SOME EFFECTS OF CHLORAMPHENICOL IN THE GUT

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The treatment of typhoid fever with chloramphenicol is now well established, but numerous writers have drawn attention not only to the high relapse rate but also to the persistence of *Salmonella typhi* in the stools of many treated cases (Good & Mackenzie, 1950; Rankin & Grimble, 1950; Ramli, 1950; Edge, 1950; Marmion, 1952). Douglas (1950) has described the development of a carrier state in two typhoid patients treated with this antibiotic. There would thus appear to be an anomalous difference between the effect of chloramphenicol on *Salm. typhi* in tissue and its effect on the same organism in the gut. An explanation of the apparent discrepancy lies in the possibility that the gut chloramphenicol is low when tissue concentration is adequate. The investigations reported here were designed to establish the amount of active antibiotic present in the stool at various dosages, and to study the daily chloramphenicol excretion in bile in one case in which the opportunity to do so presented.

METHODS AND MATERIALS

(1) *Estimating chloramphenicol in faeces*

Two g. of stool were weighed into a porcelain dish which was then placed on a sand-bath at 65° C. and the specimen dried. The dried faeces were powdered and carefully transferred to sterile 15 ml. round-bottomed centrifuge tubes. Four ml. of peptone water (Difco) were added and the meniscus marked with a diamond. The tube and its contents were kept in a boiling water-bath for 20 min., and on removal the volume was readjusted. The tube was centrifuged and the supernatant transferred to a sterile test-tube with a Pasteur pipette. The extract was returned to the water-bath for a further 15 min. A series of $3 \times \frac{3}{8}$ in. tubes were set up, 0·2 ml. of the extract was added to each, and dilutions were made with peptone water 1/1, 1/2, 1/3, 1/4, etc., to 1/10. Each tube was inoculated with a loopful of a peptone water culture of El Tor vibrio sensitive to chloramphenicol at 0·5 μg. per ml. and incubated at 37° C. for 16–18 hr. The end-point was taken as the first tube showing no growth. As the stool was originally diluted 1/2 and the El Tor vibrio was sensitive at 0·5 μg., the concentration of antibiotic present was the reciprocal of the first dilution showing no growth. The use of the El Tor vibrio for the estimation of chloramphenicol concentrations was suggested by Doorenbos & Kop (1951). It has certain characteristics which make it invaluable for this work. It grows well in Difco peptone water, producing a greasy surface pellicle, which on tapping the test-tube breaks into large flocules. It is not inhibited by the boiled extracts of body fluids or tissue and its extreme sensitivity to the antibiotic allows for the measurement of very small amounts. With the method described it was possible to measure down to 1 μg.

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(2) *Estimation of chloramphenicol in bile*

Bile was collected in sterile medicine bottles. Two ml. were transferred to a sterile test-tube and 4 ml. peptone water were added. The tube and its contents were kept in a boiling water-bath for 20 min. and on removal the meniscus was adjusted to the mark. The concentration of chloramphenicol was estimated as already described.

(3) *Titration of bacterial sensitivity to chloramphenicol*

Flasks containing 100 ml. peptone water and 10 and 50 μg. chloramphenicol per ml. were prepared. Two series of ten $3 \times \frac{3}{8}$ in. test-tubes were set up. In the first tube of one series was placed 0·1 ml. of the 10 μg. per ml. concentration, in the second, 0·2 ml., in the third 0·3 ml. and so on to 1 ml. in the tenth. The second series of tubes was treated in the same way from the flasks containing the 50 μg./ml. chloramphenicol concentration. Volumes were brought to 1 ml. and the tubes inoculated with one loopful of an overnight peptone water culture of the organism under test. The first tube showing no growth gave the end-point, measuring the resistance of the organism between 1–10 μg. in the first series and 5–50 μg. in the second.

(4) *Estimation of residual active chloramphenicol*

Three 200 ml. peptone water flasks were inoculated with a coliform bacillus and incubated at 37° C. for 16 hr. The first flask then had chloramphenicol added to give a concentration of 30 μg./ml., the second 60 and the last 100 μg./ml. The flasks were returned to the incubator. At the end of 1 day, 3 and 6 days, 15 ml. quantities of fluid were removed from each flask and Seitz filtered. A volume of each filtrate was diluted 1/5, 1/10, 1/20,..., up to 1/100 with peptone water. The tubes were now inoculated with a loopful of a peptone water culture of El Tor vibrio and the first showing no growth was taken as the end-point. As the dilutions were known and the sensitivity of El Tor vibrio is 0·5 μg./ml., simple multiplication gave the concentration of active antibiotic remaining. The difference between the residual active chloramphenicol and the original concentration of antibiotic per ml. was the inactivated portion.

(5) *Stool Cultures*

For the isolation and rough estimation of the numbers of faecal bacteria, a 3 mm. platinum loop was plunged into the sample of stool and rotated until a small lump adhered. This was spread over the surface of a plate in the usual manner. Two media were used, MacConkey's agar and blood agar. From each specimen two plates of each medium were inoculated. Growth in all areas of the plate was recorded as + + +, growth on $\frac{1}{2}$ plate as + + and growth around the area of inoculum only as +. If colonies were few enough they were counted. No growth was recorded as 0.

RESULTS

The concentration of chloramphenicol per g. wet stool in relation to dosage and its effects on the flora of the large bowel are shown in Table 1. The pre-treatment control period and post-treatment findings are not included as they showed no variation from the expected.

Table 1. *The concentration of chloramphenicol in μg./g. of wet stool in relation to dosage and stool culture*

Case no.	Dose	Day of treatment 1		2		3		4		5		6	
		Stool culture	Stool chloramphenicol (μg./g.)	Stool culture	Stool chloramphenicol (μg./g.)	Stool culture	Stool chloramphenicol (μg./g.)	Stool culture	Stool chloramphenicol (μg./g.)	Stool culture	Stool chloramphenicol (μg./g.)	Stool culture	Stool chloramphenicol (μg./g.)
1	30 mg./kg./24 hr.	+ + +	0	+ +	0	+	2	+	2	+	2	+	1
2		+ + +	1	+ +	1	+ +	1	+ +	0	+ + +	0	.	.
3		+ + +	3	+ +	1	+	1	+ +	3	+ + +	1	.	.
4	75 mg./kg./24 hr.	+ + +	0	+	6	+	7	+	7	.	.	.	.
5		(50 colonies)	5	(1 colony)	9	0	8	0	8	(19 colonies)	4	.	.
6		(3 colonies)	0	0	8	0	4	0	4	0	4	0	4
7		+ + +	2	+	4	+	4	(2 colonies)	6	0	6	0	4
8	100 mg./kg./24 hr.	+ +	3	+ +	3	+	2	+	2	+	2	.	.
9		+ + +	0	+	4	0	4	0	7	+	3	.	.
10		+ + +	0	+	5	0	3	0	4	0	7	0	5
11		+ + +	2	+ + +	8	+ + +	11	+ + +	7	+ + +	7	.	.

Case No. 8. The coliforms which persisted throughout treatment showed a resistance of 3 μg/ml.

Case No. 11. The coliforms which persisted were of two kinds, one strain resistant at 20 μg./ml. and a second at 35 μg./ml.

At a dose of 30 mg./kg./24 hr., the amount of chloramphenicol in the stool in μg./g. weight of wet faeces after the second day's treatment varied between 0 and 3 with an average of 1·3. Although the coliforms were not completely suppressed, a definite reduction at this concentration was noted. When the daily dosage was increased to 75 mg./kg. the concentration of chloramphenicol over the same period in the faeces varied between 4 and 9 μg./g. with an average of 6. The flora was completely suppressed in all but one case. At 100 mg./kg./day the chloramphenicol stool concentration in μg./g. varied between 3 and 11 with an average of 5. The coliforms were suppressed except in case 11, in which the very high resistance of the organisms to the antibiotic is explanatory.

There is a demonstrable difference in effect on the gut flora and stool chloramphenicol concentration between a daily dose of 30 mg./kg. and of 75 mg./kg., but little or no difference between 75 and 100 mg./kg. In a further series of cases treated at 100 mg./kg./day the following was observed: number of cases, 12; sterile plates from stool cultures, 10; average number of days on treatment to sterile plates, 2·7; average number of days after cessation of treatment to the return of normal flora, 1·4; number of cases in which yeasts were isolated from the stool in pure culture during treatment, 7.

Two of these patients developed a rash on the buttocks. The yeasts isolated from the stools and skin from both were identified as *Monilia albicans*. The longest period during which yeasts were isolated after treatment had been stopped was 3 days.

In three of the twelve cases, the resistance of coliforms to chloramphenicol was found to rise.

1st case. Sensitivity before treatment = 2 μg./ml. After 6 days' exposure to the antibiotic the organisms first recovered after cessation of therapy had a resistance of 10 μg./ml.

2nd case. Sensitivity before treatment = 3 μg./ml. After 7 days' therapy the organisms first isolated showed a resistance of 6 μg./ml.

3rd case. Sensitivity before treatment = 2·5 μg./ml. After 5 days' treatment the organisms first isolated showed a resistance of 7 μg./ml.

Table 2 shows the concentration of chloramphenicol found in bile following a dosage of 100 mg./kg./day. The patient was a male, weight 19 kg., admitted with acute cholecystitis for which the gall bladder was drained.

At the time of maximum biliary chloramphenicol output the liver delivered to the gut via the bile 5–6 mg. of active chloramphenicol per day on a dose of 100 mg./kg./24 hr. It has already been shown that at this intake the faeces contain 5–6 μg. active chloramphenicol per g. wet stool. The average weight of the daily stool of six children in this series was 50·5 g. The daily faecal excretion of active chloramphenicol was therefore about 250–300 μg., which is 1/20 of the bile content for the same period. The difference may be due to reabsorption into the blood

stream or inactivation by the bacteria in the gut. Estimation of residual active chloramphenicol from 16 hr. cultures exposed to a known quantity of antibiotic (see section on methods and materials for details) showed that after 3 days coliforms inactivate about 70% of added chloramphenicol. Therefore, if 250–300 μg. represent the active chloramphenicol excreted daily, the total faecal chloramphenicol by analogy must be about 0·8–1 mg. The difference between the amount of active chloramphenicol excreted in the bile and the total lost in the stool is taken to represent the quantity reabsorbed, i.e. 4–5 mg. daily. Consideration of the amounts of chloramphenicol given and the minute totals recovered from the stool confirm the statement that this antibiotic is most efficiently absorbed (Kekwick, 1952).

Table 2. *The concentration of chloramphenicol found in bile following a dosage of* 100 *mg./kg./*24 *hr.*

Day of treatment	Bile output per 24 hr. (ml.)	Chloramphenicol (μg./ml.)	Total chloramphenicol output per 24 hr. (μg.)	Stool culture	Chloramphenicol in stool (μg./g.)
1	81	6	486	+ + +	0
2	90	12	1080	.	.
3	63	37	2451	.	.
4	125	42	5250	+ + +	1
5	103	42	4326	+ + +	2
6	122	51	6222	+ + +	2
7	102	37	3374	+	7
Days after treatment					
1	77	18	1386	.	.
2	69	0	0	+ + +	3
3	.	.	.	+ + +	0

The post-chloramphenicol treatment period shown in Table 2 suggests that the amount of antibiotic present in the faeces is dependent on the quantity excreted with the bile. It will be noted that on the day after cessation of therapy the bile contained 18 μg. chloramphenicol per ml. Two days later when the bile was free of antibiotic the stool concentration was 3 μg./g.; this disappeared on the succeeding day.

DISCUSSION

On the basis of the observations reported, an explanation can be afforded for 'relapses' and for persistent excretion of typhoid bacilli in many of the cases of typhoid fever treated with chloramphenicol.

The resistance of *Salmonella typhi* to chloramphenicol varies between 0·5 and 4 μg./ml. with an average of 2·5.

A patient given 30–40 mg. of antibiotic per kg. per day will produce about 1·5 μg./gm. of wet stool, provided the liver and gall bladder function efficiently. This amount is slightly less than that required to destroy pathogenic organisms of average sensitivity swarming in the gut; at the same time the dosage produces tissue and bile levels high enough to deal with the invaders. Continuance of the antibiotic sustains tissue protection, but if the administration is stopped before body

defence mechanisms have had time to elaborate antibodies, reinfection occurs, producing the so-called relapse.

If the dosage is raised to 75 mg./kg./day the gut concentration would be in the region of 4–6 μg./g. wet stool, normally slightly in excess of the resistance of the pathogens.

In order to clear the gut of salmonellae this amount of antibiotic in theory would have to be maintained for about 7 days; *in vitro* experiments have shown that 99 % of a proliferating bacterial population are killed within a day at ordinary therapeutic concentrations, but the remaining 1 % persist in diminishing numbers for 5–7 days (Gray, 1952). The levels of 4–6 μg./g. wet stool of antibiotic can only be effective if the bacterial resistance lies between the expected limits and remains constant; if the evidence afforded by the coliforms in these experiments can be applied to the salmonellae it seems that a rise of 3–5 μg. in the resistance of some strains can be expected, and this increase might be sufficient to overcome the antibacterial effect of the very modest concentrations available. The problem of dealing with bacteria when their initial resistance is relatively high or which acquire a high resistance cannot be solved by increasing the oral dose of chloramphenicol, for it has been shown that the maximum stool concentration is reached on an intake of 75 mg./kg./day. The crux lies in the almost complete absorption of antibiotic. It would appear that some 'excretors' could only be rendered free by use of some other antibacterial compound, the absorption of which is not as complete as that of chloramphenicol.

The high concentration of chloramphenicol found in bile suggests that a permanent carrier state can be avoided only by clearing the gut of *Salm. typhi*, for it is unlikely that bacteria lying inside the gall bladder would survive the high concentration for any length of time provided that the primary focus was eliminated before treatment ended.

The following factors suggest themselves as governing the control of pathogens in the gut:

(1) The necessity for a dosage of chloramphenicol which is optimum in amount and duration for each patient.

(2) Organisms of normal sensitivity which do not decrease much during treatment.

(3) The number of bacteria in a 'resting' phase. The speed of bacterial death induced by this antibiotic apparently depends on metabolic activity—the greater the bacterial metabolic rate the more rapid the death and vice versa.

(4) The concentration of antibiotic in relation to the density of bacterial population. It has been shown that increasing bacterial populations demand increasing amounts of chloramphenicol to destroy them (Gray, 1952). These last two factors may be an explanation of the inability to achieve absolute gut sterility.

(5) A liver and gall bladder functioning efficiently.

Specific antibody production in useful amounts demands the presence of a stimulus in tissue for certain periods of time and in satisfactory quantities. Pulvertaft (1952) has shown that bacterial lysis follows exposure to chloramphenicol at effective concentrations. In treating typhoid fever with chloram-

phenicol, rapid bacterial death, accompanied by lysis of those organisms lying in tissues, probably takes place. If this released protoplasm is treated by the body in the usual manner it is probably excreted with considerable rapidity and thus lost antigenically. The continued presence of chloramphenicol in gut tissue will act as a barrier against further invasion by *Salm. typhi*; thus the pre-requisite for antibody production—the stimulus—is lost. It is therefore suggested that in any given case the amount of antibody produced will depend on the duration of the illness and its intensity prior to antibiotic treatment.

Marmion (1952), in a detailed study of the results in 330 cases of typhoid fever treated with chloramphenicol, lends support to these views. He noted that the persistence of *Salm. typhi* in the stools often heralded a relapse, that the relapse rate was highest in those cases treated in the first week of illness in contradistinction to the rate in those treated during the third week, and lastly that the agglutination titres following chloramphenicol therapy were unpredictable.

Although a daily dosage of 75 mg./kg. body weight for 7 days has been advocated in this paper, the recommendation is made with reservations. A higher incidence of 'toxic crises' may follow its use, particularly in the hands of an inexperienced clinician; secondly, the results of the effect of the antibiotic on the normal gut flora must be borne in mind.

Some of the secondary effects arising from an alteration of gut flora have been summarized by an annotation in *The Lancet* (1952). The symptoms described were nausea, epigastric discomfort, diarrhoea, perianal itching and fissuring. It was suggested that these were the result of a deprivation of the vitamin B complex due to the cessation of coliform function and the proliferation of yeasts. It will be noted in this inquiry that seven out of twelve cases treated with chloramphenicol at a daily dosage of 100 mg./kg. showed yeasts in pure culture. In two a 'monilial' rash appeared on the buttocks, and in both cases the yeasts from the stools and skin were identified as *Monilia albicans*.

No indications of vitamin B deficiency were noted. With the re-establishment of a normal gut flora the yeasts disappeared from culture, and it is assumed that they were 'swamped' by the return of the normal inhabitants. Continuance of treatment in our own cases was never prolonged beyond 7 days, but it would appear that the administration of antibiotic for long periods to patients with yeasts and a bacterial flora of normal or high sensitivity to chloramphenicol, may expose them to moniliases of the gut and perianal region.

SUMMARY

A method for the estimation of chloramphenicol in faeces and bile is described.

It has been shown that the bile contains high concentrations of the antibiotic, but the faeces small amounts. It is considered that the faecal concentration is dependent on the biliary supply.

The action of chloramphenicol on the gut flora is described and the dominance of yeasts on complete suppression of the coliforms is reported, drawing attention to the possibility of intestinal moniliasis following unrestricted use of the antibiotic.

An explanation of 'relapses' in the chloramphenicol treatment of typhoid fever is given, and the possible effect of chloramphenicol on immunity response is discussed.

It is suggested that the carrier state following typhoid fever can only be checked by clearing the gut as well as the gall bladder of *Salm. typhi*.

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