In this reaction $\Delta S°_4$ has a high value (see Table IV) for all of the analogs studied, both tetracycline and chlor-derivatives. This may be explained by the fact that when the second ligand attaches to the metal, the over-all charge of the complex becomes zero, and the number of ions in solution is decreased. This neutralization of charge and decrease in the number of ions is common to all the analogs studied and would be expected to give a positive entropy change. In addition, the conformational entropy changes between tetracycline and the chlor-analogs is not significant here, since little conformational change is needed to form an ion-pair complex bond.

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Ionization of Bases with Limited Solubility

Investigation of Substances with Local Anesthetic Activity

By IVO SETNIKAR

The deionization process of six local anesthetics, *i.e.*, procaine, lignocaine, cocaine, Rec 7-0518, Rec 7-0544, and Rec 7-0591, was investigated. The compounds are weak bases with a low or a very low solubility of the unionized form. The effects of this property on the deionization process were studied and an explanation of the irregularities of the deionization curve suggested. A method for plotting the deionization process as a straight line is described. Temperature markedly affects the ionization constant. A limited solubility of the unionized base influences its buffering capacity. Both phenomena may be relevant to tissue tolerance for solutions of these substances.

It is essential to know the ionization curve of a local anesthetic in aqueous solution in order to choose a pH of the injectable solution that is optimal both for pharmaceutical stability and for local tissue tolerance.

Ionization curves may be plotted by the conventional method (1) which, for monoprotic species, leads to the well-known S-shaped curve. Other expressions of the results lead to straight-lined representation of ionization, with the advantage of showing more clearly experimental errors or deviations from theory.

Methods of obtaining straight-line representation of ionization of weak acids or bases were presented by Hofstee (2), by Benet and Goyan (3, 4), and by Leeson and Brown (5). The methods involve recalculations of the results and are strongly influenced by experimental errors (4). More immediate and easier to apply is the method proposed by Druckrey (6, 7), based on the use of a specially designed scale for the titrant, which yields a straight-line expression of the law of mass action. This method may also be adapted for expressing with a straight line the ionization process of weak acids or bases in which the unionized form is sparingly soluble, a fact which limits its availability for the ionization equilibrium.

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THEORY

The ionization of a proton acceptor, B, is represented by

$$B + H^+ \cdot H_2O \rightleftarrows BH^+ + H_2O \quad \text{(Eq. 1)}$$

Since in a diluted aqueous solution the concentration of H_2O remains practically constant, the equilibrium of the ionization process is expressed by Eq. 2.

$$\frac{[BH^+]}{[B][H^+ \cdot H_2O]} = K' \quad \text{(Eq. 2)}$$

where K' is the apparent ionization constant, valid

for the particular system in which ionization takes place. Equation 2 describes with sufficient approximation the ionization equilibrium of a weak base, except at the boundaries of almost total ionization or unionization of the base. Without approximations the ionization equilibrium is expressed by Eq. 3, derived from Clark (8).

$$\frac{[A^-] - [H^+ \cdot H_2O] - [D^+] + [OH^-]}{([S] - [A^-] - [OH^-] + [H^+ \cdot H_2O] + [D^+]) \cdot [H^+ \cdot H_2O]} = K' \qquad \text{(Eq. 3)}$$

in which A^- and D^+ are the ions of strong acids and bases present in the solution and S is the total quantity of the base, *i.e.*,

$$[S] = [B] + [BH^+] \qquad \text{(Eq. 4)}$$

In the range of its validity Eq. 2, in logarithmic terms and substituting K_B' for $1/K'$, becomes

$$pH = pK_B' + \log \frac{[B]}{[BH^+]} \qquad \text{(Eq. 5)}$$

If B has a limited solubility, at a certain point of titration the excess of B precipitates and the concentration of the dissolved B remains constant during the rest of titration. When this phenomenon happens Eq. 5 describes the deionization process only up to the point at which B starts to precipitate. Above this point the deionization is described by:

$$pH = pK_B' + \log \frac{[C]}{[BH^+]} \qquad \text{(Eq. 6)}$$

where C is the concentration of dissolved B on saturation.

In the conditions described by Eq. 5, a straight line is obtained by plotting the pH on the ordinate and log $[B]/[BH^+]$ on the abscissa, or using charts in which the abscissa is graded to a special scale, corresponding to the values of log $[B]/[BH^+]$. Furthermore, it may be convenient to substitute $[B]/[BH^+]$ with the titrated fraction F, where F is given by $[B]/[S]$. Since $[B]$ is equal to $[A^-]$, *i.e.*, the titrating strong acid, F is also given by $[A^-]/[S]$.

Similarly, Eq. 6 is expressed by a straight line when the pH is plotted on the ordinate and log $1/[BH^+]$ on the abscissa, or using a chart in which the abscissa is graded to a special scale corresponding to the values of log $1/[BH^+]$. In this case too $1/[BH^+]$ may be substituted by the titrated fraction F. The correspondence of the logarithmic scales, with these special F scales is represented in Fig. 1. The LR scale of Fig. 1 (log $[B]/[BH^+]$) refers to Eq. 5. The L scale (log $1/[BH^+]$) refers to Eq. 6.

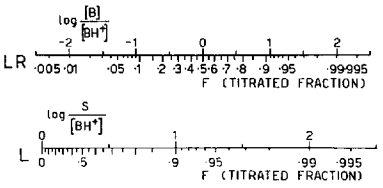

Fig. 1.—Scales of F (titrated fraction), which yield a straight-line relationship between F and pH for deionization processes of weak proton acceptors. Key: LR, scale equivalent to units of log $([B]/[BH^+])$ according to Eq. 5; L, scale equivalent to units of log $([S]/[BH^+])$ according to Eq. 6.

Equation 5 shows that $pK_B' = pH$ when $[B]/[BH^+] = 1$ or $F = 0.5$. Therefore the pK_B' can easily be found, either from the point at which $[B]/[BH^+] = 1$, or from any other point on the ionization line, since its slope, for monoprotic species, is the same on a pH *versus* LR diagram.

On the contrary Eq. 6, which may also be written as

$$pH = (pK_B' + \log C) + \log \frac{1}{[BH^+]} \qquad \text{(Eq. 7)}$$

does not yield the pK_B' value, unless C, the concentration of B at saturation, is determined.

EXPERIMENTAL

Investigated Substances.—The investigated substances were: procaine; lignocaine; cocaine; Rec 7-0518, *i.e.*, ketocaine or 2-(*N*-diisopropylaminoethoxy)-1-butyrophenone; Rec 7-0544, *i.e.*, 1-(*N*-diisopropylaminoethoxyphenyl)-butan-1-ol; and Rec 7-0591, *i.e.*, 2-(*N*-diisopropylaminoethoxy)-3-amino-1-butyrophenone. Rec 7-0518, Rec 7-0544, and Rec 7-0591 are three new local anesthetics described by Setnikar (9, 10).

The hydrochlorides of these substances were dissolved in CO_2-free glass-distilled water at a 0.1 and 0.01 M concentration and submitted to titration with 2 N and, respectively, 0.2 N carbonate-free NaOH.

Apparatus and Procedures.—The pH was measured with a Beckman Zeromatic model 96 meter, standardized against 0.05 M potassium hydrogen phthalate (pH = 4.0) and 0.01 M sodium borate (pH = 9.2). NaOH was added from a 3-ml. microburet, calibrated to 0.01 ml. Measurements were taken at 20.0° and at 37.0° on 50 ml. of the solutions of the local anesthetics under continuous and uniform agitation (magnetic stirrer). After each addition of NaOH, the pH was read when the meter had reached a stable value. The meter was read

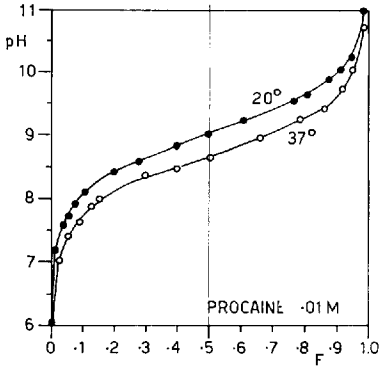

Fig. 2.—Ionization curve of 0.01 M procaine plotted by the conventional method. The titration was performed at 20.0° and at 37.0°, yielding two S-shaped deionization curves.

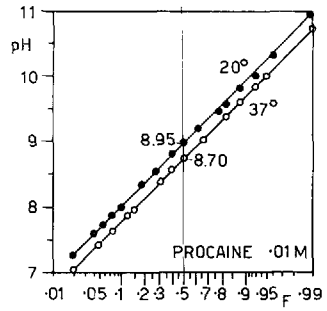

Fig. 3.—Procaine 0.01 *M*. Same data as in Fig. 2 plotted on a LR scale yielding straight-line relationships between pH and titrated fraction F. The pK_B' can be estimated from the whole titration and not only from the central data, as by the conventional method.

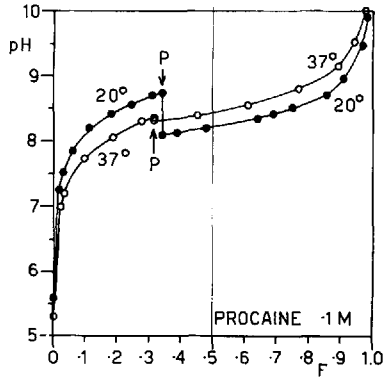

Fig. 4.—Ionization curve of 0.1 *M* procaine plotted by the conventional method. At the arrows, P, the undissociated base, starts to precipitate, markedly altering the deionization process. At this point the pH drops considerably, particularly on the curve obtained at 20°.

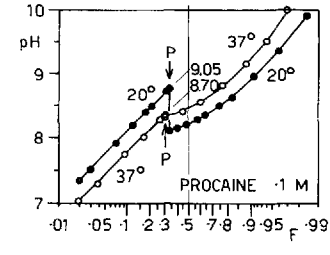

Fig. 5.—Procaine 0.1 *M*. Same data as in Fig. 4, but plotted on a LR scale for F. The initial straight-line part of the deionization process enables one to find the pK_B' values by extrapolation (9.05 at 20.0° and 8.70 at 37.0°). The data beyond the precipitation point are on a curved line, demonstrating a deviation from the theory described by Eq. 5.

10 min. after addition of the titrant when the titration was associated with precipitation.

The amounts of NaOH, corrected according to Parke and Davis (1), were expressed as fractions of the quantity necessary to titrate the whole base present in solution, and plotted on the charts *versus* the pH values.

Solubility of the Unionized Base.—Solutions of the hydrochlorides of the investigated substances, at a concentration of 0.1 *M* for procaine and lignocaine, and 0.01 *M* for the others, were deionized with a 5% excess of NaOH, filtered, or centrifuged, and the clear filtrate or supernatant acidified with HCl. These procedures were performed at 20° and at 37°. The concentrations of the substances in these acidified solutions were determined spectrophotometrically.

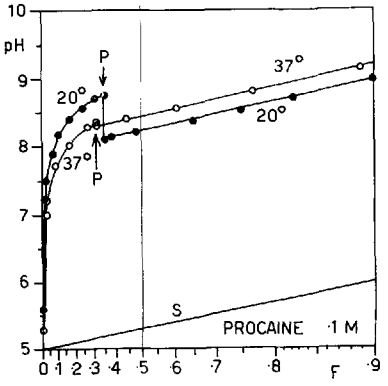

Fig. 6.—Procaine 0.1 *M*. Same data as in Figs. 4 and 5, but plotted on the L scale for F, which shows a straight-line deionization process after the precipitation point. Beyond this point the theory described by Eq. 6, therefore, applies. The line marked with S on the bottom of the figure shows the theoretical slope in these conditions.

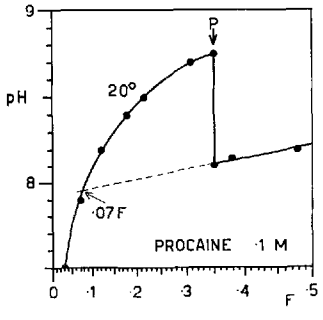

Fig. 7.—Detail of Fig. 6 showing a part of the deionization process at 20.0°. The arrow marks the point at 0.07 titration which corresponds to the maximum solubility of the unionized base in these conditions. This value is found by extrapolating the measurements after precipitation. From 0.07 F to 0.35 F, however, the base in unionized form remains still in solution, due to a phenomenon of hypersaturation. Phenomena of hypersaturation are often present, transiently as in this case or even during the whole titration, interfering with the evaluation of the maximum solubility and of the pK_B' values. They are not seen in back-titrations, *i.e.*, of the unionized base with a strong acid. In this case the dotted line is followed and the solution becomes clear only at the point in which the dotted line meets the first part of the titration curve.

RESULTS

The ionization curve for the tertiary amino group of 0.01 *M* procaine, plotted by the conventional method, is given in Fig. 2, yielding the well-known S-shaped curve. The same data plotted on the LR scale (*cf.* Fig. 1) yield a straight line with a slope of 45° (Fig. 3). In both instances the pK_B' value (second apparent ionization constant of procaine) is easily found.

The shape of the deionization curve of 0.1 *M* procaine is different, since between 0.3 and 0.4 F the unionized base precipitates and the deionization curve changes markedly. Particularly at 20.0° the pH drops by about 0.6. Then, proceeding in the titration, the pH raises again, first slowly, and then more rapidly, the final (right) part of the curve closely resembling the S-shaped deionization curve of soluble bases.

Wehr and Koelzer (11) noted that other bases with local anesthetic activity behaved in a similar complex way, but they offered no explanation.

The same data for 0.1 *M* procaine were plotted on a chart with the titrated fraction F expressed on the LR scale of Fig. 1. They yield a straight-line relationship between F and pH up to the precipitation point, *i.e.*, as far as the requirements of Eq. 5 are fulfilled (Fig. 5). Then the pattern departs markedly from the straight line and the theoretical pattern is approached again toward the end of titration.

By using a chart which yields a straight-line relationship between titrated fraction and pH when the requirements of Eq. 6 are fulfilled, it may be shown that, after precipitation, the theory described by Eq. 6 applies (Fig. 6) since the data are now on a straight line which has the theoretical slope.

The irregularities of the deionization curve of 0.1 *M* procaine shown by Fig. 4 are, therefore, related to the limited solubility of the unionized base and to the constant concentration of [B] in solution.

In the example given by 0.1 *M* procaine the solubility of B is limited but not very low. The estimation of pK_B' obtained in Fig. 5 by extrapolation of the straight-lined part of the deionization curve before precipitation may be considered reasonably precise, and so is the estimation of the maximum solubility of unionized procaine (Fig. 7).

For substances with a low solubility of the unionized base precipitation occurs after the addition of a very small amount of titrant and, due to the interference of transient hypersaturation phenomena, the evaluation of the pK_B' by extrapolation becomes rather arbitrary. An example of this is given by by the deionization curves obtained with 0.01 *M* and 0.1 *M* Rec 7-0518, which, in the unionized form, has a solubility lower than 1 mmole/L. (Figs. 8 and 10 and Table I). As shown by Figs. 9 and 11 the deionization of the Rec 7-0518 fits the theory of Eq. 6 throughout titration. In fact the relationship of the titrated fraction *versus* the pH on a chart with F on the L scale is straight-line and has the slope required by Eq. 6. Furthermore, the pH of the deionization lines of 0.1 *M* Rec 7-0518 is 1 unit higher than that of 0.01 *M* Rec 7-0518, due to the fact that log S and, therefore, also log $[BH^+]$ differ by one unit in the two conditions.

For substances with such properties both the pK_B' and the maximum solubility values may be estimated only with rough approximation. Un-

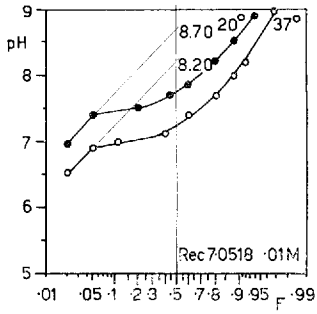

Fig. 8.—Deionization curve of 0.01 *M* Rec 7-0518 plotted on a LR scale for F. Owing to the very low solubility of the unionized form the process of precipitation starts at the very beginning of titration (in the range of 0.05 F). Taking account of the possible presence of hypersaturation phenomena, it becomes difficult to evaluate exactly the maximum solubility of Rec 7-0518. Since the calculation of the pK_B' value depends on the maximum solubility of the unionized base, the pK_B' can be estimated only with rough approximation. The figure shows how the pK_B' was estimated as 8.70 at 20.0° and as 8.20 at 37.0°.

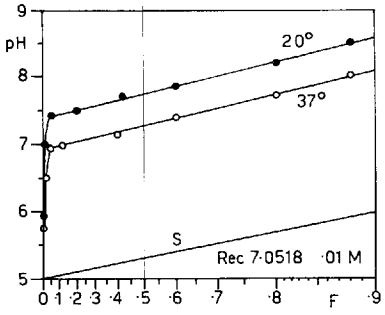

Fig. 9.—Rec 7-0518 0.01 *M*. Same data of Fig. 8 plotted on a L scale of F. After the precipitation point the theory described in Eq. 6 applies. At the bottom the theoretical slope (S) of the deionization process according to Eq. 6 is shown.

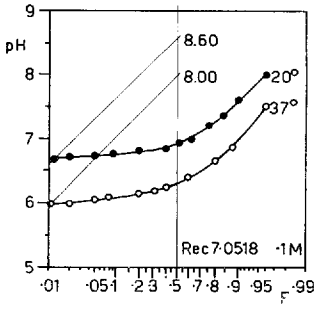

Fig. 10.—Deionization curve of 0.1 *M* Rec 7-0518 plotted on a LR scale for F. At this concentration it becomes still more difficult to evaluate the maximum solubility of the unionized form, since the precipitation obviously occurs at a lower titration point than for 0.01 *M* Rec 7-0518. Therefore, the calculation of the pK_B' value becomes still more approximate.

TABLE I.—APPARENT IONIZATION CONSTANTS OF THE INVESTIGATED BASES AND MAXIMUM SOLUBILITY OF THEIR UNIONIZED FORM AT 20.0° AND AT 37.0°[a]

		pKB′		Maximum Solubility, mmoles: From the Deionization Curves		Maximum Solubility, mmoles: Spectrophotometrically Determined			
		20°	37°	20°	37°	20°		37°	
Procaine (II)	0.01 *M*	8.95	8.70	S	S	...	..	...	..
	0.1 *M*	9.05	8.70	7	32	...	..	...	..
	BE	9.0	8.7	7	32	12	H	14	H
Lignocaine (II)	0.01 *M*	8.20 D	7.70 D	S	S	...	..	...	..
	0.1 *M*	8.00	7.70	18	16	...	..	...	..
	BE	8.1	7.7	18	16	16	..	15	..
Cocaine	0.01 *M*	8.78	8.35	2	2.5	...	..	...	..
	0.1 *M*	8.76	8.40	2	2.5	...	..	...	..
	BE	8.8	8.4	2	2.5	2	..	2	..
Rec 7-0518	0.01 *M*	8.70	8.20	0.5	0.5	...	..	...	..
	0.1 *M*	8.60	8.00	?	?	...	..	...	..
	BE	8.7	8.2	0.5	0.5	0.4		0.5	..
Rec 7-0544	0.01 *M*	9.10	8.55	0.4	0.4	...	..	...	..
	0.1 *M*	8.92	8.40	?	?	...	..	...	..
	BE	9.1	8.5	0.4	0.4	0.8	..	0.5	..
Rec 7-0591 (I)	0.01 *M*	4.60 D	4.32 D	S	S	...	..	...	..
	0.1 *M*	4.60 D	4.34 D	S	S	...	..	...	..
	BE	4.6	4.3	S	S	...	..	...	..
Rec 7-0591 (II)	0.01 *M*	9.40	9.40	0.8	0.2	...	..	...	..
	0.1 *M*	?	?	?	?	...	..	...	..
	BE	9.4	9.4	0.8	0.2	0.5	..	0.7	..

[a] BE = best estimate, based on the most reliable values; D = obtained directly, without extrapolation; S = soluble at the investigated concentration; procaine II = second deionization of procaine; lignocaine II = second deionization of lignocaine; Rec 7-0591 I = first deionization of Rec 7-0591; Rec 7-0591 II = second deionization of Rec 7-0591; H = a substantial amount of procaine (about 20%) hydrolyzes at the high pH values of some steps of the experimental conditions.

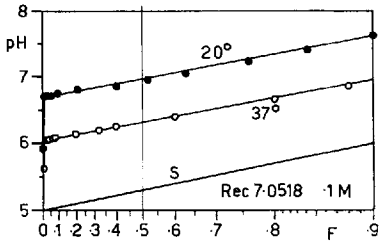

Fig. 11.—Rec 7-0518 0.1 *M*. Same data of Fig. 10 plotted on a L scale for F, in order to check the theory of Eq. 6. Besides by the straight-line alignment and by the slope of the data, the theory of Eq. 6 is verified also because the lines of Fig. 9 (Rec 7-0518 0.1 *M*) are higher by 1 pH unit than the corresponding lines of Fig. 11, as required by Eq. 6, since log S differs by 1 in the two conditions. Figure 9 becomes thus a right-hand continuation of Fig. 11.

fortunately, interference by hypersaturation phenomena and other technical difficulties make the estimation of maximal solubility by other methods problematic too. Therefore, the pK_B' values and the maximum solubility values of bases with a very small solubility of the unionized form, obtained by the described methods, must anyway be considered as very rough estimates.

The comments on Rec 7-0518 apply also to Rec 7-0544, since the solubility of its unionized form is also very low.

Rec 7-0591 has two basic radicals which ionize: one is the 3-amino group, with a pK_B' value of 4.6 at 20° and of 4.3 at 37°, and the other is the tertiary amino group in the *N*-diisopropylaminoethoxy chain, whose chemical-physical features are similar to those of the same radical in Rec 7-0518.

The results obtained with the investigated substances are summarized in Table I which leads to the following grouping.

(*a*) Substances soluble enough in the unionized form to remain in solution during the whole titration when the titration is performed on solutions with a concentration approximating that of the pharmaceutical solutions and in the range of pharmacological activity. This category includes procaine and lignocaine at 0.01 *M* concentration and Rec 7-0591 with regard to their properties during the first deionization process. The theory of Eq. 5 applies and the pH *versus* log $[B]/[BH^+]$ is a straight-lined relationship (*cf.* Fig. 3 of 0.01 *M* procaine). The pK_B' values can be determined directly.

(*b*) Substances with a more limited solubility of the unionized base. The theory of Eq. 5 applies until full saturation of the solution with the unionized base. Then the unionized form starts to precipitate and the theory described by Eq. 6 applies. This group includes 0.1 *M* procaine (Figs. 4–6), 0.1 *M* lignocaine, and 0.01 *M* and 0.1 *M* cocaine.

Maximum solubility of the unionized base and pK_B' values must be determined indirectly but still may be estimated with good approximation. Phenomena of hypersaturation are often present and can be demonstrated by plotting the data on an abscissa with a LR or L scale of Fig. 1. By the linearization so obtained it becomes possible to determine the actual concentration of soluble B during the remaining part of titration, when the excess of the unionized form precipitates.

(*c*) Substances with a low solubility of the unionized base. This group includes Rec 7-0518, Rec 7-0544, and Rec 7-0591, the last with regard to its properties during the second deionization process,

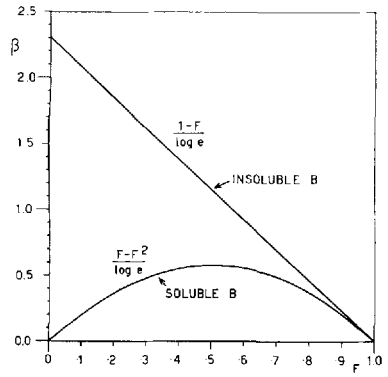

Fig. 12.—Buffering capacity β *versus* titrated fraction F of bases with insoluble unionized form or with soluble unionized form. The curves were calculated from the differential $\beta = dM/d\text{pH}$ and substituting for pH the right-hand member of Eq. 5 or, respectively, of Eq. 6. At a given concentration, the buffering capacity of bases with insoluble unionized form is much higher than that of bases with soluble unionized form. Furthermore, the maximum buffering capacity for the first type of bases is at the initial part of titration, whereas it is at the medial part of titration for the second type of bases.

Solubility of the unionized base and pK_B' values may be determined only with rough approximation.

DISCUSSION

Drop of pH Concomitant with Precipitation.—This behavior is frequently seen with substances whose unionized base is sparingly soluble, *e.g.*, 0.1 *M* procaine in Figs. 4–6. It is related to phenomena of hypersaturation of the unionized base, so that an excess of base provokes the precipitation of a part of [B] in Eq. 5, and the pH drops according to the new equilibrium described by Eq. 6. The maximum solubility of the unionized base, therefore, cannot always be deduced from the titrated fraction at the moment of precipitation, but must be calculated from the curve found after precipitation, extrapolating it in the direction of the first tract of the deionization curve, as exemplified in Fig. 7. This abrupt change of pH is never seen during the back-titration of the unionized base with a strong acid. In the back-titration the ionization curves follow the pattern of the dotted line of Fig. 7 and the solution becomes clear at the point in which the dotted line meets the first part of the ionization curve, which is then followed. This holds good for procaine and for all weak bases generally, which show the abrupt drop of pH during during deionization.

Buffering Capacity.—The tissue-tolerance for solutions with a pH different from that of the tissues depends partly on the buffering capacity of the solution, since possible damage to the tissues is related to the quantity of basic or acid radicals needed for re-equilibrating the pH. The buffering capacity is given by

$$\beta = \frac{d\,M}{d\,\text{pH}} \qquad \text{(Eq. 8)}$$

where M is the quantity of base or acid which provokes a change in pH.

For a base soluble in its unionized form, the buffering capacity β *versus* the titrated fraction F is given by the lower curve of Fig. 12 and is equal to:

$$\beta = \frac{F - F^2}{\log e} \qquad \text{(Eq. 9)}$$

The maximum value of β is at 0.5 titration, *i.e.*, when the pH = pK_B'.

A base which is insoluble in its unionized form has a much higher buffering capacity, equal to

$$\beta = \frac{1 - F}{\log e} \qquad \text{(Eq. 10)}$$

as shown by the higher curve of Fig. 12. The maximum value of β in this case is at zero titration. At a given concentration, a buffering system formed by a base with limited solubility of the unionized form is, therefore, more damaging for the tissues than a system formed by a base with a good solubility of the unionized form, when the pH differs from that of the tissues by the same degree. This explains the observation of Wehr and Koelzer (11) that low precipitation points on the pH or on the titration scale are correlated with low tolerance.

Effect of Temperature.—Conventionally pK_B' values are determined at 20° or at other temperatures close to room temperature.

As shown by Table I the pK_B' at 37° is usually lower, by a value up to 0.6, than the pK_B' at 20°. This phenomenon is related to the influence of temperature on the ionization constant of water which is 14.167 at 20.0° and 13.620 at 37°, with a drop of more than 0.5 unit.

The investigated bases have, therefore, a stronger proton-accepting property at 37° than at 20° and measurements taken at 20° or 25° can be misleading with regard to any inference on the tolerance of solutions for tissues at 37°.

It is interesting to note that for bases with a limited solubility of the unionized form an increase of the solubility of this form has an effect comparable to an increase of pK_B', *i.e.*, a shift toward a higher pH region of the whole deionization curve. The increase of temperature has, therefore, two opposite effects on the position of the deionization curve: due to the increase of the solubility of the unionized base, the deionization curve is shifted toward the top (*cf.* Eq. 6) and due to the decrease of the pK_B', the curve is shifted toward the bottom. The second effect seems usually to prevail over the first.

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