POTENTIATION OF SULPHADIMIDINE BY 2,4-DIAMINO-6,7-DI-ISOPROPYLPTERIDINE AND OTHER 6,7-DISUBSTITUTED 2,4-DIAMINOPTERIDINES AGAINST *EIMERIA* INFECTIONS OF CHICKS

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Six 2,4-diaminopteridines substituted in the 6 and 7 positions were tested as potentiators of sulphadimidine against *Eimeria tenella*. 2,4-Diamino-6,7-di-isopropylpteridine and 2,4-diamino-1'-propylindolo(2',3':6,7)pteridine were selected for further study. Both potentiated sulphadimidine against *E. tenella* and the di-isopropylpteridine potentiated it against *E. necatrix*. A 10% mixture of the di-isopropylpteridine with sulphadimidine (or other sulphonamides) appears to offer advantages in the treatment of *E. tenella* and still more of *E. necatrix*.

Coccidia are protozoal parasites of the alimentary tract of fowls. *Eimeria tenella* is the cause of caecal coccidiosis, a common and often lethal disease of young chickens. There are also several forms of intestinal coccidiosis which are becoming increasingly important in Britain as diseases of older fowls. The different forms of intestinal coccidiosis are caused by different species. A form of intestinal coccidiosis caused by *E. necatrix* is often responsible for a high death rate in flocks. Owing to their economic importance, the species *E. tenella* and *E. necatrix* were selected as the infective agents in the present work.

Certain sulphonamides exhibit pronounced anti-coccidial properties and have been used in the control of field outbreaks of caecal coccidiosis for a number of years. Horton-Smith and Taylor (1945) showed that sulphadimidine could be used successfully to treat established infections of *E. tenella* in chickens. Later Horton-Smith and Boyland (1946) and Waletzky and Hughes (1946) found that its inhibitory effect could be reversed by *p*-aminobenzoic acid. These observations suggested that "more potent inhibitors of this parasite (*E. tenella*) might be found among other more active structures known to interfere with the *p*-aminobenzoic acid-folic acid sequence" (Lux, 1954). Lux obtained encouraging results when 2,4-diaminopyrimidines or 2,4-diamino-

dihydro-s-triazines were mixed with sulphonamides, pyrimethamine being a particularly effective synergist. Joyner and Kendall (1955, 1956) confirmed this observation and showed that full therapeutic control of caecal coccidiosis was obtained with 0.005% pyrimethamine mixed with 0.05% sulphadimidine. Kendall and Joyner (1958) extended this work and showed that 2,4-diamino-6,7-di-isopropylpteridine and other folic acid antagonists potentiated sulphadimidine against *E. tenella*.

Collier, Campbell and Fitzgerald (1950) first described 2,4-diamino-6,7-di-isopropylpteridine as a vibrostatic agent, which potentiated sulphaguanidine in antibacterial activity, but not in toxicity to mice. Collier and Waterhouse (1950) described the antagonism between this pteridine and folic acid, and Collier and Phillips (1954) the antagonism between folinic acid and the pteridines used in the present investigation. Pteridines of this type were also shown to be active inhibitors of *Streptococcus faecalis* and *Staphylococcus aureus* (Collier and Waterhouse, 1952) and of *Plasmodium gallinaceum* (Bishop, 1954) and *P. berghei* (Thurston, 1954). The pteridines selected for the present trial were those shown most active in the above experiments.

We describe below extended observations showing potentiation of sulphadimidine by admixture with pteridines against *E. tenella*. We also describe the successful treatment with a

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mixture of 2,4-diamino-6,7-di-isopropylpteridine and sulphadimidine of chicks infected with a strain of *E. necatrix* not particularly susceptible to sulphonamides alone (Horton-Smith and Long, 1959).

METHODS

Chemicals.—Sulphadimidine and the six 2,4-diamino-pteridines were administered as bases and *p*-aminobenzoic acid as the acid. All the pteridines were substituted in the 6 and 7 positions, and are listed

TABLE I

EFFECTS OF SIX DIFFERENT PTERIDINES ON THE SULPHADIMIDINE TREATMENT OF CHICKS INFECTED WITH *E. TENELLA*

Groups 1 to 7 were given diets containing 0.05% sulphadimidine, and groups 1 to 6 diets containing 0.005% of the appropriate pteridine.

Group	Treatment	Experiment 1	
		No. of Birds	No. of Deaths
1	2,4-Diamino-6,7-di-isopropylpteridine+sulphadimidine	12	4
2	2,4-Diamino-6,7-di(cyclohexylmethyl)pteridine+sulphadimidine ..	12	8
3	2,4-Diamino-1'-methylindolo(2',3':6,7)pteridine+sulphadimidine ..	12	8
4	2,4-Diamino-1'-propylindolo(2',3':6,7)pteridine+sulphadimidine ..	12	2
5	2,4-Diamino-6,7-dibutylpteridine+sulphadimidine	12	9
6	2,4-Diamino-6,7-dipentylpteridine+sulphadimidine	12	9
7	Sulphadimidine alone	12	7
8	No treatment	12	12

TABLE II

EFFECTS OF 2,4-DIAMINO-6,7-DI-ISOPROPYL- AND 2,4-DIAMINO-1'-PROPYLINDOLO(2',3':6,7)-PTERIDINE ON THE SULPHADIMIDINE TREATMENT OF CHICKS INFECTED WITH *E. TENELLA*

Pteridine	Pteridine % in Diet	Sulphadimidine % in Diet	Experiment 2		Experiment 3		Total		
			No. of Birds	No. of Deaths	No. of Birds	No. of Deaths	No. of Birds	No. of Deaths	% Mortality
Di-isopropyl	0.00625	0.00625	9	7			9	7	78
	0.00125	0.0125	9	6			9	6	67
	0.0025	0.025	9	7	9	2	18	9	50
	0.005	0.05	9	1	9	2	18	3	17
	0.01	0.1			9	0	9	0	0
	0.005	—	9	7			9	7	78
	0.01	—			9	7	9	7	78
Propylindolo	0.000625	0.00625	8	6			8	6	75
	0.00125	0.0125	8	5			8	5	63
	0.0025	0.025	8	3	9	1	17	4	24
	0.005	0.05	8	1	9	1	17	2	12
	0.01	0.1			9	0	9	0	0
	0.005	—	9	7			9	7	78
	0.01	—			9	8	9	8	89
—	—	0.05	9	6	9	5	18	11	61
	—	0.1			9	2	9	2	22
	—	—	9	6	9	7	18	13	72

in Table I. The methods of synthesis of these pteridines were described by Campbell, Dunsmuir, and Fitzgerald (1950).

Tests in Chicks.—Rhode Island Red $\times$ White Leghorn chicks aged 8 to 16 days at time of infection were used in the experiments. Groups of chicks were maintained in electrically heated metal brooders with wire floors and were kept for 13 to 18 days after infection with oocysts. *E. tenella* and *E. necatrix* cultures were derived from single oocyst infections. Cultures of oocysts used for infecting chicks in these experiments were freshly collected where possible and never stored for longer than 45 days. The infective dose was 50,000–100,000 oocysts of either species. Medication commenced 48 hr. after infection with oocysts and continued for 3 days, that is, until the 5th day of the infection.

Full details of methods used in chemotherapy experiments and the composition of the diet have already been described (Horton-Smith and Long, 1956, 1959). In toxicity tests drugs were administered in the diet to uninfected birds of the same age and type, food intake being measured.

RESULTS

Therapeutic Tests against E. tenella

Comparison of Pteridines as Potentiators of Sulphadimidine.—In a preliminary experiment involving 96 chickens, the six pteridines listed in Table I were administered at a concentration of 0.005% in the diet, together with sulphadimidine at 0.05%. Only those groups of birds receiving the di-isopropyl- and propylindolo-pteridine in addition to sulphadimidine had lower mortalities than the control group receiving sulphadimidine alone (Table I).

The effects of these two active pteridines mixed with sulphadimidine were compared in two experiments involving 203 chicks (Table II). In these experiments the pteridines alone were ineffective, but mixtures of either pteridine and sulphadimidine were effective if given in sufficient concentration in the diet. The propylindolo-pteridine appeared slightly more active than the di-isopropylpteridine.

TABLE III
SUMMARY OF FOUR EXPERIMENTS ON 2,4-DIAMINO-6,7-DI-ISOPROPYLPTERIDINE AND SULPHADIMIDINE AND THEIR MIXTURES IN THE TREATMENT OF CHICKS INFECTED WITH *E. TENELLA*

Pteridine % in Diet	Sulpha- dimidine % in Diet	Experiment 4		Experiment 5		Experiment 6		Experiment 7		Total		
		No. of Birds	No. of Deaths	No. of Birds	No. of Deaths	No. of Birds	No. of Deaths	No. of Birds	No. of Deaths	No. of Birds	No. of Deaths	% Mortality
0.01	0.05			12	1	11	1			23	2	9
0.02	0.025							12	5	12	5	42
0.02	0.05			12	0	11	0	12	0	35	0	0
0.03	0.025							12	6	12	6	50
0.03	0.05			12	0	11	1	12	1	35	2	6
0.01	—	12	4			11	9			23	13	57
0.02	—	12	8			11	11	12	10	35	29	83
0.03	—					11	10	12	10	23	20	87
0.04	—	12	9							12	9	75
0.05	—			12	11					12	11	92
0.06	—	12	11							12	11	92
0.08	—	12	4							12	4	33
0.1	—	12	7	12	11					24	18	75
0.12	—	12	4							12	4	33
0.15	—			12	5	11	5	12	6	35	16	46
0.2	—			12	8					12	8	67
—	0.025							12	10	12	10	83
—	0.05					11	7	12	7	23	14	61
—	0.08			12	5	11	7			23	12	52
—	0.1	12	0	12	1	11	3	12	1	47	5	11
—	0.12	12	0	12	1					24	1	4
—	0.14	12	1							12	1	8
—	—	12	8	12	10	11	10	12	11	47	39	83

TABLE IV

EFFECTS OF 2,4-DIAMINO-6,7-DI-ISOPROPYL- AND 2,4-DIAMINO-1'-PROPYLINDOLO(2',3':6,7)-PTERIDINE ON THE SULPHADIMIDINE TREATMENT OF CHICKS INFECTED WITH *E. NECATRIX*

Pteridine		Sulpha- dimidine % in Diet	Experiment 8		Experiment 9		Total		
Name	% in Diet		No. of Birds	No. of Deaths	No. of Birds	No. of Deaths	No. of Birds	No. of Deaths	% Mortality
Di-isopropyl	0.0025	0.025	10	6			10	6	60
	0.005	0.05	10	9			10	9	90
	0.01	0.1	10	2	10	1	20	3	15
	0.015	0.15			10	0	10	0	0
	0.02	0.2	10	0	10	0	20	0	0
	0.02	—	9	4			9	4	44
Propylindolo	0.0025	0.025	9	6			9	6	67
	0.005	0.05	9	6			9	6	67
	0.01	0.1			11	9	11	9	82
	0.015	0.15			11	3	11	3	27
	0.02	0.2			11	7	11	7	64
	0.005	—	9	7			9	7	78
	—	—							
	—	0.2	9	7	11	8	20	15	75
	—	—	10	6	11	8	21	14	67

TABLE V

EFFECTS OF 2,4-DIAMINO-6,7-DI-ISOPROPYLPTERIDINE, SULPHADIMIDINE AND THEIR MIXTURES ON *E. NECATRIX* INFECTIONS OF CHICKS AND THE EFFECT OF *p*-AMINO BENZOIC ACID ON THEIR ACTION

% Drug in Diet			Experiment 10		Experiment 11		Experiment 12		Total		
Pteridine	Sulpha- dimidine	<i>p</i> -Amino- benzoic Acid	No. of Bi- ds	No. of Deaths	No. of Birds	No. of Deaths	No. of Birds	No. of Deaths	No. of Birds	No. of Deaths	% Mor- tality
0.01	0.1	—			9	1	8	1	17	2	12
0.01	0.15	—					8	0	8	0	0
0.01	0.2	—			9	1	8	0	17	1	6
0.015	0.1	—					8	0	8	0	0
0.015	0.15	—					8	0	8	0	0
0.015	0.2	—					8	0	8	0	0
0.02	0.1	—			9	1	8	1	17	2	12
0.02	0.15	—					8	0	8	0	0
0.02	0.2	—	8	0	9	0	8	0	25	0	0
—	1.0	0.01	8	7					8	7	88
0.02	0.2	0.01	8	4					8	4	50
0.01	—	—			9	4			9	4	44
0.02	—	—	8	8	9	8			17	16	94
0.04	—	—			9	5			9	5	56
—	1.0	—	8	2					8	2	25
—	0.2	—	8	8	9	5	8	4	25	17	68
—	—	—	7	7	8	6	9	5	24	18	75

2,4-Diamino-6,7-di-isopropylpteridine with Sulphadimidine.—It will be shown below that the di-isopropylpteridine is more active than the propylindolopterin as a sulphadimidine potentiator against *E. necatrix*. For this reason and because supplies were more readily available, the di-isopropylpteridine was selected for further study against *E. tenella*. The results of four experiments, involving 517 chickens, with this pteridine, sulphadimidine and their mixtures are summarized in Table III, from which it will be seen that each drug alone exerted some therapeutic action, though that of the pteridine was only just detectable at high dose levels. Mixtures of the two drugs were very much more effective, roughly equiactive treatments being: the pteridine alone, 0.15%, sulphadimidine alone, 0.08%, and a mixture of the pteridine 0.02% and sulphadimidine 0.025%.

Therapeutic Tests against *E. necatrix*

Comparison of the Di-isopropyl- and Propylindolopterin as Potentiators of Sulphadimidine.—In two experiments, involving 180 chickens, 10% mixtures of each of the pteridines with sulphadimidine were compared. The results expressed in Table IV indicate clearly that the di-isopropylpteridine strongly potentiated sulphadimidine and that the propylindolopterin potentiated it only slightly if at all. Sulphadimidine alone at 0.2% in the diet was inactive.

In a further three experiments, involving 224 chicks (Table V) the activities against *E. necatrix* infections of the di-isopropylpteridine, sulphadimidine and their mixtures were again compared and the results confirmed those shown in Table IV. Also as *p*-aminobenzoic acid is known to

antagonize sulphonamides, we investigated in one of these experiments its effect on the therapeutic action of sulphadimidine and of a mixture of the di-isopropylpteridine and sulphadimidine. There were 7 deaths in 8 birds treated with sulphadimidine and *p*-aminobenzoic acid, and 2 in the corresponding group treated with sulphadimidine alone. There were 4 deaths in a group of 8 infected birds treated with *p*-aminobenzoic acid as well as the pteridine-sulphadimidine mixture, as compared with no deaths in the group treated with the pteridine-sulphadimidine mixture alone (Table V, Experiment 10).

Toxicity Studies in Chicks

Diets containing various concentrations of a mixture of 1 part of the di-isopropylpteridine with 9 parts of sulphadimidine were fed to groups

TABLE VI
DIETARY INTAKE OF 2,4-DIAMINO-6,7-DI-ISOPROPYLPYRIDINE AND SULPHADIMIDINE BY TWO-WEEK-OLD CHICKS

Groups of 10 chicks were tested at each dose level.

% in Diet		Amount of Drug Consumed in mg.	
Pteridine	Sulphadimidine	Pteridine	Sulphadimidine
0.005	0.05	3.47	34.7
0.01	0.1	7.35	73.5
0.02	0.2	11.4	114
0.04	0.4	21.4	214
0.08	0.8	56.0	560

FIG. 1.—The effect of 2,4-diamino-6,7-di-isopropylpteridine-sulphadimidine mixtures in the diet on the weight gain of chicks during and after treatment. Treatment from Day 0 to Day 3. The horizontal line indicates the initial mean weight of all birds at Day 0 (107.8 g.). ●—● = Untreated control. ▲—▲ = Pteridine 0.005%, sulphadimidine 0.05%. ■—■ = Pteridine 0.01%, sulphadimidine 0.1%. ○—○ = Pteridine 0.02%, sulphadimidine 0.2%. △—△ = Pteridine 0.04%, sulphadimidine, 0.4%, □—□ = Pteridine 0.08%, sulphadimidine 0.8%.

of 10 two-week-old chicks for 3 days. The amount of drug consumed was calculated and the body weight of the chicks was observed. The amount of drug consumed is given in Table VI and the weight gain in Fig. 1, which shows that drug treatment at high concentrations depressed weight gain and this gradually recovered after the withdrawal of treatment.

DISCUSSION

In discussing the combined action of sulphadimidine and pteridines, the terminology described by Gaddum (1940) will be used. In this, the term "synergism" refers to any kind of joint action that is not antagonism, and "potentiation" refers to synergism that is greater than additive. From the experiments reported above it would be difficult to provide formal proof of potentiation by constructing a diagram of the type illustrated by Gaddum. However, a number of experiments strongly suggest that the pteridines potentiate sulphadimidine against both species of *Eimeria*. For *E. tenella* these experiments are Nos. 3, 5, 6, and for *E. necatrix* No. 11. In the other experiments, the mixtures generally show therapeutic action, but the absence of results at appropriate dose levels made it difficult to conclude that potentiation is present.

As strains of *E. necatrix* which do not respond to sulphonamide have been described (Horton-Smith and Long, 1959), the therapeutic action of the mixture of 2,4-diamino-6,7-di-isopropylpteridine and sulphonamide against this species might be of considerable importance. Mixtures of these drugs seem worthy of toxicological investigation with a view to field trial.

The brief experiment on toxicity of the di-isopropylpteridine-sulphonamide mixture in the diet of chicks reported above indicates some depression of growth, from which birds afterwards recovered, by a mixture fully active in the therapeutic tests. It is of interest that Collier, Hall and Waterhouse (1950) observed no potentiation by di-isopropylpteridine of sulphaguanidine toxicity in mice.

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