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THE EFFECTS OF PYRIMIDINE SULPHONAMIDE DERIVATIVES UPON THE BLOOD-CLOTTING SYSTEM AND TESTES OF CHICKS AND THE BREEDING CAPACITY OF ADULT FOWLS

BY

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Caecal coccidiosis in chickens can be effectively treated with certain sulphonamides, particularly sulphamezathine (sulphadimethylpyrimidine) (Horton-Smith and Taylor, 1943) and sulphapyrazine (Horton-Smith and Boyland, 1946). Sulphamezathine was extensively used in the treatment of outbreaks of caecal coccidiosis on poultry farms during the 1945 and 1946 seasons. On some of these farms a few chicks did not thrive during treatment and a small proportion died. The only apparent abnormality found on post-mortem examination of such chicks was the presence of haemorrhages of the intestines and occasionally of the liver and skeletal muscles. The appearance of such chicks resembled that described in vitamin K deficiency and the haemorrhagic form of sweet clover disease. In order to investigate this syndrome, sulphamezathine was dosed to chicks experimentally and was found to produce hypoprothrombinaemia in most chicks. Internal haemorrhages, similar to those found in the field, were observed in a small proportion of cases.

In the course of these experiments cockerels dosed with sulphamezathine for periods longer than 10 days showed premature development of combs and wattles; such cockerels had enlarged testes with hypertrophy of the seminiferous tubules. A brief account of these effects has been published (Asplin, Boyland, and Horton-Smith, 1946).

EXPERIMENTAL

Except where otherwise stated Rhode Island Red (R.I.R.) chicks of mixed sexes or Light Sussex × Rhode Island Red cockerels were kept on dry standard mash or placed on experimental diets when one week old. The sulphonamides were administered as solutions of the sodium salts in the drinking water. In some experiments chicks were weighed at weekly intervals to determine the effect of treatment on body growth. Blood from recently killed chicks was taken by heart puncture and placed in tubes containing sodium oxalate. The plasma was separated by centrifugation and the prothrombin time determined by a modification of the method of Witts and Hobson (1940); the only change in the method was the omission of lecithin. The prothrombin times were determined on undiluted plasma

and on plasma diluted four times with saline, in the presence of Russell viper venom (0.004 per cent (w/v)) and $CaCl_2$ (0.1 M.).

A lengthening of clotting time was observed regularly in chickens dosed with 0.2 per cent (w/v) sulphamezathine; in some cockerels, similarly dosed, there was also enlargement of the combs and wattles as shown in Fig. 1. Cockerels with enlarged combs invariably had enlarged testes, which was clearly shown when the individual gonad weights were expressed in mg. per 100 g. body weight.

TABLE I

THE EFFECT OF ADDITION OF VARIOUS SULPHONAMIDES TO THE DRINKING WATER OF GROUPS EACH OF 7 YOUNG CHICKS FOR 28 DAYS

All values are means for the groups

Drug 0·2 per cent (w/v)	Sex	Body weights at end of dosing g.	Haemoglobin g. per 100 ml.	Prothrombin time (sec.)		Gonad weight (mg.)	
				undil.	dil. × 4	actual	mg. per 100 g. b. wt.
Sulphadiazine	M	255	8·9	26·4	47·3	29·7	11·6
	F	282				136·5	48·0
Sulphathiazole	M	321	10·2	26·2	47·2	48·3	15·0
	F	284				133·7	47·1
Sulphamerazine	M	290·2	9·5	27·3	49·6	54·6	18·8
	F	228·8				122·3	53·4
Sulphamezathine	M	224·5	8·9	36·2	81·4	63·1	28·1
	F	265·5				149·5	56·3
Sulphapyrazine	M	293·4	9·3	22·9	43·0	30·9	10·5
	F	315·8				146·8	43·3
Water control	M	275·4	9·8	23·2	41·8	39·4	14·3
	F	289·2				137·2	47·5

Results of a typical experiment in which groups, each of seven 7-day-old R.I.R. chicks, were dosed with different sulphonamides are shown in Table I. Sulphamezathine treatment appears to reduce body growth more than any of the other drugs and has a much greater effect on prothrombin clotting time and testes weight than any of the other compounds tested. None of the compounds used

TABLE II

EFFECT OF ADMINISTRATION OF SULPHAMEZATHINE AND *p*-AMINOBENZOIC ACID IN DRINKING WATER FOR 28 DAYS ON TESTES WEIGHT AND PLASMA PROTHROMBIN OF COCKERELS

Treatment	No. of chicks	Prothrombin time sec. (means) undil. plasma	Testes weight (mg.) (means)	
			actual	mg. per 100 g. body wt.
Sulphamezathine 0·2%	6	43·3	51·6	24·2
Sulphamezathine 0·2% + *p*-aminobenzoic acid 0·02%	6	39·3	77·4	34·4
p-aminobenzoic acid 0·02%	6	26·3	43·8	13·7
Water control	6	26·1	34·7	13·0

in a concentration of 0.2 per cent (w/v) had any marked effect upon the blood haemoglobin concentration or female gonad weight.

The therapeutic effect of sulphamezathine or sulphapyrazine on coccidiosis is neutralized by *p*-aminobenzoic acid. The results shown in Table II demonstrate that neither the effect of sulphamezathine on the development of the testes nor on the prothrombin is neutralized by addition of *p*-aminobenzoic acid. The figures for body growth (Table III) show that sulphamezathine reduced the rate of body growth and that normal growth was not restored by addition of *p*-aminobenzoic acid. These results indicate that the effects of sulphamezathine on blood prothrombin and on body growth are not due to a reduction in vitamin synthesis owing to partial sterilization of the gut, although if *p*-aminobenzoic acid is more

TABLE III

AVERAGE WEIGHT IN GRAMS OF CHICKS AT 7-DAY INTERVALS

Treatment	No. of chicks	7 days (i.e., initial wt.)	14 days	21 days	28 days	35 days
Sulphamezathine	11	55·0	74·2	113·6	164·0	204·1
Sulphamezathine + *p*-aminobenzoic acid	12	56·2	80·8	118·3	162·5	211·9
p-aminobenzoic acid	12	54·6	97·7	144·8	226·9	277·6
Water control	12	54·9	97·3	149·0	229·7	275·8

completely absorbed than sulphamezathine the latter might still inhibit vitamin synthesis by intestinal flora ; it was, however, found that the administration of sodium succinyl sulphathiazole (0.2 per cent (w/v) of drinking water) had no effect on body growth, prothrombin time, or testes size of chicks.

TABLE IV

THE EFFECT OF VITAMIN K ON THE BLOOD PROTHROMBIN LEVELS OF CHICKS TREATED WITH SULPHAMEZATHINE

(a) Groups of 7-day-old chicks under experiment for 17 days

Food	Supplements	No. of chicks	Prothrombin time (sec.)	
			undil.	dil. × 4
Standard mash	None	8	23·3	39·4
Standard mash	Sulphamezathine (0·2%) in drinking water	8	32·9	55·5
Vitamin K deficient	None	6	53·5	82·2
Vitamin K deficient	Sulphamezathine (0·2%) in drinking water	6	46·1	71·8
Vitamin K deficient	Sulphamezathine (0·2%) in drinking water, menaphthone (10 mg. per kg.) in food	6	19·25	33·2
Vitamin K deficient	Menaphthone (10 mg. per kg.) in food	5	18·2	34·2

TABLE IV—*continued.*

(b) Groups of 8-day-old chicks under experiment for 15 days

Supplement	No. of chicks	Final body wt. (grams)	Prothrombin time		Testes weight (mg.)	
			undil.	dil. × 4	actual	mg./100 g. body wt.
Sulphamezathine, 0·2% sol.	6	125·5	36·8	54·7	28·4	21·9
Sulphamezathine, 0·2% sol. + menaphthone, 10 mg./kg. food	7	126·7	29·0	41·1	39·6	30·4
Menaphthone, 10 mg./kg. food	7	135·3	25·4	37·1	15·4	11·3
None	7	140·7	27·9	38·7	16·9	12·0

(c) Groups of 9-week-old cockerels under experiment for 26 days

Supplement	No. of chicks	Increase in wt. during expt. (g.)	Prothrombin time (sec.)	
			undil.	dil. × 4
Sulphamezathine, 0·2% sol.	10	353·6	71·1	124·6
Sulphamezathine, 0·2% sol. + synkavit 0·0025%	10	262·6	30·7	51·2
Sulphamezathine 0·3% + synkavit 0·0025%	10	257·9	30·1	56·6
None	6	388·7	33·1	60·8

The effect of sulphamezathine on blood prothrombin is similar to but not so great as that produced by vitamin K deficiency, and it was completely neutralized by the addition of menaphthone (vitamin K) to the diet (Table IVa); the effect of sulphamezathine on testes development, however, was not influenced by vitamin K (Table IVb). The effect of combining soluble vitamin K (calcium-2-methyl-1 : 4-dihydroxynaphthalinediphosphoric ether, "synkavit" Roche) and sulphamezathine in a single solution gave similar results (Table IVc). However, in a further experiment (Table VI, expt. E) three of a group of seven older chickens which received sulphamezathine (0.25 g. per kg. body weight daily), combined with vitamin K (menaphthone, 25 mg. per kg. body weight), showed intestinal haemorrhages on post-mortem examination; this indicates that haemorrhagic lesions in older birds may not be entirely due to deficiency of prothrombin.

Sulphamezathine has a rapid effect upon the blood clotting mechanism similar to that of dicoumarin (3 : 3′-methylenebis-4-hydroxycoumarin), the causative agent of sweet clover disease in cattle. Sulphamezathine appears to be quantitatively less effective than dicoumarin (Table V) because a single dose of 2 g. per kg. body weight sulphamezathine had less effect on prothrombin time than 150 mg. per kg. dicoumarin. The effect of sulphamezathine on prothrombin level disappears between four and ten days after the withdrawal of sulphamezathine.

Although the effect of sulphamezathine on blood clotting develops rapidly, the addition of varying amounts of sodium sulphamezathine to plasma *in vitro* was found to have no effect on the prothrombin time. Sulphamezathine had no

TABLE V

THE EFFECT OF LARGE DOSES OF SULPHAMEZATHINE AND DICOUMARIN ON PLASMA PROTHROMBIN OF 23-DAY-OLD CHICKS

Prothrombin times (sec.)

Sulphamezathine, 3 doses of 1 g./kg. body wt. 42, 26 and 18 hr. before sampling		Sulphamezathine, single dose of 2 g./kg. body wt. 18 hr. before sampling		Dicoumarin, 150 mg. per kg. body wt. 18 hr. before sampling		Normal control	
undil.	dil. × 4	undil.	dil. × 4	undil.	dil. × 4	undil.	dil. × 4
24·2	61·0	39·4	68·8	48·0	113·8	19·2	44·6
27·2	69·4	44·2	110·4	33·8	78·4	24·4	49·6
26·4	58·2	46·8	87·6	54·4	112·2	25·0	36·4
28·6	58·4	29·0	61·4	43·0	118·2	30·4	58·8
		30·8	69·0			25·2	42·0
Mean 26·6	61·75	38·0	79·4	44·8	105·7	24·8	46·3

direct anticoagulant action similar to that of heparin or of dyes such as chlorazol fast pink ; the effect on blood clotting *in vivo* may therefore be independent of the actual concentration of the drug at the time when the blood is taken.

Haemorrhagic lesions in chicks

A proportion of chicks dosed with sulphamezathine have developed multiple haemorrhages, while no such haemorrhages have been seen in a large number of control chicks, or in chicks treated with other sulphonamides. It is therefore clear that this syndrome is induced by sulphamezathine, and the experiments described suggest that the action is similar to that of sweet clover in producing haemorrhagic disease in cattle. In various experiments a total of ninety-one 7-day-old chicks were given a 0.2 per cent (w/v) solution of sulphamezathine for periods of 21–35 days ; intestinal haemorrhages were present in 6 cases only and there were no deaths attributable to haemorrhage. In all chicks of this group lesions were confined to the intestines ; they varied from very numerous petechiae extending throughout the greater part of the length of the intestine to relatively few larger haemorrhages of 3–5 mm. diameter. The haemorrhages were readily visible through the serosa. The lesions occurred in all layers of the bowel wall, but were most frequent in the submucosa.

Chickens treated for caecal coccidiosis on farms are usually between 6 and 12 weeks old. All field cases of sulphamezathine poisoning, characterized by haemorrhagic lesions, which have been brought to our attention have occurred in chickens of this age.

The incidence of haemorrhagic lesions shown in Table VI indicates that chickens of 6 weeks old are more susceptible to the haemorrhagic effect of sulphamezathine than are younger chicks ; for instance, in experiment E, 7 out of 13 chickens killed after 3 weeks' treatment with 0.25 g. sulphamezathine per kg.

TABLE VI

THE EFFECT OF ADDITION OF SULPHAMEZATHINE TO DRINKING WATER OF 6–12-WEEK-OLD CHICKENS

Exper.	No. of chickens	Age	Dose	Period of treatment	Effect
A	8	7 days	0·2% sol.	100 days	Two died from haemorrhage on 44th and 50th days. Six survived and remained healthy
B	9	9 weeks	0·2% sol.	97 days	None died. Precocious development of comb and wattles and male plumage
C	11	6 weeks	0·2% sol.	62 days	Four died from haemorrhage on 18th, 36th and 39th days; two killed on 63rd day showed small intestinal haemorrhages. Five normal
D	15	6 weeks	0·2% sol.	70 days	Three died, 26th, 44th and 56th days, with haemorrhages; four killed showed haemorrhages. Eight normal
E	7	6 weeks	0·25 g. per kg. body wt. daily	20 days	None died. Four showed haemorrhages when killed on 21st day
	6	6 weeks	0·25 g. per kg. body wt. daily + menaphthone 25 mg. per kg. body wt. daily	20 days	None died. Three showed internal haemorrhages when killed on 21st day
F	7	10 weeks	0·2% sol.	15 days	No deaths or gross lesions
	6	10 weeks	0·2% sol. + synkavit 50 mg. per l.	15 days	No deaths or gross lesions

body weight daily were found to possess intestinal haemorrhages. Furthermore, 9 out of the 43 chickens receiving 0.2 per cent (w/v) sulphamezathine for 62–100 days (experiments A, B, C, and D) died as the result of internal haemorrhage. Most of the chickens remained in apparently good health after prolonged administration of sulphamezathine, but post-mortem examination of them frequently revealed intestinal haemorrhages similar to those of younger chicks. The lesions in chickens dying as a result of haemorrhage were widespread and severe. Haemorrhages were invariably present in the intestinal tract; the liver also was frequently bespattered with haemorrhagic foci. Massive haemorrhages were seen in the skeletal muscles, particularly in the muscle groups of the thighs and breasts. The ventral border of the gizzard was the site of haemorrhage in two fowls. Persistent bleeding from feather follicles resulted in the deaths of two chicks. It appeared that haemorrhages might occur at any site, but were most frequent at those points particularly exposed to trauma.

Testicular hyperplasia

Precocious sexual development of cockerels was often evident after 10–14 days' dosing with sulphamezathine. With continued dosing, growth of combs and wattles proceeded rapidly, so that after 4 weeks a stage of development normally seen in cockerels of 2–3 months old was reached.

The development of secondary sexual characters was evident in only a proportion of the dosed cockerels. Macroscopic enlargement of testes was invariably present in chicks showing obvious comb and wattle development. Of the 71 chicks receiving sulphamezathine for 21–28 days, 43 had enlarged testes, 21 were approximately normal in size, and 7 were abnormally small. The testes weights of some sulphamezathine-treated chicks after 4 weeks were as much as five times greater than those of untreated control chicks. Sulphamezathine regularly inhibited the body growth of chicks (Tables I and III) and in general it was the chicks which had the best growth rates which developed testicular hyperplasia. A proportion of treated chicks grew very poorly, and in these the testes were very small and inactive.

Sulphamezathine-treated chicks which responded with testicular hyperplasia had haemoglobin and prothrombin levels not significantly different from those of chicks failing to respond in this way (Table VII).

Quantitative data on the enlargement of the testes of cockerels has been given. Table I shows that there was no comparable enlargement of the female gonad after dosing for 4 weeks with any of the sulphonamides tested, and microscopic examination revealed no abnormalities. Sulphamezathine has more effect on the testes than either sulphamerazine (sulphamethylpyrimidine) or sulphadiazine (sulphapyrimidine). Although the average testes weights of chicks dosed with sulphadiazine (Table I) do not indicate enlargement, microscopic examination revealed that the testes of some treated chicks were actively stimulated. Sulphathiazole, succinyl sulphathiazole, and sulphapyrazine had no such action.

The effect was not neutralized by simultaneous dosing of an amount of *p*-aminobenzoic acid which completely neutralized the therapeutic action of the drug in caecal coccidiosis (Horton-Smith and Boyland, 1946 ; cf. Table II) ; nor was it neutralized by menaphthone (Table IVb).

Microscopical examination of sections of the cockerel testes showed that the main action of the drug was on the seminiferous tubules which were hyperplastic (Figs. 2 and 3). The interstitial tissue remained scanty and appeared to be compressed by the proliferating tubules, but active interstitial tissue was present and this was the probable source of the male hormone. The enlarged seminiferous tubules were lined with spermatocytes in active mitotic division. In the lumen of the tubules there were degenerative forms so that the centre of the tubules sometimes contained a "necrotic area." Sometimes (as in Fig. 4) the hyperplasia occurred in a few tubules, whilst the other tubules remained almost normal and immature.

TABLE VII

A COMPARISON OF THE BODY WEIGHTS, PROTHROMBIN TIMES AND HAEMOGLOBIN LEVELS OF COCKEREL CHICKS WHICH RESPONDED TO SULPHAMEZATHINE DOSING BY PRECOCIOUS SEXUAL DEVELOPMENT, AND THOSE FAILING TO DO SO

Group of cockerels	Body weight (grams)	Testes weight mg./100 g. body weight	Prothrombin clotting times		Haemoglobin g. per 100 ml.
			undil.	dil. × 4	
Dosed sulphamezathine Good body growth Marked comb development	295	39·6	51·2	73·4	7·84
Dosed sulphamezathine Poor growth No comb development	166	8·5	48·7	68·6	7·54
Control—undosed ..	308	13·6	34·8	49·6	7·58

The stimulus to sexual development provided by sulphamezathine to cockerels disappeared upon withdrawal of the drug, so that it became impossible to distinguish between dosed and undosed cockerels 3 weeks after the end of the treatment.

Extracts of testes from normal and sulphamezathine-treated cockerels appeared to have no hyaluronidase activity. This is perhaps to be expected, as the immature testes contained no mature spermatozoa.

Microscopic examination of the thyroid glands of the sulphamezathine-dosed chicks failed to reveal any change.

The effects of certain dimethylpyrimidines on cockerel chicks

Only those sulphonamides which contain a pyrimidine ring, i.e., sulphamezathine, sulphamerazine, and sulphadiazine, appear to have any action on the testes. Sulphamezathine, which contains a dimethylpyrimidine ring, was much more active in this respect than either sulphamerazine or sulphadiazine. Groups of 7-day-old chicks were dosed with 0.2 per cent (w/v) solutions of 2-hydroxy-4:6-dimethylpyrimidine (white form), 2-hydroxy-4:6-dimethylpyrimidine (yellow form), 2-amino-4:6-dimethylpyrimidine, and 2-mercapto-4:6-dimethylpyrimidine in place of drinking water. None of these substances had any effect on testes size or prothrombin time. The mercapto compound reduced the rate of growth, but the hydroxy compounds and the amino derivative had no effect on body growth.

The effect of sulphamezathine on drakelets

A group of ten 8-day-old drakelets was dosed with 0.2 per cent (w/v) sulphamezathine in their drinking water for 28 days. Dry food of the same composition as used for chicks was made into a damp mash with sulphamezathine solution. The sulphamezathine was readily consumed. After 28 days' treatment all drakelets were killed and examined. There was no evidence of intestinal haemorrhages in any of the birds. The treatment reduced

the growth rate, but had no effect on testes weight or on prothrombin and haemoglobin levels. The sulphonamide level of the blood of ducklings dosed with 0.2 per cent (w/v) sulphamezathine (4.4 mg. per 100 ml.) was lower than that of chicks receiving the same dose.

The effect of sulphamezathine on the sex organs of male rats

Groups of ten rats weighing 50–60 g. were placed on diets containing sulphonamide (as 0.1 per cent of the dry matter of the diet). Half the rats in each group were castrated just before being put on the diets. The rats were weighed at the beginning of the experiment and at intervals throughout the period of dosing. The data (Table VIII) show that growth was considerably inhibited by sulphapyrazine—rats on this drug hardly grew at all during the last two weeks of treatment—while sulphathiazole had no, and sulphamezathine only a slight, inhibitory effect on growth.

TABLE VIII

THE EFFECT OF DOSING SULPHONAMIDES FOR 34 DAYS ON SEX ORGANS OF MALE RATS

Nature of animals	Mean body wt., g. Beginning	Mean body wt., g. End	Testes, mg. mean	Seminal vesicles and prostate, mg. mean
Undosed normal rats	64	143	850	272
Undosed castrated rats	66	146	—	90
Normal rats dosed with sulphamezathine	55	119	880	426
Castrated rats dosed with sulphamezathine	63	127	—	88
Normal rats dosed with sulphapyrazine	53	71	480	79
Castrated rats dosed with sulphapyrazine	58	86	—	90
Normal rats dosed with sulphathiazole	56	139	960	292
Castrated rats dosed with sulphathiazole	59	135	—	85

None of the drugs produced any marked enlargement of the testes of normal rats, or enlargement of the seminal vesicles and prostate of castrated rats. Sulphamezathine thus has no male hormone action on adult rats and does not cause testicular hypertrophy. On the other hand, the seminal vesicles and prostate of normal rats treated with sulphamezathine were larger, and those of rats treated with sulphapyrazine smaller, than the same organs of rats fed on normal diet or on a diet containing sulphathiazole. Thus although sulphamezathine had no effect on castrated rats and very little on the weight of testes of normal rats, it caused enlargement of the seminal vesicles and prostate presumably by stimulation of the testes.

When rats were dosed with sulphamezathine from birth, hyperplasia of the testes occurred which was obvious in rats weighing less than 50 g. Pregnant rats were fed on a diet containing 0.1 per cent sulphamezathine and the diet was given to the mother and young after birth. The weights of the testes of young rats of different ages dosed with sulphamezathine are compared with the weights of normal animals in Table IX. While the testes weight per 100 g. body weight increases from 380 to 700 mg. in normal rats from 16 to 52 g. in weight, the weights vary from 600 to 1300 mg. per 100 g. body weight in sulphamezathine-dosed rats from 17 to 54 g. body weight. Increases in size of seminal vesicles and prostate occurred in young rats, but these were less than the increases in size of these organs in adult rats dosed with sulphamezathine. The increase in testicle size (Figs. 5 and 6) is similar to the effect on the cockerel testes.

TABLE IX

THE WEIGHT OF SEX ORGANS OF YOUNG NORMAL MALE RATS AND YOUNG RATS DOSED WITH SULPHAMEZATHINE FROM BIRTH

A. NORMAL RATS

Wt. of rat, g.	16	22	29(2)	32(4)	46(2)	52(2)
Wt. of testes, mg.	61	97	120	163	303	363
Wt. of seminal vesicles and prostate, mg.	30	32	47	55	66	71
Testes wt. as mg./100 g. body wt.	380	440	410	510	660	700
Seminal vesicle and prostate wt. as mg./100 g. body wt. ..	187	146	162	172	144	196

B. RATS DOSED WITH SULPHAMEZATHINE

Wt. of rats, g.	17	29(2)	35	38	47(2)	50(2)	54
Wt. of testes, mg.	103	221	302	330	609	585	702
Wt. of seminal vesicles and prostate, mg.	34	48	65	99	94	104	93
Testes wt. as mg./100 g. body wt.	600	760	860	870	1290	1170	1300
Seminal vesicle and prostate wt. as mg./100 g. body wt. ..	200	165	186	260	200	204	173

Some figures are averages of groups of rats of the same weight. The number in such groups is indicated in parentheses.

The toxicity of sulphamezathine in adult fowls

Two groups of six mature R.I.R. pullets and cockerels were dosed by mouth with a 16 per cent (w/v) solution of sodium sulphamezathine at a rate of 0.25 g. per kg. body weight daily for 14 days. The pullets which had been in full production ceased to lay on the 3rd day. The fowls became sick and deaths of pullets occurred on the 6th, 7th, 9th, and 16th days, and of cockerels on the 7th, 12th, 13th, 15th, and 16th days. The surviving cockerel remained apparently normal and when killed on the 21st day showed no apparent lesions. The two surviving pullets were very sick on the 15th day but improved by the 21st day, when they were killed. Both had low haemoglobin concentrations (7.5 and 8.1 g. per 100 ml.). On post-mortem examination both were found to have retained fully formed eggs in the oviduct; the eggs were coated with a fibrinous deposit and appeared to have remained stationary for some time.

Obvious symptoms were only apparent during the four days prior to death: although appetite was decreased, the crop remained distended with food; the feathers around the vent were matted and stained with yellow faecal material; the excreta of some birds had a bright greenish-yellow colour which resembled that of fowls affected with fowl typhoid. Some fowls were disinclined to move about and locomotor ataxia was apparent for about 24 hours before death. The colour of the comb and wattles changed in some instances to a bright salmon-red; in one fowl the head appendages became blue and shrivelled. Body temperatures were elevated up to a maximum of 110.4° F. On examination of the cloacal mucosa petechiae could sometimes be observed. In cockerels the genital eminences became green or brown as a result of extravasated blood.

Post-mortem examination revealed widespread lesions. Haemorrhages were invariably present in the intestinal tract, extending from the duodenum to the cloaca (Fig. 7); the gizzard lining of one fowl also was involved. The haemorrhages were similar to those seen in younger chickens and varied in size from petechiae to plaques of 5 mm. diameter. The lesions in the large intestine became ulcerated as a result of erosion of the mucosa; the contents of the intestines were mucoid and sometimes bloodstained; petechiae were present in the serous membranes, in the abdominal fat, at the base of the heart, and of the gizzard. The liver was somewhat enlarged and friable; large wedge-shaped infarcts were seen; the

colour of the liver of two fowls was changed to green. The kidneys were enlarged and the colour changed as in the liver. Numerous basophilic erythrocytes appeared before death and the haemoglobin level fell sharply. The spleen was slightly enlarged in some cases, and contained subcapsular haemorrhages. The ovarian follicles were soft and flabby, and in one case yolk material was present in the peritoneal cavity. A coliform organism was isolated in pure culture from the spleens of three fowls and heart blood of one. It is probable that the infecting organisms invaded the blood from the gut through the intestinal lesions. Similar effects on the gut were produced when sulphamezathine (250 mg./kg. body weight per day) was administered by feeding in capsules (cf. Fig. 8).

The effect of sulphamezathine on the breeding capacity of fowls

Four 10-month-old R.I.R. cockerels were dosed with 0.15 g. per kg. body weight daily. Examination of semen collected from these cockerels, before dosing commenced and at intervals during treatment, failed to reveal any change in the yield of semen, or the density, form, or motility of sperms. One cockerel died after the 36th dose with lesions of sulpha-

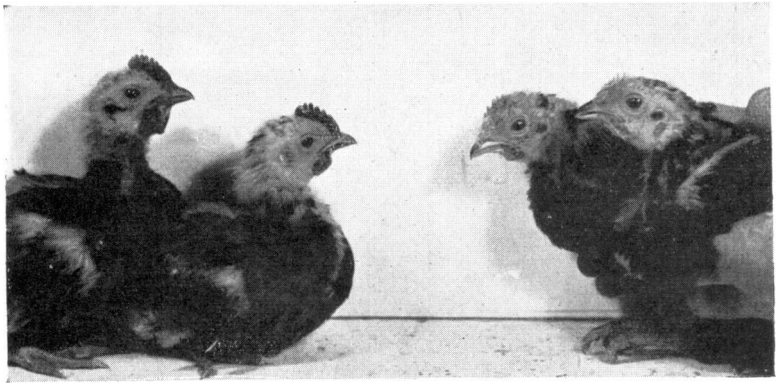

FIG. 1.—35-day-old cockerels. The two on the left were dosed with sulphamezathine for the last 28 days and show enlarged combs and wattles as compared with the two controls on the right.

mezathine poisoning. After the 50th dose the cockerels were mated to three pens (A, B and C) each of six virgin pullets. The cockerel in pen A died on the 58th day of dosing from sulphamezathine poisoning. Fourteen eggs laid by pullets in pen A between the 53rd and 58th day were incubated: 5 were fertile and 3 hatched normal chicks. Dosing of the cockerels in pens B and C continued until 102 doses had been given but had no apparent effect upon fertility.

A group of six laying hens and a cockerel were dosed with 0.2 per cent (w/v) sulphamezathine in their drinking water. Egg production ceased by the 3rd day, 2 eggs were laid on the 10th day; all six hens had resumed laying by the 15th day. During the succeeding 6 weeks 42 eggs were incubated of which 5 were infertile; 28 normal chicks were hatched from the remaining 37 fertile eggs. Thus the dosing had only a temporary effect on the egg-laying of fowls.

DISCUSSION

As sulphamezathine and sulphapyrazine are so useful in treatment of coccidiosis in chickens it is of value to know what other effects these drugs may have.

FIG. 2.—Section of testis from normal 35-day-old cockerel. H and E ×660.

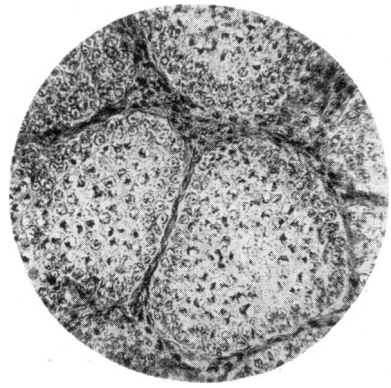

FIG. 3.—Section of testis from 35-day-old cockerel, dosed with sulphamezathine (0.2% in drinking water) for 28 days, showing hypertrophy of tubules. H and E×660.

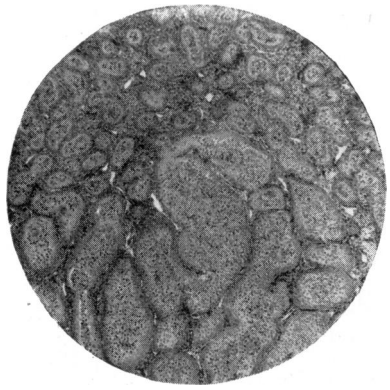

FIG. 4.—Section of testis from 35-day-old cockerel dosed with sulphamezathine showing hypertrophy of part of the tubular tissue while other tubules are not enlarged. H and E×100.

FIG. 5.—Section of normal testis from rat weighing 16g. H and E×660.

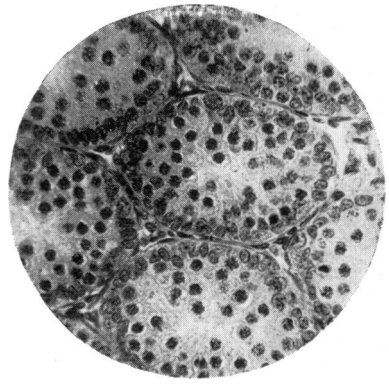

FIG. 6.—Section of testis from a rat weighing 17 g. and dosed with sulphamezathine from birth. H and E×660.

While sulphamezathine is liable to cause hyperplasia of the testes of young cockerels, lengthens the blood clotting time, reduces the rate of growth, and causes haemorrhage in the gut, sulphapyrazine has not been found to have any of these effects. Sulphapyrazine is also quantitatively more effective than sulphamezathine in curing coccidiosis, but it is not prepared and is not available in England. In spite of the pathological actions which sulphamezathine can have, it remains a valuable drug because the toxic effects are not produced unless treatment is carried on for longer than the recommended period of 7 days. Prolonged treatment has, however, been used by some farmers and a few deaths from haemorrhage have occurred.

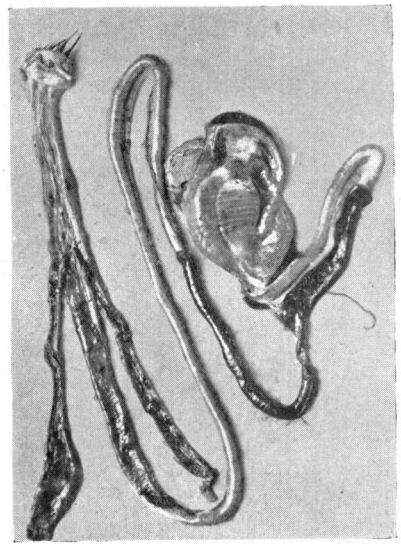

FIG. 7.—Alimentary tract from chicken dosed with sulphamezathine showing widespread haemorrhagic lesions.

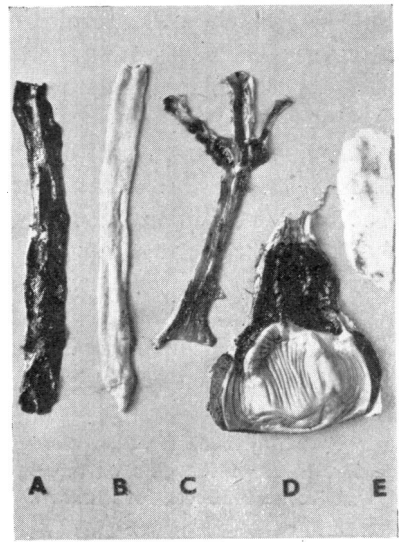

FIG. 8.—(*a*) Duodenum from chicken dosed with sulphamezathine. (*b*) Corresponding duodenum from normal chicken. (*c*) Rectum and caecum from chick treated with sulphamezathine. (*d*) Gizzard and proventriculus from chicken treated with sulphamezathine. (*e*) Omental fat with haemorrhages from chicken treated with sulphamezathine.

The increase in therapeutic effect, paralleled by an increase in effects on the blood clotting system and on the testes, which occurs when one (sulphamerazine) or two (sulphamezathine) methyl groups are introduced into the sulphadiazine molecule, may be due in part to the increased absorption of these methyl sulphapyrimidines.

The effect on blood clotting, and probably to some extent on induction of haemorrhages, can be neutralized by feeding menaphthone or by the addition of a soluble vitamin K preparation to the sulphamezathine solution. In view of this it might be advisable to incorporate a small amount of a vitamin K preparation in sulphamezathine solutions issued for treatment of caecal coccidiosis.

SUMMARY

1. Sulphamezathine, which is an excellent drug for the treatment of caecal coccidiosis in chickens, has certain undesirable side effects. These effects include : (*a*) a decrease in the rate of clotting of the blood, similar to that produced by dicoumarin, which is neutralized by vitamin K ; (*b*) induction of haemorrhages, particularly in chicks between 6 and 12 weeks old dosed for several weeks ; (*c*) a decrease in rate of growth ; and (*d*) a hyperplastic action on the testes of young cockerels, accompanied by growth of the comb and wattles and precocious sexual development.

2. Sulphapyrazine, which is somewhat more effective than sulphamezathine in treatment of coccidiosis, has none of these effects.

3. Sulphamezathine causes hyperplasia of the testes of young rats, but not of adult rats. The increase in testes size is accompanied by some increase of the seminal vesicles and prostate. The drug has no effect on the seminal vesicles and prostate of castrated rats.

4. Sulphamezathine does not appear to affect the fertility of adult cockerels or the laying capacity of fowls.

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