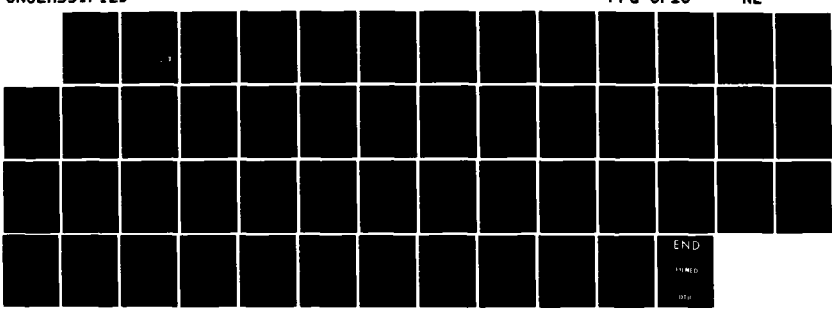
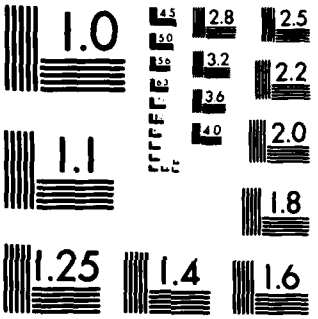
AD-A149 098 LOCAL ANESTHETIC MICROENCAPSULATION(U) BIOTEK INC WOBURN MA D L WILLIAMS ET AL. 18 MAR 83 2111-6 DAMD17-81-C-1195 1/1

UNCLASSIFIED F/G 6/15 NL





MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

AD_____

Report No. BIOTEK 2111-6

LOCAL ANESTHETIC MICROENCAPSULATION

Annual Report

David L. Williams, Ph.D.
William A. Nucefora, B.S.
David E. Creeden, B.S.
Shirley A. Odell, B.S.

March 18, 1983
(For period June 1, 1982 through February 28, 1983)

Supported by

US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-81-C-1195

BIOTEK, Inc.
21-C Olympia Avenue
Woburn, Massachusetts 01801

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A149098
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle) Local Anesthetic Microencapsulation	5. TYPE OF REPORT & PERIOD COVERED Annual June 1, 1982-February 28, 1983
	6. PERFORMING ORG. REPORT NUMBER 2111-6
7. AUTHOR(s) David L. Williams, Ph.D. Shirley A. Odell, B.S. William A. Nucefora, B.S. David E. Creeden, B.S.	8. CONTRACT OR GRANT NUMBER(s) DAMD17-81-C-1195
9. PERFORMING ORGANIZATION NAME AND ADDRESS BIOTEK, Inc. 21-C Olympia Avenue Woburn, MA 01801	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102A.3M161102BS10.DA.380
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command Fort Detrick, Frederick, MD 21701	12. REPORT DATE March 18, 1983
	13. NUMBER OF PAGES 51
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report) Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Biological & Medical Sciences - Pharmacology; Lidocaine, Bupivacaine, Etidocaine, Local Anesthetics, Encapsulating, Polymer, Polylactide

HCl

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Etidocaine-HCl-polymer core particles have been microencapsulated. Most of the product is in the anticipated 100-400 micron size range. Release rates vary from 50% release in one hour (75-105 micron, 10% coating) to only about 40% release in one day (150-425 micron, 30% coating).

Selected materials were evaluated for stability to various storage conditions and for bulk density and pore distribution.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

In vivo studies included determination of anesthesia of the peripheral nerves of guinea pigs, and the sciatic nerve of rats. The rat studies were discontinued. Circulating levels of anesthetics were measured after injection of solution and microcapsular suspensions in rabbits.

Measurement of the twitch of a guinea pig after tactile stimulation was more difficult than anticipated. Older animals were replaced with younger, more sensitive animals. For the EMG, suture needles were replaced with less traumatic electrodes, and the tactile stimulus was confined to the area of the injection. Also, the initial contact of the tactile stimulator often interferred with the measurement. In repeating the method of Bülbring and Wajda, intradermal injection of microcapsules was a problem. Finally a series of measurements were performed with the guinea pig wheal, using a hand-held needle as the stimulus and observing the twitch visually. The quantity of microcapsules was increased to compensate for the loss due to the incomplete injection of microcapsules into the intradermal space. The results of these experiments were reproducible and complete sensory blockage was achieved for several of the initial 5 minute test periods. Sensory perception slowly returned and could be approximated by a straight line function. A least squares fit allowed a mathematical analysis of the time of return of 50% of feeling, and of complete recovery. The results indicate that the addition of lidocaine microcapsules may be effective ($p<0.10$) in extending the time of anesthesia. Work continues with more potent anesthetics.

Sciatic nerve blocks were short when using slow-releasing etidocaine-HCl microcapsules. However, when using a high concentration (8% drug) of faster releasing microcapsules, many hours of nerve block were observed. Although the results were variable, individual blocks lasted as long as 2 and 4 days.

Circulating levels of lidocaine were measured ~~at USAIDR by~~ GC/MS after I.M. injection of soluble and microencapsulated drug in rabbits. Almost no circulating lidocaine was found after injection of lidocaine (base) microcapsules. For lidocaine-HCl, the solution showed peak levels at 10 minutes of 5.6 µg/ml; whereas for the same quantity of encapsulated drug the peak occurred at 1 hour and was only 0.9 µg/ml. Blood levels of CPK were also signifcantly lower when microencapsulated drug was injected. This is indicative of less local tissue damage.

Accession For	
NTIS GRA&I	X
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

Citation of commercial organizations and trade names in this report does not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

I. SUMMARY

Etidocaine-HCl-polymer core particles have been microencapsulated. Most of the product is in the anticipated 100-400 micron size range. Release rates vary from 50% release in one hour (75-105 micron, 10% coating) to only about 40% release in one day (150-425 micron, 30% coating).

Selected materials were evaluated for stability to various storage conditions and for bulk density and pore distribution.

In vivo studies included determination of anesthesia of the peripheral nerves of guinea pigs, and the sciatic nerve of rats. The rat studies were discontinued. Circulating levels of anesthetics were measured after injection of solution and microcapsular suspensions in rabbits.

Measurement of the twitch of a guinea pig after tactile stimulation was more difficult than anticipated. Older animals were replaced with younger, more sensitive animals. For the EMG, suture needles were replaced with less traumatic electrodes, and the tactile stimulus was confined to the area of the injection. Also, the initial contact of the tactile stimulator often interferred with the measurement. In repeating the method of Bülbring and Wajda, intradermal injection of microcapsules was a problem. Finally a series of measurements were performed with the guinea pig wheal, using a hand-held needle as the stimulus and observing the twitch visually. The quantity of microcapsules was increased to compensate for the loss due to the incomplete injection of microcapsules into the intradermal space. The results of these experiments were reproducible and complete sensory blockage was achieved for several of the initial 5 minute test periods. Sensory perception slowly returned and could be approximated by a straight line function. A least squares fit allowed a mathematical analysis of the time of return of 50% of feeling, and of complete recovery. The results indicate that the addition of lidocaine microcapsules may ($p<0.10$ be effective in extending the time of anesthesia. Work continues with more potent anesthetics.

Sciatic nerve blocks were short when using slow-releasing etidocaine-HCl microcapsules. However, when using a high concentration (8% drug) of faster releasing microcapsules, many hours of nerve block were observed. Although the results were variable, individual blocks lasted as long as 2 and 4 days.

Circulating levels of lidocaine were measured at USAIDR by GC/MS after I.M. injection of soluble and microencapsulated drug in rabbits. Almost no circulating lidocaine was found after injection of lidocaine (base) microcapsules. For lidocaine-HCl, the solution showed peak levels at 10 minutes of 5.6 µg/ml; whereas for the same quantity of encapsulated drug the peak occurred at 1 hour and was only 0.9 µg/ml. Blood levels of CPK were also significantly lower when microencapsulated drug was injected. This is indicative of less local tissue damage.

II. ACCOMPLISHMENTS

A. In Vitro Studies

1. Polylactide Availability

During the present contract period we have continued to use the polymer blend (poly-L(-)lactide of R.S.V. = 1.19 dl/g) which was described in the previous report (Report No. 2111-3, p 4-7). Additional polymer of 1.2±0.6 dl/g is available which can be used to obtain a blend of 1.2±0.1 dl/g. This will be blended when more polymer is required.

2. Microencapsulation of Etidocaine-HCl

In previous microencapsulations, etidocaine-HCl particles fluidized so well that agglomeration did not occur, even at high fluid flow rates. Therefore, this time a slurry of drug particles was suspended in a solution of polymer in methylene chloride, and the solvent was evaporated. The resulting film was broken up and the particles forced through a 500 micron and then a 250 micron sieve. A 15% polymer loading was anticipated. However, the final product had an assay value of 22.4%, indicating a loss of fine drug particles. This material made an excellent core particle for fluidized bed spray coating. A large fraction of the product was in the 100-400 micron size range. Table 1 summarizes the process yields, and Table 2 gives the sieving analyses of these materials.

The sieve fractions of microcapsules were tested for drug release as a function of time. The data are shown in Figures 1-3. In general, smaller microcapsules release drug more rapidly, and capsules with lower polymer coatings also release drug more rapidly.

3. Porosimetry Measurements

On similar projects, mercury porosimetry has been found to yield useful information on microcapsule morphology. A single test gives information on (1) bulk density, by a repeatable method on a small sample, (2) interparticle void volume, (3)

TABLE 1

PROCESSING SUMMARY OF ETIDOCAINE-HCl MICROENCAPSULATION

Coating Polymer (%)	Starting Weights: Starting Sample (g)	Starting Weights: Polymer Added (g)	Final Weight of Microcapsules (g)	Losses: Wurster Holdup (g)	Losses: Oversize Removed (g)	Losses: Sieve + Bag (g)	Losses: Samples Removed (g)
0-10	280[3]	31	190	121	0	-	30
10-20	160	18	182	-4	0	-	30
20-30	152	17	241[2]	-72	0	-	191 circulating 50[1] brushed
TOTALS		66		45	0	0	301[2]
	A	B	C	D	E	F	G

Material Balance In = 280 g core + 66 g polymer = 346 g

Out = 301 g samples + 45 g losses = 346 g

Yield at 30% coating is 87% based on samples/input materials[2]

$D_n = A_n + B_n - C_n$ $\qquad$ $A_{n+1} = C_n - (E_n + F_n + G_n)$

[1] Brushing of chamber yields 50 grams

[2] Includes brush-down material

[3] Core particles were 22% polymer, < 250 μm

TABLE 2

ETIDOCAINE-HCl MICROCAPSULE SIZE DISTRIBUTION

(Values are % of weight in each sieve fraction)

Microcapsule Size Range (μm)	Core Material	% Coating on Core		
		10%	20%	30%*
> 600	--	0	0	1
425-600	--	1	2	3
300-425	--	9	16	24
212-300	17	25	28	25
150-212	28	20	21	19
106-150	32	17	15	14
75-106	9	3	10	6
38-75	0	19	5	7
< 38	14	4	2	2

* Does not include brush-down material

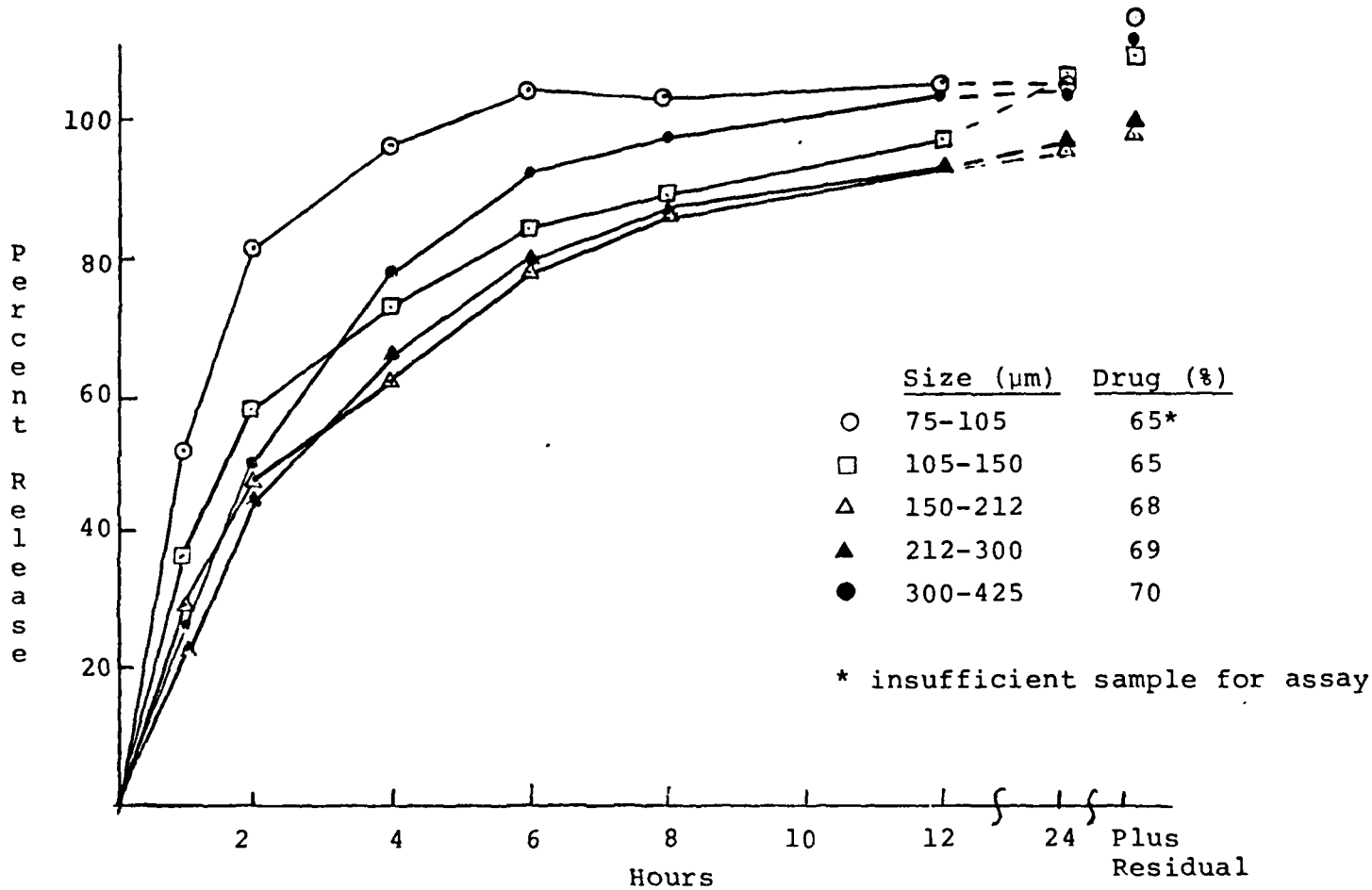


Figure 1 Etidocaine-HCl Release at 10% Coating Level (11-4-10)

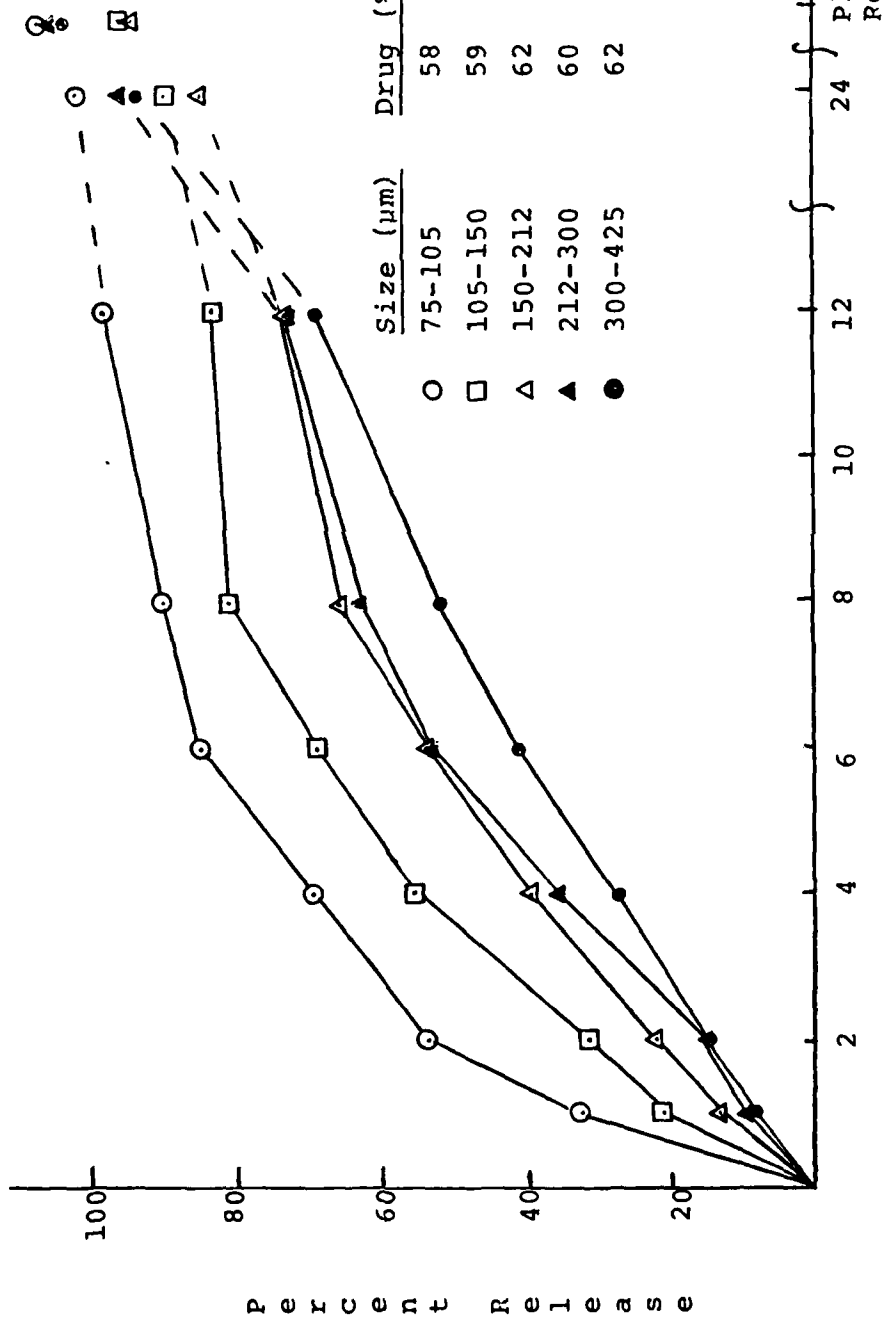


Figure 2 Etidocaine-HCl Release at 20% Coating Level (11-4-20)

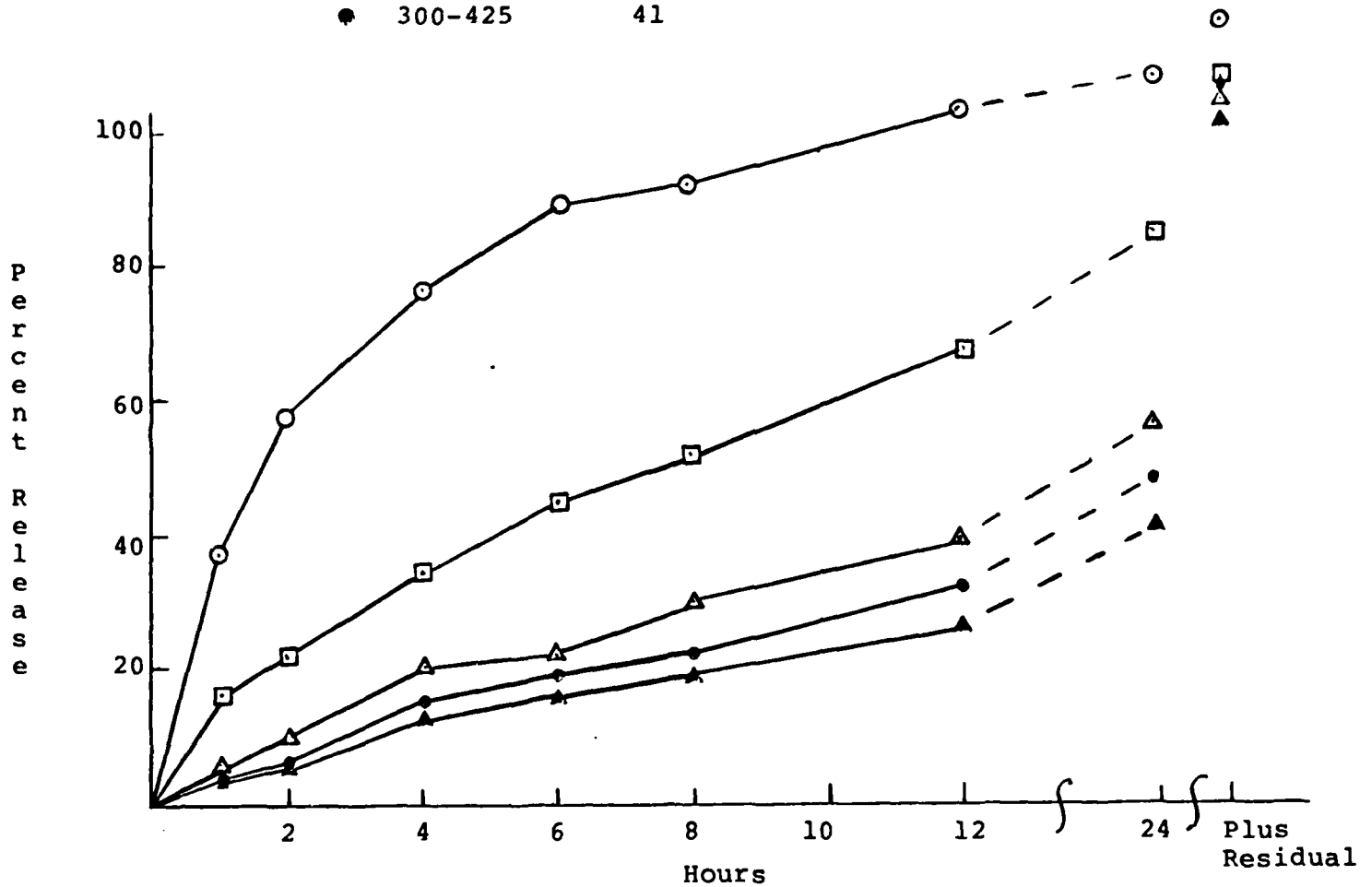


Figure 3 Etidocaine-HCl Release at 30% Coating Level (11-4-30)

pore volume, with volume associated with various restricted pore radii, and (4) skeletal density which defines closed pore volumes if the absolute density of the material is known. We sent samples of the previous batch of etidocaine-HCl microcapsules to Micromeritics Inc. who do this test on a purchase order basis.

The data on the equivalent volumes for microcapsules of 70% etidocaine-HCl are shown in Table 3. For a bed of microcapsules of 106-300 micron sieve size, we assume that volumes with equivalent pore sizes of more than 10 microns are interparticle voids. Constrictions of less than 10 microns are considered to be pore volumes.

The bulk volume of microcapsules are dependent on the method of compaction. After loading the porosimeter tube with mercury at atmosphere pressure, volumes larger than 123 microns in diameter are filled by the porosimeter. The bulk volume of the microcapsules was estimated by carefully filling a small graduated cylinder with microcapsules. Extensive tapping was avoided. The bulk volume was 2.24 cm^3. Filling a mercury penetrometer gave a bulk volume of 1.98 cm^3/gm.

Physical property information is most useful as a quality control parameter. It is also useful as a correlating parameter with either in vitro drug release or a useful clinical property.

B. Stability of Stored Microcapsules

During this report period bupivacaine-HCl microcapsules were tested after storage under various conditions for one year. The results shown in Table 4 indicate no significant degradation of the sample. The results are comparable to those for lidocaine-HCl and etidocaine-HCl samples, which were reported in the previous annual report (June 14, 1982, page 35). However, there is a possibility that the bupivacaine-HCl is releasing faster from the samples stored for one year.

C. In Vivo Studies

1. Response of Guinea Pigs to Tactile Stimulation

Experiments to demonstrate peripheral nerve anesthesia were initiated on Contract No. DAMD17-80-C-0110. Results obtained by poking a rabbit with a needle were suggestive but not especially convincing (Annual Report July 23, 1981, pp 28-31). Improvements were made by using the USAIDR tactile stimulator to deliver the stimulus and an EMG to measure the response. Last year,

TABLE 3

POROSIMETRY DATA ON
Etidocaine-HCl Microcapsules
(70% drug, 106-300 μm)

	Equivalent Volumes (cm^3/gm)	
Bulk Volume (cylinder)	2.24	
Bulk Volume (porosimeter)	1.983	
Skeletal Volume	0.994	
Absolute Volume	0.833	(assume absolute d=1.2 g/cm^3 for Etidocaine-HCl)
Intrusion Volume	0.989	
Interparticle (>10 μm)	0.726	
Pores (total <10 μm)	0.263	
1.0 - 10 μm	0.024	
0.1 - 1.0 μm	0.074	
<0.1 μm	0.165	
*Closed Pore Volume	0.161	

* Difference of Skeletal and Absolute Volumes

TABLE 4

ASSAY AND RELEASE OF MICROCAPSULES

AFTER ONE YEAR OF STORAGE

Sample	Release Time (Hrs.)	Original Percent Release	One Year Later		
			40°C Dark Not Sealed	R.T. Light Not Sealed	R.T. Light Not Sealed
Bupivacaine-HCl	1	27	36	40	36
80% drug	6	69	88	88	91
150-212 μm	24	85	92	92	97
	Assay %	(80)	79	82	79

intradermal studies with anesthetic solutions showed that the guinea pig was a better animal model than the rabbit for cutaneous anesthesia. We then proceeded with subcutaneous injection studies of anesthetic solutions and microcapsule suspensions.

Initial experiments were hampered by the guinea pig's failing health. This was finally analyzed as scurvy, even though we were using a commercially available guinea pig food. We lost 9 of 10 guinea pigs and sacrificed the last one.

During these experiments we found that we could elicit an EMG response by rapidly moving away from the table after firing the stimulator. This was caused by electrostatic charging, as demonstrated by scraping a plastic sheet across the table. The metal table was then grounded and the Centrap placed on an insulating rubber mat. This removed the spurious signal of the movement of the operator. Problems sometimes arose in control tests with the sound of the stimulator firing or the pressure of the stimulator hub. If either of these elicited a positive response the series of readings had to be discarded.

The next group of guinea pigs were heavy and quite lethargic. They rarely vocalized, even when suture needles were implanted as electrodes. Male retired breeders had been used for all of the previous experiments at BIOTEK and Northeastern. An experiment was next performed in which five guinea pigs were used on five different days. The results were variable. These animals were capable of going into a catatonic state in which neither mechanical nor hand stimulation with a needle caused any visual twitch or EMG response. With no adequate control stimulus data, no anesthetic effect could be defined. These animals were healthy but could not be used on this project.

Three additional guinea pigs were purchased at 400-450 g (36-42 days old). These pigs are more sensitive and results appeared to improve. Miniature surface electrodes were used at this time, with an EKG gel. This system gave a larger signal than the subcutaneously implanted suture needles. The system also did not subject the guinea pig to a continuous painful stimulus or stress. A depilatory cream (Nair®) had been used to remove the hair. However, the animals are very sensitive to this material and a calm guinea pig is a requirement for adequate data collection. At this time a rayon tape (Dermacel®) was used to hold the electrodes in place. This also served to keep the animal in the Centrap.

Finally it was noted that when a guinea pig is huddled in the Centrap with his legs retracted under him, he was less apt to move than if he had a paw outside the cage. Hence we gently retracted his rear leg from the trap. A one millimeter throw of a small (25 gauge) needle was quite sufficient to cause a large

EMG response under these conditions. Selected data, such as shown in Figure 4 caused us to be optimistic about this system.

During the next quarter, 50 guinea pigs tests were performed using the USAIDR tactile stimulator and analysis of the EMG recording. The guinea pigs remained in good health and were reasonably responsive to tactile stimulus. We continued to use surface electrodes. These were held in place with a rubber dam. The guinea pig is held in a Centrap, with its posterior sticking out beyond the trap. Of these 50 tests, 35 tests were performed with lidocaine and 15 with etidocaine; 30 were performed with subcutaneous injections and 20 with intradermal injections. About 50% of these tests were control anesthetic solution tests. For most of these tests a hand held needle was also used as a stimulant and a visual movement of the guinea pig was used as the indicator of anesthesia.

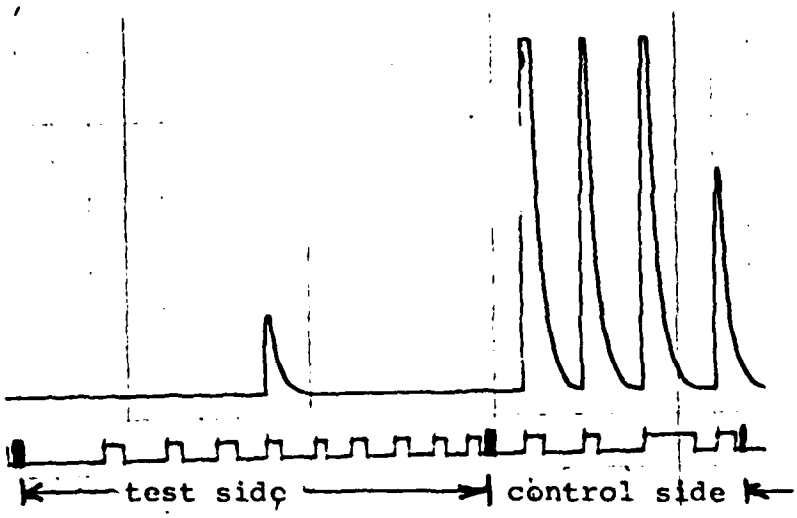
Soon after this quarter started, the concept of the lateral extent of anesthesia was eliminated and only the site of injection was stimulated. Using this method, with subcutaneous injection, the anesthesia of 10 mg of lidocaine-HCl in 0.5 ml (2% solution) was 35 ± 10 minutes (N=5). However, there was no anesthesia when lidocaine-HCl microcapsules (06-1-50, 150-212) were injected at 20 and 40 mg.

Larger quantities of the more powerful anesthetic, etidocaine were then injected. To aid in the injection, the guinea pig was anesthetized with ether or halothane. Only partial anesthesia was observed for 90 minutes with 20 mg of etidocaine-HCl in microcapsules (11-2-20, 74-105). In some experiments convulsions occurred. We now believe that these convulsions were due to a combined effect of the local anesthetic and the excitation state when coming out of the general anesthesia.

After several more (15) questionable experiments, we eliminated the general anesthetic and performed a series of 20 experiments in which we compared 1% lidocaine-HCl in solution and microcapsules (06-1-50, 150-212) as subcutaneous and intradermal injections. The subcutaneous injections were performed first with 0.5 ml of vehicle (5 mg). The results were variable. The most useful analysis was of the total number of anesthetic responses. Responses were elicited at approximately 5 minute intervals, and anesthesia was never observed after 65 minutes; hence we assumed that there were 13 possible negative responses (13 x 5 = 65 responses/group).

Table 5 shows the results of this analysis. All experiments were conducted with the USAIDR stimulator and EMG recording. In

120 minutes after injection



145 minutes after injection

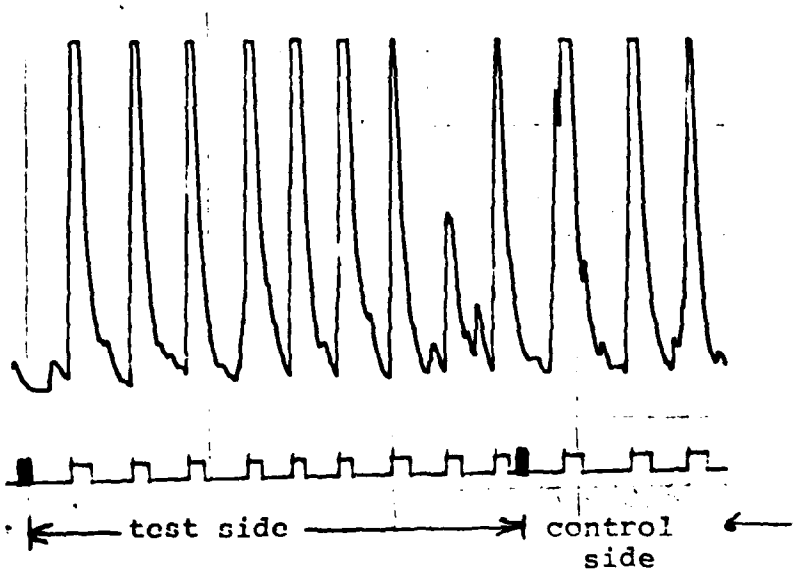


Figure 4 Integrated EMG of Guinea Pig Response (No. 8, 8/27/82) to 1 ml of 2% Lidocaine-HCl Solution Injected S.C.

TABLE 5

INJECTION OF 1% LIDOCAINE-HCl IN GUINEA PIGS

	Subcutaneous (0.5 ml, 5 mg)		Intradermal (0.25 ml. 2.5 mg)			
	Soln.	M.C.	Soln.		M.C.	
	USAIDR EMG	USAIDR EMG	USAIDR EMG	Needle Twitch	USAIDR EMG	Needle Twitch
Total negative responses	10/65	7/65	9/65	37/65	5/65	15/65
Animals with negative response	5/5	3/5	4/5	5/5	3/5	5/5
Ave. time of anesthesia (minutes)	-	-	-	51±10	-	15±10

addition, in the intradermal studies we also performed the test by the standard method, devised by Bülbring and Wajda (1945), in which a hand-held needle is used as the stimulus and the visible twitch is observed. This stimulus is weaker and the test method is more sensitive. However, we would anticipate that the mechanized stimulus method is more repeatable and reliable.

The results of this study indicate that the same quantity of lidocaine is less effective in microcapsules than in solution.* In this quantity there is no indication of a longer action of the encapsulated drug. There were insufficient negative responses with the USAIDR stimulator to obtain a reliable duration of anesthesia. One of the problems was demonstrated by using the USAIDR stimulator with the needle completely retracted. A twitch was often elicited under these conditions. However, with the tactile-visual method, durations of 51±10 minutes of anesthesia was observed with the solution and 15±11 minutes with the microcapsules.

Although we had anticipated more consistant data, we continued intradermal studies using both test methods and using more local anesthetics. These data are shown in Table 6. Since lidocaine is a less potent drug than etidocaine, and lidocaine-HCl microcapsules contain more polymer, more analgesia was achieved with etidocaine. The results indicate that large quantities of relatively rapid releasing etidocaine microcapsules can provide long term anesthesia (e.g., 4mg of microencapsulated etidocaine-HCl provided anesthesia after five hours). Unfortunately, we could not prove that this is advantageous over a solution of etidocaine. At higher concentrations of pure or encapsulated drug, tissue damage occurred.

As more animal experiments were being run in parallel the surface electrodes were sent through a switch box to the EMG. However, when one animal got loose, all recording normally stopped. Dr. Wynkoop sent us a set of Copeland-Davies surface electrodes (available from Ealing Corp., Natick, Mass.) which held better and did not cause as much trauma as the suture needles. Other experiments were performed with wound clips inserted as electrodes, and small alligator clips and light wire attached to the clip. All of these approaches present some discomfort to the animal or a possible means of unintentional disconnection.

* However later data indicated that the intradermal injection of microcapsules led to incomplete delivery of the microcapsules.

TABLE 6

INTRADERMAL STUDIES OF ANESTHESIA

	Drug	ml*	Soln. mg	M.C.** mg	Duration of Anesthesia EMG	Visual
1.	Lidocaine	0.25	2.5	2.5	:40	:45
2.	Lidocaine	0.25	2.5	5.0	:20	:45
3.	Etidocaine	0.25	2.5	-	1:10	1:20
4.	Etidocaine	0.25^{-}	-	2.5^{-}	2:00	2:20
5.	Etidocaine	0.25^{-}	-	4.0^{-}	>5:00	>5:00
6.	Etidocaine	0.15	-	6.0	?	2-5 days**
7.	Etidocaine	0.25	1.25	-	?	∿ 5:00
8.	Etidocaine	0.25	10.0	-	>6:00	1-4 days**
9.	Etidocaine	0.20	4.0	-	?	4:45
10.	Etidocaine	0.20^{-}	-	4.0^{-}	(>3:45)	6:00

* Problems of injection, marked as 0.25^{-}, etc. denotes some liquid loss from intradermal wheal

** mg of drug in M.C.
Lidocaine-HCl, M.C., 06-1-50, 150-212, 50% drug
Etidocaine-HCl, M.C., 11-2-20, 74-106, 70% drug

*** Skin blanched and scab formed indicating tissue necrosis

In the above experiments we have used primarily the USAIDR tactile stimulator or a hand held needle. The needle has been connected to the arm of a momentary-on SPST switch which is used to trigger the EMG event marker. In all of these experiments we are impressed by the fact that a calm animal is required and the best data is obtained without EMG electrodes and with a minimal, rapid, tactile stimulus.

During these experiments we suspected that intradermal injection of microcapsules might not deliver the expected quantity of material. In three experiments the residual anesthetic in the syringes was measured and only 23% of the microencapsulated anesthetic was injected. Apparently the intradermal space acts as a sieve, preventing the passage of microcapsules from the needle. Other suspending media for the microcapsules were tried, without a noticeable improvement. Injecting saline to form the wheal prior to injecting the microcapsules was unsuccessful because the volume of the initial wheal could not be returned to the syringe. The syringe became plugged with the dermal tissue. We know that the syringe delivery of microcapsules was not a problem with intraperitoneal injections in mice and it could be overcome with intramuscular injections in rabbits. At that point we had become complacent. We now measure the quantity of material left in the syringe, and calculate the amount injected intradermally.

Using the hand stimulation and visual response technique, a series of experiments were performed to evaluate the effect of a combination of lidocaine solution and microcapsules. The quantity of microcapsules was increased to compensate for the loss due to the incomplete injection of microcapsules into the intradermal space. The results of these experiments were reproducible and complete sensory blockage was achieved for several of the initial 5-minute test periods. Sensory perception slowly returned and could be approximated by a straight line function. A least squares fit allowed a mathematical analysis of the time of return of 50% of feeling (t_{50}), and complete recovery (t_0). The correlation coefficient (r) gives a value of the reproducibility of the method and the slope (m) is a measure of how quickly the anesthetic wears off, after some sensibility returns. Each experiment was performed in triplicate on separate guinea pigs. Since six needle pricks were used at each time interval, each response equalled 5.5% (1/18) of the total. Statistical significance of this type of experiment can be achieved by the analysis of the total number of anesthetic responses between groups of guinea pigs. The data for this experiment are shown in Table 7. Figure 5 shows the results of the first test condition. Surprisingly small differences are seen between the various concentrations of solutions. A suspension of microcapsules was less potent than

TABLE 7

COMPARISON OF LIDOCAINE-HCl AS SOLUTION AND MICROCAPSULE SUSPENSION

(Data from 3 guinea pigs with 0.35 ml intradermal wheals)

Test Condition	mg of Lidocaine-HCl injected* as Solution	mg of Lidocaine-HCl injected* as Microcapsules**	Total mg Drug	t_{50} min.	t_0 min.	r	m %/min.	Total Anesthetic Response
1	1.2	1.0	2.3	71	135	-0.939	-0.78	252
2	1.2	2.3	3.5	83	146	-0.951	-0.78	289
3	2.3	0.8	3.1	62	112	-0.936	-1.13	214
4	1.2	-	1.2	62	112	-0.963	-1.02	215
5	2.3	-	2.3	58	95	-0.967	-1.44	199
6	3.5	-	3.5	57	93	-0.959	-1.37	197
7	-	0.6	0.6	35	118	-0.828	-0.60	144
8	-	1.9	1.9	48	100	-0.959	-0.96	165

* Based on residual assay

** 06-1-50, 150-212 micron

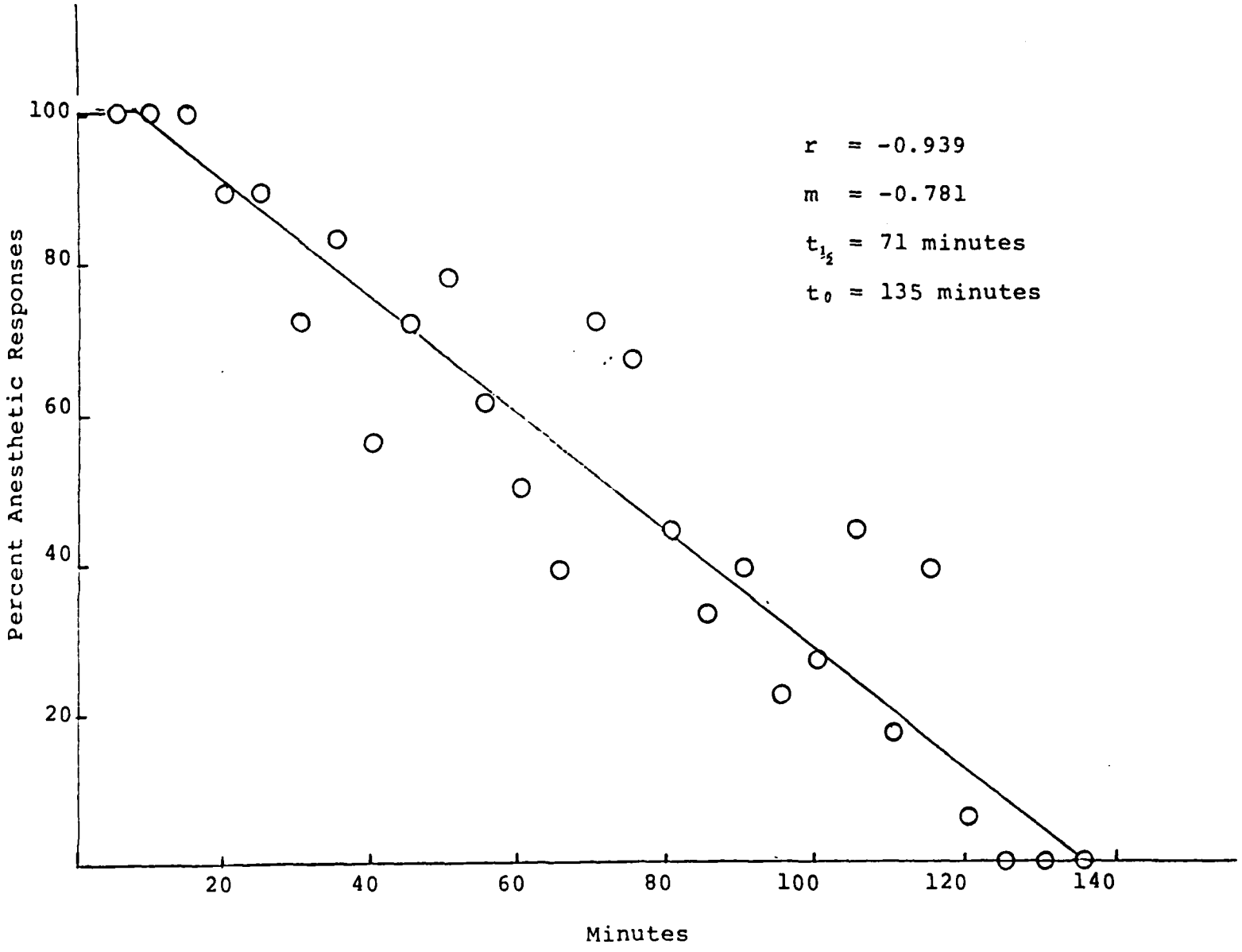


Figure 5 Anesthetic Response of Three Guinea Pigs Injected with 0.35 ml of ∿0.5% Lidocaine-HCl in Solution and in Microcapsules

the same amount of lidocaine in solution. The incorporation of microcapsules in a solution may be advantageous (comparison of 2 and 6, $t = -1.88$, $p < 0.10$). However we are looking for considerably longer periods of anesthesia. We now believe that the method of testing is reliable, but that we need more and/or more potent encapsulated anesthetics to achieve the required extended duration of anesthesia.

We are not the first to note that a calm guinea pig is necessary for the reliable measurement of pain using this procedure (Quevauviller, 1971). In the first set of the above experiments the EMG was used in addition to the visual observation of the twich. We believe that this method can be used to confirm visual observations in selected experiments. However the electrodes limit the rate at which data can be collected and analyzed.

2. Sciatic Nerve Block Experiments

In the previous report, data were presented on blocks of the sciatic nerve of rats which had been injected with 0.25 ml of 1% etidocaine-HCl as a solution or as a microencapsulated suspension (previous report, pp 59-61). The data indicated a longer duration of sciatic nerve block for rats injected with etidocaine microcapsules (175±58 minutes) than with the etidocaine solution (71±25 minutes). However, both of these mean durations were shorter than expected. These data were collected by our consultant, Dr. Gay at Northeastern University.

At BIOTEK we proceeded to increase the quantity of etidocaine, in order to achieve longer blocks. However, the conclusions of these experiments was that our technique needed improvement. Using 0.25 ml of 2% etidocaine-HCl in solution and in microcapsules, we obtained 120±91 minutes for the solution, but only 24±14 minutes for the microcapsule suspension. Doubling the concentration again for the microcapsules gave only a 61±19 minute block.

At this point the microcapsules were changed from the standard (70% drug, 106-300 μm) preparation to a faster releasing (80% drug, 74-106 μm) preparation. Results were variable, but 3 of 5 rats were blocked for more than four hours (one for more than 6 hours), when injected with 4% etidocaine microcapsules. When injecting 8% etidocaine in microcapsules 2 of 5 blocks lasted longer than 11 hours. All animals recovered by the next day.

In order to achieve better reproducibility we went to Northeastern University and worked with Dr. Gay to improve the technique. About 15 rats, which were scheduled for sacrifice, were injected with a dye supension. About 30 cut-downs were per-

formed and the method of injection was changed. Unfortunately, injection by the new technique did not improve the precision of the results. However, one rat injected with an 8% suspension of microencapsulated etidocaine, remained blocked for about 96 hours, and another for about 46 hours. Since almost all rat nerves were blocked for a few minutes after the injection, we tend to discount the hypothesis that a higher concentration is required initially in order to initiate the block.

Since the treatment of an avulsive wound would involve peripheral nerve endings rather than blockage of a nerve trunk, it was the decision of the USAIDR review committee to not fund this activity in the extended contract. However, the sciatic nerve block is a simple test with a well defined end point which can be easily performed by anyone at odd hours of the day or night. It has indicated that anesthetics with a short in vitro drug release pattern may be more useful than expected in in vivo studies.

3. Circulating Levels of Lidocaine in Rabbits

Following discussions with Dr. Judson Wynkoop and Robert Miller, a method of injection of lidocaine was developed which was similar to that used by the USAIDR personnel. Thus their previous data on blood levels of lidocaine in rabbits following intramuscular injection of various quantities of lidocaine-HCl solution could be used as control data. After reviewing the in vitro release data of various lidocaine microcapsules, we decided to perform a pilot study with three rabbits injected with lidocaine solution and three rabbits with lidocaine (base) microcapsules.

Six injection sites were chosen and marked on the thigh (2 rows of 3 sites, 1 cm between rows and 0.75 cm between sites in a row). The syringe was marked at 1.2 cm and inserted to this depth. Three milliliters were injected as 0.5 ml injections. The suspension was prepared as 213 mg of 75% lidocaine (base) microcapsules (150-212 μm) in 4 ml of HPC vehicle. This was designed to give 3 ml of a 4% lidocaine (base) injection in free and microencapsulated forms. The solution was prepared as 198 mg of lidocaine-HCl which was dissolved in 4 ml of HPC vehicle.

The blood samples were drawn at standard times at BIOTEK, and the analysis of lidocaine was measured by a GC/MS procedure at USAIDR. By omitting the GC/FID data, the averages are shown

in Table 8. Some early blood values from injections of lidocaine solutions were above the therapeutic range (>5 µg/ml) as defined from use of lidocaine to suppress ventricular arrhythmias. However, no toxic effects were noted in these rabbits. The fast decrease of blood lidocaine values is consistent with the 95 minute half life of this drug (Covino and Vassallo, 1976).

Since lidocaine is primarily metabolized by the liver, the area under the curve (AUC) should be similar for any administration of the same quantity of drug. Any difference is generally associated with a decreased bioavailability. Thus the encapsulated base showed very little bioavailability in a useful time frame (up to one day). Furthermore the small amount that was measured as circulating lidocaine was rapidly released to the circulation. We then repeated this study using a fast releasing lidocaine-HCl microencapsulated preparation.

These lidocaine-HCl microcapsules had an in vitro release of 50% in two hours (Report 2111-3, page 25). Based on our earlier measurement of blood levels of lidocaine from similar lidocaine-HCl microcapsules (DAMD17-80-C-0110, Annual Report, July 23, 1981, page 27), we anticipated measureable values of lidocaine from the microencapsulated product in this new experiment.

The injections of lidocaine-HCl solutions and microcapsule suspensions were performed as in the previous experiment. The samples were sent on October 5, 1982 by Federal Express, with dry ice to Robert Miller at Fort Meade. The suspension was prepared as 340 mg of 47% lidocaine-HCl microcapsules (150-212", Run 06-1-50) in 4 ml of HPC vehicle. This was designed to give 3 ml of a 4% lidocaine-HCl injection in free and microencapsulated form. The solution was prepared as 160 mg of lidocaine-HCl which was dissolved in 4 ml of HPC vehicle.

The intramuscular injection of lidocaine as solution and microcapsules proceeded smoothly. As in the previous experiment, the force required for the IM injection sometimes led to some sieving effects, with a packed bed of microcapsules left in the syringe. When this happened we resuspended the microcapsules in additional vehicle and re-injected. In the first experiment with these microcapsules, we weighed the solids remaining in the syringe and vial after injection of 3 ml of the suspension. We found 102 mg and calculated that 97 mg should have been left. Therefore, we believe that the method does deliver the appropriate amount of microcapsules.

It should be noted that we had agreed to use a 4% solution of anesthetic. This contains the same quantity of lidocaine as was used in the 6% study at USAIDR. Thus 3 ml of 4% should be similar to 2 ml of 6% USAIDR solution. Finally, there is less lidocaine in 4% lidocaine-HCl than in lidocaine (base). Hence a

TABLE 8

CIRCULATING LEVELS OF LIDOCAINE FOLLOWING IM INJECTION OF SOLUTIONS AND MICROCAPSULES OF LIDOCAINE BASE

Rabbit No.	Weight (kg)	Exp. Date	Serum Levels (µg/ml) at 0:10	0:30	1:00	2:00	4:00	6:00	10:00	24:00	48:00
LIDOCAINE SOLUTION INJECTION											
365	4.9	6/10	4.72	5.58	2.33	1.22	0.72	0.27	0.05	0.04	--
365	4.9	6/14	5.90	6.97	2.94	1.52	0.90	0.34	0.05	0.04	--
367	4.4	6/7	3.85	4.38	0.52	0.30	0.09	0.25	0.13	0.02	--
	→	Mean	4.82	5.64	1.93	1.01	0.57	0.29	0.08	0.03	
		SEM	0.59	0.75	0.72	0.37	0.24	0.03	0.03	0.01	
365(FID)		6/10	1.69	1.21	0.73	0.42	0.20	0.04	0.09	0.05	
LIDOCAINE MICROCAPSULE INJECTION *											
364	4.2	6/10	0.26	0.18	0.10	0.09	0.07	--	0.06	0.01	--
367	4.4	6/14	0.26	0.26	0.20	0.19	0.10	0.05	0.09	0.05	0.01
368	4.4	6/7	0.30	0.05	0.05	0.04	0.04	0.03	0.01	0.00	0.00
	→	Mean	0.21	0.16	0.12	0.11	0.07	0.04	0.05	0.02	0.01
		SEM	0.08	0.06	0.04	0.05	0.02	--	0.02	0.02	--

* Lidocaine (base) microcapsules, 11-1-30, 75% drug, 150-212 microns, 120 mg of lidocaine (base)

difference of 1.24 may be expected, based on the molecular weight difference. However, the purity and dryness of these materials will also affect this factor.

The only toxic reaction occurred with Rabbit 350 on September 3, 1982. About 7 minutes after the injection of the lidocaine solution, the rabbit went into a toxic flexion convulsion. She was removed from the restrainer and appeared quite cyanotic for several minutes (7-10 minutes). She had an arched back and neck rigidity for about 20 minutes after the injection. The rabbit was bled, outside the restrainer at about 15 minutes. She appeared to be out of danger at that time, although still convulsing.

Rabbit 190 (September 13, 1982) flailed her legs for several seconds prior to being bled at the 4 hour point. All other bleedings were unremarkable.

The GC/MS analysis of amide anesthetics and their metabolites is a time-consuming process, and the results were not immediately available to us. Fortunately, circulating levels of lidocaine are measureable using an enzyme immunoassay procedure which was developed by Syva for cardiac patient monitoring. This is a rapid, stat, procedure. We therefore used this procedure on one set of rabbits (186, 167, on 9/20/82) to generate sufficient data to indicate a release of lidocaine from these microcapsules.

We had to modify the method for use with the equipment in our laboratory. The standard method uses a pre-dilution (6-fold, 50 µl to 300 µl) of the serum. For the later time samples we omitted this dilution. The method is simple, sensitive, and specific. It is almost certain to be useless for etidocaine and will not measure any metabolites of the anesthetics. The data are shown in Figure 6. Almost no lidocaine was measureable at 10 and 24 hours. The results indicate the absence of toxic (> 5 µg/ml) concentrations of lidocaine at 10 and 30 minutes after injection when using the microencapsulated product. Local release of lidocaine during this period should produce local anesthesia, without systemic or local toxicity.

At this point future experiments were discussed with Dr. Wynkoop at USAIDR. We decided to increase the number of rabbits in this study from three to six. At this point the effect of subcutaneous injections of the same solution and microcapsule suspension would be evaluated. We have now completed the six intramuscular experiments and the three subcutaneous experiments with lidocaine-HCl as a solution and as a suspension of microcapsules.

The results of the GC/MS procedure of Leo Kazyak are shown in Table 9 and Figure 7. For calculation of the mean and for plotting, the one hour data point of Rabbit 167 on 9/20/82 was

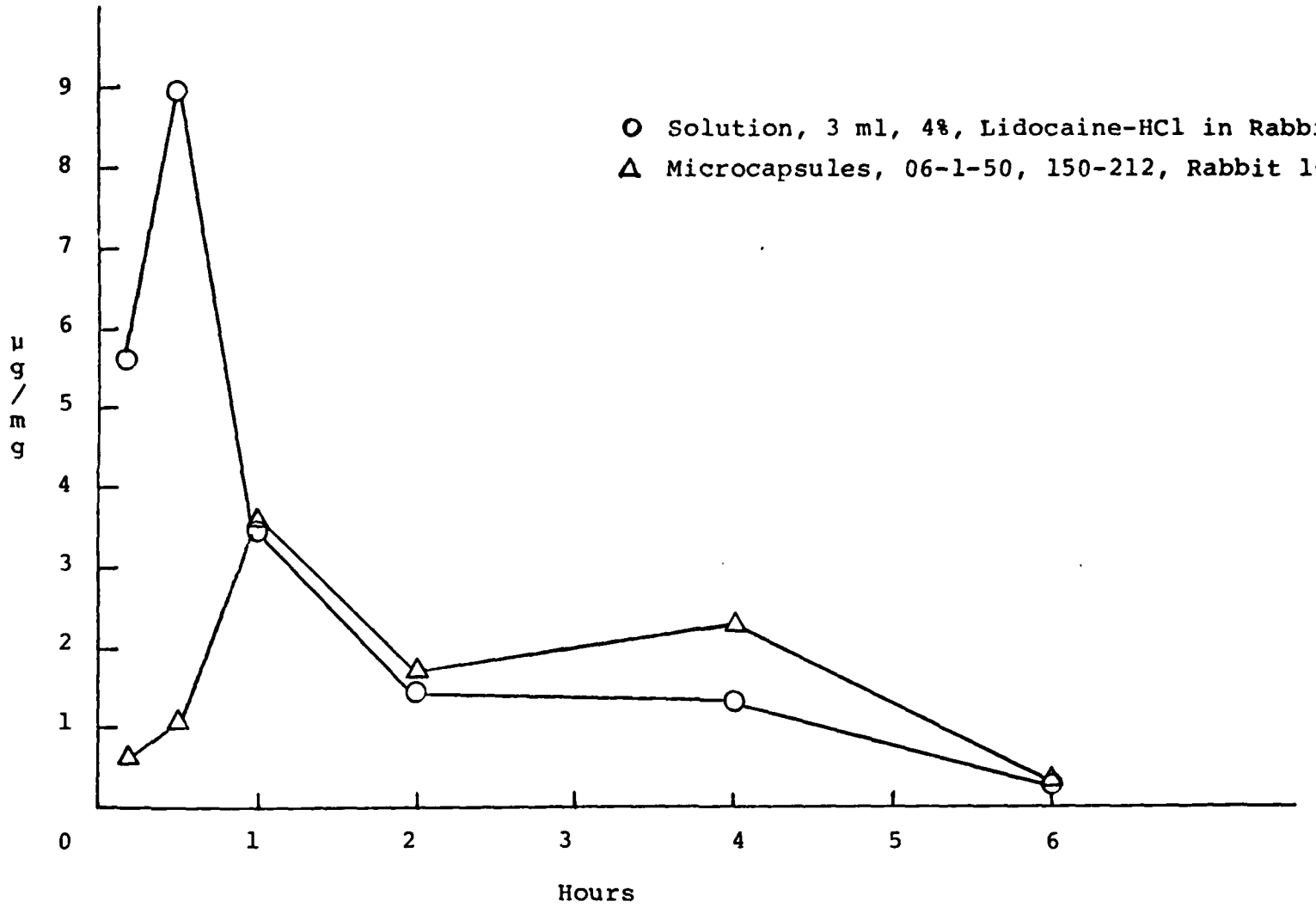


Figure 6 Serum Levels of Lidocaine after I.M. Injection of 120 mg of Drug as Solution or Microcapsules (EMIT Assay at BIOTEK)

TABLE 9

CIRCULATING LEVELS OF LIDOCAINE FOLLOWING IM INJECTION OF SOLUTIONS AND MICROCAPSULES OF LIDOCAINE-HCl

Rabbit No.	Weight (kg)	Exp. Date	Serum Levels (μm/ml) at 0:10	0:30	1:00	2:00	4:00	6:00
LIDOCAINE SOLUTION INJECTION								
167	5.2	8/31	3.83	2.16	1.09	0.78	0.22	0.03
350	4.4	9/13	8.71	1.91	1.34	0.50	0.25	0.16
186	5.3	9/20	4.19	2.88	0.59	0.25	0.17	0.06
Mean		→	5.57	2.31	1.01	0.51	0.21	0.08
SEM			±1.57	0.29	0.22	0.15	0.02	0.04
186	(EMIT)	9/20	5.6	9.0	3.4	1.4	1.3	0.3
LIDOCAINE-HCl MICROCAPSULE INJECTION*								
169	5.4	8/31	0.75	0.80	0.91	0.78	0.17	0.03
190	4.5	9/13	0.16	1.02	0.89	0.67	0.28	0.11
167	5.2	9/20	0.28	0.41	(0.15)	0.61	0.22	0.09
Mean		→	0.40	0.74	(0.90)	0.69	0.22	0.08
SEM			±0.18	0.18	----	0.05	0.03	0.02
167	(EMIT)	9/20	0.6	1.1	3.6	1.7	2.3	0.3

* Lidocaine-HCl microcapsules 06-1-50, 47% drug, 150-212 μm

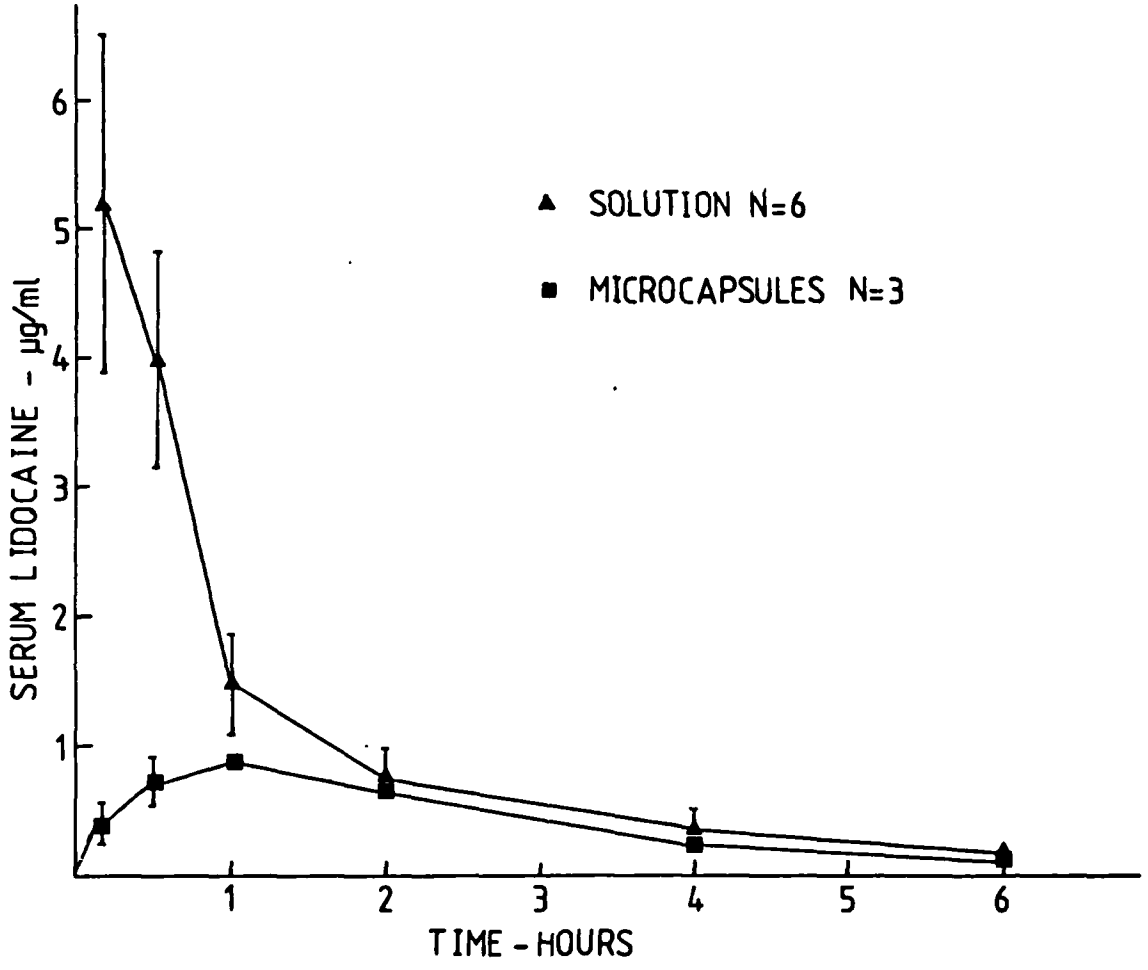


Figure 7 Serum Levels of Lidocaine After Injection of 120 mg of Lidocaine-HCl as Solution or Microcapsule Suspension (3 ml of 4% Lidocaine-HCl as Either a Solution or as a Suspension of Microcapsules of 47% Drug and 150-212 µm Were Injected I.M. in Rabbits) GC/MS Assay at USAIDR. Vertical lines are standard error of the mean.

removed. It did not seem reasonable, with respect to the other GC/MS values, nor with respect to our EMIT values. We also interchanged the ten minute data on 9/13/82 for Rabbits 350 and 190. Rabbit 350 almost died, the sample was taken about five minutes late, and probably went into the wrong tube.

Finally data obtained by BIOTEK by a GC/FID procedure using a double extraction procedure is re-plotted and shown in Figure 8. This experiment used the same microcapsules, but used only 20 mg as a subcutaneous injection (Contract DAMD 17-80-C-0110).

From all of this data, the trend is clear. The potential systemic toxicity of a rapid peak level of lidocaine is eliminated by microcapsulating the lidocaine-HCl. The anesthetic is released from the microcapsules over a period of several hours. The data is too sparse and variable to warrant a mathematical analysis, such as described by Smolen, et al, (1979), or Bjornsson and Desjerdins (USMRDC contractor, see Shand, et al, 1981). Other investigators have demonstrated the equivalency of the EMIT and a GC method (Pape, et al, 1978).

The lack of equivalency of areas under the curve (AUC) is worrisome. Lidocaine is primarily metabolized in the liver; local tissue metabolism is believed to be minimal. However the AUC for lidocaine-HCl microcapsules appears to be less than for the same quantity of drug in solution. Also, the lidocaine (base) microcapsules generated almost no bioavailable drug by the analysis of the sera collected for one day.

4. Local Toxicity (CPK Analysis)

As stated in the proposal, an increase in creatine phosphokinase (CPK) in blood is a sensitive indicator of muscle damage. This approach was used by Zener and Harrison (1974) to follow muscle damage after IM injection of a 10% lidocaine solution in human volunteers. CPK values increased about 7-fold from 40 to 300 IU/l. During the study in which 4% lidocaine was injected intramuscularly into rabbits, additional blood samples were taken for normal and 6-hour CPK values. All values were high, compared to humans, with the *norm* being about 3000 IU/l. Six hours after injection of microcapsules, the CPK was 17,000 IU/l, whereas 6 hours after the injection of a solution the CPK was 32,500 IU/l.

These tests were performed by a local clinical laboratory, using a kinetic uv determination of enzyme activity. Creatine phosphokinase (CPK) analyses were then performed at BIOTEK using the Sigma CPK-UV diagnostic kit (No. 45). The CPK activity in serum was determined at 6 and 24 hours following the intramuscular injection of 3 ml of vehicle, 4% lidocaine-HCl solution or 4% microencapsulated lidocaine-HCl. The data are shown in Table 10.

FIGURE 8

LIDOCAINE PLASMA CONCENTRATIONS AFTER S.C. IMPLANTATION OF DRUG CRYSTALS AND MICROCAPSULES

(20 mg OF LIDOCAINE-HCl NORMALIZED TO A 4 kg RABBIT) (GC/FID FOLLOW MATHER AND TUCKER, 1974)

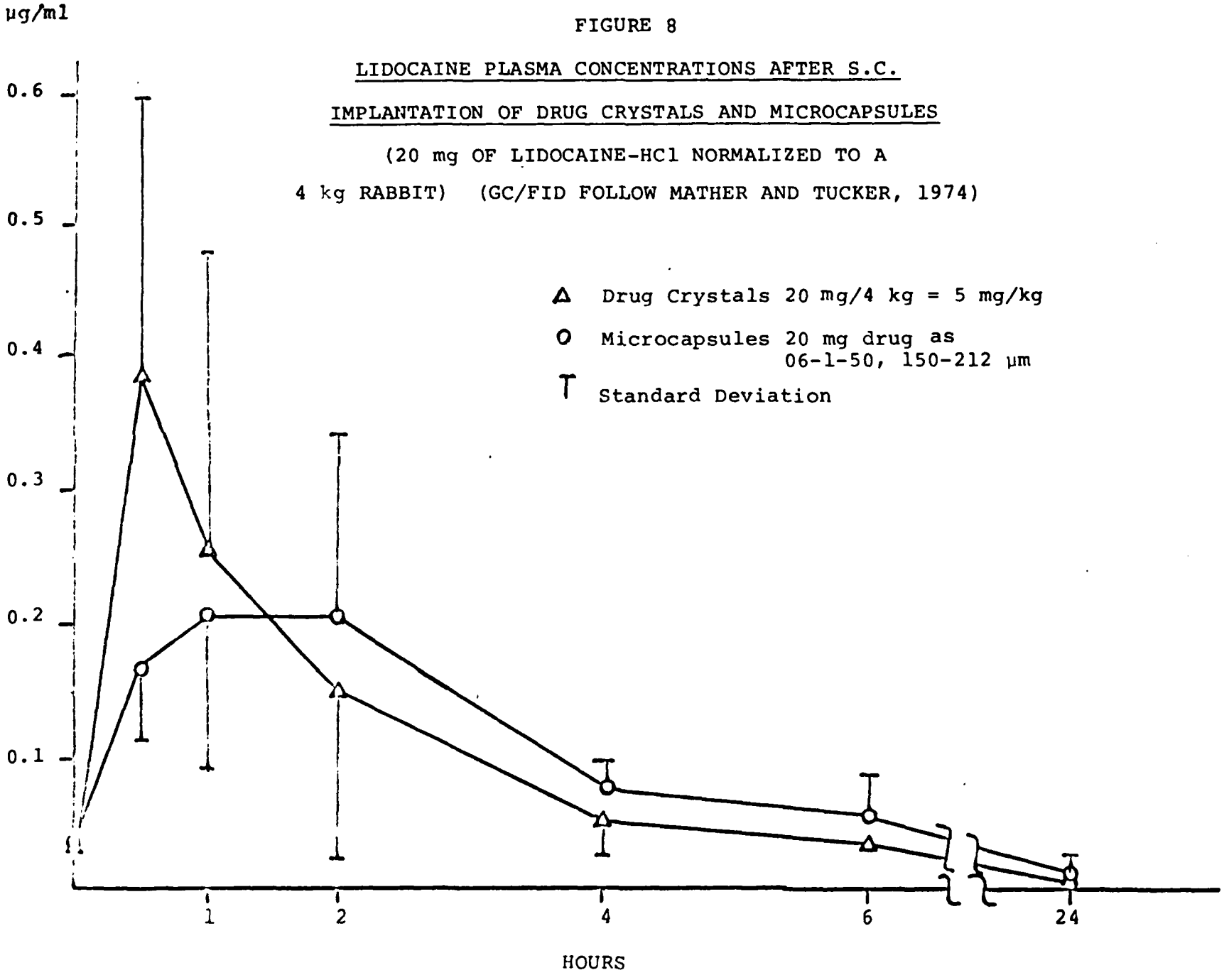


TABLE 10

LOWER LOCAL TOXICITY

Serum Levels of Creatine Phosphokinase After Injection of 120 mg of Lidocaine-HCl as Solution or Microcapsule Suspension

(3 ml of 4% Lidocaine-HCl as Either a Solution or as a Suspension of Microcapsules of 47% Drug and 150-212 μm Were Injected I.M. in Rabbits)

	Pre-Injection	24 Hours Post-Injection
Solution	345±56	4096±542
Microcapsules	377±53	1868±372
Vehicle	527±80	2228±430

Units are I.U./liter, Mean±SEM, n=6

Solution vs. Vehicle, $t = -2.7$, $p < 0.025$

The normal CPK values are much higher in rabbits (∿ 400 IU/liter) than in humans (normals 40 IU/liter). Following an intramuscular injection of hydroxypropylcellulose vehicle (0.3% in saline, using 3 ml at 6 locations), there was significant increase of CPK activity. A Student t-test of the lidocaine data indicates that the microencapsulated anesthetic is less damaging to the muscle tissue.

Data from subcutaneous administration of lidocaine solutions and microcapsules show a similar increase of CPK by lidocaine solution. However the CPK activities are significantly lower for this mode of administration. CPK values 24-hours after microcapsule injection is 410 IU/liter, and for lidocaine solution the value is 2,400 IU/liter.

III. REFERENCES

Bülbring, E. and Wajda, I. (1945), J. Pharmacal., 85, 78-84.

Covino, B.G. and Vassallo, H.G. (1976), "Local Anesthetics: Mechanisms of Action and Chemical Use, Grune and Stratton, NY, NY.

Pope, B.E. et al (1948), Clin. Chem., 24 2020-2022.

Quevauviller, A. (1971), "Local Anesthetics", Vol. 1, p. 291-318, Pergmon Press, Oxford.

Shand, D.G., et al (1981), Clin. Pharm. Ther., 29 542-457.

Smolen, V.F., Ball, L., and Scheffler, M. (1979), Pharm. Tech., 3, 89-102.

Zener, J.C., Harrison, D.C. (1974), Arch. Intern. Med., 134, 48-49.

IV. APPENDIX

A. Presented Paper

Data collected on this contract was presented at the Twelfth Annual Meeting of New England Pharmacologists. This meeting was held at the Marriot Hotel in Newton, Mass., on February 4-5, 1983.

The title, authors, and associations were listed as:

SUSTAINED RELEASE, MICROENCAPSULATED, LOCAL ANESTHETIC AGENTS

D.L. Williams, D.E. Creeden, E.S. Nuwayser, J.R. Wynkoop, L. Kazyak, D. Hadjilambris, M.H. Gay. BIOTEK, Inc., Woburn, MA 01801; U.S. Army Institute of Dental Research, Washington, D.C., 20012; Walter Reed Army Institute of Research, Washington, D.C., and Section of Pharmacology, Northeastern University, Boston, MA 02115.

The poster data is reproduced in the following pages. The message was:

1. Abstract
2. Variation of drug delivery rate by choice of anesthetic

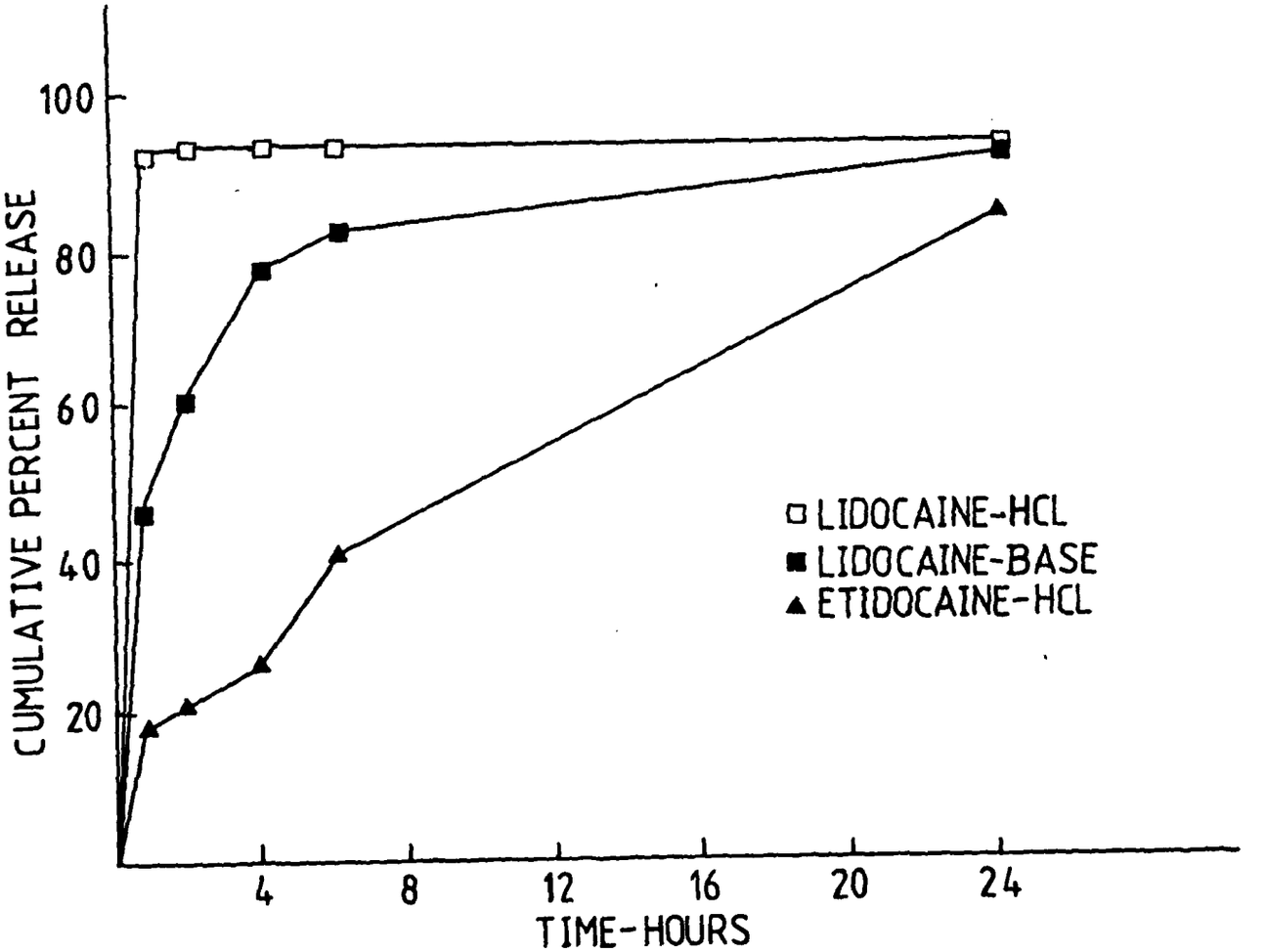
Solubility:	Lidocaine-HCl in water	570 mg/ml
	Lidocaine (base) in water	3.5
	Lidocaine-HCl in buffer	240
	Etidocaine-HCl in buffer	24
	Bupivacaine-HCl in buffer	39

3. Drug release slower from larger microcapsules.
4. Drug release slower from microcapsules with more polymer coating.
5. Drug release of microcapsules selected for *in vivo* studies.

6. Circulating levels of anesthetics shows evidence of sustained release.
7. Lower systemic toxicity of microencapsulated anesthesia.
8. Lower local toxicity of microencapsulated anesthetics.
9. Increased duration of anesthesia by encapsulated anesthetic.

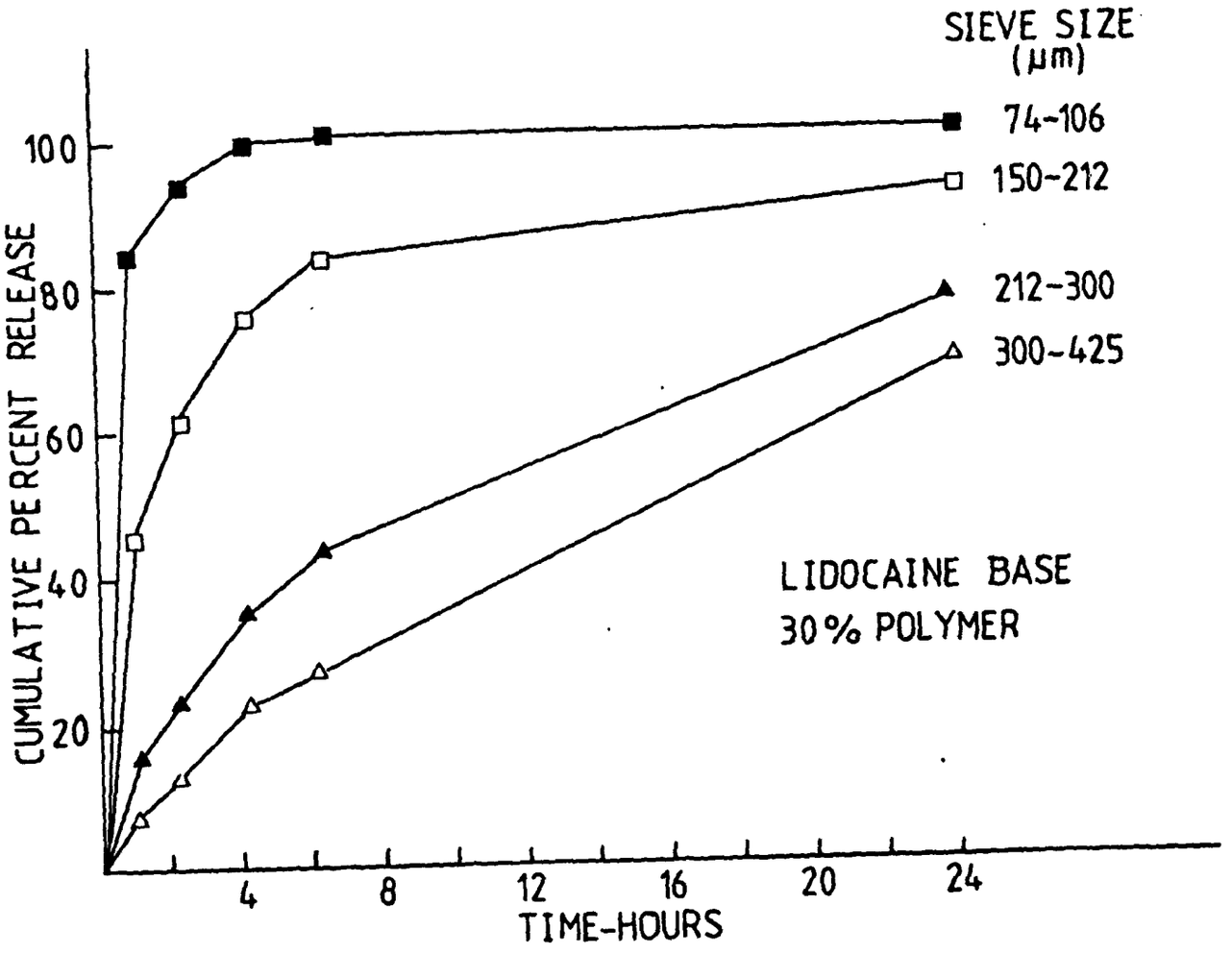
ABSTRACT

Microencapsulated local anesthetic agents were prepared by coating crystals with polylactide using the Wurster air suspension method. *In vitro* rate of release from microcapsules was proportional to the solubility of drug and inversely proportional to both the size of microcapsules and thickness of coating. Both LD-50 and median convulsant dose (CD-50) in mice were higher for suspensions of microcapsules than for solutions; 4.1, 5.0, and 6.7 times for etidocaine-HCL (ETIDO.), lidocaine-HCL (LIDO.) and bupivacaine-HCL respectively. Following I.M. injection in rabbits of 3 ml of 4% LIDO. in solution a toxic peak blood level (5.6 μg/ml) was observed at 10 min. while following microcapsules a lower peak (0.9 mg/ml) was observed at 1 hr. From 2 to 6 hrs. no differences in blood levels between solution and microcapsules were observed. Rabbit serum CPK, a measure of tissue damage, was higher following I.M. injection of 3 ml of 4% LIDO. solution than for a suspension of microcapsules. Duration of rat sciatic nerve block (0.25 ml, 1% ETIDO.) was greater for suspension of microcapsules 175 (± 26 SE) min. than for a solution, 71.0 (± 11.2 SE) min. These preliminary data suggest that microencapsulated local anesthetic agents give sustained release, lower toxicity, and longer duration than solutions. (Supported by contract #DAMD17-80-C-0110 and DAMD17-81-C-1195).



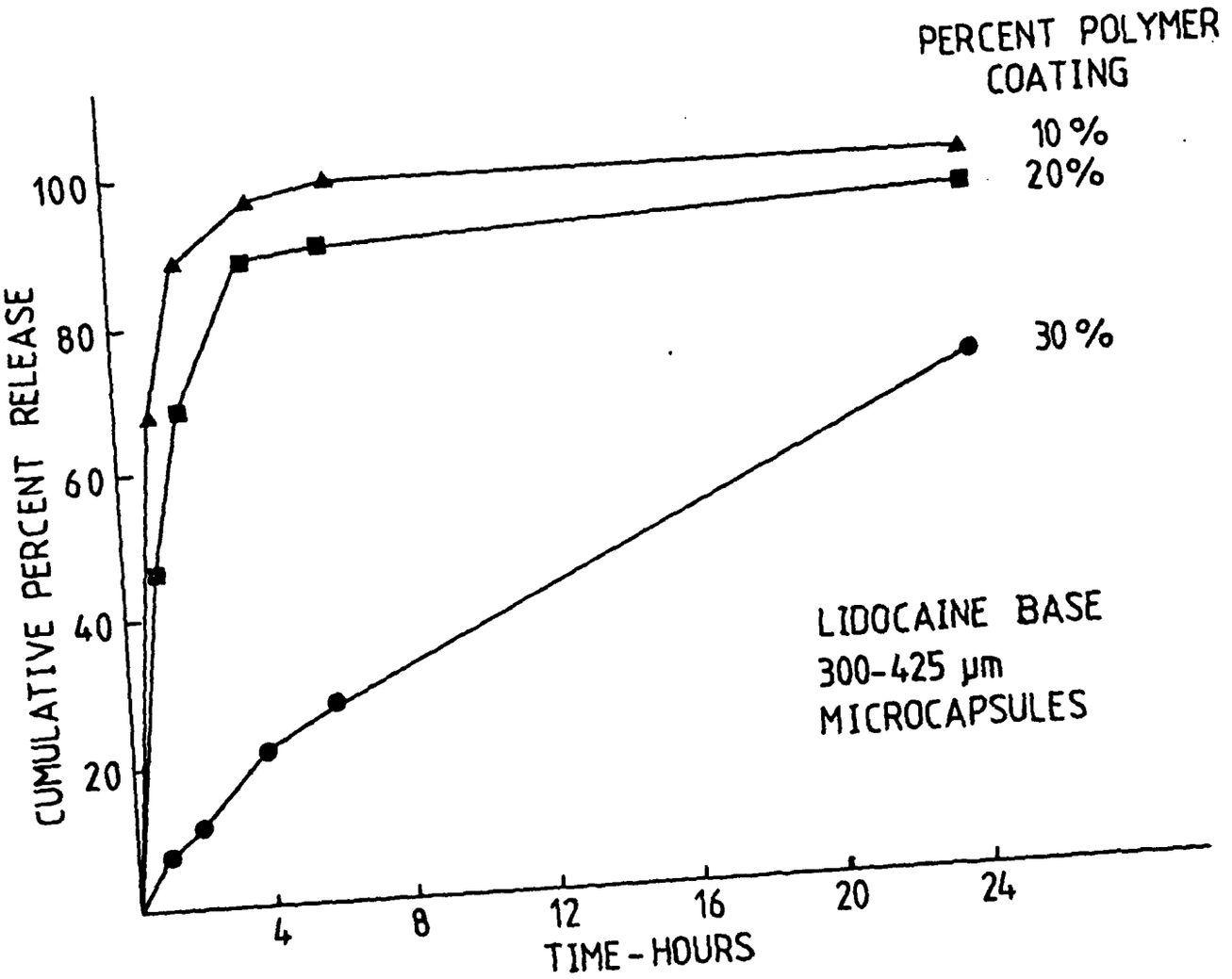
RELEASE OF VARIOUS AMIDE ANESTHETICS INTO PH 7.4 BUFFER

(30% Polymer Coating, 150-212 μm Sieve Size)

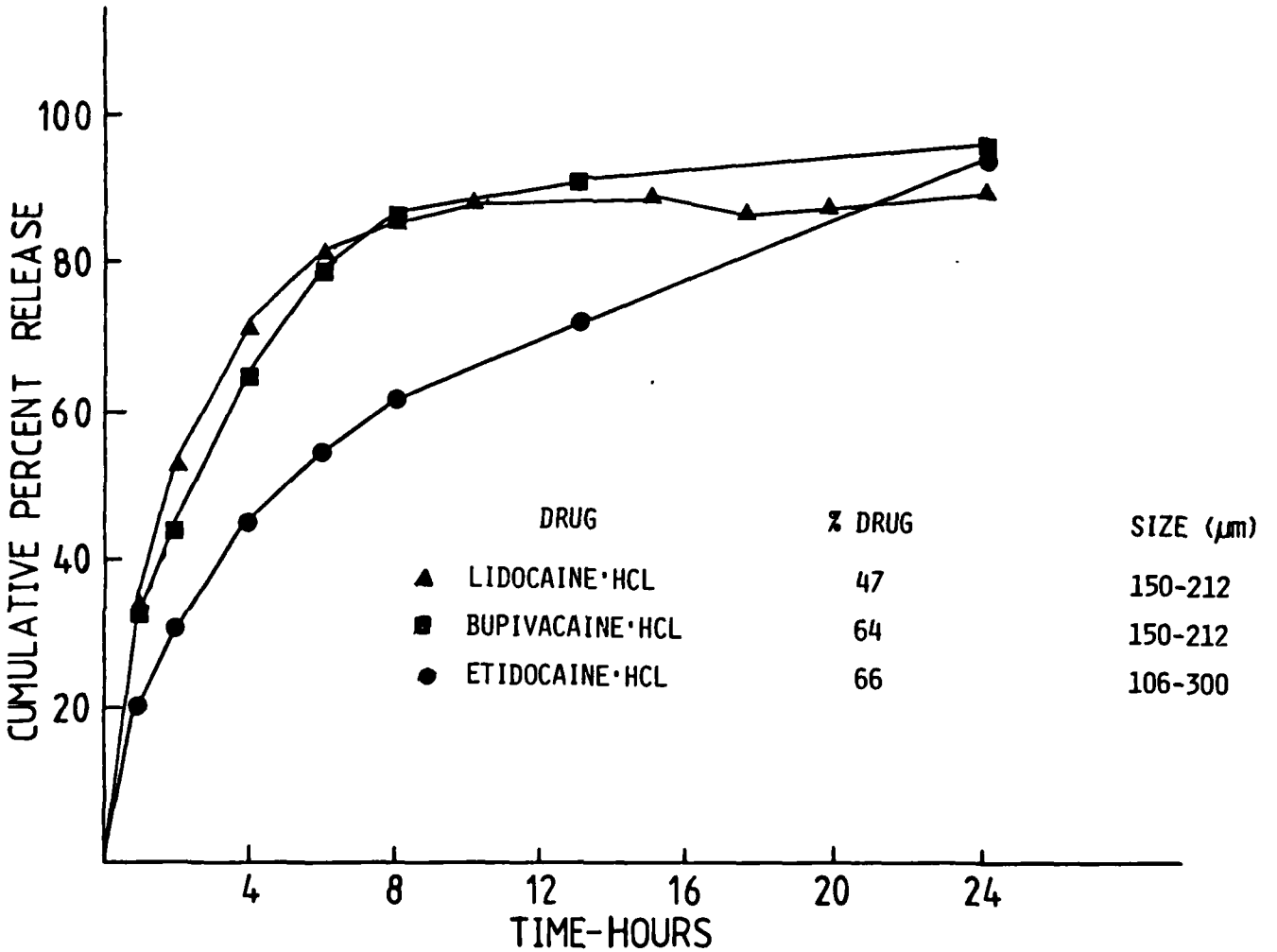


RELEASE OF ANESTHETIC FROM MICROCAPSULES INTO PHOSPHATE BUFFER

AS A FUNCTION OF SIZE

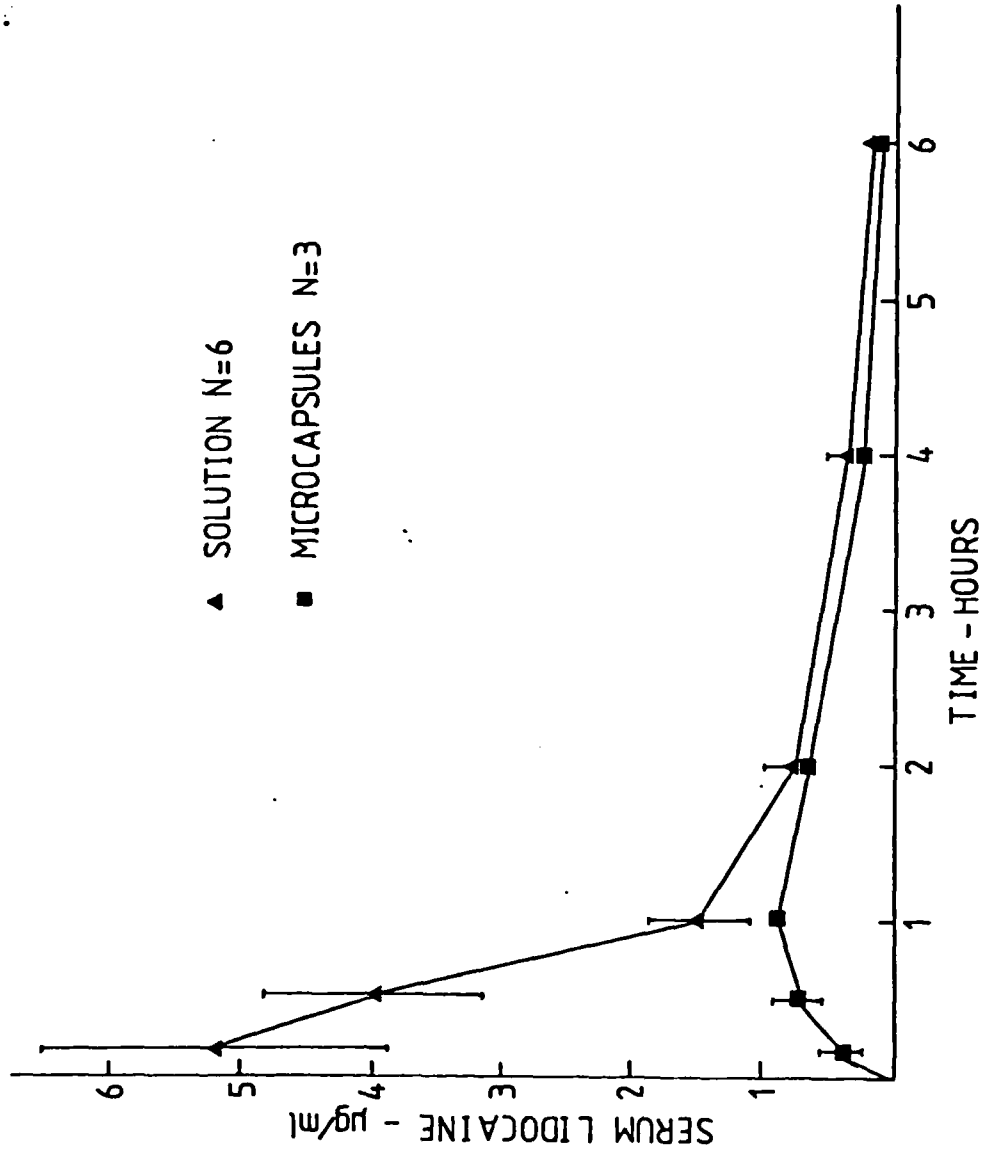


RELEASE OF ANESTHETIC FROM MICROCAPSULES INTO PHOSPHATE BUFFER AS A FUNCTION OF POLYMER COATING



MICROENCAPSULATED ANESTHETIC AGENTS SELECTED FOR IN VIVO STUDIES

(In Vitro Release Into pH 7.4 Phosphate Buffer)



SERUM LEVELS OF LIDOCAINE AFTER INJECTION OF 120 MG OF LIDOCAINE·HCL
AS SOLUTION OR MICROCAPSULE SUSPENSION
(3 ml of 4% Lidocaine·HCl as Either a Solution or as a Suspension of
Microcapsules of 50% Drug and 150-212 µm Were Injected I.M. In Rabbits)

LOWER SYSTEMIC TOXICITY

RELATIVE TOXICITY OF ENCAPSULATED AND FREE ANESTHETICS

		LD 50	CD 50	LD50/CD50
LIDOCAINE	Solution	181±12	92± 7	1.99
	Microcapsules	838±78	488±42	1.72
	M.C./Soln	4.6	5.3	
ETIDOCAINE	Solution	62± 7	47± 4	1.32
	Microcapsules	260±34	191±21	1.36
	M. C./Soln	4.2	4.0	
BUPIVACAINE	Solution	68± 5	42± 6	1.63
	Microcapsules	453±40	280±44	1.62
	M.C./Soln	6.7	6.7	

The LD 50 and convulsant dose (CD 50) were determined for solutions and suspensions of microcapsules following I.P. injection into CD-1 mice. Data is expressed as the mean dose of the drug hydrochloride in mg/kg ± SEM.

LOWER LOCAL TOXICITY

SERUM LEVELS OF CREATINE PHOSPHOKINASE AFTER INJECTION OF 120 MG OF LIDOCAINE·HCL AS SOLUTION OR MICROCAPSULE SUSPENSION

(3 ml of 4% Lidocaine·HCl as either a Solution or as a Suspension of Microcapsules of 50% Drug and 150-212 um Were Injected I.M. in Rabbits)

	PRE-INJECTION	24 HOURS POST - INJECTION
SOLUTION	345± 56	4096±542
MICROCAPSULES	377± 53	1868±372
VEHICLE	527± 80	2228±430

Units are I.U./liter, Mean ± SEM, N=6

INCREASED DURATION

DURATION OF RAT SCIATIC NERVE BLOCK FROM SOLUTION AND MICROCAPSULE SUSPENSION OF ETIDOCAINE·HCL

ANESTHETIC DURATION IN MINUTES
0.25 ML OF 1% ETIDOCAINE·HCL

SOLUTION	MICROCAPSULE SUSPENSION
71.0±11.2	175.1±26.1

$p < 0.01$

Microcapsules of 30% coating level, 106-300 μm

DISTRIBUTION LIST

Commander
US Army Medical Research and Development Command
ATTN: SGRD-RMS
Fort Detrick, Frederick, Maryland 21701-5012

Defense Technical Information Center (DTIC)
ATTN: DTIC-DDAC
Cameron Station
Alexandria, VA 22304-6145

END

FILMED

2-85

DTIC