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The Time-Concentration Curve of Local Anesthetics

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The purpose of the present investigation is to find an adequate method to measure the activities of local anesthetics, especially some alkylaminoacyl anilides (investigated by Löfgren *et al.*[1-6]; see Table 1) such that it can be used in a theoretical study of the relation between physiological activity and physicochemical properties of anesthetics. The uncritical use of data from standard pharmacological methods for such comparisons has often led to inconclusive results and contradicting theories, because the methods, as a rule, work with too complicated systems.

It has been proposed that the activity of narcotics, also termed anesthetics — in a wide sense, substances which can reduce the ability of cells to become stimulated [8] — parallels their lipid solubilities or lipid/water partition coefficients [9—12]. In such, and similar [13] quantitative comparisons, the minimum concentration (C_m) for narcotic action, *i. e.* the concentration that just fails to produce an action at infinite time, is of the greatest interest. Measurements of depth and duration (*cf.* Girouard and Robillard [14a]) of anesthesia or the action time (T), *i. e.* the time between the moment of application and the beginning activity of an anesthetic, as a rule, involve diffusivity in an uncontrolled manner. In the commonly used wheal and cornea methods [15] the anesthetic has to diffuse from the point of application to the points of action at the same time as it is diluted and transported away by the body fluids. For example, the influence of diffusion is clearly demonstrated by the prolonged duration due to increased viscosity of the injection solution [16]. Thus, because these quantities are no simple functions of concentration, they cannot be used directly in quantitative comparisons.

Another important point which is often neglected is the great influence of pH on the composition of solutions of weak acids and bases. The alkylaminoacyl anilides and other local anesthetics are amines, B, which at physio-

logical conditions are partly ionized as BH^+, the proportion between B and BH^+ being determined by the dissociation constants, K_s, and the pH [17]. It has been claimed that B should be the active principle [18–21], and further, it is known that the proportion of uncharged molecules largely affects the diffusion through biological membranes [22, 23]. The fact that anesthesia is potentiated by a stronger basicity of the anion combined with procaine [24–27], or that it decreases with increasing concentrations in the cornea [28] or linguo-maxillary [14b] tests are probably at least partly pH effects (*cf.*, however, the phosphate effect, see page 73). The uncertainty of an activity measurement, due to a pH error of the test solution, is only partly [29] decreased (in injection methods) through the buffering action of the body fluids. It follows, that quantitative activity measurements must admit careful pH control throughout the experiment.

This control can only be affected by immersing the test object in an anesthetic solution so large that the concentrations of all components remain constant during the experiment. In addition, this is the only method to eliminate the complicated diffusion phenomena of injection experiments, although these can be somewhat simplified in cases of more localized application (*e. g.* intracutaneous and eyelid wheals, cornea test).

From the above considerations it seemed convenient for the present purpose to use a modification of the old method involving block of the frog sciatic nerve [19, 30–32] through immersing it in a sufficiently large solution the pH of which was determined. This method has been elaborated most ideally but somewhat expensively by Bennett *et al.* [33] — Naturally, the pharmacologists' determinations of depth, duration and action time are extremely valuable and necessary for the *pharmacodynamical* characterization of a compound. This fact must be distinguished from the inapplicability of these determinations for certain quantitative theoretical comparisons.

THE MINIMUM CONCENTRATION (C_m)

The C_m of an anesthetic can be determined by keeping identical nerves etc. for a long time in a series of solutions with decreasing concentrations (C) [10, 11, 34]. The C_m interval thus obtained can be made arbitrarily small but the significance of the measurement is diminished by alterations in the aging specimens (*cf.* Laubender and Saum [46]).

Between C and T (*i. e.* the action time, see above) a hyperbolic relation has been shown to exist [35–42] which, at least at intermediate concentrations can be expressed [35, 36]:

$$(C - C_m)^n \ (T - T_m) = K \qquad (1)$$

This and similar [37–43] expressions are exclusively empirical, and the constants, n and K, are without biological significance. As to membrane permeation a similar expression has been derived from Fick's diffusion law [44]. The disturbing influence of a long duration on the experiment could be avoided if a C,T-curve could be extrapolated from intermediate values to $T = \infty$, where $C = C_m$. But this has not yet been possible, because eq. (1) and similar expressions do not hold at small C values [35, 46]; *cf.*, however, Adams *et al.* [42], who have extrapolated straight lines (gold-fish method), *i. e.* n in (1) $= 1$.

For the present investigation a relation between C and T was derived, which admits such an extrapolation to $T = \infty$ and an evaluation of C_m. At the same time the diffusion constant, D, of the anesthetic in nerve tissue is calculated. The expression (eq. (2), p. 63) contains no empirical or unbiological constants. (As will be shown in Figs. 3A, 4A, 8, the intermediate part of the C, T-curve derived can be taken for a straight line; *cf.* Adams *et al.*[42]).

METHODS

The method used has many features in common with those of earlier workers [33, 31] but some details should be noted.

a. *Nervus ischiadicus*-leg preparations from *Rana arvensis* were investigated. Frogs weighing 25—40 g were used. All frogs were of the same natural race, and they were kept at identical conditions. The preparing of the nerve was performed according to Abderhalden's standard methods [45], the difference being that all the muscles beneath the knee were left. In order to decrease the metabolism and drying of the specimens an ice-cold preparation table, covered with Ringer-moistened blotting-paper, was used. After the preparing was completed the specimens were immediately immersed in Ringer solution (6.5 g of NaCl, 0.14 g of KCl, 0.12 g of $CaCl_2$, *aq. ad* 1000 ml [46]) at 15° C. They were used after 30 min.

b. In a double-walled thermostate cage, cooled with streaming tap water to 15 ± 0.5° C., and with a glass frontal wall, two preparations (as a rule, the two legs of one frog) could be mounted (Fig. 1) and tested simultaneously. The stretching of the nerve was made constant through the application of a constant pull from a 2 g weight (Fig. 1). The thermostate air was moistened thoroughly: large amounts of wet blotting-paper on the floor and walls of the cage proved to be most effective.

c. The nerves were stimulated through the application of D. C. sqare wave voltages of 0.5 s duration between two Pt wire rings 0.7 cm apart (*cf.* Laubender and Saum [46]) upon which the nerve was resting, see Fig. 1; (above 0.6 cm the nerve resistance is a linear function of the electrode distance [47]). The impulses were generated by an electronic stimulator [48]. The negative electrode was proximal to the leg. A switch permitted stimulation of one or the other preparation. The rheobase, *Rh* [49, 50a], was determined. A normal *Rh* value — about 100 mV — was taken as evidence of the normal function of an unhurt nerve. Specimens with abnormal *Rh* values, or which were known to have been hurt during the preparing, were withdrawn from further investigation.

d. The nerves were anesthetized within a proximal 2 cm region (only at regions < 0.8 cm the anesthesia is influenced by the length of the region [50b, 51]) between the point

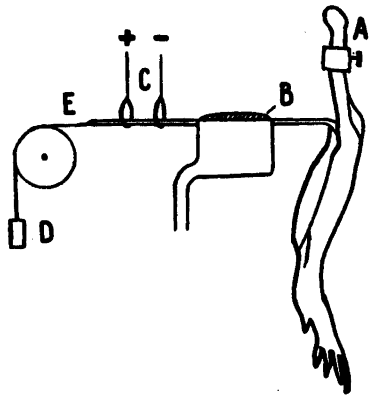

Fig. 1. Mounting of nerve-leg preparation.
A: Femur fixed in a brass holder.
B: The cup heaped up with anesthetic solution, which by reason of the paraffine does not run over.
C: Pt electrodes.
D: Weight.
E: Silk thread fixed at the end of the nerve.

of stimulation and the leg (Fig. 1), by the application of anesthetic solution in a 6 ml cup (Fig. 2; *cf.* Bennett *et. al.*[33]), which was paraffinized and provided with two diametrically situated cuts. This arrangement permitted the immersion of the nerve without hurting it through any contact with the edges of the cup. It was possible to stimulate the nerve at intervals without removing it from the anesthetic solution. Interruptions of the contact between the anesthetic solution and the test object, as a rule, are not avoidable when the test object is stimulated within the narcotized region, this giving an erroneous *C, T*-curve (*cf.* Laubender[38], Grammacioni[39]). After the application of the anesthetic solution the nerve was stimulated at 1 or 2 min intervals with one single impulse of 0.5 s duration and an amplitude somewhat greater than *Rh, i. e.* this quantity was determined continuously. In fact, *Rh* was found to be constant until block occurred. Consequently, it could also be used as evidence of normal function of the preparation during the experiment. Any drying of the nerve or the fatiguing influence of an inappropiate shape of the current impulses seem to increase *Rh* gradually. Perhaps, such effects will explain the ascending *Rh*/time curve found by other authors[46]. — The time from the application of the anesthetic until that stimulus to which there was no muscle response was noted as *T*.

e. Highly purified specimens of the anesthetics were dissolved, as chlorides or nitrates of the ions, BH^+ (*cf.* Table 1), in Ringer solution containing 0.01 mole of sodium phosphate buffer per l.* The pH at 15° C was adjusted to 7.39 by NaOH; the buffer was sufficient to keep the pH within the limits 7.39 ± 0.01 during the experiment. — The pH was measured at 25° C with a glass and a calomel electrode. Vacuum tube mV-meter (Radiometer PHM 3, Copenhagen), accuracy ± 0.002 pH. Standard: 0.05-C potassium biphthalate, pH = 4.008[52]. To the values obtained is added 0.03 to get the pH at 15° C[53].

* *I.e.* with 1.422 g of $Na_2HPO_4 \cdot 2H_2O$ and 0.276 g of $NaH_2PO_4 \cdot H_2O$ per l.

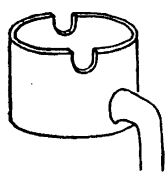

Fig. 2. Anesthetic cup with cuts and outlet for solution.

f. Every anesthetic was applied in several concentrations (Table 3), and in every concentration at least two nerves from different frogs were tested.

g. T varies as R^2 (R = nerve radius); this is found experimentally by [33] and verified by the present author (unpubl.); further, *cf.* eq. (2). This variation in T is great because R varied between 0.019 and 0.034 cm. Hence, after the completion of every experiment the mean nerve thickness within the narcotized region was determined microscopically with an ocular micrometer. The nerve was suspended horisontally and under constant pull between the objective and the stage of an ordinary microscope. Some five readings were made and averaged. By this method R could be determined within 3 %. The T values were than recalculated to a standard nerve radius, R = 0.0275 cm (Table 3), which is a mean value.

DIFFUSION THEORY

Contrary to the anesthesia of a whole nerve [54] that of a single nerve fiber follows the all-or-none law [55], and it is instantaneous after an anesthetic, at a concentration greater than C_m, has reached the nodes of Ranvier [55]. It follows, that the time before total block of the nerve, T, is due entirely to the diffusion of the anesthetic into the nerve. It is assumed that all the motoric fibers of a nerve have the same C_m, and that their distribution in the trunk includes the central part. — In order to registrate the function of all motoric elements of the nerve, whole nerve-leg preparations were made. The registration of the action current with a cathode ray oscillograph [33] would be an equivalent or preferable method. It follows that at the time (T) when the concentration, C_m, is reached at the centre of the nerve, all fibers of the trunk are blocked, *i. e.* no part of the leg responds to a stimulus.

The inner of the nerve contains a large number of small discontinuities (nerve fibers, layers of connective tissue, etc.), but, taken as a whole, it may be regarded homogeneous in the respect that an anesthetic has the same D in all parts of it. Thus, regarding the relation between C and T, the problem reduces to a special case of the heat conduction regarding the conduction of heat in an infinitely long cylinder.

Analogously to Hill's [56] calculations on the diffusion of oxygen into a cylindrical tissue, we*) arrived at the expression

$$c(t;r) = C(1-2\sum_{\nu=1}^{\infty} \varphi_\nu (r) \cdot e^{-Da_\nu^2 t})$$

where

$c(t;r)$ = concentration of the anesthetic at the time t and at the distance r from the centre of the nerve,

* The derivation of eq. (2) was kindly performed by fil. lic. H. Rådström, University of Stockholm, to whom I am greatly indebted.

C = concentration of the anestetic in the surrounding solution,

$\varphi_\nu(r) = \frac{J_0(\alpha_\nu r)}{\alpha_\nu R J_1(\alpha_\nu R)}$, J_0 and J_1 being the Bessel's functions, and α_ν the zeros of J_0,

R = the nerve radius,

D = the diffusion constant.

Thus, the concentrations in the centre of the nerve at times t are, terms of higher order being rejected:

$$c\ (t;0) = C\ (1—2\ \varphi_1\ (0)\ e^{-D\alpha_1^2 t})$$

where $\varphi_1\ (0) = 0.8$ and $\alpha_1^2 = \frac{5.76}{R^2}$

Total block occurs when $c(t;0) = C_m$, *i. e.*

$$C_m = C(1—1.6\ e^{-5.76DT/R^2}) \qquad (2)*$$

The expression (2) is used for the determination of C_m and D for local anesthetics from measurements of C, T, and R. For $C < 10C_m$, (2) holds within 1 %.

The pH of the inner of the nerve is not known exactly. If there were free exchange of ions between the surrounding fluid and the extrafibrillar, fluid-filled spaces of the nerve (as in the case of *Loligo* [57a]), these spaces would soon get the pH of the surrounding fluid. Further, at diffusion equilibrium, C would be the same in the surrounding fluid and in the extra-fibrillar spaces mentioned. But, if there were diffusion of uncharged molecules, B, only, the pH of the inner spaces of the nerve would not alter much, and, at diffusion equilibrium, only C of the free base (C_B) would be equal outside and inside the nerve. As will be shown, the latter is valid in the present case, although it is possible that BH^+ can partake in the diffusion *within* the nerve. The fact that, in the following, C and C_m refer to the total concentration outside the nerve does not influence on the calculations [*cf.* eq. (2)]. However, the more important minimum concentrations of free base, $(C_m)_B$, are calculated from the pK_s values [7] (Table 1). f_{BH^+} in physiological solutions is assumed to be 0.80 (calculated from the Debye-Hückel formula [68]).

RESULTS

With the methods presented above 22 alkylaminoacyl anilides (see Table 1) and procaine were investigated. From corresponding C and T values, listed in Table 3, $C/\frac{1}{T}$ curves were drawn, because at infinite time, *i. e.* $\frac{1}{T} = 0$,

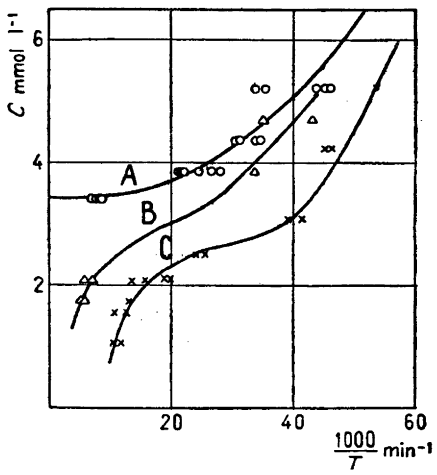

Fig. 3. $C/\frac{1}{T}$ curves of compound N.

A: Ringer without phosphate (expt. Nε) (= 0.01 C phosphate).
B: Ringer with 0.02 C phosphate.
C: 0.10 C phosphate.

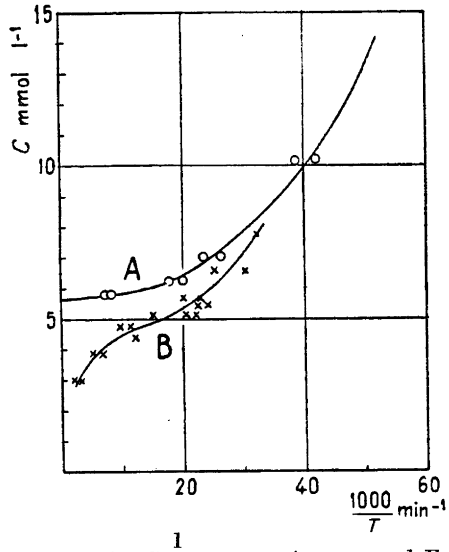

Fig. 4. $C/\frac{1}{T}$ curves of compound F.

A: 0.01 C phosphate.
B: 0.10 C phosphate.

this curve is horizontal and $C = C_m$ [eq. (2)]. Thus, C_m could be determined approximately (C'_m) through extrapolation of the curve to $\frac{1}{T} = 0$. Putting C'_m and R into eq. (2), a D value could be calculated for every C, T pair. The fact that in nearly all of the experiments, the C'_m values calculated are such that D is independent of C [*i. e.* the points $(C;\frac{1}{T})$ lie at random on both sides of the theoretical curve, Figs. 3A, 4A] is taken as evidence that within the limits of error, the experimental $C/\frac{1}{T}$ curves do not differ from the theoretical ones. Concentrations smaller than C'_m gave no anesthesia, even after long times (expts. A, C, J, K, Nε, Table 3). These experiments are important because they prove that the curve actually does not descend at small $\frac{1}{T}$ values as in the presence of higher phosphate concentrations (*cf.* p. 73; Figs. 3, 4). — It should be pointed out that the correctness of the theory cannot be proved merely by the present investigation; it can only be made probable.

If for each substance tested, the average of the D values obtained be called D', the true C_m and D values may be written $C'_m + \Delta$ and $D' + \delta$, resp. If these values are substituted into eq. (2) the expression

$$(C - C'_m)\left[\log\left(1 - \frac{C'_m}{C}\right) + 3288\ D'T - 0.204\right] = \Delta - \delta\ 3288\ T\ (C - C'_m) \qquad (3)$$

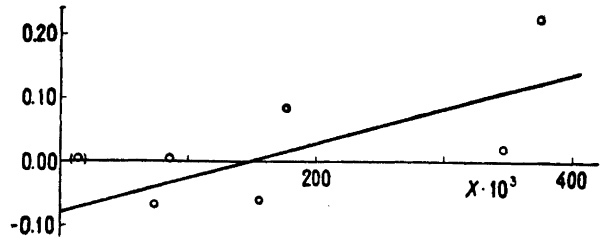

Fig. 5. Expt. F as example of correction curve. The points are obtained from original values $C'_m = 5.76$ *mmol* l^{-1}; $D' = 0.725 \cdot 10^{-5}$ cm^2 min^{-1} *put into eq. (3). From the slope and intercept of the most probable line* $-\delta = (+ 0.05 \pm 0.03)\ 10^{-5}$ *and* $\Delta = - 0.08 \pm 0.06$. *Hence,* $C_m = 5.64$ *mmol* l^{-1}; $D = 0.68 \cdot 10^{-5}$ cm^2 min^{-1}.

may be derived. Eq. (3) is of the type $x = a + by$. It is valid at small errors Δ and δ. The curves (3) were drawn for the substances investigated, and $+ \Delta$ and $- \delta$ were estimated from the intercept and the slope of the line, resp. Thus C'_m and D' could be corrected to more true values, C_m and D, at the same time as the uncertainty of these could be estimated. The C_m and D values are listed in Table 1. The mean errors of D are given; as a rule, these are about 30 % of the maximum errors obtained from the curves (3). Points at low concentrations give very uncertain D values: they were excluded from the calculations of D' and the mean error of D. In Table 1, (n) is the number of measurements used for these calculations.

During the experiments the pH of the test solutions decreased 0.00—0.01 (determined at the times, $T/2$), due to diffusion of B into the nerve. — The use of larger or, better, streaming test solutions could have removed this source of error; however, the supplies of purified substances were too limited. — It follows that C_B of the outer solution is diminished maximally about 2 % through a) diminished total concentration, b) decreased pH. This error of C_m, ΔC_m (pH), which is of a systematical character, as a rule dominates. The total error of C_m, ΔC_m (pH, D, T) depends further on uncertainty in D and T. ΔC_m (D) and $\Delta C_m(T)$ do not exceed 0.2 % and 0.5 %, resp.; only in bad cases the latter exceeds 2 %. Thus, with some higher exceptions (expts. H, J, L, R, V, W, X), the maximum errors, ΔC_m of Table 1, are given to $\pm$ 3 %.

INFLUENCE OF EXTERNAL FACTORS

a. The nature of the salt anions used (Cl^- and NO_3^-), within limits of error did not influence the properties of the curve (Table 2, expts. Nα and Nβ).

b. Within limits of error, the pH of the outer solution proved to be without influence on D, whereas C_m varies in such a way that the minimum con-

Table 1. Mol weights, dissociation constants (according to Löfgren and Östlund [7]), minimum concentrations, diffusion constants, and minimum times of local anesthetics.

Expt. no.	Anesthetic (BH^+X^-)		Synthesis Literature (1—6)	Mol. wt. of B	$pK_s = -\log \frac{a_{H^+} \cdot a_B}{a_{BH^+}}$ [from 7]	$C_m \pm \Delta C_m$ = total conc. at pH 7.39	$(C_m)_B$ = base conc.	$D \pm$ mean error (n), see text	T_m (at nerve radius R = 0.0275 cm) $= \frac{\log 1.6}{5.76 \log e} \cdot \frac{R^2}{D}$ min
	B of BH^+	X				mmol l^{-1}	mmol l^{-1}	10^{-5} cm^2 min^{-1}	
	I. $RCOCH_2N(C_2H_5)_2$; R =								
A	C_6H_5NH	NO_3	(1)	206.3	8.029	9.54 ±0.29	1.48	1.39 ±0.06 (5)	4.4
B	cyclohexyl NH	Cl	(6)	212.3	8.21	25.4 ±0.8	2.73	1.12 ±0.13 (5)	5.5
C	2-$CH_3C_6H_4NH$	NO_3	(1)	220.3	7.905	5.22 ±0.16	1.02	0.95 ±0.07 (5,8)***	6.5
D	2-$CH_3C_6H_4NCH_3$	Cl	(2)	234.3	8.74	50.8 ±1.5	1.75	0.50 ±0.03 (4)	12.3
E	3-$CH_3C_6H_4NH$	Cl	(2)	220.3	8.035	4.09 ±0.12	0.625	0.80 ±0.03 (9)	7.7
F	4-$CH_3C_6H_4NH$	Cl	(2)	220.3	8.055	5.65 ±0.17	0.832	0.68 ±0.02 (6)	9.1
G	2,3-$(CH_3)_2C_6H_3NH$	Cl	(2)	234.3	7.940	3.51 ±0.11	0.644	0.46 ±0.02 (6)	13.4
H	2,4-$(CH_3)_2C_6H_3NH$	NO_3	(2)	234.3	7.945	3.50 ±0.18	0.636	0.52 ±0.04 (4)	11.9
J	2,5-$(CH_3)_2C_6H_3NH$	NO_3	(2)	234.3	7.920	4.04 ±0.34	0.769	0.63 ±0.05 (8)*	9.8
K	3,4-$(CH_3)_2C_6H_3NH$	Cl	(2)	234.3	8.055	2.51 ±0.08	0.369	0.42 ±0.01 (9)	14.7
L	3,5-$(CH_3)_2C_6H_3NH$	Cl	(2)	234.3	8.015	2.07 ±0.12	0.329	0.47 ±0.02 (11)	13.1
M	2,6-$(CH_3)_2C_6H_3NH$	Cl,NO_3	(2)	234.3	7.855	3.79 ±0.11	0.815	1.00 ±0.06 (18)	6.2
N	2,4,6-$(CH_3)_3C_6H_2NH$ (mean values)	Cl,NO_3	(2)	248.4	7.900	3.44 ±0.06	0.680	0.89 ±0.02	6.9
	II. 2,6-derivatives others than M, N. 2,6-$(CH_3)_2C_6H_3NH$ COR_1; R_1 =								
P	$CH_2N(CH_3)_2$	Cl	(2)	206.3	7.355	11.1 ±0.3	5.14	1.9 ±0.1 (6)	3.2
Q	$CH_2N(n\text{-}C_3H_7)_2$	NO_3	(5)	262.4	7.68	0.77 ±0.02	0.224	0.43 ±0.02 (6)	14.3
R	CH_2NHCH_3	Cl	(5)	192.3	7.990	47 ±5	7.81	probably small	
S	$CH_2NHC_2H_5$	Cl	(5)	206.3	8.075	23.9 ±0.7	3.38	0.47 ±0.01 (8)	13.1
T	$CH_2NH\text{-}n\text{-}C_3H_7$	Cl	(5)	220.3	8.012	9.35 ±0.28	1.50	0.52 ±0.01 (10)	11.9
U	$CH_2NH\text{-}n\text{-}C_4H_9$	Cl	(4)	234.3	8.035	4.21 ±0.13	0.644	0.65 ±0.03 (7)	9.5
V	$CH_2NH\text{-}iso\text{-}C_4H_9$	Cl	(4)	234.3	7.830	4.1 ±0.4	0.912	1.16 ±0.20 (6)**	5.3
W	$CH(CH_3)N(C_2H_5)_2$	Cl	(3)	248.4	8.110	3.97 ±0.20	0.524	1.25 ±0.12 (4)**	4.9
X	$CH_2CH_2N(C_2H_5)_2$	Cl	(3)	248.4	8.96	36 ±4	0.761	0.30 ±0.05 (3)*	20.5
Y	*III.* Procaine	Cl		236.3	8.96 (From 67)	12.46 ±0.37	0.262	1.10 ±0.05 (9)	5.6

* Maximum error. ** Temp. error, see table 3. *** Eliminated points are regarded to some extent.

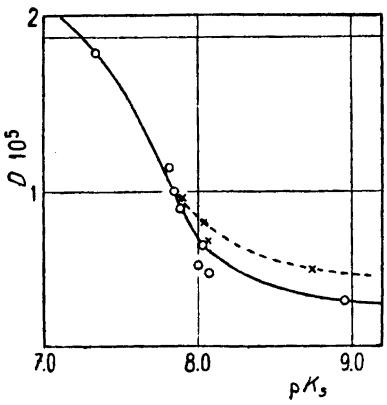

Fig. 6. D/pK_s curves of 2,6-xylidides (○) and toluidides (×).

Table 2. N-Diethylaminoaceto-2,4,6-trimethyl anilide (expt. no. N). Influence of pH, anion, and phosphate on minimum concentration and diffusion constant.

Expt. no.	Anion X^-	Conc. of phosphate buffer mol l^{-1}	pH	$C_m \pm \Delta C_m$ = total conc. mmol l^{-1}	$(C_m)_B \pm \Delta(C_m)_B$ = base conc. mmol l^{-1}	$D \pm$ mean error 10^{-5} cm^2 min^{-1}
α	Cl^-	0.01	7.39	3.41 ±0.10	0.674 ±0.020	0.95 ±0.10
β	NO_3^-	0.01	7.39	3.50 ±0.10	0.691 ±0.021	0.88 ±0.06
γ	Cl^-	0.01	6.96	8.07 ±0.24	0.678 ±0.020	0.92 ±0.02
δ	Cl^-	0.01	7.00	7.37 ±0.22	0.673 ±0.020	0.86 ±0.08
ε	Cl^-	none	7.39	3.41 ±0.10	0.674 ±0.020	0.83 ±0.03

centration of the free base, $(C_m)_B$, is constant (Table 2). This fact needs not evidence that B is the active principle of a local anesthetic (*cf.* Trevan and Boock [18]), but only that the anesthetic acts on the other side of a membrane permeable to B only. However, the fact that most general and many local anesthetics (*e. g.* alcohols [58, 59]) are unionizable molecules, makes it probable that B can anesthetize.

c. The small amounts of phosphate (0.01 moles per l) did not, within limits of error, influence the course of the curve, *cf.* Table 2, expts. Nα and Nε. In expt. Nε, where the anesthetic was dissolved in unbuffered Ringer solution, the anesthetic solution in the cup was renewed every 15 min during the experiment, so that pH should not sink appreciably. In fact the two C_m values are identical.

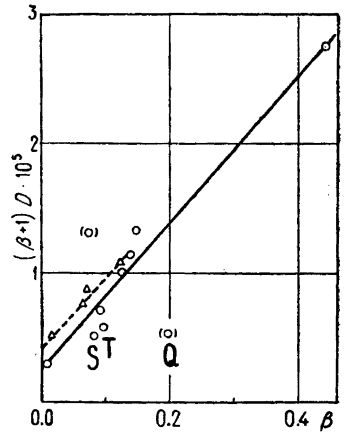

Fig. 7. Cf. Fig. 6. Curves from eq. (5). 2,6-xylidides (O) and toluidides (△). Expts. Q, S, and T (see text) are marked.

However, greater amounts of phosphate had a remarkably increasing effect on the anesthetizability of nerves (Figs. 3C, 4B). The sodium phosphate buffer used in these experiments was made according to Green [53]. It was isotonic with the Ringer and contained, per liter: 0.0213 moles of HPO_4^{2-}, 0.0787 moles of $H_2PO_4^-$, and $1.9 \cdot 10^{-3}$ moles of KCl. The 1×10^{-3} moles of Ca^{2+} required for a frog Ringer [46] could not be added, because part of it precipitated as $Ca_3(PO_4)_2$. From the solubility product of this substance, $1 \cdot 10^{-25}$, [60a] it follows that only ca. $2 \cdot 10^{-5}$ moles of Ca^{2+} can remain in solution.

In the calculation the phosphate ion activity, $a_{PO_4^{3-}}$, is determined from the expression $pH = pK_s + \log \frac{a_{PO_4^{3-}}}{c_{HPO_4^{2-}} \cdot f_{HPO_4^{2-}}}$, [17] where the 3rd dissociation constant of H_3PO_4, pK_s, is 12.32 [60b]. The activity coefficients of divalent ions, $f_{Ca^{2+}}$, and $f_{HPO_4^{2-}}$, are supposed to be ca. 0.33, calculated from the simplified form of Debye-Hückel's formula:

$$-\log f_i = 0.5\, z_i^2 \frac{1+\sqrt{I}}{\sqrt{I}}.$$ [17]

At more intermediate concentrations of phosphate (*e. g.* 0.02 moles per liter) the effect sets in later (Fig. 3B), and in the Ringer with 0.01-C phosphate *i. e.* the solution used in the head experiments, there is no influence on the narcotizability (*cf.* above), neither is Ca^{2+} precipitated at physiological concentrations. It is probable that the effect is due, at least partly, to precipitation or extraction of the cell Ca, which is necessary for irritability [61]. This explanation may also hold for some of the effects of other anions [24, 25]. Oxalate and citrate are known to prevent nerve activity [62a]. — The »phosphate anesthesia» is reversible.

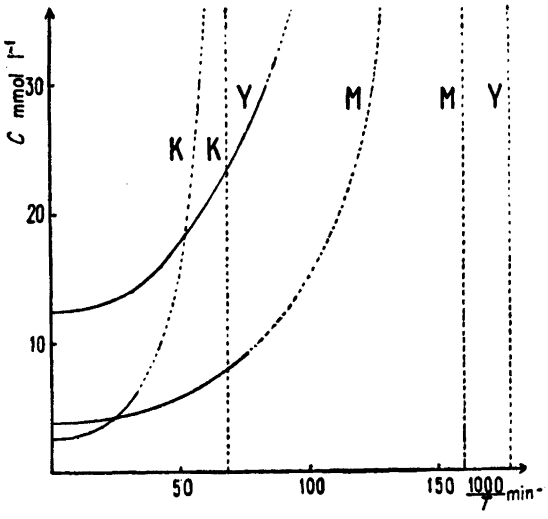

Fig. 8. Theoretical curves and asymptotes of xylocaine (M), its 3,4-isomer (K), and procaine (Y). The extrapolated parts are dotted.

d. Within a group of related substances (*e. g.* toluidides, or 2,6-xylidides) the compounds exhibit some kind of negative proportionality between D and pK_s (Table 1). Although the situation is too complicated to be analysed in detail, it is evident that the base, B, more easily diffuses through nerve tissue than does BH^+. This indicates what could be expected from histological data, namely, that only part of the cross section of the diffusion path, the fraction γ say, is open to diffusion of BH^+ (and, naturally, of B, occurrent in a concentration determined by pH and pK_s). The fraction $1-\gamma$, which is probably lipoidic, is permeated by B only. From D/pK_s diagrams (Fig. 6) it is seen that at high pK_s values, where only BH^+ diffuses, the curve tends to a lower limit, whereas at low pK_s values, where only B diffuses, it probably tends to an upper limit. If the real diffusion constants (D_{real}) are supposed to be identical in both fractions of the nerve, the effective diffusion constant of B will be D_{real}, that of BH^+ only $\gamma \cdot D_{real}$. Substituting differences for the derivates of Fick's diffusion law the expression

$$\Delta C = C\alpha^2 \left(D \Delta t - \tfrac{1}{\alpha} \Delta x\right) \tag{4}$$

can be derived, where $C = f(x) \cdot g(t)$ is the concentration and α^2 is a constant.

From $\frac{\delta c}{\delta t} = D \cdot \frac{\delta^2 c}{\delta x^2}$ the expression $\frac{1}{D \cdot g} \cdot \frac{dg}{dt} = \frac{1}{f} \cdot \frac{d^2 f}{dx^2} = \alpha^2$ is obtained.

I. e. $\frac{dg}{dt} = \frac{\Delta g}{\Delta t} = D g \alpha^2$; $f = k \cdot e^{-\alpha x}$, and $\frac{df}{dx} = \frac{\Delta f}{\Delta x} = -\alpha f$.

Because $\frac{\Delta C}{C} = \frac{\Delta g}{g} + \frac{\Delta f}{f}$, eq. (4) is valid.

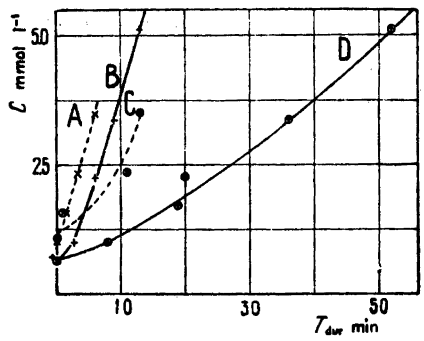

Fig. 9. Intracutaneous wheals.
A: Compound K.
B: Xylocaine.
C: Compound K, with epinephrine.
D: Xylocaine, with epinephrine.

The increase in concentration, ΔC, can be designed to consist of two parts, ΔC_B and ΔC_{BH+}. According to (4)

$$\Delta C = \Delta C_B + \Delta C_{BH+} = \alpha^2 [(C_B + \gamma C_{BH+}) D_{real} \Delta t - (C_B + C_{BH+}) \tfrac{1}{\alpha} \Delta x],$$

which can be expressed $\Delta C = \alpha^2 (C D \Delta t - C \tfrac{1}{\alpha} \Delta x)$.

$$\therefore C D = (C_B + \gamma C_{HB+}) D_{real}; \quad i.e. \frac{D}{D_{real}} = \frac{\beta + \gamma}{\beta + 1}, \tag{5}$$

where $\beta = C_B/C_{BH+}$ at the pH of the nerve, which is here assumed to be 7.00 (*cf.* Heilbrunn [62b]). If $(\beta + 1)$ D is plotted against β (Fig. 7) a straight line can be obtained, the slope of which is D_{real} and the intercept of which is $\gamma \cdot D_{real}$. D_{real} of 2,6-xylidides is about $6 \cdot 10^{-5}$ cm^2 min^{-1}, *i. e.* nearly the same as D of solutes in gels [60c] and slightly smaller than D of correspondingly large molecules in water [60d] or organic solvents [60e] (α-bromonaphthaline!). This strongly supports the theory upon which the present investigation is based. — The »hydrophilic» nerve fraction, γ, is only about 0.04.

It should be noted that different groups of anesthetics show different ability to diffuse into the nerve (*cf.* Table 1), a phenomenon which could be ascribed to specific properties. The high D of procaine when compared with its high pK_s is notable. On the other hand, high molecular weight (compound Q) and low lipid solubility (*e. g.* compounds S and T) are known to diminish diffusivity through biological membranes [63, 57b]. *Cf.* Fig. 7.

Anyhow, it is proved that D depends on pK_s. This fact in connection with the fact that D is not affected by an altered pH of the outer solution (point b. above) proves that the pH of the nerve is not altered appreciably during an experiment. Otherwise β, and, followingly, D should be altered.

Table 3. Experimental C and T values; the latter are recalculated to the nerve radius R = 0.0275 cm.

Expt.	C mmol l^{-1}	T min
A	16.7	15
	»	12
	11.1	22
	»	23
	10.0	37 ½
	9.00	>200
	7.41	>200
B	76.8	8
	»	8 ½
	57.5	13 ½
	28.7	48 ½
	»	29 ½
	25.9	82
C	6.95	19
	»	27 ½
	»	29
	6.39	34
	5.93	37
	»	40
	5.31	69 ½
	»	195
	5.29	>200
D	78.1	42
	»	48
	65.3	47
	»	48
	51.2	150
	»	203
E	9.19	19 ½
	»	20
	7.20	21
	»	21 ½
	5.45	32 ½
	5.41	27
	»	27 ½
	4.67	41
	»	42 ½
	4.28	84
	»	>120
F	10.16	24
	»	26
	7.01	38
	»	43
	6.23	50
	»	56
	5.80	120
	»	130
G	6.20	32 ½
	»	43
	5.13	42 ½
	»	45
	4.43	60
	»	64
	3.69	76 ½
	»	150
H	5.38	38
	»	43 ½
	4.27	46
	»	58
	3.70	128
	3.36	240
J	6.22	26 ½
	»	35 ½
	5.85	30
	»	34
	5.05	34
	»	36
	»	35 ½
	4.47	63
	»	200
	4.10	>200
K	5.43	35 ½
	»	36
	4.06	43 ½
	»	43 ½
	3.53	47 ½
	»	51 ½
	»	55 ½
	2.93	81 ½
	»	81 ½
	2.40	>200
L	6.27	22 ½
	»	24
	»	24 ½
	4.61	26 ½
	»	29
	3.69	34
	»	39
	2.95	47
	»	57
	2.33	77
	»	83
	1.99	140
M	8.32	12
	»	15 ½
	»	17
	6.40	(13 ½)
	»	16
	6.36	11 ½
	»	13 ½
	»	16 ½
	»	18 ½
	5.52	15 ½
	»	22 ½
	5.16	(16 ½)
	»	24 ½
	5.12	27
	»	29
	4.27	42
	»	45 ½
	4.14	40
	»	94
	3.95	53 ½
Na	4.74	21
	»	23 ½
	»	27 ½
	3.86	27 ½
	»	28

Expt.	C mmol l^{-1}	T min
	3.86	53
	3.42	165
	»	170
	»	250
β	5.40	23
	4.50	27
	»	29 ½
	»	32
	3.98	53
	»	75
	3.53	131
	»	160
γ	10.36	28
	»	29 ½
	8.78	43
	»	45
	8.35	57 ½
	»	72
δ	10.42	26
	»	29
	9.30	29 ½
	»	(42)
	8.36	40
	»	(60)
	7.87	55 ½
	»	60
	»	61
	»	66
ε	5.25	22
	»	22
	»	22 ½
	»	28 ½
	»	30
	4.42	29
	»	29 ½
	»	32
	»	32 ½
	3.89	35 ½
	»	38
	»	41
	»	45 ½

Expt.	C mmol l^{-1}	T min
	3.89	46
	»	46
	3.42	120
	»	123
	»	125
	»	137
	2.50	>210
	»	>210
P	28.2	8⅓
	»	7
	18.9	7⅔
	»	9⅓
	12.4	18
	»	18 ½
	10.7	125
Q	1.96	27
	»	30
	1.26	43 ½
	»	44 ½
	1.01	52
	»	65
R	53.6	87
	39.0	>300
S	50.8	30
	»	38
	41.2	32
	»	47
	32.5	43 ½
	»	51
	28.8	71
	»	71
T	28.7	21
	»	21 ½
	22.2	24 ½
	»	28
	18.5	31
	»	31 ½
	15.4	34
	»	37
	12.9	(60)

Expt.	C mmol l^{-1}	T min
	9.81	82
	»	85
U	7.50	22
	»	27
	6.46	30
	»	30 ½
	5.54	38
	»	45
	4.69	65
	4.25	132
V	8.71	10
	»	11 ½
	5.79	17 ½
	»	17 ½
	»	24 ½
	4.84	(19 ½)*
	»	(21)*
	»	37
W	6.88	12*
	»	13*
	4.60	24 ½
	»	25
	4.07	30*
	»	76
X	59.2	61
	42.6	86
	»	134
	22.0	>200
Y	29.7	11
	»	14 ½
	20.6	13 ½
	»	15 ½
	14.8	30
	»	30 ½
	»	31
	14.1	32 ½
	»	32 ½
	12.8	59
	»	64

* Temperature erroneously 17° C.

SOME CONNECTIONS WITH CLINICAL ANESTHESIA

a. From the C, T relation found experimentally (Figs. 3, 4, 8) and derived mathematically [eq. (2)] it follows that there is, at a given nerve radius, a shortest time (T_m), within which anestesia can be produced. T_m is proportional to $\frac{R^2}{D}$(values, see Table 1). In clinical conduction anesthesia, the rapid action of good anesthetics like xylocaine [2–5] and novocaine (Table 1, compounds M and Y) is related to the quantity, T_m. — Further, it should be noted, that it is impossible to compare the activities of a couple of anesthetics by means of their action time values at only one concentration, because the curves may intersect at some point (*cf.* Fig. 8) below which one and above which the other compound is the better anesthetic, *i. e.* has the shortest T.

b. It should be pointed out once more that, although there may be some connections of interest, the present methods to characterize anesthetics does not try to evaluate their pharmacodynamical properties. For a study of the latter the ability to narcotize sensory nerve fibers must catch the greatest interest. Especially as the ratio of motor paralysis to sensory paralysis may not always be the same [34, 64, 65], although C_m in the two cases is of the same order of magnitude. Now, for a few substances, C_m was determined in the case of intracutaneous wheals in man [58, 66]. 0.4 ml of anesthetic solutions (containing 0.85 % of NaCl and with pH = 7.4) were injected in the skin of the inside of the forearm, and the duration, T_{dur}, of the anesthesia, which occurred instantaneously, was measured (pin prick). The T_{dur}/C curves of xylocaine and its 3,4-isomer are seen in Fig. 9. Here the C_m ratio is the inverse of that found in motor paralysis. Further it is clear that T_{dur} should diminish with increasing ability of the substance to diffuse away from the point of action. If this diffusion were related to the diffusion into nerves described above, xylocaine would be expected to exert the shorter duration, which is not the case (Fig. 9). The longer remaining of xylocaine might be related to a greater vasoconstricting effect of this substance, but as the addition of epinephrine (0.002 %) does not reverse the T_{dur} ratio (Fig. 9), specific adsorption may play a role. Also in the case of D, specific properties could be traced to the 2,6-derivatives (p. 74—75).

SUMMARY

1. A method is described to determine, with high accuracy, the minimum concentration for anesthesia (C_m) of the motoric fibers of the frog sciatic nerve.

2. The action times (T) at different concentrations (C) of local anesthetics

(22 alkylaminoacyl anilides and procaine) were determined. The compounds were applied in buffered Ringer at pH 7.39.

3. Applying the theory of heat conduction in an infinitely long cylinder, C could be expressed as a function of C_m, T, D (*i. e.* diffusion constant of the anesthetic in nerve), and R (*i. e.* nerve radius). From measurements of C, T, and R the C_m of an anesthetic could be determined.

4. The validity of the theory was made probable by a. conformity of the empirical curve with the theoretical one b. agreement of known diffusion constants with the D values calculated; the latter are in fact depending on the diss. constants of the compounds, only the free base diffusing through all parts of the nerve.

5. C_m depended on the pH of the test solution, the minimum concentration of the free base being constant.

6. Phosphate at concentrations able to precipitate cell Ca increased the anesthetic activity.

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