EFFECTS ON PERIFERAL NERVE OF CONTINUOUS BLOCK BY SERIAL INJECTIONS OF LOCAL ANESTHETICS

by

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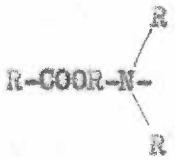
The use of local anesthetic agents in the control of pain has been accelerating since the turn of the century with the appearance and widespread use of many diversified techniques. One major factor that has held up progress in this field has been the inadequacy of the agents used. Indeed, Procaine was the first effective safe local anesthetic for infiltration and conduction anesthesia and, after the synthesis of thousands of compounds with local anesthetic properties, it remains today as the most popular and perhaps the safest of all. Procaine is still the universal standard of comparison. This situation is no better demonstrated than in the field of long-acting local anesthetics. The importance of these compounds has long been recognized in control of post-operative pain, therapy for pain syndromes, diagnostic procedures, and prolonged surgical procedures. This was first approached with the use of local anesthetics in oil with 3-6% benzyl alcohol as a solvent. This has proved unsatisfactory in that the prolonged block was caused by the degenerative effect of benzyl alcohol and the anesthetic solution (1,2). Recently a new approach has been made to the problem with the introduction of Efocaine. As will be pointed out later in this thesis, this has proved most unsatisfactory. Clinicians have therefore, in lieu of a safe long-acting anesthetic substituted serial injections of presently employed and pharmacologically and clinically approved local anesthetics. Techniques vary from intercostal block to continuous epidural and continuous spinal anesthesia. So far these have proven clinically effective with few subjective complications due to the agent employed. It is nevertheless true that local anesthetics are not evaluated by the drug houses for use in serial injections. The question arises

whether agents completely safe for single injections might be injurious if used repeatedly. This paper is an attempt to present an objective anatomical basis for an answer to this question.

CHEMICAL NATURE OF LOCAL ANESTHETICS

Most local anesthetics are tertiary aminoesters of aromatic acids. As pointed out by Goodman and Gilman (3) the basic structure can be represented as follows:



Lofgren (4) has demonstrated that local anesthetics consist of three functional parts:

1) an amino group which should be secondary or tertiary. An exception is benzocaine (ethyl p amino benzoate)

2) the intermediate (alcohol) chain

3) the aromatic radical

Modification of any of these three parts modifies the properties of the agent. The amino group seems absolutely necessary, for there are no usable local anesthetics that do not possess it. Lofgren considers this the hydrophilic part of the molecule and that it is attracted in polar association to the axon membrane. It is known that increasing the length of the intermediate chain increases the potency but also the toxicity. This is because the basicity of the compound is altered with a resulting change in the dissociation constants of both acid salt and free base of the compound. The aromatic group, as the lipophilic radical, is attracted to the lipoid part of the lipo-protein complex

on the plasma membrane. The substitution of an aliphatic for the aromatic radical produces a much inferior product.

Agents used in this study have this general structure with the exception of Xylocaine and Nupercaine. Xylocaine, rather than being an ester, is an aminoacyl amide. The same principles of structure can be applied to this compound in that it has an aromatic (lipophilic) group, an intermediate chain and an amino (hydrophilic) radical. The intermediate chain has an amide rather than an ester linkage.

Nupercaine is a quinoline derivative but also possesses the aromatic-intermediate-amino basic structure.

MODE OF ACTION

In order to understand the various theories on how local anesthetics block conduction in a nerve fiber it is necessary to review the basic physiology of conduction. As presently understood nerve depolarization occurs in the following manner (5,6,7,8).

The axon membrane is considered freely permeable to potassium ions but not as permeable to other ions. Since potassium is readily diffusible, it is mainly the difference in potassium ion concentration that determines the resting membrane potential. As the concentration of potassium on the inside of the axon membrane is many times the concentration on the outside, far more of these positive-charged ions will diffuse out than in; thus creating an excess of positivity on the outside and a negativity on the inside. When the electronegativity on the inside is such that as many potassium ions go out as come in, the resting potential is stabilized and the nerve is polarized. Leveque and others feel that acetylcholine is present at the membrane

in the lipo-protein complex. With stimulation of the axon, acetycholine is released from the complex and causes the membrane to become freely permeable to all ions. Sodium ions enter faster than potassium ions can leave and for an instant there is an electropositivity on the inside of the axon membrane. The acetylcholine that was released is rapidly hydrolyzed by the cholinesterase always present and the membrane ceases to be freely permeable to all except potassium ions. The potassium ions again migrate outward and the resting potential is reestablished. The "sodium pump" mechanism works to actively extrude the intracellular sodium and a normal sodium-potassium is reestablished.

In order to be effective a nerve block must be absolute and the narcotized zone must reduce the intensity of the nerve response in all the individual fibers of the nerve trunk below the minimal threshold strength necessary to stimulate (9). Unless this occurs conduction will go through without detrement; that is the nerve impulse in each fiber, however feeble it might become in it s passage through the narcotized zone will be restored to original intensity.

Brown (10) notes that the time of inductance (latent period) represents the time required for the local anesthetic to diffuse from the site of application to the site of action. Gasser and Erlanger (11) have pointed out that the lapse of time necessary for agents to reach each individual fiber is dependent in part on:

1) the distribution of fibers within the nerve trunk
2) the thickness of the connective tissue sheath around the trunk,

Thus the latent period is a function of diffusion time. The time

necessary for 0.01 M of Procaine to block conduction is proportional to the square of the diameter of the whole nerve. This helps to explain the reason that the small, unmyelinated, class C fibers of pain, temperature, and the autonomic system are blocked first and by a lower concentration. The large, heavily myelinated, class A fibers are blocked last and a higher concentration of the local anesthetic is required.

In connection with myelination Lussier (12) and others have demonstrated that myelinated nerves are so well insulated that they are only excitable at the nodes of Ranvier. Tasaki (13) and Kato (14) have shown that the site of action of local anesthetics on myelinated fibers is at the node of Ranvier.

To be effective clinically local anesthetics must come in contact with the nerve fiber as free base because only free base is lipoid soluble (15). However free base has a low water solubility and hence local anesthetics are prepared as salts of weak bases and strong acids and as such dissociate in aqueous solution into ions.

$$R{\equiv}NHCl \rightleftharpoons R{\equiv}NH^{+} + Cl^{-}$$

If base is added (as in the tissues) the following occurs:

$$R{\equiv}NHCl + NaHCO_3 \rightarrow R{\equiv}N \text{ (free base)} + NaCl + H_2CO_3$$

The extent of liberation of free base depends on the amount of alkalinity. The general feeling clinically seems to be that the tissues are effective buffers and no attempt is usually made to alkalinize the anesthetic solution. Alkalinization decreases the stability of the solution and the free base is more liable to precipitate out.

The mode of action of local anesthetics on nerve tissue is

not yet fully understood. This is due, in large part, to the incomplete understanding of the mechanism of nerve conduction.

One of the theories postulated is that the effect is directly on the metabolism of the axon (16). Sherif (17) showed that Cocaine hydrochloride inhibits oxygen usage in concentrations of 0.2% to 1% and inhibition increases as the concentration increases. He claimed that Procaine had a similar action. Gray (18) mentions that Tetracaine is very potent in this respect. It has been suggested that the blockage occurs at the cytochrome C-cytochrome oxidase level, but this theory has recently come under question and Larrabee proved that nerve blocks could be produced by concentrations of Cocaine that do not effect uptake of oxygen (19, 20).

Bishop (21), in 1932, found that Cocaine blocked donduction without any evidence of depolarization; in fact he found an increase in positive potential at the site of application. This has been substantiated by Bennett and Chinberg (22) in 1946 and by Shanes (8) in 1949. Straub (23) points out that both sodium and potassium permeability are decreased by Procaine but sodium to a greater extent than potassium.

Thus local anesthetics stabilize the axon in respect to permeability. They maintain the demarkation potential by raising the threshold to stimulation and preventing depolarization. The mechanism by which this occurs is not yet fully understood. The Meyer-Overton rule has long been debated in explanation of the action of local anesthetics. The distribution coefficient is felt by most to have a certain influence but it cannot alone be the decisive factor (4,16,20,24,25). Lofgren has shown that local

anesthetics with similar activities have different distribution coefficients. Skow (15,26) demonstrated that there seems to be a parallelism between the coefficient and the toxic potencies of local anesthetics but not necessarily is there a parallelism between the coefficient and blocking potency. It is generally accepted that lipoid solubility of the free base is necessary for the anesthetic to be effective. Lofgren feels that the amino or hydrophilic radical is attracted to a polar group in the membrane film. The aromatic (lipophilic) group then swings around and makes contact with the lipoid part of the lipo-protein complex. In this way penetration occurs and the membrane is stabilized against depolarization.

The increasing evidence that acetylcholine plays a major role in nerve excitation and conduction and the marked chemical similarity between local anesthetics and acetylcholine (27) have raised the possibility that competition with acetylcholine for a chemical receptor may be responsible for nerve block by local anesthetics (20,25). This action, in competing for receptors, would be similar to Curare. Nordquist (28) has demonstrated that, after block with Procaine 0.05%, a clear unblocking action was noted when a mixed solution of Procaine 0.05% and acetylcholine 0.5% was substituted. However, Lorente de No (29) has shown that acetylcholine even in massive concentrations and several hours duration could exert no excitatory action upon a fiber of the sciatic nerve of frogs. In fact, a slight increase in potential was produced. In line with present theories of nerve conduction, Gray et al (18) postulate that in vivo acetylcholine actually may be liberated in the axon membrane.

It is obvious that an understanding of the mechanism of action of local anesthetics is still in the theorotical stage. It is quite probable that an understanding of the excitation-depolarization mechanism will predate complete knowledge of the mode of action of these drugs.

NEUROTOXICITY

It is well known that local anesthetics, in proper concentration, can cause destructive lesions in nervous tissue. Numerous reports of neurologic damage following local anesthesia substantiate this. Thorsen (30), in reviewing the case histories of spinal cord damage due to Procaine, Pontocaine, Nupercaine and Decacaine, reached the conclusion shared by most, that the injuries are due to a neurotoxic and myelolytic effect of the agent involved. In support of this it is known that damage occures most commonly and are of greatest severity in the region of the cauda equina there the concentration of the drug is greatest (31). Direct trauma is an unlikely cause in view of the tens of thousands of diagnostic spinal taps that have been undertaken without incident. Rapid onset of complications after injection seems to eliminate infection as an etiologic cause and points rather to toxicity. There is considerable experimental evidence. Lundy et al (32) produced degenerative changes with paralysis in dogs with subdural injection of five cc's of 25% solution of Procaine. Davis (33) produced similar changes in dogs and cats with Nupercaine. Cotui (34) et al produced severe degenerative changes with lethal doses of Procaine, Novocaine and Nupercaine. Thus since it seems quite probable that the damage is due to the neurotoxicity of the drug, an important conclusion has been reached by Nicholson(35);

that the concentration employed in most spinal anesthetic drugs have a toxicity little short of that which would cause paralysis in a higher percentage of patients. Courville pointed out that all local anesthetics are protoplasmic poisons in proper concentrations. The chief difficulty in clinical evaluation is that the minor and transitory changes go unrecorded while the delayed ones are often unknown to the anesthetist.

Almost all interest in this problem of nerve damage has been centered on the spinal cord and spinal anesthesia. It is unfortunate that similar work has not been done on peripheral nerves. However the same principles of local anesthetic toxicity should apply to other nervous tissue. Whether repeated injections of a local anesthetic, that in single doses would prove non-toxic, would cause degenerative changes similar to those already noted by other workers with spinal anesthesia is obviously a very pertinent and pressing question to those who use serial injections.

TECHNIQUE

The experiment consisted of the injection of commonly used, longer action local anesthetics alongside the sciatic nerve of the guinea pig, serially repeated in order to mainhain the block for twenty-four or thirty-six hours depending on the anesthetic and tissue reaction. A total of ninety animals were used. A total of six animals were run in each series. The blocking technique was that of Shackell (36).. The hair of the thighs and lower back was clipped up to the iliac crests and an antiseptic solution was applied. A sterile tuberculin syringe and a#27 by three-eighth needle was used. These were resterilized between each injection. The animal was

immobilized in a specially constructed cage in a prone position and, grasping the leg with the thumbnail of the free hand on the greater trochanter, the needle was inserted inside the nail vertical to the skin surface and was advanced along the inner surface of the trochanter until the neck of the femur was encountered. Here the needle was in vertical contact with the sciatic nerve. The syringe was then moved forward so that the needle sloped posteriorly about thirty degrees and thus was in the same plane as the nerve. 0.4 cc's of the solution was injected alongside the nerve. A typical sciatic nerve paralysis occured with extention of the leg on the thigh and this was associated with an area of analgesia on the posterior-lateral surface of the thigh about two by three centimeters in extent. In most cases a control of normal saline or 1/100,000 epinepherine was injected alongside the contra-lateral nerve. Injections were spaced to maintain continuous block. Following the period of block two animals of the series of six were sacrificed and the nerves were fixed in 10% formalin. A period of twelve to fourteen days was allowed to pass and two more of the animals were sacrificed and nerves removed and fixed. This would allow time for degeneration to take place. Then, at varying periods of time, depending on the microscopic results of the fourteen day sampling, the last two animals were sacrificed and nerves fixed. The purpose of this was to note any regeneration. This was the general pattern of the animal experimentation.

Since a longer acting anesthetic solution was of greater value in this experiment, 1/100,000 epinepherine was used in most solutions. This is in accord with the probable clinical utilization

of these compounds. Twelve animals were run with controls of 1/100,000 epinepherine.

The method of preparing the nerves after fixation was as follows. The fixed tissue was placed in a small labeled gauze bag, washed overnight, and dehydrated in the following manner:

70% ethanol---½ hour

70% ethanol---1 hour

80% ethanol---1 hour

95% ethanol---1 hour

95% ethanol---1½ hours

95% ethanol---1 hour

absolute butynol---1 hour

absolute butynol---all night

methyl benzoate---until cleared

50% methyl benzoate + 50% xylol---½ hour

xylol---1 hour

xylol---2 hours

paraffin (soft)---1 hour

paraffin (hard)---1 hour

The nerves were then removed from the gauze bags and embedded and blocked in fresh paraffin. Sections were then cut with the microtome set at 10 microns. They were applied to the slide with a thin layer of egg albumin and placed in a 37 degree oven overnight. The impregnation and toning of the tissue was done by following the Bodian procedure with a counterstain of aniline blue as described by Lyons (37). The paraffin was removed from the slides with toluene and they were then passed through

100, 95, and 70% alcohol to water. The slides were placed in a solution of Winthrop's protargol made by dusting one gram of protargol over each 100 cc's of water and heating to 37 degrees. In the A series copper was added (7 grams per 50 cc's) but, following Winthrop's recommendation, it was excluded from the B series.

After twenty hours the precipitate was washed from the slides and they were rinsed in running water for one minute and then placed in the following developer for five to ten minutes:

Hydroquinone---2 grams

Sodium sulfite---10 grams

Distilled water---100 cc's

The slides were rinsed in three changes of distilled water and placed in a 1% solution of gold chloride which had been acidified with a few drops of glacial acetic acid for ten minutes. They were rinsed again and placed in 2% oxalic acid for ten minutes; were rinsed again and put in a 5% aqueous solution of hypo; rinsed again and counterstained with the following solution for ten minutes:

Aniline blue---0.1 grams

Oxalic acid---2.0 grams

Phosphomolybdic acid---15.0 grams

Water---300 cc's

The slides were then rinsed, differentiated in 95% alcohol, dehydrated in absolute butyl alcohol, cleared in toluene and mounted in neutral canadian balsam.

The sections were studied for evidence of degenerative change. These changes when present, were classified in three groups:

Grade 1.-Vacuolization of the myelin but axon still present.

Grade 2.-Group 1 changes plus dissolution of the axon.

Grade 3.-Group 1 and group 2 changes plus a disruption of the integrity of the neuralemmal sheath.

The percentage of nerve fibers damaged was determined by a count.

An effort was made to evaluate grossly the amount of tissue swelling in the animal immediately after the final injection. These were classified as follows:

None---0

Slight---+1

Moderate---+2

Marked---+3

Extreme---+4

"Slight", representing the amount of swelling seen with the saline controls is only very minimal swelling. "Marked" was swelling of such great intensity that the operator was not able to note the landmarks with assurance. "Moderate" represented any tissue swelling between 1+ and 3+.

IRRITANCY STUDIES

The trypan blue molecule is of such a size that normally it remains inside the capillary. However, in the presence of hyperemia and/or capillary injury, it will diffuse out and stain the surrounding tissue. Tainter, Throndson and Lehman (38), in 1937, utilized this principle in the investigation of irritant properties of sodium bisulfite solutions. The test was employed by Rocha e

Silva (39) and Dragstedt in studies on the effect of histamine and substances causing the liberation of histamine. Last and Loew (40) used the test to evaluate the effect of antihistamines in preventing positive results after injection of histamine. Hoppe, Alexander, and Miller (41) then utilized the test for investigation of local anesthetics. They studied Tetracaine, Dibucaine, and Procaine.

As a preliminary introduction to the irritant properties of local anesthetics, it was decided to expand the work of Hoppe et al. (41) to other local anesthetics before progressing to the guinea pig experiments. Following the technique of these workers, two to three kilogram albino rabbits were used. They were fastened in a supine position and the hair was clipped from the anterior abdominal wall. The abdominal wall was then marked off into squares of approximately sixteen square centimeters. Three tenths of a cubic centimeter of each solution was injected intracutaneously into a separate square. A dose of 1.0 cc/kilogram of 1% solution of Trypan Blue was injected intravenously fifteen minutes after the last intracutaneous injection. The sites on the abdominal wall were examined one-half, one, and three hours after injection of Trypan Blue and were graded as follows:

No color....0

Faint, but discernible blue color throughout....2

Distinct blue color throughout....4

Deep color throughout....8

Ischemic contral area surrounded by deep blue halo....16

Hoppe considers any rating over 3 as too irritating for infiltration anesthesia.

He considers the correct interpretation of these grades to be thus:

0....no irritation

1-3....mild irritation

4-7....moderate irritation

8....marked irritation

The results of the Trypan Blue tests are presented in table 1.

Table 1

NORMAL SALINE--0	2% INTRACAINE--6
95% ETHANOL--16	0.2% SYMPOCAINE--0
1.5% HEXYLCAINE--8	0.5% SYMPOCAINE--2
2% HEXYLCAINE--8	1.5% PRIMOCAINE--2
1.5% XYLOCAINE--2	0.18% PONTOCAINE--0
1/500 NUPERCAINE--2	1/100,000 EPINEPHERINE--0
2 % XYLOCAINE--4	1% NOVOCAINE--0

NORMAL SALINE CONTROLS

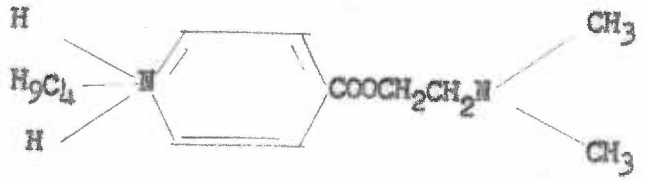
Normal saline was run as a control solution in animals A8 through A41, B1 through B6 and B 16 through B18. In this group injections were given along the right nerve at the same frequency and for the same duration as the injections of local anesthetic on the other side. Tissue swelling was mild (+) in this series of 43 animals run with saline controls. No nerves showed any signs of degeneration on microscopic examination. A 38 is shown in figure 1.

EPINEPHRINE

As pointed out by Beutner in 1948 (42) nearly all local anesthetics have a marked vaso-dilating effect sufficient to lower blood pressure in most cases. This property of vaso-dilation allows rapid absorption of the drug with increased toxicity and decreased duration of action. It is common practice today to decrease the toxicity and prolong the action of the drugs by adding epinephrine to them. Since most of the local anesthetics used in this study were combined with 1/100,000 epinephrine, a control series was run with this drug. A solution of 1/100,000 epinephrine was injected adjacent to the right nerve of animals B7 through B12 and B43 through B48. The interval between injections was the same as the interval between injections of the anesthetic used in the same series on the left nerve. Tissue swelling was mild. All nerves removed at intervals ranging from 0 to 28 days were normal on microscopic examination (see table 2). A section of nerve from B12 is shown in figure 2.

TETRACAINE

Tetracaine (Amethocaine, Pontocaine, Pantocaine) is the p-butyl amino benzoic acid ester of dimethyl amino ethanol. Its chemical structure is as follows:



Pontocaine was first synthesized by Eisleb in 1933. It has an absolute toxicity ten times that of Procaine but is used in one-tenth the concentration; so in reality its relative toxicity is

about the same. The drug is detoxified in the liver by hydrolysis. Pontocaine is one of the longer acting local anesthetics and, like the other drugs used in this project, it is compatible with epinephrine. It is marketed as the hydrochloride salt.

Despite the numerous reports of toxic reactions to Pontocaine in the literature, the belief is growing that this is a relatively safe drug for infiltration and conduction anesthesia. Bonica (43) claims that none of the fatal toxic reactions due to Pontocaine reported in the literature have occurred during this type of block. These severe reactions generally occur in surface anesthesia because of the stronger (2%) solution used and the marked vaso-dilation effect of the drug without epinephrine with the resulting increased absorption. In 3089 cases, using Pontocaine in 0.1% to 0.2% solutions, a reaction was noted in only one case; this was moderate and was due to overdosage. Pontocaine, used in one-tenth the concentration of Procaine, gives anesthesia three times as long.

The marked vaso-dilating action of Pontocaine is the basis of the contention that the drug should be used with epinephrine. Somers and Edge (44) point out that 1/100,000 epinephrine increases the potency of anesthesia two and one-half times and lowers the toxicity two and one-half times with a resulting five-fold increase in the safety margin.

The vaso-dilating effect of the drug might lead one to expect false positive results in the Trypan Blue tests (see table one). However, Pontocaine 0.18% was found to have a rating of "0" irritation.

Twelve guinea pigs (A8-A19) were run using a solution of 0.18% Pontocaine with 1/100,000 epinephrine. Injections were made

every four hours for a duration of 36 hours. Minimal tissue swelling (+) was present after the thirty-six hours of injections and no functional loss was noted. In this series four animals (A9,A11,A12,A19) were sacrificed immediately following the injections and microscopic examination revealed normal nerve in every case.

Three animals (A8, A15, A18) were sacrificed at twelve days and normal nerve was noted in all three. One animal (A17) was sacrificed at ninety days and found to have a normal nerve.

Normal saline was injected at the same frequency along the opposite nerve in all animals of this series. Microscopic study showed normal nerve in every case except in those nerves that had undergone autolysis.

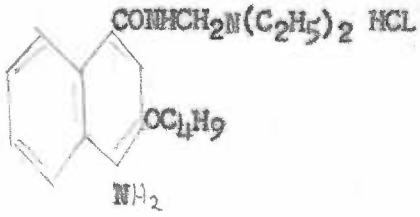
In another series (A20-A25) Pontocaine 0.3% with 1/100,000 epinephrine was run. The duration of block was thirty-six hours with injections spaced every five hours. There was moderate (++) tissue swelling and no functional disturbance was noted after the block. One animal (A23) died and the nerves underwent autolysis. A22 and A25 were sacrificed immediately and the nerve was normal in each case. A20 and A21 were sacrificed at twelve days with findings of normal nerve. A24 was sacrificed at eight days and the nerve was normal. Normal saline controls, injected every five hours on the other side, were normal.

Pontocaine 0.5% was used in B13, B14 and B15. Injections were spaced at six hour intervals but were terminated at twenty-four hours because marked (+++) tissue swelling obliterated the landmarks. No functional disturbance was noted after the injections. One animal (B15) was sacrificed immediately and showed no pathologic

change. B13 was sacrificed at fourteen days and showed 50% grade 1 changes (see figure 3). B14 was sacrificed at twenty-one days and showed normal nerve.

NUPERCAINE

Nupercaine (Dibucaine, Percaine) is a quinoline derivative of high toxicity and high anesthetic potency (3). It is the b-diethylaminoethyl amide of butyloxychinchonic acid and thus differs considerably in structure from other local anesthetics. The structural formula of the drug is as follows:



This drug was synthesized by Meischer in 1925. Its potency is described as 15 times, and its toxicity 10-15 times that of Procaine (45). It is detoxified by hydrolysis in the liver and is quite stable. Hand and Size (46) felt that Nupercaine is a good relatively safe druge when used in the proper dosage. They described the absolute toxicity as 14 times that of Procaine but the relative toxicity as only 0.71 times that of Procaine (in measurements of the LD50 in cats). Rosenbaum et al (47) described 5 cases of permanent neurologic damage due to adhesive arachnoiditis following spinal anesthesia with Nupercaine. However, Roman and Adriani (48) state that the belief that Nupercaine will cause degenerative changes in the cord more frequently than other currently employed local anesthetics is not based on fact. They had a series of 5453 cases without neurologic complications. On review of the literature from 1938

to 1946 they found that clinical damage to the cord was very rare and there was none reported over this period due to Nupercaine. However, the findings of Rosenbaum are consistent with recent findings of neurologic complications following spinal anesthesia. Interestingly, Ansbro et al (49) described three cases in which 1/200 Nupercaine plus 1% Procaine were used for continuous spinal anesthesia with injections every four hours for days. In one case these injections were carried on for 14 days (85 injections), being discontinued after onset of severe back pain and stiff neck but with no residual neurological complications. In the other two cases the block was carried on for 11 and 7 days with no complications.

The Trypan Blue experiments showed an irritancy index of 2 (mild) for 1/500 Nupercaine. This differs somewhat from the observation of Adriani that the drug may cause a slough when injected subcutaneously.

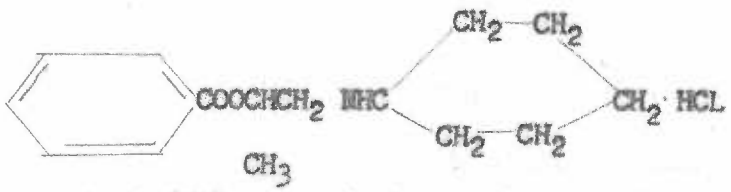
A series of six animals (A26-A31) received 1/560 Nupercaine. Injections were spaced at 7 hours for a duration of 36 hours. Saline control injections were done on the other side. Tissue swelling was mild (+) and no functional disturbance was noted. A27 and A30 were sacrificed immediately after injection and the nerves were normal microscopically. A28 and A31 were sacrificed at 12 days, A 26 and A29 at 50 days and the nerves were normal in all instances.

Two series of six animals each received injections of 1/250 Nupercaine. Injections were given at 8 hour intervals over a period of 36 hours. Moderate tissue swelling (++) was noted and there was no functional disturbance. In the first series (A32-A37), A 32 died and the nerves underwent autolysis. A37 and A34 were

sacrificed immediately. Normal nerve was found in A37, but a 15% grade 1 change in A34. A33 and A36 were sacrificed at 14 days and normal nerve was noted in A33; however, A36 showed 10-15% grade 2 change (see figure 4). A35 was sacrificed at 60 days and showed normal nerve. Control injections of saline were done on the other side. In the second series (B37-B42), B42 was sacrificed immediately and normal nerve was noted. B37 was sacrificed at 14 days and demonstrated 10% grade 1 change. B38 also was sacrifieed at 14 days, showed 5% grade 1 change. B39 showed 5% grade 1 and 5% grade 2 change after 14 days. B40 and B41, sacrificed at 120 days showed normal nerve. Primqcaine was injected in the opposite thigh of these animals and these experiments are discussed later in the paper.

HEXYLCAINE

Hexylcaine (Cyclaine) was synthesized by Cope and Hancock in 1944. It is 1-cyclohexylamino-2-propylbenzoate HCL. Its structural formula is as follows:



Beyer et al (50), in animal experiemtns, found the LD50 to be one-third of that of Procaine and 3 to 4 times that of Tetracaine. However, as he pointed out, the effective dose was one-fourth that of Procaine, tus the relative toxicity would be lower. Orkin and Ravenstein (51) describe the absolute toxicity of Cyclaine as being about three times that of Procaine and thus agrees with Beyer. Cyclaine has a short latent period and a duration three to five

times that of Procaine. Orkin claimed that it produces no irritation and reported 1113 procedures using the drug with only five toxic reactions. Jacques and Hudon (52) reported 1730 cases with no major difficulty. Blundell et al (53) feel that Cyclaine is the outstanding drug for epidural anesthesia. In comparison with Xylocaine, they claim that it produces anesthesia of longer duration, is less toxic and is of equal clinical value. The 2% solution is recommended by them, because the 1% solution is described as not strong enough to bring about muscle relaxation.

The same group (54) reported that in a series of 2000 epidural blocks (1000 with 2% Xylocaine and 1000 with 2% Cyclaine) they experienced 11 toxic reactions to Xylocaine and only one such reaction to Cyclaine. Ruben and Anderson (55) had a total of 500 cases of spinal anesthesia with Cyclaine and found it superior to Procaine. In a later article (56) these same men reported burning on injection of Cyclaine and residual soreness and discontinued the use of this drug except in pre-medicated patients. However, they then had a series of 1300 spinal anesthetics with no neurologic complications. Gordon (57) also described this tenderness at the site of injection lasting, he point out, sometimes for several days.

Thus it seems Cyclaine has been accepted with enthusiasm in the literature. Single shot anesthesia has proved very satisfactory in many large series and its toxicity is reported as low. However, the one apparent drawback of tissue irritation raises doubts of the value of the drug when administered in continuous fashion.

The results of the Trypan Blue (table 1) supported the clinical impression that Cyclaine was irritating. Both 1.5% and

2% Cyclaine showed an irritancy rating of 8 ("marked"). This is well above the acceptible figure of 3 (Hoppe).

Two series using 2% Cyclaine with 1/100,000 epinephrine were run. The first series consisted of four animals (A38-A41). Tissue swelling was marked (+++) after the block but no functional disturbance was noted. Injections were limited to twenty-four hours because of the swelling, and the injection frequency was four hours. A40 was sacrificed immediately after the injections were terminated and 40% grade 1 change was noted (see figure 5). A38 was sacrificed at twelve days and 30% grade 2 change was present on microscopic section (see figure 6). A39 was also sacrificed at twelve days and showed 40% grade 2 change. A41 caught its foot in the cage meshing and died and the nerves showed autolytic changes.

Contralateral control injections of saline resulted in normal undamaged nerves in all instances.

The second series consisted of six animals (B13-B18). B15 was sacrificed immediately and showed 15% grade 1 change. In B18, also sacrificed immediately, the nerve was normal. B13 was sacrificed at fourteen days and showed normal nerve, while B16, also sacrificed at 14 days, showed 30% grade 2 change. The nerves of B14 and B17 were damaged in preparation for sectioning and were discarded. In each of the nerves where degenerative changes were noted after 12-14 days (A38,A39,B16) the nerve was found to be grossly thickened to approximately twice its normal diameter. This was also true in the Efocaine series.

Injections of 1% Cyclaine with 1/100,000 epinephrine in six animals (B7-B12) over a 24 hour period with injections every

3 hours were done. The tissue swelling was marked (+++) but, as in the 2% solution, no functional disturbance was noted after recovery from the block. B8 died and the nerves underwent autolysis. B10 was sacrificed immediately and showed 5% grade 1 change. B9 (see figure 7) was sacrificed at 14 days and showed 10% grade 1 and 5% grade 2 change. B11 was sacrificed at 14 days and showed 5% grade 2 change. B12, also sacrificed at 14 days, demonstrated 10% grade 1 and 20% grade 2 changes. B7, sacrificed at 28 days, had a normal nerve.

Cyclaine 2% with 1/100,000 epinephrine was injected in animals B25 through B30 at 4 hour intervals for only 12 hours. Mild (+) tissue swelling was noted and there was no functional disturbance. B25 was sacrificed immediately and showed normal nerve. B29 was sacrificed at 14 days and 5% grade 1 change was found. B28 and B30, sacrificed also at 14 days, showed 5% grade 2 change. B26 and B27, sacrificed at 40 days, had normal nerves.

A single injection of 2% Cyclaine was made along the nerve on the opposite side in animals B25 through B30. No tissue swelling was noted and all nerves were microscopically normal.

EFOCAINE

Efocaine was a new idea in long-acting local anesthesia. It is a saturated solution of Procaine 1%, Procaine hydrochloride 0.25% and butyl-p-amino benzoate 5% in a solvent composed of polyethylene glycol 300 2%, propylene glycol 78% and water. It contains no vaso-constricting agent. Procaine and butyl-p-aminobenzoate are crystalline, slowly absorbed substances which are normally insoluble in water but are soluble in the solvents used in Efocaine. Propylene

glycol and polyethylene glycol are completely miscible with tissue fluids. Contact with an aqueous medium (tissue fluids) causes the Procaine base and the butyl-p-aminobenzoate to precipitate out. The procaine crystalline precipitate is slowly absorbed; theoretically giving prolonged anesthesia (58). The duration of anesthesia is described as 9 to 22 days.

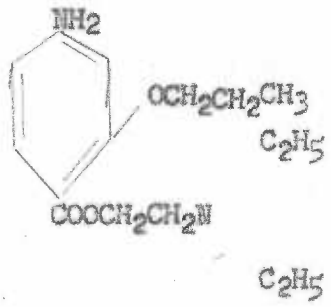
This drug was greeted with enthusiasm when it first appeared in 1952 (59,60). Ansbro et al (60) claimed that Efocaine was a local anesthetic agent without any neuro-degenerative effects or local tissue toxicity. However, soon reports of degeneration began to appear in the literature. In 1953 Nowill et al (61,62) reported spinal cord destruction with paraplegia secondary to intercostal block. They injected the sciatic nerve of rabbits with Efocaine and found degeneration in 43 out of 73. The solvents alone caused degeneration in 44 out of 73; propylenglycol alone caused degeneration in 12 out of 23 and Procaine hydrochloride caused no degeneration in 26 animals. They conclude that they could not differentiate the effect of Efocaine from the effect of the solvent experimentally and clinically. The anesthetic action of Efocaine, they felt, was more like that of alcohol than Procaine. Brittingham, Berlin, and Wolff (63) reported nervous damage following paravertebral block in 1954. Mannheimer et al (64), Moore (65), Maycut (66), and Rothman (67) reached similar conclusions. Deaton et al (68) reported the destruction of nerves in intercostal nerve block in dogs. Thus this drug has been proved many times to cause degeneration. The present study reaffirms these results.

Six guinea pigs were run with Efocaine. A single injection

was made and one animal (B4) was sacrificed at 36 hours (see figure 8). There is extensive disorganization of the nerve trunk with 50% grade 1 and 30% grade 2 changes. Four animals (B1,B2,B5,B6) were sacrificed at 14 days and all showed almost complete grade 2 change and some grade 3 change. A section of the nerve from B6 is shown in figure 9. One animal (B3) was sacrificed at 141 days and complete regeneration of the nerve was found (see figure 10). Complete loss of function was evident in the injected leg of all animals. A beginning return of function was noted in B3 after 45 days. The nerves, upon removal, were enlarged to about twice the normal diameter and bound down by adhesions. In all animals excpet B3 and B4, the foot on the injected side broke down and ulcerated.

RAVOCAINE

Ravocaine (Blockaine) is a relatively short-acting local anesthetic which has recently been presented for clinical investigation. It is diethylaminoethyl 2 propoxy-amino benzoate; thus it is propoxy-Procaine. Blockaine has the following structural formula:



Ravocaine is described by Luduena (69) as eight to nine times more active than Procaine but only twice as irritating. Its relative toxicity is about the same as that of Procaine.

Six guinea pigs (B19-B24) were run with o.5% Blockaine with 1/100,000 epinephrine. Injections were spaced every three hours

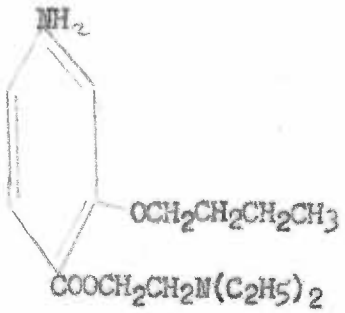
for twenty-four hours. Tissue swelling was graded as moderate (++) and there was no functional disturbance. B20 was sacrificed immediately; B19, B21 and B22 were sacrificed at fourteen days, and B23 and B24 at forty days. All sections in this series showed normal nerve.

Sympocaine 0.5% was injected in the opposite extremity in this series.

SYMPOCAINE

Sympocaine is a new local anesthetic that has only recently come under clinical investigation. It was developed in response to a desire for a short acting local anesthetic with a higher potency than Procaine (which is a weak local anesthetic) and a low toxicity. Luduena (69) feels that Sympocaine answers these requirements.

Sympocaine is 1-diethylaminoethyl-4-amino-2-butoxy benzoate HCL. The structural formula is as follows:



Thus it is 2-butoxy-procaine. Sympocaine is described by Ludena (69) after animal experiments, as being 20 times as potent as Procaine. Sadove et al (70) describes the duration as midway between thos of Procaine and Tetracaine. Luduena feels that the relative toxicity of the drug is lower than that of Procaine.

Trypan Blue experiments showed an irritation index of 0 for 0.2% Sympocaine and 2 for 0.5% solution.

0.5% Sympocaine with 1/100,000 epinephrine was used in a

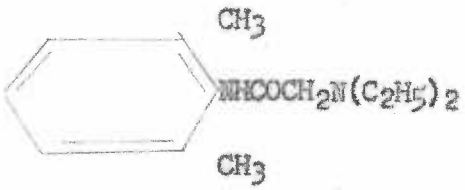
series of six animals (B19-B24). Blockaine was injected in the other extremity and the results are reported elsewhere in this paper.

No functional disturbance was noted after the block, and tissue swelling was moderate (++).

Injections were spaced at 3 hour intervals for 24 hours. B20 was sacrificed immediately and showed normal nerve. B19, B21 and B22 were sacrificed at 14 days after cessation of injections and were normal except for a 5% grade 1 change in B22. B23 and B24 were sacrificed at 40 days, and the nerves were normal.

XYLOCAINE

Xylocaine (Lidocaine) is unique in that it is an amino aceyl amide rather than an ester. It was synthesized in 1943, the preliminary data was published in 1946, and the druge was introduced by Lofgren (4) in 1948 after a comprehensive study of many compounds with similar structure. It has the following structural formula:



Lofgren describes Xylocaine as the most stable of all local anesthetics; it can be boiled for eight hours in thiety percent hydrochloric acid without change. The ester group of local anesthetics, in contrast, can barely endure the usual heat sterilization. Duration of block is three times that of Procaine. Ciocatto (71) presented the first Italian studies of the drug in 1950 in which he described rapid, complete and long lasting anesthesia without signs of toxicity or local irritation in 301 cases. Wiedling (72) summarized the properties of Xylocaine in 1952. Cox and other s

(73) described the onset of anesthesia as twice as fast as with Procaine and the relative toxicity as less than that of Procaine. He had noted only four reactions in 300 cases, and claimed that the drug was non-irritating in concentrations up to 8%. Powell and Nowill (74) point out that a higher percentage of successful blocks are possible with Xylocaine because of its greater diffusibility. Stringer (75) found the drug to be the superior one for epidural anesthesia. Southworth and Dobbs (76) reviewed 68,281 cases of block with Xylocaine and found isgnificant reactions in only .0005%. Thus Xylocaine is a drug of great stability, low toxicity and rapid action with a high percentage of successful blocks of relatively long duration.

In the Trypan Blue experiments six animals were run with 2% Xylocaine and 1/100,000 epinephrine. Injections were carried out for 24 hours and were spaced at 3 hour intervals. The tissue swelling was mild (+) and no functional disturbance was noted after the block. B36 was sacrificed immediately after the injections; B31, B32, and B33 were sacrifieced at 14 days, B34 and B35 at 120 days. In all cases normal nerve was found on microscopic examination.

INTRACAINE

Intracaine is a relatively short-acting local anesthetic. It is b-dietyl-aminoethyl-p-ethoxybenzoate and thus belongs to the general group of ester local anesthetics. Ravenstein and Cullen (77), in 1939, described the drug as slightly more toxic than Procaine but effective in lower concentrations and of longer duration. The latent period of Intracaine is shorter than that of Procaine. According to Adriani (45), the duration, toxicity and potency are about 1½ times

that of Procaine. However, McIntyre and Sievers (78) did sciatic nerve blocks with Intracaine and found them to last 4 times as long as Procaine. Abajian (79) reported its use in epidural anesthesia.

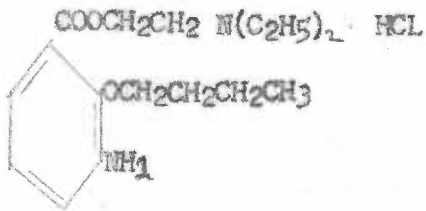
Its structural formula is as follows:

In the Trypan Blue studies Intracaine showed an irritation index of 6.

In the guinea pig experiments a series of six animals (B31-B36) was rundwith Intracaine. Injections were spaced at 2½ hours over a period of 24 hours. The amount of tissue swelling was marked (+++) but there was no functional loss. B36 was sacrificed immediately after the block and normal nerve was noted on microscopic section. B31, B32, and B33 were sacrificed at 14 days. B31 and B32 revealed 5% grade 2 changes (see figure 11) while B33 was normal. B34 and B35, sacrificed at 120 days, revealed normal nerve.

PRIMACAINE

Primacaine is a new local anesthetic agent (80), synthesized by Epstein and Myer, and only recently submitted for clinical investigation. It is 2-dietyl-aminoethyl-2-butoxy-3-amino benzoate HCL. Primacaine has the following structural formula:



It is similar to Procaine except that it has the amino group at the meta position rather than the para position and a

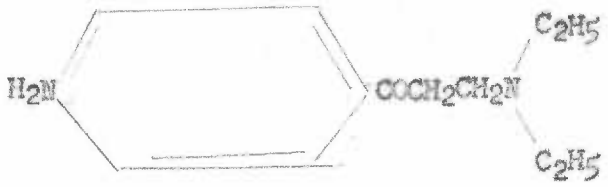
butoxy group is added at 2. It is claimed that Primacaine's toxicity is in the Procaine range and its anangesic potency is greater than that of Procaine.

The Trypan Blue test showed an irritation index of 2 (mild) for 1.5% Primocaine.

The drug firm supplied an experimental solution of Primacaine with 5% dextran on request and this was used in the guinea pig experiments. Injections were spaced at 3 hour intervals for 24 hours. Six animals (B37-B42) were employed. As seen in table 1, the tissue swelling after injection was extreme (++++) but no functional disturbance was noted. B42 was sacrificed immediately add a normal nerve was found. B37, B38, and B39 were sacrificed at 14 days and demonstrated normal nerves. B40 and B41 were sacrificed at 120 days and the nerves were normal. Nupercaine 1/250 was injected on the other side.

2-CHLOROPROCAINE

2-Chloroprocaine (Nesacaine) is the hydrochloride salt of b-diethylaminoethyl 2-chloro-4-aminobenzoate. The structural formula is:



The druge is described by Foldes (81,82) as being about twice as potent as Procaine but less toxic. This low toxicity is apparently due to the fact that the drug is hydrolized four times as fast as Procaine by the plasma Procaine esterase. Foldes reports no toxicity in 461 blocks. Lund (83) had no toxic reactions in 35

cases of chloroprocaine block in epidural anesthesia. He notes that the drug possesses most of the essentials of an ideal local anesthetic except prolonged duration: high potency, short latent period, high degree of diffusibility, and low absolute and relative toxicity.

Six animals were injected (B43-B48) with Nesacaine 3% with 1/100000 epinephrine. Duration of block was 24 hours and the injections were spaced at 3 hour intervals. Epinephrine 1/100,000 was injected at the same intervals adjacent to the nerve on the opposite side; the results of this procedure have already been discussed. A moderate (++) tissue reaction was noted after the Nesacaine block but there was no functional disturbance. 1 B43 was sacrificed immediately and normal nerve was noted on microscopic examination. 1 B45 and B46, sacrificed at 14 days, showed 10% grade 1 change and B45 also showed 5% grade 2 change. 1B44, sacrificed at 14 days, demonstrated 30% grade 2 change (see figure 12). B47, sacrificed at 14 days, showed 5% grade 1 change. B47 was also sacrificed at 14 days but the microscopic results are unknown because the sections were misplaced.

DISCUSSION

A note should be made about the animals that died prematurely. A1 through A7 were injected with 1% procaine with 1/100,000 epinephrine. The short duration of action of the drug necessitated injections ever 1½ hours. This was obviously severely traumatic to the animal and after several hours landmarks were extremely difficult to find. For these reasons it was felt impractical to use such short-acting anesthetics in this experiment. The two animals that were sacrificed immediately after injections

showed normal nerve. All the other animals in this series died two to four days following injections. These deaths were attributed to the extremely high temperatures (over 100°F) of the animal room at the time, plus the trauma of the injections. The deaths in the Pontocaine series were attributed to the same causes. It is significant in this regard that many of the stock animals died during this heat wave and that the problem was completely solved when the animals were moved to a cooler atmosphere for the first few days following injections. These deaths in no way represented reactions to local anesthetics since death occurred several days after injection in every case. It was noted that the animals exposed to this heat wave were not inclined to eat or drink and the true cause of death was probably starvation. No generalized reaction to any of the local anesthetics used was noted in any of the 87 animals used in these experiments.

The property of neurotoxicity was quite evidently demonstrated. Whether or not an anesthetic solution possesses this property is something that should concern the practitioner as much as the systemic toxicity measurements of the drug. One important fact brought out in this study is that the animal can experience quite severe nerve damage (as in the Cycaline series) without noticible loss of function in the extremity. Only in the Efocaine series was loss of function noted. It is quite probable in a certain percentage of clinical patients, nerve damage does occur but is not recognized as a clinical entity. As long as the endoneurium is intact regeneration will take place as it did in B3.

Pontocaine seems to be the most popular drug used clini-

cally for serial injection anesthesia. In evaluating the individual drugs, these experiments indicate that the clinical judgement of those men who use Pontocaine in this type of anesthesia is sound. The strength of the drug used clinically in most cases would be in the range of that used in animals A8 through A19. This concentration caused minimal tissue swelling and no degeneration. The Trypan Blud experiments showed an irritation index of 0. Only in strength of 0.5% was a grade 1 change noted in one animal, but this is well out of the range of clinically used strengths. It, however, points out the important fact that even the safest agent may be neurotoxic if used in high enough concentration.

Nupercaine has been very popular for prolonged anesthesia. In 1/500 concentration the drug causes no degeneration in 36 hours. These experiments, however, would lead to questioning the use of this drug in 1/250 concentration for prolonged serial injection anesthesia. It produces moderate tissue swelling and produces some degenerative changes after 36 hours. It is significant that B40 and B41, sacrificed at 120 days, showed normal nerve; this fact probably points to regeneration of injured nerves. With only 5-10% nerve damage and a complete regeneration at 120 days, it is doubtful that a clinical change would be noted.

The butoxy and propoxy substituted Procaine compounds (Sympocaine and Blockanine respectively) show promise. These drugs combine the properties of a greater potency than Procaine, a decreased relative toxicity, and a short latent period. There was no degeneration after 24 hours use with these compounds, except for a 5% grade 1 change in B22 (Sympocaine). Since this is a minor

change and occurs in only one animal, it may well be due to some other factor besides local anesthetic neurotoxicity (i.e. needle damage).

Xylocaine is a drug that has become very popular over a relatively short period of time. The results of this experiment seem to indicate that this popularity is justified since only mild tissue swelling, an index of 2 on the Trypan Blue experiment, and no degeneration were noted. One major objection to its use in continuous block is its short duration of action in comparison with Pontocaine. Most of the enthusiasm for Xylocaine has resulted from experience with single injection anesthesia.

Serious doubts about the use of Hexylcaine for continuous block must certainly be raised from the results of this experiment. As noted in table 2 this drug causes degenerative changes in both the 1% and the 2% solutions. Table 1 points out that the drug has an irritation index of 8 in the Trypan Blue experiments. The drug is being used extensively today for single injection anesthesia dnd is very popular for epidural anesthesia. No objection can be raised to single injections, since experiments in animals B25-B30 indicated that this would not cause degeneration. However, after 12 hours use some degenerative changes were noted. It is probable, with other drugs of equal clinical value on the market, that the popularity of this drug will soon wane.

The neurotoxicity of Efocaine is well documented and these experiments merely reaffirm the conclusions of the others. The changes seen in Efocaine are similar in type, if not in degree, to the changes seen with other local anesthetics in these experiments.

They therefore offered a means of comparison. This drug would not be so dangerous if it were viewed by the medical profession as a neurolytic drug rather than a local anesthetic.

2-chloroprocaine caused some degeneration when used for 24 hours. It would seem doubtful that this fact would negate the use of this drug since it is probable that its short duration of action would contraindicate its use as the primary agent in continuous analgesia.

Intracaine showed 5% grade 2 change in two animals and also caused marked tissue swelling and a Trypan Blue irritation index of 6. This drug is not very popular today and no doubt is being superseded by other anesthetics with similar duration of action; As with 2-chloroprocaine, Intracaine is too short acting to be of much value in continuous block. The practice of using the drug in oil seems to have faded from the clinical scene.

Nothing in this experiment contraindicates the use of Primocaine. The marked tissue swelling is probably due to the 5% dextran in the solution, since reports indicate that the drug is non-irritating. No degeneration was present. This suggests that tissue swelling was not responsible for the degeneration seen in other series. Normal findings in the saline and epinephrine control series eliminated these as causitive agents in degeneration.

CONCLUSIONS

Serious objections have already been raised concerning the us of Efocaine. On the basis of these experiments these objections should be extended to Hexylcaine when used for multiple injections. Nupercaine, among the other drugs that caused degen-

eration, is the one most likely to be used for prolonged anesthesia by means of serial injections. No objection can be found in these experiments to the 1/500 solution; however, it is doubtful that the 1/250 solution should be used. 2-Chloroprocaine and Intracaine were also shown to cause degeneration when used in clinical concentrations for a 24 hour period. Use should be restricted to single injections. Pontocaine, Xylocaine, Sympocaine, Blockaine and Primocaine are considered free of degenerative changes in clinical strengths and under the conditions of this experiment.

Probably the best drug for serial injection anesthesia available today is Pontocaine. However, there is great demand for an anesthetic of low toxicity that has a shorter latent period and a longer duration than Pontocaine. Until this drug is synthesized, it is doubtful that clinicians will be satisfied with the local anesthetics available for their use.

It is felt by this investigator that the methods of evaluating local anesthetics are not completely satisfactory in that the property of neurotoxicity is not carefully explored. It would seem desirable that an experiment of this type be run on every local anesthetic before it is placed on the market. When considering the toxicity of a local anesthetic it is essential that local nerve degeneration be considered as important, if not more important, than the systemic toxicity.

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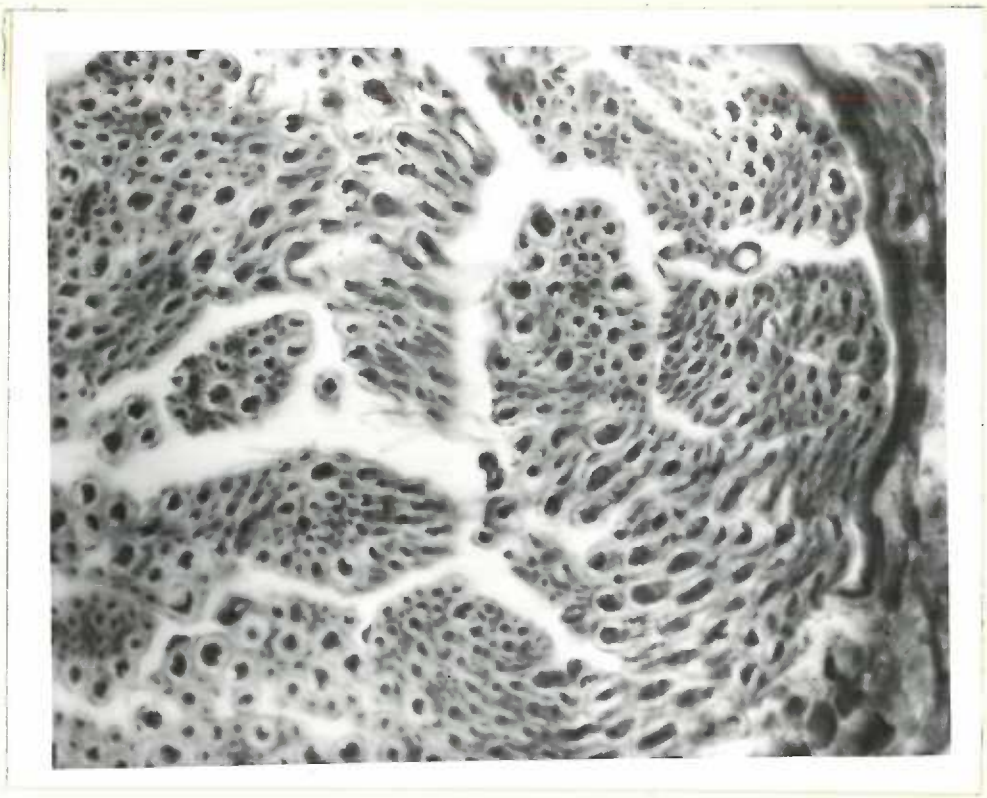
Figure 1

Section of the sciatic nerve of animal A38: run as a normal saline control with injections every 4 hours for 24 hours, and sacrificed at 12 days. Microscopically normal nerve.

Figure 2

Section of the sciatic nerve of animal B12: injected with 1/100,000 epinephrine every 3 hours for 24 hours and sacrificed at 14 days. Microscopically normal nerve.



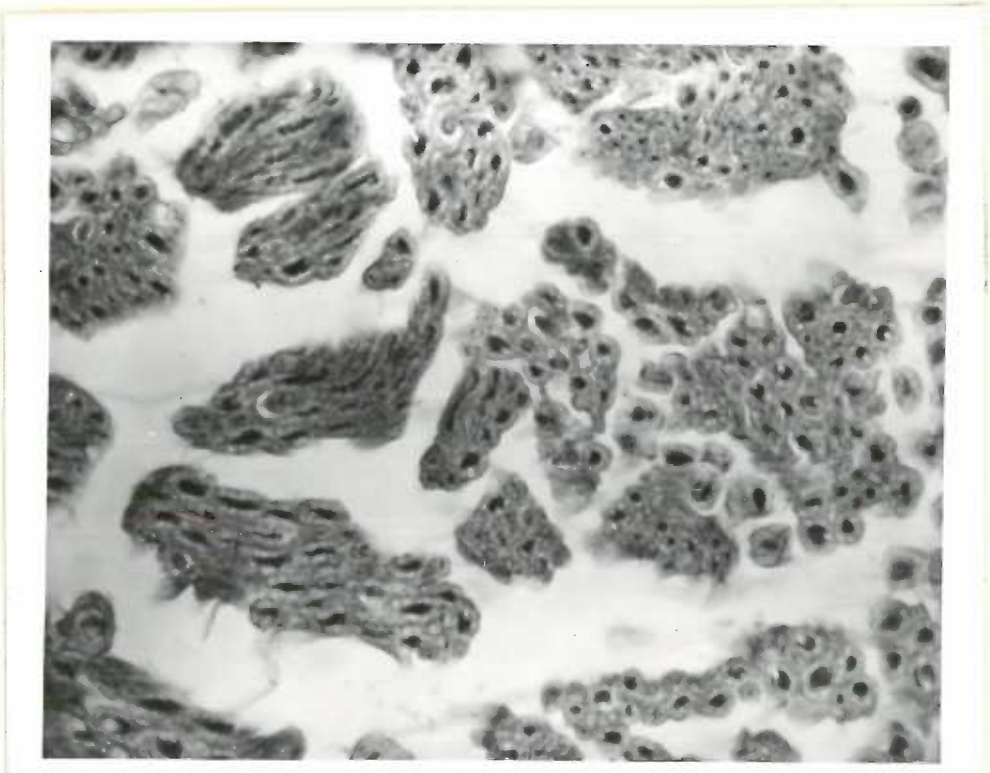
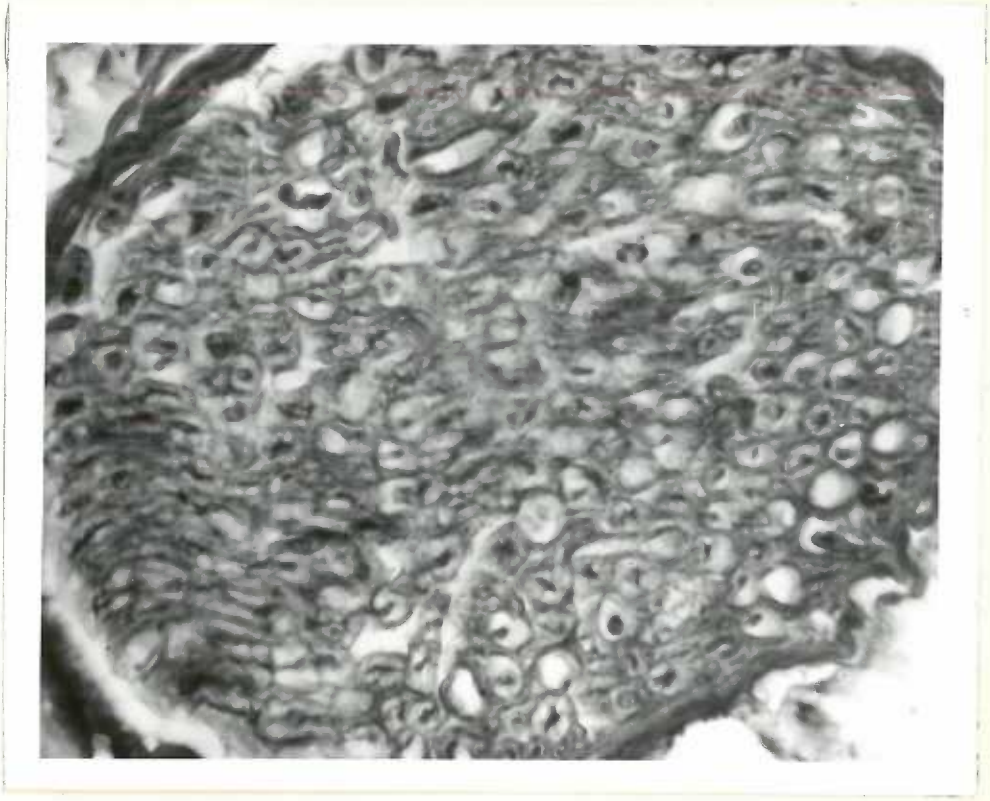


Figure 3

Animal B13: injected with 0.5% Pontocaine with 1/100,000 epinephrine every 6 hours for 24 hours and sacrificed at 14 days. Almost complete grade 1 change in this fascicle; 40% grade 1 change was present in trunk.

Figure 4

Animal A36: injected with 1/250 Nupercaine with 1/100,000 epinephrine every 8 hours for 36 hours and sacrificed at 14 days. 10-15% grade 2 change.



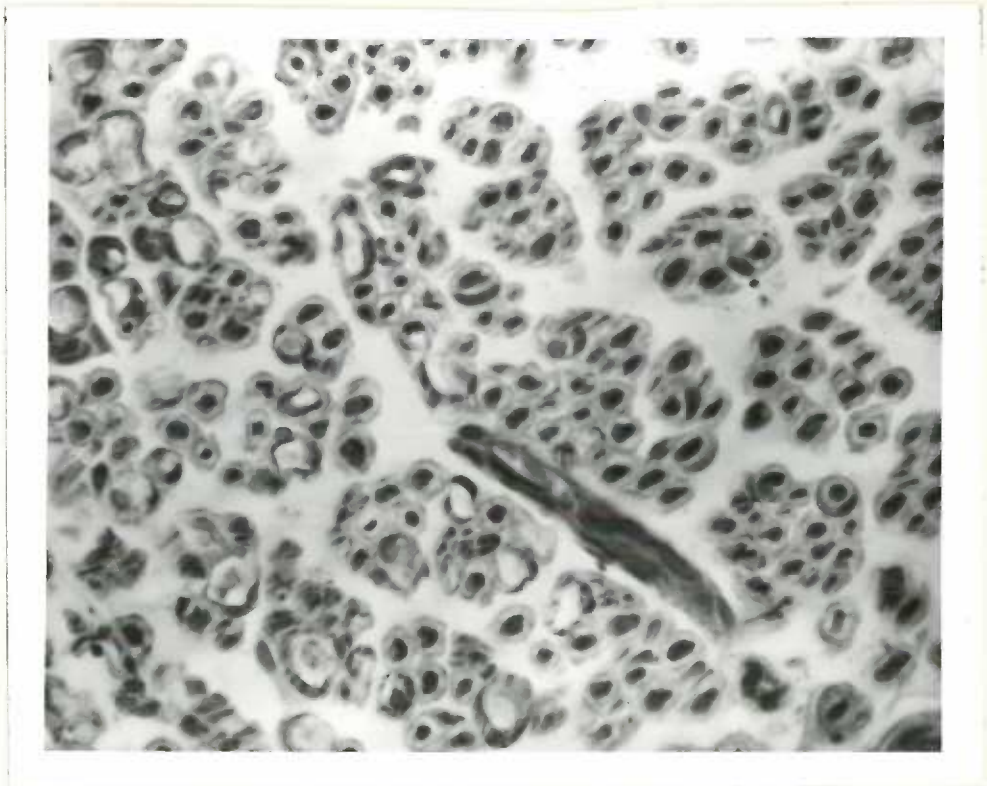
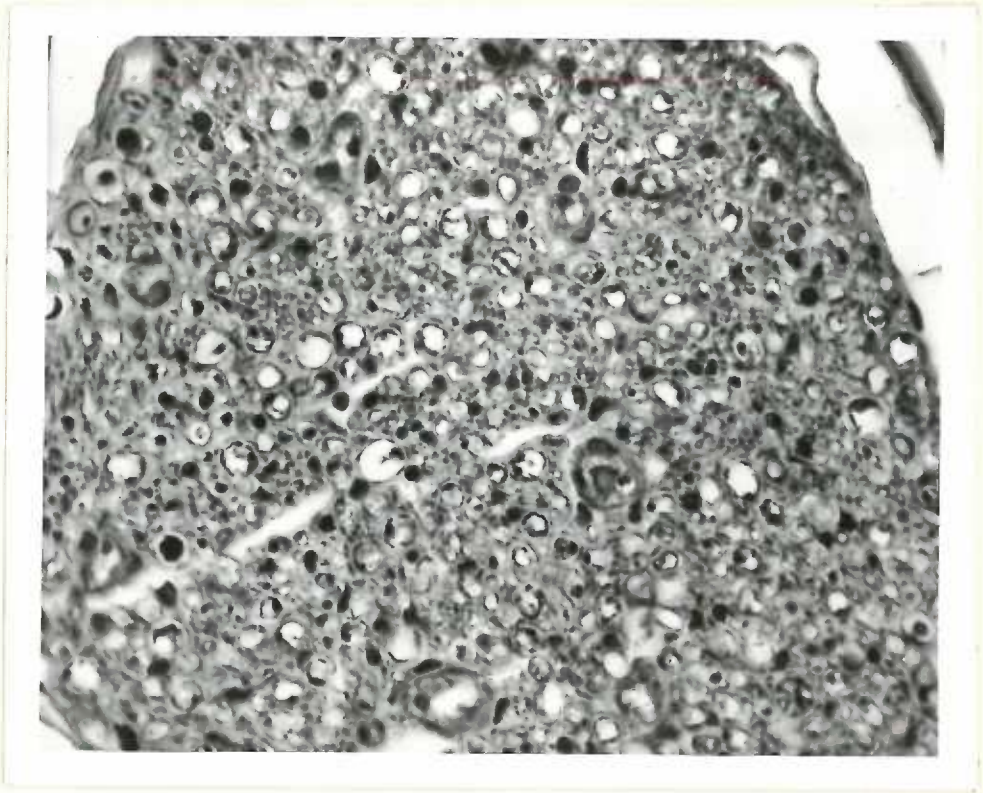


Figure 5

Animal A40: injected with Cyclaine 2% with 1/100,000 epinephrine every 4 hours for 24 hours and sacrificed immediately following injections. 40% grade 1 change microscopically.

Figure 6

Animal A38: injected with Cyclaine 2% with 1/100,000 epinephrine every 4 hours for 24 hours and sacrificed at 12 days. Microscopically 30% grade 2 change; severely damaged fascicle shown.



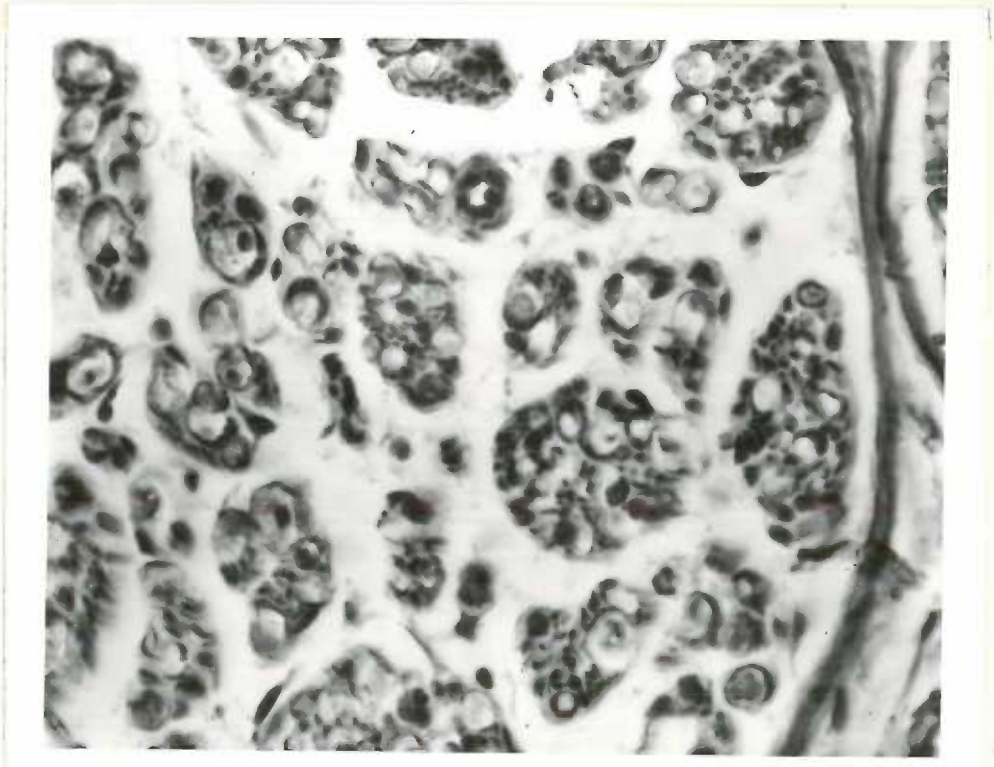
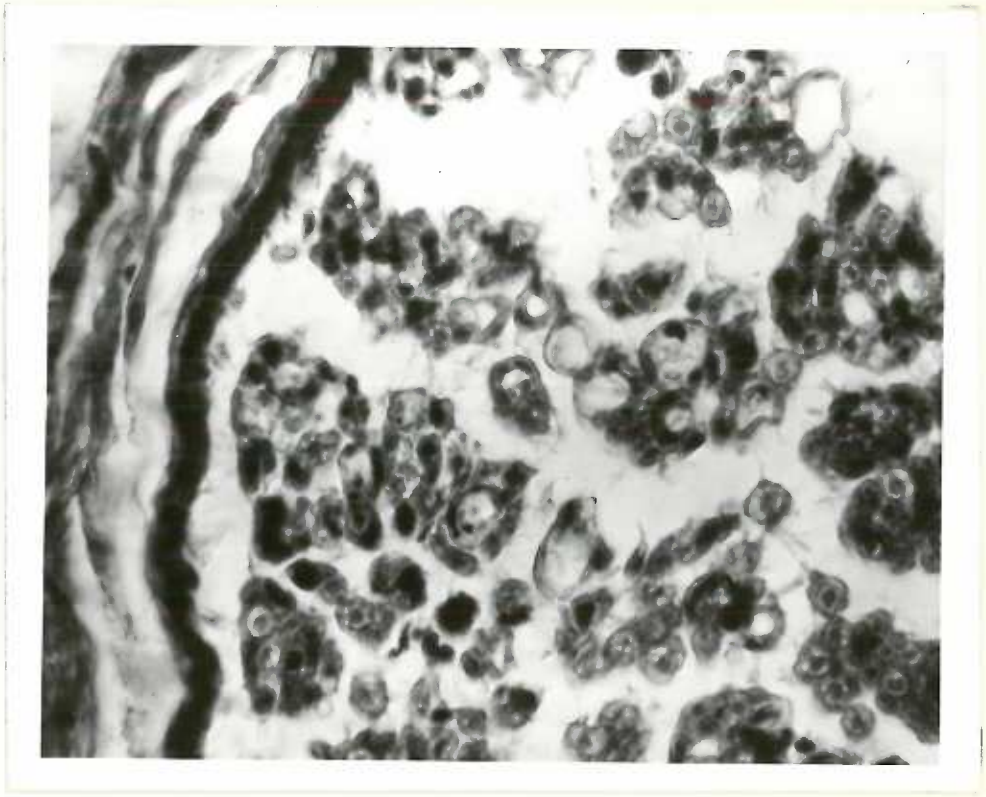


Figure 7

Animal B9: injected with Cyclaine 1% with 1/100,000 epinephrine every 3 hours for 24 hours and sacrificed at 14 days. 10% grade 1 and 5% grade 2 changes microscopically; severely damaged fascicle shown here.

Figure 8

Animal B4: given a single injection of Efocaine and sacrificed after 36 hours. Microscopically 50% grade 1 and 30% grade 2 changes.



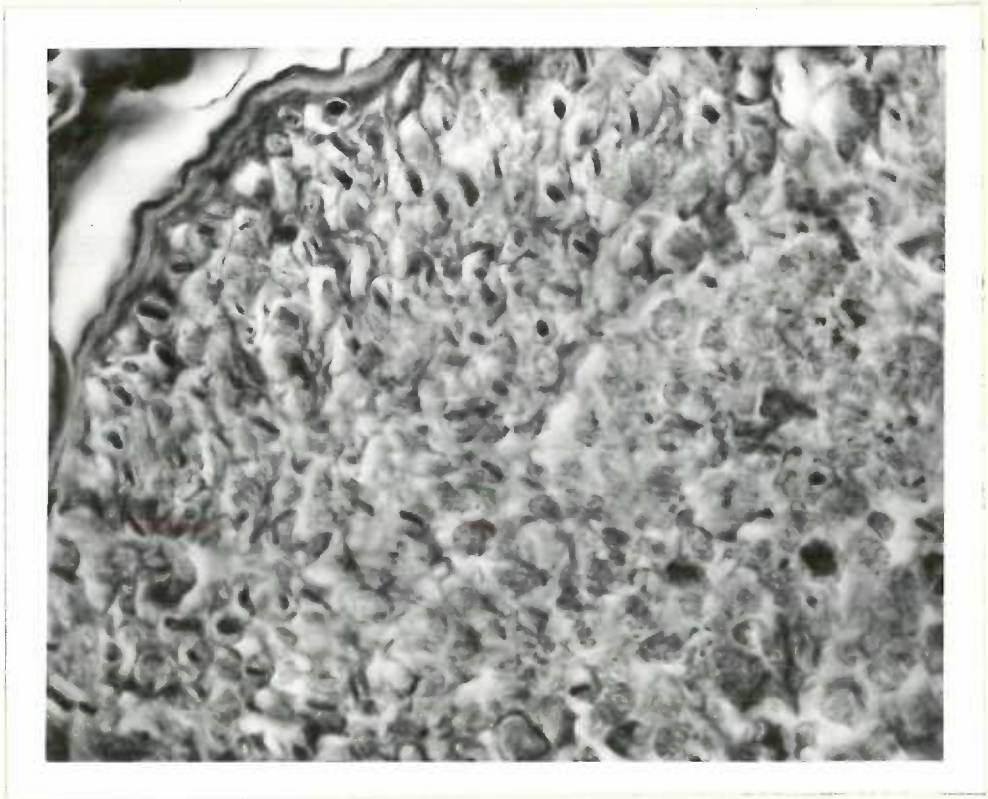
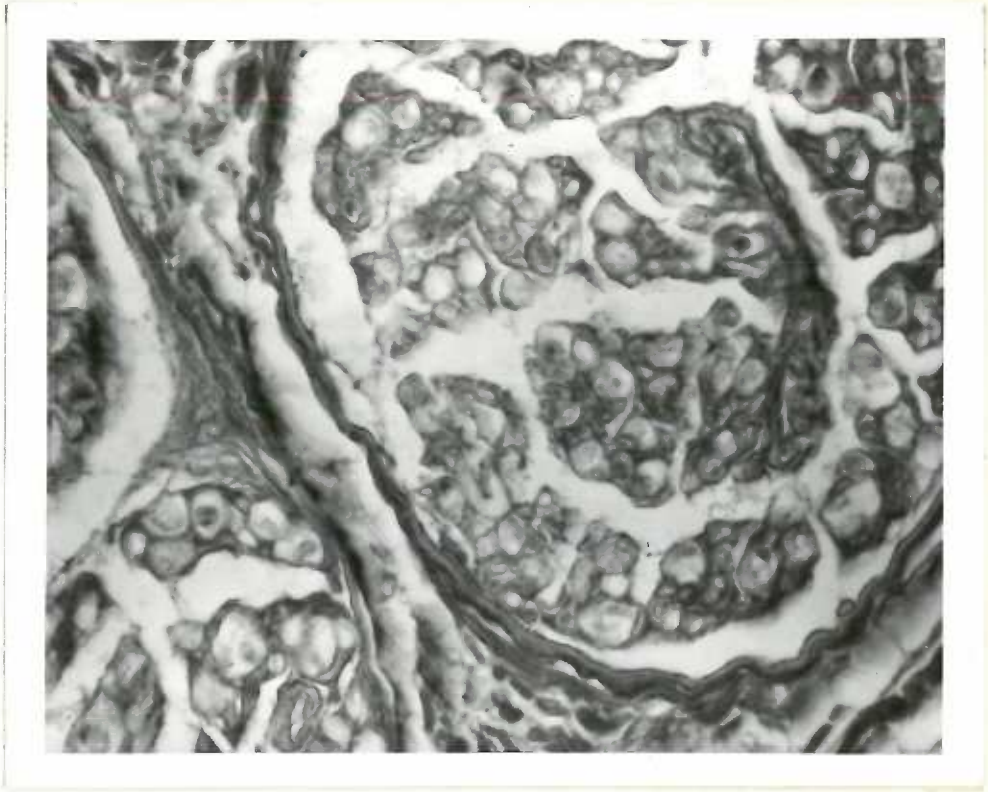


Figure 9

Animal B6: given a single injection of Efocaine and sacrificed at 14 days. Microscopically 90% grade 2 and 10% grade 3 change.

Figure 10

Animal B3: given a single injection of Efocaine and sacrificed at 141 days. Complete regeneration of the nerve has occurred.



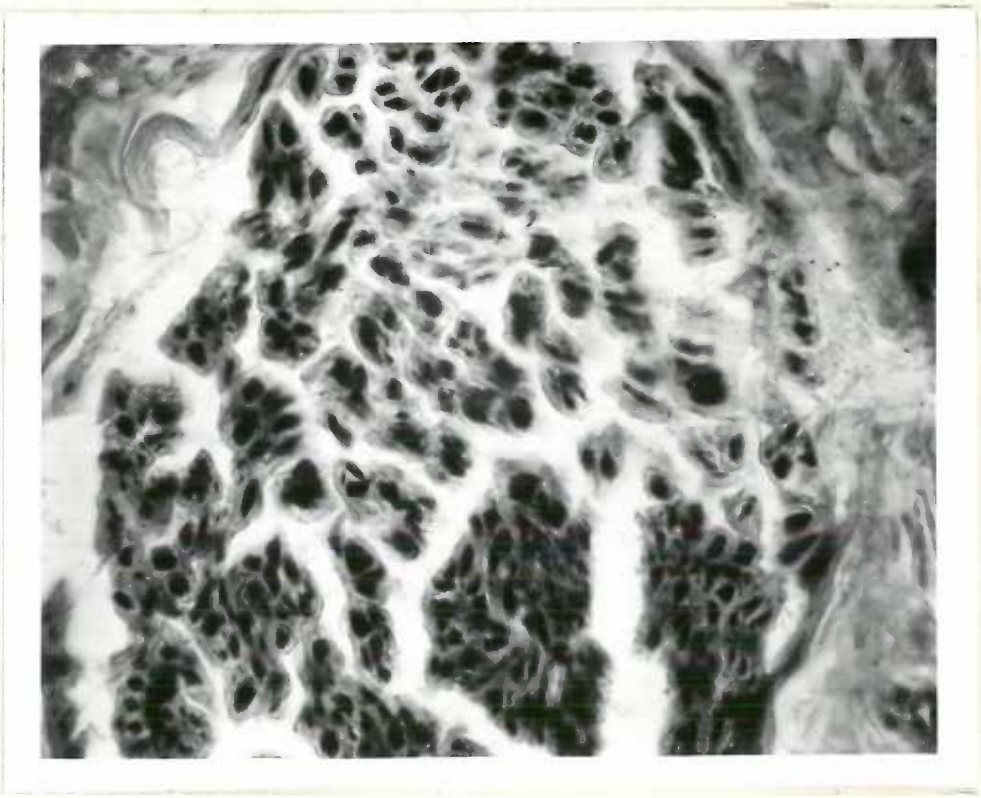
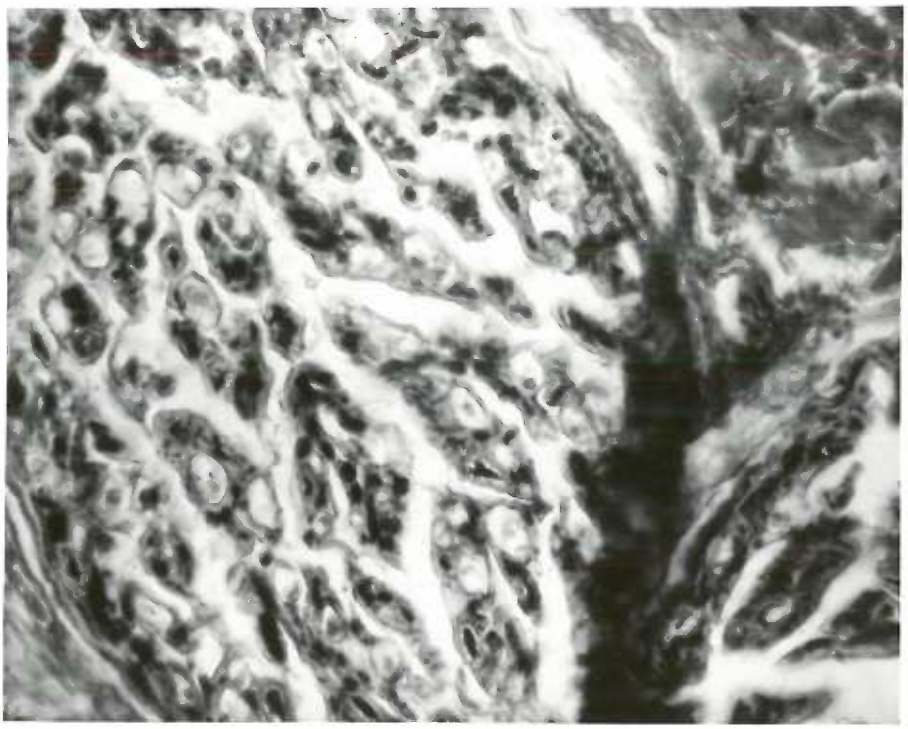


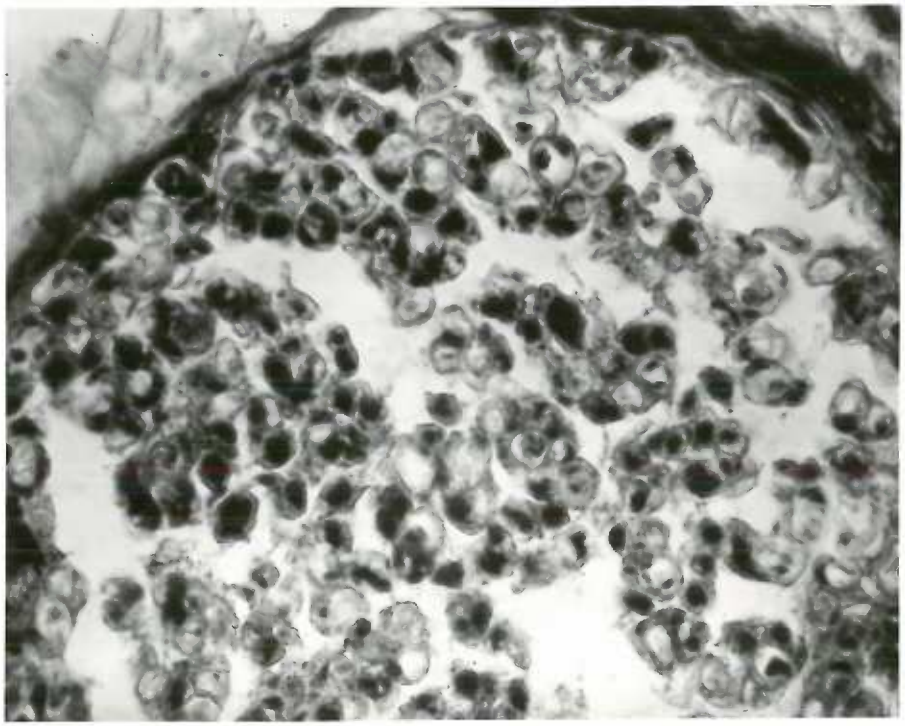
Figure 11

Animal B31: injected with Intracaine 2% with 1/100,000 epinephrine every 2½ hours for 24 hours and sacrificed at 14 days. Microscopically 5% grade 2 degeneration; severely damaged fascicle shown here.

Figure 12

Animal B44: injected with Nesacaine 3% with 1/100,000 epinephrine every 3 hours for 24 hours and sacrificed at 14 days. Microscopically 30% grade 2 change; severely damaged fascicle shown here.





G.P.#	Solution	Duration	Inject. freq.	Funct. Distur. & block	Sacrificed at	Tissue swelling	Micro.
A8	Pont. 0.18% & 1/100,000 epinephrine	36 hrs.	4 hrs.	none	12 days	+	normal nerve
A9	"	36 hrs.	4 hrs.	none	died 3 days	+	autolysis
A10	"	36 hrs.	4 hrs.	none	immediate	+	normal nerve
A11	"	36 hrs.	4 hrs.	none	died 4 days	+	autolysis
A12	"	36hrs.	4 hrs.	none	died 4 days	+	autolysis
A13	"	36 hrs.	4 hrs.	none	immediate	+	normal nerve
A14	"	36 hrs.	4 hrs.	none	died 4 days	+	autolysis
A15	"	36 hrs.	4 hrs.	none	12 days	+	normal nerve
A16	"	36 hrs.	4 hrs.	none	immediate	+	normal nerve
A17	"	36 hrs.	4 hrs.	none	90 days	+	normal nerve
A18	"	36 hrs.	4 hrs.	none	12 days	+	normal nerve
A19	"	36 hrs.	4 hrs.	none	immediate	+	normal nerve
A20	Pont. 0.3% & 1/100,000 epinephrine	36 hrs.	5 hrs.	none	12 days	++	normal nerve
A21	"	36 hrs.	5 hrs.	none	12 days	++	normal nerve

G.P.#	Solution	Duration	Iqject. freq.	Funct. Distur. ß block	Sacrifi-ded at	Tissue swelling	Micro.
A22	Pont. 0.3% ℰ ℰ/100,000 epinephrine	36 hrs.	5 hrs.	none	immediate	++	normal nerve
A23	"	36 hrs.	5 hrs.	none	died	++	autolysis
A24	"	36 hrs.	5 hrs.	none	80 days	++	normal nerve
A25	"	36 hrs.	5 hrs.	none	immediate	++	normal nerve
A26	Nupercaine 1/500	36 hrs.	7 hrs.	none	50 days	+	normal nerve
A27	"	36 hrs.	7 hrs.	none	immediate	+	normal nerve
A28	"	36 hrs.	7 hrs.	none	12 days	+	normal nerve
A29	"	36 hrs.	7 hrs.	none	50 days	+	normal nerve
A30	"	36 hrs.	7 hrs.	none	immediate	+	normal nerve
A31	"	36 hrs.	7 hrs.	none	12 days	+	normal nerve
a32	Nupercaine 1/250	36 hrs.	8 hrs.	none	died	++	autolysis
A33	"	36 hrs.	8 hrs.	none	14 days	++	normal nerve
A34	"	36 hrs.	8 hrs.	none	immediate	++	15% grade 1 change

G.P.#	Solution	Duration	Iqject. freq.	Funct. Distur. p block	Sacrifi-ced at	Tissue swelling	Micro.
A35	Nupercaine 1/250	36 hrs.	8 hrs.	none	60 days	++	normal nerve
A36	"	36 hrs.	8 hrs.	none	14 days	++	10-15% grade 2 change
A37	"	36 hrs.	8 hrs.	none	immediate	++	normal nerve
A38	Cyclaine 2% ē 1/100,000 epinephrine	24 hrs.	4 hrs.	none	12 days	+++	30% grade 2 change
A39	"	24 hrs.	4 hrs.	none	12 days	+++	40% grade 2 change
A40	"	24 hrs.	4 hrs.	none	immediate	+++	40% grade 1 change
A41	"	24 hrs.	4 hrs.	none	died	+++	autolysis
B1	Efocaine	-	single	complete	14 days	+	90% grade 2, 5% gr. 1, 5% grade 3.
B2	"	-	single	complete	14 days	+	90% grade 2, 5% grade 1, 5% grade 3.

G.P.#	Solution	Duration	Inject. freq.	Funct. Distur. p̄ block	Sacrificed at	Tissue swelling	Micro.
B3	Efocaine	-	single	complete recovery p̄ 100 days	141 days	+	complete regeneration
B4	"	-	single	complete	immed.(2d.)	+	50% grade 1, 30% grade 2.
B5	"	-	single	complete	14 days	+	70% grade 2, 30% grade 1.
B6	"	-	single	complete	14 days	+	90% grade 2, 10% grade 3.
B7	Epinephrine 1/100,000	24 hrs.	3 hrs.	none	28 days	+	normal nerve
B8	"	24 hrs.	3 hrs.	none	died	+	autolysis
B9	"	24 hrs.	3 hrs.	none	14 days	+	normal nerve
B10	"	24 hrs.	3 hrs.	none	immediate	+	normal nerve
B11	"	24 hrs.	3 hrs.	none	14 days	+	normal nerve
B12	"	24 hrs.	3 hrs.	none	14 days	+	normal nerve
B7	Cyclaine 1% ε 1/100,000 epin.	24 hrs.	3 hrs.	none	28 days	+++	normal nerve

G.P.#	Solution	Duration	Inject. freq.	Funct. distur. p block	Sacrifi-eed at	Tissue swelling	Micro.
B8	Cyclaine 1% c 1/100,000 epin.	24 hrs.	3 hrs.	none	died	+++	autolysis
B9	"	24 hrs.	3 hrs.	none	14 days	+++	10% grade 1, 5% grade 2.
B10	"	24 hrs.	3 hrs.	none	immediate	+++	5% grade 1.
B11	"	24 hrs.	3 hrs.	none	14 days	+++	5% grade 2
B12	"	24 hrs.	3 hrs.	none	14 days	+++	20% grade 2, 10% grade 1.
B13	Cyclaine 2% c 1/100,000 epin.	24 hrs.	4 hrs.	none	14 days	+++	normal nerve
B14	"	24 hrs.	4 hrs.	none	21 days	+++	slide destroyed
B15	"	24 hrs.	4 hrs.	none	immediate	+++	15% grade 1.
B16	"	24 hrs.	4 hrs.	none	14 days	+++	30% grade 2.
B17	"	24 hrs.	4 hrs.	none	21 days	+++	slide destroyed
B18	"	24 hrs.	4 hrs.	none	immediate	+++	normal nerve
B13	Pontocaine 0.5% c 1/100,000 epinephrine	24 hrs.	6 hrs.	none	14 days	+++	50% grade 1

G.P.#	Solution	Duration	Inject. freq.	Funct. distur. p block	Sacrificed at	Tissue swelling	Micro.
B14	Pontocaine 0.5% c 1/100,000 epinephrine	24 hrs.	6 hrs.	none	21 days	+++	normal nerve
B15	"	24 hrs.	6 hrs.	none	immediate	+++	normal nerve
B19	Blockaine 0.5% c 1/100,000 epinephrine	24 hrs.	3 hrs.	none	14 days	++	normal nerve
B20	"	24 hrs.	3 hrs.	none	immediate	++	normal nerve
B21	"	24 hrs.	3 hrs.	none	14 days	++	normal nerve
B22	"	24 hrs.	3 hrs.	none	14 days	++	normal nerve
B23	"	24 hrs.	3 hrs.	none	40 days	++	normal nerve
B24	"	24 hrs.	3 hrs.	none	40 days	++	normal nerve
B19	Sympocaine 0.5% c 1/100,000 epinephrine	24 hrs.	3 hrs.	none	14 days	++	normal nerve
B20	"	24 hrs.	3 hrs.	none	immediate	++	normal nerve
B21	"	24 hrs.	3 hrs.	none	14 days	++	normal nerve
B22	"	24 hrs.	3 hrs.	none	14 days	++	5% grade 1

G.P.#	Solution	Duration	Inject. freq.	Funct. distur. p block	Sacrificed at	Tissue swelling	Micro.
B23	Sympocaine 0.5% ē 1/100,000 epinephrine	24 hrs.	3 hrs.	none	40 days	++	normal nerve
B24	"	24 hrs.	3 hrs.	none	40 days	++	normal nerve
B25	Cyclaine 2% ē 1/100,000 epinephrine	4 hrs.	single	none	immediate	0	normal nerve
B26							
B26	"	4 hrs.	single	none	40 days	0	normal nerve
B27	"	4 hrs.	single	none	40 days	0	normal nerve
B28	"	4 hrs.	single	none	14 days	0	normal nerve
B29	"	4 hrs.	single	none	14 days	0	normal nerve
B30	"	4 hrs.	single	none	14 days	0	normal nerve
B25	"	12 hrs.	4 hrs.	none	immediate	+	normal nerve
B26	"	12 hrs.	4 hrs.	none	40 days	+	normal nerve
B27	"	12 hrs.	4 hrs.	none	40 days	+	normal nerve
B28	"	12 hrs.	4 hrs.	none	14 days	+	5% grade 2
B29	"	12 hrs.	4 hrs.	none	14 days	+	5% grade 1

G.P.#	Solution	Duration	Inject. freq.	Funct. Distur. & block	Sacrificed at	Tissue swelling	Micro.
B30	Cyclaine 2% c 1/100,000 epinephrine	12 hrs.	4 hrs.	none	14 days	+	5% grade 1, 5% grade 2, changes
B31	Xylocaine 2% c 1/100,000 epinephrine	24 hrs.	3 hrs.	none	14 days	+	normal nerve
B32	"	24 hrs.	3 hrs.	none	14 days	+	normal nerve
B33	"	24 hrs.	3 hrs.	none	14 days	+	normal nerve
B34	"	24 hrs.	3 hrs.	none	120 days	+	normal nerve
B35	"	24 hrs.	3 hrs.	none	120 days	+	normal nerve
B36	"	24 hrs.	3 hrs.	none	immediate	+	normal nerve
B31	Intracaine 2% c 1/100,000 epinephrine	24 hrs.	2½ hrs.	none	14 days	+++	5% grade 2
B32	"	24 hrs.	2½ hrs.	none	14 days	+++	5% grade 2
B33	"	24 hrs.	2½ hrs.	none	14 days	+++	normal nerve
B34	"	24 hrs.	2½ hrs.	none	120 days	+++	normal nerve
B35	"	24 hrs.	2½ hrs.	none	120 days	+++	normal nerve

G.P.#	Solution	Duration	Inject. freq.	Funct. distur. p block	Sacrificed at	Tissue swelling	Micro.
B36	Intracaine 2% ē 1/100,000 epinephrine	24 hrs.	2½ hrs.	none	immediate	+++	normal nerve
B37	Nupercaine 1/250	36 hrs.	8 hrs.	none	14 days	++	10% grade 1
B38	"	36 hrs.	8 hrs.	none	14 days	++	5% grade 1
B39	"	3 6hrs.	8 hrs.	none	14 days	++	5% grade 1, 5% grade 2.
B40	Nupercaine 1/250--	36 hrs.	8 hrs.	none	120 days	++	normal nerve
B41	"	36 hrs.	8 hrs.	none	120 days	++	normal nerve
B42	"	36 hrs.	8 hrs.	none	immediate	++	normal nerve
B37	Primecaine 1½% ē 5% Dextran and 1/100,000 epinephrine	24 hrs.	3 hrs.	none	14 days	++++	normal nerve
B38	"	24 hrs.	3 hrs.	none	14 days	++++	normal nerve
B39	"	24 hrs.	3 hrs.	none	14 days	++++	normal nerve
B40	"	24 hrs.	3 hrs.	none	120 ddys	++++	normal nerve
B41	"	24 hrs.	3 hrs.	none	120 days	++++	normal nerve

G.P.#	Solution	Duration	Inject. freq.	Funct. distur. p block	Sacrificed at	Tissue swelling	Micro.
Bl42	Premacaine 1½% c 5% Dextran and 1/100,000 epinephrine	24 hrs.	3 hrs.	none	immediate	++++	normal nerve
Bl43	Mesocaine 3% c 1/100,000 epin.	24 hrs.	3 hrs.	none	immediate	++	normal nerve
Bl44	"	24 hrs.	3 hrs.	none	14 days	++	30% grade 2
Bl45	"	24 hrs.	3 hrs.	none	14 days	++	10% grade 1, 5% grade 2.
Bl46	"	24 hrs.	3 hrs.	none	14 days	++	10% grade 1
Bl47	"	24 hrs.	3 hrs.	none	14 days	++	5% grade 1
Bl48	"	24 hrs.	3 hrs.	none	14 days	++	misplaced slide
Bl43	Epinephrine 1/100,000	24 hrs.	3 hrs.	none	immediate	+	normal nerve
Bl44	"	24 hrs.	3 hrs.	none	14 days	+	normal nerve
Bl45	"	24 hrs.	3 hrs.	none	14 days	+	normal nerve
Bl46	"	24 hrs.	3 hrs.	none	14 days	+	normal nerve

G.P.#	Solution	Duration	Injec. freq.	Funct. Distur. p block	Sacrificed at	Tissue swelling	Micro.
B47	Epinephrine 1/100,000	24 hrs.	3 hrs.	none	14 days	+	normal nerve
B48	"	24 hrs.	3 hrs.	none	14 days	+	normal nerve