Some metabolic relationships between host and parasite with particular reference to the *Eimeriae* of domestic poultry

By L. P. JOYNER, *Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey*

With the protozoa, attempts to analyse the relationship between host and parasite have been confined largely to those species that are extracellular and can be subjected more readily to in vitro biochemical techniques. The sporozoa, however, include intracellular protozoa of the genera *Plasmodium* (malarial parasites) and *Eimeria* (coccidia) which are of major economic importance but, because their relationship with the host is an intimate one and difficult to analyse, little is known of their precise metabolic requirements particularly with the coccidia. Both groups show a high degree of host specificity with relatively limited immunological reactions, probably resulting from extremely exacting protein requirements.

Such a close relationship between host and parasite may be affected by variations in the diet of the host. This phenomenon has been studied in some detail in the malarial parasites (McKee, 1951) but only limited progress on these lines has been made with the coccidia. In recent years great emphasis has been placed on the chemotherapy of coccidial infections, the experimental basis of which has suggested information on the metabolites that are essential for the parasites but not for the host. Thus the *Eimeriae* react to changes in their environment. Conversely, however, like all parasites that induce pathological changes in the host they ultimately impair its metabolic efficiency. The coccidia have a special significance in this respect because nearly all species of *Eimeria* invade the epithelium of the intestinal tract. Under modern intensive conditions they constitute an important factor in poultry husbandry with its emphasis on the efficiency of food conversion. I propose to confine this discussion to the six species of *Eimeria* most commonly found in the domestic fowl (*Gallus domesticus*) in which the effects of the different species vary according to the nature and location of the changes they induce in the intestinal epithelium.

The Eimeriae *as parasites of the alimentary tract of the domestic fowl*

The efficiency of transmission depends upon the numbers of oocysts produced, and a characteristic of the development of the parasite within the tissues is the multiple division (schizogony) which it undergoes. Several schizogony cycles may take place, each giving rise to a number of merozoites which are released to invade fresh cells. The effect upon the host depends primarily upon the number of cells that are invaded and the depth of penetration into the tissues. Ultimately, gametocytes are formed in the epithelium and syngamy leads to the formation of fresh oocysts. Table 1 summarizes the major effects of the common species of *Eimeria* found in chickens.

Of the series, *E. mitis* is the most benign. Here the schizonts are small and superficial and it is only when the parasites are most numerous at the gametocyte stage

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Table 1. *Effects of infection with different species of* Eimeria *in the domestic fowl*

Species	Pathogenic stages	Location in alimentary tract	Degree of penetration	Major effect on host: Heavy infections, 50 000 to 1 000 000 oocysts	Major effect on host: Light infections, up to 10 000 oocysts
E. acervulina	Schizonts and gametocytes	Upper small intestine	Superficial	Inflammation and oedema, weight loss—emaciation, limited mortality	Frequently undetectable
E. brunetti	Schizonts and gametocytes	Lower small intestine	Superficial (deeper in severe infections)	Inflammation, oedema, necrosis, weight loss, mortality	Reduced weight gain
E. maxima	Gametocytes	Small intestine	Superficial (deeper in severe infections)	Inflammation, oedema, occasional haemorrhage, weight loss, mortality	Reduced weight gain
E. mitis	Gametocytes	Small intestine	Superficial	Reduced weight gain in very young chickens	None
E. necatrix	Schizonts	Small intestine	Deep	Inflammation, haemorrhage and anaemia; mortality often severe	Reduced weight gain
E. tenella	Late schizonts	Caecums	Deep	Haemorrhage; heavy mortality	Haemorrhage, anaemia; mortality often severe

that an effect upon the growth of very young chicks (1–2 weeks old) can be demonstrated (Joyner, 1958). Of the remaining five species mentioned in Table 1, a very reliable guide to their development may be obtained from the growth curves of the host birds. With *E. acervulina* relatively heavy infections are necessary to produce significant reductions in the growth of the birds. Such infections, however, do occur in the field and are often responsible for poor growth and a high rate of culling in birds 8–12 weeks of age. The effect of moderate infections generally can be related to the degree of invasion of the absorptive surfaces of the intestine but, as indicated in Table 1, heavier infections often crowd the parasites into deeper tissues and stimulate complex inflammatory and other changes in the host.

With *E. necatrix* and *E. tenella*, however, deep penetration into the submucosa by the large schizonts is typical. The release of merozoites at maturity is accompanied by extensive tissue breakdown and by haemorrhage which may be sufficient to induce

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considerable anaemia. In a study of these two species by Joyner & Davies (1960) an attempt was made to separate haemorrhagic effects from inhibition of the growth of the host. Using a microhaematocrit technique to follow the development of anaemia and daily weight measurements to record growth of the chicks, we observed the response to a series of graded infections. With *E. necatrix*, 10 000 oocysts were sufficient to cause a pronounced fall in body-weight, whereas even 20 000 oocysts produced only a barely detectable fall in haematocrit value. It is probably because *E. tenella* mainly parasitizes caecal tissue, which is not primarily concerned with food absorption, that its effect upon growth is not so pronounced. Very light infections with only about 2000 oocysts were shown to be sufficient to cause marked anaemia without affecting weight gain.

The effects of *E. tenella* infections, however, are not confined to anaemia. Pratt (1940) demonstrated an increase in the blood sugar content during the acute stages which coincided with a reduced muscle glycogen content. Pratt (1941) attempted to explain these changes in terms of haemorrhage, but Daugherty & Herrick (1952) showed that the respiratory quotient of muscles from infected birds was reduced and demonstrated the presence in caecal homogenates of a substance capable of reducing the glucose utilization of normal chick brain. Also associated with this infection is a reduced efficiency of muscular contraction. Early and pronounced fatigue was characteristic of the muscles of infected birds (Levine & Herrick, 1954). Similarly, Schildt & Herrick (1955) showed that muscular activity of the crop and the caecums was reduced. Using cytochemical techniques Gresham & Cruickshank (1959) have shown that nucleoprotein production by macrophages in infected caecal tissue is enhanced. These changes all indicate that this species has a profound effect upon certain metabolic functions of the host, although unlike the other species it does not directly interfere with food absorption.

Some effects of variations in the diet of the host

Some of the first reports of the influence of diet on the development of coccidial infection in the fowl arose from the observations of Beach & Davis (1925) and Tyzzer (1929), who showed that the severity of infection with *E. tenella* was reduced by the incorporation of dried skim milk in the diet. One possible explanation considered was that lactose induced an acid reaction in the caecums which destroyed the merozoites. Tyzzer (1929) noted the diarrhoeic effects of milk diets but considered that their prophylactic effects were due to their high food value. For a number of years skim milk was commonly given to poultry with coccidiosis.

There is a considerable literature concerning the effects of milk diets on experimental malarial infections. (See reviews by Hawking & Terry, 1957 and by Fulton & Spooner, 1955). The theory is generally held that the effects are due to a deficiency of *p*-aminobenzoic acid and that they are reversed by the addition of this substance to the milk. In this laboratory it has been confirmed that the administration of skim milk to chicks infected with *E. tenella* markedly reduces the mortality. A *p*-aminobenzoic acid-deficient diet based upon dextrin and casein with vitamins and amino acids, similar to that used by Taylor (1957) in her experiments on *Plasmodium*

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gallinaceum in chicks, reduced the average mortality caused by *E. tenella* to 35% of that in the controls. With both the milk and the synthetic diet, the effects could not be reversed by the administration of either 0·01–0·5% *p*-aminobenzoic acid in the food or 0·075% folic acid. A combination of 0·005% *p*-aminobenzoic acid with 0·005% *p*-hydroxybenzoic acid in the food with which Taylor (1957) reversed the suppressive effects on *P. gallinaceum* in the same host also was ineffective.

The severity of the haemorrhage caused by *E. tenella* was first shown to be dependent upon the vitamin K level of the diet by Baldwin, Wiswell & Jankiewicz (1941). The mortality has been shown to be particularly severe when the diet does not contain a source of vitamin K. Supplements of grass meal, lucerne meal, extracts of chicken faeces or poultry litter have the effect of reducing the mortality due to caecal coccidiosis (Stephens, Tugwell & Harms, 1957; Stephens & Tugwell, 1959, 1960). Low concentrations of synthetic vitamin K, menaphthone sodium bisulphite, of the order of 1–4 g/ton, which now are frequently added to poultry food, also effectively reduce the severity of the disease (Otto, Jeske, Frost & Perdue, 1957). The effect can be related to the blood clotting time and is most apparent with those species causing haemorrhage—*E. tenella* and *E. necatrix*. Dicoumarol significantly ncreases the blood clotting time and with it the severity of *E. tenella* infections, an effect which can be reversed by the administration of menaphthone (Harms & Tugwell, 1956). In order to obtain a high mortality in experiments on *E. tenella* and *E. necatrix* the use of a diet without a vitamin K supplement is desirable. Such a diet, described by Joyner & Davies (1960), has been in use in these laboratories for some years. When 2 g/ton of menaphthone sodium bisulphite were added to this diet the mortality due to heavy *E. tenella* infections was reduced in three experiments from 100% to an average of 40·5%.

We have been unable to show that the giving of the vitamin to the uninfected birds receiving this diet appreciably reduces the blood clotting time. Most of the reported observations apply to the blood clotting time of infected birds and, as Tugwell, Stephens & Harms (1957) have suggested, there is a need for information on the effect of *Eimeria* infections on vitamin K requirements.

Davies (1952) noted that in cases of coccidiosis due to *E. tenella* and *E. necatrix* the liver vitamin A reserves were reduced. This finding was confirmed by Erasmus, Scott & Levine (1960) who noted also that the severity of *E. tenella* and *E. acervulina* infections was not affected by increase in the vitamin A content of the diet but that recovery was enhanced. Recently Gerriets (1961) has demonstrated an inverse relationship between losses from caecal coccidiosis and the dose of the vitamin. Further investigation of this phenomenon is required, particularly in relation to invasion of the intestinal wall where conversion of β-carotene into vitamin A occurs.

Chemotherapy and parasite metabolism

Therapeutic drugs may exert their activity by interference with the metabolism of specific nutrients which the parasites require from the host. When such activity can be reversed by restoring an excess of the essential metabolite, indirect evidence is thus obtained of the parasite's requirements and the extent to which it is dependent

for them upon the host. The results of experiments with drugs and antagonists, however, can provide only circumstantial evidence for the existence of metabolic reactions.

Sulphonamides are active against all the species of *Eimeria* found in the domestic fowl. The differing activities of compounds within this series against one species, *E. tenella*, has been related to differences in the levels absorbed into the blood (Waletzky & Hughes, 1946). Some species, however, may be more susceptible than others. Sulphaquinoxaline at the low level of 0·0125% administered continuously in the food appears to control infections of *E. acervulina* and *E. maxima* more readily than those of *E. necatrix* and *E. tenella*, for which higher doses are generally necessary. It may be that differences are due to the fact that the experimental criteria of the development of *E. acervulina* and *E. maxima*—oocysts output and weight loss—are partly due to the activity of the gametocytes (Table 1) of which the metabolic requirements may be quantitatively different from those of the schizonts of *E. tenella* which cause mortality and at the same time are most susceptible to these drugs.

It has been recognized that the therapeutic effects of sulphonamides against a variety of organisms arise from competition with *p*-aminobenzoic acid (Woods, 1940). This effect has been confirmed for *E. tenella* and *E. necatrix* and it has been shown that the coccidiostatic effects of sulphadimidine are competitively reversed by the simultaneous administration of *p*-aminobenzoic acid (Horton-Smith & Boyland, 1946; Waletzky & Hughes, 1946; Joyner & Kendall, 1956). The chick, however, appears to be able to maintain growth on a diet which induces a *p*-aminobenzoic acid deficiency in *Plasmodium gallinaceum* (Taylor, 1957). The chick's requirements for *p*-aminobenzoic acid are therefore relatively small and insensitive to drug-induced deficiencies of it.

The action of the antimalarial pyrimethamine (2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine) is concerned with the conversion of folic into folinic acid (Hitchings, 1952). The use of small amounts of this drug in combination with a sulphonamide produces a true potentiation of the effect of the sulphonamide against *E. tenella* (Kendall & Joyner, 1956). This activity is very similar to that against *P. gallinaceum*, a malarial parasite of the same host (Rollo, 1955). This potentiation arises because the two drugs are acting against stages in the same metabolic chain leading to the formation of nucleic acids. Both drugs act upon the schizonts when active nuclear division raises the demand for nucleic acid metabolites. In the malarial parasite, folic and folinic acids have only a weak effect upon the action of pyrimethamine. It has been suggested, therefore, that the malarial parasite synthesizes folic acid from *p*-aminobenzoic acid within the cell and that the preformed metabolite is not available to it (Goodwin & Rollo, 1955). Experiments with *E. tenella* have shown that with this parasite also folic acid has only a slight effect upon the activity of pyrimethamine. The chick, however, is able to absorb the preformed metabolite from the gut, and the toxicity of pyrimethamine for the chick can readily be reversed by folic acid without affecting therapeutic efficacy (Joyner, 1960). A similar type of potentiation with sulphonamides has been demonstrated with another

group of folinic acid inhibitors—the diaminopteridines (Horton-Smith, Long & Collier, 1960).

A requirement for thiamine is suggested by the work of Rogers, Clark, Pessolano, Becker, Leanza, Sarett, Cuckler, McManus, Garzillo, Malanga, Ott, Dickinson & Van Iderstine (1960) on thiamine antagonists as coccidiostats. 1-(2-alkyl-4-amino-5-pyrimidinylmethyl)-alkylpyridinium salts possess marked prophylactic activity, particularly against *E. tenella* and *E. necatrix*. These compounds are structurally related to thiamine and appear to function by a reversible thiamine-inhibition mechanism. The structurally related thiamine antagonist pyrithiamine, however, was not active at the maximum tolerated level of 0·05% in the food. Amprolium [1-(4-amino-2-*n*-propyl-5-pyrimidinylmethyl)-2-picolinium chloride hydrochloride] has a marked prophylactic activity against *E. tenella* and *E. necatrix* when 0·0125% is administered in the food. It is slightly less potent against *E. acervulina*, *E. maxima* and *E. brunetti*. This level is well tolerated by the birds. It is postulated that the drug acts by inhibiting protozoal thiamine phosphorylase located at the cell wall. At concentrations above 0·1% in the food, Amprolium is toxic for chickens. The effects may be reversed by administration of 0·01% thiamine.

The attempt to analyse the interrelation between the metabolism of species of *Eimeriae* and that of their host is made difficult by the many gaps in present knowledge. The exercise, however, is a useful one if it directs attention to possible future lines of investigation. Despite these gaps it is evident that however attractive Elton's (1949) 'food chain' theory of parasitism may be as a working hypothesis, its initial simplicity is overshadowed by the extreme complexity of the host–parasite relationship when their metabolic exchanges are examined.

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The effect of milk intake on nematode infestation of the lamb

By C. R. W. SPEDDING, T. H. BROWN and R. V. LARGE, *The Grassland Research Institute, Hurley, Berks*

It is well known that nematodes inhabiting the alimentary tract may reduce the growth rate of lambs (Kauzal, 1936; Andrews, Kauffman & Davis, 1944; Franklin, Gordon & Macgregor, 1946; Taylor, 1942, 1953; Spedding, 1953, 1955; Spedding & Brown, 1957*a*,*b*,*c*). This effect is due in part to reduction of food intake (Gordon, 1950; Gibson, 1951, 1955; Spedding, 1954) and to interference with the digestive economy of the sheep (Stewart, 1932–3; Franklin *et al.* 1946; Spedding, 1954; Shumard, Bolin & Eveleth, 1957). The presence of these parasites may thus affect the nutrition of the host. Obviously a given effect of worm infestation on the growth rate of the lamb must be both more noticeable and more serious when the growth rate is low. Nutrition may thus affect the importance of damage due to parasites.

It is frequently assumed that a poor diet results in more helminths and that a well-fed animal will not be troubled with worms (Hunter, 1953), although Gibson (1951) demonstrated that good nutrition alone cannot be relied upon to mitigate the effects of infestation with *Trichostrongylus axei*. It has been pointed out by Gordon (1957), however, that nutritional conditions in the field also affect the grazing habits of herbivores and that the provision of food supplements may reduce the intake of both grass and the infective larvae on it.

Within the sheep industry, it is probably in the production of fat lamb that worm infestation is of greatest consequence. Some lambs receive supplementary solid food and it is commonly observed that it reduces the amount they graze. For the vast majority of lambs, liquid milk is a major constituent of the diet: this paper examines the effect of milk intake on the opportunity of the lamb to acquire a serious worm burden.