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Ultrastructural studies of the effects of amprolium and dinitolmide on *Eimeria acervulina* macrogametes

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Abstract. Medication of chicks with 125 ppm amprolium or dinitolmide adversely affected oocyst sporulation of *Eimeria acervulina* (Weybridge strain). Dinitolmide delayed oocyst production and no oocyst wall formation was seen up to 168 h post infection. Both drugs caused large numbers of abnormally small wall-forming bodies to be produced in the macrogametes. In amprolium-fed chicks, abnormal oocyst wall formation was seen. It was concluded that the main drug action was against wall forming bodies of type 2.

Most anticoccidial drugs are designed to suppress the development of the schizogonous stages of the eimerian parasites and were thought to exert little effect on gametogony. However, Joyner and Norton (1977) and Mathis and McDougald (1981) demonstrated that amprolium and dinitolmide (zoalene) inhibited the sporulation of the oocysts passed by birds infected with 'drug sensitive' laboratory strains of *Eimeria acervulina*. These results suggested an effect upon the gametogonous stages and the present investigation was undertaken to study any structural alterations visible in the macrogametes of *E. acervulina* in birds fed amprolium or dinitolmide. Previous information on the process of oocyst wall formation of *E. acervulina* was reviewed by Pittilo and Ball (1984) and these data from unmedicated infected control birds are not reiterated here.

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Abbreviations. *C*, canaliculi; *CL*, cytoplasmic limiting membrane; *L*, lipid; *M1*, *M2*, *M3*, membranes; *N*, nucleus; *NU*, nucleolus; *OW*, outer oocyst wall; *PG*, polysaccharide granule; *PV*, parasitophorous vacuole; *WFB1*, wall forming bodies of type 1; *WFB2*, wall forming bodies of type 2

Materials and methods

Ross Ranger cockerels were maintained coccidia-free from 1 day old until 40 days old when they were divided into 3 groups and fed unmedicated ration or food with 125 ppm amprolium or 125 ppm dinitolmide. Then 2 days later each bird was inoculated orally with 5×10^6 sporulated oocysts (15 day old culture) of the Weybridge strain of *E. acervulina*. Two birds from each group were killed at 96, 120, 144 and 168 h post infection (hpi) and tissue prepared for transmission electron microscopy in the manner previously described (Pittilo and Ball 1979). The faeces of five birds from each group were collected daily from 4 to 9 days pi for oocyst counts and sporulation data following the methods of Long et al. (1976).

Results

Amprolium. Amprolium in the diet at 125 ppm reduced the pathological effects of severe *E. acervulina* infection and the birds produced approximately twice as many oocysts as the infected unmedicated controls (Table 1). Oocysts from the latter group sporulated well and six separate oocyst suspensions gave an average sporulation of 85% (range 65–97%). There was no delay in oocyst output from the amprolium medicated birds, but the oocysts were largely unable to sporulate and only a small percentage showed attempts at division of the sporoplasm (Table 1).

Macrogametes in the unmedicated controls showed normal morphology (Fig. 1). The most noticeable difference within the macrogametes from amprolium medicated birds was the increased number of small wall-forming bodies (Fig. 2). Some of these were similar to developing wall-forming bodies of type 2 (WFB2) but the majority

Table 1. The effect of amprolium and dinitolmide on oocyst output and oocyst sporulation of *Eimeria acervulina*

	Days after inoculation	Millions passed per bird	Percentage of oocysts			
			Sp	D. Deg.	Deg.	Unsp.
Unmedicated control	4	1.48	86	3	10	1
	5	61.20	89	2	9	0
	6	22.80	97	0	3	0
	7	10.72	79	9	10	2
	8	8.32	65	9	24	2
	9	16.64	95	1	4	0
Amprolium at 125 ppm	4	1.88	0	5	45	50
	5	112.00	0	5	69	26
	6	72.00	5	11	54	30
	7	80.00	4	6	80	10
	8	19.20	7	5	76	12
	9	12.00	0	5	85	10
Dinitolmide at 125 ppm	4	few	12	20	60	8
	5	0.18	57	33	8	2
	6	0.05	41	39	18	2
	7	0.26	52	26	17	5
	8	1.23	67	28	6	0
	9	4.67	70	27	3	0

Sp, sporulation assessed by examining 200 oocysts after 7 days at 29° C; *D.Deg.*, divided but degenerate; *Deg.*, undivided and degenerate; *Unsp.*, unsporulated

had the appearance of small wall-forming bodies of type 1 (WFB1). Material similar in electron density to the WFB1 and 2 was seen within the parasitophorous vacuole (Fig. 2). The polysaccharide granules in the macrogametes and oocysts from medicated birds were electron translucent compared with the controls (Figs. 1–6).

Up to 144 hpi, only the formation of the outer layer of the oocyst wall was seen. Material of two different electron densities was observed on either side of a double membrane which divided the outer wall (Fig. 3). In some sections these two membranes were interrupted (Figs. 4 and 5). Dispersal of WFB1 material did not necessarily form a complete outer layer (Fig. 6) and at this time (144 hpi) WFB2 were still present in the cytoplasm and had not taken part in wall production.

Dinitolmide. Dinitolmide medication delayed the peak of oocyst production, greatly reduced oocyst output and approximately halved the sporulation count (Table 1). About one third of the oocysts showed degenerative changes after division of the cytoplasm. The most prominent alteration in the macrogametes of medicated birds was the large number of small WFB (Fig. 7). Many of these were discrete (Figs. 8 and 9) whilst some were in groups (Fig. 8), a situation not encountered in the normal parasite. No oocyst wall formation was seen up to 168 hpi.

Discussion

During maturation of the macrogametes of *Eimeria* coccidia and the formation of oocysts, changes in the structure of WFB are always apparent. Thus at any one time certain variations in appearance can be attributed to different rates of development which occur in a normal population and this may be accentuated by the action of anticoccidial drugs. However, taking into account the range of WFB morphology seen in *E. acervulina* macrogametes in the unmedicated control birds from 94–144 hpi in this and previous studies (Pittilo and Ball 1984), it was clear that amprolium and dinitolmide each fed at 125 ppm affected these cell structures. In both cases a number of WFB1 and WFB2 could be distinguished, but there was an obvious increase in the number of small WFB. Although it was not clearly shown in all cases, the evidence strongly suggested that the effect was on the WFB2 rather than on the WFB1.

The absence of an oocyst wall in birds fed dinitolmide may in part be accounted for by the very small number of macrogametes that could be examined due to the drug delaying their development and greatly reducing their numbers. By contrast, partial oocyst wall formation was seen in birds fed amprolium but this was abnormal in three ways. Firstly, there was no evidence, over the time of the experiment, that WFB2 participated in oocyst wall formation. This is similar to the findings with amprolium, arprinocid and dinitolmide against *E. maxima* (Pittilo et al. 1981). Secondly, the oocyst wall, made up of WFB1 material only was incomplete at times and occasionally gave an appearance of localised deposition similar to the observation of wall formation in *E. maxima* in the presence of dinitolmide (Pittilo et al. 1981). Thirdly, the deposition of the WFB1 material differed from that described for normal *E. acervulina* macrogametes (see Pittilo and Ball 1984). Normally the material is at first deposited between the two original macrogamete limiting membranes, after which there is a new membrane synthesised which divides the WFB1 material into two layers. When the material is transposed into the outer oocyst wall layer, its electron density changes from opaque to a uniform fine granular electron-pale form. Drug treatment introduced a number of abnormalities into this sequence. In birds fed amprolium, two membranes, interrupted in places, were involved in partitioning the developing outer wall. Osmiophilic, as well as

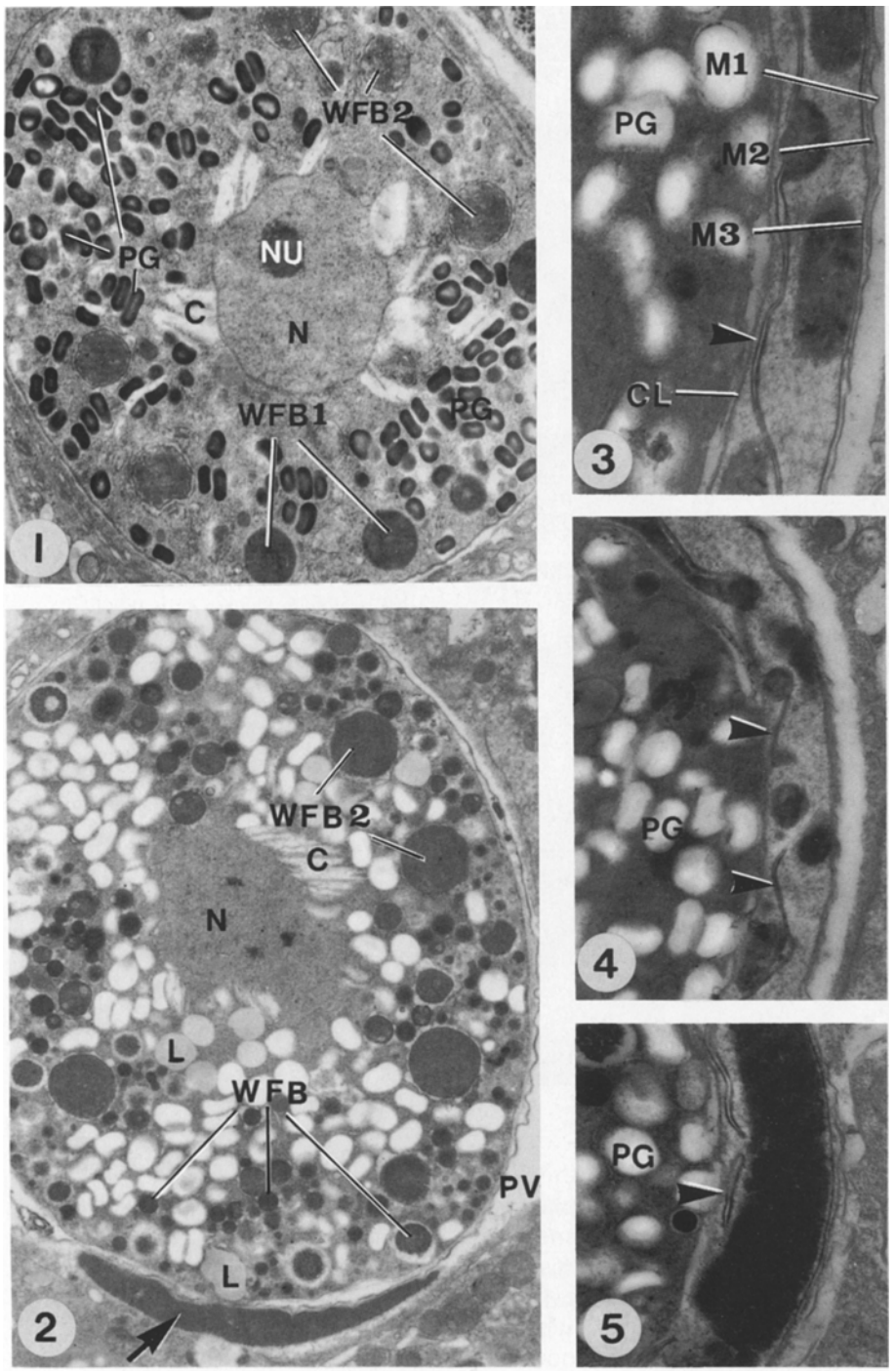

Fig. 1. Section of *Eimeria acervulina* macrogamete from unmediated infected control bird 96 hpi (hours post infection) with wall forming bodies, polysaccharide granules and canaliculi. ×6700

Fig. 2–5. Sections of *E. acervulina* stages from amprolium medicated birds

Fig. 2. Macrogamete 120 hpi with some large wall-forming bodies of type 2 and a large number of small wall forming bodies (*WFB*). Material similar to *WFB* is *arrowed*. ×7800

Fig. 3. Oocyst wall formation 144 hpi. In places the wall has three outer membranes and at least one cytoplasmic limiting membrane. The wall is divided by a double membrane (*arrowhead*) on either side of which the wall forming material is of two electron densities. ×25300

Fig. 4. The double membrane (*arrowheads*) in the oocyst wall appears to be discontinuous in places (144 hpi). ×14500

Fig. 5. Oocyst wall formation with internal discontinuous double membrane (*arrowhead*) (144 hpi). ×15000

fine granular material remained on either side of these membranes. Also, at the outer surface of the macrogamete at least one additional membrane was present which was not seen in macrogametes from unmedicated controls. The existence of membranes surrounding developing oocysts is well recognised for several *Eimeria* species (Pittilo and Ball 1980). The origin of the extra membranes seen in this study is not clear but they may be derived from the membranes surrounding the large numbers of WFB1 observed (Pittilo and Ball 1984).

Joyner and Norton (1977) considered the possibility that the absence of division of the sporoblast in oocysts from infected medicated birds could be due to a lack of fertilization, but their examination

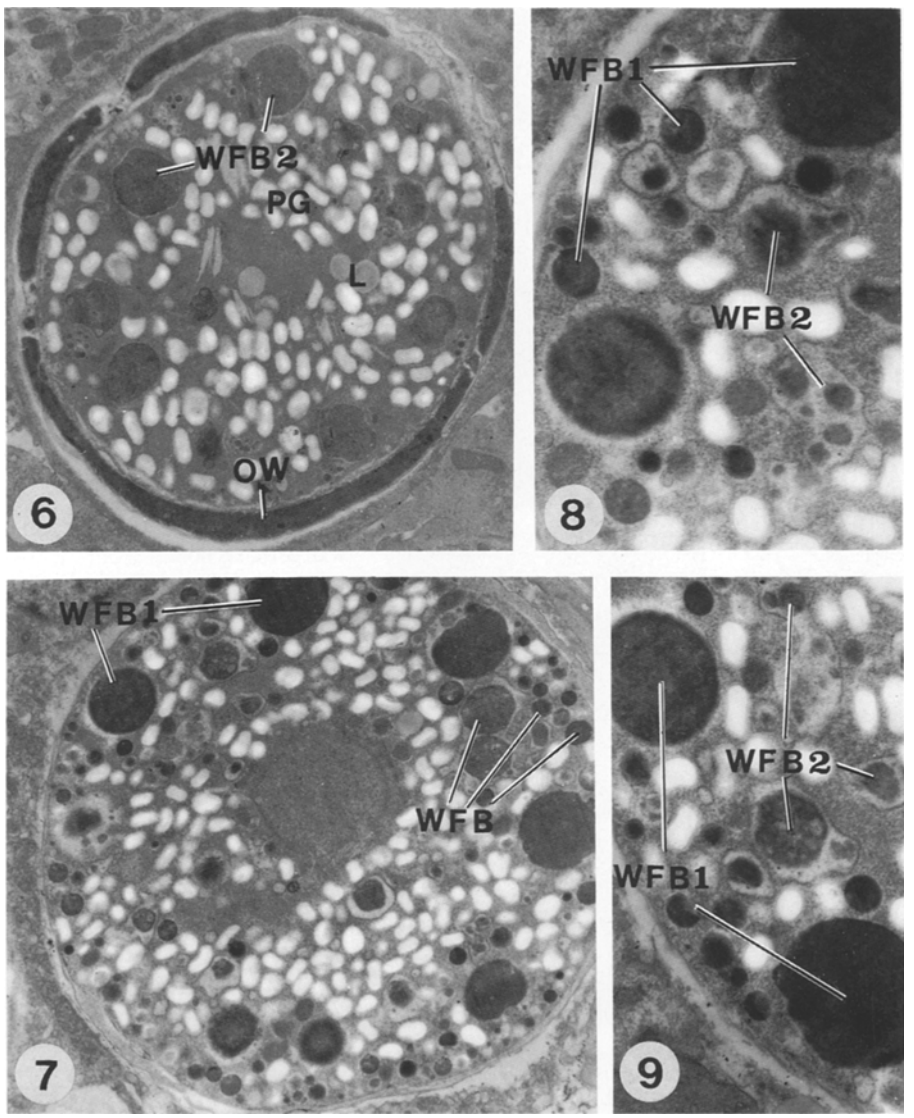

Fig. 6. Oocyst in tissue of amprolium medicated bird 144 hpi showing incomplete outer wall formation. Wall-forming bodies of type 2 have not taken part in wall formation. ×6700

Figs. 7–9. Sections of macrogametes from dinitolmide medicated birds

Fig. 7. Macrogamete with a large number of small wall-forming bodies 120 hpi. ×7500

Figs. 8 and 9. Details of portions of macrogames 120 hpi showing different appearances of the small wall-forming bodies. Fig. 8 ×16400. Fig. 9 ×13800

of tissue sections by light microscopy showed no obvious abnormalities of the microgamonts. Although not reported here, our observations confirmed these findings in that mature microgamonts and microgametes were seen when mature macrogametes were present. It is more likely that incomplete oocyst wall formation by drug action would reduce protection of the sporoplasm, resulting in incomplete sporulation. Amprolium and dinitolmide as well as arprinocid also caused changes in the structure of WFB2 of *E. maxima*. This similarly resulted in abnormal oocyst wall production (Pittilo et al. 1981). The same effects were produced in WFB in *E. tenella* treated with arprinocid (McManus et al. 1980) and in WFB2 of *E. acervulina*, *E. maxima* and *E. tenella* treated with two sym. triazinones (Mehlhorn et al. 1984). The latter authors considered that the drugs acted on the endoplasmic reticulum within which the WFB2 are formed. In the present state of knowledge this seems a logical explanation. It is tempting to speculate, from the few results to date, that compounds which adversely affect sporulation seem to have as one of their activities an action on WFB, particularly those of type 2 even though their biochemical mode of action appears dissimilar.

The electron transparency of the polysaccharide granules in the parasites from drug treated birds may be a drug related effect. However, further work is necessary to clarify this because other factors may determine the polysaccharide affinity for osmium. Variation in the electron density of polysaccharide granules is also seen in control birds (Pittilo and Ball 1984), but to a lesser extent

than in the present study. The reduced numbers of macrogametes in the dinitolmide-treated birds may have been due to a primary activity against the schizonts, as was demonstrated by Ryley and Wilson (1976) for *E. tenella*.

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