JOURNAL OF BACTERIOLOGY, Oct. 1968, p. 916-919
Vol. 96, No. 4

Lysosomes of *Toxoplasma gondii* and Their Possible Relation to the Host-Cell Penetration of Toxoplasma Parasites

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Received for publication 27 May 1968

Lysosome-like structures of *Toxoplasma gondii* were observed by means of vital staining with acridine orange and by the Gomori technique. These structures were found scattered over the cytoplasm but were often located at one end of the parasite. In comparison with parasites of the inoculum used for infecting HeLa cell cultures, the toxoplasma which had penetrated the HeLa cells revealed a markedly lower percentage of parasites showing lysosomal staining. After the penetration, the number of parasites with demonstrable lysosomes increased successively and, at the time for release of newly formed parasites (at 24 hr), the majority of the parasites demonstrated lysosome-like bodies in the cytoplasm. The observations are discussed with special reference to the mechanism of host-cell penetration.

Toxoplasma gondii seems to exert an enzymatic activity which facilitates its penetration of the host cell (4, 10, 12). An enzyme (or enzymes) responsible for this activity has not yet been characterized but can be extracted from disintegrated toxoplasma parasites. Lysosomal enzymes have been demonstrated in toxoplasma (5, 7), but their relation to the cell-penetrating capacity of the parasite is not known. In the present report, the occurrence of lysosomes in *Toxoplasma gondii* was studied before and after the cell penetration of the parasite, as well as during its intracellular reproductive phase.

MATERIALS AND METHODS

Toxoplasma parasites. Suspensions of parasites (RH strain) were prepared as described previously (10).

Cell cultures. HeLa cells were cultivated on cover slips in Leighton tubes with Eagle's medium containing 10% human serum devoid of antitoxoplasma activity. Two 1-day-old cultures were inoculated with 5-million parasites per ml in Hank's balanced salt solution (BSS), 1 ml per culture.

Staining procedures. Two staining methods were employed, one with acridine orange (3), the other according to Gomori (2). For acridine orange staining, 0.1 ml of a stock solution containing 2 mg of acridine orange in 100 ml of Parker 199 was added to the culture, giving a final concentration of about 2 μg of acridine orange per ml of fluid. The cultures were incubated at 37 C for 30 min. The fluid of the culture was then siphoned off, and droplets were placed on slides and observed by fluorescence and phase-contrast microscopy. The cover slips with the toxoplasma-infected cells were mounted with saline on slides for microscopic examination.

The Gomori technique was applied as follows. The culture fluid was removed, and 5.5% glutaraldehyde in Tyrode's solution was added for fixation of the cells on the cover slips. After 20 min at 4 C, the glutaraldehyde was removed, the cells were washed once in saline, and freshly prepared and filtered Gomori medium was supplied. Subsequently, the cells were incubated at 37 C for 3 hr, the medium was removed, and a 1% solution of HAc was added for 10 sec. The HAc solution was then replaced by distilled water saturated with H_2S. After 5 min at room temperature, the cells were washed and microscopic observations were made on wet-mounted cover slips. The same technique was employed for extracellular parasites pelleted by centrifugation.

Reading of the preparations. Cells were exposed to parasites for various periods of time. After staining, the number of extra- and intracellularly located parasites with and without staining (indicating lysosomal activity) was recorded. Usually, 100 to 200 parasites per preparation were registered. To reduce the subjective influence of the reader, series of experiments were performed on coded preparations, each time examined by different readers. Results were studied by variance analysis.

RESULTS

After vital staining with acridine orange, a number of distinct red-stained spots indicating presence of lysosomes were observed in the cytoplasm of the toxoplasma parasites. Such spots were often found located at one end of the para-

sites. The nucleus and the nucleoli of the host cells appeared green-stained. Both extra- and intracellularly located parasites took up the dye (Fig. 1 and 2).

At 1 to 6 hr after the inoculation of the cell cultures, few of the extracellular parasites (1 to 10%) showed lysosomal staining. During the reproductive phase, practically all intracellular parasites showed lysosomal staining, both when they appeared in pairs and in larger clones. Results obtained with the Gomori technique demonstrated a similar pattern (Fig. 3).

The percentage of toxoplasma parasites developing lysosomes demonstrable with acridine orange and Gomori technique during the different phases of the parasite infection of host cells is illustrated by results compiled in Fig. 4. Of the parasites used for inoculation of the cell cultures, 52% showed lysosomal staining with acridine orange. With the Gomori technique, the corresponding percentage was 74. At 40 min after inoculation, only 22% of the intracellular parasites demonstrated staining, thus suggesting presence of lysosomes. Upon further incubation, however, these became successively more frequent, and, at 24 hr, when cell-bursts and release of parasites occurred, almost all of the intracellular parasites showed stainings of lysosome-like cytoplasmic structures.

In contrast to the early period of observation, when only a few per cent of the extracellular parasites demonstrated a specific staining reaction, the picture at 24 hr changed and a majority of the free toxoplasma parasites demonstrated lysosomal staining.

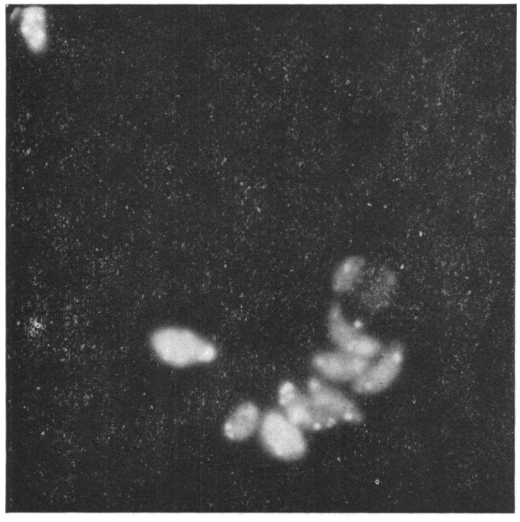

FIG. 1. *Extracellular toxoplasma parasites stained with acridine orange. Fluorescence microscopy.* × *775.*

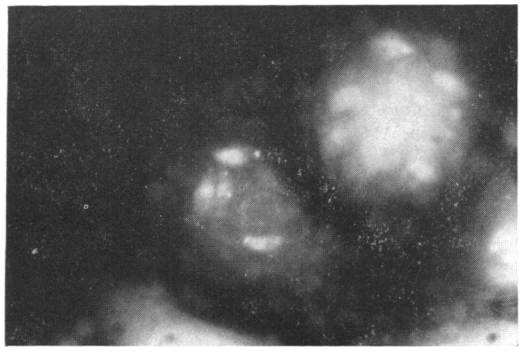

FIG. 2. *HeLa cells infected with Toxoplasma gondii and stained with acridine orange. Fluorescence microscopy.* × *775.*

DISCUSSION

The Gomori technique and vital staining with acridine orange are both generally accepted methods for demonstration of lysosomes. Vital staining of cells with low concentrations of acridine orange is thought to detect the lysosomes by the uptake of the cells of the dye and a concentration of amino acridines in the lysosomes. By the Gomori technique, acid phosphatases are histochemically detected and, thus, the lysosomes are traced. There is good evidence that, with these techniques, a reliable histochemical detection of lysosomes can be obtained (1, 13).

The present report confirms earlier observations

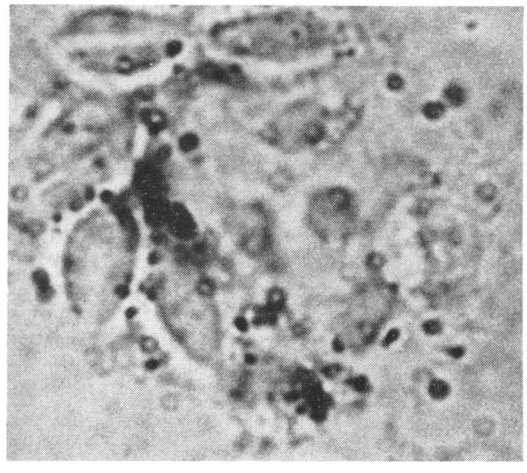

FIG. 3. *Intracellular Toxoplasma gondii stained according to the Gomori technique, demonstrating lysosomes as black spots in the cytoplasm. Light microscopy.* × *1,900.*

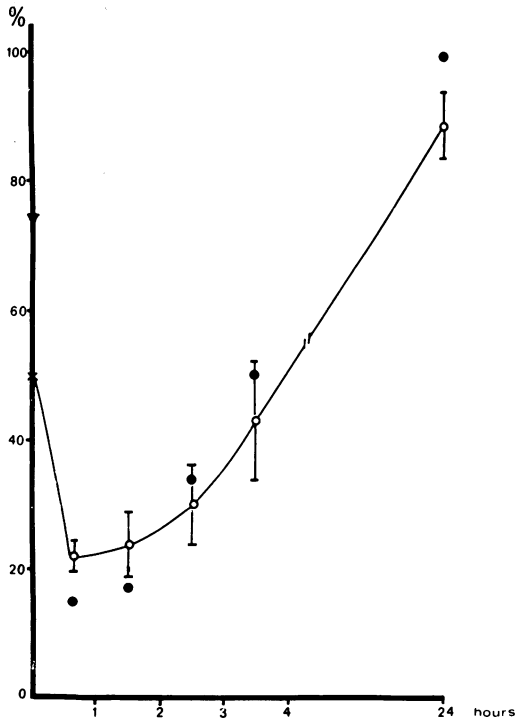

FIG. 4. *Infection of HeLa cells with Toxoplasma gondii. Percentages of intracellular parasites with lysosomes plotted against incubation time. Results obtained with acridine orange (○) are means of six experiments. Vertical bars give ± standard errors of means. Results obtained with Gomori technique (●). Percentages of stained parasites of the inocula used are plotted at zero-time.*

on the occurrence of lysosomes in *Toxoplasma gondii*. An interesting finding was that specific staining was frequently observed at one end of the parasite. Garnham et al. (4) have suggested a secretory function of a structure, referred to as the paired organelle, located at the anterior end of toxoplasma.

Our previous reports (10, 12) have described extracts of *Toxoplasma gondii* that contain an enzyme-like factor which enhances the host-cell penetration of toxoplasma. The most effective methods employed for extraction of the penetration-promoting activity from the toxoplasma parasites were similar to those used for obtaining lysosomal enzymes from mammalian cells. This observation, the findings of lysosomal enzymes and lysosomes in toxoplasma (5, 7), and the demonstrated penetration-promoting effect of certain enzymes such a lysozyme, hyaluronidase, β-glucuronidase, and β-galactosidase (9, Norrby, R. and E. Lycke, *unpublished data*) seemed to suggest a dependence upon a lysosomal activity of the parasite for its host-cell-penetrating capacity. In addition, we have found that fractions of disintegrated toxoplasma parasites contained material capable not only of opposing the effect of the penetration-enhancing factor mentioned above, but also capable of inhibiting the penetration of toxoplasma which had not been experimentally enhanced (12).

As shown by the present report, some correlation seemed to exist between the occurrence of lysosomes in toxoplasma and its host-cell penetration. Before penetration, the parasites exhibited several lysosomes in the cytoplasm, whereas, shortly after penetration, the number of parasites with lysosomes was markedly reduced. This was interpreted as lysosomal activity operating during the process of host-cell penetration. It is known that the metabolism of toxoplasma is activated inside the host cell (6). In accordance with this, we found that the number of parasites with stainable lysosomes increased gradually after the host-cell penetration to a maximum at cell-burst and release of the newly formed parasites.

Most of the studies on the penetration mechanism of toxoplasma have been performed on cultured host cells. However, it has been shown recently (11) that the penetration-enhancing factor extractable from toxoplasma parasites can increase their virulence for mice. Thus, the observations of the existence of an enzyme promoting penetration of toxoplasma may be a finding of more general importance for the understanding of the biology of toxoplasma.

ACKNOWLEDGMENTS

This study was supported by a Public Health Service research grant AI 05074-06 from the National Institute of Allergy and Infectious Diseases.

We thank Mona Andersson, Monika Niklasson, and Agnete Svensson for their skillful technical assistance.

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