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AN ATTEMPT TO ANALYZE ANTIGENS FROM SEXUAL AND ASEXUAL STAGES OF COCCIDIA BY THE INDIRECT FLUORESCENCE ANTIBODY REACTION

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Abstract. By means of the indirect fluorescence antibody reaction (IFAR) a divergent component was detected in the structure of antigens from asexual and sexual stages in the mouse species *Eimeria pragensis*. Antisera from experimentally infected mice were negative after absorption by free merozoites, if antigen from asexual stages was employed in the IFAR, but remained positive to antigen from gametocytes.

Very few data are available on the antigenic properties of various developmental stages of coccidia used in serologic reactions. Horton-Smith et al. (1963) were the first to observe in precipitation tests with chicken coccidioses that best results were obtained with antigens from asexual (schizogonial) stages. They prepared antigens for the test by the extraction of tissues containing individual developmental stages and by the extraction of oocysts. Also Augustin and Ridges (1963) prepared antigens for precipitation in a similar way with *E. meleagrimitis* from turkeys. These authors, however, assume that the number of precipitation lines was (with the species revealed in turkeys) affected rather by the quantity of inoculated oocysts than by the developmental forms of the parasite. One of the authors of the present paper (Černá 1970) studied the difference in antigenic activity of four coccidian species of the genus *Eimeria* in the IFAR. On the basis of these experiences an attempt was made to resolve the problem of the relationship of the antigenic structure of sexual and asexual coccidian stages while absorbing antisera, containing coccidian antibodies, by free merozoites. As a model for the reactions, antisera containing antibodies against the mouse coccidian *E. pragensis* were chosen, partly because with this species it is relatively easy to obtain free merozoites in the lumen of the caecum, partly because both the asexual and the sexual stages are good antigens for the IFAR.

MATERIAL AND METHODS

Obtaining free merozoites: Free merozoites *E. pragensis* were recovered from the caecum and large intestine of mice on day 5 after the inoculation of sporulated oocysts. Smears from the intestinal lumen were mixed with saline, pH 7.2–7.4 (phosphated buffer 0.01 M) and the material was incubated for 2—2 1/2 hours at a temperature of 37 °C. The majority of debris settled in the sediment; the

supernatant was transferred to other test-tubes and kept for another hour at a temperature of 37 °C. Supernatant containing predominantly free merozoites was placed in centrifugal test tubes and centrifugated for 15 minutes at 1,500 – 2,000 rpm. The sediment containing merozoites was fixed with 0.4 % neutral formalin in saline.

Antisera: Antisera were obtained from white laboratory mice experimentally infected by 50—100 thousand oocysts of *E. pragensis*.

Antigens: Antigens were prepared in a manner described in Černá's paper (1966a). For the species *E. pragensis* schizogonial antigen is recorved from the caecum or large intestine of mice 5 days p. i., gametocytic antigen 7 days p. i.

Conjugate: FITC labelled pig gamma-globulin was used against mouse gamma-globulin (commercially produced by ÚSOL, Prague). For IFAR techniques see earlier papers (Černá 1966 a, b).

Controls: Both the absorbed (see Results) and the corresponding nonabsorbed sera were kept under equal conditions and then examined repeatedly by IFAR using both antigens.

RESULTS

In a series of provisional experiments the most suitable condition of the absorption reaction of antisera by free merozoites was established. It was ascertained that for the absorption of the antiserum (i.e. for disappearance of antibodies which become fixed to asexual antigen) it is necessary to use 40 million free merozoites for 0.025 ml of the serum. The absorption proceeded best at a temperature of 37 °C within 4 hours.

After having adapted the conditions of absorption reaction, 15 antisera with titres ranging from 80 to 640 in both antigens were chosen and these antisera were—under the above-quoted conditions—absorbed by free merozoites. The absorption being finished, the antisera were again tested by the IFAR. The results are presented in the Table. In all instances (except serum M_{142}), where after the absorption a low titre(10) had been retained in the schizogonial antigen, the IFAR after antiserum absorption was negative in the asexual antigen while in the antigen from the gametocytes it continued demonstrating positivity in titres from 20 to 80.

DISCUSSION AND CONCLUSIONS

The above-cited absorption experiments confirmed that the results of serologic reactions by coccidia were influenced by the developmental stages employed as antigen for the

Table 1. Antisera of *E. pragensis* examined by the IFAR before and after absorption by free merozoites of this species

Serum no.	Original titres		Titres after absorption		Serum no.	Original titres		Titres after absorption	
	asexual	sexual	asexual	sexual		asexual	sexual	asexual	sexual
	antigen		antigen			antigen		antigen	
M_{128}	80	80	neg	20	M_{74}	160	160	neg	40
M_{129}	80	80	neg	20	M_{126}	160	160	neg	40
M_{130}	80	80	neg	20	M_{127}	160	160	neg	40
M_{75}	80	160	neg	80	M_{132}	160	160	neg	40
M_{61}	160	160	neg	40	M_{143}	320	640	neg	40
M_{69}	160	160	neg	40	M_{146}	320	640	neg	80
M_{72}	160	160	neg	40	M_{142}	640	640	10	80
M_{73}	160	160	neg	80					

reactions, which had been indicated in one of our previous papers (Černá 1970). Our experiments showed that absorption with free merozoites facilitated the separation of antibodies fixed to these merozoites from antibodies fixed to the antigen of gametocytes, and that the absorption of antisera was followed by a decrease of antigen titre to antigen from the gametocytes. This indicates that the antigenic structure of the sexual and asexual stages of coccidians contains, in addition to components specific of the individual stages, also components shared in common, which make the attachment to both antigens possible.

ПОПЫТКА АНАЛИЗА АНТИГЕНОВ, ПОЛУЧЕННЫХ ИЗ ПОЛОВОЙ И БЕСПОЛОЙ СТАДИЙ КОКЦИДИЙ НЕПРЯМЫМ МЕТОДОМ ФЛУОРЕСЦИРУЮЩИХ АНТИТЕЛ

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Резюме. Непрямым методом флуоресцирующих антител (НМФА) был обнаружен дивергентный компонент в структуре антигенов, полученных из половой и бесполой стадий паразитирующей в мышах кокцидии вида *Eimeria pragensis*. В случае применения антигена из бесполых стадий в НМФА антисыворотки от экспериментально зараженных мышей были отрицательны после абсорбции свободными мерозоитами, но оставались положительными к антигену из гаметоцитов.

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