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The Dynamics of Antibody against *Eimeria tenella* under the Fluorescent Microscope

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Abstract. The dynamics of antibody in a single sublethal infection of chickens with the species *Eimeria tenella* were studied. 15 14-day-old chickens were given orally 150,000—200,000 oocysts each for obtaining information on the first appearance of antibody and its quantitative increase. Also SHARMA et FOSTER (1963) tried to prove by indirect fluorescence the presence of antibody in the antisera of *E. tenella* on oocysts, sporocysts and sporozoites, but did not succeed in eliminating completely the nonspecific reaction. The antigens used were the asexual and sexual developmental stages on histological sections and free merozoites. The qualitative evaluation was obtained from the schizonts, on which the antibodies became first demonstrable (8 days p.i.) and where the fluorescence was intensive enough for a further titration of the sera; the gametocytes were found to be weaker antigens demonstrating a less intensive fluorescence and becoming evident as late as 15—16 days p.i. Antibodies against *E. tenella* disappeared both on the schizonts and the gametocytes 61—68 days after the infection.

Previous experiments with antibodies against rabbit coccidia performed by the method of the indirect fluorescent test (IFAT) on the developmental stages of *E. stiedae* and *E. magna* (ČERNÁ 1966a, b) have given evidence on the convenience of this method not only for the detection of coccidial antibodies but also for their quantitative evaluation and for the fact that histological sections with the developmental stages of coccidia could be used for the antigen. To confirm also the suitability of IFAT on other host species, we studied antibody production against the fowl species *E. tenella* by this method.

MATERIALS AND METHODS

Sera were obtained from 14-day-old chickens infected orally by doses of 150,000—200,000 oocysts of *E. tenella**). In this group of infected chickens blood was extracted daily from two chickens until the first appearance of antibodies; thereafter blood was drawn in intervals of 4—6 days. The infected group consisted of 15 chickens, control sera were obtained from three uninfected birds of the same

*) Obtained through the courtesy of Ing. Štrossová.

age, kept separately. For the reaction the sera were diluted in buffered saline (PBS) – starting dilution 1 : 10.

Antigens. Two antigens were used: the developmental stages on histological sections and free second generation merozoites. The histological antigens were prepared a) of the material from the caeca 5 days p.i. containing high numbers of 2nd generation schizonts, b) from material 7 days p.i. containing gametocytes of various age. (For histological methods see ČERNÁ 1966 a, b.) The antigens were controlled microscopically after staining the sections with Harris' hematoxylin. Antigen from the free merozoites was obtained by washing the second generation merozoites from the caecal lumen, where they occurred in high numbers, 5 days p.i., in saline (pH 7.2); after centrifugation at 2,500 r.p.m for 20 minutes they were transferred to slides. The material was left to dry and kept unfixed in the refrigerator at +4 °C. The conjugates used were rabbit antibodies labelled with fluorescein-isothiocyanate against chicken gamma globulins, produced by the Institute for Sera and Vaccines, Prague.

Equipment. For our observations we used the Soviet microscope ML-2.

Reaction. After removing the paraffin from the sections the serum was added and left to act for 45 min in the moist chamber at a temperature of 37 °C; after washing for 20 min in saline solution (pH 7.2), for 15 min in tap water and for 5 min in distilled water the conjugate was added and left to act for another 45 min in the moist chamber at 37 °C; the preparations were again rinsed and stained supplementary with Evans' blue (final dilution 1 : 10,000) for 15 min at 37 °C. After washing them in the same way as after the incubation with the sera and conjugates, the preparations were mounted in glycerin, pH 7.2. When using free merozoites for the antigen, the serum, the conjugate and the stain (Evans' blue) were left to act directly for the same time as on the sections. Also the rinsing was the same.

Evaluation of the reaction. Reactions, where the developmental coccidial stages were of a yellowish-green fluorescence of various intensity (+ — + + +) were considered positive. Autofluorescence of the tissue on the sections was suppressed by complementary staining with Evans' blue.

RESULTS

The appearance and course of antibodies against *E. tenella* were observed on a group of 15 chickens, aged 14 days, infected orally with a near-lethal dose of oocysts of this species. The first antibodies were detected on the antigen with the schizogonies 8 days p.i. in titers of 1 : 10 and 1 : 20. The antibody level increased very rapidly, between the 11—13th day the titers in the sera were 1 : 160 to 1 : 640.

A reaction of the same intensity of fluorescence was observed in the antigens from free, unfixed merozoites. The course of antibody formation in the chicken, attaining the maximum titer of 1 : 640 at this period, was traced in intervals of 4—6 days until the disappearance of antibodies (Fig. 1). As shown on Fig. 1, the level of fluorescent antibodies, after attaining its maximum, decreased very rapidly (after 27 days 1 : 40, after a month 1 : 20), but a low level of antibodies still persisted in the chickens 61 days p.i. A week later, no antibody was demonstrable in the merozoites and the reaction on this antigen became negative.

A different reaction with the same antisera was observed on the second histological antigen with the gametocytes of *E. tenella*. The first positive fluorescence of gametocytes was recorded 15—16 days p.i. In the sera diluted 1 : 10, the gametocytes were moderately fluorescent (intensity +), while the merozoites with the same

serum of the same dilution and at the same time showed a fluorescence of + + + and the titer of the sera attained a value of 1 : 160—1 : 320. Although the moderate fluorescence persisted also on the gametocytes to the 68th day p.i., the fluorescence was not intensive enough for the sera of the above dilution to act on the gametocytes.

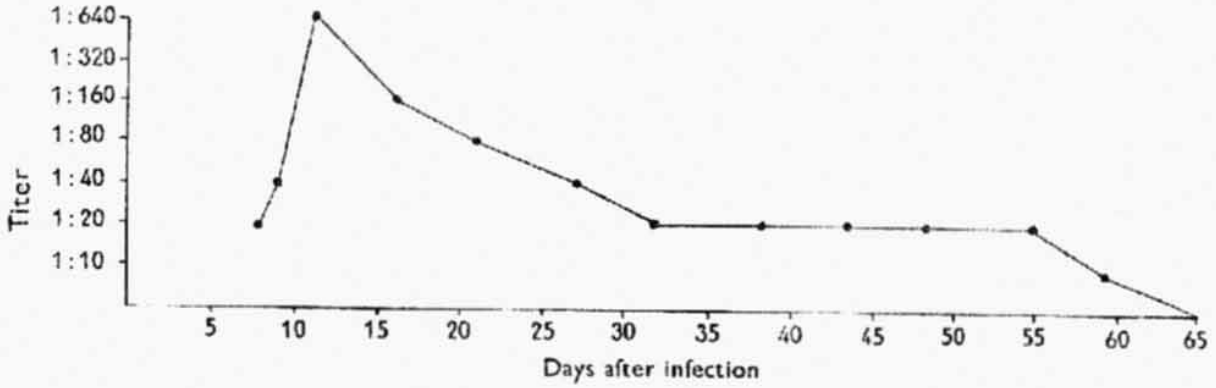

Fig. 1. Course of antibody in a chicken, experimentally infected with *E. tenella*, with the maximum titer in the antiserum.

To illustrate this fact, Tab. 1 contains also an evaluation of the intensity of fluorescence in the sera, examined at various intervals p.i. at a solution of 1 : 10 on the schizonts and gametocytes (Tab. 1).

In the uninfected chickens of the control group the reactions on both the gametocytes and the merozoites were negative throughout the course of the experiment.

Table 1. Evaluation of the fluorescence intensity in *E. tenella* antisera on the schizonts and gametocytes as antigens

Serum 1 : 10	Antigen-schizonts	Antigen-gametocytes	Number of days p.i.
1	+++	0	13
2	+++	(+)	14
3	−++	+	16
4	−++	(+)−+	21
5	++	(+)−+	27
6	+	(+)−+	41
7	+	(+)−+	61
8	0	0	72

DISCUSSION

Our results indicate that in the serum of chickens infected experimentally with *E. tenella* only antibodies against the asexual stages of the parasite could be traced quantitatively, while, contrary to that, the antigenic properties of the gametocytes were very low. Here, our results differ from those obtained from rabbits infected experimentally with the species *E. stiedae* and *E. magna* (Černá 1966 a, b),

where the young gametocytes of both rabbit species reacted to the antisera with intensive fluorescence. In this connection we compared the results obtained from the gametocytes of *E. stiedae* on the histological antigen with the gametocytes of *E. tenella* on the histological antigen. The rabbit antiserum (titer on the schizonts of *E. stiedae* 1 : 160 80 days p. i.) causes an intensive fluorescence (+ + +) on the gametocytes of *E. stiedae* (dilution 1 : 10): the antiserum from the

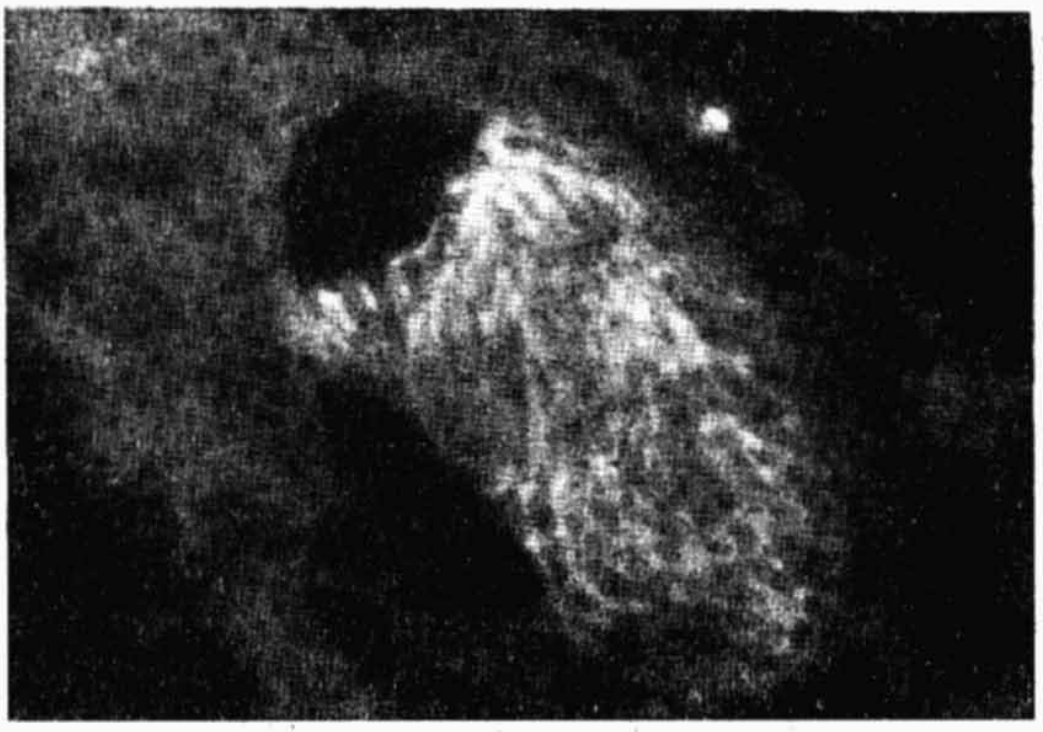

Fig. 2. Schizont of *E. tenella* in a positive fluorescent reaction. ML-2, objective × 60, ocular Hom. 5.

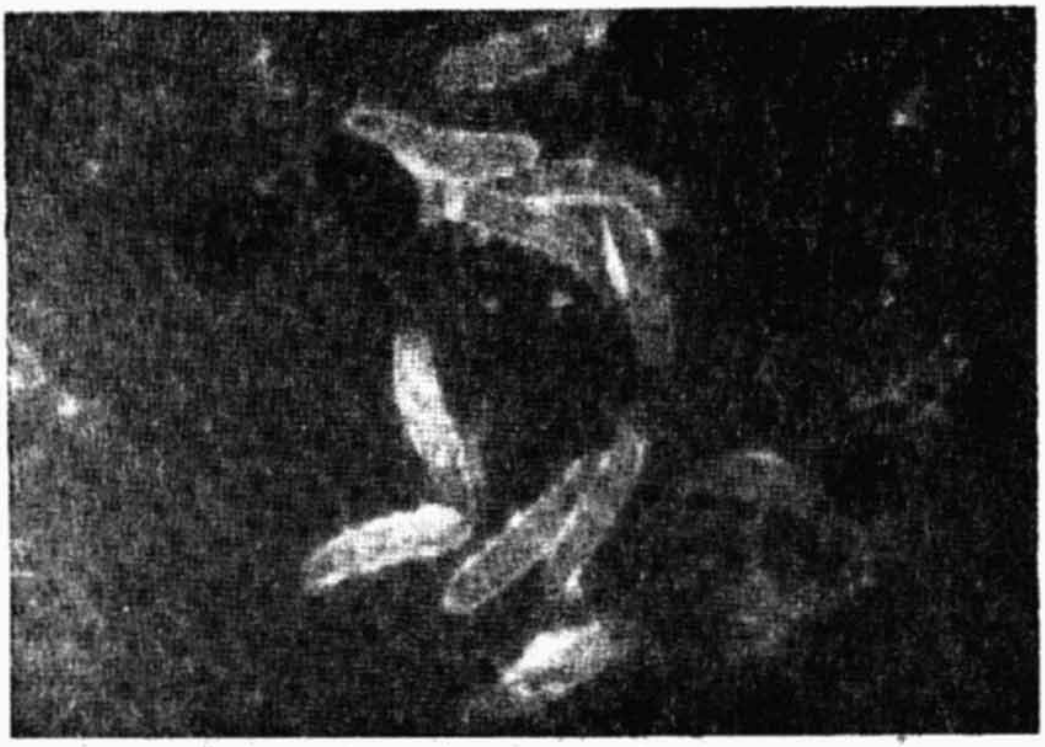

Fig. 3. Free merozoites of *E. tenella* in a positive fluorescent reaction. ML-2, objective × 60, ocular Hom. 5.

chickens (titer on the merozoites of *E. tenella* 1 : 160) evokes only a weak fluorescence (+) on the gametocytes of *E. tenella* (dilution 1 : 10). PIERCE et al. (1962, 1963) proving antibody against *E. tenella* by precipitation on agar, obtained from a comparison of antigens from asexual stages and from oocysts round the antigen from the oocysts only a single precipitation band, congruous with one of the many bands round the antigen from the merozoites. Also HORTON—SMITH et al. (1963) determined the asexual stages as more convenient antigens in the precipitation reaction in agar for the chicken coccidia *E. tenella*, *E. acervulina*, *E. necatrix* and *E. maxima*. It remains to be solved why the gametocytes of chicken species, in this case of *E. tenella*, behave like weak antigens against their own antiserum, if the gametocytes of other coccidial species (*E. stiedae*, *E. magna*) were found strongly antigenic in the IFAT. In my opinion an explanation should be looked for in the period in which the individual developmental stages are active in the cells of the host's organism. After KOTLÁN et PELLÉRDY (1936) (cit. PELLÉRDY 1965) the gamogony of *E. stiedae* starts a fortnight after infection; in our experimental rabbits we observed the first sexual stages between the 14—15 day p.i. A massive occurrence of oocysts was observed 25—27 days p.i. Contrary to that in chicks, infected with the species *E. tenella*, masses of asexual stages (mezoroites of the 2nd generation) were found 5 days p.i., on the 6th day the attacked caeca were packed with mostly gametocytes, oocyst production started between the 7—9th day after the infection. It seems that the shorter period during which the organism of the host is affected by the sexual stages of chick coccidia, finds its reflection in a reduced production of corresponding antibody.

The course of fluorescent antibodies in a one-time infection with *E. tenella* shows that these antibodies disappear in a much shorter time than in infections with the afore mentioned rabbit species. While in infections with *E. stiedae* fluorescent antibodies were present 220 days p. i., in the one-time infection with oocysts of *E. tenella* antibody production lasted only 68 days. Similar results were obtained by ROSE (1963); the rabbits in her experiments remained resistant to *E. stiedae* for two years while resistance of chicks to *E. tenella* lasted approximately two months.

CONCLUSION

The use of a histological antigen in the indirect fluorescent method has also been found suitable for detecting antibody in *E. tenella* antisera from chicks. The first antibodies were earlier demonstrable on the asexual stages than on the gametocytes. Also the intensity of fluorescence was much higher on the schizonts and free merozoites than on the gametocytes. It was possible to work out a quantitative evaluation of antibodies on the antigen with the asexual stages.

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