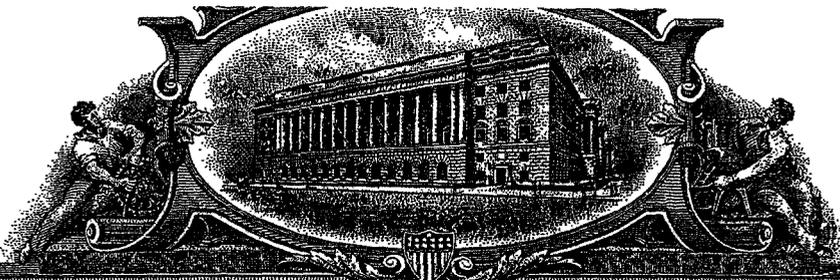




A 1185223



THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

**UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office**

June 22, 2004

**THIS IS TO CERTIFY THAT ANNEXED IS A TRUE COPY FROM THE
RECORDS OF THIS OFFICE OF A DOCUMENT RECORDED ON
October 18, 1988**

**By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS**



H. L. Jackson
**H. L. JACKSON
Certifying Officer**

ASSIGNMENT

WHEREAS, I, MICHAEL MEZEI, a citizen of Canada, residing at 14 Starling Street, Nova Scotia, Canada B3M 1V8, have jointly invented with ADRIENN GESZTES certain new and useful improvements in LIPOSOMAL LOCAL ANESTHETIC AND ANALGESIC PRODUCTS for which an application for Letters Patent of the United States was executed on even date herewith; which application was filed in the United States Patent and Trademark Office on August 26, 1988 and accorded Serial No. 236,724; and

WHEREAS, MEZEI ASSOCIATES LIMITED, a corporation duly organized under the laws of Canada, having a place of business in Nova Scotia, Canada, is desirous of acquiring the entire right, title and interest in and to the aforesaid invention and in and to any Letters Patent of the United States or any foreign country which may be granted therefor;

NOW, THEREFORE, in consideration of the sum of One Dollar (\$1.00) and other good and valuable consideration, the receipt of which is hereby acknowledged, I, the said MICHAEL MEZEI by these presents do sell, assign, and transfer unto MEZEI ASSOCIATES LIMITED, its successors, legal representatives and assigns, the full and exclusive right to the said invention as described in the said application, and the entire right, title and interest in and to any and all Letters Patent which may be granted therefor in the United States and its territorial possessions and in any and all foreign countries and in and to any and all divisions, reissues, continuations and extensions thereof;

AND I HEREBY authorize and request the Commissioner of Patents and Trademarks or any other proper officer or agency of any country to issue all said Letters Patent to said assignee;

AND I HEREBY warrant and covenant that I have full right to convey the entire interest herein assigned and that I have not

REF 5044 FMR741

executed and will not execute any instrument or assignment in conflict herewith;

AND I HEREBY agree to communicate to said assignee or its representatives any facts known to me respecting said invention, to execute all divisional, continuation, reissue and foreign applications, sign all lawful documents and make all rightful oaths relating to said invention, and to testify in any judicial or administrative proceeding and generally do everything possible to aid the said assignee to obtain and enforce said Letters Patent in the United States or any foreign country when requested so to do by said assignee.

IN WITNESS WHEREOF, I have hereunto set my hand and seal.

Sept 24 1988
(Date)

Michael Mezei
Michael Mezei

REF 5044 FRAME 742

On this 29 day of September, 1988, before me a Notary Public, personally appeared MICHAEL MEZEI, to me known and known to me to be the person of that name, who signed and sealed the foregoing instrument, and acknowledged the same to be his free act and deed.

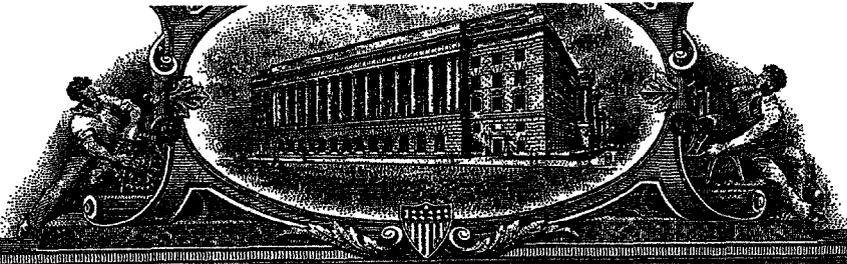
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Joseph W. Pettigrew
NOTARY PUBLIC

Joseph W. Pettigrew
JOSEPH W. PETTIGREW Public
A Barrister of the Supreme
Court of Nova Scotia
My Commission Expires:

no expiry date.

A 1184838



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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

June 21, 2004

THIS IS TO CERTIFY THAT ANNEXED IS A TRUE COPY FROM THE
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OCTOBER 18, 1988.

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS




M. K. HAWKINS
Certifying Officer

ASSIGNMENT

WHEREAS I, ADRIENNGESZTES, a citizen of Hungary, have jointly invented with MICHAEL MEZEI, a citizen of Canada, residing at 14 Starling Street, Nova Scotia, Canada, B3M 1V8, certain new and useful improvements in LIPOSOMAL LOCAL ANESTHETIC AND ANALGESIC PRODUCTS, for which an application for Letters Patent of the United States will be filed in the United States Patent and Trademark Office.

WHEREAS, MEZEI ASSOCIATES LIMITED, a corporation duly organized under the laws of the Canada, having a place of business in Nova Scotia, Canada, is desirous of acquiring the entire right, title and interest in and to the aforesaid invention as described and set forth in Exhibit "A" annexed hereto to this Assignment and in and to any Letters Patent of the United States or any foreign country which may be granted therefor;

NOW THEREFORE, in consideration of the sum of One Dollar (\$1.00) and other good and valuable consideration, the receipt of which is hereby acknowledged, I the said ADRIENNGESZTES by these presents do sell, assign, and transfer unto MEZEI ASSOCIATES LIMITED, its successors, legal representatives and assigns, the full and exclusive right to the said invention as described in the said Exhibit "A" annexed hereto and the entire right, title and interest in and to any and all Letters Patent which may be granted therefor in the United States and its territorial possessions and in any and all foreign countries and in and to any and all divisions, reissues, continuations and extensions thereof;

AND I HEREBY authorize and request the Commissioner of Patents and Trademarks or any other proper officer or agency of any country to issue all said Letters Patent to said assignee;

REF 5044 PAME743

AND I HEREBY warrant and covenant that I have full right to convey the entire interest herein assigned and that I have not executed and will not execute any instrument or assignment in conflict herewith;

AND I HEREBY agree to communicate to said assignee or its representatives any facts known to me respecting said invention, to execute all divisional, continuation, reissue and foreign applications, sign all lawful documents and make all rightful oaths relating to said invention, and to testify in any judicial or administrative proceeding and generally do everything possible to aid the said assignee to obtain and enforce said Letters Patent in the United States or any foreign country when requested so to do by said assignee.

IN WITNESS WHEREOF, I have hereunto set my hand and seal. City of ...
Embassy of the U.S.
States of America } ss

AUGUST 24, 1988
(Date)

Adrienn Gesztes
Adrienn Gesztes

On this 24th day of AUGUST, 1988, before me a Notary Public, personally appeared Adrienn Gesztes, to me known and known to me to be the person of that name, who signed and sealed the foregoing instrument, and acknowledged the same to be their free act and deed.

(SEAL)

Elizabeth Barnett
Notary Public

Elizabeth Barnett
Consul of the United States
of America

REF 5044 FRANK 744

"Schedule A"

PATENT APPLICATION

Title: THE USE OF LIPOSOMES TO ENHANCE LOCAL ANESTHETIC
EFFECT

Inventor: Michael Mezei and Adrienn Gesztes, Halifax, Canada

Assignee: Mezei Associates Ltd., Halifax, N.S. Canada

REF 5044 FRAME 745

ABSTRACT

Liposome encapsulated local anesthetic agents when applied to skin or mucous membranes provide greater local anesthesia and analgesia than the same agents incorporated in conventional vehicles i.e., ointment, cream or lotion.

BACKGROUND OF THE INVENTION

Liposomes - vesicles consisting of phospholipid membranes - have been studied in recent years to utilize them for altering the pharmacokinetic properties of drugs encapsulated into them. A few studies focused on their potential as drug carriers in topical preparations, involving corticosteroids, econazole, progesterone and methotrexate. Liposomal formulations delivered

more of these drugs into the skin than conventional vehicles, and also localized them at the desired site of action (M. Mezei in "Liposomes as Drug Carriers" ed. G. Gregoriadis, John Wiley & Sons Ltd. New York 1988 page 663-677).

Topical anesthetics are agents that reversibly block nerve conduction causing numbness and cessation of pain even after major stimuli. A topical analgesic agent is a substance which relieves pain without necessarily causing numbness, or which can relieve topical pain of minor nature, but not of a great degree (Fed. Register 44, 69768-69866, 1979). These drugs are therefore used to treat or prevent pain. For operations of peripheral and minor nature involving skin, like removal of superficial skin lesions and plastic surgery, or intradermal allergen testing, split skin grafting, treatment of painful ulcers, venipuncture - the ideal and painless way of anesthesia would be the topical application of local anesthetics.

The commercially available topical anesthetic preparations however, are not suitable for this purpose. Studies of Dalili and Adriani (Clin. Pharm. Ther. 12: 913-919, 1971) provided the first experimental evidence that manufactured preparations containing local anesthetics intended for use on the surface of the skin often lack efficacy. The preparations were tested on normal and ultraviolet light burned skin for the ability to block itching and pricking induced by electrical stimulation. The only effective preparation was one containing 20% benzocaine, the effect of which has disappeared within 60 seconds after it has

been wiped off the test site. The same authors pointed out several possible reasons for the lack of efficacy, such as low concentration of active ingredient, possible chemical change or interaction with other components and a penetration-preventing effect of the vehicles used (J. Adriani and H. Dalili. *Anesth. Analg.* 50: 834-841, 1971).

The most successful commercially available preparation for dermal anesthesia at the present is a lidocaine-prilocaine cream, first reported by Juhlin et al. (*Acta Derm Venereol.* 59: 556-559, 1979). The cream consists of the emulsion of 5% eutectic mixture of lidocaine and prilocaine bases (EMLA) in water, thickened with Carbopol^R (G.M.E. Ehrenstrom Reiz and SLA Reiz. *Acta Anaesth. Scand.* 26: 596-598, 1982). An application time of 60 minutes under occlusion is required to achieve complete anesthesia to pin-pricks, which lasts one to two hours (H. Evers et al. *Br. J. Anaesth.* 58: 997-1005, 1985).

In general, to achieve adequate local anesthesia of the skin excessive amounts of drug, prolonged application or invasive methods are required. For surgical anesthesia, the local anesthetic must be injected subcutaneously in order to reach sensory nerve endings lying in the dermis. When injecting a local anesthetic, pain is produced by the needle's penetration and by the deposition of the anesthetic solution. Distortion of the wound or performing the infiltration of large areas can also be problems in case of surgery (L. Juhlin, H. Evers, and F. Broberg. *Acta Derm. Venereol.* 60: 544-546, 1980).

In contrast to the skin, anesthesia of the mucous membrane covered surfaces can be produced by topical application of local anesthetics quickly and easily. However, rapid absorption of local anesthetic from these surfaces into the systemic circulation may cause short duration of local action, and possibly toxicity, since these drugs have low therapeutic ratios (J.A. Wildsmith, A.P. Rubin and D.B. Scott. Clin. Anaesth. 4: 527-537, 1986).

There is a need for a preparation that would be safe, yet effective on the unbroken skin, or on mucous membranes by providing a proper rate of premeation without discomfort or the risk of systemic reactions. The present invention can fulfill this need. Liposomes as drug carriers enhance penetration and localization of the drug applied topically (M. Mezei. in "Liposomes as Drug Carriers" ed. G. Gregoriadis, John Wiley & Sons, New York, 1988, page 663-677).

Local anesthetic agents have been previously encapsulated into liposomes in order to study their mechanism of action i.e. the interaction of local anesthetic with lipid bilayers, (Papahadjopoulos et al. Biochim. Biophys Acta 394: 504-519, 1975) however, no liposome encapsulated local anesthetics were tested or used for producing local anesthesia or analgesia prior to this invention. The first reports of this invention (Gesztes and Mezei, 47th International Congress of Pharmaceutical Sciences of F.I.P. Amsterdam, September 1, 1987; and Anesthesia and Analgesia, in press) relates to the superiority of liposome-

encapsulated tetracaine over the cream form tetracaine to produce anesthesia of the skin.

SUMMARY OF THE INVENTION

The present invention relates to the use of liposomes for improving the effect of local anesthetics by enhancing the penetration and localization of the anesthetic agents into and within the skin.

Local anesthetics as amphipathic agents are good candidates for entrapment in the phospholipid bilayers of the liposomes. Several anesthetic agents, e.g. benzocaine, lidocaine, prilocaine, lidocaine-prilocaine eutectic mixtures, tetracaine and dibucaine, was encapsulated into liposomes according to the method described by Mezei and Nugent (U.S. Patent 4,485,054, Nov. 27, 1984). In some cases, e.g. with benzocaine, lidocaine and dibucaine where the solubility would have restricted high enough concentration in the final product, the multiphase liposomal drug delivery system (Mezei, U.S. Patent Application, Serial No. 774.266) was utilized. Most of the time the base (and not the salt) of the anesthetic agent was used for preparing the liposomal product.

The following examples demonstrate the formulas and the activity of selected anesthetic agents in liposome versus ointment or cream form.

Example 1

Formula:

Tetracaine (base)	0.5 g
Soy phosphatidylcholine	7.0 g
Cholesterol	0.5 g
Stearic acid	0.7 g
Ethanol (95%)	10.0 ml
Propylene glycol	7.0 ml
Solution of sodium chloride (0.45%) and sodium bicarbonate (0.65%)	83.0 ml

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Tetracaine, phosphatidylcholine, cholesterol and stearic acid were dissolved in chloroform:methanol 2:1 v/v in a pear-shaped flask, and glass beads (100 g) were added. The solvent was evaporated to dryness in a rotary evaporator under reduced pressure at 30°C, until a smooth, thin lipid film was obtained on the surface of the flask and glass beads. The film was hydrated with the aqueous phase containing 0.65% NaHCO₃, 0.45% NaCl, 10% ethanol and 7% propylene glycol in distilled water, by shaking for 30 minutes in a Lab-Line Orbit Environ-Shaker at 55°C. The liposomes were separated from the glass beads by filtering through a Buchner funnel without using a filter paper.

Example 2

Formula:

Lidocaine	2.0 g
Soy phosphatidylcholine	9.0 g
Tocopherol acetate	0.24 g
Hydroxypropylmethylcellulose	1.5 g
Aqueous solution of sodium chloride (0.45%) and sodium bicarbonate (0.65%)	100.0 ml

REF 5044 FRAM751

Lidocaine, soy phosphatidylcholine and tocopherol acetate were dissolved in chloroform:methanol (2:1) in a pear-shaped flasks containing 100 g of glass beads. The solvent was evaporated in a rotary evaporator under reduced pressure at 30° until a thin, smooth film of the residue was obtained on the surface of the glass beads and the wall of the flask. The resulting lipid film was hydrated by the sodium chloride, sodium bicarbonate solution in an environment shaker at 55° for 30 minutes. The hydroxypropylmethylcellulose was added within 5 seconds after the lipid film and aqueous solution were mixed.

Example 3

Dibucaine	1.0 g
Soy phosphatidylcholine	8.0 g
Tocopherol acetate	1.0 g
Hydroxypropylmethylcellulose	1.0 g
Tween-80	1.0 g
CaCl ₂ solution 0.8 mM	100.0 ml

The method of preparation was similar as described above for example number 2; Tween-80 was added last to the liposomal product.

Example 4

In a manner similar to the preceding examples several other composition were prepared, characterized and tested, including

- (a) different local anesthetic agents
(e.g. benzocaine, prilocaine, lidocaine-prilocaine mixture) with various concentrations (0.5 to 5%);
- (b) phosphatidylcholines of different origin and various concentrations (2-15%);
- (c) cholesterol or tocopherol in different concentrations (0.5-5%);

(d) buffer solutions with various pH and electrolyte content;

(e) various viscosity inducing agents
(e.g. methylcellulose, Carbopols, etc.) and

(f) various preservatives or antioxidant agents
(e.g. benzoic acid, methyl and propyl paraben, BHA, benzylalcohol, etc.);

The efficacy of local anesthetic agents were tested in liposomal form against a commercial cream preparation or an ointment from of the same drug.

Evaluation of local anesthetic/analgesic activity.

The protocol for the human experiments was approved by the Ethics Committee for Human Research of the Faculty of Health Professions of Dalhousie University, Halifax, Canada. Healthy adult volunteers with no skin disorders or previous history of allergic sensitivity to local anesthetics were asked to participate in the study. Twelve subjects in each experimental group from 25 to 60 years of age were investigated following their written consent.

Tetracaine (0.5%) liposomes

Liposome preparation containing 0.5% tetracaine base (formula as example No. 1) and Pontocaine^R cream (tetracaine

REF 5044 FRANK 753

hydrochloride cream U.S.P., equivalent to 1% tetracaine base, manufactured by Winthrop Laboratories) were compared. A 0.2 ml volume of the liposomal preparation was applied to a 10 cm² area marked by ink on the volar surface of one forearm of the volunteers and covered with a Blenderm^R tape to form an occlusive dressing. A sample of Pontocaine^R cream was applied in the same manner to the other arm. The samples of the liposomal and commercial preparation were randomly numbered, and the number of applied preparations recorded for each subject. The identity of the preparations was not known for the subjects or for the evaluator to maintain the "double blind" study design.

The samples had been applied for 30 minutes in the first group of volunteers and 60 minutes in the second group. After this time interval the covering tape was removed and the tested area wiped dry with a tissue paper. Onset and duration of anesthesia at the test sites was tested with the pin-prick technique, described in detail by Lubens et al. (Am. J. Dis. Child. 128: 192-194, 1974). Each skin test area was pricked ten times using a relatively blunt sterile needle, which allowed the subject to discriminate between the perception of touch and pain. Ten painless pricks indicated complete anesthesia. Sensitivity was confirmed by pin-pricking near to the test site areas before application of the samples to be tested. Testing score indexes were obtained from the volunteers by noting the numbers of painfree pin-pricks out of the 10 in both test areas. Testing was done immediately after the preparations had been removed from the test site, and then at 30 min., 1h, 2h, and 4h afterwards. Results are indicated in Tables 1 and 2.

The liposome preparation containing 0.5% tetracaine base was effective in producing dermal anesthesia. After the onset of anesthesia the perception of pain was greatly reduced; although the pressure could be felt. The perception of cold was also observed to disappear at the "numb" test sites (by testing with a cold metal rod). Sensitivity of nerve fibers conveying the sensations of pain, cold, warmth, touch and deep pressure to local anesthetic action is differential. This is correlated with the fiber diameter, that increases from the fibers conveying the sensation of pain to those conveying deep pressure. Pain fibers are the first to be blocked, followed by sensations of cold, warmth, touch and deep pressure. Probably the absorbed doses were high enough only to block the pain and cold fibers, having no or little effect on touch or pressure sensations.

Duration of application influenced the intensity and duration of the effect. On removal of the preparation after 30 minutes of application, the anesthesia was less pronounced, than after one hour of application. In both cases the anesthesia improved with time. A maximum was reached in the 30 minutes and 1 hour application time groups two hours after removal of the preparation at an average painless score of 8.25 and 9.5 respectively. This level of anesthesia was maintained until the end of the experiments. Tests were conducted only up to 4 hours after removal of the preparations, but the anesthesia as reported by the volunteers to persist longer, from 5 to 8 hours, depending on the application time. Considerable interindividual variations were observed in the onset time of action. Painless scores in

REEL 5044 FRAME 755

the 30 minutes and 1 hour application time groups at the time of removal were at or above 7 in 25% and 50% of all the subjects (N=12), respectively. Pontocaine cream, the control preparation, was found to be ineffective, in agreement with findings of Dalili and Adriani (Cin. Pharm. Ther. 12: 913-919, 1971).

Statistical analysis of the data by paired t-tests indicated a statistically highly significant difference in favor of the liposomal tetracaine over the commercial preparation (Tables 1 and 2).

Liposomes with 2% lidocaine (preparation as example No. 2) were compared first to a placebo, which consisted of "empty" liposomes with the same composition as that of the active preparation (example No. 2) but without lidocaine.

Comparison of the 2% lidocaine liposomes was also carried out to a control dosage form, which contained 2% lidocaine incorporated in Dermabase^R as the vehicle. In both groups the length of application of liposomal and control preparations was one hour.

The effect of the 2% lidocaine liposomes compared to the placebo as measured by the painless scores is shown in Table 3. Similarly to the tetracaine liposomal preparation, lidocaine encapsulated in liposomes could produce anesthesia in the intact skin. The pain and cold sensations have been greatly reduced but not the perception of pressure. The intensity of the effect

again increased after the removal of the preparation, to reach its maximum one hour later. The differences between the placebo and the liposome-encapsulated lidocaine preparation were statistically significant at every time point (Table 3). The results were similar, when liposomal lidocaine was compared to lidocaine in Dermabase^R (Table 4).

CLAIMS

1. The use of liposomes for improving the effect of local anesthetics or analgesics applied topically
2. The liposomes of claim 1 can be:
 - a) multilamellar lipid vesicles
 - b) unilamellar lipid vesicles, and
 - c) classified as multiphase liposomal drug delivery system
3. The liposomal local anesthetic or local analgesic products (of claim 1) can contain:
 - a) any biologically active agents that is classified as local anesthetic or local analgesic
 - b) phospholipids of different origin and in different concentration
 - c) cholesterol other lipids and/or tocopherol in various concentrations

- d) auxiliary agents, e.g. ethanol, glycerin, propylene, glycol, viscosity inducing agents, surface active agents preservatives, antioxidants, etc.
- e) the aqueous phase can be distilled water or buffer with various pH, and electrolyte content

Table 1. Mean painless scores at different times of observation aft
 30 minutes application of 0.5% tetracaine liposome preparation
 and Pontocaine cream
 Number of volunteers=12
 Statistical analysis by paired t-tests

Time	Liposome preparation		Pontocaine cream	
	Mean	SD	Mean	SD
at removal	2.75	3.25	0.25	1.73
30 min	5.50	3.94	1.08	1.98
1 hour	6.75	3.28	1.08	1.68
2 hours	8.25	2.45	1.08	1.31
4 hours	8.33	2.31	0.25	0.62

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Table 2. Mean painless scores at different times of observation after 1 hour application of 0.5% tetracaine liposome preparation and Pontocaine cream
 Number of volunteers=12
 Statistical analysis by paired t-tests

Time	Liposome preparation		Pontocaine cream	
	Mean	SD	Mean	SD
at removal	6.25	3.65	0.08	0.2
30 min	8.08	2.27	0.41	0.9
1 hour	8.83	1.47	0.25	0.6
2 hours	9.50	0.67	0.33	1.1
4 hours	8.75	1.48	0.16	0.5

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Table 3. Mean painless scores at different times of observation after 1 hour application of 2% lidocaine liposome preparation and placebo

Number of volunteers=12

Statistical analysis by paired t-tests

Time	Liposome preparation		Placebo	
	Mean	SD	Mean	SD
at removal	4.08	4.42	1.08	1.51
30 min	6.08	4.14	1.33	1.92
1 hour	7.25	3.86	2.08	2.23
2 hours	6.16	3.35	1.58	2.81
3 hours	5.33	3.17	1.16	2.04

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Table 4. Mean painless scores at different times of observation after 1 hour application of 2% lidocaine in liposomes and Dermabase (control)
 Number of volunteers=5
 Statistical analysis by paired t-tests

Time	Liposome preparation		Dermabase (control)	
	Mean	SD	Mean	
at removal	6.2	3.56	1.8	2
30 min	7.4	3.71	2.6	1
1 hour	9.8	0.45	3.6	0
2 hours	8.6	1.14	3.2	1
3 hours	4.6	3.13	2.2	2

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