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Eimeria tenella (Sporozoa, Coccidia): Gametogony Following a Single Asexual Generation

Abstract. *Selection of* Eimeria tenella *for precociousness resulted in a strain with only one asexual generation prior to gametogony.*

Propagation in cell culture of a precocious strain (Wis-F) of *Eimeria tenella* (*1*) from the chicken cecum revealed that gametogony occurs after the first asexual generation and viable oocysts are produced in less than 90 hours. Until now the life cycle of this parasite was considered fixed, with a minimum of two generations of schizogony required prior to gametogony, resulting in a prepatent period of about 6 days.

After the appearance of first generation merozoites at 48 to 56 hours in cell cultures, clusters of intracellular zoites developed into immature macrogametocytes or microgametocytes by 64 to 72 hours. Macrogametocytes, recognized as early as 64 hours by the distinct karyosome prominent under phase-contrast microscopy or in stained material, were numerous by 72 hours (Fig. 1A). As the macrogametocytes matured, formation of peripheral, plastic granules and the oocyst wall was similar in appearance to that reported in the normal Wisconsin strain of *E. tenella* (*2*) (Fig. 1C).

Developing microgametocytes were recognized as multinucleate clumps of positively stained material among clusters of immature macrogametocytes (Fig. 1A). After 88 hours we found numerous mature microgametocytes, recognizable in living cultures by the sperm-like appearance of the active, flagellated microgametes within the cell (Fig. 1B). Some microgametes had escaped from the cells and were motile in the culture medium.

Clusters of mature oocysts were numerous after 96 hours of incubation. Transition from macrogamete to oocyst was marked by wall formation and constriction of the oocyte away from the wall. At this point in fixed, stained cultures, oocysts were easily recognized because they were impervious to the stains and have a golden appearance when viewed microscopically (Fig. 1D).

To test viability of cell culture–produced oocysts we allowed them to sporulate at room temperature for 2 days before feeding to 7-day-old chickens. Oocysts and gametocytes were found in cecal smears at 120 hours after inoculation, indicating that at least some of the oocysts were viable. For this experiment, we maintained controls similar to those described by Doran (*3*) by incubating sporulated oocysts at 40°C for 96 hours. These oocysts were not viable when fed to chickens.

Primary cell cultures were derived from kidneys of 2-week-old chicks by conventional tissue culture techniques. Leighton tubes with cover slips were inoculated with 200,000 cells per milliliter of culture medium (0.16 percent lactalbumin hydrolyzate in Earle's balanced salt mixture and 5 percent fetal calf serum) and incubated at 40°C for 3 days prior to inoculation with sporozoites.

Sporulated oocysts of the Wis-F strain were washed free of fecal debris and sterilized with sodium hypochlorite solution. Sporocysts were released by grinding in a tissue homogenizer, then excystation was effected by incubation for 2 hours in a solution of trypsin and bile (0.25 percent : 5 percent). Freshly excysted sporozoites were washed in saline, then inoculated into cultures at 200,000 per milliliter (2 ml per tube).

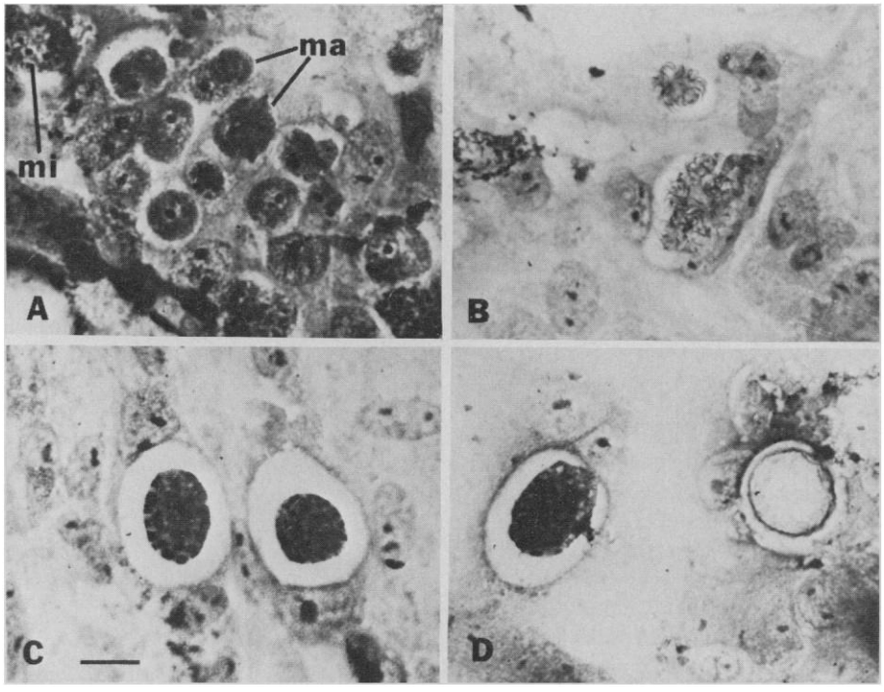

Fig. 1. Sexual development of the Wis-F strain of *Eimeria tenella* in chick kidney cell cultures. (A) Immature macrogametocytes (*ma*) and microgametocytes (*mi*) at 72 hours; (B) mature microgametes at 88 hours; (C) mature macrogametes with prominent wall-forming bodies at 88 hours; and (D) mature macrogamete and oocyst at 96 hours (scale marker, 10 μm).

We examined living cultures by phase-contrast and interference-contrast microscopy. Representative replicate cultures were fixed (Bouin's fixative) at 8-hour intervals from 16 to 114 hours and then stained with toluidine blue or hematoxylin.

Development of gametocytes in vitro after a single asexual generation is significant because it demonstrates that control of the transition from schizogony to gametogony is a genetic trait, largely independent of host influence. These experiments demonstrated that abbreviation of the life cycle was responsible for the shortened prepatent period, rather than a more rapid development of each stage as was originally suspected. Further, we observed macrogametocytes and microgametocytes within isolated clusters of parasites (presumably the progeny of a single sporozoite), suggesting that sporozoites, although haploid in chromosome number, may be bisexual.

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8 December 1975

Blockade of Prolactin Action by an Antiserum to Its Receptors

Abstract. *A guinea pig antiserum to prolactin receptors selectively inhibited the binding of [^{125}I]prolactin to its membrane receptors as well as prolactin-mediated incorporation of [^{3}H]leucine into casein and transport of [^{14}C]aminoisobutyric acid, but was without effect on the binding of [^{125}I]insulin and insulin-mediated events in explants of rabbit mammary glands maintained in culture. These findings provide direct evidence for an obligatory functional role of a membrane receptor in mediating the action of a polypeptide hormone.*

That plasma membrane receptors are involved in mediating the biological effects of polypeptide hormones is a widely accepted concept. However, the hypothesis is based principally on indirect evidence derived from studies correlating the extent of specific binding of hormones to plasma membranes or target cells with activation of adenylate cyclase or other cellular functions (*1*). A review of these data still leaves unanswered the question whether specific hormone binding sites or receptors are obligatory mediators of hormone action. To test the hypothesis more directly we have examined the question of whether antibodies to prolactin receptors would block the biological effect of the hormone. In the case of acetylcholine, Patrick *et al.* (*2*) have used a similar approach to establish the essential role of the receptor in mediating the action of this neurotransmitter. We have found that an antiserum to prolactin receptors which were purified from rabbit mammary glands not only specifically inhibited the binding of prolactin to its receptors but also selectively blocked prolactin-stimulated casein synthesis and amino acid transport in explants of rabbit mammary glands. The same antiserum, however, had no effect on insulin binding, on insulin-stimulated amino acid transport, or on glucose oxidation; nor did it bind prolactin. Our results provide evidence for an obligatory functional role of the prolactin receptor in mediating the action of this hormone.

Prolactin receptors were purified from extracts of mammary glands of pregnant rabbits by affinity chromatography (*3*) and were used to immunize guinea pigs (*4*). These antiserums to receptors have been characterized and described (*4*). One of these antiserums was used in our study to examine its effect on the binding of prolactin to membrane receptors and on prolactin-mediated biological actions in rabbit mammary glands. The isolation of rabbit mammary gland particles which contain receptors for prolactin and insulin has been described (*5*). The procedure described (*5*) for the determination of specific binding of ^{125}I-labeled ovine prolactin ([^{125}I]oPRL) and [^{125}I]insulin was also used in the study reported here. Figure 1 shows that the addition of 100 μl of diluted (1/100) guinea pig antiserum to receptor to the prolactin binding assay mixture resulted in approximately 90 percent inhibition of specific binding of [^{125}I]oPRL to membrane particles, whereas the same amount of a control guinea pig serum (CS; serum obtained from an animal which was injected with vehicle alone) had no effect. Not included in Fig. 2 is the observation (*4*) that even antiserums obtained from guinea pigs immunized with crude membrane particles isolated from either mammary gland or liver of rabbits did not inhibit the binding of [^{125}I]oPRL to membrane receptors. Moreover, the specific binding of [^{125}I]insulin to the same membranes was not affected by this antiserum to receptor (*4*). The same antiserum to the receptor not only inhibited the binding of [^{125}I]oPRL to crude particulate membrane receptors, but also to receptors solubilized by Triton X-100 and then purified by affinity chromatography (*3*, *4*). Specific binding of prolactin to membrane particles derived from several rat and rabbit tissues was also inhibited.

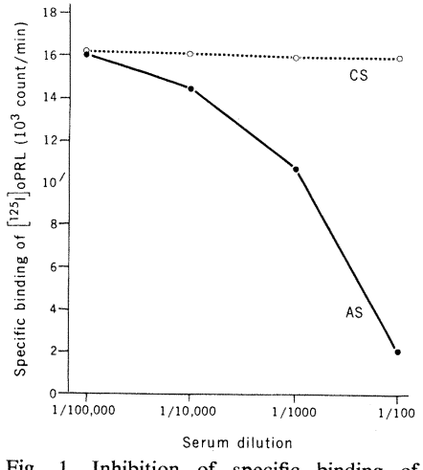

Fig. 1. Inhibition of specific binding of [^{125}I]ovine prolactin (oPRL) to membrane particles from pregnant rabbit mammary tissues by guinea pig antiserum to receptor. The preparation of crude membrane particles derived from pregnant rabbit mammary glands and the method for the determination of specific binding of [^{125}I]oPRL to membranes have been described (*5*). The incubation mixture (final volume, 0.5 ml) contained 230 μg of membrane proteins, 1.2×10^5 count/min (approximately 0.5 ng) of [^{125}I]oPRL and 100 μl of antiserum to receptor (AS) or control serum (CS) that had been previously diluted. Each point represents the mean of duplicate determinations.

To exclude the possibility that the antiserum to the receptor was binding [^{125}I]oPRL and thereby inhibiting the tissue binding of prolactin, additional experiments were performed with the use of a double antibody immunoprecipitation procedure. Trace amounts (~ 0.5 ng) of [^{125}I]oPRL were incubated with increasing concentrations of the antiserum to receptor for 3 days at 4°C, at which time excess rabbit antiserum to guinea pig γ-globulin was added to precipitate guinea pig antibodies. Virtually no [^{125}I]oPRL was precipitated even when the guinea pig antiserum to the receptor was used at a concentration of 10 percent by volume. When the same experiment was repeated with specific antiserum to oPRL in place of the antiserum to the receptor, 90 percent of the [^{125}I]oPRL was precipitated when the antiserum dilution was 1/1000.