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SHORT COMMUNICATION

AUTORADIOGRAPHIC STUDIES ON INTRACYTOPLASMIC MULTIPLICATION OF *TOXOPLASMA GONDII* IN FL CELLS

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Toxoplasma gondii is known to be a typical obligate intracellular parasite and multiplies well in the cytoplasm of cultured FL cells derived from human amnion cells (KISHIDA, 1962). Poxviruses also multiply only in the cytoplasm of cells and synthesize viral DNA in the " B " type inclusions (KATO *et al.* 1960 a, b). PLT(psittacosis-lymphogranuloma venereum-trachoma) group agents are other examples which contain DNA and multiply only in the cytoplasm of cells. It is noteworthy that the meningopneumonitis agent which belongs to the PLT group, does not take up ^{3}H-thymidine during the stage of multiplication. However infected cells showed remarkable incorporation of ^{3}H-cytidine (TAMURA, 1962) and ^{3}H-uridine (KATO *et al.*, 1963 b) into both the DNA and the RNA of the cytoplasmic inclusions. The other difference between poxviruses and the PLT agent seems to be with regard to nuclear DNA synthesis in the cells during multiplication of the agents. On viral multiplication poxviruses definitely suppress host nuclear DNA synthesis (KATO *et al.*, 1960 a, b; KATO *et al.* 1964), while on multiplication the meningopneumonitis agent does not affect nuclear DNA synthesis appreciably except in the terminal stage of infection (KATO *et al.*, 1963 b). Another interesting subject is the nucleo-cytoplasmic interaction in cells parasitized with one of the pathogenic Protozoa, *Toxoplasma gondii*.

Toxoplasma gondii (RH strain) was kindly given by Dr. S. TAKADA (Department of Medical Zoology, Medical School, Osaka City University). FL cells, at a density of 3×10^5 cells per ml, suspended in LE medium (Earle's balanced salt solution containing 0.5 per cent lactalbumin hydrolyzate) with 10 per cent calf serum, penicillin (100 units per ml) and streptomycin (100 μg per ml), were put into 14 Leighton tubes containing coverslips. The cells were allowed to become attached and grow for 2 days at 37°C. Then 12 out of 24 tubes were infected with about 10^7 *Toxoplasma gondii* per tube. The infected Leighton tubes were divided into three groups. Two tubes being free from *Toxoplasma* were named the fourth group. Fourty eight hours later the coverslips of the first group were taken and observed under the phase contrast microscope. As shown in Fig. 1, about 50 per cent of the cells were found to contain some *Toxoplasma gondii* and many of them showed a cluster formation with a specific arrangement which indicates the active multiplication of *Toxoplasma* in the FL cells.

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The second group of cells were kept in medium containing ^{3}H-thymidine (1 μc/ml) for 1 hour. Then dipping autoradiography was carried out using Kodak NTB2, as reported in our previous paper (KATO *et al.* 1963 a). The exposure time was 22 days at 4°C in a cold room. After development and fixation, the autoradiograms were stained with Giemsa solution. None of the *Toxoplasma gondii* in the cytoplasma were labeled, while more than 50 per cent of the nuclei of both cells containing a cluster of *Toxoplasma* and of cells without any *Toxoplasma* were heavily labeled (Fig. 2).

The third group of cells were kept in medium containing ^{3}H-uridine (5 μc/ml). A part of the sample was treated with 1 N HCl at 60°C for 15 min to remove RNA. Then autoradiography was carried out. The exposure time was 4 days at 4°C in a cold room. Most of the *Toxoplasma* in the cytoplasm and most of the nuclei of the host cells were well labeled (Fig. 3). HCl-treated preparations showed a definite decrease in the number of silver grains both in the *Toxoplasma* and in the nuclei of the host cells. However, a certain number of silver grains remained in about 30 per cent of the *Toxoplasma* (Figs. 4 and 5). These results suggest that most of the silver grains in the *Toxoplasma* are derived from ^{3}H-uridine incorporated into RNA while a few seem to be derived from ^{3}H-uridine transported into DNA via ^{3}H-intermediates.

To test whether the DNA of *Toxoplasma* was derived from host nuclei or not, the fourth group of cells previously labeled with ^{3}H-thymidine (1 μc/ml) for 24 hours were challenged by *Toxoplasma gondii*. Fourty eight hours later the coverslips were taken and autoradiography was carried out. Autoradiogram of the preparations showed that host nuclear DNA was not transferred to *Toxoplasma gondii*.

These results obtained with *Toxoplasma* were in exact accordance with those on the meningopneumonitis agent. The results also suggest an essential difference in the nature of the host-parasite interactions in these two microorganisms and viruses. A comparison of the nucleic acid metabolism in cells parasitized with various agents is shown in Table 1.

Summary

1. *Toxoplasma gondii* does not take up ^{3}H-thymidine during intracytoplasmic multiplication. 2. Extensive incorporation of ^{3}H-uridine into *Toxoplasma* was demonstrated. Most of the uridine was incorporated into RNA. However a certain amount was definitely

TABLE 1 *Comparison of nucleic acid metabolism in the cells parasitized with various agents*

Parasites examined		Hosts examined	Incorporation of ^{3}H-T into the site of multiplication of the agent in the cytoplasm	Incorporation of ^{3}H-U into the site of multiplication of the agent in the cytoplasm	Incorporation of ^{3}H-T into the nucleus of the cell during multiplication of the agent	Transfer of host nuclear DNA into the site of multiplication of the agent
Group	Agents					
Virus	Variola Cowpox Ectromelia	FL cells	+	DNA only	− or ±	−
PLT	Meningopneumonitis	L cells	−	both DNA and RNA	+*	−
Protozoa	*Toxoplasma gondii*	FL cells	−	both DNA and RNA	+*	−

^{3}H-T : ^{3}H-thymidine ^{3}H-U : ^{3}H-uridine

* Except in the terminal stage of infection.

transported into the DNA of *Toxoplasma* via ^{3}H-intermediates. 3. Multiplication and metabolism of *Toxoplasma gondii* do not affect nuclear DNA synthesis appreciably.

Addendum

During preparation of this manuscript a report by MATSUBAYASHI and AKAO (1965) appeared in which evidence was obtained by electronmicroscopic autoradiography that *Toxoplasma gondii* takes up ^{3}H-thymidine during multiplication in abdominal monocytes of mouse. However, we cannot explain this difference between their data and our own.

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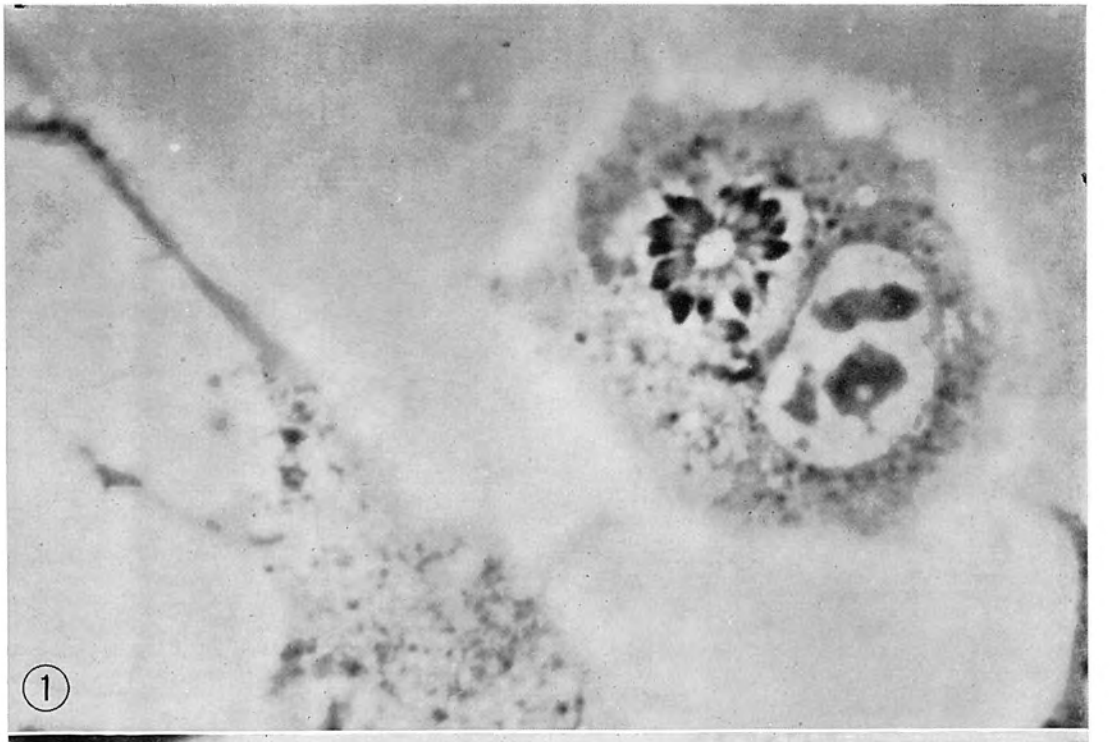
FIGURE 1 A cluster formation of *Toxoplasma gondii*, showing specific arrangement which indicates the active multiplication of the agents in the cytoplasm of the FL cell, observed under a phase contrast microscope.

FIGURE 2 Autoradiogram of ^{3}H-thymidine incorporated into FL cells parasitized with *Toxoplasma gondii*. Stained with Giemsa solution. No *Toxoplasma gondii* was labeled, while two nuclei were heavily labeled.



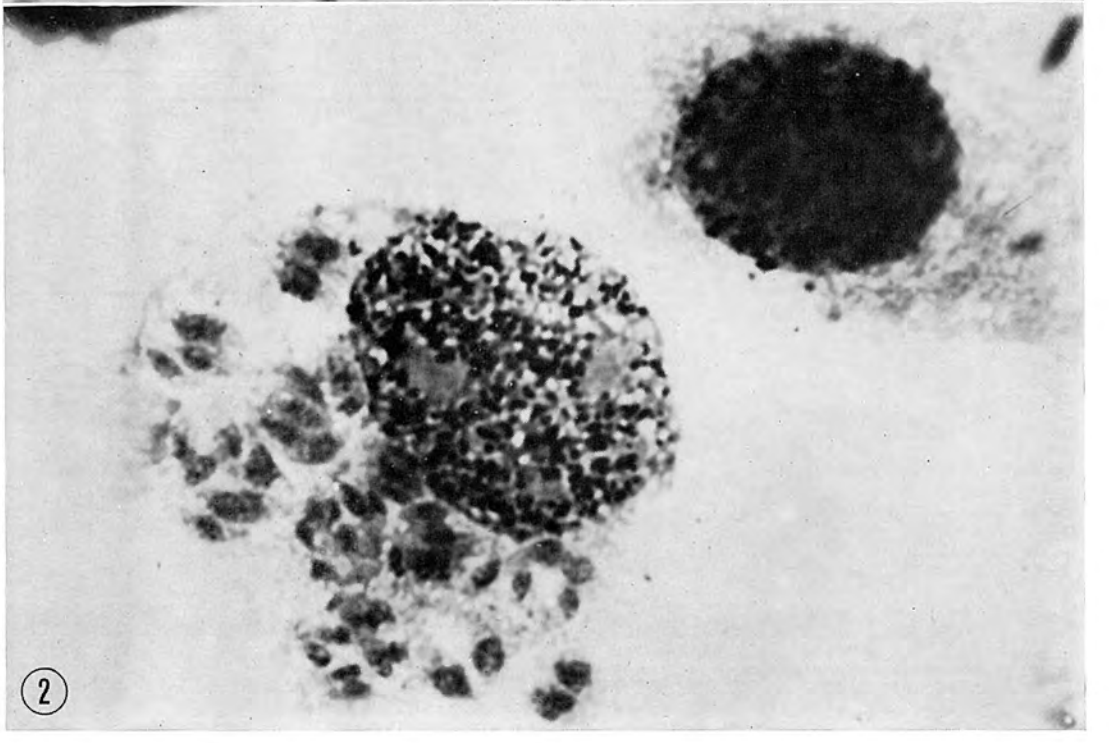
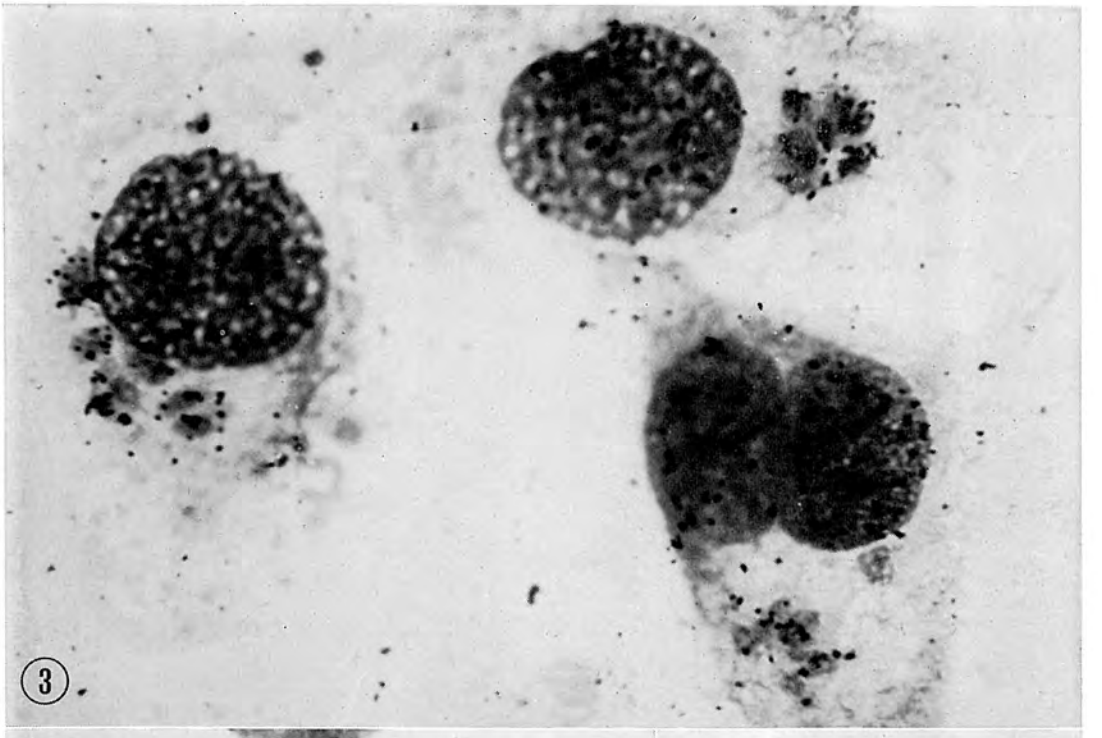


FIGURE 3 Autoradiogram of ^{3}H-uridine incorporated into FL cells parasitized with *Toxoplasma gondii*, stained with Giemsa solution. Most *Toxoplasma* and all four nuclei were well labeled.

FIGURE 4, 5 Autoradiogram of ^{3}H-uridine incorporated into FL cells parasitized with *Toxoplasma gondii*, treated with 1 N HCl at 60°C for 15 min. Stained with Giemsa solution. Only some *Toxoplasma gondii* were labled, indicating the DNA synthesis of the agents.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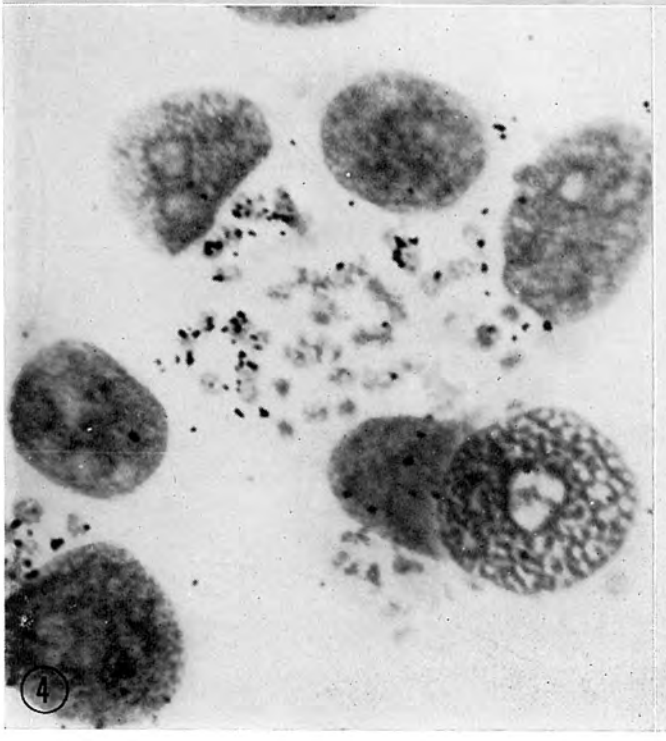
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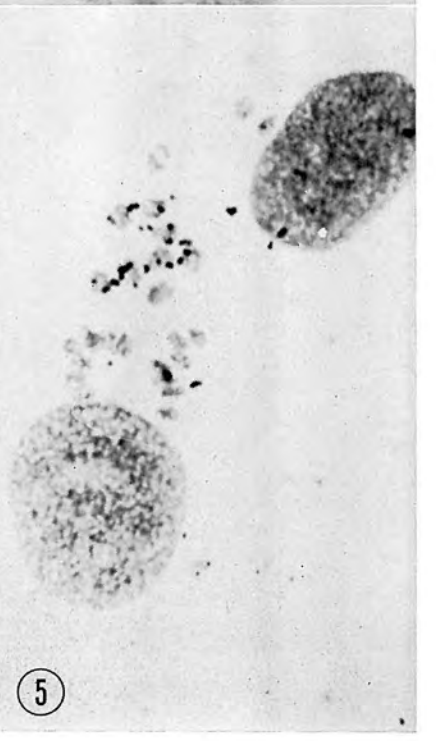
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