Drug Absorption IV: Influence of pH on Absorption Kinetics of Weakly Acidic Drugs

W. G. CROUTHAMEL, G. H. TAN, L. W. DITTERT, and J. T. DOLUISIO

Abstract □ This paper reports the absorption kinetics of sulfaethidole and barbital from the rat stomach and rat small intestine *in situ*. The absorption rates varied with pH for both drugs. With both drugs, absorption from the intestines proceeded 10 times faster at a given pH than from the stomach. Rate constants for the transport of unionized and ionized drug moieties were determined from the stomach and from the intestine; in all cases, significant transport of ionized drug was noted.

Keyphrases □ Pharmacokinetics, gastrointestinal absorption, pH effect—sulfaethidole and barbital, rats □ Drug absorption, gastrointestinal, pH effect—sulfaethidole and barbital, rats □ pH-partition coefficients—gastrointestinal drug absorption *in situ*, rats □ Membrane transport, gastrointestinal drug absorption—ionized and unionized sulfaethidole rate constants, pH effect

Since the pH-partition hypothesis of gastrointestinal drug absorption was first proposed by Shore *et al.* (1), reports have appeared both agreeing and disagreeing with the theory. For example, Hogben *et al.* (2) found that the gastric absorption of several drugs appeared to obey the pH-partition theory, whereas the intestinal absorption of the same drugs deviated from the theory. The latter deviations were explained by hypothesizing the existence of a layer at the intestinal mucosal surface with a pH different from that of the bulk intestinal lumen fluid. If the "virtual pH" at the membrane surface was assumed to be 5.3, the compounds studied by Hogben *et al.* (2) obeyed the pH-partition theory. Other investigators (3, 4) postulated virtual pH's at 6 or 7 to explain intestinal absorption data.

Factors such as drug binding to intestinal mucosa or the absorption of ionized drug may also be responsible for deviations from the pH-partition hypothesis. For example, Kakemi *et al.* (5) found that the absorption-rate constants for a series of barbituric acids in an *in situ* rat intestinal preparation did not always follow the expected changes when pH was altered; they suggested that binding to intestinal mucosa also had to be taken into account.

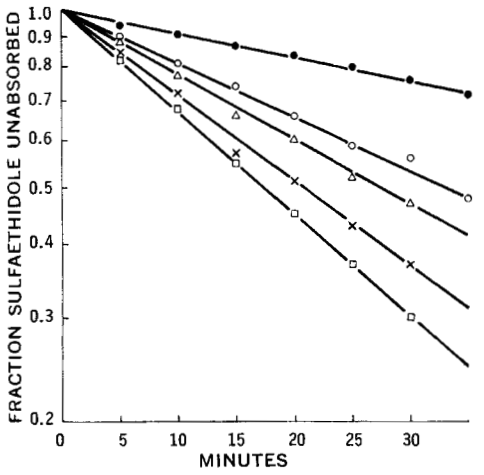

Figure 1—*Semilogarithmic plots of the disappearance of sulfaethidole from the lumen of rat intestine* in situ *at various lumen solution pH's. Key:* ●, *pH 8.6,* $t_{1/2}$ = *81 min.;* ○, *pH 6.1,* $t_{1/2}$ = *34 min.;* △, *pH 5.75,* $t_{1/2}$ = *28 min.;* ×, *pH 5.45,* $t_{1/2}$ = *20 min.; and* □, *pH 5.1,* $t_{1/2}$ = *17 min.*

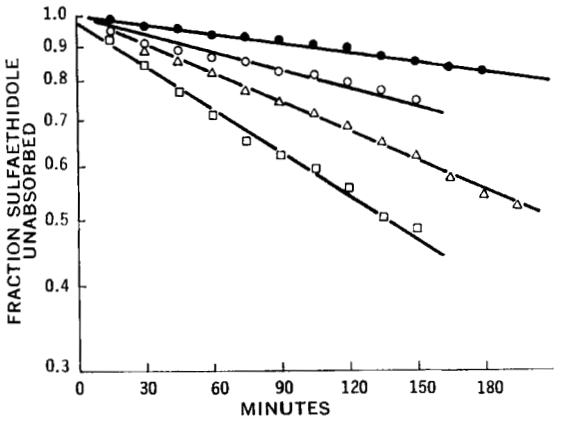

Figure 2—*Semilogarithmic plots of the disappearance of sulfaethidole from the lumen of rat stomach* in situ *at various lumen solution pH's. Key:* ●, *pH 8.3,* $t_{1/2}$ = *637 min.;* ○, *pH 5.9,* $t_{1/2}$ = *325 min.;* △, *pH 5.35,* $t_{1/2}$ = *210 min.; and* □, *pH 3.45,* $t_{1/2}$ = *143 min.*

Nogami *et al.* (6) showed that the ionized as well as the unionized forms of sulfonamides were absorbed through isolated rat intestinal segments *in vitro*, and Turner *et al.* (7) tested the pH-partition hypothesis experimentally in everted intestinal sacs *in vitro*. The latter authors concluded that the virtual pH hypothesis of Hogben *et al.* (2) appeared to be inoperative in *in vitro* preparations, and such preparations do not always mimic *in vivo* behavior, especially with regard to the transport of ions which occurs to a much greater degree *in vitro* than *in vivo*.

If the pH-partition hypothesis is correct, acidic drugs might be expected to be absorbed preferentially from the stomach and basic drugs preferentially from the intestines, since at these sites the respective drug classes would be ionized to a lesser degree than at other gastrointestinal sites. However, anatomical and functional differences such as total surface area, blood supply, and transit time greatly influence the rate and extent of absorption at the two sites; in actual practice, the intestines may be the primary absorption site for both weak acids and weak bases. The purpose of the present investigation was to study the influence of pH on the rates of absorption of weakly acidic drugs from *in situ* rat stomach and small intestinal preparations (8). It was expected that the results would reveal differences between the two absorption sites with regard to kinetics of drug transfer of unionized and ionized drug species.

Table I—Sulfaethidole Absorption Rates ($k_{app.}$) and Fraction of Drug Unionized (f_U) at Various pH Values

Average pH (Range)	Buffer[a]	Fraction Unionized (f_U)	Half-Life, min.	$k_{app.}$, min.$^{-1}$
Rat Intestine *In Situ*				
5.20 (5.0–5.4)	C	0.67	17	0.0408
5.10 (5.0–5.2)	C	0.72	17	0.0408
5.45 (5.3–5.6)	P	0.53	21	0.0330
5.45 (5.3–5.6)	P	0.53	20	0.0346
5.75 (5.7–5.8)	P	0.36	28	0.0248
5.80 (5.7–5.9)	P	0.33	29	0.0239
6.05 (6.0–6.1)	P	0.22	30	0.0231
6.10 (6.0–6.2)	P	0.20	34	0.0204
8.60 (9.0–8.2)	B	0.001	81	0.0086
8.45 (9.0–7.9)	B	0.001	79	0.0088
Rat Stomach *In Situ*				
3.45 (3.0–3.9)	C	0.99	143	0.00485
5.15 (5.3–5.0)	C	0.69	156	0.00444
4.65 (5.3–4.0)	C	0.88	150	0.00462
4.90 (5.3–4.5)	C	0.80	150	0.00462
5.50 (5.7–5.3)	C	0.50	246	0.00282
5.45 (5.7–5.2)	C	0.53	243	0.00285
5.35 (5.7–5.0)	C	0.59	210	0.00330
6.40 (6.5–6.3)	C	0.11	338	0.00205
6.35 (6.5–7.2)	C	0.12	323	0.00215
5.90 (6.0–5.8)	C	0.28	325	0.00213
5.85 (6.0–5.7)	C	0.31	275	0.00252
8.20 (9.0–7.5)	B	0.002	690	0.00100
8.30 (9.0–7.6)	B	0.002	637	0.00109

[a] C = McIlvaine's citrate–phosphate buffer (14), P = 0.09 *M* phosphate buffer made isotonic with NaCl, and B = Clark and Lubs borate buffer (14).

EXPERIMENTAL

Apparatus and Reagents—All chemicals were reagent grade except sulfaethidole[1] and sodium barbital[2]. A Beckman Zeromatic II pH meter and a Cary model 15 spectrophotometer were used. The perfusion and drug solutions used were described in a previous communication (8). The initial concentrations of sulfaethidole and sodium barbital were approximately 2.5×10^{-3} and 5×10^{-3} *M*, respectively.

Test Animals—Male Sprague-Dawley albino rats, weighing 220–260 g., were fasted for 15–20 hr. prior to use.

Procedure—The procedure for studying drug disappearance from the *in situ* rat stomach and rat small intestinal lumen was described previously (8).

Analytical Methods—Barbital was extracted from the samples with chloroform and then from the chloroform into a pH 9.4 borate buffer; the absorbance of the buffer solution was determined at 242 nm. Sulfaethidole was analyzed by the method of Bratton and Marshall (9).

RESULTS AND DISCUSSION

Semilogarithmic plots, showing the disappearance of sulfaethidole from rat intestinal lumen *in situ* at average pH's between 5.15 and 8.52 and from rat gastric lumen *in situ* at average pH's between 3.45 and 8.30, are shown in Figs. 1 and 2. In both preparations, all disappearances followed first-order kinetics, and the disappearance-rate constants increased (half-lives decreased) as the pH was lowered. Similar results were obtained with barbital. With both drugs, absorption from the intestines proceeded about 10 times faster at a given pH than from the stomach, reflecting the difference in surface area of these two organs. The half-lives and apparent first-order rate constants for the disappearances of sulfaethidole and barbital are summarized in Tables I and II. In all cases, the pH of the lumen solutions shifted several tenths of a unit during the experiment, and the average of the initial and terminal pH's was used to calculate the fraction of drug in the unionized form during the experiment. These fractions are also shown in Tables I and II.

[1] Smith Kline & French Laboratories.
[2] Merck & Co.

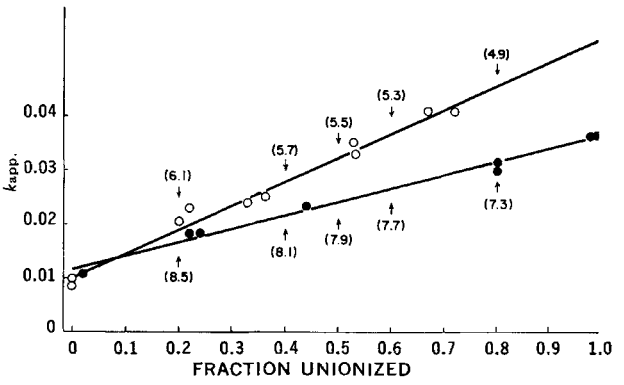

Figure 3—*Plots showing the relationship between* $k_{app.}$, *the rate constant controlling overall disappearance of drug from rat intestinal lumen* in situ, *and the fraction of drug present in the unionized form. Key:* ○, *sulfaethidole; and* ●, *barbital. The numbers in parentheses are pH's at various points on the respective curves.*

If it is assumed that both the unionized and the ionized drug species are capable of being absorbed and that the apparent first-order absorption-rate constant ($k_{app.}$) at a given pH is the sum of the rate constants attributable to the two species at that pH, then the influence of pH on the rate of absorption of a weak electrolyte can be expressed as (10):

$$k_{app.} = f_U k_U + f_I k_I \qquad \text{(Eq. 1)}$$

where k_U and k_I are the rate constants controlling absorption of the unionized and ionized species, respectively, and f_U and f_I are the fractions of the drug present in the unionized and ionized forms, respectively. Since:

$$_I = (1 - f_U) \qquad \text{(Eq. 2)}$$

Eq. 1 can be rewritten:

$$k_{app.} = k_I + f_U(k_U - k_I) \qquad \text{(Eq. 3)}$$

Table II—Barbital Absorption Rates ($k_{app.}$) and Fraction of Drug Unionized (f_U) at Several pH Values

Average pH (Range)	Buffer[a]	Fraction Unionized (f_U)	Half-Life, min.	$k_{app.}$, min.$^{-1}$
Rat Intestine *In Situ*				
6.10 (6.0–6.2)	P	0.98	19	0.0365
6.10 (6.0–6.2)	P	0.98	19	0.0365
6.05 (6.0–6.1)	P	0.99	19	0.0365
7.30 (7.5–7.1)	P	0.80	23	0.0301
7.30 (7.5–7.1)	P	0.80	22	0.0315
7.70 (8.1–7.3)	P	0.44	30	0.0231
8.40 (8.5–8.3)	P	0.24	38	0.0182
8.45 (8.5–8.4)	B	0.22	38	0.0182
9.70 (10.0–9.4)	B	0.02	65	0.0107
Rat Stomach *In Situ*				
3.20 (3.00–3.40)	C	1.00	222	0.00312
3.20 (3.00–3.40)	C	1.00	201	0.00345
5.95 (6.00–5.90)	P	0.99	232	0.00299
5.90 (6.00–5.80)	P	0.99	240	0.00289
6.00 (6.00–6.00)	P	0.99	260	0.00267
6.40 (7.00–5.80)	B	0.97	232	0.00299
6.45 (7.00–5.90)	B	0.96	242	0.00289
7.45 (8.00–6.90)	B	0.74	290	0.00239
7.45 (8.00–6.90)	B	0.74	315	0.00220
7.50 (8.00–7.00)	B	0.71	375	0.00185
7.85 (8.50–7.20)	B	0.53	340	0.00204
7.85 (8.50–7.20)	B	0.53	330	0.00210
7.85 (8.50–7.20)	B	0.53	380	0.00182
7.85 (8.50–7.20)	B	0.53	320	0.00217
7.85 (8.50–7.20)	B	0.53	390	0.00180
8.20 (9.00–7.40)	B	0.33	390	0.00178
8.40 (9.00–7.80)	B	0.24	465	0.00149

[a] C = McIlvaine's citrate–phosphate buffer (14), B = Clark and Lubs borate buffer (14), and P = 0.09 *M* phosphate buffer made isotonic with NaCl.

Table III—Rate Constants (min.$^{-1}$) for Absorption of Unionized (k_U) and Ionized (k_I) Sulfaethidole and Barbital from Rat Gastrointestinal Segments *In Situ*

Drug	pKa	Gastrointestinal Segment	Range of f_U Studied	k_U	k_I	k_U/k_I
Sulfaethidole	5.5	Intestine	0.002–0.72	0.055	0.0099	5.55
		Stomach	0.002–0.99	0.0051	0.0012	4.25
Barbital	7.9	Intestine	0.02–0.99	0.037	0.012	3.08
		Stomach	0.24–1.00	0.0029	0.0009	3.22

According to Eq. 3, a plot of $k_{app.}$ *versus* f_U is a straight line, with intercepts of k_I (at $f_U = 0$) and k_U (at $f_U = 1.0$) and a slope equal to $(k_U - k_I)$.

Recently Suzuki *et al.* (11, 12) proposed physical models for drug absorption. The data shown in Tables I and II are consistent with their Model III (12) where the membrane phase is heterogeneous (aqueous and lipid), simulating a membrane consisting of lipoidal cells in an aqueous intercellular fluid environment. For this model, it would be expected that the unionized moiety could pass with differing ease through both the aqueous and nonaqueous environments while the ionized moiety could pass through only the aqueous. Hence, Eq. 1 can be modified to:

$$k_{app.} = f_U(k_{U,lipid} + k_{U,aqueous}) + f_I k_I \qquad \text{(Eq. 4)}$$

Equation 4 could then be modified to:

$$k_{app.} = k_I + f_U(k_{U,lipid} + k_{U,aqueous} - k_I) \qquad \text{(Eq. 5)}$$

Thus, a plot of $k_{app.}$ *versus* f_U should yield a straight line with intercepts of k_I (at $f_U = 0$) and $k_{U,lipid} + k_{U,aqueous}$ (at $f_U = 1.0$), and a slope of $(k_{U,lipid} + k_{U,aqueous} - k_I)$ if the physical model is correct. Equations 3 and 5 both yield linear plots, and the present data fit both equations. Equation 5 assumes a heterogeneous physical model where ionized drug transport occurs through aqueous portions of the membrane. Equation 3 assumes that ionized drug passes the same barrier(s) as the unionized moiety but with differing ease. Consequently, Eqs. 3 and 5 lead to slightly different interpretations, but both cases provide for absorption of the ionized drug moiety. Equation 3 is probably the more correct expression. For the data reported in this paper, the value of $k_{U,lipid}$, calculated from Eq. 5 by assuming that $k_{I,aqueous} = k_{U,aqueous}$, would be approximately 26% greater than the values of k_U calculated with Eq. 3 and reported in Table III.

Plots according to Eq. 3 for the absorption of sulfaethidole and barbital from the rat intestine *in situ* are shown in Fig. 3. Similar plots for the rat stomach are shown in Fig. 4. The rate constants, k_U (Eq. 3) and k_I, for the two drugs were calculated by regression analysis and are summarized in Table III. In all cases, the correlation coefficient was greater than 0.96. The ionized forms of both sulfaethidole and barbital are absorbed at significant rates from both the rat gastric and intestinal *in situ* preparations. The unionized forms of these drugs are absorbed 3–5 times faster than the ionized forms at both sites. The ratios of k_U/k_I shown in Table III for sulfaethidole are greater than those for barbital, suggesting that pH would have a greater influence on the absorption rate of sulfaethidole than on that of barbital.

The larger ratio for sulfaethidole is probably due to a greater partition coefficient for the drug into the absorbing membrane. True partition coefficients for the two drugs were calculated from apparent coefficients at the indicated pH's using a separator shake-out method (13). The chloroform–water true partition coefficients were: sulfaethidole, 2.7 (pH 5.6 and 6.6); and barbital, 0.8 (pH 7.7 and 8.5). The *n*-octanol–water true partition coefficients were: sulfaethidole, 7.5 (pH 6.0 and 7.0); and barbital, 3.4 (pH 8.0 and 9.0). Thus, partitioning data in chloroform–water and *n*-octanol–water systems agree with kinetic data obtained in the *in situ* preparation.

The results shown in Figs. 3 and 4 and in Table III can be rationalized completely on the basis of the pKa's of the drugs and the pH's of the bulk lumen solutions as these factors affect the fractions of the drugs present in their unionized forms. For sulfaethidole and barbital in the *in situ* rat gastric and intestinal preparations, there is no evidence of the existence of a "virtual pH" layer at the absorbing membrane surface different from the pH of the bulk lumen solution. If such a layer exists, it is likely that changes in the pH of the bulk solution would have little or no effect on the absorption rate. On the other hand, transfer of sulfaethidole and barbital ions made significant contribution to the overall absorption of these drugs, which suggests that in rationalizing deviations from the traditional pH-partition hypothesis, serious consideration must be given to absorption of drug ions.

The significance of the absorption of the ionized moieties can be illustrated by the relatively rapid intestinal absorption of sulfaethidole at pH 6.1. At this pH, $f_I = 0.80$ and $f_U = 0.20$ for sulfaethidole. Since the apparent rate constants for drug absorption are equal to $f \times k$, it can be calculated that the apparent rate constants for the intestinal absorption of ionized and unionized sulfaethidole are 0.0079 and 0.011 min.$^{-1}$, respectively. Consequently, the absorption of sulfaethidole ions accounts for 42% of the total absorption at this pH. Thus, even though k_U and k_I greatly favor the preferential absorption of unionized drug, under certain pH conditions a rather significant degree of absorption may be due to the transport of ionized drug.

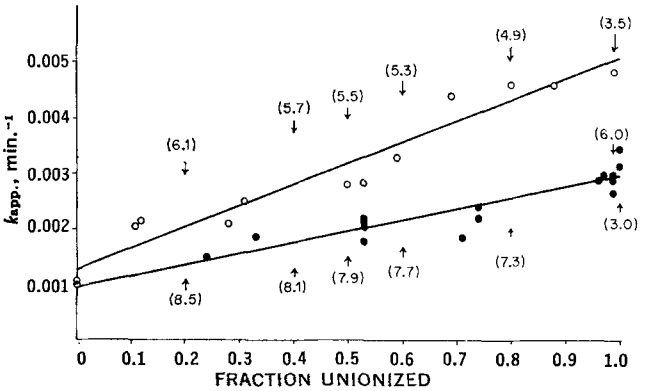

Figure 4—*Plots showing the relationship between* $k_{app.}$, *the rate constant controlling overall disappearance of drug from rat gastric lumen* in situ, *and the fraction of drug present in the unionized form. Key:* ○, *sulfaethidole; and* ●, *barbital. The numbers in parentheses are pH's at various points on the respective curves.*

REFERENCES

(1) P. A. Shore, B. B. Brodie, and C. A. M. Hogben, *J. Pharmacol. Exp. Ther.*, **119**, 361(1957).

(2) C. A. M. Hogben, D. J. Tocco, B. B. Brodie, and L. S. Schanker, *ibid.*, **125**, 275(1959).

(3) J. G. Wagner, *Drug Intel.*, **2**, 244(1968).

(4) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.*, **12**, 421(1964).

(5) K. Kakemi, T. Arita, R. Hori, and R. Konishi, *ibid.*, **15**, 1883(1967).

(6) H. Nogami, M. Hanano, and J. Watanabe, *ibid.*, **10**, 1161 (1962).

(7) R. H. Turner, C. S. Mehta, and L. Z. Benet, *J. Pharm. Sci.*, **59**, 590(1970).

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

(9) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).

(10) G. Levy and S. P. Gucinski, *J. Pharmacol. Exp. Ther.*, **146**, 80(1964).

(11) A. Suzuki, W. I. Higuchi, and N. F. H. Ho, *J. Pharm. Sci.*, **59**, 644(1970).
(12) *Ibid.*, **59**, 651(1970).
(13) D. R. Reese, G. M. Irwin, L. W. Dittert, C. W. Chong, and J. V. Swintosky, *J. Pharm. Sci.*, **53**, 591(1964).
(14) "Documenta Geigy," Geigy Pharmaceuticals, Basle, Switzerand, 1962, p. 314.

ACKNOWLEDGMENTS AND ADDRESSES

Received September 14, 1970, from the *University of Kentucky, College of Pharmacy, Lexington, KY 40506*

Accepted for publication March 15, 1971.

This investigation was supported, in part, by a research grant from McNeil Laboratories, Fort Washington, PA 19034

Toxicity of Ethylene Chlorohydrin II: Subacute Toxicity and Special Tests

W. H. LAWRENCE, K. ITOH*, J. E. TURNER, and J. AUTIAN

Abstract □ Ethylene chlorohydrin may be found as a reaction product when plastic items are sterilized with ethylene oxide. A previous publication presented results of an acute toxicity study of ethylene chlorohydrin in animals and tissue culture. This paper presents additional toxicity information derived from a study of rats receiving the compound intraperitoneally three times a week for 3 months, and from a similar study in which the compound was administered daily for 1 month. No adverse effects were observed in the 1-month study from 6.4 mg./kg. daily of ethylene chlorohydrin (one-tenth of the acute LD_{50}), or from 12.8 mg./kg. administered three times a week for 3 months. Additionally, special tests were included to determine the effect of the compound upon: cardiovascular, respiratory, and neuromuscular functions in rabbits; sensitizing potential in guinea pigs; and pentobarbital sleeping time in mice.

Keyphrases □ Ethylene chlorohydrin—subacute toxicity, cardiovascular, respiratory, neuromuscular, sensitizing potential, sleeping time effects □ Ethylene oxide sterilization—ethylene chlorohydrin toxicity evaluation □ Toxicity, subacute—ethylene chlorohydrin evaluation, rats

During the late sixties, a growing awareness of a lack of pertinent information relating to the toxicity of ethylene chlorohydrin (2-chloroethanol) prompted a number of studies. For many years, industrial workers have experienced local and systemic adverse reactions from improper handling of this compound; more recently, the treatment of foods and the sterilization of polymeric substances with ethylene oxide have resulted in the formation of ethylene chlorohydrin when chlorides were present even in minute amounts in the exposed material. The acquisition of additional toxicological data is essential to alert medical care personnel to the potential hazards associated with ethylene chlorohydrin when hospitalized patients come in contact with ethylene oxide-sterilized devices, to warn the general public of the dangers of consuming ethylene oxide-treated foods, and, finally, to inform those who come in contact with large quantities of the chemical in industry.

In a previous paper (1), results of an acute toxicity study of ethylene chlorohydrin gave LD_{50} values within a rather narrow range (64–98 mg./kg.) when the compound was administered to mice, rats, rabbits, and guinea pigs, which were independent of the route of administration. The compound was easily absorbed through the intact skin; thus, the LD_{50} produced by topical administration was comparable to that from intraperitoneal or oral administration. Its vapors, when mixed with air, killed 50% of the test animals after 13.3 min. of exposure. The compound was found to be highly irritating intradermally or from ophthalmic application. Little irritation was noted, however, when the compound was applied to the skin of rabbits.

Because it is possible for people to be repeatedly exposed to ethylene chlorohydrin in very small concentrations by the parenteral, dermal, or oral[1] routes, it was deemed appropriate to extend the previous studies into an investigation of subacute toxicity and to conduct certain other special studies.

EXPERIMENTAL

Materials—Ethylene chlorohydrin[2] was used.

Subacute Toxicity Studies—*Phase One: 12-Week Study*—In these experiments, male Sprague-Dawley rats were used, having initial weights of 57–64 g. In the first series of experiments, two dose levels were administered intraperitoneally to groups of 12 rats. The dose levels were based upon one-fifth and one-half the acute LD_{50} dose or 12.8 and 32.0 mg./kg., respectively. When possible, the compound was administered as such or, when necessary, it was diluted in distilled water and administered using microliter syringes. The respective dose levels were administered three times a week (Monday, Wednesday, and Friday) over a 12-week period. A control group of rats received normal saline in a volume equivalent to the test samples and at the same time intervals. The animals were housed two in a cage and given food and water *ad libitum*.

All animals were observed daily to assess general health and mortalities; they were weighed three times per week, just prior to injection of the chlorohydrin, and the weights were recorded. Food consumption was measured during the 1st, 2nd, 5th, 9th, and 12th weeks. Hematologic tests were performed on six rats selected at random from each test group and saline controls at the end of the 12th week. Several drops of blood were obtained from each animal by clipping the end of the tail for determination of the following:

[1] In addition to the possibility that it may be contained in certain foods, some hospitals sterilize plastic drinking tumblers and pitchers with ethylene oxide and then fill these with drinking water, which, of course, contains chlorides and possibly fluorides.

[2] Matheson, Coleman, and Bell, East Rutherford, N. J. The liquid was established as being more than 99% ethylene chlorohydrin.