Table IV—Analysis of Variance for 2-hr Data, Study II, Antipyresis in Rats

Source	*df*	*MS*	*F*
Treatments	20	1.310	9.51
Control *versus* preparations	1	3.197	23.2
Among preparations	4	0.078	0.57
Regression	1	20.566	149.3
Deviation from parallelism	4	0.216	1.57 N.S.
Deviation from linearity	10	0.126	0.92 N.S.
Error	143	0.138	
Total	163		

and dissolution characteristics of the various preparations (7), additives that alter the absorptive processes in the GI tract (8), particle size (9, 10), fasted *versus* nonfasted subjects (7), and normal *versus* febrile (disease) states (11). In these studies, nonuniformity of dose was controlled by testing all preparations at equivalent aspirin doses. The disintegration parameter was controlled by allowing complete disintegration of tablets prior to dosing the animal. Since it has been pointed out that the amounts of buffering agents contained in analgesic products are probably insufficient in quantity to affect drug action or gastric pH (8, 12), this parameter was considered insignificant. Finally, all animals were fasted and febrile. However, one parameter was not controlled: particle size.

The particle size of a drug influences dissolution or disintegration rates of tablets (9) and absorption from the GI tract (10, 13). Study 2 attempted, in part, to control particle size more closely. In this second study, in which testing parameters were more tightly controlled, the data obtained indicated statistically significant antipyresis for all test preparations, and no statistical or pharmacological differences could be determined among the various test preparations.

Relatively few reports have been published wherein an attempt was made to ascertain the variability of pharmacological response relative to controlled particle sizes. One report (11) detailed the variability of responses not only due to different particle sizes but also due to differences in dissolution rates and absorption rates, all of which are interdependent. An interesting factor was noted, *i.e.*, that the plasma concentration and time course activity of aspirin not only differs with the particle size of aspirin crystals but also differs between febrile and normal rabbits; febrile rabbits obtain faster, higher, and longer blood levels than the nonfevered controls.

Additionally, data reported (11) for the antipyretic activity of various aspirin preparations of different particle sizes in fevered rabbits correlate with the present data for fevered rats dosed with random particle-size aspirin. Itami *et al.* (11) concluded that the data supported the theory that as the particle size of aspirin decreases, antipyretic activity is more pronounced and more rapid in onset. Study 2 results also supported this theory, particularly at low dose levels where the antipyretic activity (Table II) was markedly different than the activity of the larger sized aggregates (Table I).

On the other hand, the favorable improvement in the physical properties of aspirin crystals may be reversed when subjected to the tableting process (9), a factor that also could compromise much of the pharmacological benefits derived from smaller crystal size. Thus, the recent suggestion (14) that aspirin tablets be crushed or chewed to a fine powder prior to being swallowed merits some attention.

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Effect of Temperature and Relative Humidity on Nitrazepam Stability in Solid State

D. GENTON and U. W. KESSELRING ˣ

Abstract □ The decomposition of a 1% dilution of nitrazepam in microcrystalline cellulose was established by quantitative determination of the two main breakdown products, 2-amino-5-nitrobenzophenone and 3-amino-6-nitro-4-phenyl-2(1*H*)-quinolone, using *in situ* diffuse reflectance measurements on thin-layer chromatograms. The decomposition and formation rate constants of nitrazepam and of the breakdown products, respectively, were determined at four temperatures and six relative humidities. By means of a three-parameter regression equation, it was possible to correlate quantitatively the decomposition constant of nitrazepam to both temperature and relative humidity.

Keyphrases □ Nitrazepam, solid—decomposition in microcrystalline cellulose, effect of temperature and humidity □ Decomposition—nitrazepam in solid state in microcrystalline cellulose, effect of temperature and humidity □ Stability—nitrazepam in solid state in microcrystalline cellulose, effect of temperature and humidity □ Anticonvulsants—nitrazepam in solid state in microcrystalline cellulose, decomposition, effect of temperature and humidity □ Solid state—decomposition, nitrazepam in microcrystalline cellulose, effect of temperature and humidity

Stability studies of drugs in pure form and in solid dosage forms were reviewed and summarized previously (1, 2). Apparently, no workers have attempted to combine the influences of both temperature and humidity (or water content) in a unique mathematical expression.

The purpose of this work was to assess quantitatively

Table I—Sample Storage Conditions

Temperature[a]			
42.5°	55.0°	73.8°	83.4°
Relative Humidity[b], %			
29.9	30.6	32.1	29.3
38.6	55.3	40.9	38.1
47.9	49.2	51.7	53.1
60.6	60.3	59.3	60.2
66.3	67.5	69.0	70.0
82.2	82.7	83.8	83.8

[a] Mean values of 30 control measurements taken with a precision thermometer in 40 days. The corresponding standard deviations are ±0.6, ±0.1, ±1.4, and ±1.3° for 42.5, 55.0, 73.8, and 83.4°, respectively. [b] Precision was better than ±2% using a SINAequiHygroscope type eZFBA-PP, Sina AG, Zurich, Switzerland.

the separate and combined influences of temperature and humidity on the decomposition of a 1% dilution of nitrazepam in microcrystalline cellulose.

EXPERIMENTAL

Compounds—Nitrazepam[1] (1,3-dihydro-7-nitro-5-phenyl-2*H*-1,4-benzodiazepin-2-one) (I), 2-amino-5-nitrobenzophenone[1] (II), 3-amino-6-nitro-4-phenyl-2(1*H*)-quinolone[1] (III), and microcrystalline cellulose[2] were used as received. All reagents were of analytical grade or Pharm. Helv. VI quality.

Sample Storage Conditions—Individual samples of about 300 mg of microcrystalline cellulose containing 1.00% nitrazepam were stored as described previously (3) at relative humidities of about 30, 40, 50, 60, 70, and 80% (4) and at around 40, 55, 70, and 85° (Table I).

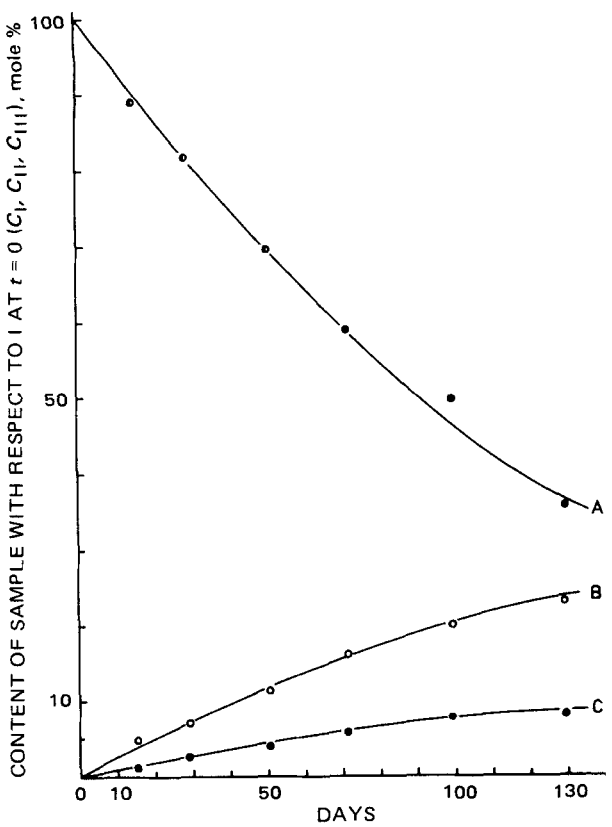
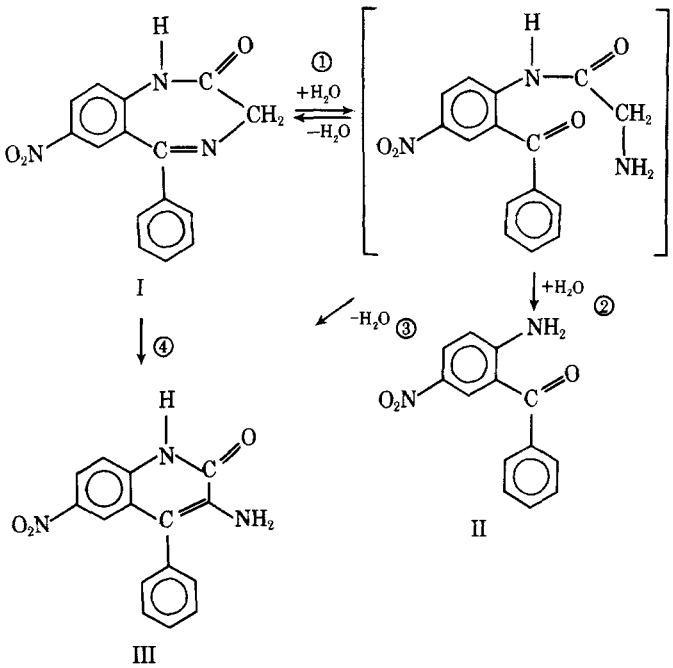


Figure 1—*Nitrazepam stability at 38.1% relative humidity and 83.4°. Key: A, decomposition of I; B, formation of II; and C, formation of III.*

[1] F. Hoffmann–La Roche & Co. SA., Basel, Switzerland.
[2] Avicel PH-101, Interchemie, Zurich, Switzerland.



Scheme I

Quantitative Analysis—The quantitative analysis consisted essentially of extraction of I–III with methanol, their separation by TLC, and their quantitative determination by *in situ* diffuse reflectance measurements.

Extraction—Methanol, 3.00 ml, was added to the sample and vigorously shaken for 10 min. After the microcrystalline cellulose formed a deposit, the liquid layer was collected and centrifuged for 5 min. The clear solution, transferred to a hermetically sealable test tube, constituted the sample solution and eventually was diluted for quantitative determination, depending on the degree of decomposition.

Separation by TLC—The stationary phase was 0.40-mm kieselgel GF_{254}[3]-coated plates, dried for 12 hr at room temperature and activated just before use at 105° for 10 min. The mobile phase was benzene–ethyl acetate–acetic acid (60:36:4). The chamber saturation time was 1 hr, and the development time was 1 hr for the whole plate (about 18 cm).

Standard solutions, which were stable for about 6 months if stored away from light and at 4°, were at concentrations of 0.04–0.26, 0.02–0.10, and 0.02–0.10 mg/ml in methanol for I, II, and III, respectively.

One plate was used for the assay of each substance. With a precision syringe, seven spots (five standards and two samples) of 5 μl, one every 2.5 cm, were applied in increasing order of standard concentration with the samples placed third and fifth. The developed chromatograms were dried for 40 min in a cold air stream. The spots were located by visualizing with UV light at 254 nm.

Quantitative Evaluation—The spots were quantitatively evaluated by diffuse reflectance measurements[4] at 265, 365, and 295 nm for I, II, and III, respectively. The instrument conditions were: diaphragm opening, 12 mm; amplification, 10/10/I/A; scanning speed of the chromatogram, 3 cm/min; and chart flow (log scale), 10 cm/min. The peak surfaces, determined by direct integration[5], were linearly related to concentration in the ranges of 0–1.3 μg for I and 0–0.5 μg for II and III.

Kinetics (5)—The following decomposition scheme, reported previously (6–8) and confirmed by this work, shows the main decomposition routes of nitrazepam in the solid state under the influence of temperature and humidity (Scheme I). The bimolecular reactions 1 and 2 are acid–base catalyzed and depend on the amount of water available. The main decomposition product is II in aqueous solution and III in the solid state, with the II–III ratio depending on the availability of water (6). For low water concentrations, reactions 2 and 3 are competitive with preferential formation, especially in the solid state, of the quinolone derivative. The isomerization reaction 4 occurs only around and above the fusion temperature.

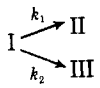
[3] E. Merck, Darmstadt, Germany.
[4] Zeiss PMQ II densitometer, Carl Zeiss, Oberkochen, Germany.
[5] W + W model 3212 recorder, Electronic Inc., Basel, Switzerland.

Table II—Experimental Values of k_1 and k_2

Temperature	Relative Humidity, %	k_1, day^{-1} × 10^5	y_0[a], %	r^2[b]	k_2, day^{-1} × 10^5	y_0[c], %	r^2[b]
42.5°	38.6	—	—	—	2.83	0.07	0.946
	47.9	2.69	0.01	0.998	2.97	0.08	0.900
	60.6	6.77	−0.25	0.887	11.67	−0.13	0.968
	66.3	6.09	−0.10	0.960	26.81	0.01	0.992
	82.2	5.30	−0.19	0.654	21.43	0.48	0.952
55.0°	30.6	—	—	—	4.42	0.03	0.981
	49.2	6.28	−0.06	0.867	12.48	−0.05	0.960
	55.3	11.03	−0.31	0.976	17.28	0.07	0.978
	60.3	14.73	−0.20	0.955	29.01	0.41	0.985
	67.5	32.26	−0.25	0.895	86.14	−0.27	0.993
	82.7	26.92	−0.47	0.810	101.11	0.18	0.966
73.8°	32.1	7.55	0.02	0.850	14.89	0.45	0.858
	41.0	19.10	−0.08	0.927	37.51	0.25	0.982
	51.7	29.05	0.02	0.950	84.99	0.48	0.988
	59.3	90.43	0.46	0.965	282.32	0.27	0.994
	69.0	246.89	0.47	0.979	482.52	0.25	0.996
	83.8	130.84	0.03	0.971	487.64	−0.17	0.989

[a] y_0 = y-intercept (*cf.*, Eq. 4); theoretically zero. [b] r^2 = coefficient of determination. [c] y_0 = y-intercept (*cf.*, Eq. 5); theoretically zero.

Nitrazepam (I) decomposes in two pseudo-first-order parallel reactions to II and III according to Scheme II:



Scheme II

and:

$$C_I = C_{I_0}e^{-Kt} \quad \text{(Eq. 1)}$$

$$\log C_I = 0.4343Kt + \log C_{I_0} \quad \text{(Eq. 2)}$$

$$K = k_1 + k_2 \quad \text{(Eq. 3)}$$

$$C_{II} = \frac{k_1}{K} C_{I_0}(1 - e^{-Kt}) \quad \text{(Eq. 4)}$$

$$C_{III} = \frac{k_2}{K} C_{I_0}(1 - e^{-Kt}) \quad \text{(Eq. 5)}$$

where k_1 = formation constant of II, k_2 = formation constant of III, K = decomposition constant of I, C = mole percent content of the sample with respect to I at time t, and C_{I_0} = mole content of I at time t = 0 (=100%).

The value of K is obtained from the slope of the regression line calculated through Eq. 2. The obtained K values are used in Eqs. 4 and 5, whose corresponding regression lines reveal k_1 and k_2.

Table III—Experimental Values of K

Temperature	Relative Humidity, %	K, day^{-1} × 10^5	Confidence Interval, p = 0.05, day^{-1} × 10^5	n[a]	r^2[b]
42.5°	38.6	2.83	0.94	7	0.946
	47.9	5.98	1.72	5	0.976
	60.6	18.74	2.67	11	0.965
	66.3	32.68	3.08	7	0.993
	82.2	26.73	1.83	13	0.987
55.0°	30.6	4.42	1.30	5	0.975
	49.2	19.57	5.85	6	0.956
	55.3	28.72	2.18	10	0.990
	60.3	43.95	3.27	12	0.988
	67.5	120.64	27.55	4	0.984
	82.7	127.47	10.19	11	0.988
73.8°	32.1	24.82	4.27	7	0.979
	41.0	57.46	6.62	11	0.980
	51.7	125.29	21.83	7	0.978
	59.3	371.29	50.14	11	0.977
	69.0	729.96	55.91	5	0.990
	83.8	618.65	65.92	10	0.985
83.4°	29.3	567.83	89.54	7	0.981
	38.1	777.17	76.50	7	0.992
	53.1	1990.92	260.56	9	0.978
	60.2	3037.61	497.55	6	0.986
	70.0	5580.19	1253.77	3	0.999
	83.8	4612.95	1295.45	5	0.977

[a] n = number of experimental points. [b] r^2 = coefficient of determination.

RESULTS

Substances II and III are the only important decomposition products of I; both have to be followed to establish the decomposition kinetics of I (Figs. 1 and 2). Substances II and III were considered stable, although a few unidentified compounds (seemingly breakdown products of II and III) were observed in samples stored at 73.8 and 83.4° and high humidities. The appearance of these unidentified products was automatically detected on the chromatograms and helped establish the time limits for the system for the calculation of rate constants.

At 83.4°, the instability of II and III was such that only the disappearance constant K could be calculated (Tables II and III). For temperatures up to 83.4° and relative humidities up to 70%, the rate constants

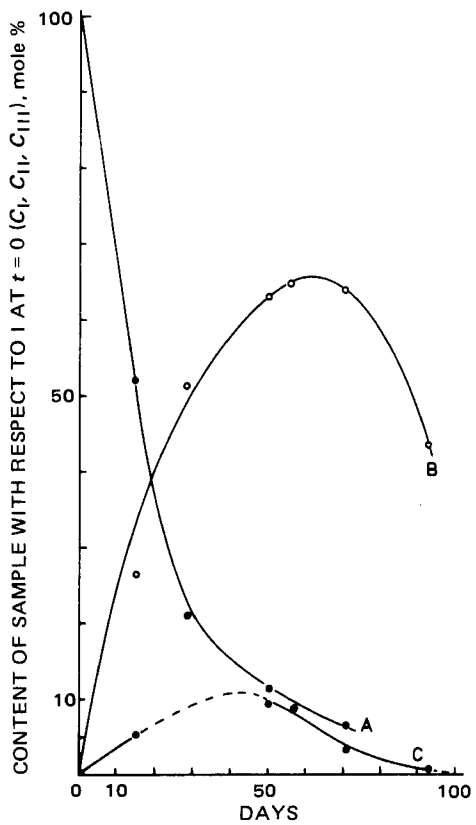


Figure 2—*Nitrazepam stability at 83.8% relative humidity and 83.4°. Key: A, decomposition of I; B, formation and decomposition of II; and C, formation and decomposition of III.*

Table IV—Regression Line Parameters for Logarithm $K \times 10^5$ as a Function of Relative Humidity

Temperature	42.5°	55.0°	73.8°	83.4°
Slope	0.03838	0.03718	0.04052	0.02492
Confidence interval, $p = 0.05$	0.00393	0.00960	0.00749	0.00348
y-Intercept	−1.0437	−0.5316	0.08549	1.9849
Confidence interval, $p = 0.05$	0.2141	0.5193	0.3917	0.1819
Coefficient of determination	0.988	0.980	0.990	0.994

followed an exponential pattern proportional to relative humidity. At 80% relative humidity, they diminished significantly. At about 75% relative humidity, an instability maximum was apparent at all temperatures (Fig. 3).

The parameters defining the regression equations relating log K, for a given temperature, to relative humidities are summarized in Table IV.

The slope values, discarding the result for 83.4°, were all within the confidence interval of each other. Therefore, if the straight lines are considered to be parallel with a slope equal to the mean value of the observed slopes at the three temperatures, then:

$$\log K \times 10^5 = 0.03869\% \text{ relative humidity} + A \qquad \text{(Eq. 6)}$$

where A represents a constant depending on temperature. Its values are obtained by putting the mean $\bar{x}, \bar{y}$ values of the regression lines for 42.5, 55.0, and 73.8° in Eq. 6. This approach yields $A = -1.0602$, -0.6105, and 0.1785 for the three temperatures, respectively. Treating the variation of A as a linear function of the reciprocal temperature, one obtains an Arrhenius-like relationship:

$$A = -4.3576 \times 10^3 \frac{1}{T} + 12.7207 \qquad \text{(Eq. 7)}$$

If Eqs. 6 and 7 represent truly straight lines, the decomposition constants of nitrazepam must be linearly related to both relative humidity and the reciprocal absolute temperature, in which case one might as well calculate directly the equation of the corresponding regression plane:

$$X_1 = a + bX_2 + cX_3 \qquad \text{(Eq. 8)}$$

where $X_1 = \log K \times 10^5$, $X_2 = 10^3\ T^{-1}$ (K), X_3 = relative humidity (percent), a = constant depending on the system, b = partial regression coefficient of log K on T^{-1} (relative humidity = constant), and c = partial regression coefficient of log K on relative humidity (T = constant).

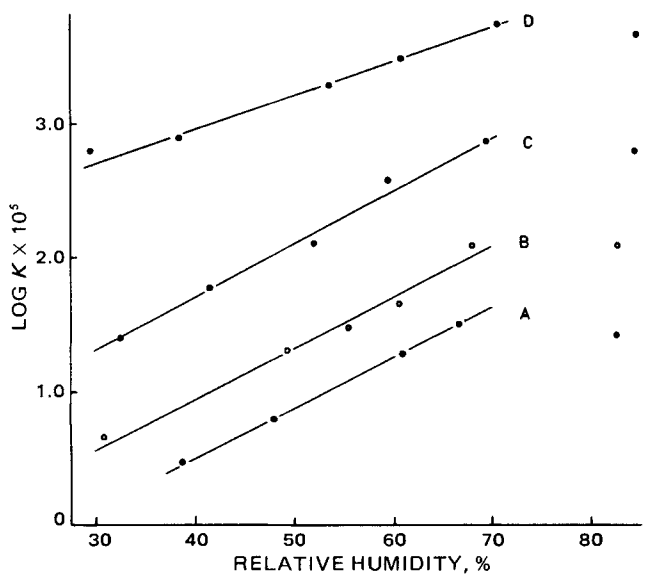


Figure 3—*Logarithm of the nitrazepam decomposition constant,* K, *as a function of relative humidity at various temperatures. Key: A, 42.5°; B, 55.0°; C, 73.8°; and D, 83.4°.*

Table V—Values of $t_{0.9}$ for Various Climatic Conditions

Climatic Condition	log $K \times 10^5$	$K \times 10^5$	$t_{0.9}$, years
North of Europe 18°; 50% relative humidity	−0.331	0.46	62.8
Central Europe 20°; 65% relative humidity	0.353	2.25	12.9
Tropical climate 30°; 70% relative humidity	1.040	11.0	2.7

The coefficients a, b, and c are found by solving the following system of equations (9):

$$\sum X_1 = aN + b\sum X_2 + c\sum X_3 \qquad \text{(Eq. 9)}$$

$$\sum X_1X_2 = a\sum X_2 + b\sum X_2^2 + c\sum X_2X_3 \qquad \text{(Eq. 10)}$$

$$\sum X_1X_3 = a\sum X_3 + b\sum X_2X_3 + c\sum X_3^2 \qquad \text{(Eq. 11)}$$

where N represents the number of storage conditions defined by temperature and relative humidity. Thus, the final result is obtained for all storage conditions where $T < 75°$ and relative humidity <80% (N = 14):

$$X_1 = 12.783 - 4.3804X_2 + 0.03877X_3 \qquad \text{(Eq. 12)}$$

or:

$$\log K \times 10^5 = 12.783 - 4.3804 \times 10^3T^{-1} + 0.03877\ (\%\text{ relative humidity}) \qquad \text{(Eq. 13)}$$

with a regression coefficient of $R_{1.23} = 0.995$. Figure 4 represents the plane corresponding to Eq. 13. Extrapolation with respect to T and interpolation with respect to relative humidity of the plane regression equation yields K for any other desired climatic condition. Therefore, the time interval $t_{0.9}$, after which only 90% of the initial nitrazepam concentration is left, may be calculated (Table V).

DISCUSSION

The presented results do not suggest adherence to any published model. In particular, the rate of disappearance of nitrazepam in a 1% dilution in microcrystalline cellulose apparently does not follow a sigmoid decomposition pattern like aspirin (10), and it does not reach an equilibrium level like thiamine hydrochloride in microcrystalline cellulose (11). Although this latter compound displays an instability maximum at a water content of about 5%, no comparison can be made with the instability maximum shown for the nitrazepam system at 75% relative humidity; no relationship between water content and relative humidity has yet been established, and thiamine hydrochloride is water soluble

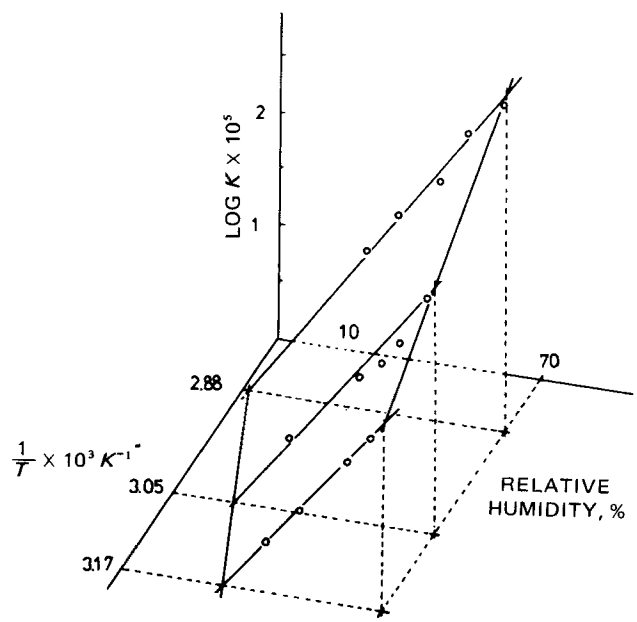


Figure 4—*Variation of log* K *as a function of relative humidity and reciprocal temperature.*

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.

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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.