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DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188

AD-A199 168

2b. DECLASSIFICATION/DOWNGRADING SCHEDULE		1b. RESTRICTIVE MARKINGS	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)		3. DISTRIBUTION/AVAILABILITY OF REPORT This document has been approved for public release; its distribution is unlimited.	
6a. NAME OF PERFORMING ORGANIZATION US Army Institute of Dental Research		6b. OFFICE SYMBOL (if applicable) SGRD-UDZ	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Walter Reed Army Medical Center Washington, DC 20307-5300		7b. ADDRESS (City, State, and ZIP Code)	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION US Army Medical Research & Development Command		8b. OFFICE SYMBOL (if applicable) SGRD-ZA	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Ft. Detrick Frederick, MD 21701-5012		10. SOURCE OF FUNDING NUMBERS	
		PROGRAM ELEMENT NO. 61102A	PROJECT NO. 3M161102BS10
		TASK NO. DA	WORK UNIT 506 ACCESSION NO. DA302908
11. TITLE (Include Security Classification) (U) Analysis of the Ability of DMSO and Lidocaine to Penetrate Dentin			
12. PERSONAL AUTHOR(S) C.E. Hastings Jr., J.O. Hollinger, J.W. Vincent and D.R. Swayze			
13a. TYPE OF REPORT FINAL	13b. TIME COVERED FROM 8410 TO 8701	14. DATE OF REPORT (Year, Month, Day) 88 01 15	15. PAGE COUNT 16
16. SUPPLEMENTARY NOTATION			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	
06	01		
06	16		
		Dimethyl Sulfoxide; Lidocaine; Topical Anesthesia; Dental Pain; Neurophysiology.	
19. ABSTRACT (Continue on reverse if necessary and identify by block number)			
Commonly used topical anesthetics in dentistry lack the ability to penetrate beyond the outer layers of the oral mucosa and must be applied for relatively long periods of time in order to see an affect. This is unacceptable in a dental emergency. Dimethyl sulfoxide (DMSO) is an analgesic drug that is readily absorbed through the skin and mucous membranes and which can also increase the penetration of other drugs across biologic barriers. Our current research is to develop a fast acting topical analgesic compound using DMSO plus a topical anesthetic that could be applied to the dentin of a painful tooth. The "Pashley" tooth chamber was used to evaluate the ability of DMSO, lidocaine, and their combination to penetrate 0.5 mm human dentin <u>in vitro</u>. Three concentrations (100, 90, and 75%) of DMSO were evaluated, using three teeth per concentration. DMSO (500 ul) was added to side A of the split chamber and samples removed from side B at times 0, 14, 30, 45, and 60 min and evaluated by gas chromatography. Flow rates for DMSO through dentin were not directly proportional to the concentration of DMSO. A 10 or 25% reduction in DMSO concentration			
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED	
22a. NAME OF RESPONSIBLE INDIVIDUAL COL Harold E. Plank, DC		22b. TELEPHONE (Include Area Code) 202-576-3484	22c. OFFICE SYMBOL SGRD-UDZ

ANALYSIS OF THE ABILITY OF DMSO AND LIDOCAINE
TO PENETRATE DENTIN

FINAL REPORT

January 1988

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In conducting research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources National Research Council.

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ABSTRACT

Commonly used topical anesthetics in dentistry lack the ability to penetrate beyond the outer layers of the oral mucosa and must be applied for relatively long periods of time in order to see an affect. This is unacceptable in a dental emergency. Dimethyl sulfoxide (DMSO) is an analgesic drug that is readily absorbed through the skin and mucous membranes and which can also increase the penetration of other drugs across biologic barriers. Our current research is to develop a fast acting topical analgesic compound using DMSO plus a topical anesthetic that could be applied to the dentin of a painful tooth. The Pashley tooth chamber was used to evaluate the ability of DMSO, lidocaine, and their combination to penetrate 0.5mm human dentin in vitro. Three concentrations (100, 90, and 75%) of DMSO were evaluated, using three teeth per concentration. DMSO (500 ul) was added to side A of the split chamber and samples removed from side B at times 0, 14, 30, 45, and 60 min and evaluated by gas chromatography. Flow rates for DMSO through dentin were not directly proportional to the concentration of DMSO. A 10 or 25% reduction in DMSO concentration resulted in a 24 or 48% reduction, respectively, in flow rate indicating a need to use DMSO in a high concentration. Lidocaine HCl and lidocaine base (both at 20%) were combined with either DMSO (100%) or an aqueous solution and evaluated as before using the "Pashley" tooth chamber. Four teeth were used per treatment. Lidocaine HCl more easily penetrated dentin than lidocaine base. DMSO has little effect on the penetration of lidocaine base but decreased the penetration of lidocaine HCl. A preliminary in vivo study in cats produced negative results.

INTRODUCTION

Pulpal anesthesia is usually induced by injection of local anesthetics into the oral mucosa adjacent to a painful tooth. Many times due to poor penetration and varying absorption rates, profound pulpal anesthesia is not obtained by this method leading to pain sensation by the patient. Topically applied anesthetics produce no pulpal anesthesia as they lack the ability to penetrate to the pulp when applied to the oral mucosa. A local anesthetic that could be applied topically to a cavity in a tooth, diffuse across dentin directly to the pulp and anesthetize pulpal pain fibers would be of great use in both diagnostic and therapeutic dentistry.

Cocaine was used in the nineteenth and early twentieth centuries to produce pulpal anesthesia when applied to cavities in teeth. Since that time, local anesthetics used in dentistry have received little attention as to topical application directly in cavities of teeth. One problem with the topical use of local anesthetics could be a slow penetration across dentin. Dimethyl sulfoxide (DMSO) is a reported analgesic and anti-inflammatory drug that is readily absorbed across skin, mucous membranes, and dentin and in addition has the ability to increase the penetration of other drugs across biologic barriers.

Dimethyl sulfoxide (DMSO) has been used to treat several types of neurological pathoses. Paul (1967) topically applied DMSO for treating trigeminal neuralgia. De la Torre et al. (1975) indicated that DMSO can reduce lesions in the white matter of experimental spinal cord injury in the dog with protection against permanent paraplegia. Brown (1967) reported that a 70% DMSO carbinal gel topically applied was effective in treating musculoskeletal pain.

DMSO is an analgesic and anti-inflammatory drug that is readily absorbed through the skin and distributed to the brain (Haigler and Spring, 1981). The small nerve fibers (A-delta and C) will not transmit an action potential (AP) when DMSO affects nerve membrane. Large fibers, the A-beta and gamma-types, are also susceptible to DMSO AP blockade (Becker et al., 1969).

Haigler and Spring (1981) have postulated that DMSO may have a mechanism of action similar to morphine, that is: produce a direct or indirect action on central nervous (CNS) opiate receptors. Such an action would result in release of endogenous enkephalins or endorphins from nerve terminals, thereby producing analgesia.

The significant quality of DMSO that is to be considered in this study is DMSO's analgesic effect when topically applied to a painful, carious lesion in the tooth. DMSO is believed to affect either peripheral nerve receptors or the axons themselves (Becker et al. 1969). Because small peripheral nerve fibers (A-delta and C's) are important in centripetal pulpal pain conduction, blocking the action potential of these fibers by DMSO would be expected to alter pain sensibility.

Extremely important consideration with any drug is its systemic effects. Kolb et al. (1967) have reported that when a 90% DMSO (^{35}S or ^3H labeled) solution was applied cutaneously to human subjects, radioactivity appeared in the systemic circulation within five minutes, and reached a plateau in four to six hours. This level was maintained for one and a half to three days. The maximum concentration in the systemic circulation of rats (18 mg%) following topical application was achieved in ten minutes, and the half life was ten hours (Denko et al., 1967). Haigler and Spring (1981) mentioned that following IV administration of DMSO to rhesus monkeys, the pons, medulla, and spinal cord were found to contain

the highest body levels of this agent.

A lidocaine plus DMSO solution would essentially be an anesthetic dissolved in an anesthetic possibly increasing the total amount of anesthetic penetrating dentin per unit time. In addition, the DMSO could enhance the penetration rate of lidocaine to the pulpal nerves. the objective of this study was to measure the diffusion rate through dentin of lidocaine and DMSO and to compare the diffusion rates of lidocaine in DMSO and aqueous carrier solutions.

MATERIALS AND METHODS

The experimental methodology is outlined in Figure 1. Freshly extracted human third molars were stored in phosphate buffered saline (PBS) (pH = 7.4) at 4°C until used. A 0.5 mm dentin disk was cut from each tooth using a slow-speed diamond-blade saw and the smear layer was removed from both surfaces by placing them in a 50% citric acid solution for two minutes. The dentin disks were placed into a "Pashley" tooth diffusion chamber (Fig. 1) that was constructed of Delrin^R and silicone o-rings to prevent damage by DMSO. Test solutions were added to chamber A on the occlusal side of the tooth at a volume of 250 ul. A cover was placed over chamber A to prevent evaporation of the test solutions. The pulpal side of the tooth was continuously rinsed by PBS (pH = 7.4) at a rate of 0.1 ml per minute and samples collected every 15 minutes in a fraction collector.

In the first study, 100, 90 and 75% solutions of DMSO were prepared by mixing (v/v) HPLC grade DMSO (Fisher Scientific) with double distilled water as needed. In the second study, the four test solutions consisted

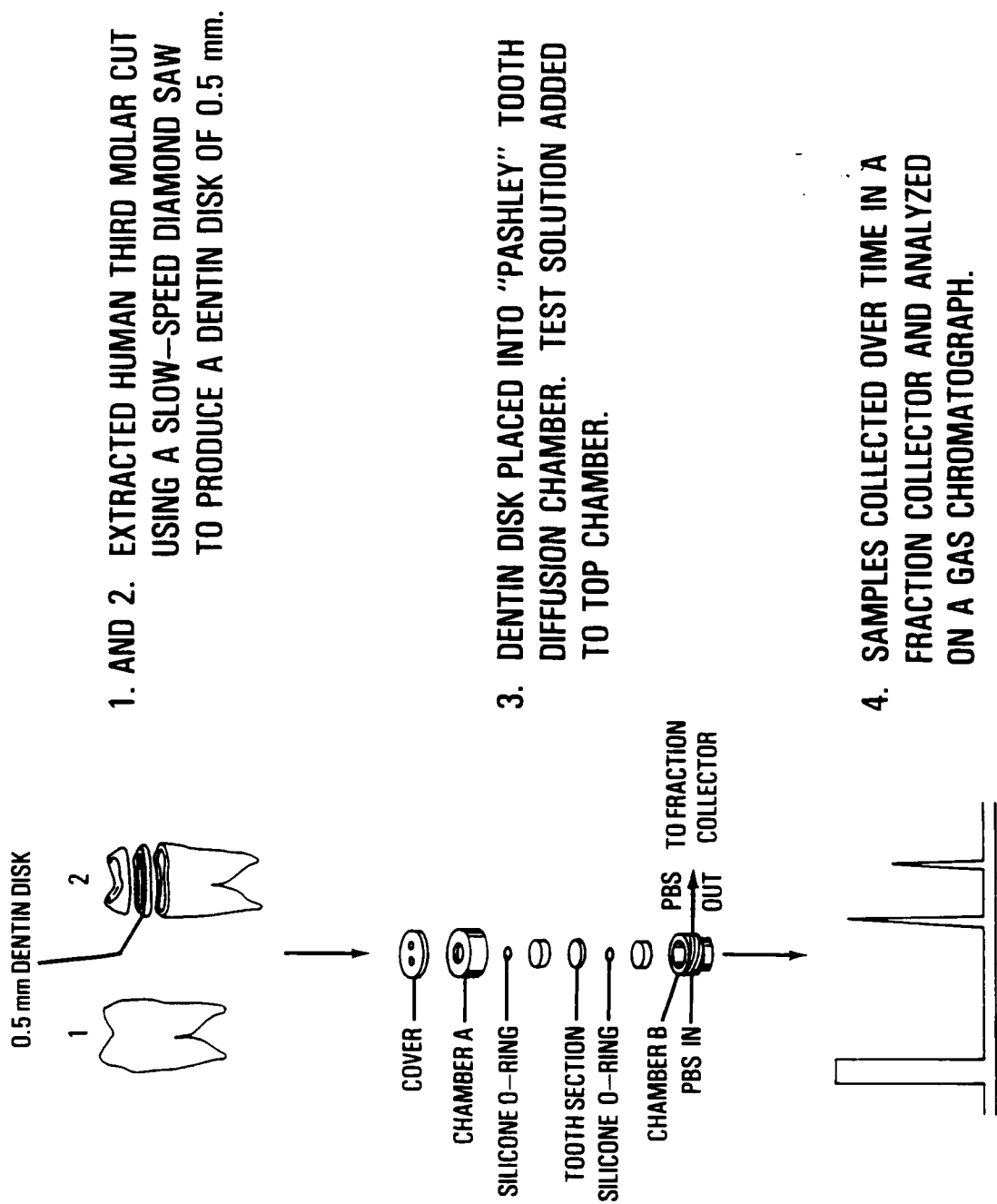


FIGURE 1

of 20% lidocaine HCl (w/v) in distilled water and in 100% DMSO and 20% lidocaine base in distilled water and 100% DMSO. Three replicates were used per test solution.

The samples were analyzed for lidocaine or DMSO concentration by gas chromatography using a SIGMA 2000 capillary gas chromatograph equipped with a Perkin-Elmer 7500 computer for data computation, reintegration, and analysis. For lidocaine analysis, a 30 m X 0.25 mm I.D. fused silica capillary column coated with OV-17 was used. The carrier gas was helium, the injector and detector temperatures were both 280°C. The following temperature program was used: two min. at 100°C, increase 30°C to 250°C, 8 min. at 250°C. A splitless injection of a 5 ul sample was used. One ml was removed from each 15 min fraction collector sample and extracted in a gladd centrifuge tube by adding 0.5 ml chloroform, 300 ul of 2M Tris base, and 300 ul of a 5 g/l mepivacaine HCl solution which acted as the internal standard. The mixture was vortexed for one min and centrifuged at 2,000 rpm for 15 min. A 5 ul sample from the chloroform layer was injected onto the GC.

For the DMSO analysis, a 30 m X 0.25 mm I.D. Spelcowax 10 fused silica capillary column was used. The injector and detector temperatures were both 250°C and the oven was isothermally at 225°C. Helium was used as the carrier gas with a splitless injection of 5 ul of sample. Aqueous samples were taken directly from the reaction collector and injected onto the GC.

Flow rates of lidocaine and DMSO vs time were determined and treatment groups compared using a one or two-way Analysis of Variance. A t-test was used to check for significant differences ($p < 0.05$).

RESULTS

The data for the DMSO experiment is reported as a flux ($\text{ug min}^{-1} \text{cm}^{-2}$) through 0.5 min dentin disks (fig. 2). Large variances were seen in the flux vs time data. No significant differences were seen between any of the three groups due to the large variances but a trend was seen between the 100% DMSO and the two lower concentrations. The 100% DMSO flux remained noticeably higher than the other two concentrations throughout the two hour measurement. Flux equilibrium was attained at 60 min for the 75 and 90% concentrations of DMSO with values of 171 and 209 $\text{ug min}^{-1} \text{cm}^{-2}$ respectively. Equilibrium occurred at 90 min for 100% DMSO with a value of 305 $\text{ug min}^{-1} \text{cm}^{-2}$. The 90 and 75% DMSO equilibrium flux values were 69 and 56% of that for 100% DMSO.

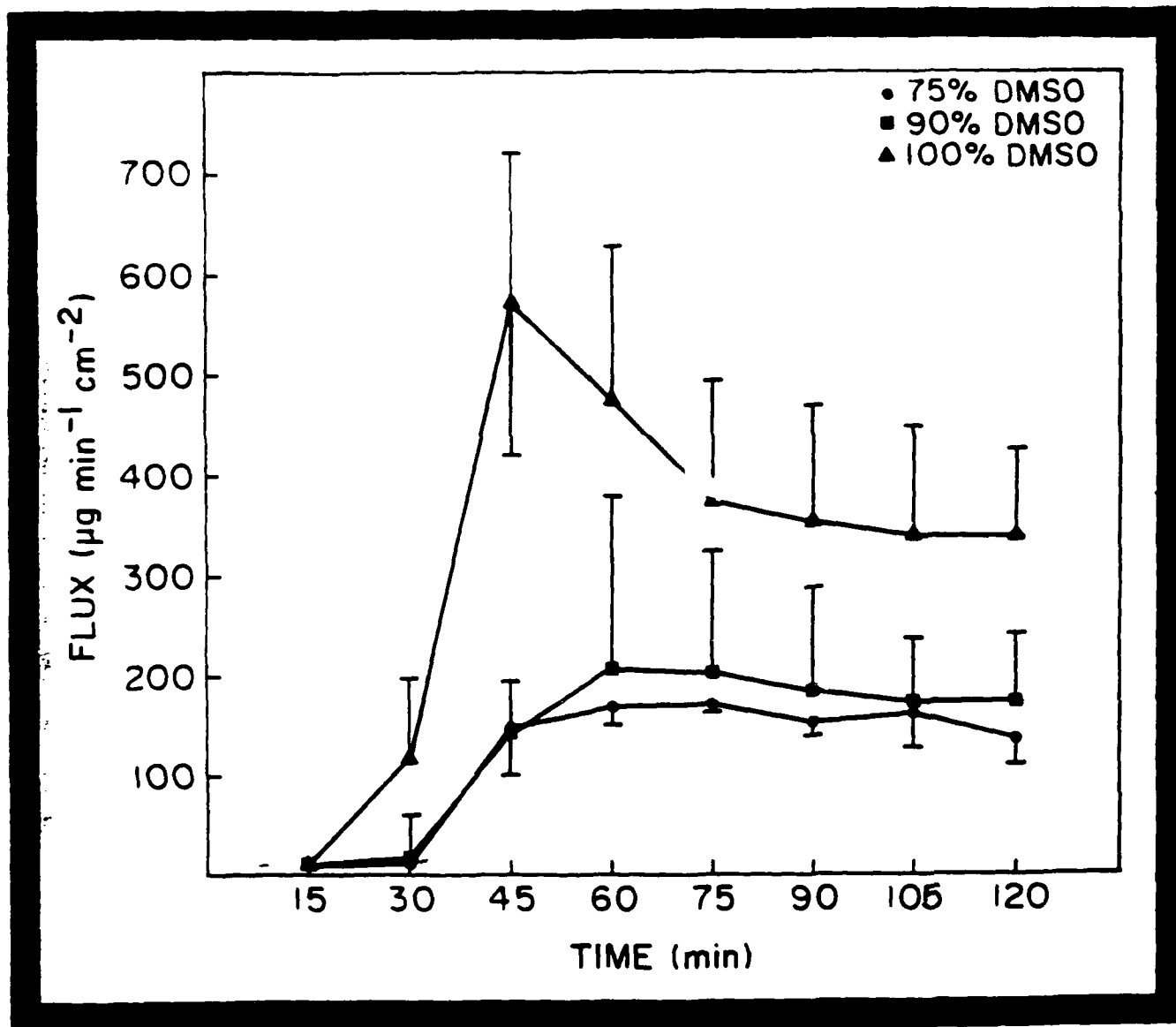
The lidocaine data is reported as flux ($\text{ug min}^{-1} \text{cm}^{-2}$) of lidocaine through 0.5 min of dentin (fig. 3). Flux equilibrium was reached at 45 min for the two lidocaine preparations with values of 54 and 24 $\text{ug min}^{-1} \text{cm}^{-2}$ respectively for the DMSO and alcohol preparations. No significant differences in flux were seen between the two lidocaine base preparations at any time during the two hour study. Equilibrium was attained at 60 min for lidocaine HCl in DMSO at 180 $\text{ug min}^{-1} \text{cm}^{-2}$, and at 90 min for lidocaine HCl in H_2O at a higher flux of 517 $\text{ug min}^{-1} \text{cm}^{-2}$. Both lidocaine HCl preparations were significantly above the lidocaine base solutions between 45 and 120 min ($p \leq 0.05$).

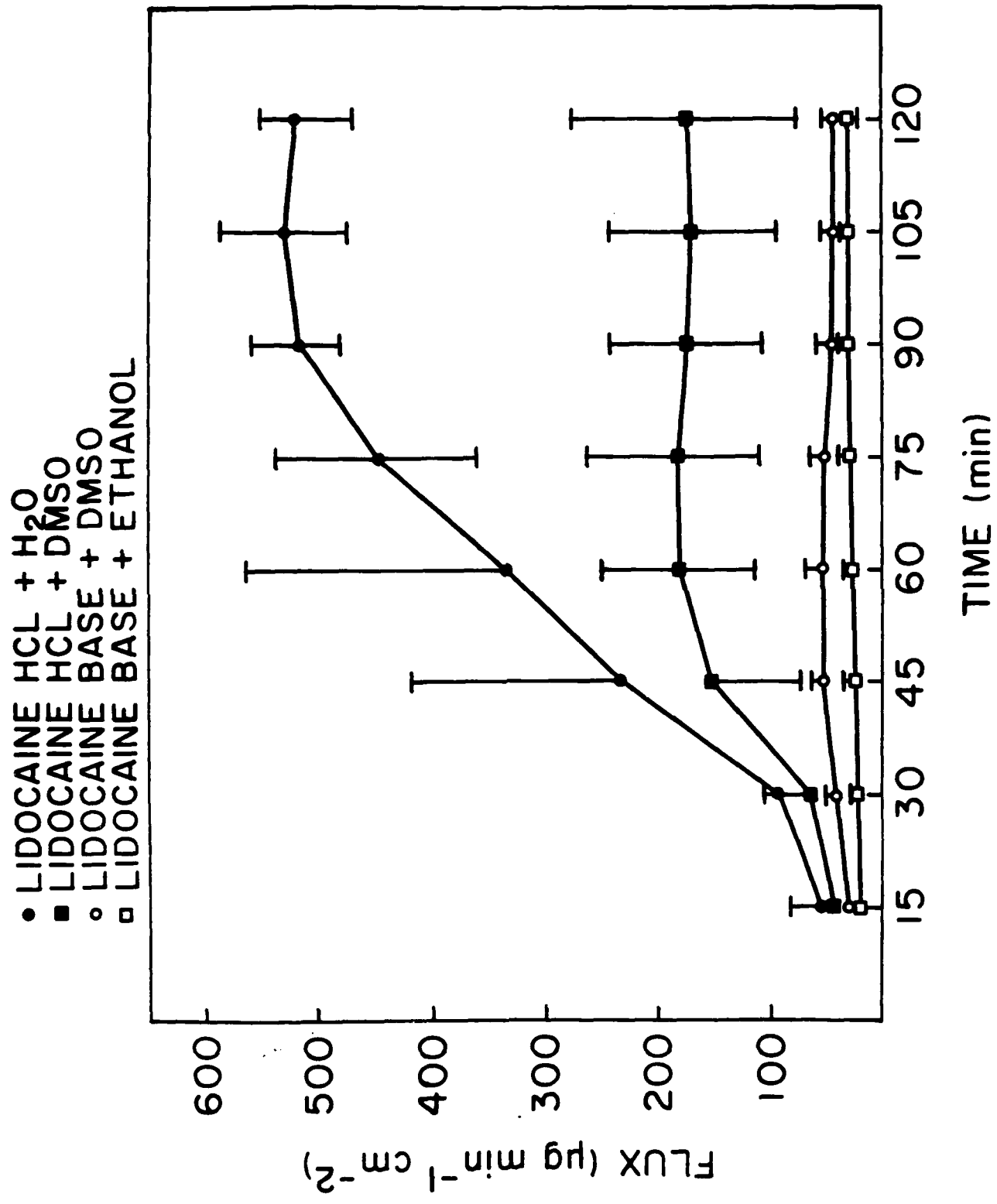
DISCUSSION

The flux of DMSO through dentin was not directly proportional to the concentration of DMSO applied. A large drop in equilibrium flux was seen between 100% and 90% DMSO, indicating a need to use DMSO in as high a

FIGURE 2. THE EFFECT OF CONCENTRATION ON DENTIN PENETRATION BY DMSO.

DATA POINTS ARE MEANS $\pm$ STANDARD DEVIATIONS ($n = 3$)





concentration as possible to maximize dentin penetration. This is of concern since DMSO at high concentrations is cytotoxic to skin, oral mucosa, and certainly to pulp. DMSO at 100% could conceivably be used in tooth cavities however, as its concentration will be diluted as it passes through dentin possibly reaching non-toxic concentrations at the pulp.

DMSO is a polar solvent of moderately high dielectric constant which dissolves both organic and inorganic reagents. Both lidocaine HCl and base at concentrations of 20% were easily dissolved in DMSO. DMSO is a relatively small molecule and would be thought to penetrate dentinal tubules easily but it inhibited the penetration of lidocaine. When the permeability for 100% DMSO at equilibrium is computed according to the equation given by Pashley and Livingston (1978), a value of $3.72 \times 10^{-4} \text{ cm min}^{-1}$ is obtained. This value is 32% of the permeability value for water ($1.17 \times 10^{-3} \text{ cm min}^{-1}$) reported by Pashley (1). This correlates well with the lidocaine flux of the DMSO preparation being 34% of that of the water preparation. The lower dentinal permeability of DMSO slowed the penetration of lidocaine proportionately. The reasons for the slower penetration of DMSO compared to water could be that DMSO is a larger molecule or that greater adhesion or absorption by the dentin occurred.

This study observed the penetration of dentin in an area devoid of organic matter. As solutions move closer to the pulp, they encounter tubules that are increasingly filled with odontoblastic processes which offer an entirely different diffusion barrier. DMSO has been shown to penetrate biologic membranes and to facilitate the movement of other drugs across these barriers (Wood and Wood, 1975). Once at the region of the odontoblastic processes, the DMSO could enhance the penetration of lidocaine into the pulp.

A preliminary effort was made to evaluate the in vivo effect of the lidocaine-DMSO combination using cats. A total of eight animals were used. Each animal was premedicated with ketamine. Alpha-chloralose (50 mg/kg i.v.) was administered through the femoral vein to induce anesthesia and lactated Ringer's solution was also administered by that route for fluid replacement. A femoral artery was also cannulated with the catheter tip retrogradely positioned in the abdominal aorta for the continual monitoring of mean and pulsatile arterial pressure. Breathing was assisted with a positive pressure respirator. A rectal thermistor continually monitored body temperature and auxiliary heat was provided as needed. An ECG monitor was attached with standard limb leads. Heart rate was also monitored. At the end of the experiments the cats were euthanitized with 10 ml saturated KCl i.v.

Dental pain was induced by perfusing a hyperosmotic (4 M sucrose) solution into prepared dental cavities so that nociceptors in the pulp were activated. An attempt was made to relate blood pressure and heart rate to the induction of dental pain. Bipolar recording electrodes were placed on the sympathetic chain between the T_2 and T_3 connecting rami to determine if dental pain will evoke reflex activation of sympathetic cardiac efferents.

Approximately 5 ml of the stimulating solution was introduced to the prepared cavities and left undisturbed for two minutes and the cavity was then washed with Ringer's solution. Action potentials ranging from 2.0 to 9.0 microvolts were recorded for a two minute control period prior to stimulation. Approximately 20

ul of a solution containing physiological saline, 50 percent by volume medical grade DMSO and 20 percent by volume lidocaine base, was used as the anesthetizing solution.

The results suggested that there was a direct correlation between blood pressure and heart rate and evoked pain potentials. However, the potentials obtained between control and experimental levels were not significant. Considerable difficulty was encountered with background interference and it was concluded that the Faraday cage constructed to isolate the experiment from surrounding noise levels was inadequate. The experiments were, therefore, terminated. No useful data were obtained.

CONCLUSIONS

The penetration through dentin of DMSO is greatly diminished by a reduction in concentration from 100% indicating a need to use as high a concentration as possible for use as a local anesthetic. DMSO inhibited the flux of lidocaine through dentinal tubules presumably due to a decreased permeability for the DMSO itself compared to water. DMSO may still be useful as a carrier for lidocaine if it can enhance lidocaine penetration through odontoblastic processes in deeper dentin. A preliminary attempt at in vivo evaluation in cats did not produce any useful results due to inadequate isolation of the experimental setup from surrounding electrical interference.

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