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THE RELATION OF HYDROGEN ION CONCENTRATION TO THE ACTION OF THE LOCAL ANÆSTHETICS.

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Gros (1912), Regnier (1924–1925), Copeland (1925) and others have shown that alkaline solutions of local anæsthetics are active in higher dilutions than acid solutions. Many other salts of physiologically active bases are also potentiated by alkali in the same way, *e.g.* quinine (Brown and Henry, 1923).

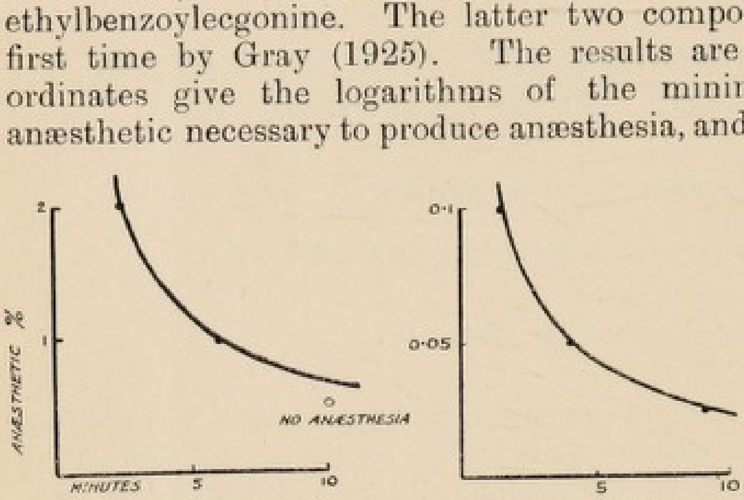
Gros suggests that the free base is the only active constituent, and that alkalinization produces increased anæsthetic action by virtue of the increase in the amount of base set free. He suggests that in the form of free base, all anæsthetics are much of the same activity, and that a correlation exists between the local anæsthetic action and the solubility of the base in lipoid solvents.

Regnier (1925[2]), on the other hand, on the ground that the addition of alkali to a saturated solution of cocaine base in water increases the anæsthetic action, considers that all the forms, ion, base and salt in which cocaine occurs in aqueous solution are active, and that the action of alkali is directly on the tissues concerned.

We have investigated the action of a series of local anæsthetics on the rabbit's cornea under slightly different conditions, and have determined the dissociation constants with a view to comparing the relative activities of the free bases at the same hydrogen-ion concentration.

The experiments have been carried out with solutions of the hydrochlorides of the base dissolved in Clark and Lub's buffer mixtures. In the stronger solutions necessary at the more acid reactions, the reaction of the buffer was shifted towards the acid side. The actual reaction was estimated colorimetrically. The smallest concentration of anæsthetic which will produce anæsthesia in ten minutes was determined at each pH, the necessary dilutions being carried out with buffer solutions. The time limit of ten minutes was adopted because it was found that further dilution beyond the concentration necessary to produce anæsthesia in that time did not cause anæsthesia at all (see Fig. 1). The dilutions were tested in a series, each dilution being half the concentration of the previous one, and the end-point taken as that which produced a patchy anæsthesia in ten minutes, or alternatively, that concentration intermediate between that which produced complete anæsthesia in rather less than ten minutes and that which produced no anæsthesia at all. It is of great importance, for the reasons stated below, to use heavily buffered solutions, and to instil the solution continuously during the specified time, testing the response of the corneal reflex to light touch during instillation.

The effect of hydrogen-ion concentration has been examined for cocaine, novocaine, β-eucaine, stovaine, conessine, benzylbenzoyl-ecgonine and phenylethylbenzoylecgonine. The latter two compounds were synthesized for the first time by Gray (1925). The results are plotted in Fig. 2, where the ordinates give the logarithms of the minimum molar concentrations of anæsthetic necessary to produce anæsthesia, and the abscissæ the pH. The dots give the actually observed points of the experiments with cocaine, which were typical of the series. Fig. 2 shows that over a large range of concentration the relation between the minimum concentration required to obtain anæsthesia is linear, but in the neighbourhood of pH 7 to 8 diminution of the pH commences to have less effect, so that the curves become convex towards the abscissæ. The straight parts of these curves are parallel, with the exception of that for conessine, the straight part of which has approximately twice the slope of the other.



pH 6·2 pH 8·2

FIG. 1.—Curves showing times of onset of anæsthesia with novocain at different pH's.

The reason for choosing a logarithmic scale for the concentrations is that the concentrations distinguishable are members of a geometrical series. It is just as easy to distinguish 0·005% from 0·01% cocaine at pH 8·2, as it is to distinguish 0·5% from 1% at pH 5·2.

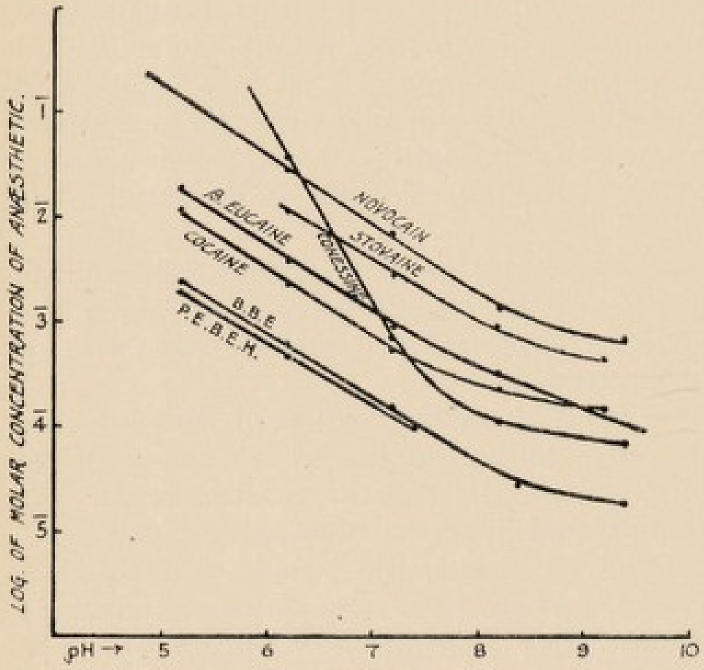


FIG. 2.—Curves showing the relation between anæsthesia and pH.

To estimate how far these results agree with Gros's hypothesis that the active constituent of a solution of cocaine is the base, necessitates the determination of the amount of undissociated base present in the solution at each hydrogen-ion concentration. We have worked out electrometrically with the glass electrode (Kerridge, 1926) the neutralization curves for the substances used in this investigation. The results are given in Fig. 3. The solutions are M/300 in concentration, stronger solutions of some of the substances (benzylbenzoyl-ecgonine-hydrochloride, for example) giving precipitates of base with very small additions of soda. 10 c.c. quantities were titrated with 0·56N soda (free from carbon dioxide), the soda being added from a micrometer syringe (Trevan, 1925), volume changes during the titration being, therefore, reduced to negligible amounts, since 10 c.c. of M/300 required only about 0·03 c.c. of 0·56 N soda for half neutralization. This amount can be measured with the micrometer syringe to 1 part in 300, which is more than sufficient for the purpose—a 1% error in the amount of soda added for half neutralization giving an error in the estimate of pK of 0·01 of a pH unit. Each point on the curve was estimated on a separate sample of the stock solution, the requisite amount of soda being added to every sample immediately before the determination of its pH. The precipitation of base prevents the working out of the whole titration curve with several of these substances, but sufficient of the titration curve can be obtained for the half-neutralization point to be determined, except for phenylethylbenzoylecgonine hydrochloride. The pH at this point

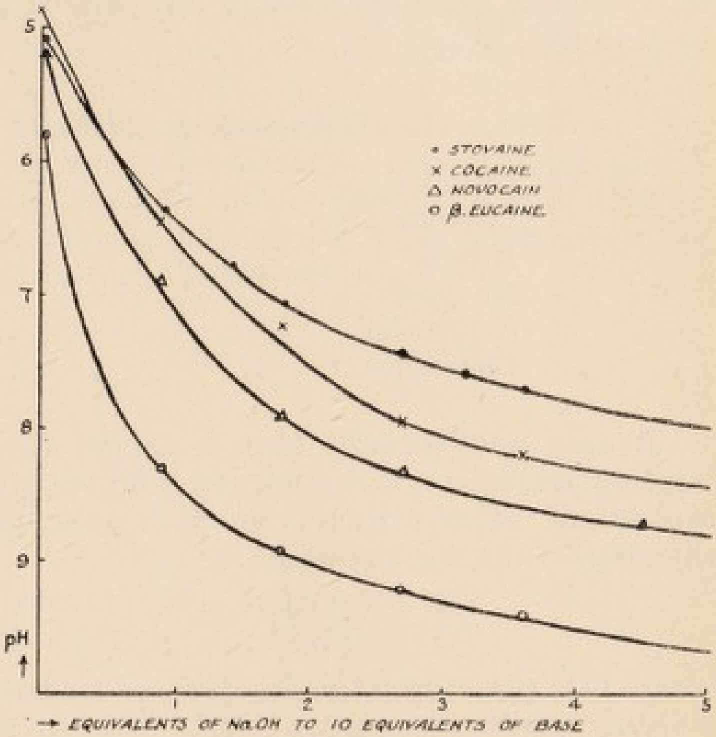


Fig 3.—Titration curves of anæsthetics.

Table I.—*Effect of Reaction on the Anæsthetic Action of Cocaine on the Cornea of the Rabbit.*

pH.	Concentration.	Time for anæsthesia.	End-point.
5·2	1·0%	4′+	
	0·5%	9′+	
	0·25%	10′−	0·375%
6·2	0·15%	4′+	
	0·075%	10′±	0·075%
7·2	0·2%	1′+	
	0·1%	1′+	
	0·05%	1′+	
	0·05%	1′+	
	0·025%	7½′+	
	0·025%	7½′+	
	0·0125%	10′−	0·019%
8·2	0·01%	7′+	
	0·005%	10′−	0·0075%
9·4	0·01%	4½′+	
	0·005%	108′−	0·0075%

when subtracted from 14·14 gives the pK (negative log of dissociation constant) with sufficient accuracy for our purposes. Fig. 3 gives the titration curves, and Table II the estimated pK for each. There is sufficient agreement between the estimated pK for novocaine and cocaine obtained by us and those obtained by Kolthoff (1925).

TABLE II.—*Dissociation Constants (pKs) for Anæsthetics.*

M/300	Stovaine	6·14
,,	Cocaine	5·69 (Kolthoff gives 5·59).
,,	B.B.E.	5·69
,,	Novocain	5·29 (,, ,, 5·15).
,,	β-Eucaine.	4·49
,,	Conessine	pK_1 *ca* 4·6

If only undissociated base is active, the amount of base present in the limiting active concentrations at different pH's will be the same. The concentrations of anæsthetic which contain equal quantities of base can be calculated from the equations:—

$$[B^+] \times [OH^-] = K \times [BOH] \quad . \quad . \quad . \quad 1$$

$$[B^+] + [BA^-] + [BOH] = C \quad . \quad . \quad . \quad 2$$

where C is the limiting molecular concentration to produce anæsthesia at any hydroxyl ion concentration [OH] (the square brackets represent molecular concentration), $[B^+]$ is the molecular concentration of basic ion, [BA] that of salt. As a first approximation [BA] may be ignored, the dissociation of the salt into ions being taken as complete. Starting with the point at pH 7 on the anæsthetic curve for novocain, the amount of base in the limiting concentration may be calculated. Then the molar concentrations at pH 5, 6, 7 and 8, which contain the same amount of base, can be calculated (see Table III and Fig. 4). The curve is practically a straight line at 45° to the axis, but the slope becomes less between 8 and 9, where [BOH] becomes a large fraction of C. Similar curves drawn parallel to this, but separated by horizontal distances equal to the differences of pK, would represent the theoretical log C/pH curves for the same concentration of base as novocain for the other anæsthetics.

TABLE III.—*Values of the Molecular Concentrations of Novocaine at Different pHs, containing the Amount of Base Necessary to Produce Anæsthesia at a pH of* 7. (0·00013.)

pH.	B.	C (B BOH).	Log. C.
5	0·65554	0·656	$\bar{1}$·8167
6	0·065554	0·657	$\bar{2}$·818
7	0·0065554	0·0668	$\bar{3}$·825
8	0·00065554	0·0078	$\bar{4}$·894
9	0·000065554	0·00019	$\bar{4}$·287

In the first place the slope of the observed anæsthetic curves is not so great as the slope of the theoretical curve for solutions containing equal concentrations of undissociated base.

There are four possibilities which might explain the discrepancy:

(*a*) The effect of the reaction may be directly on the tissue, rendering it more permeable to base, ion and salt alike. The linear relationship between the pH and log C would then only be a striking but accidental coincidence. For this explanation to be valid the increase in permeability with increase in pH for each component of the solution, ion, base and undissociated salt must be exactly the same, which seems an unlikely coincidence when we consider the very different solubilities of each component in fat solvents.

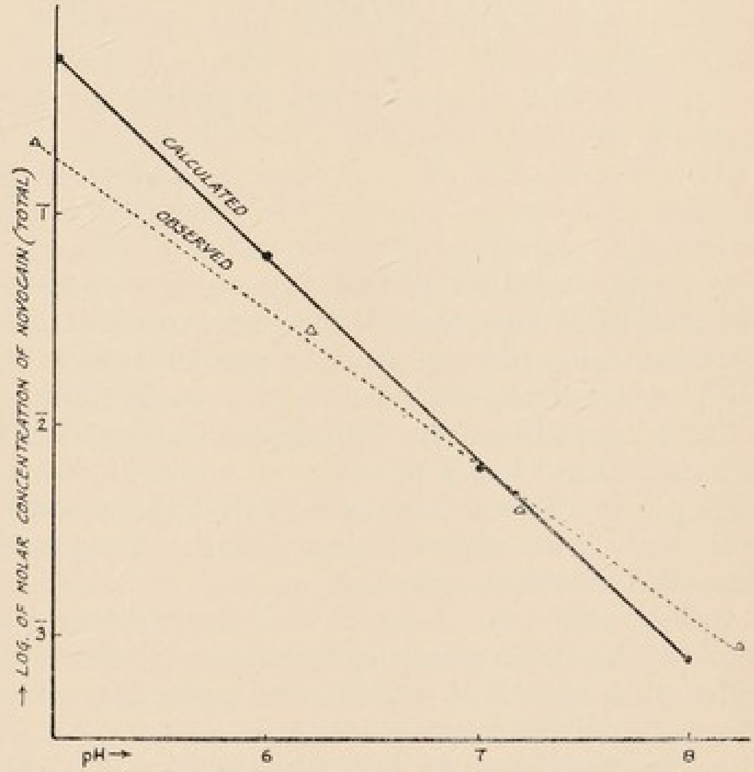


Fig. 4.—Curves showing the relation between the observed and calculated anæsthetic limits for novocain.

In addition to these arguments, if we take a substance, dissociation of which does not take place at the reactions worked with, namely, benzyl alcohol, no alteration in activity is produced by variations in reaction. The minimal concentration of benzyl alcohol for anæsthesia of the cornea is 1–2%, whether the pH is 6, 7 or 8; certainly no difference occurs of the order observed with the anæsthetic bases. The other possibilities to explain the discrepancy in Fig. 4 all involve the assumption that the base is the most active constituent, and they are:

(*b*) The ion has an activity which is a small fraction of that of the base.

(*c*) The undissociated salt of the local anæsthetic has anæsthetic activity.

(*d*) The discrepancy is due to the actual pH in the tissues around the nerve-fibre differing from the pH of the applied solution, being less alkaline on the alkaline side of pH 7 and less acid on the acid side of pH 7. If this effect increases with increased distance from neutrality, the result will be similar to that observed. The limiting concentration will be under-estimated on the acid side and over-estimated on the alkaline side, since when the solution applied is on the side of pH 7 there will be a larger proportion of base set free, the solution diffusing into the less acid corneal epithelium, and on the alkaline side a smaller proportion.

Explanation (*b*) is, we think, eliminated by the following argument: Let the anæsthetic activity of the ion [B] be $\frac{1}{r}$ times the anæsthetic activity of the base [BOH]. Then if the ion could be obtained free from base, the concentration of ion necessary to produce anæsthesia would be r times the concentration of base necessary. The anæsthetic activity of a mixture of base of concentration $[\mathrm{BOH}_x]$ and ion of concentration $[\mathrm{B}_x]$ would be equivalent to a concentration of base of $[\mathrm{BOH}_x + \frac{1}{r}\mathrm{B}_x]$. Then in two solutions of the salt of equal anæsthetic activity at different pH's x and y—

$$[\mathrm{BOH}_x] + \frac{1}{r}[\mathrm{B}_x] = [\mathrm{BOH}_y] + \frac{1}{r}[\mathrm{B}_y],$$

where $[\mathrm{BOH}_x]$ is the concentration of base at pH_x,
,, $[\mathrm{B}_x]$,, ,, ion ,, pH_x,
,, $[\mathrm{BOH}_y]$,, ,, base ,, pH_y,
,, $[\mathrm{B}_y]$,, ,, ion ,, pH_y.

$$\therefore \frac{1}{r}\left\{[\mathrm{B}_x]-[\mathrm{B}_y]\right\}=[\mathrm{BOH}_y]-[\mathrm{BOH}_x],$$

$$\therefore r = \frac{[\mathrm{B}_x]-[\mathrm{B}_y]}{[\mathrm{BOH}_y]-[\mathrm{BOH}_x]}.$$

In the solution just strong enough to cause anæsthesia at pH 5, there is 0·0000367 M base and 0·1884 M ion present, calculated from equations 1 and 2, and the value of r obtained by substitution in equation is approximately 4000. Whereas if the concentrations are worked out for pH 7 and 8, the value of r is only 5·1, and the curve connecting log C and pH, if r had been constant, would therefore have had a pronounced convexity towards the abscissæ between pH 5 and pH 8. Since it is unlikely that there would be such a large change in the value of r with alteration in pH, the conclusion seems inevitable that the ion has no anæsthetic activity.

The explanation (*c*) of the discrepancy by the possession of anæsthetic activity by the undissociated salt is eliminated by a similar argument, which will show that this would also make the relation between log C and pH a curved and not a straight line.

We are therefore left with explanation (*d*), namely, that the reaction to which the nerve-fibre is exposed is nearer neutrality than that of the applied solution; so that the nerve-ending will be exposed to a smaller base concentration on the alkaline side and larger on the acid side, than that which is calculated from the observed pH and limiting concentration.

That variations of reaction do take place in the corneal tissues on application of acid on alkaline fluids to the eye can be demonstrated in several ways:

1. If a 1% solution of neutral red is applied to the cornea, changes in colour of the stained corneal epithelium can be demonstrated when buffered solutions of various pH's are applied to the eye.

2. The reaction of the aqueous humour in a pithed cat becomes more alkaline when alkaline buffer solution is applied to the cornea for a period of 10 minutes.

3. If a 1% solution of cocaine hydrochloride brought to a pH of 3 to 4 with HCl is applied to the eye, as long as the instillation is continued, even for a prolonged period, no anæsthesia develops; but within half a minute of the

cessation of instillation complete anæsthesia develops, and persists for many minutes. The explanation we offer of this observation is that although base diffuses into the cornea during the instillation of the acid solution, the concentration is not sufficient to induce anæsthesia at the acid reaction set up in the cornea. Some of the hydrochloride itself diffuses in. When the instillation ceases, the reaction slowly returns to normal, and the salt which has diffused in sets free more base, owing to the alteration of reaction.

4. The changes in reaction are also shown by the following experiment: A solution of 0·03% benzylbenzoylecgonine hydrochloride in saline, this solution having a pH of 6·43, was instilled into both conjunctival sacs of a rabbit. After 10 minutes' instillation there was no anæsthesia, but almost complete anæsthesia of both corneas developed in about 30 seconds after instillation had ceased. The right eye was then washed with a buffer solution of pH 4, and the left eye with a buffer solution of pH 8. The anæsthesia in the right eye entirely disappeared, while that in the left eye became complete.

These last two experiments show that there is a constant tendency for the reaction of the corneal tissue to return to normal. In consequence, the reaction in the tissue at any time is the result of a balance between the influx of buffers from the applied solution and from the surrounding tissue. We have no information as to the shape of the titration curve of the tissues, but, as was pointed out to us by Dr. Gaddum, if it approximates to a straight line, as it is probable that it does over the reactions studied, then the discrepancy between tissue pH and pH of the applied solution would be proportional to the difference of the pH of the applied solution from 7, or whatever the normal pH of the cornea is.

We think that in this way we can explain the observation of Regnier, that the addition of alkali to a solution of cocaine base in water enhances its anæsthetic power. The concentration of cocaine base necessary to produce anæsthesia is very low and has practically no buffering action. The reaction of the superficial corneal tissues will therefore be little displaced from neutrality, and some of the cocaine will therefore combine to form a salt. On the addition of alkali to the base, the reaction of the corneal epithelium will become definitely shifted towards the alkaline side, and less cocaine will combine with the anions of the tissue and a higher concentration of base will be in contact with the nerve-fibre.

The figures for conessine have not so far been discussed, but they corroborate the theory that the base is the only active fraction in an interesting manner. The first part of the curve is practically a straight line. Conessine is diacidic, and the theoretical curve can be developed in the same manner as for the mono-acidic bases.

$$K_2\,[B\ (OH)_2] = [B\ OH] \times [OH]$$
$$K_1\,[B\ OH] = [B''] \times [OH]$$
$$\text{Therefore}\quad [B''] - \frac{K_1K_2\,[B\ (OH)_2]}{[(OH)]^2} = K_wK_1K_2\,[B\ (OH)_2]\ [H]^2$$
$$\therefore\quad \text{Log}\ [B''] = \log K_1K_2K_w + \log\ [B\ (OH)_2] - 2\ \text{pH}.$$

For small concentrations of $[BOH]$ and $[B(OH)_2]$, $[B'']$ may be taken as equal to the total concentration of alkaloid, and the slope of the first part of the anæsthetic curve for conessine should be twice that of the anæsthetic curve for the monacidic bases, if the active component of conessine solution is the undissociated base $B\ (OH)_2$. This is approximately the case, as will be seen from Fig. 2. The straight line part of the curve extends to pH 7. In order to obtain pH 7·2 in a solution of conessine hydrochloride, it is necessary to add only 2% of the total quantity of soda which is required to set free the whole of the base as $B\ (OH)_2$. Above this pH the slope of the curve becomes less owing to the relation between B'' and the total alkaloid concentration becoming appreciably less than one. We were unable to obtain values for K_2 for conessine, as precipitation of base occurred in M/300 solution at a pH of 9·1, but pK_1 is approximately 4·6.

The order of the activities of the various substances tried is not the same as the order of their pKs. The most easily dissociated, stovaine, with a pK of 6·14, is not by any means the most active. In Table IV the order of activities is given, in which allowance is made for the difference in dissociation, and the amount of base present in the limiting concentrations at pH 7 is calculated in every case. pH7 is chosen, because the reaction in or around the nerve-fibres is probably near that of the applied solution when the latter is neutral.

TABLE IV.—*The Molecular Concentrations of Free Base Contained in the Highest Dilution Producing Anæsthesia at pH 7.*

Anæsthetic.	C.	BOH.
β-Eucaine	0·0000324	0·00000308
B.B.E.	0·000168	0·00000783
Cocaine	0·000708	0·0000331
Novocain	0·00668	0·000128
Stovain	0·00133	0·000162

These figures then represent the relative anæsthetic activity, what may be called the molecular anæsthetic power, of the bases of the different substances tried. The fact that the bases are so active suggested to Gros that their solubility in oily media may "explain" their action. We tried to determine the partition coefficients of the substances between ether and water. The various experimental difficulties encountered destroyed our confidence in the results obtained, but the figures at any rate gave no indication of differences of partition coefficients of the magnitudes necessary to account for the wide differences in Table IV.

There are therefore two methods of attack for the synthesis of new local anæsthetics. One method is to diminish the basic dissociation constant, and render it possible to obtain a higher concentration of base of the same molecular anæsthetic power, and the other method is to produce structural changes in the molecule, which, while not necessarily diminishing the dissociation constant, might confer increased molecular anæsthetic power on the substance.

It is of course difficult to predict what effect a given structural change will produce on the dissociation constant, and impossible to make any sort of confident prediction of the effect of a structural change on the physiological similarity of such dissimilar substances as novocain and cocaine. Changes in both will generally result from any structural change. But Gray's attempt to augment the physiological activity of cocaine by the substitution of the benzyl and phenyl ethyl group, which were shown by Macht (1918) and Hjort and Eagan (1919) to have some anæsthetic action of their own, has resulted in two compounds for which the molecular anæsthetic value is considerably higher than that for cocaine, while in the case of the benzyl compound the dissociation constant is not appreciably altered. One interesting point which comes out is that the anæsthetic action of β-eucaine, when reckoned as concentration of free base necessary to produce the effect, is even greater than that of cocaine.

CONCLUSIONS.

(1) The effect of a series of local anæsthetics on the rabbit's cornea at different hydrogen ion concentrations is given.

(2) Plotting the logarithms of the concentration of the minimal effective concentrations against pH is shown to give lines which for all the mono-acidic local anæsthetics are straight and parallel between pH 5 and pH 8.

(3) It is shown that this relationship is consistent with the view that the only active constituent of a solution of local anæsthetic is the free base and not the ion or the undissociated salt.

(4) The relationship between the anæsthetic action and pH for a diacidic base, conessine, is shown to confirm this view.

(5) Further evidence that the influence of pH is due to dissociation of base is drawn from the fact that variations of pH have no influence on the anæsthetic activity of benzyl alcohol on the cornea. It is shown that variations in the dissociation of the basic anæsthetics do not account for the whole of the difference between them, and that therefore there must be some other relationship between the actual molecular structure of the base itself and its anæsthetic power.

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