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THE INFLUENCE OF ALPHA-TOCOPHEROL ON BARBITURATE ANESTHESIA

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THE INFLUENCE OF ALPHA-TOCOPHEROL ON BARBITURATE ANESTHESIA

By

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Thesis presented to the faculty
of Yale University School of
Medicine in candidacy for the
degree of Doctor of Medicine.

Department of Pharmacology
School of Medicine
Yale University
1953

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INTRODUCTION

A. Purpose and Scope

Giarman and Flick (8), proceeding on the assumption that it should be possible, with natural or artificial antioxidants, to control enzymatic oxidation at various stages in the biotransformation of certain barbiturates found that various oxidation-reduction systems markedly prolong the duration of anesthesia induced by thiopental ("Pentothal"). It is the purpose of this work to attempt to define and extend the study of such substances.

In view of the results of the above workers it was decided to study the effects of α-tocopherol along these lines, since it is known that α-tocopherol is a powerful antioxidant in vivo. In an attempt to elucidate further the mechanism of action of α-tocopherol in this regard, it seemed advisable to study the interaction with certain antagonistic and synergistic substances.

Because of the known differences in the dependence or independence of action of certain barbiturates upon their rate of metabolic transformation this study was designed to include several pertinent barbiturates.

Such an investigation has a two-fold objective. Its practical one is the hope that a non-toxic substance may be brought to light which would potentiate or antagonize the effect of an intravenous anesthetic agent. Academically it is hoped that the results of this investigation may eludicate the fundamental mode of action as well as the factors involved in the fate and distribution of the anesthetic agents in the living organism.

Mice were used in this study as the experimental animal, although a few experiments made use of the rabbit. The advantage of using mice

in such an investigation lies in the fact that a good sampling of a population may be made at a relatively low cost. Furthermore in the mouse the relatively low variability in the loss and regaining of the righting reflex under the influence of a fixed anesthetic makes possible the observation of sharp beginning and end points for determination of the duration of anesthesia.

B. Historical Background

1. Distribution and Fate of Barbiturates

The duration of effect of non-volatile anesthetic agents like the barbiturates is determined in general by their metabolic transformation and excretion (1).

It is believed that the longer acting barbiturates, barbital and phenobarbital for example, are excreted essentially unchanged by the kidneys, while an ultra-short-acting barbiturate like thiopental is rapidly detoxified by the liver and excreted (3,4). Shideman, Kelly and Adams (2) in this regard found that 90 percent sub-total hepatectomy or carbon tetrachloride damage to the liver in rats prolonged anesthesia following intravenous thiopental by as much as 800 percent. The implication from this work is that thiopental is an ultra-short-acting barbiturate simply by virtue of its relatively rapid degeneration in the organism.

Recently, Brodie and co-workers (4,5) have presented important evidence leading them to conclude that thiopental is only slowly degraded <u>in vivo</u> (about 10% per hour), and that its ultra-short action is due chiefly to its rapid distribution from aqueous media of the body to fatty depots. These workers found, for example, that the plasma concentration of thiopental declines rapidly for 30 minutes after an intravenous

injection of thiopental followed by a slow, gradual decline for 24 hours. It is postulated that an equilibrium between plasma and tissue is established primarily, and that the secondary slow decline is due to the slow metabolism of thiopental. The highest concentrations (up to 70%) of thiopental were found in the fat stores of the body, leading to the suggestion that it is distribution into fat which regulates the duration of action of this agent. This mechanism has been shown to hold for certain other short-acting barbiturates, but not for oxygen analogues, such as pentobarbital ("Nembutal") (4). Rather, the short action of pentobarbital, it is suggested, is due to its rapid metabolic transformation in the organism (5).

Herman and Wood (6), in keeping with Brodie's hypothesis, compared the amount of thiopental needed for anesthesia in a group of fat rats as compared to a group of lean litter mates. They found a 5 percent decrease in the length of anesthesia at a fixed thiopental dosage, or a 40 percent increase in the amount of thiopental required for a given duration of anesthesia in the fat group of rats. In sharp contrast, the duration of pentobarbital anesthesia was not influenced by the amount of body fat.

2. Substances which Influence the Duration of Barbiturate Anesthesia

There have been many reports in the literature concerning exogenous chemical substances which influence the duration of thiopental anesthesia. Such substances have been extremely diverse and have had no particular chemical property in common.

Lamson, Grieg and Robbins (7) found that glucose and related compounds increased the pentobarbital anesthesia time in dogs and guinea pigs. Flick (8), studying thiopental anesthesia in mice, failed to observe

these effects with glucose, but did observe similar effects with sodium lactate.

Winter (9) reported that antihistamines prolong the anesthetic effect of hexobarbital ("Evipal") in mice. Such agents as "Pyribenzamine", "Neo-Antergan", and "Benadryl" were included in this study.

Richards, Bertcher and Taylor (10) found that guinea pigs recovering from barbiturate anesthesia can be returned to sleep by intraperitoneal injections of such diverse substances as NaSCN, NaCl (15% solution), Na_2SO_4, urea, Na cyclohexylsulfamate. Cyclic anesthesia such as the above could also be obtained with intracardiac injections of 0.1 c.c. to 1.0 c.c. of distilled water or blood. They found that resumption of sleep occurred at a blood barbiturate level below the level on awakening. It was suggested that a change in the permeability of the blood-brain barrier should be kept in mind as an explanation for this action.

Wooster and Sunderman (11) found that such vasodilatory agents as nitroglycerin, butyl nitrite, and sodium nitrite, administered intraperitoneally prolong the sleeping time induced by thiopental in mice.

Richards, Kueter and Klatt (12) found a prolonged pentobarbital anesthesia time in vitamin C-deficient rats but no change in the anesthesia time of barbital and thiopental in these animals. Higgens and Mann(13) reported essentially the same findings in vitamin B-deficient rats.

Westfall (14) showed that pyruvic acid and pyruvic acid polymers reduced the duration of anesthesia in rabbits anesthetized with barbiturates.

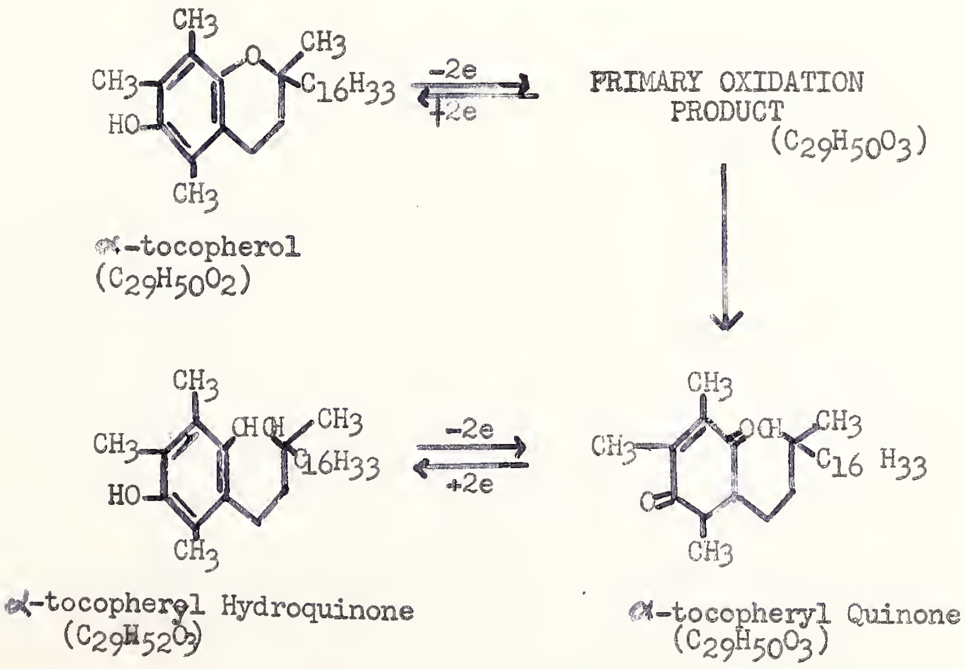
Giarman, White and Flick (15) have shown that tetraethylthiuram disulfide ("Antabuse") prolonged the anesthesia time induced by intravenous thiopental in mice by as much as 60 fold. Graham, Tweed and Allmark (17) have reproduced this effect of "Antabuse" in rats. They failed, however,

to prevent the "Antabuse" effect with sodium ascorbate just as Giarman and co-workers (15) failed to prevent it with "Ferro-ascorbin" (ascorbic acid and ferrous sulfate).

Giarman and Flick (18) have suggested that agents which are capable of prolonging thiopental anesthesia may be found among compounds which establish oxidation-reduction systems _in vivo_. These workers have reported that two oxidation-reduction systems, ascorbic acid-dehydroascorbic acid and cysteine-cystine, prolong thiopental anesthesia in the mouse. The ascorbic acid-dehydroascorbic acid was found to be the more potent of the two and was found to be effective in the production of cyclic anesthesia. Three to six cycles of anesthesia were observed without further addition of thiopental.

3. α-tocopherol

It is believed that α-tocopherol acts as a non-specific physiological antioxidant, which enters into an oxidation-reduction system _in vivo_ as follows: (19)



In normal animals it has been found that α-tocopherol modifies the metabolism of lipids and phospholipids, enhances phosphorylation and improves the metabolism of carbohydrates (20. In regard to the influence on lipid metabolism, Dam has shown that pathological peroxidation occurs in the fat of vitamin E deficient chicks (21). Dam has indicated that this peroxidation can be returned to a normal state of metabolism by the administration of vitamin E and other oxidation-reduction substances such as methylene blue and vitamin K (22).

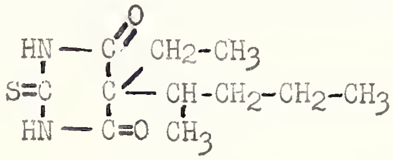
α-tocopherol phosphate <u>in vitro</u> is not an antioxidant and possesses no oxidation-reduction potential (20). Engle has shown, however, that this ester is readily hydrolized <u>in vivo</u> and has the biological activity of the unesterified compounds, entering into the above oxidation-reduction system(23).

It is conceivable, therefore, that α-tocopherol is potentially capable of modifying the duration of barbiturate anesthesia because of these two fundamental properties:

1. Its antioxidant property which might inhibit bio-oxidation of the barbiturates.
2. Its influence on fat metabolism which suggests a possible effect on the distribution of the thiobarbiturates into fat.

EXPERIMENTAL METHODS AND RESULTS

The barbiturate used chiefly in this study was thiopental which is a thiobarbiturate of the following chemical formula:



Giarman and Flick found that 30 mgm. of thiopental per kilogram of body weight produced a convenient duration of anesthesia for studies of this type. Therefore, this dosage was used throughout these experiments. A 1.5% solution was prepared each day by dissolving 150 mgm. of the sodium salt of thiopental in 10 c.c. of distilled water; thus, 0.002 c.c. of this solution per gram of body weight yielded the desired dosage.

The mice were held stationary for an intravenous injection by placing them in a 1 inch by 4 inch glass tube which had a 1 hole rubber stopper at one end and a notched cork at the other end to allow protrusion of the tail. The glass tube was attached by a clamp to a ring stand at a 45% angle. The tail was prepared for injection by sponging it with xylol and flicking it lightly. In this manner the lateral veins were easily visable and a short no. 27 gauge needle could be easily inserted.

The intravenous injections were made slowly and uniformly over a period of approximately 5 seconds. The volume of material injected intravenously was always less than 0.1 c.c. The experimental animals showed no ill effects from the injections except the occasional production of necrotic areas at the site of the injection.

The α-tocopherol phosphate solutions were prepared by dissolving a weighed amount of the dry material in distilled water. This solution and

control saline and water solutions were injected intraperitoneally 5 minutes before the thiopental or other barbiturate was administered intravenously. Exceptions to this sequence of injections occurred in those experiments where compounds antagonistic or synergistic to α-tocopherol were sought.

The duration of anesthesia was measured from the end of the thiopental or other barbiturate injection to the time when the righting reflex returned in the animal. Care was taken not to stimulate the animals unduly after the induction of anesthesia.

All the experiments were performed on adult mice (Webster strain) averaging approximately 25 grams in weight.

Experiment I. Thiopental Controls

Several mice were given thiopental intravenously without premedication with every new solution of thiopental prepared. In this way, several controls were obtained on each experimental day. These data are recorded in Table I in the Appendix. The mean sleeping time for this population was 5.86 minutes.

Experiment II. Dosage-response Relationship of the Influence of α-tocopherol on Thiopental Anesthesia

Giarman and Flick have shown that certain oxidation-reduction systems are effective in prolonging thiopental anesthesia, e.g. cysteine-cystine and ascorbic acid-dehydroascorbic acid (18). It was expected therefore, that the agent under study in this work, α-tocopherol phosphate, which is known to act *in vivo* as an

α-tocopherol-α-tocopheryl-hydroquinone oxidation-reduction system might have a similar effect.

Solutions of α-tocopherol phosphate in distilled water were prepared in the following percentages: 0.156, 0.3125, 0.625, 1.25 and 2.50. These solutions were administered intraperitoneally to different groups of mice 5 minutes before thiopental anesthesia in the following incremental dosages: 7.8, 15.6, 31.25, 62.5, 125 and 250 mgm. per kilogram.

The results of this experiment are expressed graphically in Figure I, the raw data for which may be found in the Appendix. The augmented sleeping times should be contrasted with the mean sleeping time of the controls (Experiment I) which is 5.86 minutes.

It may be observed from this curve that there is a distinct prolongation of the duration of anesthesia at the lowest dose, 7.8 mgm. per kilogram, which effect is augmented at a rapid rate of change in the succeeding higher dosage levels up to 62.5 mgm. per kilogram. The next two dosage levels, 125 and 250 mgm. per kilogram, indicate a much less marked rate of increase in effect.

Experiment III. The Effect of -tocopherol Acetate Administered Orally

It has been shown that α-tocopherol phosphate does not act as an antioxidant and has no oxidation-reduction potential *in vitro* (20). However, this phosphate ester has the convenient property of water solubility. Further, Engle has shown that the esters of α-tocopherol are readily hydrolyzed to the free acid in the living organism (23).

It seemed necessary, nevertheless, to compare the results of Experiment II with those arising from an experiment involving

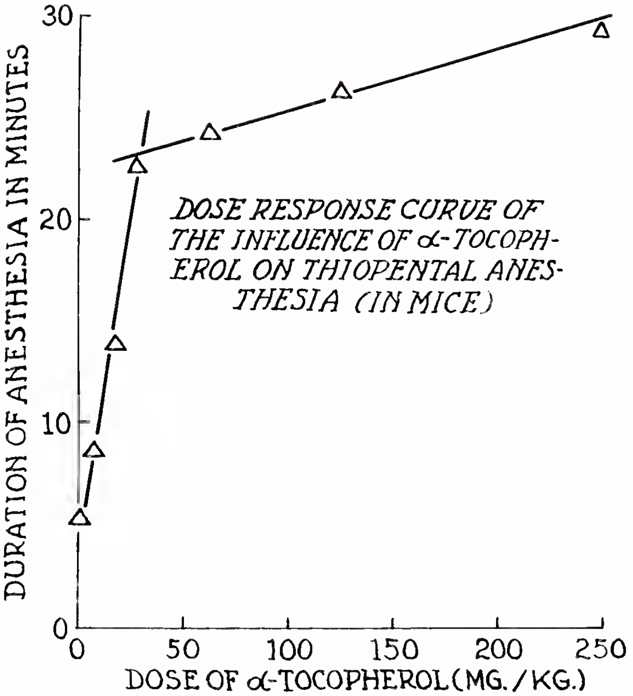

DOSE RESPONSE CURVE OF THE INFLUENCE OF α-TOCOPHEROL ON THIOPENTAL ANESTHESIA (IN MICE)
DURATION OF ANESTHESIA IN MINUTES
30
20
10
0
0
50
100
150
200
250
DOSE OF α-TOCOPHEROL (MG./KG.)

Figure I

α-tocopherol acetate administered orally on a sub-acute dosage regime prior to the intravenous injection of thiopental.

A 2% solution of α-tocopherol was prepared by dissolving 200 mgm. dl-α-tocopheryl acetate in 10 c.c. of peanut oil. This was administered orally to 15 mice by means of a silver abscess cannula which was carefully inserted through the esophagus into the stomach. The total doses used were 200, 400, and 800 mgm. per kilo., divided into convenient daily amounts. These dosage regimes were assumed to provide persistent, high tissue levels.

The dosage regimes and results are presented in Table VIII of the Appendix.

From the results it was apparent that dl-α-tocopheryl acetate administered orally in an oily base has little or no significant effect on the duration of thiopental anesthesia, in marked contrast to the activity observed in Experiment II. Neither the dosage regime nor the time interval between the last oral administration of α-tocopherol and the intravenous injection of thiopental exerted any influence on these results.

Experiment IV. Control Material

Richards, Bertcher and Taylor (10) have reported that various organic and inorganic substances can cause guinea pigs to return to sleep following awakening from barbiturate anesthesia. By intracardial injection small volumes (0.1 to 1.0 c.c.) of water, blood and glucose were effective. The injection of these agents did not influence the decline of the blood barbiturate level but permitted sleep to recur at progressively lower levels. It was postulated that hypertonicity

played a contributory role in the effect of the intraperitoneal injections.

Richards reported, further, that distilled water and normal saline are effective in prolonging the duration of thiopental anesthesia.

Accordingly, it seemed necessary to study as control substances distilled water, isotonic (0.9%) saline, and hypertonic (3%) saline, in the same manner as the α-tocopherol was studied.

Five adult mice were injected intraperitoneally with each of the above materials 5 minutes before intravenous thiopental. The volume injected was the same as that used for the α-tocopherol injections.

The data from this study may be found in Table IX of the Appendix.

It was observed that premedication with these substances in the same volume as the α-tocopherol solutions had no effect on the duration of thiopental anesthesia. The duration of anesthesia following premedication with each of the materials was approximately equal to that observed in the animals receiving no premedication.

Experiment V. Synergism and Antagonism of the α-tocopherol Effect

A. Methionine

It has been shown that methionine increases the anti-oxidant activity of α-tocopherol by more than three fold. Since one hypothesis concerning the mechanism of the α-tocopherol effect is based on its anti-oxidant property, it seemed fruitful to study the interaction of methionine and α-tocopherol on thiopental anesthesia.

For this experiment a 5% solution of methionine was prepared by dissolving 500 mgm. of meonine* in 10 c.c. of distilled water. It was

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* Dl-Methionine (Wyeth)

found that at this concentration the material would remain in solution at approximately 100°F. The methionine, at a dose level of 500 mgm. per kilo. was injected intraperitoneally 5 minutes before the intravenous thiopental in the control experiment.

The experimental animals were given 500 mgm. per kilo. of methionine 5 minutes before the α-tocopherol phosphate at 250 mgm. per kilo. which, in turn, was followed in 5 minutes by the intravenous thiopental. The results of these experiments are summarized in the table below.

Number of animals	Time Sequence	Premedication sequence	Dosage	Duration of anesthesia
30	0 min.	thipental	30 mg./kg.	5.86 min.
5	0 min. 5 min.	methionine thiopental	500 mg./kg. 30 mg./kg.	5.5 min.
8	0 min. 5 min. 10 min.	methionine α-tocopherol thiopental	500 mg./kg. 250 mg./kg. 30 mg./kg.	40.5 min.
15	0 min. 5 min.	α-tocopherol thiopental	250 mg./kg. 30 mg./kg.	30.91 min.

In view of the results of Experiment II which indicated a dual mechanism of action related to dosage, it was decided to study the effect of methionine on lower doses of α-tocopherol. Animals were premedicated with methionine solution at dosage of 500 mgm. per kilo. prior to the administration of increasing doses (7.8, 15.6, 31.25 mgm. per kilo.) of α-tocopherol and the standard dose of thiopental, in the same sequence as above, ie.:

1. 0 time - methionine
2. 5 minutes - α-tocopherol
3. 10 minutes - thiopental

The results are summarized below.

Premedication	Duration of anesthesia
methionine 500 mg./kg. & α-tocopherol 31.25 mg./kg.	19.61 min.
methionine 500 mg./kg. & α-tocopherol 15.6 mg./kg.	6.61 min.
methionine 500 mg./kg. & α-tocopherol 7.8 mg./kg.	6.38 min.

When these results are compared with those found in Experiment II it is observed that enhancement of the α-tocopherol effect by methionine occurred only in the high dose range of α-tocopherol.

B. Calcium and Magnesium

Ames and Risley (20) have shown that α-tocopherol phosphate, acting as a non-specific protein inhibitor markedly inhibits several non-related enzyme systems <u>in vitro</u>, including the succinoxidase system. It was shown further by these workers that excess calcium ions completely remove the α-tocopherol inhibition of the succinoxidase system.

Moreover Brodie and coworkers (24) have indicated that an enzymatic oxidative mechanism operates in the living organism to break down thiopental into an inactive carboxylic acid end product.

It was decided in this investigation, therefore, to study the effect of calcium ions on the prolongation of thiopental anesthesia, demonstrated in Experiment II. For this purpose a 10% solution of calcium gluconate* was used. This was administered intraperitoneally at a dosage of approximately 1000 mgm. per kilo. It was found necessary to administer

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* Neocalglucon (Sandoz)

this material 15 minutes before the α-tocopherol phosphate to prevent precipitation of the calcium complex of α-tocopherol in the peritoneal cavity.

Furthermore it is a well-known pharmacological fact that a mutual antagonism exists between calcium and magnesium ions in the living organism. In certain experiments, therefore, we endeavored to seek an influence of magnesium on any inhibition of the α-tocopherol effect by calcium. In these experiments 100 mgm. per kilo. of magnesium sulfate (in 1% solution) was administered intraperitoneally 5 minutes before the administration of the calcium gluconate. The results are summarized below.

Number of animals	Time Sequence	Premedication sequence	Dosage	Duration of anesthesia
30	0 min.	thiopental	30 mg./kg.	5.86 min.
5	0 min.	Ca. gluconate	1000 mg./kg.	
	5 min.	thiopental	30 mg./kg.	6.60 min.
6	0 min.	Mg. sulfate	100 mg./kg.	
	5 min.	thiopental	30 mg./kg.	6.91 min.
5	0 min.	Ca. gluconate	1000 mg./kg.	
	15 min.	α-tocopherol	250 mg./kg.	
	20 min.	thiopental	30 mg./kg.	11.25 min.
5	0 min.	Mg. sulfate	100 mg./kg.	
	15 min.	α-tocopherol	250 mg/kg.	
	20 min.	thiopental	30 mg./kg.	16.61 min.
10	0 min.	Ca. gluconate	1000 mg./kg.	
	15 min.	Mg. sulfate	100 mg./kg.	
	20 min.	α-tocopherol	250 mg./kg.	
	25 min.	thiopental	30 mg./kg.	28.41 min.

From this experiment the following may be observed:

1. Calcium and magnesium ions per se have no significent effect upon the anesthesia induced by thiopental.

2. Calcium ions markedly inhibit the α-tocopherol potentiation of thiopental anesthesia. Magnesium ions also inhibit the α-tocopherol effect.
3. Calcium and magnesium ions in the same animal antagonize each other so that the α-tocopherol potentiation of thiopental anesthesia in that animal is the same as when α-tocopherol alone is used.

Experiment VI. Failure to Produce Cyclic Anesthesia with α-tocopherol

Levi (24) found that the administration of myanesin to dogs or rabbits at the time the animal was awakening from barbiturate anesthesia caused a brief return of anesthesia.

Lamson, Greig and Robbins (7) reported the production of cyclic anesthesia in dogs using glucose and lactate. When the animals awakened from an initial dose of pentobarbital, an intravenous injection of glucose (10 c.c. of a 50% solution) caused a return of anesthesia for a relatively long period of time.

Richards, Bertcher and Taylor (10) found that in guinea pigs recovering from barbiturate anesthesia (thiopental, hexabarbital and pentobarbital) the intraperitoneal injection of various unrelated organic and inorganic compounds in relatively high concentration caused a reinduction of anesthesia.

Giarman and Flick (18) found that in mice awakening from a 30 mgm. per kilo. dose of thiopental the intraperitoneal injection of ascorbic acid, dehydroascorbic acid the procedure could be repeated (without

further thiopental) producing from 3 to 6 cycles of anesthesia.

On the basis of these findings and the ability of α-tocopherol to prolong markedly thiopental anesthesia,it was decided to determine its ability to produce cyclic anesthesia in the mouse.

A group of mice were injected with thiopental at the standard dosage of 30 mgm. per kilo. and the duration of anesthesia recorded. Immediately on awakening the animals received 250 mgm. per kilo. of α-tocopherol phosphate intraperitoneally. A second group of mice received 500 mgm. per kilo. intraperitoneally of α-tocopherol phosphate on awakening from thiopental anesthesia.

The re-induction of anesthesia was not accomplished in either group of animals, as seen in Table X of the Appendix.

Experiment VII. The Influence of α-tocopherol on the Duration of Thiopental Anesthesia in Rabbits

Lamson and coworkers (7), found a marked species difference in their study of non-hypnotic substances which lead to cyclic anesthesia upon awakening from barbiturate anesthesia. The ease of re-induction of anesthesia and the duration of sleep was found to be greater in guinea pigs than in dogs.

Richards and coworkers (12) were also able to demonstrate a difference between guinea pigs and rabbits in the susceptibility to the re-induction of barbiturate anesthesia with inorganic substances.

Other workers in our laboratories have shown that premedication with α-tocopherol before thiopental anesthesia in the dog does not significantly prolong the duration of anesthesia, as it does in the mouse.

In view of these apparent species differences it was decided to study the effect of α-tocopherol on the duration of thiopental anesthesia in the rabbit.

Procedure: Two rabbits weighing 2.3 kilo. and 2.8 kilo were injected with 12.5 mgm. per kilo. of thiopental intravenously into the lateral ear vein. The duration of anesthesia was then recorded.

After a lapse of 4 days they were given intravenously 25 mgm. per kilo. of α-tocopherol 5 minutes before the same dosage of intravenous thiopental.

Two days after this the procedure was repeated using 50 mgm. per kilo. instead of 25 mgm. per kilo. of α-tocopherol 5 minutes before the intravenous thiopental.

The results of these studies are as follows:

1. Average duration of anesthesia with no premedication-<u>14.75</u> minutes
2. Average duration of anesthesia following 25 mgm. per kilo. of α-tocopherol-<u>17.</u> minutes
3. Average duration of anesthesia following 50 mgm. per kilo. of α-tocopherol-<u>16.25</u> minutes.

It is apparent that α-tocopherol had no significant effect on the duration of thiopental anesthesia in the rabbit.

Experiment VIII. The Influence of α-tocopherol on Duration of Anesthesia Induced by Other Barbiturates

Brodie and co-workers (4,5) have shown that thiopental is oxidized slowly in the living organism and is excreted as a carboxylic acid at a rate of 10% per hour. He postulates that its ultra-short action is

dependent on its rapid localization in the fat stores of the body. He could demonstrate this same fat localization for certain thio-barbiturates (Surital and Kenithol).

The tissue distribution of pentobarbital was demonstrated as being equal in fat and other tissues. Therefore it was postulated that the duration of action of pentobarbital is largely dependent on its rate of metabolism.

In the light of these differences in the relative dependence of duration of action upon fat distribution and rate of metabolism, it was decided to study the effect of α-tocopherol on certain other barbiturates.

The procedure of this experiment was as follows:

A. Hexobarbital (Evipal)

A 2.5% solution of hexobarbital was prepared and administered intravenously at a dosage of 50 mgm. per kilo. in a manner identical with that used in the thiopental study.

α-tocopherol (dosage 250 mgm. per kilo.) was administered 5 minutes before the intravenous hexobarbital in the experimental animals. Sleep was immediate in control as well as experimental animals.

B. Secobarbital (Seconal)

A 5.0% solution of secobarbital (Seconal) was prepared and administered intravenously at 25 mgm. per kilo. The experimental animals were given α-tocopherol at 250 mgm. per kilo. as above.

It was interesting that anesthesia in the control animals was preceded by 30 to 90 seconds of hyperactivity and in three cases failure to achieve anesthesia was observed. In contrast, the animals premedicated with α-tocopherol were unconscious instantaneously following the completion of the secobarbital injection.

C. Pentobarbital (Nembutal)

A 3% solution of pentobarbital was prepared and administered at a dose of 30 mgm. per kilo. The experimental animals were premedicated with 250 mgm. per kilo. of α-tocopherol intraperitoneally, as above. Again a sharp contrast in the onset of anesthesia was noted in the control and the experimental animals as in the secobarbital experiment.

The results of these experiments are summarized in graph form in Figure II. The raw data are recorded in the Appendix. It is apparent that the effect of α-tocopherol on the duration of pentobarbital anesthesia is equivalent to its effect on thiopental anesthesia. The same is true for secobarbital but curiously the effect is not apparent with hexobarbital.

D. Dosage-response Relationship of the Influence of α-tocopherol on Pentobarbital Anesthesia

In view of the results of Experiment II which indicated a dual mechanism of action of thiopental related to dosage of α-tocopherol, it was decided to determine whether a similar dual mechanism might hold for pentobarbital. Therefore it was necessary to study the effect of lower doses of α-tocopherol on pentobarbital anesthesia.

The following procedure was used in these experiments:

Groups of animals were premedicated with increasing doses (7.8, 15.6, 31.25, 62.5 and 1.25 mgm. per kilo.) of α-tocopherol 5 minutes before the standard dose (30 mgm. per kilo.) of pentobarbital.

The results of this experiment in graph form produced a dosage-response curve nearly identical with the dosage-response curve for thiopental in Figure I.

It was noted that there was an inverse relationship between the dose of α-tocopherol and the lag observed between completion of the injection of pentobarbital and the onset of anesthesia.

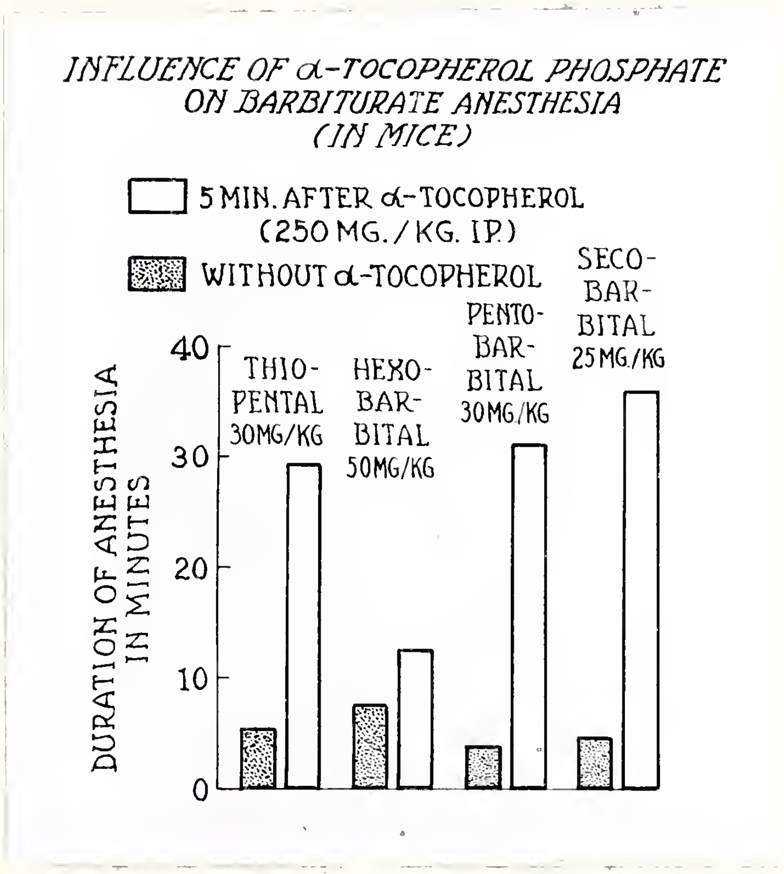

Figure II

DISCUSSION

The data presented here indicate clearly that α-tocopherol prolongs the duration of thiopental anesthesia. While such a phenomenon has been observed before with other oxidation-reduction compounds very few suggestions as to the mechanism of this effect have been presented. Several possibilities for explaining the α-tocopherol effect may be considered.

1. α-tocopherol, by virtue of its antioxidant property, can inhibit the biological oxidation of barbiturates thereby prolonging their action.

2. α-tocopherol may influence the distribution of the barbiturates, altering particularly the distribution equilibrium between the aqueous and fat compartments of the body.

   a. Possible effect on the permeability of the blood-brain barrier.

   b. Possible influence on transfer of barbiturates between extracellular fluid and the brain cells.

3. α-tocopherol may antagonize the barbiturate at the enzymic site of anesthesia.

4. Miscellaneous mechanisms.

It has been shown by Brodie and others that the barbiturates undergo metabolic transfer in the body by way of side-chain oxidation. It is believed that this oxidation process goes through a succession of alcohol, aldehyde, and is finally excreted as a carboxylic acid. It is conceivable therefore that an antioxidant material introduced at any point

in this metabolic sequence could prolong the existance of the unchanged drug or one or more of the potentially active intermediate compounds with a resulting increase in the duration of anesthesia.

Our finding, using several pertinent barbiturates, have not given us any direct evidence that this indeed is the mechanism of action. Indirect evidence is found, however, in the fact that anesthesia produced by pentobarbital is prolonged by α-tocopherol in a manner similar to thiopental. The relative dependence of the duration of action of pentobarbital on its rate of metabolic oxidation has been demonstrated by Maynert and Van Dyke (25) and Brodie et al. (4). Another point of evidence that α-tocopherol acts as a blocking agent at some point in the oxidation of the barbiturates is the fact the methionine, which is known to enhance the antioxidant activity of α-tocopherol, acts in a synergistic manner with α-tocopherol in prolonging thiopental anesthesia. It would seem, therefore, that a study of the influence of α-tocopherol on the rate of metabolic oxidation of those barbiturates known to be largely dependent upon this oxidation for their duration of action, would be an interesting continuation of this study.

The demonstration by Brodie that the thiobarbiturates are rapidly distributed into the fat stores of the body and the postulation that their ultra-short action is dependent upon this distribution brings to mind the possibility that α-tocopherol may alter distribution of these barbiturates. This possibility is even more attractive when one considers the property of α-tocopherol of influencing the metabolism of lipids and phospholipids (26). In addition, Dam has demonstrated that the

pathologic peroxidation of lipids that occurs in vitamin E defficient rats can be returned to normal with α-tocopherol, another indication of a direct relationship between α-tocopherol and fat metabolism.

It is interesting to postulate that α-tocopherol, in a manner unknown in our present state of knowledge, may alter the metabolism of fat so that the barbiturate agent is maintained in a higher concentration in the more effective aqueous compartment of the body.

The biphasic curve obtained from the dosage-response relationship of the influence of α-tocopherol on the duration of thiopental anesthesia could be interpreted to indicate a dual mechanism of action for α-tocopherol, one acting at the lower dosages, below 31 mgm. per kilo., and the other being more effective at the higher dosages. There is no direct evidence, however, that either is a distribution effect; although one might well be such an effect, while the other might be concerned with transformation of the drug as argued above.

As mentioned earlier, Brodie has demonstrated that the oxygen analogue of thiopental, i.e. pentobarbital, is not localized in the fat stores and its duration of action is probably not dependent on this factor. We were able to demonstrate, however, that the effect by α-tocopherol on the duration of pentobarbital anesthesia was equivalent to its effect on thiopental anesthesia. Using pentobarbital in our studies on dosage effect with α-tocopherol, we obtained a biphasic curve quite similar to that obtained when thiopental was used. One is further encouraged to consider a dual mechanism of action for α-tocopherol, one on the metabolism of the barbiturates and the other on their distribution.

Another mechanism concerned with altered distribution which would influence the duration of anesthesia is referable to changes in permeability of the blood-brain barrier. This barrier is constituted by the walls of capillaries throughout the substance of the brain and it is easily understood that any change in the permeability characteristics of this diffusion barrier would affect the quantity of barbiturate entering the brain cell and therefore the duration of action of the barbiturate. α-tocopherol may be implicated as inducing an effect on permeability since it has been demonstrated by Dessert and Miller (28) to inhibit hyaluronidase which is in turn reported by Duron-Reynals (29) to affect the permeability of the vascular system. An even more fundamental distribution relationship is that found between the cerebral extracellular solution and the brain substance itself. Research on this aspect of the mechanism of α-tocopherol on barbiturate action is non-existant and any investigation would be of prime importance.

In any evaluation of α-tocopherol's effect on the enzyme systems involved in barbiturate anesthesia one must discuss its possible action in each of the theories concerning the cellular activity of barbiturates in the brain.

Govier and Gibbons (35) in studying the effect of pentobarbital on pyruvic acid metabolism in brain tissue found that barbiturates blocked the oxidative reactions of pyruvate. These workers demonstrated further that this blocking of oxidation of pyruvate acted as a barrier to acetylcholine synthesis with a resulting lack of activity at the neural junction.

Ames and Risely (20) have shown evidence that a marked decrease in the cholinesterase content of tissue exists in vitamin E deficiency.

Furthermore Torda and Wolff (31) have shown that α-tocopherol in very low concentrations is able to stimulate acetylcholine synthesis. All of these studies imply a close association between α-tocopherol and acetylcholine synthesis.

Another widely accepted theory is that the barbiturates in some manner inhibit the activity of the respiratory enzymes in the brain and thereby delay the utilization of energy released by the oxidation of glucose. This inhibition is thought to occur in the production of high energy phosphate bonds in the D.P.N. (diphosphopyridine nucleotide) system. Succinoxidase is a necessary enzyme in this transfer of energy. In this regard Ames and Risely (20) have shown that α-tocopherol inhibits the succinoxidase system.

α-tocopherol has also been shown to reduce markedly the activity of D.P.N.-ase (32). Since calcium ions are known to be necessary for the activity of the D.P.N.-ase system the mechanism of action of the α-tocopherol in this regard may be the reduction of the calcium ion concentration by the formation of an insoluble calcium compound, thus indirectly inactivating the D.P.N.-ase system.

This calcium antagonism of α-tocopherol was evident in our experiments. The prolonged duration of thiopental anesthesia following α-tocopherol was nearly completely removed by the addition of excess calcium ions in the form of calcium gluconate. This action of calcium to reverse the effect of α-tocopherol upon thiopental anesthesia could be antagonized by the addition of magnesium ions, as expected.

While it seems probable that α-tocopherol is involved in these enzyme systems it is apparent that this action of α-tocopherol would tend to protect the enzymes from inactivation and should therefore antagonize rather than prolong the action of the barbiturate. Thus, it is not possible at this stage of our knowledge to implicate the effect of α-tocopherol on barbiturate anesthesia by this mechanism.

It has been suggested by other workers, including Richards (10), that the hypertonicity of the various substances which influence barbiturate anesthesia alters the vascular dynamics of the organism resulting in a prolongation of anesthesia. Our negative results with normal saline, hypertonic saline, and distilled water, given in a manner similar to α-tocopherol, would tend to discount this effect.

Another suggestion has been that these materials stimulate a release of epinephrine in the organism which may be responsible for the prolongation of anesthesia (34). Our work did not include an investigation of this aspect of the problem.

SUMMARY

Anesthesia produced in adult white mice by thiopental at a dosage of 30 mgm. per kilo. produced an average sleeping time of 5.86 minutes. It was demonstrated that premedication with α-tocopherol intraperitoneally at low dosages markedly prolongs this sleeping time. The dosage-response curve of this study indicates the possibility of a dual mechanism of action for α-tocopherol, one predominating at low doses up to 35 mgm. per kilo. and another at the higher doses. Maximum effects occurred at 250 mgm. per kilo. of α-tocopherol with a six-fold prolongation of anesthesia.

Control material (distilled water, normal saline and hypertonic saline) in equivalent doses were without effect on the duration of thiopental anesthesia.

α-tocopherol acetate administered orally to mice in high doses had no effect on the duration of thiopental anesthesia.

In rabbits, intravenous α-tocopherol had no effect on thiopental anesthesia.

The effect of materials known to be antagonistic (calcium gluconate) and synergistic (methionine) with α-tocopherol was studied.

Finally the effect of α-tocopherol on other short-acting barbiturates was studied. At a dosage of 250 mgm. per kilo. of α-tocopherol, secobarbital and pentobarbital were prolonged about seven-fold. Hexobarbital anesthesia was not significently prolonged.

The dosage-response relationship of the influence of α-tocopherol on the duration of pentobarbital anesthesia was investigated and was

found to produce a curve similar to that found with thiopental anesthesia.

Several possible mechanisms for this effect of α-tocopherol on barbiturate anesthesia were discussed.

APPENDIX

Experiment I. Thiopental Controls

Duration of anesthesia in control mice using 30 mgm. per kilo. of thiopental in a 1.5% solution without premedication. These controls were done at intervals from October 1951 through June 1952.

Table I

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	26	4.58
2.	27	1.16
3.	27.5	7.75
4.	28	7.83
5.	24	8.50
6.	32	3.75
7.	29	4.16
8.	31	7.25
9.	26	5.16
10.	31	4.33
11.	24	10.50
12.	23	7.00
13.	28	11.75
14.	23.5	6.75
15.	24	4.25
16.	27	6.58
17.	32	7.08
18.	25	8.58
19.	32.5	5.16
20.	32	4.16
21.	30	4.75
22.	34	4.83
23.	33	3.50
24.	27	4.16
25.	39	6.08
26.	30	5.50
27.	24	3.75
28.	22	7.75
29.	31	4.83
30.	26	4.41

Average duration of anesthesia: 5.86 minutes
± 0.83 min. (95% Confidence Limits)

Experiment II. Dosage-response Relationship of the Influence of α -tocopherol on Thiopental Anesthesia, (30 mgm. per kilo.)

Part 1. 7.8 mgm. per kilo. of α -tocopherol phosphate
0.005 c.c. per gram of body weight of a 0.156% solution

Table II

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	32	6.33
2.	27	5.83
3.	28	11.50
4.	24	12.75
5.	25	7.00

Average duration of anesthesia: 8.68 minutes

Part 2. 15.6 mgm. per kilo of α -tocopherol phosphate
0.01 c.c. per gram of body weight of a 0.156% solution

Table III

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	29	11.50
2.	22	5.25
3.	23	13.75
4.	32	19.00
5.	23	18.75

Average duration of anesthesia: 13.65 minutes

Part 3. 31.25 mgm. per kilo. of α -tocopherol phosphate
0.01 c.c. per gram of body weight of a 0.3125% solution

Table IV

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	30	13.00
2.	34	33.50
3.	32	27.16
4.	31	9.08
5.	31	24.75
6.	26	25.25
7.	27	17.16
8.	24	31.25
9.	26	37.00

Average duration of anesthesia: 24.24 minutes

Part 4. 62.50 mgm. per kilo. of α -tocopherol phosphate
0.01 c.c. per gram of body weight of 0.625% solution

Table V

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	28	25.50
2.	30	24.00
3.	27	23.75
4.	28	36.25
5.	27	8.00
6.	25	21.66
7.	22	61.66
8.	26	9.50
9.	32	26.20

Average duration of anesthesia: 26.36 minutes

Part 5. 125.0 mgm. per kilo. of α-tocopherol phosphate
0.01 c.c. per gram of body weight of 1.25% solution

Table VI

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	24	17.50
2.	32	30.00
3.	18	18.00
4.	24	11.75
5.	30	27.50
6.	24	49.16
7.	23	36.66
8.	26	41.50
9.	37	16.86
10.	34	16.33

Average duration of anesthesia: 26.52 minutes

Part 6. 250.0 mgm. per kilo. of α-tocopherol phosphate
0.01 c.c. per gram of body weight of 2.5% solution

Table VII

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	27	33.25
2.	26	25.50
3.	25	20.13
4.	27	18.75
5.	25	26.16
6.	29	30.66
7.	35	35.16
8.	34	45.83
9.	30	33.00
10.	30	53.50
11.	29	32.00
12.	30	36.00
13.	28	14.00
14.	35	23.33
15.	30	36.50

Average duration of anesthesia: 30.91 minutes

Experiment III. The Effect of α-tocopherol Acetate Administered Orally

2.0% solution of dl-α-tocopherol acetate in peanut oil was administered orally in the following dosages and time sequence before intravenous thiopental, 30 mgm. per kilo.

Table VIII

Mouse no.	Weight in grams	Dosage Regime	Total Dosage	Time since last 100 mg./kg.dosage	Thiopental	Duration of anesth.
1.	26	100 mg/kg. q.d.x2	200 mg/kg.	3 hrs.	30 mg/kg.	1.58
2.	28	100 mg/kg q.d.x2	200 mg/kg.	3 hrs.	30 mg/kg	12.37
3.	29	100 mg/kg q.d.x4	400 mg/kg	2 hrs.	30 mg/kg	7.88
4.	29.5	100 mg/kg q.d.x4	400 mg/kg	2 hrs.	30 mg/kg	7.91
5.	30	100 mg/kg q.d.x4	400 mg/kg	2 hrs.	30 mg/kg	5.83
6.	25	100 mg/kg q.d.x4	400 mg/kg	2.5 hrs.	30 mg/kg	6.13
7.	29	100 mg/kg q.d.x4	400 mg/kg	2.5 hrs.	30 mg/kg	6.71
8.	24	100 mg/kg q.d.x4	400 mg/kg	3 hrs.	30 mg/kg	5.31
9.	26	100 mg/kg q.d.x4	400 mg/kg	3 hrs.	30 mg/kg	5.65
10.	25	100 mg/kg q.d.x4	400 mg/kg	3.5 hrs.	30 mg/kg	4.83
11.	32	200 mg/kg q.d.x4	800 mg/kg	1 hr.	30 mg/kg	3.50
12.	34	200 mg/kg q.d.x4	800 mg/kg	1 hr.	30 mg/kg	5.75
13.	30	200 mg/kg q.d.x4	800 mg/kg	1.5 hr.	30 mg/kg	2.00
14.	38	200 mg/kg q.d.x4	800 mg/kg	1.5 hr.	30 mg/kg	12.00

Experiment IV. Control Material

Distilled water, isotonic (0.9%)saline and hypertonic (3%) saline were administered intraperitoneally 5 minutes prior to 30 mgm. per kilo. of thiopental intravenously.

Table IX

Mouse no.	Weight in grams	Premedication	Duration of anesthesia in minutes
1.	30	.30 c.c. dist.H_2O	5.00
2.	37	.37 "	2.75
3.	28	.28 "	4.25
4.	32	.32 "	4.58
5.	27	.27 "	3.50
		Average duration of anesthesia:	4.02
1.	30.5	.30 c.c. isot.saline	4.58
2.	22	.22 "	7.25
3.	28	.28 "	8.16
4.	30	.30 "	4.00
5.	32	.32 "	4.83
		Average duration of anesthesia:	5.76
1.	23	.30 c.c. 3% saline	8.75
2.	28	.30 "	4.33
3.	29	.30 "	5.75
4.	30	.30 "	4.66
5.	32	.32 "	5.25
		Average duration of anesthesia:	5.74

Experiment VI. Failure to Produce Cyclic Anesthesia with α-tocopherol

Effect of α-tocopherol in dosages of 250 and 500 mgm. per kilo. intraperitoneally in the production of cyclic anesthesia using thiopental at 30 mgm. per kilo.

Table X

Mouse no.	Weight in grams	Dosage thiopental	Duration of anesthesia in min.	Dosage -tocopherol	Duration of reinduction anesth.
1.	35	30 mg./kg.	6. ---	250 mg./kg.	-- 0
2.	32	"	4.75 ---	"	-- 0
3.	30	"	7.75 ---	"	-- 0
4.	32	"	3.25 ---	500 mg./kg.	-- 0
5.	31	"	11.50 ---	"	-- 0
6.	31	"	5.25 ---	"	-- 0

Experiment VIII. The Influence of α-tocopherol on Duration of Anesthesia Induced by other Barbiturates

Part 1. Pentobarbital (Nembutal)

a. Duration of anesthesia using 30 mgm. per kilo. of pentobarbital intravenously in a 3% solution without premedication

Table XI

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	30	0
2.	23	0
3.	27	0
4.	23	4.25
5.	32	6.75
6.	25	0
7.	23	1.50
8.	24	2.75
9.	21	0
10.	20	0

Average duration of anesthesia: 3.81 minutes

b. 30 mgm. per kilo. of pentobarbital intravenously; premedication--250 mgm. per kilo. α-tocopherol intraperitoneally

Table XII

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	32	18.50
2.	34	38.00
3.	36	32.50
4.	23	42.00
5.	24	35.00
6.	29	41.00
7.	23	15.00
8.	28	41.50
9.	29	31.00
10.	26	16.00

Average duration of anesthesia: 31.05 minutes

Part 2. Secobarbital (Seconal)

a. Duration of anesthesia using 25 mgm. per kilo. of secobarbital intravenously without premedication

Table XIII

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	22	7.50
2.	23	5.75
3.	20	0
4.	31	5.75
5.	36	0
6.	33	3.50
7.	34	4.50
8.	28	3.75
9.	33	2.25
10.	23	0

Average duration of anesthesia: 4.71 minutes

b. 25 mgm. per kilo. of secobarbital intravenously; premedication--250 mgm. per kilo. α-tocopherol intraperitoneally

Table XIV

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	30	38.00
2.	35	36.50
3.	35.5	54.75
4.	35	22.25
5.	25	23.50
6.	31	27.50
7.	36	24.25
8.	32	44.00
9.	22	25.50
10.	36	35.50
11.	33	29.50

Average duration of anesthesia: 36.13 minutes

Part. 3. Hexobarbital (Evipal)

a. Duration of anesthesia using 50 mgm. per kilo. of hexobarbital intravenously without premedication

Table XV

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	31	8.50
2.	30	3.33
3.	29	14.75
4.	31	15.25
5.	30	6.16
6.	37	6.25
7.	21	6.50
8.	27	7.25
9.	25	4.16
10.	26	6.50

Average duration of anesthesia: 7.87 minutes

b. 50 mgm. per kilo. of hexobarbital intravenously; premedication--250 mgm. per kilo α-tocopherol intraperitoneally

Table XVI

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	31	8.75
2.	29	11.16
3.	38	21.50
4.	25	9.83
5.	23	22.25
6.	21	10.08
7.	23	5.25
8.	24	12.50
9.	22	6.25
10.	25	17.00

Average duration of anesthesia: 12.45 minutes

Part 4. Dosage-response relationship of the influence of α -tocopherol on pentobarbital anesthesia

a. 7.8 mgm. per kilo. α-tocopherol phosphate
0.005 c.c. per gram of body weight of a 0.156 % solution

Table XVII

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	34	7.25
2.	35	9.50
3.	31	0.
4.	32	4.00
5.	34	14.75

Average duration of anesthesia: 7.10 minutes

b. 15.6 mgm. per kilo. α-tocopherol phosphate
0.01 c.c. per gram of body weight of a 0.156% solution

Table XVIII

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	24	27.00
2.	23	0.0
3.	28	25.50
4.	27	16.25
5.	25	23.25

Average duration of anesthesia: 18.4 minutes

c. 31.25 mgm. per kilo. α -tocopherol phosphate
0.01 c.c. per gram of body weight of a 0.3125% solution

Table XIX

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	25.5	40.30
2.	28	34.00
3.	24	45.75
4.	26.5	30.25
5.	23	17.50

Average duration of anesthesia: 33.60 minutes

d. 62.5 mgm. per kilo. α-tocopherol phosphate
0.005 c.c. per gram of body weight of a 1.25% solution

Table XX

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	23	35.25
2.	24	41.50
3.	23	23.50
4.	24	14.75
5.	27	31.25

Average duration of anesthesia: 29.25 minutes

e. 125 mgm. per kilo. α-tocopherol phosphate
0.01 c.c. per gram of body weight of a 1.25% solution

Table XXI

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	22	17.75
2.	22	26.00
3.	24	35.00
4.	24	37.50
5.	22.5	21.50

Average duration of anesthesia: 27.55 minutes

f. 250 mgm. per kilo. α-tocopherol phosphate
0.01 c.c. per gram of body weight of a 2.5% solution

Table XXII

Mouse no.	Weight in grams	Duration of anesthesia in minutes
1.	32	18.50
2.	34	38.00
3.	36	32.50
4.	23	42.00
5.	24	35.00
6.	29	41.00
7.	23	15.00
8.	28	41.50
9.	29	31.00
10.	26	16.00

Average duration of anesthesia: 31.05 minutes

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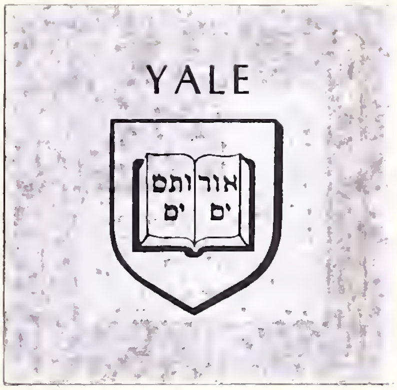
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