Brit. J. Pharmacol. (1946), **1**, 139.

SULPHONAMIDES IN THE TREATMENT OF CAECAL COCCIDIOSIS OF CHICKENS

BY

C. HORTON-SMITH AND E. BOYLAND

From the Veterinary Laboratory, Ministry of Agriculture and Fisheries

(Received April 3, 1946)

Beneficial results obtained with sulphamezathine (sulphadimethylpyrimidine) and sulphadiazine (sulphapyrimidine) in the treatment of caecal coccidiosis in chickens have been reported by Horton-Smith and Taylor (1942, 1943, 1945), who found the mortality among treated chicks to be reduced by 50 to 73 per cent of that among untreated controls in induced epidemics. Hawkins (1943) also obtained satisfactory results when a saturated sulphamezathine solution was substituted for drinking-water 98 hours after infection of chicks. Ripsom and Herrick (1945) found sulphadiazine to be effective when administered in the food, and Swales (1944) found that both sulphamezathine and sulphamerazine (sulphamethylpyrimidine) had a definite curative effect upon established infections even up to the time when intestinal haemorrhage appeared.

Although good results were obtained in the treatment of caecal coccidiosis in chickens by dosing with sulphamezathine incorporated in the food and as a saturated solution in drinking-water, neither of these methods of dosing was perfect. It is difficult in practice to obtain a completely uniform mixture of a small amount of drug with dry food. The low solubility of the drug makes the preparation of saturated solutions of sulphamezathine from the powder troublesome, and such solutions are apt to vary in strength according to the hardness or softness of the water used and the method of preparing the solution. Sulphapyrazine and all the sulphapyrimidine derivatives are more soluble in hard water than in distilled or soft water because the calcium salts are more soluble than the free drugs. Thus a saturated solution in distilled water contained 0.06 per cent sulphamezathine, while a similar solution made in Weybridge tap water at the same time contained 0.13 per cent.

EXPERIMENTAL

I. *The Sodium Salt of Sulphamezathine as a Convenient and Effective Means of Administering the Drug*

1. *Method of Preparation.*—The readily soluble sodium salts of sulphonamides can be prepared by dissolving the drugs in a little over the theoretical amount of sodium hydroxide solution or sodium carbonate solution. If sodium carbonate is used the solution must be boiled. A concentrated stock solution of sodium sulphamezathine was prepared

by dissolving 160 g. of sulphamezathine in 200 ml. of 3N.NaOH (12 per cent) and diluting to one litre. Such a solution is marketed by Imperial Chemical (Pharmaceuticals) Ltd. The solution was diluted, just before use, to the concentration required. With hard waters the diluted material slowly produces a deposit of calcium carbonate on exposure to air. An additional advantage of the sodium salts is that one equivalent of alkali is given with the drug, so that the sulphonamides and their acetyl derivatives are unlikely to be deposited in the kidney.

2. *Therapeutic Experiments with Sodium Sulphamezathine after Artificial Infection.*—The satisfactory action of sodium sulphamezathine was demonstrated in the following experiments. In each of four experiments one-week-old Light Sussex × Rhode Island Red cockerels were infected by one administration into the crops by pipette of equal heavy infective doses of sporulated oocysts of *Eimeria tenella.* Groups of chicks were given access to different concentrations of sodium sulphamezathine at intervals of 24, 48, 72 and 96 hours after infection. Treatment, which consisted of substituting the solutions to be tested for the ordinary drinking-water, was carried on for approximately seven days after the death of all the controls. The percentage mortality from acute caecal coccidiosis is shown in Table I.

TABLE I

PERCENTAGE MORTALITY FROM CAECAL COCCIDIOSIS AMONG GROUPS OF 50 CHICKS AFTER DELAYED TREATMENT WITH VARIOUS STRENGTHS OF SODIUM SULPHAMEZATHINE SOLUTION

Time that treatment was delayed after infection (in hours)	Percentage mortality at various strengths of sodium sulphamezathine solution, and in control groups.					
	Water control	0·025 per cent	0·05 per cent	0·1 per cent	0·15 per cent	0·2 per cent
24	82	40	14	0	not tested	0
48	100	not tested	14	0	0	0
72	90	not tested	46	26	10	0
96	100	not tested	76	not tested	not tested	50

The surviving chicks were in good condition when they were killed 16 days after the deaths of the last controls. Post-mortem examination revealed scattered lesions of coccidiosis in the caeca of chicks receiving the 0.025, 0.05, 0.1 and 0.15 per cent solutions at all times after infection and in all groups treated from the ninety-sixth hour after infection. The experiments point to the 0.2 per cent solution as being the most effective and reliable in controlling the disease. (Percentage strength of all solutions is in grams per 100 ml.)

3. *Sodium Sulphamezathine in the Control of an Induced Epidemic.*—A single experiment was carried out with a view to testing the efficacy of three strengths of sodium sulphamezathine solution in controlling such an epidemic as might occur in the field.

Sixty-eight three-week-old chicks were placed on sawdust litter in each of four pens. The litter of each pen was infected with equal quantities of a heavy suspension of sporulated oocysts of *Eimeria tenella.* Blood appeared in the faeces of the chicks of each pen five days after the infection of the litter; the chicks were then randomized by transferring 17 chicks (i.e., a quarter of the number in each pen) to each of the other three pens. Each pen then contained equal numbers of chicks made up of 17 chicks originally present plus 17 from each of the other three pens. This procedure was adopted in an attempt

to correct differences in the original distribution of the oocysts and therefore in infections of the chicks. The chicks in three pens were then given 0.05, 0.1 and 0.2 per cent solutions of sodium sulphamezathine respectively in the place of drinking-water. The chicks in the fourth pen served as controls and continued to receive ordinary drinking-water. The results of this experiment are summarized in Table II.

TABLE II

CONTROL OF AN INDUCED EPIDEMIC OF CAECAL COCCIDIOSIS PRODUCED BY *Eimeria tenella* IN GROUPS OF 68 CHICKS BY THE SUBSTITUTION OF SODIUM SULPHAMEZATHINE SOLUTION OF DIFFERENT STRENGTHS FOR THE DRINKING-WATER

No. of days after commencement of treatment.	Deaths from caecal coccidiosis among chicks on various percentages of sodium sulphamezathine solution and in a control group.			
	0·05 per cent	0·1 per cent	0·2 per cent	Water
0	5	2	3	3
1	3	3	–	7
2	2	–	–	5
3	–	–	–	3
4	–	–	2	2
5	–	–	1	1
6	4	–	–	2
7	3	3	–	11
8	7	2	–	14
9	4	2	–	3
10	–	–	–	3
11	–	–	–	1
12	–	–	–	1
13	–	–	–	–
14	–	–	–	–
Total number of deaths	28	12	6	56

The results compare favourably with those previously reported by Horton-Smith and Taylor (1945) for sulphamezathine, and again show the superiority of the 0.2 per cent solution.

4. *Duration of Treatment with Sodium Sulphamezathine.*—An experiment was carried out to determine the minimum time of treatment necessary for effective results. Four groups of 13 chicks each were heavily infected with sporulated oocysts of *Eimeria tenella.* Three groups were given 0.2 per cent sodium sulphamezathine 48 hours after being infected. Ordinary drinking-water was substituted for the solution 24, 48 and 72 hours respectively after the deaths of the fourth group of untreated controls which succumbed to acute caecal coccidiosis on the fifth day. Two deaths from coccidiosis occurred in the 24-hour group and one in the 48-hour group. No deaths occurred in the group that was returned to water three days after the deaths of the controls. The surviving chicks were killed 16 days after the deaths of the controls, and post-mortem findings showed them to be normal apart from some minor lesions in a few of the caeca. From these results it would appear that a treatment carried on for three days after the last deaths from coccidiosis is probably sufficient to control an outbreak of the disease.

Recent work (Asplin, Boyland, and Horton-Smith, 1946) has shown that there are dangers in using sulphamezathine over long periods. If young chicks receive sulphamezathine for two or more weeks the blood-clotting time is lengthened, possibly owing to the decreased synthesis of vitamin K in the gut. In a few cases multiple petechial

haemorrhages of the intestines have been found post mortem after prolonged dosing. Dosing with sulphapyrimidines, particularly sulphamezathine, causes hyperplasia of the seminiferous tubules of the testes of cockerels. The testicular enlargement is accompanied by the precocious development of the comb and wattles. It is recommended that the duration of treatment with sulphamezathine should not exceed one week.

II. *Comparison of Various Sulphonamides with Sodium Sulphamezathine in the Treatment of Caecal Coccidiosis*

1. *Method Used in Tests.*—The therapeutic effects of solutions of sulphaguanidine and of the sodium salts of sulphadiazine, sulphamethylpyrimidine, sulphapyrazine, and sulphathiazole were compared with those obtained with solutions of sodium sulphamezathine as substitutes for drinking-water. Equal heavy infective doses of sporulated oocysts of *Eimeria tenella* were administered to groups of chicks which later were given the different drugs at different concentrations. One group, the control, remained on drinking-water. In each case one group was treated with sodium sulphamezathine and served as a standard for comparison. The results of these trials are shown in Table III.

2. *Tests made with Sulphapyridine and Sulphaguanidine.*—A test was carried out with a ration to which 1 per cent sulphapyridine was added. Groups of chicks had access to the medicated ration 48 hours before and 48 hours after being heavily infected with sporulated oocysts of *Eimeria tenella.* The treatment did not prevent deaths from coccidiosis ; 9 of the 10 chicks in each of the treated groups and in the untreated control group succumbed.

Levine (1941), Farr and Allen (1942), and Horton-Smith (1942) have shown that chicks receiving a ration containing 1 to 2 per cent of sulphaguanidine are protected against infection with caecal coccidiosis provided treatment is instituted before the ingestion of the infective dose of oocysts. In view of these findings an experiment was carried out with sulphaguanidine incorporated in the ration on lines similar to those described in Section I for sodium sulphamezathine. Fifty-four chickens were placed in a single pen and the litter was infected. Thirteen days later 10 chickens died of acute caecal coccidiosis. The remaining 44 chickens were then distributed in two groups of 22 chickens each. One group was treated with 2 per cent sulphaguanidine in the food and the other continued to receive a normal ration. Treatment exerted little or no effect on the course of the infection, as 20 of the treated and 21 of the untreated chickens succumbed to the disease.

3. *Resistance of Chickens to Caecal Coccidiosis after Recovery due to Treatment with the Sodium Salts of Sulphamezathine and Sulphapyrazine.*—Previous work (Horton-Smith and Taylor, 1945) showed that a strong immunity to coccidiosis developed in chicks which had survived a previous epidemic as a result of treatment. A single experiment was carried out to find whether similar results were obtained when solutions of sodium sulphamezathine and sodium sulphapyrazine were used in treatment.

Three heavily infected groups of 10 chicks each were treated with 0.05, 0.1, and 0.2 per cent sodium sulphamezathine respectively. Another group of 10 chicks was treated with 0.1 per cent sodium sulphapyrazine solution. Treatment was commenced 24 hours after the chicks had been infected. A fifth group received a similar infection, but was maintained on water and served as a control group for the first part of the experiment. A sixth group was not infected, maintained on water, and served as a control for the second part of the experiment. Only one of the chicks from the 0.05 per cent sodium sulphamezathine group died from acute caecal coccidiosis as compared with 10 chicks from the control group. All surviving chicks were restored to water five days after the deaths of the controls. Five days later all the chicks, together with the second control group,

TABLE III

COMPARISON OF SODIUM SULPHAMEZATHINE AND OTHER SULPHONAMIDES INTRODUCED INTO THE DRINKING-WATER IN THE TREATMENT OF CAECAL COCCIDIOSIS.

Test substance (as Na-Salt, except Sulphaguanidine)	Time Treatment was delayed after Administration of Oocysts (in hrs.)	Percentage Strength of solution of Test Substance	Mortality after Treatment with Test Substance	Control Groups				
				Mortality after Treatment with Sodium Sulphamezathine of Various Strengths				Mortality among Untreated Control Chicks
				·025	·05	0·1	0·2	
Sulphadiazine (a)	24	0·025	13/16	11/16	3/16	–	–	16/16
	24	0·05	13/16					
		0·10	0/10					
(b)	24	0·05	1/8	2/7	0/7	–	–	8/8
		0·10	2/7					
Sulphathiazole	24	0·10	10/10	-	–	0/10	0/10	10/10
		0·30	7/10					
Sulphamethyl-pyrimidine (Sulphamerazine)	24	0·05	9/13	2/13	0/13	0/13	–	13/13
		0·1	8/13					
		0·2	0/13					
Sulphapyrazine	24 (1)	0·025	1/7	2/7	1/7	0/7	–	9/10
		0·05	0/7					
		0·1	0/7					
	24 (2)	0·1	0/10	4/10	1/10	0/10	–	10/10
		0·2	0/10					
	48	0·025	1/6	6/6	0/6	0/6	–	5/6
		0·05	1/6					
		0·1	0/6					
	72 (1)	0·2	0/10	4/10	1/10	0/10	–	9/10
	72 (2)	0·025	2/7	6/7	3/7	3/7	–	6/7
		0·05	2/7					
		0·1	0/7					
Sulphaguanidine	24	0·1	4/8	–	–	–	–	6/8
	48	0·1	10/16	–	–	–	–	–
	72	0·1	14/16	–	–	6/16	4/16	–

received heavy doses of sporulated oocysts. The results of this experiment are set out in Table IV.

TABLE IV

RESISTANCE OF CHICKS, WHICH HAD SURVIVED INFECTION AS A RESULT OF TREATMENT WITH SODIUM SULPHAMEZATHINE AND SODIUM SULPHAPYRAZINE, TO A SECOND HEAVY DOSE OF OOCYSTS ADMINISTERED FIVE DAYS AFTER THEIR RETURN TO WATER

Solution or Water	No. of Chicks in Group	Deaths during Treatment	Deaths from Infection after 5 days on Water
Sulphamezathine 0·05%	10	1	1
„ „ 0·1%	10	0	1
„ „ 0·2%	10	0	1
Water (1st controls)	10	10	–
Water (2nd controls)*	8	–	7
Sulphapyrazine 0·1%	10	0	3
Water (1st controls)	10	10	–
Water (2nd controls)*	10	–	9

*The second controls remained uninfected until the chicks which survived the first infection as a result of treatment had received their second heavy infection of oocysts.

4. *Discussion of Results of the Comparisons Made.*—Sulphapyridine, sulphathiazole, and sulphaguanidine were all ineffective in treatment of established infections. Sulphadiazine and sulphamethylpyrimidine are both less effective than sulphamezathine. Thus in Table III it will be seen that the mortality was similar in groups treated with 0.1 per cent sulphadiazine and 0.025 per cent sulphamezathine. Similarly, the concentration of sulphamethylpyrimidine required for complete protection (0.2 per cent) was much higher than that of sulphamezathine (0.05 per cent) in groups of chicks dosed at the same time. These results suggest that sulphadiazine and sulphamethylpyrimidine have only about one-quarter of the therapeutic effect of sulphamezathine. The results obtained with sulphadiazine were rather erratic.

The results were interesting in that they showed one sulphonamide, sulphapyrazine, to be more effective than sulphamezathine. In comparative experiments 0.1 per cent sulphapyrazine and 0.2 per cent sulphamezathine have prevented symptoms in almost all chicks even when treatment has been delayed to 72 hours after infection. In practice it is recommended that infections should be treated by the substitution of 0.2 per cent sodium sulphamezathine or of 0.1 per cent sodium sulphapyrazine for the drinking-water as soon as coccidiosis is diagnosed. The work of Asplin, Boyland, and Horton-Smith (1946) has shown that sulphapyrazine and sulphathiazole have no ill effect on the clotting power of the blood or on the testes comparable with that of sulphamezathine.

III. *Concentration of the Drugs in the Blood*

In an endeavour to gather some information on the mode of action of sulphonamides on the parasite within the epithelial cells we made a study of the concentrations of the various drugs in the blood of chickens of different ages and at various times of day (Tables V and VI).

The concentrations of the sulphonamides in the blood of chickens were estimated by a modification of the method described by Bratten and Marshall (1939). Blood was taken

from the wing vein of adult birds and from the hearts of recently killed young chicks. The blood (0.5 ml.) was allowed to haemolyse in 6.5 ml. of distilled water for fifteen minutes, after which 1.0 ml. of 30-per-cent trichloracetic acid was added and the precipitated proteins were removed by filtration. The amount of free drug was estimated by comparison of the colour produced in 2 ml. of the filtrate after the addition of 0.2 ml. of 0.1 per cent sodium nitrite, 0.2 ml. of 0.5 per cent ammonium sulphamate, and finally 0.2 ml. of 0.1 per cent N-(1-naphthyl)-ethylene diamine hydrochloride, with the colour developed by similar treatment of standard solutions. The amount of total drug was estimated by the same procedure after heating 2 ml. of the blood filtrate with 0.2 ml. of 2N hydrochloric acid at 100° C. for twenty minutes. The "free drug" refers to that which is estimated directly and is probably present as such in the blood. The "total" figures refer to the amount estimated after hydrolysis, and include the drug which is acetylated or in other combined forms.

The individual variations in concentration of sulphamezathine in the blood of groups of chickens (Table V) are considerable, but the differences between

TABLE V

CONCENTRATIONS OF SULPHAMEZATHINE AND SULPHADIAZINE IN MG. PER 100 ML. OF BLOOD OF CHICKENS RECEIVING DIFFERENT STRENGTHS OF THESE DRUGS IN WINTER

Drug	Times Samples were Taken	Concentration of Drug in the Drinking-water								
		0·05 per cent			0·1 per cent			0·2 per cent		
		Free Drug	Total Drug	Percentage of Total Present as Free Drug	Free Drug	Total Drug	Percentage of Total Present as Free Drug	Free Drug	Total Drug	Percentage of Total Present as Free Drug
Sulphamezathine	7.30 a.m.	1·4	2·0	70	4·9	6·3	78	8·5	9·9	86
		2·5	3·2	78	3·3	4·3	77	5·7	5·8	98
		1·9	2·4	79	3·9	5·2	75	6·3	8·0	79
		1·6	2·5	64	2·3	2·5	92	5·5	7·1	78
Mean		1·8	2·5	73	3·6	4·6	80	6·5	7·7	85
	3.30 p.m.	3·6	6·1	59	9·1	9·9	93	17·9	19·6	91
		2·0	2·2	91	5·7	5·8	98	10·0	12·0	83
		2·7	4·4	62	4·3	6·6	65	15·8	17·3	91
		2·5	2·9	86	4·9	5·1	96	12·2	14·6	83
Mean		2·7	3·9	75	6·0	6·8	88	14·0	15·9	87
Sulphadiazine	Morning	1·5	2·7	56	1·6	1·7	94	10·3	11·1	93
		1·4	2·5	56	3·8	5·0	76	3·0	3·1	97
		2·6	2·7	96	1·5	3·2	47	0·5	0·6	83
		3·1	3·4	91	7·3	8·1	90	0·4	0·7	57
Mean		2·1	2·8	75	3·5	4·5	76	3·5	3·8	82
	Evening	4·9	5·9	83	1·4	2·4	58	1·3	2·4	54
		5·4	6·7	81	1·3	2·6	50	12·6	15·9	79
		3·7	3·9	95	9·5	9·8	97	14·1	4·7	96
		4·1	4·6	89	6·7	8·5	79	6·1	6·3	97
Mean		4·5	5·3	87	4·7	5·9	71	8·3	9·8	82

different levels of dosing and between morning and evening are quite clear and greatly exceed individual variation. The concentrations of sulphadiazine showed much individual variation except when a low level (0.05 per cent in drinking-water) was administered. There was also considerable variation in curative effect with sulphadiazine ; the two effects may be related and be due to some individual chickens not drinking or not absorbing the more concentrated sulphadiazine solutions. The blood concentrations given in subsequent tables and Figure 1 are

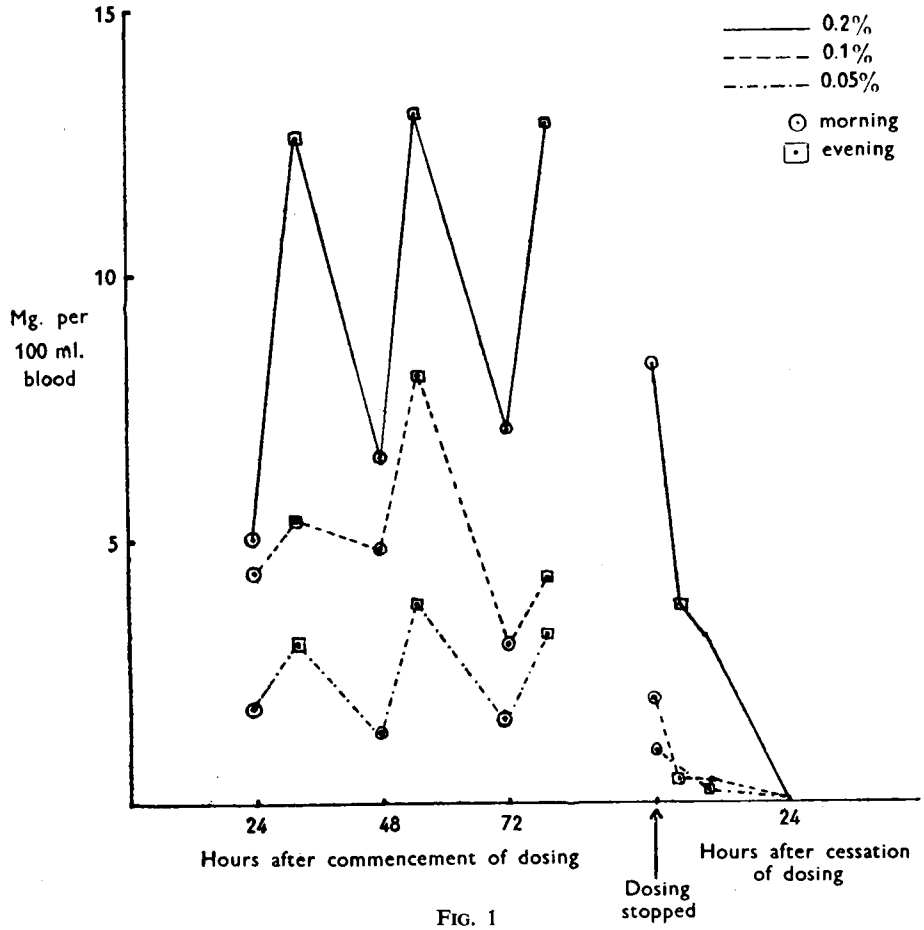

FIG. 1

all mean values for four chickens sampled at the same time. The individual variations in blood concentrations in the case of the other drugs examined were much less than with sulphadiazine. The variation in concentration of sulpha-mezathine with time of day and the fall in concentration after cessation of dosing are shown in Table V (one-week old chicks) and Figure 1 (twelve-week old chickens).

TABLE VI

CONCENTRATIONS OF SULPHAMEZATHINE, SULPHAPYRAZINE AND SULPHATHIAZOLE (IN MG. PER 100 ML.) IN THE BLOOD OF CHICKS IN WINTER AND SUMMER. CONCENTRATIONS WERE DETERMINED AT LEAST 48 HOURS AFTER COMMENCEMENT OF DOSING AND ARE MEAN VALUES FOR FOUR CHICKS SAMPLED AT THE SAME TIME.

Drug and Season When Dosed		Age of Chickens and Time of Day Samples were Taken	Concentration of Drug in Drinking Water								
			0·05%			0·10%			0·20%		
			Free Drug	Total Drug	Percentage of Total Present as Free Drug	Free Drug	Total Drug	Percentage of Total Present as Free Drug	Free Drug	Total Drug	Percentage of Total Present as Free Drug
Sulphamezathine	Winter	One week old:									
		Morning	1·8	2·4	75	3·7	5·2	71	7·1	9·7	73
		Evening	3·0	3·8	79	5·8	9·4	62	10·1	13·0	78
		Two weeks old:									
		Morning	2·0	2·8	72	3·6	5·0	72	9·3	13·9	67
		Evening	3·1	4·2	72	5·2	7·9	66	9·3	13·1	71
		Twelve weeks old:									
		Morning	1·4	2·3	61	3·9	5·6	70	6·6	7·8	85
		Evening	3·4	5·2	65	5·7	8·4	68	10·4	14·4	72
	Summer	Two weeks old:									
		Morning	3·9	4·2	93	8·0	10·2	78	10·9	11·9	91
		Evening	3·3	4·0	83	8·6	11·0	78	10·5	12·6	83
		Twelve weeks old:									
		Morning	1·9	2·8	68	5·1	5·5	93	9·2	10·2	90
		Evening	2·0	3·0	66	6·1	6·4	95	9·2	10·2	90
Sulphapyrazine	Summer	Two weeks old:									
		Morning	5·7	6·4	89	8·7	9·4	92	16·8	17·9	94
		Evening	3·9	4·5	87	6·0	6·6	91	14·4	15·6	92
		Twelve weeks old:									
		Morning	1·7	2·0	85	3·7	4·3	86	9·9	11·3	88
		Evening	2·7	3·6	75	4·2	4·8	87	10·3	12·0	86
Sulphathiazole	Summer	Two weeks old:									
		Morning	1·0	1·1	91	0·9	0·9	100	2·4	2·6	92
		Evening	1·1	1·5	73	0·9	1·4	65	0·9	1·5	60

When the drug was administered as a 0.2 per cent solution the blood concentration was very rarely below 5 mg. per 100 ml. As the most satisfactory therapeutic results were obtained by dosing with the 0.2 per cent solution it appears that a blood concentration of 5 to 10 mg. per 100 ml. is necessary for optimal results. There was a tendency for the blood concentrations to be higher in younger chicks when dosed in summer. The blood concentrations are of the order of one-twentieth of the concentration of drug in the drinking-water. Table VI indicates the mean values of concentrations obtained in chicks of different ages in winter and summer. The blood concentrations varied with the concentrations of drug in the drinking-water and to some extent with the time of day. Samples of blood taken at 7.30 a.m. (G.M.T.) in the winter had consistently lower concentrations than similar samples taken at 3.30 p.m. (G.M.T.). This is presumably due to the chicks drinking less during the hours of darkness, so that the blood concentrations fall during the night. Similar determinations carried out in the summer (June) showed no regular variations between morning (6.30 a.m., G.M.T.) and afternoon (2.30 p.m., G.M.T.) values.

The concentrations of sulphapyrazine in young chicks (two weeks old) were considerably higher than in older (twelve weeks old) chickens treated at the same time. The difference with age is similar but much greater than that noticed with sulphamezathine. The concentrations of sulphapyrazine were higher than those for comparable dosage levels of sulphamezathine and the higher therapeutic action is probably due to the higher blood concentrations obtained. It is remarkable that sulphapyrazine should be so well absorbed when it is poorly absorbed from the alimentary tract of mammals (cf. Robinson *et al.*, 1943)

The differences in blood concentrations obtained in chickens and canaries after oral administration of sulphonamides have been described by Marshall (1945). In his experiments sulphapyridine derivatives appeared to be more easily absorbed and more slowly excreted than was sulphathiazole, and the antimalarial activity was correlated with the attainable blood concentration. In our experiments the values for sulphathiazole, which are given in Table VI, show that this drug is poorly absorbed or quickly excreted, and possibly has no therapeutic action for this reason.

Sulphapyridine, given as 1 per cent of the chickens' ration, was fairly well absorbed. The blood concentrations were as follows: morning values were, free 6.2 and total 6.6 mg. per 100 ml., while evening values were, free 6.5 and total 7.6 mg. per 100 ml. Sulphapyridine is ineffective in curing the disease presumably because it does not inhibit the growth of coccidia.

Sulphaguanidine is relatively poorly absorbed from the alimentary tract of mammals and is therefore used in the treatment of intestinal diseases. When a solution (0.1 per cent) of this drug was given to chicks the blood concentrations appeared to rise very slowly (Table VII). Even so, the concentrations obtained after five days were insufficient to have a curative or prophylactic effect as experiments have shown. When 1 to 2 per cent of sulphaguanidine was administered in

the food it was found to have a prophylactic effect, and under these conditions the blood concentrations were quite high and appeared to be rapidly attained. The method of determination would not differentiate between sulphaguanidine and any derivative (with an intact aminophenyl group) which might be formed from sulphaguanidine either in the gut or in the body.

TABLE VII

CONCENTRATION OF SULPHAGUANIDINE IN THE BLOOD OF CHICKS AT DIFFERENT TIMES FOLLOWING THE COMMENCEMENT OF DOSING

Concentration of Drug and the Means of Dosing	Time after Commencement of Dosing in Hours	Sulphaguanidine mgs. per 100 ml. Blood	
		Free	Total
0·1% in drinking-water	16	1·8	2·1
	26	1·9	2·6
	42	2·2	2·5
	66	2·6	3·2
	84	3·3	4·5
	108	3·2	3·7
	192	4·0	4·4
1% in food	24	7·6	8·1
	96	5·7	7·5
	120	5·3	6·1
2% in food	24	20·0	21·8
	96	20·9	24·6
	120	19·5	27·3

IV. *Antagonism of Sulphonamide Action by* p-*Aminobenzoic Acid*

The bacteriostatic action of sulphonamides on streptococci has been shown to be inhibited *in vitro* by *p*-aminobenzoic acid (Woods, 1940), and in view of experiments on the neutralization of the therapeutic action of sulphonamides in small animals (Selbie, 1940) it would seem probable that a similar mechanism would operate *in vivo*. An experiment was carried out to test this assumption in the case of avian coccidiosis. Groups of chicks, heavily infected with coccidia, were given (1) sulphamezathine and sulphapyrazine solutions, (2) these solutions with the addition of different concentrations of *p*-aminobenzoic acid (PAB), and (3) PAB only. As *Eimeria tenella* cannot be grown *in vitro* the effect can only be shown in infected chicks.

The results (Table VIII) show that the therapeutic effect of 0.2 per cent sulphamezathine was largely neutralized by the presence of 0.01 per cent PAB and that of 0.1 per cent sulphamezathine by 0.005 per cent PAB. This means that the therapeutic action of ten molecules is neutralized by one molecule of PAB. The action of 0.05 per cent sulphapyrazine was neutralized by 0.002 per cent PAB. Compared with the effect of sulphanilamide on streptococci *in vitro*, where the amount of PAB required to neutralize the effect of the drug is small (i.e., one molecule of PAB nullifying the effect of several thousand molecules of the drug), the amount of PAB required to neutralize sulphamezathine or sulphapyrazine is large (i.e., ten molecules are neutralized by one molecule), the ratio

being of the same order as that found in the treatment of streptococcal infections of mice (Selbie, 1940). The action of sulphamezathine in the animal appears to be dependent on the use of PAB by the coccidia.

TABLE VIII

THE EFFECT OF DIFFERENT CONCENTRATIONS OF PAB ON THE THERAPEUTIC EFFECT OF SULPHAMEZATHINE AND SULPHAPYRAZINE

Percentage Concentrations of Drugs and of PAB			Mortality
Sulphamezathine	Sulphapyrazine	PAB	
Exp. A.			
–	–	0·1	6/6
0·2	–	–	0/7
0·2	–	0·1	8/8
0·2	–	0·01	5/8
0·2	–	0·001	1/7
Exp. B.			
Controls –	–	–	10/10
0·1	–	–	0/10
–	–	0·02	10/10
0·1	–	0·02	10/10
0·1	–	0·01	10/10
0·1	–	0·005	6/10
0·1	–	0·002	6/10
–	0·05	–	0/10
–	0·05	0·005	8/10
–	0·05	0·002	9/10

DISCUSSION

The results show clearly that a protozoal infection can be cured by certain heterocyclic sulphonamides and that the therapeutic action resembles the antibacterial action of sulphonamides in being neutralized by *p*-aminobenzoic acid. The two most effective drugs, sulphamezathine and sulphapyrazine, differ from each other in some properties to a greater extent than does sulphamezathine from the less effective compounds like sulphadiazine and sulphamethyldiazine. Thus sulphapyrazine is much less soluble in neutral solution than the three sulphapyrimidine compounds. The effects of the different drugs are summarized in Table IX.

The difference in the therapeutic efficiencies of the drugs is no doubt partly due to differences in absorption or excretion ; thus, with the effective compounds, the therapeutic value increases with the concentration of the drug in the blood for a given dosage level. But absorption (or delay in excretion) is not the only factor, because sulphapyridine is absorbed but has no therapeutic action ; it may be assumed that this drug is not toxic to the parasite. Consideration of the similarity of the effects of sulphathiazole and sulphadiazine on the growth of bacteria *in vitro* would suggest that the variation in, or lack of, effect on coccidia

is due to the poor absorption of the drug by the host. In our present state of knowledge of sulphonamide metabolism the unpredictable variations in absorption between different drugs in different species, and in chickens of different ages, can only be found by trial. From the fact that only drugs which are absorbed appear to be effective in the treatment of chickens already infected, and consider-

TABLE IX

EFFECT OF DIFFERENT SULPHONAMIDES ON INFECTIONS OF *Eimeria tenella* IN CHICKENS

Drug	Effect on *Eimeria tenella* Infection	Number of Hours after Infection that Treatment was Delayed.
Sulphanilamide	No prophylactic effect (Levine, 1939)	–
Sulphapyridine	No prophylactic effect although absorbed	–
Sulphathiazole	1-2% was effective when administered in food before or at time of infection (Ripsom and Herrick, 1945). Dosing gives low blood concentrations.	–
Sulphaguanidine	2% in food had prophylactic effect if fed to chickens before infection.	–
Sodium Sulphadiazine	0·1% in drinking-water had some therapeutic effect.	24*
Sodium Sulphamerazine	0·2% in drinking-water had some therapeutic effect.	24*
Sodium Sulphamezathine	0·2% in drinking-water had excellent therapeutic effect.	24–72
Sodium Sulphapyrazine	0·1% in drinking-water had excellent therapeutic effect.	24–72

*Longer delays in treatment were not carried out with these sulphonamides.

ing that the life-cycle of the protozoon is principally intracellular, it would seem that effective treatment for caecal coccidiosis consists in attacking the parasite in the tissues.

SUMMARY

1. Caecal coccidiosis in chickens caused by the protozoon *Eimeria tenella* can be effectively treated by substituting solutions of 0.2 per cent sodium sulphadimethylpyrimidine (sodium sulphamezathine) or 0.1 per cent sodium sulphapyrazine for drinking-water. Sulphadiazine and sulphamethylpyrimidine (sulphamerazine) will also cure the infection but are not so reliable as the other drugs. Sulphathiazole and sulphapyridine are completely ineffective in preventing symptoms of caecal coccidiosis in infected chicks.

2. The relative therapeutic effectiveness of sulphapyrazine and the sulphapyrimidines depends upon the blood concentrations obtained. Effective doses result in blood concentrations of 5 to 10 mg. per 100 ml.

3. Chickens which survive an infection of caecal coccidiosis as a result of treatment with sulphamezathine or sulphapyrazine are resistant to subsequent infections with *Eimeria tenella* within the period tested.

4. The action of sulphamezathine and sulphapyrazine on coccidia in the chicken is antagonized by *p*-aminobenzoic acid.

REFERENCES

Asplin, F. D., Boyland, E., and Horton-Smith, C. (1946). *Biochem. J.*, **40**, ii.
Bratton, A. C., and Marshall, E. K. (1939). *J. biol. Chem.*, **128**, 537.
Farr, M. M., and Allen, R. W. (1942). *J. Amer. vet med. Ass.*, **100**, 47.
Hawkins, P. A. (1943). *Poult. Sci.*, **22**, 459.
Horton-Smith, C. (1942). *Vet. Record*, **54**, 259.
Horton-Smith, C., and Taylor, E. L. (1942). *Vet. Record*, **54**, 516.
Horton-Smith, C., and Taylor, E. L. (1943). *Vet. Record*, **55**, 109.
Horton-Smith, C., and Taylor, E. L. (1945). *Vet. Record*, **57**, 35.
Levine, P. P. (1939). *Cornell Vet.*, **29**, 309.
Levine, P. P. (1941). *Cornell Vet.*, **31**, 107.
Marshall, P. B. (1945). *J. Pharmacol.*, **84**, 1.
Ripsom, C. A., and Herrick, C. A. (1945). *Jour. Parasit.*, **31**, 98.
Robinson, H. J., Siegel, H., and Graessie, O. B. (1943). *J. Pharmacol.*, **79**, 354.
Selbie, F. R. (1940). *Brit. J. exp. Path.*, **21**, 90.
Swales, W. E. (1944). *Canad. J. Res. D.*, **22**, 131.
Woods, D. D. (1940). *Brit. J. exp. Path.*, **21**, 74.

ACKNOWLEDGEMENTS

We are indebted to Messrs. Imperial Chemical (Pharmaceuticals), Ltd., for a supply of sulphamezathine, and to Messrs. May & Baker and Messrs. Burroughs Wellcome for other sulphonamides used in these experiments.