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Initial And Challenge Infection With Trichobilharzia Ocellata In Anas Rubripes

Manfred Ernst Rau

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INITIAL AND CHALLENGE INFECTION WITH
TRICHOBLHARZIA OCELLATA
IN ANAS RUBRIPES

by

Manfred E. Rau

Department of Zoology

Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Ontario
September 1969
ABSTRACT

Progressive changes in the host-parasite relationship between *Trichobilharzia ocellata* and the black duck (*Anas rubripes*) during the course of initial and challenge infections were followed by means of daily counts of miracidia from viable eggs passed with the droppings of the host. During the acute phase of infection following single, massive cercarial exposure of 1- and 9-week-old ducks, egg passage approximated the path of a normal curve although a state of reduced immunologic competence, as manifested by a pronounced extension of the acute phase of egg passage, was encountered in 3 of 6 ducks exposed at the age of 1 week. In contrast, when comparable numbers of cercariae were applied as a series of small exposures over a period of 51 consecutive days, apparently only few reached the stage of egg passage. Where definite patterns of egg passage could be distinguished, these again approximated the course of a normal curve descending rapidly despite continued cercarial exposure once an early peak had been reached.

Ducks receiving adult worms by intravenous injection exhibited often highly erratic patterns of egg passage, which, although always low grade, at times persisted for extended periods. Previous exposure to *T. ocellata* conferred substantial and lasting protection from subsequent homologous
challenge after immunologically significant periods, regardless of the method of initial exposure.
ACKNOWLEDGMENTS

The author is indebted to Dr. T.K.R. Bourns, Associate Professor of Zoology, University of Western Ontario, for suggesting this problem and for encouragement and constructive criticism throughout the course of this investigation.

Gratitude is due to Dr. A.W.A. Brown, Professor and Head of the Department of Zoology for making available laboratory facilities.

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To Mr. W. Carrick, Director of the Niska Waterfowl Research Station, Guelph, Ontario, the author owes thanks for providing experimental birds for this study.

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INTRODUCTION

Research into the biology of *Trichobilharzia ocellata* (La Valette, 1854) Brumpt, 1931 (Trematoda, Digenea, Schistosomatidae) has been stimulated primarily by this parasites close relationship to members of the genus *Schistosoma*, causative agents of bilharziasis in man, and by its etiological role in schistosome dermatitis or swimmers' itch. Bilharziasis today constitutes the most important public health problem in many areas of the world, conservative estimates fixing the number of infected persons at 180 to 200 million. Not only has intensive research failed to provide highly effective means of prevention and cure for this disease, but extension of irrigation projects and concentration of human populations are actually increasing its incidence and distribution. Schistosome dermatitis occurs when cercariae of avian parasites commit "biological errors" by invading man instead of their normal definitive hosts (ducks in the case of *T. ocellata*). This condition is worrisome not only in a medical and economic sense, but also because of its effect on the recreational value of waterways across the temperate portions of the earth.

The schistosomes are a somewhat unusual group. Like other Digenea, they pass through a relatively complex life cycle which involves asexual multiplication in a molluscan
intermediate host and sexual reproduction in a vertebrate definitive host. Unlike the others, however, the schistosomes are dioecious and characteristically inhabit the circulatory system and tissues of the definitive host. It follows, then, that in addition to the usual adaptations whereby trematodes compensate for the hazards of their life cycles, special adaptations must have evolved to allow schistosomes to cope with the difficulties of initial access, maturation, mate finding, and egg dispersal, which their unusual habitat imposes. One such adaptation seems to be the prolonged and tenacious persistence of patency so characteristic of the human bilharziases, a feature associated with their resistance to control measures. In contrast, while T. ocellata was being established in culture in this laboratory it was noted that infected ducks underwent what seemed to be self-cure a short time after the infections had become established. These observations prompted the author to study the situation in detail with a view to documenting the events of primary and challenge infections as evidenced by the passage of viable eggs. It was hoped that some understanding of the underlying mechanisms and of the over-riding significance of these events might be elucidated. It should be pointed out that unlike most laboratory studies on schistosomes where abnormal hosts have been used, the present work relates to normal (Anas rubripes), (McMullen and Beaver, 1945) and near-normal (domestic duck) vertebrate hosts.
MATERIALS AND METHODS

I. Sources of Animals:

a) Parasite:

_T. ocellata_ was established in culture in our laboratory in 1965 using cercariae from naturally-infected snails, _Lymnaea stagnalis appressa_, from Peterborough, Ontario. The parasite has been maintained by serial passage through laboratory-reared snails and ducks without the introduction of new field-collected cercariae.

b) Birds:

All wild species of birds were purchased as hatchery-reared stock from the Niska Waterfowl Research Station, Guelph, Ontario. Domestic ducks were either hatched in the laboratory or purchased from a commercial hatchery (Webfoot Farms, Elora, Ontario) as day-old ducklings. All birds with experience outside a brooder cage were tested for trematode egg passage prior to use and all were found to be negative.

II. Exposure of Hosts to Cercariae of _T. ocellata_.

Birds were exposed to pooled cercariae from 10 to 40 snails within 1 hr of cercarial emergence. In preparation, the birds' feet were freed of accumulated skin debris and feces by prolonged soaking in tap water and by a final rinse in distilled water. Three methods of exposure were employed, the choice of method being dictated by the nature of the experiment.
a) The "Stopper and Bottle" Method:

When massive but uncounted exposures were required the bird was kept in a padded, dark box for 90 min with one foot held securely in a bottle by means of a split rubber stopper so that the foot was immersed in about 170 ml of cercarial suspension.

b) The "Truss" Method:

In some cases involving massive exposure it was necessary to know the number of cercariae that penetrated the bird's foot. Ducks to be exposed with this in mind were trussed to a padded board (Fig. 1) with the left foot in about 120 ml of cercarial suspension. In this way splashing and spilling was avoided during the 90 min exposure period.

Since most cercariae release their tails during penetration (Appendix I), it was possible to determine the numbers that actually penetrated the hosts by counting the excess tails remaining in the jars (Appendix II). Staining with purpurin and fixation in Davidson's A. solution facilitated this process.

c) The "Coverglass" Method:

Small daily exposures were conducted by transferring low numbers of counted cercariae to the web of the host's foot on an inverted coverglass. The coverglass was lifted after 5 min and the number of cercariae which had transferred to the web was determined. This procedure was repeated until the desired number of cercariae had been applied. The toes were
Figure 1. Exposure of a black duck to cercariae of *T. ocellata* by the "Truss" method.
then firmly taped together, folding the web into a pocket which was kept moist for 2 hr. A given web was never exposed on consecutive days.

III Eggs and Miracidia:

a) Collection of Eggs:

Individual exposed birds were kept in barred, wire mesh-floored cages suspended above plastic trays containing 4L of 0.85% NaCl solution (Fig. 2). Evaporation did not concentrate the solution beyond 0.90% (Appendix III). Because the numbers of viable eggs passed with hourly stool samples fluctuated widely (Appendix IV), feces were collected over 24-hr periods throughout the course of the experiments.

b) Processing of Feces:

Feces were resuspended as the trays were removed and tilted to an angle of approximately 50°. The suspension was allowed to settle for approximately 15 min before the supernatant was carefully decanted. If the supernatant remained turbid the residue was washed with 0.85% saline until it remained almost clear. Tests showed that only 0.05% of the miracidia were lost by this procedure (Appendix V).

c) Hatching of Eggs:

The residue was transferred to specially designed flasks built to the author's specifications by the Kimble Glass Company, Toledo, Ohio (Fig. 3a). These flasks concentrate miracidia in a side arm as do those of McMullen and Beaver (1945) (Fig. 3b), but differ from the latter in several
Figure 2. Accommodation of ducks during the experimental period.
c, cage; t, tray; wt, watering trough; f, food dish.
respects. An increase in volume to 2L and the adoption of the erlenmeyer shape allowed the fecal sediment to disperse in a thin layer over the bottoms of the flasks, greatly facilitating the escape of hatching miracidia. The lower extension of the vertical element of the sidearm was designed to trap miracidia which were losing their motility or their tactic responses after reaching the sidearm, while the stopcock facilitated the removal of large numbers of miracidia.

Certain departures from the traditional mode of operation of the apparatus were also developed. These were designed to prevent antagonistic action of the negative geotactic and positive phototactic responses of the miracidia and, where possible, channel these responses to reinforce each other. The original design relied on the positive phototaxis being stronger than the negative geotaxis so that the miracidia were drawn from their vertical migration into the horizontal element of the sidearm. It was found, however, that a large proportion of miracidia of T. ocellata failed to respond to the light under these conditions. Instead, they accumulated in the neck of the flask above the opening to the sidearm. This situation was remedied by tilting the flask and by introducing an airlock above the insertion of the sidearm. Miracidia were then guided into the gently upward sloping sidearm by the action of the light. Thus the airlock made it possible to dispense with the masking of the body of the flask, allowing both light and gravity to act on the eggs and the miracidia.
Figure 3a. Diagram of the modified McMullen and Beaver flask. b, body; n, neck; st, rubber stopper; hs, horizontal element of sidearm; vs, vertical element of sidearm; a, air lock; wl, water level; sc, stopcock; l, light source.

Figure 3b. Diagram of the original McMullen and Beaver flask. wl, water level; m, masking.
The actual protocol was as follows: after washing the feces into the flask, it was topped up with tap-water at 23°C to a point just below the insertion of the sidearm. Floating debris was agitated, allowed to consolidate in the neck of the flask, and removed with a spoon. A rubber stopper was inserted into the mouth of the flask, and the flask was filled through the sidearm to the final levels as shown in Figure 3a. Debris, which occasionally rose to block the opening to the sidearm, was resuspended by swirling the fluid in the neck of the flask. By inclining the flask slightly the rising debris could then be made to collect on the side opposite to the opening where it did not interfere with miracidial migration. The flask was then returned to its previous position. The sediment was gently agitated from time to time to free any trapped miracidia.

d) Concentration of Miracidia:

The vertical element of the sidearm was examined for miracidia over a 4 hr period. Low numbers were pipetted individually from the sidearm while heavy miracidial suspensions were drained through the stopcock to be counted. Tests with introduced miracidia showed that 100% of the organisms were recovered by this method (Appendix VI).

e) Counting of Miracidia:

In some cases miracidia were counted immediately, in others they were preserved in 3 parts of tincture of merthiolate to 1 part of miracidial suspension. In either case the
suspension was distributed in droplets onto a clear plastic surface where the number in each droplet was counted under the microscope X 6.3. Data were recorded in terms of the number of miracidia per duck per day.

IV Adult Worms:

a) Recovery of Adult Worms:

Adult worms were obtained from adult blue-winged teal and domestic ducklings by perfusion of lung, 8 days after exposure to cercariae (Ellis, 1968). Donors received 4 units/gm body wt of sodium heparin (Connaught Medical Research Laboratories, Toronto, Ontario) followed 1 min later by an overdose of the anaesthetic "L.A. Thesia" (Haver-Lockhart Laboratories, Kansas City, Missouri). The breast and abdomen of the donor were then plucked and wetted with 70% ethanol in preparation for incision. The body cavity was opened along the ventral mid-line just to the right of the sternum, from the base of the neck to the level of the mid-abdominal region. After the incision had been retracted widely to expose the heart and associated bloodvessels, the heart was freed of the pericardium, and the insertion of the pulmonary artery was located by carefully teasing away fat which usually surrounded it. A broad ligature was looped loosely around the ventricles just below the atria after which the heart was fixed in position by inserting a hook into the ventricular apex and applying mild traction. A curved hypodermic needle (23G) attached to a length of polyethylene tubing was inserted into the tip of
the right ventricle and passed anteriad until it slipped into the lumen of the pulmonary artery. Another hypodermic needle (24G) with tubing was inserted into the apex of the left ventricle and carefully guided anteriad into the left atrium. The ligature was then tightened to hold the needles in place and to seal the atrio-ventricular passages.

Perfusion medium (Appendix VII) at 40°C was drawn into the syringe of the perfusion apparatus, a modification of that used by Duvall and DeWitt (1967), and then forced, via the polyethylene tubing, through the pulmonary vein so that flow through the capillary bed of the lung was in the opposite direction to the normal flow of blood. The perfusate was drained through the pulmonary artery into a 300 ml separatory funnel where it was allowed to settle for 3 min before the sediment was removed with a pasteur pipette to a petri dish. Here the sediment was diluted with one part of buffered balanced saline and examined for adult worms under a dissecting microscope X 20. Worms were removed individually with a finely drawn pasteur pipette, counted, and transferred to a plastic hypodermic syringe containing 1.25 cc of the recipient's plasma at a temperature of 40°C. The syringe was fitted with a 23G hypodermic needle, cleared of air, and inverted to allow the worms to settle into close proximity of the port.

b) Injection of Adult Worms:

The worms were injected into the vena magna of the right tarso-metatarsus. After injecting the contents of the
syringe, blood was withdrawn and injected several times to ensure that all worms had been expelled. Two minutes later, the needle was withdrawn and a small pad of packed cotton was firmly taped over the puncture to prevent haemorrhage. The assembly was examined for residual worms by repeated rinsing into a petri dish.
RESULTS

1. The Course of Initial Infection:

a) Adult Black Ducks Exposed Once to Cercariae:

Five male and 5 female black ducks were exposed once to large numbers of cercariae by the "truss" method at the age of 9 weeks (Ducks 1 to 10, Experimental Group I). Cercarial penetration was accompanied by local inflammation, engorgement of the blood vessels of the web, and formation of subcutaneous haemorrhages, a condition which persisted for several days. The prepatent period was marked by two critical periods of illness, approximately 2 and 5 days after exposure. These were characterized by general listlessness, compulsive swallowing, respiratory distress, and occasional mild pulmonary haemorrhage. The severity of these symptoms varied, so that at times they could be detected only when the ducks were excited or under stress.

Infections in Experimental Group I attained patency between 13 and 14 days after exposure. Subsequent egg passages although highly variable in terms of total numbers (Fig. 4a to 13a), revealed a basic pattern which in composite view (Fig. 14) approximated a normal curve. Egg passage followed normalcy closely during its rapid ascent to a peak between 5 and 6 days after patency and during most of its precipitous descent. However, after 10 days of patency, the curve of egg passage departed from normalcy to decrease
more gradually to reach a minimal level approximately 25 days after the onset of patency.

The normal character of the individual curves was, in many cases, distorted by bursts of egg passage separated by periods of sometimes severe depression creating a series of peaks. In most ducks this acute period of egg passage, and especially the major recurrent peaks, were marked by the passage of blood and mucus which contained large number of eggs and sometimes small tissue fragments. Infections were considered to have terminated after seven consecutive days of zero egg passage. Thus, the last viable eggs were passed 33 to 138 days after exposure.

b) Week-old Ducklings Exposed Once to Cercariae:

Three male and 3 female week-old black ducks were exposed once to large numbers of cercariae by the "truss" method (Ducks 11 to 16 Experimental Group II). The clinical symptoms following exposure were essentially identical to those shown by the ducks of Experimental Group I but were more sever and prolonged, Ducks 11, 12, and 15 dying 47, 55, and 35 days after exposure.

Two distinct patterns of egg passage were exhibited by the birds of Experimental Group II. Ducks 11, 14 and 16 (Figs. 15, 18, 20, and in composite, Fig. 21a) developed patent infections 1 to 2 days earlier than the adults of Experimental Group I, but the subsequent events were almost identical. In both groups the passage of viable eggs reached a peak 5 to 6 days after the onset of patency and declined
Figure 4a. Duck 1, Experimental Group I. (A. rubripes, 9-week-old male). Passage of viable eggs during the course of initial infection following single massive exposure to cercariae of *T. ocellata*.

Figure 4b. Passage of viable eggs by Duck 1 following homologous challenge 53 days after initial exposure.
Figure 5a. Duck 2, Experimental Group I.  
(A. rubripes, 9-week-old male).  
Passage of viable eggs during the course of initial infection following single massive exposure to cercariae of T. ocellata.

Figure 5b. Passage of viable eggs by Duck 2 following homologous challenge 87 days after initial exposure.
Figure 6a. Duck 3, Experimental Group I. (A. rubripes, 9-week-old female). Passage of viable eggs during the course of initial infection following single massive exposure to cercariae of T. ocellata.

Figure 6b. Passage of viable eggs by Duck 3 following homologous challenge 59 days after initial exposure.
Figure 7a. Duck 4, Experimental Group I. (A. rubripes, 9-week-old female). Passage of viable eggs during the course of initial infection following single massive exposure to cercariae of T. ocellata.

Figure 7b. Passage of viable eggs by Duck 4 following homologous challenge 71 days after initial exposure.
Figure 8a.  Duck 5, Experimental Group I.  
(Anas rubripes, 9-week-old male).  
Passage of viable eggs during 
the course of initial infection 
following single massive exposure 
to cercariae of T. ocellata.

Figure 8b.  Passage of viable eggs by Duck 5 
following homologous challenge 
159 days after initial exposure.
Figure 9a. Duck 6, Experimental Group I. (A. rubripes, 9-week-old female). Passage of viable eggs during the course of initial infection following single massive exposure to cercariae of T. ocellata.

Figure 9b. Passage of viable eggs by Duck 6 following homologous challenge 65 days after initial exposure.
Figure 10a. Duck 7, Experimental Group I. (A. rubripes, 9-week-old male). Passage of viable eggs during the course of initial infection following single massive exposure to cercariae of T. ocellata.

Figure 10b. Passage of viable eggs by Duck 7 following homologous challenge 90 days after initial exposure.
Figure 11a. Duck 8, Experimental Group I. (A. rubripes, 9-week-old female). Passage of viable eggs during the course of initial infection following single massive exposure to cercariae of *T. ocellata*.

Figure 11b. Passage of viable eggs by Duck 8 following homologous challenge 50 days after initial exposure.
Figure 12a. Duck 9, Experimental Group I. (A. rubripes, 9-week-old female). Passage of viable eggs during the course of initial infection following single massive exposure to cercariae of T. ocellata.

Figure 12b. Passage of viable eggs by Duck 9 following homologous challenge 85 days after initial exposure.
Figure 13a. Duck 10, Experimental Group I. (A. rubripes, 9-week-old male). Passage of viable eggs during the course of initial infection following single massive exposure to cercariae of *T. ocellata*.

Figure 13b. Passage of viable eggs by Duck 10 following homologous challenge 55 days after initial exposure.
Figure 14. Composite pattern of egg passage during the course of initial infection of Ducks 1 to 10, Experimental Group I. (Figs. 4a to 13b).
precipitously to low levels after 10 days of patency. Egg passage of Ducks 12, 13, 15 (Figs. 16, 17, 19, and in composite Fig. 21b), on the other hand, rose rapidly following the onset of patency between 13 and 14 days after exposure, but persisted at near-peak levels for approximately 8 days before declining gradually.

c) Relationship Between Cercarial Exposure and Penetration

To assess possible relationships between exposure intensity and cercarial penetration, the size of the cercarial dose and the corresponding extent of cercarial penetration was examined in the case of Ducks 1 to 8, 10, and 40. The relationship between exposure intensity and the percentage of cercariae penetrating was shown to be linear (5% level; F=8.6, df1,8.) Furthermore, the relationship is inverse, the greater the exposure intensity, the lower the absolute number and percentage of cercariae that succeeded in penetrating. Thus, beyond the 5000 cercariae level, even massive increases in exposure intensity failed to elicit an increase in the number of penetrating cercariae above its highest count of 1100 (Table I).

d) Relationship Between Cercarial Penetration and Egg Production

The numbers of cercariae entering Ducks 1 to 8, and 10 were compared with corresponding numbers of viable eggs produced per penetrating cercaria, a measure of reproductive
Figure 15. Duck 11, Experimental Group II. (A. rubripes, week-old male). Passage of viable eggs during the course of initial infection following single massive exposure to cercariae of T. ocellata.
Figure 16. Duck 12, Experimental Group II. (A. rubripes, week-old male). Passage of viable eggs during the course of initial infection following single massive exposure to cercariae of T. ocellata.
Figure 17. Duck 13, Experimental Group II. (A. rubripes, week-old male). Passage of viable eggs during the course of initial infection following single massive exposure to cercariae of T. ocellata.
Figure 18.  Duck 14, Experimental Group II.  
(A. rubripes, week-old female). 
Passage of viable eggs during 
the course of initial infection 
following single massive exposure 
to cercariae of T. ocellata.
Figure 19. Duck 15, Experimental Group II. *(A. rubripes, week-old female).* Passage of viable eggs during the course of initial infection following single massive exposure to cercariae of *T. ocellata.*
Figure 20. Duck 16, Experimental Group II. (A. rubripes, week-old female). Passage of viable eggs during the course of initial infection following single massive exposure to cercariae of T. ocellata.
Figure 21a. Composite pattern of egg passage of Ducks 11, 14, and 16, Experimental Group II (Figs. 15, 18, and 20).

Figure 21b. Composite pattern of egg passage of Ducks 12, 13, and 15, Experimental Groups II (Figs. 16, 17, and 19).
success of the resident parasite population. Peak reproductive success was reached at cercarial penetrations of approximately 350 to 450 (Ducks 5, 6, Table I). Below this level of penetration (Duck 8, Table 1) and above (Ducks 1, 2, 3, 4, 7, 10, Table I), reproductive success seemed greatly reduced. The trend toward reduced reproductive success at high penetration intensities was substantiated by a highly significant difference between the means of grouped data from Ducks 5 and 6, and data from Ducks 1, 2, 3, 4, 7, and 10. The difference between the means of data from Ducks 5 and 6, and the reproductive success of Duck 8, however, was not significant.

e) Adult Black Ducks Exposed Daily to Cercariae:

Five male and 4 female adult black ducks (Ducks 17 to 25, Experimental Group III) were exposed to small numbers of cercariae by the "cover-glass" method on 51 consecutive days. Each of 7 ducks was exposed to 25 cercariae, and 1 duck each to 20 and 10 cercariae per day. The course of infection was followed throughout the period of daily exposure and beyond for a total of 70 to 100 days.

Ducks 24 and 25, (Figs. 29a and 30a) receiving 10 and 20 cercariae per day respectively, and Ducks 20 and 21 (Figs. 25a, 26a), each receiving 25 cercariae per day, passed no viable eggs whatsoever. Ducks 18, 19, and 23, (Figs. 23a, 24a, 28a) passed viable eggs only occasionally, and always in extremely low numbers. Only Ducks 17 and 22 (Figs. 22a, 27a)
TABLE I

Relationship Between Exposure Intensity, Cercarial Penetration and the Number of Miracidia Produced Per Cercaria Entering

<table>
<thead>
<tr>
<th>Duck</th>
<th>Exposure</th>
<th>Number Entering</th>
<th>% Entering</th>
<th>Miracidia Per Cercaria Entering</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1699</td>
<td>160</td>
<td>9.4</td>
<td>21.7</td>
</tr>
<tr>
<td>6</td>
<td>5777</td>
<td>364</td>
<td>6.3</td>
<td>259.3</td>
</tr>
<tr>
<td>5</td>
<td>19185</td>
<td>406</td>
<td>2.1</td>
<td>175.7</td>
</tr>
<tr>
<td>10</td>
<td>2187</td>
<td>552</td>
<td>25.2</td>
<td>15.6</td>
</tr>
<tr>
<td>3</td>
<td>9458</td>
<td>565</td>
<td>6.0</td>
<td>5.2</td>
</tr>
<tr>
<td>40</td>
<td>6798</td>
<td>611</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4107</td>
<td>822</td>
<td>20.0</td>
<td>3.7</td>
</tr>
<tr>
<td>7</td>
<td>5078</td>
<td>825</td>
<td>16.3</td>
<td>11.6</td>
</tr>
<tr>
<td>1</td>
<td>2585</td>
<td>1063</td>
<td>41.1</td>
<td>1.4</td>
</tr>
<tr>
<td>2</td>
<td>5787</td>
<td>1100</td>
<td>19.1</td>
<td>.8</td>
</tr>
</tbody>
</table>
also receiving 25 cercariae per day, exhibited a pattern of egg passage approximating that of the ducks of Experimental Group I. Patency, however, was slightly delayed.

f) Adult Ducks Parasitized by Implanted Adults.

Ten ducks were injected intravenously with unsexed, live, 8-day-old adult worms (Ducks 26 to 35, Experimental Group IV). Eight of these birds, 7 black ducks (Ducks 26, 28 to 33) and one Rouen (Duck 27), were injected with two doses of 100 adult worms each, at intervals of 42 to 57 days. Ducks 34 and 35, Rouens, each received one dose of 10 worms. Three black duck controls (Ducks 36, 37, 38) were injected with corresponding doses of worm-free transfer medium, consisting principally of donor plasma, some red blood cells, and balanced buffered saline solution.

The pattern of egg passage from ducks receiving adult worms was quite variable. In the case of Duck 30 (Fig. 35a), egg passage commenced 5 days after the introduction of the worms and continued for 7 days, following a clearly defined curve. Egg passage in Ducks 27, 28, and 34, (Figs. 32a, 33a, 39a), although delayed by 2 to 3 days, followed a pattern quite similar to that of Duck 30. Ducks 26, 29, 31, 32, 33, and 35, (Figs. 31a, 34a, 36a, 37a, 38a, 40a), on the other hand, exhibited quite a different pattern. After a delay in patency of 0 to 18 days, egg passage followed a highly erratic course, terminating after 20 to 84 days of patency.

Of the 8 birds receiving a second injection of 100
Figure 22a. Duck 17, Experimental Group III. (A. rubripes, adult female). Passage of viable eggs during the course of initial infection induced by a series of 51 exposures to 25 cercariae of T. ocellata.

Figure 22b. Passage of viable eggs by Duck 17 following homologous challenge 99 days after the first exposure.
Figure 23a. Duck 18, Experimental Group III. 
(A. rubripes, adult female). 
Passage of viable eggs during the course of initial infection induced by a series of 51 exposures to 25 cercariae of T. ocellata.

Figure 23b. Passage of viable eggs by Duck 18 following homologous challenge 99 days after the first exposure.
Figure 24a. Duck 19, Experimental Group III. (A. rubripes, adult male). Passage of viable eggs during the course of initial infection induced by a series of 51 exposures to 25 cercariae of T. ocellata.

Figure 24b. Passage of viable eggs by Duck 19 following homologous challenge 99 days after the first exposure.
Figure 25a. Duck 20, Experimental Group III. (A. rubripes, adult male). Passage of viable eggs during the course of initial infection induced by a series of 51 exposures to 25 cercariae of T. ocellata.

Figure 25b. Passage of viable eggs by Duck 20 following homologous challenge 70 days after the first exposure.
Figure 26a. Duck 21, Experimental Group III.
(A. rubripes, adult male).
Passage of viable eggs during the course of initial infection induced by a series of 51 exposures to 25 cercariae of T. ocellata.

Figure 26b. Passage of viable eggs by Duck 21 following homologous challenge 100 days after the first exposure.
Figure 27a. Duck 22, Experimental Group III. 
(A. rubripes, adult male).
 Passage of viable eggs during the course of initial infection induced by a series of 51 exposures to 25 cercariae of T. ocellata.

Figure 27b. Passage of viable eggs by Duck 22 following homologous challenge 100 days after the first exposure.
Figure 28a. Duck 23, Experimental Group III. (A. rubripes, adult female). Passage of viable eggs during the course of initial infection induced by a series of 51 exposures to 25 cercariae of T. ocellata.

Figure 28b. Passage of viable eggs by Duck 23 following homologous challenge 100 days after the first exposure.
Figure 29a. Duck 24, Experimental Group III. (A. rubripes, adult female). Passage of viable eggs during the course of initial infection induced by a series of 51 exposures to 10 cercariae of T. ocellata.

Figure 29b. Passage of viable eggs by Duck 24 following homologous challenge 100 days after the first exposure.
Figure 30a. Duck 25, Experimental Group III. (A. rubripes, adult male). Passage of viable eggs during the course of initial infection induced by a series of 51 exposures to 20 cercariae of T. ocellata.

Figure 30b. Passage of viable eggs by Duck 25 following homologous challenge 98 days after the first exposure.
Figure 31a. Duck 26, Experimental Group IV. (A. rubripes, adult). Passage of viable eggs during the course of initial infection induced by intravenous injection of 8-day-old adult T. ocellata. ai, second adult injection; cc, cercarial challenge.

Figure 31b. Passage of viable eggs by Duck 26 following homologous cercarial challenge.
Figure 32a. Duck 27, Experimental Group IV. (Rouen, adult). Passage of viable eggs during the course of initial infection induced by intravenous injection of 8-day-old adult T. ocellata. ai, second adult injection; cc, cercarial challenge.

Figure 32b. Passage of viable eggs by Duck 27 following homologous cercarial challenge.
Figure 33a. Duck 28 Experimental Group IV. (A. rubripes, adult). Passage of viable eggs during the course of initial infection induced by intravenous injection of 8-day-old adult T. ocellata. ai, second adult injection; cc, cercarial challenge.

Figure 33b. Passage of viable eggs by Duck 28 following homologous cercarial challenge.
Figure 34a. Duck 29, Experimental Group IV. (A. rubripes, adult).
Passage of viable eggs during the course of initial infection induced by intravenous injection of 8-day-old adult T. ocellata.
ai, second adult injection; cc, cercarial challenge.

Figure 34b. Passage of viable eggs by Duck 29 following homologous cercarial challenge.
Figure 35a. Duck 30, Experimental Group IV. (A. rubripes, adult).
Passage of viable eggs during the course of initial infection induced by intravenous injection of 8-day-old adult T. ocellata.
ai, second adult injection; cc, cercarial challenge.

Figure 35b. Passage of viable eggs by Duck 30 following homologous cercarial challenge.
Figure 36a. Duck 31, Experimental Group IV.
(A. rubripes, adult).
Passage of viable eggs during
the course of initial infection
induced by intravenous injection
of 8-day-old adult T. ocellata.
ai, second adult injection;
cc, cercarial challenge.

Figure 36b. Passage of viable eggs by Duck 31
following homologous cercarial
challenge.
Figure 37a. Duck 32, Experimental Group IV.
(A. rubripes, adult).
Passage of viable eggs during the course of initial infection induced by intravenous injection of 8-day-old adult T. ocellata.
ai, second adult injection; cc, cercarial challenge.

Figure 37b. Passage of viable eggs by Duck 32 following homologous cercarial challenge.
Figure 38a. Duck 33, Experimental Group IV. (A. rubripes, adult).
Passage of viable eggs during the course of initial infection induced by intravenous injection of 8-day-old adult T. ocellata. ai, second adult injection; cc, cercarial challenge.

Figure 38b. Passage of viable eggs by Duck 33 following homologous cercarial challenge.
Figure 39. Duck 34, Experimental Group IV. (Rouen, adult).
Passage of viable eggs during the course of initial infection induced by intravenous injection of 8-day-old T. ocellata and following homologous cercarial challenge.

Figure 40. Duck 35, Experimental Group IV. (Rouen, adult)
Passage of viable eggs during the course of initial infection induced by intravenous injection of 8-day-old T. ocellata and following homologous cercarial challenge.
adult worms only Duck 26 (Fig. 31a) exhibited egg passage which could be attributed to that source. Eggs were suddenly passed 20 days after the second injection and continued to be passed for approximately 19 days. However, relatively few eggs were passed.

II Homologous Challenge Infections:

a) Fifty to 159 Days After Single Exposure:

Ducks 1 to 10 (Experimental Group I) were challenged by cercariae of the homologous parasite 50 to 159 days after the initial exposure. In 9 of 10 ducks (Ducks 2 to 10, Figs. 5b to 13b), erratic, low-grade passage of viable eggs occurred during the prepatent period, a phenomenon hereafter referred to as "flushing". Only 3 times was this period of egg passage extended beyond the 13th day after exposure, the expected time of patency, persisting in these cases (Ducks 6, 9, 10, Figs. 9b, 12b, 13b) until days 19, 17, and 18 respectively. Mild respiratory distress, uni- or bilateral opacity and profuse lacrimation of the eyes associated with intense irritation sometimes accompanied this release of viable eggs, usually 2 to 5 days after exposure (Ducks 2, 3, 5, 7).

Eight of the 10 ducks exhibited no egg passage beyond that associated with the prepatent "flushing" phenomenon, over a period of approximately 85 days of examination. Only 2 birds (Ducks 4, 8, Figs. 7b, 11b) passed significant numbers of
viable eggs during the usual period of patency. Egg passage
did not follow the established pattern of initial infection
of this group, however, being highly erratic and low-grade.
b) Eleven to Sixteen Months After Single Exposure:

Ten 9-week-old black ducks (Ducks 39 to 48, Experimental Group V) were exposed once to large numbers of cercariae
by the "truss" method and challenged after 11 to 16 months
with another single, massive dose. Feces of all but one bird
(Duck 39) were examined for viable eggs for several days just
prior to challenge and the total period of examination ex-
tended over a period of 40 to 69 days beyond the date of the
challenge exposures.

Sporadic, low-grade passage of viable eggs was
encountered in 5 of the 9 ducks prior to exposure (Ducks
42, 43, 45, 46, 47, Figs. 44, 45, 47, 48, 49). Egg passage
exhibited by Duck 39 (Fig. 41) the day of challenge and
throughout the prepatent period of the challenge indicated
that it, too, probably passed viable eggs during the pre-
challenge period.

The phenomenon of "flushing", so characteristic of the
Experimental Group I challenges could not be demonstrated
because of the masking effect of the persisting levels of
pre-exposure egg passage. However, in the 5 birds (Ducks 40,
41, 42, 44, 48, Figs. 42, 43, 44, 46, 50) where this masking
did not occur, "flushing" was not observed. Ocular involve-
ment on the other hand was noticed in several birds.

The pattern of egg passage established during the
Figure 41. Duck 39, Experimental Group V. (A. rubripes, adult). Passage of viable eggs following homologous challenge 16 months after initial exposure.

Figure 42. Duck 40, Experimental Group V. (A. rubripes, adult). Passage of viable eggs following homologous challenge 11 months after initial exposure.
Figure 43.  Duck 41, Experimental Group V.  
(A. rubripes, adult).  
Passage of viable eggs following homologous challenge 16 months after initial exposure.

Figure 44.  Duck 42, Experimental Group V.  
(A. rubripes, adult).  
Passage of viable eggs following homologous challenge 16 months after initial exposure.
Figure 45. Duck 43, Experimental Group V. (A. rubripes, adult). Passage of viable eggs following homologous challenge 14 months after initial exposure.

Figure 46. Duck 44, Experimental Group V. (A. rubripes, adult). Passage of viable eggs following homologous challenge 14 months after initial exposure.
Figure 47. Duck 45, Experimental Group V. (A. rubripes, adult). Passage of viable eggs following homologous challenge 11 months after initial exposure.

Figure 48. Duck 46, Experimental Group V. (A. rubripes, adult). Passage of viable eggs following homologous challenge 11 months after initial exposure.
Figure 49. Duck 47, Experimental Group V. (A. rubripes, adult).
Passage of viable eggs following homologous challenge 11 months after initial exposure.

Figure 50. Duck 48, Experimental Group V. (A. rubripes, adult).
Passage of viable eggs following homologous challenge 16 months after initial exposure.
pre-exposure and prepotent period of the challenge infection persisted, for the most part, throughout the periods of observation. Two possible exceptions occurred in Ducks 42 and 46 (Figs. 44, 48) which exhibited brief flurries of egg passage late in the challenge infection. However, even this departed radically from the pattern of a second infection as seen in Ducks 4 and 8 (Experimental Group I), persisting for only 5 and 4 days respectively.

c) After Multiple Exposure Initial Infection:

Ducks 17 to 25 (Experimental Group III) were challenged 70 to 100 days after the first of a series of 51 consecutive exposures to small numbers of cercariae. No viable eggs whatsoever were passed by 8 of the 9 birds. Only Duck 25 (Fig. 30b) passed 3 viable eggs each on days 18 and 21 following challenge exposure. The "flushing" phenomenon was observed in only 1 bird (Duck 17, Fig. 22b), although ocular involvement and respiratory distress did occur in several birds 2 to 5 days after challenge.

d) After Implanted Adult Initial Infection:

Ducks 26 to 35 (Experimental Group IV) whose initial infection had been induced by the injection of live, mature adult worms, were challenged percutaneously by the "stopper and bottle" method 51 to 72 days after the first injection.

Ducks 27, 28, and 31 (Figs. 32b, 33b, 36b) exhibited no egg passage whatsoever subsequent to the challenge exposure, while Ducks 30 and 32 (Figs. 35b, 37b) passed only 3 and 1 eggs
respectively. Egg passage following the cercarial challenge of Duck 29 (Fig. 34b) was attributable to the continuation of the initial infection. Only 4 birds, Ducks 26, 33, 34 and 35 (Figs. 31b, 38b, 39b, 40b) developed definite second infections. However, in all cases, egg passage by previously infected birds was dwarfed by that of the injection controls (Ducks 36, 37, 38) which rose to heights of approximately 5000, 2000, and 3000 viable eggs within 17 days of the challenge exposure. Although no "flushing" was noted, ocular involvement and respiratory distress were quite severe in 3 birds (Ducks 26, 27, 33).

e) Shortly After the Injection of Adult Worms:

Three adult black ducks (Ducks 42, 51, 51, Experimental Group VI) were challenged with a single massive dose of cercariae only 5 days after the intravenous injection of 26, 28, and 100 mature adult worms respectively. All birds developed infections upon challenge which were undistinguishable from initial infections from massive cercarial exposure.
DISCUSSION

The efficiency of adaptations which allow parasites to persist within individual members of the host population must be measured, on final analysis, in terms of their contributions to the reproductive success of the parasites. Similarly, host reactions, whether directed against cercariae, schistosomules, adults, or eggs, are of biological importance only insofar as they interfere with this process. Consequently one would expect biologically significant fluctuations in the equilibrium of the host-parasite relationship to be reflected in the passage of viable eggs, the latter becoming, then, a useful gauge. Strange to relate, few authors have considered the passage of viable eggs by infected hosts, despite the foregoing and despite the fact that it has long been recognized that eggs of the human schistosomes are the primary pathogenic agents in Bilharziasis.

I Initial Infection:

Data presented by authors who did follow egg passage show that initial infections by several species follow a pattern similar to that of T. ocellata in the black duck (see Figs. 4a to 12a), apparently ending in self cure. Such a series of events has been reported from the rhesus monkey (Macaca mulatta) by Cram and Files (1947), Standen (1949), Meleney and Moore (1954), Naimark et al. (1960), Smithers and Terry (1965a), Ritchie et al. (1966), and McMullen et al.
(1967) with *S. mansoni*, by Vogel (1949) with *S. japonicum*, and by Meloney and Moore (1954) with *S. haematobium*. Similar work by Meisenhelder and Thompson (1963) with the rhesus and African green monkey (*Cercopithecus aethiops*), and work by Moore and Sandground (1956), Kuntz (1961), and Kloetzl (1967) with smaller mammals has provided further insight into this phenomenon.

Although precise comparison of the present work with the literature was often difficult in view of the great variation in sampling procedures and egg-counting techniques employed by the various authors, several points of common agreement have emerged.

a) Initial Infection Following Massed Cercarial Exposure:

With few exceptions the acute phase of the infection accounts for a large proportion of the total number of eggs passed. Although egg counts during this period are often quite irregular, the pattern of egg passage has a tendency to follow the course of a normal curve, the period of rapid decline which concludes the acute phase being succeeded in turn by a longer phase of much more gradual decline.

During the acute phase, eggs of *T. ocellata* are passed in bursts (Fig. 7), separated at times by 1 or 2 days. This pattern can probably be explained by the fact that the adult schistosomes do not lay their eggs directly into the intestinal lumen, but into the tissues of the intestinal wall where most of them are at least temporarily trapped. This
explanation was substantiated by the fact that no eggs, viable or otherwise, could be detected in the gut contents of the black duck killed on day 13, whereas immense numbers of eggs were seen in the intestinal wall itself. Progressive irritation and sensitization of the mucosa by the eggs and their products (Ellis 1968, unpublished thesis), may give rise to the formation of egg abscesses which sporadically rupture, releasing large numbers of eggs into the lumen. The idea of eggs being discharged as a result of mucosal erosion is further supported by the fact that flecks of mucus, blood, and some tissue debris which are frequently passed with the droppings during peak periods characteristically contain considerably larger numbers of eggs than the accompanying sample of feces. A similar mechanism was proposed by Liu and Bang (1950) for *S. japonicum* in *Macacus philippinensis* and by Moore and Sandground (1956) for *S. japonicum* and *S. mansoni* in the hamster. Moore and Sandground (1956) stated that this variation in the pattern of egg passage was accentuated in the case of animals infected with *S. japonicum* since many eggs were usually expelled from the uterus of the female at one time so that eggs are released into the intestinal lumen in clusters. A mechanism of this kind is highly unlikely in the case of *T. ocellata* since there is never more than one intra-uterine egg in this species. (Ellis, 1968, unpublished thesis).

The precipitous reduction in numbers of eggs leaving the host each day once a peak in egg counts is reached, is
intriguing. This phenomenon has been observed in simians
(Meisenhelder and Thompson, 1963; Standen, 1949; Jachowski
et al., 1963; and Naimark et al., (1960) as well as in man
(Kloetzel, 1962). Kloetzel (1967) working with S. mansonii
in hamsters showed that this drop was not attributable to a
decline in oviposition, nor was it the reflection of a sudden
decrease in the adult population within the intestine and
mesenteries. Instead, it was due to a retention of eggs in
host tissue. The mechanisms by which eggs of the human
schistosomes cross the barrier between the venules and the
lumen of the intestine remains unknown. However, they must
be rather inefficient since, according to the estimates of
Weinmann and Hunter (1961) and Moore and Sandground (1956),
between 78 and 90.4% of all eggs produced by S. mansonii in
the hamster never reach the intestinal lumen. Furthermore,
Kuntz (1961) reported that in all of his experimental animals,
as the infection continued beyond the period of peak egg
production, there was a definite increase in the percentage
of non-viable eggs being passed. The eggs of T. ocellata,
on the other hand, seem to be able to overcome this tissue
barrier quite effectively. Although the percentage of viable
eggs passed with the droppings had decreased from approximately
99% of 104 eggs examined on day 15 to approximately 40% of
40 eggs on day 23 indicating that some eggs had been retained
in host tissues until rendered non-viable by age or by some
host reaction as suggested by Kuntz (1961), probably very few
eggs were affected since autopsy of 1 black duck and 2 Rouens, 21 days after exposure, revealed only low numbers of eggs in the small intestine. Autopsy 35 days after exposure yielded only occasional, heavily-encapsulated eggs, usually from the muscularis of the intestine. Histologic examination (Ellis, 1968, unpublished thesis) confirmed and added to these observations. Thus, on day 21 the entire small intestine of a black duck contained only 75 eggs and 33 adults, and birds examined at later dates yielded no adults at all.

This vast difference in the efficiency of egg elimination between *S. mansoni* and *T. ocellata* is probably a reflection of differences in their respective sites of ovisposition. Size restricts *S. mansoni* adults to relatively large peripheral veins of the small intestine and the associated vessels of the mesenteries so that after deposition, eggs of *S. mansoni* must still overcome a relatively thick barrier of host tissue. Furthermore, many eggs are swept to the liver by the portal blood flow. The minute adults of *T. ocellata*, on the other hand, may actually leave the blood vessels and invade the tissue of the mucosa even as far as the tips of villi, a characteristic it shares with *T. szidati* (Neuhaus, 1952). Eggs deposited in such sites are firmly lodged in tissue and are, at times, separated from intestinal crypts or the lumen proper by only the thickness of a few cells (Ellis, 1969, personal communications). It is conceivable that under such conditions tissue reactions might actually
accelerate the process of egg release instead of retarding it as in the case of the human schistosomes. In this connection, it is of interest to note that for the most part, only eggs trapped in the more peripheral layers of the small intestine apparently, remain in situ long enough to become encapsulated by concentric fibrosis as described by Lichtenberg (1964). However, although the position of the worms close to the intestinal lumen may have overcome the tissue barrier to egg passage, it may also have exposed the adults to a relatively unstable environment. It is conceivable that as mucosal erosion and hemorrhage progress, a proportion of these worms might either be expelled with mucosal debris, or be forced to retreat to more peripheral layers of the intestine or, perhaps, even the liver itself, resulting in the observed rapid decline in the number of eggs passed with the feces. Although it is tempting to suggest that these events are assisted by an immunological response, there is at present no conclusive evidence that adult schistosomes are affected in vivo by an immune mechanism (Smithers, 1967). It is, however, of interest that the duck host has responded immunologically as manifested by the occurrence of the Hoepli phenomenon, an in vivo antigen-antibody reaction (Lichtenberg, et al., 1966). Perhaps the combination of antibody and the precarious lodgement of the parasite in the eroding mucosa may be sufficient to tip the balance in favour of the host. Similar action of a host response against a
physiologically ill-adapted parasite was proposed by Smithers and Terry (1965b) to explain the elimination of *S. mansoni* adults from the liver of the albino rat.

In any event, not all adults are eliminated during this phase of the infection, a resistant minority being eliminated only very slowly. Whether these adults owe their resistance to innate properties or to the chance occupation of sites which are favourable to their survival, remains unanswered.

The commonly observed phase in which the number of eggs eliminated with the droppings declines gradually may be attributed in some cases to the slow, irregular release of tissue-trapped eggs. According to the estimates of Kagan and Lee (1952), and Maldonado (1959), mammalian schistosome eggs remain viable in host tissue for 3 weeks or longer. However, since eggs of *T. ocellata* are first deposited in the intestinal wall on days 9 and 10 and approximately 60% of eggs passed on day 23 are inviable, it seems that the process in this host-parasite system is much more rapid. In the case of most of the ducks of Experimental Group I then, egg passage during this last phase of the infection must have been, for the most part, attributable to active egg-laying by a residual population of adult worms. The persistence of egg laying attributable to residual adults varies with the particular host-parasite system. According to Liu and Bang (1950), *M. philippinensis* still passed viable eggs of *S. japonicum*, 4 1/2 years after the monkeys had been infected.
Similarly, in the case of *S. mansoni*, *C. aethiops* (Vogel, 1958), and baboons (*Papio hamadryas*) (Newsome, 1956), pass eggs for several years, while the rhesus monkey gradually eliminates the residual population over a period of 1 to 2 years. Also, as demonstrated by Meisenhelder and Thompson (1963), the level of egg passage varies with the host species. Autopsy of black ducks more than 6 months after exposure revealed small numbers of residual adults of *T. ocellata* in liver parenchyma, and even 11 months after exposure some ducks of Experimental Group III still passed a few viable eggs. Early reports on bird schistosomes suggested a life span of something less than a year. In view of the difficulties in finding adults, however, and a recent report by Litvishko (1967), who estimated the life span of *Bilharziella polonica* to be 6 1/2 years, it would seem likely that bird schistosomes also live for years, possibly, as in the case of deer mice (*Peromyscus maniculatus*) infected with *S. douthitti* (Kagan et al., 1954), as long as the host itself. Obviously then, the cessation of egg passage by birds of Experimental Group I cannot be interpreted as the termination of the infection proper.

In black ducks exposed to single massive doses of cercariae the clinical manifestations of infection were most noticeable during prepatency, the 2 critical periods, approximately 2 and 5 days after exposure coinciding with the passage of schistosomules through the lungs. However, as the infections
progressed the 9-week-old ducks recovered completely and, except for the flecks of blood and mucus in the droppings and a temporary reduction in feeding during periods of peak egg passage, no further symptoms of illness were noticed. In contrast, ducklings (Experimental Group II) exhibited much more severe symptoms during the prepatent period, the course of infection being marked by progressive weakening and emaciation, culminating in the deaths of 3 of the 6 ducklings (Ducks 11, 12, 15). Ducks 11, 14, and 16 (Figs. 15, 18, 20) exhibited patterns of egg passage essentially indistinguishable from those of the adults of Experimental Group I. The course of infection in Ducks 12, 13, and 15, on the other hand, was characterized by a pronounced extension of the acute phase of egg passage. Furthermore, the levels of egg passage during the phase of gradual decline was approximately twice as high and the duration of the phase was much longer than in infections of 9-week-old ducks. This suggests that at least some week-old ducklings are considerably less competent in dealing with massive infection of T. ocellata than are older birds. Although this enhanced competence of older birds might well be a reflection of the state of organization of their connective tissues (Lewert and Lee, 1954; Lewert and Mandlowitz, 1960), the possibility of an immunological basis presents itself. Whether the greater susceptibility of the ducklings is the result of age-dependent immunological incompetence, or whether the comparatively massive burden of the infection impeded the development of immunity through its debilitating effects (Zaiman et al.,
1961; Lichtenberg et al., 1963), or paralysis of the response cannot be answered with any degree of confidence. However, age resistance has been demonstrated by Ritchie et al. (1963b) in the hamster, while lability of the immune response was reported by Vogel (1958).

The extension of the acute phase of infection and the relatively high level of egg passage during the phase of gradual decline in these ducklings is of particular interest in view of reports by Ritchie et al. (1966) and McMullen et al. (1967). These authors reported that after heavy S. mansoni infections in M. mulatta the numbers of eggs dropped precipitously after 100 days, and eggs were seen infrequently after 400 days. In the case of light infections, however, egg passage persisted at low levels for longer periods. According to the postulates of Dineen (1963), this would indicate that light infections failed to provide the minimal level of antigenic information necessary for the stimulation of a significant immune response. Massive infection, on the other hand, would confront the host with a "gross antigenic insult", well beyond the level of "subliminal tolerance", resulting in rapid elimination. In contrast, even the lightest infections in Experimental Group I followed a course of egg passage similar to that of heavily infected monkeys, while the ducklings of Experimental Group II, although often passing even larger numbers of viable eggs, exhibited a pattern of egg passage which approximated that of lightly infected simians. This may indicate that the level of
subliminal tolerance is considerably higher in 3 of the 6 ducklings of Experimental Group II than in older ducks (Experimental Group I), probably as a result of a state of impaired immunologic competence. As a result, infections of the two groups, although comparable in the magnitude of egg passage, may exhibit strikingly different patterns of egg passage.

b) Relationship Between Exposure Intensity, Cercarial Penetration and the Number of Miracidia Produced Per Penetrating Cercaria.

Further insight into the host-parasite relationship was derived from an examination of the reproductive success of the parasite in relation to the intensity of cercarial invasion. Although the well-documented variability of cercarial infectivity (Evans and Stirewalt, 1951; Sadun and Bruce, 1964, Smithers and Terry, 1965a), idiosyncrasies of the host response (Naimark et al., 1960; Smithers, 1967), and the small sample size, allow only guarded interpretation of the data, the highly significant differences between the mean number of miracidia per penetrating cercaria produced at low intensities of cercarial penetration (364 and 406 cercariae) and at high intensities (552 to 1100 cercariae), indicates that when large numbers of cercariae penetrate a host, their subsequent reproductive success is drastically reduced. Again, this may indicate that as the flow of antigenic information from the parasite to the host increases,
the host reaction becomes more pronounced. Comparisons of these data with the literature is difficult because of differences in the criteria used. Only Kuntz (1961) considered egg passage at all, stating that as a rule, the hosts with the greater number of parasites recovered at autopsy had passed larger numbers of eggs, but that this relationship did not always hold, since on several occasions hosts with only few schistosomes passed numerous eggs.

Stirewalt et al. (1951) reported that groups of albino rats exposed to 100, 200, and 500 cercariae of *S. mansoni* exhibited worm recoveries of 9.5, 6.0 and 3.2% respectively, 4 weeks after infection. Ritchie et al. (1963b), on the other hand, working with the same system, reported that in the 4th week of infection relatively uniform rates of worm recovery were obtained with the number of cercariae at exposure ranging from 116 to 1000, while at 8 weeks a mean recovery rate of 16% was obtained from animals exposed to 250 cercariae, compared to 7.6% and 6.3% for 500 and 1000 cercariae respectively. Their data indicate that at identical exposure intensities the rats used by Ritchie et al. (1963b) seemed to be more susceptible to infection than those used by Stirewalt et al. (1951). This may explain the delay in the onset of worm elimination presented in the report of Ritchie et al. In the albinomouse the percentage recovery of worms at autopsy was similar regardless of exposure intensity within the range of 20 to 140 cercariae per animal
(Sadun et al., 1958). Peña de Grimaldo and Kershaw (1961), however, found that at higher intensities, (100 to 5000 cercariae per animal) factors inhibitory to the schistosomes limited parasitization. Furthermore, as the intensity of infection increased from 100 to 15000 worms the size of the schistosomes decreased, females being more severely affected. A concomitant change in the sex ratio favouring male worms supported this observation. Although no data are available, it is conceivable that such a decrease in the number of females and a simultaneous interference with their growth would be, at least in part, reflected in a decrease in the reproductive efficiency of the parasite.

However, an earlier protective response had already been brought to bear on the infective organisms during the phase of skin penetration effectively limiting the size of the invading population. The number of penetrating cercariae never rose above 1100, the level reached after exposure to approximately 5800, although some birds were exposed to as many as 19000 cercariae. This mechanism is probably akin to the resistance imparted to mouse skin immediately following cercarial penetration. Stirewalt (1953) showed that challenging cercariae applied to the skin of a white mouse within 24 hours of the initial exposure penetrated relatively poorly, and that comparatively few adult worms were subsequently recovered at autopsy. This resistance was ascribed to a pronounced and often extensive thickening of the corneal
layers above the horizontal, subcorneal migration path (Stirewalt, 1958). In mice this hyperkeratosis was observable almost as soon as cercarial penetration began and secretions made contact with the skin, thus interfering with later penetration of other cercariae. This partial barrier to penetration was effective for less than 2 days (Stirewalt, 1963). Cercariae of *T. ocellata* were observed to accumulate gradually on the foot of the duck during the 90 minute exposure period, providing ample time and opportunity for the development and action of hyperkeratosis. Indeed, evidence of hyperkeratosis was observed in section 3 hr after exposure (Ellis, 1969, personal communications). The phenomenon of "collaboration" by several penetrating cercariae at one site of entry through the corneal layer as reported by Griffiths (1953) and Stirewalt and Hackey (1956) in *S. mansoni* was not observed. However, facilitation of penetration by the collaboration of several cercariae may well be of importance during the migration through the Malpighian layer. According to Ellis (1968, unpublished thesis), there is no evidence, however, that the skin of the duck presents a serious barrier to the penetration of cercariae during massive exposures as was observed by Rai and Clegg (1968) in their study of *Austrobilharzia terrigalensis* in its natural host, the seagull.

c) Initial Infection Following Multiple Exposure:

Collaboration may account in part for the relatively
layers above the horizontal, subcorneal migration path (Stirewalt, 1958). In mice this hyperkeratosis was observable almost as soon as cercarial penetration began and secretions made contact with the skin, thus interfering with later penetration of other cercariae. This partial barrier to penetration was effective for less than 2 days (Stirewalt, 1963). Cercariae of *T. ocellata* were observed to accumulate gradually on the foot of the duck during the 90 minute exposure period, providing ample time and opportunity for the development and action of hyperkeratosis. Indeed, evidence of hyperkeratosis was observed in section 3 hr after exposure (Ellis, 1969, personal communications). The phenomenon of "collaboration" by several penetrating cercariae at one site of entry through the corneal layer as reported by Griffiths (1953) and Stirewalt and Hackey (1956) in *S. mansoni* was not observed. However, facilitation of penetration by the collaboration of several cercariae may well be of importance during the migration through the Malpighian layer. According to Ellis (1968, unpublished thesis), there is no evidence, however, that the skin of the duck presents a serious barrier to the penetration of cercariae during massive exposures as was observed by Rai and Clegg (1968) in their study of *Austrobilharzia terrigalensis* in its natural host, the seagull.

c) Initial Infection Following Multiple Exposure:

Collaboration may account in part for the relatively
great success of massed cercarial invasion, as reflected in the passage of viable eggs, compared with the poor success of similar numbers of cercariae applied in small daily increments over a period of 51 days. Since as few as 10 adult worms implanted into the venous circulation of normal ducks will pass an abundance of viable eggs over a prolonged period, egg passage by birds of Experimental Group III indicates severe reductions in the reproductive success of the invading populations. Initial invading parasites may be faced with the problems of mate-finding, while later waves may be confronted by an antibody response which could make their successful establishment progressively more difficult. Since given areas of the ducks’ webs were exposed at intervals of 4 days, hyperkeratosis probably did not contribute significantly to this process of resistance. Where definite patterns of egg passage could be distinguished (Ducks 17, 22), no significant differences between these and the patterns exhibited by the birds of Experimental Group I could be detected. The patterns again approximated a normal curve, rising rapidly to a peak and then descending equally precipitously to negligible levels despite continued exposure to doses of 25 cercariae per day. Similar patterns were recorded by Naimark et al. (1960), Ritchie et al. (1966), and McMullen et al. (1967) from rhesus monkeys exposed repeatedly to small doses of S. mansonii, and by Vogel and Minning (1953) from monkeys exposed to S. japonicum. However, the success of these exposures, as judged by the passage of eggs,
was greater than that of single, massed exposures to similar numbers of cercariae (Naimark et al., 1960). Caution must again be exercised in making comparisons for Naimark et al. (1960) did not consider the viability of the eggs passed. Such considerations might well make these data compatible.

d) Initial Infection Following Implantation of Adult Worms:

Ducks which had received living adult worms exhibited egg passage which, although always low-grade, either approached the pattern established by birds of Experimental Group I, or followed a highly erratic course, often over prolonged periods. Similar patterns of egg passage were reported by Smithers and Terry (1967) following the injection of living adult *S. Mansoni* into rhesus monkeys. Since neither the percentage maturation of the invading cercariae of Experimental Group I nor the percentage survival of implanted adults is known, no fruitful comparisons can be made. However, the fact that periods of egg passage following implantation of adults were often prolonged, indicates that the resident adult population is antigenically below the "subliminal tolerance" level proposed by Dineen (1963).

It is of interest to note that in all birds, except perhaps Duck 26, a second dose of 100 living adult worms produced no increase in the level of egg passage. Thus, effective resistance to adult worms had already developed 50 to 70 days after the first injection. This may indicate that the decline in the level of egg passage following the peak is
not merely a reflection of the natural termination of the
infection, but a manifestation of conditions unsuitable to the
persistence of the parasite within the host.

II Homologous Challenge:

As mentioned in communications by various authors,
relative acquired resistance to schistosomes can indeed be
induced in the commonly used laboratory animals (Stirewalt,
1963). The term "relative" must, however, be emphasized,
since on the whole, the numerous attempts to demonstrate
resistance to schistosomes have been met with only partial
success. Rodents such as mice and hamsters are relatively
inefficient at resisting reinfection. Thus, Kagan (1952)
demonstrated that mice infected with *Schistosomatium
douthitti* develop a partial resistance to challenge infection
after 30 to 60 days. This resistance was manifested in a
statistically significant reduction in the number of worms
recovered at autopsy, and a stunting of worms in the challeng-
ing infection as compared to control mice. Purnell (1966)
was able to show partial resistance conferred on hamsters
by low-level infections with cercariae of *S. haematobium.*
The resistance was reflected in a significant reduction both
in worm burden and the length of male worms in the challenging
infections. Similar degrees of resistance were reported in
a variety of host-parasite associations by Olivier and
Schneidermann (1953), Lin et al. (1954), Lurie and De Meillon
(1957), and Sadun and Lin (1959). Among primates *P.
hamadryas* (Newsome, 1956) and *C. aethiops* (Ritchie et al.,
1964) proved to be unsuitable for the study of acquired resistance. Only the rhesus monkey has thus far shown spontaneous cessation of egg passage and substantial resistance to challenge following active infections with *S. mansonii* (Cram and Files, 1947; Standen, 1949; Meleney and Moore, 1954; Newsome, 1956; Naimark et al., 1960; Smithers and Terry, 1965a; Ritchie et al., 1966; McMullen et al., 1967), with *S. haematobium* (Standen, 1949; Meleney and Moore, 1954), and with *S. japonicum* (Vogel and Minning, 1953; Sadun et al., 1961).

a) Challenge Following Initial Infection Induced by Massed Cercarial Exposure.

In a similar fashion, but associated with the normal host (McMullen and Beaver, 1945), the black duck has been shown in this study to develop almost complete protection against homologous challenge following initial infection induced by massed cercariae of *T. ocellata*. Eight of 10 birds were completely refractory to reinfection, while the other 2 birds (Ducks 4, 8) exhibited very low-grade infections. The initial infections of both ducks reveal some peculiarities which may, in part, explain their relatively weak resistance to challenge. Very few cercariae penetrated Duck 8, and the passage of small numbers of viable eggs during the ensuing brief infection indicated that few adult worms survived.

These factors, in conjunction with the brief period between initial and challenge exposure, may have resulted
in little antigenic information being contributed toward the development of resistance (Hunter et al., 1962; Ritchie et al., 1963). Relatively large numbers of cercariae, on the other hand, penetrated Duck 4. However, the pattern of egg passage of the initial infection exhibited a slight extension (Fig. 7) which may indicate reduced efficiency of the mechanism of resistance. This, by the same token, may have allowed the development of the weak, second infection.

In those few cases where second infections developed, passage of viable eggs was characteristically delayed, low-grade, erratic, and of relatively long duration, indicating that few cercariae of the challenge exposure matured. This was confirmed by the recovery of relatively low numbers of immature worms from the livers of previously exposed birds 12 days after challenge, a time when worms of initial infections had already reached sexual maturity. Furthermore, no eggs were found in the intestinal wall of 2 previously exposed birds 17 and 27 days after challenge. This agrees with Naimark et al. (1960) who found that in experiments with S. mansoni in the rhesus monkey not more than 10% of the schistosomules of the challenge exposure reached the liver, and these after some delay. Furthermore, these authors noted stunting and a decrease in the number of worms as the time between challenge and necropsy increased, while McMullen et al. (1967) found no evidence that any of the cercariae from the final exposures reached adulthood. According to
Olivier and Schneidermann (1953), and Vogel and Minning (1953) there is a high mortality of schistosomules as they are passing through the lungs of resistant animals. Magalhães (1959) has shown in mice that the retention and destruction of schistosomules of *S. mansoni* occurs in pulmonary lesions characterized by diffuse congestion and diffuse leucocytic infiltration of the capillaries probably caused by an allergic phenomenon. No inflammatory reaction occurred in the lungs of the control mice.

Whether a similar mechanism is at work in resistant ducks cannot be answered at this time. It is of interest to note, however, that a hypersensitivity, probably of the delayed type has developed, as indicated by the often pronounced cloudiness of the eyes which occurred in several ducks approximately 2 to 5 days after the massive challenge exposure. Hypersensitivity is probably also associated with the phenomenon of "flushing" which is thought to be a release of tissue-trapped eggs during the prepotent period following challenge exposure. The relationship of hypersensitivity, as indeed of all serological reactions, to resistance to schistosomiasis is obscure. Reagin-like antibodies associated with the immediate type of hypersensitivity have been implicated in the acquired resistance of rats to *S. mansoni* (Ogilvie, 1964; Ogilvie *et al.*, 1966). However, similar antibodies in rhesus monkeys failed to show protective action (Ogilvie *et al.*, 1966; Hsü and Hsü, 1966; Edwards *et al.*, 1967). It is becoming increasingly clear in immunologi-
cal studies on helminths that there is a functional distinction between antibodies demonstrated by serological techniques on the one hand and protective immunity on the other (Kagan, 1958; Smithers, 1962; Sadun, 1963; Jachowski et al., 1963; Jaimes and Lichtenberg, 1965). It is also interesting to note that monkeys bearing unisexual infections of S. mansoni failed to show the increase in serum gamma globulin which is characteristic of monkeys with a bisexual infection (Smithers and Walker, 1961). The fact that these monkeys nevertheless developed effective resistance indicated that protection is independent of increases in gamma globulin levels.

In any event, whatever the nature of the mechanism which prevented the schistosomules of the challenge from maturing, this mechanism did not at the same time destroy residual adults, nor did it prevent them from continuing to pass viable eggs with the feces of their hosts. Thus, several ducks of Experimental Group V continued to pass small numbers of viable eggs in the face of maximal resistance to reinfection. Similar observations were reported by Smithers and Terry (1965a), Ritchie et al. (1966), and Smithers (1967) with S. mansoni in the rhesus monkey. What role these residual worms play in the maintenance of resistance in the black duck cannot be answered. Their effect on the invading schistosomules may be direct, e.g. pheromone-like in action, or indirect, by providing the host with the necessary antigenic information. In any event, 11 to 16 months after
the initial exposure the level of resistance had not noticeably decreased. This persistence of resistance is in agreement with the data from rhesus monkeys but is in marked contrast with the gradual loss of acquired resistance against schistosomes in rodents in spite of the residual adult population (Lin et al., 1954; Ritchie et al., 1963b). A similar loss of resistance was encountered in Macacus sinicus infected with S. spindale (Fairley et al., 1930).

While it has been demonstrated that in rhesus monkeys the adults of S. mansoni apparently provide the host with the necessary antigenic information (Smithers and Terry, 1967), evidence from S. japonicum presented by Hsü et al. (1962) suggests that the schistosomule is of great immunologic importance. Kagan (1952), on the other hand, has presented circumstantial evidence that the stimulus is provided by the egg in infections of mice with S. douthitti, while the observations by Li (1958) on the inhibitory action of worms in copula on unpaired females may suggest "pheromone-like" control.

b) Challenge Following Initial Infection Induced by Multiple Exposures:

There is general agreement that multiple exposures to small doses of cercariae of S. mansoni or S. japonicum will induce strong resistance in the rhesus monkey (McMullen et al., 1967; Naimark et al., 1960; Vogel and Minning, 1953). In mice, repeated exposures to S. douthitti seemed to produce a more intense resistance than a single infection (Kagan, 1952).
Lurie and DeMeillon (1957), Thompson (1954), and Olivier and Schneidermann (1953) reported similar results from mice and *S. mansoni*, and Wang *et al.* (1958) from mice and rabbits exposed to *S. japonicum*. Similar enhancement of resistance can be induced by a single intraperitoneal injection of cercariae of *S. mansoni* into mice, since cercariae leave this site over a relatively long period according to Moore and Meleney (1955). Thus, immunologic stimulation of the lungs and liver is prolonged (Frick *et al.*, 1965).

All ducks of Experimental Group III were solidly resistant to challenge 70 to 100 days after the initial exposure. This is of special interest since egg passage during the initial infection of these ducks receiving a series of small doses of cercariae, suggested that in some birds few, if any, worms had matured to lay eggs. This is confirmed by the reports of Smithers and Terry (1965a), Ritchie *et al.*, 1963a; Ritchie *et al.* (1966), McMullen *et al.* (1967), and Smithers (1967), who state that resistance develops eventually, regardless of infection intensity.

c) Cercarial Challenge Following Infections Induced by the Transfer of Adult Worms:

In the duck even small numbers of living mature adult worms injected intravenously into normal hosts, thus completely avoiding exposure to cercariae or migrating schistosomules, provided an adequate stimulus for the production of powerful resistance which, although not always complete, prevented the
maturation of massive cercarial challenges (Experimental Group V). The stimulus, however, must act over immunologically significant periods. Thus, the presence of adults in the circulation for 5 days prior to challenge did not inhibit reinfection (Experimental Group VI). This finding argues strongly against the action of a "pheromone-like" mechanism of resistance to reinfection, at least at that adult concentration. Furthermore, the transfer of donor plasma and cells had no obvious effects on the fate of challenge exposures after immunologically significant periods, indicating that these cells do not interfere with the metabolism of the worms of the challenging infection (Oliver-González, 1967; Smithers, 1968) nor was significant immunologic competence transferred (Gray, 1966). Smithers and Terry (1967), working with the S. mansoni - rhesus monkey system, reached similar conclusions. Although their criteria in judging resistance were somewhat less severe, these authors showed that the adult worm provided the major stimulus to resistance. Furthermore, these authors pointed out that the schistosome egg is probably of little importance in the development of resistance since one of their monkeys which had received live, half-worms by injection never passed eggs with the feces. Subsequent challenge exposure, however revealed a very high state of resistance. Similar observations on ducks receiving a series of small doses of cercariae of T. ocellata (Experimental Group III) reinforced the strong resemblance between
maturation of massive cercarial challenges (Experimental Group V). The stimulus, however, must act over immunologically significant periods. Thus, the presence of adults in the circulation for 5 days prior to challenge did not inhibit reinfection (Experimental Group VI). This finding argues strongly against the action of a "pheromone-like" mechanism of resistance to reinfection, at least at that adult concentration. Furthermore, the transfer of donor plasma and cells had no obvious effects on the fate of challenge exposures after immunologically significant periods, indicating that these cells do not interfere with the metabolism of the worms of the challenging infection (Oliver-González, 1967; Smithers, 1968) nor was significant immunologic competence transferred (Gray, 1966). Smithers and Terry (1967), working with the S. mansonii - rhesus monkey system, reached similar conclusions. Although their criteria in judging resistance were somewhat less severe, these authors showed that the adult worm provided the major stimulus to resistance. Furthermore, these authors pointed out that the schistosome egg is probably of little importance in the development of resistance since one of their monkeys which had received live, half-worms by injection never passed eggs with the feces. Subsequent challenge exposure, however revealed a very high state of resistance. Similar observations on ducks receiving a series of small doses of cercariae of T. ocellata (Experimental Group III) reinforced the strong resemblance between
two host-parasite systems.

Until now, no other experimental animal which allows the full development of a schistosome infection could equal the rhesus in its ability to acquire resistance against this parasite. However, monkeys are expensive, difficult to handle, and hard to keep, and certain dangers are inherent in culturing pathogenic organisms. Furthermore, infections of the human schistosomes are slow to mature. By contrast, Trichobilharzia-bird systems which are relatively inexpensive to maintain and which are free from pathological hazards, involve much more rapid events (e.g. patency being reached by T. ocellata in 12 to 14 days as shown in the present work and at similar intervals by T. szidati (Neuhaus, 1952), by T. brevis (Basch, 1966), and by T. cameroni (Wu, 1953). It becomes apparent then, that study of host-parasite relationships of bird schistosomes are not only worthwhile in their own right but also that they may prove to be useful models for more general schistosome research.
The Tail-shedding Phenomenon Exhibited by Cercariae during Skin-penetration

Cercariae of _T. ocellata_ were pipetted onto the web of a young Rouen and observed by transmitted light under a dissecting microscope X31.25 and X50.00 during the process of skin penetration. All of 367 cercariae thus observed shed their tails before having fully penetrated the skin of the duck.
Evaluation of the Counting Method
for Cercariae.

To establish the degree of accuracy of this counting procedure, freshly emerged cercariae of *T. ocellata* were quickly pipetted onto a petri dish which was lightly coated with human sebum, and immediately scanned for dissociated tails and bodies. After most cercariae had shed their tails, the suspension was stained with purpurin and fixed in Davidson's A solution. The numbers of cercarial tails and bodies were determined using a hand counter. Since only whole cercariae had been transferred to the plate, the number of tails was expected to equal the number of bodies. Three independent determinations yielded identical results, 466 bodies and 466 tails.
Concentrating Effect of Evaporation on the 0.85% Saline Solution.

To assess the concentrating effect of evaporation on 4 L. of 0.85% saline solution over a period of 24 hr, 5 trays were prepared as usual. The reductions in volumes due to evaporation were determined and the new concentrations calculated. After 24 hr a loss of 210 to 223 c.c of water had increased the concentration of the solution from 0.85% to between 0.897 and 0.900% saline.
Variation in Egg Passage during a 24-hour Period.

Feces were collected over 24 consecutive 1 hr periods and processed immediately. The number of miracidia appearing in the sidearm was plotted against time (Figure 1, Appendix IV).

The pattern of egg passage was highly erratic fluctuating between 0 and 60 eggs per hr.
Figure 1. Variation in egg passage during a 24-hour period.
Test of the Efficiency of Separation of Egg-bearing Sediment and the Supernatant by Decanting.

The efficiency of the decanting technique was tested by pouring supernatant from egg-bearing sediment into a tray containing the washed residue of feces of an unexposed duck. The suspension was allowed to settle, and the decanting procedure was repeated. Both residues were processed in the usual manner. The number of viable eggs transferred by decanting was established and expressed as a percentage of the total number of viable eggs.

Percentage of viable eggs transferred by decanting.

<table>
<thead>
<tr>
<th>Total No. of viable eggs</th>
<th>No. of viable eggs transferred</th>
<th>% transferred</th>
</tr>
</thead>
<tbody>
<tr>
<td>9173</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10494</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>58000*</td>
<td>28</td>
<td>0.05</td>
</tr>
<tr>
<td>16769</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*estimate

Thus, approximately 0.0 to 0.05% of the viable eggs are lost during the decanting process.
Efficiency of Miracidial Concentration

To test the efficiency of the modified McMullen and Beaver flask in concentrating miracidia, feces of uninfected, hatchery-reared ducks were processed like those of exposed birds. Counted numbers of miracidia were introduced at the bottom of the flask just above the sediment. Their appearance in the sidearm was timed with a stop watch.

All of 204 introduced miracidia appeared in the vertical element of the sidearm within 23 minutes. (Fig. 1, Appendix VI).
Figure 1. Timing of the arrival of introduced miracidia in the sidearm.
Composition of Perfusion Medium.

Tris-Citratred Buffered Balanced Saline Solution

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>5.270</td>
</tr>
<tr>
<td>KCl</td>
<td>0.260</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.110</td>
</tr>
<tr>
<td>MgSO₄ · 7H₂O</td>
<td>2.470</td>
</tr>
<tr>
<td>Na₂ HPO₄</td>
<td>0.032</td>
</tr>
<tr>
<td>K H₂PO₄</td>
<td>0.040</td>
</tr>
<tr>
<td>Tris (Hydroxy-Methyl)-Amino-Methane</td>
<td>1.920</td>
</tr>
<tr>
<td>Citric Acid (1H₂O)</td>
<td>(\nu_1) *</td>
</tr>
</tbody>
</table>

The solution was sterilized by filtration through Millipore (GS).

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*Citric acid was added to the solution until pH 7.8 was reached.*
REFERENCES


, 1969. Personal communication.


Olivier, L. and M. Schneidermann, 1953. Acquired resistance to Schistosoma mansoni infection in laboratory animals. 2: 298-305.


