

## DEVELOPMENT OF SEXUAL STAGES AND OOCYSTS. FROM THE 2ND GENERATION OF *EIMERIA TENELLA* MEROZOITES IN TISSUE CULTURES

In coccidia grown in tissue cultures only the development of single asexual stages has been described till now (PATTON W. H., Science 150/No. 3697: 767—769, 1965, STROUT R. G., Exp. Parasit. 17: 241—246, 1965, BEDRNÍK P., Folia parasitol. (Praha) 14: 361—363, 1967.) The development of sexual stages and of oocysts in a tissue culture was for the first time observed in our experiments.

Cells derived from 10-day-old chicken embryos and established in a monolayer on a cover slip, were infected with 2nd generation of *Eimeria tenella* merozoites. The merozoites were obtained from the contents of chicken caeca on the 5th day following the artificial infection. The monolayer tissue cultures with coccidia were fixed in Schaudin's solution and stained with Harris's and Mayer's haematoxylin. All methods used were described in detail by Bedrník.

Simultaneously with the development of the 3rd generation of merozoites also the sexual stages, i.e. the macro- and microgametocytes, the macro- and microgametes were developing from the inoculated second-generation merozoites in the tissue culture. Only sporadically oocysts were found as well. The sexual stages were most frequently observed 24 and 48 hours after inoculation. The number of microgameto-

cytes considerably exceeded that of macrogametocytes.

While the number of the third-generation merozoites, developed in the tissue culture, was considerable enough, the number of the sexual stages was rather small. They were noted in three of the four experiments conducted, but only in one instance they occurred in a larger number.

The development of sexual stages does not take place in fibroblasts being strictly limited to islands of epithelial-like cells.

Tissue cultures derived from 10-day-old chicken embryos contain several types of different cells. Islands of epithelial-like cells in which the development of the sexual stages from the second generation of merozoites takes place, are occurring rather scarcely, and, in the individual experiments, quite irregularly. They will probably be the main factor conditioning the development of sexual stages. Outside these small islands sexual stages have never been observed. To determine the origin of these cells and to multiply them in a sufficient quantity, will be a further step towards successful cultivation of coccidia in tissue cultures.

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