An evaluation of the absorption characteristics of different chloramphenicol preparations in normal human subjects

The absorption characteristics of four different chloramphenicol preparations were compared in normal adult volunteers by means of blood level measurements and urinary excretion of chloramphenicol and its metabolites following administration of single 0.5 Gm. oral doses. Products of two manufacturers were compared in the first two studies, while a third study involved a comparison of four commercial lots of chloramphenicol. Product A produced colorimetric and microbiologic plasma levels which were nearly double those of B; the area under the mean time-concentration curve was nearly twice as great, and peak blood levels were reached earlier. Product C showed absorption characteristics similar to those of B. Product D showed a greatly extended period of absorption with maximum plasma levels about one quarter those of A. In vitro dissolution tests indicated that products B, C, and D went into solution more slowly than A. These observations emphasize the need for caution in assuming that absorption characteristics are the same for different chloramphenicol preparations containing identical amounts of drug.

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Preparations of drugs which are poorly soluble in water may differ considerably in their rates of dissolution, and as a result may show marked differences in absorption from the intestinal tract.[2, 6, 14, 16] Kakemi and colleagues[12] studied the effect of the particle size of chloramphenicol upon intestinal absorption in rabbits, finding that blood level peaks were somewhat lower and occurred later with preparations of larger particle size, although the total amount of drug absorbed was about the same for all. We examined several chloramphenicol preparations in gelatin capsules which were obtained from a local pharmacy. In vitro dissolution rate measurements indicated that some of these preparations were not going into solution as rapidly as others.[1] Direct microscopic examination revealed that large crystals were present in some preparations, while others consisted mainly of very small particles. Under the circumstances, it was felt that a definitive absorption study should be conducted in human subjects, using blood levels and urinary excretion measurements to evaluate the absorption characteristics of these preparations.

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Methods

Three trials were carried out from July to September, 1967, with chloramphenicol products purchased from open stock in local pharmacies. Trials 1 and 2 were conducted with 5 subjects per drug preparation in each study, using products from Manufacturers A and B. In the third trial, 10 subjects each took a preparation from Manufacturers A, B, C, or D.

Product A was Chloromycetin Kapseals, 250 mg., manufactured by Parke, Davis & Company; Lot No. P13215A, with an expiration date of October, 1971, was used in Trials 1 and 2, Lot No. P13542A, with an expiration rate of April, 1972, in Trial 3. Preparations B, C, and D were all 250 mg. chloramphenicol capsules from different manufacturers. One lot number of B was used in Trials 1 and 2, a second in Trial 3. C and D were used in Trial 3 only.

Trial 1 was carried out at the Detroit House of Correction, Plymouth, Michigan. Trials 2 and 3 were carried out at the Florida State Prison, Raiford, Florida. In all cases, the volunteers were adult men judged to be normal from their medical history and physical examination. Other details about the subjects are given in Table IV. For Trial 3, laboratory tests were carried out for hematopoietic, hepatic, and renal function, to establish that these parameters were within normal limits.

In all studies, collections of urine were made over a 24 hour period before medication, and heparinized venous blood samples were also obtained. Each subject was then given a single 500 mg. dose of chloramphenicol (2 capsules) by mouth (after breakfast in Trial 1, before breakfast in Trials 2 and 3). Blood specimens were taken at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after medication. Urine collections were made at 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 12, 12 to 24, and 24 to 48 hours (24 to 48 hour specimens were not taken in the first study, however). Blood specimens were refrigerated immediately after collection and centrifuged within a few hours. The plasma was carefully separated from the red cells, frozen, and delivered to the Ann Arbor or Detroit laboratories of Parke, Davis & Company for analysis. Urine volumes were measured in a graduated cylinder and recorded; a 2 ounce sample was retained for analysis.

Analysts were not informed of the identity of the samples being assayed. The urine and plasma specimens were assayed for total nitro compounds* with the use of the titanous chloride reduction procedure described by Glazko, Wolf, and Dill,[8] modified by the addition of potassium fluoride to complex excess reducing agent.[3] Corrections were made for aromatic amines, and results were calculated in terms of chloramphenicol equivalents. The plasma specimens were also assayed for chloramphenicol by a turbidimetric procedure using *S. sonnei* as the test organism.[11] High blanks were encountered in Trial 1, and there was insufficient sample to repeat the assays with a modified technique. In Trials 2 and 3, the plasma specimens were heated to 56° C. for 30 minutes to prevent inhibition of the test organism by immune mechanisms. All microbiologic assays were performed by the Quality Control group (W. C. A.); colorimetric assays for Trials 1 and 2 were carried out by the Chemical Pharmacology group (A. J. G.), and for Trial 3, by the Biopharmaceutics group (A. W. K.). Standard errors were calculated for small numbers of samples (N-1); Student's t test was applied to evaluate the significance of the differences between means, and probability estimates were based upon the t values.[2a]

Results

Trial 1. The plasma levels obtained by colorimetric assay in the first trial are shown in Table I (Preparation A versus B). The analytic results are presented for the individual subjects to bring out the

*The term "total nitro compounds" refers to chloramphenicol plus its metabolic products which contain the nitro group (colorimetric assay); "aromatic amines" refers to diazotizable amines which are determined prior to reduction of the nitro group; "unchanged drug" refers to free chloramphenicol (microbiologic assay).

Table I. *Plasma levels (colorimetric) in normal human subjects receiving single 0.5 Gm. doses of chloramphenicol A and B (Trial 1)*

Hours after medication	Preparation A							Preparation B						
	Subject No.					Mean	S.E.	Subject No.					Mean	S.E.
	1 30, 77.5, C*	2 27, 80.3, C	5 34, 82.5, C	7 26, 63.9, N	8 24, 72.1, C			3 27, 72.6, N	4 24, 75.3, C	6 29, 71.2, N	9 29, 79.4, N	10 39, 75.7, C		
	(μg/ml.)	(μg/ml.)	(μg/ml.)	(μg/ml.)	(μg/ml.)	(μg/ml.)	(μg/ml.)	(μg/ml.)	(μg/ml.)	(μg/ml.)	(μg/ml.)	(μg/ml.)	(μg/ml.)	(μg/ml.)
Predrug	0.0	0.2	0.0	0.2	0.0	0.1	0.0	0.1	0.9	0.0	0.7	0.4	0.4	0.2
0.5	8.2	12.6†	1.1	1.4	7.1	6.1	2.2	0.1	0.0	0.4	1.5	0.1	0.4	0.3
1	8.9†	10.9	7.4	3.1	8.6	7.8	1.3	6.0†	0.2	0.0	4.2†	5.6	3.2	1.3
2	7.4	9.3	9.7†	4.8	9.2†	8.1	0.9	3.9	0.1	0.1	3.9	1.5	1.9	0.9
4	5.1	6.9	6.9	8.2†	6.9	6.8	0.5	3.4	1.9†	5.0	2.7	8.0†	4.2	1.1
6	3.7	4.2	4.6	5.9	4.0	4.5	0.4	1.8	0.4	6.3†	1.6	4.6	2.9	1.1
8	2.3	3.0	3.2	4.5	3.0	3.2	0.4	1.6	0.0	4.9	0.8	3.5	2.2	0.9
12	0.8	1.3	1.5	1.9	1.2	1.3	0.2	1.2	0.0	2.3	0.1	1.5	1.0	0.4
24	0.1	0.0	0.1	0.1	0.0	0.1	0.0	0.3	0.0	0.1	0.0	0.0	0.1	0.1

All values corrected for predrug blanks.

*Age (years), body weight (kilograms), and race.

†Maximum level.

marked differences in time required to reach maximum plasma levels. For Preparation A, peak levels of 8.2 to 12.6 μg per milliliter (mean = 9.7) were reached in 0.5 to 4 hours after medication. Preparation B produced maximum plasma levels of 1.9 to 8.0 μg per milliliter (mean = 5.3) in 1 to 6 hours after medication. The difference between the means of the maximum plasma levels attained with each preparation was statistically significant ($p < 0.001$); however, the differences between the means for each time period were not as significant because of the variable absorption pattern in different individuals. These differences were significant only in the early periods (0.5 to 2 hours after medication). The area under the time-concentration curve for Preparation B averaged about 60 per cent of that obtained with A; this difference again was more pronounced in the early time periods (e.g., 0 to 4 hours), in which the area under the curve for B represented only 34 per cent of that under the blood level curve for A.

The mean urinary excretion data obtained in Trial 1 are shown in Fig. 1. The maximum excretion rate for Preparation A (46.5 mg. per hour) occurred in the 2 to 4 hour period after medication, with a mean 24 hour recovery representing 76 per cent of the dose. The maximum excretion rate for Preparation B (31.5 mg. per hour) occurred in the 4 to 6 hour period, with a mean recovery of only 46 per cent of dose. The amount of drug excreted in 24 hour urine with Preparation B was about 60 per cent of A, confirming the blood level data in the same subjects.

Trial 2. The plasma levels obtained for each subject by colorimetric assay are plotted on a semilog scale in Fig. 2 to illustrate the major differences existing between Preparations A and B. Preparation A produced peak blood levels of 9.2 to 12.1 μg per milliliter (mean = 9.9 μg per milliliter) in 30 minutes to 1 hour after medication; Preparation B produced peak levels of 3.2 to 5.8 μg per milliliter (mean = 4.1) in 2 to 6 hours after dosing. The difference

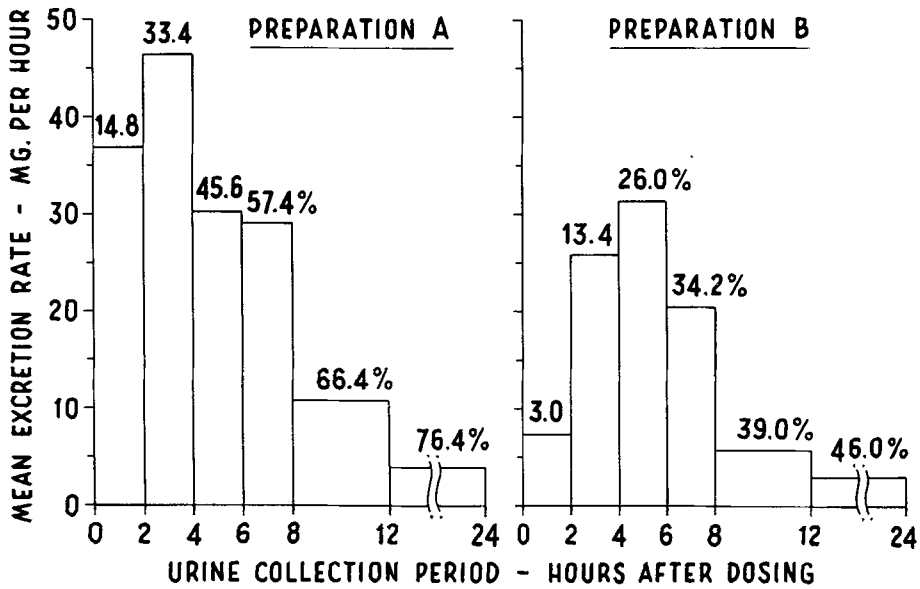

Fig. 1. Urinary excretion of total nitro compounds in human subjects receiving single 0.5 Gm. oral doses of chloramphenicol *Preparations A and B* (Trial 1; colorimetric assay). Bar graph indicates *mean excretion rate* (5 subjects) expressed as milligrams of chloramphenicol equivalents excreted per hour. Figures at top of each bar indicate the cumulative per cent of dose accounted for by the urinary excretion data. All values were corrected for normal predrug blanks.

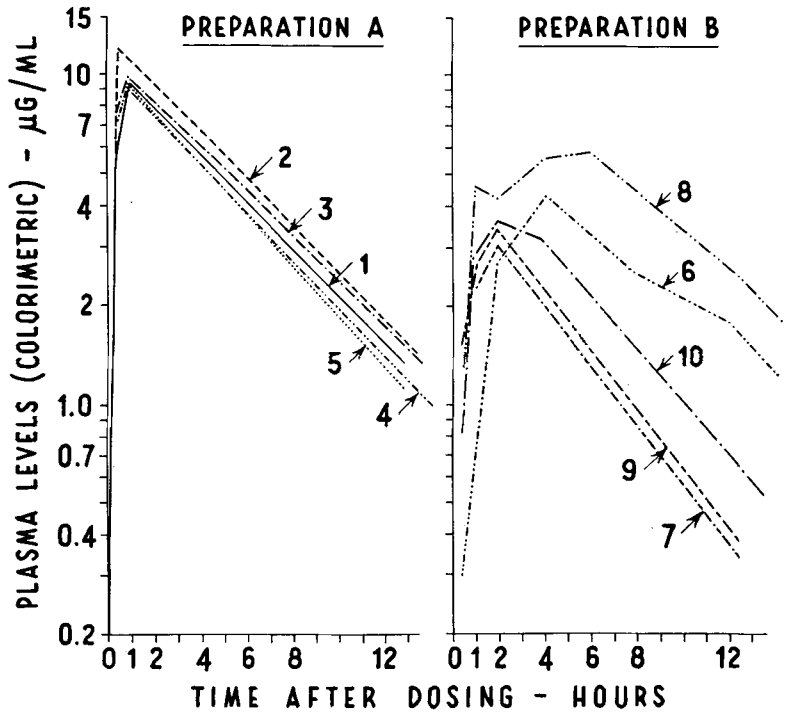

Fig. 2. *Plasma levels* (logarithm scale) for individual subjects receiving single 0.5 Gm. oral doses of chloramphenicol *Preparations A and B* (Trial 2; colorimetric assay). Best straight line drawn through plasma level data for each subject after maximum plasma levels had been attained.

Table II. *Plasma levels for groups of five human subjects receiving 0.5 Gm. oral doses of chloramphenicol Preparations A and B (Trial 2)*

Time after dosing (hours)	Colorimetric* (mean ± S.E.)		Microbiologic† (mean ± S.E.)	
	A (μg/ml.)	B (μg/ml.)	A (μg/ml.)	B (μg/ml.)
Predrug	0.2 ± 0.0	0.2 ± 0.1 (N.S.)‡	< 1.25 ± 0.0	< 1.3 ± 0.0 (N.S.)
0.5	8.4 ± 1.0	0.8 ± 0.3 (p < 0.001)	6.5 ± 0.9	< 1.5 ± 0.1 (p < 0.001)
1	9.6 ± 0.3	3.0 ± 0.5 (p < 0.001)	6.8 ± 0.4	2.4 ± 0.4 (p < 0.001)
2	7.9 ± 0.3	3.4 ± 0.3 (p < 0.001)	5.4 ± 0.2	2.7 ± 0.2 (p < 0.001)
4	6.0 ± 0.3	3.5 ± 0.7 (p = 0.01)	4.7 ± 0.3	3.3 ± 0.4 (p < 0.05)
6	4.5 ± 0.2	2.6 ± 1.1 (N.S.)	3.3 ± 0.3	2.7 ± 0.7 (N.S.)
8	3.0 ± 0.2	2.0 ± 0.6 (N.S.)	3.3 ± 0.4	2.1 ± 0.2 (p < 0.05)
12	1.4 ± 0.1	1.2 ± 0.4 (N.S.)	< 1.8 ± 0.4	1.7 ± 0.1 (N.S.)
24	0.2 ± 0.1	0.2 ± 0.1 (N.S.)	< 1.3 ± 0.0	< 1.7 ± 0.3 (N.S.)
Maximum levels	9.9 ± 0.6	4.1 ± 0.5 (p < 0.001)	7.2 ± 0.7	3.4 ± 0.5 (p < 0.01)

*Assay values corrected for individual predrug blanks.

†Microbiologic assay will not detect < 1.25 μg per milliliter.

‡Probability values estimated from Student's t test. N.S. = not significant (p > 0.05).

Table III. *Urinary excretion of total nitro compounds in human subjects receiving 0.5 Gm.*

Collection period (hours)	Preparation A							
	Subject No.							
	1 29, 72.3, C* (mg.)	2 22, 70.5, C (mg.)	3 33, 65.9, C (mg.)	4 34, 85.0, C (mg.)	5 30, 72.3, C (mg.)	Mean (mg.)	S.E. (mg.)	Cumulative (% of dose)
Predrug (24 hr.)	(1.2)	(4.0)	(0.0)	(0.6)	(2.2)	(1.5)	(0.8)	
0–2	103	94	88	97	89	94	2.8	18.8
2–4	98	101	101	107	127	107	5.3	40.2
4–6	21	72	60	62	48	53	8.8	50.8
6–8	38	60	40	51	47	47	4.0	60.2
8–12	71	55	79	59	68	66	4.3	73.4
12–24	41	60	48	46	19	43	6.7	82.0
24–48	7	14	14	8	11	11	1.5	84.2
0–48	379	456	430	430	409	421		
(% of dose)	75.8	91.2	86.0	86.0	81.8	84.2		

*Age (years), body weight (kilograms), and race.

between the means of the peak plasma levels for the two preparations was statistically significant ($p < 0.001$). With Preparation A, the plasma levels were fairly close together at different time periods, and the apparent biologic half-life was about 4.5 hours. Preparation B showed a relatively wide spread between the plasma levels for individual subjects; but the half-life values were not greatly different from those observed with Preparation A.

The mean values for colorimetric assays at each time period are shown in Table II, together with probability estimates based upon Student's t test for significance of the differences between the means. Highly significant differences were observed at 0.5, 1, and 2 hours ($p < 0.001$), probable significant differences at 4 hours ($p \cong 0.01$), and no significant differences were found thereafter ($p > 0.05$). The area under the plasma level curve for Preparation B was about 50 per cent of the area under Curve A at 12 hours, showing good agreement with the observations in Trial 1.

The mean values for microbiologic assays are also shown in Table II. Preparation A produced peak microbiologic assays of 6.1 to 9.9 μg per milliliter (mean = 7.2) in 30 minutes to 1 hour; Preparation B produced peak levels of 2.5 to 4.8 μg per milliliter (mean = 3.4) in 2 to 6 hours after medication. The difference between the means of the peak plasma levels for the two preparations was significant ($p < 0.01$). However, the differences between the means taken at different time periods were significant only in the first few hours (0.5 to 4 hours). The plasma levels obtained by microbiological assay were approximately 75 per cent of the colorimetric levels over the first 6 hour period after medication.

The urinary excretion data obtained in Trial 2 are shown in Table III. The total urinary excretion of nitro compounds over a 48 hour period after dosing accounted for 76 to 91 per cent of dose in the subjects receiving Preparation A (mean = 84 per cent). The subjects receiving Preparation B excreted 35 to 72 per cent of the administered dose in the same time period (mean = 52 per cent). Application of Student's t test indicated highly significant differences between the means for the 0 to 2 and 2 to 4 hour urine specimens ($p < 0.001$), reflecting the correspondingly greater plasma levels obtained with Preparation A in the early time periods.

doses of chloramphenicol Preparations A and B (Trial 2)

Preparation B							
Subject No.							
6 *49, 85.5, C* *(mg.)*	*7* *36, 87.3, C* *(mg.)*	*8* *24, 80.0, N* *(mg.)*	*9* *38, 71.4, N* *(mg.)*	*10* *32, 80.9, N* *(mg.)*	*Mean* *(mg.)*	*S.E.* *(mg.)*	*Cumulative* *(% of dose)*
(4.1)	(2.7)	(2.7)	(1.9)	(4.6)	(3.2)	(0.5)	
4	40	42	40	20	29	7.5	5.8
37	55	67	33	41	47	6.3	15.2
57	36	64	27	34	44	7.2	24.0
43	30	68	13	31	37	9.1	31.4
53	29	82	4	30	40	13.1	39.4
63	79	12	48	30	46	11.8	48.6
24	6	25	9	17	16	3.8	51.8
281	275	360	174	203	259		
56.2	55.0	72.0	34.8	40.6	51.8		

Table IV. *Age, weight, sex, and race of subjects (Trial 3)*

Subject No.	*Age (years)*	*Weight (Kg.)*	*Race and sex**
1	35	55.9	C, m
2	34	69.5	N, m
3	34	73.6	N, m
4	41	77.4	C, m
5	24	69.0	C, m
6	48	72.7	N, m
7	31	78.2	C, m
8	29	68.6	N, m
9	49	85.9	C, m
10	41	63.6	N, m
11	38	70.9	C, m
12	25	68.2	C, m
13	22	69.0	C, m
14	49	80.0	C, m
15	35	73.6	C, m
16	28	64.1	C, m
17	33	83.6	C, m
18	22	66.4	N, m
19	29	69.5	C, m
20	35	80.0	C, m
21	29	68.2	C, m
22	21	62.7	C, m
23	33	65.0	C, m
24	35	70.9	C, m
25	50	84.1	C, m
26	30	70.9	C, m
27	35	80.9	C, m
28	27	85.0	C, m
29	27	69.0	C, m
30	37	79.5	N, m
31	26	69.5	C, m
32	33	84.1	C, m
33	27	76.4	C, m
34	35	67.3	C, m
35	19	77.7	C, m
36	30	65.0	N, m
37	22	72.7	C, m
38	22	68.2	C, m
39	25	86.4	N, m
40	25	65.9	C, m

*C = Caucasian; N = Negro; m = male.

Trial 3. With the preceding observations clearly indicating that chloramphenicol Preparation A produced higher plasma levels and greater urinary output than Preparation B, a more comprehensive trial was set up in which four different chloramphenicol preparations were administered as single 0.5 Gm. doses to groups of 10 normal adult subjects. The age, body weight, race, and sex of each subject is shown in Table IV. The mean plasma levels and standard errors for the colorimetric assays are shown in Fig. 3. The colorimetric assays are also presented in Table V together with probability values calculated from Student's t test for significance of the differences between means.

In all 10 subjects receiving Preparation A, plasma levels (colorimetric assay) reached maximum values within 2 hours of medication (8.9 to 12.9 μg per milliliter; mean = 10.9). Four subjects showed peak levels at 30 minutes, 4 had peak levels at the 1 hour sampling period, and 2 showed peak levels at 2 hours. Preparation B produced peak levels in most subjects in 2 to 4 hours (2.2 to 8.2 μg per milliliter; mean = 5.2); peaks occurred in 1 subject each at 1 hour and 6 hours. Preparation C produced peak levels ranging from 4.3 to 10.7 μg per milliliter (mean = 6.3) at 1 and 2 hours after dose, with 1 subject at 4 hours. Preparation D produced the lowest levels of all, and the time periods required to reach peak levels also covered a wider range. Peak plasma levels ranged from 0.7 to 5.1 μg per milliliter (mean = 2.7), occurring mainly at 2 hours (6 subjects), with 1 subject each at 0.5 to 1 hour, 4 to 6 hours, 6 hours, and 8 hours. Application of Student's t test to the differences between the mean values for Preparation A and those of the other preparations tested indicated that the differences were significant at all time periods.

With the use of the colorimetric data, the areas under the mean blood level curves, expressed as per cent of the area under Curve A, were 53 per cent for B, 61 per cent for C, and 34 per cent for D. The half-life values estimated from the slopes of the semilog plots were nearly identical for these drug preparations, indicating that the rate of removal of drug from the blood, once it was absorbed, did not differ greatly with the type of preparation used.

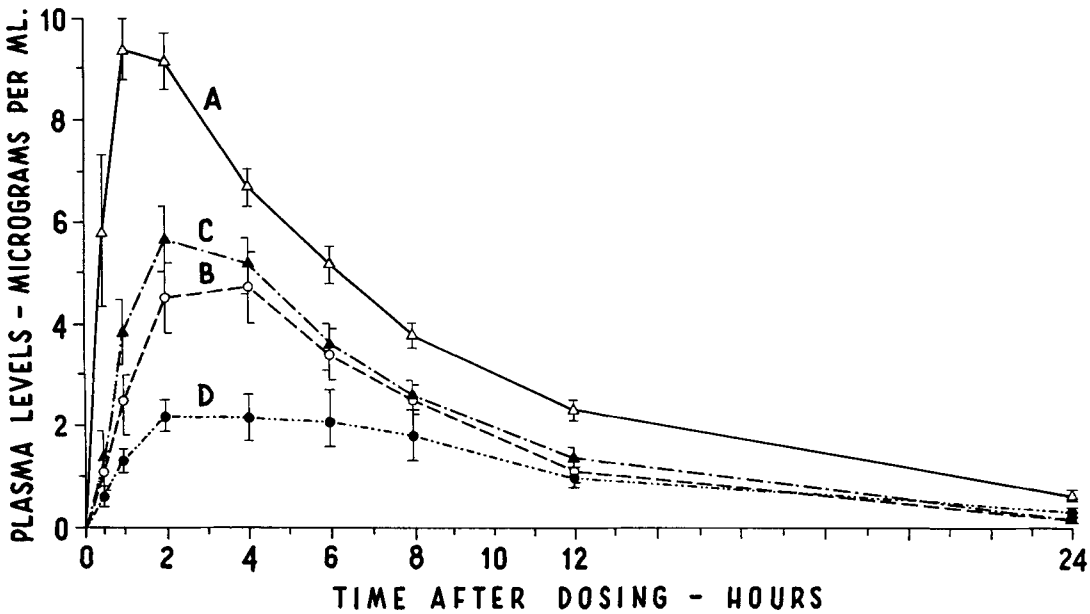

Fig. 3. Mean *plasma levels* for groups of 10 human subjects receiving single 0.5 Gm. oral doses of chloramphenicol Preparations *A, B, C, or D* (Trial 3; colorimetric assay). Vertical lines represent one standard error on either side of the mean.

Table V. *Plasma levels (colorimetric assay) for groups of ten human subjects receiving single 0.5 Gm. oral doses of chloramphenicol (Trial 3)*

Time after medication (hours)	*Mean plasma levels ± S.E. (μg/ml.)*			
	A	*B*	*C*	*D*
0.5	5.8 ± 1.1	1.1 ± 0.4 ($p = 0.001$)†	1.4 ± 0.5 ($p < 0.01$)	0.6 ± 0.2 ($p < 0.001$)
1	9.4 ± 0.6	2.4 ± 0.6 ($p < 0.001$)	3.9 ± 0.7 ($p < 0.001$)	1.3 ± 0.2 ($p < 0.001$)
2	9.1 ± 0.6	4.5 ± 0.7 ($p < 0.01$)	5.7 ± 0.7 ($p < 0.01$)	2.2 ± 0.3 ($p < 0.001$)
4	6.7 ± 0.4	4.7 ± 0.7 ($p < 0.05$)	5.2 ± 0.5 ($p < 0.05$)	2.2 ± 0.5 ($p < 0.001$)
6	5.2 ± 0.3	3.4 ± 0.5 ($p < 0.01$)	3.6 ± 0.5 ($p < 0.02$)	2.1 ± 0.5 ($p < 0.001$)
8	3.8 ± 0.2	2.5 ± 0.3 ($p < 0.01$)	2.6 ± 0.3 ($p < 0.01$)	1.8 ± 0.5 ($p < 0.01$)
12	2.3 ± 0.2	1.1 ± 0.1 ($p < 0.001$)	1.4 ± 0.2 ($p < 0.01$)	1.0 ± 0.2 ($p < 0.001$)
24	0.6 ± 0.1	0.2 ± 0.1 ($p = 0.01$)	0.2 ± 0.1 ($p < 0.01$)	0.3 ± 0.1 ($p < 0.05$)
Peak levels*	10.9 ± 0.5	5.2 ± 0.7 ($p < 0.001$)	6.3 ± 0.6 ($p < 0.001$)	2.7 ± 0.4 ($p < 0.001$)

*Mean of the maximum plasma levels in each series.

†Significance of difference between the means (versus A), evaluated by Student's t test.

Table VI. *Plasma levels (microbiologic assay) for groups of ten human subjects receiving single 0.5 Gm. doses of chloramphenicol (Trial 3)*

Time after dosing (hours)	*Mean plasma levels ± S.E. (μg/ml.)*			
	A	*B*	*C*	*D*
0.5	4.1 ± 0.9	< 1.4 ± 0.1 ($p < 0.01$)†	< 1.4 ± 0.1 ($p < 0.01$)	< 1.25 ($p < 0.01$)
1	5.6 ± 0.6	< 1.6 ± 0.2 ($p < 0.001$)	< 2.3 ± 0.3 ($p < 0.001$)	< 1.25 ($p < 0.001$)
2	4.9 ± 0.3	< 2.6 ± 0.4 ($p < 0.001$)	3.2 ± 0.6 ($p = 0.02$)	< 1.5 ($p < 0.001$)
4	3.7 ± 0.2	2.7 ± 0.4 ($p < 0.05$)	2.9 ± 0.4 (N.S.)	< 1.8 ($p < 0.001$)
6	2.6 ± 0.2	2.0 ± 0.2 ($p = 0.05$)	2.2 ± 0.2 (N.S.)	< 1.6 ($p < 0.01$)
8	2.0 ± 0.1	1.7 ± 0.1 ($p = 0.05$)	< 1.6 ± 0.1 ($p = 0.01$)	< 1.4 ($p < 0.001$)
12	< 1.4 ± 0.1	< 1.4 ± 0.1 (N.S.)	< 1.4 (N.S.)	< 1.5 (N.S.)
24	< 1.3 ± 0.0	< 1.3 ± 0.0 (N.S.)	< 1.25 (N.S.)	< 1.25 (N.S.)
Peak levels*	6.9 ± 0.5	3.0 ± 0.4 ($p < 0.001$)	3.6 ± 0.6 ($p < 0.001$)	2.1 ± 0.3 ($p < 0.001$)

*Mean of the maximum plasma levels in each series.

†Significance of difference between the means (versus A), evaluated by Student's t test. N.S. = not significant.

The mean values for the microbiologic assays at different times are shown in Table VI. These generally paralleled the colorimetric assays, although at a considerably lower level (50 to 60 per cent). The rate of decline in the microbiologic assays appeared to be about the same as in the colorimetric series. Although the peak plasma levels attained by the individual subjects were significantly greater with A in all cases ($p < 0.001$), the mean values for the different times after medication were significantly greater only in the early time periods (0.5 to 2 hours for Preparations B and C; 0.5 to 6 hours for D).

The urinary excretion data obtained in Trial 3 are shown in Table VII, expressed as the mean and standard error of chloramphenicol equivalents (in milligrams) excreted in each time period. For Preparation A there was a mean recovery representing 85 per cent of the dose, whereas it was 60, 70, and 33 per cent of the dose for Preparations B, C, and D, respectively. Preparation A produced a significantly higher urinary excretion of nitro compounds than the other preparations; Preparation D showed the lowest urinary output. The maximum excretion rate (in milligrams per hour) for Preparation A occurred at 2 to 4 hours in most subjects; Preparations B and C showed a greater number of subjects with maximum excretion rates in the 4 to 6 hour period; Preparation D showed consistently lower urinary excretion rates, although these also occurred in the early time periods.

Discussion

Two different lots of chloramphenicol Preparations A and B were used in these studies, each preparation producing about

Table VII. *Urinary excretion of total nitro compounds* in human subjects following single oral doses of 0.5 Gm. of chloramphenicol (Trial 3)*

Collection period (hours)	Chloramphenicol excretion (mean of 10 subjects ± S.E.)			
	A (mg.)	B (mg.)	C (mg.)	D (mg.)
Predrug (24 hr.)	5 ± 1	4 ± 1 (N.S.)†	5 ± 1 (N.S.)	4 ± 1 (N.S.)
0-2	57 ± 10	24 ± 6 ($p < 0.02$)	32 ± 7 ($p = 0.05$)	12 ± 2 ($p < 0.001$)
2-4	95 ± 12	68 ± 10 (N.S.)	83 ± 14 (N.S.)	35 ± 6 ($p < 0.001$)
4-6	67 ± 6	65 ± 9 (N.S.)	90 ± 12 (N.S.)	34 ± 7 ($p < 0.01$)
6-8	60 ± 6	52 ± 8 (N.S.)	52 ± 6 (N.S.)	20 ± 7 ($p < 0.001$)
8-12	62 ± 4	53 ± 9 (N.S.)	56 ± 9 (N.S.)	33 ± 9 ($p < 0.01$)
12-24	75 ± 7	36 ± 6 ($p < 0.001$)	53 ± 8 ($p = 0.05$)	33 ± 9 ($p < 0.01$)
24-48	24 ± 4	9 ± 2 ($p < 0.01$)	17 ± 3 (N.S.)	12 ± 2 ($p < 0.02$)
Total (mg.)	424 ± 14	302 ± 40 ($p = 0.01$)	350 ± 32 ($p = 0.05$)	165 ± 33 ($p < 0.001$)
% of dose	85 ± 3	60 ± 8	70 ± 6	33 ± 7
Range (%)	68-99	39-91	37-104	12-69

*All values corrected for predrug blanks.

†Significance of the difference between means (versus A), evaluated by Student's t test. N.S. = not significant.

the same absorption pattern in each trial. However, Preparation B consistently produced lower plasma levels than A, indicating that intestinal absorption differed. Although the urinary excretion data indicated somewhat lower excretion of nitro compounds than with Preparation A, there was considerable variation between individual subjects, and the differences were not significant. In the third trial, Preparation C produced plasma levels which were similar to those of Preparation B; whereas Preparation D produced distinctly lower plasma levels and urinary excretion. In the clinical trials with chloramphenicol which we reported some 20 years ago, plasma levels in human subjects were found to be directly proportional to dosage, while the urinary excretion of nitro compounds indicated essentially complete intestinal absorption.[9] The absorption characteristics of Preparation A in the current studies, evaluated by colorimetric plasma levels, were much the same.

The present studies were carried out with 0.5 Gm. doses of chloramphenicol, not by choice, but in accordance with the limitations set up by the FDA. None of the studies included a cross-over design, since not more than one dose was permitted in normal human subjects. As a result, the statistical evaluation of data was dependent upon the mean values obtained with different subjects, and a cross-over comparison of different preparations could not be made. Due to the low doses given,

certain differences appeared which require further explanation. In the present studies, microbiologic assays were considerably lower than anticipated, representing only 50 to 60 per cent of the colorimetric assay values. Griffith[10] has also reported low microbiologic assay values in plasma from human subjects receiving single 0.5 Gm. oral doses of chloramphenicol, representing about 55 per cent of the peak colorimetric levels. Most of our earlier observations were carried out at higher dose levels (e.g., 1.5 Gm. of chloramphenicol), and the microbiologic assays generally represented 90 to 95 per cent of the colorimetric assays in the first few hours after dose, dropping to 80 per cent in 6 hours and 60 per cent in 12 hours after dose.[3] This appeared to be due to a slightly longer half-life for chloramphenicol metabolites than for unchanged drug. However, the plasma half-life of chloramphenicol at the 0.5 Gm. dose level appeared to be about the same as at higher doses. The wide discrepancy between colorimetric and microbiologic assays at the 0.5 Gm. dose level was evident at all time periods. This could be due in part to a lack of specificity in colorimetric assays, with normal variations in the blank being proportionately greater at low plasma levels; the microbiologic assays were also subject to greater errors at low drug levels, and the sensitivity of the assay procedure was inadequate to detect chloramphenicol levels below 1.25 μg per milliliter.

The dissolution rate of poorly water-soluble drug preparations appears to relate to particle size, which obviously, directly relates to the total surface in contact with the surrounding liquid.[16] The early work of Reinhold and colleagues[14] with different preparations of sulfadiazine clearly indicated the importance of particle size to absorption rate. In some of our early work with diphenylhydantoin,[2] significant differences were found in the plasma levels of human subjects receiving 0.4 Gm. oral doses of the sodium salt compared with crystals of free acid. The salt was converted to the poorly soluble acid form in the stomach, where the rate of solution appeared to be dependent upon the particle size of the preparations. In other experiments with different particle-size preparations of chloramphenicol,* we found that the absorption of the larger crystals took place slowly in man; but over a 24 to 48 hour period most of the administered dose was accounted for by the urinary excretion of nitro compounds, indicating eventual complete absorption. Similar observations were reported by Kakemi and colleagues[12] with different preparations of chloramphenicol in rabbits. In the present study, Preparations A, B, and C showed relatively good absorption as indicated by the excretion of nitro compounds; but Preparation D showed a significantly lower urinary excretion of chloramphenicol and its metabolites which can be explained only by incomplete absorption.

A detailed in vitro study of the physical and pharmaceutical factors influencing the availability of the drug from these chloramphenicol preparations has been carried out by our group and will be reported elsewhere.[1] Preparation A showed the greatest rate of solution; Preparation C went into solution at about half this rate; while Preparations B and D showed the lowest dissolution rates.

The variations observed in the absorption of the same chloramphenicol preparation in different individuals are of interest because of the factors which may be involved. The presence or absence of bile in the intestinal tract undoubtedly contributes to the dissolution of drugs and their subsequent absorption. Chloramphenicol is absorbed by simple diffusion mechanisms in the intestinal tract, but absorption from the stomach appears to be minimal.[7] In common with many other drugs, chloramphenicol appears to have an effect on active transport mechanism in the small intestine of the rat.[4, 5] In attempting to extend these observations to in vivo

*Unpublished observations.

experiments, high oral doses of chloramphenicol in laboratory animals produced a significant delay in the gastric emptying time, resulting in delayed absorption of test substrates from the intestinal tract.* It seems likely that some of the variations in absorption encountered in different individuals may be due largely to this factor.[13] A better comparison between different preparations would result if the stomach could be bypassed entirely, with the drugs being placed directly in the intestine. However, even with the handicap of peroral administration, the present study reveals marked differences in the absorption characteristics of these chloramphenicol preparations. Although "equivalent" in the chemical sense, it is evident that they will not produce equivalent plasma levels in man.[15]

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