A DETERMINATION OF THE EFFECTIVE SURFACE AREA OF A SUBCUTANEOUSLY IMPLANTED SUSPENSION

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A DETERMINATION OF THE EFFECTIVE SURFACE AREA
OF A SUBCUTANEOUSLY IMPLANTED SUSPENSION

BY

PETER JAMES BLANDING

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
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ABSTRACT

The effective surface area of a parenteral drug in suspension, that which is exposed to body fluids at the injection site, is a major determinant of its in vivo absorption rate. Precise methods of determining the effective surface area without disturbing the in vivo system have not been developed.

A method to estimate the effective surface area of a subcutaneously injected suspension based on the urinary excretion of drug from solid disk implants of known surface area is presented. Standard curves for the mean surface area of from two to four subcutaneously implanted cylindrical disks of pure sulfadiazine versus cumulative urinary sulfadiazine excretion to 48 hours were developed for three test animals. By applying the urinary excretion data obtained following the subcutaneous injection of an aqueous suspension of sulfadiazine to the appropriate standard curve for area, a preliminary estimate of the apparent or effective in vivo surface area for the suspension formulation was obtained.

Improvements in the experimental methodology which would control certain biopharmaceutical factors related to parenteral drug absorption from subcutaneous sites, and increase the statistical significance of the surface area estimate are suggested.
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### LIST OF SYMBOLS

| Symbol | Definition |
|--------|------------|
| \( \varphi \) | Density |
| A | Area of disk at any time following implantation |
| \( \bar{A} \) | Mean surface area of implanted drug |
| \( A_i \) | Initial disk surface area |
| \( A_f \) | Final disk surface area |
| \( D^0 \) | Disk diameter at implantation |
| \( H^0 \) | Disk height at implantation |
| \( k, k_1-k_5 \) | Rate constants |
| \( \bar{R} \) | Mean absorption rate |
| \( \bar{R}/\bar{A} \) | Mean absorption rate per mean area |
| S | Amount of sulfadiazine distributed in body fluids |
| \( S_{ae} \) | Amount of acetylated sulfadiazine distributed in body fluids |
| \( S_{ae} \) | Cumulative amount of acetylated sulfadiazine excreted via the urine |
| Se | Amount of unmetabolized sulfadiazine excreted via the urine |
| Si | Amount of sulfadiazine at the absorption site |
| Su | Amount of unchanged and acetylated sulfadiazine distributed to an unknown body compartment |
| \( S_{ue} \) | Cumulative amount of free and metabolized sulfadiazine excreted by non-urinary route(s) |
| Sw | Specific surface |
| t, ti | Time |
| V | Disk volume |
| W | Weight of disk at any time after implantation |
| Wi | Initial disk weight |
| Wf | Final disk weight |
I. INTRODUCTION

Drug implants have been widely used in cancer, endocrine, nutrition, chronic toxicity and other studies where prolonged drug action is being investigated. The clinical use of solid implants is not without disadvantage, however. A surgical procedure is required for dosage administration or removal, which is usually less convenient to the physician and patient than an injection at the same site (1). Cosmetically, the suspension is unnoticed, whereas the solid dose form would be more likely to be noticed and would therefore be less acceptable. Parenteral dose forms are thus usually designed as suspensions rather than implants when prolonged action is desired.

The purpose of this project was to develop a preliminary method of determining the apparent or effective surface area of a subcutaneously injected suspension in vivo. While there is presently no published method to determine the effective surface area of a parenteral drug in suspension at the site of injection, the mathematical relationships (i.e., models, equations, rate constants and analog computer techniques) dealing with the absorption of certain solid implanted drugs of known geometric design have been investigated by Ballard and others (2-6). The application of certain specifically related principles derived from
these studies applied to systems of pure drug in aqueous suspension would be an extension of the classical work on the physical and biological properties of injectable procaine penicillin G suspensions published by Ober et al. (7) in 1958.

The accepted assumptions underlying the processes involved with drug absorption from the implantation site are: 1) that the absorption rate is in part proportional to the effective solid surface area exposed to the surrounding tissues and in part due to the intrinsic physical properties of the drug, i.e., solubility, pKa, diffusion layer pH and diffusion coefficient (2-4), 2) that the absorption or disappearance of drug from a solid implant or parenteral depot mimics a zero-order process which is dissolution rate limited, (i.e., where the rate-determining step is the dissolution of drug from the solid form) (8,9); 3) that the effective or apparent surface area of a depot whose geometry is ill-defined is less than the total true surface area of all the particles making up the suspension as compared to the area of all the particles as measured in vitro by gas adsorption techniques (10); and 4) that the amount of drug and metabolite excreted over time, in this case of sulfadiazine and its acetylated metabolite, is directly proportional to the amount absorbed (11).
If a drug is formulated into a compressed pellet of known geometric shape and dimension, which can be implanted and then removed before it is completely absorbed, the absorption rate can be estimated if certain quantitative information (e.g., changes in pellet dimensions with respect to time) is known. Accordingly, for a disk shaped implant, the weight, \(W\), at any time following implantation \((t)\) is (3):

\[
W = \frac{\pi \rho}{4} (D_0 - kt)^2 (H_0 - kt) \tag{Eq. 1}
\]

Where \(\rho\) = the apparent density, \(D_0\) is the initial diameter of the disk, \(H_0\) its initial height and \(k\) the absorption constant having units of length \(\times\) time\(^{-1}\).

The area, \(A\), of a disk at any time after implantation is (12):

\[
A = \pi \left[ (D_0 - kt)^2 + \frac{\pi}{2} (D_0 - kt)(H_0 - kt) \right] \tag{Eq. 2}
\]

In both these equations it is assumed that the shape of the implant is not distorted during the implantation time, and that \(D_0\) and \(H_0\) are both \(\geq kt\).

The absorption constant, \(k\), for a geometrically defined drug in pellet form at a particular absorption site in a given animal species can be determined using equation 1 if the pellet dimensions, density, initial and final weights, and implantation time are known. The absorption constant can then be used to aid in determining the probable absorption rates of other geometric forms, e.g., a
sphere, of the same drug under similar conditions.

One of the problems encountered in developing and evaluating test suspension formulations is that, upon injecting the dose, there is no way to predict the geometric shape of the drug depot in contact with the body fluids at the injection site. Once the vehicle has migrated from the suspension in situ, the suspended injection resembles the geometrically defined pellet with regard to all characteristics except shape. The shape of the implanted or injected suspension could theoretically vary from a flat or very thin sheet (high area/volume ratio) at one extreme, to a perfect sphere (minimum area/volume ratio) at the other. In practice, the actual shape is determined by such factors as the injection technique, dose formulation and the site of implantation, and is within these two extremes.

The "effective" surface area, that which is immediately exposed to the action of circulating biological fluids, is smaller than the true area of the sum total of all the drug particles in suspension. This effective area would be extremely difficult to determine in vivo without mechanically disturbing the system. Thus, one cannot use the mathematical equations presented previously to predict the suspension weight or area at any time after implantation because the exact shape of the depot
at the site is not known at any given time after the injection. Since the net absorption rate is presumed to be directly proportional to the effective area, which cannot be determined with certainty, absorption rate predictions cannot be made accurately. If the effective or apparent surface area of the suspension depot could be estimated, absorption rates for different formulations of the same drug in suspension would then allow the product formulator to select the most appropriate formulation with the desired characteristics.

In this study, an in vivo system of implanted drug in aqueous suspension was studied in order to develop a correlation between a physical property (effective surface area) and its biological parameter (absorption and urinary excretion) in the same test animal.

Accordingly, two, three and four solid cylindrical disks of pure sulfadiazine of known surface area and weight were implanted on separate occasions and correlated with the amount of drug and metabolite excreted over time in the urine. Ballard has shown (5) that if the cumulative amount of drug excreted in the urine after implantation is plotted against time, a line with progressively decreasing slope results, indicating that the excretion rate diminishes with time. This decrease
in rate should be associated with a reduced pellet surface area. The absorption rate of various numbers of implanted pellets per unit time can be determined by removing the pellets and weighing them. The amounts of unchanged sulfadiazine and its metabolites were measured according to a colorimetric assay procedure developed by Bratton and Marshall (13). Since the absorption rate of the implants is assumed to be proportional to the effective surface area exposed, a plot of the mean surface area of each trial of pellets versus the total amount of drug and metabolite excreted in 48 hours post pellet implantation should result in a linear relationship. This graph is, in effect, a standard curve for apparent or effective surface area. Following this procedure, an aqueous suspension of sulfadiazine of known weight, whose estimated surface area is between two and four pellets on the standard curve, was implanted subcutaneously. The cumulative excretion of the drug and metabolite was followed until the implanted dose was completely absorbed and excreted. The value for the cumulative amount of drug excreted 48 hours after implantation of the suspension can then be applied to the least squares regression line on the standard curve for area generated with the disk. Thus, the solid-equivalent or apparent mean surface area for the drug in suspension should be determined for the specific formulation in the test animal observed.
II. MATERIALS AND METHODS

Thin cylindrical disks of drug grade sulfadiazine\(^1\) without further purification were prepared. About 50 mg of the powder was compressed at 7158 kg/cm\(^2\)\(^2\) on a Carver Laboratory press\(^2\) modified to allow the use of standard tableting machine punches and dies. The prepared disks had a mean diameter of 0.6369 cm (0.6350 cm to 0.6398 cm) and a mean height of 0.1123 cm (0.0940 cm to 0.1341 cm) when measured by a micrometer. The disks were weighed on a standard analytical balance\(^3\) and were between 41.14 mg and 56.82 mg (mean = 48.93 mg) in weight. Thus, the disks had a calculated mean density of 1.37 g/cm\(^3\) prior to implantation.

The sulfadiazine powder used to prepare the solid disks was also used without modification to prepare the aqueous suspension for subcutaneous implantation. An independent microscopic analysis was performed to determine the average particle surface area and specific surface of the powder\(^4\) (See Appendix). The apparent

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1. Pfaltz and Bauer, Inc., 126-02 Northern Blvd., Flushing, New York 11358.
2. Carver Laboratory Press Model C, Fred S. Carver, Inc., subsidiary of Sterling, Inc., Fountain Blvd., Menomonee Falls, Wisconsin 53057
3. Type H-16, Metler Instrument Corp., Hightstown, New Jersey.
4. Courtesy, Dr. T. J. Rockett, Associate Professor of Materials and Chemical Engineering, College of Engineering, University of Rhode Island, Kingston, Rhode Island 02881
powder density estimated independently by pycnometer was \( 1.43 \text{ gm/cm}^3 \).

Quantities of twenty percent (w/w) sulfadiazine suspension were prepared as needed by adding 10.0 ml of normal saline by pipette to 2.5 gm of sulfadiazine powder accurately weighed. No binders, diluents, excipients or lubricants were added. The resultant suspension was shaken on a Burrell shaker\(^5\) at maximum rotation (10 degrees) for one hour.

On separate occasions, two, three, and four solid disks and a known weight and volume of 20\% (w/w) aqueous suspension of sulfadiazine were implanted into each of three male Sprague-Dawley rats, A-379 gm, B-375 gm, and C-359 gm (mean weight 371 gm) according to the method of Ballard and Nelson \(^2\). During the experiments the animals were fed standard laboratory chow once daily and water ad libitum. They were placed in separate stainless steel cages from which the urine samples and cage washings could easily be collected.

Immediately prior to a sulfadiazine disk implantation trial, the animal was lightly anesthetized with ether and a ventral midline incision of suitable length was made in the abdominal skin. The subcutaneous tissue surrounding

---

5. Burrell Corporation, 2223 Fifth Avenue, Pittsburgh, PA 15219.
the incision was teased apart to provide from two to four sites for the implantation of the disks as appropriate. Following this, the disks were implanted in two, three, or four corner sites, the implantation time was noted, and the incision was closed by suturing.

After the 48 hours urine sample collection, the animal was reanesthetized, the sutures were cut and the disks were located manually and removed by palpation without the aid of forceps. The time of disk removal was noted, the implantation site was resutured and the site was not reused. The mean implantation time for the nine disk implant trials was 48.31 (range 47.98-48.63) hours.

The extracted disks were placed briefly on a filter paper which had been previously wetted with 3% (v/v) hydrogen peroxide solution in order to remove any closely adhering tissue. The disks were then allowed to air dry for 24-48 hours prior to measurement of their final weights and dimensions.

The suspensions were deposited utilizing a similar procedure. Following light ether anesthesia, a subcutaneous injection of 0.125 ml of the suspension depot was made in the midventral abdominal wall using a previously tared glass syringe6 fitted with a 21 gauge 38 mm needle6. The

6. Becton Dickinson and Company, Box 183, Rutherford, New Jersey 07070.
time of implanting the depot was noted. To estimate
the amount of suspension deposited, the following
gravimetric technique was used. The tared syringe
containing a quantity of sulfadiazine suspension was
placed on its side (in order to assure that suspended
solid drug that settled would do so on the length of
the syringe wall) and weighed. An approximate 0.125
ml sample was quickly injected first into the animal;
the syringe was reweighed and a second 0.125 ml of the
sample was injected into a volumetric flask. The syringe
was once again weighed.

Thus, the weight of suspension injected into the
test animal could be determined by the difference be-
tween the initial weighing of the syringe and total
contents minus the second weighing. The third weighing
determined the weight of an equivalent volume of sus-
pension delivered to the flask which was later analyzed
to provide an additional estimate of the amount of
sulfadiazine injected into the test animal.

In order to assess sulfadiazine excretion, urine
samples with distilled water cage washings were collected
at approximately 12 hour intervals for 96 hours after
separate implantations of the two, three, and four disks.
Samples were collected at approximately 24 hour intervals
for 240 hours following subcutaneous injection of the
suspension. Because the sulfadiazine suspension remaining to be excreted 48 hours after injection could not be easily recovered from the site, it was necessary to collect urine samples for 10 days following subcutaneous administration. During both the disk and suspension experiments the animals were induced to void at the appropriate times by introducing 0.5 ml of ether into the cages. Repetition produced voiding if the initial attempt was unsuccessful.

Enough distilled water was added to each 12 or 24 hour sample with cage rinsings to bring the final volume to 400 ml which was then well-stirred. A 90-120 ml portion of this dilution was frozen (-17°C) for future assay.

Urine was assayed for total (total = free + acetylated) sulfadiazine according to the colorimetric assay procedure of Bratton and Marshall (13). Reagents for the determination of sulfadiazine in the urine included 4N hydrochloric acid7, 0.1% sodium nitrite8 which was freshly prepared daily, 0.5% ammonium sulfamate8, and 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride8 which was stored in a dark bottle in the refrigerator and prepared each week.

In this assay, protein-free urine is treated with nitrous acid to diazotize free sulfonamide. Excess nitrous

7. Allied Chemical Corp., Industrial Chemical Div., P.O. Box 6, Solvay, New York 13209.
8. Fisher Scientific Co., 711 Forbes Avenue, Pittsburgh, Pennsylvania 15219.
acid is destroyed with ammonium sulfamate and the diazo-
tized sulfonamide is coupled with N-naphthylethlenediamine
to form a stable red-violet color. The absorbance is known
to follow Beer's Law and is compared with stock standard
solutions of sulfadiazine at 545 nm using a Spectronic 20
colorimeter9 fitted with a constant voltage transformer9.

In this experiment previously frozen samples of urine,
which had been diluted to 400 ml, are thawed and further
diluted with distilled water as required. To ten milli-
liter portions of the dilution to be assayed is added
0.5 ml of 4 N hydrochloric acid. For determining total
sulfadiazine (free drug plus its acetylated metabolite),
the acidified sample is then placed in a boiling water
bath for one hour. One milliliter of 0.1% sodium nitrite
is added to diazotize the primary amine. After three
minutes, 1 ml of 0.5% ammonium sulfamate is added to
destroy any excess nitrous acid present. After two minutes,
1 ml of 0.1% N-(1-naphthyl)-ethlenediamine dihydrochloride
is then read at 545 nm against a distilled water blank.

Blank (drug-free) urine collected prior to drug
implantation or injection is treated and analyzed in the
same manner as samples containing sulfadiazine. Corrected
values for the absorbance of each sample are used to

9. Bausch & Lomb, Inc., Analytical Systems Division,
820 Linden Avenue, Rochester, New York 14625.
calculate the cumulative amounts of total sulfadiazine excreted in each 24 hour period.
III. RESULTS

Tables I and II give the measured height, diameter and weight of each of the sulfadiazine disks before and after implantation. From these data, the volume, area, and density were calculated. These values are also included in Tables I and II.

Tables III, IV, and V provide urinary excretion data for total sulfadiazine for rats A, B and C, respectively, at various time periods.

From the disk implant and urinary excretion data obtained, the mean absorption rates per mean area, $\bar{H}/\bar{A}$, and absorption constant, $k$, can be calculated for each implant trial. These values are given in Tables VI, VII, and VIII.

The values found by assay for the amount of total sulfonamide excreted in 48 hours versus the mean surface area of the implanted disks are given and also plotted, and the appropriate least squares regression line has been constructed, for each animal in Figures 1, 2, and 3.

The extrapolated mean surface area of the suspension (Tables VI, VII and VIII) was found by using the equation of the least squares line and the weight of total sulfadiazine assayed at 48 hours following suspension implantation.
| Pellet Number | Height (cm) | Diameter (cm) | Weight (mg) | Volume ($\text{cm}^3$) | Density ($\text{g/cm}^3$) | Area ($\text{cm}^2$) |
|---------------|-------------|---------------|-------------|----------------------|--------------------------|-------------------|
| 1.            | 0.1102      | 0.6372        | 47.76       | 0.03514              | 1.359                    | 0.8584            |
| 2.            | 1.190       | 0.6392        | 52.89       | 0.03819              | 1.385                    | 0.8076            |
| 3.            | 1.290       | 0.6383        | 56.82       | 0.04128              | 1.377                    | 0.8987            |
| 4.            | 1.263       | 0.6366        | 55.79       | 0.04020              | 1.388                    | 0.8892            |
| 5.            | 1.324       | 0.6350        | 55.30       | 0.04193              | 1.319                    | 0.8975            |
| 6.            | 1.066       | 0.6365        | 47.32       | 0.03392              | 1.395                    | 0.8495            |
| 7.            | 1.098       | 0.6371        | 48.10       | 0.03500              | 1.374                    | 0.8573            |
| 8.            | 1.019       | 0.6373        | 44.26       | 0.03251              | 1.362                    | 0.842             |
| 9.            | 1.041       | 0.6371        | 46.12       | 0.03319              | 1.39                     | 0.8450            |
| 10.           | 1.275       | 0.6373        | 55.77       | 0.04067              | 1.371                    | 0.8933            |
| 11.           | 1.251       | 0.6362        | 55.10       | 0.03977              | 1.386                    | 0.8858            |
| 12.           | 0.979       | 0.6360        | 41.71       | 0.0311               | 1.341                    | 0.831             |
| 13.           | 0.985       | 0.6362        | 44.32       | 0.03131              | 1.415                    | 0.8327            |
| 14.           | 1.042       | 0.6361        | 44.19       | 0.03113              | 1.335                    | 0.8438            |
| 15.           | 1.270       | 0.6387        | 54.30       | 0.04069              | 1.334                    | 0.8956            |
| 16.           | 1.183       | 0.6360        | 52.54       | 0.03758              | 1.398                    | 0.8718            |
| 17.           | 0.988       | 0.6370        | 41.61       | 0.03149              | 1.322                    | 0.8351            |
| 18.           | 1.115       | 0.6372        | 48.28       | 0.03556              | 1.358                    | 0.8610            |
| 19.           | 1.341       | 0.6350        | 58.19       | 0.04247              | 1.371                    | 0.9009            |
| 20.           | 1.158       | 0.6363        | 50.36       | 0.03682              | 1.368                    | 0.8675            |
| 21.           | 0.982       | 0.6390        | 42.77       | 0.03149              | 1.358                    | 0.8385            |
| 22.           | 0.940       | 0.6398        | 41.14       | 0.03022              | 1.361                    | 0.8319            |
| 23.           | 0.973       | 0.6360        | 41.29       | 0.03091              | 1.336                    | 0.8298            |
| 24.           | 1.215       | 0.6350        | 54.04       | 0.03848              | 1.404                    | 0.8758            |
| 25.           | 1.145       | 0.6355        | 50.43       | 0.03632              | 1.389                    | 0.863             |
| 26.           | 1.029       | 0.6393        | 44.63       | 0.03303              | 1.351                    | 0.8487            |
| 27.           | 1.052       | 0.6365        | 46.10       | 0.03347              | 1.377                    | 0.8467            |

Mean 0.1123 0.6369 48.93 0.0357 1.368 0.8592
### TABLE II

Data on Sulfadiazine Disks After Implantation

| Pellet Number | Height (cm) | Diameter (cm) | Weight (mg) | Volume (cm³) | Density (gm/cm³) | Area (cm²) |
|---------------|-------------|---------------|-------------|--------------|-----------------|------------|
| 1             | 0.1023      | 0.6320        | 42.15       | 0.03209      | 1.313           | 0.8305     |
| 2             | 1.131       | 6355          | 47.12       | 3587         | 1.313           | 8602       |
| 3             | 1.190       | 6279          | 48.26       | 3685         | 1.31            | 8540       |
| 4             | 1.179       | 6280          | 47.40       | 3652         | 1.298           | 8521       |
| 5             | 1.250       | 6291          | 50.35       | 3885         | 1.293           | 8687       |
| 6             | 1.040       | 6246          | 42.69       | 3187         | 1.34            | 8169       |
| 7             | 1.062       | 6305          | 45.17       | 3316         | 1.362           | 8348       |
| 8             | 0.857       | 6183          | 33.65       | 2573         | 1.308           | 767        |
| 9             | 0.816       | 6199          | 35.94       | 2463         | 1.459           | 7626       |
| 10            | 1.216       | 6311          | 49.16       | 3804         | 1.292           | 8667       |
| 11            | 1.181       | 6343          | 47.55       | 3732         | 1.274           | 8673       |
| 12            | 0.858       | 6255          | 32.57       | 2637         | 1.235           | 7832       |
| 13            | 0.932       | 6280          | 38.91       | 2887         | 1.348           | 8034       |
| 14            | 0.947       | 6277          | 37.64       | 2931         | 1.284           | 8057       |
| 15            | 1.178       | 6320          | 47.61       | 3696         | 1.288           | 8613       |
| 16            | 1.100       | 6301          | 46.86       | 3430         | 1.366           | 8414       |
| 17            | 0.893       | 6298          | 35.57       | 2782         | 1.279           | 7997       |
| 18            | 1.013       | 6310          | 42.41       | 3168         | 1.339           | 8262       |
| 19            | 1.288       | 6323          | 55.07       | 4044         | 1.352           | 8839       |
| 20            | 1.118       | 6322          | 45.07       | 351          | 1.284           | 8499       |
| 21            | 0.905       | 6294          | 35.88       | 2816         | 1.274           | 8012       |
| 22            | 0.896       | 6281          | 35.08       | 2776         | 1.264           | 7965       |
| 23            | 0.850       | 6311          | 35.12       | 2659         | 1.221           | 7942       |
| 24            | 1.146       | 6317          | 48.44       | 3592         | 1.343           | 8543       |
| 25            | 1.060       | 6317          | 44.62       | 3221         | 1.343           | 8372       |
| 26            | 0.961       | 6287          | 38.84       | 2983         | 1.302           | 8107       |
| 27            | 1.002       | 6317          | 41.09       | 314          | 1.333           | 8257       |

Mean 0.1040 0.6293 42.60 0.03236 1.315 0.828
### TABLE III

Cumulative Total Sulfadiazine Excretion in Mg After Implantation Rat-A

| Time (hr.) | Two Pellets | Three Pellets | Four Pellets | Suspension |
|------------|-------------|---------------|--------------|------------|
| 0-24       | 3.81        | 8.84          | 11.65        | 1.91       |
| 24-48      | 7.02        | 12.13         | 22.38        | 5.59       |
| 48-72      | 9.5         | 14.13         | 26.51        | 6.31       |
| 72-96      | 9.86        | 15.00         | 27.39        | 6.84       |
| 96-120     |             |               |              | 8.08       |
| 120-144    |             |               |              | 8.31       |
| 144-168    |             |               |              | 8.58       |
| 168-192    |             |               |              | 8.81       |
| 192-216    |             |               |              | 9.02       |
| 216-240    |             |               |              |            |
| Total      | 9.86        | 15.00         | 27.39        | 9.02       |
### TABLE IV

**Cumulative Total Sulfadiazine Excretion in Mg After Implantation**  
**Rat-B**

| Time (hr.) | Two Pellets | Three Pellets | Four Pellets | Suspension |
|------------|-------------|---------------|--------------|------------|
| 0-24       | 3.63        | 10.27         | 10.95        | 9.64       |
| 24-48      | 7.52        | 18.01         | 18.96        | 12.43      |
| 48-72      | 10.01       | 21.47         | 23.73        | 13.55      |
| 72-96      | 11.44       | 22.3          | 25.32        | 14.19      |
| 96-120     | -           | -             | -            | 14.61      |
| 120-144    | -           | -             | -            | 14.61      |
| 144-168    | -           | -             | -            | -          |
| 168-192    | -           | -             | -            | -          |
| 192-216    | -           | -             | -            | -          |
| 216-240    | -           | -             | -            | -          |
| Total      | 11.44       | 22.3          | 25.32        | 14.61      |
TABLE V
Cumulative Total Sulfadiazine Excretion in Mg After Implantation Rat-C

| Time (hr.) | Two Pellets | Three Pellets | Four Pellets | Suspension |
|------------|-------------|---------------|--------------|------------|
| 0-24       | 2.58        | 7.63          | 8.24         | 8.59       |
| 24-48      | 6.40        | 14.29         | 17.13        | 10.77      |
| 48-72      | 6.91        | 18.30         | 22.6         | 11.57      |
| 72-96      | -           | 20.40         | 24.13        | 12.11      |
| 96-120     |             |               |              | 12.39      |
| 120-144    |             |               |              | 12.75      |
| 144-168    |             |               |              |            |
| 168-192    |             |               |              |            |
| 192-216    |             |               |              |            |
| 216-240    |             |               |              |            |
| Total      | 6.91        | 20.40         | 24.13        | 12.75      |
### Table VI

**Sulfadiazine Implant Data - Rat A**

| Dimension or Property          | Mean of Two Pellets (#1,#2) | Mean of Three Pellets (#3-#5) | Mean of Four Pellets (#6-#9) | Suspension |
|-------------------------------|-----------------------------|-------------------------------|-------------------------------|------------|
|                               | Initial                     | Final                         | Initial                      | Final      | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| Height (H) cm                 | .1146                       | .1077                         | .1292                        | .1206      | .1056   | .0944 |
| Diameter (D) cm               | .6382                       | .6338                         | .6366                        | .6283      | .6370   | .6233 |
| Weight (W) gm                 | .05033                      | .04464                        | .05597                       | .04867     | .04645  | .03936|
| Estimated Area (A) cm<sup>2</sup> | .8696                      | .8454                         | .8951                        | .8583      | .8485   | .7953 |
| Estimated Volume (V) cm<sup>3</sup> | .0367                      | .0340                         | .0411                        | .0374      | .0337   | .0288 |
| Apparent Initial Density (\(\rho\)) gm/cm<sup>3</sup> | 1.37                        | 1.36                          | 1.38                         | 1.38       |        |      |
| Implantation Time (t) hr      | 48.37                       | 47.98                         | 48.30                        | 48.30      |        |      |
| Initial time of Implantation | 10:22 A.M.                  | 9:46 A.M.                     | 10:47 A.M.                   | 4:11 P.M.  |        |      |
| Total Weight Lost gm          | .01138                      | .02190                        | .02835                       | .02739     | .00684 |
| Wt SDZ Assayed @ 96 hr gm     | .00986                      | .01500                        | .02739                       | .00684     |        |      |
| \(\frac{R/A \times 10^{-4}}{4}\) gm/hr/cm<sup>2</sup> | 1.37                        | 1.74                          | 1.78                         | 1.78       |        |      |
| \(k \times 10\) length X time<sup>-1</sup> | 2.02                        | 2.58                          | 2.61                         | 3.29       |        |      |
| \(\frac{R/A/\rho}{k}\)        | .495                        | .496                          | .494                         | .494       |        |      |
| Mean Surface Area over 48 hr/pellet (\(A_i+A_f/2\)) cm<sup>2</sup> | .8575                      | .8767                         | .8219                        | .8219      |        |      |
| Total Apparent Surface Area over 48 hr (A) cm<sup>2</sup> | 1.72                        | 2.63                          | 3.29                         | 1.75       |        |      |
TABLE VII  
Sulfadiazine Implant Data - Rat B

| Dimension or Property | Mean of Two Pellets (#10, #11) | Mean of Three Pellets (#12-#14) | Mean of Four Pellets (#15-#18) | Suspension |
|-----------------------|---------------------------------|---------------------------------|---------------------------------|------------|
|                       | Initial            | Final          | Initial            | Final          | Initial            | Final          |                 |
| Height (H)            | cm                 | .1263          | .1199          | .1002          | .0912          | .1139          | .1046          |
| Diameter (D)          | cm                 | .6368          | .6327          | .6361          | .6271          | .6372          | .6307          |
| Weight (W)            | gm                 | .05544         | .04836         | .04341         | .03637         | .04918         | .04361         |
| Estimated Area (A)    | cm²                | .8895          | .8670          | .8358          | .7974          | .8659          | .8322          |
| Estimated Volume (V)  | cm³                | .0402          | .0377          | .0318          | .0282          | .0363          | .0327          |
| Apparent Initial      | Density (ρ)        | gm/cm³         | 1.38            | 1.36            | 1.35            |                 |
| Implantation Time (t) | hr                 | 48.30          | 48.32          | 48.25          |                 |                 |
| Initial time of Implantation |            | 9:33 A.M.      | 2:17 P.M.      | 12:20 P.M.     | 2:55 P.M.      |                 |
| Total Weight Lost     | gm (Wt-Wf)         | .01146         | .02110         | .02428         |                 |                 |
| Wt SDZ Assayed @ 96 hr gm |            | .01144         | .02230         | .02532         | .01419         |                 |
| R/A X 10⁻⁴            | gm/hr/cm²         | 1.67           | 1.78           | 1.48           |                 |                 |
| k X 10⁻⁴              | length X time⁻¹    | 2.46           | 2.63           | 2.21           |                 |                 |
| R/A/ρ                 | k                 | .492           | .498           | .496           |                 |                 |
| Mean Surface Area over 48 hr/pellet (Ai+Af/2) cm² | .8783 | .8106 | .8491 |
| Total Apparent Surface Area over 48 hr (A) cm² | 1.76 | 2.45 | 3.40 | 2.27 |
| Table VIII: Sulfadiazine Implant Data – Rat C |
|------------------------------------------------|
| Mean of Two Pellets (#19-#20) | Mean of Three Pellets (#21-#23) | Mean of Four Pellets (#24-#27) | Suspension |
|---------------------------------|---------------------------------|---------------------------------|------------|
| **Dimension or Property**       | **Initial** | **Final** | **Initial** | **Final** | **Initial** | **Final** | **Wt SDZ Assayed @ 96 hr gm** |
| Height (H) cm                   | 0.1250      | 0.1203    | 0.0965      | 0.0884    | 0.1110      | 0.1042    |
| Diameter (D) cm                 | 0.6357      | 0.6323    | 0.6383      | 0.6295    | 0.6366      | 0.6310    |
| Weight (W) gm                   | 0.05428     | 0.05007   | 0.04173     | 0.03536   | 0.04880     | 0.04325   |
| Estimated Area (A) cm²          | 0.8842      | 0.8669    | 0.8334      | 0.7971    | 0.8585      | 0.8319    |
| Estimated Volume (V) cm³        | 0.0396      | 0.0378    | 0.0309      | 0.0275    | 0.0353      | 0.0326    |
| Apparent Initial Density (ρ) gm/cm³ | 1.37      | 1.35      | 1.38      |           |           |           |
| Implantation Time (t) hr        | 48.63       | 48.32     | 48.35      |           |           |           |
| Initial time of Implantation    | 9:46 A.M.   | 2:36 P.M. | 12:45 P.M. | 2:55 P.M. |           |           |
| Total Weight Lost gm            | 0.00842     | 0.01912   | 0.02221    |           |           |           |
| Wt SDZ Assayed @ 96 hr gm       | 0.00691*    | 0.0204    | 0.02413    | 0.01211   |           |           |
| $\frac{R/A}{k} \times 10^{-4}$ gm/hr/cm² | 0.989     | 1.62      | 1.36      |           |           |           |
| $k \times 10^{-4}$ length X time⁻¹ | 1.46       | 2.41      | 1.99      |           |           |           |
| $\frac{R/A}{\rho} \times k$     | 0.494       | 0.498     | 0.495     |           |           |           |
| Mean Surface Area over 48 hr/pellet (A₁+Af/2) cm² | 0.8755     | 0.8153    | 0.8452     |           |           |           |
| Total Apparent Surface Area over 48 hr (A) cm² | 1.75        | 2.45      | 3.38      | 2.27      |           |           |

* Cumulative value @ 72 hrs; no 96 hr sample
FIGURE 1. STANDARD CURVE FOR MEAN SURFACE AREA FOR RAT A

| No. Pellets | 2 (●) | 3 (▲) | 4 (■) | Suspension (▲) |
|-------------|-------|-------|-------|---------------|
| $\bar{A}$   | 1.72  | 2.63  | 3.29  | 1.75 (est.)   |

Mg. total SDZ 7.02 12.13 22.38 5.59
Excreted to 48 hours

$m = 0.0968$
$b = 1.207$
$r^2 = 0.9228$
$t(n-2) = 3.445$
FIGURE 2. STANDARD CURVE FOR MEAN SURFACE AREA FOR RAT B

m = .1113
b = .8868
r² = .7356
t(n-2) = 1.6683

| No. Pellets | 2(C) | 3(▲) | 4(■) | Suspension(★) |
|-------------|------|------|------|---------------|
| A           | 1.76 | 2.45 | 3.40 | 2.27 (est.)   |
| Mg. total SDZ | 7.52 | 18.01 | 18.96 | 12.43 |

No. pellets excreted to 48 hours (mLilligrams)
FIGURE 3. STANDARD CURVE FOR MEAN SURFACE AREA FOR RAT C

\[
m = 0.1383 \\
b = 0.7827 \\
r^2 = 0.885 \\
t(n-2) = 2.7686
\]

| No. Pellets | 2(●) | 3(▲) | 4(■) | Suspension(●) |
|-------------|------|------|------|---------------|
| \( \bar{A} \) | 1.75 | 2.45 | 3.38 | 2.27 (est.) |

Mg. total SDZ 6.4 14.29 17.13 10.77
excreted to 48 hours

TOTAL SULFADIAZINE EXCRETED TO 48 HOURS (MILLIGRAMS)
IV. DISCUSSION

The quantitative aspects of subcutaneous drug absorption and the physiochemical factors which affect this process have been reviewed by Schou (14), Ballard (2,15), and Ritschel (16). The evaluation of parenteral suspended or solid implanted dose formulations depends in part upon the determination of the surface area of the solid particles of the depot exposed to the surrounding tissue and fluids once the suspension vehicle has migrated from the injection site (15). The influence of crystalline particle size on surface area and on drug absorption was demonstrated in 1955 by Foglia (17). He showed that by increasing the surface area of parahydroxypropiophenone (PHP) through a particle size reduction from $10,000 \mu^3$ to $2000 \mu^3$, pharmacological activity could be produced in rats in parenteral doses of 0.05 mg where no activity was demonstrated in doses as high as 120 mg using the larger particle size.

In 1958, Ober and co-workers (7) correlated the rheological and physical properties of intramuscularly injected aqueous suspensions of procaine penicillin G with injectability and clinical response in rabbits. In addition to specifying the optimum ranges for specific surface and particle size distribution of the antibiotic powder and structural breakdown point of the suspension formulation, the investigators used a 2% gelatin gel as a model for
screening test preparations. They also demonstrated the formation of a spherical depot or "thixotropic pellet" at the intramuscular injection site.

Presently, various methods are commonly used to assess the absorption rates of drugs from intramuscular and subcutaneous sites. Monitoring of the pharmacological response of certain drugs or serum level determinations of drugs and their biologically active metabolites are often used in this regard. In 1969, Baldridge (18) recommended the assessment of urinary drug and/or metabolite concentrations as a simple procedure to assess drug absorption over time. The absorption rate of sulfadiazine from pellet implants and subcutaneous injections is readily determined via the urinary excretion method. The excretion of sulfadiazine and its acetylated metabolite by the rat occurs primarily via the urine according to a first order kinetic process.

A pharmacokinetic model for the absorption, distribution, metabolism, and excretion of sulfadiazine in rats has been presented by Ballard and Goyan (6):

![Pharmacokinetic model diagram]

\[ Sa \xrightarrow{k_1} Sae \xleftarrow{k_2} S \xrightarrow{k_3} Se \xleftarrow{k_4} Sue \xrightarrow{k_5} Su \]

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In this model, Si is the amount of drug in the disk or injection at the implantation or injection site, respectively, S is the amount of free drug distributed in body fluids of the animal, Se is the cumulative amount of free drug excreted unchanged in the urine, Sa is the amount of acetylated drug in the fluids of distribution in the animal, and Sae is the cumulative amount of acetylated drug excreted into the urine up to any time. Su and Sue are, respectively, the amount of sulfadiazine and/or its metabolite(s) distributed to a hypothetical unknown compartment, and the cumulative amount excreted to any time by a non-urinary route.

The rate constant, k, is a mean absorption constant having dimensions of length x time$^{-1}$. The rate constants, $k_1$, $k_2$ and $k_3$ are first order rate constants for acetylation, urinary excretion of acetylated and urinary excretion of free drug, respectively, in units of time$^{-1}$. The constant $k_4$ is the first order constant for the elimination of free drug by a route other than the urinary one, or is the formation constant of an undetected metabolite, and $k_5$ is the first order constant for the elimination of that metabolite by some route, if appropriate.

The mean constant, k, for each animal was estimated from the following equation by the method of successive approximations using data on the initial and final mean weights, and initial mean disk heights, diameters, densities,
and total implantation time, \( t \) (6):

\[
Si = \frac{\pi \varphi}{4} \left( D^0 - kt \right)^2 \left( H^0 - kt \right)
\]

\( Si \) = amount (weight) of drug

\( \varphi \) = apparent density of implanted disks

\( D^0 \) = initial (mean) diameter of disks

\( H^0 \) = initial (mean) height of disks

\( t \) = implantation time (hrs)

The mean absorption rate per mean area, \( \bar{R}/\bar{A} \), per disk, was estimated from the following equation:

\[
\bar{R}/\bar{A} \text{ per disk} = \frac{(W_i - W_f)/t_i}{(A_i + A_f)/2}
\]

where \( W_i \) and \( W_f \) are the initial and final mean disk weights for each separate implantation trial, \( t_i \) is the implantation time and \( A_i \) and \( A_f \) are, respectively, the initial and final mean areas of the implanted disks.

Values for the absorption constants, \( k \), and the mean absorption rates per mean surface area, \( \bar{R}/\bar{A} \), which were obtained for 2, 3, and 4 implanted disks of sulfadiazine in

---

NOTE: For cylindrical disks, the mean absorption rate, \( \bar{R}/\bar{A} \), absorption constant, \( k \), and pellet density, \( \varphi \), are theoretically related in the following manner:

\[
\frac{R/A}{\varphi} = \frac{k}{2}
\]

Thus, \( \bar{R}/\bar{A}/\varphi/k \) should equal 0.5. Values closely approximating 0.5 using the above equation were calculated from the appropriate data (Tables VI-VIII).
rats A, B and C are generally similar to those previously reported in studies (2,5) utilizing a similar experimental design.

As a first approximation, the mean absorption rate of a subcutaneous implant at any time is considered to be proportional to the surface area exposed to body fluids at the implantation site (2). When the in vivo absorption rate of sulfadiazine from implanted disks is approximated by urinary excretion of sulfadiazine and its metabolite unexpected variations in the results may occur. These may depend partly upon the influence of certain biopharmaceutical and physiological factors inherent to the model and partly to the experimental design.

The pH of body fluids (i.e., urine and plasma) is known to affect the excretion rate and plasma half-lives of drugs which are weak acids and bases. Sulfadiazine, an organic weak acid, has a pKa' of 6.28 at 28°C (12). According to the pH partition hypothesis (19), a large portion of sulfadiazine would exist in the ionized form if the urine were previously rendered alkaline (pH 8) with sodium bicarbonate. The urinary excretion rate of sulfadiazine would be increased due to a reduction in renal tubular reabsorption. In this experiment the pH of the urine was not controlled.

The time of urine sample collections for each separate disk and injection trial was dependent upon the time of disk
implantation or suspension injection. The time of implantation and injection varied from 9:33 A.M. to 4:11 P.M. Dettli and Spring (20) have shown that urinary pH variations in man over a 24 hour period may be of such magnitude as to result in significant alterations in urinary sulfonamide excretion rates. It is possible that urine pH variations could have affected sulfadiazine excretion rates for different implant trials in the present experiment. Consecutive pellet implantation and subcutaneous injection trials should be performed and urine samples collected at roughly the same time of day. Additionally, the urine pH of each sample should be monitored to detect significant variations that might lead to changes in drug excretion rates.

Body movement has been shown to affect the absorption of drugs from pellets. Ballard (21) reported a statistically significant increase in the absorption of procaine penicillin G implants from rats subjected to exercise. Theoretically, increased body movement can result in increased drug dissolution due to a stirring effect. In the present experiment, body movement was not controlled.

At different sample times, voiding may not have been complete, and therefore some drug may have remained in the bladder. Incomplete bladder emptying at the 48 hour collection of this experiment would have resulted in inappropriately low cumulative sulfadiazine excretion. The problem
of incomplete voiding is lessened somewhat when data are collected over long time intervals (6). Also, in this experiment, water was continuously available to the animals. Baldridge (18) has recommended administering water just prior to drug dosing and routinely thereafter to maintain urinary flow rate and enhance drug excretion.

Significant migration of the implanted disks occurred during the experiment. In a fourth experimental animal, during one of the disk implant removal procedures, two adjacent disks were discovered to be overlapping. It was impossible to determine either the initial time of this occurrence or the resultant decrease in total implant surface area. Hence, a data point on the standard curve for this animal was unobtainable and, since time did not permit repetition of the particular trial, previous data were invalidated.

In a recent report by Kent (22) wherein six Delman-dinone acetate pellets of approximately 28 mg were implanted subcutaneously in rats, the author utilized separate implantation sites for each disk. When more than one pellet is to be implanted, it would be advantageous to make separate incisions both to facilitate identification and to minimize implant migration.

Since only three data points were determined for the surface area versus urinary sulfadiazine excretion plots,
it was not possible to perform standard statistical analysis on the least squares curve obtained. Values for total sulfadiazine excretion in animals B and C for the three and four disk trials are very close together considering the difference in mean implant surface areas exposed (Figures 2,3). Unfortunately, it cannot be determined whether urinary sulfadiazine excretion to 48 hours was inappropriately high during the trial with three implanted disks, or if excretion during the trial with four disks was inappropriately low. Urinary excretion values would be higher than expected if residual sulfadiazine from the immediately previous implantation trial was present. Had time permitted, a better fit of the least squares curve could have been obtained if another implantation trial utilizing a single 50 mg disk of sulfadiazine had been performed. Additional data points would also be obtainable if disks of other diameters could have been prepared. Various combinations of such disks would permit additional data points on the standard curve which were intermediate in surface area to those obtained by combining single disks of the same dimensions. The appropriate number of trials could then be made until the necessary degree of statistical significance was attained.

Another problem area within the experimental methodology occurred with regard to the injection of the sulfadiazine suspension. While the subcutaneous injection was being made
into animal A, an unknown volume of suspension leaked from the injection site. Thus, while the precise volume and weight of suspension injected into animal A could not be determined, it was significantly less than the 0.125 ml injected into the other two animals. The leaked portion was collected from the site on filter paper for later analysis in order to estimate the amount of suspension injected, however, a precise determination could not be made. The amount of total sulfadiazine excreted up to 48 hours following the partial injection of the suspension in rat A was considerably less (about 5.6 mg) when compared with 12.4 mg and 10.8 mg excreted over the same time interval by rats B and C, respectively. This difference was reflected in the mean surface area estimates which for rat A (1.78 cm$^2$) was 26% less than for rat B (2.27 cm$^2$) and rat C (2.27 cm$^2$). The mean surface area determination for rat A is further suspect since it occurs outside the boundaries of the experimental data for rat A used to construct the standard curve for surface area. Extrapolation of the mean surface area of the suspension injected into animal A is possible only with the assumption that the standard curve for rat A is linear in the region below the data point corresponding to a cumulative total sulfadiazine excretion of about 7 mg.

Despite the above factors which theoretically or actually affected the urinary excretion of sulfadiazine,
values for the cumulative amount of drug eliminated at the end of 48 hours provide the best approximation of a standard curve for surface area. The majority of the effect on sulfadiazine urinary excretion can be related to the surface area of the disks or injections exposed to the animal body fluids at the site of implantation.

The results obtained indicate that a preliminary estimate of the effective surface area of 0.125 milliliters of the 20% w/w sulfadiazine suspension prepared would be about 2 cm², or an area approximated by from two to three 50 mg cylindrical disks of pure sulfadiazine, each with an average surface area of 0.86 square centimeter.

The approximate weight of sulfadiazine suspension injected into animals A and B was 96.18 and 92.72 mg, respectively. This weight corresponds to 19.23 and 18.54 mg of sulfadiazine powder. The surface area per milligram of powder was estimated via quantitative microscopy to be 8.933 square centimeters per milligram (See Appendix). The calculated total initial surface area of the powder in aqueous suspension using this method is 8.933 cm²/mg times the appropriate weight of powder injected, or 171.78 cm² for rat A and 165.61 cm² for rat B. Thus, the estimated effective surface area value of 2.27 cm² determined for both animals using the proposed in vivo method is less than 2% of the total surface area of the drug crystals in suspension initially injected as calculated microscopically.
V. SUMMARY

1. Thin cylindrical disks of pure sulfadiazine of known surface area and weight were implanted into three test animals. Total urinary sulfadiazine excretion was followed to assess the absorption rates of the sulfonamide from subcutaneous tissue.

2. Standard curves for mean disk surface area versus cumulative sulfadiazine excretion to 48 hours were prepared. A quantity of sulfadiazine in aqueous suspension was then injected subcutaneously and its cumulative urinary total sulfadiazine excretion to 48 hours used to obtain a preliminary estimate from the standard curve of the effective in vivo surface area of the injected suspension in the test animal observed.

3. The reliability of the proposed method to accurately estimate the effective mean suspension surface area cannot be established from the present data. However, the validity of the data could be increased by modifying the experimental method to: 1) decrease the influence of the many physiochemical factors which combine to influence the rates of drug absorption and excretion, and 2) increase the statistical significance of the data obtained.
VI. APPENDIX

**Determination of the Specific Surface of the Sulfadiazine Powder Used in the Preparation of Implanted Disks and Suspension**

The specific surface, $(S_w)$, the surface area per unit weight of a substance, was determined for the drug grade sulfadiazine powder by micrometric analysis. Gross photomicroscopic observation of the 20% aqueous suspension of the powder revealed uniformly suspended needle-shaped crystals. Direct visual microscopic analysis of the dry powder in Cargille immersion oil revealed needle-shaped crystals uniform in size with an occasional large particle. One hundred particles were selected at random and their lengths and widths determined. It was assumed that the height of each crystal was equal to its width. The powder density was estimated by pycnometer to be $1.43 \text{ gm/cm}^3$. A summary of the properties of the sulfadiazine powder based upon the analysis of 100 randomly selected particles is as follows:

| Property                              | Value                      |
|---------------------------------------|----------------------------|
| Average particle length               | $1.086 \times 10^{-3}\text{cm}$ |
| Average particle width                | $3.655 \times 10^{-4}\text{cm}$ |
| Average particle height               | $3.655 \times 10^{-4}\text{cm}$ |
| Density                               | $1.43 \text{ gm/cm}^3$      |
| Particle volume                       | $1.451 \times 10^{-10}\text{cm}^3$ |
| Weight per particle                   | $2.076 \times 10^{-10}\text{gm}$ |
| Particles per gram                    | $4.817 \times 10^{9}$       |
| Surface area per particle             | $1.8546 \times 10^{-6}\text{cm}^2$ |
| Surface area per gram powder          | $8933 \text{ cm}^2/\text{gm}$ |
| Surface area per milligram of powder  | $8.933 \text{ cm}^2/\text{mg}$ |
VII. LITERATURE CITED

(1) J. A. Monteleone, Pediatrics, 43, 294(1969).
(2) B. E. Ballard and E. Nelson, J. Pharmacol. Exp. Ther., 135, 120(1962).
(3) B. E. Ballard and E. Nelson, J. Pharm. Sci., 51, 915(1962); 53, 1414(1964).
(4) B. E. Ballard and E. Nelson, Am. J. Vet. Res., 23, 678(1962).
(5) B. E. Ballard and E. Nelson, J. Pharm. Sci., 52, 301(1963).
(6) B. E. Ballard and J. E. Goyan, Med. Bio. Engng., 4, 483(1966).
(7) S. S. Ober, H. C. Vincent, D. E. Simon, and K. J. Frederick, J. Am. Pharm. Ass., 47, 667(1958).
(8) C. J. Eastland, J. Pharm. Pharmacol., 3, 942(1951).
(9) J. G. Wagner, J. Pharm. Sci., 50, 359(1961).
(10) P. H. Emmett, in "Catalysis," P. H. Emmett, Ed., Reinhold, New York, N.Y., 1954, pp31-74.
(11) E. R. Garrett, J. Pharmacokin. Biopharm., 1, 341 (1973).
(12) B. E. Ballard, Doctor of Philosophy Dissertation, University of California, Ca., 1961.
(13) C. Bratton and E. K. Marshall, J. Biol Chem., 128, 537(1939).
(14) J. Schou, Pharmacol. Rev., 13, 441(1961).
(15) B. E. Ballard, J. Pharm. Sci., 57, 357(1968).
(16) W. A. Ritschel, in "Drug Design," E. Ariens, Ed., Academic Press, New York, N.Y., 1973, pp. 75-92.
(17) V. C. Foglia, J. C. Penhos, and E. Montuori, Endocrinology, 57, 559(1955).
(18) J. L. Baldridge, Paren. Drug. Ass. Bull., 23, 40 (1969).

(19) W. A. Ritschel, in "Perspectives in Clinical Pharmacy," 1st ed., D. E. Francke and H. A. Whitney, Jr., Eds., Drug Intelligence Publications, Hamilton, Illinois, 1972, p. 325.

(20) L. Dettli and P. Spring, Helv. Med. Acta., 33, 291 (1967).

(21) B. E. Ballard, J. Pharm. Sci., 55, 515 (1966).

(22) J. A. Kent, J. Pharm. Sci., 55, 89 (1976).
VIII. REFERENCES

Baldridge, J. L. 1969. Application of urinary excretion studies to dosage form evaluation. Par. Drug Ass. Bull. 23:40-47.

Ballard, B. E. 1981. Doctor of Philosophy Dissertation, University of California, San Francisco, California.

Ballard, B. E. 1966. Effect of physical activity on the absorption rates of procaine penicillin G implants. J. Pharm. Sci. 55:515-16.

Ballard, B. E. 1968. Biopharmaceutical considerations in subcutaneous and intramuscular drug administration. J. Pharm. Sci. 57:357-78.

Ballard, B. E. and Goyan, J. E. 1966. Application of analog computer techniques to in vivo drug kinetic studies. Med. & Biol. Engng. 4:483-90.

Ballard, B. E. and Nelson, E. 1962. Physiochemical properties of drugs that control absorption rate after subcutaneous implantation. J. Pharmacol. Exp. Ther. 136:120-27.

Ballard, B. E. and Nelson, E. 1953. Absorption and excretion of sulfadiazine after subcutaneous implantation of disks in rats, J. Pharm. Sci. 52:301-3.

Ballard, B. E. and Nelson, E. 1964. Absorption of implanted solid drug, J. Pharm. Sci. 51:915-25, 53:1414.

Bratton, C. and Marshall, E. K. 1939. A new coupling component for sulfanilamide determination, J. Biol. Chem. 128:537-50.

Foglia, V. G., Penhos, J. C. and Montuori, E. 1955. Relation of crystal size to estrogenic activity of parahydroxypropiophenone. Endocrinology 57:559-65.

Dettli, L. and Spring, P. 1967. Diurnal variations in the elimination rate of a sulfonamide in man, Helv. Med. Acta. 33:291-306.
Eastland, C. J. 1951. Some aspects of modern formulation. J. Pharm. Pharmacol. 3:942-58.

Emmett, P. H. 1954. in Catalysis, Chapter 2, p. 31. New York: Reinhold.

Garrett, E. R. 1973. Classical pharmacokinetics to the frontier. J. Pharmacokinet. Biopharm. 1:341-61.

Kent, J. S. 1976. Implant pellets I: Effects of compression pressure on in vivo dissolution of desminon acetate pellets. J. Pharm. Sci. 65: 89-92.

Monteleone, J. A. 1969. Hypertensive encephalopathy with overdosage of desoxycorticosterone. Pediatrics 43: 294-95.

Ober, S. S., Vincent, H. C., Simon, D. E., and Frederick, K. J. 1958. A rheological study of procaine S depot preparations. J. Am. Pharm. Ass. 47:667-75.

Ritschel, W. A. 1972. in Perspectives in Clinical Pharmacy, Chapter 17, p. 325. Hamilton, Illinois: Drug Intelligence Publications.

Ritschel, W. A. 1973. in Drug Design, Chapter 3, p. 75. New York: Academic Press.

Schou, J. 1961. Absorption of drugs from subcutaneous connective tissue. Pharmacol. Rev. 13:441-64.

Wagner, J. G. 1961. Biopharmaceutics: absorption aspects. J. Pharm. Sci. 50:359-87.