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The effect of feeding diets containing permitted antibiotics on the faecal excretion of *Salmonella typhimurium* by experimentally infected chickens

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SUMMARY

Groups of 45 chickens were fed continuously on diets containing 10 or 100 mg./kg. of different 'growth-promoting' antibiotics and infected orally with a nalidixic acid-resistant mutant of *Salmonella typhimurium*. The amount of *S. typhimurium* organisms excreted in their faeces was estimated by culturing them at weekly intervals and in a standard manner on plates of brilliant green agar containing sodium nalidixate, both direct and after enrichment in selenite broth.

All of four groups fed diets containing 100 mg./kg. of nitrovin in three different experiments excreted much larger amounts of *S. typhimurium* than did groups fed antibiotic-free diets. In some, but not all, experiments, larger amounts were also excreted by groups fed diets containing 10 mg./kg. of nitrovin or 10 or 100 mg./kg. of flavomycin or tylosin. Feeding diets containing 10 or 100 mg./kg. of virginiamycin or bacitracin either did not influence or slightly ncreased the amount of *S. typhimurium* excreted.

Two groups fed continuously on diets containing 100 or 500 mg./kg. of sulphaquinoxaline for 44 days excreted smaller amounts of the *S. typhimurium* organisms than did groups fed antibiotic-free diets; no sulphonamide-resistant organisms of the *S. typhimurium* strain were isolated from the faeces.

INTRODUCTION

Following upon the report of the Swann Committee (Report, 1969), legislation was introduced in Britain in March 1971 prohibiting the routine use of 'therapeutic' antibiotics, except sulphonamides, as feed additives for growth promotion in animals. Sulphonamides were exempted because it was considered then that their use as feed additives was essential for the prophylaxis of coccidiosis in poultry; sulphaquinoxaline is the sulphonamide principally used for that purpose. Other 'non-medical' antibiotics came into use as feed additives for growth promotion. These included bacitracin, virginiamycin, nitrovin and flavomycin. Although these antibiotics are not active against enterobacteria, they are active against many of the other bacteria that inhabit the alimentary tract. It is possible, therefore, that their use as feed additives might disturb the ecological balance in the alimentary tract in favour of the enterobacteria, particularly the salmonellas.

Consequently, it was decided to determine whether they do indeed have this effect in chickens.

The work was facilitated by employing a nalidixic-acid resistant mutant of *Salmonella typhimurium* for infecting chickens and a brilliant green-based medium containing sodium nalidixate and novobiocin, for culturing their faeces. This permitted a quantitative assessment to be made of the salmonella content of the faeces because very few faecal bacteria grow on this medium and the colonies of those that do can easily be differentiated visually from the colonies of the infecting strain. The macrolide, tylosin, was included in this study because not only does its antibiotic spectrum resemble that of bacitracin, virginiamycin, nitrovin and flavomycin but it is used in some countries as a feed additive for disease control and growth promotion. Sulphaquinoxaline which, unlike the other antibiotics, is active against enterobacteria was also studied.

MATERIALS AND METHODS

The kind of chickens, their management and the methods of infecting them with the nalidixic acid-resistant (*nal*^{r}) mutant of *S. typhimurium* and of estimating its concentration in the faeces were those employed in studying the effect of 'therapeutic' antibiotics on the excretion of salmonellas by experimentally infected chickens (Smith & Tucker, 1975). The only important difference was that the groups of approximately 45 chickens were fed on antibiotic-containing diets for the whole of their lives, including the period before they were infected when 3 days old by the oral administration of the *nal*^{r} mutant of *S. typhimurium*. Disks containing 300 μg. of compound sulphonamides (Oxoid) were placed on the plates of MacConkey agar and brilliant green agar (Oxoid, CM263) containing sodium nalidixate (20 μg./ml.) and novobiocin (1 μg./ml.) used for estimating the amount of *Escherichia coli* and the *S. typhimurium* strain in the faeces of the chickens fed diets containing sulphaquinoxaline; antibiotic disks were not placed on the plates used for this purpose in the case of chickens given the other antibiotic-containing diets. The amount of *E. coli* that grew on the MacConkey agar plates and of *S. typhimurium* that grew on the brilliant green agar plates was recorded as described in the previous paper (Smith & Tucker, 1975).

RESULTS

The faecal excretion of Salmonella typhimurium *by chickens fed diets containing bacitracin, virginiamycin, flavomycin, tylosin and nitrovin*

The results of examining the faeces of groups of 45 chickens fed continuously on diets containing bacitracin, virginiamycin, flavomycin, tylosin or nitrovin and infected with the *nal*^{r} strain of *S. typhimurium* are summarized in Table 1. The amount of *S. typhimurium* excreted by the groups fed diets containing virginiamycin, bacitracin, flavomycin, tylosin or 10 mg./kg. of nitrovin was similar to, or slightly greater than, the amount excreted by the two groups fed antibiotic-free diets; the amount excreted by the group fed 100 mg./kg. of nitrovin was much

Table 1. *The isolation of* Salmonella typhimurium *from the faeces of groups of experimentally infected chickens fed diets containing* 10 *or* 100 *mg./kg. of different antibiotics*

	% of chickens from which *S. typhimurium* was isolated when fed diets containing																							
	Virginiamycin at (mg./kg.)				Bacitracin at (mg./kg.)				Flavomycin at (mg./kg.)				Tylosin at (mg./kg.)				Nitrovin at (mg./kg.)				No antibiotics‡ group			
	10		100		10		100		10		100		10		100		10		100		A		B	
Time* (days)	D†	T†	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T
5	100	100	95	100	88	100	95	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
12	95	100	95	100	88	100	89	100	95	100	97	100	95	100	91	100	95	100	100	100	97	100	100	100
18	40	86	58	87	77	82	91	100	69	100	58	87	75	100	68	100	67	97	73	97	50	100	69	100
25	21	88	68	93	49	91	64	97	23	77	46	86	68	100	54	95	31	74	71	97	35	71	50	82
32	5	43	31	80	17	73	32	79	29	72	25	71	62	95	42	82	10	46	59	97	5	18	24	56
39	3	15	12	63	22	61	18	54	18	46	29	73	40	86	23	64	10	29	40	95	12	31	27	58
46	0	15	6	37	8	21	8	46	13	24	14	39	31	66	19	43	5	11	42	77	5	22	16	41
53	0	0	3	23	3	8	5	23	11	30	5	25	17	54	15	31	0	8	0	35	0	3	0	25
60	0	0	0	0	0	3	0	21	11	13	5	21	3	36	8	40	0	8	14	52	0	3	0	13
67	0	5	0	3	0	0	3	13	11	13	0	3	8	29	3	15	0	5	10	31	0	0	0	3
74	0	3	0	5	0	0	5	5	16	16	7	7	5	12	15	17	0	0	5	26	0	0	0	3
81	0	0	0	3	0	0	0	5	5	5	7	7	3	12	8	15	0	3	21	37	0	0	0	0
88	0	3	0	3	0	0	3	8	0	3	7	7	0	3	3	7	0	0	7	37	0	0	0	3
95	0	0	0	0	0	0	5	8	3	3	5	5	0	5	3	3	0	0	5	15	0	0	0	0
102	0	0	0	0	0	0	0	5	3	5	5	5	3	5	3	5	0	0	10	15	0	0	0	0
109	0	0	0	0	0	0	3	5	0	0	0	5	0	0	0	0	0	0	3	3	0	0	0	0

* After the chickens were infected when 3 days old; antibiotic-containing food was fed from day 1.

† D = *S. typhimurium* isolated by direct culture; T = by direct culture or following enrichment in selenite broth. At the commencement of the experiment each group consisted of 45 chickens.

‡ Two groups, A and B, were given food not containing antibiotics.

greater. No obvious difference was noted between any of the groups of chickens in this experiment or between those in subsequent experiments in regard to the *Escherichia coli* content of their faeces.

The results of repeating the above experiment with the omission of one group fed the antibiotic-free diet and the groups fed on 100 mg./kg. of virginiamycin and bacitracin are summarized in Table 2; the experiments employing 100 mg./kg. of virginiamycin and bacitracin were repeated separately. The amount of *S. typhimurium* organisms excreted by the groups fed 10 mg./kg. of virginiamycin and bacitracin was only slightly greater than that of the group fed the antibiotic-free diet. The amount and duration of excretion of these organisms by the groups fed flavomycin, tylosin and nitrovin was much greater, this being especially so in the case of both groups fed the tylosin-containing diets and the group fed the diet containing 100 mg./kg. of nitrovin. In the repeat experiments employing 100 mg./kg. of virginiamycin and bacitracin, there was a slight increase in the amount of *S. typhimurium* excreted by the two groups given food containing one or other of these antibiotics compared with a group given the antibiotic-free diet, the difference between the results for these two groups and the results for the group given the antibiotic-free diet resembling that between the group given food containing 10 mg./kg. of bacitracin and the group given antibiotic-free food in Table 2.

The particular concentrations of nitrovin, tylosin and flavomycin which appeared most favourable to the survival of *S. typhimurium* in the alimentary tract of chickens in the previous experiments were studied further, each antibiotic-containing diet and an antibiotic-free diet being fed to two groups of 45 chickens; because the results for each group given the same diet closely resembled each other they have been amalgamated in Table 3. Once again, the rate, amount and duration of *S. typhimurium* excretion was much greater in the groups fed diets containing 100 mg./kg. of nitrovin than in the groups given antibiotic-free diets. The pattern of salmonella excretion by the groups fed diets containing 10 mg./kg. of flavomycin differed little from that of the groups fed antibiotic-free diets. The pattern did differ in the case of the groups fed 10 mg./kg. of tylosin in that in the examinations performed on day 19, 26 and 33 they had higher concentrations of *S. typhimurium* in their faeces than the groups fed antibiotic-free diets. In this particular experiment, each chicken was killed after the third consecutive weekly examination at which no *S. typhimurium* organisms were demonstrated in its faeces and its caecal contents cultured. At these examinations it was uncommon to find *S. typhimurium*, even after selenite enrichment, in the caecal contents of the chickens from all except the nitrovin group; it was frequently isolated from the caecal contents of this group, often in high concentrations.

The faecal excretion of S. typhimurium *by chickens fed diets containing sulphaquinoxaline*

The results of examining the faeces of groups of 45 chickens fed diets containing 100 or 500 mg./kg. of sulphaquinoxaline and infected with the *nal*^{r} *S. typhimurium* strain are summarized in Table 4. The rate and amount of excretion of

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Table 2. *The isolation of* Salmonella typhimurium *from the faeces of groups of experimentally infected chickens fed diets containing* 10 *or* 100 *mg./kg. of different antibiotics*

	% of chickens from which *S. typhimurium* was isolated when fed diets containing																	
	Virginiamycin at 10 mg./kg.		Bacitracin at 10 mg./kg.		Flavomycin at (mg./kg.)				Tylosin at (mg./kg.)				Nitrovin at (mg./kg.)				No antibiotics	
					10		100		10		100		10		100			
Time (days)	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T
5	94	100	97	100	97	100	94	100	97	100	97	100	94	100	97	100	91	100
14	88	97	86	100	90	100	73	97	97	100	79	100	94	100	91	97	94	97
21	30	67	36	71	80	93	58	88	88	91	57	78	78	100	76	93	50	73
28	48	69	20	40	50	97	38	69	64	97	32	63	49	85	65	86	27	31
35	0	11	14	43	46	86	47	75	54	90	25	44	45	94	52	85	4	8
42	14	17	4	16	42	88	55	88	70	100	63	94	82	89	83	100	8	8
49	0	0	0	28	38	78	41	77	80	100	57	94	23	65	70	97	4	4
56	0	7	0	7	15	40	28	60	67	97	57	94	20	50	65	97	0	0
63	0	4	7	14	7	75	36	55	76	96	50	88	20	48	97	97	0	4
70	4	7	0	0	7	7	10	40	83	90	73	94	0	29	25	71	0	0
77	0	0	0	0	3	3	3	7	66	86	53	97	0	0	37	75	0	0
84	0	0	0	0	14	14	3	7	40	82	79	96	0	4	4	35	0	0
91	0	4	0	0	0	0	0	7	11	32	41	58	0	0	8	35	0	0
98	0	0	0	0	0	0	0	3	11	18	4	18	0	0	8	32	0	0
105	0	0	0	0	0	0	3	7	7	7	0	10	0	0	4	28	0	0
112	0	0	0	0	0	0	0	3	7	7	4	4	0	0	4	4	0	0

For details and abbreviations see Table 1.

Table 3. *Concentration of* Salmonella typhimurium *organisms in the faeces of chickens fed diets containing selected concentrations of flavomycin, tylosin and nitrovin*

Time (days)	% of chickens whose faeces had the following concentrations of *S. typhimurium* when fed diets containing: Flavomycin at 10 mg./kg. > +	> 50	D*	T*	Tylosin at 10 mg./kg. > +	> 50	D	T	Nitrovin at 100 mg./kg. > +	> 50	D	T	No antibiotics > +	> 50	D	T
5	17	56	89	93	10	65	91	98	9	56	98	100	9	41	97	97
10	8	43	98	98	9	44	95	100	8	67	98	98	3	50	89	97
19	2	14	73	86	19	57	91	100	5	36	83	98	2	19	83	91
26	0	4	55	72	14	38	70	86	13	79	95	100	0	7	45	76
33	3	8	35	53	10	19	53	81	19	48	89	97	2	2	19	40
40	0	3	13	20	3	10	16	23	2	31	75	85	0	0	13	28
47	0	2	8	20	0	0	8	22	0	28	60	90	0	0	3	13
54	0	0	0	6	0	0	8	12	3	16	52	90	0	2	3	6
61	0	0	3	3	0	0	2	2	0	4	32	57	0	0	0	2
68	0	0	2	5	0	0	0	2	0	10	40	78	0	0	2	5
75	0	0	2	2	0	0	0	3	0	2	30	52	0	0	2	2
82	0	0	4	5	0	0	0	2	0	2	16	65	0	2	5	5
89	0	0	2	3	0	0	0	0	0	8	20	31	0	0	3	3
96	0	0	0	3	0	0	0	0	0	4	11	24	0	0	2	3
103	0	0	0	0	0	0	0	0	0	0	9	17	0	0	2	3
110	0	0	0	0	0	0	0	0	0	2	8	15	0	0	2	3
117	0	0	0	0	0	0	0	0	0	0	2	3	0	0	2	2

Each of the four diets was given to 45 chickens; each pair of groups is considered as one in the table.

* T = *S. typhimurium* isolated by selenite enrichment or direct culture. D = isolated by direct culture; 50 = 50 colonies of *S. typhimurium* grew on the culture plate used for this purpose; + = the culture plate was covered with discrete colonies.

For other details and abbreviations see Table 1.

Table 4. *Concentration of* Salmonella typhimurium *organisms in the faeces of groups of chickens fed diets containing sulphaquinoxaline*

	% of chickens whose faeces had the following concentrations of *S. typhimurium* organisms when fed diets containing															
	Sulphaquinoxaline at 100 mg./kg.				No antibiotics				Sulphaquinoxaline at 500 mg./kg.				No antibiotics			
Time (days)	> +	> 50	D	T	> +	> 50	D	T	> +	> 50	D	T	> +	> 50	D	T
2	48	80	100	100	28	86	100	100	5	54	97	100	8	31	85	97
9	23	74	100	100	5	59	100	100	5	24	89	95	0	27	89	97
16	7	17	50	97	15	44	70	97	0	0	18	26	0	3	46	73
23	3	13	43	63	8	18	61	84	3	5	20	24	0	0	22	49
30	0	13	26	40	3	16	43	65	0	0	3	20	0	0	20	33
37	0	7	17	40	0	3	43	65	0	0	0	3	0	0	3	11
44	0	7	13	20	3	18	24	43	0	0	0	0	0	0	3	11
	Medicated food replaced by ordinary food on the 46th day															
48	0	3	10	10	0	8	11	21								
56	0	7	7	13	0	3	5	13								
61	3	3	3	7	3	3	3	13								

For details and abbreviations see Tables 1 and 3.

The first 'no antibiotic' group served as a control for the group fed the diet containing 100 mg./kg. of sulphaquinoxaline and the second for the group fed the diet containing 500 mg./kg. of sulphaquinoxaline, both dietary concentrations being studied on different occasions.

S. typhimurium organisms was less in the groups given the sulphaquinoxaline-containing diets than in their corresponding non-antibiotic-fed groups. After the change to antibiotic-free food on the 46th day, the rate and amount of *S. typhimurium* organisms excreted by the group previously fed on the diet containing 100 mg./kg. of sulphaquinoxaline continued to decline; the group given 500 mg./kg. was not examined after the 44th day.

DISCUSSION

None of the groups of chickens fed diets containing virginiamycin, bacitracin, flavomycin, tylosin or nitrovin excreted smaller amounts of the infecting strain of *S. typhimurium* in their faeces than the groups fed antibiotic-free diets did. They either excreted similar amounts, slightly greater amounts or much greater amounts; those that excreted greater amounts often remained carriers for longer periods. By contrast, the groups fed diets containing sulphaquinoxaline excreted smaller amounts of *S. typhimurium* than their non-antibiotic-fed control groups did. This was not unexpected bearing in mind that this agent is known to have some activity against salmonellas and that no resistant organisms of the infecting strain arose in their faeces. Salmonellas are resistant to all the other antibiotics studied but some of the other bacteria that inhabit the alimentary tract are sensitive to them. It is probable, therefore, that any effect these antibiotics would have on the excretion of the infecting strain of *S. typhimurium* would be the consequence of their activity against those sensitive alimentary bacteria that antagonize or compete in some way with the *S. typhimurium* organisms. The extent of this effect would depend on, amongst other things, the fate of the antibiotics in the alimentary tract, the degree and range of their antibacterial activity, the ease with which bacteria become resistant to them and the composition of the bacterial flora of the alimentary tract. It did not amount to much in the groups of chickens fed diets containing virginiamycin or bacitracin, the amount of *S. typhimurium* they excreted, at the most, being only slightly greater than that of the groups fed antibiotic-free diets. It did, though, in all four groups fed diets containing 100 mg./kg. of nitrovin, the amount of *S. typhimurium* they excreted being much greater than that excreted by the groups fed antibiotic-free diets. It also did in some, but not in all, of the groups fed diets containing flavomycin, tylosin or 100 mg./kg. of nitrovin, the differing results obtained with the same antibiotic only being noted when the tests with that antibiotic were performed on different occasions. These differing results may well be a reflexion of a difference in the alimentary bacterial flora of the chickens employed on the different occasions. The fact that the infecting strain of *S. typhimurium* was often found in high concentrations in the caeca of chickens fed diets containing 100 mg./kg. of nitrovin when it had not been found in their faeces at the three previous weekly examinations suggests the bacterial flora of the caeca may be important in this respect.

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