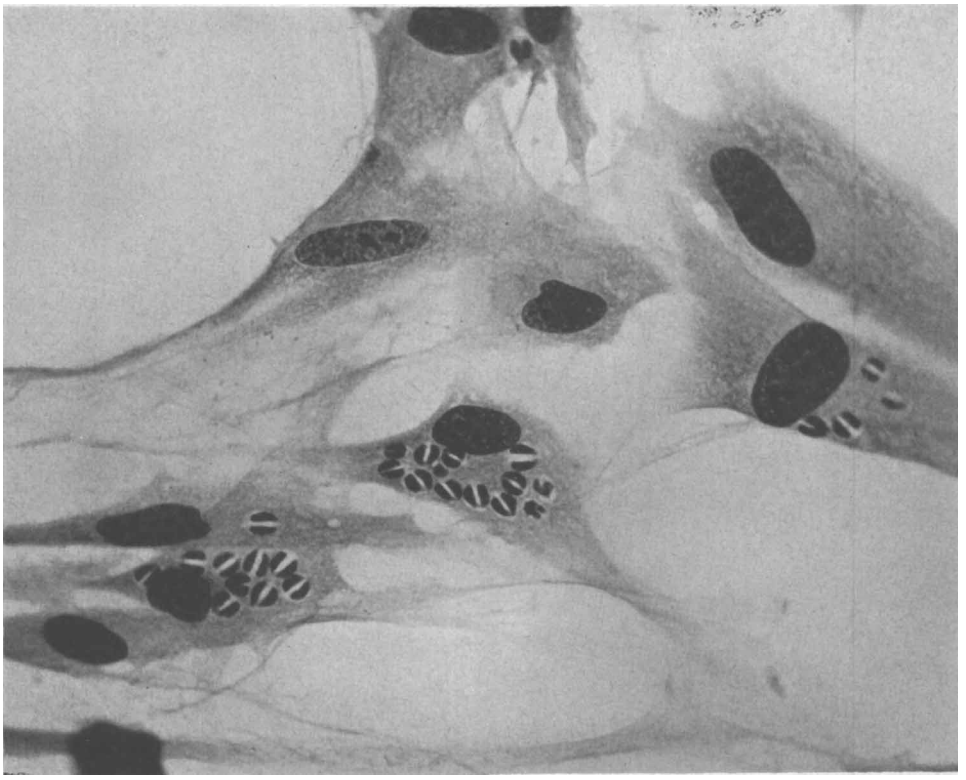

FIG. 2. *Toxoplasma* in roller culture of human uterus 4 days after inoculation. Coverslip preparation. Giemsa. × 600.

um in inoculated and in control cultures was also present, reflecting the marked destruction of tissue in infected tubes. Characteristically, the number of organisms in the medium declined rapidly once the tissues were destroyed.

In addition to the observations made during passage of the two strains, a series of ancillary experiments have been performed that will be described in detail elsewhere. Certain of the findings may be summarized as follows:

1) In confirmation of earlier reports, multiplication of *T. gondii in vitro* has not been observed in the absence of living cells. Negative results were obtained following inoculation of tubes containing medium alone, or of cultures in which the tissue phase had previously been killed with heat. 2) While study of stained preparations is not complete, all types of cells present in the mouse embryo cultures appear susceptible to the cytopathic effect of *T. gondii*. Evidence of renewed cell growth in cultures following generalized specific destruction was not noted during an extended period of observation. 3) No evidence has yet been educed indicating that a soluble toxic substance is responsible for the cytopathic changes. When the medium in uninfected cultures was replaced by medium from heavily infected cultures that had been rendered free of organisms by centrifugation, no obvious changes were noted in the metabolic activity or growth rate of the tissues. 4) No significant alteration of virulence of *T. gondii* for mice has been observed thus far in the course of serial cultivation *in vitro*. Tenfold dilutions of fluids from the 7th roller tube passage of the RH strain in mouse tissue were inoculated into roller tubes and also intraperitoneally in mice; the ID_{50} and LD_{50} titers respectively were of the order of 10^{-6} per ml. Two titrations performed on infected fluids from subsequent passages again indicated an LD_{50} titer for mice of the order of 10^{-6}; however, the ID_{50} titers in roller cultures of these materials were significantly lower.

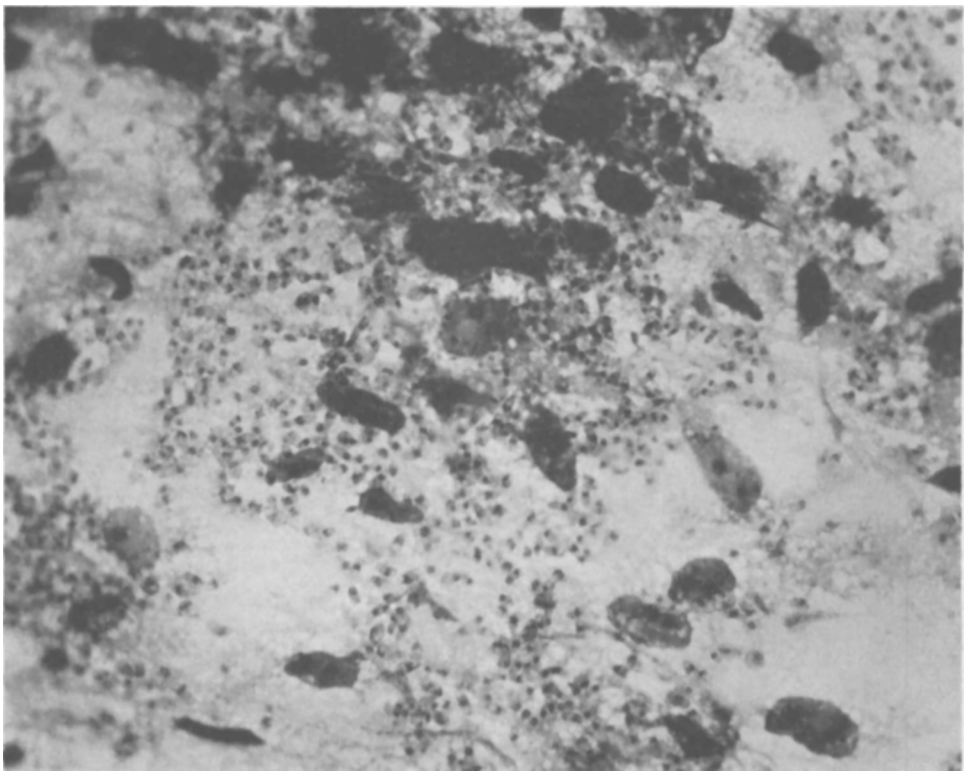

FIG. 3. Roller culture of human foreskin 7 days after inoculation with *Toxoplasma*, showing extensive tissue destruction and numerous extracellular organisms. Coverslip preparation. Giemsa. × 600.

B. *Growth of the RH strain in various human tissues.* This phase of the study was designed to determine if *T. gondii* derived from infected mouse embryo tissue cultures could multiply in various human tissues *in vitro*. Growth of *Toxoplasma* was readily demonstrated in roller tubes containing coverslip preparations of human uterus (Fig.2), foreskin, or embryonic skin-muscle. In each of these, as in mouse tissues, multiplication was associated with a generalized cytopathic effect (Fig. 3). Furthermore, the organism was propagated for 3 passages in roller cultures of human myometrium, during which time its pattern of multiplication and cytopathogenicity generally paralleled that in mouse embryo cultures.

Discussion. Multiplication of *T. gondii* has been conclusively demonstrated in roller tube cultures of mammalian cells. The findings serve indirectly to confirm Lock's(7) note on the growth of this organism in embryonic rat heart tissue. That *T. gondii* grows readily in cultures of various human tissues has been determined. Moreover, so far as we are aware, organisms of the RH strain have for the first time been grown in tissues similar in origin to those from which they were originally isolated some 15 years ago.

Although previous workers have noted degenerative changes in cultures infected with *Toxoplasma*, the relationship between multiplication of this organism *in vitro* and concomitant destruction of actively proliferating tissue has not been generally recognized. As in the case of certain viruses, the cytopathogenic attributes of *Toxoplasma* may be utilized as an indicator of specific biological activity in cultures. The large number of organisms released into the fluid phase of the cultures suggests that such material may provide a useful source of antigen for various immunologic procedures. In contrast to the organisms in infected mouse peritoneal fluid,

many of which are intracellular, those in tissue culture fluid are almost completely extracellular.

The demonstrated ability of *Toxoplasma* to multiply *in vitro* in mammalian cells also suggests that this organism may be encountered on occasion by those utilizing tissue culture procedures for the isolation of viruses from man. In such cultures, the phenomena associated with the multiplication of *Toxoplasma* might be erroneously ascribed to bacterial contamination or to viral cytopathogenicity.

Summary. The RH strain of *Toxoplasma gondii* has been propagated for 15 serial passages and a second strain for 7 passages in roller tube cultures of mouse embryonic tissues. Multiplication of the RH strain was also observed in roller cultures of human foreskin, uterus and embryonic skin-muscle tissues. In each instance, the appearance of organisms in the culture was associated with extensive degenerative changes and ultimate destruction of the tissue. At the point of apparent maximum release, the culture fluid contained large numbers of the protozoa.

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Epidemic Histoplasmosis and Airborne *Histoplasma capsulatum.* (20789)

MARTHA J. IBACH, HOWARD W. LARSH, AND MICHAEL L. FURCOLOW.
From the Communicable Disease Center, Public Health Service, Department of Health, Education, and Welfare, University of Kansas Medical Center, Kansas City, Kansas.

Several authors(1.2) have suggested that infection with *Histoplasma capsulatum* is acquired by the airborne route. This route of infection is supported by the primary localization in the lungs, the frequency of pulmonary involvement, the small size of the majority of the spores(3). and the occurrence of laboratory infections(4). This evidence is further supported by the recent report of a number of epidemics of histoplasmosis(5.6) where infections appeared to have been acquired from the air. *H. capsulatum* has been isolated from man, as well as from wild and domesticated animals, and from soils collected in various areas of the United States(6,7). The study reported in this paper represents the first isolation of the fungus from the atmosphere. An epidemic of histoplasmosis in a farm family near Atchison, Kansas, afforded an excellent opportunity to culture the air for the presence of *H. capsulatum* in a specific locality. The entire family of 5 became ill with an influenza-like illness about 10 days after cleaning an unused chicken house. One patient required hospitalization for 62 days. *H. capsulatum* was isolated from farm animals and the soil both inside and immediately outside of the chicken house. All 5 persons involved in the epidemic had pulmonary lesions demonstrable by X-ray and all of them had initially positive serological test for histoplasmosis, followed by falling titers at later examinations. A similar epidemiological picture was found near Beverly, Mo. A tenant farmer had cleaned an abandoned chicken house and contracted histoplasmosis as shown