Does Repeated Heat Sterilization of Local Anesthetic Drugs Affect Potency?

L. Donald Bridenbaugh, M.D., and Daniel C. Moore, M.D.

Samples of all the commonly used local anesthetic drugs were autoclaved for periods varying from 30 minutes to 3 hours. These were subsequently assayed for potency. None of the drugs tested showed any appreciable loss of potency following autoclaving at 18 pounds pressure at 260–275° F. for 3 hours.

IN RECENT years, the necessity for heat sterilization of local anesthetic drugs has been recognized. In some hospitals, if a sterilized ampule is not used it may be reautoclaved for a subsequent procedure; thus, some ampules may be subjected to several autoclavings before use. However, as newer local anesthetic agents have become available, their tolerance to heat sterilization has not been adequately evaluated. The following questions may be raised: can all local anesthetic agents be heat sterilized without loss of anesthetic potency; if so, how many times? This study was undertaken to answer these questions and to evaluate our method of heat sterilizaiton of local anesthetic drugs.

Method of Study

The commonly used local anesthetic agents were selected for testing, *i.e.*, niphanoid crystals of tetracaine (Pontocaine),* and solutions of each of the following: procaine (Novocain), piperocaine (Metycaine), dibucaine (Nupercaine), chloroprocaine (Nesacaine) mepivacaine (Carbocaine), lidocaine (Xylocaine), and hexylcaine (Cyclaine). Six samples of each drug were selected and numbered 1 through 6. The samples were subjected to heat sterilization in the unopened bottle or ampule as received from the pharmaceutical company. With the exception of one sample of each drug which served as a control, all others were placed in a regional block tray and autoclaved at 260–275° F. at 18 pounds pressure for 30 minutes. The trays were then cooled, one sample of each was removed and the time and number recorded. This process was repeated for each drug until there were samples which had been autoclaved for 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 180 minutes, respectively. This was equivalent to autoclaving the drug from one to six times under this system of sterilization. The numbered samples were then sent to the various drug manufacturers for assay of potency in a blind study.

Results

Procaine (2 per cent, 30 ml. vial), mepivacaine (2 per cent, 50 ml. vial), and two samples of tetracaine (20 mg. and 250 mg. ampules of niphanoid crystals) were sent to the manufacturer for assay. The results were reported as follows:

Procaine. Four milliliters samples were made alkaline with 5 ml. of 10 per cent sodium carbonate solution and extracted 3 times with 10-ml. volumes of chloroform. Thirty milliliters acetone was added to these extracts and the resulting solution titrated with 0.1*N* acetous perchloric acid. Only intact procaine is measured in this technique. The percentage concentrations of the samples of procaine assayed were:

Sample	Percentage Procaine
3	Control— 1.91
4	30 minutes—1.91
6	60 minutes—1.91
5	90 minutes—1.92
2	120 minutes—1.90
1	180 minutes—1.89

Accepted for publication December 20, 1963.
The authors are in the Department of Anesthesiology, The Mason Clinic, Seattle, Washington.

* Tetracaine crystals were used because this is the drug form used and recommended in our institution for regional anesthesia.

The conclusion of the assay was: "Since one of the samples presumably represents a non-autoclaved control, it follows that the slightly low assay values have nothing to do with the autoclaving; that is, there is no evidence of decomposition."

Tetracaine. Since there was a large number of tetracaine samples, selection for assay was made as follows: melting points were taken of the contents of each ampule. Those having a high melting point (145°–150° C.) were put aside as being of unquestioned excellence. Those with lower melting points were subjected to the more meaningful assay for p-butylaminobenzoic acid (BABA), the hydrolysis product of tetracaine. Two of the 20-mg. size ampules and 5 of the 250-mg. size ampules were thus examined. The results are shown in table 1. These melting points demonstrate an interesting characteristic of tetracaine hydrochloride. This drug exists in three polymorphic crystalline forms melting, respectively, at about 134° C., 130° C., and 149° C. It generally consists of the highest melting form. Obviously, at autoclaved temperatures some conversion to the lower melting forms occurs. However, further check was made for BABA. The values indicate negligible decomposition, if any, produced by autoclaving.

Mepivacaine (2 per cent). Five-milliliter samples were made alkaline with 5 ml. of 10 per cent sodium chloride and the solution extracted 4 times with 15 ml. of methylene

TABLE 1. Results of Assays of Tetracaine after Autoclaving

	Melting Point (degrees centigrade)	Percentage Apparent BABA
Tetracaine 20 mg.		
Control	145.6–146.8	
30 minutes	148.8–150.8	
60 minutes	145.2–146.6	
90 minutes	130.8–134.8	0.07
120 minutes	146.6–148.4	
180 minutes	137.3–139.6	0.3
Tetracaine 250 mg.		
Control	121.8–133.2	0.01
30 minutes	130.8–134.2	0.02
60 minutes	135.4–142.2	0.02
90 minutes	129.4–135.4	0.02
120 minutes	146.8–149.2	0.01
180 minutes	124.8–134.0	0.02
Pure drug	145.0–150.0	0.02

TABLE 2. Results of Assays of Mepivacaine After Autoclaving

Sample Number	Time Autoclaved	Percentage Concentration of Drug	Melting Point of Extracted Mepivacaine Base
1	180 minutes	1.95	In each case, the melting point of the extracted mepivacaine base was between: 150°–153° C.
2	Control	1.88	
3	90 minutes	1.92	
4	60 minutes	1.91	
5	30 minutes	1.99	
6	120 minutes	2.00	

TABLE 3. Results of Second Study of Stability of Mepivacaine During Autoclaving

Autoclaved Sample	Weight of Base (Gm.)	Equivalent of Mepivacaine HCl (Gm.)	Concentration of Drug Percentage	Melting Point of Extracted Mepivacaine Base (degrees centigrade)
1	0.0882	0.1014	2.03	149.6–151.6
2	0.0878	0.1010	2.02	149.1–151.3
3	0.0873	0.1003	2.01	149.3–151.0
4	0.0870	1.1000	2.00	149.3–150.9
5	0.0873	1.1003	2.01	149.3–151.2
6	0.0871	1.1001	2.00	149.3–151.3
1	0.0864	0.0993	1.98	149.1–151.0
2	0.0871	0.1001	2.00	149.2–151.0
3	0.0870	1.1000	2.00	149.2–151.0
4	0.0882	0.1014	2.03	149.1–151.5
5	0.0874	0.1004	2.01	149.0–151.2
6	0.0878	1.1010	2.02	149.3–151.5

TABLE 4. Results of Assays of Chloroprocaine After Autoclaving

Sample	Time Autoclaved	Batch	Percentage Concentration Chloroprocaine HCl	Percentage Hydrolysis
1	120 minutes	6009-0043	1.88	3.58
2	Control	6009-0043	1.99	1.88
3	90 minutes	6009-0043	--- Broken ---	
4	180 minutes	6012-05—	1.90	4.68
5	30 minutes	6012-0525	1.97	3.09
6	60 minutes	6012-0525	1.95	3.65

chloride. These were pooled, dried with sodium sulfate, the sodium sulfate washed with several small portions of solvent. All solvent portions were then pooled and evaporated. The residues were weighed and the melting points taken (table 2). The values for samples 2, 3, and 4 were slightly low, but it was thought unlikely that this indicated decomposition. More likely some analytical variation was involved. In order to verify this supposition that only analytical variations were involved, additional assay was thought desirable. In another test of stability twenty-four 50-ml. commercial vials of 2 per cent mepivacaine hydrochloride solution were used. Twelve were reserved as controls and twelve were autoclaved at 260° F. for 180 minutes at 20 pounds pressure. The autoclaved samples were slightly discolored. Six of the control vials and six of the autoclaved vials were assayed as before (table 3). These results apparently confirm the impression that mepivacaine was not appreciably altered by heat sterilization and that minor variations in concentration were due to analytical variation.

Chloroprocaine. The autoclaved vials of 2 per cent chloroprocaine were sent to the manufacturer for assay. Sample 3 was broken in transit. The results of the analyses were reported as shown in table 4. REPORT: "It appears that this experiment was set up with materials from two different lots. It would be desirable, if possible, to set up an experiment of this type with materials from the same lot. Label identifications were difficult to read because of the breakage of a sample in transit." Because of the request for assay of samples all from the same lot six more bottles of 2 per cent chloroprocaine were autoclaved in the same manner. The assay was repeated and the results obtained as shown in table 5. REPORT: "Since all of these vials came from our Lot 1421, the initial assays on this lot were: percentage chloroprocaine HCl = 1.97. Apparently there was no significant loss of potency from the repeated sterilization."

TABLE 5. Assays of Samples of Same Lot of Chloroprocaine After Autoclaving

Sample	Time Autoclaved	Percentage Chloroprocaine HCl	Percentage Hydrolysis
1	90 minutes	1.95	3.28
2	30 minutes	1.98	3.11
3	120 minutes	1.98	3.95
4	60 minutes	1.95	2.83
5	Control	1.96	1.10
6	180 minutes	1.90	4.44

Lidocaine (2 per cent). Lidocaine was subjected to both chemical and biological assay by the manufacturer (table 6). These results further support the established findings that lidocaine solutions, without epinephrine, may be reautoclaved without deterioration in biological potency. The difference in the duration of motor block for the two sites of injection is due to the fact that the degree of vascularity of the two sites is different.

Hexylcaine (2 per cent). The samples were assayed by the manufacturers for intact hexylcaine hydrochloride and total hexylcaine hydrochloride (table 7).

Piperocaine (1.5 per cent 200 ml. vial) forwarded to the manufacturer and analyzed for potency (method not reported). The results are shown in table 8. Since the theoretical value is 15 mg./ml. (1.5 per cent), no significant decomposition is indicated during autoclaving of these samples.

TABLE 6. Chemical and Biological Assays of Lidocaine After Autoclaving

Sterilizing Time	Sample	Percentage HCl Lidocaine	pH Value
Chemical Assay: (Tests performed at room temperature of 73° F.)			
Control	4	1.98	6.62
30 minutes	5	1.98	6.60
60 minutes	1	1.98	6.62
90 minutes	6	1.98	6.61
120 minutes	3	1.98	6.60
180 minutes	2	1.98	6.61

Sample	Sterilizing Time	Cage	Inj. Vol. (ml.)	Leg Inj.	Site of Inj.	Freq. Motor Block	Av. Duration Block (min.)
Biological Assay (white rat sciatic nerve injection)							
4	Control	D	0.1	R	Hip	12/12	104
5	30 minutes	E	0.1	R	Hip	12/12	84
1	60 minutes	A	0.1	R	Hip	12/12	109
6	90 minutes	F	0.1	R	Hip	12/12	101
3	120 minutes	C	0.1	R	Hip	12/12	102
2	180 minutes	B	0.1	R	Hip	12/12	104
4	Control	D	0.2	L	Midthigh	6/6	103
5	30 minutes	E	0.2	L	Midthigh	5/6	88
1	60 minutes	A	0.2	L	Midthigh	5/6	85
6	90 minutes	F	0.2	L	Midthigh	5/6	84
3	120 minutes	C	0.2	L	Midthigh	6/6	90
2	180 minutes	B	0.2	L	Midthigh	6/6	94

Dibucaine (1:1500, 20-ml. ampule, each ml. containing 0.667 mg.) samples were sent to the manufacturer (method of assay not reported). The results are shown in table 9. It is important to note that dibucaine in heavy solution should be resterilized only once because further sterilization results in a darkening of the solution due to carmelization of the dextrose.

TABLE 7. Results of Assays of Hexylcaine After Autoclaving

Ampule	Sterilizing Time	Intact Hexylcaine Hydrochlodride	HCl Total (mg./ml.)
4	Control	18.3	19.6
6	30 minutes	17.8	19.7
5	60 minutes	16.8	19.9
2	90 minutes	17.5	19.6
1	120 minutes	17.3	19.6
3	180 minutes	--- Broken ---	

Discussion

With the publication of the law suit of Wooley and Roe versus the Ministry of Health,[1, 2] in which the legal decision stated that it was necessary to heat sterilize ampules of local anesthetics used for spinal anesthesia, the autoclaving of local anesthetics became established. However, there appeared to be no uniform method—some autoclaving the

TABLE 8. Results of Assays of Piperocaine After Autoclaving

Bottle	Autoclaving Time	Assay Value (mg./ml.)	pH
1 (Lot 703119)	Control	14.7	3.47
2 (Lot 703119)	180 minutes	14.7	3.50
3 (Lot 703119)	90 minutes	14.7	3.40
4 (Lot 703119)	60 minutes	14.8	3.50
5 (Lot 703119)	120 minutes	14.7	3.39
6 (Lot 727715)	30 minutes	14.5	3.37

TABLE 9. Results of Assays of Dibucaine After Autoclaving

Ampule	Time Autoclaved	Potency (mg./ml.)
1	90 minutes	0.65[5]
2	120 minutes	0.65[5]
3	Control	0.65[5]
4	60 minutes	0.65[5]
5	180 minutes	0.64[5]
6	30 minutes	0.64[4]

ampules in which the lumbar puncture sets,[3] some autoclaving only the ampules for spinal anesthesia, others advocating autoclaving all drugs and equipment used for regional anesthesia.[4, 5, 6] Time and pressure techniques varied from 10 pounds for 15 minutes to 20 pounds for 45 minutes at the same temperature.[7] In 1954, Whittet[8] attempted to determine the effects of multiple autoclaving of local anesthetic drugs for periods of from 1 to 6 hours. There was deterioration within 3 hours. The results of our study vary from Whittet's study in that the tetracaine tested did not alter in potency after three hours of autoclaving. This may be due to the fact that both of the samples of tetracaine tested consisted of niphanoid crystals, while that tested by Whittet was in solution, hence less stable.

In the authors' hospital, both heavy dibucaine (1:200 in 6.0 per cent glucose) and heavy tetracaine (1 per cent in 10 per cent dextrose) are not autoclaved more than twice because of carmelization of the glucose and subsequent discoloration if heated longer than two hours.

The results of the present study indicate that all of the commonly used local anesthetic drugs can be heat sterilized (18 pounds pressure, 260–275° F. for 30 minutes) at least 6 times, for a total time of at least three hours without loss of potency. Gerlich *et al.*[9] determined that sterilization could be accomplished at 15 pounds pressure and 250° F. temperature for 15 minutes. The system of heat sterilization using 18 pounds pressure at 260–275° F. for 30 minutes is recognized as being longer than necessary for sterilization of the drug. However, it was adopted because this is the routine for autoclaving surgical packs at our institution. Consequently, the regional block trays containing the local anesthetic drugs could be included along with the surgical packs, thus decreasing the work necessary, and eliminating any special routine for sterilization. This system of autoclaving and reautoclaving of drugs has been in practice at our institution since 1953 and the drugs used in 30,979 cases without any case of infection or clinically observed loss of potency.

Summary

Heat sterilization of local anesthetic drugs has been established a necessity. In order to determine whether the newer anesthetic agents could be autoclaved without loss of potency and, if so, how many times, samples of all of the commonly used local anesthetic drugs were autoclaved for periods varying from 30 minutes to 3 hours. These were subsequently assayed by their manufacturers for potency. None of the agents tested showed any appreciable loss of potency following autoclaving at 18 pounds pressure, 260–275° F. for 3 hours.

The authors wish to express their gratitude to the research laboratories of Sterling-Winthrop Institute, Strasenburgh Laboratories, Merck Sharp & Dohme Research Laboratories, Astra Pharmaceutical Products, Inc., Eli Lilly and Company, and Ciba Pharmaceutical Company for their cooperation and aid in assaying the drug samples for potency following sterilization.

References

1. Medico-Legal: Spinal anaesthetics appeals fail, Brit. Med. J. 1: 940, 1953.
2. Medico-Legal: Contaminated spinal anaesthetic, Brit. Med. J. 2: 1165, 1953.
3. Walton, F. A.: Correspondence, ANESTHESIOLOGY 13: 441, 1952.
4. Bridenbaugh, L. D., and Moore, D. C.: Is heat sterilization of local anesthetic drugs a necessity?, J.A.M.A. 168: 1334, 1958.
5. Young, J. A., Collette, T. S., and Brehm, W. F.: Sterility of multiple dose vials after repeated use, Amer. Surg. 24: 811, 1958.
6. De Jong, R. H.: Multiple autoclaving of commercial local anesthetic solutions containing dilute epinephrine, ANESTHESIOLOGY 24: 582, 1963.
7. Carter, A. B., Herbert, C. L., DeWald, W. S., and Talley, A. W.: Multiple autoclaving of drugs used in spinal anesthesia, ANESTHESIOLOGY 15: 480, 1954.
8. Whittet, T. D.: Effect of autoclaving in ampoules of local analgesics, Anaesthesia 9: 271, 1954.
9. Gerlich, N. A., Nicholas, P. S., and Ballinger, C. M.: Heat sterilization of spinal anesthesia ampules (abstract), ANESTHESIOLOGY 18: 164, 1957.