THE STABILITY OF DIOTHANE SOLUTION. II.*

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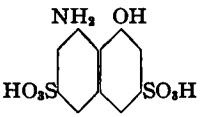
In a previous paper (1) it was reported that prolonged aging or heating of diothane solutions produces a very slight degree of decomposition, yielding a substance containing a primary amino group which may be detected by diazotization and coupling with beta-naphthol. It was pointed out that such a substance could be either an aminobenzoate, formed by rearrangement, or aniline, formed by hydrolysis. A final decision as to the nature of the alteration product was not reached, but it was inferentially shown that its concentration probably did not exceed 1:100,000.

A detailed study of the hydrolysis of diothane by extreme conditions has shown that aniline is, in fact, produced when the hydrolysis is carried out with alcoholic potash. It has been possible to develop a method whereby aniline can be quantitatively recovered. While the details of this experimental work will be published elsewhere, the conclusions are reported in support of the belief that the primary amino compound detected is in all probability aniline.

When diothane solutions are diazotized and coupled with beta-naphthol, as previously reported, the color produced is concentrated upon the precipitate of diothane free base which forms as a result of the alkalinization of the solution. This renders small amounts of color readily detectable but does not make quantitative color comparison easy. The reaction when modified by conducting it in alcohol, in which the free base is soluble, gave a homogeneous color which, however, was not sufficiently intense for colorimetric comparison in the low aniline concentrations encountered.

The standard colorimetric procedure for small amounts of aniline (development of a yellow color with bleaching powder solution) (2) proved to be inapplicable in the presence of diothane. When this reaction was potentiated with phenol (3), the typical blue color appeared but not in sufficient strength for purposes of quantitative comparison.

A diazotization method has been developed using H-acid



instead of beta-naphthol according to the suggestion of Minaev, Svetlyakov and Frolov (4). This reaction was carried out in the presence of alcohol and proved to be of the utmost sensitivity. Concentrations of aniline as low as 1:10,000,000 can be detected qualitatively, which considerably exceeds the 1:500,000 limit claimed by the Russian investigators.

In applying this reaction ten cc. of 1% diothane solution are placed in a test-tube, 0.1 cc. of 0.5*N* HCl is added, the solution is cooled in an ice-bath and 0.1 cc. of a 10% aqueous solution of sodium nitrite is added. The solution is kept cold for 10 minutes and then made alkaline with 0.5 cc. of a 5% aqueous solution of sodium bicarbonate. The precipitate of diothane base is dissolved by the addition of 8 cc. of alcohol and then 0.2 cc. of a fresh 3% solution of H-acid is added. The H-acid solution is made by dissolving 0.75 Gm. of sodium bicarbonate in 50 cc. water and

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adding 1.5 Gm. of H-acid. Five minutes after the addition of the H-acid solution to the diothane base solution the color is compared with that of a standard aniline solution which has been coupled with H-acid in exactly the same way and at practically the same time.

The standard aniline solution is made by the addition of a known amount of redistilled aniline to a fresh 1% diothane solution. This diothane solution itself should give no color when coupled with H-acid in the manner described above, indicating that it contains no aniline, or at least that the concentration of aniline present is less than the limiting sensitivity of the test.

Lovibond tintometer readings have been taken of all test colors wherever possible, and it was noticed that the color of a given solution deepened upon standing over night. This deepening in color was not detectable for two hours after the test was completed, but sixteen hours or more later was noticeable. The H-acid solution itself darkens with time, and this fact is at least a partial explanation of the darkening of the test colors with age.

RESULTS.

The above procedure has been applied to several 1% diothane solutions which had been aged for varying periods and to others which had been sterilized at 100° C., both with and without the addition of acid, for varying periods of time. The results are summarized in the following tables.

TABLE I.—AGING OF DIOTHANE SOLUTIONS.

Age (Months).	Estimated Aniline Content.
Fresh	Neg. (<1:10,000,000)
7	1:350,000
9	1:200,000
19	1:80,000

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

Length of Sterilization (Hours).	Estimated Aniline Content.
1	1:150,000
$4^1/_2$	1:40,000
$18^1/_2$	1:20,000

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

Length of Sterilization (Hours).	Estimated Aniline Content.
1	1:200,000
$4^1/_2$	1:60,000
$18^1/_2$	1:30,000

The solution, the testing of which is reported in Table II, was unacidified and had a p_H of about 5.0. The solutions, the testing of which is reported in Tables I and III, were manufacturer's stock solutions, the p_H of which had been adjusted to 4.5–4.7, during manufacture. It is evident that the addition of excess acid inhibits the development of aniline on sterilization.

The samples which were sterilized for $18^1/_2$ hours became slightly yellow and the free base, when precipitated, was yellow. There is, of course, no need for such lengthy sterilization in practice, and such discolored solutions would automatically be discarded by the careful user.

It is of interest to estimate the percentage decomposition of the diothane solution which is necessary to produce the amount of aniline actually detected. Assuming that 1 molecule of aniline is developed by the decomposition of 1 molecule of diothane (complete hydrolysis would yield twice this ratio), complete decomposition of a 1% solution of diothane would lead to an aniline concentration of 1

part in 464. This would mean that a concentration of aniline of 1:80,000 as found would represent a decomposition of only 0.6% of the diothane present. Expressed in another way, this would mean that a 1% solution of diothane which had been aged for over $1^1/_2$ years would still contain at least 0.99% of diothane. The sample sterilized for $18^1/_2$ hours still contained over 0.98% of diothane.

This estimation of the maximum amount of diothane which would be decomposed is in harmony with the previously published fact (5) that diothane solutions when sterilized for prolonged periods do not show a perceptible loss in anesthetic activity. We have found that, so long as the diothane solution remains clear and colorless, neither aging nor sterilization affects the local anesthetic potency.

The aging of diothane solutions is being further studied with a view to correlating aniline formation with p_H changes in order more fully to elucidate the nature of the alterations taking place.

SUMMARY.

1. Aging or prolonged heating of diothane solutions produces a very small amount of aniline. The maximum concentration produced by sterilization ($18^1/_2$ hours at 100°) is 1:20,000 in an unacidified solution; in the acidified solution as furnished by the manufacturers the maximum is 1:30,000. Ordinary solutions show a concentration of about 1:350,000. This change is too slight to affect the local anesthetic potency.

2. Addition of acid inhibits the formation of aniline in diothane solution.

3. So long as a diothane solution is clear and colorless its local anesthetic potency is unchanged by aging or sterilization. Solutions which have for *any* reason become cloudy or discolored should not, of course, be used.

4. A very delicate colorimetric method for the estimation of small amounts of aniline has been employed. It is capable of detecting aniline in concentrations of 1:10,000,000.

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HONORING CRAWFORD W. LONG.

The centennial of graduation from University of Georgia by Crawford W. Long, physician-pharmacist, was celebrated in Athens, March 30th. Many references to him may be found in the JOURNAL; among them: 13, 51 (1924); 15, 317 (1926); 17, 517 (1928).
