An Evaluation of the Biological Availability of Chloramphenicol*

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SUMMARY

The biological availability of chloramphenicol from different commercial preparations was investigated. Methods used to evaluate the *in vitro* release were disintegration, de-aggregation and dissolution tests and particle size measurement. Considerable differences between the rate of release of the antibiotic from the different capsule preparations were detected. These differences can be attributed to the formulation used during the preparation of the different products. The absorption characteristics of chloramphenicol from 3 brands of chloramphenicol capsules and 3 chloramphenicol powder samples from different sources were tested on 6 healthy male subjects in a cross-over trial. Each subject received 500 mg as a single oral dose at intervals of one week. Significant differences between the average amount of chloramphenicol excreted in the urine from the different preparations were noted 1 - 2 hours after administration of the dose. The experimental results were used to compute the absorption rate of the antibiotic from different preparations. The differences observed in the *in vitro* release were confirmed by the *in vivo* results. The differences between the release of the antibiotic from the different capsule preparations could be ascribed to the method of formulation of the antibiotic which is poorly soluble in water.

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The necessity to assure the biological availability of drugs from drug products has been emphasized to a great extent in the past decade. Several examples of drug products which had little or no therapeutic effect have been reported.[1-6] The hazards of therapeutic non-equivalency of drug products are obvious and it is, therefore, necessary to emphasize the need for caution in assuming that absorption characteristics are the same for a drug from different preparations.

Using dogs as experimental animals the *in vivo* absorption of chloramphenicol from capsules, each originating from 4 different manufacturers, has been studied. No significant difference in serum concentrations and rate of elimination of the drug from the blood was noted.[7] Glazko *et al.*[5] and Aguiar *et al.*[8] studied the *in vitro* dissolution and de-aggregation as well as the *in vivo* absorption of chloramphenicol from 4 different chloramphenicol preparations in human adults. A distinct variation in the intestinal absorption pattern of chloramphenicol from the different preparations was noted, and these differences can be correlated with the differences in dissolution and de-aggregation rate of the different preparations.

Martin *et al.*[9] concluded from their experiments with 3 different chloramphenicol capsule products that the rate of absorption for 1 product was considerably faster than with the other 2 products. When the contents of these 2 products were recrystalized, no difference was observed. It was, therefore, concluded that the particle size of the drug influenced the dissolution and absorption rate of the drug. The absorption of chloramphenicol from two different preparations in 20 male volunteers receiving each a dose of 1,0 g chloramphenicol every 6 hours for 48 hours, was studied.[10] Significantly higher blood concentrations of chloramphenicol from one preparation were observed during the first hour; but 18 hours after the first administration the serum concentration from the other preparation was greater. After reformulation of the one preparation, it produced similar blood concentrations of chloramphenicol to the other preparation.

In an extensive comparison between 5 different capsules, 4 different tablets and 5 different suspensions, it was found that there were significant differences in the rate of absorption of chloramphenicol from the gastro-intestinal tract. In all the cases there was a close correlation between absorption rate and dissolution rate.[11] From the results in the quoted literature it is apparent that the formulation of chloramphenicol into a drug product plays a very important part in the release characteristics of the drug from the particular dosage form.

For this reason the bio-availability of chloramphenicol from different capsules on the South African market was studied. The use of certain *in vitro* methods are suggested for quality control purposes so as to predict the *in vivo* absorption rate from commercially available products.

EXPERIMENTAL METHODS

Assay of Chloramphenicol Capsules

Chemical method: The chloramphenicol content in the different capsules was determined by the method described in the *British Pharmacopoeia* (B.P.) (1968).

Microbiological method: The microbiological assay was done by using *Bacillus subtilus* as test organism. The method used complied with the method of the B.P. (1968).

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Disintegration Test

The disintegration time of the different capsules was determined according to the B.P. (1968) specifications, using a Manesty tablet disintegration test unit without a disc. A capsule was placed in each of the 6 tubes containing 250 cm^3 of simulated gastric fluid without pepsin.[12] Five, ten and fifteen minutes after the unit was put into operation, 5 cm^3 aliquots of each tube were withdawn, centrifuged at 2 000 rpm for 2 minutes, and 2 cm^3 of the supernatant was diluted appropriately with simulated gastric fluid and assayed spectrophotometrically at 278 nm. After the removal of a sample the same amount of gastric fluid was replaced. The disintegration time of each capsule was recorded. This procedure was repeated for each of the capsule products

De-aggregation Rate Determination

The method used was essentially the same as reported by Aguiar *et al.*[8] For each determination 1 litre simulated gastric fluid without pepsin[12] and presaturated with chloramphenicol at 37°C was added to a 2-litre beaker placed in a constant-temperature bath set at 37 ± 0,5°C. The solution was stirred at 100 rpm with a blade-stirrer without creating a vortex. The solution circulated via a plastic tube, with a 0,3 cm internal diameter, through a flow cell located in a Beckman DBG spectrophotometer. The flow rate of the medium through the cell was determined and kept constant for each determination at 60 cm^3 per minute. Gastric fluid presaturated with chloramphenicol at room temperature, was used as a blank for the determinations. A Beckman recording unit was connected to the spectrophotometer, set on the linear registration position and adjusted to record 100% light transmittance at 650 nm. After the medium was allowed to reach the determined temperature, 2 capsules were placed in the beaker. A stopwatch was started simultaneously to check the time on the recorder. The de-aggregation pattern for each capsule was recorded for 60 minutes.

Dissolution Rate Determination

The apparatus used was essentially the same as the one previously reported.[13] One litre of simulated gastric fluid without pepsin[12] was placed in a 2-litre beaker in a constant-temperature bath at 37 ± 0,5°C and stirred at 100 rpm. One capsule was placed in the capsule holder and placed in position. A filter was connected to a plastic tube to withdraw 2 cm^3 samples at predetermined time intervals. The same volume of dissolution medium was replaced via the filter after removal of each sample. The samples were diluted appropriately with dissolution medium and assayed spectrophotometrically at 278 nm.

Particle Size Determination

The particle size of the chloramphenicol in the different samples was determined with a Zeiss Particle Size Analyzer TGZ3.[14] Microscopic slides were prepared for each sample and recorded photographically. After appropriate enlargement, the photographs were used to determine the particle size distribution. The average particle diameter was calculated according to the appropriate equation.[15]

Absorption Rate Determination

Six healthy male volunteers were used in a cross-over study testing chloramphenicol capsule A, B and C as well as chloramphenicol powder sample A, B and C. The subjects weighed 71 - 75 kg and were between 20 and 23 years of age. All of them had a good medical record and had no history of renal dysfunction. Urine samples were collected 24 hours before each medication to serve as a blank. All subjects received a single oral dose corresponding to 500 mg chloramphenicol of each product at intervals of one week between each dose. Each chloramphenicol capsule was administered eventually to each of the 6 subjects, but each chloramphenicol powder sample was administered to only 2 subjects. The subjects received the dose together with a glass of water half an hour before a light breakfast. Urine samples were collected at 0,5; 1; 2; 3; 4; 6; 8; 12; 24; and 48 hours after dosage. After measuring the volume of the samples, it was kept at 4°C before analysis. The amount of chloramphenicol in the urine was determined by a colorimetic method basically the same as described by Glazko *et al.*,[16] and modified by the Food and Drug Administration.[17] A digital computer was used to calculate the absorption rates and other pharmacokinetic parameters for chloramphenicol from the cumulative amount of chloramphenicol excreted in the urine. The following pharmacokinetic model was used to describe the absorption and excretion of chloramphenicol:

$$A\text{—lag} \xrightarrow{k_1} B \xrightarrow{k_2} U$$

where A = amount drug in gastro-intestinal tract,
k_1 = rate constant for absorption of drug from the gastro-intestinal tract.
k_2 = rate constant for elimination of drug into urine,
B = amount of drug in body,
U = amount of drugs in urine.

The following differential equations were used to describe the model:[18]

$$\frac{dA}{dt} = -k_1A$$

$$\frac{dB}{dt} = k_1A - k_2B$$

$$\frac{dU}{dt} = k_2B$$

RESULTS AND DISCUSSION

Chloramphenicol Content

The results of both chemical and microbiological assays of each capsule are given in Table I. Only capsule B

did not comply with the requirements of the B.P. (1968) in that each chloramphenicol capsule should contain between 92,4 and 107,5% of the label claim. However, if the microbiological assay results are considered applicable to the same limits, then capsules B, C and D do not comply with these standards. It has been proved that no known chemical assay method for chloramphenicol is capable of determining the amount of active chloramphenicol.[19,20] The only method of determining the amount of active antibiotic is a microbiological assay. By using the official chemical assay method, inactive degradation products of chloramphenicol, such as those present in capsule D, were assayed as active chloramphenicol and this is the reason why this capsule wrongly complied with the standards, but failed to do so with a microbiological assay.

TABLE I. ASSAY OF CHLORAMPHENICOL CAPSULES

	Amount of chloramphenicol per capsule (mg)			
Capsule	Spectrophotometric (mg)	% of 250 mg	Microbiological (mg)	% of 250 mg
A	**235,4**	**94,16**	**240**	**96,0**
B	**217,7**	**87,08**	**230**	**92,0**
C	**243,0**	**97,21**	**230**	**92,0**
D	**243,5**	**97,40**	**220**	**88,0**
E	**235,7**	**94,28**	**240**	**96,0**

Disintegration Time

The results of the disintegration and dissolution tests are given in Fig. 1. The average disintegration time for capsules A, B, C, D, and E was 4,65 ± 0,3; 6,75 ± 2,1; 7,7 ± 1,8; 4,68 ± 0,3 and 6,55 ± 0,2 minutes, respectively. Capsule A and D disintegrated faster than any of the other capsules while capsule C had the slowest disintegration rate. All the capsules disintegrated within the 15 minutes required by the B. P. (1968). The chloramphenicol in capsule E dissolved faster than any of the other capsules while only approximately 50% of the chloramphenicol in capsule B dissolved in 15 minutes. No correlation existed between the disintegration time and the amount of chloramphenicol dissolved. A true indication of the dissolution rate of chloramphenicol from a capsule was not possible by using the disintegration apparatus. These results were comparable to those reported elsewhere.[21]

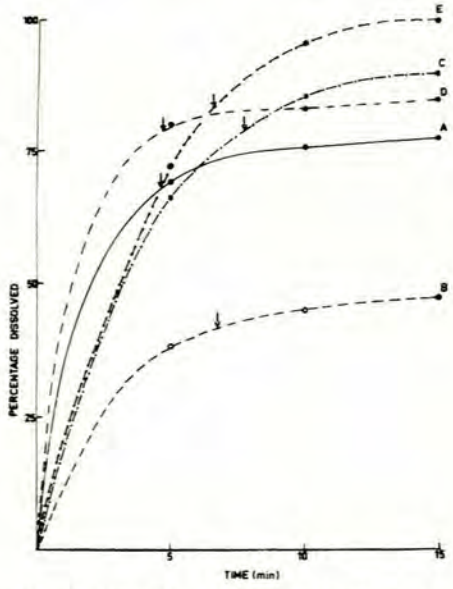

Fig. 1. Dissolution rate of chloramphenicol capsules as determined with disintegration apparatus. Arrows indicate disintegration time.

Determination of De-aggregation Rate

In Fig. 2 the percentage transmission is plotted against time. A higher percentage transmission is obtained with a higher de-aggregation state. The de-aggregation rate of each capsule can be evaluated from the slopes of the curves, or it can be expressed by the de-aggregation $T_{50\%}$ which in this case is the time required to reach half of the de-aggregation state of the final value obtained after 60 minutes for each capsule. For capsules A, B, C, D and E the de-aggregation- $T_{50\%}$ was respectively 2,0; 7,4; 11,0; 2,8 and 13,5 minutes. A correlation existed between the disintegration time and de-aggregation- $T_{50\%}$ for the capsules.

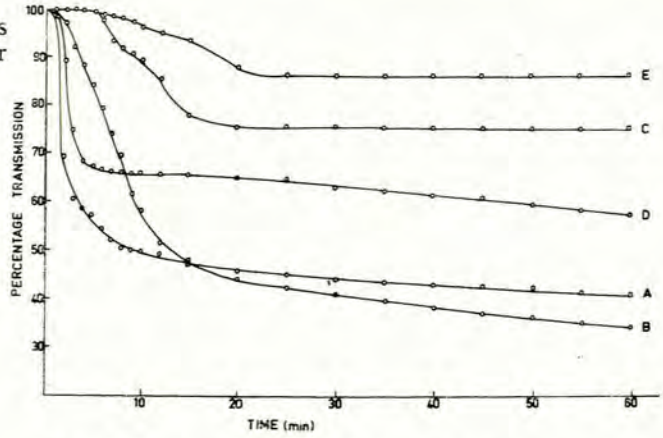

Fig. 2. De-aggregation rate of chloramphenicol in different capsules.

Dissolution Rate Studies

The results of the dissolution rate determinations are given in Fig. 3. The rate of dissolution for chloramphenicol from each capsule can be evaluated from the slope of the curve or can be expressed as the dissolution- $T_{50\%}$ which is the time required to dissolve half the amount (125 mg) of chloramphenicol in a capsule.

Capsules A, B, C and D had a dissolution- $T_{50\%}$ of 2,4; 17,6; 33,0 and 3,6, respectively. Capsule E was still an intact capsule after 90 minutes in the dissolution beaker and only approximately 23% chloramphenicol dissolved during this period. A correlation existed both between the disintegration time and de-aggregation and dissolution rates for capsule A, B, C and D.

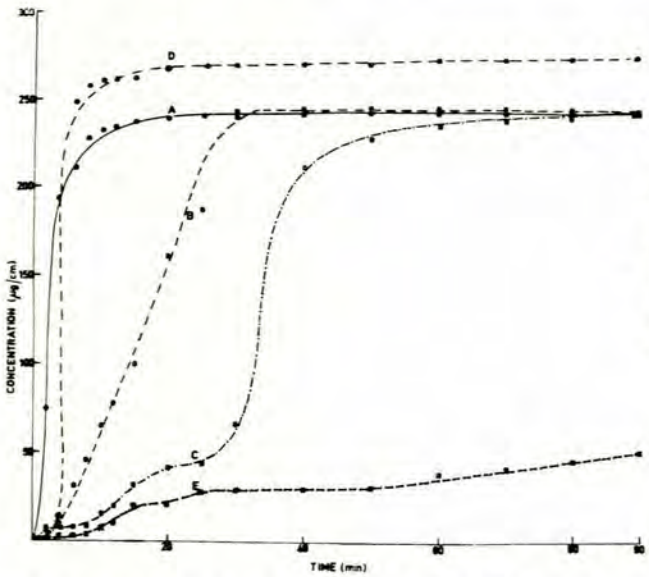

Fig. 3. Dissolution rate of chloramphenicol in different capsules.

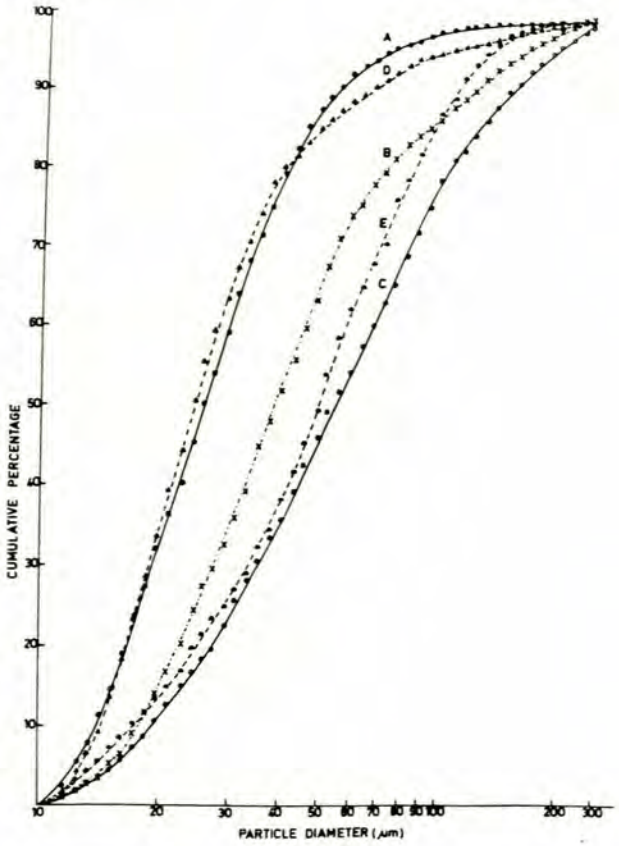

Fig. 4. Particle size distribution of chloramphenicol samples.

Particle Size Distribution

The particle size distribution of the different chloramphenicol powders are plotted on semilogarithmic paper in Fig. 4. The calculated average particle diameter was 41,3 μm, 71,1; 93,4; 49,6 and 72,5 μm for chloramphenicol powder samples A, B, C, D and E, respectively. The particle size distribution of sample A and D was almost similar.

In Vivo Determinations

The experimental results, the calculated excretion rate and cumulative amount chloramphenicol excreted for capsules A, B, and C are illustrated in Fig. 5.

From Fig. 5 it can be concluded that capsule A released its contents faster than capsule B or C, if the lag time is taken into account. In Table II the average amount of chloramphenicol excreted after administering capsule A, B, and C are given as well as the calculated parameters for the different capsules. The only statistically significant differences between the capsules, as expressed by the amount excreted, were noticed 1 hour after ingestion of the antibiotic. Capsule A proved to have the fastest rate of absorption and this can be correlated with the results of the *in vitro* tests.

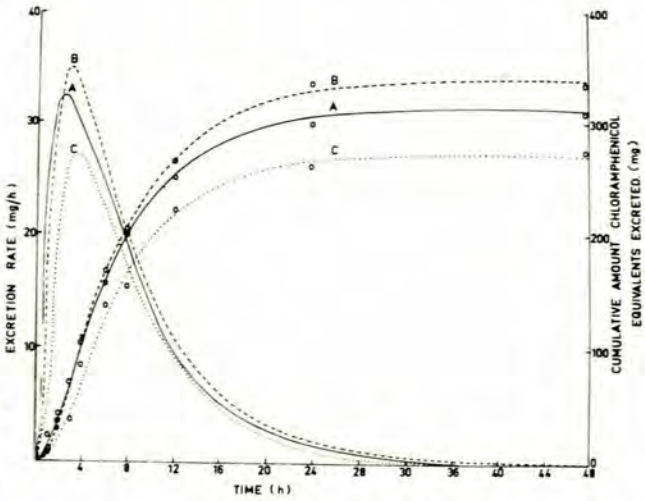

Fig. 5. Computed curves and experimental data points for urinary excretion of total chloramphenicol equivalents of capsules A, B and C (dose 500 mg).

TABLE II. EXPERIMENTAL RESULTS AND CALCULATED PARAMETERS FOR CHLORAMPHENICOL CAPSULES

	Average cumulative amount excreted after 500 mg doses		
Time (h)	Capsule A (mg)	Capsule B (mg)	Capsule C (mg)
0,5	1,9 ± 3,7	0,8 ± 0,9	1,5 ± 2,8
1	*21,4 ± 19,2	*4,9 ± 4,8	*6,4 ± 10,9
2	49,8 ± 21,2	36,4 ± 20,6	28,4 ± 31,9
3	72,5 ± 34,1	69,3 ± 23,1	36,6 ± 39,5
4	93,3 ± 33,3	106,0 ± 45,2	85,8 ± 48,8
6	156,8 ± 75,7	166,8 ± 39,3	136,4 ± 54,7
8	191,0 ± 76,1	204,1 ± 44,4	154,1 ± 64,8
12	267,4 ± 129,5	265,5 ± 65,7	221,8 ± 83,2
24	303,3 ± 134,6	330,9 ± 117,1	263,2 ± 123,2
48	310,4 ± 133,9	335,2 ± 118,4	273,2 ± 122,8
	42,1%	67,04%	54,6%
Calculated parameters			
$t_1/_2$ (h)	4,07	4,60	4,08
T max. (h)	2,99	2,51	3,06
C max. (mg)	38,98	35,05	27,00
Lag. (h)	0,00	0,34	0,27
k_1 (h^{-1})	0,58	0,83	0,53
k_2 (h^{-1})	0,17	0,15	0,17

* Significant differences between average amount excreted evaluated with Student's *t*-test ($P>0,05$).

TABLE III. EXPERIMENTAL RESULTS AND CALCULATED PARAMETERS FOR CHLORAMPHENICOL POWDERS

	Average cumulative amount excreted after 500 mg doses		
Time (h)	Powder A (mg)	Powder B (mg)	Powder C (mg)
0,5	*1,4 ± 0,8	*4,5 ± 0,3	*0,6 ± 0,6
1	27,1 ± 14,5	*29,8 ± 3,5	*5,5 ± 4,8
2	*67,0 ± 2,1	67,4 ± 6,5	*56,6 ± 0,9
3	101,7 ± 7,0	105,3 ± 21,6	92,9 ± 3,1
4	126,5 ± 24,3	164,0 ± 12,3	136,2 ± 5,8
6	174,9 ± 17,1	220,6 ± 37,6	197,8 ± 13,5
8	210,1 ± 18,7	265,3 ± 46,1	221,3 ± 4,4
12	254,1 ± 16,9	289,8 ± 70,6	266,5 ± 28,3
24	305,6 ± 20,4	311,2 ± 92,0	308,7 ± 59,7
48	316,9 ± 31,6	314,4 ± 95,3	316,5 ± 67,5
	63,4%	62,9%	63,3%
Calculated parameters			
$T_1/_2$ (h)	4,89	3,31	3,82
T max. (h)	1,14	2,20	0,92
C max. (mg)	38,24	48,32	48,20
Lag. (h)	0,00	0,00	0,64
k_1 (h^{-1})	2,74	0,65	3,36
k_2 (h^{-1})	0,14	0,30	0,18

* Significant differences between average amount excreted evaluated with Student's *t*-test ($P>0,05$).

The results of the excretion of the different chloramphenicol powder samples are given in Fig. 6. From this figure and the calculated parameters in Table III it is obvious that chloramphenicol from powder sample A was absorbed faster than from both B and C. Likewise, this difference was only significant during the first hour after administration. This could be correlated with the average particle size of the different chloramphenicol powder samples.

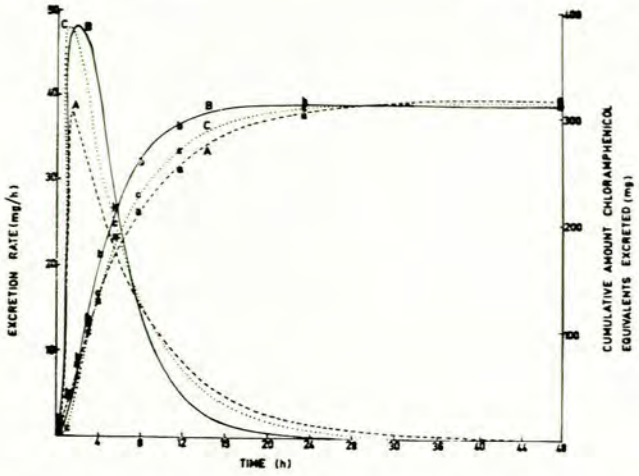

Fig. 6. Computed curves and experimental data points for urinary excretion of total chloramphenicol equivalents of powders A, B and C (dose 500 mg).

CONCLUSION

The formulation of chloramphenicol in the different capsules had an influence on the biological availability of the antibiotic. Particle size, apparent presence of a dispersing or wetting agent and other adjuvants used had an influence on the dissolution rate of chloramphenicol from the capsules. The storage conditions of the capsules also had an influence on the release rate, since it was brought to our attention that capsule E had been stored in an open container for several months before the tests were conducted. Moisture obviously had an influence on the dissolution rate of this capsule.[22] As the *in vitro* results can be correlated with the *in vivo* results, it seems obvious that *in vitro* tests can be used to test the biological availability of chloramphenicol. The results obtained in this study again emphasize the need for efficient quality control methods during and after the product is manufactured. Factors that could limit the biological availability of a drug from a drug product should be investigated thoroughly so that the optimum biological availability can be achieved at any time.

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