but nitrazepam is not. Kinetically limiting factors could be different in the two systems.

Nevertheless, there is now enough evidence to conclude that the rate of decomposition of pure and compound drugs in the solid state may be influenced as much by water as by temperature and that it is possible to relate the decomposition constants simply and simultaneously to temperature and to a term reflecting the influence of water. Relative humidity seems to be a good way to deal with this problem; combined with temperature, it can characterize climatic conditions and allows establishment of the corresponding $t_{0.9}$ values directly from experimental results.

REFERENCES

(1) J. T. Carstensen, *J. Pharm. Sci.*, **63**, 1 (1974).

(2) J. T. Carstensen, "Theory of Pharmaceutical Systems," vol. II, Academic, New York, N.Y., 1973, p. 294.

(3) F. Tripet and U. W. Kesselring, *Pharm. Acta Helv.*, **50**, 318 (1975).

(4) H. Landolt and R. Börnstein, "Physikalisch-Chemische Tabellen," Springer, Berlin, Germany, 1912, pp. 361, 419, 427.

(5) Z. G. Szabo in "Chemical Kinetics," vol. 2, C. H. Bamford and C. F. H. Tipper, Eds., Elsevier, New York, N.Y., 1969, p. 14.

(6) W. Mayer, S. Erbe, and R. Voigt, *Pharmazie*, **27**, 32 (1972).

(7) W. Mayer, S. Erbe, G. Wolf, and R. Voigt, *ibid.*, **29**, 700 (1974).

(8) J. T. Carstensen, K. S. E. Su, P. Maddrell, J. B. Johnson, and H. N. Newmark, *Bull. Parenteral Drug Assoc.*, **25**, 193 (1971).

(9) M. R. Spiegel, "Théorie et applications de la statistique," Ediscience SA., Paris, France, 1972, p. 222.

(10) L. J. Leeson and A. M. Mattocks, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 329 (1958).

(11) J. T. Carstensen, M. Osadca, and S. H. Rubin, *J. Pharm. Sci.*, **58**, 549 (1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received December 8, 1975, from the *Ecole de Pharmacie, 3, Place du Château, CH-1005 Lausanne, Switzerland.*

Accepted for publication June 28, 1976.

The authors thank M. Soliva for helpful discussions. They are indebted to Hoffmann–La Roche & Co. SA., Basel, Switzerland, and to the Swiss National Foundation for Scientific Research (Grant 3.730.72) who in part supported this work.

[x] To whom inquiries should be directed.

GI Drug Absorption in Rats Exposed to Cobalt-60 γ-Radiation III: *In Situ* Intestinal Absorption

MICHAEL E. BRADY [*] and WILLIAM L. HAYTON [x]

Abstract □ The absorption rate of sulfanilamide, bretylium tosylate, sulfisoxazole acetyl, and riboflavin from the *in situ* small intestine was studied in rats exposed to 850 rad of cobalt-60 γ-radiation. Compared to absorption from the intestine of sham-irradiated animals, the absorption rate of sulfanilamide declined following irradiation. It declined to a minimum that was 65% of the control value at 4 days postirradiation before it began to return toward the control value. The reduced absorption rate of sulfanilamide in irradiated animals was accompanied by, and appeared to result from, a reduction in the absorption rate of water from the intestinal drug solution. The sulfisoxazole acetyl absorption rate was reduced compared to the control rate at both 1 and 5 days postirradiation. While this reduced absorption rate at 5 days postirradiation may have resulted from a reduced absorption rate of water, postirradiation water absorption rates at 1 day were similar in irradiated and control animals. The absorption rates of bretylium and riboflavin were not affected by exposure of animals to radiation 1 or 5 days previously. The permeability of the small intestinal epithelium to the drugs studied appeared to be reduced slightly following its exposure to ionizing radiation. The reduced permeability is apparently an indirect effect of the reduced absorption rate of water.

Keyphrases □ Absorption, GI—sulfanilamide, bretylium tosylate, sulfisoxazole acetyl, and riboflavin, effect of γ-radiation on rate, rats □ Radiation, gamma—effect on rate of GI absorption of sulfanilamide, bretylium tosylate, sulfisoxazole acetyl, and riboflavin, rats □ Sulfanilamide—GI absorption, effect of γ-radiation on rate, rats □ Bretylium tosylate—GI absorption, effect of γ-radiation on rate, rats □ Sulfisoxazole acetyl—GI absorption, effect of γ-radiation on rate, rats □ Riboflavin—GI absorption, effect of γ-radiation on rate, rats

The bioavailability of drugs administered orally was altered in rats exposed to 850 rad of γ-radiation (1, 2). The alterations in bioavailability in irradiated animals compared to controls appeared to result primarily from a pronounced reduction in the gastric emptying rate. The effects of irradiation on bioavailability depended upon the physicochemical properties of the drug, the dosage form, and the time postirradiation at which the drug was administered.

While reduced gastric emptying appeared to be the principal mechanism by which irradiation of the gut altered bioavailability, damage resulting from irradiation of the intestine (3, 4) can also affect the absorption of orally administered drugs by alteration of the permeability of the intestinal epithelium. In irradiated rats, the apparent decrease in the absorption rate of sulfanilamide from the *in situ* intestine (5) and the altered permeability of the *in vitro* intestine to several drugs (6, 7) indicate that the permeability of the intestinal epithelium may be altered directly by its exposure to ionizing radiation. The purpose of this study was to explore further the effects of prior whole body exposure to 850 rad of γ-radiation on the apparent permeability of the *in situ* rat intestine to drugs.

EXPERIMENTAL

Materials—The chemicals and reagents used were described previously (1).

Intestinal Absorption Rate—Male Sprague–Dawley rats, 170–250 g, were either irradiated or sham-irradiated by a procedure described previously (1). At various times following irradiation, equal numbers of irradiated and sham-irradiated animals were fasted overnight, anesthetized with 1.3 g of ethyl carbamate/kg, and prepared surgically for the determination of the drug absorption rate from 7.0 ml of a solution instilled into the cannulated small intestine (8, 9). The drugs[1], at initial

[1] The following drugs were used: sulfisoxazole acetyl, Hoffmann–La Roche, Nutley, N.J.; bretylium tosylate, Burroughs Wellcome Co., Research Triangle Park, N.C.; 2-^{14}C-*d*-riboflavin, Amersham/Searle, Arlington Heights, Ill.; and sulfanilamide, Sigma Chemical Co., Irvington, N.Y.

Table I—Drug Absorption from the *In Situ* Rat Intestine following Exposure to 850 rad of Cobalt-60 γ-Radiation or Sham Irradiation

Drug (Initial Concentration)	Day Post-irradiation	Absorption Rate Constant[a,b], min^{-1}		Volume of Water Absorbed[b,c], ml/hr		Gut Length[b], cm		Final pH of Drug Solution[b,d]	
		Sham	850 rad	Sham	850 rad	Sham	850 rad	Sham	850 rad
Sulfanilamide (60 μg/ml)	1	0.0395 ± 0.00755	0.0445 ± 0.00412	3.9 ± 1.0	4.2 ± 0.7	85.6 ± 2.88	84.8 ± 3.63	6.6 ± 0.03	6.6 ± 0.07
	4	0.0410 ± 0.00518	0.0268[e] ± 0.00566	3.2 ± 1.4	−0.4[e] ± 1.3	90.0 ± 6.30	87.0 ± 4.90	6.3 ± 0.12	6.4 ± 0.15
Bretylium (0.70 mg/ml)	1	0.00[f]	0.00[f]	—[g]	—[g]	84.3 ± 2.63	84.0 ± 3.37	6.6 ± 0.05	6.6 ± 0.04
	5	0.00[f]	0.00[f]	—[g]	—[g]	86.0 ± 1.41	87.5 ± 2.08	6.6 ± 0.05	6.7 ± 0.08
Sulfisoxazole acetyl (60 μg/ml)	1	0.156 ± 0.0111	0.138[e] ± 0.0150	4.5 ± 1.8	4.0 ± 1.5	87.9 ± 3.13	87.1 ± 2.04	6.7 ± 0.06	6.6 ± 0.08
	5	0.133 ± 0.0067	0.120[e] ± 0.0097	4.0 ± 0.6	1.7[e] ± 0.9	89.3 ± 3.98	89.4 ± 4.67	6.5 ± 0.09	6.5 ± 0.06
Riboflavin (20 μg/ml)	1	0.00155 ± 0.00081	0.00151 ± 0.00048	4.3 ± 0.5	3.2[e] ± 0.7	86.2 ± 4.32	85.8 ± 4.30	7.2 ± 0.11	7.1 ± 0.17
	5	0.00121 ± 0.00046	0.00167 ± 0.00085	4.1 ± 0.9	2.5[e] ± 1.3	85.3 ± 3.61	88.2 ± 3.19	7.0 ± 0.18	6.9 ± 0.08

[a] Corrected for loss of drug due to sampling. [b] Mean of five or more animals (four with bretylium) ± *SD*. [c] This volume is the difference between the volume of 0.9% NaCl solution added to maintain the volume of the drug solution constant and the volume of drug solution removed as samples. [d] Initial pH was 6.8, except for sulfanilamide at 4 days postirradiation where the pH was 6.5. [e] Significantly different ($p < 0.05$) from corresponding sham-irradiated animals. [f] Value was negative but not significantly different from zero. [g] Not recorded.

concentrations determined by assay sensitivity and drug solubility, were dissolved in 67 m*M* sodium phosphate buffer made isotonic with sodium chloride. The volume of the drug solution in the intestine was maintained at 7.0 ml by addition of 0.9% NaCl solution immediately prior to removal of each sample.

The rectal temperature of each animal was monitored[2] throughout the experiment and maintained at 37° by a rheostatically controlled heating pad. At the conclusion of each experiment, the following data were recorded: the volume of water absorbed[3], the pH of the intestinal drug solution, and the length of intestine perfused.

Absorption rate constants were estimated from the slopes of lines fitted by least squares to plots of the logarithm of drug concentration in the intestinal drug solution *versus* time. Each rate constant was corrected for the loss of drug due to sampling[4], and absorption half-lives were calculated from the corrected value of the rate constant.

Analytical Methods—To separate sulfisoxazole acetyl from sulfisoxazole present due to hydrolysis, samples of intestinal perfusate were made alkaline by addition of 0.6 *M* pH 8.0 sodium phosphate solution and extracted with chloroform. Sulfisoxazole acetyl in the chloroform layer was determined by spectrophotometry[5] at 278 nm. Methods for the determination of the other drugs and radioactivity were described previously (1). Tonicity (osmolality) was measured by dew point depression using an osmometer[6].

RESULTS AND DISCUSSION

A preliminary study showed that the absorption rate of sulfanilamide from the *in situ* rat intestine decreased to a minimum at 4–5 days following exposure of the animals to 850 rad of γ-radiation (Fig. 1). Therefore, additional experiments were performed at 4 days postirradiation (5), and the results showed a statistically significant decrease in the rate of sulfanilamide absorption in irradiated animals compared to controls (Table I). The reduced absorption rate could not be attributed to differences in the length of the intestine perfused nor to differences in the intestinal perfusate pH (Table I). The decrease in the apparent permeability of the intestinal mucosa to sulfanilamide at 4 days postirradiation did not affect significantly the overall *in vivo* rate or extent of absorption of sulfanilamide, since the slow step in its absorption tended to be gastric emptying (2).

The decreased rate of sulfanilamide absorption from the intestine of irradiated animals was accompanied by a similar decrease in the absorption rate of water from the intestine (Fig. 1 and Table I). The water absorption rate affects the rate of intestinal drug absorption directly due to a solvent drag effect (10–12). To examine the role of water absorption in the reduced rate of sulfanilamide absorption, the rate of water absorption from the *in situ* intestine was varied by altering the sodium chloride concentration in the intestinal perfusate (Table II). The ab-

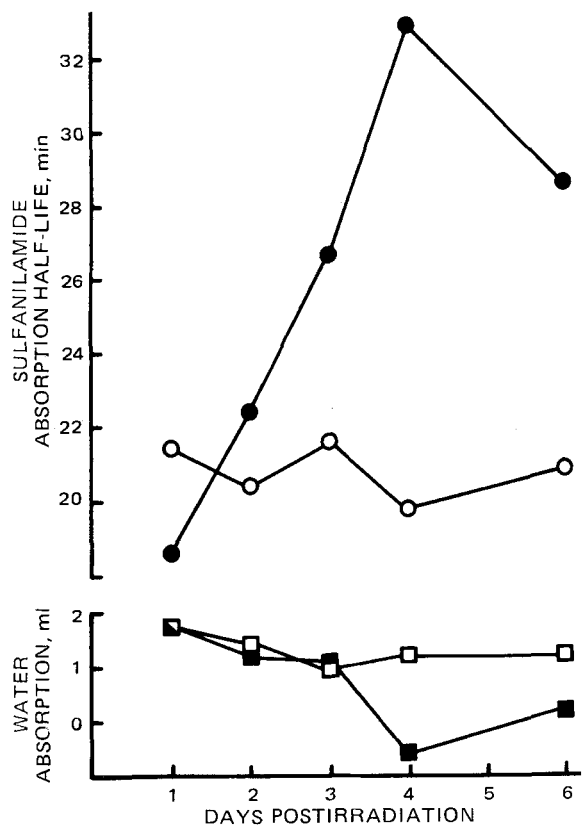

Figure 1—*Sulfanilamide (initial concentration of 60 μg/ml) absorption half-life from the* in situ *rat intestine at various times following irradiation. Key: ●, irradiated (850 rad); and ○, sham irradiated. Also shown is the volume of water absorbed over the 30-min absorption experiment. Key: ■, irradiated (850 rad); and □, sham irradiated. Each point represents the mean of two animals.*

[2] Tele-Thermometer model 44TD, Yellow Springs Instrument Co., Yellow Springs, Ohio.

[3] This volume is the difference between the volume of 0.9% NaCl solution added to maintain the volume of the drug solution constant and the volume of drug solution removed as samples.

[4] Rate constants were corrected by subtracting the quantity: sample size in milliliters/(sampling interval in minutes × 7 ml).

[5] Beckman DU (Beckman Instruments, Fullerton, Calif.), with a Gilford model 2000 multiple-sample recorder (Gilford Instrument Laboratories, Oberlin, Ohio).

[6] Vapor pressure osmometer, model 1500, Wescor, Logan, Utah.

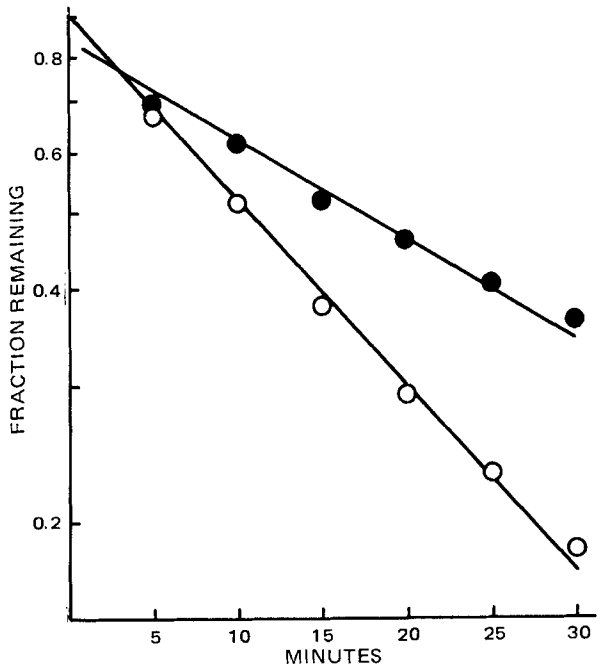

Figure 2—*Absorption of sulfanilamide (initial concentration of 60 μg/ml) from the* in situ *rat intestine under conditions of net water flux. Key: ●, hypertonic (548 mOsm/kg) perfusate; and ○, hypotonic (232 mOsm/kg) perfusate. Each point represents the mean of four animals.*

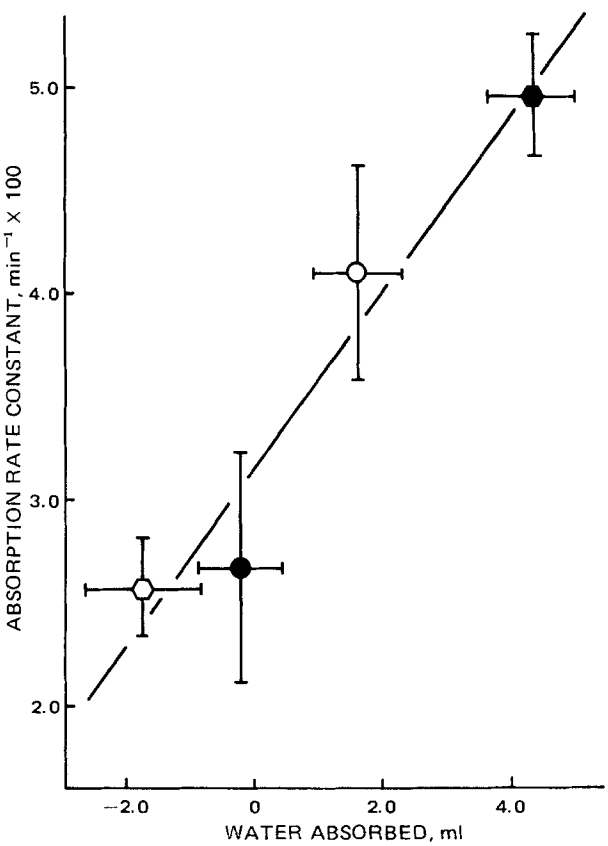

Figure 3—*Relationship between water flux and the rate constant for absorption of sulfanilamide (initial concentration of 60 μg/ml) over 30 min from the* in situ *rat intestine. Key: ○, sham-irradiated animals perfused with isotonic solution; ●, nonirradiated animals perfused with hypotonic solution (232 mOsm/kg); ○, nonirradiated animals perfused with hypertonic solution (548 mOsm/kg); and ●, irradiated animals perfused with isotonic solution at 4 days postirradiation.*

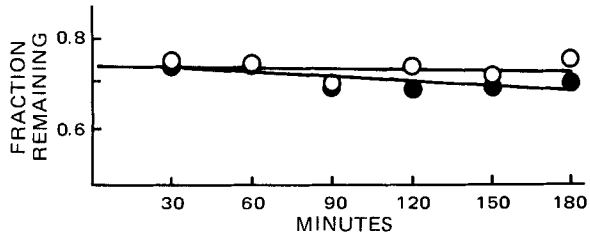

Figure 4—*Effect of 850 rad of γ-radiation on the absorption of bretylium (initial concentration of 0.70 mg/ml) from the* in situ *rat intestine at 5 days postirradiation. Key: ●, irradiated; and ○, sham irradiated. Each point represents the mean of four animals.*

sorption rate of sulfanilamide was significantly greater from a hypotonic than from a hypertonic perfusate (Fig. 2).

In nonirradiated animals, there was a linear relationship between the sulfanilamide absorption rate constant and the water absorption rate (Fig. 3). This relationship was similar to that reported for several other drugs (10–12). When the mean absorption rate constant of sulfanilamide in irradiated animals was plotted at the corresponding rate of water absorption, it was nearly on the line (Fig. 3). Thus, the reduction in the absorption rate constant of sulfanilamide in irradiated animals at 4 days postirradiation apparently resulted indirectly from a reduction in the water absorption rate and not from a direct alteration in the permeability of the intestinal mucosa to the drug. The possibility remains, however, that the rates of absorption of both water and sulfanilamide depend on a third factor affected by radiation.

Irradiation did not appear to alter the permeability of the intestinal epithelium to bretylium (Table I). No measurable absorption of bretylium occurred at 1 or 5 days postirradiation (Fig. 4). The initial relatively rapid decline in the fraction of bretylium remaining in the drug solution (Fig. 4) was possibly due to binding of the drug to the surface of the epithelial mucosa (13).

In a previous study (1), poor absorption of orally administered bretylium was demonstrated by low recovery of the drug in the urine of irradiated and sham-irradiated rats. The data reported here indicate that epithelial permeability is the rate-controlling step that limits both the rate and extent of drug absorption. In addition, this study indicates that the barrier properties of the intestinal epithelium to the transfer of positively charged compounds are not altered by prior exposure of the intestine to radiation.

Sulfisoxazole acetyl, a highly lipid-soluble drug, disappeared rapidly

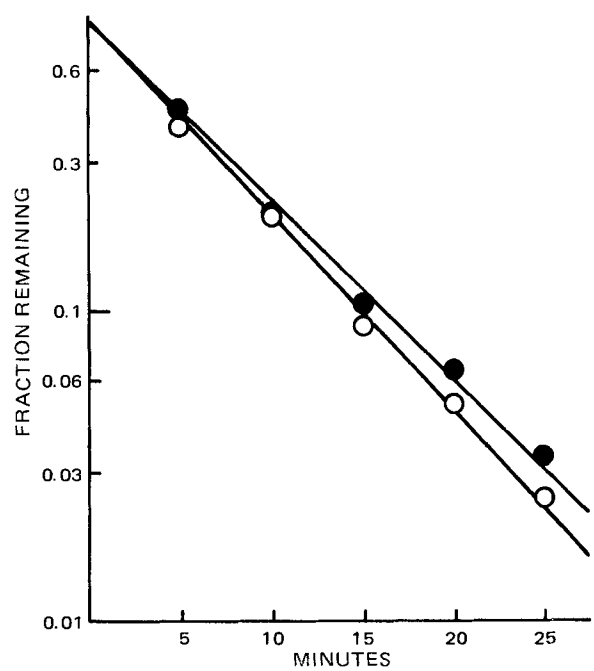

Figure 5—*Effect of 850 rad of γ-radiation on the absorption of sulfisoxazole acetyl (initial concentration of 60 μg/ml) from the* in situ *rat intestine at 5 days postirradiation. Key: ●, irradiated; and ○, sham irradiated. Each point represents the mean of at least five animals.*

Table II—Effect of Tonicity on the Absorption Rate Constant of Sulfanilamide[a] from the *In Situ* Rat Intestine

Tonicity, mOsm/kg	Absorption Rate Constant[b,c], min^{-1}	Volume of Water Absorbed[c,d], ml/hr	Gut Length[c], cm	Final pH of Drug Solution[c,e]
232	0.0497 ± 0.00299[f]	8.7 ± 1.4[f]	88.5 ± 4.80	6.6 ± 0.10
314 (Isotonic)	0.0410 ± 0.00518	3.2 ± 1.4	90.0 ± 6.30	6.3 ± 0.12
548	0.0258 ± 0.00236[f]	−3.5 ± 2.7[f]	88.5 ± 2.65	6.7 ± 0.08

[a] Initial concentration 60 μg/ml. [b] Corrected for loss of drug due to sampling. [c] Mean of four or more animals ± *SD*. [d] This volume is the difference between the volume of 0.9% NaCl solution added to maintain the volume of the drug solution constant and the volume of drug solution removed as samples. [e] Initial pH was 6.8, except for isotonic solutions where the pH was 6.5. [f] Significantly different ($p < 0.02$) from the isotonic value.

from the intestinal drug solution (Fig. 5). While the analytical method was specific for sulfisoxazole acetyl, deacetylation may have occurred simultaneously with absorption. When sulfisoxazole acetyl was incubated with intestinal epithelium, hydrolysis was accelerated compared to control studies in which the incubation medium did not contain intestinal epithelium (14). Thus, the rapid rate of disappearance of sulfisoxazole acetyl from the intestinal drug solution may reflect both rapid absorption and hydrolysis (15, 16).

Prior exposure of animals to 850 rad of γ-radiation reduced slightly, but significantly, the apparent absorption rate constant for sulfisoxazole acetyl at both 1 and 5 days (Table I). The reduction in the absorption rate of sulfisoxazole acetyl in irradiated animals was not due to a reduction in the length of intestine perfused nor to differences in the drug solution pH (Table I). Nor did a reduced rate of water absorption account entirely for the reduced rate of sulfisoxazole acetyl absorption in irradiated animals, since the water absorption rate at 1 day postirradiation was similar in irradiated and control animals (Table I).

Sulfisoxazole acetyl may penetrate the intestinal epithelium from solution so rapidly that blood flow to the intestine is the rate-controlling step in its absorption. Exposure to radiation reduced the rate of blood flow to the intestine of rats (17) and dogs (18) exposed 2–3 days previously. Absorption limited by blood flow was demonstrated for other rapidly absorbed drugs from the perfused *in situ* rat intestine (12, 19). The reduction in the sulfisoxazole acetyl absorption rate observed here was not clinically significant, since the intestinal absorption rate of the drug was still very high in irradiated animals.

Riboflavin disappeared slowly from the intestinal drug solution (Fig. 6). The absorption rate of the vitamin was not significantly different from that of the control at 1 or 5 days postirradiation (Table I). The initial rapid decline in the riboflavin concentration in the intestinal solution was similar to that observed for bretylium (Fig. 4) and may be attributed to binding of riboflavin to the intestinal mucosa rather than to absorption. In irradiated animals, the volume of water absorbed was lower than in sham-irradiated animals at 1 and 5 days postirradiation. The reduced rate of water absorption was not accompanied by a corresponding decrease in the rate of riboflavin absorption (Table I).

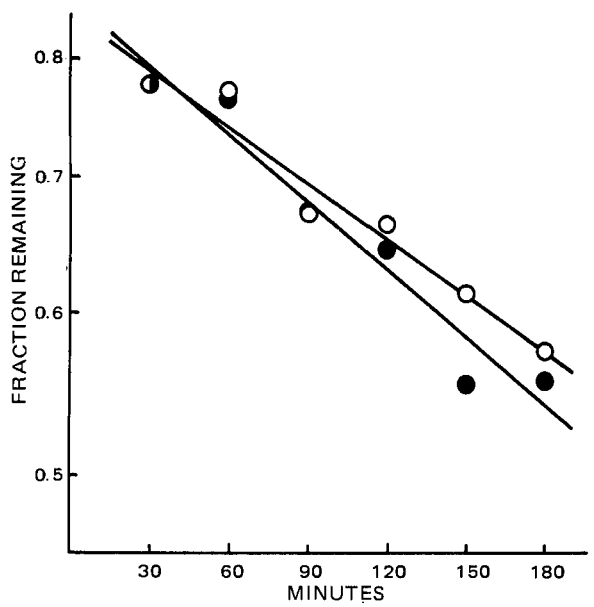

Figure 6—*Effect of 850 rad of γ-radiation on the absorption of riboflavin (initial concentration of 20 μg/ml) from the* in situ *rat intestine at 5 days postirradiation. Key: ●, irradiated; and ○, sham irradiated. Each point represents the mean of at least five animals.*

In general, prior irradiation of the small intestine tends to reduce its apparent permeability to drugs in an indirect manner by reducing the rate of water absorption by the intestine. In addition, the drug absorption rate may be reduced following irradiation due to a reduction in mesenteric blood flow, particularly for rapidly absorbed drugs. The magnitude of the observed radiation-induced reduction in the intestinal absorption rate was relatively small. If such a reduction in absorptive capacity occurred in humans following radiation therapy, it would not be clinically significant, although higher doses of radiation might have a more pronounced effect on intestinal drug absorption.

REFERENCES

(1) M. E. Brady and W. L. Hayton, *J. Pharm. Sci.*, **66**, 361 (1977).

(2) *Ibid.*, **66**, 366 (1977).

(3) H. R. Withers, *Cancer*, **28**, 75 (1971).

(4) K. E. Carr and P. G. Toner, *Virchows Arch. B*, **11**, 201 (1972).

(5) W. L. Hayton, *J. Pharm. Sci.*, **63**, 645 (1974).

(6) M. J. Mattila, S. Takki, and L. R. Holsti, *Arzneim.-Forsch.*, **18**, 889 (1968).

(7) M. J. Mattila, L. R. Holsti, V. M. K. Venho, and S. Takki, *ibid.*, **20**, 533 (1970).

(8) J. T. Doluisio, N. F. Billups, L. W. Dittert, E. T. Sugita, and J. V. Swintosky, *J. Pharm. Sci.*, **58**, 1196 (1969).

(9) W. L. Hayton and G. Levy, *ibid.*, **61**, 362 (1972).

(10) H. Ochsenfahrt and D. Winne, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **281**, 175 (1974).

(11) *Ibid.*, **281**, 197 (1974).

(12) S. Kojima and J. Miyake, *Chem. Pharm. Bull.*, **23**, 1247 (1975).

(13) R. M. Levine, M. R. Blair, and B. B. Clark, *J. Pharmacol. Exp. Ther.*, **114**, 78 (1955).

(14) S. Mizukami and K. Nagata, *Ann. Rep. Shionogi Res. Lab.*, **6**, 58 (1956); through *Chem. Abstr.*, **51**, 4560b (1957).

(15) R. E. Notari, J. L. DeYoung, and R. H. Reuning, *J. Pharm. Sci.*, **61**, 135 (1972).

(16) D. Perrier and M. Gibaldi, *ibid.*, **62**, 225 (1973).

(17) G. Janossy, *Acta Med. Acad. Sci. Hung.*, **26**, 13 (1969).

(18) J. Kabal, S. J. Baum, and L. J. Parkhurst, *Radiat. Res.*, **50**, 528 (1972).

(19) H. Ochsenfahrt and D. Winne, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **279**, 133 (1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 29, 1976, from the *College of Pharmacy, Washington State University, Pullman, WA 99164.*

Accepted for publication July 1, 1976.

Presented in part at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, San Francisco meeting, April 1975.

Abstracted from a dissertation submitted by M. E. Brady to Washington State University in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported in part by funds provided for biological and medical research by Washington State University Initiative Measure No. 171.

The authors appreciate the gifts of sulfisoxazole acetyl provided by Dr. W. E. Scott, Research Division, Hoffmann-La Roche Inc., and bretylium tosylate provided by Dr. R. A. Maxwell, Burroughs Wellcome Co.

* AFPE Paul M. Scott Memorial Fellow. Present address: College of Pharmacy, University of Cincinnati, Cincinnati, OH 45221.

ˣ To whom inquiries should be directed.