Brit. J. Pharmacol. (1956), **11**, 454.

THE MODE OF ACTION OF A MIXTURE OF PYRIMETHAMINE AND SULPHADIMIDINE ON *EIMERIA TENELLA*

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(RECEIVED SEPTEMBER 1, 1956)

It has been suggested by Joyner and Kendall (1955) that a combination of pyrimethamine and sulphadimidine is likely to be of practical value for the control of caecal coccidiosis caused by *Eimeria tenella* in chicks. Further knowledge of its mode of action, and the phases of the life history of the parasite that are most susceptible, is therefore desirable.

The development of *Eimeria tenella* includes two schizogonous cycles before the formation of gametocytes and oocysts. Studies by Horton-Smith and Taylor (1945) and Horton-Smith and Boyland (1946) have shown that sulphonamides inhibit the second generation schizonts by interference with *p*-aminobenzoic acid metabolism. The extension of the work of Wehr and Farr (1947) by Kendall and McCullough (1952) has shown that for satisfactory therapy this is the only stage which should be controlled. Inhibition of earlier stages, which occurs with certain doses of sulphonamides, leads to incomplete protection, and there are further deaths after stopping the drug. In the early experiments with pyrimethamine and sulphadimidine (Joyner and Kendall, 1955) there was some evidence that the mixed drugs might exert a specific effect upon the second generation schizonts.

The experiments described in this paper were designed to investigate this point in greater detail and to attempt an assessment of the role of the two drugs as inhibitors of the two metabolites—*p*-aminobenzoic acid and folic acid—which they are believed to affect (Goodwin and Rollo, 1955).

METHODS

Rhode Island Red or White Leghorn chicks, reared in isolation from adventitious infection, were inoculated orally with sporulated oocysts of *E. tenella* in numbers sufficient to kill at least 70% of untreated birds. The chicks, which were 1 to 3 weeks old, were kept in heated wire-floored brooders and allowed unlimited food and water. The diet was composed of a proprietary chick mash in which the drugs were incorporated during the experimental period. The food-drug mixtures were prepared with the aid of a mechanical mixer.

Folic acid and *p*-aminobenzoic acid were given twice daily, by intraperitoneal injection. The first dose was given 5 hr. after beginning therapy, and the second dose 5 hr. after ending it.

RESULTS

The Effect Upon Different Stages of the Parasite.—The development of *E. tenella* follows a regular course after infection with a single dose of oocysts. The first schizogony starts at about the 48th hour and the second shortly after the 72nd hour. It follows that by beginning drug treatment at different intervals after infection, and observing the effects upon the development of the disease, an indication may be obtained of the relative susceptibility of the different developmental stages of the parasite.

TABLE I

EFFECT OF TREATMENT AT DIFFERENT STAGES OF *E. TENELLA* INFECTION IN CHICKS

A mixture of 0·004% pyrimethamine and 0·05% sulphadimidine was given in the food at different stages in the development of *E. tenella* infection. All groups, each of 14 chicks, were infected on day "0." Drug treatments covered successive periods of 3 days, from 3 days before infection (Group A) to 7 days after (Group H). The deaths in each group are tabulated

Day after Infection	Treatment with Drugs on Days:								
	−3 to 0 A	−2 to 1 B	−1 to 2 C	0 to 3 D	1 to 4 E	2 to 5 F	3 to 6 G	4 to 7 H	Controls J
5	5	3					1	6	6
6	1	3	7	1				1	3
7			1	3					1
8				2					
9					1				
Total deaths	6	6	8	6	1	0	1	7	10

In the experiment recorded in Table I, 8 groups of 14 chicks were given 3-day courses of treatment with a mixture of 0.004% pyrimethamine and 0.05% sulphadimidine in the food; treatment began at different times during the development of the infection. A further group was kept untreated as a control.

Mortality was relatively unaffected when treatment ended on the day of infection, or was delayed until the fourth day. In both these groups of

chicks (A and H), as in the control group (group J), the greatest number of deaths occurred on the fifth day.

Deaths were progressively delayed in groups B, C, and D when therapy was stopped 1, 2, and 3 days after infection respectively. Almost complete control of the infection was obtained in groups E, F, and G when treatment ended 4, 5, and 6 days after infection.

It may be concluded that treatment given during the first 72 hr. retards the development of the early schizonts, whereas the effect upon the later schizonts is lethal. This is in agreement with the preliminary studies reported by Kendall (1956).

Inhibition by Metabolites.—Studies with other micro-organisms have led to the conclusion that pyrimethamine interferes with the conversion of folic to folinic acid, and in some organisms its action can be reversed by simultaneous administration of either of these metabolites (Goodwin and Rollo, 1955).

As the action of sulphadimidine on *E. tenella* can be reversed by *p*-aminobenzoic acid (Horton-Smith and Boyland, 1946) we decided to compare the inhibitory effects of folic acid and *p*-aminobenzoic acid against pyrimethamine and sulphadimidine given either separately or in combination.

Table II shows that both metabolites antagonized pyrimethamine. In experiment A, 0.01% pyrimethamine was completely antagonized by 100 mg./kg. folic acid. With the higher dose of the drug, however, neither metabolite caused complete antagonism.

TABLE II

ANTAGONISM OF THE THERAPEUTIC EFFECT OF PYRIMETHAMINE AND SULPHADIMIDINE ON *E. TENELLA* BY FOLIC ACID AND *p*-AMINOBENZOIC ACID

Expt.	Metabolite Given by Injection	Mortality, % with					
		No Drug	Pyrimethamine in the Food (%)		Sulphadimidine in the Food (%)		
			0·01	0·02	0·1	0·2	0·4
A	Folic acid: 100 mg./kg.	90	92	25	—	—	—
	p-Aminobenzoic acid: 100 mg./kg.	100	—	40	83	100	92
	25 ,, ..	—	—	30	75	83	45
	Nil	80	33	8	42	0	0
B	Folic acid: 100 mg./kg.	—	50	33	75	33	17
	50 ,, ..	—	67	42	83	42	—
	p-Aminobenzoic acid: 100 mg./kg.	—	83	42	—	—	—
	25 ,,	—	92	17	—	—	—
	Nil	100	67	8	67	0	0

The antagonism by *p*-aminobenzoic acid was marked even with relatively high doses of sulphadimidine—well above those which in this series of experiments were required for complete protection against *E. tenella* infection. With folic acid there was some antagonism of the action of the sulphonamide, but much less than with *p*-aminobenzoic acid.

It was not possible from the data obtained with pyrimethamine to decide whether the action of either metabolite was competitive, but with sulphadimidine there was evidence for competition by both folic acid and *p*-aminobenzoic acid. For example, in experiment A, with 0.4% sulphadimidine in the food, a reduction in the dose of *p*-aminobenzoic acid from 100 mg./kg. to 25 mg./kg. resulted in a reduction in the degree of antagonism. With doses of *p*-aminobenzoic acid of 25 mg./kg. in the same experiment, antagonism was reduced when the amount of sulphadimidine in the food was increased from 0.2% to 0.4%. In experiment B, with folic acid in a dose of 100 mg./kg. the degree of inhibition varied inversely with the amount of sulphadimidine administered.

TABLE III

ANTAGONISM OF THE THERAPEUTIC EFFECT OF MIXTURES OF PYRIMETHAMINE AND SULPHADIMIDINE ON *E. TENELLA* BY FOLIC ACID

Experiment No.:	1	2	3	4
Pyrimethamine in food (%)..	0·004	0·004	0·004	0·005
Sulphadimidine ,, ,, ,, ..	0·05	0 05	0·04	0·05
	% Mortality			
Folic acid 100 mg./kg. ..	8	0	61	60
No metabolite	0	0	13	0
Untreated control	75	100	73	92

Synergism between sulphadimidine and pyrimethamine was apparent when relatively small doses of the two drugs were given together. Thus in the experiment illustrated in Table III, in which mixtures of sulphadimidine and pyrimethamine were given, almost complete therapeutic control was obtained with doses between one-fifth and one-eighth of those used to obtain the same effect in the experiments recorded in Table II. It might be expected, therefore, that correspondingly lower doses of the metabolites would be necessary to antagonize the action of the drugs—that is, the synergistic combination might be more readily antagonized by the metabolites.

Antagonism of mixtures of pyrimethamine and sulphadimidine using large doses of folic acid proved, however, to be incomplete and irregular, as shown in the four experiments recorded in Table III. Only in experiments 3 and 4 was the

percentage mortality appreciably raised by the administration of folic acid simultaneously with the drugs.

Antagonism of the effects of the mixture by *p*-aminobenzoic acid occurred more regularly, but as much of the metabolite was required when the mixture was used as when large doses of sulphadimidine were given alone. This is demonstrated in Table IV, where groups of chicks were given either 0.3% sulphadimidine, or a mixture of 0.05% sulphadimidine and 0.004% pyrimethamine. Both treatments gave complete protection (A, Table IV), and increasing doses of *p*-aminobenzoic acid progressively reduced the effects of treatment (B, C, and D).

The dose of *p*-aminobenzoic acid required to increase the mortality with either drug treatment to one half of that in the untreated controls (E, Table IV) was estimated by plotting the results

TABLE IV

ANTAGONISM ON *E. TENELLA* OF THE THERAPEUTIC EFFECT OF SULPHADIMIDINE, ALONE AND IN COMBINATION WITH PYRIMETHAMINE, BY *p*-AMINOBENZOIC ACID

Group	*p*-Aminobenzoic Acid Injected Twice Daily mg./kg.	% Mortality	
		0·3% Sulphadimidine	0·05% Sulphadimidine + 0·004% Pyrimethamine
A	0	0	0
B	10	25	8·3
C	25	41·6	16·6
D	50	66·7	58·3
E	Untreated control mortality, 83%		

graphically. It was found that 25 mg./kg. of *p*-aminobenzoic acid was required to produce this effect when 0.3% sulphadimidine alone was given. Similarly, 42.5 mg./kg. *p*-aminobenzoic acid was required when 0.05% sulphadimidine was given together with 0.004% pyrimethamine.

It was concluded that folic acid was a weak antagonist of both pyrimethamine and sulphadimidine. *p*-Aminobenzoic acid was an active antagonist of sulphadimidine, but resembled folic acid in having only a slight inhibitory effect upon pyrimethamine (Table II). When reduced doses of the two drugs were given together, there was no apparent increase in sensitivity to the antagonistic effects of the metabolites (Tables III and IV).

DISCUSSION

It is evident from Table I that the combination of low doses of pyrimethamine and sulphadimidine affects the development of *E. tenella* in the same way as the other effective drugs so far investigated —sulphaquinoxaline (Cuckler and Ott, 1947), nitrofurazone (Horton-Smith and Long, 1952), and sulphadimidine itself (Horton-Smith and Taylor, 1945).

The sensitivity of the late schizonts is probably due to several physiological factors. The intensely active division in this stage of the life-cycle of *E. tenella* must result in extreme susceptibility to any metabolic deficiency induced by drug action. If the difference in susceptibility to drugs of the early and late schizonts is due to quantitative differences in metabolic activity, then the earlier stages, which divide less actively, would be expected to be less sensitive. This, in fact, has been shown to occur (see Table I). It is also possible that qualitative metabolic differences exist; the different location of the first and second generation schizonts in the caecal tissue, and the different course of development of the resultant merozoites, lend support to this possibility.

The results of experiments with drugs and antagonists can provide only circumstantial evidence for the existence of metabolic reactions. The results of the experiments with folic acid and *p*-aminobenzoic acid described in this paper do, however, suggest similarities between the metabolism of *E. tenella* and plasmodia, to which they are systematically related.

Experiments with a number of organisms have provided evidence for the belief that pyrimethamine interferes with the conversion of folic to folinic acid (Hitchings, 1952). The success with which pyrimethamine can be antagonized by these metabolites seems to depend upon whether or not the organism ordinarily synthesizes them within the cell. The effect of pyrimethamine on *Streptococcus faecalis*, which utilizes exogenous sources of folinic acid, is easily reversed by the metabolite.

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 within the cell, and that the preformed metabolite is not available to it (Goodwin and 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. Therefore, like the plasmodia, *E. tenella* probably is unable to utilize preformed folic acid. It is likely that the slight antagonistic effect of folic acid upon sulphadimidine is due to the breakdown within the chick of folic acid to *p*-aminobenzoic acid. Rollo (1955) has suggested this for *Plasmodium gallinaceum*, where a similar phenomenon has been observed.

The slight antagonistic activity of *p*-aminobenzoic acid against the action of pyrimethamine

on *E. tenella* is in agreement with the experience of Thurston (1954) with *P. berghei*, and of Rollo (1955) with *P. gallinaceum*, but it has not been possible to conclude from the data presented above that the antagonism with *E. tenella* is competitive.

The comparison of the antagonism of *p*-aminobenzoic acid to sulphadimidine alone, and to mixtures of this sulphonamide and pyrimethamine, is of considerable interest. Both drug treatments give almost complete control of the infection, but when used together the amount of the sulphonamide required is very much reduced. It would therefore be expected that much smaller amounts of *p*-aminobenzoic acid would be required to antagonize the combination. Table IV shows that, on the contrary, almost the same dose of *p*-aminobenzoic acid is required to antagonize either 0.3% sulphadimidine or 0.05% sulphadimidine together with 0.004% pyrimethamine.

It has not so far proved possible to induce any marked degree of drug-resistance in *E. tenella* which would provide further information on the mode of action of drugs (Goodwin and Rollo, 1955). Theories of drug action must therefore remain conjectural and based mainly on analogy with other organisms. All the evidence so far available indicates that pyrimethamine and sulphonamides act upon the second generation schizonts of *E. tenella* in the same way as they act upon the schizonts of some species of *Plasmodium*.

Summary

1. A mixture of 0.004% pyrimethamine and 0.05% sulphadimidine in the food was administered to chicks at different periods after infection with *E. tenella*. Treatment of the early schizonts earlier than the 72nd hr. after infection resulted in a retardation of development. Treatment of the later schizonts resulted in complete suppression.

2. Antagonism of the effects of pyrimethamine and sulphadimidine was studied by the simultaneous administration of either folic acid or *p*-aminobenzoic acid. Folic acid was a weak antagonist of therapeutic amounts of both pyrimethamine and sulphadimidine. *p*-Aminobenzoic acid was an active antagonist of sulphadimidine, but resembled folic acid in having only a slight inhibitory effect upon pyrimethamine. When reduced doses of both drugs were given there was no apparent increase in the inhibitory effects of the two metabolites.

References

Cuckler, A. C., and Ott, W. H. (1947). *J. Parasit.*, **33**, Sec. 2 (Suppl.), 10.

Goodwin, L. G., and Rollo, I. M. (1955). *The Chemotherapy of Malaria, Piroplasmosis, Trypanosomiasis and Leishmaniasis* in *Biochemistry and Physiology of Protozoa*, **11**, Ed. Hutner, S. H., and Lwoff, A., New York: Academic Press.

Hitchings, G. H. (1952). *Trans. R. Soc. trop. Med. Hyg.*, **46**, 467.

Horton-Smith, C., and Boyland, E. (1946). *Brit. J. Pharmacol.*, **1**, 139.

—— and Long, P. L. (1952). *Brit. vet. J.*, **108**, 47.

—— and Taylor, E. L. (1945). *Vet. Rec.*, **57**, 35.

Joyner, L. P., and Kendall, S. B. (1955). *Nature, Lond.*, **176**, 975.

Kendall, S. B. (1956). *Proc. R. Soc. Med.* (in press).

—— and McCullough, F. S. (1952). *J. comp. Path.*, **62**, 116.

Rollo, I. M. (1955). *Brit. J. Pharmacol.*, **10**, 208.

Thurston, J. P. (1954). *Parasitology*, **44**, 99.

Wehr, E. E., and Farr, M. M. (1947). *Proc. helm. Soc. Wash.*, **14**, 12.