GROWTH OF THE SINGAPORE STRAIN OF **TOXOPLASMA GONDII** IN PRIMARY MONKEY KIDNEY TISSUE CULTURES

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INTRODUCTION

Toxoplasma gondii like viruses do not grow in non-viable media. However, unlike viruses which show specific cellular tropism, the parasites will grow in a variety of tissue culture systems. The cell lines which are known to support *Toxoplasma gondii* include chick embryonic tissue (Guimaraes and Meyer, 1942), bone marrow (Holz and Albrecht, 1953), embryonic rat heart (Lock, 1953), Hela (Balducci and Tyrrell, 1956), retinoblastoma cells (Hogan et al, 1960), and trypsinized kidney tissue cells (Cook and Jacobs, 1957).

Recently a strain of *Toxoplasma gondii* was isolated from a pig brain in Singapore (Zaman et al, 1968). It was felt that a study of the growth response of this strain in monkey kidney tissue cells will be of interest, particularly to find out whether or not the strain encysts in tissue cultures.

MATERIAL AND METHODS

Primary monkey kidney (MK) tissue cultures were prepared from Malayan *Macaca irus*. The trypsinized MK cells suspended in growth medium were dispensed in Leighton tubes with flying coverslips. The growth medium consisted of Hanks's balanced salt solution (Hanks's BSS), 0.5% lactalbumin hydrolysate, 5.0% ox serum, 0.22% phenol red-sodium bicarbonate, 250 units penicillin and 250μ mg streptomycin per ml medium. Each tube seeded with 2.0 ml of MK cell-suspension (approximately 250,000 cells per ml) was allowed to grow at 37°C. Confluent sheets of cells were formed on the coverslips after 5-6 days incubation. Prior to use, the growth medium was removed and replaced with Earle's maintenance medium containing Earle's balanced salt solution, 0.5% lactalbumin hydrolysate, 0.22% phenolized sodium bicarbonate and antibiotics.

The *Toxoplasma* were maintained in white swiss mice by intraperitoneal inoculation of parasites every 4-5 days. The parasites for inoculation into tissue cultures were harvested by injecting 2.0 ml Hanks's BSS intraperitoneally and withdrawing the contents. The parasite counts were made in a haemocytometer and appropriate dilutions made using Hanks's BSS. An inoculum containing 50,000 organisms was introduced into each tissue culture tube with a pasteur pipette. The tissue culture tubes used as controls received inoculum of Hanks's BSS prepared in a similar manner but from uninfected mice. The cultures were examined periodically by removing the coverslips and staining them in Giemsa. Examinations were made after 1 day, 2 days, 4 days, 8 days and 16 days from the date of inoculation.

RESULTS

Tubes examined 1 day after inoculation showed a light infection with a few parasites scattered singly or lying in pairs in the cytoplasm of the infected MK cells. After 2 days, a greater number of parasites were seen. The distribution of the parasites was uneven as some host cells were lightly infected (1-3 parasites per cell) while others were more heavily infected (50 or more parasites per cell). After 4 days, growth of the *Toxoplasma* was more marked with extensive involvement and degeneration of host cells. The heavily infected cells showed up to a few hundred parasites which were usually lying in pairs (Fig. 1). After 8 days, the arrangement of parasites in the infected host cells changed and the parasites were found more often in chain formations or loosely arranged circles (Fig. 2). After 16 days, a number of cells were seen in which the circular arrangement of the parasites had changed even further to form more compact masses or clumps (Fig. 3). In some of the infected cells, the parasite masses were surrounded by darkly staining areas of cytoplasm which separated them from the rest of the cytoplasm (Fig. 4).

DISCUSSION

The parasites appeared in the tissue culture cells as early as 12 hours after inoculation. It is not certain whether this is due to active penetration of the MK cells by the parasite or due to the phagocytosis of the parasites by the MK cells. In the early stages of infection, most of the

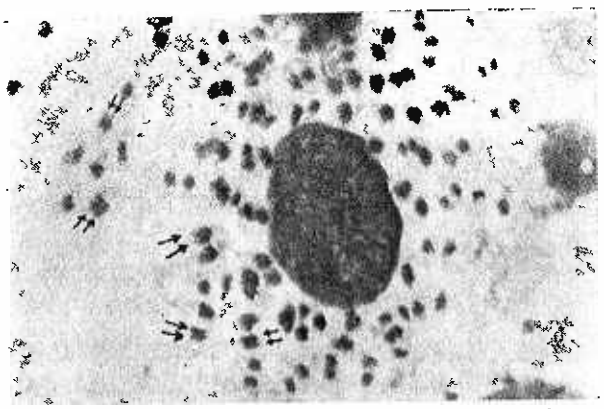

Fig. 1. 4 days after inoculation. Cytoplasm contains a large number of *Toxoplasma*. Many of these are in pairs (marked by arrows). Photographed at × 1000. Stained with Giemsa.

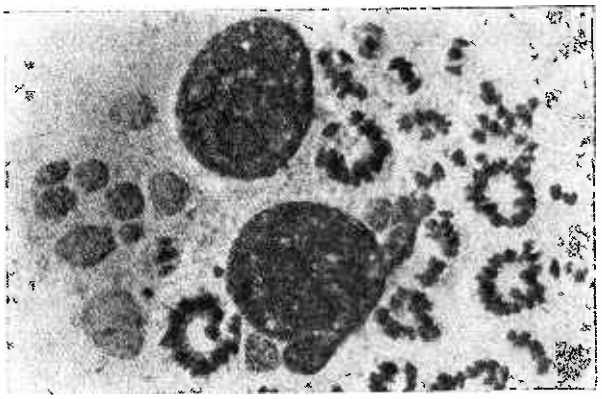

Fig. 2. 8 days after inoculation. Cytoplasm contains a large number of *Toxoplasma*. Parasites are found in chains or loosely arranged circles. Photographed at × 1000. Stained with Giemsa.

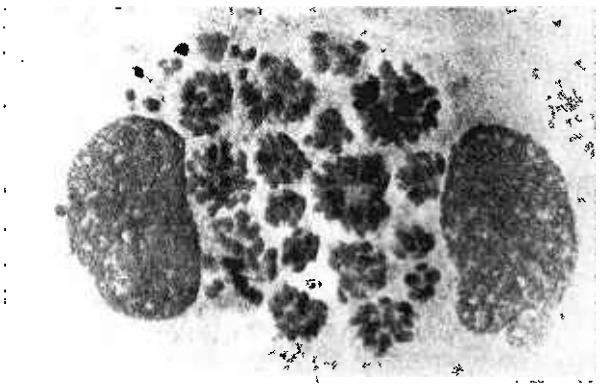

Fig. 3. 16 days after inoculation. Cytoplasm contains large masses of parasites. Photographed at × 1000. Stained with Giemsa.

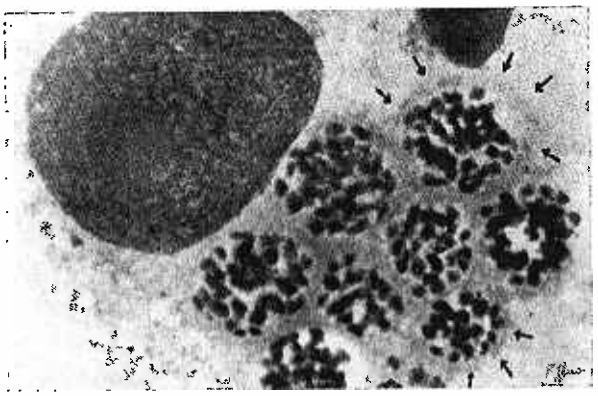

Fig. 4. 16 days after inoculation. Cytoplasm contains six masses of parasites surrounded by darkly staining areas, indicated by arrows. Photographed at × 1000. Stained with Giemsa.

parasites are seen in pairs indicating binary fission as a mode of reproduction. In the later stages, chains and circles of parasites start appearing in the infected cells. The aggregation of parasites in the form of circles or "rosettes" has been described previously (Hogan, 1962), using the RH strain of *Toxoplasma*. The presence of chains probably indicates the continuation of the reproductive process but the mechanism by which the circles appear is not clear. It is possible that the chains curl up to form circles and the curling up process could be due to the adherence of the proximal end of the parasite to each other producing a fanning effect. The circular arrangement of the parasites is followed by their condensation into tight aggregates or clumps. In the early stages these clumps do not have any structure resembling a cyst wall but later on a zone of demarcation develops between the aggregates and the rest of the cytoplasm indicating that cyst formation does occur in tissue cultures. In conclusion, it could be stated that the tissue cultures provide a convenient method of studying various development stages of *Toxoplasma* and that the Singapore strain encysts in primary monkey kidney tissue cultures.

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