SOME PHYSIO-PATHOLOGICAL CHANGES ASSOCIATED
WITH EXPERIMENTAL EIMERIA BRUNETTI
INFECTION IN THE CHICKEN

By

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INTRODUCTION
Intestinal disease can result in diarrhoea of varying severity and consequent
changes in the concentration of plasma proteins and electrolytes. In mammals
such electrolyte alterations, particularly raised potassium levels, have been
attributed as the likely cause of death in the calf and pig (Fisher and McEwen,
1967; Patterson, Allen, Berrett, Sweasey, Thurley and Done, 1969). Fitzgerald
(1964, 1967) also demonstrated in the calf, that extracellular potassium rose
to toxic levels approximately 3 weeks after experimental infection with Eimeria
bovis. In the fowl the physio-pathological changes, subsequent to parasitic
infection, and their importance relative to mortality have only been described
in a few reports. Beg and Clarkson (1970) described a fall in plasma protein
concentration coincident with the development of acute caecal lesions during
infection of the fowl with Histomonas meleagridis. During experimental infection
of the chicken with Eimeria tenella and E. acervulina Oikawa, Kawaguchi and
Tsunoda (1971) described a decline in erythrocyte count, haemoglobin and
total protein concentration. These changes occurred following the appearance
of bloody diarrhoea and intestinal lesions. By contrast, infection with Eimeria
brunetti is characterized by a severe diarrhoea, but the faeces are not obviously
blood tinged and there has been no study of the consequent physio-pathological
changes.

This paper describes the protein and electrolyte alterations subsequent to
E. brunetti infection at 2 different dose rates and discusses their pathological
significance and possible contribution to mortality.

MATERIALS AND METHODS

Birds. Seventy 16-week-old White Link hybrid cockerel chickens (Sterling) were
fed ad lib on a standard ration (Joyner and Davies, 1960) and reared in strict isolation.
They were weighed 4 days before inoculation and divided at random into groups
3 days before infection. Thereafter, they were weighed daily from day 2 of the infec-
tion until day 14 when the survivors were killed and autopsied.

Infection. The oocysts used in this experiment were of the Weybridge strain of
E. brunetti. Fresh suspensions of sporulated oocysts were administered orally in a
similar manner to that described previously (Hein, 1973). Two doses of oocysts were
selected for inoculation based on the results of earlier dose response studies, viz.
3.2 x 10^5 oocysts which causes high morbidity and low mortality and, 1.28 x 10^6
oocysts which leads to both high morbidity and mortality. Twenty-six birds were
infected at the higher dose level and 20 at the lower. Corresponding control groups contained 14 and 10 birds respectively. Separate groups of chickens were inoculated on each occasion to confirm the pathogenicity of the inoculum. In another experiment total body and organ weights of similar groups of birds were determined at autopsy 7 and 14 days after infection.

**Blood samples.** Heparinized blood was collected from the wing vein 2 days before and 3, 5, 7, 10 and 13 days after infection. Serum samples were collected from clotted blood for total protein estimation and stored at −20 °C. prior to electrophoretic separation of the protein fractions.

**Analytical procedures.** Packed cell volume, potassium, chloride and total protein concentrations were estimated by routine methods of this laboratory (Patterson, Allen, Berrett, Ivins and Sweasey, 1968). The protein fractions were separated electrophoretically (Kohn, 1957; Patterson, Sweasey, Hebert and Carnaghan, 1967) for at least 4 chickens in each group. The identification and definition of the various protein fractions under these particular conditions have been described by Patterson et al. (1967). In this experiment, however, the β₄ and γ₁ globulin fractions were combined and considered as a single fraction, as were the α fractions.

**RESULTS**

**Clinical Effects**

Typical symptoms of severe coccidiosis with marked anorexia, depression, cyanosis, loss in weight and severe diarrhoea were evident during the acute phase of the disease between day 4 and day 8. Changes were more severe in the group receiving the higher dose of oocysts. The majority of deaths occurred on day 6. Mortality was 10 per cent. and 45 per cent. in the chickens given 3.2 × 10⁵ and 1.28 × 10⁶ oocysts respectively. Oocyst production was high on days 6, 7 and 8 with a total output of approximately 45 million oocysts per bird in each group.

**Data Presentation**

The differences in mean level between each of the 2 infected groups of birds and their corresponding control groups at the various sampling dates are shown in Fig. 1 for total protein, the second globulin (β₁) protein fraction and packed cell volume. The level of significance at which the change in mean level of an infected group from one sampling to the next differed from the change in its control group is indicated by one or more asterisks. The results for sodium, potassium and chloride are presented in a similar manner in Fig. 2. The initial values of these parameters in the 2 control groups are included in Table 1. Fig. 3 shows the difference in mean body weight of the infected groups from their own control groups. In this instance the number of asterisks indicates the level of significance of the difference in actual mean weight of controls and infected at each particular sampling.

**Plasma Proteins**

There was a progressive decline in the total serum protein concentration 3 days after infection at the higher rate (Fig. 1). This fall continued to a minimum value on day 7 then increased by day 10, but on day 13 the total protein concentration had fallen again and was similar to that observed on day 7.
Five days after infection the serum protein concentration of the birds which ultimately died was similar to the concentration in those birds which survived. In the group of birds which had received the lower dose there was a similar decline in the total protein concentration, which was at a minimum 7 days after infection.

During the course of the infection the concentration of all plasma protein fractions, including albumin, fell with the exception of β1 globulin fraction. Apart from the latter, the proportionate fall of each fraction was similar. The concentration of the β1 globulin fraction increased significantly in both groups of birds by day 5, then returned to normal 13 days after infection.

![Fig. 1. Total protein, β1 globulin and packed cell volume, difference from control values: (○) Birds receiving 3.2 x 10^6 oocysts; (●) Birds receiving 1.28 x 10^6 oocysts; × Change sig. different from controls, P < 0.05; × × change sig. different from controls, P < 0.01; × × × change sig. different from controls, P < 0.001.](image-url)
Fig. 2.—Plasma electrolytes, difference from control values. (○) Birds receiving $3.2 \times 10^5$ oocysts; (●) birds receiving $1.28 \times 10^6$ oocysts. For $P$, see legend to Fig. 1.

Fig. 3. Changes in body weight during infection: (○) Birds receiving $3.2 \times 10^5$ oocysts; (●) birds receiving $1.28 \times 10^6$ oocysts. For $P$, see legend to Fig. 1.
EXPERIMENTAL E. brunetti INFECTION IN THE CHICKEN

TABLE 1

<table>
<thead>
<tr>
<th>Control group</th>
<th>Na (mg/100 ml)</th>
<th>K (mg/100 ml)</th>
<th>Cl (mg/100 ml)</th>
<th>PCV (%)</th>
<th>Total protein (g/100 ml)</th>
<th>Albumin (%) of total</th>
<th>Protein fractions (%) of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>156.4</td>
<td>4.89</td>
<td>116.9</td>
<td>34.3</td>
<td>4.47</td>
<td>8.4</td>
<td>12.8</td>
</tr>
<tr>
<td>Low dose controls</td>
<td>151.5</td>
<td>4.96</td>
<td>121.2</td>
<td>34.3</td>
<td>5.11</td>
<td>10.1</td>
<td>13.1</td>
</tr>
</tbody>
</table>

These values did not vary significantly throughout the subsequent 15 days of observation.† 6 birds examined in exp. 1 and 4 in exp. 2.

Plasma Electrolytes

Alterations in the plasma electrolyte concentration occurred in both groups of infected chickens within 5 days of infection and appeared to be dose-dependent (Fig. 2). Plasma sodium levels declined in both groups 5 days after infection, to reach a minimum at day 7 and returned to normal by day 13. Alterations in plasma chloride were similar. On the other hand, there was a large variation of the individual plasma Cl concentrations in the high dosed group on the 5th day after infection, and thus the decline between day 3 and 5 was not significant. Potassium concentrations, however, increased during the same period, while the packed cell volume (Fig. 1) increased initially and then declined. This decline continued until day 13 when the study terminated.

Effect on Body Weight

The changes in body weight are shown in Fig. 3. The mean body weight began to decrease on day 3 or 4 and reached a minimum value on day 7 or 8 (P < 0.001), thereafter the mean weight of the survivors increased rapidly. Weight loss was far greater in the high dose group during the acute phase of the disease and at day 13 they were significantly lower than the non-infected chickens (P < 0.001) and those infected at the lower level (P < 0.001). The weights of the various body organs and tissues including the eviscerated carcass, skin and appendages, heart, liver, proventriculus, gizzard and intestine declined significantly by day 7 in both infected groups (P < 0.001). The values indicated that the decline in total body weight could not be attributed to any particular tissue or organ.

DISCUSSION

The clinical symptoms observed following experimental infection with E. brunetti appeared to correlate with marked alterations in total serum protein and electrolyte concentrations. Maximum changes were coincident with the acute damage caused by the second stage schizonts and early gametogenic stages of the parasite. Similarly Pout (1969) had noted the association between
the clinical symptoms, the stage of parasitic development and the severe tissue reaction, which occurred between the 3rd and 7th day post-infection. This was followed by resolution in the surviving birds. Beyond the 3rd day the decline in total protein in the higher dose group was associated with the onset of the severe intestinal lesions.

During experimental infection with either E. acervulina or E. tenella the plasma protein concentration likewise declined up to the 6th day post infection (Oikawa et al., 1971). Caecal histomoniasis in the fowl also resulted in a significant decline in plasma protein concentration and in particular the albumin fraction (Beg and Clarkson, 1970). Nevertheless, in this experiment because of haemoconcentration, which was evident as a rise in packed cell volume on day 5, the total extent of the decline in protein concentration may have been obscured. As the lesion became more acute on day 6 and 7, packed cell volume also fell suggesting that whole red blood cells were also now being lost at the site of intestinal lesions. During the phase of recovery when fluid was being retained within the plasma compartment there was a further fall both in PCV and total protein concentration presumably because synthesis, to replace lost red blood cells and plasma protein, was delayed compared to the ability for rehydration. The increase in the \( \beta \) globulin fraction at day 5 may indicate the synthesis of protein by the liver in response to intestinal damage as observed in mammals at the time of severe cell destruction (Darcy, 1964, 1968; Allen, 1971).

Alterations in plasma electrolytes were similar to those observed during acute calf enteritis (Fisher, 1965) and coccidiosis (Fitzgerald, 1967). Thus, there was a fairly rapid fall of sodium and chloride concentrations coincident with the appearance of the severe mucoid diarrhoea and a rise in plasma potassium. During the acute diarrhoeic phase fluid and electrolyte loss into the intestine accounted for the lowered extracellular sodium and chloride, whereas the increased plasma potassium probably arose by increased permeability of cellular membranes allowing release of potassium from the high intracellular concentration into the extracellular fluid. In those birds which died, plasma protein and electrolyte concentrations were not significantly different from those birds which survived; although in some individuals the increase in potassium and fall in sodium concentration was particularly large. Since the majority of deaths occurred during the period 12 to 24 h. after the sample collection on day 5 further detailed examination of changes in the immediate pre-terminal phase are necessary to ascertain the exact role of electrolytes as the cause of fatalities.

Preston-Mafham and Sykes (1967) demonstrated that, during E. acervulina infection, 70 per cent. of weight loss during the clinical stage of infection could be attributed to a reduced food intake. Thus complete inappetence for one day, as observed in our chickens, could clearly contribute a part of the observed weight loss of 450 g. in the severely affected chickens. Changes in water consumption could also represent another significant loss and the balance of weight loss could be attributed to the rapid and severe dehydration which occurred coincident with the diarrhoea. Rehydration probably contributes considerably to the rapid regain in weight. The exact cause of death is still not explained,
but as well as the severe hypoproteinaemia and electrolyte disturbance which were dependent on the number of parasites administered, the drastic reduction in extracellular fluid may result in the onset of an irreversible shock situation.

SUMMARY

Chickens aged 16 weeks were inoculated with either \(3.2 \times 10^5\) or \(1.28 \times 10^6\) oocysts of *Eimeria brunetti*. During the acute phase of the infection total protein, sodium and chloride concentrations declined, potassium levels increased, while the packed cell volume (PCV) increased initially and then decreased. These changes appeared to be dose dependent. Mortality was 10 per cent. and 45 per cent. in the low and high dosed groups respectively. In the surviving chickens electrolyte values returned to normal by day 10. The PCV continued to fall after clinical recovery and normal plasma protein levels were not regained by day 13 in the higher dose group given \(1.28 \times 10^6\) oocysts. The progressive alteration in plasma proteins, electrolytes and the PCV were attributed to the severe enteritis associated with the development of *E. brunetti*. In surviving birds the return of plasma electrolytes to normal concentrations corresponded to the time of resolution of intestinal lesions. The weight of organs examined at autopsy indicated that the decline in body weight could not be attributed to any particular tissue or organ. Rapid and severe dehydration appeared to be a significant factor in the loss in body weight, while rapid rehydration probably contributes to the rapid initial recovery in weight. The complete inappetence of the severely affected chickens confirmed that reduced food consumption is another important factor in weight loss in coccidiosis.

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REFERENCES


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