LIGNOCAINE HYDROCHLORIDE AND ITS SOLUTIONS: PURITY, TESTS, STABILITY AND ASSAY

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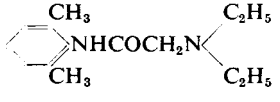
Received June 21, 1955

Introduction

Lignocaine (I), like cinchocaine (II), is an amide and thus differs from the large group of local anæsthetics which are aromatic esters of tertiary amino-alcohols and to which procaine and cocaine belong. While cinchocaine resembles this latter group in that it is the amide of an aromatic acid and possesses a tertiary nitrogen atom in the non-acidic portion of the molecule, lignocaine, on the other hand, has its tertiary nitrogen atom in the acidic portion of the molecule and is the substituted anilide of an aliphatic acid; in this way it resembles the analgesic acetanilide.



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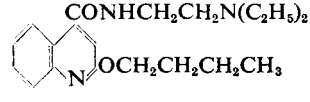

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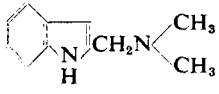

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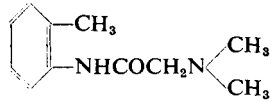

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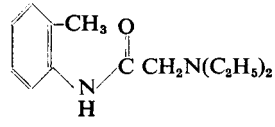


(V)

The first synthesis of lignocaine, in 1943 by Löfgren[1], followed from an earlier observation made by Erdtman and Löfgren[2] that 2-dimethylaminomethyl indole (III) possessed considerable local anæsthetic activity as did 2-dimethylaminoacetamido-1-methylbenzene (IV) in which the nitrogen containing ring of the indole group has been opened. The structural similarity of these earlier compounds to lignocaine can easily be seen if the latter is written as in (V).

Ehrenberg[3] using conduction measurements on frog muscle-nerve preparations showed that lignocaine was 3·3 times as active as procaine at *p*H 7·39 and has a shorter latency time. Compared with procaine it has been claimed that lignocaine is more toxic[4] but may be used in the same concentrations, is less irritating when injected into the tissues[5,6,7] and gives a deeper anæsthesia with more rapid onset and more extensive spread[8,9]. Lignocaine can be used for surface as well as infiltration anæsthesia without, but preferably with, adrenaline. As a local anæsthetic for use in dental and oral surgery it may be said to rival procaine.

Lignocaine hydrochloride is used clinically in aqueous solution as follows. (1) 1 per cent. usually containing 1 in 200,000 adrenaline for nerve block. (2) 2 per cent. usually containing 1 in 80,000 to 1 in 50,000

adrenaline for dental surgery and infiltration anæsthesia. (3) 4 per cent. for surface anæsthesia.

Probably the above mentioned slight advantages of lignocaine over procaine would not have led to its wide-spread use had it not been for one other important chemical characteristic. While almost all other local anæsthetics are relatively easily hydrolysed in solution, particularly at neutral or higher *p*H values, the lignocaine molecule is extremely resistant to hydrolysis by both acid and alkali[10]. This is the more surprising since acetanilide and most other amides readily undergo hydrolysis. These interesting considerations, coupled with the fact that requests were received from the Dental Department of this University for information about the stability, methods for analysis and the detection of decomposition in neutral or alkaline solutions of lignocaine, caused us to undertake an investigation of such solutions similar to the investigations carried out previously in this department relating to other local anæsthetics[11,12,13]. The results of this work, which was commenced several years ago, are reported in this paper.

Experimental

Purity of Materials

All melting points recorded in this paper are uncorrected.

Lignocaine and its Hydrochlorides

At the commencement of this work neither lignocaine nor its salts were available commercially, but only solutions of the hydrochloride. A sample of lignocaine was therefore synthesised by the method described by Löfgren[1]. After six recrystallisations from light petroleum (b.pt. 40° to 60°) the melting point was 67° C. (Löfgren[1] 67° C.). Found: C, 71·9; H, 9·6; N (Dumas), 11·9 per cent.; calculated for $C_{14}H_{22}ON_2$, C, 71·8; H, 9·46; N, 11·96 per cent.

A portion of this material was retained as an analytical reference sample of the free base while the remainder was converted, as described by Löfgren[1], into the anhydrous hydrochloride. After three recrystallisations from anhydrous ethyl methyl ketone and drying at 65° C. under reduced pressure over phosphorus pentoxide the melting point was found to be 128° C. (Löfgren[1] 128 to 129° C.). Found: C, 61·9; H, 8·85; N, 10·23 per cent.; calculated for $C_{14}H_{22}ON_2$,HCl, C, 62·1; H, 8·56; N, 10·34 per cent.

After reserving a portion as analytical standard the rest of the hydrochloride was converted into the monohydrate by recrystallisation from moist ethyl methyl ketone and drying under reduced pressure at room temperature. The melting point was found to be 77° C. (with decomposition). Found: C, 58·45; H, 8·75; N, 9·40 per cent.; calculated for $C_{14}H_{22}ON_2HCl{\cdot}H_2O$, C, 58·2; H, 8·72; N, 9·70 per cent. Later, during the course of the work, lignocaine base, the anhydrous hydrochloride and the monohydrate of the hydrochloride, all of good purity (Table I), became available commercially.

2-*Amino*-1 : 3-*dimethylbenzene* (*ADB*). A sample of ADB of commercial quality was purified by fractionating 3 times under reduced pressure. The fraction selected distilled at 56° C./2 mm. The hydrochloride, obtained by passing dry hydrogen chloride into a solution of the amine in sodium-dried ether, was purified by recrystallisation from

TABLE I

PURITY OF COMMERCIAL LIGNOCAINE AND ITS HYDROCHLORIDE

Commercial subs.	m.pt. ° C.	Mixed m.pt. ° C. with pure salts	Per cent. lignocaine HCl from ionisable Cl per cent.
Lignocaine base	67·0	67·0	—
Anhydrous hydrochloride ..	128·0	128·0	99·8
Hydrochloride monohydrate ..	77·0	77·0	100·2

butan-1-ol. The pure ADB hydrochloride melted at 234° C. (with decomposition). Found: C, 60·95; H, 7·66; N, 8·88; Cl, 22·49 percent.; calculated for $C_8H_{12}NCl$, C, 60·80; H, 7·80; N, 9·35; Cl, 22·50 per cent.

Diethylaminoacetic acid (*DEAA*) was prepared according to the method of Heintz[14] with the following modifications. The reaction between monochloroacetic acid and diethylamine was carried out in absolute ethanol which, after completion of the reaction, was distilled together with any excess of diethylamine. After drying under reduced pressure over phosphorus pentoxide the DEAA was separated from diethyl-ammonium chloride by extraction with ether in a continuous extractor of the type described in the British Pharmacopœia, 1953. It was purified by recrystallisation from either benzene or ethyl methyl ketone and melted at 130° C. (Bowman and Stroud[15] 131° C.).

ASSAY PROCESSES FOR LIGNOCAINE, ITS SALTS AND SOLUTIONS

As with other basic substances and their salts the assay processes for lignocaine may be divided into two parts, (*a*) isolation of the base, usually by making a solution of a salt alkaline and shaking with a suitable organic solvent, and (*b*) estimation of the isolated base. The latter will be discussed first.

(i) *The Kjeldahl Process*

A sample of pure lignocaine base was assayed for total nitrogen by the Kjeldahl process as modified by Middleton and Stuckey[16] (British Pharmaceutical Codex, 1954, method I). Multiplying the percentage total nitrogen found by the factor 0·02343 gave in 4 determinations 99·95, 99·43, 99·87 and 100·03 (relative mean deviation 0·19) as the percentage purity of the sample.

(ii) *The Reineckate Method*

The reineckate method of assay adapted for lignocaine and described by Örtenblad and Jonsson[17] was found to be satisfactory within the usual

limitations of a colorimetric process. Recoveries of 98·95 per cent. and 100·7 per cent. were obtained.

(iii) *Weighing the Recovered Base*

Attempts to estimate lignocaine base in solution in organic solvents by distilling the latter, drying at 100° C. and weighing the residue led to low and variable results. This was shown to be due to the volatility of lignocaine base both alone and in steam. A sample of pure lignocaine base heated at 100° C. gradually lost weight, the loss amounting to 13 per cent. in 5 hours. Another sample of lignocaine base was subjected to steam distillation. The distillate gave a positive reaction with potassio-mercuric iodide solution, but no reaction for ADB when tested by the sensitive colour reaction described below.

(iv) *Titrating the Base*

The curve shown in Figure 1 was obtained by dissolving 0·2110 g. of pure lignocaine base in 20 ml. (an excess) of 0·1084N hydrochloric acid and titrating with 0·1089N sodium hydroxide. The *p*H at half neutralisation, i.e., the pKa value of lignocaine, was found to be 7·90 at room temperature (Lofgren[10] pKa 7·85). Possible indicators for the back titration of excess acid in a solution of lignocaine base in hydrochloric acid are therefore bromocresol green and methyl red.

Results of quintuplicate determinations obtained by using both these indicators and also a mixture containing 3 parts of 0·1 per cent. bromocresol green and 1 part of 0·2 per cent. methyl red are shown in Table II.

TABLE II

ASSAY OF A SAMPLE OF PURE LIGNOCAINE BY TITRATION USING DIFFERENT INDICATORS

Indicator	Percentage lignocaine indicated	Mean	Relative mean deviation
Methyl red	99·34 99·29 99·30 99·40 99·70	99·4	0·11
Bromocresol green	100·00 100·00 100·50 99·86 100·23	100·12	0·20
Methyl red and bromocresol green ..	100·45 100·30 100·30 100·50 100·30	100·37	0·084

Extraction of Lignocaine from Alkaline Solution by Means of Volatile Solvents

In all experiments 10 ml. of a 2 per cent. solution of lignocaine hydrochloride was made alkaline with 0·5 g. sodium carbonate (anhydrous) and the precipitated base extracted with several quantities of volatile solvent. After washing the combined extracts with 5 ml. of water and washing the

latter with 5 ml. of volatile solvent the combined extracts were shaken with 10 ml. of 0·1N hydrochloric acid. After separation, the excess acid was titrated with 0·1N sodium hydroxide using bromocresol green as indicator. Using 3 quantities of 20 ml. each of a mixture of 3 parts of chloroform and 1 part of *iso*propanol as volatile solvent, low results showing between 94·4 per cent. and 96·6 per cent. recovery of the lignocaine were obtained. The following modifications were tried without any improvement in the results obtained.

(1) 5 extractions with the same volatile solvent, (2) the use of 0·5 g. sodium hydroxide in place of sodium carbonate to render the solution alkaline, (3) the use of chloroform only in place of chloroform-*iso*propanol mixture.

Washing the volatile solvent with two 5 ml. quantities of water after the extraction with hydrochloric acid and adding the washings to the acidic solution before back titrating, as was to be expected, slightly, but only slightly, lowered the percentage recovery[12]. It was found that approximately 100 per cent. recovery could be obtained by any of the following modifications, (*a*) using 3 quantities of 20 ml. each of ether as the volatile solvent, (*b*) extracting the chloroform or chloroform-*iso*-propanol solutions of the base with one 20 ml. or two 10 ml. quantities of 0·1N hydrochloric acid, with careful separation of the acidic layer and omission of the washing of the volatile solvent with water before back-titrating the excess hydrochloric acid, or (*c*) evaporation of the chloroform or chloroform-*iso*propanol extracts followed by solution of the residue in 10 ml. 0·1N hydrochloric acid and back-titration of the excess acid with 0·1N sodium hydroxide using bromocresol green as indicator. The results obtained by these modifications are shown in Table III.

TABLE III

SATISFACTORY METHODS FOR THE ESTIMATION OF LIGNOCAINE DISSOLVED IN A VOLATILE SOLVENT BY TITRATION TO BROMOCRESOL GREEN

Method	Percentage recovery lignocaine	Mean
Ether as volatile solvent + 10 ml. 0·1N HCl	99·6 99·7 100·1	99·8
$CHCl_3$-*iso*propanol + 20 ml. 0·1N HCl	99·9 99·7	99·8
$CHCl_3$ but evaporation before addition of 10 ml. 0·1N HCl	99·9 99·7 99·6	99·7

It may be mentioned that if, in method (*b*) after separation of the hydrochloric acid, the volatile solvents were washed with water and the washings added to the acidic extract before back titration low results were again obtained. Thus the low results at first obtained must be due to pecularities in the acid-base partition between chloroform and water. Solid anhydrous lignocaine hydrochloride was found to be insoluble in ether but soluble to the extent of 1·95 per cent. in dry chloroform at room

temperature. It was also shown that when lignocaine hydrochloride solutions are shaken with chloroform both lignocaine and chloride ions could be recovered from the chloroform layer. This presumably contributes to the cause of the low results using chloroform. The difficulty may be overcome by providing a considerable excess of hydrochloric acid in the aqueous phase. This was confirmed by the results shown in Table IV. In these experiments 10 ml. quantities of a 2 per cent. solution of lignocaine hydrochloride were treated with various quantities of 0·1N hydrochloric acid solution and shaken with 30 ml. of chloroform and *iso*propanol mixture. 4 ml. or more of 0·1N excess acid has to be present in the aqueous solution to prevent an increase of acidity on shaking with the mixed organic solvents. On repeating these experiments but using ether as organic solvent in no case did shaking with the ether increase the titratable acidity.

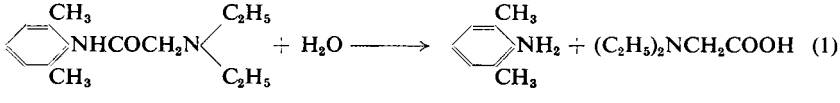
TABLE IV

INCREASE IN ACIDITY ON SHAKING LIGNOCAINE HYDROCHLORIDE SOLUTIONS WITH CHLOROFORM/*iso*PROPANOL MIXTURE AND VARIOUS QUANTITIES OF STANDARD ACID

Amount of 0·1N hydrochloric acid (in ml.) added to a 2 per cent. solution of lignocaine hydrochloride (10 ml.)	Titre of 0·1N sodium hydroxide (in ml.)		Difference
	After shaking with solvent chloroform/ *iso*propanol mixture	Omitting shaking with solvent chloroform/ *iso*propanol mixture	
1	1·14	1·03	0·11
2	2·12	2·06	0·06
3	3·12	3·09	0·03
4	4·12	4·12	0·00
5	5·14	5·14	0·00
10	10·28	10·28	0·00

THE STABILITY OF LIGNOCAINE IN SOLUTION

To establish the stability of lignocaine in solution it was first necessary to obtain a process which could be shown to be capable of detecting a slight degree of decomposition. In solution lignocaine would be expected to decompose by hydrolysis as follows:



(1)

It was to be expected that on such hydrolysis the *p*H of the solution would fall since ADB is a very weak amine while DEAA is a disubstituted amino-acid of which a 2 per cent. solution in water shows a *p*H of 6·5. In fact, two solutions containing DEAA, and lignocaine and ADB hydrochlorides in the proportions corresponding to 1 per cent. and 50 per cent. decomposition showed *p*H values of 3·7 and 2·8 respectively, whereas the original solution had a *p*H of 4·8. This small fall in *p*H cannot be used as an indicator of slight decomposition. It was considered possible that the two *ortho*-methyl groups might well suppress the usual diazo reaction of the aromatic amino group and so interfere with the detection of ADB. In the first place therefore some commercial ADB was diazotised and coupled with 2-naphthol when a vivid red colour developed.

The reaction would detect 100 μg., but not 10 μg., of ADB in 1 ml. of solution. If a 2 per cent. solution of anhydrous lignocaine hydrochloride undergoes hydrolysis to the extent of 1 per cent., calculation shows that the resultant solution will contain 89·5 μg. of ADB per ml. Thus 1 per cent. but not 0·1 per cent. decomposition would be detected by the simple diazotisation test.

A 2 per cent. solution of lignocaine hydrochloride made alkaline to *p*H 7·3 with phosphate buffer (solution F, Table VI) was heated in an autoclave at 116° C. for 30 minutes. Subsequent test by diazotisation showed no colouration and therefore less than 1 per cent. decomposition. Further, 100 per cent. of the lignocaine originally present was recovered by the assay process described above. Other similar but more drastic attempts failed to bring about hydrolysis detectable in this way.

Finally some hydrolysis was obtained by two methods. Two 0·1 g. quantities of lignocaine base, known to be pure, were dissolved, one in 25 ml. of 50 per cent. sulphuric acid and the other in 20 per cent. ethanolic potash. Both solutions were heated in an autoclave at 116° C. for 5 hours. The acidic solution was treated with excess sodium hydroxide and the bases extracted with ether and transferred to 0·1N hydrochloric acid. The alkaline solution was diluted with water and most of the ethanol removed by evaporation. The bases were extracted with ether and transferred to 0·1N hydrochloric acid. The simple diazo colour test described above indicated that in the 20 per cent. ethanolic potash lignocaine was hydrolysed to the extent of approximately 1 per cent. while in 50 per cent. sulphuric acid between 1 per cent. and 5 per cent. had been hydrolysed. All attempts to isolate a sample of ADB from the residual liquids failed but a sample of pure lignocaine m.pt. 67° C. was readily obtained.

Later the two solutions were examined using the more sensitive colour test described below. The material heated in an autoclave in 50 per cent. sulphuric acid showed 3 per cent. decomposition, and that similarly heated with ethanolic potash showed 0·5 per cent. decomposition. These figures can only be considered to be approximate owing to the difficulty of isolating small quantities of ADB from solutions containing large amounts of acid or alkali.

These preliminary experiments appeared to justify the conclusions that the lignocaine molecule in aqueous solution is resistant to heat, acid and alkali, but when decomposition does occur it is by the hydrolysis shown in equation (1). Later quantitative experiments described below confirmed these conclusions. To estimate the degree of decomposition it is necessary to know (*a*) either the lignocaine originally added or (*b*) one of the hydrolysis products. (*a*) The amount of lignocaine originally present in the solution may be obtained by estimation of the total bases, i.e., lignocaine plus ADB, or the undecomposed lignocaine, i.e., the lignocaine but not the ADB. Since one is usually concerned with solutions showing less than 50 per cent. decomposition titration methods are sufficiently accurate for this section of the work. (*b*) DEAA in solutions of relevant concentrations has a *p*H value approximating to 7 so that

titration methods are not to be recommended; it does not give the ninhydrin reaction and no other colour reactions could be traced in the literature. ADB is too weak a base to be titrated accurately but it can be estimated colorimetrically.

Estimation of Undecomposed Lignocaine

Various pK values have been reported for ADB. Thomson[18] found the pKa 25° C. for the free base in 75 per cent. ethanol to be 3·42, and in 50 per cent. ethanol 3·19. Spryskov[19] using an aqueous solution of the hydrochloride found pKb 10·39.

ADB in mixtures of lignocaine and ADB should not therefore interfere with the estimation of lignocaine by addition of excess hydrochloric acid and back titration with alkali provided that a suitable indicator is chosen. That this is true is shown in Figure 2, where the titration of a mixture of

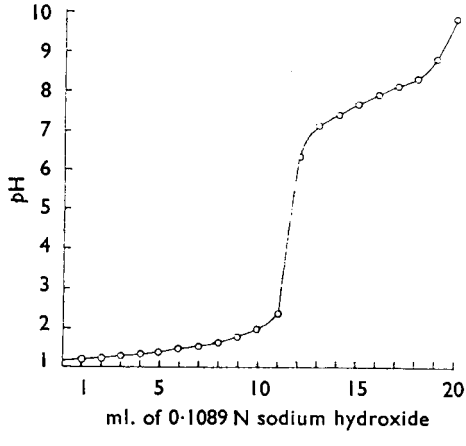


FIG. 1. Titration of lignocaine base (0·2110 g.) dissolved in 20 ml. of 0·1084N hydrochloric acid with 0·1089N sodium hydroxide.

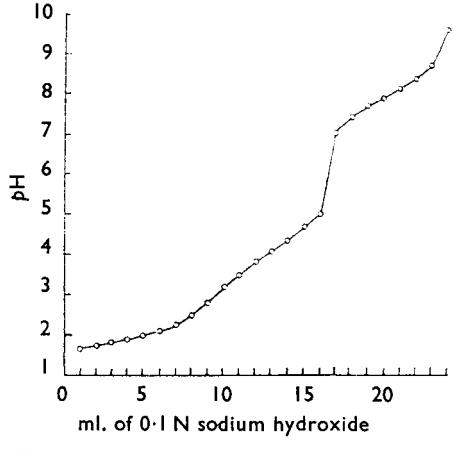


FIG. 2. Titration of 2-amino-1:3-dimethylbenzene hydrochloride (0·1 g.) and lignocaine base (0·1 g.) dissolved in 20 ml. of 0·1N hydrochloric acid with 0·1N sodium hydroxide.

0·1 g. of ADB hydrochloride and 0·1 g. lignocaine in 20 ml. of 0·1N hydrochloric acid with 0·1N sodium hydroxide is shown. Comparison of Figures 1 and 2 show that while the end-point for lignocaine alone is *p*H 4 to 6, for lignocaine in presence of ADB it is over the range 5 to 7. It was in fact found that chlorophenol red (*p*H range 4·8 to 6·4) gave accurate results for the lignocaine only in the mixture, while methyl red or bromocresol green gave results approximately 2 per cent. high. No attempt was made to estimate the ADB as well as the lignocaine by titration; examination of Figure 2 shows that there would be no satisfactory end-point even if a suitable indicator in the *p*H range 2 to 3 could be found.

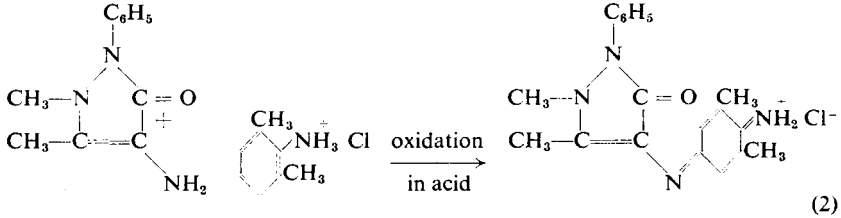
(*b*) *Colorimetric Assay of ADB*

Estimation of ADB by diazotisation and coupling in alkaline solution would be undesirable on account of the precipitated lignocaine which

would render the solution turbid. To avoid turbidity would necessitate a difficult separation of the two bases.

Following the method suggested by Bratton and Marshall[20] for sulphonamides and modified by Brodie and Axelrod[21] for aniline, ADB was estimated by diazotisation, removal of excess nitrous acid with ammonium sulphamate and coupling with *N*-(1-naphthyl)-ethylenediamine dihydrochloride in acidic solution. This process gave only a faint colour with 100 μg. ADB per ml. and so was unsuitable for the estimation of less than 1 per cent. decomposition of lignocaine solutions. With larger quantities of ADB, owing to insolubility, the coupling reagent had to be dissolved in ethanol and it was found that the colour developed varied with the proportion of ethanol present. Diazotisation methods were therefore abandoned.

In 1938 Eisenstædt[22] showed that aromatic amines combine with 4-amino-2:3-dimethyl-1-phenyl-5-pyrazolone(4-amino-phenazone) with loss of 4 hydrogen atoms in the presence of potassium ferricyanide as an oxidizing agent to give blue-red dyes of the indamine type. This reaction was found to occur with ADB and may be represented as follows:



(2)

Neither lignocaine nor acetylated ADB gave any colour under the conditions used. The absorption spectrum of the colour produced showed maximum absorption in the region of 540 mμ. Estimations were subsequently carried out on the Spekker absorptiometer using a Chance OGI filter.

The effect of *p*H is shown in Figure 3. As the *p*H increased from 1·5 to 5·5 the colour and therefore the optical density at 540 mμ of both test and blank increased. Up to *p*H 2·9 the colour of the test increased more rapidly than that of the blank. Over *p*H 3·2 the colour of the blank began to increase more rapidly than that of the test. Thus the greatest difference between test and blank occurred between those two *p*H values and *p*H 3·1 was chosen as the optimum.

At all *p*H values the colour reached a maximum and then faded. At low *p*H values the maximum colour was reached in 5 to 15 minutes and remained fairly constant at the maximum for a period of 5 to 10 minutes. At *p*H 5·5 the maximum was reached in 4 minutes and rapid fading set in at once. At *p*H 3·05 the maximum was reached in 5 to 10 minutes and remained fairly constant over that period of time as shown in Figure 4. For a concentration of 20 μg. per ml. of ADB, which is suitable for the colorimetric assay as described below, 0·05 ml. of 3 per cent. 4-aminophenazone solution and 0·7 ml. of 2 per cent. potassium ferricyanide

solution were required for each 1 ml. of solution examined. More 4-amino-phenazone resulted in increased optical density in the blank without increasing the excess of optical density in the test. Increase in the quantity of potassium ferricyanide solution added resulted in a slight increase in

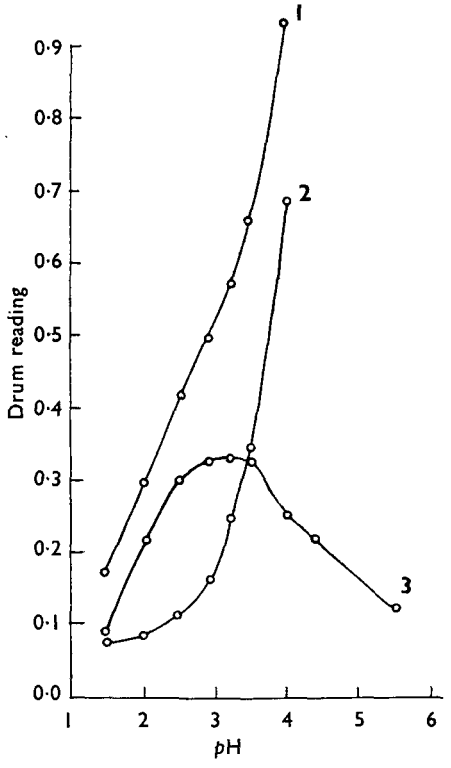


FIG. 3. Effect of *p*H on the development of the colour in the colorimetric assay of 2-amino-1 : 3-dimethylbenzene.

1. Variation in extinction of the test solution when compared with water.
2. Variation in extinction of blank when compared with water.
3. Variation in extinction of test solution when compared with blank.

Quantity of ADB in test solution, 20 μg.
Quantity of 3 per cent. solution of 4-amino-phenazone, 0·3 ml.
Quantity of 2 per cent. solution of potassium ferricyanide, 0·7 ml.

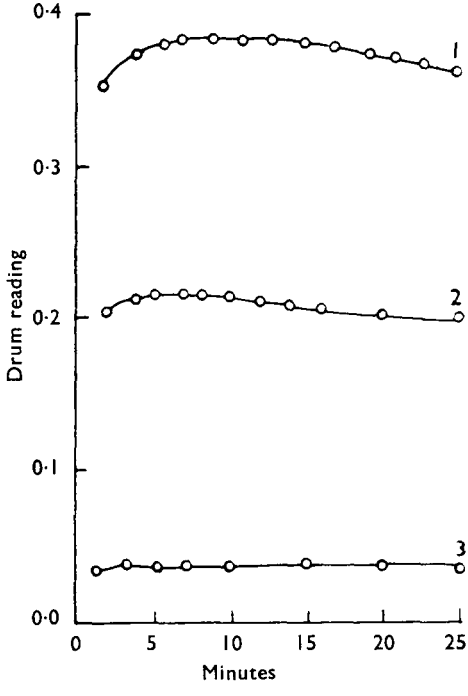


FIG. 4. Stability of colour in the colorimetric assay of 2-amino-1 : 3-dimethylbenzene.

1. Extinction of test solution when compared with water. Concentration of ADB is 20 μg.
2. Extinction of test solution when compared with water. Concentration of ADB is 10 μg.
3. Extinction of blank when compared with water.

colour in the blanks with only a slight corresponding increase in colour in the test. Extraction of the colour with chloroform, as recommended by Ettinger, Ruchhoft and Lishka[23] for the estimation of phenol by a similar process, was found to be unsatisfactory because the colour in the chloroformic extract faded rapidly.

The Process Recommended

(*a*) *Assay for undecomposed lignocaine.* Make alkaline 10 ml. of a solution containing the equivalent of 0·2 g. of lignocaine, by addition of 0·5 g. of sodium carbonate (anhydrous) and extract the precipitated bases by shaking with three quantities of 20 ml. each of ether. Wash the mixed ethereal extracts with 5 ml. of water and wash this in turn with 5 ml. of ether before discarding it. Shake the mixed ethereal solutions successively with 10 ml. 0·1N hydrochloric acid and two 5 ml. quantities of water. Titrate the excess acid in the mixed aqueous extracts with 0·1N sodium hydroxide using chlorophenol red as indicator. 1 ml. 0·1N hydrochloric acid = 0·02888 g. $C_{14}H_{22}ON_2HCl{\cdot}H_2O$, = 0·02708 g. $C_{14}H_{22}ON_2HCl$, = 0·02343 g. $C_{14}H_{22}ON_2$.

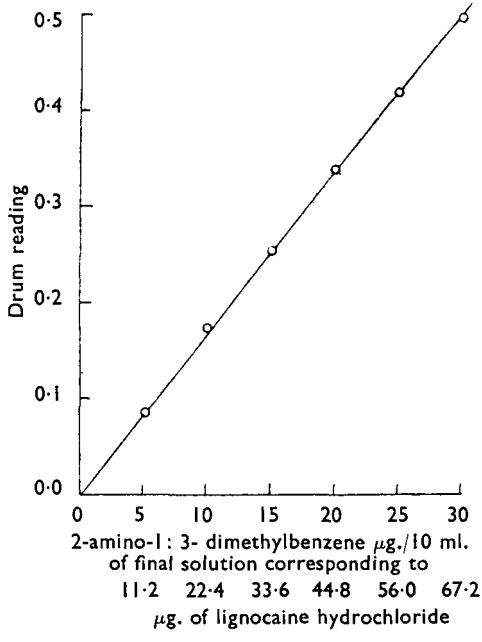


FIG. 5. Calibration curve of 2-amino-1 : 3-dimethylbenzene.

(*b*) *Assay for ADB resulting from decomposition of lignocaine.* Take 1 ml. of solution previously diluted if necessary so as to contain approximately 20 μg. of ADB per ml. Add 8·25 ml. B.P. phthalate buffer *p*H 3·1, 0·05 ml. 3 per cent. solution of 4-amino-phenazone and 0·7 ml. 2 per cent. solution of potassium ferricyanide, mixing thoroughly after each addition. At the same time carry out a blank determination using 1 ml. of water. Between 5 to 10 minutes later, i.e., during the period of maximum colour intensity, measure the optical density in a Spekker absorptiometer using a 1 cm. cell and a Chance OGI green filter. Any other suitable instrument may be used.

From a calibration graph (see Fig. 5) read off the mg. of decomposed lignocaine, corresponding to the resultant ADB, per ml. of the solution tested. From (*a*) and (*b*) the concentration of lignocaine or its hydrochloride in the original fresh solution and the percentage decomposition which it has undergone may be calculated.

The accuracy of the above process was assessed by analysing mixtures of lignocaine hydrochloride, ADB hydrochloride and DEAA of known composition and mixed in the proportions which would result from a 2 per cent. solution of lignocaine hydrochloride undergoing decomposition to the extent of 10 and 50 per cent. respectively. The results are shown in Table V.

TABLE V

ASSAY OF SOLUTIONS CORRESPONDING TO 2 PER CENT. SOLUTION OF LIGNOCAINE HYDROCHLORIDE AFTER 10 PER CENT. AND 50 PER CENT. DECOMPOSITION

	Per cent. composition corresponding to					
	10 per cent. decomposition			50 per cent. decomposition		
	Made up	Found by analysis	Per cent. recovery	Made up	Found by analysis	Per cent. recovery
Lignocaine hydrochloride ..	1·8000 g.	1·7950 g.	99·7	1·000 g.	0·9980 g.	99·8
ADB hydrochloride	0·1164 g.	0·1192 g.	102·4	0·5822 g.	0·5995 g.	103·0
DEAA	0·0969 g.	—	—	0·4844 g.	—	—

SOLUTIONS OF LIGNOCAINE HYDROCHLORIDE SUITABLE FOR USE IN DENTAL SURGERY

The preparation and stability of solutions for dental work will be described here. The pharmacological experiments and clinical trials are proceeding and will be reported later.

All the quantities of materials to be used in preparation of the solutions described refer to the dried anhydrous substances, sodium chloride, sodium phosphate, sodium acid phosphate and anhydrous lignocaine hydrochloride. This has been done so that dry ampoules may easily be made if required. All freezing points quoted are uncorrected for disengaged ice[24]. Lignocaine base was found to be soluble in water at room temperature to the extent of 0·38 per cent., the *p*H of the saturated solution being 9·85. On addition of 0·1N sodium hydroxide to 10 ml. of a 2 per cent. solution of lignocaine hydrochloride at room temperature the solution became turbid due to separation of free base at *p*H 7·8 after the addition of 3·8 ml. of the alkali solution.

The Effect of Temperature on Alkaline Buffered Solutions of Lignocaine Hydrochloride

All the alkaline solutions described below became turbid on heating but became clear again on cooling. That it was lignocaine base which separated was shown by removing some of the separated material and, after dissolving in dilute hydrochloric acid, obtaining a precipitate with potassio-mercuric iodide solution. This same phenomenon occurred previously with amylocaine[12] and was attributed to alteration of the dissociation constant of water with temperature. Probably changes, with

increase of temperature, of the hydrolysis and dissociation constants of the local anæsthetic salt are also involved.

Isotonicity. Figures for the depression of the freezing point by lignocaine hydrochloride could not be found in the published literature. Experiments using the Beckmann apparatus showed that a 2 per cent. solution of the anhydrous salt possessed an average depression of 0·278° C. This corresponds to the figure, subsequently adopted by the British Pharmaceutical Codex, 1954, of 0·130° C. for the depression produced by 1 per cent. of the monohydrate. It had previously been ascertained[11] that isotonic solutions of anhydrous sodium phosphate and anhydrous sodium acid phosphate contain respectively 2·2 per cent. and 2·0 per cent. of the salts.

Formulation of solutions of lignocaine hydrochloride for use in surgery. The composition of the six solutions formulated is shown in Table VI.

TABLE VI

PERCENTAGE COMPOSITION OF LIGNOCAINE SOLUTIONS FORMULATED

Solution	A	B	C	D	E	F
Lignocaine HCl (anhydrous) ..	2·0	2·0	2·0	2·0	2·0	2·0
NaCl	0·478	0·366	—	—	—	—
Na_2HPO_4	—	0·262	0·800	1·10	0·889	0·977
NaH_2PO_4	—	—	0·289	—	0·200	0·112
*p*H	4·8	7·1	7·1	7·5	7·2	7·3

Availability of anæsthetic base in the solutions. 10 ml. quantities of the various solutions were each shaken with 3 successive quantities of 10 ml. each of ether. The free base in the first and in the mixed second and third ethereal extracts was estimated as described earlier. The *p*H of the aqueous solution was determined electrometrically before and after extraction with ether. The results obtained are recorded in Table VII.

TABLE VII

PERCENTAGE OF THE TOTAL BASE REMOVED BY ETHER FROM SOLUTIONS OF LIGNOCAINE HYDROCHLORIDE

Solution	A	B	C	D	E	F
1st extraction	1·5	20·0	44·9	55·5	48·0	50·6
2nd and 3rd extractions ..	1·2	2·8	15·2	17·3	18·9	17·6
Total 1st, 2nd and 3rd extractions	2·7	22·8	60·1	72·8	66·9	68·2
Initial *p*H	4·8	7·1	7·1	7·5	7·2	7·3
*p*H after extraction	2·95	3·2	5·3	6·1	5·55	6·05

TABLE VIII

PERCENTAGE DECOMPOSITION OF LIGNOCAINE SOLUTIONS ON HEATING IN AN AUTOCLAVE

	Approximate per cent. decomposition		*p*H		
Solution	30 min.	3 hours	Initial	30 min.	3 hours
C	0·03	0·05	7·1	7·1	7·1
F	0·03	0·05	7·3	7·3	7·3

Stability on sterilisation and storage. The most alkaline and heavily buffered solutions C and F were autoclaved for 30 minutes and for 3 hours at 115° C. The resultant decomposition and *p*H changes are shown in Table VIII.

The result obtained by analysis of the solutions after storage at room temperature for 33 and 84 weeks respectively are shown in Table IX.

TABLE IX

PERCENTAGE DECOMPOSITION OF LIGNOCAINE SOLUTIONS AT ROOM TEMPERATURE

Solution	Approx. per cent. decomposition after		*p*H		
				After	
	33 weeks	84 weeks	Initial	33 weeks	84 weeks
A	0·005	0·005	4·8	5·3	5·5
B	0·005	0·005	7·1	7·05	7·15
C	0·015	0·15	7·1	7·0	7·2
D	0·015	0·15	7·5	7·3	7·5
E	0·02	0·02	7·2	7·2	7·3
F	0·02	0·02	7·3	7·2	7·4

DISCUSSION

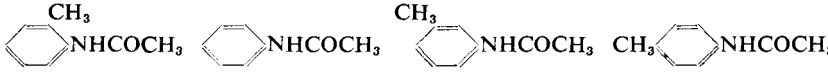
Commercially available samples of lignocaine and its hydrochloride were found to be as pure as samples prepared in the laboratory by synthesis and purified by repeated recrystallisations.

In the assay of lignocaine salts and solutions two facts should be borne in mind:—(*a*) the free base is volatile at 100° C. alone and in steam so that methods involving recovery from volatile solvents by evaporation of the latter and drying the residue at 100° C. or over should be avoided. (*b*) The hydrochloride is soluble in chloroform. There are three satisfactory methods available for the estimation of recovered lignocaine base. The Kjeldahl process is accurate but tedious. The colorimetric reineckate method is accurate but unless the estimation of very small quantities is involved it has the disadvantages common to colorimetric processes. The most convenient method is titration of the excess acid added to the base with standard alkali using bromocresol green as indicator. If this method is used it is unnecessary to evaporate the organic solvent in which the base is usually, at this stage, dissolved provided that ether is used. If chloroform is employed either it must be removed by evaporation or considerable excess acid must be added to extract the lignocaine base and the chloroformic solution must not subsequently be washed with water (Table IV). The physico-chemical behaviour of the hydrochloride of a base soluble in a volatile solvent has been discussed by Davis[25] and Moede and Curran[26]. These workers showed that the hydrochloride of a base soluble in chloroform could undergo dissociation into free base and hydrogen chloride. Such behaviour almost certainly accounts for the difficulties introduced by using chloroform in the extraction of lignocaine solutions. The assay process found to be most satisfactory was thus similar to that now official in the British Pharmaceutical Codex, 1954.

Solutions of lignocaines have been reported to be exceptionally stable. Löfgren[10] claimed that such solutions "endured eight hours' boiling with

30 per cent. aqueous hydrochloric acid or lengthy boiling with strong alcoholic caustic potash."

The stability of lignocaine to hydrolysis by acid or alkali is undoubtedly due to the presence of the two methyl groups in the *ortho*- position to the diethylaminoacetamido group. Thus 2-acetamido-1 : 3-dimethyl benzene, a compound structurally similar to lignocaine, was found by Nölting and Pick[27] to undergo no decomposition when heated in strong hydrochloric acid solution although at 200° C. in 70 to 75 per cent. sulphuric acid it was hydrolysed, after prolonged heating, into ADB and acetic acid. Davis[28] calculated from experimental data that the corresponding monomethyl derivative VI would require 17·16 hours refluxing in alkaline solution for 50 per cent. hydrolysis. On the other hand, compounds VII, VIII, and IX, under similar conditions required only 3·79, 3·89 and 3·90 hours respectively for 50 per cent. hydrolysis. The unreactivity of groups which have other groups in the *ortho*-position is a general property known as the *ortho*-effect[29a].



(VI) (VII) (VIII) (IX)

Originally attributed to simple steric hindrance[29b] the *ortho*-effect is now thought [30,31], to be due to reduced mesomerism between the benzene nucleus and substituent resulting from a loss of planarity between the nucleus and substituents caused by the *ortho*-groups—in the case of lignocaine, the *ortho*-methyl groups. This steric inhibition of mesomerism has recently been renamed the secondary steric effect[32] and has been demonstrated in lignocaine by Löfgren[10].

Preliminary experiments soon confirmed the stability of the lignocaine molecule, but it was also established that when lignocaine in solution does decompose it is hydrolysed as shown in equation (1).

Local anæsthetics of the procaine type are easily hydrolysed, particularly in alkaline solutions, and the object of the previous research work carried out with them in this department was to obtain a quantitative estimate of the degree of hydrolysis under various conditions and then to formulate solutions and devise methods of sterilisation and storage which would result in a maximum of activity combined with a minimum of decomposition. In the case of lignocaine the object was different. The molecule is stable. A colorimetric test was elaborated which would detect even less than 0·1 per cent. of decomposition in a 2 per cent. solution of lignocaine hydrochloride. This test was then applied to show that under any of the usual conditions of preparing, sterilising and storing such solutions there was not more than 0·1 per cent. of decomposition.

Reactions based upon diazotisation and coupling, which, owing to the presence of undecomposed lignocaine, have to be carried out under acid conditions, were not sufficiently sensitive to detect the concentration of ADB corresponding to 0·1 per cent. decomposition of a 2 per cent. solution of lignocaine. A colour reaction represented by equation (2) was found to be of the required sensitivity as can be seen from Figure 5.

The accuracy of the method devised for the estimation of the degree of decomposition in lignocaine solutions was tested on solutions containing lignocaine hydrochloride, ADB hydrochloride and DEAA in proportions corresponding to 10 and 50 per cent. decomposition of a 2 per cent. solution of lignocaine hydrochloride. The results, shown in Table V, indicate the success of the method.

Although not applicable without considerable modification and consequent loss of accuracy, the method was used to show that heating lignocaine in an autoclave with 50 per cent. sulphuric acid for 5 hours at 116° C. caused approximately 3 per cent. decomposition, while similar treatment with 20 per cent. ethanolic potash caused approximately 0·5 per cent. decomposition.

Usually amides are more readily hydrolysed by alkalis than acids[33]. The reverse phenomenon in the case of lignocaine may probably be attributed to the inhibited mesomerism in that substance (*ortho*-effect) giving rise to higher electron densities at the amide nitrogen and acyl carbon atoms. Such conditions would facilitate the electrophilic attack by a proton and water molecule at the two points required for the hydrolysis.

The advantages of using in dental surgery local anæsthetics in buffered alkaline solutions have been stated previously[11,34]. More recent work[3] has confirmed the enhanced activity of solutions of local anæsthetics at higher *p*H values although there has been a suggestion[35], based perhaps on a not very critical examination of the data, that the local anæsthetic cation as well as the free base may have anæsthetic activity. Previously the relatively easy hydrolysis of the local anæsthetics as well as the instability of adrenaline at *p*H values over 7 has prevented the general use of alkaline buffered solutions which had to be freshly prepared involving the employment of two solution cartridges or dry ampoules. It was obviously of interest to investigate the pharmacological activity and clinical use of alkaline buffered solutions of the very stable lignocaine hydrochloride.

Since lignocaine resembles procaine in potency it was decided to prepare solutions for surgical use similar to those previously described for procaine[11], i.e., 2 per cent. solutions made alkaline and buffered with sodium phosphate alone or in admixture with sodium acid phosphate and, if necessary, rendered isotonic by addition of sodium chloride. Adrenaline was omitted for two reasons, (*a*) lignocaine has been recommended for use without adrenaline and (*b*) the dispensing and storage of alkaline solutions of adrenaline constitute a separate problem which has been previously investigated and discussed[11,36]. It was decided that adrenaline would be added if required immediately before use, at any rate in the preliminary experiments, in the Dental Hospital.

Six different solutions, A to F, having the percentage compositions shown in Table VI were formulated and examined. All contained 2 per cent. of lignocaine hydrochloride. A was made isotonic by the addition of sodium chloride. B was a similar solution brought to *p*H 7·1 by addition of sodium phosphate before being made isotonic by addition of sodium chloride. Thus the buffering capacity was the minimum consistent with the *p*H. On heating it became turbid at 65° C. Solution C

like B had a *p*H of 7·1 but it had the maximum buffering capacity consistent with isotonicity. On heating it became turbid at 43° C. D had maximum buffering capacity and maximum *p*H consistent with the use of sodium phosphate as the alkalising agent. It had a *p*H of 7·5 and on heating became turbid at 34° C., i.e. below body temperature. E and F were similar to C and D in having maximum buffering capacity consistent with isotonicity but, by varying the proportions of sodium phosphate and sodium acid phosphate, the *p*H values were respectively 7·2 and 7·3. On heating solution E became turbid at 37° C. and solution F at 34° C.

If the current belief that on reaching the tissues a local anæsthetic base must dissolve in fatty tissue before anæsthesia is attained is true, then clearly when the base is removed the acid with which it was associated will be liberated in the tissues. Unless this acid is neutralised by the alkalinity and buffering capacity of the blood supply the *p*H of the tissues will fall and the further supply of anæsthetic base will be restricted until the *p*H is raised again. The effects of rendering alkaline and buffering solutions of lignocaine hydrochloride are clearly shown in Table VII. The base available to the tissues, estimated in these experiments by shaking with ether, is greatly increased and the fall in *p*H after removal of the base is lessened.

All six solutions proved to be remarkably stable. Heating in an autoclave for three hours at 115° C. or storage for over 18 months at room temperature in all instances brought about less than 0·1 per cent. of decomposition (Table VIII and IX). On autoclaving the more alkaline buffered solutions for 30 minutes almost the whole of the lignocaine came out of solution. Solutions C and F on removal from the autoclave both showed an oily layer consisting of free base. With occasional shaking during cooling this redissolved to yield a perfectly clear solution.

The pharmacological activity and clinical suitability of the different solutions are at present being examined. It is intended that the results of these investigations shall be published later.

Summary

1. Samples of lignocaine and its anhydrous and monohydrated hydrochloride have been synthesised, purified and used as analytical standards.

2. A number of assay processes for lignocaine, its salts and solutions have been examined and are reported upon.

3. A method for determining the percentage decomposition of solutions of lignocaine has been described.

4. Formulæ for alkaline buffered solutions of lignocaine hydrochloride suitable for clinical trial have been suggested. Their stability and properties have been examined.

This work was carried out at the request of the British Pharmacopœia Commission.

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DISCUSSION

The paper was presented by MR. J. GRUNDY.

DR. W. MITCHELL (London) asked whether the authors had tried extracting the lignocaine base from chloroform with sulphuric acid. He would be surprised if the sulphate were soluble in chloroform.

MR. T. D. WHITTET (London) said that he had examined the effect of prolonged sterilisation of lignocaine solutions and his results were largely in agreement with those of the authors. After six hours autoclaving at 115° C. there was slight discoloration. The solutions were estimated by extraction of the base with chloroform, dissolving in sulphuric acid, back titration with alkali, using as an indicator a solution of bromocresol blue and cresol red. The *p*H of the solutions showed no change. The low figure for *p*H given by the authors was rather surprising as he had found it to be about 6·2 both in commercial samples and in his own. As a further check the melting point of the base was determined and no significant difference was observed. Some pharmacological tests were also carried out, and a slight lowering of analgesic potency was found by the corneal reflex method, and there was a slight increase in toxicity in rabbits.

DR. G. E. FOSTER (Dartford) said that as lignocaine was often used with adrenaline could the colour reaction be used in the presence of adrenaline.

DR. L. SAUNDERS (London) asked whether the authors had considered using ion exchange resins to simplify some of the extraction procedures in the analysis.

MR. J. GRUNDY, in reply, said that he had carried out experiments using sulphuric acid to extract the lignocaine base from a chloroform solution. Low results were obtained similar to those when using hydrochloric acid for the extraction. That was presumably due to lignocaine sulphate being soluble in chloroform and subsequently dissociating in that solvent. He found that there was no difference in toxicity between an ordinary aqueous solution of the hydrochloride and alkaline buffered solutions. Why Mr. Whittet should obtain a rather high value for the *p*H of an aqueous solution of the hydrochloride was difficulty to understand. It was quite possible that the presence of adrenaline would interfere in the estimation of lignocaine in a solution containing both. He had not considered the use of ion exchange resins to separate the decomposition products of lignocaine.