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Neurotoxicity of Intrathecal Local Anesthetics in Rabbits

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The authors developed a new method of intrathecal local anesthetic injection in rabbits in order to study the relationship between anesthetic concentration and impaired neurologic function. They found that none of the local anesthetics studied produced persistent neurologic damage in concentrations used clinically. However, lidocaine and tetracaine can be prepared in high concentrations (far exceeding those clinically used) that will produce extensive irreversible neurologic injury and histologic changes. This was also true for sodium bisulfite, an antioxidant used in a number of commercially prepared local anesthetic solutions. Pure solutions of relatively insoluble local anesthetics (bupivacaine and 2-chloroprocaine) failed to produce comparable neurologic or neuropathologic changes when tested at concentrations up to their solubility limits. Extensive neurologic impairment was not necessarily accompanied by equally extensive lesions in the spinal cord and nerve roots. (Key words: Anesthetics, local: bupivacaine; 2-chloroprocaine; lidocaine; tetracaine. Spinal cord: toxicity, local anesthetics. Toxicity: spinal cord, local anesthetics.)

CONCERN about the neurotoxic potential of local anesthetics was heightened in 1980 when Ravindran[1] and Reisner[2] independently reported cases of persistent neurologic deficits following regional anesthesia with 2-chloroprocaine. However, despite the fact that all commercially available local anesthetics now appear to have been studied by subarachnoid injection into animals, few studies have addressed the question of permanent neurologic injury. The purpose of this study was to compare the neurotoxic potential of 2-chloroprocaine with a number of other clinically used local anesthetics, with the use of a new intrathecal injection model in rabbits.

The earliest report of intrathecal injection of local anesthetics into rabbits was by van Lier[3] in 1907. Bieter *et al.*[4–7] studied local anesthetics extensively between 1936 and 1939 and showed that permanent paralysis occurred in many rabbits that survived the intrathecal injection of concentrated solutions. Subsequent authors, including Co Tui,[8] Loomis,[9] Luduena,[10–12] Berman and Murray,[13] and Adams *et al.*[14] used Bieter's model or modifications of it to study further effects of intrathecal local anesthetic injection in rabbits. Wang *et al.*[15] has recently described implantation of subarachnoid catheters in rabbits to permit serial local anesthetic injections.

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Methods

Site of Injection

A model suitable for the study of neurotoxic effects of drugs administered intrathecally should be free of spinal cord and spinal nerve root trauma produced by placement of the needle. Therefore, we chose an injection site lower in the spinal canal than other investigators have used. We found that between the fused first and second sacral vertebrae, a 22-gauge needle could consistently be advanced into the subarachnoid space via a paramedian approach. Passing a needle through the ligamentum flavum covering this space produces a characteristic "feel." An assistant restrained the rabbit on a tabletop with a 10-cm-diameter rolled towel beneath its lower abdomen. The operator palpated the iliac crests, identified the prominent spinous process of the first sacral vertebra 1.5–2 cm caudal to those landmarks, and inserted the needle through the skin 0.5 cm lateral to the midline and 1 cm caudal to the first sacral spinous process. The needle was directed toward the midline and "walked" cephalad until it entered the S1–S2 interspace, penetrating the ligamentum flavum (fig. 1).

Criteria for Correct Needle Placement

As noted by previous investigators, we found the volume of cerebrospinal fluid (CSF) in rabbits is so small that none is seen in the hub of a needle correctly placed in the subarachnoid space.[16]

To minimize needle manipulation, and the possibility of direct needle trauma, we confirmed our clinical impression of correct needle placement with the use of a nerve stimulator. A low-intensity stimulus (1 Hz, duration 1 ms, intensity 0.2 mA) delivered through an electrically insulated needle (Teflon®-coated 22-gauge short bevel, tip exposed), using a second subcutaneous needle as a ground lead, produced a characteristic bilateral hind limb twitch only when the needle tip was within the subarachnoid space.

Volume of Injectate

In a series of preliminary experiments, we found that a volume of 5 μl/cm of cord length (average 0.15 ml/rabbit) of 0.5% Gentian Violet injected over 1 s as de-

scribed spread in the subarachnoid space to stain the spinal cord as far cephalad as the upper lumbar or lower thoracic region. That volume (which is one-fourth the volume used in most previous studies—see "Discussion") was chosen for injection of subsequent local anesthetic solutions.

Cord length measured from the base of the skull to the lumbosacral interspace varied from 28 to 30 cm and the volumes of local anesthetic test solutions administered ranged from 0.14 to 0.15 ml. Each solution studied was injected in five rabbits; thus, a total of 215 rabbits was used.

Solution Preparation

Solutions were prepared immediately before injection by dissolving the crystalline local anesthetic HCl salt in distilled water and sterilizing the solution with a 0.22 mM Millipore® filter. The compounds used were obtained as follows: tetracaine and bupivacaine from Breon Laboratories Inc., lidocaine from Astra Pharmaceutical Products Inc., and 2-chloroprocaine from Penwalt Pharmaceutical Division.

Neurologic Examination

After injection, each rabbit was examined immediately, at 5 and 30 min, at hourly intervals for 6 h, then daily for 5–7 days. The examinations scored three neurologic functions (ability to hop, "toe spread," and cutaneous sensation) as 0—normal, 1—diminished, or 2—absent. The ability to hop was evaluated by placing the rabbit on the floor and observing its attempts to ambulate. "Toe spread" is a characteristic toe abduction in the hind limbs observed when a normal animal is first lifted from a surface. Cutaneous sensation was evaluated by seeking an aversive response to a 200-volt two-point skin stimulator progressing from sacral to thoracic dermatomes. The amount of neurologic abnormality caused by the test solutions was determined by totaling the three examination scores for all the five rabbits in each test group. Each animal was provided with food, water, and any necessary supportive care (*e.g.*, bladder expression) during the time of the study. During the entire series of neurologic examinations, the observers were "blinded" to the identity of the solutions under study.

Neuropathologic Examination

After the observation period, the animals were killed and the spinal cords and attached nerve roots were immediately removed and preserved for light microscopic examination. Examiners classified each cord as normal or abnormal in relation to three possible types of lesion: cauda equina damage, central necrosis of the cord and subpial vacuolation and chromatolysis. All histologic specimens were examined by two neuropathologists, who were unaware of the drug protocol used until the entire series had been completed.

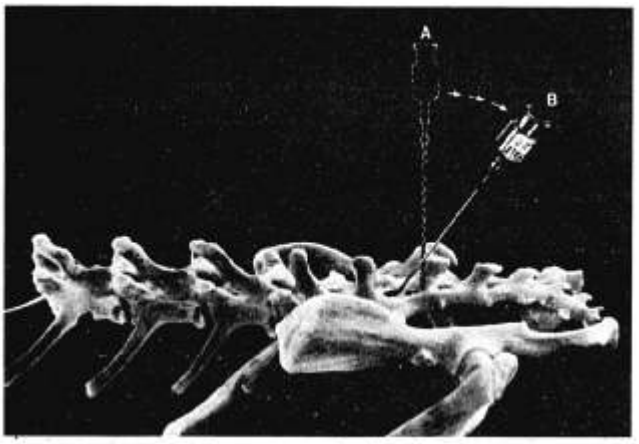

FIG. 1. A 22-g, 3.8 cm (1.5 inch) needle is placed through the skin 0.5 cm lateral to the midline and 1.0 cm caudal to the first sacral spinous process. It is advanced from this paramedian position until it contacts the lamina of the second sacral vertebra (Needle A). The tip is directed toward the midline and "walked" cephalad until it enters the interspace between the fused first and second vertebrae (Needle B).

Determination of Minimum Reversible Blocking Concentration

We determined the minimum reversible blocking concentration (MRC) for each agent, defined as the concentration at which 50% of the rabbits injected had reversible abolition of "toe spread" develop. We found this sign the most sensitive of the three neurologic elements tested. For each concentration point of each drug, five rabbits were examined before injection to confirm the presence of the response, then reexamined at 5 min after injection to determine whether "toe spread" had been blocked by the solution being investigated. To determine MRC, we plotted the 5 min responses from each five-animal group against the concentrations being studied. We then estimated from each graph the approximate point at which 50% of the animals would have been blocked.

Determination of Minimum Irreversible Blocking Concentration

We attempted to establish the concentration of each agent capable of producing persistent nerve block by defining its minimum irreversible blocking concentration (MIC)—the concentration of each agent capable of producing in 50% of the rabbits a block of the "toe spread" response that persisted beyond 48 h. The MIC was determined graphically as described above for the MRC.

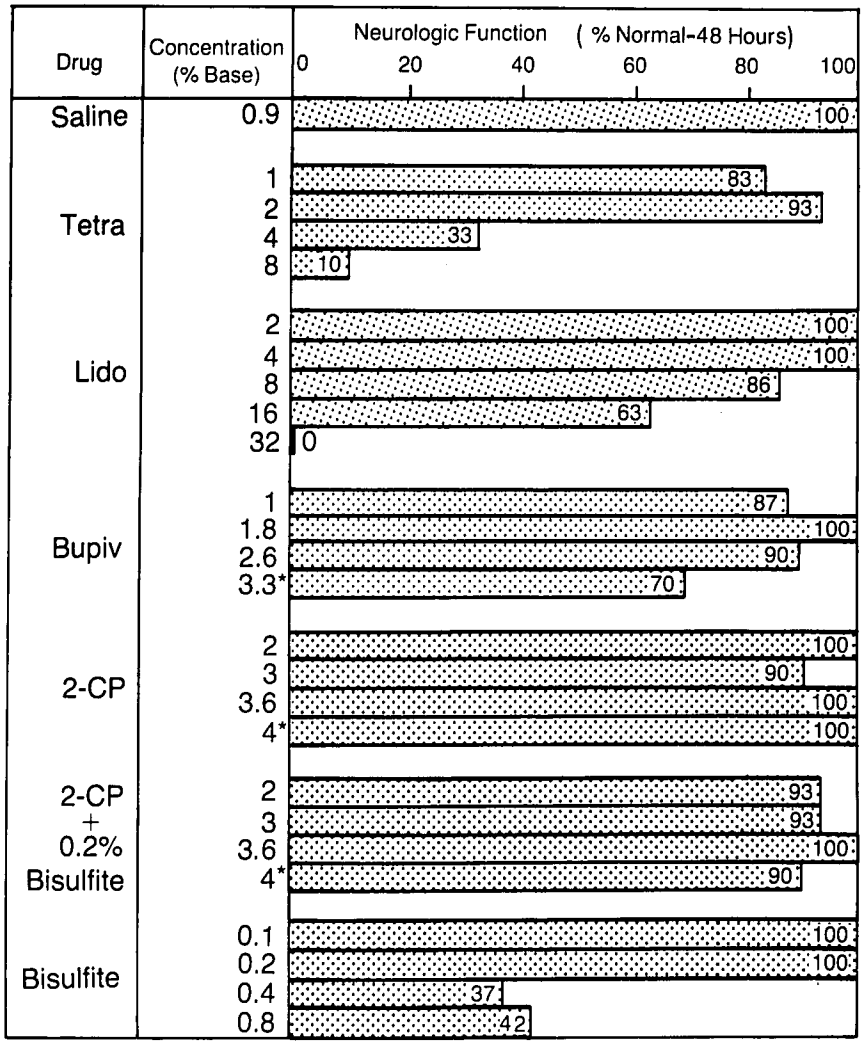

FIG. 2. Neurologic function as a per cent of normal 48 h after intrathecal injection using a three-component neurologic examination (ability to hop, "toe spread," and cutaneous sensation). Each bar represents the cumulative scores of five rabbits. See "Methods" for details of examination. *Approximate solubility limit.

Results

NEUROLOGIC FUNCTION

No cutaneous anesthesia or impairment of hop or "toe spread" was seen at any time in the control animals injected with saline, indicating that mechanical trauma does not occur during injection. All animals receiving a local anesthetic solution that resulted in a "block" displayed absence of cutaneous sensation to the upper lumbar or lower thoracic area, absence of hop, and absence of "toe spread." Onset of these deficits started to become apparent within 10 s following injection and returned to normal over a 2–4-h period, except in those animals in which persistent neurologic deficits developed. No animals lost function in the forelimbs, nor was there evidence of respiratory distress or shock (as evidenced by full peripheral pulses) while the blocks were in effect. Even those animals with extensive persistent neurologic deficits continued to drink and eat normally when returned to their cages. Figure 2 shows neurologic function 48 h after injection of test solutions. All deficits present at 48 h persisted until the animals were killed at 5–7 days.

Persistent neurologic deficits were not seen with clinically used concentrations of tetracaine, lidocaine, bupivacaine, chloroprocaine, chloroprocaine + 0.2% bisulfite, or bisulfite alone. Such deficits did occur with higher concentrations of tetracaine, lidocaine, and bisulfite.

*p*H AND OSMOLALITY OF INJECTION SOLUTIONS

In accord with common clinical practice, we used unbuffered distilled water to dissolve the anesthetic agents. The *p*H values and the osmolality of the solutions are listed in table 1. No correlation was seen between the *p*H or osmolality and the neurologic and neuropathologic effects of the solutions. For instance, the *p*H of 32% lidocaine and 4% chloroprocaine + bisulfite were 3.2 and 3.3, respectively. However, the lidocaine solution pro-

duced complete impairment of neurologic function, while the chloroprocaine showed only 10% abnormality.

Comparison also can be made between solutions with similar osmolality such as 8% tetracaine, which caused extensive neurologic damage, and 4% chloroprocaine, which caused none.

Determination of MRC and MIC

The minimum reversible blocking concentration (MRC) and minimum irreversible blocking concentration (MIC) data are presented in figure 3, and the estimated values for MRC and MIC are shown in table 2. Because bupivacaine was relatively insoluble, we could not achieve a stable unsaturated solution above 3.3%. Because irreversible block produced by this concentration did not approach 50%, we were unable to estimate the MIC for bupivacaine. As with bupivacaine, 2-chloroprocaine was relatively insoluble and could not be administered in concentrations above 4%, a concentration that did not approach 50% irreversible block.

Since sodium bisulfite is not a local anesthetic agent, we did not attempt to establish a concentration at which neurologic function is reversibly blocked. Higher concentrations of bisulfite did produce enough persistent impairment of neurologic function to permit an estimate of MIC.

Neuropathologic Findings

Microscopic examination of all spinal cords and attached nerve roots disclosed several different types of damage (table 3). These included damage to the cauda equina with axonal degeneration, areas of central necrosis within the cord, and subpial vacuolation.

We found no correlation between lesions in the cauda equina and the extent or type of functional loss. Some neurologically normal animals had marked histologic changes in the cauda equina; other animals were neurologically incapacitated but showed no abnormality in the cauda equina.

Marked central cord necrosis was present in 10 animals in our series. In seven of these, severe neurologic deficit was present as well. This lesion was associated with only four compounds: bisulfite (three animals), 2-chloroprocaine with bisulfite (three animals), 8% tetracaine (three animals), and 32% lidocaine (one animal).

Local damage at the surface of the cord (subpial vacuolation) was seen primarily in those animals with significant neurologic deficit. This was most notably present in some of the damaged animals that had received the highest concentrations of the soluble agents (lidocaine and tetracaine).

No histologic evidence of infection was present.

TABLE 1. *p*H and Osmolality of Injectates

Drug Conc. (% base)	*p*H	Osmolality mOsmol/kg
Lidocaine HCl		
0.1	4.8	28
0.2	4.6	37
0.4	4.4	40
0.8	4.4	60
2	4.5	130
4	4.2	262
8	4.0	519
16	3.8	989
32	3.2	1,997
Bupivacaine HCl		
0.01	7.0	16
0.02	6.0	20
0.04	5.9	26
0.08	5.8	29
0.16	5.6	34
1	4.5	64
1.8	4.4	112
2.6	4.1	163
3.3	4.0	196
Na bisulfite		
0.1	3.3	49
0.2	3.3	120
0.4	3.3	177
0.8	3.4	236
Tetracaine HCl		
0.01	4.8	23
0.02	5.0	24
0.04	5.1	25
0.08	5.1	27
1	4.9	55
2	4.8	92
4	4.6	183
8	4.0	239
2-chloroprocaine HCl		
0.2	6.6	54
0.4	6.5	64
0.8	5.8	81
2	5.4	138
3	5.2	188
3.6	5.1	220
4	5.1	242
2-Chloroprocaine HCl + 0.2% Na bisulfite		
2	3.5	151
3	3.4	191
3.6	3.3	212
4	3.3	241

Comparison of Neurologic Function and Histologic Normality

Although, in general, there is increasing neurologic injury and histologic damage with increased concentration of local anesthetics, there are isolated exceptions showing poor correlation between neurologic and histologic change.

Discussion

Because the rabbit has been a suitable model in the past and is inexpensive, easily available, and relatively

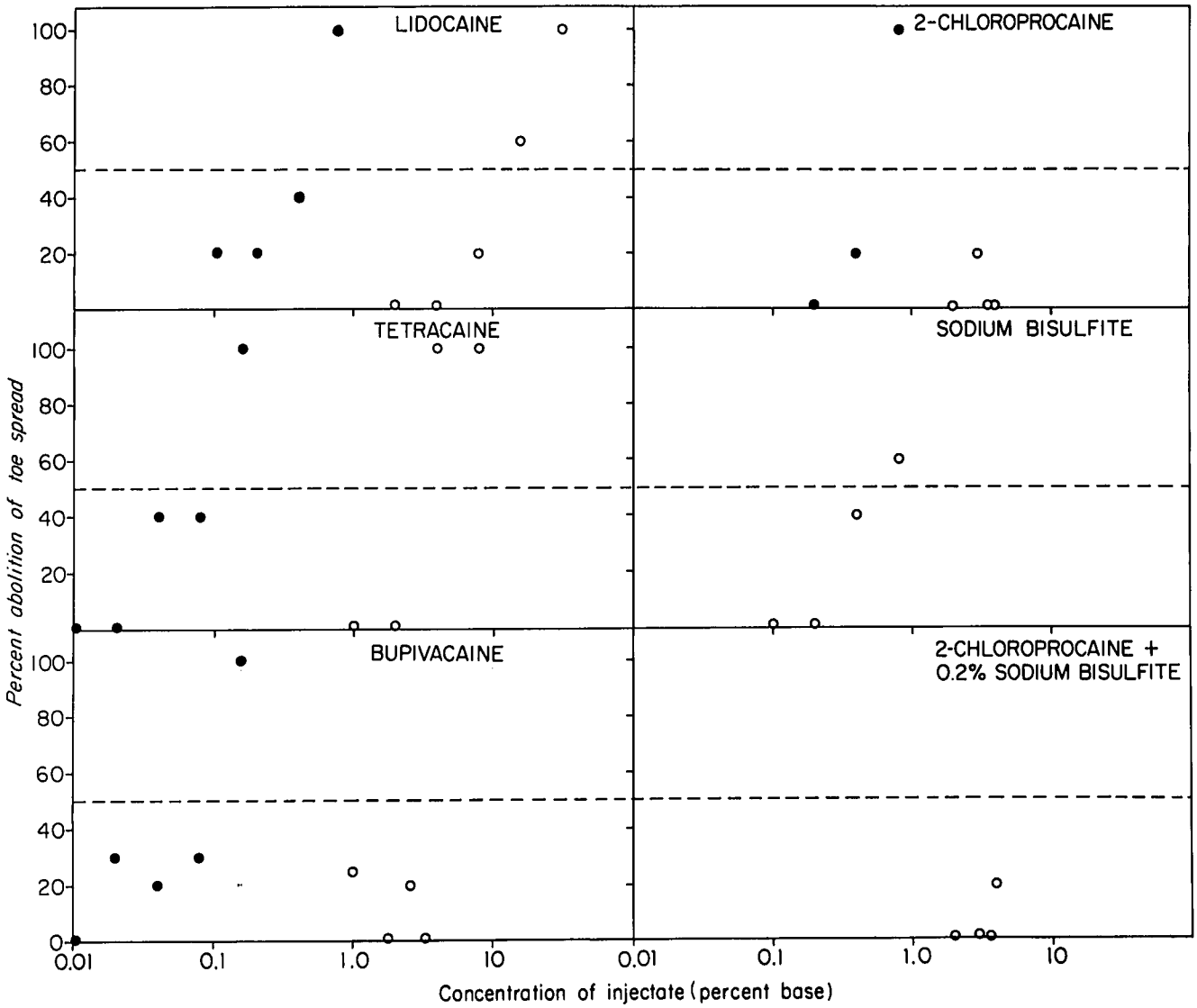

FIG. 3. Concentration of injectate (logarithmic scale) causing reversible and irreversible abolition of "toe spread." ● Reversible abolition of "toe spread" was visible by 5 min following injection but did not persist. O Irreversible abolition of "toe spread" was still present 48 h following injection. Each concentration point is derived from the responses from a group of five animals. For a complete description of the measurement, see "Methods."

convenient to work with, we chose to pursue the development of an alternate rabbit model for the study of intrathecal drug effect. On the basis of our preliminary dissections and dye volume studies, we made three major changes in the model as established by Bieter in 1936[4] and modified by Luduena in 1957.[11] First, since the rabbit's spinal cord extends to the mid-sacrum, we minimized cord trauma by injecting at the first sacral interspace instead of one or two spaces higher as all previous authors have done. Second, since our objective was to bathe the lower portion of the cord with local anesthetic solution without causing respiratory paralysis, shock, and immediate death, we used a volume of solution that was only one-fourth that used in most previous studies. Nevertheless, our dye studies showed that this volume of injectate is sufficient to bathe the caudal, sacral, lumbar, and often the lower thoracic portion of the spinal cord. Third, we performed lumbar puncture with an electrically insulated needle and a precisely calibrated nerve stimulator to produce a bilateral lower extremity twitch with very low current to confirm our clinical judgment that the needle had entered the subarachnoid space.

We found that needle placement could be accomplished with little apparent distress in the unanesthetized rabbit. An assistant could easily immobilize a rabbit on a tabletop in a position that facilitated identification of the surface

anatomy of the lower lumbar and sacral areas. Since we saw neither neurologic deficits nor histologic damage with clinically used doses of the agents studied, we believe that the incidence of mechanical trauma to the spinal cord and spinal nerves with this technique of subarachnoid injection is extremely low.

Although the absence of CSF in correctly placed needles was of initial concern, bilateral hind limb twitch with electrical stimulation followed by injection of local anesthetic produced spinal anesthesia. Further, postmortem examinations confirmed that dye injected with the use of our criteria for correct needle placement was seen only in the subarachnoid space and only spread to levels similar to the blocks produced by equal volumes of local anesthetics.

Though we did not shave or disinfect the skin before injection in this study, we saw neither gross nor microscopic evidence of infection in any animal. We believe those measures are unnecessary.

Our model provides well-defined criteria for quantitative assessment of sensory and motor block. We found the examinations reproducible on different occasions and with different examiners. In particular, the "toe spread" represents a very sensitive indicator of neurologic function. It was typically the first form of impairment noted with onset of a block and was also the last function to return during block recession. Many rabbits judged to have return of a normal hop still had impairment of "toe spread" for an additional period of time during block recession.

Although exposed to concentrated solutions of potent local anesthetic agents producing profound spinal anesthesia, no animals appeared to suffer respiratory failure or cardiovascular collapse. This is an important consideration in an animal model proposed for the study of the neurotoxicity, since hypoxia or hypotension might contribute to neuronal injury.

This study shows that two local anesthetics generally thought safe for intrathecal use in humans (lidocaine and tetracaine) can produce dose-related neural injury in concentrations greater than those used clinically. Their high solubility makes these concentrations possible. This finding confirms the incidental observations of Bieter[4] in 1936 made during lethal dose studies that rabbits surviving injections of high concentrations of tetracaine remained neurologically impaired.

Two other clinically used local anesthetics (2-chloroprocaine and bupivacaine) failed to produce consistent neurologic injury. The relative insolubility of these agents made it impossible to study them in concentrations equivalent to those of lidocaine and tetracaine, which produced injury.

Sodium bisulfite, an antioxidant added to the commercial preparation of 2-chloroprocaine and some other local anesthetic agents, showed a degree of neurotoxicity at concentrations of two times and four times that used in commercial solutions of local anesthetics. That finding is consistent with the work of Wang,[15] who found with his model of sequential intrathecal rabbit injections that bisulfite, but not pure 2-chloroprocaine, was capable of producing neural injury.

TABLE 2. Estimated Values of Minimum Reversible Concentration (MRC) and Minimum Irreversible Concentration (MIC) for the Agents Studied

Drug	MRC Per Cent Base	MIC Per Cent Base
Lidocaine	0.2–0.4	7.6–10.6
Tetracaine	0.04–0.07	2.5–5.0
Bupivacaine	0.04–0.09	*
2-Chloroprocaine	0.4–0.5	*
Bisulfite	*	0.4–0.8

* Not established. MRC and MIC ranges were estimated from data in figure 3.

TABLE 3. Distribution of Histologic Lesions

	Number of Animals with Lesion		
	Cauda Equina	Central Necrosis	Subpial Vacuolation
Saline	0	0	0
Tetracaine			
1%	1	0	0
2%	3	0	0
4%	1	0	0
8%	3	3	2
Lidocaine			
2%	0	0	0
4%	2	0	0
8%	0	0	2
16%	2	0	3
32%	5	1	3
Bupivacaine			
1%	2	0	0
1.8%	2	0	0
2.6%	2	0	0
3.3%	4	0	1
2-Chloroprocaine			
2%	1	0	0
3%	3	0	1
3.6%	2	0	0
4%	2	0	0
2-Chloroprocaine +0.2% bisulfite			
2%	3	2	1
3%	5	0	0
3.6%	1	0	0
4%	3	1	0
Bisulfite			
0.1%	3	0	0
0.2%	0	0	0
0.4%	4	2	0
0.8%*	2	1	0

There were five animals in each group except (*) in which there were four animals.

Gisson *et al.*¶ have reported that the combination of 0.2% sodium bisulfite and low *p*H (<3.2) produced profound and persistent block in isolated rabbit vagus nerve *in vitro* where the *p*H was controlled. In our study, *p*H in the subarachnoid space after injection is unknown and impossible to accurately estimate because of clearance from the injection site by tissue uptake and dilution by CSF. Therefore, it is not known what role the *p*H of our injected solutions played in producing neurologic damage.

Although a number of neuropathologic lesions were seen at 5–7 days, these did not correlate well with neurologic findings and were not useful in elucidating a mechanism of neural injury. This finding is consistent with previous work by Berman and Murray.[13] We conclude that the considerable expenditure of time and resources to harvest and examine histologic material by light microscopy at a fixed interval from the date of injection provides insufficient information to justify continuing the practice. It might be more productive for future investigators to expose animals to a known injurious concentration of local anesthetic agent (such as 16% tetracaine), then to kill animals sequentially to permit studying the histologic evolution of the lesions.

References

1. Ravindran RS, Bond VK, Tasch MD, Gupta CD, Luerssen TG: Prolonged neural blockade following regional analgesia with 2-chloroprocaine. Anesth Analg 59:447–451, 1980
2. Reisner LS, Hochman BN, Plumer MH: Persistent neurologic deficit and adhesive arachnoiditis following intrathecal 2-chloroprocaine injection. Anesth Analg 59:452–454, 1980
3. van Lier EH: Histologischer Beitrag zur Rückenmarksanästhesie. Beitr zur Klin Chir 53:413, 1907
4. Bieter RN, Cunningham RW, Lenz O, McNearney JJ: Threshold anesthetic and lethal concentrations of certain spinal anesthetics in rabbits. J Pharmacol Exp Ther 57:221–244, 1936
5. Bieter RN, McNearney JJ, Cunningham RW, Lenz O: On the duration of spinal anesthesia in the rabbit. J Pharmacol Exp Ther 57:273, 1936
6. Bieter RN: Applied pharmacology of local anesthetics. Am J Surg 34:500–510, 1936
7. Cunningham RW, Bieter RN: Experiments on the potentiation of procaine spinal anesthesia in rabbits. J Pharmacol Exp Ther 66:410–422, 1939
8. Co Tui FW, Preiss AL, Borchan I, Nevin MI: Local nervous tissue changes following spinal anesthesia in experimental animals. J Pharmacol Exp Ther 81:209–217, 1944
9. Loomis TA, Spielmeyer R: Local anesthetic activity and toxicity of Di (B-Diethylaminoethyl) ester of 1-Benzyl 2,5-Dicarboxy Pyrrolidine. Yale J Biol Med 18:165–169, 1946
10. Luduena FP, Hoppe JO: 2-Alkoxy benzoate and thiolbenzoate derivatives as local anesthetics. J Pharmacol Exp Ther 117: 89–96, 1956
11. Luduena FP: Experimental spinal anesthesia. Arch Int Pharmacodyn Ther 109:143–156, 1957
12. Luduena FP: Duration of local anesthesia. Annu Rev Pharmacol 9:503–520, 1969
13. Berman ML, Murray WJ: Effect of intrathecal epinephrine on rabbit spinal cord. Anesth Analg 51:383–385, 1972
14. Adams HJ, Mastri AR, Eicholzer AW, Kilpatrick G: Morphologic effects of intrathecal etiodocaine and tetracaine on the rabbit spinal cord. Anesth Analg 53:904–908, 1974
15. Wang BC, Hillman DE, Spielholz NI, Turndorf H: Chronic neurological deficits and nesacaine-CE—an effect of the anesthetic, 2-chlorprocaine, or the antioxidant, sodium bisulfite? Anesth Analg 63:445–447, 1984
16. Craige EH: A laboratory guide to the anatomy of the rabbit, second edition. Toronto, University of Toronto Press, 1966, p 83

¶ Gissen AJ, Datta S, Lambert D: The chloroprocaine controversy II. Is Chloroprocaine neurotoxic? Regional Anesthesia 9:135–145, 1980.