Immunity to Coccidiosis: Gut Permeability Changes in Response to Sporozoite Invasion

Circulating antibodies demonstrable by agglutination, precipitation, lysis, complement fixation and fluorescent staining are associated with the infection of many host species with their parasitic *Eimeria*, but their role in resistance to re-infection is unclear. Serum from infected or recently recovered animals has an adverse effect on stages of the specific parasite exposed to it in vitro; lysis is observed in the presence of complement and, in its absence, other morphological changes coupled with reduced infectivity (see review by ROSE[1]). However, protection from infection by the transfer of large amounts of such serum (or globulin fractions) from recovered to susceptible animals has not been recorded[2–6] except when the infection was induced i.v.[7]. In the latter case, the time interval elapsing between infection and the injection of serum was critical; if the administration of serum was delayed until the invasive stages had penetrated host cells, no protection occurred. This suggests that humoral antibodies can be effective in resistance to coccidiosis, provided that they are brought into direct contact with the organism and we have postulated that this might occur if the invasion of the gut of a previously infected animal is accompanied by an increase in local permeability, possibly due to a hypersensitivity-type reaction[1].

Intravenously injected pontamine sky blue (PSB) has recently been used to demonstrate changes in gut permeability during primary infections of chickens with *Eimeria acervulina* and *E. praecox*[8,9]. We have now used this method to study gut permeability in resistant and susceptible chickens given *E. acervulina*, *E. maxima*, *E. acervulina* or *E. praecox*.

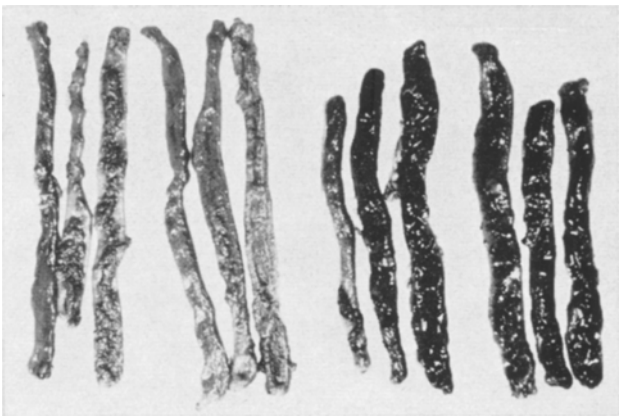
The chickens used for the *E. praecox* and *E. acervulina* experiments were Light Sussex (L.S.) and for the *E. maxima* infections either Brown Leghorn (B.L.) or Rhode Island Red (R.I.R.). They were kept in coccidia-free conditions until infected and fed a standard ration free from coccidiostats; the methods of handling and enumerating the parasites have been described[10,11]. In each experiment half the birds were immunized by crop inoculation once or twice with a suspension of viable oocysts to provide the 'resistant' groups. After an interval these and the un-immunized controls ('susceptible' groups) were given large numbers of oocysts of the relevant species. Pontamine sky blue (PSB) was injected i.v. as a 2% solution at the rate of 40 mg/kg 20 min before the birds were killed by the i.v. injection of pentobarbitone sodium solution ('Sagatal', May and Baker). The intestines were removed and a length from the gizzard to approximately mid-intestine opened up and the surface of the mucosa and contents examined for blue staining. This varied from an intense blue-black to a pale-greenish blue appearance and was graded accordingly from ++++ to (+). Colour photographs were taken and at the conclusion of all experiments, the grading was reassessed after examination of all the colour slides.

Pieces of gut were taken from some birds not used in the dye tests towards the end of infection to check resistance and susceptibility. These were fixed in formal sublimate and stained with hematoxylin and eosin for histological examination.

Results are presented in the Table. They show that at $3^1/_2$ and 7 h after infection, there was a leak of dye in the susceptible and immune groups. Thin sections taken at this time (by Dr. D. L. LEE of this Department) showed that sporozoites were penetrating or had penetrated the host cells. Thus the penetration of the host cells by sporozoites provoked a reaction resulting in increased vascular permeability. This occurred in most chickens irrespective of whether they had been previously infected or not, thus it was not exclusively the result of sensitization. There was, however, some indication, especially in the *E. maxima* infected group, that dye leakage was greater in the immunized group and this was particularly so at $3^1/_2$ h after infection.

One of the many properties of cortisone and its derivatives is to decrease capillary permeability (see review KASS and FINLAND[12]), consequently a group of birds immunized with *E. maxima*, together with a susceptible control group, were given daily injections of 1 mg betamethasone ('Betsolan', Glaxo) for 6 days (the last injection being on the day of testing for dye leakage) and the tests carried out as before. Results are at the foot of the Table and in the Figure; they show that the very considerable leak of dye in the immunized group could be completely suppressed by betamethasone treatment. In this experiment, there was very little leak of dye in the susceptible group indicating that the increased vascular permeability found in the immunized group was a consequence of previous contact with the parasite.

The experiments demonstrate a possible means by which any anti-parasitic factors present in serum may gain access to the invasive stages. Parasites could be



Dye-leakage in intestines of 4 resistant birds, with and without betamethasone treatment, 3.5 h after challenge dose of 5×10^6 *E. maxima* oocysts.

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Dye (pontamine sky blue) staining of intestines of birds at $3^1/_2$ or at 7 h after infection with *E. praecox*, *E. acervulina* or *E. maxima*. Dye injected 20 min before killing

Details of immunizing infections	No. and species of oocysts used for challenge infection	Group	Dye staining of intestines at 2 time intervals after infection			
			3.5 h		7.0 h	
L.S. given 10^6 *E. praecox* oocysts at 7 and 11 weeks	10^7 *E. praecox* at 14.5 weeks	susceptible	+++ —		+ —	
		resistant	+++ ++		(+) (+)	
L.S. given 10^6 *E. acervulina* oocysts at 7 and 11 weeks	10^7 *E. acervulina* at 14.5 weeks	susceptible	++ ++++		++ +	
		resistant	+++ +++		++ ++	
B.L. given 500 *E. maxima* oocysts at 3 weeks	10^7 *E. maxima* at 7 weeks	susceptible	? ?	(+) (+)	(+) (+)	? ?
		resistant	(++) (++) (++)	++ (++)	(+) (++) —	— —
R.I.R. given 5000 oocysts at 4.5 weeks and 10,000 oocysts at 10.5 weeks	5×10^6 *E. maxima* at 14 weeks	susceptible	+ — —	— — —	+++ (++) +	? — —
		susceptible + betamethasone	— — —	— — —	— — —	— — ?
		resistant	++++ ++++ (+)	(+) ++	+++ ++ (+)	(+) (++)
		resistant + betamethasone	— — —	— — —	— — —	— — —
None	None		— —	— —	— —	—

+ values indicate intensity of staining from ++++ = blue-black to (+) = pale blue-green, ? = doubtful. Each grading represents one bird.

affected in the lumen or on the mucous surfaces and possibly within host cells if these also are affected by the conditions accompanying sporozoite penetration. It is known that some sporozoites invade the immune host but fail to complete their life cycles[13,14] and it has also been suggested that there may be, in addition, a cut-down in the numbers of sporozoites which succeed in penetrating (freshly invaded sporozoites are always difficult to find in immune hosts). Parasiticidal factors in the intestinal mucus of immune birds have not been demonstrated[2,15], but the results obtained here indicate how such factors might pass from the circulation in response to the stimulus of invasion. A proportion of affected sporozoites might still be capable of penetration but not of further development whilst the remainder would fail to penetrate. The exudate shown to occur in un-immunized birds should prove relatively harmless to the parasite as normal serum, has, if anything, a beneficial effect on invasive stages[15], although there is some conflicting evidence[2,16].

This mechanism may also play a part in the extreme host-specificity of the *Eimeria*; *E. stiedae* sporozoites penetrate the epithelial cells of chicken intestine, an abnormal host, but fail to develop and we have shown that the sera of adult duck, turkey, quail, chick, pig, cattle, rabbit, horse, cat, dog and human lyse *E. tenella* sporozoites. A vascular leak in response to sporozoite penetration would allow the 'hostile' serum of the abnormal host contact with the parasite[17].

Zusammenfassung. Mit einer neuen Methode wird gezeigt, dass die Invasion des Darmepithels von immunen Hühnern durch Sporozoiten verschiedener *Eimeria*-Arten zu einer vermehrten Durchlässigkeit der Gefässe führt.

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