The Circumoval Precipitate and Cercarienhüllen
Reaktion of Austrobielharzia variglandis

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ABSTRACT: The Circumoval Precipitin Reaction (COP) and the Cercarienhüllen Reaktion (CHR) were characterized for the marine avian schistosome, Austrobielharzia variglandis, utilizing sera from infected and artificially immunized chickens.

Circumoval precipitins were present in chicken sera 25 days following infection. No positive COP reactions were observed for eggs incubated in sera from animals artificially immunized with cercariae or adults. Complement was not essential in the development of positive COP reactions.

A positive CHR was observed in chicken serum collected 25 days following exposure to cercariae, but no reaction occurred in 14-, 32-, and 39-day sera. Sera from chickens artificially immunized with cercarial or adult homogenates elicited a positive CHR.

Normal chicken serum and normal guinea pig serum were cercaricidal. The role of complement in cercaricidal action was verified, but its participation in CHR formation is uncertain.

The Circumoval Precipitate (COP), first described for Schistosoma mansoni by Oliver-Gonzalez (1954), is typified by the appearance of a precipitate contiguous with the eggshell following incubation in immune serum. Newsome (1958), Yogore et al. (1968), and others have described the COP for other mammalian schistosomes but it has never been reported for avian forms.

The Cercarienhüllen Reaktion (CHR) of Vogel and Minning (1949) is characterized by the appearance of a sheath external to the cercarial tegument following incubation in immune serum. The CHR is well documented for mammalian schistosomes (Stirewalt and Evans, 1955; Ratanaret-Brokelman, 1972, and others). It has also been reported for three freshwater avian schistosome cercariae, Trichobilharzia ulvae, T. physellae, and T. stagnicola (Hendricks and Cort, 1956) and for an unknown marine avian form (Leflore and Martin, 1972).

In the present study, the COP and CHR were characterized for the marine avian schistosome, Austrobielharzia variglandis (Miller and Northup, 1926; Penner, 1953), using sera from infected and artificially immunized birds.

Materials and Methods

To obtain cercariae for infections, naturally infected Nassarius obsoletus were isolated in dishes of seawater the afternoon of the day preceding exposure of chicks. Twelve-day-old white leghorn chicks were collectively exposed to approximately 4,000–5,000 cercariae by wading (5 birds/battery jar) for 90 min.

Adult worms were obtained from chicks infected 4 weeks or longer. Organs from these birds were teased apart and refrigerated overnight in normal saline. Worms obtained were washed six times in saline followed by three times in distilled water, frozen with Cryokwik (Damon/TEC Division, Needham Hts., Massachusetts), lyophilized, and stored at 4°C in capped vials.

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Table 1. Immunization schedule for artificially immunized chickens.

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<th>Day 1</th>
<th>Day 14</th>
<th>Day 21</th>
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<tr>
<td><strong>Controls</strong> (2 birds)</td>
<td>*FCA, intramuscular, 0.2 ml/thigh</td>
<td>FCA, subcutaneous, 0.1 ml amounts in 4 different locations</td>
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<tr>
<td><strong>Cercarial</strong></td>
<td>Cercarial homogenate in FCA, 1:1 (v/v), intramuscular, 0.2 ml/thigh</td>
<td>Cercarial homogenate in FCA, 1:1 (v/v), subcutaneous, 0.1 ml in 4 different locations</td>
<td>Cercarial homogenate only, intravenous, 0.4 ml</td>
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<tr>
<td><strong>immunization</strong></td>
<td></td>
<td></td>
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<td>(2 birds)</td>
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<tr>
<td><strong>Adult worm</strong></td>
<td>Adult worm homogenate in FCA, 1:1 (v/v), intramuscular, 0.2 ml/thigh</td>
<td>Adult worm homogenate in FCA, 1:1 (v/v), subcutaneous, 0.1 ml in 4 different locations</td>
<td>Adult worm homogenate only, intravenous, 0.4 ml</td>
</tr>
<tr>
<td><strong>immunization</strong></td>
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<td>(2 birds)</td>
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* FCA = Freund’s complete adjuvant.

Eggs were isolated from infected chicken livers using the method of James and Colley (1974). To prevent hatching, however, 0.85% rather than 1.7% saline was used. Eggs were stored in the dark in saline at 4°C and used within 3 days.

Seawater containing cercariae was filtered through four layers of cheesecloth and then through a Millipore apparatus fitted with a 47 mm, 8 μm filter. The filter with adherent cercariae was weighed, placed in a petri dish, frozen with Cryokwik, and stored at −20°C until needed. Approximately 30 mg (wet weight) defrosted cercariae were flushed from the filter paper with 1 ml ice cold 0.85% saline and homogenized with a Kontes hand driven tissue grinder. This suspension was used as antigen for artificial immunization. Adult whole worm antigen was similarly prepared using 10 mg lyophilized worms.

Cercariae used for the CHR were collected as above but were not frozen. When seawater was added to a dish containing a processed filter, cercariae were released and removed from solution with a pipette.

Experimentally infected chickens were bled by cardiac puncture at 14 (7 birds), 25 (11 birds), 32 (6 birds), and 39 days (5 birds) postinfection. Eight uninfected birds of the same age as the 39-day postinfection group were also bled. Six 14-day-old white leghorn chicks were immunized as shown in Table 1. All artificially immunized chickens were bled 1 week following their last injection and controls were bled 2 weeks after their last injection. Sera from similarly treated animals were pooled, preserved with sodium azide (0.10%) and stored in small aliquots at −20°C.

All COP and CHR tests were done in triplicate on covered 3 × 1 inch glass slides using a drop of serum handled in each of the following ways: (1) untreated, (2) inactivated (incubated at 56°C for 30 min), and (3) inactivated plus complement (one drop guinea pig serum). COP preparations were examined at 24 and 72 hr. They were scored as weak if the length of the precipitate chain was less than half the diameter of the egg and strong if longer. CHR preparations were observed at 1, 4, and 24 hr. A strong positive reaction was always indicated by a thick, sharply defined envelope surrounding the entire cercaria, whereas a weak positive reaction resulted in a thinner, more delicate appearing envelope that sometimes was confined to the tail only.
Figures 1–6. Photomicrographs of COP responses of *A. variglandis* eggs. 1. Eggs incubated in 39-day postinfection chicken serum for 72 hr. Note that smaller immature egg shows no COP. ×420. 2. Egg incubated in 25-day postinfection chicken serum for 72 hr. Weak positive reaction. ×490. 3. Egg incubated in 25-day postinfection inactivated chicken serum for 72 hr. Weak positive reaction. ×420. 4. Egg incubated in 32-day postinfection chicken serum for 72 hr. Strong positive reaction; note segmentation. ×420. 5. Egg incubated in 32-day postinfection chicken serum for 72 hr. Strong positive reaction; note outline of developing miracidium. ×490. 6. Egg incubated in 39-day postinfection chicken serum for 72 hr. Strong positive reaction. ×380.
Results

COP

No positive COP response occurred in sera from control animals or from those immunized with cercarial or adult homogenates. Dead, empty, vacuolated, or immature eggs rarely showed a reaction whereas those containing a well-formed miracidium usually did (Fig. 1). Positive reactions occurred in sera from experimentally infected chickens harboring worms for 25, 32, and 39 days (Figs. 2–6) but serum obtained 14 days postinfection was negative. Most precipitates exhibited a typically segmented appearance. The percentage of eggs showing a positive reaction increased with the age of the infection. In 25-, 32-, and 39-day postinfection sera the mean percent positives were 14.0, 24.6, and 26.7, respectively. Inactivation of these sera reduced the percentages to 7.9, 12.1, and 11.7. Addition of complement did not restore full activity.

CHR

Positive CHR activity was never observed in serum from control animals. Although most cercariae incubated in control serum from uninfected birds were not affected after 24 hr (Fig. 7), a few became motionless, and developed oral deposits and curled furcae within 1 hr. When inactivated, this serum was harmless to cercariae but became strongly cercaricidal when complement was added, suggesting a cercaricidal effect of guinea pig serum. Serum taken from experimentally infected chickens at 14, 32, and 39 days was also cercaricidal. Massive agglutination and positive CHR activity occurred within 24 hr when cercariae were incubated in 25-day postinfection serum (Fig. 8) but these effects were not observed at 1 or 4 hr. Agglutination and CHR activity were lost after inactivation and were not restored by adding complement. No activity was observed with 32- or 39-day sera. Artificially immunized control (FCA only) chicken serum was cercaricidal. In such serum most cercariae became spastic within 1 hr and a sticky deposit appeared at the oral end. A fine granular precipitate then appeared around the body, furcae curled, and motion ceased (Fig. 9). This effect did not occur in inactivated serum, but was restored by adding complement. Cercariae incubated in serum from chickens artificially immunized with cercarial antigen became very “sticky” within the first hour of incubation and at 24 hr all displayed definite sheaths and tended to agglutinate (Fig. 10). Inactivation of such serum resulted in the appearance of a flocculent precipitate that adhered to cercariae and hindered observation. Weak envelopes, however, were often evident (Fig. 11). Addition of complement to such serum did not restore original activity but an even more copious precipitate developed. Weak envelopes occurred occasionally in chicken anti-adult serum (Fig. 12). Envelopes were not observed with anti-adult inactivated plus complement sera, but massive precipitates occurred at the oral end (Fig. 13).

Discussion

Uninfected control serum presumably contains no specific antibody (circum-oval precipitin) against eggs, and negative results can be expected. Only after eggs begin to collect in tissues of infected birds can COP positive serum be anticipated. Our results indicate that the formation of a response occurs between

14 and 25 days postinfection. Our observation that all eggs showing a positive COP contained a live miracidium is consistent with previous findings (Oliver-Gonzalez, 1954; Newsome and Robinson, 1956; Newsome, 1958).

When COP positive sera utilized in this study were inactivated, the percentage
of positive reactions was about half that seen for noninactivated serum. The addition of complement to inactivated serum did not, however, restore original activity. This suggests that another heat labile factor is involved but is weakened by heating.

The failure of sera from any artificially immunized chicken to elicit a COP response indicates that circumoval precipitin is evoked exclusively by egg antigens. This conclusion agrees with the findings of Oliver-Gonzalez (1954) that eggs absorb circumoval precipitins but adult or cercarial tissue does not.

The cercaricidal effects of all control chicken sera and all inactivated control sera (guinea pig serum added) are consistent with the findings of Kagan and Levine (1956) and Standen (1952). Inactivated control sera were not cercaricidal, suggesting a role for complement. Observations by Standen (1952) and Machado et al. (1975) support this view.

Serum dilution delays CHR envelope formation and enhances agglutination (Stirewalt and Evans, 1955). Hendricks and Cort (1956) and LeFlore and Martin (1972) also observed retarded sheath formation in diluted antisera. Austrobilharzia variglandis cercariae agglutinated strongly and formed envelopes slowly in 25-day chicken serum thereby suggesting a low CHR antibody titer.

The role of complement in anticercarial and anti-adult serum is unclear as addition of it to inactivated serum did not restore agglutinating or CHR activity.

A positive CHR response with chicken anti-adult serum was not surprising as schistosome adults and cercariae are known to share antigens (Kemp et al., 1974).

The lack of any CHR or agglutinating activity in 32- and 39-day sera is believed to be indicative of a decreased titer.

**Literature Cited**


**Survey or Taxonomic Papers**

Authors submitting manuscripts of a survey or taxonomic nature for publication in the Proceedings of the Helminthological Society of Washington are urged to deposit representative specimens in a recognized depository such as the National Parasite Collection at Beltsville, Maryland and include the accession numbers in the manuscript.