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The effect of antibiotic therapy on the faecal excretion of *Salmonella typhimurium* by experimentally infected chickens

By H. WILLIAMS SMITH and J. F. TUCKER
Houghton Poultry Research Station, Houghton, Huntingdon, PE17 2DA

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SUMMARY

Chickens in groups of 40 were infected orally with a nalidixic acid-resistant mutant of *Salmonella typhimurium* and then fed continuously on diets containing ampicillin, chloramphenicol, furazolidone, neomycin, oxytetracycline, polymixin, spectinomycin, streptomycin or a mixture of trimethoprim and sulphadiazine. The amount of *S. typhimurium* excreted in their faeces was estimated at intervals by culture on brilliant green agar containing sodium nalidixate, both direct and after enrichment in selenite broth; the amount of *Escherichia coli* excreted was estimated by culture on MacConkey agar. The feeding of diets containing 500 mg./kg. of ampicillin, furazolidone, neomycin, polymixin, spectinomycin or streptomycin or 100 mg./kg. of trimethoprim and 500 mg./kg. of sulphadiazine for 46 days reduced to a varying degree the amount of *S. typhimurium* and *E. coli* excreted, the greatest reduction in *S. typhimurium* being brought about by the last treatment. The effect was less obvious when the concentration of the antibiotics in the food was decreased fivefold. An important reason for the very limited effect of some of the antibiotics was the emergence of antibiotic-resistant populations of *S. typhimurium* and *E. coli*. High concentrations of antibiotic-resistant organisms also arose in the faeces of the chickens fed diets containing tetracyclines and chloramphenicol, treatments which had no apparent effect on the amount of *S. typhimurium* and *E. coli* excreted. Much of the antibiotic resistance encountered was determined by R factors, a particular R factor usually being found in the *E. coli* populations of individual chickens before it was found in their *S. typhimurium* populations. No *S. typhimurium* or *E. coli* were isolated that possessed R factors determining resistance to polymixin, furazolidone or trimethoprim. No *S. typhimurium* or *E. coli* were isolated that were polymixin-resistant and no *S. typhimurium* that were furazolidone-resistant. The few trimethoprim-resistant *S. typhimurium* isolated were thymine-dependent.

The feeding of diets containing the higher concentrations of trimethoprim/sulphadiazine, neomycin, furazolidone or ampicillin for 9 days reduced the amount of *S. typhimurium* excreted. After the withdrawal of these diets, the amount of *S. typhimurium* excreted increased to the numbers found in chickens given ordinary diets throughout; the chickens that had been given trimethoprim/sulphadiazine or furazolidone did not remain faecal excreters of *S. typhimurium* longer than the chickens that had been given ordinary diets. Similar results were obtained

with trimethoprim/sulphadiazine when the start of the 9-day treatment period was delayed for an extra 9 days or when it was extended to 18 days.

INTRODUCTION

The available evidence indicates that the administration of antibiotics to human beings naturally infected with salmonellas of the food-poisoning types usually prolongs the carrier rate (Dixon, 1965; Rosenstein, 1967; Aserkoff & Bennett, 1969; Clementi, 1973). In experimentally infected chickens, too, antibiotic administration either prolongs the carrier rate (Garside, Gordon & Tucker, 1960) or does not significantly abbreviate it (Olesiuk, Snoeyenbos & Smyser, 1973). In all these reports faecal specimens were classed as positive irrespective of the numbers of salmonella organisms they contained. Because the culture media employed permit salmonellas to be isolated from faecal specimens containing only a few of these organisms per g. (Smith, 1952), many of the individuals classed as faecal excreters might have been only lightly infected. What risk such individuals pose to the community in which they live is debatable. Certainly, they would be less of a danger than individuals whose faeces were heavily infected. It is possible then that even though antibiotic therapy might prolong the carrier state it could have an overall beneficial effect if it significantly decreased the *amount* of salmonella organisms excreted. Because of this, it was decided to study the effect of antibiotic therapy on the excretion of salmonella organisms by experimentally infected chickens employing cultural methods that provided a quantitative assessment of faecal infection. This was made possible by infecting chickens orally with a nalidixic acid-resistant mutant of *Salmonella typhimurium* and culturing their faeces in a standard manner on a brilliant green agar containing sodium nalidixate and novobiocin. Few faecal organisms grew on this medium and the colonies of those that did could easily be differentiated visually from those of the *S. typhimurium* strain.

MATERIALS AND METHODS

Chickens

These were from a salmonella-free Light Sussex flock. They were kept under good hygienic conditions in groups of 40 on wire-mesh floors in identically constructed pens in an animal house maintained at 21° C. During the first 3 weeks of life additional heating was provided by suspending an infra-red brooding lamp over each pen. They were fed *ad libitum* on a diet of the following composition: wheat meal, 40 %; maize meal, 40 %; British white-fish meal, 20 %; mineral and vitamin supplement, 0·25 %. When required, antibiotics, as pre-mixes, were incorporated in the food by means of a mechanical mixer.

Bacteria

A smooth nalidixic acid-resistant (*nal*^{r}) mutant of an antibiotic-sensitive *S. typhimurium* strain, F98 of phage type 14, was used throughout for infecting

chickens; it was maintained at 5° C. on Dorset egg medium. It was employed as a broth culture (Oxoid, CM67) incubated at 37° C. for 24 hr. and containing approximately 10^9 viable organisms per ml.

The determination of the effect of antibiotics on the faecal excretion of S. typhimurium *and* Escherichia coli

Groups of 40 chickens, 3 days old, were given 0·3 ml. of a broth culture of the *S. typhimurium* strain directly into the crop by means of a Pasteur pipette passed down the oesophagus (the resulting infection was accompanied by little or no mortality). Three days later the diet of each group was changed from ordinary food to food containing antibiotics in concentrations of 100 or 500 mg./kg.; one or two groups in each experiment were fed on ordinary food throughout. Before infection, and at frequent intervals afterwards, faecal swabs were taken from the cloaca of all chickens and inoculated in a standard manner on to half of the surface of plates of MacConkey agar (Oxoid, CM7) and brilliant green agar (Oxoid CM263), the latter medium containing 20 μg./ml. of sodium nalidixate and 1 μg./ml. of novobiocin. A disk containing an antibiotic was then placed in the middle of the inoculated area of each plate, the antibiotic being the one that was given to the chicken from which the particular faecal specimens had been obtained. Faecal swabs taken from groups that were being fed on ordinary diets were also inoculated on an extra MacConkey agar and brilliant green agar plate and disks containing all the antibiotics being studied in the particular experiment were placed upon them. After incubation at 37° C. for 24 hr., the amount of growth of *E. coli* on the MacConkey agar plates and of *S. typhimurium* on the brilliant green agar plates was recorded according to the following notation: + + + + = confluent; + + + = almost confluent; + + = partly confluent; + = numerous mainly discrete colonies; ± = numerous discrete colonies; 50 = approximately 50 colonies; 5 = approximately 5 colonies; 1 = approximately 1 colony.

The amount of resistant growth around the antibiotic disks was also recorded. In selected cases, the antibiotic resistance pattern of pure cultures obtained from this resistant growth was determined; as was its transmissible or non-transmissible nature.

After being inoculated on the MacConkey and brilliant green agar plates in the manner described above, the faecal swabs were incubated in selenite broth (Oxoid, CM39a) at 37° C. for 24 hr. and then subcultured on brilliant green agar. All batches of selenite broth, brilliant green agar and MacConkey agar used for examining faecal specimens from chickens treated with trimethoprim were supplemented with thymine, 60 μg./ml., to ensure adequate growth of trimethoprim-resistant organisms that were thymine-dependent.

Antibiotic sensitivity tests

These were performed by the disk method of Smith (1970) using an Oxoid Multodisk (1744E) composed of eight disks containing (i) streptomycin, 25 μg., (ii) ampicillin, 25 μg., (iii) oxytetracycline, 50 μg., (iv) chloramphenicol, 50 μg., (v) neomycin, 30 μg., (vi) nalidixic acid, 30 μg., (vii) furazolidone, 15 μg., and

(viii) sulphonamides, 300 μg., and three separate disks containing spectinomycin, 25 μg., sulphamethoxazole/trimethoprim, 23·75 μg., and 1·25 μg., respectively and trimethoprim, 1·25 or 10 μg. The eight disks on the multodisk were also available separately for placing on the primary culture plates of the chickens' faeces.

Transfer of antibiotic resistance in vitro

This was performed by the method described by Smith (1970), a *nal*^{r} *lac*$^{-}$ *E. coli* K12 strain and the *nal*^{r} strain of *S. typhimurium* used for infecting the chickens being used as recipients when the donor was an *E. coli* strain and a rifampicin-resistant mutant of the K12 strain when an antibiotic-resistant form of the *S. typhimurium* strain was the donor.

RESULTS

The faecal excretion of Salmonella typhimurium *and* Escherichia coli *by chickens fed continuously on diets containing* 100 *mg./kg. of different antibiotics*

The effect on faecal excretion of *S. typhimurium* and *E. coli* of feeding diets containing 100 mg./kg. of different antibiotics to groups of 40 chickens that had been infected orally with the nalidixic acid-resistant mutant (*nal*^{r}) of *S. typhimurium* is summarized in Tables 1 and 2; the diet of one group contained 500 mg./kg. of sulphadiazine in addition to 100 mg./kg. of trimethoprim (Septrin, Burroughs Wellcome & Co. Ltd.). The diet containing trimethoprim/sulphadiazine had the most marked depressant effect on the faecal excretion of *S. typhimurium* and *E. coli*. No faecal excreters of *S. typhimurium* were found at 21 days in the group of chickens given these agents and only a few were found during the next six weekly examinations, including the three performed after the medicated diets were replaced by ordinary food on the 46th day. Their effect on *E. coli* was noticeable on the second day but after the ninth day the amount of *E. coli* in the faeces of the chickens in this group commenced to increase because of the emergence of trimethoprim/sulphadiazine-resistant organisms. By the 35th day practically all the *E. coli* organisms in the faeces of these chickens was resistant to both antibiotics; this resistance was of the mutational kind. Neomycin also depressed the excretion of *S. typhimurium* and *E. coli*, the effect on *E. coli* being no longer apparent after the 41st day because of the emergence of neomycin-resistant *E. coli*; no neomycin-resistant *S. typhimurium* were isolated from any of these chickens. Furazolidone may have had a slight depressant effect on *S. typhimurium* excretion but it had no noticeable effect on *E. coli* excretion or in giving rise to resistant organisms. At 2 days, ampicillin depressed the excretion of *S. typhimurium* (not noticeable from Table 1) and *E. coli* and at 15 days spectinomycin depressed *S. typhimurium* excretion. The concentrations of these organisms in both the ampicillin and spectinomycin groups then increased due to the emergence of resistant populations. Neither streptomycin, polymixin, chloramphenicol or oxytetracycline had any obvious effect on *S. typhimurium* excretion; of these

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

	% of chickens from which *S. typhimurium* was isolated when fed diets containing																			
	T/S†		Fur		Neo		Pol		Spe		Str		Chl		Tet		Amp		Nil	
Time* (days)	D‡	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T
0	89	100	88	100	82	100	93	100	89	100	89	100	89	100	79	100	97	100	97	100
2	89	100	69	87	92	100	96	100	87	100	96	100	96	100	79	93	93	100	100	100
9	14	41	71	93	75	100	80	97	87	100	79	93	96	100	93	97	89	100	100	100
15	0	3	60	89	22	40	69	85	20	40	35	64	86	100	60	63	82	100	100	100
21	0	0	28	68	7	29	32	60	32	48	37	67	60	76	43	53	60	97	70	97
28	0	0	26	45	4	11	28	54	72	80	15	42	36	60	34	62	41	69	61	84
35	0	0	26	41	0	15	38	55	61	84	20	46	29	50	27	53	30	57	43	65
41	0	0	0	27	4	4	22	29	40	68	20	35	13	38	7	28	7	34	24	43
	Medicated food replaced by ordinary food on day 46																			
48	0	3	0	27	0	0	22	26	40	64	27	46	10	20	10	13	27	47	11	21
56	7	11	0	13	4	4	9	18	36	52	11	40	8	13	7	11	7	7	5	13
61	0	7	0	3	0	0	0	18	0	24	8	23	0	4	0	11	0	4	3	13

At the commencement of the experiment each group consisted of 40 chickens.

* After the commencement of feeding medicated diets, 3 days after orally infecting the chickens when 3 days old with *S. typhimurium*.

† T/S = trimethoprim/sulphadiazine; Fur = furazolidone; Neo = neomycin; Pol = polymixin; Spe = spectinomycin; Str = streptomycin; Chl = chloramphenicol; Tet = oxytetracycline; Amp = ampicillin; Nil = no antibiotics. The T/S diet contained 100 mg./kg. of trimethoprim and 500 mg./kg. of sulphadiazine.

‡ D = *S. typhimurium* isolated by direct culture; T = by direct culture or following enrichment in selenite broth.

Table 2. *Concentration of* Escherichia coli *organisms in the faeces of groups of chickens fed diets containing 100 mg./kg. of different antibiotics*

Time (days)	% of chickens whose faeces had the following concentrations of *E. coli* organisms when fed on diets containing																			
	T/S		Fur		Neo		Pol		Spe		Str		Chl		Tet		Amp		Nil	
	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50
0	55	100	64	97	54	100	65	100	51	100	43	100	67	100	47	100	65	100	65	100
2	4	97	48	100	35	90	58	100	38	100	54	100	73	100	38	97	0	3	65	100
9	0	23	41	100	25	90	83	100	48	100	39	100	64	100	52	100	64	97	61	100
15	7	41	43	100	4	93	22	100	24	92	7	97	68	100	26	97	40	100	73	100
21	7	41	47	100	10	90	16	100	16	100	7	93	36	100	47	100	57	100	48	100
28	10	37	56	100	4	90	29	100	28	100	0	88	36	100	55	100	55	100	35	100
35	21	62	22	100	12	96	22	96	32	100	4	88	36	100	45	100	67	100	48	100
41	5	75	15	95	10	100	30	100	65	100	10	80	42	100	45	100	40	100	25	100
	Medicated food replaced by ordinary food on day 46																			
48	10	95	45	100	30	100	57	100	75	100	45	100	55	100	55	100	62	100	40	100
56	25	100	15	100	30	100	11	100	60	100	15	100	21	100	20	100	70	100	15	100
61	25	100	40	100	54	100	22	100	35	100	45	100	42	100	40	100	42	100	35	100

++ = partly confluent *E. coli* growth on MacConkey agar plate inoculated with faecal swab; 50 = approximately 50 *E. coli* colonies on the plate. For other details see Table 1.

Table 3. *The isolation of* Salmonella typhimurium *from the faeces of groups of experimentally infected chickens fed diets containing* 500 *mg./kg. of different antibiotics*

	% of chickens from which *S. typhimurium* was isolated when fed diets containing																			
	T/S*		Fur		Neo		Pol		Spe		Str		Chl		Tet		Amp		Nil	
Time (days)	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T
0	87	100	100	100	97	97	87	95	92	100	87	100	89	100	92	97	60	83	85	97
2	85	97	8	26	75	87	57	70	85	97	85	97	85	97	92	92	48	58	85	97
9	52	72	12	25	36	42	70	81	67	92	57	87	85	100	89	95	34	66	89	97
16	20	45	15	15	5	26	10	16	0	20	17	27	67	97	49	72	37	63	46	73
23	5	8	10	13	8	10	0	3	0	0	0	10	31	57	27	38	53	79	22	49
30	0	5	5	15	3	10	0	0	5	12	0	5	10	33	10	13	24	48	20	33
37	3	3	0	5	0	8	0	0	0	0	0	5	3	18	5	10	30	51	3	10
	Medicated food was replaced by ordinary food on day 40																			
44	0	5	0	0	3	3	0	0	0	0	3	8	0	16	5	10	10	51	3	10

* Twenty mg./kg. of trimethoprim and 100 mg/kg. of sulphadiazine, not 500 mg./kg. of either agent.
For other details and abbreviations see Table 1.

Table 4. *Concentration of* Escherichia coli *organisms in the faeces of groups of chickens fed diets containing 500 mg./kg. of different antibiotics*

Time (days)	% of chickens whose faeces had the following concentrations of *E. coli* organisms when fed on diets containing: T/S*		Fur		Neo		Pol		Spe		Str		Chl		Tet		Amp		Nil	
	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50
0	38	100	67	100	26	100	26	100	38	100	38	97	63	100	50	100	25	97	74	100
2	20	77	6	77	5	8	3	28	8	18	3	30	23	78	23	72	5	57	31	100
9	8	95	8	92	0	0	0	7	10	89	18	75	19	89	13	90	76	100	32	100
16	15	87	5	87	0	0	11	11	18	97	5	57	26	100	14	72	50	100	27	100
23	45	95	13	52	0	0	0	8	20	78	11	95	75	95	28	97	37	89	30	100
30	45	100	0	62	0	3	3	28	16	68	10	87	71	100	32	32	29	100	30	100
37	23	90	6	100	0	0	0	22	13	73	7	74	34	100	23	100	35	100	48	100
	Medicated food was replaced by ordinary food on day 40																			
44	16	62	22	96	26	85	48	93	26	62	6	65	32	100	40	85	12	100	50	100

* Twenty mg./kg. of trimethoprim and 100 mg./kg. of sulphadiazine, not 500 mg./kg. of either agent.
For other details and abbreviations see Tables 1 and 2.

three antibiotics only streptomycin depressed *E. coli* excretion. Oxytetracycline and streptomycin, in addition to ampicillin and spectinomycin, profoundly influenced the emergence of resistant populations of *S. typhimurium* and *E. coli*, and so did chloramphenicol in the case of *E. coli*.

All the resistant organisms of *S. typhimurium* that were examined from the ampicillin and spectinomycin groups possessed an R factor determining resistance to ampicillin (A), streptomycin (S), sulphonamides (Su), tetracyclines (T), chloramphenicol (C) and spectinomycin (Sp). *S. typhimurium* organisms possessing this and T, ST and SuT R factors were also found in the tetracycline group; at the 35th day all the *S. typhimurium* organisms in about 30 % of the faecal specimens obtained from this group were tetracycline-resistant. Most of the resistance in the *S. typhimurium* populations of the streptomycin group was of the mutant type but some was associated with ST R factors. All the different patterns of antibiotic resistance found in the *S. typhimurium* organisms isolated from the ampicillin, spectinomycin, tetracycline and streptomycin groups, including those determined by R factors, were also found in the *E. coli* isolated from these groups; resistant organisms eventually dominated their *E. coli* populations. In the ampicillin group, the ASSuTCSp R factor was detected at the same time, on the ninth day, in *E. coli* and in *S. typhimurium*. In the other three groups, however, R factors determining particular patterns of antibiotic resistance were always detected in *E. coli* at least a week before they were detected in *S. typhimurium*. *In vitro*, these R factors could be transmitted from the *E. coli* strains to the *S. typhimurium* strain in addition to *E. coli* K12. No antibiotic-resistant *S. typhimurium* organisms were ever isolated from the faecal specimens taken from the group fed antibiotic-free diets. Apart from sulphonamide resistance, the incidence of antibiotic resistance in the *E. coli* in these specimens was very low.

The faecal excretion of Escherichia coli *and* Salmonella typhimurium *by chickens fed continuously on diets containing* 500 *mg./kg. of different antibiotics*

The results of repeating the previous experiment but with the dietary concentration of the antibiotics, except trimethoprim/sulphadiazine, increased from 100 to 500 mg./kg. are summarized in Tables 3 and 4; the concentrations of trimethoprim and sulphadiazine studied were 20 and 100 mg./kg. respectively instead of 100 and 500 mg./kg. respectively. These lower concentrations of trimethoprim and sulphadiazine depressed *S. typhimurium* and *E. coli* excretion to some extent. The depressant effect on *E. coli* was no longer apparent after the 16th day due to the emergence of mutants resistant to trimethoprim and sulphonamides. It was maintained throughout on *S. typhimurium* even though small numbers of trimethoprim-resistant organisms that were thymine-requiring (*thy*$^-$) were isolated from some faecal specimens towards the end of the experiment. The higher concentrations of furazolidone, neomycin, polymixin, spectinomycin and streptomycin employed in this experiment had a more pronounced depressing effect on *S. typhimurium* excretion than the lower concentrations had in the previous experiment; no antibiotic-resistant *S. typhimurium* organisms were isolated from any of the chickens to which these antibiotics were administered.

A great reduction in the concentrations of *E. coli* also occurred in the neomycin and polymixin groups and a lesser one in the furazolidone group; furazolidone-resistant mutants were isolated from a small number of specimens from the furazolidone group. A great reduction in the *E. coli* concentrations also occurred in the spectinomycin and streptomycin groups by the second day. Afterwards, the concentrations in both these groups increased greatly due to the emergence of resistant organisms; those examined from the spectinomycin group were mutants and most of those examined from the streptomycin group possessed an SSuT R factor. Oxytetracycline and chloramphenicol did not depress *E. coli* and *S. typhimurium* excretion. At the 16th day and subsequently the *E. coli* present in most of the specimens examined from the groups given these two antibiotics were predominantly antibiotic-resistant. Those tested from the tetracycline group possessed a T R factor and those from the chloramphenicol group an ASSuTCSp R factor. No resistant *S. typhimurium* organisms were isolated from the tetracycline group but resistant ones possessing the ASSuTCSp R factor found in the *E. coli* at the 16th day were isolated from a few of the chickens in the chloramphenicol group at the 30th and 37th days. In the early part of the experiment a pronounced depression of both *E. coli* and *S. typhimurium* excretion occurred in the ampicillin group. This was followed by an increase in the concentrations of *E. coli* at the ninth day and of *S. typhimurium* at the 23rd day, the increase in each case coinciding with the emergence and rise to dominance of organisms possessing an ASSuTCSp R factor. These resistant *E. coli* and *S. typhimurium* attained concentrations higher than the *E. coli* and *S. typhimurium* concentrations in the chickens given antibiotic-free food, a situation that persisted in the case of *S. typhimurium* to the end of the experiment.

The faecal excretion of Escherichia coli *and* Salmonella typhimurium *by chickens fed for* 9 *or* 18 *days on diets containing* 500 *mg./kg. of different antibiotics*

The effect on the faecal excretion of *E. coli* and *S. typhimurium* of feeding diets containing 500 mg./kg. of different antibiotics for 9 days only are summarized in Tables 5 and 6; the trimethoprim/sulphadiazine diet contained 100 mg./kg. of trimethoprim and 500 mg./kg. of sulphadiazine. Some reduction in the faecal concentrations of *E. coli* was found in all except the streptomycin group, the reduction being most obvious in the trimethoprim/sulphadiazine group and least obvious in the tetracycline and chloramphenicol groups. The lack of response in the streptomycin group was associated with the early emergence of a predominantly streptomycin-resistant *E. coli* flora. A similar situation arose in the spectinomycin group during the early part of the treatment period and in the ampicillin and tetracycline groups during the later part. The increased *E. coli* concentrations found in a few of the chickens in the trimethoprim/sulphadiazine group at 9 days was due to the emergence of an *E. coli* flora composed principally of trimethoprim/sulphadiazine resistant mutants. When the antibiotic-containing diets were replaced by ordinary food at 9 days, the *E. coli* concentrations in the chickens in all the groups that had been fed antibiotic-containing diets returned to approximately that found in the chickens that had been fed on ordinary food throughout.

Table 5. *The isolation of* Salmonella typhimurium *from the faeces of groups of experimentally infected chickens fed diets containing* 500 *mg./kg. of different antibiotics for* 9 *days*

Time (days)	% of chickens from which *S. typhimurium* was isolated when fed diets containing																			
	T/S*		Fur		Neo		Pol		Spe		Str		Chl		Tet		Amp		Nil	
	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T
0	100	100	100	100	100	100	100	100	100	100	100	100	94	94	100	100	100	100	100	100
2	85	97	72	88	100	100	100	100	97	97	100	100	97	100	97	100	91	91	100	100
4	64	88	79	100	84	84	90	90	97	97	74	100	93	93	97	100	73	80	100	100
7	12	33	81	100	87	100	97	100	97	100	97	97	84	100	97	100	73	86	100	100
9	6	16	41	56	63	90	79	100	93	100	79	100	79	100	88	97	69	79	100	100
	Medicated food replaced by ordinary food on day 9																			
16	87	91	50	70	74	87	79	100	77	100	68	100	47	100	85	85	81	85	77	100
23	42	90	30	46	72	96	69	90	81	93	96	100	59	91	77	83	44	81	28	96
30	7	33	4	16	48	74	56	71	57	57	54	82	20	54	30	50	28	72	14	39
37	11	26	7	10	16	58	22	37	23	43	22	53	10	33	9	18	4	32	7	14
44	0	0	0	0	8	56	22	50	10	23	17	34	3	7	3	9	0	20	0	0
51	0	0	3	10	8	26	4	32	7	14	4	27	7	14	3	3	8	8	0	0
58	0	0	0	0	4	8	8	16	3	3	0	17	3	3	3	3	0	0	0	0
65	0	0	0	3	4	4	4	16	3	3	4	13	3	3	3	3	0	0	0	0

* 500 mg./kg. of sulphadiazine and 100 mg./kg. of trimethoprim.
For other details and abbreviations see Table 1.

Table 6. *Concentration of* Escherichia coli *organisms in the faeces of groups of chickens fed diets containing 500 mg./kg. of different antibiotics for 9 days*

	% of chickens whose faeces had the following concentrations of *E. coli* organisms when fed diets containing																			
	T/S*		Fur		Neo		Pol		Spe		Str		Chl		Tet		Amp		Nil	
Time (days)	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50	> ++	> 50
0	41	100	30	100	70	97	70	97	27	93	41	100	52	97	47	100	57	100	52	100
2	6	18	18	44	30	60	3	30	9	30	30	60	24	78	17	56	0	0	55	100
4	0	13	25	51	3	13	7	24	27	80	66	100	42	90	21	38	0	4	55	100
7	0	6	41	93	0	16	13	46	30	90	62	100	19	69	37	72	0	4	33	100
9	13	13	10	86	7	37	7	76	30	100	68	100	12	63	37	97	35	52	45	100
Medicated food replaced by ordinary food on the ninth day																				
16	6	94	27	100	19	100	11	93	16	97	23	100	10	90	43	97	34	96	17	100
23	6	100	23	100	13	71	11	93	13	87	20	100	13	97	18	94	19	92	14	97
30	18	100	40	100	4	88	22	97	16	97	17	92	38	100	9	100	8	92	7	100
37	0	100	23	100	0	92	11	85	23	100	13	81	4	100	0	100	4	100	11	100

* 500 mg./kg. of sulphadiazine and 100 mg./kg. of trimethoprim.
For other details and abbreviations see Tables 1 and 2.

Table 7. *Concentration of* Salmonella typhimurium *organisms in the faeces of chickens fed diets containing 500 mg./kg. of furazolidone or 100 mg./kg. of trimethoprim and 500 mg./kg. of sulphadiazine for 9 days*

Time (days)	% of chickens whose faeces had the following concentrations of *S. typhimurium* when fed diets containing: Furazolidone > +	Furazolidone > 50	Furazolidone D	Furazolidone T	Trimethoprim/sulphadiazine > +	Trimethoprim/sulphadiazine > 50	Trimethoprim/sulphadiazine D	Trimethoprim/sulphadiazine T	No antibiotics > +	No antibiotics > 50	No antibiotics D	No antibiotics T
0	3	24	82	94	13	49	90	95	8	47	87	97
2	2	12	46	73	2	10	56	70	10	41	97	97
4	2	4	31	44	0	10	26	56	10	53	94	100
7	0	6	40	53	2	2	3	28	3	50	90	97
9	2	13	27	50	0	0	2	9	2	36	85	100
Medicated food replaced by ordinary food												
11	19	47	84	89	0	23	54	80	17	48	96	100
14	23	48	96	100	11	47	86	86	14	54	90	97
16	19	61	91	93	9	45	83	94	2	20	83	91
23	2	16	36	67	0	18	41	61	0	4	32	57
30	0	2	5	25	0	2	11	28	2	2	16	45
37	0	0	7	18	0	4	9	21	0	0	4	15
44	0	0	5	7	0	0	2	4	0	0	2	9
51	0	0	2	5	0	0	0	2	0	2	2	5
58	0	0	0	2	0	0	0	0	0	0	0	2
65	0	0	0	0	0	0	0	0	0	0	2	5

Each of the three diets was given to two groups of 40 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 by mainly discrete colonies.

Table 8. *Concentration of* Salmonella typhimurium *organisms in the faeces of chickens fed diets containing* 100 *mg./kg. of trimethoprim and* 500 *mg./kg. of sulphadiazine for nine or* 18 *days*

Time after discontinuation of treatment (days)	% of chickens whose faeces had the following concentrations of *S. typhimurium* when fed diets containing: Trimethoprim/sulphadiazine for 9 days*				Trimethoprim/sulphadiazine for 18 days				No antibiotics			
	> +	> 50	D	T	> +	> 50	D	T	> +	> 50	D	T
0	0	1	1	4	0	0	2	4	0	7	42	67
3	0	2	6	9	1	6	30	45	2	17	51	62
4	1	4	12	20	0	6	30	55	0	14	42	59
5	0	4	16	34	0	12	37	55	2	7	50	72
6	0	4	13	27	2	15	42	55	0	6	48	64
7	1	3	11	30	0	1	29	51	0	7	42	56
10	0	2	19	30	0	3	22	46	0	7	42	62
17	0	4	8	16	0	1	9	21	0	6	25	35
24	0	1	12	20	0	0	5	8	0	1	11	16
31	0	1	2	5	0	0	1	2	0	2	6	16
38	0	1	3	7	0	1	2	5	0	2	5	13

* Commencing 11 days, instead of the customary 2 days, after infection.
For other details see Table 7.

The greatest depression in *S. typhimurium* excretion occurred in the trimethoprim/sulphadiazine group, some depression also occurring in the furazolidone, neomycin and ampicillin groups. Resistant *S. typhimurium* organisms were only found in the streptomycin and spectinomycin groups. Within 1 or 2 weeks of the withdrawal of the antibiotic-containing foods, the concentrations of *S. typhimurium* increased in those groups in which it had previously been depressed. The increase was such that in all but the furazolidone group the concentrations became as great as those in the group that had been given ordinary food throughout. The upsurge, however, quickly abated. Even so, chickens in some of the antibiotic-fed groups remained excreters of *S. typhimurium* for longer periods of time than did chickens in the group given ordinary food throughout.

When the above experiment was repeated, similar results, in general, were obtained. One exception was that a great depression of *E. coli* occurred in the streptomycin group associated with the non-emergence of resistant organisms; no *E. coli* were isolated from the faeces of any of the chickens in this group or in the neomycin group at the seventh and ninth days. After the withdrawal of antibiotic-containing food a resurgence of *S. typhimurium* to a concentration as high as that in the group fed ordinary food throughout again occurred in those groups, except the furazolidone group, in which before withdrawal there had been a depression in the concentrations of these organisms. The resurgence soon subsided. When the experiment was concluded on the 93rd day, 15 % of the chickens in the group fed ordinary diets throughout were still excreting *S. typhimurium* organisms in their faeces. Only in the polymixin group was the faecal excreter rate higher at this time (43 %) – the trimethoprim/sulphadiazine group had ceased to excrete *S. typhimurium* by the 65th day.

Because of the increased excretion of *S. typhimurium* that occurred in these two experiments after the withdrawal of the antibiotic-containing food, two groups of chickens were given a nine-day course of food containing 500 mg./kg. of furazolidone and two a similar course of food containing 100 mg./kg. of trimethoprim and 500 mg./kg. of sulphadiazine. Their faeces and those of two groups given ordinary diets throughout were examined much more frequently in the period immediately after the withdrawal of antibiotic-containing diets than had been the case in the previous experiments. Because the results for each group given the same diet closely resembled each other, they are amalgamated in Table 7. After the suppression of excretion of *S. typhimurium* during the period of administration of the antibiotic-containing diets, the amount of salmonella excretion increased rapidly after their withdrawal, reaching a peak 4–6 days later in the case of the furazolidone groups and the trimethoprim/sulphadiazine groups. The peaks were approximately equal to the concentrations of *S. typhimurium* excreted at those particular times by the groups fed ordinary diets. The amount of *S. typhimurium* excreted by the groups fed all three diets then decreased with time at a similar rate; the duration of excretion was no greater in the groups that had been given antibiotic-containing diets than in the groups given ordinary food throughout. No antibiotic-resistant *S. typhimurium* were isolated from any of the chickens in this experiment.

The results of increasing the treatment period with trimethoprim/sulphadiazine from 9 to 18 days and of delaying the start of a 9-day treatment period from the customary 2 to 11 days after infection are summarized in Table 8. As in the previous experiment, the amount of *S. typhimurium* excreted decreased considerably during the treatment period but increased afterwards, the increase being more noticeable in the groups treated for 18 days. The increase did not exceed the amount found at that time in the groups fed ordinary diets and at the end of the experiment the faecal excreter rates in the treated groups was no higher than that in the groups fed ordinary diets. No salmonella organisms resistant to trimethoprim or sulphadiazine were isolated from any of the chickens used in this or the previous experiment.

DISCUSSION

The amount of *Salmonella typhimurium* excreted by the experimentally infected chickens was reduced to a variable extent by feeding them for 46 days on diets containing 500 mg./kg. of neomycin, spectinomycin, streptomycin, polymixin, ampicillin, furazolidone or a mixture of 100 mg./kg. of trimethoprim and 500 mg./kg. of sulphadiazine, the last treatment having the greatest effect. Only trimethoprim/sulphadiazine and neomycin apparently reduced the amount of excretion when the concentrations of the antibiotics in the food was decreased fivefold and only trimethoprim/sulphadiazine, neomycin, furazolidone and ampicillin when the period of treatment was reduced to 9 days.

An important reason why some of the antibiotics had such a limited effect in reducing the amount of *S. typhimurium* excreted, and an obvious danger to their use in this manner in practice, was the emergence and rise to dominance of antibiotic-resistant *S. typhimurium* organisms. This occurred in the chickens given ampicillin, spectinomycin and streptomycin but never in those given neomycin, polymixin or furazolidone. Sometimes it happened in chickens given chloramphenicol or oxytetracycline, antibiotics which had little or no effect in reducing the amount of *S. typhimurium* excreted even when the organisms remained sensitive to these antibiotics throughout the treatment period. All the trimethoprim-resistant *S. typhimurium* examined were thymine-requiring mutants, a fact that probably accounted for their concentrations in the faeces always being low (Smith & Tucker, to be published). However, R factors determining trimethoprim resistance exist and so do *Salmonella* strains that are resistant to neomycin and furazolidone. It is possible then that if diets containing trimethoprim/sulphadiazine, neomycin or furazolidone were fed for long periods of time to naturally infected chickens under field conditions antibiotic-resistance could be a problem. To what extent this would also apply in the case of polymixin is open to question because polymixin resistance in salmonellas appears to be a rare phenomenon.

When the period of administration was reduced to 9 days, antibiotic resistance, not unexpectedly, was much less common and some of the antibiotics, notably trimethoprim/sulphadiazine, brought about an appreciable reduction in the faecal excretion of *S. typhimurium*. This at a time when high concentrations were being excreted by the non-antibiotic-fed chickens. Although, after the withdrawal of

antibiotic-containing food, the concentrations of *S. typhimurium* increased to figures similar to those found in the non-antibiotic-fed chickens they quickly decreased and there was no suggestion of a prolongation of carrier rate in the groups that had been treated with trimethoprim/sulphadiazine and furazolidone. It is conceivable then that under certain conditions short courses of trimethoprim/sulphadiazine, for example, might be of value during periods when animals or human beings are excreting high concentrations of salmonellas in their faeces, a period when they would be most dangerous as a source of infection for other individuals.

Although many of the antibiotics, particularly at the higher dietary concentrations, brought about a profound reduction in the concentrations of faecal *E. coli*, this was usually short-lived because of the emergence of populations of *E. coli* that were antibiotic-resistant. Most of this resistance, in the chloramphenicol, oxytetracycline, streptomycin, spectinomycin and ampicillin groups was due to R factors, and R factors determining the same patterns of antibiotic resistances were often found in their *S. typhimurium* populations. Because these R factors were usually found in the *E. coli* populations of individual chickens before they were found in their *S. typhimurium* populations, it is logical to assume that small numbers of *E. coli* possessing these R factors were present in the alimentary tracts of some of the chickens at the start of the experiments or gained access to them some time afterwards. There they ultimately achieved dominance owing to the selection pressure provided by the antibiotic-containing food. Later they transferred their R factors to the *S. typhimurium* organisms in their alimentary tracts. These R^+ *S. typhimurium* organisms in turn became dominant, again owing to the selection pressure provided by the antibiotic-containing food. It is noteworthy that the *S. typhimurium* and/or *E. coli* organisms that achieved dominance in the alimentary tract of several of the groups of chickens possessed an R factor determining resistance to ampicillin, streptomycin, sulphonamide, tetracycline, chloramphenicol and spectinomycin and that their dominance was the result of exposing them to any *one* of five of the antibiotics to which the R factor determined resistance.

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