The effect of intestinal coccidiosis (*Eimeria acervulina*) on blood and tissue ascorbic acid concentrations

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1. Two experiments were conducted to investigate the effect of intestinal coccidiosis (*Eimeria acervulina*) on blood plasma and tissue ascorbic acid (AA) concentrations in chicks, and a third experiment was carried out to examine the role of dietary AA in this infection.

2. Experimental infection with intestinal coccidiosis (*Eimeria acervulina*) brought about a depletion in the concentration of AA in blood plasma, duodenum, jejunum, ileum, liver and the adrenal glands. Generally, these changes were obtained after the prepatent period of 4–5 d of the infection.

3. Dietary AA at 1000 mg/kg prevented the depletion in plasma and tissue AA levels but did not significantly affect adrenal weight.

The first indication of a possible requirement for exogenous ascorbic acid (AA) in poultry was made by Holst & Halbrook (1933) who observed that chicks suffering from a scurvy-like disease recovered completely when a diet rich in AA was fed, and they concluded that chickens require AA under certain stress conditions in which synthesis of the vitamin may be impaired. Bell *et al.* (1941) supported the previously mentioned observation and reported a dramatic recovery in laying hens showing symptoms of leg-weakness, by treatment with AA. In rats, which, like the chicken, also synthesize AA, an exogenous supply of the vitamin has been shown to be beneficial under certain stress situations: Malathi & Ganguly (1964) demonstrated a better resistance to vitamin A deficiency when exogenous AA was supplied; Galal *et al.* (1974) reported that AA reduced the number of gastric ulcers. In rabbits, Zohdy *et al.* (1974) indicated that AA tended to abolish electroencephalographic changes induced by experimental atherosclerosis. The purpose of the following experiment was to examine the changes in AA concentrations in blood plasma and tissues of male chicks infected experimentally with intestinal coccidiosis (*Eimeria acervulina*) and to see whether supplementation with dietary AA has any beneficial effects. This disease was chosen as a form of stress because it is reproducible experimentally, but it is not lethal. The infection involves mainly the upper part of the small intestine (up to the yolk stalk or Meckel's diverticulum) and it was here that depletion of AA occurred with hot climates as a stress factor (Kechik, 1971).

MATERIALS AND METHODS

Expt 1

Eighteen, 7-d-old cockerels of a medium hybrid strain were used in this experiment. They were allowed *ad lib.* access to food and water and continuous artificial light was used. The food was a commercial-type chick crumb without added coccidiostat. Twelve chicks were infected orally with two million sporulated oocysts of *E. acervulina* and the remaining six were kept separately as controls. On day 5 of the infection, six of the infected and three of the control chicks were killed and the duodenum, jejunum, liver and adrenal glands immedi-

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ately removed for determination of AA, using the method of Roe & Kuether (1943). Dry weights were obtained by placing in an oven at 105° for 24 h. The remaining six infected and three controls were killed on day 8 of the infection and the previously mentioned procedure was followed. The tissues were examined on the days mentioned because on the fifth day the infection was at its maximum, with the release of oocysts in the gut lumen, while, on the eighth day the infected birds were recovering from the disease as judged by body-weight gain and histologically.

Expt 2

The aim of this experiment was to investigate whether older birds respond in a similar manner in their tissue AA concentration. The last segment of the small intestine (ileum) and blood plasma which were not examined in the previous experiments were also examined and observations were made on the fourteenth day when the birds would be expected to have recovered completely from the disease. For this purpose, twenty-four 14-week-old cockerels of the same strain as in Expt 1 were used. A grower's ration without added coccidiostat was fed throughout the experiment. No artificial lighting was applied. Fifteen birds were orally infected with eight million sporulated oocysts of *E. acervulina*. The remaining nine birds were housed separately and were maintained as normal controls. Five infected and three control birds were killed on day 5 and the remaining birds on day 8 and on day 14. However, due to the deaths of three infected birds during the course of the experiment, only four and three birds respectively, were killed from the infected group, on the eighth and fourteenth days of infection.

Expt 3

The purpose of this experiment was to determine whether supplementary dietary AA would prevent or limit any change in the concentration of AA in the tissues and plasma and to determine whether plasma and tissue AA concentrations would be restored naturally 3 weeks after the infection. It is known that birds infected with coccidiosis consume less food than non-infected birds and, since food restriction alone may influence tissue AA concentration (Kechik, 1971), it was decided to compare all observations with pair-fed controls. Body-weight gains were also recorded. Seventy-five 4-week-old cockerels of the same strain were used and were divided into three groups of twenty-five. A commercial-type chick mash ration without added coccidiostat was fed *ad lib*. The first and second groups were orally infected with four million sporulated oocysts of *E. acervulina* after the latter group had been fed with a diet containing 1000 mg AA/kg for 5 d before the infection, until the end of the experiment. Measurements of food consumption were made daily at the same time from the first day of infection. The amount of food consumed by the first-infected group (without AA) in 24 h was given to the third group (pair-fed controls). On days 5, 8, 14 and 21 after the infection five birds each were randomly selected from both the infected groups for blood and tissue sampling, as well as for observations on body-weight gains. A similar procedure was also carried out with the pair-fed group, on days 6, 9, 15 and 22, The remaining five birds in each of the three groups were kept as spares. From here on, a similar procedure was followed as in Expt 2.

RESULTS

Expt 1

When the ratios, dry weight: wet weight of different tissues, except the adrenals, were examined (Table 1) it was observed that the moisture content of the duodenum and jejunum of the infected chicks was significantly higher ($P < 0{\cdot}001$) than the non-infected controls; in the liver the differences were non-significant. For this reason, it was thought justifiable to express the final result on tissue AA concentration in mg/kg dry weight tissue.

Table 1. *Expt* 1. *Values for dry weight: wet weight in chicks infected with intestinal coccidiosis* (Eimerial acervulina)†

(Mean values for six birds)

Day of infection	Duodenum	Jejunum	Liver
0	0·226	0·224	0·247
5	0·193***	0·201***	0·242 NS
8	0·197***	0·207***	0·239 NS

NS, Not significant.
Values were significantly different from those of controls: *** $P < 0.001$.
† For details of procedures, see p. 97.

Table 2. *Expt* 1. *Effect of* Eimeria acervulina *infection on tissue ascorbic acid concentration (mg/kg dry weight tissue) and on adrenal weight, in chicks*

(Mean values with their standard errors for six birds)

	Tissue ascorbic acid concentration (mg/kg dry wt tissue)						Adrenal (mg/kg wet wt tissue)		Adrenal weight (g)	
	Duodenum		Jejunum		Liver					
Day of Infection	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	1675	102	1749	152	799	15	1459	107	0·012	0·001
5	1731	82	1542	76 NS	895	69 NS	1645	47 NS	0·012	0·001
8	1416	59 NS	1140	27**	501	14***	992	21**	0·014	0·001NS

NS, Not significant.
Values were significantly different from those of controls: ** $P < 0.01$; *** $P < 0.001$.

In the duodenum (Table 2) the AA concentration fell on the eighth day of infection but this fall was non-significant. In the jejunum the depletion of the vitamin was highly significant ($P < 0.01$) on the eighth day of infection and was present, but non-significant, on the fifth day. The liver and the adrenals also showed highly-significant depletions in the AA concentration on the eighth day of infection ($P < 0.001$ and $P < 0.01$ respectively), but the differences on the fifth day were non-significant. Adrenal weight did not show any significant changes.

Expt 2

As in Expt 1, it was observed here that the moisture content of the different tissues, except the liver, was significantly higher in infected birds compared with the controls (Table 3). Table 4 shows that significant depletion of AA in the duodenum and jejunum occurred on the fifth and fourteenth days of infection but not on the eighth day, in contrast to the previous experiment although both tissues did show lower AA concentrations on the eighth day. In the ileum a depletion of the vitamin occurred on day 8 (non-significant) and day 14 ($P < 0.01$) of the infection. It is interesting to note that as in Expt 1, there was a depletion of the vitamin from the liver on the eighth day, and also on the fourteenth day of the infection. In the adrenal glands the AA concentration did not show any significant fall on the eighth day, but fell significantly only on the fourteenth day ($P < 0.05$). The differences in plasma AA levels between the infected and normal control birds were also significant on the eighth day ($P < 0.05$) and fourteenth day ($P < 0.001$), but not on the fifth day after the infection. It was also observed that the weight of the adrenal glands of birds killed on the fifth and fourteenth days was significantly greater than the non-infected controls ($P < 0.01$ and $P < 0.05$ respectively). The adrenal weight on the eighth day of infection was heavier than

Table 3. *Expt* 2. *Values for dry weight: wet weight in chicks infected with intestinal coccidiosis* (Eimeria acervulina)

(Mean values for the number of birds given in parentheses)

Day of infection	Duodenum	Jejunum	Ileum	Liver
0 (9)	0·226	0·214	0·213	0·268
5 (5)	0·192***	0·185***	0·197***	0·265
8 (4)	0·183***	0·183***	0·200*	0.270
14 (3)	0·196***	0·201*	0·202*	0·275 NS

NS, Not significant.
Values were significantly different from those of controls: * $P < 0.05$; *** $P < 0.001$.

Table 4. *Expt* 2. *Effect of* Eimeria acervulina *infection on plasma and tissue ascorbic acid concentration, and on adrenal weight in cockerels*

(Mean values with their standard errors for number of birds given in parentheses)

Day of infection	Plasma Ascorbic acid (mg/100 ml)	Tissue ascorbic acid concentration (mg/kg dry wt tissue)				Adrenal (mg/kg wet wt tissue)	Adrenal weight (g)
		Duodenum	Jejunum	Ileum	Liver		
0 (9)	1·383 ±0·094	1823 ±88	1835 ±82	1713 ±98	1209 ±73	1238 ±49	0·147 ±0·007
5 (5)	1·533 ±0·200 NS	1381 ±62**	1468 ±42**	1713 ±19	1279 ±112	1218 ±63	0·185 ±0·010**
8 (4)	0·903 ±0·142*	1507 ±104 NS	1555 ±89 NS	1428 ±102 NS	771 ±48**	1097 ±73 NS	0·187 ●0·037 NS
14 (3)	0·444 ±0·150***	1238 ±289*	1136 ±288**	982 ±219**	555 ±127**	838 ±184*	0·174 ±0·006*

NS, Not significant.
Values were significantly different from those of controls: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

the controls, but due to the wide variation between the individual weights the result was not statistically significant ($P < 0.05$).

Expt 3

Values for dry:wet weight (Table 5) of different tissues of infected birds examined on the fifth day of infection were almost similar to those seen in previous experiments, compared with pair-fed controls. The differences on the eighth, fourteenth and twenty-first days of infection were non-significant. The AA concentration of the duodenum, jejunum and ileum (Table 6) of the infected birds not supplemented with AA did not show any significant depletion on all the periods examined compared with pair-fed controls, except on days 14 and 21 when the ileum and jejunum respectively demonstrated a significant fall in the concentration of the vitamin ($P < 0.05$). In the liver, a slight fall in the concentration ($0.05 < P < 0.1$) was observed in the infected birds on the fourteenth and twenty-first days. The adrenal glands, like the liver, also showed a slight depletion in AA concentration on the fifth and fourteenth days. A similar fall in the plasma AA level of infected birds was observed on day 8 ($0.05 < P < 0.1$). Although the previously mentioned changes in AA concentrations did not reach the same levels of significance as obtained in previous experiments, there was generally a tendency for the infected birds to show lower AA concentrations during the periods examined.

Table 5. *Expt* 3. *Values for dry weight: wet weight in cockerels infected with intestinal coccidiosis* (Eimeria acervulina)

(Mean values for five birds)

Day of infection	Treatments	Infected (no ascorbic acid)	Infected (1000 mg ascorbic acid/kg)	Pair-fed control	'*F*' value	LSD ($P < 0.05$)
5	Duodenum	0·194	0·196	0·240	66·145***	0·010
	Jejunum	0·200	0·203	0·233	10·100**	0·018
	Ileum	0·208	0·208	0·232	8·147**	0·015
	Liver	0·246	0·252	0·259	4·272*	0·010
8	Duodenum	0·205	0·206	0·215	2·108 NS	—
	Jejunum	0·210	0·211	0·211	0·026 NS	—
	Ileum	0·217	0·217	0·210	0·975 NS	—
	Liver	0·267	0·262	0·254	3·330 NS	—
14	Duodenum	0·225	0·224	0·228	0·264 NS	—
	Jejunum	0·229	0·231	0·224	0·841 NS	—
	Ileum	0·237	0·232	0·220	2·196 NS	—
	Liver	0·253	0·258	0·251	1·152 NS	—
21	Duodenum	0·232	0·232	0·226	0·921 NS	—
	Jejunum	0·225	0·231	0·222	3·365 NS	—
	Ileum	0·237	0·230	0·219	3·497 NS	—
	Liver	0·252	0·256	0·251	0·583 NS	—

LSD, Least significant differences; NS, not significant.
Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

It is also shown in Table 6 that birds infected with intestinal coccidiosis and given AA did not demonstrate any depletion of the vitamin in plasma or tissues. The weight of the adrenals was also found to be significantly heavier in both the infected groups, with or without dietary AA, on the fifth day of infection ($P < 0.05$).

DISCUSSION

Infection with intestinal coccidiosis, caused by *E. acervulina*, has been shown to deplete plasma and tissue AA concentrations in immature chicks. The variation in results may be attributed to the use of different infective dosages, the age of the oocyst culture and to the response shown by birds of differing ages. For example, in Expt 3 when the plasma and tissue AA concentrations of the infected birds did not differ very significantly from controls, it was known that the culture had been stored for 1 month and the severity of the infection was not as great as expected. This group also did not show the increased tissue moisture after day 5, typical of previous infections. Observations on food consumption and body-weight changes of all groups of birds did not differ significantly which also supports the previously mentioned contention, since the most obvious effect of a severe infection of *E. acervulina* is a reduction in body-weight gain and food intake (Preston-Mafham & Sykes, 1970). However, in general it has been shown quite conclusively that the AA concentration of tissues and plasma is depleted in *E. acervulina*-infected birds. An interesting feature of the present studies is that, generally the depletion was observed only after the prepatent period of 4–5 d when the birds were recovering from the infection, although on one occasion the duodenum and jejunum demonstrated a highly significant depletion on the fifth day. This earlier depletion of the vitamin might be due to the more severe infection; indicated by complete anorexia for a few days, and the occurrence of deaths, a feature not observed in

Table 6. *Expt* 3. *Effect of dietary ascorbic acid on plasma and tissue ascorbic acid concentrations and on adrenal weight of* Eimeria acervulina *infected cockerels*

(Mean values with their standard errors for five birds)

Day of infection	Tissue	Ascorbic acid concentration (mg/kg dry wt tissue)						'F' value	LSD ($P < 0.05$)
		Infected (no ascorbic acid)		Infected (1000 mg ascorbic acid/kg)		Pair-fed control			
		Mean	SE	Mean	SE	Mean	SE		
5	Blood plasma	1·117	0·127	1·389	0·236	1·076	0·089	1·086 NS	—
	Duodenum	1928	63	2156	108	2249	165	1·896 NS	—
	Jejunum	1758	98	2020	114	1755	66	2·568 NS	—
	Ileum	1581	64	1876	120	1738	132	1·816 NS	—
	Liver	1138	18	1329	64	1156	83	2·920[a]	190
	Adrenal (mg/kg wet wt)	1625	117	1522	29	1925	142	3·797[a]	331
	Adrenal weight (g)	0·034	0·001	0·035	0·002	0·024	0·002	4·604*	0·009
8	Blood plasma	0·844	0·063	1·845	0·086	1·117	0·128	29·044***	0·296
	Duodenum	1911	833	2455	513	2063	156	6·969**	328
	Jejunum	1678	77	2238	37	1830	102	14·212***	237
	Ileum	1500	94	2433	117	1705	90	23·553***	311
	Liver	1073	71	1565	87	1213	49	12·710**	219
	Adrenal (mg/kg wet wt)	1214	65	1500	65	1357	70	4·577*	205
	Adrenal weight (g)	0·041	0·002	0·037	0·001	0·039	0·002	0·434 NS	—
14	Blood plasma	1·188	0·125	1·981	0·069	1·287	0·055	23·843***	0·273
	Duodenum	2035	118	2610	32	2061	98	12·813***	279
	Jejunum	1731	61	2105	77	1871	82	6·533*	228
	Ileum	1563	96	1986	7	1789	79	7·891**	221
	Liver	1307	89	1782	78	1550	108	6·567*	285
	Adrenal (mg/kg wet wt)	1348	401	1544	70	1509	55	3·448[a]	173
	Adrenal weight (g)	0·039	0·003	0·045	0·003	0·049	0·001	2·678 NS	—
21	Blood plasma	1·149	0·098	1·929	0·168	1·269	0·072	12·270**	0·370
	Duodenum	1905	374	2408	102	1875	76	15·274***	236
	Jejunum	1655	27	2091	39	1820	57	26·436***	132
	Ileum	1511	74	2276	123	1621	75	19·550***	288
	Liver	1401	36	1816	146	1661	96	4·148*	317
	Adrenal (mg/kg wet wt)	1416	93	1799	33	1447	54	5·757*	200
	Adrenal weight (g)	0·049 ± 0·004		0·049 ± 0·003		0·056 ± 0·006		0·690 NS	—

LSD, Least significant difference; NS, not significant.
Levels of significance: [a] $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

other trials. Mortality is not a usual feature of *E. acervulina* infection although it has been known to occur.

The depletion of adrenal AA during coccidiosis differs from the claim of Challey (1960, 1966) to have obtained a significant increase in AA concentration of the adrenal gland on the fourth, fifth and sixth days of infection. Although caecal coccidiosis (*E. tenella*) was used in these experiments which may influence the results, a close study of his values (1960) showed that there was in fact a significant decrease of the vitamin on the third and fourth

days. The increase in adrenal weight is in agreement with Challey (1960) who observed a tendency (non-significant) for the adrenal weights to be heavier in the infected birds from the fifth to the eighth day of infection. A similar increase in adrenal AA concentration and adrenal weight was shown by Larsh (1963) during infections with trichinosis (*Trichinella spirallis*) in hamsters.

It was shown that high levels of dietary AA prevented the depletion of plasma and tissue AA during infection in Expt 3. Hill & Garren (1958) reported a depletion of plasma AA in chicks infected with fowl typhoid (*Salmonella gallinarum*) and the supplementation with dietary AA at 1000 mg/kg in the diet also prevented this fall. In contrast, an increase in whole blood AA in chicks infected with *Capillaria obsignata* was observed by Chubb *et al.* (1964).

It is generally believed that in mammals AA is required for the formation, maintenance and repair of intercellular materials (Bourne, 1953; Gould & Woessner, 1957; Gould, 1958; Antonowicz & Kodicek, 1968) and that it is essential for wound healing and similar tissue repair after trauma and infections (Goldsmith, 1961; Gould, 1961; Chatterjee, 1967). Since intestinal coccidiosis in the fowl involves necrosis and sloughing of the intestinal epithelium (Levine, 1961), it is most likely that during recovery there would be increased tissue utilization of AA for the repair of the previously mentioned lesions. Although the domestic fowl is normally able to synthesize AA for its requirements, it is possible either that, after an infection this synthetic ability is impaired, or that the normal rate of synthesis is not sufficient to meet an increased demand. The present studies seemed to indicate that the increased requirement of AA for intestinal repair is derived from tissue stores, e.g. liver, and an exogenous supply of dietary AA is able to prevent the depletion of the vitamin.

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