THE SENSITIVITY TO ANTIBIOTICS OF STRAINS OF *BACT. COLI* ASSOCIATED WITH INFANTILE GASTRO-ENTERITIS

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Various workers (Bray, 1945; Giles and Sangster, 1948; Taylor, Powell and Wright, 1949; Rogers, Koegler and Gerrard, 1949; Beeuwkes, Gijsberti Hodenpijl and Ten Seldam, 1949; Giles, Sangster and Smith, 1949; Smith, 1949; Magnusson, Laurell, Frisell and Werner, 1950; Kirby, Hall and Coackley, 1950; Smith, Galloway and Speirs, 1950; Kauffmann, 1950; Neter and Shumway, 1950; Taylor, 1951; Rogers, 1951; Drimmer-Herrnheiser and Olitzki, 1951; Orskov, 1951; Braun and Henckel, 1951; Modica, Ferguson and Ducey, 1952; Shanks and Studzinski, 1952; and Laurell, 1952) have shown that in Great Britain, Denmark, Sweden, Holland, Germany, Palestine and the U.S.A. certain specific serological types of *Bact. coli* can be isolated from the faeces of cases of infantile gastro-enteritis, of which epidemiological evidence suggests they may be the cause. In fact a recent editorial article in the *Lancet* (1952) summarizing the position as regards the 0111 B4 type states that there is now good evidence that the 0111 B4 type of *Bact. coli* may cause infantile gastro-enteritis both as a sporadic and an epidemic disease.

These organisms have been described under various names, but where possible, they have now been classified in the Kauffmann (1947) schema according to their antigenic structure as follows:

Type	Sub-types Described		
First type 0111 B4	H- H2 H12 H7	non-motile sucrose-positive sucrose-negative H10 H26	
Second type 055 B5	H2	H6 H7	
Third type 026 B6	H- H11	non-motile	
Taylor's types not yet classified serologically	Vincent E611 E990		

Several papers have been published (Magnusson *et al.*, 1950; Rogers *et al.*, 1949; Neter and Webb, 1951; Lowdon and McNeill, 1951; Alexander, Benjamin, Maslen and Roden, 1952) on the results

of antibiotic therapy in this disease. Some workers have found certain antibiotics gave, clinically, some benefit, but in other controlled experiments the results have not been promising.

It was therefore decided to investigate the action of the antibiotics *in vivo* and *in vitro* from the point of view of their bacteriostatic and bactericidal action. In hospital wards, in particular, the amount of cross infection associated with the presence of the specific types of *Bact. coli* is very great, and there is every need to utilize all available means for its control. No attempt has been made, however, to evaluate the therapeutic action of any of the antibiotics as infantile gastro-enteritis is now, for the most part, a mild disease, and when severe cases do occur adjustment of the water balance gives excellent results.

Methods

The preliminary identification of the specific coliform organisms on plates inoculated with the faeces of infants is difficult because there is no distinctive fermentation reaction. Culture on MacConkey agar, on which, it has been suggested, the organisms have a distinct colonial appearance, is the method preferred, the appearance depending, however, on the composition of the medium. In this laboratory the first examination of the colonies is made by taking a sweep from the plate culture with a platinum loop and emulsifying the material in a drop of 0 K anti-serum diluted 1 in 4 with saline, this method of slide agglutination being effective if the specific organisms are profuse. It is not effective if the specific colonies are greatly outnumbered by other coliforms, and roughness may cause spurious slide agglutination and may be revealed by a control slide test in 1 in 500 acriflavine in saline. If the sweep from the original plate gave a positive slide agglutination test several colonies could usually be identified quickly and picked on to agar tubes for further serological and biochemical tests. If, however, the original sweep was negative then seven individual colonies were inoculated in parallel lines across an agar plate and these subcultures after incubation were examined by slide agglutination.

The biochemical tests on 0111 B4 strains were remarkably constant, and the fact that the H2 variety ferments

TABLE 1

IN VITRO BACTERIOSTATIC ACTION OF ANTIBIOTICS ON BACT. COLI ASSOCIATED WITH INFANTILE GASTRO-ENTERITIS

Strains	No. tested	No. of Strains Inhibited after Incubation for 24 Hours at 37°C. in Antibiotic (concentration µg./ml.)						No. of Strains Inhibited after Incubation for 48 Hours at 37°C. in Antibiotics (concentration µg./ml.)					
		50	25	12.5	6.25	3.1	1.5	50	25	12.5	6.25	3.1	1.5
0111 B4 H2 ..	24	12	6	4	0	(1) Dihydro-streptomycin							
0111 B4 H12 ..	24	15	14	6	2	0	0	8	6	1	0	0	0
055 B5 H6 ..	24	14	11	7	6	1	0	10	8	0	0	0	0
								15	7	5	3	0	0
0111 B4 H2 ..	24	24	18	18	8	(2) Aureomycin hydrochloride							
0111 B4 H12 ..	24	24	24	24	15	3	0	18	10	6	2	0	0
055 B5 H6 ..	24	24	21	21	14	6	0	20	12	12	3	0	0
						9	0	19	17	10	3	0	0
0111 B4 H2 ..	24	24	24	24	22	(3) Chloromycetin							
0111 B4 H12 ..	24	24	24	24	6	2	0	24	24	20	4	2	0
055 B5 H6 ..	24	24	24	23	17	2	0	24	24	18	3	0	0
						9	0	24	24	22	7	0	0
0111 B4 H2 ..	24	24	24	22	22	(4) Terramycin hydrochloride							
0111 B4 H12 ..	24	24	24	24	18	6	2	24	24	20	10	6	0
055 B5 H6 ..	24	24	24	22	17	15	1	24	18	18	18	12	0
						10	0	24	24	21	17	8	0

saccharose and that H12 does not was of great value in the preliminary orientation of these sub-varieties. Rare strains of the two serological sub-types also fail to produce gas. The sugar reactions of the 055 B5 strains were also uniform and any deviation made re-investigation of the strains necessary.

The actions of dihydro-streptomycin, aureomycin, chloromycetin, and terramycin on 0111 B4 and 055 B5 were tested in tubes containing 1 ml. of broth and quantities of antibiotic ranging from 50 to 1.5 µg., one drop of a 1 in 10 dilution of a 24-hour broth culture of the strain to be tested being added to each tube. Inhibition of growth was noted after incubating for 24 hours at 37°C. and at the same time a subculture was made from each tube showing no visible growth onto a MacConkey plate to see if there had been any bactericidal effect. The tubes were again incubated for a further period of 24 hours and then re-examined for bacteriostatic and bactericidal effects.

In vitro Sensitivity of Strains to Antibiotics

Twenty-four strains of each of the 0111 B4 H2, 0111 B4 H12 and 055 B5 H6 varieties were tested for sensitivity to the various antibiotics. These strains included nine from the collection of Dr. Joan Taylor; the rest were obtained locally. The bacteriostatic effects of antibiotics are recorded in Table 1 and the bactericidal in Table 2. There was no evidence that any one of the varieties was more sensitive than another to any one antibiotic in regard either to its bacteriostatic or to its bactericidal power. The most inhibitory antibiotic both at 24 hours and at 48 hours was terramycin, followed by chloromycetin, then aureomycin and finally by dihydro-streptomycin. Very few strains were inhibited by 1.5 µg. per ml. and then only by terramycin after incubation for 24 hours but not after 48 hours. Most strains were

TABLE 2

IN VITRO BACTERICIDAL ACTION OF ANTIBIOTICS ON BACT. COLI ASSOCIATED WITH INFANTILE GASTRO-ENTERITIS

Strains	No. Tested	No. of Strains Killed after Incubation for 24 Hours at 37°C. in Antibiotic (concentration µg./ml.)						No. of Strains Killed after Incubation for 48 Hours at 37°C. in Antibiotic (concentration µg./ml.)					
		50	25	12.5	6.25	3.1	1.5	50	25	12.5	6.25	3.1	1.5
0111 B4 H2 ..	24	6	2	0	0	(1) Dihydro-streptomycin							
0111 B4 H12 ..	24	7	4	0	0	0	0	7	5	0	0	0	0
055 B5 H6 ..	24	10	2	2	1	0	0	8	3	0	0	0	0
								12	2	2	1	0	0
0111 B4 H2 ..	24	0	0	0	0	(2) Aureomycin hydrochloride							
0111 B4 H12 ..	24	0	0	0	0	0	0	2	0	0	0	0	0
055 B5 H6 ..	24	0	0	0	0	0	0	1	0	0	0	0	0
								2	1	0	0	0	0
0111 B4 H2 ..	24	0	0	0	0	(3) Chloromycetin							
0111 B4 H12 ..	24	0	0	0	0	0	0	3	1	0	0	0	0
055 B5 H6 ..	24	0	0	0	0	0	0	2	1	0	0	0	0
								1	0	0	0	0	0
0111 B4 H2 ..	24	0	0	0	0	(4) Terramycin hydrochloride							
0111 B4 H12 ..	24	0	0	0	0	0	0	4	1	0	0	0	0
055 B5 H6 ..	24	0	0	0	0	0	0	3	1	0	0	0	0
								8	0	0	0	0	0

inhibited by the larger concentrations of all antibiotics except dihydro-streptomycin, in which many strains grew freely even in concentrations of 50 and 25 μ g. per ml. As seen in Table 2 aureomycin, chloromycetin and terramycin were not completely bactericidal in any concentration after 24 hours' incubation and killed only a few strains in the higher concentrations after 48 hours. When a drop from each tube was plated on MacConkey medium after incubating for 24 hours the number of colonies growing from the broth containing 50 μ g. per ml. of antibiotic was small compared with the number growing from loopfuls taken from the tubes with lower concentrations. On the other hand although dihydro-streptomycin was the least inhibitory it was most bactericidal. It apparently killed about half the strains which were inhibited at 24 hours and about three-quarters of the strains inhibited at 48 hours. Similar results were obtained on 12 strains of *Bact. coli* picked at random from faecal cultures from conditions other than infantile gastro-enteritis.

One strain of anaerogenic 0111 B4 H12 obtained from Dr. Taylor and three strains of anaerogenic 0111 B4 H2 isolated originally from cases in Edinburgh were also tested in the same way and all seemed to be slightly more sensitive to the bacteriostatic action of the drugs than the members of the same serological type producing gas.

In vivo Sensitivity of Strains to Antibiotics

As mentioned previously, it is not proposed to evaluate the effects of antibiotics in the treatment of infantile gastro-enteritis but simply to review the cases to see if these drugs caused the disappearance of the serological types of *Bact. coli* from the faeces. The cases included were those in which only one drug or antibiotic had been employed for treatment throughout the course of the illness.

All the patients were treated in a similar fashion and on admission were starved for 24 hours or longer during which period the milder cases received half strength Ringer lactate solution orally in quantity depending on the weight and based on the formula of 100 ml. per pound per day, while if dehydration was severe the same fluid and same relative quantity was given intravenously by drip, using a polythene cannula. Oral feeding was gradually introduced starting with one-quarter strength milk feeds, increasing through half, three-quarters, to full strength over a period of four days. In the treatment of these cases the first essential is to restore the fluid balance and in severe cases the intravenous infusion of fluid is probably the most important life-saving therapeutic aid available.

Immediately a patient was admitted a specimen

of faeces was sent to the laboratory for examination, and if this showed a specific serological variety of *Bact. coli* and if the case was not to be used as a control, then the appropriate drug was given as follows:

Sulphamezathine	.. ..	0.5 g. to begin with followed by 0.25 g. every four hours orally
Penicillin	.. ..	50,000 units to begin with followed by 25,000 four-hourly parenterally
Polymyxin	.. ..	50 mg. in six-hourly doses orally
Dihydro-streptomycin	.. ..	20 mg. per kg. in six-hourly doses parenterally
Aureomycin hydrochloride	.. ..	10 mg. per kg. in six-hourly doses orally
Chloromycetin	.. ..	50 mg. per kg. in six-hourly doses orally
Terramycin hydrochloride	.. ..	50 mg. per kg. in six-hourly doses orally

The courses of treatment lasted six days in all, and thereafter a specimen of faeces was sent for bacteriological examination while further specimens were sent twice weekly until the patient was discharged from hospital. If, however, the faeces were still positive, then a second course of treatment was instituted, and if necessary a third.

It is customary to divide cases of gastro-enteritis into varying degrees of severity, mild, moderate and severe, but as the severity of the disease has greatly diminished they have been classified as mild and severe. Those with no symptoms of illness and yet showing the specific *Bact. coli* varieties in the faeces have been designated carriers. Comparatively few cases infected with the 0111 B4 H2 variety have been treated, due to the virtual disappearance of this organism, while cases infected with the new type 026 B6 have only been encountered nine times and the only organism prevalent has been the 055 B5 with the H6 antigen.

Table 3 shows the results in the cases infected with the 0111 B4 H2 strains. The cases treated with each drug, however, were so few that no definite conclusion could be drawn except to say that the organisms disappeared from the faeces in the control cases in about the same time as they disappeared from those of the treated ones.

There were 140 cases infected with the 055 B5 H6 strains, 102 clinical cases and 38 carriers, the particulars of this group being given in Table 4. In the investigation of the duration of infectivity the period of illness before admission has not been taken into account, and it has been assumed that the specific coliform organisms are unlikely to remain longer in the intestine of the clinical case than in the intestine of the temporary carrier. As the course of treatment lasted six days and as a further two days were allowed for the flora of the intestine to resume some semblance of normality, the minimum period in which a case could become negative was eight days. In the control series of cases the average duration

TABLE 3
DISAPPEARANCE OF *BACT. COLI* 0111 B4 FROM CASES OF GASTRO-ENTERITIS AND CARRIERS

	Type of Case							
	Mild		Severe		Carriers		All	
	No. in Series	Days Infective	No. in Series	Days Infective	No. in Series	Days Infective	No. in Series	Days Infective
Controls	12	9	3	12	3	9	18	9
Sulphamezathine	2	7	—	—	1	6	3	7
Polymyxin	6	9	—	—	—	—	6	9
Aureomycin hydrochloride	2	12	—	—	—	—	2	12
Totals	22		3		4		29	

of infectivity after admission was 16 days. There appeared to be a slight reduction in this period after the administration of sulphamezathine and streptomycin, but none after penicillin, and only a slight reduction after polymyxin. Aureomycin was only used in a few cases but chloromycetin and terramycin showed little evidence of being any more effective. These results do not mean that the effect of giving an antibiotic or suppressive drug was negligible, for while the patient was under treatment the coliform flora of the intestine could frequently no longer be cultured on MacConkey plates and the organisms obtained, if any, belonged to the *Proteus* group. This inhibition must therefore limit the spread of cross infection.

The Effect of Terramycin on the Faecal Flora of Children in an Institution

On June 25, 1951, an outbreak of gastro-enteritis occurred in Thorngrove Home, a residential nursery where there were 21 infants whose ages ranged from 1½ to 10 months. Three moderately severe clinical cases were admitted to the gastro-enteritis unit at the City Hospital, and among the 18, housed in two rooms at Thorngrove Home, seven developed loose stools and loss of weight—mild symptoms of gastro-enteritis. The faeces from all cases were then examined for agglutinable *Bact. coli* and it was found that the specimens from three cases admitted to the

City Hospital, from seven mild cases and from five children without clinical symptoms in Thorngrove Home, all showed the 055 B5 H6 type of *Bact. coli* (Table 5). Under these circumstances it was not thought advisable to admit any more cases to hospital but simply to treat all 18 cases (12 infected and six non-infected) remaining in Thorngrove Home with terramycin to see what would happen to the specific type of *Bact. coli*. Accordingly each child was given 100 mg. of terramycin in the elixir form by mouth four times daily for six days. Two days later, in order to give time for the disappearance of some of the effects of the antibiotics, the faeces were again examined. The flora of nine of the specimens consisted mainly of organisms belonging to the *Proteus* group, but the other nine were fairly normal so far as coliforms were concerned, and one of these showed the 055 B5 H6 type again. No further treatment was administered and the faeces were examined eight days later. This time the coliform flora in all specimens were normal and seven of the previously positive cases again showed the 055 B5 H6 type in the faeces. A further course of terramycin was given to each of the seven positive cases and two days after its termination all specimens from previous positive and negative cases alike were examined. This time four specimens from cases positive at the previous examination were still positive and in addition the faeces of three of the

TABLE 4
DISAPPEARANCE OF *BACT. COLI* 055 B5 H6 FROM THE FAECES OF CASES OF GASTRO-ENTERITIS AND CARRIERS

Treatment	Type of Case							
	Mild		Severe		Carriers		All	
	No. in Series	Days Infective	No. in Series	Days Infective	No. in Series	Days Infective	No. in Series	Days Infective
Control	20	18	6	14	7	12	33	16
Sulphamezathine	5	12	3	14	1	13	9	13
Penicillin	5	19	3	20	—	—	8	19
Polymyxin	10	9	6	15	1	25	17	12
Dihydro-streptomycin	7	13	4	20	4	19	15	16
Aureomycin hydrochloride	2	18	1	12	4	10	7	12
Chloromycetin	13	17	8	13	14	12	35	14
Terramycin hydrochloride	8	14	1	8	7	20	16	16
Total	70		32		38		140	

TABLE 5
PLATE CULTURES ON MACCONKEY MEDIUM FROM 18 PATIENTS FROM THORNGROVE HOME TESTED BY 'SWEEP'
AND COLONY SLIDE AGGLUTINATION TESTS USING 055 B5 0B ANTISERUM

Examination	Date	'Sweep Test'*				Colony Agglutination Test†								Acriflavine Control Tests	
		(degree of agglutination)				(seven tested from each plate)									
		10	2	1	5	7	6	5	4	3	2	1	0		
First after outbreak	25.6.51	10	2	1	5	10	0	0	0	1	1	0	6	Colonies rough	on one plate
Terramycin to all															
Second	2.7.51	0	0	1	17	0	0	0	0	0	0	1	17	Colonies rough	on one plate
Third	10.7.51	4	1	1	12	0	3	0	0	0	3	1	11	No rough colonies on plates	
Terramycin to all previous positives															
Fourth	17.7.51	6	1	1	10	3	2	1	0	0	0	1	11	Colonies rough	on two plates
Terramycin to all previous positives															
Fifth	31.7.51	1	1	3	13	1	0	0	0	0	1	0	16	Colonies rough	on three plates
Sixth	9.8.51	1	1	0	16	1	0	0	0	0	1	0	16	No rough colonies on plates	

* Plate cultures giving slide agglutinations.

† Colonies giving positive slide agglutinations.

originally infected children were again positive. A fourth course of terramycin was then given to all positive cases, and after this was completed specimens on two occasions from all 18 children showed only two individuals still carrying the organisms. These two children were then transferred to the gastro-enteritis unit at the City Hospital for isolation where a further course of treatment eliminated the specific type. All the children thrived well while taking terramycin, and despite the uncertainty as to its effect from the therapeutic aspect there is no doubt that the giving of the drug inhibited the growth of the organisms, thereby reducing the chances of cross-infection.

Also in Table 5 are tabulated the findings after seven colonies of *Bact. coli* from a MacConkey plate from each faecal specimen had been examined serologically. To begin with the specimens from 10 out of 12 positive cases (seven clinical cases and three carriers) showed apparently pure cultures of the 055 B5 H6 type, while the specimens from two carriers showed three and two colonies out of the seven examined. Later, after further treatment with terramycin, the clinical cases still gave the higher proportion of positive colonies and at the end of the period of observation one was still showing a pure culture. Finally, none of the six negative cases at the first examination later became positive.

Discussion

The degree of the bacteriostatic or bactericidal action of an antibiotic *in vitro* is determined by such factors as the number of organisms employed, the age of the culture, the composition and hydrogen

ion concentration of the culture medium, the temperature and duration of exposure during incubation, and the concentration of the antibiotic. Furthermore these factors can probably be made less variable under experimental conditions in the laboratory than in an *in vivo* test, when, for example, the inhibition or clearance of certain types of *Bact. coli* from the intestines of animals or man is attempted.

The *in vitro* experiments demonstrate that, though the strains of *Bact. coli* are frequently markedly inhibited by low concentrations of antibiotics for the first 24 hours of incubation at 37° C., this action is not convincingly maintained up to periods of 48 hours except where the concentration of antibiotic amounts to 25 or 50 µg. per ml. Furthermore, the bactericidal action is less marked than the bacteriostatic, and dihydro-streptomycin, which was the least effective in its inhibitory action, was most effective in its germicidal capacity. The difference between the concentration of antibiotic necessary to produce bacteriostasis and a germicidal action is less with this substance than with the other antibiotics if the organism is sensitive. It is possible that during incubation *in vitro* at 37° C. for more than a short period there is a considerable deterioration in the small amount of antibiotic contained in the culture fluid. Experiments by Bryson and Demerec (1950) have shown, however, that when terramycin is diluted in broth and incubated at 37° C. there is a slight deterioration in its inhibitory effect after 48 hours, still more after 72 hours, while at 96 hours it is almost ineffective.

Though the tests on children have not been

satisfactory from the point of view of clearing the intestines rapidly of specific types of *Bact. coli* this may to some extent be due either to inadequate dosage or to too short a period of treatment. The faeces in the control cases, both clinical and carrier, became negative almost as soon as in those treated with an antibiotic. An infectivity period of 16 days after admission seems to be about the average for this type of infection, though some treated cases have been found to remain positive for over three weeks and certain carriers for as long as five. One fact, however, is manifest and that is, while a child is undergoing treatment with aureomycin, chloromycetin, or terramycin, the *Bact. coli* flora and particularly the specific types under investigation are very markedly suppressed and this in itself is bound to limit the possible spread of cross infection. Administration of the drugs for a longer period might produce additional advantageous results. In the test trial of terramycin on children in an institution the dosage given was less than in the gastro-enteritis unit and the period over which it was administered was considerably extended by waiting for complete bacteriological results to become available.

Drug resistance has not been studied but the work of others has shown that coliform organisms may develop resistance to streptomycin very rapidly, whereas with aureomycin, chloromycetin and terramycin resistance develops much more slowly. No ill effects have been noted following the elimination of the coliform flora from the intestines and the establishment of members of the *Proteus* group, and the withdrawal of the antibiotic soon permits the coliform organisms to predominate again. In addition the infants treated with antibiotics have shown no complicating side effects such as involvement of the mucous membrane of the mouth, tongue, anus and vagina. Following on terramycin therapy, in particular, the weight of an infant seems to increase at a slightly greater rate than in an untreated child.

At the best, therefore, the newer antibiotics now in use cannot be expected to do more than produce bacteriostasis in the intestine of members of the *Bact. coli* group. The high concentration necessary for bactericidal action can be obtained only in the test tube and is unlikely to be obtained, after oral administration, in the gastro-intestinal tract. Further effectiveness of the bacteriostatic action in the bowel is probably influenced by many factors connected with the composition of its contents.

Summary

Tests have been carried out *in vitro* and *in vivo*

on the effect of antibiotics on the specific types of *Bact. coli* associated with infantile gastro-enteritis.

The *in vitro* tests showed that low concentrations of aureomycin, chloromycetin and terramycin had a marked inhibitory action on growth over a period of 24 hours at 37° C. and very much less at 48 hours. Dihydro-streptomycin was less effective.

The bactericidal action of aureomycin, chloromycetin and terramycin was relatively weak and was only evident in high concentrations of 25 and 50 µg. per ml. and then only on the more susceptible strains. Dihydro-streptomycin, in the higher concentrations, had a bactericidal action on a greater number of strains.

The *in vivo* tests with the antibiotics have shown that suppression of the coliform flora of the intestine could be effectively obtained, these organisms being largely replaced by members of the *Proteus* group.

So long as oral administration was continued the specific types of *Bact. coli* were held in check but with the cessation of treatment they tended to re-appear.

Antibiotic therapy did not appear to lessen the total period of infectivity with the specific coliform types, though presumably while being employed it limited the possibility of cross-infection simply by reducing the number of organisms excreted.

Owing to the present mildness of infantile gastro-enteritis no attempt has been made to evaluate the therapeutic action of the antibiotics studied.

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