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Resistance to *Eimeria tenella* and its Transference from one Caecum to the other in Individual Fowls

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Summary. Infections were produced in patent caeca by administering oocysts of *Eimeria tenella per os* to fowls in which one caecum was ligated. The caeca were later challenged with sporozoites and it was found that a resistance to infection had been acquired by the previously uninfected ligated caeca. The observations indicate that resistance to caecal coccidiosis develops in areas not previously exposed to parasitism by *E. tenella*. It is suggested that the immunity acquired by the ligated caeca is mediated through the circulation either by humoral antibodies or by lymphoid cells, or by a combination of both.

INTRODUCTION

It is well established that, provided adequate numbers of sporulated oocysts are administered, fowls will develop a firm resistance to *E. tenella*. The stage of the life cycle most concerned with inducing this resistance in the host is the second generation schizont (Horton-Smith, 1947). The criterion for assessing resistance is usually the survival rate of fowls after challenge with large numbers of sporulated oocysts at different times after primary infection.

Waletzky and Hughes (1949) have criticized the validity of this method. Caecal coccidiosis produces occluding cores, thickening of the caecal walls and the absence of normal caecal contents. These sequelae may persist for some time after clinical disease has subsided, the duration being dependent upon the severity of the infection and, therefore, upon the size of the dose of oocysts administered. These authors state that retained caecal cores developing from a primary infection may sometimes endure for as long as 3 weeks and may prevent the initiation of a secondary infection by preventing the entry of sporozoites into the caeca. Therefore, the so-called challenge by a secondary infection may, in fact, be no challenge at all, with the result that false assumptions may be made concerning the immunity developed.

The objections raised by Waletzky and Hughes do not apply to the present experiments where one caecum is ligated and severed from the intestinal tract before primary infection of the patent caecum with oocysts and therefore remains free from infection. Both caeca are subsequently exposed through abdominal incisions, when the caeca may be examined visually and challenged by the injection of sporozoites into their lumina.

While the present work was proceeding, Burns (1958, 1959) also used fowls with ligated caeca in a study of the immune response. Sporozoites were used to induce immunity in the ligated caecum, the non-ligated caecum subsequently being challenged by giving oocysts 12-17 days later. Burns concluded that resistance to coccidiosis was, at least in part, established by humoral mechanisms as yet incompletely understood. The same technique was

adopted at the outset of the present work but was subsequently abandoned because cores which formed in the ligated caeca could not be discharged and even caused rupture of the caecal walls in several instances.

The objects of this investigation were to ascertain whether the resistance developing in a patent caecum was shared by the ligated caecum, and, if this were so, to determine the approximate time in which the ligated caecum developed appreciable resistance and the duration of this resistance; and to compare the time required for the acquisition of resistance in non-ligated caeca with that required by fowls with ligated caeca. Although transference of resistance has been demonstrated, the mechanism of the acquired immunity has not yet been considered. Although the so-called transfer may be a manifestation of a general resistance, the elucidation of the process is difficult owing to the fact that the intracellular cycle of *E. tenella* is typically restricted to the caeca and that consequently the response to parasitism cannot be studied in other sites.

MATERIALS AND METHODS

White Leghorn × Rhode Island Red fowls of mixed sexes were used in all experiments. The fowls were kept on wire floors in electrically heated units and their freedom from coccidial infections was confirmed before immunization. The caeca were ligated and severed from the intestinal tract in fowls aged 9–10 days, which will be referred to as 'operated chickens'. Infection was initiated on the 14th day of age. Fowls which received an initial infection with oocysts *per os* form the 'oocyst-infected group'.

At a later date this group and other groups not previously infected, which will be termed 'control groups', were infected with sporozoites (in one experiment, oocysts were used) as a 'challenging' infection. Fowls in the oocyst-infected and control groups were always of the same age at challenge. Oocysts of *E. tenella* used in these studies were derived from a single cell isolate and the suspensions were stored at room temperature in 2.5 per cent potassium bichromate and used before they were 1 month old. An initial dose of 60,000 sporulated oocysts was administered *per os* to each fowl of the relevant groups in all experiments. For challenge of resistance in Experiment 3, 120,000 oocysts were given.

Preparation and administration of sporozoites. Sporozoites for injection into the lumina of the caeca were prepared from the intestinal washings of starved susceptible fowls dosed $1\frac{1}{4}$–2 hours previously with $3–8 \times 10^6$ sporulated oocysts. The sporozoites were, as far as possible, freed from intestinal debris by centrifugation and then stored at 37° until required. The sporozoite suspensions were used within $2\frac{1}{2}$ hours of their preparation during which time they invariably produced infections in susceptible fowls. In experiments 1 and 2, 20,000 sporozoites were injected into the tip of the caeca in a 0.1–0.15 ml. suspension; the volume was kept small to prevent over-distension. As a precaution against bias the caeca of different oocyst-infected and control fowls were injected alternately.

Operative technique. All fowls were anaesthetized with ether. After de-feathering the anterior abdominal wall, the skin surface was washed with 70 per cent alcohol. A small sheet of sterile lint covered the abdomen, legs and lower thorax. An incision was made through the skin and abdominal muscles to the right of the middle line. The two caeca and the lower intestine were withdrawn through the incision and the right caecum emptied by gentle pressure. The right caecum was then severed from the intestine between two ligatures tied near the junction of the right caecum with the large intestine. Precau-

tions were taken to ensure that there was no contamination of the adjacent peritoneum during and after section of the caecum. The cut ends were washed with 70 per cent alcohol followed by normal saline. The gut was returned to the abdominal cavity and the wound closed with through-and-through sutures. When the abdominal cavity was opened for the second time to inject the sporozoites, the incision was made to the left of the middle line and the caeca, with the lower end of the small intestine, were withdrawn. Routine single injections of streptomycin, 5000 units per fowl, were administered on the day of the operation and the day following.

AUTOPSY OF FOWLS AFTER INFECTION WITH SPOROZOITES. The fowls in all experiments were killed on the 6th day after receiving the challenge doses of sporozoites. With the exception of Experiment 3, in which no ligation was made and oocysts were used for challenge, survival or mortality were not used in assessing resistance in operated fowls. When the same number of oocysts was used, the chances of killing fowls with one ligated caecum are approximately half as great as killing fowls with non-ligated caeca, owing,

TABLE 1

SCHEME OF GRADING OF LESIONS AND EXTENT OF PARASITISM

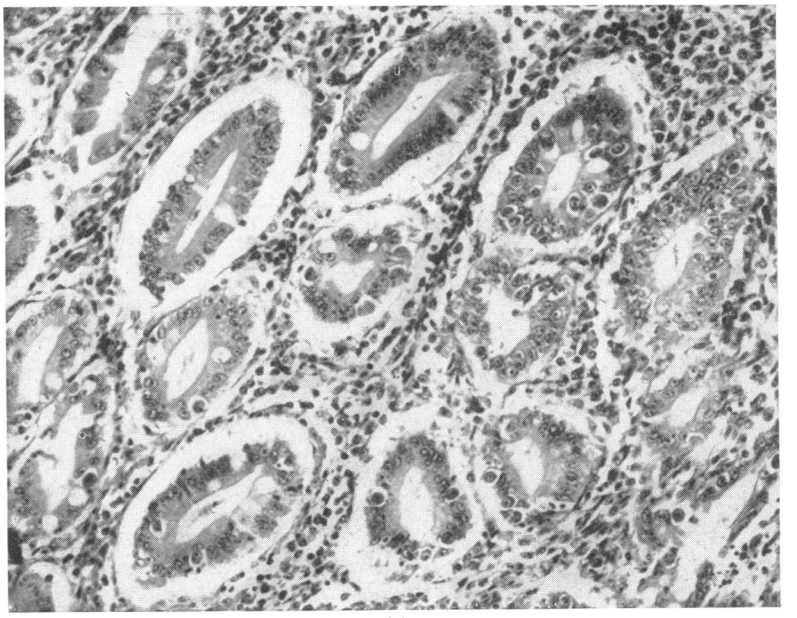
Extent of lesions and parasitism	*Macroscopical grading of lesions*	*Microscopical grading of parasitism*
–	No detectable lesions	No coccidial stages found
+	Small numbers of lesions	Small numbers of gametocytes found by careful search (see Fig. 1*a*)
++	Moderate numbers of lesions with some haemorrhage	Small numbers of second schizonts and/or gametocytes in scattered groups with some associated tissue damage (see Fig. 1*b*)
+++	Numerous lesions and haemorrhages	Numerous widely distributed gametocytes in localized foci with appreciable tissue damage (see Fig. 1*c*)
++++	Numerous lesions with severe haemorrhage and caecal enlargement	Numerous schizonts and/or gametocytes with widespread tissue damage (see Fig. 1*d*)

possibly, to the reduced surface of caecal wall available for parasitism. In all experiments the mortality arising from the initial dose was 37.6 per cent in groups with one ligated caecum and 76 per cent in fowls in which the caeca were not ligated.

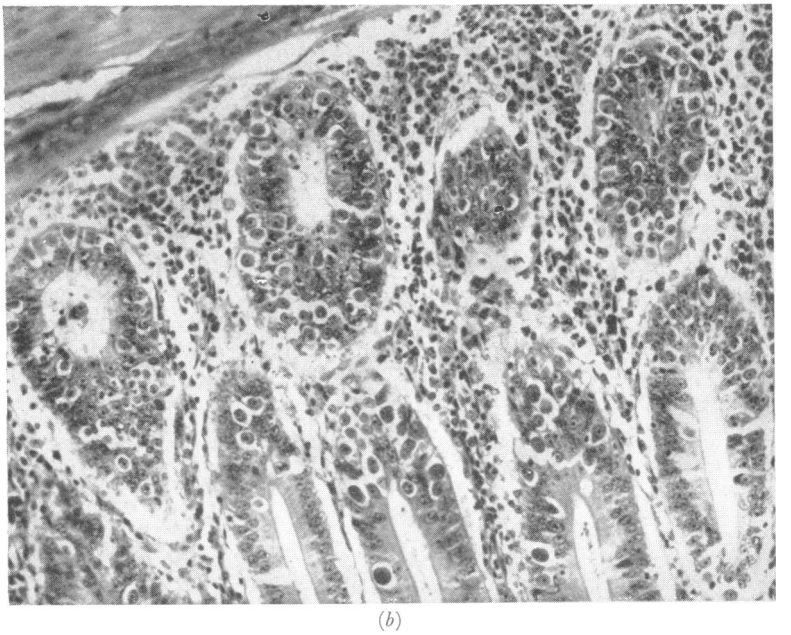
The main criteria used for determining the resistance developed were macroscopical lesions and the extent of parasitism shown in the walls of stained sections of the caeca viewed under low power (× 150 diameter). In sparsely parasitized tissue, careful searches were made for coccidia under high power (× 600 diameter).

Series of sections for microscopical study were prepared from tissues fixed in formal sublimate sectioned at 5 μ thickness and stained with haematoxylin and eosin or picro-Mallory.

The grading of lesions and extent of parasitism is not a simple matter and the scheme in Table 1 was adopted as conveying the data most conveniently and no account was taken of changes, mostly cellular infiltration, in the submucosa in awarding these scores. Photomicrographs are included to illustrate more clearly the system of grading used (Fig. 1).

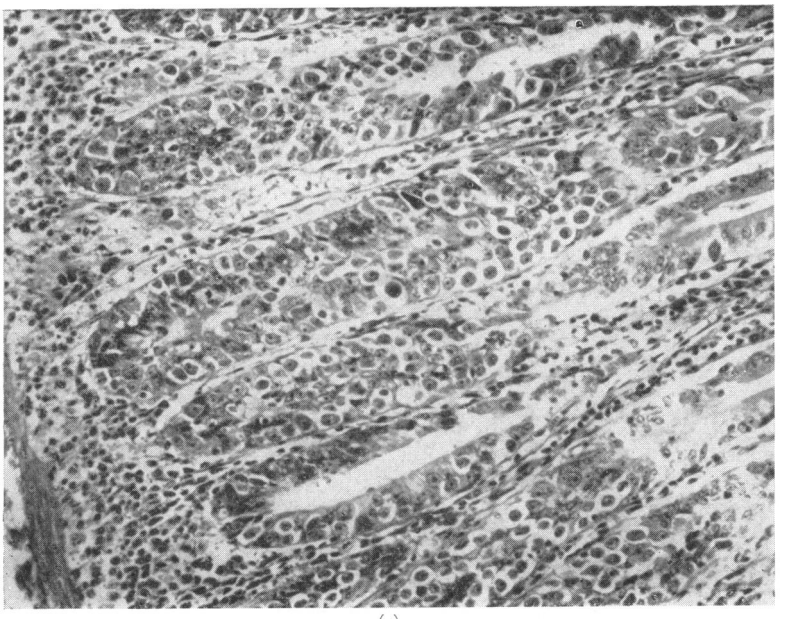


(*a*)

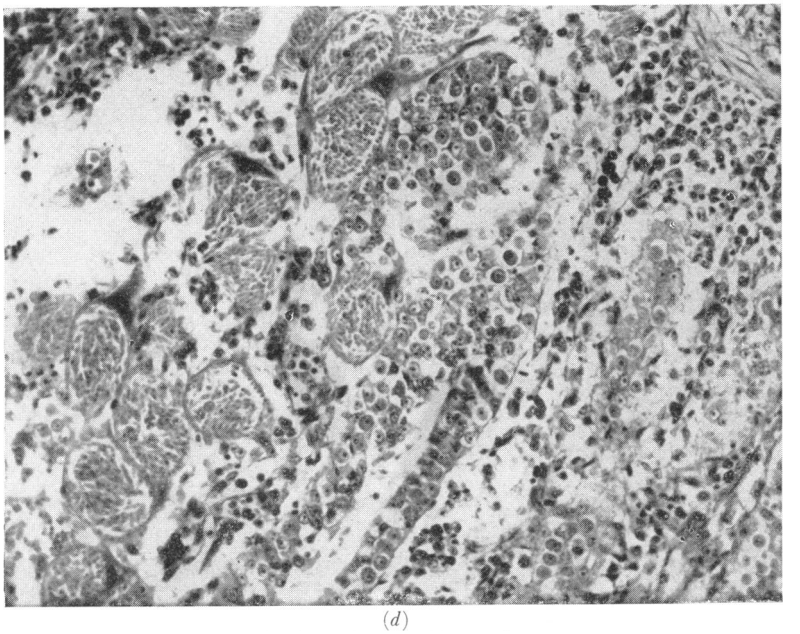


(*b*)

FIG. 1. Four photomicrographs (*a*), (*b*), (*c*), (*d*) of sections of caeca to illustrate scheme of grading.
(*a*) — Small numbers of gametocytes in deep glands and villi. Grading = +
(*b*) — Gametocytes in scattered groups with some associated tissue damage. Grading = ++



(*c*)



(*d*)

(*c*) — Numerous widely distributed gametocytes with appreciable tissue damage. Grading = +++
(*d*) — Numerous second-generation schizonts and gametocytes with gross tissue damage. Grading = ++++

RESULTS

OOCYST-PRODUCED INFECTION OF THE PATENT CAECUM AND CHALLENGE OF THIS AND THE LIGATED CAECUM 21 DAYS LATER

Two groups of fowls were used. Group 1 contained eighteen and Group 2, ten operated fowls. Six of the oocyst-infected fowls in Group 1 died from the infection. Examination post mortem revealed no infections in the ligated caeca of these six fowls. The fowls of the control Group 2 were not infected with oocysts.

Twenty-one days after the oocysts had been given to Group 1, the ligated and non-ligated caeca of both groups were infected with 20,000 sporozoites. The fowls were killed 6 days later and the observations made are shown in Table 2.

TABLE 2

RESULTS AT AUTOPSY 6 DAYS AFTER THE INJECTION OF SPOROZOITES INTO THE CAECA OF BIRDS IN GROUP 1 WHICH HAD RECEIVED DOSES OF OOCYSTS 21 DAYS PREVIOUSLY, AND CONTROL GROUP 2 OF COMPARABLE AGE

Grading of lesions	*Oocyst-infected Group 1 (twelve birds)*				*Controls Group 2 (ten birds)*			
	Macroscopical examination		*Microscopical examination*		*Macroscopical examination*		*Microscopical examination*	
	Ligated caecum	*Open caecum*	*Ligated caecum*	*Open caecum*	*Ligated caecum*	*Open caecum*	*Ligated caecum*	*Open caecum*
++++	0	0	0	0	8	9	5	5
+++	0	0	1	0	1	0	5	4
++	1	1	0	1	0	1	0	1
+	0	0	0	0	1	0	0	0
−	11	11	11	11	0	0	0	0

Note: Two of the twelve fowls in Group 1 showed the presence of a few oocysts in the immunized caecum (the result of the primary infection; when examined 6 days after the challenge dose).

Comparisons of the two groups showed significant differences in the macroscopical appearance as well as in the extent of the parasitism. The results show that in the operated oocyst-infected fowls the resistance has also been shared by the ligated caecum.

OOCYST-PRODUCED INFECTION OF THE PATENT CAECA AND CHALLENGE OF THE LIGATED CAECA AT DIFFERENT TIMES

This experiment was designed to determine the approximate time at which a significant resistance develops in the ligated caecum after the oocysts were administered. Twenty-seven operated oocyst-infected fowls were divided into three groups of nine fowls, 1a, 1b and 1c, which were subsequently challenged with sporozoites at 7, 14 and 21 days respectively. Another twenty-nine operated control fowls were divided among three similar groups, 2a, 2b and 2c and infected with sporozoites in similar numbers and at the same time as Groups 1a, 1b and 1c. They confirmed the infectivity of the sporozoites and the susceptibility of the operated fowls. Two fowls, one from Group 1c and one from Group 2b, were culled before the injection with sporozoites and therefore do not appear in the results.

Another three groups, each consisting of three or four fowls in which no ligations were performed, were used for additional confirmation of the infectivity of the sporozoites.

TABLE 3

RESULTS AT AUTOPSY 6 DAYS AFTER THE LIGATED CAECA WERE CHALLENGED WITH SPOROZOITES AND AT 13, 20 AND 27 DAYS AFTER GROUP 1 HAD PREVIOUSLY RECEIVED DOSES OF OOCYSTS. THE AGES OF BIRDS IN THE a, b AND c GROUPS ARE COMPARABLE

Fowl number	*Oocyst-infected Group 1*						*Control Group 2*					
	Number of days between Group 1 receiving oocysts and the injection of sporozoites to Groups 1 and 2											
	7 (1*a*)		14 (1*b*)		21 (1*c*)		7 (2*a*)		14 (2*b*)		21 (2*c*)	
	Macro	*Micro*	*Macro*	*Micro*	*Macro*	*Micro*	*Macro*	*Micro*	*Macro*	*Micro*	*Macro*	*Micro*
1	+	++++	−	−	−	++	++++	++++	++++	++++	++++	++++
2	+	+++	++	+++	−	−	++++	++++	++++	++++	++++	++++
3	+	++	−	−	−	++	++++	+++	++++	++++	++++	++++
4	++++	++++	−	++	−	++	++++	++++	++++	++++	++++	++++
5	+	++	−	−	+	++	++++	++++	++++	++++	++++	++++
6	++	++	−	−	++++	+++	+++	++++	++++	++++	++++	++++
7	+	+	+	++	++++	++++	++	++++	++++	++++	++++	++++
8	++	+	−	−	−	−	++++	++++	++++	++++	++++	++++
9	++	++	−	−			++++	++++	++++	++++	++++	+++
10											++++	++++
No. negative	0	0	7	6	5	2	0	0	0	0	0	0

These groups received no oocysts but sporozoites were introduced into a single caecum at the same time as the test groups received their sporozoite injection. The caeca of these fowls were found to be heavily infected at autopsy and there was evidence of migration of coccidial stages from one caecum to the other in every case.

The results of the macroscopical and microscopical examinations of the caeca of Groups 1a, 1b and 1c, 2a, 2b and 2c, are presented in Table 3.

The fowls of the control Groups 2a, 2b and 2c showed a greater preponderance of acute infections with related heavy parasitism than the oocyst-infected Groups 1a, 1b and 1c. Also, the fowls receiving the challenge doses of sporozoites 7 days after the oocysts were given were more heavily infected than those challenged on the 14th and 21st days. There also appeared to be a slightly diminished resistance on the 21st day as shown by the macroscopical and microscopical findings at autopsy. Second-generation schizonts and

TABLE 4

RESULTS OF AUTOPSY 6 DAYS AFTER CHALLENGE WITH 120,000 OOCYSTS

Grading of lesions	*Oocyst-infected Groups 1, 2 and 3*			*Control Groups 1a, 2a and 3a*		
	Days after administration of oocysts to Groups 1, 2 and 3 that fowls were challenged					
	14	21	28	14	21	28
	Number of fowls in each group					
	14	8	13	13	6	11
++++	0	1D	0	3+10D	3+3D	4+5D
+++	0	1	4	0	0	2
++	2	0	2	0	0	0
+	3	3	5	0	0	0
−ve	9	3	2	0	0	0

D = Deaths from acute caecal coccidiosis 5-6th day of infection.

developing gametocytes were the predominant stages in sections taken from the sporozoite-infected caeca of fowls which had received no oocysts. It was interesting to observe a greater number of mature 2nd-schizonts in sections of caeca taken from the control fowls than in those taken from the oocyst-infected fowls.

THE INFECTION AND CHALLENGE OF PATENT CAECA WITH OOCYSTS

A large number of deaths resulted in the oocyst-infected group, 127 fowls dying from acute caecal coccidiosis within a group of 167. Thirty-five of the surviving fowls were divided among three groups, 1, 2 and 3. At the same times as these groups were challenged with oocysts on the 14th, 21st and 28th days respectively after receiving an infecting dose of oocysts, thirty susceptible fowls, distributed in three control groups, 1a, 2a and 3a, were given numbers of oocysts equalling those used for challenge of Groups 1, 2 and 3. This

experiment was not concerned with the transfer of immunity from one caecum to the other but rather with the duration of a resistance developing from a single infection for comparison with the results obtained from fowls with ligated caeca treated in the same way. The results of the macroscopical and microscopical examinations are given in Tables 4 and 5.

A study of these tables shows that higher lesion and parasitism scores were allotted to groups challenged at 28 days after receiving the infecting doses of oocysts than in the groups challenged at the 14th and 21st days. Higher scores were also obtained at the examination of the groups challenged at 21 days than in those challenged at 14 days. On the whole, these results are comparable with those obtained with operated fowls challenged by sporozoites.

TABLE 5

SUMMARY OF THE MICROSCOPICAL FINDINGS ON SECTIONS TAKEN 6 DAYS AFTER CHALLENGE WITH 120,000 OOCYSTS

Extent of parasitism	*Oocyst-infected Groups 1, 2 and 3*			*Control Groups 1a, 2a and 3a*		
	Days after primary infection of Groups 1, 2 and 3 with oocysts that fowls were challenged					
	14	21	28	14	21	28
	Number of fowls examined					
	14	8	13	7	3	9
++++	0	1	3	5	3	9
+++	0	0	7	2	0	0
++	2	2	2	0	0	0
+	4	3		0	0	0
−ve	8	2	1	0	0	0

This experiment might be criticized on the grounds that it did not take into account the objections raised by Waletzky and Hughes. In the experiments with operated fowls which had been given 60,000 oocysts, caecal cores were found in 22 per cent of those examined at the time of challenge with sporozoites 14 or 21 days later. Bearing in mind the incidence of cores when considering the results of the present experiment in which oocysts were used for challenge as well as infecting doses, and assuming that about one-quarter of the fowls had retained cores at challenge, a sufficiently wide margin exists in the negatives to cover possible misinterpretation of the results.

DISCUSSION

When the oocyst-produced infection was confined to the patent caecum, the acquired resistance shown at challenge was afforded to both caeca, of which one was ligated and therefore deprived of any direct organic continuity apart from those systems (e.g. the blood) in which *E. tenella* is known not to occur.

Whereas the mechanism of the resistance is the subject of further work now in progress, it is clear from these results that resistance can be transferred to the isolated caeca and that the acquired resistance to *E. tenella*, under these experimental conditions, is not local in the sense that it is confined only to those areas of the caeca which had been previously infected. The immunity developing in the isolated caeca apparently attains its optimum at or about the 14th day after the other caecum received the products of its single dose of oocysts. When the immune status of the fowls was challenged on the 21st day, there was some evidence of a slightly diminished resistance. These observations received confirmation in Experiment 3 when fowls were used in which the caeca were not ligated and had been infected and subsequently challenged with oocysts. No detectable infection or only light infections were produced by challenge at 14 days, whereas after challenge at 21 or 28 days, heavier infections were observed, from which it might be assumed that the immunity acquired under these experimental conditions is of short duration. These observations confirm the view that immunity can be preserved over long periods only in fowls which are regularly picking up infective oocysts.

A comment has been made that greater numbers of mature 2nd-schizonts seemed to develop in susceptible than in partially resistant caeca, in which large numbers of developing gametocytes are observed in association with relatively few schizonts. This may be accounted for by the fact that it is this stage more than any other which promotes resistance in the host. Therefore, in partially resistant fowls, fewer schizonts develop and less tissue damage ensues, so that the second-generation merozoites have more adjacent cells available for penetration and development to gametocytes on the 6th day of the infection. This might also account in fact for Brackett and Bliznick's observation (1952) that more oocysts are produced per oocyst fed in light infections than in heavy ones.

The asexual stages of the coccidial cycle are concerned with the immune response of the host (Kendall and McCullough, 1952). The second-generation schizont is probably the stage most concerned (Horton-Smith, 1947). This stage is not reached until the 4th to 5th day after the introduction of oocysts and, as far as is known, stages preceding the 2nd-schizont do not exert as marked an effect on the development of immunity. It is therefore hardly to be expected that fowls challenged on the 7th day after the administration of oocysts, i.e. approximately 2–3 days after second-generation schizogony has begun, would have developed substantial immunity. Challenges given at 14, 21 and 28 days may be challenging an immunity which has been developing for 9–10, 16–17 and 23–24 days.

The results show that an infection, limited to one of the two caeca of a susceptible fowl, with a single dose of sporulated *E. tenella* oocysts *per os*, will induce a resistance to sporozoites subsequently injected into the lumina of both caeca. This suggests that the acquired immunity of the occluded caeca is mediated through the circulation either by humoral antibodies or by lymphoid cells or by a combination of both.

However, more conventional immunological investigation now being carried out must be completed before the more precise nature of the immunity and the mechanism of its transference to the ligated caecum can be established.

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