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Comparative Development of *Eimeria uzura* and *E. tsunodai* from Japanese Quails in Cultured Cells

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Introduction

Although many papers have been published on the cultivation of coccidia in cultured cells (Trager and Krassner, 1967; Taylor and Baker, 1968; Doran, 1973; Piekarski, 1974), no research has been done as yet on the comparative development of *Eimeria* species from Japanese quail (*Coturnix coturnix japonica*) in cultured cells. The present investigation was conducted to determine the degree to which *Eimeria uzura* (Tsunoda and Muraki, 1971) and *Eimeria tsunodai* (Tsutsumi, 1972) from Japanese quails would develop in cultured cells from whole embryos of quail (QE), whole embryos of chicken (CE), and chick kidney (CK), as well as in the established cell line cultures of baby hamster kidney (BHK).

Materials and Methods

Oocysts of *Eimeria uzura* and *E. tsunodai* were supplied by courtesy of Dr. K. Tsunoda, Chief of 1st Resarch Division, of National Institute of Animal Health, Japan. They were inoculated into Japanese quails and obtained from their droppings at the height of oocyst production. The dropping with oocysts were passed through a 100-mesh sieve and the oocysts were allowed to sporulate 1n 2.0% postassium dichromate at 25 C for 4-6 days. Sporulated oocysts were collected by sugar floatation and centrifugation.

They were sterilized in Purelox (5% sodium hypochlorite) for 30 min and washed three times with sterile PBS (pH 6.0). The pellets of oocysts were ground with a teflon homogenizer at 1.00 rpm for 10 min to release the sporocysts. Sporozoites were released from the sporocysts after the treatment with 0.5% trypsin (Difco 1 : 250) and 3.0% quail bile in Earle's balanced salt solution at 39 C for 2h. Sporozoites were then separated from debris and excystation fluid by centrifugation with Earle's solution.

A concentration of $2.0\text{-}3.0\times10^5$ sporozoites per 1.0 ml was obtained by diluting the suspension with serumfree culture medium. Leighton tube (15×150 mm) containing a coverslip with cultured monolayer cells each were inoculated with 1.0 ml of sporozoite suspension per one tube. They were incubated at 37 C. The coverslips were removed from the tubes at various intervals after incubation, stained with Giemsa solution, and examined by bright-field microscopy.

Primary cell cultures of QE, CE, and CK and the established cell line culture of BHK were used in this study. The methods used to obtain and maintain culture cell were

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similar to those described by youngner (1954). The cell growth medium employed was LE medium (Earle's solution containing 0.5% lactalbumin hydrolysate) supplemented with 5% fetal calf serum. The medium contained 100 units/ml penicillin and 100 μg/ml streptomycin.

Results

Freshly excysted sporozoites of *Eimeria uzura* and *E. tsunodai* were inoculated into Leighton tubes containing cultured cells of QE, CE, CK, and BHK and observed over a period of 10 days.

Development of Eimeria tsunodai in cultured cells.

E. tsunodai developed to mature first-generation schizonts in all kinds of cultured cells employed in this investigation. Freshly excysted sporozoites were 11.5×2.5 μm in size on average. Penetration of host cells by sporozoites was observed 24h after inoculation, regardless of a variety of cultured cell used. Especially, sporozoite penetrated into QE cells was observed usually 24h after inoculation had an large refractile body and nucleus (Fig. 1). As is seen in the other *Eimeria* species (Speer *et al.*, 1970; Speer and Hammond, 1970; Speer and Hammond, 1971; Sampson *et al.*, 1971; Doran and Augstine, 1973), each intracellular sporozoite usually lay in the vicinity of the host cell nucleus and was surrounded by a parasitophorous vacuole (Figs. 1–3).

Many sporozoites, up to 16, were frequently observed to have penetrated into one host cell (Fig. 3). It was common for a single host cell to contain 2 or 3 sporozoites. Intracellular sporozoite was 10.0×2.0 μm in size on the average and had a refractile body, a nucleus and a nucleous.

The refractile body was over one half as long as the sporozoite body (Figs. 1, 2). Intracellular sporozoites were shorter and thicken rods than extracellular ones. U-shaped sporozoites often appeared in *Eimeria* species from such mammals as mice (Kelley and Youssef, 1977) and ground squirrels (Speer *et al.*, 1970; Sper and Hammond, 1970) were not observed on this study. Transformation of sporozoite into trophozoite was usually initiated by a gradual increase of sporozoite in size. Fig. 4 showed the differentiation of trophozoites in QE cells.

Trophozoites were observed in QE cells 48h to 72h after inoculation of sporozoites and in CE, CK, and BHK cells 72h to 96h after the inoculation.

Immature schizonts appeared in QE cells 72h after the inoculation and in CE, CK, and BHK cells 96h to 120h after the inoculation. In BHK cell cultures, only one immature schizont was present at 120h. It contained 8 or more nuclei (Fig. 5). Mature schizonts were observed in QE cell 72h, in CE and CK cells 96h, and in BHK cells 120h after the inoculation. They were 15.0×14.0 μm in size on the average, containing many merozites and one residual body. The merozoites were arranged radially in a rosette from around the central residual body (Fig. 6). No crescent-shaped body (Fayer and Hammond, 1967; Clark and Hammond, 1969) was seen in any of the developmental stage of *E. tsunodai*.

Development of Eimeria uzura in cultured cells.

Eimeria uzura rapidly entered cells and developed into mature schizonts only in QE cells. Freshly excysted sporozoite was 8.5×1.5 μm in size on the avarage and had a nucleus located near the obtus end of the body and a refractile body occupying approximately half the length of the body. Sporozoites penetrated into cultured cells of all types 24 to 96h after inoculation of sporozoites. After its penetration, each sporozoite settled down adjacent to the nucleus of the host cell and formed a parasitophorous vacuole around itself (Figs. 6, 7). The occurrence of one or more sporozoites was common in a single host cell. Intracellular sporozoite was 9.5×3.5 μm in size on the average and contained a refractile body and nucleus. More intracellular sporozoites were found in

QE and CE cell than in CK and BHK cells. The intracellular sporozoites become shorter, wider, and more blunt at the anterior end with the lapse of time. No U-shaped sporozoites were observed in the culture of any cell type, as in the case of *E. tsunodai*.

Large number of sporozoites entered into all types of cells, but only a few of them were transformed into trophozoites did. Occasionally, intracellular sporozoites in BHK cell cultures (Fig. 7). Transformation of sporozoites to trophozoites began by the enlargement of sporozoites with an increase in size of the nucleus followed by nuclear division (Figs. 8, 9). Those trophozoites sppeared in QE cells 48h to 72h, and in CE, CK, and BHK cells 72 to 120h after sporozoites inoculation.

Trophozoites were found to developed into immature schizonts in QE cells 72h, in CE and CK cells 72h to 120h, and in BHK cells 120 to 168h after the inoculation. Thus, the rate of development of schizonts was lower in the established BHK cell line than in the primary cell cultures of QE, CE and CK. Immature schizonts usually had 6 or more nuclei (Figs. 10, 11).

Mature first-generation schizonts were observed in QE cell culture 120h after sporozoite inoculation, but were not in any culture of cells of other types. Mature schizonts were 15.0×13.5 μm in size on the average and contained many merozoites and one residual body (Fig. 12).

Effect of two Eimerian species on the cultured cells.

Cultured cells of any cell types were degenerated in parasites of two species 6–10 days after sporozoite inoculation. Degeneration was heavier in QE cell than cells of any others. *E. uzura* appeared to have a less effect on the cultured cells than dose *E. tsunodai*. The enlargement of nuclei of the host cell was pronounced in cell harboring *E. tsunodai*. The multiple nuclei in a host cell, which had been observed frequently in cells inocultated with *E. tsunodai* sporozoites (Fig. 2) were not seen in cells harboring *E. uzura*.

Discussion

It was proved that *Eimeria uzura* and *E. tsunodai* from Japanese quails could develop in cultured cells. The results of the present investigation indicated that of the cultured cells examined so far, primary cell from the whole embryo of quails (QE) provided the most favorable environment for the development of *E. uzura* and *E. tsunodai*. These views are supported by the findings as to the rate of development and the presence of schizonts having reached maturity. These Eimerian species from Japanese quails, however, differed from each other in their ability to develop in cultured cells. It was found that more mature schizonts of *E. uzura* developed in QE cell than in any cell of other types, and that schizonts of *E. tsunodai* showed no difference among four types of culture cells in the state of their development. Numerous trophozoites of *E. tsunodai* were also produced in cell cultures, whereas a relatively few trophozoites of *E. uzura* were seen. In some species of *Eimeria* from the chicken, Long (1966) and Long and Millard (1976) suggested that the development in cultured cells might be closely related to their site-specificity. The difference in their ability to develop in cultured cells between two species of *Eimeria* from Japanese quails may be related to the behavior of each species in the host.

The developmental stage of *E. uzura* in cultured cells resembled that in quails, as reported by Tsunoda (1971). Tsunoda found that almost all sporozoites had grown into immature schizonts and some schizonts further into mature ones containing 6 to 12 merozoites in Japanese quails similar to those observed in the present *in vitro*. The schizonts of *E. tsunodai*, however, were a little smaller and more variable in size in cultured cells than in the host quails. When mature schizonts occurred in QE cells, their average size was 15.0×14.0 μm, whereas mature schizonts in Japanese quails showed an aver-

age sizie of 28.5×22.5 μm (Tsutsumi, 1972).

In Japanese quails, *E. uzura* seen 48 h after oocysts inoculation were immature schizonts. In cultured cells schizonts did not usually appear untile 72 h after sporozoite inoculation. An immature schizonts of *E. tsunodai* was found in Japanese quails 24 h after oocysts inoculation (Tsutsumi, 1972). Immature schizonts did not usually appear in cultured cells until 72 h after sporozoite inoculation. Thus, it was thought that the development usually occurs a little more slowly in cultured cell than in the definitive host.

Summary

Monolayer primary cultures of cells from whole embryo of Japanese quail (QE), whole embryo of chicken (CE), and chick kindney (CK), as well as established cell line cultures of baby hamster kidney (BHK), were inoculated with freshly excysted sporozoites of *Eimeria uzura* and *E. tsunodai* from Japanese quails and observed for 10 days. Interacellular sporozoites of *E. tsunodai* developed into mature schizonts in QE cells 72 h, in CE and CK cells 96 h, and in BHK cells 120 h after sporozoite inoculation. In QE cells, relatively numerous mature schizonts were observed. They were 15.0×14.0 μm in size, containing many merozoites and one residual body. Sporozoites of *E. uzura* rapidly penetrated into all types of cultured cells 24 h to 96 h after inoculation, but developed into mature schizonts only in QE cell 120 h after inoculation. Nature schizonts were 15.0×13.5 μm in size. Degenerative changes usually occurred in the cultured cells and in the parasites 6–10 days after two Eimerian species of sporozoites inoculation. Degeneration was heavier in QE cell than cells of any others. *E. uzura* appeared to have a less effect on the cultured cells than dose *E. tsunodai*.

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日本ウズラ由来 Eimeria の培養細胞内における発育について

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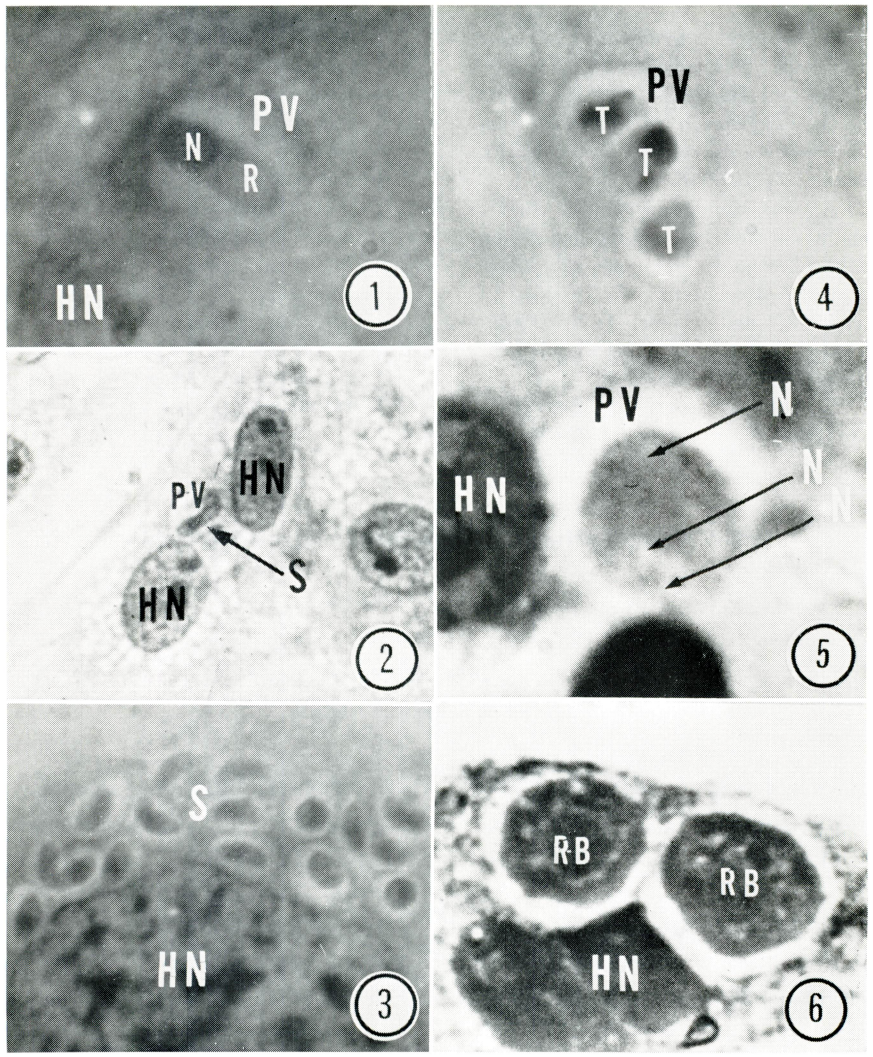
日本ウズラ *Coturnix coturnix japanica* に寄生する2種の *Eimeria* の Oocyst から人工脱殻して得た sporozoites を各種培養細胞に接種し，その発育態度について観察した．

盲腸粘膜寄生性で急性病状を呈する *Eimeria tsunodai* (Tsutsumi, 1972) は，ウズラ胎児培養細胞内 (QE) では接種72時間後には多数の成熟 schizonts が観察された．しかしながらニワトリ胎児培養細胞 (CE)，ニワトリ腎培養細胞 (CK) では96時間，ハムスター幼児腎培養細胞 (BHK) では120時間後にようやく schizonts が見出され，その数も少なかつた．

また小腸・十二指腸粘膜寄生性で慢性病状を呈する *Eimeria uzura* (Tsunoda and Muraki, 1971) の場合は，各培養細胞に侵入した sporozoits は96時間後までは認められたが，その後成熟 schizonts が見出されたのは QE 細胞のみであつた．

一方，2種のウズラ由来 *Eimeria* を接種した各培養細胞は，6日後には細胞変性が始まつたが，その度合は特に QE 細胞で著しかつた．また *E. tsunodai* 接種の培養細胞は *E. uzura* に比して細胞変性の度合が大で，宿主細胞の核の肥大化・多核化は *E. tsunodai* 接種時に多く観察された．

これらの成績から，ウズラ由来 *Eimeria* の培養細胞内の発育態度は，培養細胞の種類によつて異なり，また *Eimeria* の種類によつても差があることが知られた．



Explanation of Figures

Figs. 1–6 Photomicrographs of developmental stages of *Eimeria tsunodai* in cell culture.

Fig. 1 Intracellular enlarged sporozoite; note prominent nucleus. QE cell culture, 24 h. after inoculation. ×1,500

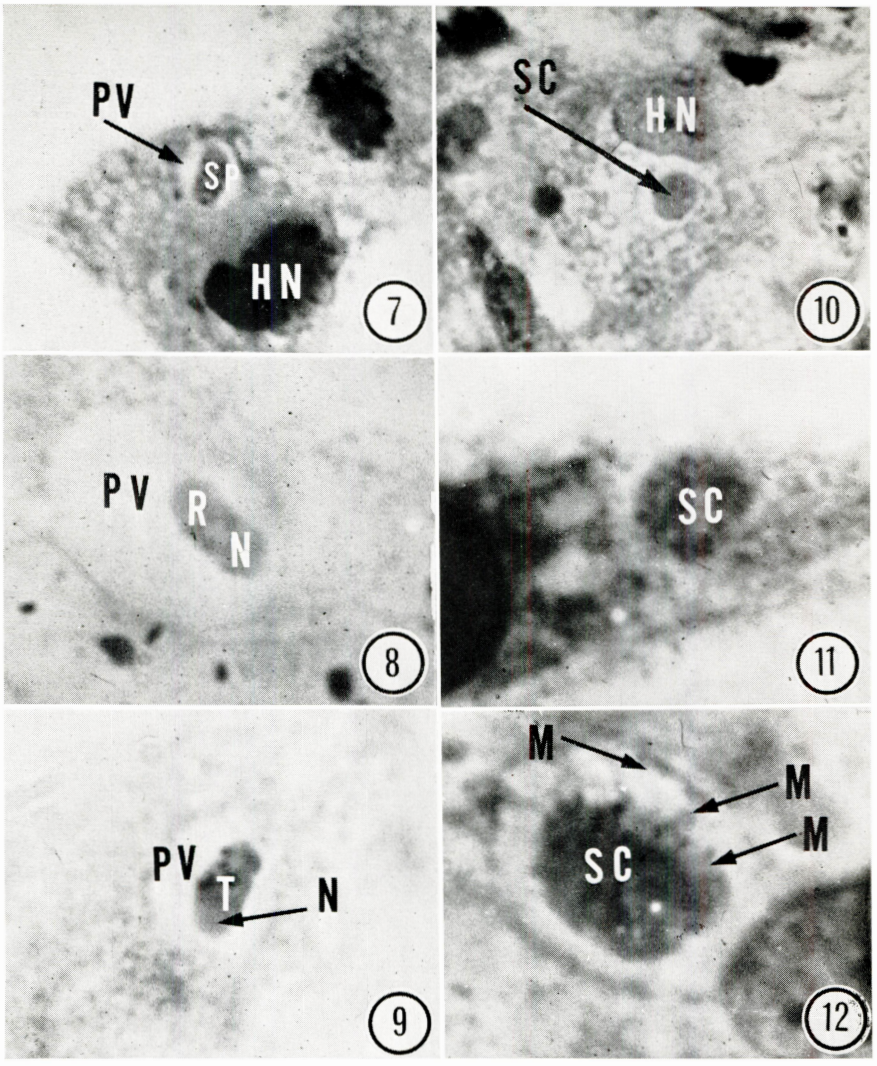
Fig. 2 Intracellular sporozoite; note prominent parasitophorous vacuole. CE cell culture, 48 h. after inoculation. ×1,000

Fig. 3 Many sporozoites around the host cell nucleus; note clear zone of parasitophorous vacuole, BHK cell culture, 48 h. after inoculation. ×,1000

Fig. 4 Trophozoites; note each enlarged nucleus, QE cell culture, 72 h. after inoculation. ×1,000

Fig. 5 Immature schizont; note many nuclei, BHK cell culture, 120 h. after inoculation. ×1,500

Fig. 6 Two mature schizonts; note each residual body, QE cellc ulture, 72 h. after inoculation. ×1,500



Explanation of Figures

Figs. 7–12 Photomicrographs of developmental stages of *Eimeria uzura* in cell cultures

Fig. 7 Intracellular sporozoite; note the small body. BHK cell culture, 72 h. after inoculation. ×1,000

Fig. 8 Intracelluar enlarged sporozoite; note prominent nucleus and parasitophorous vacuole. CE cell culture, 72 h. after inoculation. ×1,500

Fig. 9 Trophozoite with enlarged nucleus. QE cell culture, 72 h. after inoculation. ×1,500

Fig. 10 Immature schizont of small type. CE cell culture, 72 h. after inoculation. ×1,000

Fig. 11 Immature schizont; note many nuclei. BHK cell culture, 120 h. after inoculation. ×1,500

Fig. 12 Ruptured mature schizont. QE cell culture, 120 h. after inoculation. ×1,500

All figures are stained with Giemsa's.

Abbreviations Used in the Figures:

HN — nucleus of host cell.
M — merozite.
N — nucleus of parasite.
R — refractile body.
RB — residual body
PV — parasitophorous vacuole
SC — schizont
SP — sporozoite
T — trophozoite