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STOOL CONCENTRATIONS AND ABSORPTION OF CHLORAMPHENICOL AND ITS PALMITATE IN BABIES

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For a period after its introduction chloramphenicol was widely used in the treatment of enteral infections because of its bacteriostatic action against a wide range of Gram-negative organisms. But reports of its efficacy in the treatment of some of these infections, especially infantile gastro-enteritis, have been conflicting (Rogers, Koegler, and Gerrard, 1949; Smellie, 1950; Cerruti and Scarzella, 1950; Shanks and Studzinski, 1952). Stool concentrations have, therefore, been assayed in a series of infants under treatment in the hope that the results might lead to a better understanding of the role of chloramphenicol in treatment. Welch (1950) has made a few studies on normal adults, but these are not necessarily applicable to infants.

Early in this study it became apparent that chloramphenicol palmitate differed from the crystalline form in its pharmacological behaviour. The palmitate, which was introduced primarily as a palatable form for children, is an ester which contains 57.6% by weight of chloramphenicol and possesses practically no antibiotic activity. Its action depends on the release of the free alcoholic form by the intestinal lipases (Ross, Burke, and Rice, 1952). In view of this difference in behaviour two series of infants were treated, one with the crystalline form and one with the palmitate, and the stool and serum levels of each group were compared. Because of its potential toxicity the popularity of chloramphenicol has fallen, but an annotation in the *Lancet* (1953) gives a guide to its use by stating that "in moderate dosage and for short administration it is safe enough."

MATERIAL AND METHODS

Clinical Material.—25 infants, of ages ranging up to 15 months, were studied. They suffered from a variety of conditions, and some had loose stools.

Dosage.—Infants on crystalline chloramphenicol were given 160 mg./kg./day divided into four doses. Those on palmitate received a dose which could release 160 mg./kg./day of active chloramphenicol from the ester (4 ml. of suspension is equivalent to 125 mg. of crystalline chloramphenicol).

Sampling Methods.—Venous blood and stools were obtained at approximately the same time each day, after the infants had been on treatment for at least 24 hr. Stools were obtained by introducing a short sterilized rubber tube into the rectum. If the bowel contents were fluid they flowed out easily. If formed, the rectal reflex, excited by the passage of the tube, caused the early passage of a specimen. Stools were thus obtained fresh and uncontaminated.

24-hr. urine specimens were collected from six male babies, through a sterilized system, for assay of urinary excretion. Collection was not begun until at least 24 hr. after the start of treatment. Fluid intake was standardized according to body weight, and no case showed evidence of fluid imbalance.

Assay Methods.—The following preliminary treatment of the specimens was always carried out within half an hour of completing the collection.

1. *Blood Serum.* Venous blood, which had been withdrawn under sterile conditions, was allowed to clot in a plain sterile tube. The specimen was then centrifuged, and the supernatant serum removed by pipette and transferred to another plain sterile tube. This tube was then incubated at 56° C. for 30 min. to inactivate enzymes which might destroy the chloramphenicol.

2. *Stool.* 2 g. of moist stool (in some instances this was fluid) was emulsified with 10 ml. of peptone water and boiled in a water-bath for exactly 20 min. This process inactivated the enzymes present and sterilized the specimen. The emulsion was then centrifuged to yield a supernatant fluid suitable for Seitz filtering. This fluid was transferred to the chamber of a centrifuge Seitz filter, and a clear sterile filtrate suitable for assay was obtained.

3. *Urine.* These specimens were inactivated by incubating at 56° C. for 30 min. and Seitz filtered before assay.

On a few occasions it was inconvenient to start immediate assay. The fluid was therefore stored in a refrigerator until the actual assay was carried out.

Serial double dilutions in peptone water of the fluid for assay were set up in 3-in. × ⅛-in. test-tubes with a final volume of 1 ml. in each tube. Two tubes, one for growth control and one for sterility control, were added. The tubes were inoculated with a 2.5-mm. loopful of an overnight broth culture of El Tor vibrio and incubated overnight. The highest dilution to show no growth was read as containing 0.5 μg./ml. of chloramphenicol and the original concentration calculated from this. For example, in the following experiment there was no growth in tube 3 and growth in tube 4:

Tube ...	1	2	3	4	5	6
Dilution ...	1/2	1/4	1/8	1/16	1/32	1/64
Growth ...	−	−	−	+	+	+

The concentration in tube 3 is taken as 0.5 μg./ml., and as the contents of this tube have been diluted eight times the original concentration will be (8 × 0.5) or 4 μg./ml. Although the accepted method is to record the concentration as 4 μg./ml., it is apparent that the actual concentration lies between 4 and 8 μg./ml.

By using a different method of diluting the fluid for assay, in which the differences in dilutions were much less, it was possible to reach a more accurate end-point. This method was used to assay the urine concentrations and to test the sensitivity of the organism.

The vibrio was tested at intervals and found to remain constantly sensitive to 0.5 μg./ml. and insensitive to 0.4 μg./ml. of chloramphenicol. The accuracy of the method was tested by assaying normal stools with a known amount of chloramphenicol added. Six normal stools were also assayed and found to have no recordable antibiotic activity.

It was not possible to record stool levels below 6 μg./g. because of the necessary dilution of the stools with peptone water.

Results

Serum and Stool Levels.—The serum levels are compared in Table I and the stool levels in Table II. These indicate that chloramphenicol palmitate yields lower serum levels and higher, though scattered, stool levels than does the crystalline form.

The mean value of each group of serum and stool levels has been calculated to compare these differences more easily. As the recorded levels in each group rise in geometric progression (as a result of the double dilutions) the geometric mean of each group was calculated according to the formula:

$$\text{Log } G = \frac{(\log x_1 + \log x_2 + \ldots\ldots + \log x_n)}{N}$$

where G is the geometric mean.

N is the number of values in the series and $x_1, x_2 \ldots\ldots x_n$ are the individual values. It was pointed out previously how, although a value is recorded as 4 μg./ml., it lies in reality between 4 and 8 μg./ml. For the purposes of mathematical accuracy this value is taken as the geometric mean between 4 and 8, i.e., 5.7 μg./ml. Thus each individual value in the above series $x_1, x_2 \ldots\ldots x_n$ is taken as the geometric mean between it and the next highest value. Values of less than 6 μg./g. presented a problem, as it is not possible to calculate a geometric mean between 0 and 6. The arithmetic mean of 3 was assumed as a reasonable figure. Thus on a comparable dosage the mean levels are:

(*a*) Serum. On chloramphenicol palmitate, 7.1 μg./ml. On crystalline chloramphenicol, 18.6 μg./ml.

A t-test shows that the difference between the means of the two samples is highly significant ($P<0.01$).

(*b*) Stools. On chloramphenicol palmitate, 33.1 μg./g. On crystalline chloramphenicol, <6 μg./g.

There is again a highly significant difference between the means of the two samples.

As a test of the consistency and reliability of the serum and stool findings, it was found possible, by assaying these levels in individual babies, to decide which form of chloramphenicol was being given. In addition, in four babies a change from one form of drug to the other was always reflected in an appropriate change in the serum and stool levels.

Urine Levels.—The percentage of the 24-hr. oral dose excreted in the urine in the same period was 10, 7 and 6% respectively in three babies on

Table I

SERUM LEVELS OF CHLORAMPHENICOL IN TWO GROUPS OF BABIES TREATED WITH COMPARABLE DOSES OF THE CRYSTALLINE AND PALMITATE FORMS

Form of Chloramphenicol	No. of Cases with Serum Levels per ml. of				
	2 μg.	4 μg.	8 μg.	16 μg.	32 μg.
Crystalline ..	0	1	3	9	1
Palmitate ..	2	9	6	1	0

Table II

STOOL LEVELS OF CHLORAMPHENICOL IN TWO GROUPS OF BABIES TREATED WITH COMPARABLE DOSES OF THE CRYSTALLINE AND PALMITATE FORMS

Form of Chloramphenicol	No. of Cases with Moist Stool Levels per g. of							
	< 6 μg.	6 μg.	12 μg.	24 μg.	48 μg.	96 μg.	192 μg.	384 μg.
Crystalline ..	14	1	1	0	0	0	0	0
Palmitate	2	5	5	5	4	3	1	2

crystalline chloramphenicol, and 4.1, 2.3 and 1.2% in three babies on chloramphenicol palmitate.

Urine levels closely reflect serum levels. This small experiment served to check whether, in fact, higher urine levels did occur in the group on crystalline chloramphenicol as compared with that on the palmitate. Though the results are too few for statistical analysis they do suggest a higher urinary excretion of the crystalline form. They therefore add weight to the serum and stool findings.

Discussion

These results, taken in conjunction with published serum absorption curves (Ross, Burke, and Rice, 1952 ; O'Brien, 1953), indicate that crystalline chloramphenicol is effectively absorbed from the bowel giving high serum and urine levels. In only two out of sixteen cases was it possible to record stool concentrations above 6 μg./g. It may be, however, that a proportion of the drug is excreted in an inactive form, since it is known that intestinal and bacterial enzymes may inactivate it.

The palmitate, in contrast, yields low serum and urine levels and high though widely scattered stool levels.

These findings have practical applications which may be summarized as follows:

(1) Where a pathogen lies in the body tissues or urinary tract, then, if chloramphenicol is the drug of choice, the crystalline form should be used.

(2) Where a pathogen lies in the lumen of the bowel, the palmitate is preferable. In this situation, however, the antibiotic of choice should be bactericidal in action.

Summary

In comparable doses, in babies, chloramphenicol palmitate yields lower serum and urine levels and higher stool levels than does the crystalline form.

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