Anaesthetic effect of Sodium-Thiopental by bath on the three different body weights of Rainbow trout (*Oncorhynchus mykiss,* Walbaum, 1792)

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Abstract

The aim of this study was the efficacy of sodium-thiopental as an aneasthetic by bath on three different body weights of rainbow trout (*Oncorhynchus mykiss*). During the experiment, aeration had done and physicochemical properties of water such as dissolved O_2, temperature and pH were recorded. Trouts were sorted into three groups with different mean body weights of 8.31±0.36g, 22.58±0.61g and 81.61±1.76g. Every three groups were anaesthetized by bath, with 100 mg/L (100 ppm) of sodium-thiopental concentration. The results showed that the induction and recovery times, being significantly affected by sodium-thiopental, so that, these were shorter in the heavier fish. Anaesthesia duration for the fish with different mean body weights of 8.31±0.36g, 22.58±0.61g and 81.61±1.76g were thiopental with the weight of fish. So that the elapsed and recovery times were shorter in more weight fish than lighter ones.271±64.88, 240±3.33 and 231±76.47 seconds respectively. Also, recovery times were 448±67.81, 415±10.80 and 383±57.55 seconds respectively. Monitoring for 2 hours was shown that all fish have been alive. So, 100mg/L concentration of sodium thiopental is safe in the trout. The results of the present study showed that there was an inverse relationship between the elapsed and recovery times of sodium.

Keywords: Fish, Rainbow trout, *Oncorhynchus mykiss*, Sodium-thiopental, Anesthesia, Aquaculture, Pharmacology

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Introduction

Anaesthesia had been one of the problems of ancient medicine. For years, humans were unaware of the presence of anaesthetics, but today these drugs have important applications in medicine, veterinary and aquaculture. Anaesthetics are necessary for some procedures on aquatic animals such as reducing stress, handling, transporting, physical examination, surgical, blood sampling ,egg extraction (artificial reproduction), drug or vaccine injection, biopsy and biometry (Marking and Meyer, 1985; Gilderhus and Marking, 1987, Brown, 1988; Boyer *et al*., 2009). The efficiency, safety and harm of anaesthetics depend on fish species, fish size, physicochemical properties of water (water quality), environmental factors, type of anaesthetics and methods of delivery anaestetics (Schoettger and Julin, 1967; Bailey *et al*., 2014; Balko *et al*., 2018). There are different compounds as anesthetics in fish, such as: ether (Anonymous, 1954), tricaine methanesulfonate (MS-222, Finquel) (Watson, 1961; Schoettger and Julin, 1967; Massee *et al*., 1995), quinaldine (2-methylquinoline) (Muench, 1958; Schoettger and Julin 1969; Moring, 1970; Massee *et al*., 1995), sodium bicarbonate (Booke *et al*., 1978; Peake, 1998), metomidate, benzocaine, chrobutanol, clove oil, eugenol, Iso-eugenol, 2-phenoxyethanol, quinaldine-sulphate (Mattson and Riple, 1989; Massee *et al*., 1995; Woody *et al*., 2002; Soltani *et al*., 2007; Sneddon, 2012), 4-styrylpyridine (4-SP) (Howell and Thomas, 1964), carbon dioxide, salt (NaCl), etomidate, chlorotone, chlorobutanol, tobacco juice (Nicotine) (Marking and Meyer, 1985), halothane, etorphine (Mehrabi, 2002) alphaxalone/alphadolone, chlorbutanol, diazepam, ethanol, chloral hydrate, lidocaine, methylparafynol, metheylpentynol, pentobarbitone, procaine, propandid, propoxate, sodium amytal, sodium bicarbonate, tertiary-amyl alcohol, urethane (Brown, 1988). Some of them are effective while others have ambiguous anaesthetics effects. Clove and its essence applied as an anaesthetic in some aquatic animals (Soltani *et al*., 2007; Boyer *et al*., 2009). Clove is herbal which make anaesthesia in the fish as natural form (flower) (Mehrabi, 2002; Soltani *et al*., 2007; Boyer *et al*., 2009). In Iran, dry flower buds of the clove tree grind and use an anesthetic in fish (3-30 grams of clove powder in 20 liters of water) at hatcheries. It can make anaesthesia in small and large fish up to 5 Kg effectively. Clove is organic, natural, inexpensive anaesthetic in rainbow trout. Anaesthetics could be divided into two categories including products of synthetic (chemical) and natural (plant-based) (Aydin and Barbas, 2020). Some anaesthetics are effective while others have ambiguous anaesthesia effects. Thiopental sodium has introduced to the medical profession in 1934 and has the advantages of rapid action and relatively short duration (Dickie, 1948). Sodium thiopental had known as trade names such as sodium-pentothal (Pentothal®), thiopentone and trapanal. Its chemical structure showed in Figure 1. Thiopental

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is a lipophilic barbiturate and penetrating brain tissue rapidly following intravenous administration, a characteristic favouring their use for the induction of anaesthesia. In human, thiopental use for general anaesthesia as an intravenous agent, it usually occurs in less than 30 seconds. Induction dose is 3-5 mg/Kg for thiopental and its duration of action is 5-10 minutes.

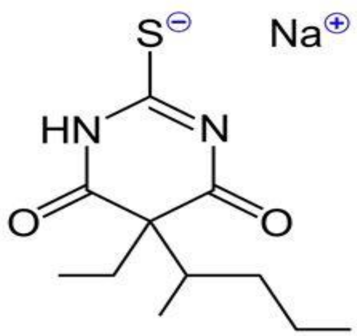

Figure 1; Chemical structure of Sodium thiopental, formula: C11H17N2NaO2S. (©http://en.wikipedia.org/wiki/Sodium_thiopental)

Thiopental may reduce respiration and %85 of the drug bind with protein. It metabolized by the liver but has a long elimination half-time. Solutions of thiopental sodium for intravenous injection have a pH range of 10–11 to maintain stability. In man, the effects of thiopental anaesthesia on the cardiovascular system are benign in comparison with those of the volatile anaesthetic agents (Brunton *et al*, 2018; Katzung, 2018). Na-thiopental is excreted by the kidneys in an unidentified form. Sodium-thiopental unlike the slower-acting barbiturates, may be excreted from the kidney unchanged over two to three days (Dickie, 1948).

Material and methods

Fish

The trial was done on rainbow trout (*Oncorhynchus mykiss,* Walbaum, 1792). The size of fish was ranging from 8.31±0.36 to 81.61±1.76 grams. They grouped into 3 treatment group (each group including 10 fish) based on their weight (Table 3). Fish captured from concreted ponds (size: ~6×40 meters) and sorted in 3 weight types, each group keep in a plastic basin (water).

Drug

Sodium-thiopental applied as an anaesthetic drug. Exactly, 2 gram pure powder of sodium-thiopental (Medical grade) solved in 20 liters of freshwater of spring in Iran. Its final concentration was 100 ppm equal to $100 mgL^{-1}$.

Water quality

Properties of spring water (freshwater) were: temperature=11°C, pH=7.6, dissolved oxygen 6 mgL^{-1}.

Methods

In a basin, 2g Na-thiopenthal powder dissolved in a 20L of water (spring water). Its final concentration was 100 ppm equal to $100 mgL^{-1}$. For each treatment, 10 fish caught and put up in the plastic basin (volume=20 liters). It contained anaesthetic solution (100mg drug which has dissolved in water), and elapsed time recorded for each fish while each stage (1 to 4) of anaesthesia happened (based on Table 2). At the end of the anesthesia period, the fish were weighed rapidly and transferred to a water bath without anesthesia to

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determine the recovery time (Table 3). In each treatment, the fish were monitored to determine the survival rate for two hours.

Anesthesia in fish has different stages such as 0, I, II, III and IV. Stage 0 is the normal fish (non-anesthetized). Stage III may have 3 planes (consist of plane 1, 2 and 3), stage III is surgical anaesthesia; while, stage IV (Table 1) causes death finally (Brown, 1988; Sneddon, 2012).

Table 1: Anaesthesia stages[1] in fish.

Stage	Plane	Level of Anaesthesia	General Demeanour	Activity	Equilibrium	Gill Ventilation Rate	Reactivity	Heart Rate	Muscle Tone	Examples of Procedures
0		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	
I		Lightly sedated	Disorientated	Reduced	Normal	Normal	Reduced	Normal	Normal	
II		Excitation	Agitated	Increased	Difficulty	Increased	Increased	Increased	Normal	
III	1	Light anaesthesia	Anaesthetized	None	Loss	Decreased	Reflex responses	Regular	Decreased	Weight; close visual inspection; external noninvasive tags, gill scrape
	2	Surgical	Anaesthetized	None	Loss	Shallow	None	Reduced	Decreased	Invasive tags; tissue removal; injection; blood sampling; gill biopsy, lesion dressing, recovery surgery
	3	Deep	Anaesthetized	None	Loss	Rare movements	None	Reduced	Relaxed	Non-recovery surgery
IV		Overdose	Apparently dead	None	Loss	None	None	Cardiac failure	None	

[1]Cited by: Sneddon, 2012; It has a few differences from Brown, 1988.

Table 2: Recovery stages[1] in fish.

Stage	Activity
I	Effect of anaesthesia going to be decreased, and the first movement such as tail movement (Slow-single movement) observed in fish while the fish is still on the floor. This stage shows that deep anaesthesia had finished.
II	Fish start to swimming but hasn't equilibrium completely. It tries to go away from touching but can't swim strongly. Fish swims to the left or right side and it settled sometimes.
III	Fish have a normal position and can swim rapid, strong with complete equilibrium.

[1]It is based on observations of this research.

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Table 3: Effect of 100 mg/l Sodium-thiopental concentration on each stage of induction times and recovery times in rainbow trout (*Oncorhynchus mykiss*).

Treatment	Fish number	Fish (*Genus Sp.*)	Mean of fish weight (Grams)	Minimum Weight of fish (Grams)	Maximum Weight of fish (Grams)	Na-Thiopental Concentration (ppm) Dissolved in Water	Start time[2]	Elapsed time to cause Stage[3] II of Anesthesia (Seconds)	Elapsed time to cause Stage III-Plane1 of Anesthesia (Seconds)	Elapsed time to cause Stage III-Plane2 Anesthesia (Seconds)	Elapsed time to cause Stage[4] III-Plane3 Anesthesia (Seconds) [Muscle relaxation]	Efficiency (Percent of fish which had been anaesthetized)	Anaesthesia duration[5] (Seconds)	Full recovery time[6] (Seconds)	Total duration[7] (Minutes)
1	10	OM[1]	8.31± 0.36	7.8	8.9	100	0	50±0.94	120±3.46	150±5.65	601±99.49	100	271±64.88	448±67.81	17.29
2	10	OM	22.58± 0.61	21.7	23.6	100	0	55±3.33	150±4.78	240±5.77	720±160.69	100	240±3.33	415±10.80	18.55
3	10	OM	81.61± .76	79.2	84.6	100	0	90±2.49	154±4.59	345±27.08	800±24.03	100	231 +76.47	383±57.55	19.43

[1] – *Oncorhynchus mykiss* (Walbaum-1979): (Common name: rainbow trout).

[2] - Start time: It shows the time that fish transfer to the basin containing the anaesthesia (Na-thiopental).

[3] - Anaesthesia stages (Sneddon, 2012).

[4] – Fish have been relaxed at the bottom of the basin and haven't moved. They didn't reflex to touching.

[5]- Anaesthesia duration: It started at the time of transferring anaesthetized fish from anaesthetic solution (settled at the basin bottom) to a basin with clean water-without drug, and it finished while the first muscle movement (especially tail movement) showed in fish.

[6]- It was elapsed time from the introduction of fish to clean water (basin contain freshwater without drug) to time that fish have swam normally and strongly.

[7] - It was elapsed time from the exposure fish to anaesthesia (basin contain water and anaesthesia) to full recovery time, when fish have swimming normal strongly.

Recovery

Symptoms of anaesthesia appeared in the fish after they were exposed to the aqueous solution of sodium-thiopental concentration (100ppm). They were under deep anaesthesia while they were completely immobilized and settled on the bottom of the basin and without any reaction to the external stimuli. After ensuring deep anaesthesia in the fish, they were transferred to a basin containing drug-free water to measure recovery time. The criterion of the end of the anaesthesia period was the first movement of the fish (deep gill movement or especially tail movement). The criterion of full consciousness was a fish swimming in full balance and easily escaping from the external stimuli.

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Ethical reason

In aquaculture and for artificial reproduction of the fish (rainbow trout), the technique of artificial insemination is used. Based on this, the breeders are examined and the male and female breeders which can produce sperm and eggs are separated from the fish flock. Then, by hand and pressing the chest area to the tail of the fish, sperm and eggs are artificially extracted from the fish. This operation is not pleasant for alert fish. Therefore, the use of anaesthesia can greatly reduce the pain and discomfort of the fish. Accordingly, anaesthetic study not only promotes the development of pharmacology for aquaculture but also may provide a suitable drug to reduce stress and pain during artificial fish reproduction. On the other hand, anaesthetics help us to reduce stress and irritation in fish during handling, transferring and clinical procedures. Therefore, there are important reasons for doing this research so it wasn't unethical. On the other hand, the sampling was very low. Each treatment contains 10 fish only while 30 fish generally is the need for statistical analysis. If less than 10 fish were used, the results would be unreliable. Adaptation time was also considered for the sampled fish to reduced stress in fish. After the test all fish was alive and they were returned in the pool to have natural living in the flock (Table 1).

Results

The results determined that Na-thiopental can cause anaesthesia in the fish (*Oncorhynchus mykiss*) as an aqueous solution (100 mgL^{-1}=100 ppm). At the end of the experimental period, the survival rate of all fish under anesthesia was 100% recorded. No any mortality were recorded even 2 hours after the end of experiment. In this research, three different body weight fish groups tested (Table 3), each treatment contains 10 fish sample. Therefore, the fish gradually lost their balance for about 50 to 90 seconds and began to swim erratically, then, they became completely inactive and anaesthetized and they settled at bottom of the pan. The results showed that Na-thiopental (100 mgL^{-1}) concentration cause anaesthesia in fish with three different weights (8.31, 22.58 and 81.61 grams), which were 10, 12 and 13 minutes respectively. Anaesthesia durations were 4.31, 4.00 and 3.51 minutes respectively. The recovery time gained completely after 7.28, 6.55 and 6.23 min respectively. In summary, Na-thiopental solution (100 ppm) in water caused anaesthesia in fish after 10-13 minutes by the estimate and its effects remaining for 3.51 to 4.31 minutes while fish is out of reach of the drug. Fish recovered to normal condition after 6-8 minutes by estimate. These results are shown in Table 3.

After the ending of anaesthesia-duration, this trial shows that 3 stages of recovery are recognizable in the fish (*Oncorhynchus mykiss*). The stages explained in Table 3.

On the other hand, administration of the drug by intramuscular injection did not cause any anaesthetic effect in the fish. Therefore, the exact details of this experiment were not mentioned.

Statistical analysis had done by GraphPad®–Prism-8 software. It done by one-way-ANOVA method and tuckey's test. The results show that "Elapsed time to cause Stage III-Plane3 Anesthesia", "Anaesthesia duration" and "Full recovery time" were haven't equal in the 3 treatments. The P-values mentioned in Tables 4 and 5.

Table 4: Results of statistical analysis (One way anova).

Row	Compared Factor[1]	Compared treatments	*P* value	Significant	Result
1	Induction time (Stage III-Plane3) Anesthesia [Muscle relaxation]	T1 vs. T2	0.0569	No	"Elapsed time" has equality for fish of T1 and T2.
		T1 vs. T3	0.0011	Yes	"Elapsed time" hasn't equality for fish of T1 and T3.
		T2 vs. T3	0.2522	No	"Elapsed time" has equality for fish of T2 and T3.
2	Anaesthesia duration	T1 vs. T2	0.4654	No	"Anaesthesia duration" has equality for all fish of T1, T2 and T3.
		T1 vs. T3	0.2870	No	
		T2 vs. T3	0.9358	No	
3	Full recovery time	T1 vs. T2	0.3418	No	"Recovery time" has equality for fish of T1 and T2.
		T1 vs. T3	0.0239	Yes	"Recovery time" hasn't equality for fish of T1 and T3.
		T2 vs. T3	0.3635	No	"Recovery time" has equality for fish of T2 and T3.

[1]It had done by GraphPad-Prism-8 software with One-Way ANOVA method and Tuckey's test.

Table 5: Results of statistical analysis (Correlation).

Row	Compared Factor[1]	Treatments	Pearson r	Result
1	Correlation between "Elapsed time to cause Stage III-Plane3 Anesthesia" and weight.	T1	0.898	Elapsed time to cause anesthesia had increased by fish weight increasing.
		T2	0.931	
		T3	0.797	
2	Correlation between "recovery duration" and weight.	T1	-0.934	Recovery duration had decreased by fish weight increasing.
		T2	-0.787	
		T3	-0.919	
3	Correlation between "anaesthesia duration" and "recovery duration".	T1	0.931	There is positive correlation between "anaesthesia duration" and "recovery duration" in each treatments.
		T2	0.772	
		T3	0.981	

[1]It had done by GraphPad-Prism-8 software with Pearson test

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Chart 1:
Statistical analysis of elapsed time to cause stage III-plane3 anesthesia.

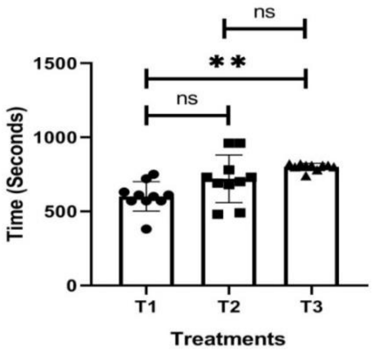


Chart 2:
Statistical analysis of anaesthesia duration.

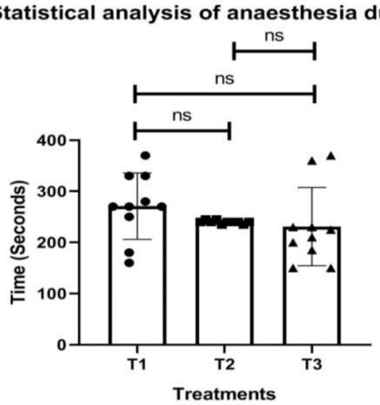
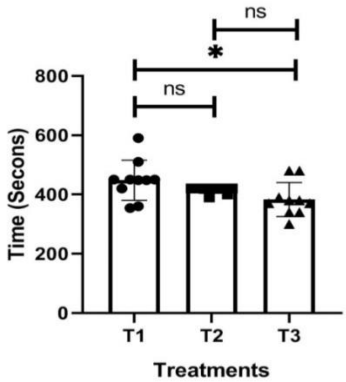


Chart 3:
Statistical analysis of recovery time.



Discussion

The present study showed that the induction and recovery times, being significantly affected by sodium-thiopental, so that, these were shorter in the heavier fish. Anaesthesia stage 4 for the fish with three different mean body weights were 10, 12 and 13.3 min respectively. These results are in agreement with the results of obtained Hikasa *et al*. (1986). They showed that the mean times required to induction fish exposed to 200 ppm and 300 ppm of thiopantal at 10ºC were 21.9 min and 15.2 min respectively; and 17.5 and 13.3 min at 20ºC. Other results of the present study obtained were the recovery times which were 7.46 min, 6.91 min and 6.38 min respectively. These results are inconsistent with the results obtained Hikasa *et al*. (1986). They stated that the recovery times on adult carp (Cyprinus carpio) exposed to 200 ppm and 300 ppm of thiopantal at 10ºC were 76.4 min and 83.0 min, respectively; while at 20ºC were 50.2 min and 70.0 min in 200 and 300 ppm respectively. These results indicated that the recovery time on adult carp with 200 ppm and 300 ppm concentration of sodium thiopental was very long. While, the present study showed less than 8 min generally. This difference suggests that the anaesthesia effect of sodium thiopental can depend on the fish species, the size and water temperature. Due to the huge differences

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between the results of these two studies regarding the recovery time of sodium thiopental, more research is suggested with different fish species, different sizes and different temperatures.

Walter and Florence (1978) evaluated the effects of surfactants on the passive transfer of thiopentone (Thiopantal) across epithelial membranes of goldfish at the concentration of 0.01%. It shows that Thiopental can transfer across the epithelial membrane. The present research shows that Thiopental can cause anaesthesia in fish while it is soluble in water. Therefore, thiopental enters to body fish by gill. So, thiopental penetrates to blood circulation through gill and effect the nervous system of the fish.

Sevcik, 1980 evaluated the effect of Thiopental at the cellular level, He claimed that Thiopental depolarizes the nerve fibers of the giant squid and produces spontaneous repetitive discharges (Sevcik, 1980). So, it shows that Thiopantal is effective on squid nerves. Dodson *et al*. (1990) studied potencies of several drugs to binding to the torpedo (fish) acetylcholine receptor. Although, they didn't study anaesthetic effects of drugs they evaluated the effects of drugs on the receptors. They evaluated binding powers of the drugs and expressed their potential as ranking order: pentobarbitone, secobarbitone> thiopentone (Thiopental)> DMBB> butabarbitone - phenobarbitone> aprobarbitone> allylbarbitone. Based on their research Thiopental have a moderate potential to binding with acetylcholine receptor. Base on the researches Thiopental is effective on the nerve cell. The present research claimed that Thiopental has anaesthetic effects. However, all researches show similar features of a drug.

Hikasa *et al*. (1986) studied the anaesthetic effects of tricaine methanesulfonate (MS-222), eugenol (FA-100) and Sodium-Thiopental (RABONAL) on adult carp (*Cyprinus carpio*). They applied them to carp by immersing the fish in the water-solution contains drugs and claimed that Thiopantal (200 to 300 ppm) shortened the time required to induce each anaesthetic stage and delayed recovery with dose depended on manner. They claimed that MS-222 caused an increase in respiratory rates while eugenol and Thiopantal decreased respiratory rates. They mentioned high water temperature (20°C) augmented the anaesthetic effects of agents and facilitated the recovery. They had applied Thiopental as a soluble form in water. We applied thiopental as a soluble form in water, too. Therefore, Thiopental as aqueous not only can cause anaesthesia in carp but also it can cause anaesthesia in rainbow trout. They had applied Thiopental as 200-300 ppm concentration for carp between 700-750g. We applied it as 100 ppm for rainbow trout between 8-80g. It shows that Na-Thiopental concentration for anaesthesia can be lower in small fish. Anaesthesia happened after ~13-22 minutes in carp while the present research shows that anaesthesia

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happened in rainbow trout after ~10-13 min.

The last point is that Thiopantal is a medical drug with potential anaesthesia effects on human (Katzung, 2018), so its public use probably needs some limitations to prohibit its abusers.

Finally, according to our findings, it could be concluded that:

1- Na-Thiopantal could cause anaesthesia in fish as a soluble form in water.
2- Applied dose was 100 PPM for fish size between 8-80g. It caused about 5 min anaesthesia in the fish.
3- It is a safe drug for the fish (*O. mykiss*) without mortality.

Conclusion

The results determined that Na-thiopental can cause anaesthesia in the fish by the soluble form in water. Therefore, it absorbed through gills of fish and transferred by gill blood circulation in fish body probably and make anaesthesia in fish by the effect on CNS. It is an effective anaesthetic drug in the aquaculture because all fish (%100) had been anaesthetized. It is a safe drug for fish because all (%100) fish (samples) were survival and recovered safely after the trials. Sodium-thiopental is a barbiturate, which can cause anaesthesia not only in human (Katzung, 2018) but also in the fish (Oncorhynchus mykiss). It can prescribe by gill route as a soluble drug in water. Suitable concentration to make anaesthesia is about 100 ppm (100 mg L-1) for fish with a weight range between 8-80 grams. Less or higher concentrations didn't test by this research because some limitaions. However, Na-thiopental can be a drug choice for safe anaesthesia in fish. Due to the huge differences between the results of these two studies regarding the recovery time of sodium thiopental, more research is suggested with different fish species, different sizes, different temperatures and different doses of sodium-thiopenthal.

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