resentative of skin with regard to drug penetration, it nevertheless provides the requirements for the present comparative study where the following assumptions were made:

1. Skin transport is passive.
2. The skin parameters relevant to drug penetration are not influenced by the vehicle.
3. The vehicle composition does not change significantly during release.

Derivation of Eq. 5 from Higuchi's Eq. 1 (11) was possible because an inverse proportionality was observed between the diffusivity of drug in gels and the viscosity of the respective solvents. Such a relationship is justifiable considering the current theories on diffusion in diluted gels, as long as the viscosity of each solvent is accepted as the microscopic viscosity of the corresponding gel and self-aggregation or complexation of the drug is excluded. All of the gels studied in the present work (except, perhaps, the gel containing sorbitol) met the requirement of Eq. 5 that the molecular volume of the components of the liquid phase be substantially smaller than that of the diffusing drug. The cases of molecular volume of solvent approaching or exceeding that of the drug might deserve investigation for the applicability of Eq. 5 or 6.

It is hoped that the present study will help to rationalize the compounding of pharmaceutical gels and, furthermore, that the suggested principles and procedures for controlling the vehicle parameters will be useful in *in vivo* studies intended to assess the effects of excipients on skin permeability to drugs.

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Effect of Particle Size on Ophthalmic Bioavailability of Dexamethasone Suspensions in Rabbits

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Abstract □ Three suspensions of 0.1% [^{3}H]dexamethasone were prepared with mean particle sizes of 5.75, 11.5, and 22.0 μm. The suspensions were dosed topically to the right eyes of rabbits. Their bioavailability was compared by measuring aqueous humor and corneal levels over 5 hr. A statistically significant rank-order correlation was observed between increasing drug levels and decreasing particle size.

Keyphrases □ Dexamethasone—ophthalmic suspensions, bioavailability, effect of particle size, rabbits □ Bioavailability—dexamethasone, ophthalmic suspensions, effect of particle size, rabbits □ Ophthalmic preparations—dexamethasone suspensions, bioavailability, effect of particle size, rabbits

In the development of an aqueous suspension for topical use in the eye, the size of the suspended particles often is governed by their irritation potential. Although the particle size is an important consideration in irritation and comfort, the ophthalmic bioavailability of the drug can be influenced by particle size according to one or two possible *in vivo* mechanisms. If the particle induces tearing, rapid drainage of the instilled dose could reduce bioavailability (1, 2). In addition, the dissolution rate of particles residing in the conjunctival sac just after dose instillation should influence ophthalmic bioavailability.

Few published articles have indicated the importance of particle size in ophthalmic bioavailability. Sieg and Robinson (2) studied the bioavailability of a 0.1% ophthalmic fluorometholone suspension and demonstrated that the particles were retained within the conjunctival sac longer than the corresponding saturated solution and contributed significantly to the quantity of drug penetrating the cornea. By comparing the area under the aqueous humor–time curve for the 0.1% suspension and the saturated solution, ~78% of the area was determined to come from the retained particles. Therefore, the dissolution rate for poorly soluble drugs may influence the rate and extent of penetration into eye fluids.

The present study was conducted in rabbits to determine the importance of particle size in the ophthalmic bioavailability of a 0.1% dexamethasone suspension. Dexamethasone was chosen because of its clinical significance and because of the availability of tritiated dexa-

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methasone, which permits determination of low drug levels in aqueous humor.

EXPERIMENTAL

Materials—Two millicuries of tritiated dexamethasone dissolved in benzene–ethanol (9:1) was obtained commercially[1]. Nontritiated dexamethasone[2] was used as received. Other chemicals were reagent grade.

Preparation of Particle-Size Dexamethasone Fractions—Tritiated dexamethasone in benzene–ethanol (9:1) was evaporated to dryness using a nitrogen stream and was redissolved in 0.5 ml of absolute alcohol. An alcoholic solution containing 120 mg of nontritiated dexamethasone in 5.6 ml was heated to 65° and was added to 0.4 ml of the tritiated alcoholic dexamethasone solution. This supersaturated solution was transferred to a test tube, submerged in an ice bath at 0°, and sonified[3] for 10 min until crystallization occurred.

Crystals were harvested 2, 9, and 24 hr following sonification by removing 2 ml of the resulting suspension at each time increment and filtering through a 0.45-μm membrane[4]. The three fractions of dexamethasone crystals were air dried and removed carefully from the membrane. The dried microfine powder was assumed to contain tracer homogeneously throughout its crystalline structure.

Suspension Preparation—Five milliliters of 0.1% dexamethasone suspension was prepared separately from the three microfine powder fractions using a vehicle consisting of 0.9% NaCl, 0.05% polysorbate 80, and water for injection. Each suspension was sonified for 30 sec to achieve uniform dispersion of the particles and was stored in several small vials at 4° for <4 weeks.

Particle-Size Measurement—Several suspensions were formulated with narrow and nearly nonoverlapping particle-size distributions using an adapted sonification technique (3–5). The suspensions were formulated as simply as possible. Viscosity-inducing agents were omitted because their effect on particle-size growth is not known. Particle-size distributions of each suspension were performed with a research microscope[5]. The eye-piece micrometer had been calibrated previously using a stage micrometer.

At least 300 particles were measured for each determination of the particle-size distribution. Microscopic examinations confirmed that the particles were well dispersed with no evidence of agglomeration. At the end of the study, the suspensions were examined again for the mean particle size and distribution; no differences were noted as a function of storage conditions.

Procedure—New Zealand White rabbits without observable eye defects, 1.5–3.0 kg[6] and 2–3 months old, were used. Four to six rabbits were studied at each time interval for each preparation. The animals were randomized with respect to the formulation each received. A single 50-μl dose[7] was administered to the right eye. The lower lid was pulled away from the globe, and the drop was allowed to fall onto the cornea, with the excess falling into the conjunctival sac. The eyelid was returned carefully to its normal position.

The rabbits were sacrificed by rapid injection of 20 cm of air into the marginal ear vein at intervals of 0, 0.33, 0.67, 1, 1.67, 2.5, and 5 hr. Aqueous humor samples of about 0.15 ml were removed from the anterior chamber with a 27-gauge needle attached to a 1-ml tuberculin syringe and were added quantitatively to a counting vial[8] in volumes of 0.125–0.175 ml. Ten milliliters of scintillation fluid[9] was added to each vial.

Immediately following removal of the aqueous humor samples, 8-mm corneal buttons were removed with the use of a trephine[10], blotted once on each side, weighed, and immediately place in a counting vial containing 1.0 ml of tissue solubilizer[11]. The mixture was allowed to stand overnight to permit dissolution of the cornea. Ten milliliters of scintillation fluid[12] then was added to each vial. The vials were allowed to adapt to the dark overnight before counting. A minimum of 10,000 counts was obtained (2% counting error) for each sample. The internal standardization method (6) was used to determine counting efficiencies and, therefore, permitted the quantity of steroid[13] present in each sample to be calculated.

Table I—Particle Statistics for Dexamethasone Suspensions

Suspension	d_{lm}[a], μm	d_{vn}[b], μm	d_{vs}[c], μm	Number of Particles per Dose[d]	*CV* for Randomized System[e], %
A	2.19	3.50	6.75	1,114,200	0.095
B	8.80	9.90	11.50	49,200	0.450
C	18.93	20.4	22.0	5,630	1.3

[a] Length number diameter or arithmetic mean, $\Sigma nd/\Sigma n$. [b] Volume number diameter, $(\Sigma nd^3/\Sigma n)^{1/3}$. [c] Volume surface diameter, $\Sigma nd^3/\Sigma nd^2$. [d] Calculated from the particle number, $6/\pi d_{vn}^3 p$. [e] Calculated from $\sigma = \sqrt{N}$ (from Ref. 7).

RESULTS AND DISCUSSION

The specific activities of Suspensions A, B, and C were 10.3, 11.4, and 11.2 μCi/mg, respectively. The radiochemical purity of each tracer before and after preparation of each suspension as well as at the end of the study was approximately 98%[14]. A particle-size analysis was performed on each suspension prior to use (Fig. 1).

The particle statistics and diameters of each 0.1% dexamethasone suspension are shown in Table I. The mean volume surface diameter, d_{vs}, which emphasizes surface characteristics (7), was used to express the mean particle size. Measurements of 5.75, 11.5, and 22.0 μm were obtained for Suspensions A, B, and C, respectively. The d_{vs} parameter was considered appropriate since the GI absorption rate of poorly soluble, weakly acidic, and, particularly, neutral drugs has been shown to be dissolution rate limited (8–10). After sonification, crystallization continued. The

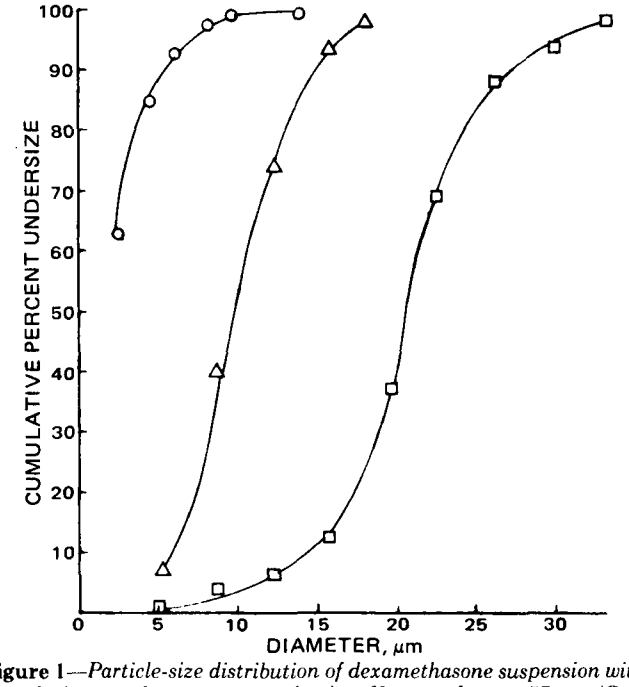

Figure 1—*Particle-size distribution of dexamethasone suspension with cumulative number percent undersize. Key:* ○, d_{vs} = *5.57 μm (Suspension A);* △, d_{vs} = *11.5 μm (Suspension B); and* □, d_{vs} = *22.0 μm (Suspension C).*

[1] Lot 1022-145, [6,7-^{3}H]dexamethasone, New England Nuclear, Boston, Mass.

[2] Lot 18C-01302, Sigma Chemical Co., St. Louis, Mo.

[3] Model W140 sonifier cell disruptor (Heat Systems-Ultrasonics, Plainview, N.Y.) equipped with a QBSP6 probe (Branson Sonic Power Co., Danbury, Conn.)

[4] Millipore.

[5] Bausch & Lomb Optical Co., Rochester, N.Y.

[6] Three rabbits weighed 3 kg, and the remainder weighed between 1.5 and 2.2 kg; data from the larger rabbits fell within the range of data from the smaller rabbits.

[7] Eppendorf pipet, Brinkmann Instruments, Westbury, NY 11590.

[8] Low-potassium glass vials with polyseal cone-lined screw caps, Packard Instrument Co., Downers Grove, IL 60515.

[9] Insta-gel, Packard Instrument Co., Downers Grove, IL 60515.

[10] Martinez disposable corneal trephine, 8 mm, Storz Surgical Instruments, St. Louis, MO 63122.

[11] Soluene 350, Packard Instrument Co., Downers Grove, IL 60515.

[12] Dimilume-30, Packard Instrument Co., Downers Grove, IL 60515.

[13] Quantification of total quantities of tracer does not permit differentiation between metabolites and intact drug. Therefore, *steroid,* as used here, should be considered apparent.

[14] Fifty microliters of each suspension was dissolved in an equal quantity of methanol and chromatographed with chloroform–methanol (9:1). The radiochemical purity was determined using a model 7230 radiochromatographic scanner, Packard Instrument Co., Downers Grove, IL 60515.

Table II—Dexamethasone Concentration in Aqueous Humor and Cornea following Topical Application of 0.1% Suspensions of Varying Mean Particle Size

		Micrograms per Milliliter of Aqueous Humor				Micrograms per Gram of Cornea			
t, hr	Number of Eyes	Suspension A[a]	Suspension B[b]	Suspension C[c]	p	Suspension A[a]	Suspension B[b]	Suspension C[c]	p
0.33	5	0.079 (0.016)[d]	0.061 (0.043)	0.024 (0.006)	<0.01[e]	5.8 (2.56)	4.11 (0.79)	2.50 (1.68)	<0.05
0.67	6	0.215 (0.151)	0.093 (0.013)	0.0696 (0.011)	<0.01[e]	5.01 (2.84)	3.46 (1.18)	2.05 (0.62)	<0.025[e]
1.0	6	0.249 (0.069)	0.103 (0.037)	0.064 (0.023)	<0.01	4.56 (1.14)	2.24 (0.84)	0.412 (0.81)	<0.01
1.67	5	0.193 (0.112)	0.139 (0.083)	0.082 (0.03)	NS	2.81 (2.47)	2.39 (1.22)	1.40 (0.93)	NS
2.5	5	0.106 (0.054)	0.098 (0.020)	0.037 (0.018)	<0.01[e]	1.44 (0.75)	1.44 (0.31)	0.519 (0.25)	<0.025
5.0	4	0.035 (0.091)	0.021 (0.009)	0.009 (0.003)	NS[e]	0.576 (0.36)	0.371 (0.19)	0.221 (0.09)	NS

[a] Mean particle size = 5.75 μm. [b] Mean particle size = 11.5 μm. [c] Mean particle size = 22.0 μm. [d] Number in parentheses represent 1 *SD*. [e] Determined by one-way analysis of variance for heterogeneous variances; all other data sets were determined by the standard one-way analysis of variance for homogeneous variances. NS = not significant or $p > 0.05$.

particle size increased after 2 hr. Relatively narrow distributions were achieved, but some overlap occurred. Removal of crystals from the filter after drying and subsequent handling produced some particle comminution that contributed to this overlap.

The averaged aqueous humor levels of dexamethasone obtained after dosing with the 0.1% preparations are shown in Fig. 2. A rank-order correlation is observed between increasing drug levels and decreasing particle size.

Because of the small sample size used at certain time intervals, a heterogeneous variance resulted. The assumption of homogeneous variances, which is required by a standard one-way analysis of variance, was tested with the use of Bartlett's test of homogeneity of variances (11). Of the 12 time points, five produced χ^2 distributions of $p < 0.05$, indicating heterogeneous variances for these data sets. The remaining seven data sets could be treated with the standard one-way analysis of variance (Table II).

When an equal number of cases (or eyes) is used in each treatment population, the effect of heterogeneous variances is less important in producing erroneous F distributions (12, 13). Nevertheless, an analysis of variance for heterogeneous variances was applied to the appropriate data sets (12, 13). This method differs from the standard method in that the means are weighted according to the reciprocal of the sample variance, and a corrected error mean square term takes the weighting into account (12). An analysis of variance indicated a statistically significant difference for the aqueous humor levels of dexamethasone at each sampling time except the 1.67- and 5-hr intervals. Table II lists the probability values calculated for the data.

Although dissolution rates were not determined for each formulation, the results agree with the expected findings. Suspension A, with its smaller particle size, would be expected to have a more rapid dissolution rate and, providing absorption is dissolution rate controlled, would show a higher C_{max} and an earlier time to peak than Suspension B or C. In comparing Suspensions B and C, Suspension B, which had a smaller particle size, showed a higher C_{max} but had an identical apparent time to peak. More frequent sampling may have permitted a separation of time to peak for Suspensions B and C. The area under the aqueous humor–time curves through the five sampling periods diminished for Suspensions A–C. This effect would occur if the residence time of particles in the conjunctival sac for each suspension was relatively short in comparison to their respective dissolution rates.

Figure 3 shows similar results obtained for corneal levels of drug with time. A statistical difference also was determined between Suspensions A–C for corneal drug levels for most sampling times (Table II). An apparent time to peak was observed at 0.33 hr; however, earlier sampling periods might have indicated an earlier time to peak. The area under each curve shows reduced absorption as the particle size increased.

Suspensions A and B, but not C, showed a parallel decline from 2.5 to 5 hr for the aqueous humor levels. For the corneal data, Suspensions A and C, but not B, showed a parallel decline. The reason for this observation may be related to each suspension; however, more data points in this region are needed to draw a firm conclusion.

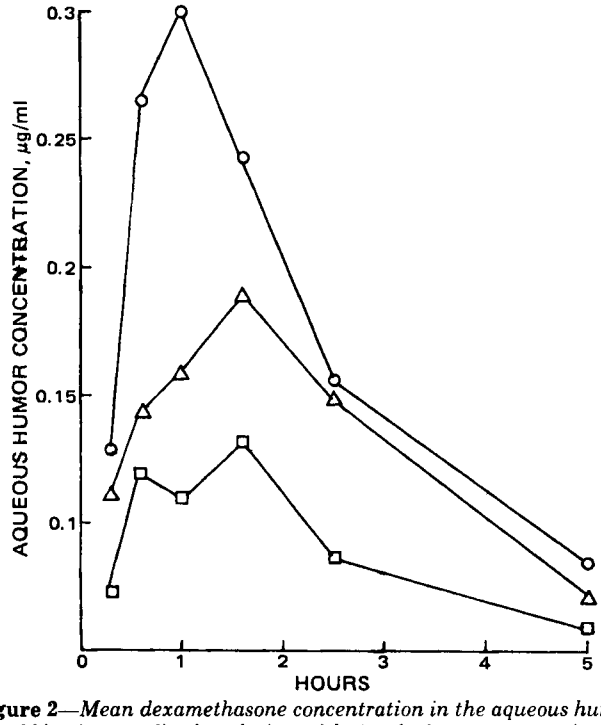

Figure 2—*Mean dexamethasone concentration in the aqueous humor of rabbits* (n = 4–6) *after dosing with 50 μl of 0.1% suspensions of varying particle size. Key:* ○, d_{vs} = *5.75 μm (Suspension A);* △, d_{vs} = *11.5 μm (Suspension B); and* □, d_{vs} = *22.0 μm (Suspension C).*

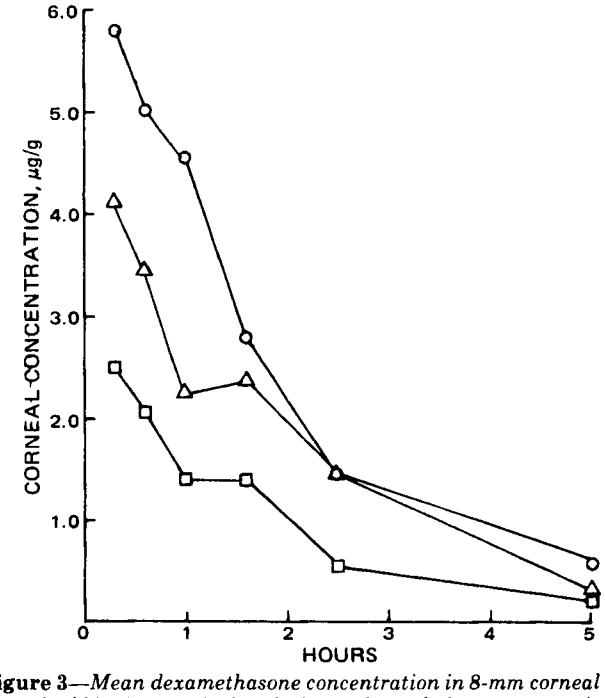

Figure 3—*Mean dexamethasone concentration in 8-mm corneal buttons of rabbits* (n = 4–6) *after dosing with 50 μl of 0.1% suspensions of varying particle size. Key:* ○, d_{vs} = *5.75 μm (Suspension A);* △, d_{vs} = *11.5 μm (Suspension B); and* □, d_{vs} = *22.0 μm (Suspension C).*

According to the data presented, Figs. 2 and 3 suggest that as the particles increase in size, the *in vivo* dissolution rate decreases such that the particles are removed from the conjunctival sac before dissolution is completed. Therefore, both the rate and the extent of penetration into aqueous humor are decreased.

It is possible that as particle size increases, a potential for discomfort is created. As a consequence, tearing could be induced and a progressively decreasing residence time might occur. No clear distinction has been made regarding an upper limit in particle size that would be considered comfortable and, therefore, would not induce tearing. Sieg and Robinson (2) stated that the particle size should be <10 μm to minimize particle irritation in the eye. However, shape and concentration are additional factors that make it difficult to select a specific particle size above which irritation or discomfort might result. From observing the animals during the study, there was no reason to believe that dexamethasone induced tearing at a concentration of 0.1% and an average size of 5.75–22 μm. Nevertheless, the present data do not rule out this possibility.

A potential source of variability between ophthalmic suspensions with different particle sizes could be differences in the amount of the administered dose. Table I lists the volume number diameter, d_{vn}, from which the number of particles per dose can be calculated. In low-strength suspensions such as 0.1% dexamethasone, as the drug particle size increases the number of particles per dose falls rapidly (*i.e.*, inversely with the cube of d_{vn}), potentially increasing the standard deviation of the drug concentration in a randomized dose. The last column in Table I illustrates this point.

Although a limited number of animals was used in the study, the results suggest that ophthalmic dexamethasone suspensions can be optimized for bioavailability by using suspensions with particles as small as possible. This approach would promote a rapid dissolution rate and reduce the chance of tearing and, therefore, would minimize rapid drainage as well as the variability in the quantity of dose administered.

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Pharmacokinetics and Bioavailability of Cimetidine in Humans

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Abstract □ Cimetidine given orally without food after an overnight fast produces a blood concentration curve with a pronounced second peak that does not appear after parenteral administration or when the drug is taken with food. The following interpretation of this kinetic phenomenon is proposed: 1. The drug cumulates in a tissue or organ that is well perfused in the first-pass transfer. 2. The hepatic parenchymal tissue and the bile phase are the most likely storage areas. 3. The high capacity of the cumulation may be due to the formation of conjugates or other modifications of the drug with a pronounced affinity for the hepatic–biliary system. 4. The rate of cumulation is much higher in the first-pass transfer than from the systemic circulation, possibly due to the difference in the drug concentrations and the conjugation rate. 5. The cumulation appears to occur by a competitive process. 6. Absorbed elements of food seem to compete in this process. 7. The second peak apparently is the result of a rapid release of drug and bioreversible drug compounds from the hepatic–biliary system with subsequent reabsorption. 8. This release may occur spontaneously but appears to be triggered by food intake. A pharmacokinetic model constructed according to this interpretation showed good agreement with data from oral, intravenous, and intramuscular administration. The special problems associated with the evaluation of bioavailability in the presence of reabsorption are discussed.

Keyphrases □ Cimetidine—pharmacokinetics, bioavailability, humans □ Pharmacokinetics—cimetidine, humans □ Bioavailability—cimetidine, humans

Blood levels of cimetidine in humans after oral dosing (1–8) and intravenous administration (2, 5, 6) have been analyzed with respect to the pharmacological response (2), influence of a meal on absorption (3), biliary distribution and secretion (4), and bioavailability (1, 5, 6). The drug shows unusual pharmacokinetic behavior in producing a significant secondary peak in the drug concentration profile after oral dosing on a fasting stomach but not after intravenous administration (5–7). No attempts have been made to describe the pharmacokinetics of the phenomenon.

A conventional two-exponential model has been applied (6), but such a model does not account for the secondary peaks. Bodemar *et al.* (8) stated that: "A second absorption peak could be explained by enterohepatic circulation of cimetidine, although a preliminary report by Spence *et al.* (4) seems almost to exclude this possibility." Considering the possibility of delayed absorption of some of the cimetidine or a varying absorption rate at different segments of the GI tract (6), the same authors (1) stated that: "Calculations from the present results indicate that the consideration from a hypothetical delayed absorption is as much as 50% of total *AUC* in some patients. Delayed absorption of this magnitude, however, is unlikely and this second peak following oral administration of cimetidine remains to be explained."

This study was intended to evaluate the pharmacokinetics of cimetidine and to explain its kinetic discrepancy using the data of Walkenstein *et al.* (5). It is proposed that the phenomenon can be described best in terms of discontinuous reabsorption. The special problems associated with the evaluation of bioavailability in the presence of reabsorption are discussed.

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