LOCAL ANESTHETIC ACTIVITY OF PARTIALLY HYDROLYZED SOLUTIONS OF TETRACAINE HYDROCHLORIDE

D. R. VanDeripe and G. K. W. Yim, Ph.D.

The United States Army Medical Service has stocked large quantities of 1 per cent tetracaine hydrochloride ampuls. After about 10 months storage, however, a slight degree of hydrolysis (3–4 per cent) occurs and crystals of *p*-butylaminobenzoic acid form in the ampuls. Spinal anesthetic failures, delayed and/or incomplete anesthesias and postspinal paraplegias have been attributed to the use of these "crystal-containing" solutions. We have compared the local anesthetic activities and toxicities of partially hydrolyzed and freshly prepared tetracaine solutions, and found that the results obtained do not support the contention that the partially hydrolyzed state of the solution was at fault.

Experimental

Two different "decomposed" solutions were used; one was obtained from crystal-containing ampuls of commercial tetracaine hydrochloride. These ampuls had been rejected by the United States Army Medical Service because of spinal anesthetic failures following the use of other ampuls from the same lot. The solution obtained from these ampuls assayed (spectrophotometrically) at 0.97 per cent tetracaine.[1] The other partially hydrolyzed tetracaine solution was an "artificially decomposed" solution; hydrolysis was induced by heating ampuls of freshly prepared tetracaine solution until crystals were formed. This solution, which assayed at 0.96 per cent tetracaine, was used to avoid possible complications arising from the varied compositions of different commercial tetracaine solutions (some contained preservatives and/or buffers).

The results obtained with the commercial solution are given in table 1. The intraperitoneal LD_{50}'s were determined in Sprague-Dawley rats weighing between 130 and 160 Gm. Ten or more animals were used at each dose. The 24 hour LD_{50} values and their 95 per cent confidence intervals were determined by the graphical method of de Beer.[2] That the partially hydrolyzed solution was slightly less toxic than the freshly prepared solution is in line with previous findings.[3]

The local anesthetic activities were determined using the guinea pig wheal method of Bulbring and Wajda.[4] Both solutions were diluted 20 times and injected intradermally into the shaven backs of guinea pigs. The resulting wheals were then pricked with a pin 36 times during the next 30 minutes, and the number of times the animal did not respond to the stimulus were summed. In 10 tests with each solution, the mean number of negative responses were very close: 25.4 (commercial) and 26.3 (freshly prepared).

The results obtained using the "artificially decomposed" solution are given in table 2. Onsets and durations of spinal anesthesia in rabbits were recorded using the procedure employed by Bieter *et al.* with the urethral reflex and point of Luduena and Hoppe.[5, 6] The partially hydrolyzed solution was as effective in inducing spinal anesthesia as the fresh solution. In 10 rabbits, the onsets of anesthesia of both solutions were within three minutes. The mean duration of action of the partially hydrolyzed solution was slightly longer. No

TABLE 1

Activity of Partially Hydrolyzed Commercial and Freshly Prepared Tetracaine Solution

Activity Tested	Hydrolyzed Solution	Fresh Solution
Intraperitoneal LD_{50} in rats	31.2 ± 2.3 mg./kg.	27.3 ± 4.0 mg./kg.
Local anesthetic potency in guinea pigs	25.4 + 2.1 responses*	26.3 ± 1.5 responses*

* Number of negative responses to 36 pinpricks.

Accepted for publication September 21, 1959.
Dr. Yim is Assistant Professor of Pharmacology, Purdue University School of Pharmacy, Lafayette, Indiana. Mr. VanDeripe's present address is Department of Pharmacology, Northwestern University Medical School, Chicago, Illinois.

TABLE 2

ACTIVITY OF "ARTIFICIALLY DECOMPOSED" AND FRESHLY PREPARED TETRACAINE SOLUTION

Activity Tested		Hydrolyzed Solution (minutes)	Fresh Solution (minutes)	Activity Ratio (Fresh 100)
Spinal anesthesia in rabbits	Onset	3	3	100
	Duration	144 ± 3.7	123 ± 31.7	117
	Delayed toxicity	none	none	100
Plexus anesthesia in frogs	Onset	****	****	120
Corneal anesthesia in guinea pigs	Duration	63.8 ± 3.5	62.0 ± 3.1	103

delayed toxic reactions were noted with either solution during the 21-day postspinal observation period.

Onsets of anesthesia were measured in the frog lumbar plexus preparation described by Bulbring and Wajda.[4] Three concentrations (0.01, 0.05, and 0.25 per cent) of both solutions were compared in a 6-point assay.[7] The treatments were coded and randomly assigned, 12 frogs per dose-groups. Although the partially hydrolyzed solution was estimated to be 1.2 times as potent as the fresh solution, the magnitude of the 5 per cent fiducial limits, 0.4–3.8, emphasizes the lack of precision of this assay method.

The study of the durations of corneal anesthesia, using the method described by Beyer *et al.*[8] gave much more uniform results. The mean durations of sensory anesthesia obtained when the solutions were instilled into the conjunctival sacs of 20 guinea pigs were remarkably close. The method was later shown to be sensitive enough to differentiate between two suprathreshold concentrations of tetracaine when 1.0 and 0.75 per cent freshly prepared solutions were instilled in opposite eyes of 12 guinea pigs. A mean difference of 11.1 minutes was observed ($t_{11} = 3.28$, $p < .01$).[9]

DISCUSSION

The results indicate that the partially hydrolyzed state of the tetracaine solutions was probably not a causative factor of the spinal anesthetic failures and postspinal sequelae reported following the use of such solutions. This is not surprising in view of the fact that the concentration of unhydrolyzed tetracaine remaining is still 3–4 times that which has produced satisfactory anesthesia.[10, 11, 12] It would then seem that faulty injection technique would account for the failures.

Spinal anesthetic failures, however, have been noted following the injection of *crystal-free* solutions of tetracaine hydrochloride in 3 patients in which the technique was carefully checked.[13] Spinal fluid was aspirated before and after the injection, a good indication that the local anesthetic agent was deposited in the subarachnoid space. On testing the solution remaining in the 3 crystal-free ampuls used in these patients, we obtained profound corneal anesthesia in guinea pigs. The remaining solution from the 3 ampuls, when pooled and injected into a rabbit, produced successful spinal anesthesia, as did solutions from other ampuls of the same lot. These failures, obtained in spite of the proper injection of active solutions, indicate that further studies on the role of spinal fluid *p*H and other deviations in these patients are warranted.[14-17]

SUMMARY

Partially "decomposed" tetracaine hydrochloride solutions, with small amounts of *p*-butylaminobenzoic acid crystallized out, showed no appreciable change in toxicity, potency and onset or duration of action in experimental laboratory animals. Crystal-free solutions of tetracaine, which failed to produce spinal anesthesia clinically, showed potent local anesthetic activity in rabbits. These results do not support the contention that the partially hydrolyzed state of the crystal-containing commercial tetracaine hydrochloride solutions is responsible for the spinal anesthetic failures, incomplete anesthesias, and delayed

toxicities sometimes observed following the use of these solutions.

This study was supported in part by a grant from the United States Medical Procurement Agency.

REFERENCES

1. Ellin, R. I., and Kondritzer, A. A.: Spectrophotometric determination of tetracaine and phenylephrine hydrochloride, J. Am. Pharm. A. (Scient. Ed.) **41**: 71, 1952.
2. de Beer, E. J.: Calculation of biological assay results by graphic methods. All-or-none type of response, J. Pharmacol. & Exper. Therap. **85**: 1, 1945.
3. Communications from Winthrop-Stearns, Inc., Rensselear, N. Y. (1954).
4. Bulbring, E., and Wajda, I.: Biological comparison of local anesthetics, J. Pharmacol. & Exper. Therap. **85**: 78, 1945.
5. Bieter, R. N., Cunningham, W., Lenz, O., and McNearney, J. J.: Threshold anesthetic and lethal concentrations of certain spinal anesthetics in rabbit, J. Pharmacol. & Exper. Therap. **57**: 221, 1936.
6. Luduena, F. P., and Hoppe, J. O.: 2-Phenyl-4-thiazolidone derivatives as local anesthetics, J. Am. Pharm. A. (Scient. Ed.) **40**: 132, 1951.
7. Burn, J. H.: Biological Standardization, ed. 2. London, Oxford Medical Publication, 1950, p. 86.
8. Beyer, K. H., Latven, A. R., Freyburger, W. A., and Parker, M. P.: Comparative study of activity and toxicity of hexylcaine (1-cyclohexylamino-2-propylbenzoate); new local anesthetic agent, J. Pharmacol. & Exper. Therap. **93**: 388, 1948.
9. Snedecor, G. W.: Statistical Methods, ed. 5. Ames, Iowa, Iowa State College Press, 1956, p. 160.
10. Lund, P. C., and Rumball, A. C.: Hypobaric pontocaine spinal anesthesia, 1640 consecutive cases, ANESTHESIOLOGY **8**: 181, 1947.
11. Cooper, M. P.: Low dosage pontocaine anesthesia in genito-urinary surgery, Anesth. & Analg. **18**: 291, 1939.
12. Lorham, P. H.: Minimal anesthesia for urologic surgery using small doses of pontocaine-glucose solution, Anesth. & Analg. **29**: 111, 1950.
13. Personal Communications from R. L. Thompson, LCDR, Medical Service Corps, United States Navy, United States Naval Hospital, Great Lakes, Illinois, September 8, 1957, June 6 & 10, 1958.
14. Black, W. R., and Walters, G. A. B.: "Rachiresistance" and spinal anesthesia, Brit. M. J. **1**: 218, 1937.
15. Brockmeyer, M. L., and McGowan, T. S.: Prolonged spinal anesthesia, use of intrathecal vasoconstrictor substances, Surg., Gynec. & Obst. **88**: 528, 1949.
16. Cohen, E. N., and Knight, R. T.: Hydrogen ion concentration of spinal fluid and its relation to spinal anesthetic failures, ANESTHESIOLOGY 8: 594, 1947.
17. Heard, K. M.: Influence upon spinal anesthesia of certain characteristics of spinal fluid, Anesth. & Analg. **17**: 121, 1938.

CHOICE OF ANESTHESIA Experiments with the excision of methylcholanthrene sarcoma in 76 rats, and of Brown-Pearce tumours in 46 rabbits under local and general anaesthesia make it possible to state that the methods of anesthesia in these operations on rats and rabbits in no way influence the development of recurrences and metastases, and that the danger of implantation of individual cells during operations carried out under local anaesthesia as compared with general anaesthesia has been exaggerated. (*Kiyashov, A. P.: Influence of Local Anaesthesia on Development of Recurrences and Metastases of Tumours in Experimental Animals, Eksper. Khir. 8: 56, 1958.*)

ANTIPYRINE Injections of antipyrine for anaesthesia according to Topchibashev's method is recommended. Thirty-eight operations were performed under this type of anaesthesia. No intraoperative or postoperative complications were observed. It is pointed out that this type of anaesthesia produces a complete painlessness at operations on jaws and oral cavity, but preserves the glottic reflex, thus preventing the danger of aspiration. (*Ismailov, A. A.: Analgesine (Antipyrine) Anaesthesia in Operations on Jaws and Oral Cavity, Az. Med. Zh. 3: 89, 1958.*)