Development of a System for Studying Subcutaneous Absorption Kinetics of Local Anesthetic Amines

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Abstract □ In the search for optimum conditions (pH 7.40, isotonic solution) for the study of subcutaneous absorption of local anesthetics using the system of Ballard and Menczel, unexpectedly wide pH variations (6.26–8.40) were consistently observed. All isotonic buffer systems examined failed to maintain a constant pH in the absorption cell for the desired duration (5–7 hr.). It was demonstrated that subcutaneous tissue liberates and absorbs ions (Na^+, K^+, Cl^-, and HCO_3^-), apparently in an attempt to produce an ionic composition in the solution similar to that of interstitial extracellular fluid. Additional experiments indicate that the high pH (above 7.40) results from the loss of carbon dioxide from the cell and that the liberation of bicarbonate ion by the tissue is time dependent. Constancy of pH was achieved using a slightly hypertonic tromethamine buffer (pH 7.95 ± 0.05, buffer capacity = 0.225).

Keyphrases □ Absorption, subcutaneous—local anesthetic amines, pH control in the absorption cell □ Subcutaneous absorption—local anesthetic amines, pH control in the absorption cell □ Anesthetic amines, local—*in vitro* subcutaneous absorption, pH control

As pointed out by several authors (1–3), very little is known about the factors affecting drug absorption from parenteral sites of administration. An understanding of these factors is especially important with local anesthetics, since they are injected at more parenteral sites than any other class of drugs. Also, whereas most drugs, independent of the route of administration, reach their target organs *via* the bloodstream, local anesthetics are delivered directly to the neural structure to which their action is confined. Therefore, tissue penetration becomes an important parameter influencing speed of onset, depth and duration of anesthesia, tissue irritation, and systemic toxicity. Although its importance is recognized (4, 5), no general method exists that allows quantitation of penetration. The major obstacle to such studies has been a suitable *in vivo* system.

In the present studies, absorption through subcutaneous connective tissue was selected since it directly pertains to local anesthetics used in infiltration and indirectly to those employed in various types of nerve block (connective tissue is thought to be the main diffusion barrier in the peripheral nerve).

The absorption kinetics of a drug from subcutaneous tissue can be studied by following either the appearance of the drug in blood or the disappearance from the site or both. These approaches are not equivalent. The first, used by several groups (6–9) to examine the influence of substances like hyaluronidase and histamine on the absorption rate of drugs (when the substances and drug are administered in the same solution), is complicated by drug disposition. Even though alterations in the initial rise result mainly from changes in absorption, careful interpretation is necessary, especially if quantitative data are desired. The difficulty in obtaining quantitative information has led others to use the second approach and to study the disappearance of drug from the site of administration, a procedure that more readily allows accurate kinetic treatment. Thus, Sund and Schou (10) developed an experimental method of measuring absorption rates from rat muscle; this method was used recently by Kakemi *et al.* (11). This approach involves ligation and removal of the muscle (extensor quadriceps femoris) at the end of the absorption period, followed by analysis of the remaining drug. Although this method presents several advantages, it is seriously limited by an inability to define and control the area for absorption. Without this control, it is difficult to examine quantitatively the subcutaneous absorption kinetics of drugs since the absorption rate is directly proportional to the area available for absorption.

To overcome the surface area problem, Ballard and Menczel (12) developed a system where the area of tissue exposed to the drug solution is maintained constant. However, these authors worked with benzyl alcohol, a neutral molecule which, unlike most drugs that are acids or bases, is not subject to the expected pH-partition effect (13). Accordingly, while the method of Ballard and Menczel was selected for the present investigation with local anesthetic amines, several preliminary studies were undertaken to control the pH in the absorption cell. These studies, which revealed an unexpected behavior of the subcutaneous tissue, constitute the subject of this report.

EXPERIMENTAL

Solutions—The following solutions were used:

1. Isotonic sodium chloride (0.9%)
2. Isotonic dextrose (5%)
3. Isotonic sodium phosphate buffers:
   - pH 7.420: monosodium phosphate 0.306 g./100 ml.; disodium phosphate 1.470 g./100 ml.
   - pH 7.560: monosodium phosphate 0.252 g./100 ml.; disodium phosphate 1.522 g./100 ml.
4. Isotonic sodium succinate buffer:
   - pH 5.37: monosodium succinate 0.757 g./100 ml.; disodium succinate 1.275 g./100 ml.
5. Tromethamine [tris(hydroxymethyl)aminomethane] buffer:
   - pH 7.950: tromethamine 5.955 g.; dilute hydrochloric acid (10%) 2.85 ml.; sodium chloride 0.2015 g.; distilled water *q.s.* to 100 ml.

Methods—All pH measurements were made on the expanded scale of a pH meter[1], standardized with National Bureau of Standards certified buffers. Usually, the original and solution exposed to subcutaneous tissue were read together.

[1] Beckman Expandomatic.

Table I—pH Studies with Isotonic Saline and Dextrose Solutions

Animal	Solution	pH Beginning of Exposure	pH End of Exposure	Exposure Time, min.
1	Saline	6.265	8.400	150
2[a]	Dextrose	6.480	7.990	90
			8.092	180
			8.630	300
3a[b]	Saline	5.70	7.48	63
3b[b]	Saline	5.70	7.62	63
3c[b]	Saline	5.78	7.79	64
3d[b]	Saline	5.70	7.70	63
4a[b]	Saline	6.010	7.990	125
4b[b]	Saline	5.990	7.890	121

[a] In this animal, the exposure time is cumulative; *i.e.*, the same solution was removed from the cell at the indicated times and the pH was measured. [b] In these experiments, a fresh saline solution was utilized for each exposure time.

Surgical Techniques—Sprague-Dawley female rats (350–400 g.) were used, one rat per experiment. Anesthesia was induced with ethyl ether and maintained with ethyl carbamate injected intraperitoneally (1 mg./g. body weight followed by 0.5 mg./g. 2 hr. later). After incision of the abdominal skin, a circular area (3-cm. diameter) of subcutaneous tissue was exposed and the subcutaneous absorption cell (2.1 cm. high and 1.8 cm. diameter) was fixed to the moist tissue with a silicone adhesive. After a few minutes, 1.8 ml. (Animals 1–8 and 24–28), 2.0 ml. (Animals 9–13 and 15–22), or 2.5 ml. (Animals 14 and 23) solutions were introduced and the timer was started. The solution was agitated by a vibrating stirrer, with four threads of polyethylene 50 attached to the end.

RESULTS AND DISCUSSION

pH Variations in Subcutaneous Cell—The main objective of this study was to establish a system that could be used to examine the kinetics of penetration of a series of *meta*-substituted 2-diethylaminoacetanilides. These local anesthetics are weak amines (pKa 8.0 ± 0.05 at 24°) and should be subject to pH-partition effects. Therefore, a system had to be developed in which a constant pH is maintained throughout the experiment.

Initially, pH variations were noted in the subcutaneous absorption cell containing unbuffered solutions. The results with isotonic sodium chloride and dextrose solutions are presented in Table I. As expected, the pH of the saline solution changed over 2.5 hr. (Animal 1). However, the final pH (8.40) was much higher than expected on the basis of physiological pH values of blood and other body fluids. Similar behavior was observed in Animals 2, 3, and 4. Since the absorption rates of the drugs under study were unknown in the early stages, this pH phenomenon was investigated further over longer periods. In Animal 2, the same isotonic dextrose solution was removed from the cell at different times and the pH was measured. The kinetics of this pH effect appear to be quite complex. The pH change is rapid over the 1st hr. and then slows. To test whether the tissue's ability to raise the pH is impaired after the 1st hr., a saline solution was left in contact with the subcutaneous tissue for four equal periods of approximately 1 hr. (63 ± 1 min.); at the end of each period, the solution was replaced by fresh saline. The data (Table I, Animals 3 and 4) show that the tissue was able to produce comparable pH increments repetitively over a sustained period.

Table II—pH Studies with Isotonic Phosphate and Succinate Buffers

Animal	Solution	pH Beginning of Exposure	pH End of Exposure	Exposure Time, min.
5	Phosphate	7.422	7.573	120
6	Phosphate	7.440	7.628	123
7[a]	Succinate	5.371	5.605	60
			6.015	150
			7.760	300
8a[b]	Phosphate	7.560	7.600	105
8b	Phosphate	7.560	7.610	105
9	Phosphate	7.560	7.930	360

[a] Same type of experiment as for Animal 2. [b] Same type of experiment as for Animal 3 or 4.

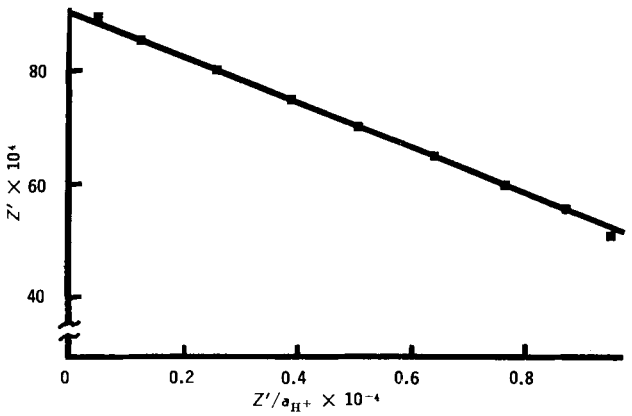

Figure 1—*Titration of 1.8 ml. of isotonic saline after a 75-min. exposure to subcutaneous tissue (Animal 11, pKa 6.51).*

Attempts with isotonic buffers to override the pH rise and maintain the pH constant failed. A buffer was considered acceptable if the pH increment, after a 5-hr. exposure, was not larger than 0.1 pH unit. Table II shows the results of those studies. An isotonic phosphate buffer (pH 7.440) was considered unsatisfactory because the pH increment was already 0.19 pH unit after 2 hr. (Animal 6). Evidently, an isotonic succinate buffer (pH 5.37) is inadequate even for 1 hr. Several experiments of the type exemplified by Animals 8 and 9 show that by using a slightly alkaline isotonic phosphate buffer (pH 7.56), it is feasible to maintain a constant pH for 1–2 hr. This would be useful for drugs that are essentially absorbed within this period, but unfortunately this proved not to be the case for the local anesthetics under study. Instead of empirically trying several other isotonic buffer systems, the cause of the pH rise was investigated further.

Cause of pH Rise—When 2 ml. of isotonic saline was exposed to the subcutaneous tissue (Animal 10) for 77 min. and then titrated with 0.001 *N* HCl, approximately 100 times more acid (7.3 μM) was needed to bring the pH from 8.306 to 5.200 than was calculated for an unbuffered solution (0.06 μM). These data suggest that a buffer system is formed in the absorption cell.

In Animal 11, three titrations were performed with 0.1 *N* HCl on solutions after 75-, 38-, and 18-min. exposures. The data, treated according to the method of Leeson and Brown (14) (except that Z'/a_{H^+} was calculated instead of $Z'[H^+]$) and plotted as Z' *versus* Z'/a_{H^+}, gave pKa values of 6.51, 6.41, and 6.26 (Fig. 1). This would seem to indicate that the solution contains significant amounts of a weak acid, possibly as a salt with a strong base. It is also possible that more than one chemical species is present.

In a further experiment (Animal 12), 2 ml. of normal saline, after 1-hr. exposure to subcutaneous tissue, was titrated with 0.1 *N* NaOH and revealed the presence of significant amounts of a species with a pKa of 9.90. A similar solution from the same animal was titrated in both directions, first with 0.1 *N* NaOH up to pH 10.920 and then with 0.1 *N* HCl down to pH 4.280. These titrations yielded pKa's of 6.25 and 10.19, respectively.

Although theoretically several ways of explaining these results exist, one possibility, liberation of bicarbonate ion from the subcutaneous tissue, appeared most reasonable. Several facts favor this hypothesis.

1. Carbonic acid is a dibasic acid whose dissociation constants [$pK_1 = 6.37$, $pK_2 = 10.33$ (15)] agree with those obtained in Animal 12, taking into account that the latter are apparent (hybrid) pKa's measured at very high ionic concentrations.

2. The alkalinity of the saline solutions is probably due to bicarbonate rather than to carbonate since equivalent amounts of sodium hydroxide and hydrochloric acid were required for complete neutralization.

3. The hydrogen-ion concentration of a solution of an acid salt such as sodium bicarbonate is given by $[H^+] = (K_1K_2C/K_1 + C)$, where C is the salt concentration and K_1 and K_2 have their usual meaning. When $K_1 < 0.01C$, this equation reduces to $[H^+] = K_1K_2$

Table III—pH Changes of Isotonic Saline when Exposed to Subcutaneous Tissue (Animal 13)

Exposure Time, hr.	0	3	4	5	6	7
pH	5.750	8.195	8.160	8.248	8.190	8.250

which, converted into pH notation, yields:

$$pH = \frac{1}{2} pK_1 + \frac{1}{2} pK_2 \qquad \text{(Eq. 1)}$$

Hence, at sufficiently high concentrations, the pH of the solution is concentration independent. For sodium bicarbonate, this pH is approximately 8.35 and exists at concentrations of 4×10^{-5} *M* and above. This predicted pH agrees with the results obtained in Animals 1 and 2 (Table I). An additional experiment (Animal 13) in which 2 ml. of saline was introduced in the cell and the pH was measured at different times demonstrated that this maximal pH (Table III) is reached within 3 hr. and maintained thereafter. Furthermore, titration of the solution at the end of the experiment demonstrated that the condition $K_1 < 0.01C$ did apply. Thus, 2 ml. of solution required 0.340 ml. of 0.1 *N* NaOH (*i.e.*, equivalent to 1.7×10^{-3} *M* bicarbonate) for complete neutralization.

4. Direct measurements confirmed the presence of bicarbonate (Animal 14). Isotonic dextrose solution (2.5 ml.) was exposed to the subcutaneous tissue for 3 hr. Of the 2.4 ml. recovered, 1.1 ml. was titrated with 0.1 *N* HCl and Na^+, K^+, Cl^-, and HCO_3^- were determined in the remainder. The titrant (0.100 ml. of 0.10 *N* HCl) required for complete neutralization agreed closely with the ionic determination (10 meq.) of HCO_3^-. The other ions were present in the following concentrations: Na^+ = 48 meq./l., K^+ = 1.5 meq./l., and Cl^- = 37 meq./l. Not only is the expected neutrality ensured, but the relative concentrations of these ions are very similar to those of interstitial extracellular fluid (16) (Table IV).

To gain further insight into the phenomenon, 2 ml. of saline solution was exposed to the subcutaneous tissue for four consecutive 1-hr. periods (Animal 15, Table V). At the end of each hour, the saline solution was titrated and replaced by fresh solution. After the fourth period, the animal was sacrificed (by injection of air into the heart) and the whole experiment was repeated in the dead animal. Under *in vivo* conditions, bicarbonate was liberated at a relatively constant rate of approximately 0.0044 meq./hr. After death, the ability to liberate bicarbonate seems to be impaired, with the rate of appearance of bicarbonate decreasing with time after death. This finding agrees with the observation that the pH of an isotonic succinate buffer (pH 5.371) remained constant (pH 5.425) after a 4-hr. exposure in a dead animal, whereas the same buffer gave pH readings of 5.605, 6.015, and 7.760 after 1-, 2.5-, and 5-hr. exposures, respectively, under previous *in vivo* conditions (Animal 16). This unique behavior of the dead animal opened the possibility of studying the effect of pH on the kinetics of subcutaneous absorption, and such studies will be presented in a later report.

Mechanism for Liberation of Bicarbonate—As was noted, the ionic compositions of the exposed dextrose solution and interstitial extracellular fluid are strikingly similar (Table IV). This is quite interesting if one recalls that extracellular fluid is made up of plasma, interstitial and lymph fluid, connective tissue and cartilage, bone, and transcellular fluids. Interstitial fluid is interposed between the rapidly circulating plasma and the cells. It flows slowly through tissue interstices and bathes the cells, but it is in rapid equilibrium with the blood plasma through an incessant transcapillary interchange of water, electrolytes, and dissolved substances. The possibility that this observation (Table IV) results from a simple dilution effect with interstitial extracellular fluid can be easily discounted since 1.25 ml. of interstitial extracellular fluid is required to yield the observed ionic concentration, but no significant volume change in the subcutaneous cell was found. However, mixing of the subcutaneous cell contents with interstitial extracellular fluid with continual lymphatic drainage cannot be discounted.

Table IV—Relative Ionic Composition of an Isotonic Dextrose Solution Exposed to Subcutaneous Tissue and of Interstitial Extracellular Fluid (I.E.C.F).

Ion	Isotonic Dextrose, meq./l.	I.E.C.F.[a], meq./l.	Isotonic Dextrose[b] / I.E.C.F.
Na^+	48	145.1	0.33
K^+	1.5	4.1	0.37
Cl^-	37	115.7	0.32
HCO_3^-	10	29.3	0.34

[a] From *Reference 16.* [b] Although these results were obtained from only one experiment (Animal 14), the data for Animals 21 and 23 (Table VI) show that the relative ionic composition of the corresponding dextrose solutions is also very similar to that of the interstitial extracellular fluid.

Table V—Rate of Bicarbonate Liberation under *In Vivo* and Postmortem Conditions (Animal 15)

Period Number	Exposure Time, min.	HCO_3^-, meq.	Conditions
1	60	0.0047	*In vivo*
2	60	0.0041	*In vivo*
3	60	0.0041	*In vivo*
4	60	0.0046	*In vivo*
5	60	0.0045	Postmortem
6	60	0.0021	Postmortem
7	60	0.0015	Postmortem
8	60	0.0010	Postmortem

Additional experiments were conducted in an attempt to elucidate this ionic phenomenon (Table VI). After 3 hr. of contact with an isotonic potassium chloride solution, the cell gained 34 meq. Na^+ and 8 meq. HCO_3^- and lost 36 meq. K^+ and 13 meq. Cl^- (Animal 17). In a duplicate experiment, the tissue exhibited the same behavior (Animal 18). It also follows that maximal changes should be observed with a potassium bicarbonate solution, since its ionic composition is furthest from that of interstitial extracellular fluid. This was indeed noticed (Animals 19 and 20). Thus, within 5 hr. the subcutaneous cell gained 55 meq. Na^+ and 45 meq. Cl^- and lost 54 meq. K^+ and 55 meq. HCO_3^- (Animal 20). In an additional experiment, after 5 hr. of contact with an isotonic dextrose solution, the tissue liberated the four ions in concentrations approximately half of that of interstitial extracellular fluid. During the same time, the glucose concentration dropped to 2.92% (Animal 21). The preceding experiments with isotonic dextrose, potassium chloride, and potassium bicarbonate support the hypothesis that the ionic composition within the subcutaneous cell changes toward that of interstitial extracellular fluid. If the subcutaneous tissue transports ions for the sole purpose of establishing and/or maintaining in its environment the ionic composition of interstitial extracellular fluid, then one would expect no net change of ions in a solution with an interstitial extracellular fluid composition. Essentially no change in the composition of 2 ml. of Krebs–Henseleit (17) buffer after 5 hr. of

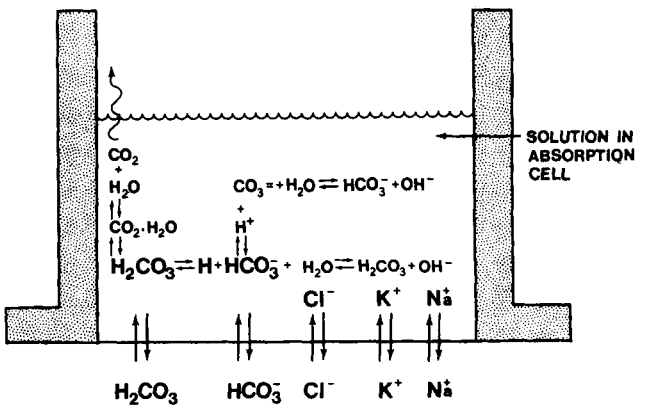

Figure 2—*Diagram depicting some of the ionic events occurring in the subcutaneous absorption cell.*

Table VI—Ionic Composition in the Absorption Cell before and after Exposure to Subcutaneous Tissue

Animal	Initial Composition[a], meq./l. Na^+	K^+	Cl^-	HCO_3^-	Dextrose, g. %	Exposure Time, min.	Final Composition[a], meq./l. Na^+	K^+	Cl^-	HCO_3^-	Dextrose, g. %
17	2	145	157	0	—	180	36	109	144	8	—
18	3	146	160	0	—	90	53	110	151	10	—
19	2	149	0	142	—	300	52	100	41	132	—
20	2	148	0	155	—	300	57	94	45	100	—
21	2	0	5	0	5	305	72	2.6	59	15	2.92
22	165	7.5	125	33	—	300	165	6.5	120	31	—
23	0	0	0	0	5	120	52	3.5	42	8	—

[a] The determination of the concentration of ions was performed by the clinical laboratory (Moffit Hospital, San Francisco, Calif.), with a precision probably no better than 5%. This explains why in some cases the anionic and cationic concentrations are not equivalent.

exposure supports this contention (Animal 22). To eliminate the possibility that the observed ion phenomena are due to experimental artifacts such as irritation of the tissue caused by the glass subcutaneous cell or the silicone adhesive, an isotonic dextrose solution was exposed to the subcutaneous tissue without the use of a glass cell (Animal 23). The skin was excised over a 3-cm. length and carefully separated from the adjacent subcutaneous tissue. By clamping the edges of the skin upward, a natural containment for the solution was formed. After a 2-hr. exposure, 52 meq. Na^+, 3.5 meq. K^+, 42 meq. Cl^-, and 8 meq. HCO_3^- were liberated. These experiments demonstrate that the subcutaneous tissue liberates and absorbs ions so that the ionic composition of the adjacent solution becomes similar to that of interstitial extracellular fluid and the liberation of bicarbonate is not an isolated process but is part of this general phenomenon.

There is still another chemical species, carbonic acid (H_2CO_3 or CO_2H_2O), which plays an important role in the observed pH of the subcutaneous cell (Eqs. 2–5). Plasma and interstitial extracellular fluid contain bicarbonate and carbonic acid in a ratio (20:1) compatible with pH 7.40. From all of the previous ionic experiments, the pH measurements in the cell, and especially the similar ionic compositions of interstitial extracellular fluid and the solution in the cell, the pH of the subcutaneous cell should be around 7.40. Instead, one generally observes a pH of 8.30, probably because liberated carbon dioxide is lost from solution to air, down its concentration gradient. As carbon dioxide disappears from solution, the net reaction (Eq. 4), the rate of which is affected if carbonic anhydrase is present, shifts to the right and the solution becomes more alkaline due to the hydrolysis of carbonate (Eq. 5). The pH of the solution is then given by Eq. 1 (Fig. 2):

$$HCO_3^- = H^+ + CO_3^{-2} \qquad \text{(Eq. 2)}$$

$$HCO_3^- + H_2O = H_2CO_3 + OH^- \qquad \text{(Eq. 3)}$$

$$2HCO_3^- = H_2CO_3 + CO_3^{-2} = H_2O + CO_2 + CO_3^{-2} \qquad \text{(Eq. 4)}$$

$$CO_3^{-2} + H_2O = HCO_3^- + OH^- \qquad \text{(Eq. 5)}$$

To test this hypothesis, in Animal 17 the potassium chloride solution was covered with mineral oil to prevent contact with air. The pH after 3 hr. was 7.490, in good agreement with the expected 7.40, taking into account that in transfer of the solution (for the pH reading) there is an appreciable loss of carbon dioxide. The bicarbonate content in the cell was not significantly affected by preventing contact with air (Table VI). This is consistent with Eq. 3, which shows that a significant pH increase (7.4 to 8.4) corresponds to a relatively minor change in bicarbonate content. In other words, within the pH range considered, the hydrogen-ion concentration is a very sensitive index of changes in bicarbonate content.

Table VII—pH Studies with a Slightly Hypertonic Tromethamine Buffer

Animal	pH Beginning of Exposure	pH End of Exposure	Exposure Time, min.
24	7.960	7.965	150
25	7.991	7.995	216
26	7.975	8.040	300
27	7.915	7.905	300
28	7.985	7.950	360

Previous experiments showed that even after several hours the ionic concentration in the cell is only a fraction of the interstitial extracellular fluid. The ions are liberated by the tissue cells, but the primary reservoir is plasma which delivers ions to the cells through the surrounding perivascular fluid (interstitial extracellular fluid). There are several possible rate-limiting steps in the observed rate of transport (blood flow to the tissue and movement of interstitial extracellular fluid to the subcutaneous absorption cell), but it is not possible with the data obtained in the present investigation to decide which is the rate-limiting process.

The usefulness and applicability of the subcutaneous model used in the present studies might be appreciably enhanced if absorption of local anesthetics can be studied under physiological conditions (pH 7.30–7.40, isotonic solution). This investigation shows that this will be possible only for drugs that are essentially absorbed in 1–2 hr. around physiological pH. However, for most of the local anesthetics under study, preliminary experiments showed absorption to be relatively slow; it was estimated that a constant pH would be required for at least 5 hr. Consequently, the only recourse was to strike a balance between the tonicity and pH (physiological conditions) requirements on one hand and the needed buffer capacity and limited drug solubility (especially at higher pH's) on the other. A slightly hypertonic tromethamine buffer (pH 7.95 ± 0.05, buffer capacity = 0.225) was selected for future studies and allowed an adequate pH control for 5–6 hr. (Table VII).

Several other alternatives should be mentioned, even though they appear to be impractical. One possibility (tested in Animal 17) would be to cover the drug solution with a layer of oil to minimize carbon dioxide loss, thereby hoping to maintain a pH as near to 7.40 as possible. However, this solution is quite unrealistic, since most drugs would partition into the oil layer at the same time as they are absorbed, which complicates any quantitative subcutaneous kinetic study. Another approach to maintain pH 7.40 in the subcutaneous cell would be to devise a system whereby the partial pressure of carbon dioxide in the air above the solution is in equilibrium with the required concentration of carbon dioxide in solution. In addition to the experimental difficulties in building such a system, this approach is hampered by the incomplete knowledge about the reaction of hydration of carbon dioxide and especially on the state of carbon dioxide in water. Perhaps metabolic inhibitors such as ouabain would prevent all transport processes and thereby allow the maintenance of pH 7.40 with suitable isotonic buffers. However, the possible effects of ouabain on drug absorption should also be considered.

This investigation of the ion-movement phenomenon allowed an elucidation of the mechanism of liberation of bicarbonate. At the same time, it represents direct evidence of the relatively strong buffer capacity of subcutaneous connective tissue. Most commercial solutions of local anesthetics have a pH between 5 and 7, mainly because of solubility limitations. The tissue buffer capacity can be relied on to neutralize solutions of local anesthetics soon after injection, thereby increasing the diffusion rate of the drug from the injection site to the nerve membrane.

Mention should also be made of the fact that this subcutaneous absorption system might offer a simple and practical approach to the quantitative study of ion-movement phenomena under *in vivo* conditions.

In summary, it appears that, with the present system, absorption studies of local anesthetics will not be possible under physiological conditions. Evidently, subcutaneous tissue is the site of various ion-

movement phenomena and it liberates and absorbs ions (Na^+, K^+, Cl^-, and HCO_3^-) to maintain in its environment an ionic composition similar to that of interstitial extracellular fluid. However, it is possible to perform studies under controlled pH conditions, if one compromises tonicity (tromethamine buffer, pH 7.95 ± 0.005, buffer capacity = 0.225). It also appears that studies at various pH's will be possible using the same system under postmortem conditions.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 26, 1971, from the *College of Pharmacy, University of Washington, Seattle, WA 98105, and the †School of Pharmacy, Department of Pharmacy, University of California at San Francisco, San Francisco, CA 94122

Accepted for publication March 28, 1972.

Presented in part to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Washington, D. C. meeting, April 1970.

Abstracted in part from a thesis submitted by René H. Levy to the Graduate Division, University of California at San Francisco, in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported by National Institutes of Health Training Grant No. 5 T01 GM 00728 from the National Institute of General Medical Sciences and by research funds from the Academic Senate Committee on Research, University of California at San Francisco.

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Enzyme Induction of Organic Nitrates I: Nitroglycerin *In Vivo* Experiments

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Abstract □ Upon oral administration of nitroglycerin–proxyphylline tablets with timed release to humans, it was observed that the nitroglycerin blood levels declined after the 5th day. Experiments in rabbits and mice were performed to clarify whether this phenomenon is caused by enzyme induction. Oral administration of 0.1 and 0.2 mg./kg. nitroglycerin to rabbits caused a decrease in the peripheral maximal temperature rise. The decline in pharmacological action depends on dosage and apparently follows first-order kinetics. Pretreatment of mice with either nitroglycerin or pentobarbital caused a similar decrease in pentobarbital sleeping time, indicative of enzyme induction. However, in contrast to the hepatic response elicited by barbiturate pretreatment in which the liver weights increased, nitroglycerin caused a significant decrease, thereby suggesting a different mechanism of action for the vasodilator.

Keyphrases □ Enzyme induction of organic nitrates—nitroglycerin, rabbits, mice □ Nitroglycerin—enzyme induction, rabbits, mice □ Vasodilation, nitroglycerin—enzyme induction, rabbits, mice

Although nitroglycerin is well established in the therapy of angina pectoris, its oral absorption has long been questioned. Salter (1) stated that nitroglycerin is not decomposed by the gastric juice, but its rate of absorption is slow. Sollmann (2) mentioned that nitroglycerin is more potent when administered buccally instead of orally, because by the latter route nitroglycerin is absorbed into the portal circulation and then destroyed by the liver. That nitroglycerin is also absorbed—at least in the rabbit—when given orally was shown by Turner (3), Lorenzetti *et al.* (4), and Bogaert *et al.* (5). Ritschel and Clotten (6) proved oral absorption of nitroglycerin in humans, calculating (7) a biological availability of orally given nitroglycerin of 36 and 55% for doses of 0.8 and 1.6 mg., respectively, to adults compared to the blood levels upon buccal absorption.

Since nitroglycerin is especially valuable when used prophylactically (8, 9), several oral timed-release nitroglycerin preparations have been developed, clinically tested, and marketed (10–18). By using an oral timed-release proxyphylline preparation (19–22), nitroglycerin was incorporated into this formula and biopharmaceutically evaluated (6, 7); it was found that the nitrate plasma level decreased after the 5th day (Fig. 1). This observation led to the suspicion of a possible enzyme induction (23).

To study the possibility of enzyme induction (23), further experiments were performed using a pharmacological parameter other than plasma levels, because there seems to be no correlation between hypotensive