extraction of belladonna root and explained the results as follows: when no moistening liquid is used all of the menstruum must pass through the whole column of drug, while in case of a moistened drug each part of the moistening liquid goes through only that portion of the drug lying between it and the bottom of the percolator.

In comparing the present results on jalap with the previous results on belladonna root a difference is noted. That is, in case of jalap there is more resin in the first portion of percolate when the amount of moistening liquid is increased from 0 to 250 cc.; in case of belladonna root there was no increase in the percentage of alkaloids in the reserve portion when the amount of moistening liquid was similarly increased. In other words, the efficiency of extraction of jalap but not of belladonna root was increased by moistening with a moderate quantity of liquid. As the proportion of moistening liquid was further increased, there was a decrease in efficiency of extraction of both drugs.

The decrease in efficiency of extraction when excessive proportions of moistening liquid are used is readily understandable on the basis of the above-mentioned explanation of Husa and Yates. No definite hypothesis can be offered at this time to explain why small quantities of moistening liquid increase the efficiency of extraction of jalap but not of belladonna root; possibly the more uniform packing which is obtained with a moistened drug is more important for jalap than for belladonna root.

SUMMARY.

Percolation experiments indicate that the efficiency of extraction of jalap is greater when 250 cc. of moistening liquid is used for 1000 Gm. of drug than when the drug is packed in the dry state. As the proportion of moistening liquid is further increased there is a decrease in efficiency of extraction.

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THE STABILITY OF DIOTHANE SOLUTIONS. III.*,[1]

BY E. S. COOK AND T. H. RIDER.

It has been shown previously (1), (2) that diothane solutions when subjected to lengthy aging or sterilization undergo a slight alteration characterized by the liberation of aniline. The percentage decomposition was found to be very small—not more than 2.25% under the most stringent conditions—and is insufficient to affect the local anesthetic potency.

An extemporaneously prepared diothane solution shows no physical or chemical alteration or change in local anesthetic potency over a period of a few days if stored in pyrex containers. However, if such a solution is kept for longer periods of time, a slight change in p_H is observed together with the precipitation of the free base of diothane. We have followed the p_H changes of a number of 1% diothane solutions which were stored in pyrex bottles. The variation of p_H with aging of a typical solution is shown in Table I. The p_H determinations were made with the *quinhydrone electrode.*[2]

* Scientific Section, A. PH. A., Dallas meeting, 1936.

[1] From the Research Laboratories of The Wm. S. Merrell Company, Cincinnati, Ohio.

[2] See page 223 for foot-note.

TABLE I.—AGING OF DIOTHANE SOLUTION IN PYREX.

Age (Months).	p_H.
Fresh	4.90
4	4.76
7	4.72
9	4.70
20	4.60

This experiment, and others not reported for lack of space, indicate that extemporaneously prepared 1% diothane solutions, when aged in pyrex, become slightly more acid and apparently tend to reach a constant p_H value. As will be pointed out later, this slight increase in acidity together with the concomitant slight precipitation of diothane free base is due to the liberation of hydrochloric acid from the salt and is not incompatible with the presence of aniline which is formed by a second type of decomposition. That the precipitate is diothane free base is shown by the fact that it redissolves upon the addition of excess acid and by melting point determinations.

It seems evident that the solution could be rendered stable by the addition of enough acid to the fresh solution to bring the p_H to approximately 4.6 by the quinhydrone electrode, or 4.8 by the glass electrode. This is, in fact, the case, and the solutions so prepared have remained without precipitation and without sensible alteration of p_H for many months. Hence, it is the practice to adjust the p_H to 4.8 by the glass electrode during the manufacture of all diothane solutions.

The changes which take place slowly on aging should occur rapidly when the solution is heated. This we have shown to be true with regard to aniline formation (1). The effects of sterilization at 100° C. on unacidified and acidified solutions in ampuls of soft glass are shown in Tables II and III. Included are figures on aniline formation obtained by our previously described method (2).

TABLE II.—STERILIZATION OF DIOTHANE SOLUTIONS IN SOFT GLASS (NO ACID ADDED).

Length of Sterilization (Hours).	p_H.	% Aniline Present.	% Decomposition.[1]
0	4.91	0	0
1	5.33	0.00067	0.30
$4^1/_2$	5.37	0.0025	1.13
$18^1/_2$	5.50	0.0050	2.25

[1] We have also employed the hydrogen electrode. The quinhydrone electrode has proved to be more rapid and to give quite reproducible results if the readings are made rapidly and the same technique is always employed. However, if diothane solutions are allowed to remain for several minutes in contact with the quinhydrone, a drifting of p_H may occur. This has led us to believe that a true equilibrium is not reached or that chemical reaction may take place between quinhydrone and diothane. Thus, while it is stressed that the quinhydrone values are reproducible, apparently they are influenced by several factors and are not an accurate measure of p_H. That this is the case is demonstrated by the fact that the glass electrode, which recently has become available to us, gives values which are 0.2 p_H unit higher than those obtained with the quinhydrone although both electrodes check on the same buffer solution. A similar behavior has been observed with procaine. It should be noted that this discrepancy does not affect the conclusions drawn in this paper since the same relative differences are found with both electrodes. Unless otherwise stated, the p_H values reported in this paper were obtained with the quinhydrone electrode.

TABLE III.—STERILIZATION OF DIOTHANE SOLUTIONS IN SOFT GLASS (ACID ADDED).

Length of Sterilization (Hours).	p_H.	% Aniline Present.	% Decomposition.[1]
0	4.71	0	0
1	4.98	0.0005	0.23
4½	5.00	0.0017	0.75
18½	5.25	0.0033	1.50

[1] The percentage decomposition in these tables is based upon one molecule of diothane yielding one molecule of aniline. Complete hydrolysis of the molecule would actually give twice this ratio and the percentage decomposition would be half the values given.

It is seen that, on sterilization in ampuls of soft glass, an *increase* in p_H takes place. Since the concentration of aniline formed is insufficient to affect the p_H, the only reasonable explanation is that the glass liberates alkali. That this is true was demonstrated by sterilizing distilled water, from which the carbon dioxide had been boiled, for various lengths of time in the same ampuls and noting the p_H changes (glass electrode) as shown in Table IV.

TABLE IV.—STERILIZATION OF DISTILLED WATER IN SOFT GLASS AT 100° C.

Length of Sterilization (Hours).	p_H.
0	7.1
1	7.5
4½	7.5
18½	7.7

The addition of acid to diothane solutions is seen not only to prevent precipitation of the free base but also to inhibit the formation of aniline to some extent.

The phenomena accompanying the aging or sterilization of diothane solutions, *viz.*, the decrease in p_H in non-alkaline containers, the precipitation of diothane free base and the formation of aniline, can be accounted for by two reactions: (1) hydrolysis of the salt (diothane hydrochloride) to give diothane free base and hydrochloric acid; (2) saponification of the free base to give piperidinopropanediol (or monophenylurethane), carbon dioxide and aniline:[1]

(1) $C_6H_{10}NCH_2CH(OCONHC_6H_5)CH_2OCONHC_6H_5 . HCl \rightarrow C_6H_{10}NCH_2CH(OCONHC_6H_5)CH_2OCONHC_6H_5 + HCl$

(2) $C_6H_{10}NCH_2CH(OCONHC_6H_5)CH_2OCONHC_6H_5 + 2H_2O \rightarrow C_6H_{10}NCH_2CHOHCH_2OH + 2C_6H_5NH_2 + 2CO_2$

These reactions readily explain the beneficial effect of the addition of hydrochloric acid which tends to repress the hydrolysis by common ion effect. The presence of alkali, on the other hand, produced diothane free base by the actual neutralization of the acid portion of the salt, and we may infer that diothane solutions sterilized in pyrex ampuls would show even less decomposition than that reported. In the preparation of diothane ampuls it was found that the ordinary resistant glass ampuls of commerce contain more than a desirable amount of alkali and it was necessary to employ ampuls of special resistant glass.

[1] The primary saponification product would theoretically be phenyl carbamic acid, $C_6H_5NHCOOH$. This, of course, is incapable of existence in the free state and decomposes to carbon dioxide and aniline.

In confirmation of these reactions it may be noted that the writers have carried them out, using alcoholic potash as the saponifying agent. We have obtained aniline quantitatively and have isolated piperidinopropanediol from the reaction mixture (unpublished work).

The amount of change occurring on normal aging or sterilization, while it can be observed chemically, is too slight to alter the physiological activity beyond the experimental error of measurement. No change has been found in local anesthetic potency (3), and the aniline produced is quite insufficient to produce toxic symptoms (4). Of course, if diothane solutions are stored in non-alkali free glass, the precipitation of free base may be so great as to alter the concentration notably and thus reduce the local anesthetic potency.

This behavior is not unique to diothane: all local anesthetics of the amino ester type on which data are available show similar hydrolytic decomposition. Cocaine hydrochloride, for example, has been shown by numerous workers (5), (6), (7), (8), (9) to hydrolyze in solution with the liberation of hydrochloric acid, methyl alcohol, benzoic acid, ecgonine and the intermediate benzoyl and methyl ecgonines. This process is accompanied by a drop in p_H to 3.5–4 for a 1% solution, and by adjusting the p_H to this value minimum hydrolysis results on sterilization (7), (8). The decomposition of cocaine may be so extensive as to cause a 50% loss in anesthetic activity.

Similar hydrolysis with lowering of p_H occurs on aging or sterilization of procaine hydrochloride solutions yielding not only hydrochloric acid, *p*-aminobenzoic acid and β-diethylaminoethanol but also ammonia (5), (10). Schou and Abildgaard found 5% decomposition of procaine solution which had been aged for seven years or sterilized for 20 minutes at 120°. This decomposition was reduced to 2% when p_H was lowered to 4.35 with hydrochloric acid (0.001*N*), and this concentration of acid is recommended for the preparation of stable solutions. No significant decomposition took place when procaine solutions with this amount of acid present were sterilized for 30 minutes at 100°. The so-called alkaline solutions of procaine which are made up extemporaneously decompose rapidly, and the commercial solutions on the market claiming to be alkaline appear to have decomposed with the production of an acid p_H as indicated by the examination of several open market purchases.

Stovaine (5), (11), alypine (11), etc., also undergo similar changes.

In all of the above cases, unlike diothane, the free base is somewhat soluble in water and frequently does not precipitate upon alkalinization. There is no visible change in such solutions to indicate decomposition.

DISCUSSION.

The three individually published papers on the stability of diothane solutions complete a primary study of this problem.

All studies of the stability of diothane solutions are made unique by the almost total insolubility of diothane free base in water. This not only makes the p_H adjustment of diothane solutions a matter of considerable importance, but also helps to explain the very low concentration of degradation products which can be developed in a diothane solution, such degradation products being in most cases secondary decomposition products of anesthetic free bases.

It has been pointed out that the decomposition of diothane accompanied by the formation of aniline is not extensive enough to affect the results of biologic tests, either of toxicity or potency.

Diothane solutions are unusually stable under ordinary storage conditions and conditions of practical sterilization when compared to other anesthetics and any significant deterioration under adverse conditions is clearly marked by discoloration, precipitation or both.

Precipitation of diothane hydrochloride solution which is indicative of alkalinization and not deterioration, may be prevented or reversed by slight acidification of the solution to a true p_H of 4.8; for all practical purposes this may be attained by adding one drop of dilute hydrochloric or dilute acetic acid to each 500 cc. of one per cent solution as prepared.

SUMMARY.

1. As is typical of solutions of salts of weak bases and strong acids, diothane hydrochloride solutions are partially hydrolyzed to the free base and hydrochloric acid. On prolonged aging or heating, the traces of hydrolytic free base undergo partial degradation with the liberation of aniline. In pyrex or other highly resistant glass a lowering of p_H occurs with aging unless the original p_H was 4.8 (glass electrode) or less. If the container is of alkaline glass, the p_H shows a progressive increase with attendant precipitation of diothane free base. These changes do not involve more than a very small percentage of the diothane present and do not measurably affect the local anesthetic potency. If the solution is sufficiently alkalinized to precipitate significant quantities of the free base, the anesthetic activity obviously is lessened owing to the decrease in concentration of the solution.

2. The addition of hydrochloric acid to p_H 4.8 (glass electrode) prevents the precipitation of free base and inhibits the formation of aniline, provided the solution is stored in containers of resistant glass.

3. Changes of similar nature take place with solutions of other local anesthetics of the amino ester type.

4. Diothane solutions which for any reason have become colored or cloudy should not be used.

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