TABLE VI—MESTRANOL ASSAY OF VARIOUS TABLETS BY GLC

Lot	Storage Conditions[a]	Original Granulation[b] U.V. Assay, mcg./ Tablet	mcg./ Tablet by GLC	% Recovery
A	6 wk. at 100/80	51.1	48.0	94
B	6 wk. at 100/80	51.3	51.8	100.9
C	3 mo. at 100/80	51.3	51.3	100.0
D	6 mo. at 37°	80.0	84.3	105.4
E	7.5 mo. at RT	49.9	51.6	103.4
F	12 mo. at RT	56.0	55.1	98.4
G	12 mo. at RT	56.0	55.0	98.2
H	13 mo. at RT	51.5	52.2	101.4
I	18 mo. at RT	57.6	56.0	97.2

[a] 100/80 refers to 100°F. and 80% humidity. RT refers to room temperature. [b] Within acceptable region of the theoretical value.

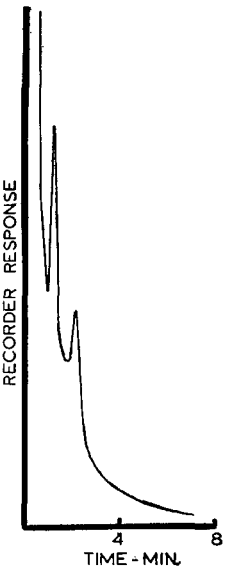

Fig. 4—GLC separation of a complex between polyvinylpyrrolidone and magnesium stearate.

REFERENCES

(1) Kober, S., *Biochem. Z.*, **239**, 209(1931).
(2) Hensghen, C., and Jehotte, J., *J. Pharm. Belg.*, **12**, 418(1957).
(3) Longecker, H., *Acta Endocrinol.*, **37**, 14(1961).
(4) McKerns, K. W., *Biochem. Biophys. Acta*, **69**, 417 (1963).
(5) Schmulowitz, J. J., and Wylie, H. B., *J. Lab. Clin. Med.*, **21**, 210(1935).
(6) Talbot, N. B., Wolfe, J. K., MacLachlan, E. A., Karush, F., and Butler, A. M., *J. Biol. Chem.*, **134**, 319 (1940).
(7) Bender, A. E., and Wilson, A., *Biochem. J.*, **41**, 423 (1947).
(8) Libermann, S., *J. Clin. Invest.*, **31**, 341(1952).
(9) Mitchell, F. L., and Davies, R. E., *Biochem. J.*, **56**, 690(1954).
(10) Rehm, C. R., and Smith, J. B., *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 386(1960).
(11) Urbanyi, T., and Rehm, C. R., *J. Pharm. Sci.*, **55**, 501(1966).
(12) Slaunwhite, W. R., Engle, L. L., Olmsted, P. C., and Carter, P., *J. Biol. Chem.*, **191**, 627(1951).
(13) Boscott, R., *Nature*, **162**, 577(1948).
(14) Huttenraugh, R., and Keiner, I., *Pharmazie*, **20**, 242 (1965).
(15) Sjostrom, E., and Nykanen, L., *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 321(1957).
(16) Kadin, H., Ugolini, M. S., and Roberts, H. R., *J. Pharm. Sci.*, **53**, 1313(1964).
(17) Schulz, E. P., *ibid.*, **54**, 144(1965).
(18) Klein, S., James, A., and Tuckerman, M., *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 314(1960).
(19) Talmage, J. M., and Penner, M. H., and Geller, M., *J. Pharm. Sci.*, **54**, 1194(1965).
(20) France, J. T., and Knox, B. S., *J. Gas Chromatog.*, **4**, 183(1966).

Diffusion of Drugs Across the Isolated Mesentery

By LATIF S. SHENOUDA* and ALBERT M. MATTOCKS†

In order to obtain knowledge of the diffusion rates of pentobarbital, salicylic acid, and urea across the peritoneal membrane under ideal conditions, experiments with isolated rabbit mesentery were conducted. Diffusion coefficients of the drugs were applied to evaluate rates of dialysis at various conditions of pH. Pentobarbital and salicylic acid diffused across the isolated mesentery in both ionic and nonionic form, with no significant difference in rate due to pH. *In vivo* tests also make it appear that the ionized form of drugs may diffuse across the peritoneal membrane. These results suggest that the research for agents to promote dialysis should include substances other than alkalizing agents.

THE REMOVAL of drugs in poisonings by peritoneal dialysis has been the subject of many studies. Salicylates and barbiturates have been most commonly investigated due to the frequency with which they are involved in poisoning cases. The rates at which these drugs have been removed by peritoneal dialysis with the usual dialysis fluids have not been encouraging, and it is desirable that improved fluids and techniques be developed for this purpose. To proceed logically in this effort a more thorough knowledge is required of the mechanism by which drugs pass the membrane and the maximum rates which might be attained.

Berndt and Gosselin (1–3) indicated that the diffusion of ions and molecules through the peritoneal membrane may take place through pores.

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Knochel *et al.* (4, 5) and Nahas and co-workers (6, 7) assumed that only the unionized forms of drugs pass the membrane and on this basis explained the effects of alkaline dialysis fluids on diffusion rates. Engel and Kerekes (8) claimed that the diffusion of a compound from the blood stream into the peritoneal cavity depends primarily on the chemical structure of the compound rather than its physical state.

The present investigation was undertaken to learn more of the nature of drug diffusion across the peritoneum *via* direct measurements on isolated membranes. The rabbit mesentery was selected for study, since the mesentery is the portion of the peritoneum thought to be most effective in dialysis. It was planned to determine the diffusion rates of pentobarbital and salicylate in solution at different pH values, thus to determine whether both ionized and unionized forms of the molecules diffuse. From the diffusion constants obtained, one might predict the maximum rates obtainable under ideal conditions. Urea, being unionized over a wide range of pH, was included in the study to evaluate direct effects of pH on the membranes.

MATERIALS AND METHODS

In order to obtain quantitative measurements on small volumes of dialysate, radioactive compounds were used: pentobarbital-2-^{14}C,[1] salicylic acid-1-^{14}C,[2] and urea-^{14}C.[3] Potassium chloride, A.R., was employed for calibration purposes. All other chemicals were of analytical reagent quality.

White grid Millipore membranes were selected for measurement of diffusion coefficients. It was felt that they would provide fast rates of diffusion and would fit the apparatus designed for animal membranes. The Millipore membranes used in this study had the following specifications: 37 mm. diameter, 0.45 ± 0.02 μ pore size, 150 ± 10 μ dry thickness, and porosity of 0.79 (9).

Isotonic solutions were used in all studies involving pentobarbital, salicylic acid, and urea. These solutions contained 0.005 moles/L. of $Na_2HPO_4 \cdot 7\ H_2O$, calculated quantities of 1 *N* HCl or NaOH to give the required pH, and sufficient NaCl to make the solution isotonic. The pH of all solutions were tested by measurement just prior to and following dialysis experiments.

The diffusion apparatus was constructed from two 125-ml. conical flasks. A 4-cm. tube with an expanded ground joint was sealed to the side of each flask. These joints could be clamped together, enclosing the membrane, by either a threaded or screw type clamp. The inside diameters of the joints were accurately measured. Stirring in both flasks was effected by glass stirrers driven by variable speed motors controlled by rheostats. The diffusion apparatus was mounted in a water bath at 37° ± 0.1°. A diagram of the diffusion apparatus is shown in Fig. 1.

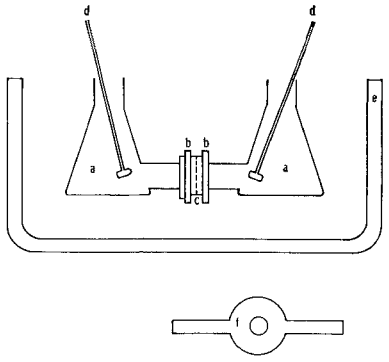

Fig. 1—Diffusion apparatus. Key: a, *conical flasks;* b, *aluminum clamp;* c, *membrane;* d, *glass stirrers;* e, *water bath;* f, *Teflon gaskets for mesentery membrane.*

EXPERIMENTAL PROCEDURE

Diffusion Through Millipore Membranes—A Millipore membrane, which had been previously soaked in water for at least 12 hr., was mounted between the two compartments and the joints were clamped. Then the apparatus was immersed in the water bath, leveled, and locked in position with a clamp. One hundred and fifty milliliters of potassium chloride solution in double distilled water, or of the drug in the isotonic solution of particular pH, was introduced into one compartment. A volume of double distilled water, in the case of KCl diffusion, or isotonic solution of the same pH, in the case of other substances, was introduced into the second compartment. Three different volumes, 150, 155, and 158 ml., were used in the latter compartment, depending on its capacity, in order to bring the liquid inside to the same level as that of the source solution. All solutions were equilibrated at 37° before use. The liquids in both compartments were stirred vigorously without creation of a vortex. Periods of 30 min. in the case of diffusion of potassium chloride, urea, and salicylic acid, and 90 min. in the case of pentobarbital, were allowed before the first sample was withdrawn from the sink compartment for analysis. Additional samples were removed every 10 min. for a period of 90 min. for potassium chloride diffusion, every 15 min. for a period of 2.5 hr. for urea, every 30 min. for a period of 4 hr. for salicylic acid, and every 30 min. for a period of 6 hr. for pentobarbital. Samples were withdrawn with a calibrated pipet which delivered 0.128 ml.

The Fajan's titration method (10) was used to assay potassium chloride samples. A manually operated microburet was utilized to deliver the 0.1 *N* silver nitrate titrant. Radioactivity measurement was employed with the samples withdrawn during diffusion of pentobarbital, salicylic acid, and urea. Each sample was added to a counting vial which contained 10 ml. of dioxane phosphor solution. The latter was prepared by dissolving 7.0 Gm. PPO, 2,5-diphenyloxazole, scintillation grade, 0.05 Gm. POPOP, (1,4-bis 2,5-diphenyloxazolyl)-ben-

[1] Supplied through the courtesy of Abbott Laboratories, North Chicago, Ill.
[2] Nuclear Chicago Corp., Des Plaines, Ill.
[3] Tracerlab, Waltham, Mass.

zene, scintillation grade, 50 Gm. of naphthalene, and 50 ml. of water in *p*-dioxane to make 1 L. The vials were counted in a Tri-Carb liquid scintillation counter using the average of three sets of counts. Suitable blanks and standards were counted with each set of tests. Results were calculated as the amount diffused over the given time interval.

Diffusion Through Isolated Mesenteric Membrane—Albino rabbits were sacrificed with intravenous pentobarbital. Immediately after death, the abdomen was opened and the broad, fan-shaped fold of the peritoneum connecting the convolutions of the jejunum and ileum with the posterior wall of the abdomen was separated. The separated tissue was immediately placed in normal saline solution and used for diffusion or stored in the refrigerator until used. When tissue was stored for longer than 24 hr., the saline was replaced daily. Membranes properly stored were not found to change in diffusion properties over periods up to 15 days. In several cases duplicate tests were performed on the same membrane to confirm this.

A piece of the mesenteric membrane freed from fat and blood vessels was mounted between a pair of Teflon gaskets, $^1/_{16}$ in. thickness, care being taken to avoid stretching the membrane. These gaskets had an outside diameter of $^7/_{16}$ in. and center opening which exposed an area of membrane of 1.266 cm.^{2} for diffusion.

The mesenteric membrane mounted between the Teflon gaskets was firmly clamped in the diffusion apparatus by means of a screw type clamp and the membrane was tested to insure that there was no leakage before use. The diffusion procedure was the same as previously described.

Since it was of some concern that the isolated membrane might in some way function differently from that in the live animal, a simple *in vivo* test was made to see whether both ionic and nonionic forms of a drug may diffuse. For this purpose it was decided to measure the transfer in the reverse direction, from peritoneum to blood, since pH of the dialysis fluid can be altered and buffered. Two tests were run on rabbits with pentobarbital in the dialysis fluid, using 100 mg. ^{14}C tagged pentobarbital in 150 ml. of intraperitoneal injection. In one rabbit an acid fluid consisting of isotonic phosphate buffer, pH 2.6, 0.15 *M*, was used; in the second an isotonic THAM solution, 0.15 *M*, was used. The concentration of nonmetabolized drug in the dialysate and pH of the dialysate were determined in samples removed in approximately 10-min. intervals over a period of about 100 min. Pentobarbital was measured by the method of Brodie *et al.* (11) but utilizing radioactive counting of the extracts rather than spectrophotometric measurement.

Results and Treatment of Data—The rate of diffusion between two well-stirred compartments separated by a membrane can be represented by Eq. 1. The detailed derivation of this equation is discussed by Lueck *et al.* (12).

$$\log_{10}\left[\left(1+\frac{V_1}{V_2}\right)C_1-\frac{V_1}{V_2}C_0\right]= -\left(\frac{V_1+V_2}{V_1V_2}\right)\frac{DA\epsilon}{2.303h}t+\log_{10}C_0 \quad \text{(Eq. 1)}$$

where

V_1 = volume of solution in source compartment in ml.,
V_2 = volume of solution in sink compartment in ml.,
C_1 = concentration of diffusing substance in source compartment at time, t,
C_0 = initial concentration of penetrant in source compartment,
D = diffusion coefficient of the penetrant in cm.^{2}/min.,
A = surface area of membrane in cm.^{2},
ϵ = porosity of membrane,
h = thickness of the membrane in cm.

In special cases where $V_1 = V_2$, Eq. 1 reduces to

$$\log_{10}(2C_1-C_0)=-\frac{2DA\epsilon}{2.303Vh}t+\log_{10}C_0 \quad \text{(Eq. 2)}$$

A plot of lefthand side of Eqs. 1 or 2 *versus* time should yield a straight line. From the slope of this line and known values of the cell constant the diffusion coefficient can be readily calculated.

The cell constant is obtained from diffusion measurements of a substance of a known diffusion coefficient with the same apparatus and membrane by dividing the slope of the line, from Eq. 1, by D. Thus,

$$\text{cell constant}=\frac{V_1+V_2}{V_1V_2}\cdot\frac{A\epsilon}{2.303h} \quad \text{(Eq. 3)}$$

or

$$\frac{2A\epsilon}{2.303hV} \quad \text{(Eq. 4)}$$

where $V_1 = V_2$.

The diffusion of 1 *M* potassium chloride through Millipore membranes was measured and using the known diffusion coefficient of 1.4356×10^{-3} cm.^{2}/min. (13) the cell constant was calculated. The mean value of nine experiments was 0.7264 cm.$^{-2}$ with a standard deviation of 0.0054 cm.$^{-2}$. With same apparatus and same type of membrane the diffusion of pentobarbital, salicylic acid, and urea was measured, and their diffusion coefficients were calculated as described above. These values, each the average of six measurements, were: for pentobarbital, 5.917×10^{-4} cm.^{2}/min.; salicylic acid, 6.901×10^{-4} cm.^{2}/min.; and urea, 1.018×10^{-3} cm.^{2}/min. Diffusion measurements at several pH values revealed no significant difference in diffusion coefficients with the Millipore membranes. A typical plot of diffusion data by Eq. 2 is presented in Fig. 2.

Measurements were then made of the diffusion of pentobarbital, salicylic acid, and urea through isolated mesentery at several pH values in isotonic solution. The data were plotted according to Eqs. 1 or 2, and results were calculated as permeability coefficients, $\frac{D\epsilon}{h}$, by dividing the slopes by the area of membrane exposed and multiplication by 2.303 and the appropriate volume factors. Coefficients obtained are presented in Tables I–III. Typical diffusion curves are shown in Figs. 3–5.

These data demonstrate that the three compounds diffused quite rapidly across the mesentery. For pentobarbital, the data indicated a slight

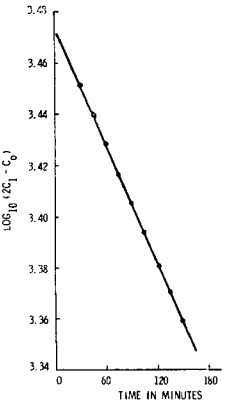

Fig. 2—Typical diffusion curve with Millipore membrane. Urea at pH 7.4.

decrease in permeability with increase in pH, but the differences are not significant when examined by statistical analysis. Using a value of 7.66 for the apparent dissociation constant of pentobarbital, from Krahl (14), it is estimated that the compound is completely dissociated at pH 11.7, 84.6% at pH 8.4, 35.5% at pH 7.4, and completely undissociated at pH 3.7. If the only species to diffuse were the undissociated molecule, one would expect great differences in diffusion rates with the different pH values used. Thus, one must conclude that both ionic and nonionic forms diffuse with approximately equal facility.

The diffusion rates of salicylic acid show that this compound diffused equally rapidly at pH 3.0 and 7.4. Salicylic acid is 50% dissociated at pH 3.0 and completely dissociated at pH 7.4. Thus, both species appear to diffuse equally well across the mesentery.

The permeability coefficients for urea at different pH values were essentially the same, as seen in Table III. This indicates that there was no appreciable effect of pH on the membranes, urea being undissociated at the pH range studies.

In several cases urea was run on membranes which had been previously used for pentobarbital. The ratios of diffusion rates of pentobarbital to urea were used to determine whether the membrane had been affected by the experimental conditions. These ratios were comparable to the ratio of the individual diffusion coefficients of the compounds, indicating no change in the performance of the membranes.

Although it is not known whether pores exist in the mesentery membrane, it is of interest to calculate the "apparent porosity" of these membranes. Such values give one a means of comparing penetrability of mesentery with other membranes and of predicting amounts which might diffuse under a variety of conditions. "Apparent porosities" can be calculated from average permeability coefficients, the measured diffusion coefficients for the three compounds, and the value of 38.5 μ for the thickness of the membrane (15). These calculations gave an average porosity of 29%, which is quite high as compared to most membranes. Calculating porosities from permeabilities with the different drugs, the values from pentobarbital data ranged from 22 to 36%, those from urea, 20 to 35%, and from salicylic acid, 29 to 39%. These differences in values for different drugs were not significantly greater than the variations between individual membranes with a single drug.

Results of the *in vivo* tests are presented in Table IV and curves of dialysate concentrations with time are shown in Fig. 6. From the data it is seen that the pentobarbital diffused rapidly at both high and low pH. This suggests that both ionic and nonionic forms diffuse, as was found with the isolated membrane.

TABLE I—PERMEABILITY COEFFICIENTS FOR PENTOBARBITAL THROUGH ISOLATED RABBIT MESENTERY AS A FUNCTION OF pH AT 37° C.

Series[a]	pH	Permeability Coefficient cm. min.$^{-1}$ × 10^3 Expt. *1*	Expt. *2*	Expt. *3*
B_{1-1}	3.7	55.8	53.9	...
B_{2-1}	3.7	37.4	52.8	...
	7.4	44.2	44.9	46.8
	11.7	40.7	43.0	44.2
B_{2-2}	3.7	50.1	45.7	...
	7.4	58.7	53.6	53.2
	8.4	43.0	48.5	...
B_{3-1}	3.7	34.0	...	...
	7.4	33.0	...	...
	8.4	32.0	...	...
B_{3-2}	3.7	42.5	...	...
	7.4	39.2	...	...
	8.4	37.5	...	...

[a] The first subscript represents rabbit number, and second subscript represents membrane number.

TABLE II—PERMEABILITY COEFFICIENTS FOR SALICYLIC ACID ACROSS ISOLATED RABBIT MESENTERY AS A FUNCTION OF pH AT 37° C.

Series[a]	pH	Permeability Coefficient cm. min.$^{-1}$ × 10^3
B_{4-1}	3.0	59.3
	7.4	66.0
B_{4-2}	3.0	60.6
	7.4	56.7
B_{5-1}	3.0	71.1
	7.4	68.0
B_{5-2}	3.0	53.6
	7.4	55.4

[a] First subscript represents rabbit number, and the second designates membrane number.

TABLE III—PERMEABILITY COEFFICIENTS FOR UREA THROUGH ISOLATED RABBIT MESENTERY AS A FUNCTION OF pH AT 37° C.

Series[a]	pH	Permeability Coefficient cm. min.$^{-1}$ × 10^3
B_{2-2}	7.4	79.9
B_{3-1}	7.4	52.3
B_{3-2}	7.4	67.8
B_{3-3}	3.7	69.5
	7.4	66.0
	8.4	68.6

[a] The first subscript represents rabbit number, and second subscript represents membrane number.

DISCUSSION AND CONCLUSIONS

This work clearly demonstrates that both ionic

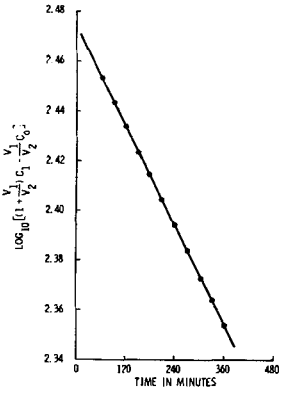

Fig. 3—Typical diffusion curve for pentobarbital with mesentery membrane, pH 7.4.

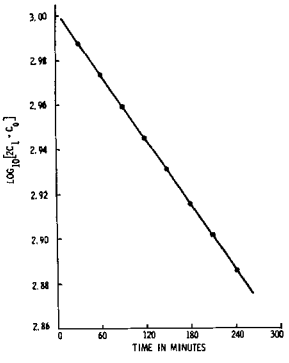

Fig. 4—Typical diffusion curve for salicylic acid with mesentery membrane, pH 7.4.

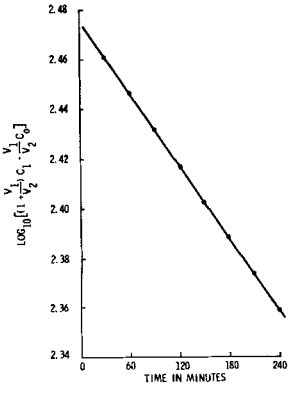

Fig. 5—Typical diffusion curve for urea with mesentery membrane, pH 7.4.

Table IV—Diffusion of Pentobarbital Across Peritoneum from Acid and Alkaline Peritoneal Fluid

Time, min.	Concn. Drug in Fluid, mcg./ml.	pH of Fluid	Concn. Unionized Drug, mcg./ml.
	THAM[a]		
0	666	8.7	59
15	347	8.6	35
25	261	8.5	34
35	168	8.2	37
45	120	8.0	38
55	89	8.0	28
65	73	7.8	31
75	64	7.8	28
85	55	7.8	24
95	40	7.7	19
	Acid Phosphate[b]		
0	666	2.6	666
8	533	2.6	533
15	443	2.6	443
25	341	2.6	341
35	302	2.6	302
60	270	2.6	270
70	224	2.6	224
80	222	2.6	222
90	209	2.6	209
100	188	2.6	188

[a] Animal No. 19, weight 4.18 Kg. [b] Animal No. 21, weight 3.56 Kg.

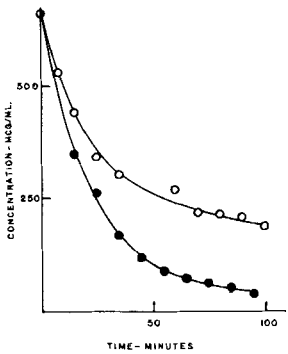

Fig. 6—Drop in concentration of pentobarbital in peritoneal fluid with time. Key: ○, acid fluid; ●, alkaline.

and nonionic forms of salicylate and pentobarbital diffuse across the isolated mesenteric membrane. Since the same buffer is on both sides of the membrane in the *in vitro* tests it is inconceivable that neutralization at the surface or within the membrane could occur. Whether this truly represents the transport in the live animal is difficult to test directly, since under all biological conditions the blood and extracellular fluid must be maintained at nearly normal pH, and the buffer substances of the blood may maintain neutrality within the membrane. The *in vivo* test strongly indicates, however, that the ionic form of pentobarbital does diffuse across the peritoneal membrane, since otherwise the concentration gradient of unionized molecules in the alkaline fluid would be low enough to have a pronounced effect on the dialysis rate. Rather than retard the diffusion, the THAM appeared to increase the rate. Other data (16) on effect of THAM on dialysis of urea show an increased rate of dialysis which cannot be attributed to pH effect.

These data suggest that the effects of THAM on rates of removal of drugs by peritoneal dialysis are not primarily a pH effect and that the search for new agents to improve the rates of dialysis should go beyond the testing of alkalizing substances.

REFERENCES

(1) Berndt, W. O., and Gosselin, R. E., *Am. J. Physiol.*, **200**, 454(1961).
(2) Berndt, W. O., and Gosselin, R. E., *Biochem. Pharmacol.*, **8**, 359(1961).
(3) Berndt, W. O., and Gosselin, R. E., *Am. J. Physiol.*, **202**, 761(1962).
(4) Knochel, J. P., Clayton, L. E., Smith, W. L., and Barry, K. G., *J. Lab. Clin. Med.*, **64**, 257(1964).
(5) Knochel, J. P., and Barry, K. G., *ibid.*, **65**, 361(1965).
(6) Nahas, G. G., Gjessing, J., Verosky, M., and Mark, L. C., *Trans. Am. Soc. Artif. Int. Organs*, **10**, 345(1964).
(7) Nahas, G. G., Gjessing, J., Giroux, J. J., Verosky, M., and Mark, L. C., *Clin. Pharmacol. Therap.*, **6**, 560(1965).

(8) Engel, D., and Kerekes, A., *Klin. Wochschr.*, **5**, 1709 (1962); through *Chem. Abstr.*, **21**, 133(1927).

(9) Millipore Technical Brochure, MF-64, Millipore Filter Corp., Bedford, Mass., 1964, pp. 2–7.

(10) Vogel, A. I., "A Textbook of Quantitative Inorganic Analysis," 3rd ed., John Wiley & Sons, Inc., New York, N. Y., 1961, pp. 79–81.

(11) Brodie, B. B., Burns, J. J., Mark, L. C., Lief, P. A., Berstein, E., and Papper, E. M., *J. Pharmacol. Exptl. Therap.*, **109**, 26(1963).

(12) Lueck, L. M., Wurster, D. E., Higuchi, T., Lemberger, A. P., and Busse, L. W., *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 694(1957).

(13) Shenouda, L. S., "The Diffusion of Drugs and Chemicals Through Isolated Rabbit Mesentery," Doctoral Dissertation, The University of Michigan, Ann Arbor, Mich., 1965.

(14) Krahl, M. E., *J. Phys. Chem.*, **44**, 449(1940).

(15) Gosselin, R. E., and Berndt, W. O., *J. Theoret. Biol.*, **3**, 487(1962).

(16) McLean, W. M., Poland, D. M., Cohon, M. S., and Mattocks, A. M., unpublished data.

Absorption and Activity of Some Derivatives of Griseofulvin

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Some derivatives of the 4′-keto group of griseofulvin were prepared which had increased water solubility and greater absorption potential. The derivatives, which exhibited little or no *in vitro* activity against *Microsporum gypseum*, were found to be converted to griseofulvin *in vivo*. After intravenous administration to rabbits, several of the derivatives were rapidly converted to griseofulvin. Griseofulvin plasma levels resulting from oral doses of the derivatives were found to be equal to, or in most instances significantly higher than, the levels produced by equal doses of griseofulvin, but of shorter duration. Evidence for the degradation of the derivatives in the gastrointestinal tract is presented.

PREVIOUS REPORTS have shown griseofulvin to be irregularly and incompletely absorbed from the gastrointestinal tract of man (1–3) and laboratory animals (4, 5). The incomplete absorption, which often produces low blood and tissue levels, appears to be a result of the slow rate of dissolution of the drug in the gastrointestinal fluids due to its extremely low solubility in water. Fischer and Riegelman (6) have shown that the solution-rate-limited absorption of griseofulvin in rabbits was by a pseudo zero-order process and that the drug was eliminated rather rapidly ($t^1/_2$ = 77 ± 14 min.) from the blood following intravenous injection.

Numerous attempts have been made to increase blood levels resulting from an oral dose of the drug. By increasing the specific surface area of orally administered griseofulvin threefold, many workers have reported experimental animal and human peak blood levels to be approximately twice those obtained with the same dose of regular particle size griseofulvin (7–14). However, Crounse (13) has observed that a double dose of microcrystalline griseofulvin did not significantly raise serum levels in some individuals and that these levels may still fall short of the proper inhibitory levels for the antibiotic. The administration of surfactants with an oral dose of griseofulvin generally has produced erratic results in animals (9, 12) and has not significantly increased blood levels in man (14). The oral administration of griseofulvin with lipids or after meals with high fat content has been shown to increase blood levels (9, 13, 15); however, the mechanism by which absorption is facilitated by lipids has not been clarified.

Although many attempts have been made to increase griseofulvin absorption by various methods, no previously published report has investigated the preparation and administration of derivatives of griseofulvin having increased water solubility and greater absorption potential. The derivatives were chosen so that active griseofulvin would be a possible product of their enzymatic metabolism in the body. The more water-soluble derivatives were expected to be absorbed more completely from the gastrointestinal tract, and if they were converted rapidly to griseofulvin in the body, increased blood levels of the antibiotic could be expected. In this manner the concept of drug latentiation was employed to increase plasma levels of griseofulvin following the oral administration of some derivatives of the 4′-carbonyl group of griseofulvin.

The derivatives of griseofulvin (I) selected to be prepared and tested were griseofulvin-4′-alcohol (II), griseofulvin-4′-oxime (III), griseofulvin-4′-carboxymethoxime (IV), and griseofulvin-4′-hemisuccinate (V). Griseofulvin-4′-

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