AD-A130 839 LOCAL ANESTHETIC MICROCAPSULES(U) BIOTEK INC WOBURN MA 1/1
D L WILLIAMS ET AL. 15 APR 81 2106-1 DAMD17-80-C-0110

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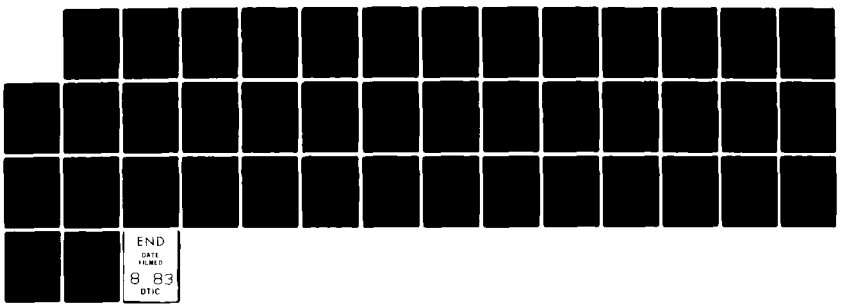

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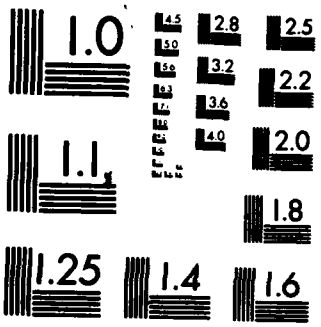

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LOCAL ANESTHETIC MICROCAPSULES

Annual Summary Report

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James H. Kerrigan, B.S.
William A. Nucefora, B.S.

April 1981

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-80-C-0110

Biotek, Inc.
Woburn, Massachusetts 01801

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REPORT DOCUMENTATION PAGE

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1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A130839 / 3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Local Anesthetic Microcapsules	5. TYPE OF REPORT & PERIOD COVERED Annual 1 July 1980-30 March 1981
	6. PERFORMING ORG. REPORT NUMBER 2106-1
7. AUTHOR(s) David L. Williams, Ph.D. James H. Kerrigan, B.S. William A. Nucefora, B.S.	8. CONTRACT OR GRANT NUMBER(s) DAMD 17-80-C-0110
9. PERFORMING ORGANIZATION NAME AND ADDRESS BIOTEK Inc. 21-C Olympia Avenue Woburn, Mass. 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 April 15, 1981
	13. NUMBER OF PAGES 42
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

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

Three kilograms of poly-L(-)lactide has been synthesized for this contract and for Contract No. DAMD 17-80-C-0093. This polymer has a viscosity (R.S.V.) of 1.19 dl/g, a molecular weight (MW_w) of 45,700 and a polydispersity of 2.14. Material from a previous contract was used for preliminary experiments.

Bupivacaine was not commercially available in the pure form and etidocaine was substituted. A purchase order for the synthesis of bupivacaine has since been issued to Chem Biochem Research, Inc. Delivery of 1 kg is expected by April 30,1981.

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Both lidocaine (HCl) and etidocaine (HCl) have been successfully microencapsulated using the Wurster coating process. Samples were taken at 10, 20, 30, 40, and 50% polymer loading (60% also for etidocaine). These samples were sieved and various size fractions were analyzed for drug release. Large microcapsules containing 50% lidocaine are required to deliver drug in vitro at the target rate (e.g. 150-212 μm deliver 36% in 1 hour, 54% in 2 hours, 72% in 4 hours, and 82% in 6 hours). Etidocaine microcapsules release drug more slowly (e.g. 70% drug in 74-106 μm microcapsules deliver 17% in 1 hour, 24% in 2 hours, 39% in 4 hours, and 48% in 6 hours). Appropriate samples are being stored for stability testing.

Microcapsules from a previous program were used to develop animal model studies. Both lidocaine and etidocaine can be measured in blood by gas chromatography. Tactile response was measured after subcutaneous implantation of lidocaine crystals (neat) and microcapsules. A longer duration of anesthesia was observed for the encapsulated product. Circulating levels of lidocaine, after intramuscular implantation of microcapsules, showed a more constant level of the anesthetic than was found after an IM injection of pure lidocaine.

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I. SUMMARY

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II. ACCOMPLISHMENTS

A. Material Preparation and Selection

1. Polymer Preparation

Poly-L(-)lactide of a reduced viscosity of about 1.0 dl/g has been prepared and used by us for several government contracts. This was the polymer used for the preparation of lidocaine microcapsules by the Wurster process under Contract No. DAMD 17-79-C-9019. It was also the polymer used for much of the steroid encapsulation work performed under Contract No. NO1-HD-3-2738. The preparation of this polymer was described in detail in the annual report. The same method was used in the present contract.

The preparation of the polylactide was performed as follows: The lactide dimer was obtained from Boehringer-Ingelheim through Henley and Company. This dimer was purified by repeated recrystallizations from ethyl acetate shortly before use. Reagent Grade solvents were used for all operations. The lactide dimer was heated in a 120°C oil bath while stirring the melt, in vacuum, for 30 minutes to remove traces of volatile materials. Dry nitrogen was then introducted to release the vacuum. Next the bath temperature was raised to 180°C. To this mixture was added 0.2 ml of stannous octoate catalyst which is 6% in mineral oil. Within about 15 minutes after the catalyst addition, the polymer mixture reaches a maximum temperature. After about 30 minutes, the reaction is stopped by removing the mixture from the oil bath, and the polymer is allowed to cool to room temperature. The polymer block is dissolved in methylene chloride and the solution is decanted and treated with three volumes of isopropanol, by slow addition to a stirred solution.

Two kilograms of poly-(L-)lactide which has a reduced specific viscosity (R.S.V.) between 1.0 and 1.5 dl/g are required for this contract. A parallel contract (DAMD 17-80-C-0110) also required an equivalent amount of polymer of the same R.S.V. To improve the characterization and reproducibility of both programs, it was decided to combine the requirements of both programs in one blend of a number of batches of polymer.

Approximately 1,200 grams of poly-(L-)lactide (R.S.V.=1.0 to 1.2 dl/g) was available from a previous contract (DAMD 17-79-C-9020). This material was used for preliminary experiments on both contracts to quantify operating parameters.

Because of the narrow range of the specified R.S.V. and the excessive heat generated in large batches, preparation of this quantity of polymer was a lengthy process. A total of 12 batches (see Table I) of dimer have been polymerized for these two contracts. Four of these were in the right range and eight were not. Five of the eight batches were too high and three were too low. Two of the out-of-range batches were exchanged with a similar project which had two batches which fell within our range but which were out of range for that application.

All six batches of polymer were blended together after dissolving in methylene chloride. A total of 19.3 liters of this polymer solution was precipitated by slow addition of isopropyl alcohol in the ratio of 3 to 1 (57.9 liters IPA). Due to the large volumes this had to be carried out in three batches.

After precipitation, the polymer was removed from the solution by vacuum filtration, the pieces were pressed with rubber dam material in the Buchner funnel to remove most of the solvent. The polymer was then placed under vacuum to remove the residual solvent. Due to the low volatility of isopropanol and the large volume of polymer, the drying process took two weeks. When the polymer chunks were sufficiently dry, they were ground in a blender to a uniform size, shaken in a large bag and again placed under vacuum to complete the drying. Three random samples of this final mixture were taken and their viscosities determined in duplicate. The viscosity was found to be 1.19 ± 0.03 dl/g and the total polymer obtained was 3,048 grams which was a 90% yield.

TABLE I

POLYMERS PREPARED FOR COMBINED PROGRAM

Polymer No.	Starting Grams Dimer	Approximate R.S.V.
5-6-6	574	1.25✓
5-6-10	649	1.02✓
5-6-15	582	0.57
5-6-18	600	2.91
5-6-19	400	> 3.0
5-6-21	665	1.08✓
5-6-23	617	> 3.0
5-6-25	637	2.23
5-6-27	500	0.72
5-6-29	500	2.08+
5-6-30	500	1.57✓
5-6-31	300	0.92
7-12-2	500	1.40✓
7-12-7	500	1.29✓
TOTAL	7,524	

✓ used in blend (total of 3,388 gm)

+ exchanged with NIH program for last two polymers on list

The polymer is presently being stored under argon in the freezer. The polymer was placed in plastic bags into one-gallon cans which are tightly sealed (paint cans). The air was removed by vacuum and replaced by argon, just prior to sealing. As mentioned in the proposal, this procedure has been standardized, but is presumably much more stringent than necessary to prevent polymer degradation.

A sample of polymer was sent to Cambridge Analytical Associates for determination of molecular weight distribution by gel permeation chromatography. Samples were dissolved in hot trichlorobenzene. Duplicate injections of the sample were run 12 hours apart to determine whether the high temperature analysis would degrade the polymer. After 12 hours, no evidence of polymer degradation was evident. A series of μ Styragel columns (10^3, 10^4, 10^5, 10^6A) from Waters Associates were used at 145°C. The flow rate was 1.5 ml/min. and the change of refractive index was used for detection. The molecular weight determination was made by reference to polystyrene standards.

Data from the chromatograms of the polymer were reduced by standard methods. The results are shown in Table II. The weight-average molecular weight of 45,700 is higher than expected for this viscosity of polymer (Nuwayser, et al, 1976). However, provided later experiments are repeated with the same technique, any degradation can be quantitated.

2. Availability of Bupivacaine

Several efforts have been made to obtain bupivacaine for use on this program. Early lack of success led us to propose the use of mepivacaine as a substitute since it has a similar structure (see Table III). This drug was available from Sterling Organics for $190/kg. After the contracting agency requested bupivacaine, a more intense search was launched.

Breon Laboratories, a division of Sterling Drug, sells bupivacaine, under the trade-name, Markaine and appropriate officials were contacted. Col. Posey also attempted to obtain bupivacaine, but without success. In June, 1980, Mr. Meehan wrote that the drug could only be supplied in ampoules of 30 ml (saline), containing 0.75% bupivacaine hydrochloride. The cost would be $16,355/kg (0.75% in saline).

TABLE II

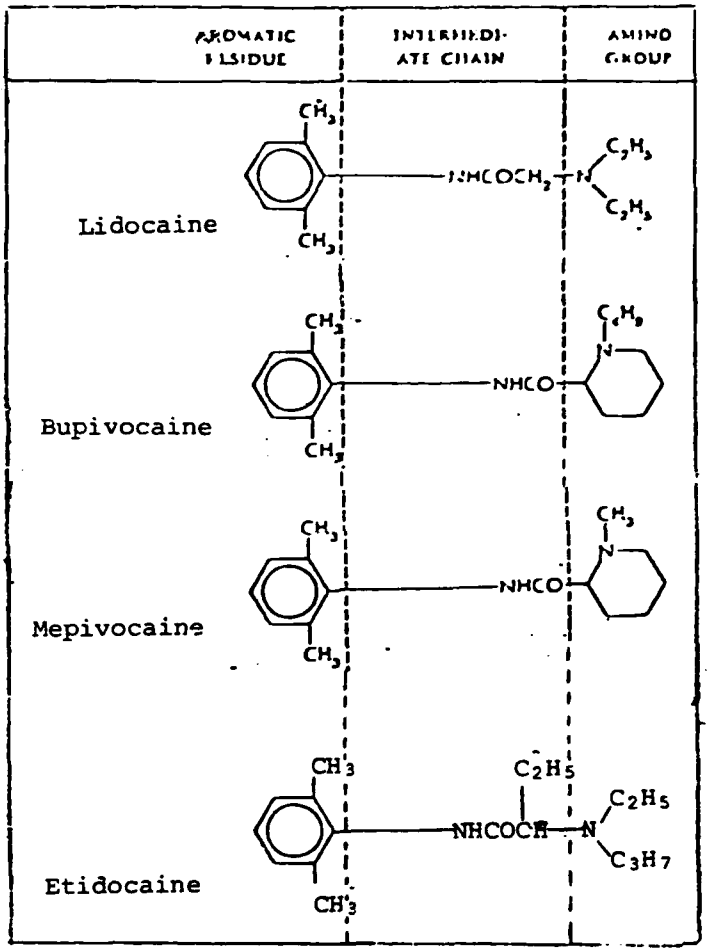
POLYMER MOLECULAR WEIGHT DISTRIBUTION

Sample: Poly-(L-)lactide (R.S.V. = 1.19 dl/g)

Number Ave. MW (MW_n)	21,500
Weight Ave. MW (MW_w)	45,700
$\Sigma n_i M_i^3 / \Sigma n_i M_i^2$ (MW_z)	60,700
Polydispersity (MW_w/MW_n)	2.14

TABLE III

CHEMICAL STRUCTURE OF LOCAL ANESTHETICS



Bofors was suggested as an alternative drug company source, and Mr. William Hahn was contacted in Bofors-Lakeway. Bofors does not sell bupivacaine in this country.

For the purposes of this research, drugs need not be sterile or USP grade. Thus drugs can be bought as chemicals from laboratory supply houses. However, no company carried bupivacaine as a stock item.

Several companies will synthesize chemicals that are not in their catalogue. Calls were placed to several such companies, giving details of the patent procedures (e.g., U.S. Patent 2,955,111, 1960, assigned to Bofors), and suggesting an alternate route using commercially available starting materials (2-pipecolinic acid and 2,6-dimethylaniline). Other companies were contacted which advertise world-wide connections for pharmaceuticals and other chemicals.

The only positive reply was from Chem Biochem Research, Inc., Salt Lake City, Utah. Their reply is attached as an appendix. They quote a price of $3,500-4,000/kg. Recently the contract was modified and a purchase order was placed for 1 kilogram of bupivacaine hydrochloride. We anticipate delivery by April 30, 1981.

3. Substitution of Etidocaine

Etidocaine is also a long-acting, potent, local anesthetic. It is sold by Astra under the tradename, Duranest. This drug is still under patent protection. Etidocaine had been considered for the present program and is being used in the companion program (Contract No. DAMD 17-80-C-0093). The structure of etidocaine is shown in Table III.

The possible use of etidocaine in this program was discussed with George Camougis, Ph.D., BIOTEK consultant, and Helen Vassallo, Ph.D., director of clinical research, Astra, Framingham, MA. Etidocaine and bupivacaine are the most practical (low toxicity) local anesthetics (Camougis, p.c.). Both etidocaine and bupivacaine are 4 times as potent as lidocaine on an isolated nerve trunk (frog nerve), but clinically about twice as much etidocaine (vs. bupivacaine) is administered for pain control (Vassallo, p.c.). This may be due to lipid solubility differences.

Efforts were then made to obtain a kilogram of etidocaine from Astra Pharmaceutical Products, Inc. At the request of Dr. Vassallo a suggested protocol was submitted with a statement assuring Astra that the drug will not be used in humans or food-producing animals. This request for etidocaine was granted (gratis), with the understanding that Astra be informed of the results of our studies.

B. MICROENCAPSULATION EXPERIMENTS

Lidocaine was encapsulated as soon as the final polymer was available. This run was made in Wilmington. After the Wurster equipment was moved to Woburn and reassembled, etidocaine was microencapsulated. Both runs followed the general method used for L-8(50%) of Contract No. DAMD 17-79-C-9019. In this previous run material from two separate 10% polymer runs were blended as the starting material for L-8. In the present experiments, the runs were continued after removal of the oversized material.

1. Lidocaine Microencapsulation

For lidocaine, the material was Sterling Lot N111RF. Methylene chloride was Baker Reagent, Lot 025824. The coating polymer solution concentration was 3% (w/v). The solution was added at 20 ml/min. up to a loading of 10% polymer on drug. The spraying rate was then dropped to 4.5 ml/minute. A 2850/50 Spraying Systems nozzle was used throughout, as in the previous contract work. The atomizing pressure was maintained at 25 psi, as in the previous work.

Table IV shows the processing losses. Oversize material was removed at each coating level, as shown in this table. Equipment holdup, losses, and sample removal lowers the yield from that expected from a continuous operation. However, the overall yield is 27.5%, of 50% coated drug in microcapsules of less than 250 μm. The overall coating time was 20 hours for this experiment. However, we believe that the spraying rate can be increased from 4.5 to 9 ml/min. for the 10 to 50% coating.

TABLE IV

PROCESSING SUMMARY OF LIDOCAINE MICROENCAPSULATION

Process Polymer %	Starting Sample g	Polymer Added g	Final Weight MC g	Final Weight (Drug) (g)	Losses Equipment Holdup g	Losses Oversize Removal g at (μm)	Samples Removed g
0-7	400	30	259	(238)	92	69 (150)	10
7-20	259	33	275	(220)	6	1 (250)	10
20-30	275	40	273	(191)	13	19 (250)	10
30-40	273	43	259	(155)	10	37 (250)	10
40-50	259	51	220	(110)	0	63 (250)	27 (220)
TOTALS		197			121	189	287

Material Balance: (in) 400 g (drug) + 197 g (polymer) = 597 g
(out) 287 g (samples) + 310 g (losses) = 597 g

Yield at 50% Coating (< 250 μm): 110:400 = 27.5%

Table V shows the size range of microcapsules at each of the coating levels. In the initial coating stage, considerable agglomeration occurs. This is the reason for sieving the material at 150 μm after about 10% polymer is added. Thereafter the material is more uniform and spherical. This leads to less agglomeration, although the particle size continues to rise at a faster rate than expected for overcoating of a constant number of particles.

2. Etidocaine Microencapsulation

During the reassembly of the Wurster unit, several improvements were made in the system. However, for comparison of etidocaine to lidocaine, it was important to change as few processing variables as possible. Etidocaine HCl was used as received from Astra Pharmaceuticals Products (Lot 11P). In this experiment we had better control and accounting procedures for losses.

At the 50% coating level a large sample was removed (110 gm) and the remaining material was coated with an additional 10% of polymer, as rapidly as possible. The coating procedure was successful, indicating that the equipment can maintain a fluidized bed with only 165 grams in the coating chamber. Furthermore, the rate of solution addition was raised slowly to 10 ml/minute without having the bed collapse. The critical processing indicator is negative pressure at the nozzle. At the temperature of these experiments (38°C at the nozzle), a flow of 10 ml/minute is practical.

The summary of the processing losses is shown in Table VI. The sieve analysis of these materials is shown in Table VII.

C. <u>In Vitro</u> Drug Release Studies

Drug release rates are determined by suspending a known mass of microcapsules in a known volume of an aqueous solution and periodically measuring the concentration of the drug in solution by spectrophotometry. From the solution volume and concentration, the quantity of drug released is computed. This quantity divided by the length of time since the last measurement is the average drug release rate over the time interval.

TABLE V

LIDOCAINE MICROCAPSULE SIZE DISTRIBUTION

(Values are % of weight in each sieve fraction)

Particle Size Range (μm)	% Drug in Microcapsule				
	93	80	70	60	50
> 212	24.6	0.9	13.9	25.1	24.2
150-212	6.3	13.8	20.9	26.3	34.6
125-150	8.2	17.6	13.3	17.4	11.4
106-125	17.4	35.5	10.5	11.6	11.9
38-106	40.1	30.4	36.1	14.6	6.8
< 38	3.4	1.9	5.6	5.0	11.7

TABLE VI

PROCESSING SUMMARY OF ETIDOCAINE MICROENCAPSULATION

Process Polymer %	Starting Sample g	Polymer Added g	Final Weight MC g	Final Weight (as drug) (g)	Wurster Holdup g	Oversize Removed g (μm)	Sieve + Bag g	Samples Removed g
0-11	400	49	385	(343)	64	23 (150)	13	10
11-20	338	31	332	(266)	37	1 (250)	4	10
20-30	316	40	311	(218)	45	2 (250)	2	10
30-40	297	42	314	(188)	25	48 (250)	3	10
40-50	251	50	275	(138)	26	20*(250)	2	110
50-60	165	40	201	(80)	4	35*(250)	2	(201)
TOTALS		252			201	74	26	351

Material Balance: (in) 400g drug + 252 g polymer = 652 g
(out) 351 g (samples) + 201 (Wurster) + 74 (oversize) + 26 (bag) = 652 g

Yield of material at 50% polymer: 138 g/400 g = 34.5%
30% polymer: 218 g/400 g = 54.5%

* Based on sieve analysis of sample, material not actually removed

TABLE VII

ETIDOCAINE MICROCAPSULE SIZE DISTRIBUTION

(Values are % of weight in each sieve fraction)

Particle Size Range (μm)	% Drug in Microcapsule				
	80	70	60	50	40
>300	2.2	1.9	6.5	4.0	4.3
212-300	3.3	5.5	15.4	15.8	26.7
150-212	15.2	20.3	24.2	34.2	41.2
106-150	26.5	21.9	28.2	23.9	18.7
74-106	17.2	28.5	9.3	12.1	7.1
38-74	33.5	21.2	15.8	9.8	1.8
< 38	2.1	0.7	0.5	0.1	0.1

* 40% sample is removed from base of coating chamber. Other samples are total (i.e., after brush-down of equipment, expansion chamber, filters, rotating nozzles (tree), sides of coating chamber, etc.)

The suspending solution for these studies is water buffered with phosphate (pH 7.4). These solutions are thermostated at 37°C in a metabolic shaker bath. The vessel used for the release studies is a specially designed L-shaped test tube. The shape of this container promotes good mixing of the release solution when used in a metabolic shaker and thereby reduces local drug concentration gradients in the solution, which might affect the release rate. The microcapsules are placed in a tea-bag like structure constructed from fine polyester mesh for convenience in separating the microcapsules from the suspending solution. Lidocaine, etidocaine, and bupivacaine have absorption bonds in the ultraviolet, which are used to monitor concentrations of the drug in solution.

As a screening procedure, one sieve fraction (106-125 μm) of each percent coating level is used for drug release studies, as well as several of the sieve fractions of the 50% or 60% coated samples. Samples showing potentially useful release rates are re-examined to give triplicate data.

1. Lidocaine Release Studies

Lidocaine is soluble in pH 7.4 buffer and samples can be removed, read at 262 nm and returned to the diffusion cell. Because of the microencapsulation process the percent drug may not be the same for all sizes of particles. Therefore, samples of each size range are assayed for percent drug, and the percent of drug released is based on this assay value.

The results of the drug release studies for the first series of experiments are shown in Table VIII. Since relatively slow release was obtained only with the largest microcapsules which were coated to 50%, these microcapsule tests were repeated. In the first test, Sorenson's buffer was prepared at 0.067 molar phosphate. In the repeat tests, Fisher's prepared buffer was used at 0.05 molar. Table IX and Figure 1 show the results of these diffusion experiments. A sample of the 150-212 μm microcapsules were sent to Colonel Posey on February 27, 1981, for use in in-house animal studies.

Recently larger microcapsules were sieved and tested for rate of drug release. The larger, 250 to 300 μm, microcapsules have significantly slower release rates. This is the result of a single experiment. Whether this release would be even slower <u>in vivo</u> and would give sufficient anesthesia for several additional hours can only be determined by animal studies.

TABLE VIII

LIDOCAINE RELEASE FROM MICROCAPSULES

OF VARIOUS SIZES AND COATING LEVELS

Size Range (μm)	Coating Values	Assay Value	% Released at Hours 1	2	4	6	24
106-125	93	95.3	92.8				
106-125	80	82.0	99.7				
106-125	70	79.6	81.9				
106-125	60	73.9	93.0	94.7			
106-125	50	49.6	88.4	90.8	95.1	97.0	
125-150	50	46.0	68.8	78.8	84.2	87.4	89.7
150-212	50	46.7	43.7	56.2	71.6	79.2	83.7

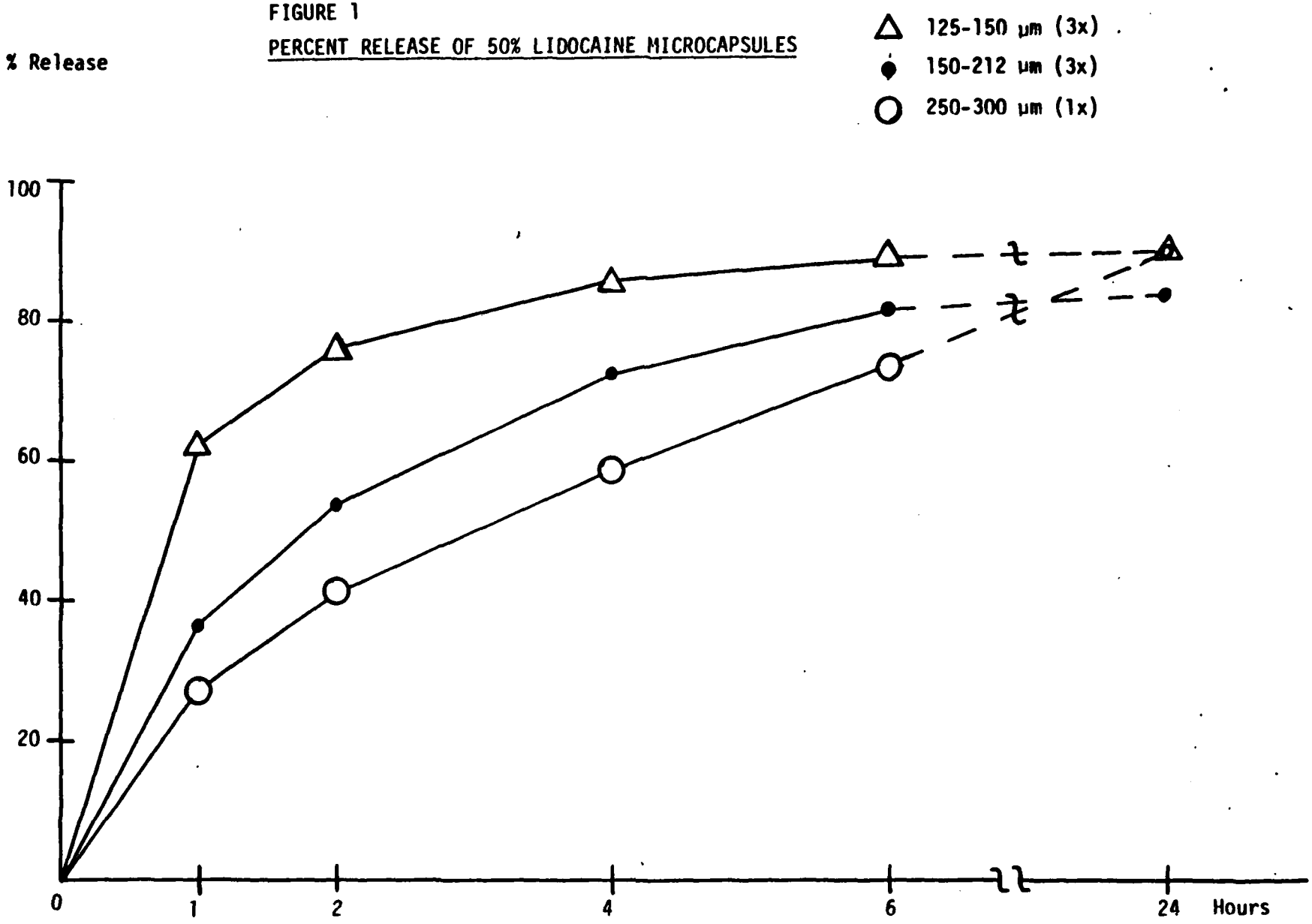
TABLE IX

LIDOCAINE RELEASE OF 50% COATED MICROCAPSULES

Size Range (μm)	Test	% Release at Hours 1	2	4	6	24
125-150	1	68.8	78.8	84.2	87.4	89.7
	2	53.7	72.7	84.1	86.7	86.7
	3	63.6	77.3	88.9	91.5	94.7
	Mean	62.0	76.3	85.7	88.5	90.3
	± S.D.	7.7	3.2	2.7	2.6	4.1
150-212	1	43.7	56.2	71.6	79.2	83.7
	2	28.9	50.7	71.6	78.6	81.4
	3	36.2	53.9	73.6	86.7	84.8
	Mean	36.3	53.6	72.3	81.5	83.3
	± S.D.	7.4	2.8	1.2	4.5	1.7

FIGURE 1

PERCENT RELEASE OF 50% LIDOCAINE MICROCAPSULES



2. Etidocaine Release Studies

Etidocaine is less soluble in water and buffer than is lidocaine. The lipophilic nature of the drug is used to an advantage in maintaining the drug at the site of injection for a longer period of time. The partition coefficients for the various drugs between n-heptane and buffer (pH 7.2), are:

Drug	K
lidocaine	2.9
etidocaine	141
bupivacaine	27.5

(Covino and Vassallo, 1976). Acidifying the buffer solution was suggested (Vassallo, p.c.) prior to making ultraviolet absorption measurements. This procedure has been followed but it does not negate the requirement to change the buffer solution as the drug reaches the saturation value. In the first set of experiments this problem was not realized and several values may be low due to drug saturation of the buffer solution. Aliquots were withdrawn, acidified, read, and discarded. However, the solutions were not changed nor was the buffer volume replaced. In Figures 2-6 points marked with an (S) are believed to be within 80% of solution saturation.

Figures 2 and 3 show the effect of polymer loading on the drug release characteristics for 150 to 212 μm and 212 to 310 μm microcapsules. These large microcapsules were chosen because of the fast release of small lidocaine microcapsules. Figures 4 and 5 show the effect of size on the 50% and 40% drug microcapsules. The probable reason for the almost total release of the 74 to 106 μm, 40%-etidocaine microcapsules is that few microcapsules were available in this sieved sample, and a small quantity of etidocaine was, therefore, used for this release study.

With this information another drug release study was performed in which the solutions were changed after the two and six hour measurements. The results are shown in Figure 6. This release approximates the *in vivo* targeted release, and was achieved with small microcapsules containing as much as 70% drug.

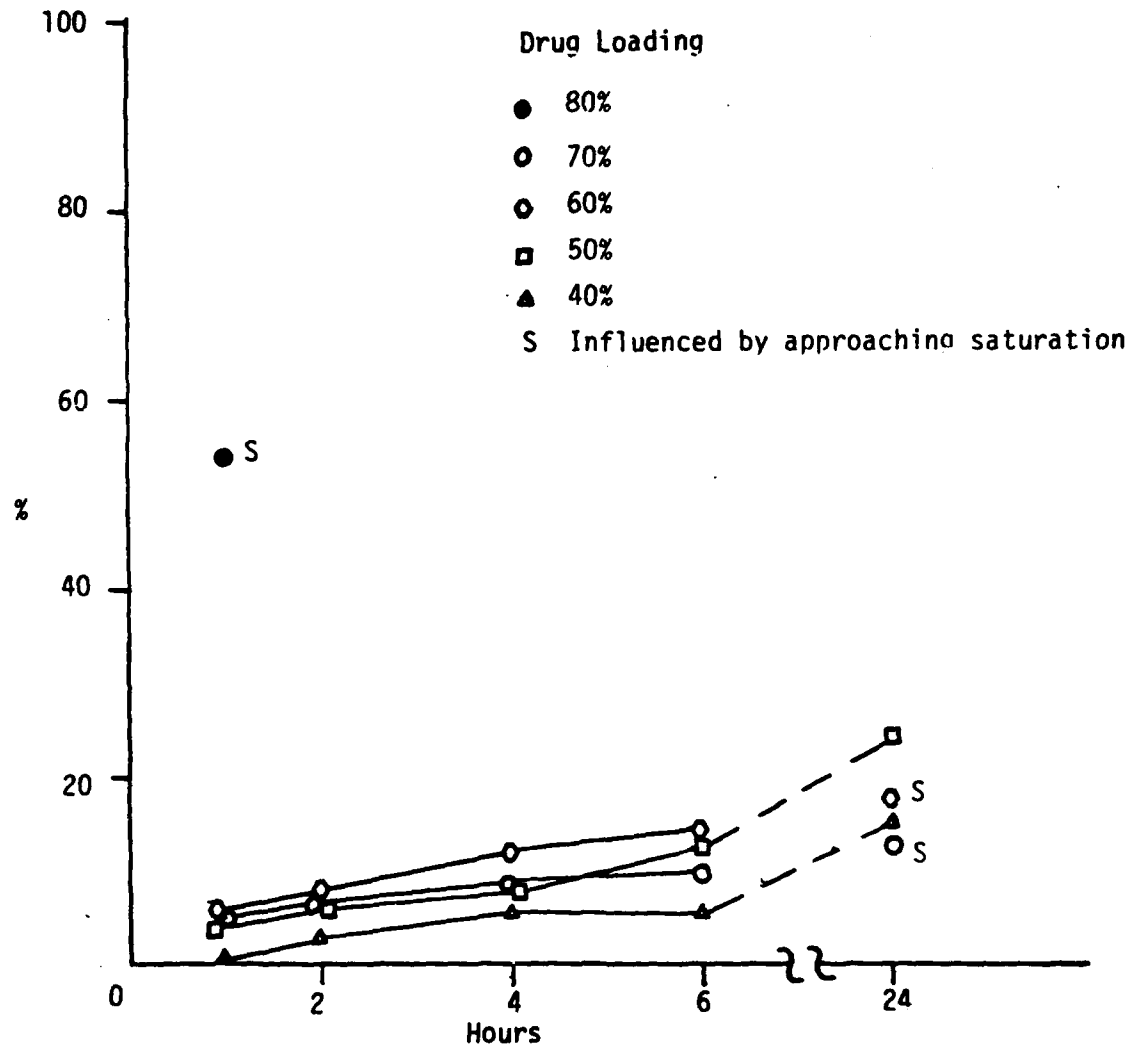


FIGURE 2 PERCENT RELEASE OF 150-212 μm MICROCAPSULES CONTAINING VARIOUS AMOUNTS OF ETIDOCAINE (S-probable slow release due to approach of solution saturation)

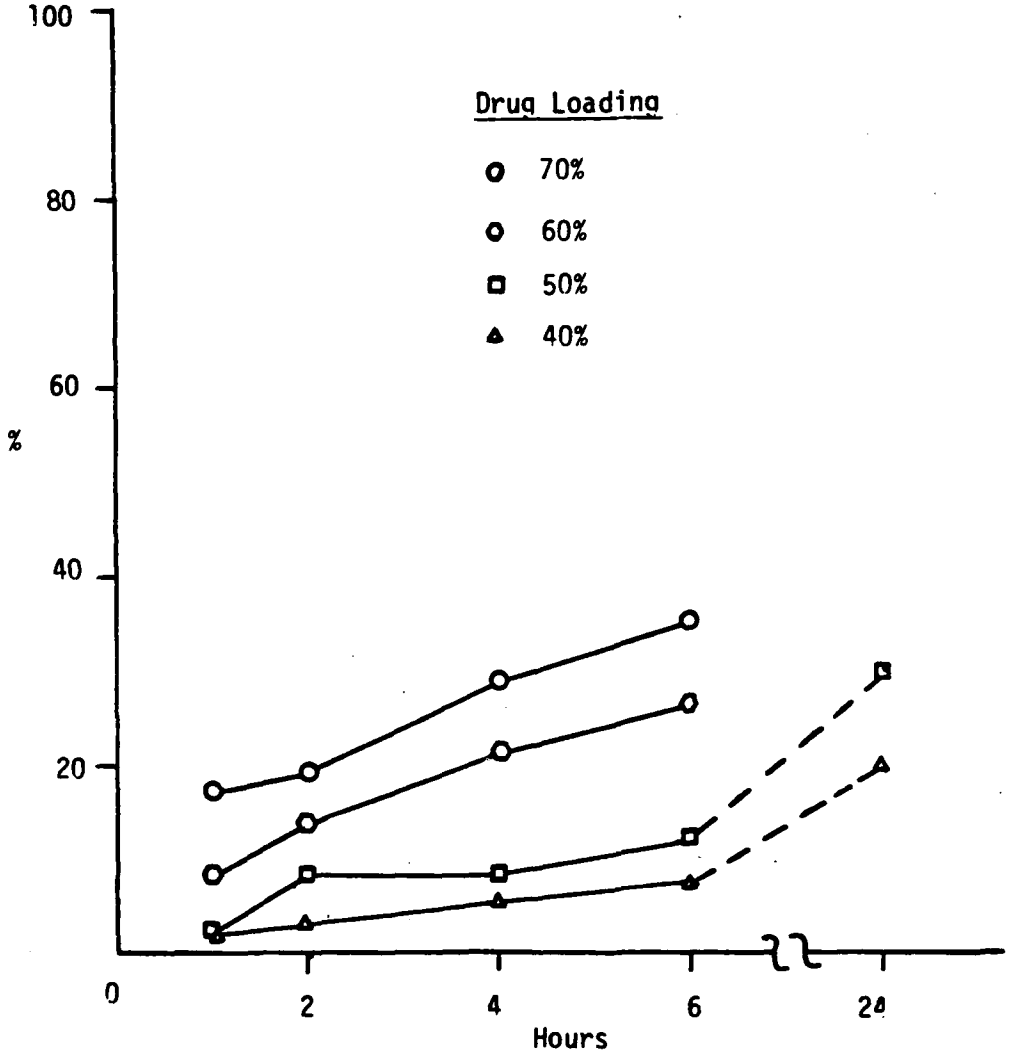


FIGURE 3 PERCENT RELEASE OF 212-300 μm MICROCAPSULES CONTAINING VARIOUS AMOUNTS OF ETIDOCAINE

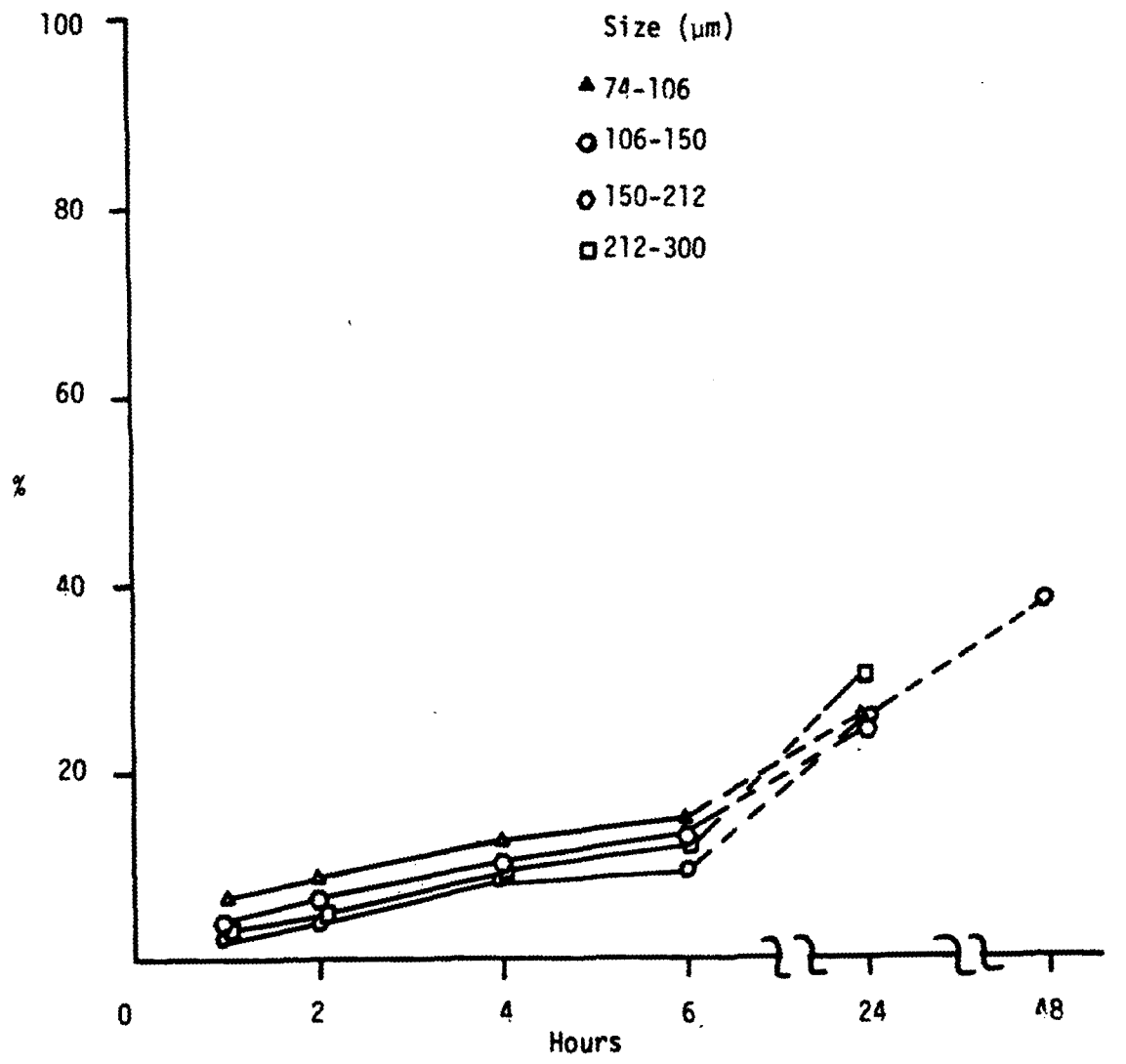


FIGURE 4 PERCENT RELEASE OF 50% ETIDOCAINE MICROCAPSULES OF VARIOUS SIEVE SIZES

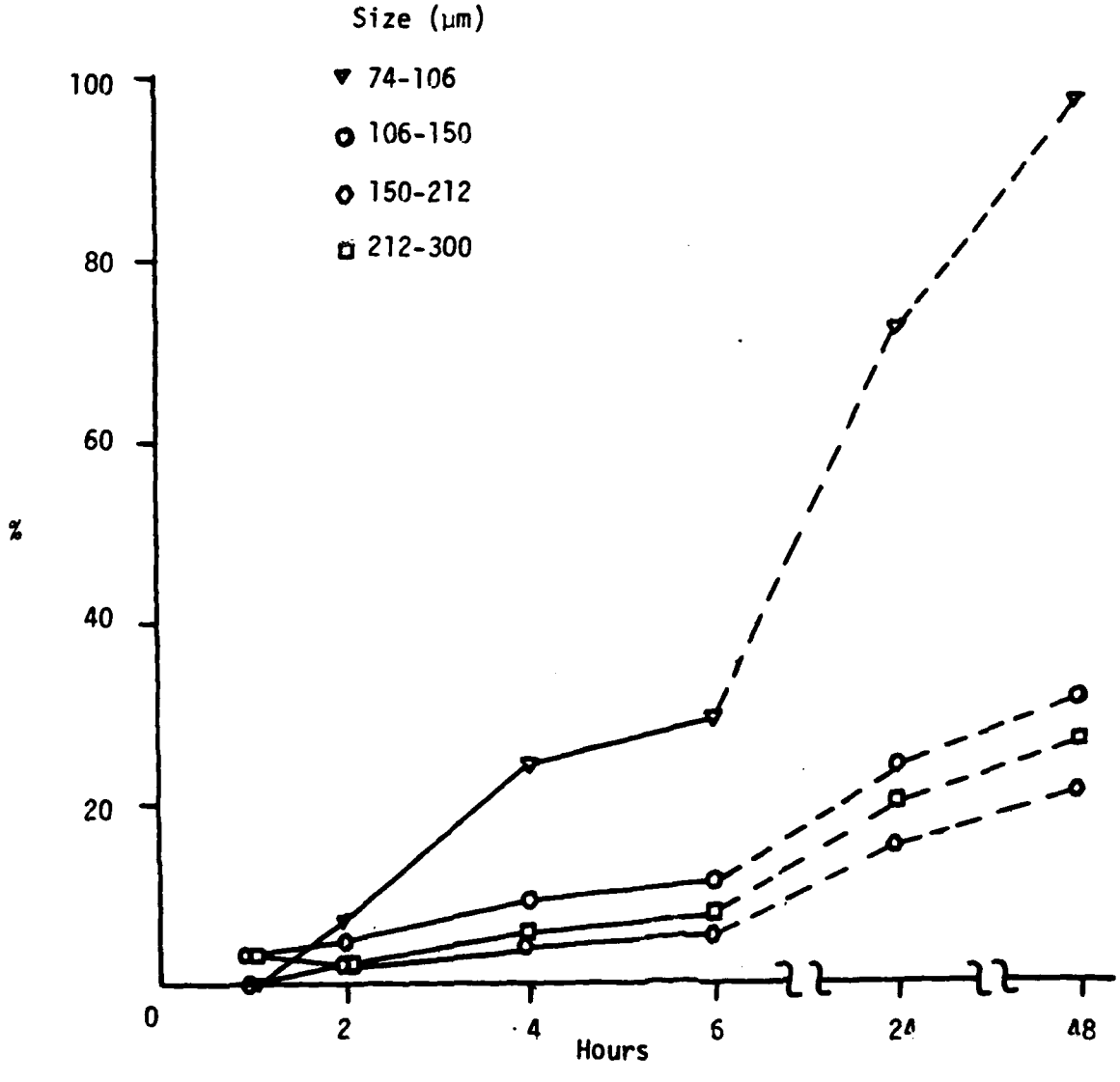


FIGURE 5 PERCENT RELEASE OF 40% ETIDOCAINE MICROCAPSULES OF VARIOUS SIZE RANGES

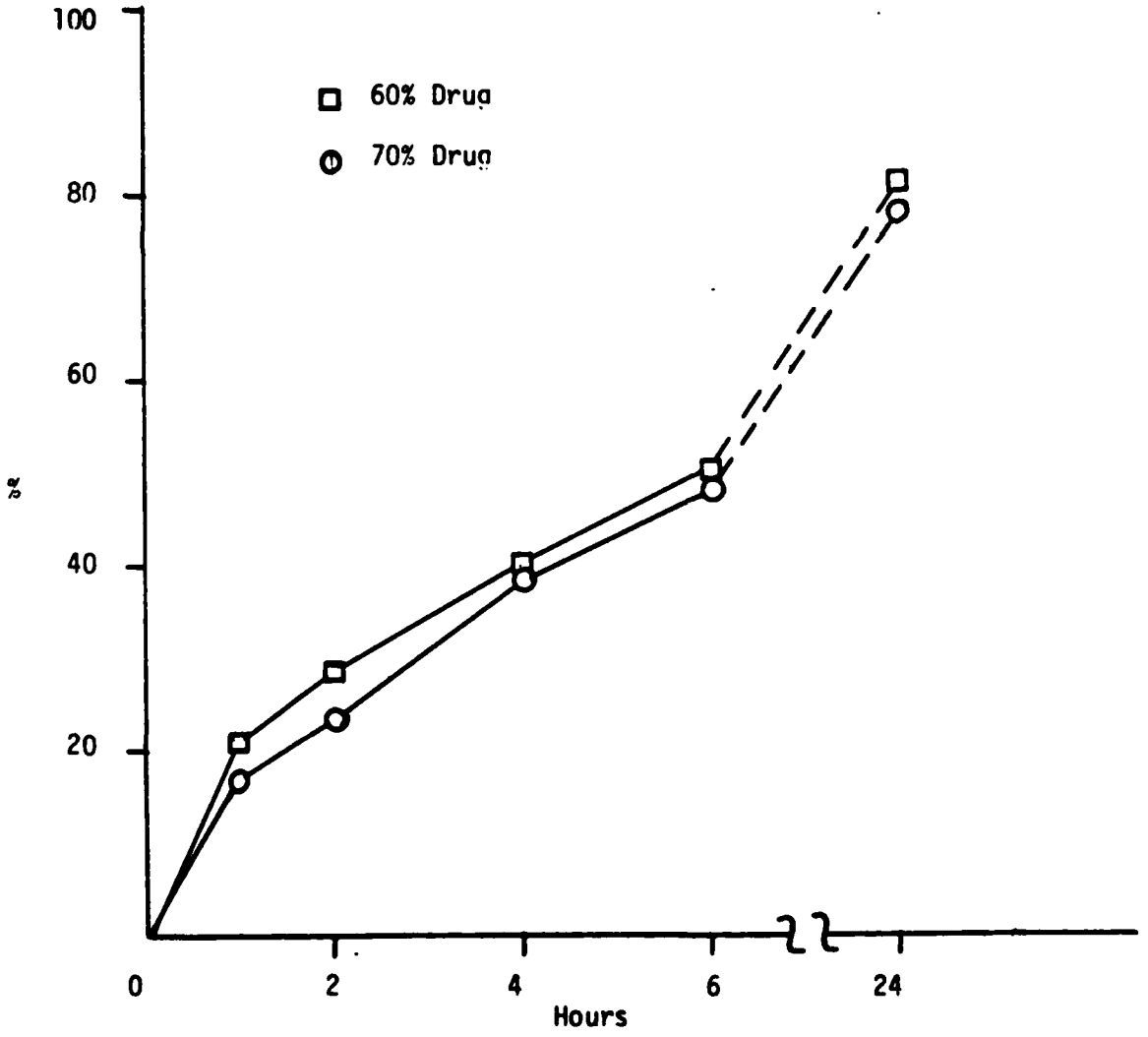


FIGURE 6 PERCENT RELEASE OF 106 to 74 μm ETIDOCAINE MICROCAPSULES OF VARIOUS PERCENT LOADINGS

D. Sample Storage

Microcapsules of 50% lidocaine and 50% etidocaine (212 to 150 μm) are being prepared for storage under the six conditions which were proposed (Table X). Two separate containers of each drug are being prepared. One container will be opened at the end of the contract year for assay and release testing. The other containder holds twice as much material and will be held for two later analyses. American Can Company has graciously supplied sufficient M-1173 61-0.93004 heat-sealable bags for dessicant storage. Pouches consist of Bartuf/Poly/Foil/ L.D.P.E. Ambient moisture is achieved in bottles capped with glass wool. Absence of light is achieved with black tape. Temperatures are 40°C (chemical oven), ambient (laboratory), 4°C (refrigerator).

E. In Vivo Evaluation of Lidocaine Microcapsules

1. Introduction

The rabbit has been evaluated as a model system for lidocaine release from microcapsules in vivo. Creation of a simulated avulsive wound was achieved surgically in the rabbit thigh. Blood levels of lidocaine and tactile stimulation of the wound were monitored after instillation of the microcapsules in the avulsive wound. Controls were run using no anesthetic and lidocaine crystals.

2. Experimental Animals and Procedures

These experiments were performed before the polymer and lidocaine microcapsules from this program were available. Therefore, these in vivo tests used L-8-50% - 125-149 μm microcapsules from Contract No. DAMD 17-79-C-9019.

TABLE X

SAMPLE STORAGE CONDITIONS

CONDITION	TEMPERATURE	HUMIDITY	LIGHT
1	40°C	Ambient	None
2	40°C	Desiccate	None
3	Ambient	Ambient	None
4	Ambient	Ambient	Ambient
5	4°C	Ambient	None
6	4°C	Desiccate	None

Four adult New Zealand white rabbits (4-5 kg) have been used for this study. To date, ten experiments have been conducted which evaluate the effect of pure lidocaine and microencapsulated lidocaine on localized analgesia as well as circulating blood levels. One experiment has been performed using etidocaine hydrochloride crytals. Under short-acting general anesthesia, using aseptic techniques, one thigh muscle area is exposed (3.0 cm) using a surgical scalpel through the skin and fascia. In the initial experiments, an avulsive wound (2.0 cm long; 3-4 mm deep) was created along the longitudinal axis of the muscle mass by blunt dissection using large tissue forceps. Hemostasis in the area of the wound was maintained, with little blood loss, using maceration and compression (forceps) needed to create an avulsive wound.

Various doses of lidocaine or lidocaine microcapsules (10-80 mg) were instilled in the wound on an individual rabbit basis and maximum use of the animal was achieved by using the opposite thigh of these rabbits at a later date. After administration of each drug, the immediate area of the wound and the surroundings (1-3 cm away from the wound, muscle and skin) were examined for response to tactile stimuli (needle pricking at various time intervals) over the first six hours and again at 24 hours. Blood was drawn at 0, 1, 2, 4, 6, and 24 hours after drug administration to obtain serum levels of the drug.

3. Results of Tactile Stimulation

The results to date suggest that various doses of pure lidocaine and lidocaine microcapsules inhibit the response of the surgical area to tactile stimulation (muscle twitch) in and near the avulsive wound. Exterior to the wound, positive response to tactile stimulation was more apparent. Response of a control rabbit (avulsive wound and no drug) showed mixed response to tactile stimulation directly in the wound. Partial response was noted in one rabbit. Destruction of nerve endings during creation of the artificial wound may account for the lack of response to tactile stimulation in the wound area in the rabbit. Later studies used the response to tactile stimulation, following instillation of the lidocaine microcapsules in a localized subcutaneous area. This procedure avoids surgical manipulation of the muscle mass.

The results of these last three experiments are shown in Table XI. Biotal (10 mg/kg) (a short-acting barbiturate) was injected (IV), and the thigh area shaved (Aster Clipper; #40 blade). A 1.0 cm incision, through the skin and fascia was produced with a surgical scalpal and iris scissors. A 1 cm subcutaneous pocket was created, using the blunt end of a pair of forceps. Crystalline drug or microcapsules (dry) were poured into the pocket and the incision closed with one suture (4-0) to retain the drug. Using a felt-tip marking pen, circles were drawn at 1, 2, and 3 cm from the center of the incision (Figure 7). Tactile response was evaluated at various points on each circle using a sterile 20 gauge, 1-inch hypodermic needle. Total and partial responses to the needle stimulation were recorded at each circle at various time periods after drug administration. The response of rabbits following the subcutaneous administration of the drugs was recorded and later plotted (Figure 8). Partial response along the circumference is plotted as fractional centimeters.

The rabbit subcutaneous thigh model shows potential as a method for evaluating long-term lidocaine release from microcapsules. Re-evaluation of the method of stimulation, however, may be needed to quantify drug-induced supression of sensory response. A device which exerts a certain force during "pin pricking" is currently being investigated for repeatable stimulation to the area.

4. Measurement of Circulating Blood Levels of Anesthetics

The original proposal stated that circulating blood levels of lidocaine would be measured concurrently with observation of local anesthesia (lack of response to tactile stimulus). Measurements were to be performed by enzyme immunoassay using the Syva reagents for lidocaine. Unfortunately no antibody has been developed, to our knowledge, for either bupivacaine or etidocaine. Therefore, other assay methods were considered. The method of Mather and Tucker (1974) was chosen for evaluation since it has been tested for lidocaine and bupivacaine, and the method should be valid for etidocaine. BIOTEK has a

TABLE XI

LIDOCAINE AS RELATIVE PEAK HEIGHTS (L/E* x1000)

Time (Hr.)	Experiment No. 1 80 mg Lidocaine as Solution I.M. Injection	Experiment No. 2 10 mg Lidocaine As Implanted Microcapsules
1/2	-	2.8
1	1,364	7.2
2	908	11.7
4	168	7.4
6	56	2.6
24	-	0

* E = Etidocaine at 1.25 µg/ml

FIGURE 7

SCHEMATIC DRAWING OF RABBIT THIGH MODEL FOR TACTILE STIMULATION FOLLOWING LOCAL ANESTHETIC ADMINISTRATION SUBCUTANEOUSLY

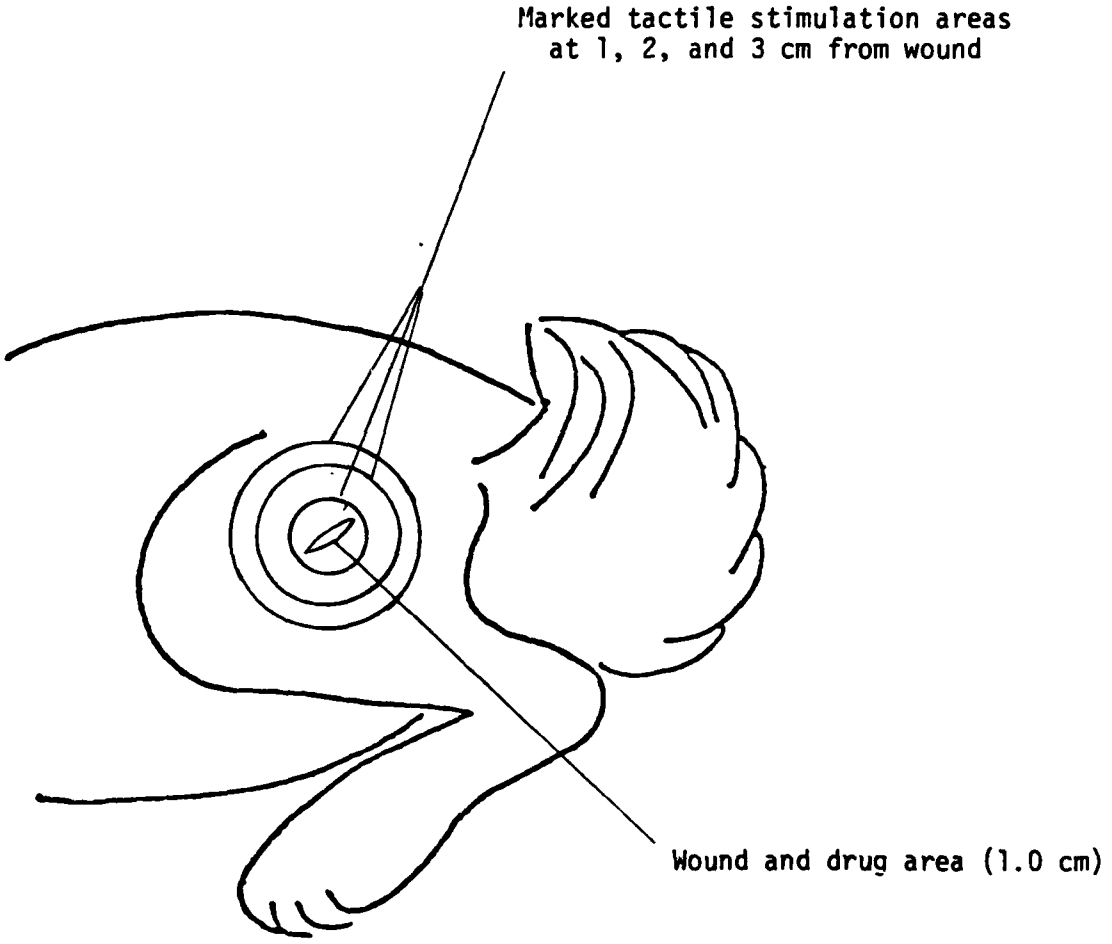
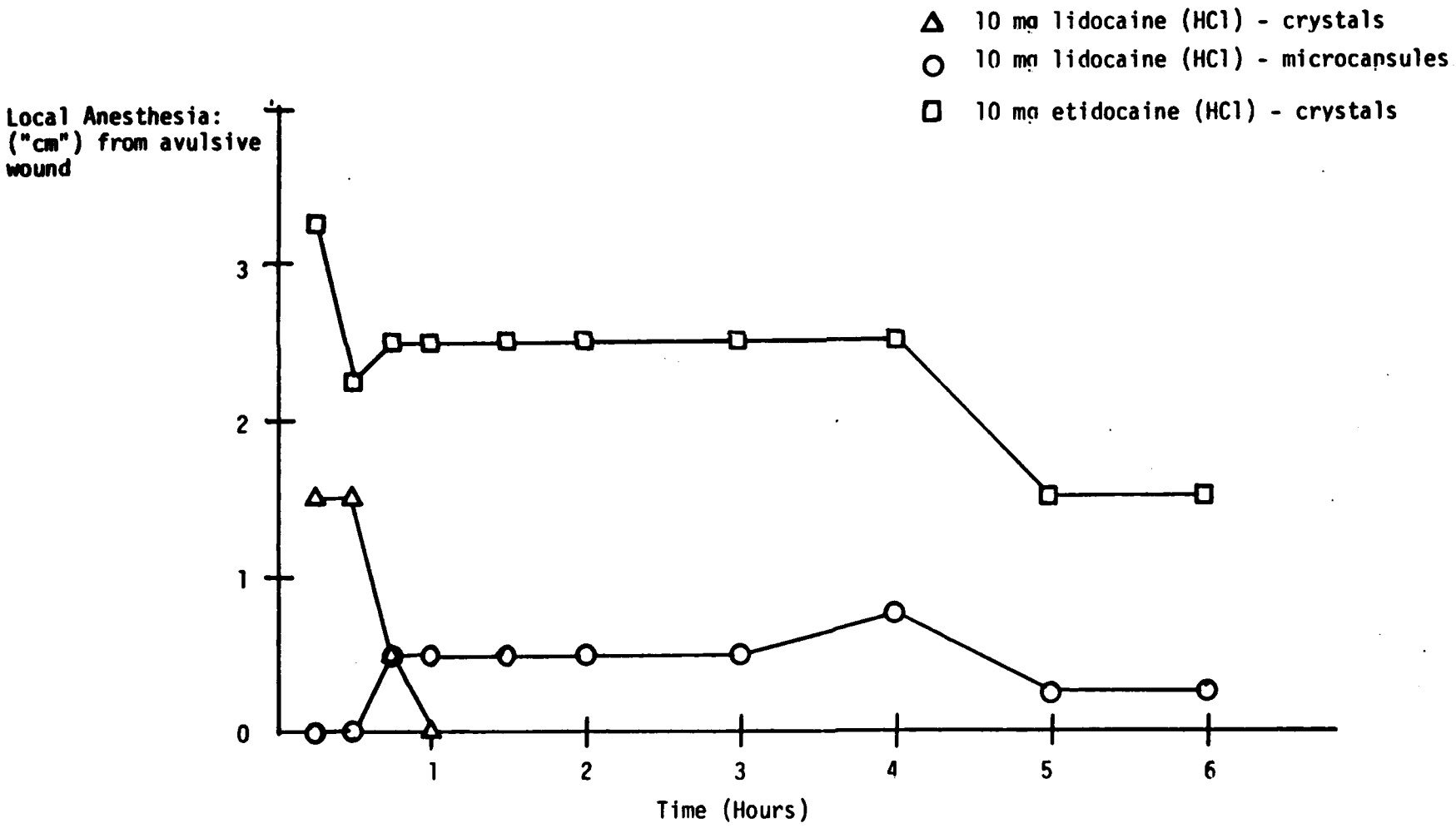


FIGURE 8 RESULTS OF TACTILE STIMULATION OF SUBCUTANEOUS IMPLANTATIONS OF LIDOCAINE OR ETIDOCAINE VS. TIME



Perkin Elmer 990 gas chromatograph and a 6-ft., 10% OV-17 column available. Good separation and detection of lidocaine and etidocaine was achieved Figure 9). Using these two drugs, the one not being measured is added as the internal standard.

A background sample of blood was used to generate a standard curve for the extraction procedure with varying amounts of lidocaine in the presence of an internal standard of etidocaine. The etidocaine standards for this run came out ± 3.9% based on peak area. From the graph, Figure 10, it can be seen that both peak areas and peak heights show excellent linearity versus the amount of lidocaine added to the 1 ml blood sample. Therefore, the peak height can be used to calculate the amount of circulating lidocaine.

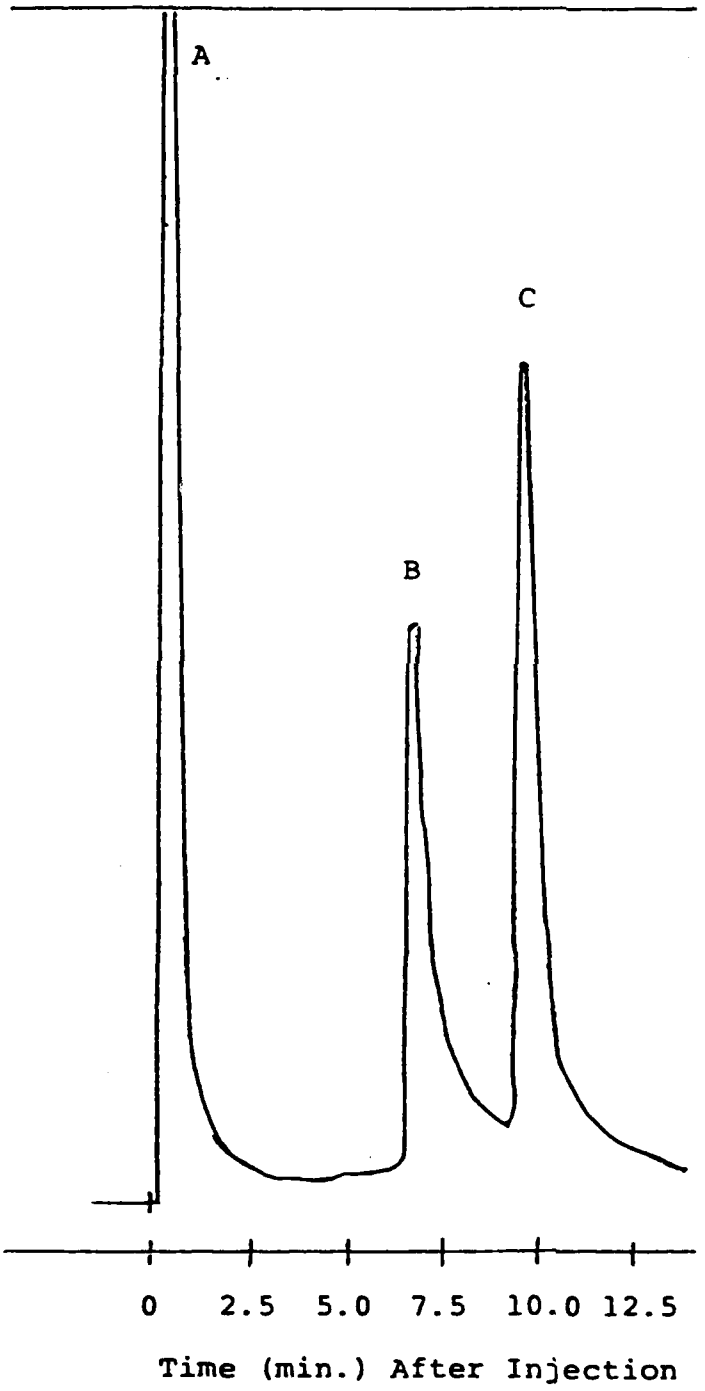
An important point on both graphs is the X-intercept. This value is 0.149 µg for peak area and 0.154 µg for peak height. This sets a theoretical lower limit of lidocaine detection at about 0.15 µg/ml. This could be due to solubility phenomena (distribution coefficient) in the extraction procedure.

During the development of the *in vivo* procedures, it was found that ketamine (Ketaset^{R}) could not be used as the general anesthetic since it gave two peaks on the gas chromatograph, one of which had the same retention time as lidocaine. Furthermore, since the general anesthetic was present in much larger concentration than lidocaine, the lidocaine peak was totally masked. Ether is an acceptable general anesthetic, but is difficult to work with. Therefore, thiamylal sodium (Bio-tal^{R}, a short acting barbituate) was tried. This was found to provide good short-acting anesthesia and showed no conflicting peaks in a chromatogram of extracted plasma which was taken one hour after thiamylal injection.

In the first rabbit experiment a 4% lidocaine solution was prepared and 1 ml was injected intramuscularly into each thigh of a 4.8.kg New Zealand white rabbit (80 mg lidocaine). The amount of lidocaine in the blood was monitored at 0, 1, 2, 4, and 6 hours. The results are shown in Table XI and Figure 11.

Figure 9

DETECTION AND SEPARATION OF LIDOCAINE/ETIDOCAINE
ON 10% OV-17 COLUMN @ 210°C
(1.0 µl of solution containing 2µg of each drug)



Peak	Assignment
A	Solvent - $MeCl_2$
B	Lidocaine - 2µg
C	Editocaine - 2µg

	Format	y = mx + b y = L/E at E= 1.25µg x = L (µg/ml)
□	Peak Height	m = 0.9086 b = -0.14 r = 0.9996 x (y=o) = 0.154
O	Peak Area	m = 0.6739 b = -0.1005 r = 0.99989 x (y=0) = 0.149

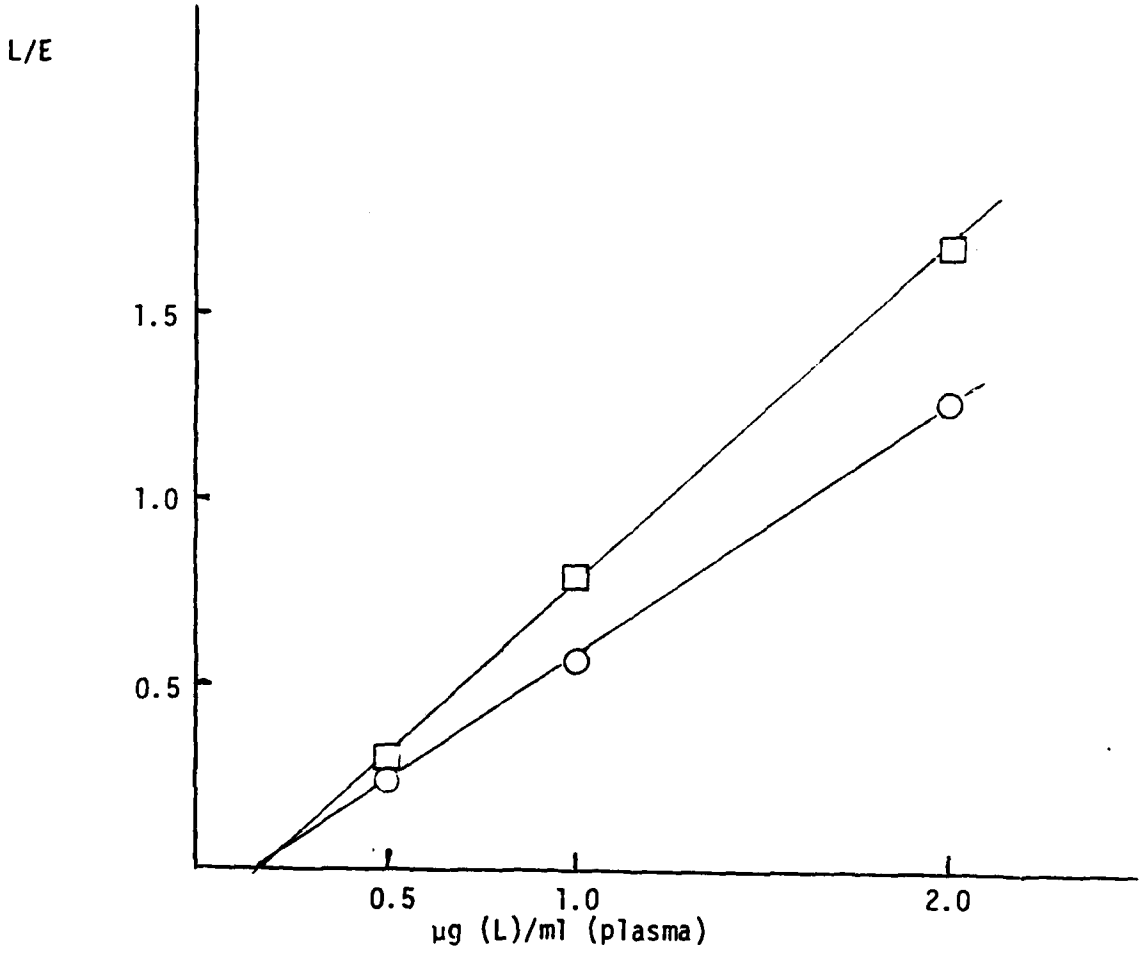


Figure 10 Standard Curve for Lidocaine (L) in Plasma with Etidocaine (E) as Internal Standard

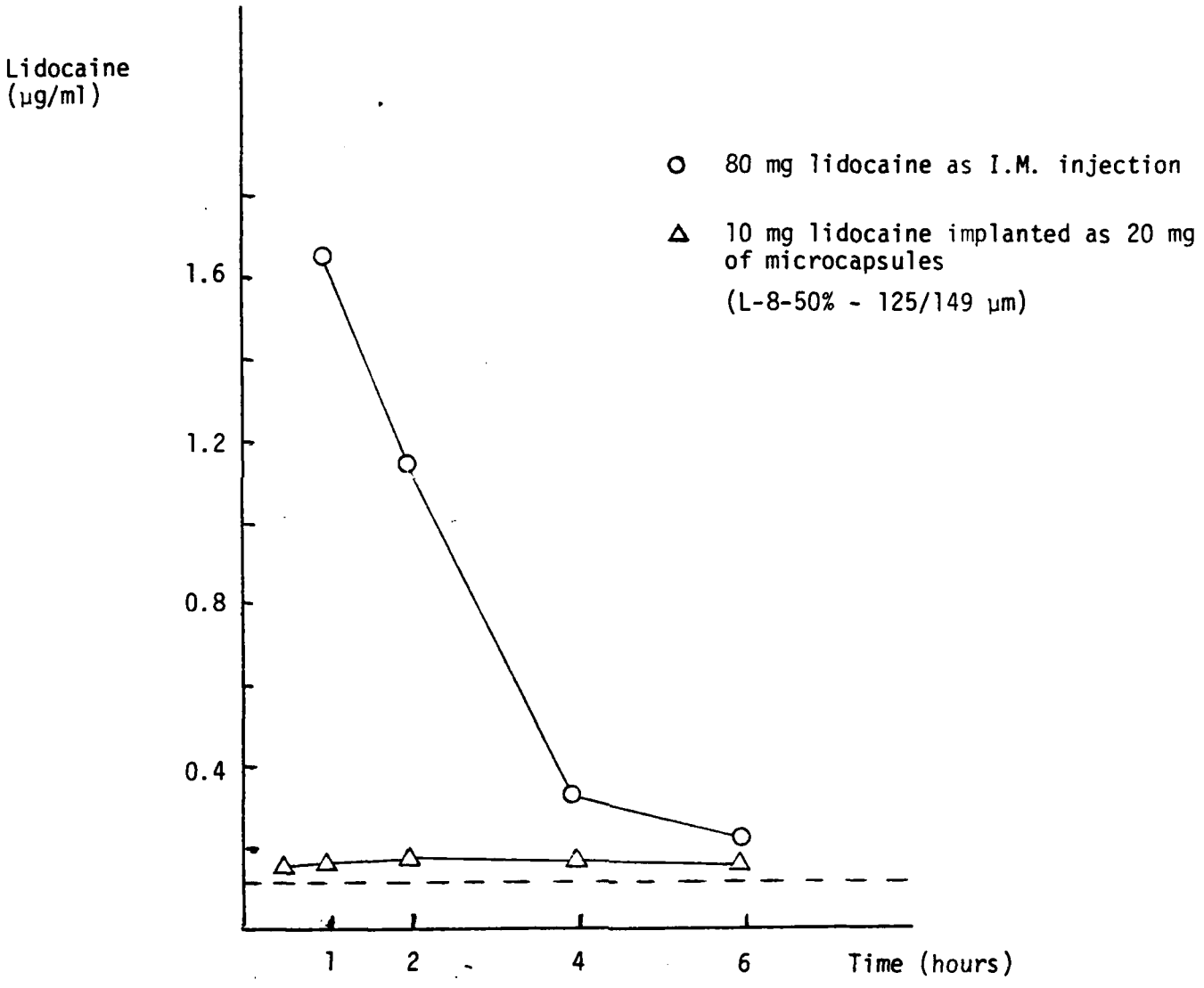


Figure 11 Circulating Levels of Lidocaine after Intramuscular Injection or Microcapsule Implantation (Using Linear Regression Equation of Figure 10, Dashed Line is Limit of Analytical Method)

In a later experiment another New Zealand white rabbit (4.6 kg) had 20 mg of lidocaine microcapsules (10 mg of lidocaine) implanted in a blunt dissection wound in the thigh. Blood levels were analyzed at 0, 1/2, 1, 2, 4, 6, and 24 hours post-implantation. The results of this experiment are also described in Table XI and Figure 11. In this experiment ether was used as the general anesthetic.

Detection of lidocaine in the serum, after administration of drug to the wound, shows circulating levels which are distinguishable from residual general anesthetics. The data indicates that lidocaine is slowly released *in vivo* from the microcapsules of Contract No. DAMD 17-79-C-9019 (L-8-50%-124/149).

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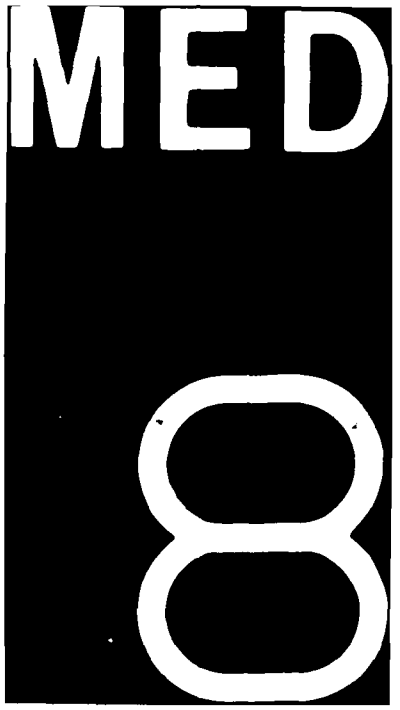
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