does not appear to alter their amino acid–acceptor activity. If this observation is extended to other tRNA's, it appears that DiHU-'s in region IV may not be involved in tRNA-synthetase interaction. Also, the results of Zachau *et al.* (*4*) show that the two yeast serine-tRNA's have different sequences in the nonhydrogen-bonded parts of region II. These two serine-tRNA's still have the same amino acid–acceptor activity.

The interaction of tRNA and aminoacyl-tRNA synthetase can occur in several ways: (i) the enzyme can recognize a specific nucleotide sequence or sequences in more than one part of the molecule; (ii) the enzyme can recognize the conformation of a certain region or regions of the tRNA molecule; and (iii) the enzyme-tRNA interaction can be dependent upon the conformation of the entire tRNA molecule. These and other observations (*15*), coupled with the results presented here, make it very unlikely that the specific sequence of nucleotide is primarily involved in the enzyme-tRNA interaction. It is then likely that the conformation of the tRNA plays an important role in conferring the required specificity for the interaction with specific synthetase. The nonhydrogen-bonded regions of tRNA must contribute in the formation of specific conformation of tRNA molecules, since hydrogen-bonded regions are deemed unlikely to be involved in this type of interaction by various studies. Indirect evidence for the involvement of conformation of tRNA in interaction with synthetase has been shown in two cases. Penswick (*16*) demonstrated that yeast $tRNA^{Ala}$ retains its acceptor activity after being cleaved by ribonuclease T_1 within the anticodon, as long as the two halves remain bonded together. Second, Cramer *et al.* (*17*) showed that inactivation of yeast serine-tRNA synthetase occurs at a higher temperature than that at which serine is incorporated into seryl-$tRNA^{Ser}$. (We have observed that such is also the case with $tRNA^{Tyr}$ in *E. coli.*) They have also indicated that this may be due to partial loss of the tRNA conformation and the tRNA is therefore incapable of interacting with synthetase.

Since yeast $tRNA^{Tyr}$ and *E. coli* $tRNA^{Tyr}$ do not interact with the corresponding heterologous synthetases, it is possible that they have different conformations in addition to the shown differences in their primary structures. The major differences in conformation between these two tRNA's may be contributed by the additional nucleotides in region II. It would be interesting to determine the contribution of the conformation of region II in a given tRNA in conferring that tRNA with its observed species specificity.

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1. Abbreviations: tRNA, transfer ribonucleic acid; Tyr, tyrosine; Ala, alanine; Ser, serine; p and - indicate a phosphate group, on the left side they represent a 5′-phosphate, and on the right side a 3′-phosphate; A, adenosine; C, cytidine; G, guanosine; U, uridine; Ψ, pseudouridine; T, ribothymidine; DiHU, 4,5-dihydrouridine; 4ThU, 4-thiouridine; OMeG, 2′-*O*-methylguanosine; *N*-MeG, N^2-methylguanosine; DiMeG, N^2-dimethylguanosine; 5MeC, 5-methylcytidine; 1MeA, 1-methyladenosine; iPA, N^6-(Δ^2-isopentenyl)adenosine; the subscript OH is used to emphasize the presence of a 3′-hydroxyl group; DEAE, diethylaminoethyl.
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Gametogony of Eimeria tenella (Coccidia) in Cell Cultures

Abstract. *Mature male and female gametocytes of* Eimeria tenella, *a coccidium of the chicken ceca, developed in primary cultures of chick embryonic kidney cells inoculated with sporozoites. Immature gametocytes appeared in the cultures after approximately 144 hours of incubation at 41°C. Mature micro- and macrogametes were present from 160 to 190 hours after inoculation.*

We have obtained gametogony in cultured cells inoculated with motile *Eimeria tenella* (coccidia) sporozoites. Until now, only limited asexual development in vitro after inoculation with sporozoites has been reported (*1*), although Bedrnik (*2*) demonstrated development of sexual stages and oocysts after inoculation of cell cultures with second generation *E. tenella* merozoites from the ceca of chickens.

Young male and female gametocytes appeared in the cultures after approximately 144 hours of incubation. Until then it was not possible to differentiate between immature macrogametocytes, immature microgametocytes, and immature schizonts. We identified the young microgametocytes by the numerous football-shaped clumps of nuclear material that stained dark pink with Giemsa's stain against a blue staining residual cytoplasmic mass. As the gametocyte matured, the nuclear material elongated, as though being pulled from either end, and then curled into comma-shaped microgametes, usually first seen at the periphery of the cytoplasmic mass. After 160 to 190 hours of incubation, microgametes too numerous to count filled the entire gametocyte (Fig. 1A). The mature microgametocyte, averaging 17.6 by 20.0 μ (range: 11.5 by 15.4 μ to 26.9 by 35.5 μ) had indistinct walls, and free microgametes protruded into the surrounding vacuole. The presence of large, cytoplasmic plastic granules that stained positively with periodic acid–Schiff reagent (PAS) and bromphenol blue identified the macrogametocytes, which averaged 16.5 by 22.8 μ (range: 15.3 by 19.2 μ to 18.2 by 28.8 μ). These plastic granules did not stain with methyl green–toluidine blue but appeared as vacuoles at the periphery of the cytoplasmic mass (Fig. 1B). Another identifying characteristic of the maturing macrogametocyte was the basophilic karyosome that became prominent with toluidine blue or Giemsa's stain.

The schizogonic cycle in vitro closely paralleled that in vivo. Within an hour after inoculation many sporozoites had invaded cells, although penetration of the cells continued for 24 hours. By 18 hours we saw trophozoites. Many first generation schizonts were mature at 48 hours, and second generation troph-

ozoites and immature schizonts appeared by 72 hours. Second generation merozoites were produced approximately 96 to 120 hours after inoculation.

Primary cultures derived from kidneys of 17-day-old chick embryos were the only cells used. Kidneys were digested with trypsin (*3*). The 2-ml cultures (250,000 cells per ml) grew on cover slips (9 by 5 mm) in Leighton tubes for 48 hours. At that time we inoculated the incomplete monolayer with sporozoites suspended in the medium used throughout, Eagle's minimum essential medium with newborn calf serum (10 percent). The medium was changed 4 or 5 days after inoculation.

We sterilized the sporulated *E. tenella* oocysts by repeated washings in chlorine solution (50 parts per million) followed by rinses in sterile distilled water. After culture in thioglycollate broth verified sterility, we excysted the oocysts by suspending them in 2 ml of 0.25 percent trypsin and 5.0 percent bile (*4*); we then ground the oocysts in a tissue homogenizer for 2 minutes. After the addition of 8 ml of trypsin-bile, the mixture was incubated for 2 to 4 hours at 41°C. After centrifugation at 300*g* for 5 minutes, we replaced the supernatant with 10 ml of fresh medium. The sporozoites were counted by hemacytometer and diluted with medium to 100,000 per milliliter; 2 ml of this suspension were placed in each Leighton tube and incubated at 40° to 41°C. Seventy-two hours after inoculation we began to withdraw cultures at 24-hour intervals up to 10 days. The cultures stained with Giemsa or toluidine blue were fixed with osmium tetroxide fumes and methanol. Slides stained with PAS or Feulgen's stain were fixed in neutral buffered formalin or Carnoy's fixative.

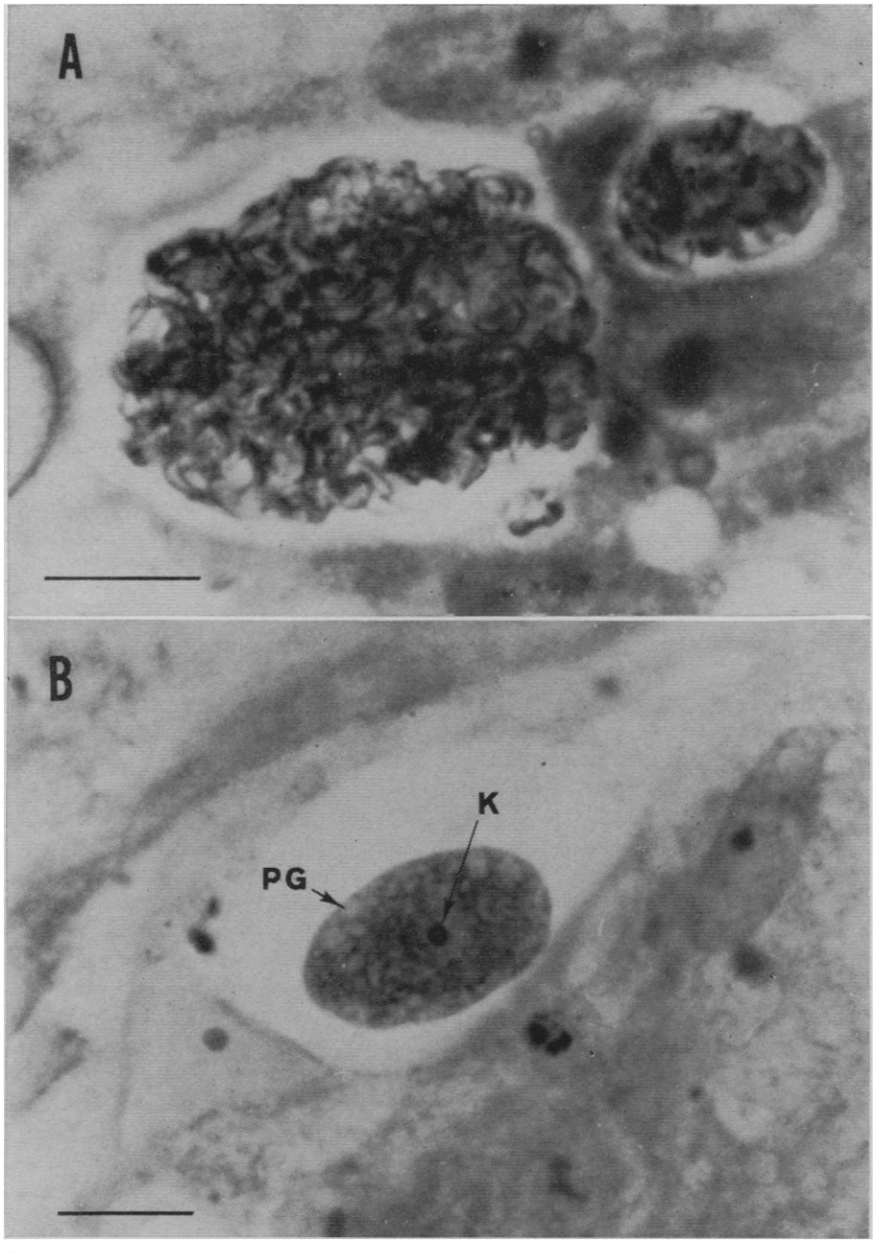

Fig. 1. Mature male and female gametocytes of *Eimeria tenella* in chick embryonic kidney cells inoculated with motile sporozoites. The scale in each photograph is 10 μ. (A) Two microgametocytes 216 hours after inoculation. The large mature gametocyte contains many microgametes, whereas the smaller gametocyte has microgametes developing at the periphery of the cytoplasmic mass. Giemsa's stain. (B) Female gametocyte after 168 hours in culture. The karyosome (*K*) is clearly visible, whereas the plastic granules (*PG*) appear as vacuoles with methyl green–toluidine blue stain.

The production of gametocytes and gametes in cell cultures inoculated with sporozoites is significant, for it demonstrates that a system in vitro is capable of supporting all the developmental stages in the life cycle of the sporozoan parasite *Eimeria tenella*. Gametogony occurred repeatedly in each culture attempt, though the number of mature gametocytes that eventually developed from sporozoites was extremely small.

Although the reason for the transition from schizogony to gametogony in vivo is not clear, the stimulus that occurs in the cell culture system is apparently the same as that in the natural host. Asexual development did not continue indefinitely, nor did it appear to be prolonged in the absence of humoral influences. In fact we observed that the life cycle in vitro corresponded closely to that in vivo, although gametogony was somewhat delayed in the cell culture system.

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