The nutritive value of colostrum for the calf

14. Further studies on the effect of antibiotics on the performance of colostrum-deprived calves*

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The beneficial action of certain antibiotics, particularly chlortetracycline (aureomycin) and oxytetracycline (terramycin) on the growth and well-being of the young calf has been reviewed by Lassiter (1955), and also by Roy, Shillam, Palmer & Ingram (1955) who showed that under their conditions aureomycin given by mouth tended to reduce the mortality rate of colostrum-deprived calves. Earlier, Kastelic, Bentley & Phillips (1950) in Wisconsin, in studies with synthetic milks for newborn calves that had received no colostrum, observed that dihydrostreptomycin afforded some measure of protection against *Escherichia coli* infections. Taylor (1955) observed that 50,000 i.u. (34 mg) penicillin added twice daily to the diet of colostrum-fed calves reduced the number of deaths from scouring associated with an *E. coli* septicaemia. Neither this amount of penicillin nor 100 mg chlortetracycline daily affected the incidence or duration of scouring. In a further experiment, in which white scours was artificially induced in colostrum-deprived calves, 100 mg chlortetracycline/day significantly reduced the number of deaths associated with an *E. coli* septicaemia during the first 4 weeks of life.

Roy, Palmer, Shillam, Ingram & Wood (1955) have demonstrated that when a large number of newborn calves are passed through a calfhouse, there is a gradual 'build-up' of scouring and mortality associated with certain serological types of *E. coli*. The stages in this progressive 'build-up' are denoted by the terms low, moderate or high 'infection'. The experiment of Roy, Shillam *et al.* (1955) in which chlortetracycline reduced scouring and increased live-weight gain of colostrum-deprived calves was conducted under conditions of moderate 'infection'. The further experiment described now was made to find if chlortetracycline was equally as effective under conditions of high 'infection' and, at the same time, to compare its efficiency with that of penicillin in the control of scouring. Penicillin has in general been found to be detrimental to the growth of the young calf (Bloom & Knodt, 1952; Knodt & Bloom, 1952; Voelker & Jacobson, 1953), but Gardner, Nevens, Folkerts & Johnson (1952) and Taylor (1955) found that penicillin had no effect on growth and Kon, Oliver, Porter & Ridler (1953) obtained a slight, not-significant beneficial effect.

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Since Smith & Crabb (1956*a*) have obtained some evidence that strains of *E. coli* in the faecal flora have a greater resistance to chemotherapeutic agents after their prophylactic use, a study of this aspect of antibiotic therapy was also made.

The experiment reported in this paper was thus planned to compare the performance of colostrum-deprived calves when given chlortetracycline and penicillin both singly and in combination under conditions of high 'infection' and to investigate the sensitivity to both antibiotics of strains of *E. coli* isolated from calves on the various treatments.

METHODS

Plan of experiment

The experiment was done in the late spring months of 1955 after 142 calves, many of which had died, had passed through the calfhouse since the previous autumn. Twenty-four Shorthorn and twenty-four Ayrshire bull calves in a randomized block design were used. Five blocks consisted of Shorthorns and five of Ayrshires evenly distributed throughout the experiment. Treatment 31 was omitted from the last block of each breed, owing to a shortage of calves. The plan was as follows:

Treatment no.	Initial diet	Antibiotic given (for quantity see below)
31	No colostrum	None
32	No colostrum	Chlortetracycline
33	No colostrum	Penicillin
34	No colostrum	Chlortetracycline and penicillin
35	6 pt. colostrum	None

Diets

Basic diet

The calves were reared for 3 weeks on bulked whole milk from the Institute herd; the daily allowance was 1 lb./10 lb. live weight, except when scouring occurred (Roy, Shillam *et al.* 1955).

Colostrum

Colostrum was collected, stored and fed in the same manner as in previous experiments (Roy, Shillam *et al.* 1955).

Antibiotics

Chlortetracycline. One-third of the daily allowance of 22·8 g Aurofac D (Lederle Laboratories Inc.) containing 5 g chlortetracycline/lb. was added to the milk at each of the three daily feeds during the first 5 days of life and 11·4 g Aurofac D at the 6.30 a.m. feed during the remainder of the experimental period. Thus, calves on treatments 32 and 34 received 250 mg chlortetracycline daily for the first 5 days and 125 mg daily thereafter.

Penicillin. 200 g procaine penicillin (Glaxo Laboratories Ltd) were mixed with 2·5 kg lactose, and 6·0 g of the mixture were added to the milk during the first 5 days of life and 3·0 g during the remainder of the experimental period in the same manner as for chlortetracycline. Thus, calves on treatments 33 and 34 received 250 mg penicillin daily for the first 5 days and 125 mg daily thereafter.

Calves

Collection and management of the calves were as in earlier experiments (Roy, Shillam *et al.* 1955).

Autopsy. Autopsies were carried out on all the calves that died.

Measurements of the small intestine. All surviving calves were slaughtered at the end of the experimental period and their alimentary tracts examined. Since there is evidence that antibiotics reduce the thickness of the wall of the small intestines of the chick and pig (see p. 213), measurements of the lengths and weights of the small intestines of all surviving calves were made. The small intestine was detached from its mesentery and laid out on a wet metal table to avoid any artificial stretching. After the length had been measured between the pyloric sphincter and the ileo-caecal valve, the small intestine was cut into yard lengths, slit open, and after the contents had been removed under a gentle stream of water it was allowed to drain for 15 min and then weighed.

Isolation of Escherichia coli *strains.* Strains of *E. coli* were isolated from the ileal contents of the surviving calves slaughtered at the end of the experimental period, from the heart blood of those calves dying of a septicaemic infection and from the mesenteric lymph nodes or ileal contents of those with a localized intestinal infection. One or more different strains from each calf were stored on plain agar slopes in the dark until required for tests of antibiotic sensitivity and serological typing.

Tests of antibiotic sensitivity

The sensitivity of each strain of *E. coli* isolated was tested by a tube method against penicillin, chlortetracycline and a mixture of both antibiotics, 'Benzylpenicillin' (Burroughs Wellcome & Co.) tablets containing 10,000 i.u. penicillin, and chlortetracycline hydrochloride (Lederle Laboratories Inc.) of 95·3 % potency being used. A ratio of 1 i.u. (0·7 μg) penicillin to 1 μg chlortetracycline was used in the mixture of penicillin and chlortetracycline. Two-fold serial dilutions of the antibiotic or mixture of antibiotics were prepared in bulk in one batch of meat-extract broth at pH 7·4 and distributed with sterile precautions into series of tubes in 1 ml. amounts. In calculating the concentration of antibiotics, the final addition of one standardized drop of inoculum and the slight impurity of the chlortetracycline were taken into account.

All tests with one antibiotic or the mixture of antibiotics were made on the day of preparation of the solutions and all strains were tested on the same day. Each series of tubes was inoculated with one standardized drop of a diluted 24 h meat-broth culture of *E. coli* so that each tube received about 20,000 organisms. The tubes were incubated at 37° for exactly 24 h and then examined for presence or absence of growth by visual inspection of the tubes for turbidity. The lowest concentration of antibiotic completely inhibiting growth was recorded. The method of testing strains and the size of bacterial inoculum used were similar to those employed by Smith (1954). Statistical analysis of the results was made after transformation of the concentration of antibiotic to the logarithmic scale.

RESULTS

Performance of the calves. The results are given in Table 1. All untreated colostrum-deprived calves died, whereas all except one of the calves given colostrum survived. Of ten colostrum-deprived calves given chlortetracycline alone, seven survived, but of the same number of colostrum-deprived calves given penicillin alone, only two survived. With the mixture of penicillin and chlortetracycline five calves survived. Thus, chlortetracycline alone significantly reduced the mortality rate of colostrum-deprived calves whereas penicillin had little or no effect.

The two surviving calves given penicillin alone had a greater incidence of a high rectal temperature (> 102·8° F.) than the surviving calves on the other three treatments and there was also a tendency for the incidence of scouring of calves given penicillin alone to be greater and the live-weight gain to be lower.

There was no significant difference in the observed weight gains of the calves on the four treatments as given in Table 1 and shown graphically in Fig. 1, but when the mean daily weight gains were analysed by multiple covariance and adjusted for the affecting variables, log 'occupation time',* birth weight and total milk consumption, colostrum-deprived calves given chlortetracycline alone gained significantly more weight ($P < 0·05$) than untreated colostrum-fed calves. The relevant partial regression coefficients with their standard errors are given below; the adjusted mean values are given in Table 1.

	General mean	Partial regression coefficient with its standard error
Live-weight gain/day (lb.)	0·278	—
Log 'occupation time' (days)	2·338	−1·621 ±0·753*
Birth weight (lb.)	85·52	−0·0248 ±0·0038***
Total milk consumption (pt.)	126·96	+0·0205 ±0·0027***

* Significant at $P < 0·05$. *** Significant at $P < 0·001$.

As was found earlier (Roy, Shillam *et al.* 1955), the mean time between birth and complete passage of the meconium of the colostrum-fed calves was greater than that of colostrum-deprived calves whether or not they were given antibiotics.

Autopsy. Table 2 shows the post-mortem findings on calves that died. Contrary to the findings of the earlier experiment under conditions of moderate 'infection' (Roy, Shillam *et al.* 1955), the death of all calves in the present experiment was associated with an *E. coli* infection only.

All the eight calves given penicillin that died succumbed to an *E. coli* septicaemia with or without polyserositis; this condition is usually encountered in colostrum-deprived calves and in those colostrum-fed calves that are affected by certain strains of *E. coli* against which colostrum contains no antibodies, e.g. strain R.V.C. 330 (Wood, 1955). On the other hand, the three calves that died in the chlortetracycline-fed group showed lesions of a localized intestinal infection, a post-mortem finding mainly restricted to colostrum-fed calves that die once the 'infection' has built up in

* 'Occupation time' is defined as the number of days that the calfhouse has been occupied after a period of vacancy (see Roy, Palmer *et al.* 1955).

Table 1. *Comparison of the performance (values with their standard errors†) of colostrum-deprived calves given chlortetracycline, penicillin or a mixture of chlortetracycline and penicillin with that of calves given colostrum or deprived of it*

	Treatment no.					
	31	32	33	34	35	
	No colostrum				Colostrum	
	No antibiotic	Chlortetracycline	Penicillin	Chlortetracycline and penicillin	No antibiotic	Significance of differences between treatments
Calves:						
No. used	8	10	10	10	10	—
No. died	8	3	8	5	1	31 > 32**, 31 > 35***, 33 > 35**
Mean age at death (days)	5 ± 1	7 ± 1	6 ± 1	8 ± 1	7‡	32 > 31*
Mean live-weight gain/day of surviving calves (lb.)	—	0·47 ± 0·12	0·01 ± 0·23	0·11 ± 0·14	0·23 ± 0·11	—
Mean no. of days on which surviving calves scoured	—	3 ± 0·7	8 ± 2·5	5 ± 2·7	4 ± 0·6	—
Mean no. of days on which surviving calves had a high rectal temperature (> 102·8° F.)	—	2 ± 0·9	6 ± 2·0	1 ± 0·9	2 ± 0·8	32 + 34 + 35 < 33*
Adjusted mean live-weight gain/day of surviving calves (lb.)§	—	0·39 ± 0·06	—‖	0·23 ± 0·07	0·21 ± 0·05	32 > 35*
Mean time between birth and complete passage of meconium (h)	28·2 ± 2·5	29·8 ± 2·0	33·5 ± 1·8	32·3 ± 1·9	43·8 ± 2·0	31 < 35***, 32 < 35***, 33 < 35**, 34 < 35***

* Significant at $P < 0·05$. ** Significant at $P < 0·01$. *** Significant at $P < 0·001$.

† In calculating the standard errors the arrangement in blocks has been ignored. ‡ One calf only.

§ See p. 206. ‖ Values not included in analysis of multiple covariance.

the calfhouse and usually associated with particular strains against which colostrum similarly is devoid of antibodies, e.g. strains R.V.C. 95A and 118A (Ingram, Lovell, Wood, Aschaffenburg, Bartlett, Kon, Palmer, Roy & Shillam, 1956). Four of the five calves that died after being given both chlortetracycline and penicillin showed lesions of a septicaemia and polyserositis.

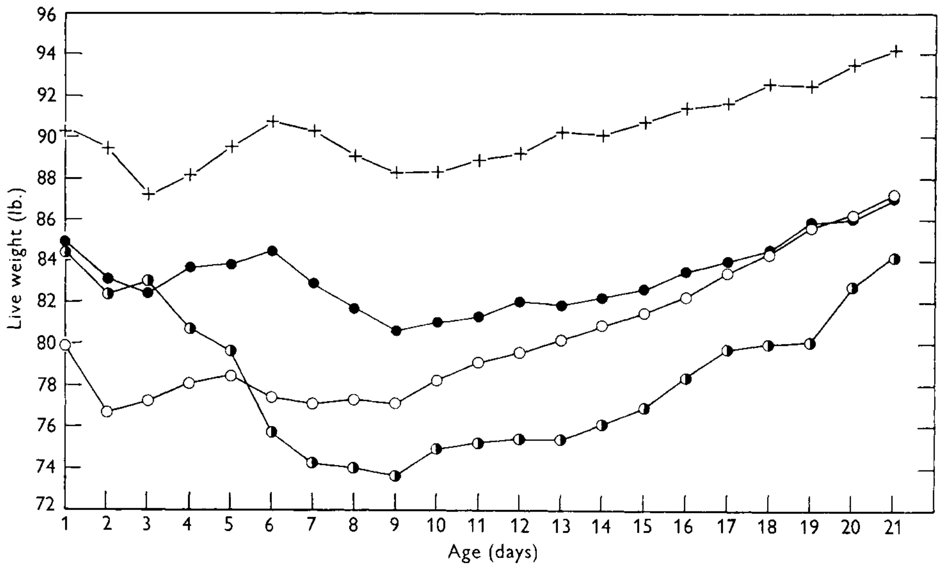

Fig. 1. Mean growth rates of colostrum-deprived calves given supplements of chlortetracycline and penicillin, either singly or together, and of colostrum-fed calves. +—+, colostrum; ○—○, chlortetracycline; ◑—◑, penicillin; ●—●, chlortetracycline and penicillin.

Table 2. *Post-mortem findings in the calves*

		No. of calves that died on treatment no.				
		31	32	33	34	35
		No colostrum				Colostrum
Post-mortem findings		No antibiotic	Chlortetra-cycline	Penicillin	Chlortetra-cycline and penicilin	No antibiotic
E. coli	septicaemia	5	0	3	0	0
	septicaemia and polyserositis	2	0	5	4	0
	localized intestinal infection	1	3*	0	1	1

* *Achromobacterium* sp. in the heart blood only of one calf (probably a post-mortem or agonal invader).

Measurements of small intestine. Table 3 shows the lengths and weights of the small intestines of the surviving calves. Both these measurements were related to the body-weight of the calf at 3 weeks of age, but even after adjustment for body-weight neither was affected by antibiotic treatment. However, the ratio of weight to length was significantly smaller ($P < 0.05$) for calves that had received penicillin with or without chlortetracycline than for those that had received chlortetracycline alone.

Tests of antibiotic sensitivity. One hundred and fifteen strains of *E. coli* were tested for their in vitro sensitivity to chlortetracycline, penicillin and a mixture of the two antibiotics, and the results are summarized in Table 4. With the mixture of antibiotics, no definite evidence of synergistic or antagonistic action was obtained and in most instances the result was the same as that with the more effective antibiotic alone, usually chlortetracycline.

All strains of *E. coli* encountered in this experiment were very resistant to penicillin. Strains isolated from untreated colostrum-deprived calves were significantly more sensitive to penicillin ($P < 0.05$) than strains isolated from untreated colostrum-fed calves. A similar trend was apparent in sensitivity to chlortetracycline but the difference was not significant.

Table 3. *Values with their standard errors for measurements of small intestines of the experimental calves*

	Treatment no.				
	32	33	34	35	
	No colostrum			Colostrum	Significance of difference between treatments
	Chlortetra-cycline	Penicillin	Chlortetra-cycline and penicillin	No antibiotic	
No. of calves	7	2	4†	9	—
Mean body-weight (lb.)	87·2 ± 5·6	84·1 ± 7·4	81·4 ± 10·4	95·1 ± 4·9	—
Mean length of small intestine (cm)	1503 ± 65	1520 ± 122	1443 ± 86	1532 ± 57	—
Mean weight of small intestine (g)	763 ± 47	719 ± 88	689 ± 62	836 ± 42	—
Adjusted mean length of small intestine (cm)‡	1516 ± 55	1553 ± 104	1494 ± 75	1492 ± 51	—
Adjusted mean weight of small intestine (g)‡	776 ± 30	752 ± 55	741 ± 40	795 ± 27	—
Ratio, weight:length (g/cm)	0·509 ± 0·010	0·475 ± 0·037	0·477 ± 0·008	0·550 ± 0·038	33 + 34 < 32*

* Significant at $P < 0.05$.
† Values for one calf are not included as complete intestine was not available.
‡ Adjusted for body-weight.

Of the sixty-five strains of *E. coli* isolated from calves either at post-mortem or at slaughter that had received no penicillin during life, all but five showed a minimum inhibitory concentration (M.I.C.) between 32 and 128 i.u. penicillin/ml. Of these five more resistant strains, four required 1024 i.u. penicillin/ml. for growth inhibition and all were isolated from calves in the last five blocks of the experiment. The fifty strains of *E. coli* isolated from calves that had received penicillin either with or without chlortetracycline showed M.I.C.'s of penicillin between 32 and 2048 i.u./ml. Thus the mean M.I.C. of penicillin for strains isolated from penicillin-fed calves was 176·1 i.u./ml., whereas for strains from calves that had not received penicillin the value was 52·5 i.u./ml. This difference in resistance to penicillin was highly significant ($P < 0.001$). Although strains isolated from calves given both chlortetracycline and penicillin appeared to be more resistant to penicillin than those obtained from calves given penicillin alone, the difference was not significant.

Table 4. *In vitro tests of sensitivity to penicillin and chlortetracycline of strains of* E. coli *isolated from calves at death or at slaughter at 3 weeks of age*

(*a*) Penicillin

Calves: Treatment no.	No.	No. of strains isolated	No. of strains for which minimum inhibitory concentration of penicillin (i.u./ml.) was: 8·0	16·0	32·0	64·0	128·0	256·0	512·0	1024·0	2048·0	Mean minimum inhibitory concentration of penicillin: Transformed values (log) with their standard errors	Corresponding values in i.u./ml.	Significance of difference between treatments
31 (deprived of colostrum, no supplement)	8	29	0	0	10	15	4	0	0	0	0	1·593 ± 0·034	39·2	31 < 32**, 31 < 35*
35 (given colostrum, no supplement)	10	15	0	0	3	7	4	0	0	1‡	0	1·756 ± 0·093	57·0	35 > 31*
32 (deprived of colostrum, chlortetracycline supplement)	10	21	0	0	2	13	2	0	1‡	3‡	0	1·871 ± 0·102	74·3	32 > 31**
All calves without penicillin	28	65	0	0	15	35	10	0	1‡	4‡	0	1·721 ± 0·044	52·5	31 + 35 + 32 < 33 + 34***
33 (deprived of colostrum, penicillin supplement)	10	29	0	0	3	10	1	0	6	9	0	2·195 ± 0·109	156·8	—
34 (deprived of colostrum, penicillin and chlortetracycline supplement)	8†	21	0	0	2	2	3	3	6	4	1	2·315 ± 0·111	206·6	—
All calves given penicillin ± chlortetracycline	18	50	0	0	5	12	4	3	12	13	1	2·246 ± 0·078	176·1	33 + 34 > 31 + 35 + 32***
Total	46	115												

(*b*) Chlortetracycline

Calves: Treatment no.	No.	No. of strains isolated	No. of strains for which minimum inhibitory concentration of chlortetracycline (μg/ml.) was: 0·5	1·0	2·0	4·0	8·0	16·0	32·0	64·0	128·0	256·0	Mean minimum inhibitory concentration of chlortetracycline: Transformed values (log) with their standard errors	Corresponding values in μg/ml.	Significance of difference between treatments
31 (deprived of colostrum, no supplement)	8	29	0	0	0	17	10	1	0	0	1‡	0	0·628 ± 0·055	4·3	—
35 (given colostrum, no supplement)	10	15	0	0	0	8	5	1	1	0	0	0	0·652 ± 0·066	4·5	—
33 (deprived of colostrum, penicillin supplement)	10	29	0	0	1	16	8	2	0	0	2‡	0	0·670 ± 0·074	4·7	—
All calves without chlortetracycline	28	73	0	0	1	41	23	4	1	0	3‡	0	0·650 ± 0·039	4·5	31 + 35 + 33 < 32 + 34***
32 (deprived of colostrum, chlortetracycline supplement)	10	21	0	0	0	0	5	1	1	10	4	0	1·455 ± 0·096	28·5	32 < 34**
34 (deprived of colostrum, chlortetracycline and penicillin supplement)	8†	21	0	0	0	0	0	1	2	8	6	4	1·799 ± 0·068	63·0	34 > 32**
All calves given chlortetracycline ± penicillin	18	42	0	0	0	0	5	2	3	18	10	4	1·627 ± 0·064	42·4	32 + 34 > 31 + 35 + 33***
Total	46	115													

* Significant at $P < 0·05$. ** Significant at $P < 0·01$. *** Significant at $P < 0·001$. † Strains of *E. coli* from two calves were lost.
‡ Strains of *E. coli* isolated during the second half of the experimental period.

With the exception of three strains isolated from calves in the last three blocks of the experiment, the seventy-three strains of *E. coli* isolated from all calves that had not received chlortetracycline were sensitive to chlortetracycline and showed M.I.C.'s between 2·0 and 32·0 μg/ml. However, the forty-two strains isolated from calves that had received chlortetracycline either with or without penicillin showed a marked increase ($P < 0.001$) of resistance to chlortetracycline with values within the range 8·0–256·0 μg/ml. for inhibition of growth. Thus the mean M.I.C. of chlortetracycline for strains isolated from chlortetracycline-fed calves was 42·4 μg/ml., whereas for strains isolated from calves that had not received chlortetracycline the value was 4·6 μg/ml. Those strains isolated from calves given both chlortetracycline and penicillin had a greater resistance ($P < 0.01$) to chlortetracycline than those isolated from calves given chlortetracycline alone, the M.I.C. values being 63·0 and 28·5 μg/ml. respectively. Similarly, those strains isolated from calves given chlortetracycline alone were more resistant to penicillin ($P < 0.01$) than those isolated from unsupplemented calves.

Table 5. *Tests of sensitivity to chlortetracycline of strains isolated from chlortetracycline-fed calves that survived and from those that died*

Calves given chlortetracycline	No. of calves	No. of strains	No. of strains for which the minimum inhibitory concentration of chlortetracycline (μg/ml.) was: 0·5	1·0	2·0	4·0	8·0	16·0	32·0	64·0	128·0	256·0
Survived	7	15	0	0	0	0	4	0	1	7	3	0
Died	3	6	0	0	0	0	1	1	0	3	1	0

The results in Table 5 show that strains isolated from chlortetracycline-fed calves that survived did not differ in sensitivity to chlortetracycline from those isolated from calves that were given chlortetracycline and died. However, strains from the calves that died were isolated when the mean age of the calves was 7 ± 0.7 days, whereas strains from those that survived were isolated at the end of the 3-week experimental period.

DISCUSSION

The results of this experiment have shown clearly that the giving of chlortetracycline at a moderately high level will reduce the mortality of colostrum-deprived calves under conditions of high 'infection', whereas penicillin has little or no effect. Moreover, all penicillin-fed calves that died showed at post-mortem an *E. coli* septicaemia with or without polyserositis, a condition found with one exception in all the non-supplemented colostrum-deprived calves that died, whereas those calves that died after being given chlortetracycline showed only an *E. coli* localized intestinal infection, the usual finding in colostrum-fed calves that succumb. The ineffectiveness of penicillin was borne out by the greater incidence of a high rectal temperature for the two surviving penicillin-fed calves and by a tendency for these calves to have a higher incidence of scouring and lower weight gains. Fig. 1 shows that the body-weights of these two calves fell rapidly owing to profuse scouring during the first 7 days of life.

Furthermore, as might be expected, all strains of *E. coli* encountered were very resistant to penicillin.

The improvement in weight gains brought about by chlortetracycline was such that calves, even though deprived of colostrum, gained more weight when given chlortetracycline than calves given colostrum but no chlortetracycline. However, it must be remembered that colostrum-fed calves received only 6 pt. of colostrum, and it is possible that this weight difference would not have occurred if the amount and period of feeding of colostrum had been increased and if the conditions of 'infection' had been lower.

The use of penicillin in combination with chlortetracycline appeared to affect the efficacy of chlortetracycline. Thus, there was a tendency for the mortality rate of calves given a combination of the two antibiotics to be higher than for those given chlortetracycline alone and also an indication that weight gains were lower and incidence of scouring higher in the penicillin plus chlortetracycline group. The finding that four of the five deaths of calves given both antibiotics were due to a septicaemia whereas in the three chlortetracycline-fed calves that died the infection was localized in the intestine suggests that penicillin was affecting the action of chlortetracycline.

Although no direct antagonistic effect of chlortetracycline and penicillin was found in the in vitro sensitivity tests on individual strains, there is some indication from the total results that antagonism was in fact occurring. Results of the sensitivity tests demonstrated the significantly greater resistance to chlortetracycline of strains of *E. coli* isolated from calves given both penicillin and chlortetracycline than of strains obtained from calves given chlortetracycline alone. Similarly, strains isolated from calves given chlortetracycline alone were more resistant to penicillin than those from calves given no antibiotic. Antagonism between penicillin and chlortetracycline might be expected for Jawetz, Gunnison, Bruff & Coleman (1952) suggested that as a general rule bactericidal drugs such as penicillin and bacteriostatic drugs like chlortetracycline show this phenomenon when used together. In vitro tests by Elek, Hilson & Jewell (1953) showed that with seventeen strains of *E. coli* from urinary infections in man, penicillin and chlortetracycline exhibited antagonism during the inhibition of growth of three strains, whereas there was no interaction in the remaining fourteen. Antagonism of penicillin and chlortetracycline has also been demonstrated with other organisms against which penicillin was the more effective drug (Gunnison, Jawetz & Coleman, 1950; Gunnison, Coleman & Jawetz, 1950; Hunter, 1950; Bartell, 1952).

The minimum inhibitory concentrations of penicillin and chlortetracycline for all strains isolated in the first half of the experiment from calves given no antibiotic, namely 32–128 i.u./ml. and 2·0–32·0 μg/ml. for penicillin and chlortetracycline respectively, were within the same range as those found by Smith (1954) who used fifty-eight strains of *E. coli*, some of which had been isolated from calves used in our earlier experiments. The five strains from calves given no penicillin that were inhibited by 512 and 1024 i.u. penicillin/ml. and the three strains from calves given no chlortetracycline that were inhibited by 128 μg chlortetracycline/ml. may be naturally more resistant strains or, since they were isolated from calves during the second half of the experiment, they may have acquired increased resistance after passage through calves that had been given the respective antibiotics. The higher resistance to penicillin or chlortetracycline of strains from penicillin- or chlortetracycline-fed calves might result from an acquired resistance or from the fact that resistant strains were becoming dominant owing to the elimination of more susceptible ones.

By means of 'phage typing of strains, Smith & Crabb (1956*a*, *b*) were able to show that oral streptomycin therapy in calves permitted streptomycin-resistant strains already present in the intestinal flora to become more dominant in the faeces; there was no evidence of mutation. Such firm evidence for selection of resistance was lacking for chlortetracycline; the only chlortetracycline-resistant strains of *E. coli* isolated were from two farms where chlortetracycline supplements had been in routine use during the 1st month of life over the previous 2 years. However, the inference from our results is that strains of *E. coli* more resistant to both chlortetracycline and penicillin were becoming established in the calfhouse at the end of the experiment which lasted over a period of 108 days. It must, however, be borne in mind that the amounts of antibiotic incorporated in the milk fed to the calves in this experiment approached therapeutic levels and were far greater than those used as a normal prophylactic food supplement.

The higher resistance to penicillin of strains isolated from colostrum-fed calves compared with that of strains obtained from colostrum-deprived calves may have been a chance effect, although a similar slight trend was noticeable with chlortetracycline.

The reduction in the weight: length ratio of the small intestine of the calves given penicillin with or without chlortetracycline compared with those given chlortetracycline alone is of some interest. It has been established that penicillin reduces the weight of the intestine of the chick in the presence of the 'growth-depressing infection' (Gordon, 1952; Coates, Davies & Kon, 1955) and a similar reduction in weight of intestine has been found in chicks given chlortetracycline (Pepper, Slinger & Motzok, 1953). With pigs, Braude, Coates, Davies, Harrison & Mitchell (1955) and Taylor & Harrington (1955) found a reduction in the weight of the small intestine when chlortetracycline had been given, but Taylor & Harrington (1955) found only a non-significant reduction when the supplement was penicillin. In chlortetracycline-supplemented calves as compared with control calves Rusoff, Landagora & Hester (1954) found a decrease in thickness of the duodenal and jejunal sections of the intestinal wall and an increase in the ileal section. In all these experiments the effect on the small intestine was accompanied by a beneficial action of the antibiotic in increasing weight gains. Our experiment has indicated that chlortetracycline had no effect in reducing the weight: length ratio of the small intestine over a 3-week period although its beneficial effect in controlling *E. coli* infections was clear. Penicillin, on the other hand, had no beneficial effect on mortality and scouring, but nevertheless reduced the weight: length ratio of the small intestine. This reduction in weight: length ratio in penicillin-fed calves may, however, have been associated with their higher incidence of scouring, although this relationship was not statistically significant.

SUMMARY

1. Forty-eight newborn bull calves, twenty-four Shorthorns and twenty-four Ayrshires were used in an experiment to find the effect of giving chlortetracycline (as Aurofac D, Lederle Laboratories Inc.), penicillin (procaine penicillin) and a combination of the two antibiotics to colostrum-deprived calves.

2. Of thirty-eight calves that were deprived of colostrum, ten were given chlortetracycline, ten were given penicillin, ten were given chlortetracycline and penicillin and eight received no antibiotic. The allowance was 250 mg/day of each antibiotic for the first 5 days of life and 125 mg/day for the remainder of the 3-week experimental period. The ten remaining calves received 6 pt. colostrum, and all were reared on whole milk throughout the experiment.

3. Chlortetracycline when given alone significantly reduced mortality whereas penicillin had no effect. The results of the post-mortem findings on those calves given both antibiotics suggest that the beneficial effect of chlortetracycline was antagonized by penicillin.

4. Live-weight gain of the colostrum-deprived calves given chlortetracycline was significantly greater than that of the colostrum-fed calves. The incidence of a high rectal temperature was greater in surviving calves given penicillin than in surviving calves on the other treatments. There was some indication that the incidence of scouring was also higher in the penicillin-fed calves.

5. Strains of *E. coli* isolated from penicillin-fed calves at death or at the end of the experimental period were more resistant to penicillin in vitro than strains isolated from calves that had not received penicillin. Similarly, strains isolated from chlortetracycline-fed calves were more resistant to chlortetracycline than strains isolated from calves not given chlortetracycline. Strains obtained from calves that received both chlortetracycline and penicillin were more resistant to chlortetracycline than strains isolated from calves given chlortetracycline alone.

6. The weight:length ratio of the small intestine was smaller for calves given penicillin with or without chlortetracycline than for calves given chlortetracycline alone.

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REFERENCES

Bartell, P. (1952). *J. infect. Dis.* **90**, 273.
Bloom, S. & Knodt, C. B. (1952). *J. Dairy Sci.* **35**, 910.
Braude, R., Coates, M. E., Davies, M. K., Harrison, G. F. & Mitchell, K. G. (1955). *Brit. J. Nutr.* **9**, 363.
Coates, M. E., Davies, M. K. & Kon, S. K. (1955). *Brit. J. Nutr.* **9**, 110.

Elek, S. D., Hilson, G. R. F. & Jewell, P. (1953). *Brit. med. J.* ii, 1298.
Gardner, K. E., Nevens, W. B., Folkerts, T. & Johnson, B. C. (1952). *J. Anim. Sci.* **11**, 761.
Gordon, H. A. (1952). In *A Colloquium 'Studies on the Growth Effect of Antibiotics in Germ-free Animals'*. Notre Dame, Indiana: Lobund Institute, University of Notre Dame (Mimeo.).
Gunnison, J. B., Coleman, V. R. & Jawetz, E. (1950). *Proc. Soc. exp. Biol., N.Y.*, **75**, 549.
Gunnison, J. B., Jawetz, E. & Coleman, V. R. (1950). *J. Lab. clin. Med.* **36**, 900.
Hunter, J. H. (1950). *J. Amer. med. Ass.* **144**, 524.
Ingram, P. L., Lovell, R., Wood, P. C., Aschaffenburg, R., Bartlett, S., Kon, S. K., Palmer, J., Roy, J. H. B. & Shillam, K. W. G. (1956). *J. Path. Bact.* **72**, 561.
Jawetz, E., Gunnison, J. B., Bruff, J. B. & Coleman, V. R. (1952). *J. Bact.* **64**, 29.
Kastelic, J., Bentley, O. G. & Phillips, P. H. (1950). *J. Dairy Sci.* **33**, 725.
Knodt, C. B. & Bloom, S. (1952). *J. Dairy Sci.* **35**, 675.
Kon, S. K., Oliver, J., Porter, J. W. G. & Ridler, B. (1953). *Proc. Nutr. Soc.* **12**, x.
Lassiter, C. A. (1955). *J. Dairy Sci.* **38**, 1102.
Pepper, W. F., Slinger, S. J. & Motzok, I. (1953). *Poult. Sci.* **32**, 656.
Roy, J. H. B., Palmer, J., Shillam, K. W. G., Ingram, P. L. & Wood, P. C. (1955). *Brit. J. Nutr.* **9**, 11.
Roy, J. H. B., Shillam, K. W. G., Palmer, J. & Ingram, P. L. (1955). *Brit. J. Nutr.* **9**, 94.
Rusoff, L. L., Landagora, F. T. & Hester, H. H. (1954). *J. Dairy Sci.* **37**, 654.
Smith, H. W. (1954). *Vet. Rec.* **66**, 42.
Smith, H. W. & Crabb, W. E. (1956*a*). *Vet. Rec.* **68**, 274.
Smith, H. W. & Crabb, W. E. (1956*b*). *J. gen. Microbiol.* **15**, 556.
Taylor, J. H. (1955). The effect of adding an antibiotic supplement to the diet of animals with special reference to the mode and site of action. Ph.D. Thesis, University of Reading.
Taylor, J. H. & Harrington, G. (1955). *Nature, Lond.*, **175**, 643.
Voelker, H. & Jacobson, N. L. (1953). *J. Dairy Sci.* **36**, 592.
Wood, P. C. (1955). *J. Path. Bact.* **70**, 179.

Flour and bread, prepared with or without treatment with chlorine dioxide, as long-term sources of vitamin E for rats

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The destruction of vitamin E in flour by chlorine dioxide was first reported by Moran, Pace & McDermott (1953). Chemical estimations of total tocopherols indicated a loss of 70% after the application of the improver at the rate of 30 p.p.m., which was the commercial level current at the time of their experiments. Later the same authors (Moran *et al.* 1954) mentioned similar experiments in which a loss of 95% of the total reducing substances in the unsaponifiable matter of the wheat oil was observed. According to chemical estimations by Frazer, Hickman, Sammons & Sharratt (1956), flour of 78% extraction rate contained 1·5 mg α-tocopherol/100 g, which was reduced to 0·2 mg after treatment with chlorine dioxide at the normal level. Their value for the untreated flour was about three times higher than would be expected, in our experience, for flour of 80% extraction rate. The destruction of the vitamin by chlorine dioxide was confirmed in biological tests on rats, which had smaller testes when their diet had contained bread crumbs made from ClO_2-treated flour than when the crumbs had been made from untreated flour.