An Attempt to Detect the Specific Antibody to Coccidia (*Eimeria tenella*) at the Infected Sites of Caecum in Immunized Chicken

Hideaki TOJO, Yoshihiko NAKANISHI and Kiyohiko OGAWA

(*Laboratory of Animal Reproduction*)

(Received for publication August 31, 1973)

INTRODUCTION

PIERCE *et al.* (6) and ROSE and LONG (8) have shown that fowls can acquire active immunity to *Eimeria tenella* as the result of the infection with graded doses of oocysts and that during the period of immunization precipitins can usually be detected. BURNS and CHALLEY (1) and HORTON-SMITH *et al.* (4) have demonstrated that the immunity to *E. tenella* actively acquired by one caecum of the young fowls can be transferred to the other, non-infected, caecum. The results of these studies suggest the existence of a humoral basis for the immunity to coccidiosis.

In contrast, most attempts to transfer passively with serum the resistance to coccidiosis, which has been acquired as a result of previous infection, have failed.

Hence the significance of circulating antibodies in resistance to re-infection is not yet well-known. To explain the mechanism of this resistance, PIERCE *et al.* (7) pointed as one of the relevant factors the permeability and concentration of antibodies at the site of infection.

The present study was attempted to demonstrate the above suggestion by detecting the specific antibody's presence to *E. tenella* at the infected sites of caecum in immunized chicken. Detection of the specific antibody in tissues was carried out by the indirect fluorescent antibody technique.

MATERIALS AND METHODS

Preparation of conjugated antibody

Immune serum was prepared from male White Leghorn chickens given the graded infection of oocysts (8) and *r*-globulin was then fractionated by the precipitation with ammonium sulphate. To obtain the anti-chicken *r*-globulin serum, most of these *r*-globulin fractons were used as antigen for injection to rabbits. The rabbit anti-chicken r-globulin serum was prepared by multiple intramuscular and subcutaneous injections, over a 3-month period, of the purified chicken *r*-globulin, each dose consisting of 50 *mg r*-globulin as a 1 % solution in Freund's adjuvant injected into four sites. The *r*-globulin was similarly obtained from rabbit antiserum.

On the other hand, some of them were used for the conjugation with fluorescein iso-

thiocyante (FITC). The chicken *r*-globulin was conjugated in sodium carbonate bicarbonate buffer (*pH* 9.5), adding 1 *mg* FITC to each 100 *mg* *r*-globulin, for 8 hours at 4°*C*. Excess FITC was removed by passing the crude conjugate down a Sephadex G-25 column, 0.005 M phosphate buffer (*pH* 7.2). The conjugate was then fractionated by chromatography on DEAE-cellulose with the same buffer, the fractions having a fluorescein-protein molar ratio between 2 and 4 being used for immunofluorescence. The purified conjugate obtained was filtrated with milipore membrane after the absorption with tissue powders and then stored at −20°*C* without preservatives until the time for use.

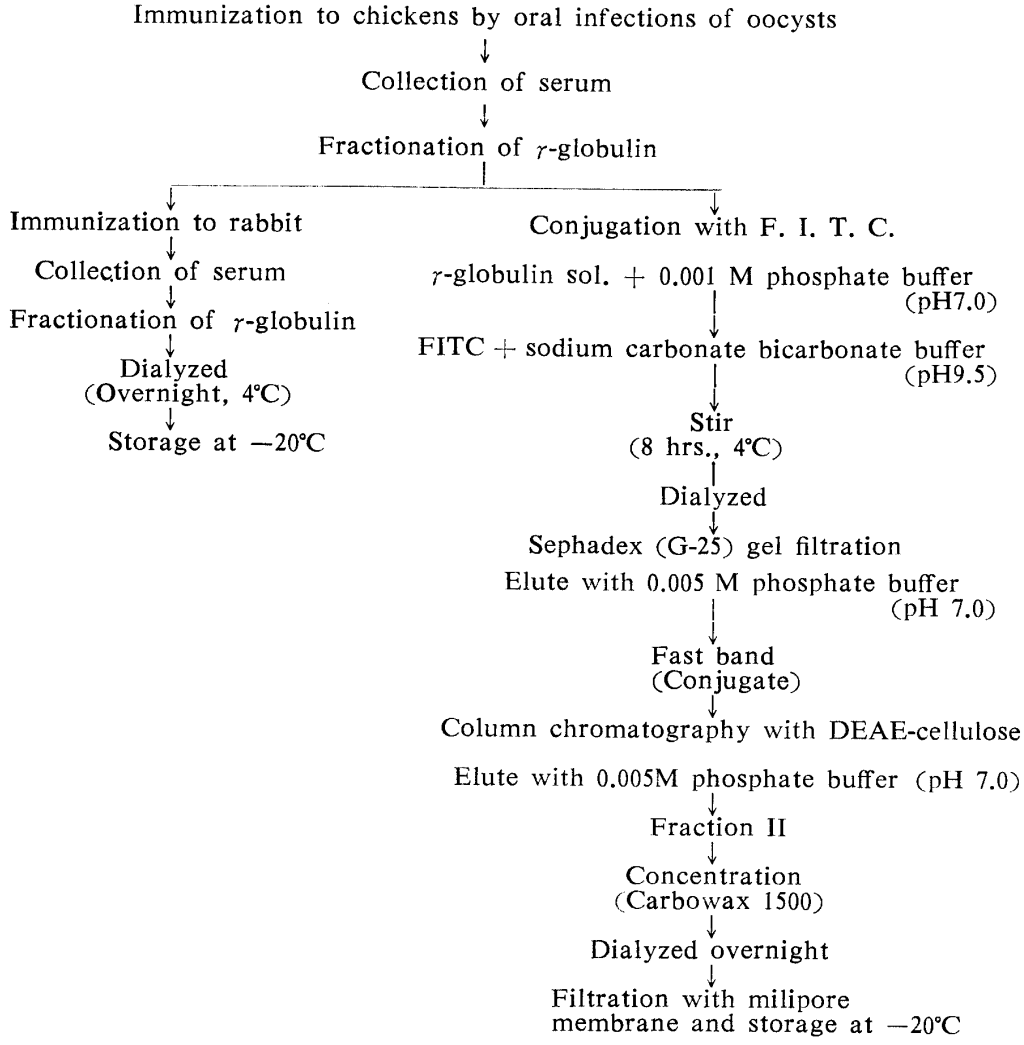


Fig. 1. Preparation of conjugated antibody.

Preparation and staining of tissue sections

The caecal ducts were removed from chickens immunized by the graded infections with oocysts. The small blocks of them were fixed in cold 95 % ethanol in dry ice at −70°*C* for 1.5 *hr*. These blocks were completely fixed and dried in the process as described at Fig. 2. Each of tissues was embedded in 57°*C* melting-point paraffin wax.

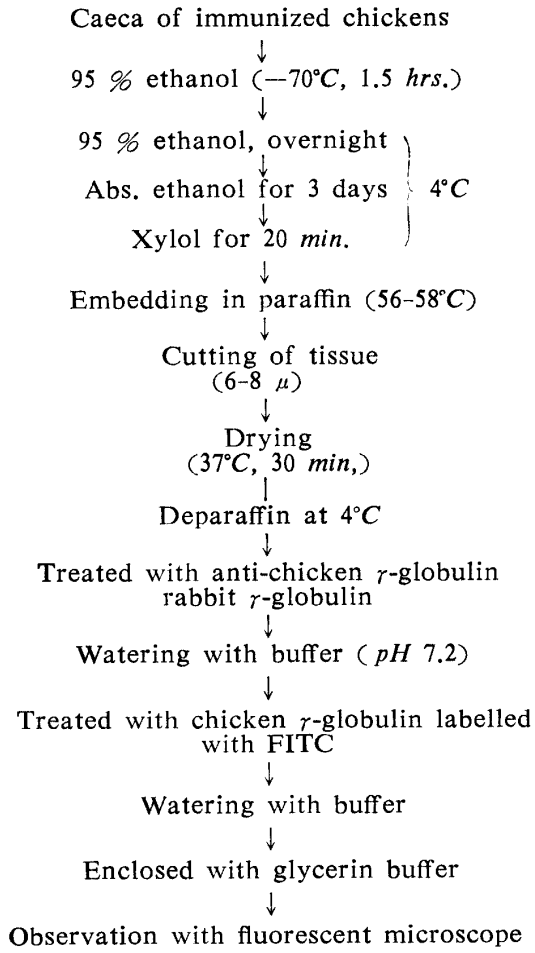


Fig. 2. Preparation and staining of caecal tissue sections.

Sections cut at 6 to 8μ were floated out xylol on microscpe slide, dried, fixed in 95 % cold ethanol and dried again. The fluorescent staining of tissue sections was conducted by the indirect method. Some appropriate tissue sections were first covered with about 0.5 *ml* of its specific anti-chicken *r*-globulin rabbit *r*-globulin solution and incubated in moist Petri dish for an hour at 37°C. The cover-slips containing the sections were then put into Coplin jars containing phosphate buffer (*pH* 7.0), and washed to remove the rabbit *r*-globulin solution which had not reacted. After the third washing the sections were dried, covered with about 0.5 *ml* FITC-labelled chicken *r*-globulin solution, placed in moist Petri dish and incubated for an hour at 37°C. After further washing and drying, the sections were mounted in buffered glycerol (*pH* 9.5) on glass slides and were immediately observed for fluorescence. Control preparations from non-infected chickens were treated in a similar manner and run simultaneously with all experimental tissue sections. Fluorescenence was observed with Nikon FL type fluorescent microscope, using a 200 W mercury vapour lamp with excitation filter UV or BV.

RESULTS AND DISCUSSION

To examine the presence of specific antibody to parasites in the conjugate prepared from immune chicken serum, the conjugate was applied to slides with parasite smears obtained from gut lumen of chicken while infection. The existence of the specific antibody to parasites was certainly indicated in this conjugate. Moreover it was assumed that its titre should be very high as the strong fluorescence was detected at the surface of parasites in the mucous epithelial cells (Fig. 3 and Fig. 4).

All tissue sections of caeca were stained by the indirect method. In caecal tissues of immunized chickens, the specific fluorescence was observed to be distributed at the epithelium of the mucous membrane, the lymphoid tissue and the inner walls of capillaries in the submucosa, but not at the intestinal glands and the muscular regions. However all of these fluorescences were not always of sufficient intensity, Furthermore some differences in the intensity of fluorescence could be distinguished among these sites though negligible (Fig. 5-Fig. 8).

In contrast, no specific fluorescene could be observed at all the control tissue sections prepared from non-immunized chickens.

From the results, it was suggested that the specific antibody to *E. tenella* could be distributed at the infected sites of caecum.

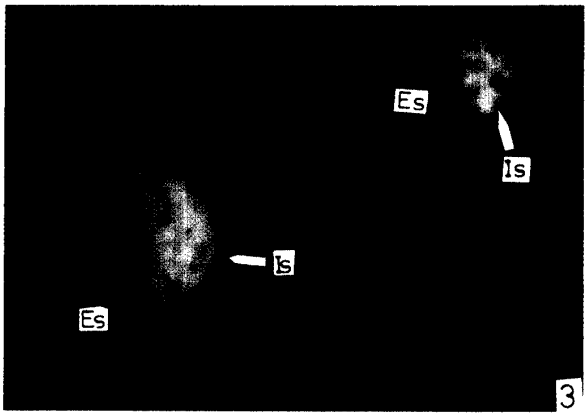
It is well established that, provided adequate numbers of sporulated oocysts are administered, fowls will develop a solid resistance to *E. tenella*. Also it has been shown that circulating antibodies demonstrable by agglutination (5. 10), precipitation (6), lysis complement fixation (2. 3. 5. 10) and fluorescent staining (11) appeared in the chicken serum after infection with oocysts of *E. tenella*.

Nevertheless the immune mechanisms in coccidiosis have long evaded the efforts of many investigators trying to clarify them. BURNS and CHEUEY and HORTON-SMITH *et al.* have demonstrated that resistance acquired by one caecum as the results of a primary infection with *E. tenella*, can be transferred to the other caecum, surgically isolated from the gut lumen and non-infected. These results suggest that circulating atibodies may play a role in the immunity to coccidiosis.

On the other hand, many investigators have failed to transfer a passive immunity to coccidiosis by the donation of serum or *r*-globulin from immunized chickens. TOJO and OKAMOTO (9) have reported that the removal of the Bursa of Fabricius, which reduce antibody productions in birds, showed no affection to the acquired immunity to coccidiosis (*E. acervulina*). Also they (10) have shown that the presence of lysin was evidently not required for immunity, since chickens immunized by injection of parasites extract produced lytic sera and yet remained susceptible to oral infection with oocysts of *E. tenella*.

Thus, the significance of circulating antibodies in the immunity to coccidiosis is not yet clear. PIERCE *et al* (7) suggested to regard the permeability of the dnıibody to the caecal mucous and the concentration of the antibody at the site of infection as relevant factors in this immunity.

In this study, it was assumed that the specific antibody to *E. tenella* existed at the infected sites of caecum, although there might be a possibility that the antibodies



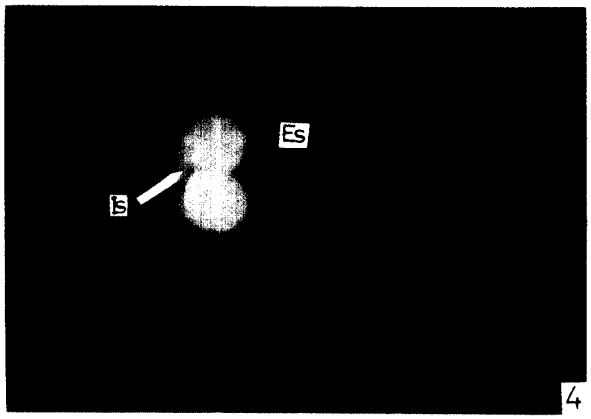
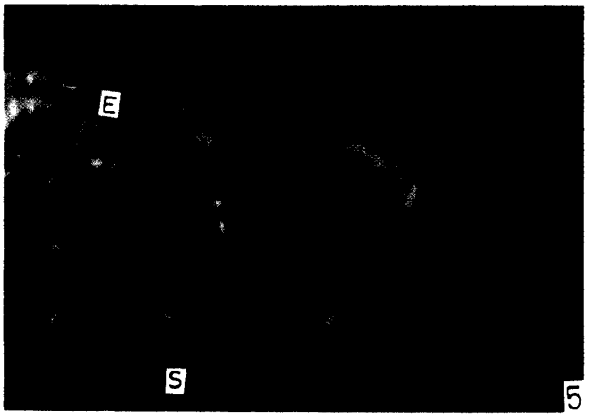


Fig. 3. The specific fluorescence at the surface of the parasite (inadult schizont) in the epithelial cells. (× 600) Is : Inadult schizont, Es : Epithelial cell.
Fig. 4. The specific fluorescence at the surface of two parasites in the epithelial cell. (× 600) Is : Inadult schizont, Es : Epithelial cell.



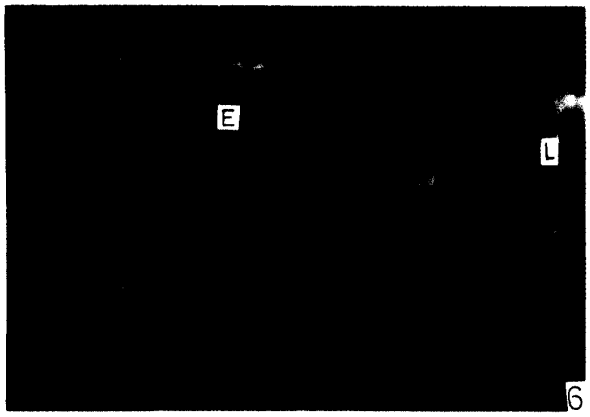
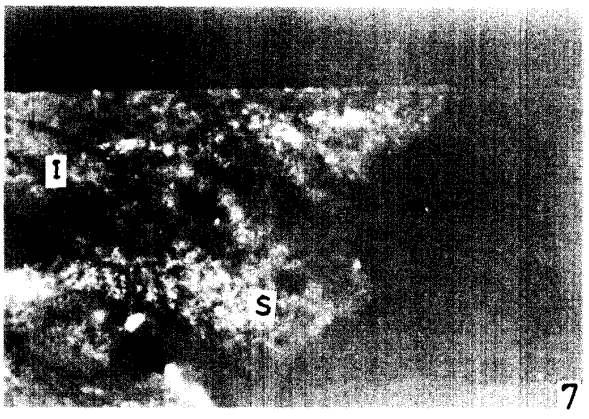


Fig. 5. The specific fluorescence at the epithelium of the mucous membrane. (× 400) E : Epithelium, S : Submucosa.
Fig. 6. The specific fluorescence at the lymphoid tissue. (× 400) E : Epithelium, L : Lymphoid tissue.



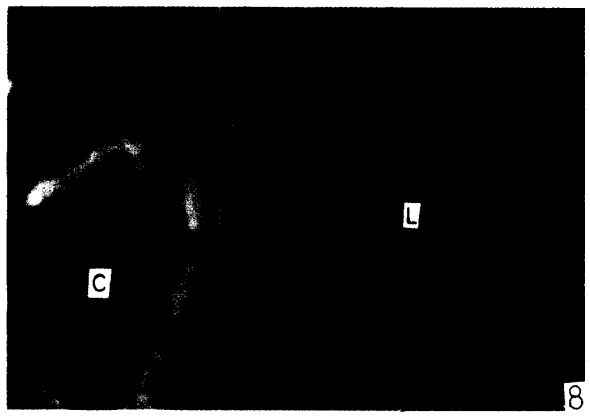


Fig. 7. The specific fluorescence at the submucosa. (× 400) I : Intestinal gland, S : Submucosa.
Fig. 8. The specific fluorescence at the inner wall of capillary. (× 600) C : Capillary, L : Lymphoid tissue.

to invaded antigens other than coccidia were stained at the caecal tissues. This observation is to support the previous suggestion by Pierce *et al.* (7). In addition, the fact that the specific antibody was detected at the epithelium of caecum may also indicate that the invasion of parasites is to be blocked at the epithelium of caecum. The results of the present study suggest that the invaded parasites can be defeated at the infected sites of caecum in immunized chicken by these antibodies at the time of re-infection. However it was left un-asscertained whether these antibodies were produced by the immuno-competent cells at the infected sites or were concentrated after having passed through the blood circulation.

Hereafter it comes to be important to investigate the possible concentraiton of the specific antibody at the infected sites.

SUMMARY

The present study was conducted to investigate the possible presence of some specific antibody to coccidia (*Eimeria tenella*) in the caecal tissue prepared from chickens immunized previously by oral infection with oocysts. The presence of specific antibody in the tissue sections was detected by the indirect immunofluorescent staining. The results obtained are as follows.

In the caecal tissue sections of immunized chickens, the specific fluorescence was observed at the following sites; the epithelium of the mucous membrane, the lymphoid tissue and the inner walls of capillaries in the submucosa, but not at the intestinal glands and muscular regions.

In contrast, no specific staining was noticeable in control tissue sections. The results of the present study indicate that the specific antibody to *E. tenella* is to exist at the infected sites of caecum in the immunized chicken.

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