STUDIES OF *TOXOPLASMA GONDII* IN CELL CULTURES BY MEANS OF IRRADIATION EXPERIMENTS*

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OBSERVATIONS on the multiplication of *Toxoplasma gondii* in different cell types of human origin were described in previous reports (Sourander, Lycke and Lund, 1960 ; Lund, Lycke and Sourander, 1961). The present report deals with observations of parasite-cell interactions after irradiation of cell cultures and parasites prior to the inoculation and the effect of the irradiation on parasite-infected cells. This was done in order to see whether inhibition of mitotic activity affected parasite propagation.

MATERIAL AND METHODS

The parasites used were obtained from freshly harvested peritoneal exudate of Swiss albino mice infected with the RH strain of *Toxoplasma gondii.*

The cell cultures were HeLa or Detroit-6 cells cultures in Gey chambers as described previously. The medium was Hanks' balanced salt solution to which was added 10 per cent human or calf serum, which was dye test negative, 0·5 per cent lactalbumin hydrolysate, 100 I.U. penicillin, and 100 μg. streptomycin. The cells were carefully rinsed 2 hr. after the inoculation of the parasites and the medium was renewed to avoid toxic effects of the mouse exudates on the cell cultures. The cultures were incubated at 37° and the medium was replaced with fresh medium every second or third day. Infected cultures were examined daily in a phase contrast microscope and photographs were taken.

The dye tests were performed according to the methods described by Sabin and Feldman (1948). As source for accessory factor fresh human sera were accepted if they were dye test negative and enabled a positive reference serum to react with a constant dye test titre in repeated tests.

The antiserum used was either a human serum, which was positive in the dye test in dilutions of 1/8000 or more or a rabbit hyper-immune serum also giving a dye test titre of 1/8000. The rabbit sera were produced by intravenous inoculation of rabbits with 0·1 ml. of a mouse exudate diluted 1/100. Irradiated and non-irradiated toxoplasma parasites were tested simultaneously as antigens to obtain titres against the reference antisera.

Irradiation technique.—In preliminary experiments the irradiation was performed by means of a conventional 250 kV X-ray unit, parasites and cells were exposed to doses varying from 600–3000 r. Later gamma rays from a 1·5 MV. ^{60}Co source were used. With this method doses varying from 30,000–120,000 rad were employed.

RESULTS

In a first series of experiments the toxoplasma parasites were irradiated. The parasite-containing peritoneal mouse exudate was divided in two portions. One portion was irradiated and the other served as a non-irradiated control. Both were used as dye test antigens and also inoculated into groups of cell cultures.

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When 600–3000 r of X-ray irradiation were given irradiated parasites did not behave differently from non-irradiated ones with regard to infection and multiplication rates as well as antigenic properties in the dye test. The X-ray unit could not be used for exposures longer than 30 min. and therefore doses exceeding 3000 r could not be obtained.

On the other hand the high intensity of the gamma irradiation did not allow doses below 30,000 rad to be used if the exposure time should not be inconveniently short. Using the gamma irradiation it was found that the irradiation did not change the stainability of the parasites or the antigenic properties as measured by the dye test. Results of experiments reported previously (Lund, Lycke and Hahn, 1960) indicated that loss of infectivity of parasites due to thermal inactivation is reflected in reduction of the number of stainable parasites. Therefore inactivation of parasites due to heat during the experimental procedure was considered negligible.

Parasites irradiated with 30,000 rad or more penetrated the cells just as untreated ones. But whereas non-irradiated parasites multiplied intracellularly and caused cell burst within 48 hr. the irradiated parasites did not divide. Only in a few cases abortive multiplications of irradiated parasites were found. Clones consisting of more than 4 parasites were never observed. After 14 days cultures inoculated with non-irradiated exudate showed parasites still in active stages of multiplication as revealed by figures of parasite divisions, cells with clones of numerous parasites, bursting cells and free extracellular parasites in the culture fluid. At this time no parasites could be detected in cultures inoculated with portions of the irradiated exudate (Fig. 1 and 2).

Two days old HeLa or Detroit-6 cells were exposed to 600–3000 r. After 48 hr. a number of giant cells were observed in the irradiated cultures. The number of such atypical cells increased gradually. However a great number of cells seemed to be morphologically unchanged. The cultures were infected with toxoplasma 48 hr. after X-ray treatment. Microscopic examinations on the following days revealed no differences between irradiated and non-irradiated cultures with respect to parasite multiplication. The development of parasites in the giant cells proceeded in the same way as in the non-irradiated cells.

When cell cultures were exposed to 30,000–120,000 rad of gamma-irradiation not only inhibition of mitotic activity was observed but grave cell damage was demonstrable already on the day following the irradiation. With 120,000 rad most cells degenerated without giant cell formation. After a couple of days no living cells could be observed in the cultures. Exposure to 60,000 rad caused death of most cells but also some giant cell formation. Such cells survived for a couple of weeks. After exposure to 30,000 rad finally most cells survived. The giant cell formation was very pronounced and these cells could be maintained for at least 4 weeks.

When cells irradiated with the gamma beam were infected with parasites, the multiplication of parasites proceeded at the same rate as in untreated cultures resulting in clone formation and cell rupture. Even the dying cells which had been treated with 120,000 rad allowed parasite multiplication. If parasites formed in irradiated cultures were passed over to new cultures they proved infective. (Fig. 3–5.)

The cultures were irradiated with 30,000 rad 3 hr. or 3 days after the inoculation of parasites. This treatment inhibited the parasite multiplication as well as

the mitotic activity of the cells. The giant cells formed survived for more than 3 weeks but no parasite multiplication was observed in the cells after the irradiation. The parasites that had penetrated or been formed in the cells before the irradiation lost their normal appearance. Thus, no internal organization could be detected, they became rounded with less distinct boundaries, and gradually the single parasites within a clone could not be distinguished. Finally there remained only dark bodies and granules from the disintegrated parasites. (Fig. 6–8.)

DISCUSSION

The electronmicroscopic studies performed by Gustafson, Agar and Cramer (1954) among others, have given evidence for a complex internal organization of the toxoplasma parasite. The parasite has a nucleus containing DNA (Sabin 1942) and a nucleolus. The cytoplasm contains regularly demonstrable structures such as the toxonemes (Gustafson *et al.*, 1954).

The parasite multiplies by binary fission as already described by Cross (1947). Thus it possesses many characteristics of a complete cell.

The mitotic activity of the mammalian cell may be affected by ionizing radiation. The chemical structure of cellular proteins and therefore also the antigenic properties are on the other hand not affected by doses sufficient to inhibit mitotic activity. For such reasons it seemed of interest to study the effects of irradiation on toxoplasma parasites. The experimental results reported showed that high doses of irradiation did not inhibit penetration into the cells but prevented multiplication of the parasites. Inhibition of multiplication was also seen of such parasites as were intracellular at the time of irradiation. However the antigenic properties utilized in the dye test remained unaffected. Thus toxoplasma reacted to irradiation with regard to antigenic properties and mitotic capability in the same way as mammalian cells.

Nothing is known about the reasons for the toxoplasma being an obligate intracellular parasite. It seems however that the intracellular metabolites necessary for parasite multiplication must be amply supplied, because parasite multiplication and growth may proceed without decrease in rate until the cytoplasm of the host cell is completely occupied by parasites (Lund, Lycke and Sourander, 1961).

EXPLANATION OF PLATES.

FIGS. 1–8.—Phase contrast micrographs of living cells.

FIG. 1.—HeLa cells with clones of toxoplasma parasites one week after inoculation of the culture with non-irradiated parasites, ×466.

FIG. 2.—HeLa cells one week after infection with parasites which had been irradiated with 30,000 rad high energy gamma rays prior to inoculation of the culture. No clones are seen, but single parasites are found in cytoplasmic vacuoles, ×500.

FIG. 3.—Detroit-6 cells, ×312.

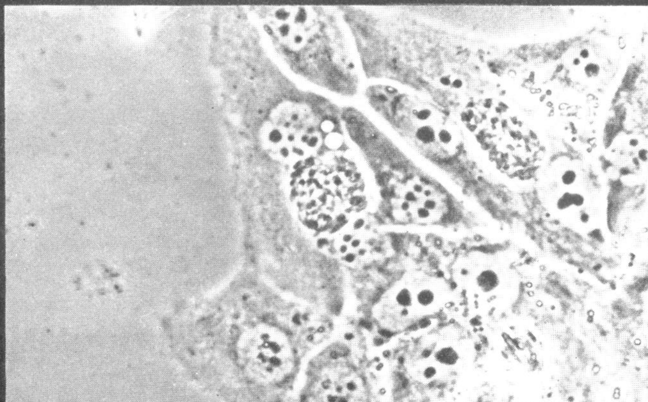
FIG. 4.—Detroit-6 cells 2 weeks after irradiation with 30,000 rad. Note the pronounced giant cell formation with swelling of the nuclei, ×312.

FIG. 5.—HeLa cells 2 weeks after irradiation with 30,000 rad. Many giant cells, some of them with multiple nuclei are seen, ×234.

FIG. 6.—HeLa cells one week after inoculation of the culture with toxoplasma. Many stages of infection are seen including rupture of parasitized cells and reinfection of other cells, ×545.

FIG. 7.—HeLa cells irradiated 3 hr. after infection. Dose 30,000 rad. One week after irradiation atypical parasites are found, but no clone formation, ×545.

FIG. 8.—HeLa cells irradiated 3 days after infection. Dose 30,000 rad. One week after irradiation disintegrated clones are seen, ×545.



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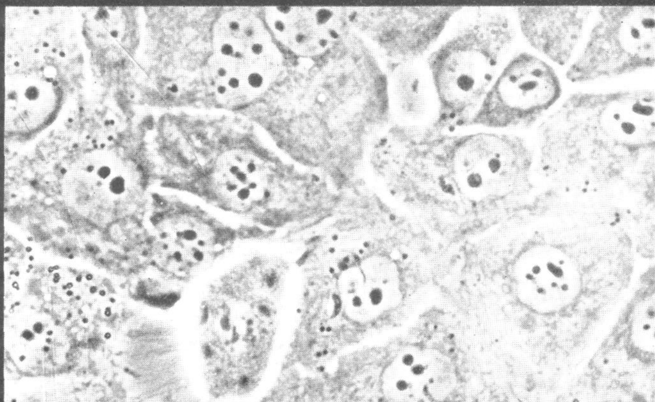

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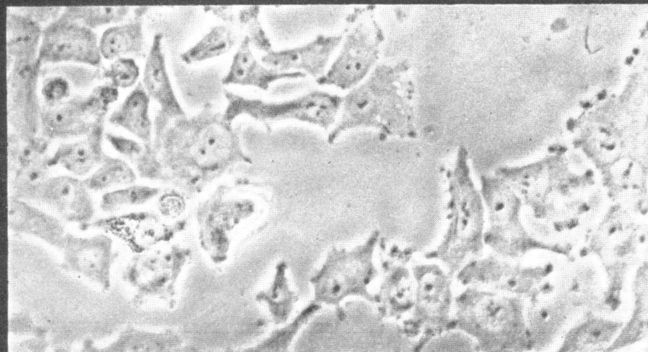

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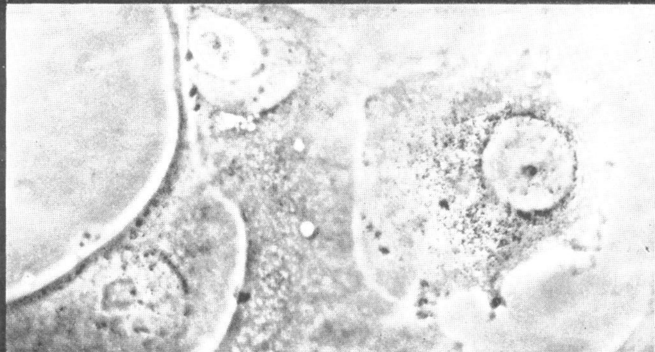

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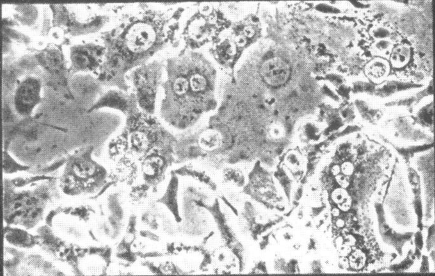

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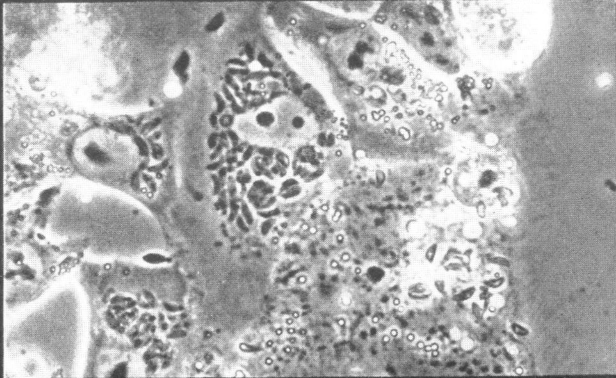

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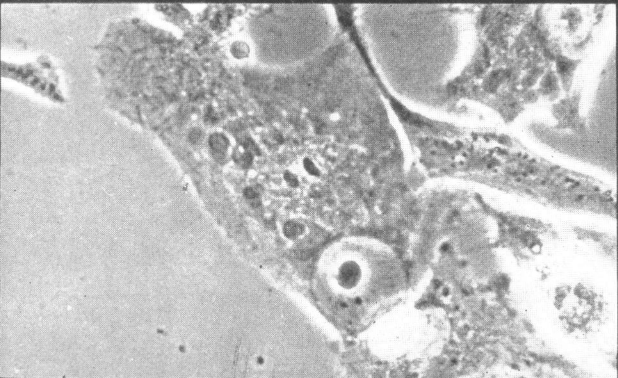

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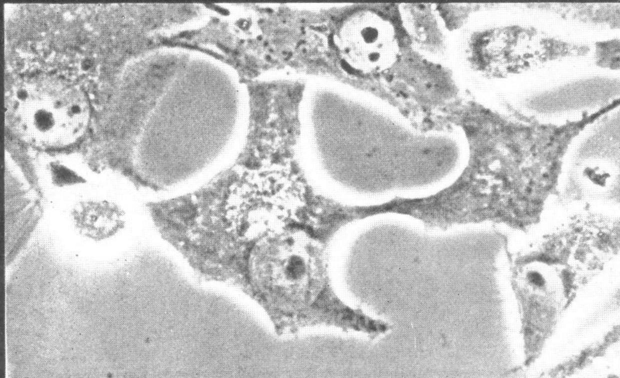

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As a first attempt to reveal on which cell activities parasite multiplication is dependent, the propagation of parasites in irradiated and that in non-irradiated cells was compared. It was found that such metabolic activities of the cytoplasm as may be directed from the nucleus and depend on its mitotic capability, did not influence the multiplication of the parasites.

SUMMARY

Toxoplasma parasites were irradiated with high energy gamma rays. It was found that irradiation inhibited the mitotic activity of the parasites. The antigenic properties of the parasites studied by the dye test were unaffected. Irradiated host cells without mitotic capacity supported multiplication of parasites just as did the non-irradiated cells.

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