since the chance of collision between reactive species is reduced.

It has been shown previously (2) that dispersions containing aliphatic aldehydes in excess of their solubility, *i.e.*, emulsions, have similar rate constants, provided the saturation ratio is the same. With benzaldehyde and *p*-methylbenzaldehyde, however, agreement between rate constants in dispersions having the same saturation ratio is confined to the solubilized state. With emulsions, the rate constant falls progressively with increase in surfactant concentration. Carless and Swarbrick (7) have reported a similar pattern for the oxidation of emulsions of benzaldehyde in betaines. They pointed out that the term "emulsion" is used to cover all forms of dispersion in which oil is present in excess of its solubility in a surfactant and that in a ternary system the nature of the separating phase or phases may vary in composition with the concentration of each component, even though the saturation ratio is the same.

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Effect of Particle Size on Gastrointestinal Absorption of Sulfisoxazole in Dogs

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After oral administration of sulfisoxazole to dogs, the blood level is affected by the particle size of the drug. Equations which describe this change in the range of experimental testing were derived empirically. Statistical evaluations and probabilities of error are given. The effect of particle size on the blood level of sulfisoxazole in dogs is due largely to the dissolution rate of the drug in the gastrointestinal tract of the animal. The possibility of controlling blood level by controlling the particle size content of a single dose is evident. Faster and higher blood levels of sulfisoxazole can be obtained by reduction of particle size. The proportion of the dose which is absorbed does not change; however, the rate at which this proportion is absorbed does change with a change of particle size.

THE PARTICLE size of some medicaments has been observed to affect their biological activity (1, 2). A reduction of the particle size of a drug increases the specific surface area and usually results in an increase in both the dissolution rate and the solubility. The logical assumption follows that if more of a medicament is in solution and if solution is effected at a faster rate in the gastrointestinal tract, a more rapid rate of absorption will result (1–3). It is reasonable to expect that those drugs which are practically insoluble present a case in which reduction of particle size may be advantageous. Such has proved to be the case with griseofulvin (4). There is also the possibility that sustained-release medication can be produced by increasing the particle size of some medicaments.

In view of the fact that most of the studies regarding the effects of particle size in biological systems have been brief, a carefully controlled statistically analyzed study of this phenomenon

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TABLE I.—RESULTS OF PARTICLE SIZE ANALYSES OF SULFISOXAZOLE CRYSTALS

Sample	Mean Diam. (d_{vs}), μ	Method of Analysis
A	1.58	Coulter counter
A	1.08	Coulter counter
A	1.72	Coulter counter
A	1.56	Coulter counter
A	2.62	Coulter counter
A (av.)	1.7[a]	Coulter counter
B	26.6	Coulter counter
B	41.57	Coulter counter
B	35.71	Coulter counter
B	45.44	Coulter counter
B	37.21	Coulter counter
B	43.73	Coulter counter
B	39[b]	Coulter counter
C (av. of 1000)	95[b]	Microscopic

[a] Average per cent above 5 μ, 7.8; average per cent above 10 μ, 1.0. [b] Particle size distribution approximates the Poisson type.

was performed. This paper reports observations on the absorption of sulfisoxazole, not reported previously in this respect, in the mongrel dog. The studies reported here substantiate theoretical data (3) and add to the overall picture of the influence of particle size on gastrointestinal absorption.

EXPERIMENTAL

Preparation and Analysis of Sulfisoxazole Crystals.—The production of small crystals (less than 10 μ in diameter) from supersaturated solutions in an ultrasonic field has been investigated previously (5). The Rapisonic Mark V[1] was used to produce the smaller crystals (sample *A*, Table I). This instrument consists of a motor driven gear pump with an ultrasonic bell. The bell is a stainless steel pipe with an inlet from the pump and an outlet containing a back pressure valve. Inside the bell is a jet with a stainless steel blade located in front of the jet and mounted on an adjustable holder. The distance of the blade from the jet can be regulated by a micrometer outside and at the end of the bell. The production of sound waves is accomplished as the pressurized jet stream strikes the edge of the blade at an optimum distance from the jet and under the influence of an optimum back pressure. A dry ice–acetone bath was constructed around the feed funnel leading to the pump.

The following procedure was used. (*a*) A 10% solution of sulfisoxazole in ethanol was prepared at a temperature of 70°. (*b*) The ultrasonic instrument was cooled to about −20° by circulating previously cooled ethanol through it. (*c*) After removal of the ethanol, the 10% solution was transferred to the Rapisonic, and insonation and cooling of the solution was effected simultaneously, resulting in the formation of low micron crystals. (*d*) After 3 min. of insonation and cooling from 70° to approximately −15°, the crystals were collected on a suitable filter and dried in an air circulating oven (60°).

The medium size crystals (sample *B*) were prepared using a portable stirrer and an ice bath to agitate and cool, respectively. The largest crystals (sample *C*) were prepared in a similar manner to sample *B*, except that the solution was allowed to cool to room temperature (no cooling bath) with occasional stirring using a glass rod.

Samples *A* and *B* were analyzed with a Coulter counter[2] and sample *C* was analyzed using a microscope. The calculated mean diameter is a weighted mean (d_{vs}) obtained by multiplying the number of particles in each size interval by the diameter of the respective intervals, summing these products, and dividing this sum by the total number of particles counted.

Since two methods of analysis were used, the mean values were equated as follows. The Coulter counter was standardized using spherical pollen grains and polystyrene spheres. Since the sulfisoxazole crystals are cylindrical and since the Coulter evaluation is based on the change of resistance in proportion to the volume of the particle, the diameter involved is that which would be assumed if the particles were spheres. The problem involves a measuring of the particles measured under the microscope and a calculation of the diameter of the sphere having the same volume.

The length, l, and the width or diameter, d_c, of the circular end of 1000 particles were measured under a microscope equipped with a calibrated micrometer disk. The equation used to calculate the diameter, d_s, of a sphere having the same volume is derived as follows: the volume of a cylinder is equal to $\pi r_c^2 l$, where r_c is the radius of the circular end. If $r_c = {}^1/_2 d_c$, then $r_c^2 = {}^1/_4 d_c^2$. Thus, volume of a cylinder in terms of diameter equals $\pi\ {}^1/_4 d_c^2 l$. Since the volume of a sphere $= {}^1/_6\ \pi d_s^3$ and since one wishes to equate the volume of the cylindrical particles to the volume of a sphere of equal volume, one can equate the two expressions as follows:

$$\pi\ {}^1/_4 d_c^2 l = {}^1/_6 \pi d_s^3$$

After rearranging and solving for d_s we obtain the equation:

$$d_s = (1.5 d_c^2 l)^{1/3}$$

After performing this calculation for each of 1000 particles and averaging the d_s values, a mean diameter (d_{vs}) for sample *C* was obtained. The results of particle size analyses are summarized in Table I.

Biological Procedures.—Five mongrel dogs, weighing 10 to 20 Kg., were used in this study. The dogs were fed the same type of food on a regular schedule during the experimental period. On the day of testing, food was given just after administration of the drug conforming to the regular feeding pattern. The interval between test days was from 5 to 7 days, thus allowing previous doses of the sulfisoxazole to be eliminated completely from the system. For a given drug and test animal, rate constants for absorption, metabolic alterations, and excretion were assumed to be the same. By keeping the experimental procedures as constant as possible, any difference in blood level, after separate administration of the various particle sizes, could be attributed to the differences in particle size.

Doses of 60 mg./Kg. of the sulfisoxazole crystals

[1] Manufactured by Sonic Engineering Co., Norwalk, Conn.

[2] Manufactured by Coulter Electronics, Inc., Chicago, Ill.

TABLE II.—BLOOD LEVEL DATA AND RESULTS OF STATISTICAL CALCULATIONS 0.5 HR. AFTER ORAL ADMINISTRATION OF SULFISOXAZOLE TO DOGS

Sample Av. Diam. (d_{vs}) μ	*A* 1.7	*B* 39	*C* 95
Blood levels of repeated trials, mg. %	2.38	0.26	0.79
	3.62	4.45	0.39
	0.35	1.30	1.00
	1.20	0.50	0.31
	0.65	0.75	0.90
	1.05	0.32	0.34
	7.00	0.44	3.70
	0.44	0.98	0.28
	5.59	3.46	0.38
	0.50	0.81	2.61
	4.50	0.44	...
	...	0.55	...
	...	4.00	...
Mean blood level, mg. %	2.48	1.4	0.74
$F_{A,B,C}$ ratio	2.852	$P > 0.05$	...
$t_{A,B}$	1.367	$P < 0.1$	$P > 0.05$
$t_{B,C}$	1.1	$P < 0.2$	$P > 0.1$

TABLE III.—BLOOD LEVEL DATA AND RESULTS OF STATISTICAL CALCULATIONS 1 HR. AFTER ORAL ADMINISTRATION OF SULFISOXAZOLE TO DOGS

Sample Av. Diam. (d_{vs}) μ	*A* 1.7	*B* 39	*C* 95
Blood level of repeated trials, mg. %	3.80	0.61	2.83
	5.88	6.50	1.13
	1.90	4.10	3.84
	5.88	2.36	0.31
	4.07	3.17	2.75
	3.00	0.43	0.44
	6.90	2.15	5.60
	3.30	5.25	0.50
	7.63	6.00	0.38
	1.50	1.40	3.70
	6.20	3.30	...
	...	5.00	...
	...	3.85	...
Mean blood level, mg. %	4.55	3.39	2.15
$F_{A,B,C}$ ratio	3.95	$P < 0.05$	...
$t_{A,B}$	1.468	$P < 0.1$	$P > 0.05$
$t_{B,C}$	1.65	$P < 0.1$	$P > 0.05$

were administered in gelatin capsules. At 0.5, 1, 2, 4, 8, and 12 hr. after administration, blood samples were taken and analyzed in duplicate by the Bratton-Marshall procedure for free sulfonamide (6). Since several analyses for free and total sulfonamide were performed over a wide range of blood levels and no difference was observed, only the procedure for free sulfonamide was performed. These procedures were repeated at random among the five dogs 10 to 13 times with each particle size.

RESULTS AND DISCUSSION

The blood level data and results of statistical calculations 0.5, 1, 2, 4, 8, and 12 hr. after oral administration of sulfisoxazole to dogs are listed in Tables II–VII.

After an analysis of variance to see if particle size affects blood level, it was found that particle size significantly affects blood level at each of the testing times at the level of confidence stated in terms of P, the probability of error. Tests of significance of differences (t test) were performed for the means nearest to each other. Since the question here is whether one mean is either greater than or less than the other, a one-tailed test is appropriate (7). The level of significance is stated in terms of the probability of error.

The behavior of the blood level as a function of time for the three particle sizes studied is shown in Fig. 1. The outstanding features to be noted for the different particle size curves are: (*a*) the smaller particle, sample *A*, results in a faster and higher blood level initially, followed by a sharp drop; (*b*) as the particle size increases, the rise in blood level is less sharp, the peak is later, and the drop in blood level is slower.

As a check to see if the same proportion of the oral dose for each particle size is absorbed in the period of testing, the areas under each curve (see Fig. 1) were determined using a planimeter. Sam-

TABLE IV.—BLOOD LEVEL DATA AND RESULTS OF STATISTICAL CALCULATIONS 2 HR. AFTER ORAL ADMINISTRATION OF SULFISOXAZOLE TO DOGS

Sample Av. Diam. (d_{vs}) μ	*A* 1.7	*B* 39	*C* 95
Blood levels of repeated trials, mg. %	6.37	2.35	3.88
	6.69	5.70	2.75
	5.75	3.75	4.50
	8.50	4.45	0.70
	4.25	5.55	3.62
	3.20	1.05	1.05
	5.50	4.50	5.40
	5.00	5.50	2.75
	6.13	5.75	0.44
	5.60	3.10	3.90
	6.00	5.50	...
	...	4.70	...
	...	4.20	...
Mean blood level, mg. %	5.73	4.3	2.9
$F_{A,B,C}$ ratio	8.319	$P < 0.01$	...
$t_{A,B}$	2.074	$P = 0.025$	...
$t_{B,C}$	2.08	$P = 0.025$	...

TABLE V.—BLOOD LEVEL DATA AND RESULTS OF STATISTICAL CALCULATIONS 4 HR. AFTER ORAL ADMINISTRATION OF SULFISOXAZOLE TO DOGS

Sample Av. Diam. (d_{vs}) μ	*A* 1.7	*B* 39	*C* 95
Blood levels of repeated trials, mg. %	5.38	5.30	3.45
	4.75	3.84	3.05
	4.50	4.37	3.00
	5.22	3.60	2.75
	3.20	3.23	2.40
	4.30	3.81	3.30
	3.75	4.20	4.00
	4.25	4.70	4.00
	5.40	3.00	3.30
	4.50	3.46	3.25
	4.70	3.30	...
	...	3.62	...
	...	5.00	...
Mean blood level, mg. %	4.54	3.95	3.25
$F_{A,B,C}$ ratio	9.557	$P < 0.01$	...
$t_{A,B}$	4.79	$P < 0.0005$	...
$t_{B,C}$	11.75	$P < 0.0005$	...

TABLE VI.—BLOOD LEVEL DATA AND RESULTS OF STATISTICAL CALCULATIONS 8 HR. AFTER ORAL ADMINISTRATION OF SULFISOXAZOLE TO DOGS

Sample Av. Diam. (d_{vs}) μ	*A* 1.7	*B* 39	*C* 95
Blood levels of repeated trials, mg. %	1.47	1.60	2.70
	1.30	1.53	3.00
	0.98	1.45	2.70
	2.95	0.95	3.05
	1.40	1.25	2.90
	1.25	2.75	2.80
	1.75	2.75	2.40
	1.40	3.05	3.75
	1.40	1.75	3.00
	3.30	4.20	2.30
	3.10	3.75	...
	...	2.60	...
	...	3.00	...
Mean blood level, mg. %	1.84	2.35	2.86
$F_{A,B,C}$ ratio	4.053	$P < 0.05$	...
$t_{A,B}$	3.116	$P < 0.005$	...
$t_{B,C}$	5.269	$P < 0.0005$	...

TABLE VII.—BLOOD LEVEL DATA AND RESULTS OF STATISTICAL CALCULATIONS 12 HR. AFTER ORAL ADMINISTRATION OF SULFISOXAZOLE TO DOGS

Sample Av. Diam. (d_{vs}) μ	*A* 1.7	*B* 39	*C* 95
Blood levels of repeated trials, mg. %	0.70	0.36	1.40
	0.60	0.30	2.40
	0.62	0.40	3.05
	1.00	0.60	1.58
	0.59	1.65	2.40
	0.85	1.25	4.00
	0.64	0.98	1.75
	0.55	0.84	2.75
	0.70	2.75	2.00
	1.40	3.00	3.30
	1.75	0.55	...
	...	1.40	...
	...	2.85	...
Mean blood level, mg. %	0.85	1.30	2.46
$F_{A,B,C}$ ratio	11.743	$P < 0.01$	...
$t_{A,B}$	1.636	$P < 0.1$	$P > 0.05$
$t_{B,C}$	3.05	$P < 0.005$	...

ples *A*, *B*, and *C* yielded 1021, 968, and 955 integral units, respectively. Since the 12-hr. sample is not at zero for any particle size and since the level for sample *C* is greater than that of sample *B* and sample *B* is greater than sample *A*, this difference in area would probably be equalized as the blood level approaches zero. Analysis of samples in preliminary studies taken 16 and 20 hr. after administration indicated only traces of sulfisoxazole. The conclusion is that the same proportion of the dose of each particle size is absorbed into the blood. However, the rate or the time required for absorption of this proportion is different.

Figure 2 shows that a plot of the logarithm of the mean of the blood level as a function of particle diameter is linear. The greatest deviation of points from linearity occurred at 0.5 and 8 hr. after administration. The slope of each time constant line changes gradually from a negative value to a positive value as time increases. The effect of the particle size on blood level at a constant time can be expressed easily by an algebraic equation of the straight line form. Let m equal the blood level in milligram percentage, and P equal particle diameter in microns, then

$$\log m = aP + b \qquad \text{(Eq. 1)}$$

where a = slope = $d \log m/dP$ and b = the intercept on $\log m$ axis. For a given drug and time, a and b are constants.

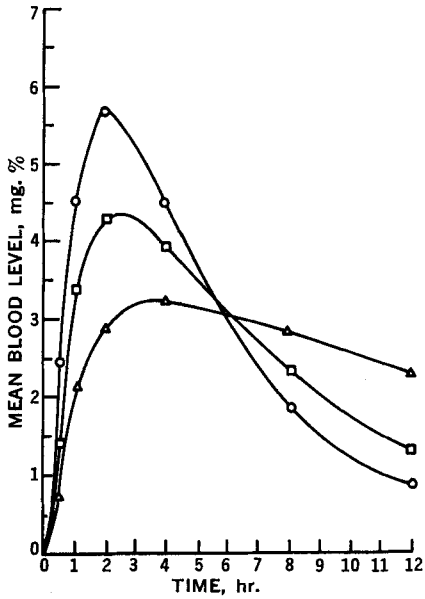

Fig. 1.—Blood level as a function of time and particle size after oral administration of sulfisoxazole to dogs. Key: ○, sample *A* (1.7 μ); □, sample *B* (39 μ); Δ, sample *C* (95 μ).

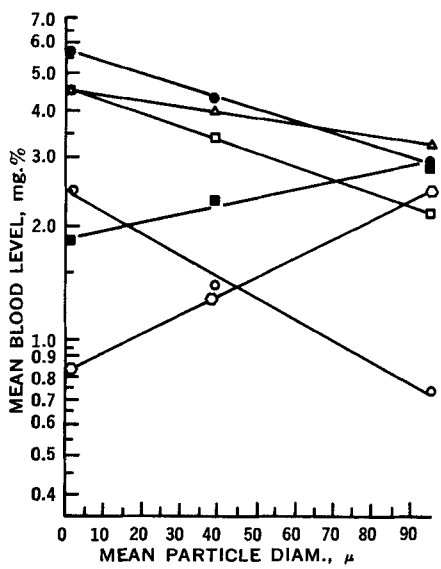

Fig. 2.—Blood level as a function of particle size at various times after administration of sulfisoxazole to dogs. Key: ○, 0.5 hr.; □, 1 hr.; ●, 2 hr.; Δ, 4 hr.; ■, 8 hr.; ○, 12 hr.

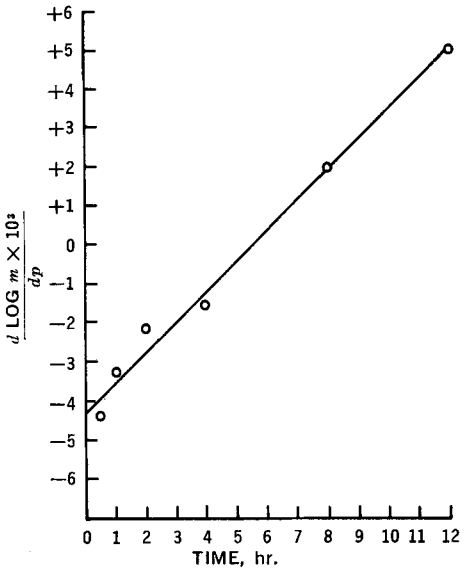

Fig. 3.—Rate of change of blood level due to particle size as a function of time after oral administration of sulfisoxazole to dogs. m, Mean blood level (milligrams per cent); P, mean particle diameter (μ).

To obtain the rate of change of blood level with particle size ($d \log m/dP$) as a function of time, a plot of $d \log m/dP$ times 10^3 *versus* T time in hours on a linear graph is also linear (see Fig. 3). The factor 10^3 was used for the convenience of plotting whole numbers. The equation for the line in Fig. 3 is

$$\frac{d \log m}{dP} \times 10^3 = a_1 T + b_1 \qquad \text{(Eq. 2)}$$

where $a_1 = 0.781$ = slope and $b_1 = -4.32$ = intercept.

Rearrangement and integration of Eq. 2 between limits yields

$$10^3 \cdot \int_{m_1}^{m_2} d \log m = \int_{P_1}^{P_2} dP\,(a_1 T + b_1) \qquad \text{(Eq. 3)}$$

$$10^3 \cdot \log \frac{m_2}{m_1} = (a_1 T + b_1)(P_2 - P_1) \qquad \text{(Eq. 4)}$$

If P_1, T, P_2, and m_1 are known, we can calculate m_2 with Eq. 4 and thus predict the blood level produced with another particle size, P_2, at a particular time, T, after oral administration. It can be said that the above equations hold for sulfisoxazole blood levels in dogs with the range of particle sizes and testing times used, *i.e.*, 1.7 to 95.0 μ and 0 to 12 hr. Furthermore, it is contended that this is the workable range of size, since with smaller particles other factors, such as surface energy, cause increasing difficulties in production and handling of the drug, and the advantage of further reduction decreases (4).

Kakemi *et al.* (3) studied the effect of particle size on the blood level of sulfaethylthiadiazole and derived a theoretical equation to calculate the blood level at time, T, after giving a definite dose of a definite particle size. Kakemi's work was based primarily on the measurement of dissolution rates, whereas the present study is based on measurement of particle sizes. Using their theoretical data of blood levels at various times with the three particle sizes and treating these data in the same manner as the data obtained in the present study with sulfisoxazole, it is found that the same type relationships hold true, *i.e.*, the log of the blood level *versus* particle diameter at the various testing times is linear, and the slopes of these lines plotted against time are linear. In Eq. 2 for sulfaethylthiadiazole, $a_1 = 0.69$ and $b_1 = -2.37$. By comparing the blood level data of sulfisoxazole to those theoretically calculated for a similar type drug, based on the dissolution rate of the drug, it is obvious that there is much similarity between the two sets of data. It can be concluded that the difference in blood levels obtained in the present study with sulfisoxazole particles of different sizes is due largely to the dissolution rate of the drug.

The possibility of controlling blood levels by controlling the particle size content of a single dose is evident after an examination of Fig. 1. Perhaps enough of the small crystals could be used to obtain a rapid therapeutic blood level, and a sufficient amount of larger crystals could be added to maintain the level over a period of 8 to 12 hr. Further studies are indicated in this area.

SUMMARY

The blood level of sulfisoxazole after oral administration to dogs is affected by the particle size of the drug. Statistical evaluations and probabilities of error are given. Equations to describe this change in the range of experimental testing were empirically derived. The data indicate that the proportion of the dose which is absorbed does not change appreciably; however, the rate at which this proportion is absorbed does change with a change in particle size.

The close correlation between this work and that of Kakemi (3) indicates that the effect of particle size on the blood level of sulfisoxazole in dogs is due largely to the dissolution of the drug in the gastrointestinal tract of the animal, assuming the rate constants for absorption, excretion, and metabolic alterations remain the same.

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