A STUDY OF THE POTENTIATION OF PENTOBARBITAL
ANESTHESIA BY GLUCOSE AND ITS METABOLITES

Dissertation

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By

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A STUDY OF THE POTENTIATION OF PENTOBARBITAL
ANESTHESIA BY GLUCOSE AND ITS METABOLITES

INTRODUCTION AND LITERATURE SURVEY

Interest in this work was first stimulated by a report by Lamson and his co-workers (1). These investigators were studying shock following hemorrhage in dogs, the dogs being anesthetized by pentobarbital sodium (the sodium salt of ethyl-1-methylbutyl-barbituric acid). On one occasion, when glucose was to be administered to an anesthetized dog, the animal suddenly recovered sufficiently to respond when spoken to, and to try to get off the operating table. However, glucose was administered intravenously, and the dog instantly relaxed, went into a deep sleep, and continued to sleep for an hour. On recovery, another injection of glucose solution again produced anesthesia, this time for a period of forty-five minutes. This procedure was continued with periods of anesthesia of thirty minutes, twenty minutes, ten minutes, and five minutes resulting. Eventually anesthesia could no longer be produced by the injection of glucose solution. The rather surprising fact remained, however, that glucose apparently had potentiated the anesthetic effect of pentobarbital.

These workers then undertook more carefully controlled studies, and reported that all dogs do not respond as the first one had responded, that rats fail to show the

"glucose effect", that rabbits and hamsters do, but only after a delay in the onset of anesthesia following glucose. Guinea pigs gave the most consistent positive response. It was also found that the effect is produced by a number of glucose metabolites, but not by sucrose or sodium chloride. This led Lamson to the belief that the "glucose effect" is a true potentiation of pentobarbital, and not merely an osmotic effect.

Many reports have appeared in the literature indicating that glucose is involved in the action of anesthetics. For example, it has been known for some time that the administration of the volatile general anesthetics brings about a rise in the glucose level of the blood (2,3). Everett states, in addition, that anesthetics and hypnotics cause an increase in adrenalin secretion, which, in turn, elicits an increase in blood glucose. Barbiturates rapidly deplete the hepatic glycogen in patients with liver disease.

That the barbiturates influence the level of glucose in the blood has been amply demonstrated. Weiss (4) reported that barbiturates cause a rise in blood glucose. Tsai and Yi (5) reported similar results, and found that the rise was due, in part, to increased glycogenolysis, as well as to an inhibition of glycogen formation caused by the barbiturate. Vigante (6) found that phenobarbital, in small doses, lowered blood sugar, but increased blood

sugar when given in larger doses. Sodium bromide increased blood sugar, regardless of the dose. Atnan and Fenz (7) believe that Evipal causes an increase in the level of blood glucose in both the diabetic and non-diabetic patient. The increase in the non-diabetic patient is greater than in the diabetic. Hrubetz and Blackberg (8) found that pentobarbital and Amytal cause no change in the blood glucose level, phenobarbital causes none in the first hour, while Pentothal and Seconal do cause a rise in the first hour of anesthesia. Satomi (9) is of the opinion that hypnotics generally cause an increase in blood sugar, and a parallel increase in blood lactic acid. Pentothal causes only a slight blood sugar increase, according to the report of Laporta and Stefanelli (10). Dietrich (11) reported that Nembutal causes a slight rise in the blood glucose level in cats. LaBarre and Vesselovsky (12) found a similar response in dogs under barbital anesthesia, and believe that barbital causes a decreased insulin secretion.

All writers do not agree with these reports. Horn (13) is of the opinion that Evipal does not produce any change in the glucose content of the blood, while Sato and Degshe (14) reported that barbital had no effect on blood glucose. Pentothal has been reported by Stern and his co-workers (15) to bring about a fall in blood sugar, and Colldahl (16) believes that all hypnotics have this effect.

Blood glucose levels throughout the period of

anesthesia, and following recovery from anesthesia, have also been studied. Blackberg and Hrubetz (17) reported that blood sugar levels showed increased variability during the period of maximum depression, and a definite drop at the time of recovery. Following an extensive survey of volatile and barbiturate anesthetics, Maloney and co-workers (18) concluded that Pentothal produces hyperglycemia by depressing the glycogenic activity of the liver and by inducing glycogenolysis. Liver glycogen depletion was increased by prolonging anesthesia. These workers also found that Pentothal produces a fall in blood sugar at the time of recovery from anesthesia of short duration, but in prolonged anesthesia the blood sugar level was restored to its pre-anesthetic level in twenty-four hours. It is their opinion that the injection of glucose prior to anesthesia does not alter the carbohydrate mobilization in Pentothal anesthesia.

Adams (19) found that the blood glucose levels in rabbits prior to, during, and at the time of recovery from, anesthesia were 109, 110, and 90 mg. per 100 c.c., respectively, and that the decreased level was synchronous with recovery. It is interesting to compare his values with those obtained in an earlier portion of this work (20), in which it was found that thirteen of eighteen rabbits used showed an increase in blood sugar fifteen minutes after the onset of pentobarbital anesthesia. All eighteen showed

a decreased level at recovery. The increase averaged 6.5 percent, while at recovery the average level was 12.2 percent below normal. The blood sugar level during the course of anesthesia showed an irregular, but gradual decrease following the initial increase.

During investigation of a problem unrelated to potentiation of the barbiturates, Clay and Nelson (21) made an observation which provided evidence of a relationship between blood glucose levels and duration and depth of anesthesia. Adrenalectomized rats were maintained on a diet of 5 percent glucose exclusively. A week after adrenalectomy, pentobarbital sodium in a dose of 3 mg. per 100 Gm. rat was injected intraperitoneally. This dose is normally sufficient to produce a period of anesthesia of about an hour and a half. However, of thirty adrenalectomized rats, nine died, and the remainder did not recover from anesthesia for from five to seven hours. Adrenalectomy does cause some debility in rats, yet it seems more likely that the diet of glucose, and the resulting high level of blood sugar, had some effect on the action of the drug.

Glucose is by no means the only substance reported as a potentiating agent of the barbiturates. Many otherwise unrelated compounds are reputed to have this one property in common. In addition, several physiological and pathological conditions exert a profound effect upon the

course of barbiturate anesthesia. Norton and DeBeer (22) showed that sub-hypnotic doses of unsymmetrical aryl-alkyl ureas potentiate barbiturate hypnosis in mice, while most of these urea derivatives possess little or no hypnotic action in themselves. The degree of hypnosis was related to the size of the dose. Evidence indicated true potentiation and not merely summation.

Adrenalin potentiates barbiturates, according to Leimdorfer and Metzner (23). These researchers found that the intracisternal injection of adrenalin alone produces surgical anesthesia in dogs, but the dose required is much less if a small amount of pentobarbital is injected intraperitoneally several hours prior to adrenalin administration. Griffith (24) reported that the intravenous injection of adrenalin into Dial-anesthetized cats at the rate of 0.0004 mg. per Kg. per minute for five minutes causes hyperglycemia of the same magnitude as the intravenous injection of 0.05 Gm. of glucose per Kg. per minute for five minutes. Everett (2) states that anesthetics and hypnotics cause an increase in adrenalin secretion, which, in turn, increases blood glucose. From these observations, one may surmise that the potentiation of barbiturates by adrenalin might be due to its hyperglycemic effect. Holck (25), however, found that the administration of adrenalin twenty minutes prior to Sodium Evipal injection did not increase the period of hypnosis, nor effect the fatal dose of Sodium Evipal.

A number of workers have studied the effect of insulin on barbiturate action. Holck (25) found insulin to be inactive as a potentiating agent for Sodium Evipal. However, Ratcliff et al (26) reported that in insulin-treated animals there was a need for more frequent administration and greater quantities of Pentothal to produce deep surgical anesthesia than was required in animals not given insulin. Insulin decreases blood sugar, while increasing the level of liver glycogen, so it is quite possible that insulin exerts its influence on anesthesia by altering the glucose-glycogen ratio. Booker, French and Molano (27) give emphasis to this possibility. They reported that blood sugar rises and liver glycogen falls in prolonged Pentothal anesthesia. They believe that Pentothal "embarrasses" the liver, disrupting enzyme systems, thus causing the glucose and glycogen changes. Insulin administration aids glycogen formation during prolonged anesthesia.

Sollmann (28) states that anesthesia by the barbiturates does not decrease insulin secretion. However, LaBarre and Vesselovsky (12) are of the opinion that barbital does decrease the secretion of insulin. Andreeva (29) made the interesting observation that the symptoms of insulin-induced hypoglycemia can be delayed by Dial and Medinal, and that the fractional introduction of Medinal completely prevents convulsions, even after the blood sugar level has dropped sharply.

De Boer (30) studied the effect of thiamin hydrochloride upon pentobarbital anesthesia, and found that it prolongs the sleeping time from normal doses of the barbiturate, and increases the mortality rate from lethal doses. These observations were made on normal rats, on young ovariectomized females, and on testectomized males.

Following an extensive survey of barbiturates and thio-barbiturates, and the effect of pituitary preparations upon their anesthetic activity in rats, Masson (31) reported no significant effects upon phenobarbital, Ipral, barbital, Alurate, and Dial. Pituitary slightly prolongs the duration of anesthesia with Phanodorn, thioethamyl, Neonol, Delvinal, Nostal, and Amytal, and greatly prolongs the duration (two to five times) with Pentothal, Seconal, allyl pental, Nembutal, Evipal, Pernostan, and Sigmodal. There is no effect on chloral. Masson suggested an action through the liver. In a subsequent report, Masson (32) states that suspensions of cattle anterior pituitary, thymus, pancreas, liver, kidney, spleen, testis, and brain, all increase the duration of pentobarbital anesthesia. It is doubtful that the effect is due to the injection of foreign protein, because egg albumin, casein digest, blood serum, and gelatin have little or no activity.

The antihistaminics have been studied as possible potentiating agents of barbiturates. Winter (33) tested Pyribenzamine, Benadryl, Neo-antergan, and R.P.3277, and

found that Neo-antergan and Pyribenzamine potentiate Evipal and pentobarbital by 10 percent or more. However, Cronheim and Ehrlich (34) were unable to show any appreciable potentiation of pentobarbital with various antihistaminics in tests on mice.

In a recent report, Krantz and Fassel (35) discussed their observation that narcosis produced by phenobarbital sodium in the white rat is prolonged and intensified by the parenteral and oral administration of iodides. The compounds tested were sodium iodide and Organidin, the latter being an organic iodine compound. In addition, it was found that the intraperitoneal injection of solutions of sorbital, glycerin, and sucrose prolongs and intensifies the narcosis produced by phenobarbital sodium. The authors expressed the opinion that the potentiation shown by substances exhibiting high osmotic pressure when injected into the peritoneal cavity is due to osmotic pressure changes in the animal. They speculated that fluid is drawn from the extracellular spaces, tissues, and the blood, and that the concentration of the narcotic agent in the circulating blood is thereby increased. Through this mechanism, the barbiturate concentration in the brain may be increased.

Ethyl alcohol and the barbiturates are both depressant to the central nervous system, so their combined action might be expected to be synergistic. Dille and Ahlquist

(36) made a study of the combined effects of alcohol and the barbiturates, and found, not simple synergism, as might be anticipated, but very definite potentiation. These workers reported that potentiation is greatest with small doses, provided each of the small doses alone is sufficient to produce some effect. The two drugs were eliminated normally, therefore potentiation was not believed to be due to altered elimination of either drug by the other. The authors suggested that the combined action is on sleep centres, each drug sensitizing these centres to the effect of the other. Fondal and Kochakian (37) reported that the addition of ethyl alcohol to liver, kidney, and brain tissue slices from adult male rats produced an increase in oxygen consumption, followed by a progressive inhibition as the ethyl alcohol concentration was increased.

An interesting observation was made by Giarman and his co-workers (38) during their investigation of Antabuse (tetra-ethylthiuriam disulphide), in that they found the drug capable of increasing the effect of ethyl alcohol and thiopental. This action is due to its blocking the oxidation of ethyl alcohol at the acetaldehyde stage, and of thiopental which is detoxified by oxidation in the side chain. Xanthine oxidase may be involved in these oxidative processes. These investigators also studied ascorbic acid, cysteine, and other reducing agents and found that all of them potentiate thiopental anesthesia. They feel that

such potentiation is due to the anti-oxidant action of the potentiating agents. Greig (39) reported similar results with ascorbic acid.

Wooster and Sunderman (40) found the recovery time of white mice anesthetized with pentobarbital sodium to be prolonged significantly by the intraperitoneal injection of nitroglycerin, butyl nitrate, diethyleneglycol dinitrate and sodium nitrite.

Some of the volatile general anesthetics have been suggested as potentiating agents of the barbiturates. Koppanyi, Dille, and Linegar (41) reported that chloroform has this action. Carbon tetrachloride has the same action, according to reports by Cameron and Saram (42), and Pratt (43). Farson, Carr, and Krantz (44) state that cholesterol potentiates barbiturates. Acetylcholine and ergotamine tartrate also potentiate barbiturates as reported by De Nito (45). Shideman, Kelly, and Adams (46) are of the opinion that carbon tetrachloride potentiates thiopental due to the fact that it causes liver damage. Similar potentiation is produced by sub-total hepatectomy, and by the diminished blood flow to the liver resulting from an Eck fistula. It was the conclusion of these writers that the conditions described hindered Pentothal destruction which is a function of normal liver.

Other surgical procedures have resulted in increased barbiturate activity. Cameron (47) found that castration

rendered animals more susceptible to barbiturate depression. In adult female rats, thyroidectomy produces a prolongation of sodium pentobarbital anesthesia (48). Thyroid and thiouracil feeding, however, have no effect on pentobarbital anesthesia, nor is the effect found in thyroidectomized dogs.

The relationship between acidosis and anesthesia has been studied. De Boer (49) investigated the effect of diet upon thiopental anesthesia in rabbits. Animals on a diet of oats slept much longer than those fed rabbit chow or a combined diet of oats and alfalfa. A diet of oats renders the urine acid, while the other diets result in the excretion of an alkaline urine. Sodium bicarbonate fed orally to rabbits on a diet of oats appears to reduce the duration of hypnosis. In contrast with this report, Whitehead and his co-workers (50) found that with the establishment of acidosis by carbon dioxide inhalation, the plasma level of thiopental decreases by as much as 50 percent even though the intravenous injection of the anesthetic is continued during this time. Upon the withdrawal of carbon dioxide, followed by the administration of oxygen, the plasma level of thiopental rises concurrently with the blood pH, even in those animals which receive no additional thiopental sodium for periods of thirty to sixty minutes.

Peterson, Shideman and Johnson (51) found that dogs

subjected to anoxia do not recover from thiopental anesthesia until the plasma level of the drug falls 19 to 44 percent lower than the level at which normal dogs recover. They believe that potentiation of barbiturate anesthesia can be accomplished by any means through which central anoxia is produced.

Various metabolites of glucose seem to be influencing factors in barbiturate anesthesia, but there is little agreement on their effect. Lamson, Greig, and Hobdy (52) made an extensive study of many of the agents reported to potentiate barbiturate anesthesia. Glucose was found to return dogs, hamsters, rabbits, pigeons, chickens, and guinea pigs to anesthesia, but produced no effects in the mouse, rat, goldfish, or tadpole. Believing that the guinea pig gives more consistent results than other experimental animals, it was used throughout the remainder of their investigations. The following substances were found to be "glucose effect" positive: glucose, levulose, galactose, arabinose, honey, glucose-1-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, pyruvate, lactate, citrate, alpha-ketoglutarate, succinate, fumarate, malate, ascorbate, baker's yeast, brewer's yeast, glutamic acid, malonate, beta-glycerophosphate, glycerol, triacetin, acetate, and epinephrine. Thiamin, nicotinic acid, and riboflavin are ineffective individually, but give a positive response when combined. Sucrose, nicotinamide, pantothenic

acid, glycine, alanine, butyrate, sodium chloride, pitressin, adenosine-triphosphate, diphosphopyridine nucleotide, and cocarboxylase are "glucose effect" negative. None of the substances tested cause anesthesia without barbiturate. Rats do not respond to glucose but do to lactate and pyruvate.

These workers found, in addition, that the occurrence of the "glucose effect" is confined to hexobarbital, pento-barbital, barbital, secobarbital, and thiopental, and does not occur following ether, alcohol, chloroform, chloral, chlorobutanol, glucochloralose, or paraldehyde.

Studies on pooled brains at the time of awakening show that anesthesia ends when the level of hexobarbital falls to 30 mcg. per Gm. of brain, if glucose is not given. However, if glucose, or one of the other potentiating agents is given, the animals return to sleep, but the brain level of hexobarbital is not raised. On the contrary, it gradually decreases to a level of 20 mcg. per Gm. of brain, at which level the animals again awaken.

Glucose and other effective substances decrease the anesthetic lag and the median anesthetic dose of barbital.

As possible explanations of this "glucose effect", Lamson et al suggested that the metabolites produce anesthesia at a time when only a fraction of the barbiturate has penetrated the brain. They may cause an increased permeability of brain to the drug, thus allowing an

anesthetic level to be arrived at more rapidly. Lamson and his associates also found that lactate, pyruvate, and glutamate cause an increased rate of barbital entry into the brain. Glucose does not have this effect, yet it, too, potentiates barbital.

The same workers reported that anticholinesterase agents increase barbital penetration into the brain, while acetylcholine decreases penetration of barbital into the brain. They believe that pyruvate and alpha-ketoglutarate inhibit choline acetylase, that is, inhibit acetylcholine formation, and that lactate and glutamate are converted to pyruvate and alpha-ketoglutarate.

These opinions are not shared by all investigators. Westfall (53) is of the definite belief that pyruvates, pyruvic acid, and pyruvic acid polymers are antagonistic to pentobarbital, and has reported a number of experiments that indicate his conclusions are sound. Schack and Goldbaum (54) have reported that succinate has no effect whatever either on the duration of barbiturate anesthesia, or on blood levels of barbiturate. Adams and Larson (55) studied the analeptic effect of succinate, malonate, citrate, and acetate on male Wistar rats under pentobarbital anesthesia and found that succinate has slight, if any, analeptic effect. None of the salts used have any significant effect on the duration of the loss of the righting reflex, except citrate, which prolongs it

considerably.

Pharmacology has as its primary function the search for the mode of action of drugs. The barbiturates are depressants to the central nervous system, and have been reported to cause their depression by interfering with the ability of the brain to metabolize glucose. The precise mechanism of this action has not been definitely established. However, it can safely be assumed that there is an intimate relationship between the depressant action of the barbiturates and the availability of glucose. If the mechanism by which glucose, its metabolites, and the other suggested potentiating substances exert their influence on the barbiturates can be found, it is quite likely that that discovery will shed further light on the mode of action of these important derivatives of barbituric acid. That possibility alone makes this investigation worthwhile. However, additional justification is provided by the fact that such potentiation has pharmaceutical and therapeutic applicability; in the formulation of barbiturate preparations, in the treatment of barbiturate toxicity, and possibly in the therapy of conditions necessitating the continuous use of the barbiturates. It is hoped that this report will help provide the ultimate answer so that its applications can be fully realized.

EXPERIMENTATION

Throughout the experimental work to be described, certain procedures and conditions were maintained:

1. The anesthetic used in all cases was pentobarbital, (Veterinary Nembutal, Abbott), a solution containing 60 mg. of pentobarbital in each c.c.
2. Rabbits and rats were the only experimental animals used.
3. When not being used for experimental purposes, the animals were housed in an animal room in which a temperature of 76°F. to 78°F. was maintained. The diet of the rabbits consisted solely of Eshelman's Rabbit Food; that of the rats of Purina Dog Food. Water was provided freely.
4. The animals were deprived of food, but not of water, for from eighteen to twenty-four hours prior to use. This procedure was followed in an effort to obtain basal blood glucose and liver glycogen levels.
5. The anesthetic was administered by intraperitoneal injection, as were all other substances being investigated.
6. In those instances when sleeping times were under investigation, the return of the righting reflex was chosen as the end point.

EXPERIMENTS TO DETERMINE GLUCOSE
POTENTIATION IN RATS

EXPERIMENT 1

Many of the workers who have studied potentiation of the barbiturates used rats as their experimental animals. However, Lamson et al (1,52) are quite emphatic in their reports that the "glucose effect", is not apparent in rats. Because it was desired to use rats in this work, if possible, it was necessary to carry out preliminary experiments to determine whether rats would give the desired response to glucose administration.

Procedure:- White rats, ranging in weight from 100 Gm. to 180 Gm., following the usual period of food deprivation, were given pentobarbital by intraperitoneal injection. The dosage was 2.75 mg. per 100 Gm. rat. Upon return of the righting reflex, 2 c.c. of a 50 percent solution of glucose were administered intraperitoneally, and the rats observed for a return to anesthesia.

The duration of both periods of anesthesia was recorded, and is given in Table 1.

EXPERIMENT 2

This second experiment had the same purpose as the first, that is, the establishing of the occurrence of the "glucose effect" in rats. The procedure, however, was somewhat different.

Table 1

Effect of Glucose on Rats Recently Recovered
from Pentobarbital Anesthesia

Number and Sex	Duration of Anesthesia following Pentobarbital	Effect of Glucose	Duration of Anesthesia following Glucose
1 F.	135 minutes	Anesthesia	33 minutes
2 F.	114 "	Anesthesia	38 "
3 F.	97 "	Anesthesia	26 "
4 F.	64 "	Anesthesia	39 "
5 F.	61 "	Anesthesia	24 "
11 F.	83 "	Anesthesia	21 "
12 F.	45 "	No Anesthesia	0 "
13 F.	45 "	Anesthesia	15 "
14 F.	36 "	Anesthesia	13 "
15 F.	37 "	Anesthesia	20 "
16 F.	48 "	Anesthesia	14 "
Mean	69.5 "		22.1 "
S.E.	10.1		3.5
6 M.	32 minutes	Anesthesia	16 minutes
7 M.	26 "	Anesthesia	25 "
8 M.	17 "	Anesthesia	23 "
9 M.	No Anesthesia	Anesthesia	12 "
10 M.	37 minutes	Anesthesia	5 "
Mean	22.4 "		16.2 "
S. E.	6.6		3.7

In Table 1 it is interesting to note that glucose caused anesthesia in one animal which had become depressed, but not anesthetized, following the administration of pentobarbital.

Procedure:- Starved white rats were anesthetized by the intraperitoneal administration of pentobarbital, and the period of time from the onset of anesthesia to the return of the righting reflex was recorded. The animals were then rested for a number of days to prevent the development of barbiturate tolerance. They were then anesthetized again, using pentobarbital in the same dosage as that used previously. However, the pentobarbital injection was followed immediately by the injection of 2 c.c. of 50 percent glucose. Again a record was made of the duration of the loss of the righting reflex.

The results of this experiment appear in Table 2.

These two experiments show rather conclusively that the "glucose effect" does occur in rats. On the basis of these results, it was decided that rats were suitable experimental animals for this study. It had been demonstrated earlier (20) that rabbits responded similarly to glucose. As a result, both were used in subsequent experimentation.

Table 2

A Comparison of the Duration of the Loss of
the Righting Reflex following Pentobarbital
Alone, and Pentobarbital and Glucose

Number and Sex	Sleeping Time following Pentobarbital Alone	Sleeping Time following Pentobarbital and Glucose
1 F.	33 minutes	101 minutes
2 F.	51 "	70 "
3 F.	49 "	89 "
4 F.	44 "	66 "
5 F.	48 "	106 "
6 F.	49 "	114 "
7 F.	30 "	67 "
8 F.	42 "	61 "
9 F.	19 "	97 "
10 F.	No Anesthesia	75 "
16 F.	71 minutes	71 "
17 F.	42 "	45 "
18 F.	55 "	82 "
19 F.	74 "	96 "
20 F.	81 "	94 "
21 F.	No Anesthesia	74 "
22 F.	70 minutes	60 "
23 F.	39 "	57 "
24 F.	55 "	64 "
25 F.	53 "	65 "
Mean	45.2 "	77.7 "
S. E.	4.8	4.0
11 M.	22 minutes	45 minutes
12 M.	30 "	37 "
13 M.	No Anesthesia	47 "
14 M.	39 minutes	53 "
15 M.	24 "	31 "
26 M.	24 "	52 "
27 M.	39 "	68 "
28 M.	29 "	57 "
29 M.	22 "	54 "
30 M.	31 "	38 "
Mean	26.0 "	48.2 "
S.E.	3.5	3.4

THE EFFECT OF GLUCOSE METABOLITES ON
BLOOD GLUCOSE

A complete investigation of the existence of, and the mechanism of, the potentiation of the barbiturates by the host of substances reported to have this action, is beyond the scope of the present work. Such an investigation would require considerable time and the combined efforts of many workers. Hence it was decided to confine this study to the role of glucose and some of its metabolites in pentobarbital anesthesia, with primary emphasis on glucose itself. While pentobarbital was chosen as the barbiturate anesthetic to be studied, it is most likely that the other short and ultra-short acting barbiturates would yield comparable results, as most of them are handled similarly by the body.

Glucose and its metabolites are so closely related in carbohydrate metabolism, even being interchangeable under suitable conditions, that the possibility of their having a common mechanism of potentiating barbiturates immediately comes to mind. Lamson (56) has, in fact, expressed the opinion that they all act through glucose. This opinion appeared to be a logical one, and led to the following experimental procedures.

EXPERIMENT 3

Female rabbits, previously starved for eighteen to twenty-four hours, were used because of the ease of withdrawing blood from the marginal ear veins. The purpose

of the study was to find the effect of glucose and a number of its metabolic degradation products upon the level of blood glucose.

Procedure:- A small incision was made in the marginal ear veins of rabbits, and 0.1 c.c. of blood withdrawn. Using this blood, the normal blood sugar level was determined by the micro method of Folin-Malmros (57). Three cubic centimetres of a 20 percent solution of the substance to be studied were then administered intraperitoneally. At periods of ten, twenty-five, forty, and sixty minutes following the injection, 0.1 c.c. of blood was withdrawn, and the glucose content determined by the same method as before.

In order that a comparison of blood glucose levels could be made more easily, the results shown in the following tables have been expressed as percentages of normal. That is, in each case, the normal glucose content of the blood has been expressed as 100 percent, and the content at other times expressed as a percent of that normal level. The actual values of blood sugar, expressed as mgs. glucose per 100 c.c. blood, are also shown.

The substances investigated included:

- (i). Sodium Lactate
- (ii). Sodium Citrate
- (iii). Sodium Acetate
- (iv). Sodium Pyruvate

(v). Sodium Succinate

(vi). Sodium Chloride (0.9 percent)

(vii). Sodium Chloride (20 percent)

(viii). Glucose (2 c.c. 50 percent)

The solutions of sodium chloride were used as controls. The 20 percent solution being strongly hypertonic, was used as such in an attempt to find its effect, if any, on blood glucose. In addition, it was hoped that its hypertonicity would give an indication of the validity of the hypothesis proposed by Krantz and Fassel (35), who suggested that potentiation is brought about by the action of substances exerting strong osmotic action. The solution containing 0.9 percent sodium chloride was used solely as a control to indicate whether a part of the change in blood glucose level could be attributed to the response of the animal to the handling and manipulation accompanying injection.

The solution of glucose was used for the obvious purpose of comparison.

The results of these observations are shown in Tables 3, 4, 5, 6, 7, 8, 9, and 10.

The maximum effect of each metabolite, and of glucose are illustrated graphically in Figure 1. The mean effect shown by each, over the one hour period of study, is illustrated in Figure 2.

Table 3

The Effect of Sodium Lactate on Blood

Glucose Levels in Rabbits

Number and Sex	Normal Level	Ten Minutes	Twenty-five Minutes	Forty Minutes	Sixty Minutes	Mean
1 F.	130 100.0	142 109.2	130 100.0	161 123.8	163 125.4	114.4
2 F.	130 100.0	127 97.7	130 100.0	123 94.6	121 93.1	96.4
3 F.	79 100.0	91 115.2	99 125.3	117 148.1	101 127.8	129.1
4 F.	117 100.0	154 131.6	134 114.5	118 100.9	119 101.7	112.2
5 F.	118 100.0	126 106.8	124 105.1	150 127.1	131 111.0	112.5
6 F.	123 100.0	141 114.6	129 104.9	149 121.1	144 117.1	114.4
7 F.	134 100.0	168 125.4	143 106.7	156 116.4	150 111.9	115.1
8 F.	143 100.0	180 125.9	156 109.1	152 106.3	144 100.7	110.5
9 F.	101 100.0	123 121.8	131 129.7	123 121.8	116 114.9	122.1
10 F.	117 100.0	126 107.7	126 107.7	131 112.0	120 102.6	107.5
11 F.	118 100.0	114 96.6	135 114.4	125 105.9	123 104.2	105.2
12 F.	110 100.0	125 113.6	127 115.5	132 120.0	114 103.6	113.2
Mean	100.0	113.8	111.1	116.5	109.5	112.7
S.E.	0.0	3.1	2.7	4.0	3.0	2.3

Upper figures show glucose expressed as mg. per 100 c.c.
Lower figures show glucose expressed as percent of normal.

Table 4

The Effect of Sodium Citrate on Blood

Glucose Levels in Rabbits

Number and Sex	Normal Level	Ten Minutes	Twenty-five Minutes	Forty Minutes	Sixty Minutes	Mean
1 F.	159 100.0	154 96.9	143 89.9	138 86.8	132 83.0	89.2
2 F.	153 100.0	185 120.9	155 101.3	174 113.7	169 110.5	111.6
3 F.	108 100.0	129 119.4	110 101.9	108 100.0	119 110.2	107.9
4 F.	106 100.0	125 117.9	110 103.8	130 122.6	123 116.0	115.1
5 F.	116 100.0	135 116.3	144 124.1	172 148.3	156 134.5	130.8
6 F.	106 100.0	106 100.0	118 111.3	123 116.0	133 125.5	113.2
7 F.	117 100.0	122 104.3	133 113.7	143 122.2	125 106.8	111.8
8 F.	108 100.0	133 123.1	133 123.1	125 115.7	160 148.1	127.5
9 F.	145 100.0	143 98.6	141 97.2	148 102.1	147 101.4	99.8
10 F.	164 100.0	196 119.5	185 112.8	200 122.0	182 111.0	116.3
11 F.	117 100.0	112 95.7	132 112.8	133 113.7	138 117.9	110.0
12 F.	134 100.0	145 108.2	167 124.6	148 110.4	146 109.0	113.0
Mean	100.0	110.1	109.7	114.5	114.5	112.2
S.E.	0.0	3.0	3.2	4.3	4.7	3.1

Upper figures show glucose expressed as mg. per 100 c.c.
Lower figures show glucose expressed as percent of normal.

Table 5

The Effect of Sodium Acetate on Blood

Glucose Levels in Rabbits

Number and Sex	Normal Level	Ten Minutes	Twenty-five Minutes	Forty Minutes	Sixty Minutes	Mean
1 F.	114 100.0	129 113.2	116 101.8	125 109.6	129 113.2	109.5
2 F.	86 100.0	96 111.6	85 98.8	98 114.0	103 120.0	111.1
3 F.	155 100.0	180 116.1	158 101.9	152 98.1	167 107.7	106.0
4 F.	144 100.0	167 116.0	160 111.1	159 110.4	174 120.8	114.6
5 F.	138 100.0	179 129.7	152 110.1	157 113.8	190 137.7	122.8
6 F.	140 100.0	163 116.4	152 108.6	179 127.9	180 128.6	120.4
7 F.	124 100.0	143 115.3	152 122.6	131 105.6	145 116.9	115.1
8 F.	144 100.0	154 106.9	144 100.0	143 99.3	141 97.9	101.0
9 F.	116 100.0	119 102.6	133 114.7	123 106.0	136 117.2	110.1
10 F.	96 100.0	98 102.0	131 136.5	116 120.8	123 128.1	121.9
11 F.	119 100.0	123 103.4	125 105.0	121 101.7	123 103.4	103.4
12 F.	115 100.0	118 102.6	120 104.3	117 101.7	122 106.1	103.7
Mean	100.0	111.3	109.6	109.1	116.5	111.6
S.E.	0.0	2.4	3.1	2.5	3.3	2.1

Upper figures show glucose expressed as mg. per 100 c.c.
Lower figures show glucose expressed as percent of normal.

Table 6

The Effect of Sodium Pyruvate on Blood

Glucose Levels in Rabbits

Number and Sex	Normal Level	Ten Minutes	Twenty-five Minutes	Forty Minutes	Sixty Minutes	Mean
1 F.	147 100.0	152 103.4	159 108.2	156 106.1	160 108.8	106.6
2 F.	154 100.0	155 100.6	233 151.3	156 101.3	160 103.9	114.3
3 F.	115 100.0	123 107.0	124 107.8	118 102.6	118 102.6	105.0
4 F.	130 100.0	135 103.8	142 109.2	142 109.2	141 108.5	107.7
5 F.	102 100.0	125 122.5	111 108.8	116 113.7	115 112.7	114.4
6 F.	106 100.0	138 130.2	118 111.3	112 105.7	111 104.7	113.0
7 F.	115 100.0	118 102.6	133 115.7	123 107.0	118 102.6	107.0
8 F.	118 100.0	124 105.1	124 105.1	121 102.5	118 100.0	103.2
9 F.	108 100.0	138 127.8	123 113.9	160 148.1	148 137.0	131.7
10 F.	129 100.0	112 86.8	142 110.1	174 134.9	134 103.9	108.9
11 F.	111 100.0	142 127.9	154 138.7	145 130.6	124 111.7	127.2
12 F.	154 100.0	147- 95.5	125 81.1	167 108.4	163 105.8	97.6
Mean	100.0	109.6	113.4	114.2	108.5	111.4
S.E.	0.0	4.1	4.9	4.3	2.8	2.8

Upper figures show glucose expressed as mg. per 100 c.c.
Lower figures show glucose expressed as percent of normal.

Table 7

The Effect of Sodium Succinate on Blood

Glucose Levels in Rabbits

Number and Sex	Normal Level	Ten Minutes	Twenty-five Minutes	Forty Minutes	Sixty Minutes	Mean
1 F.	177 100.0	148 83.6	133 75.1	148 83.6	147 83.1	81.4
2 F.	148 100.0	156 105.4	133 89.9	153 103.4	132 89.2	97.0
3 F.	100 100.0	106 106.0	110 110.0	125 125.0	127 127.0	117.0
4 F.	138 100.0	143 103.6	149 108.0	160 115.9	139 100.7	107.1
5 F.	164 100.0	182 111.0	204 124.4	190 115.9	174 106.1	114.4
6 F.	138 100.0	160 115.9	179 129.7	144 104.3	139 100.7	112.7
7 F.	163 100.0	138 84.7	124 76.1	136 83.4	140 85.9	82.5
8 F.	110 100.0	124 112.7	127 115.5	139 126.4	143 130.0	121.2
9 F.	140 100.0	142 101.4	147 105.0	163 116.4	142 101.4	106.1
10 F.	155 100.0	172 111.0	192 123.9	179 115.5	164 105.8	114.1
11 F.	144 100.0	153 106.3	131 91.0	148 102.8	127 88.2	97.1
12 F.	141 100.0	164 116.3	182 129.1	148 105.0	143 101.4	113.0
Mean	100.0	104.8	106.5	108.1	101.6	105.3
S.E.	0.0	3.1	5.6	4.0	4.3	3.7

Upper figures show glucose expressed as mg. per 100 c.c.
Lower figures show glucose expressed as percent of normal.

Table 8

The Effect of Glucose on Blood

Glucose Levels in Rabbits

Number and Sex	Normal Level	Ten Minutes	Twenty-five Minutes	Forty Minutes	Sixty Minutes	Mean
1 F.	116 100.0	156 134.5	212 182.8	169 145.7	163 140.5	150.9
2 F.	107 100.0	141 131.8	201 187.9	148 138.3	154 143.9	150.5
3 F.	120 100.0	185 154.2	149 124.2	186 155.0	148 123.3	139.2
4 F.	109 100.0	172 157.8	160 146.8	160 146.8	137 125.7	144.3
5 F.	127 100.0	222 174.8	200 157.5	205 161.4	174 137.0	157.7
6 F.	130 100.0	185 142.3	180 138.5	175 134.6	133 102.3	129.4
7 F.	116 100.0	171 147.4	175 150.9	163 140.5	193 166.4	151.3
8 F.	122 100.0	175 143.4	169 138.5	156 127.9	179 146.7	139.1
9 F.	113 100.0	143 126.5	128 113.3	138 122.1	131 115.9	119.5
10 F.	131 100.0	131 100.0	157 119.8	153 116.8	139 106.1	110.7
11 F.	125 100.0	161 128.8	180 144.0	157 125.6	146 116.8	128.8
12 F.	111 100.0	154 138.7	167 150.5	125 112.6	111 100.0	125.5
Mean	100.0	140.0	146.2	135.6	127.1	137.2
S.E.	0.0	5.3	6.5	4.3	5.8	4.2

Upper figures show glucose expressed as mg. per 100 c.c.
Lower figures show glucose expressed as percent of normal.

Table 9

The Effect of 20 Percent Sodium Chloride
on Blood Glucose Levels in Rabbits

Number and Sex	Normal Level	Ten Minutes	Twenty-five Minutes	Forty Minutes	Sixty Minutes	Mean
1 F.	164 100.0	132 80.5	148 90.2	145 88.4	159 97.0	89.0
2 F.	109 100.0	110 100.9	118 108.3	119 109.2	148 135.8	113.6
3 F.	168 100.0	165 98.2	160 95.2	145 86.3	154 91.7	92.9
4 F.	138 100.0	143 103.6	120 87.0	112 81.2	121 87.7	89.9
5 F.	118 100.0	124 105.1	147 124.6	134 113.6	129 109.3	113.2
6 F.	122 100.0	118 96.7	129 105.7	133 109.0	101 82.8	98.6
7 F.	105 100.0	110 104.8	146 139.0	116 110.5	118 112.4	116.7
8 F.	131 100.0	97 74.0	143 109.2	117 89.3	105 80.2	88.2
9 F.	159 100.0	160 100.6	196 123.3	179 112.6	142 89.3	106.5
10 F.	150 100.0	155 103.3	165 110.0	106 70.7	127 84.7	92.2
Mean	100.0	96.7	109.3	97.1	97.1	100.1
S.E.	0.0	3.4	5.2	4.8	5.4	4.2

Upper figures show glucose expressed as mg. per 100 c.c.
Lower figures show glucose expressed as percent of normal.

Table 10

The Effect of 0.9 Percent Sodium Chloride
on Blood Glucose Levels in Rabbits

Number and Sex	Normal Level	Ten Minutes	Twenty-five Minutes	Forty Minutes	Sixty Minutes	Mean
1 F.	105 100.0	84 80.0	99 94.3	93 88.6	90 85.7	87.2
2 F.	97 100.0	85 87.6	91 93.8	90 92.8	90 92.8	91.8
3 F.	159 100.0	125 78.6	133 83.6	132 83.0	128 80.5	81.4
4 F.	120 100.0	126 105.0	123 102.5	121 100.8	126 105.0	103.3
5 F.	133 100.0	120 90.2	129 97.0	128 96.2	142 106.8	97.6
6 F.	136 100.0	129 94.9	133 97.8	138 101.5	132 97.1	97.8
7 F.	125 100.0	112 89.6	109 87.2	107 85.6	114 91.2	88.4
8 F.	120 100.0	102 85.0	104 86.7	102 85.0	104 86.7	85.9
9 F.	128 100.0	116 90.6	118 92.2	118 92.2	120 93.8	92.2
10 F.	135 100.0	126 93.3	124 91.9	123 91.1	127 94.1	92.6
Mean	100.0	89.5	92.7	91.7	93.4	91.8
S.E.	0.0	2.3	1.8	2.0	2.6	2.0

Upper figures show glucose expressed as mg. per 100 c.c.
Lower figures show glucose expressed as percent of normal.

Maximum Effects on Blood Sugar following
Injection of Various Glucose Metabolites

FIGURE 1

Legend

A Glucose

B Sodium Lactate

C Sodium Citrate

D Sodium Acetate

E Sodium Pyruvate

F Sodium Succinate

G Sodium Chloride (20 percent)

H Sodium Chloride (0.9 percent)

Mean Effects on Blood Sugar following
Injection of Various Glucose Metabolites

FIGURE 2

Legend

A Glucose	E Sodium Pyruvate
B Sodium Lactate	F Sodium Succinate
C Sodium Citrate	G Sodium Chloride (20 percent)
D Sodium Acetate	H Sodium Chloride (0.9 percent)

The Folin-Malmros micro method of determining glucose concentration is a colorimetric method. It is based upon the ability of glucose to reduce potassium ferricyanide, in alkaline solution, to ferrocyanide, and determining the transmission of light through the resulting blue solution. This latter determination can be made on any one of a number of instruments. The results shown in Tables 3 to 10 were made by use of a Duboscq Colorimeter. Comparison was made against the color developed in a solution containing 100 mg. of glucose per 100 c.c.

Following injection of the various metabolites of glucose, the blood levels of these metabolites will rise. The possibility came to mind that the apparent changes in the glucose level might be due to a reaction of the metabolites with potassium ferricyanide. To find if this might be so, solutions of some of the metabolites were treated exactly as the blood samples had been treated. These consisted of 20 percent sodium acetate, 20 percent sodium citrate, 20 percent sodium lactate, and glucose solution containing 20 mg. per 100 c.c. With the exception of glucose, none of the solutions produced a blue color with the Folin-Malmros reagents. Hence it can safely be assumed that the results shown in the previous tables indicate changes in the blood glucose levels.

A STUDY OF GLUCOSE METABOLITES AS
POSSIBLE POTENTIATING AGENTS

EXPERIMENT 4

Despite the extensive study and report of Lamson et al (52), it has not been generally agreed that metabolites of glucose do potentiate barbiturate depression. This lack of agreement made additional study necessary to arrive at definite conclusions. In an attempt to clarify the picture, and to show the way for further study, a preliminary experiment on rabbits was undertaken to find the effect, if any, of lactate, citrate, and acetate, upon anesthesia.

Procedure:- The procedure used here was similar to that used in Experiment 1, Rabbits were anesthetized by the intravenous injection of pentobarbital. On return of the righting reflex, 3 c.c. of a 20 percent solution of either sodium lactate, sodium citrate, or sodium acetate, were administered by intraperitoneal injection.

Results:- All three of the compounds administered resulted in the righting reflex being lost again. With sodium lactate, the loss was for periods ranging from eleven to thirty-five minutes; with sodium citrate, eight to thirty-seven minutes; and with sodium acetate, it was for sixteen to fifty-three minutes. However, even though the animals made no attempt to assume an upright position, they were not anesthetized by the metabolites of glucose. They were

depressed, but responded to stimuli such as pinching the feet.

EXPERIMENT 5

Because of the fact that lactate, citrate, and acetate did cause some depression, but not anesthesia, it was not possible to decide whether potentiation of the barbiturate had occurred. In order to obtain more information about these and other suggested potentiating agents, a number of experiments were planned.

Procedure:- Rats were anesthetized by the intraperitoneal injection of pentobarbital, in a dosage of 4.5 mg. per 100 Gm. of rat. Upon recovery of the righting reflex, 3.0 c.c. of a 20 percent solution of sodium lactate were given intraperitoneally.

Results:- Thirteen rats were used in this series. All became depressed following the administration of lactate, and lost the righting reflex again. However, there was no anesthesia, as the animals could be aroused by handling. Later, tremors developed followed by violent tonic convulsions, and death. All thirteen of the animals were dead within two hours after injection of the sodium lactate.

A similar experiment was carried out using sodium citrate instead of lactate. Because of the undesirable results obtained with lactate, it was decided to use graded doses of the citrate solution, ranging from 1.0 c.c. to 3.0 c.c. Here too, death resulted in all rats injected,

regardless of dose. Death was preceded by symptoms which were identical with those observed following administration of lactate, that is, tremors, cyanosis, convulsions, and then death.

EXPERIMENT 6

Procedure:- The procedure was identical with that described above, except that the dose of pentobarbital was reduced to 3.0 mg. per 100 Gm. of rat. Upon recovery of the righting reflex, sodium acetate solutions of varying strengths were injected. The volumes injected, and the strengths of the solutions used, are given, along with the results, in Table 11.

Table 11

The Effect of Sodium Acetate Injection
into Rats Recovered from Anesthesia

Number and Sex	Sleeping Time	Dose of Acetate per 100 Gm. Rat	Results
1 F.	89 Mins.	1 c.c., 10 percent	No Depression
2 F.	86 "	1 c.c., 10 percent	No Depression
3 F.	52 "	1 c.c., 5 percent	No Depression
4 F.	65 "	1 c.c., 5 percent	No Depression
5 F.	70 "	1 c.c., 5 percent	No Depression
6 F.	99 "	2 c.c., 10 percent	Reflex lost
7 F.	101 "	2 c.c., 10 percent	Reflex lost
8 F.	84 "	2 c.c., 10 percent	Reflex lost
9 F.	54 "	1 c.c., 5 percent	Reflex lost
10 F.	110 "	2 c.c., 10 percent	Reflex lost
11 F.	79 "	1 c.c., 10 percent	No Depression
12 F.	88 "	2 c.c., 10 percent	Reflex lost

EXPERIMENT 7

Procedure:- The fourth substance tested was sodium pyruvate. The procedure was unchanged, except that, upon recovery, the rats received a smaller dosage of sodium pyruvate than had been the case with lactate, citrate, and acetate. The dosage of pyruvate was 2 c.c. of a 10 percent solution per 100 gm. of rat.

Results:- In this series of thirteen rats, none was returned to anesthesia. However, all of the rats were depressed to the point of returning to sleep without losing the righting reflex.

EXPERIMENT 8

As mentioned previously, Lamson et al (52) reported definite potentiation in rats, with lactate, pyruvate, and other metabolites, but not with glucose itself. The results described here indicated that some depression resulted from the administration of the metabolites, but a return to anesthesia did not occur with any except glucose. Because of these entirely contrary results, it was felt that additional experimentation was not only warranted, but necessary. The first metabolite to be subjected to further investigation was sodium lactate, one of the metabolites reported by Lamson to be able to cause a return to anesthesia in rats.

Procedure:- Rats were anesthetized in the usual manner, and

allowed to recover. One cubic centimetre of a 20 percent solution of sodium lactate was given intraperitoneally. A control series of rats received a similar injection of lactate without prior anesthesia.

Results:- Twelve rats were given lactate following anesthesia. Of these, two lost the righting reflex, but were not anesthetized, as they responded to a pinch of the tail. The remaining animals were depressed, but did not lose the righting reflex. All of the rats in the control group were depressed by the lactate. However, none lost the righting reflex, although they had difficulty in maintaining equilibrium, and appeared to be sleeping while standing. If their tails were pinched, they were awakened immediately, and moved around their cages awkwardly.

Similar results were observed with slightly larger doses of lactate. Six rats which received 1.5 c.c. of 20 percent sodium lactate upon recovery from pentobarbital anesthesia all again lost the righting reflex. They remained in a state of extreme depression for more than seven hours. During this time all developed tremors, became cyanotic, and urinated excessively. The control group, which had not been anesthetized, all exhibited the same symptoms. Shortly after the administration of lactate, they showed considerable difficulty in remaining upright. The animals crouched in the corners of their cages, unable to walk. This condition was followed by

tremors, cyanosis, violent, irregular convulsions, and in one case, death. The others recovered about nine hours after receiving the lactate injection.

EXPERIMENT 9

The similarity of response to sodium lactate following anesthesia, and to sodium lactate without previous anesthesia, indicated that the symptoms observed were possibly due to the lactate rather than to the barbiturate. To explore this possibility, six rats were each given 2 c.c. of 20 percent sodium lactate, and an additional six rats each received 3 c.c. of 20 percent sodium lactate, all without previous anesthesia. All twelve rats died within thirty minutes, after showing the same symptoms as those previously reported, that is, depressed respiration, cyanosis, tremors, coma, and finally a violent tonic convulsion just prior to death.

Similar doses of sodium citrate produced identical results, as did the injection of 1 c.c. of 20 percent solutions of sodium succinate and sodium pyruvate.

EXPERIMENT 10

It has been established that the sodium salts of acetic, citric, lactic, and a number of other organic acids are useful in the treatment of acidosis (58). While the anionic portions of the sodium salts are almost completely oxidized to carbon dioxide and water, the sodium ion

concentration increases to cause alkalosis. The symptoms of alkalosis are essentially those observed and reported above. Sollmann (59) states that alkalosis results from the administration of salts of organic acids, with such symptoms as tetany, anoxia of the tissues, and a considerable increase in urine flow.

It now appeared desirable to determine whether the depression occurring in rats might be due to an excess of sodium rather than being a true potentiation of the barbiturate. To do this, four groups each of three rats was used.

Procedure:- The rats in the first group were anesthetized, allowed to recover, and then received 1.5 c.c. of 20 percent sodium chloride per 100 Gm. of rat, by intraperitoneal injection. Members of the second group were also anesthetized, and, on recovery, received 1.5 c.c. of 20 percent sodium carbonate per 100 Gm. of rat. The rats in the third group were injected with 1.5 c.c. of 20 percent sodium chloride per 100 Gm. of rat, without previous anesthesia, while the fourth group received 1.5 c.c. of 20 percent sodium carbonate per 100 Gm. of rat, without previous anesthesia.

Results:- The results obtained were similar to those observed when sodium salts of organic acids were injected. The animals became depressed shortly after the injection of either sodium chloride or sodium carbonate, regardless

of whether they had been anesthetized previously or not. The depression increased to the point of the righting reflex being lost, but anesthesia did not occur. All of the animals, in all four groups, died within one to three hours.

EXPERIMENT 11

Lamson and his associates (52) used 2.0 M. solutions of the glucose metabolites, and reported that potentiation of barbiturate anesthesia accompanied their administration. In the experiments described above, either 5, 10, or 20 percent solutions were used. In an attempt to duplicate the results observed by Lamson, 2.0 M. solutions of sodium lactate and of sodium citrate were given, in graded doses, to rats.

Procedure:- Four groups, each of six rats, were used. Three rats of each group were anesthetized, and allowed to recover. The remaining three rats in each group served as controls. All six rats in the first group received 0.25 c.c. of a 2.0 M. solution of sodium lactate per 100 Gm. of rat; those of the second group received 0.5 c.c. of the same solution per 100 Gm. of rat; the third group each received 0.75 c.c. per 100 Gm., and the fourth group 1.0 c.c. per 100 Gm.

A similar series of rats was used to determine the effects of administering 2.0 M. sodium citrate.

Results:- The results are shown in Table 12.

In this, and subsequent tables, the term "slight

Table 12

The Effect of 2.0 M. Sodium Lactate upon
Rats Following Recovery from Anesthesia

Number and Sex	Prior Treatment	Dose of 2.0 M. Sodium Lactate per 100 Gm. rat	Result
1 F.	Anesthetized	0.25 c.c.	Slight depression and Tremors.
2 F.	Anesthetized	0.25 c.c.	Slight depression and Tremors.
3 F.	Anesthetized	0.25 c.c.	Slight depression and Tremors.
4 F.	Control	0.25 c.c.	No Effect
5 F.	Control	0.25 c.c.	No Effect
6 F.	Control	0.25 c.c.	Slight Depression.
7 F.	Anesthetized	0.50 c.c.	Slight depression and Tremors.
8 F.	Anesthetized	0.50 c.c.	No Effect
9 F.	Anesthetized	0.50 c.c.	No Effect
10 F.	Control	0.50 c.c.	No Effect
11 F.	Control	0.50 c.c.	Slight depression
12 F.	Control	0.50 c.c.	Slight depression
13 F.	Anesthetized	0.75 c.c.	Depression
14 F.	Anesthetized	0.75 c.c.	Depression. Violent Tremors.
15 F.	Anesthetized	0.75 c.c.	Depression
16 F.	Control	0.75 c.c.	Slight depression
17 F.	Control	0.75 c.c.	Depression
18 F.	Control	0.75 c.c.	Depression
19 F.	Anesthetized	1.0 c.c.	Very depressed. Violent Tremors
20 F.	Anesthetized	1.0 c.c.	Very depressed
21 F.	Anesthetized	1.0 c.c.	Very depressed. Tremors. Cyanosis.
22 F.	Control	1.0 c.c.	Depression
23 F.	Control	1.0 c.c.	Very depressed
24 F.	Control	1.0 c.c.	Very depressed

depression" is used to describe the condition wherein the animals appear lethargic, remain standing, move only when touched, or otherwise stimulated. "Depression" describes

a condition in which the rats do not lose the righting reflex, but exhibit the symptoms of what is generally referred to as general malaise, that is, they walk with difficulty, show little response to touch, and appear to be very sick. Those animals described as "very depressed" lose the righting reflex and lie on their sides.

Sodium lactate appeared to have a somewhat greater depressive effect upon rats which had been anesthetized prior to lactate injection, than it had on the control groups. None of the animals, however, was returned to anesthesia, and all responded when their tails were pinched.

All of the animals receiving sodium lactate were observed the following morning. By this time they had recovered fully, but all had urinated excessively during the night.

Regardless of dose, 2.0 M. sodium citrate caused the death of all the animals in all groups within two minutes of injection. Death was preceded by a violent tonic convulsion.

EXPERIMENT 12

In another attempt to produce potentiation of barbiturate anesthesia, it was decided to reduce the concentration of the glucose metabolites, and use graded series of doses of the weaker solutions.

Procedure:- Rats were anesthetized with pentobarbital, and allowed to recover. Upon recovery, the rats were given an

intraperitoneal injection of one of the metabolites. The dosage of the metabolites ranged from 0.25 c.c. per 100 Gm. of rat, to 1.75 c.c. per 100 Gm. of rat. All solutions used were 10 percent in strength. Control animals received similar doses of metabolites, without having undergone previous anesthesia. In this series, the metabolites used included sodium acetate, sodium lactate, sodium pyruvate, and sodium succinate.

Results are shown in Tables 13, 14, 15, and 16.

Table 13

The Effect of 10 Percent Sodium Acetate upon Rats
following Recovery from Anesthesia

Number and Sex	Prior Treatment	Dose of 10 Percent Sodium Acetate per 100 Gm. Rat	Result
1 F.	Anesthetized	0.25 c.c.	No Effect
2 F.	Anesthetized	0.25 c.c.	No Effect
3 F.	Control	0.25 c.c.	No Effect
4 F.	Anesthetized	0.75 c.c.	Slight depression
5 F.	Anesthetized	0.75 c.c.	Slight depression
6 F.	Control	0.75 c.c.	No Effect
7 F.	Anesthetized	1.25 c.c.	No Effect
8 F.	Anesthetized	1.25 c.c.	Depression
9 F.	Control	1.25 c.c.	Slight depression
10 F.	Anesthetized	1.75 c.c.	Very depressed. Convulsions
11 F.	Anesthetized	1.75 c.c.	Very depressed. Convulsions
12 F.	Control	1.75 c.c.	Slight depression

Table 14

The Effect of 10 Percent Sodium Lactate upon Rats
following Recovery from Anesthesia

Number and Sex	Prior Treatment	Dose of 10 Percent Sodium Lactate per 100 Gm. Rat	Result
1 F.	Anesthetized	0.25 c.c.	No Effect
2 F.	Anesthetized	0.25 c.c.	Slight depression
3 F.	Control	0.25 c.c.	No Effect
4 F.	Anesthetized	0.75 c.c.	Slight depression
5 F.	Anesthetized	0.75 c.c.	Slight depression
6 F.	Control	0.75 c.c.	No Effect
7 F.	Anesthetized	1.25 c.c.	Slight depression
8 F.	Anesthetized	1.25 c.c.	Slight depression
9 F.	Control	1.25 c.c.	Slight depression
10 F.	Anesthetized	1.75 c.c.	Slight depression
11 F.	Anesthetized	1.75 c.c.	Depression
12 F.	Control	1.75 c.c.	Depression

In all cases the animals were observed for two to three hours following administration of the glucose metabolite. The result shown in Tables 13, 14, 15, and 16 is the most extreme symptom shown by each rat. Those which became depressed reached that state gradually, as did those showing extreme depression and convulsions. All except those exhibiting no effect urinated excessively during the period of observation. None of the rats became anesthetized.

Table 15

The Effect of 10 Percent Sodium Pyruvate upon Rats
following Recovery from Anesthesia

Number and Sex	Prior Treatment	Dose of 10 Percent Sodium Pyruvate per 100 Gm. Rat	Result
1 F.	Anesthetized	0.25 c.c.	No Effect
2 F.	Anesthetized	0.25 c.c.	No Effect
3 F.	Control	0.25 c.c.	No Effect
4 F.	Anesthetized	0.75 c.c.	No Effect
5 F.	Anesthetized	0.75 c.c.	Slight depression
6 F.	Control	0.75 c.c.	No Effect
7 F.	Anesthetized	1.25 c.c.	Slight depression
8 F.	Anesthetized	1.25 c.c.	Slight depression
9 F.	Control	1.25 c.c.	Depression
10 F.	Anesthetized	1.75 c.c.	Slight depression
11 F.	Anesthetized	1.75 c.c.	Slight depression
12 F.	Control	1.75 c.c.	No Effect

Table 16

The Effect of 10 Percent Sodium Succinate upon Rats
following Recovery from Anesthesia

Number and Sex	Prior Treatment	Dose of 10 Percent Sodium Succinate per 100 Gm. Rat	Result
1 F.	Anesthetized	0.25 c.c.	No Effect
2 F.	Anesthetized	0.25 c.c.	No Effect
3 F.	Control	0.25 c.c.	No Effect
4 F.	Anesthetized	0.75 c.c.	No Effect
5 F.	Anesthetized	0.75 c.c.	Slight depression
6 F.	Control	0.75 c.c.	No Effect
7 F.	Anesthetized	1.25 c.c.	Slight depression
8 F.	Anesthetized	1.25 c.c.	Depression
9 F.	Control	1.25 c.c.	No Effect
10 F.	Anesthetized	1.75 c.c.	Depression
11 F.	Anesthetized	1.75 c.c.	Slight depression
12 F.	Control	1.75 c.c.	Depression

EXPERIMENT 13

The screening procedures described in Experiment 12 were carried out in an attempt to find a dose of sodium lactate, acetate, pyruvate and succinate which would exert a depressive action but would not cause death. Generally, doses of 1.25 c.c. per 100 Gm. of rat appear to have such an effect, so there did not seem to be any reason for using doses considerably smaller or larger than this in subsequent investigations.

In every prior experiment in which the sodium salts of organic acids were given to rats, all the symptoms of alkalosis later became apparent. The possibility existed that these compounds did potentiate barbiturate anesthesia, but the effects were being clouded by the development of alkalosis. A second possibility was that their only action was to produce alkalosis. If the compounds could be administered in such a way that alkalosis symptoms did not result, it would probably then be possible to evaluate their influence upon the barbiturates. To this end, a buffered solution of sodium lactate was prepared and used.

Procedure:- A 10 percent, buffered, solution of sodium lactate was prepared by combining 7.1 c.c. of 70 percent sodium lactate, 6.0 c.c. of 0.5 M. potassium acid phosphate, 31.5 c.c. of 0.5 M. disodium phosphate, and making the total volume up to 50 c.c. This combination yielded a 10 percent solution of sodium lactate, having a pH of 7.3.

Three groups, each of six rats, were used. Four members of each group were anesthetized and allowed to recover, while the other two members of each group served as controls. One cubic centimetre of the buffered lactate solution per 100 Gm. of rat was administered to each animal of the first group. Those of the second group received 1.5 c.c. per 100 Gm. of rat, while 2.0 c.c. per 100 Gm. of rat were given to each rat in the third group. Administration in all cases was by intraperitoneal injection.

Results:- In Table 17, results are shown in two columns. One column, numbered 1, shows the results immediately following administration of the lactate solution, and for two hours after administration. In the second column, numbered 2, are shown the observations made about ten hours after the lactate was given.

Here, as in previous experiments of this type, there was not a return to anesthesia in the previously anesthetized rats. Even animals which were extremely depressed would respond to a pinch of the tail. After ten hours, all animals, regardless of the quantity of lactate given, had urinated excessively.

Table 17

The Effect of Buffered, 10 Percent, Sodium Lactate, upon Rats

Number and Sex	Prior Treatment	Dose of 10 Percent, Buffered, Sodium Lactate, per 100 Gm. of Rat	Results	
			1	2
1 F.	Anesthetized	1.0 c.c.	Depression	Recovered
2 F.	Anesthetized	1.0 c.c.	Depression	Recovered
3 F.	Anesthetized	1.0 c.c.	Depression	Recovered
4 F.	Anesthetized	1.0 c.c.	Depression	Recovered
5 F.	Control	1.0 c.c.	Slight depression	Recovered
6 F.	Control	1.0 c.c.	No Effect	Recovered
7 F.	Anesthetized	1.5 c.c.	Very depressed	Recovered
8 F.	Anesthetized	1.5 c.c.	No Effect	Recovered
9 F.	Anesthetized	1.5 c.c.	Very depressed	Recovered
10 F.	Anesthetized	1.5 c.c.	Very depressed	Recovered
11 F.	Control	1.5 c.c.	Depression	Recovered
12 F.	Control	1.5 c.c.	Depression	Recovered
13 F.	Anesthetized	2.0 c.c.	Very depressed	Slight depression
14 F.	Anesthetized	2.0 c.c.	Depression	Dead
15 F.	Anesthetized	2.0 c.c.	Very depressed	Dead
16 F.	Anesthetized	2.0 c.c.	Depression	Slight depression
17 F.	Control	2.0 c.c.	Depression	Very depressed
18 F.	Control	2.0 c.c.	Depression	Very depressed

EXPERIMENT 14

Since the attempt to prevent alkalosis symptoms by using phosphate buffers was not successful, it was hoped that buffer pairs could be formed by combining the sodium salt of the glucose metabolite with the free acid. However, at pH 7.3, the buffering power of the combinations was negligible. Nevertheless, two combinations were tried.

Procedure:- Equal volumes of 20 percent sodium citrate and 20 percent citric acid were combined, as were equal volumes of 20 percent sodium lactate and 20 percent lactic acid. Rats which had not been subjected to previous anesthesia were given intraperitoneal injections of the mixtures.

Results:- Results are shown in Table 18.

Table 18

The Effect of Acid-Salt Mixtures upon Rats

1. Sodium Lactate-Lactic Acid

<u>Number and Sex</u>	<u>Dose of Mixture per 100 Gm. Rat</u>	<u>Result</u>
1 F.	0.50 c.c.	Depression
2 F.	1.0 c.c.	Depression
3 F.	1.5 c.c.	Depression

2. Sodium Citrate-Citric Acid

1 F.	0.50 c.c.	Depression
2 F.	1.0 c.c.	Death
3 F.	1.5 c.c.	Depression

EXPERIMENT 15

Alkalosis is not necessarily accompanied by a change in the pH of the blood, in that the blood buffers may be able to compensate for the increase in the "alkali reserve". However, it does not follow that a pH change cannot occur during alkalosis. As the symptoms of alkalosis developed after the injection of sodium salts of the glucose metabolites, investigation of blood pH values became necessary.

Procedure:- Three groups, each of eight rats, were used. All were anesthetized by the intraperitoneal injection of 3 mg. of pentobarbital per 100 Gm. of rat. Members of the first group received no further treatment. The rats in the second group each were given 1 c.c. of a 20 percent solution of glucose intraperitoneally. To each animal in the third group 1 c.c. of 20 percent sodium lactate was administered, also by intraperitoneal injection. The symptoms of alkalosis developed within twenty minutes in those animals receiving sodium lactate. Hence, each animal in each group was bled twenty minutes after receiving its injection. This was done by quickly slashing the throat, and allowing the blood to drain into a porcelain evaporating dish containing 0.1 c.c. of heparin sodium solution. The blood was immediately transferred to the cup of a Beckman model pH meter, and the pH of the blood determined.

Results:- The pH values of the blood are shown in Tables

19, 20, and 21. There was not a significant difference in the pH of the blood, regardless of the substance administered to the rats.

Table 19

The pH of the Blood of Rats Anesthetized
by Pentobarbital

<u>Number</u>	<u>Blood pH</u>
1	7.47
2	7.45
3	7.45
4	7.45
5	7.47
6	7.33
7	7.49
8	7.42
Mean	7.44

Table 20

The Effect of Glucose upon the pH of the Blood
of Rats Anesthetized by Pentobarbital

<u>Number</u>	<u>Blood pH</u>
1	7.49
2	7.41
3	7.42
4	7.45
5	7.42
6	7.51
7	7.43
8	7.43
Mean	7.45

Table 21

The Effect of Sodium Lactate upon the pH of the
Blood of Rats Anesthetized by Pentobarbital

<u>Number</u>	<u>Blood pH</u>
1	7.43
2	7.47
3	7.44
4	7.46
5	7.41
6	7.43
7	7.42
8	7.49
Mean	7.44

Despite the lack of change in the pH of the blood, there can be little doubt that sodium lactate injection results in alkalosis in rats. Blood buffers are capable of overcoming changes in the pH of the blood, even though the symptoms of alkalosis become evident. The mechanisms involved will be discussed more fully later.

THE EFFECT OF GLUCOSE UPON PENTOBARBITAL
LEVELS IN THE LIVER, BRAIN, AND BLOOD

The barbiturates are classified as central nervous system depressants. As such, their concentration in the brain is very likely of considerable significance in the production of anesthesia.

It has been generally agreed that, with few exceptions, the liver is the chief organ of detoxification of the barbiturates. Sollmann (60) has published a list of the barbiturates so destroyed; pentobarbital is included in that list. Kahn (61) used rat liver slices in his study of pentobarbital detoxification, while Kelly and Shideman (62) reported the liver to be the chief organ of detoxification of thiopental. There is some evidence that the accumulation of Pentothal in the liver decreases as the glycogen concentration increases. (26).

The blood concentration of any drug is a measure of its rate of absorption.

Because of the significance of these three sites, the measurement of the levels of pentobarbital in the blood, liver, and brain indicates the rates of absorption, distribution, and detoxification. Glucose, too, is connected with the blood, brain, and liver. Glucose is the required metabolite of nervous tissue. As glycogen, glucose is stored in the liver. The blood always contains glucose. These facts make an interrelationship between glucose and

pentobarbital possible. In addition, it has been shown that there is an inverse relationship between pentobarbital uptake, and glucose uptake, at the liver. (20). In studies on minced liver tissue, there was evidence that, when glucose and pentobarbital are both added to such tissue, glucose uptake is rapid, and pentobarbital detoxification slow during the first half hour. However, during the second half hour, pentobarbital uptake increases in rate, while the uptake of glucose decreases from that occurring during the first half hour. The evidence obtained in these preliminary investigations indicated the advisability of more detailed studies on

- (i) the rate of uptake of pentobarbital by the liver during pentobarbital anesthesia,
- (ii) the effect of added glucose upon liver uptake of pentobarbital,
- (iii) blood concentrations of pentobarbital during anesthesia,
- (iv) the effect of added glucose upon blood concentrations of pentobarbital,
- (v) pentobarbital levels in the brain during anesthesia, and
- (vi) the effect of added glucose upon the brain levels of pentobarbital.

The Effect of Added Glucose upon
Liver Uptake of Pentobarbital

EXPERIMENT 16

Procedure:- By intraperitoneal injection, starved rats were given a solution containing 3 mg. of pentobarbital per c.c., the dosage being 1 c.c. per 100 Gm. of rat. At periods of either ten, thirty, or sixty minutes after injection, the rats were quickly killed by a blow on the head. Other rats were allowed to recover, and killed similarly at the time of recovery. The trauma resulting from a blow on the head might be expected to cause a change in the liver glycogen-blood glucose relationship. Such is not the case, however, as Chantraine has shown (3).

Immediately after death, the liver was removed, and sliced with a sharp razor blade. Portions of the liver slices were then used for the determination of both glycogen and pentobarbital concentrations.

(a) Procedure for the Determination of Liver Glycogen:

The procedure followed was a modification of the one described by Koch and Hanke (63), and was as follows:

- (i) Five cubic centimetres of 30 percent potassium hydroxide were placed in a centrifuge tube, and weighed.
- (ii) Approximately 3 Gm. of rat liver slices were added to the potassium hydroxide, and the tube again

weighed. The weight of liver added was then recorded.

- (iii) The tube was immersed in boiling water, and heated until homogeneous (about twenty minutes).
- (iv) To precipitate the glycogen, 5 c.c. of 95 percent ethyl alcohol were added, the solutions mixed, and heated on a water bath to boiling.
- (v) The tube was cooled to room temperature, and centrifuged thirty minutes.
- (vi) Following centrifugation, the supernatant liquid was poured off, and the remaining alcohol expelled by warming the tube.
- (vii) Three cubic centimetres of 0.6 N. hydrochloric acid were added to the glycogen in the tube, and heated under an air reflux for two to two and a half hours.
- (viii) Two drops of phenol red were now added, and the solution neutralized with 10 percent sodium hydroxide.
- (ix) The volume was made up to 10 c.c.
- (x) Aliquots of 0.1 c.c. of the final solution were used to determine the quantity of glucose.

Determinations were made by the Folin-Malmros micro method (57). Readings were made on a Cenco-Sheard-Sanford photelometer which had been calibrated previously against known glucose solutions in

concentrations ranging from 10 mg. per 100 c.c. to 500 mg. per 100 c.c.

(xi) The readings obtained were corrected to show the quantity of glycogen present in each gram of liver.

(b) Procedure for the Determination of Liver Pentobarbital:

(i) A weighed portion of rat liver slices was added to 20 c.c. of 0.9 percent sodium chloride solution in a Waring Blendor, and homogenized for two minutes.

(ii) The total volume was adjusted to 25 c.c.

(iii) A 5 c.c. aliquot was added to 25 c.c. of ethylene dichloride in a separatory funnel. Goldbaum (64), who devised the method, suggested that chloroform be used as extracting solvent. However, ethylene dichloride is equally efficient, and does not cause emulsification as readily as chloroform.

(iv) The liver homogenate was extracted for five minutes.

(v) The ethylene dichloride layer was filtered through Celite¹, which removes particulate matter, to obtain a 20 c.c. volume of filtrate.

(vi) The filtrate was shaken carefully with 5 c.c. of 1.0 N. sodium hydroxide, for five minutes, in a separatory funnel.

1. Celite is an asbestos filtering aid which is manufactured by Johns-Manville.

- (vii) The sodium hydroxide layer, now containing the pentobarbital from the liver, was placed in a centrifuge tube, and centrifuged two minutes to cause separation of any residual ethylene dichloride.
- (viii) The content of pentobarbital was determined spectrophotometrically in a Beckman D.U. Spectrophotometer. Readings were made at 255 millimicrons, and the concentration of barbiturate obtained by comparison with a standard curve. This curve was established by using the readings obtained with known solutions of pentobarbital in sodium hydroxide, ranging in strength from 1 mg. per 100 c.c. to 10 mg. per 100 c.c.
- (ix) The readings were corrected to show the amount of pentobarbital present in each gram of liver.

A second series of animals was used to find the effect of glucose upon the levels of liver glycogen and liver pentobarbital. The procedures were identical to those outlined, except that glucose, 1 c.c. of a 50 percent solution per 100 Gm. of rat, was administered simultaneously with the pentobarbital.

Normal liver glycogen was determined in unanesthetized rats, which had been starved for the usual period, killed, and the livers treated as outlined above.

Blanks were determined by using the livers of

unanesthetized rats and extracting exactly as was done with the anesthetized rats. The blank values were used to correct the liver pentobarbital readings.

Results:- The results of the liver glycogen studies appear in Tables 22 and 23, and graphically in Figure 3. Pentobarbital levels are shown in Tables 25 and 26 and graphically in Figure 4.

Normal levels of liver glycogen in starved rats are shown in Table 24.

Table 22

Levels of Liver Glycogen During Pentobarbital Anesthesia

Induced by Pentobarbital Alone

Number	Ten Minutes		Thirty Minutes		Sixty Minutes		At Recovery	
1	1.47	mg./Gm.	1.69	mg./Gm.	1.56	mg./Gm.	6.20	mg./Gm.
2	1.44	"	1.58	"	1.67	"	5.78	"
3	1.34	"	1.58	"	1.56	"	5.97	"
4	1.57	"	1.58	"	1.67	"	6.05	"
5	1.61	"	3.49	"	3.03	"	4.90	"
6	1.53	"	4.04	"	2.79	"	4.79	"
7	1.66	"	4.22	"	2.90	"	4.86	"
8	1.61	"	4.04	"	3.01	"	4.90	"
9	2.37	"	1.66	"	4.13	"	7.11	"
10	2.27	"	1.26	"	3.06	"	6.43	"
11	2.53	"	1.38	"	3.28	"	6.10	"
12	2.05	"	1.26	"	2.94	"	6.51	"
Mean	1.79	"	2.32	"	2.63	"	5.80	"
S.E.	0.12	"	0.36	"	0.24	"	0.23	"

The average sleeping time of those animals allowed to recover was one hundred and thirty three minutes.

Table 23

Levels of Liver Glycogen During Pentobarbital Anesthesia
Induced by Pentobarbital and Glucose

Number	Ten Minutes	Thirty Minutes	Sixty Minutes	At Recovery
1	4.31 mg./Gm.	4.87 mg./Gm.	6.99 mg./Gm.	9.87 mg./Gm
2	4.44 "	4.65 "	6.40 "	9.42 "
3	4.36 "	4.76 "	6.23 "	9.10 "
4	4.22 "	4.46 "	6.48 "	9.16 "
5	3.62 "	7.62 "	6.43 "	9.93 "
6	3.34 "	7.50 "	6.57 "	9.77 "
7	3.74 "	7.39 "	6.24 "	9.67 "
8	4.35 "	7.19 "	6.29 "	10.03 "
9	3.38 "	3.90 "	5.30 "	10.54 "
10	3.49 "	3.08 "	5.27 "	10.41 "
11	3.18 "	3.69 "	5.15 "	10.47 "
12	3.50 "	3.50 "	5.12 "	10.27 "
Mean	3.83 "	5.22 "	6.04 "	9.88 "
S.E.	0.14 "	0.51 "	0.19 "	0.14 "

The average sleeping time of those animals allowed to recover was one hundred and seventy-seven minutes.

Table 24

Normal Liver Glycogen Levels

Number	Glycogen Level
1	2.40 mg./Gm.
2	2.34 "
3	2.34 "
4	2.45 "
5	2.25 "
6	1.82 "
7	1.82 "
8	1.74 "
Mean	2.15 "
S.E.	0.10 "

Table 25

Levels of Pentobarbital in the Liver During Anesthesia

Induced by Pentobarbital Alone

Number	Ten Minutes	Thirty Minutes	Sixty Minutes	At Recovery
1	334 mcg./Gm.	154 mcg./Gm.	143 mcg./Gm.	38 mcg./Gm.
2	362 "	137 "	77 "	39 "
3	283 "	160 "	148 "	39 "
4	322 "	101 "	175 "	26 "
5	200 "	28 "	105 "	52 "
6	179 "	47 "	102 "	102 "
7	261 "	70 "	135 "	130 "
8	260 "	66 "	130 "	47 "
9	287 "	228 "	385 "	75 "
10	320 "	218 "	257 "	114 "
11	296 "	252 "	276 "	139 "
12	315 "	217 "	322 "	148 "
13	---	212 "	28 "	---
14	---	108 "	50 "	---
15	---	108 "	89 "	---
16	---	138 "	100 "	---
17	---	145 "	126 "	---
18	---	152 "	177 "	---
19	---	182 "	201 "	---
20	---	176 "	156 "	---
21	---	170 "	182 "	---
22	---	198 "	182 "	---
23	---	238 "	158 "	---
24	---	200 "	150 "	---
Mean	284 "	154 "	161 "	79 "
S.E.	15.5 "	12.7 "	16.9 "	12.6 "

The average sleeping time of those animals allowed to recover was one hundred and thirty-three minutes.

Table 26

Levels of Pentobarbital in the Liver During Anesthesia
Induced by Pentobarbital and Glucose

Number	Ten Minutes	Thirty Minutes	Sixty Minutes	At Recovery
1	212 mcg./Gm.	102 mcg./Gm.	51 mcg./Gm.	89 mcg./Gm.
2	175 "	117 "	86 "	103 "
3	177 "	198 "	92 "	78 "
4	176 "	227 "	107 "	92 "
5	167 "	7 "	125 "	93 "
6	159 "	82 "	138 "	88 "
7	212 "	98 "	131 "	103 "
8	174 "	199 "	115 "	81 "
9	231 "	46 "	54 "	---
10	222 "	69 "	77 "	---
11	161 "	116 "	68 "	---
12	180 "	80 "	62 "	---
Mean	187 "	112 "	92 "	90 "
S.E.	7.3 "	19.3 "	9.0 "	2.7 "

The average sleeping time of those animals allowed to recover was one hundred and seventy-seven minutes.

Effect of Added Glucose upon Liver Glycogen
in Rats Anesthetized with Pentobarbital

FIGURE 3

Legend

Pentobarbital Alone _____

Pentobarbital and Glucose -----

Effect of Added Glucose upon Liver Pentobarbital Levels
in Rats Anesthetized with Pentobarbital

FIGURE 4

Legend

Pentobarbital Alone —————

Pentobarbital and Glucose - - - - -

The Effect of Added Glucose upon Blood

Levels of Pentobarbital

EXPERIMENT 17

Procedure:- (i) A solution containing 3 mg. of pentobarbital per c.c. was injected intraperitoneally into starved rats. The dosage was 1 c.c. per 100 Gm. of rat.

- (ii) Ten minutes after injection the rats were killed by severing the carotid arteries by quickly drawing a razor blade across the throat.
- (iii) The blood was allowed to drain into a porcelain evaporating dish containing 0.1 c.c. of heparin sodium.
- (iv) One-tenth cubic centimetre of the blood was used to determine the blood glucose content. The Folin-Malmros micro method was followed.
- (v) The blood remaining in the evaporating dish was measured accurately, and assayed for pentobarbital, using Goldbaum's technique (64).
- (vi) Both glucose and pentobarbital readings were corrected to show the amount of each present per 100 c.c. of blood.

Similar determinations were made on rat's blood at periods of thirty and sixty minutes after the injection of pentobarbital, as well as at the time of recovery from anesthesia.

To demonstrate the effect of added glucose upon blood levels of pentobarbital, series of rats were treated as above, except that the original injection contained both 3 mg. of pentobarbital and 1 c.c. of 50 percent glucose per 100 Gm. of rat.

Blanks were obtained by removing the blood of unanesthetized rats. To determine normal blood glucose levels, 0.1 c.c. of this blood was assayed for glucose. The remainder was extracted as previously and read on the Beckman D.U. spectrophotometer.

Results:- Results of blood glucose determinations are shown in Tables 27, 28 and 29. A chart showing changes in the glucose level during anesthesia is given in Figure 5. Pentobarbital levels appear in Tables 30 and 31, and are shown graphically in Figure 6.

Table 27
Normal Blood Glucose Levels
in Starved Rats

<u>Number</u>	<u>Blood Glucose</u>
1	90 mg./100 c.c.
2	62 "
3	86 "
4	78 "
5	75 "
6	67 "
Mean	79 "
S.E.	2.9 "

Table 28

Blood Glucose Levels During Pentobarbital Anesthesia,

Induced by Pentobarbital Alone.

Number	Ten Minutes	Thirty Minutes	Sixty Minutes	At Recovery
--------	----------------	-------------------	------------------	----------------

All values are expressed as mg. per 100 c.c. of blood.

1	84	100	82	80
2	92	96	78	78
3	92	105	72	88
4	101	86	78	79
5	96	101	75	86
6	74	92	86	76
7	96	103	84	--
8	86	92	75	--
Mean	90	97	79	81
S.E.	2.9	2.2	1.5	1.9

The average sleeping time of those animals allowed to recover was one hundred and eighty-five minutes.

Table 29

Blood Glucose Levels During Pentobarbital Anesthesia,

Induced by Pentobarbital and Glucose.

Number	Ten Minutes	Thirty Minutes	Sixty Minutes	At Recovery
--------	----------------	-------------------	------------------	----------------

All values are expressed as mg. per 100 c.c. of blood.

1	450	404	425	255
2	440	355	416	247
3	255	465	420	207
4	410	450	390	215
5	390	335	328	229
6	375	306	360	236
7	335	410	379	---
8	445	415	322	---
Mean	388	393	380	232
S.E.	27.7	23.2	16.9	8.4

The average sleeping time of those animals allowed to recover was two hundred and sixteen minutes.

Table 30

Blood Pentobarbital Levels During Pentobarbital Anesthesia,
Induced by Pentobarbital Alone.

Number	Ten Minutes	Thirty Minutes	Sixty Minutes	At Recovery
All values are expressed as mg. per 100 c.c. of blood.				
1	2.79	1.12	0.31	0.04
2	0.80	0.19	0.19	0.41
3	1.43	0.87	1.22	0.54
4	1.79	0.60	0.74	0.19
5	1.91	2.10	1.85	0.39
6	0.60	0.49	2.02	0.17
7	2.14	1.97	0.99	----
8	0.84	2.06	0.77	----
Mean	1.54	1.18	1.01	0.29
S.E.	0.27	0.28	0.24	0.08

The average sleeping time of those animals allowed to recover was one hundred and eighty-five minutes.

Table 31

Blood Pentobarbital Levels During Pentobarbital Anesthesia,
Induced by Pentobarbital and Glucose.

Number	Ten Minutes	Thirty Minutes	Sixty Minutes	At Recovery
All values are expressed as mg. per 100 c.c. of blood.				
1	3.08	4.68	2.25	1.05
2	4.93	8.87	4.83	1.01
3	3.35	5.59	2.45	0.69
4	4.72	1.36	3.54	1.58
5	6.09	2.20	2.88	0.32
6	6.07	2.89	3.18	0.88
7	3.59	3.56	4.50	----
8	5.01	2.05	4.80	----
Mean	4.61	3.90	3.55	0.92
S.E.	0.42	0.87	0.38	0.19

The average sleeping time of those animals allowed to recover was two hundred and sixteen minutes.

The Effect of Added Glucose Upon Blood
Levels of Glucose and of Pentobarbital
at Onset of Pentobarbital Anesthesia.

EXPERIMENT 18

The time required for the animals to become anesthetized was usually about five minutes. This was true, whether pentobarbital was given alone, or along with glucose. As the blood levels of both glucose and of pentobarbital were higher during the course of anesthesia, it seemed of value to check the blood levels of both at the time of onset of anesthesia.

Procedure:- The procedure followed was similar to that in Experiment 16. One series of rats received only pentobarbital; another received pentobarbital and glucose simultaneously. The dosages were 3 mg. of pentobarbital per 100 Gm. of rat, and 1 c.c. of 50 percent glucose per 100 Gm. of rat, respectively. At the time of the loss of the righting reflex, the animals were killed, and bled, as before. Blood glucose, and blood pentobarbital levels were determined as previously.

Results:- Blood levels of glucose and of pentobarbital following the administration of pentobarbital alone are shown in Table 32, along with the time required for the induction of anesthesia. Levels following pentobarbital and glucose injections appear in Table 33, accompanied by the times required for the induction of anesthesia. The

appropriate values are also included in Figures 5 and 6.

Table 32

Blood Levels of Pentobarbital and Glucose at the Time of Onset of Anesthesia, following Pentobarbital Alone.

Number	Induction Time	Glucose Level	Pentobarbital Level
1	4 minutes	85 mg./ 100 c.c.	0.49 mg./ 100 c.c.
2	4 "	82 "	0.93 "
3	4 "	73 "	1.35 "
4	6 "	83 "	1.80 "
5	5 "	90 "	1.55 "
6	5 "	95 "	1.51 "
7	5 "	84 "	1.63 "
8	4 "	73 "	0.52 "
Mean	4.6 "	83 "	1.22 "
S.E.		2.8 "	0.21 "

Table 33

Blood Levels of Pentobarbital and Glucose at the Time of Onset of Anesthesia, following Pentobarbital and Glucose.

Number	Induction Time	Glucose Level	Pentobarbital Level
1	3 minutes	306 mg./ 100 c.c.	3.17 mg./ 100 c.c.
2	7 "	285 "	4.01 "
3	5 "	205 "	3.13 "
4	4 "	263 "	2.95 "
5	4 "	330 "	1.96 "
6	3 "	270 "	3.36 "
7	4 "	256 "	2.93 "
8	3 "	229 "	3.01 "
Mean	4.1 "	268 "	3.07 "
S.E.		16.9 "	0.24 "

Effect of Added Glucose upon Blood Glucose
in Rats Anesthetized with Pentobarbital

FIGURE 5

Legend

Pentobarbital Alone _____
Pentobarbital and Glucose - - - - -

Effect of Added Glucose upon Blood Pentobarbital Levels
in Rats Anesthetized with Pentobarbital

FIGURE 6

Legend

Pentobarbital Alone _____

Pentobarbital and Glucose - - - - -

The Effect of Added Glucose upon Brain

Levels of Pentobarbital

EXPERIMENT 19

Procedure:- (i) By intraperitoneal injection, starved rats were given a solution containing 3 mg. of pentobarbital per c.c., the dosage being 1 c.c. per 100 Gm. of rat.

(ii) Ten minutes after the injection, the rats were killed by a blow on the head, and the brains removed and sliced.

(iii) A weighed portion of brain slices (about 1.5 Gm.) was added to 20 c.c. of 0.9 percent sodium chloride solution in a Waring Blendor, and homogenized for two minutes.

(iv) The volume was made up to 25 c.c.

(v) Five cubic centimetre aliquots were extracted with ethylene chloride, similar to the manner in which the rat liver homogenate was extracted.

(vi) The pentobarbital levels were determined as before, and the readings corrected to show the quantity of pentobarbital present in each gram of brain.

Blanks were determined using the brains of unanesthetized rats, and used to correct the pentobarbital readings.

Other brain pentobarbital assays were made at periods of thirty, and sixty minutes after the injection of the

anesthetic agent, and at the time of recovery from anesthesia.

A second series of rats was used to find the effect of glucose upon the levels of pentobarbital in the brain. The procedure was unchanged, except that an injection of 1 c.c. of 50 percent glucose solution per 100 Gm. of rat accompanied the pentobarbital injection.

Results:- Results are shown in Tables 34 and 35, and graphically in Figure 7.

Attempts to determine the level of glucose in the brain were unsuccessful. It was hoped that such determinations could be made, and the glucose level correlated with the level of barbiturate. However, either because glucose was present in quantities too small to be measured, or in a form which is not measureable by the method used, the quantity of glucose in the brain could not be determined.

Table 34

Levels of Pentobarbital in the Brain During Anesthesia
Induced by Pentobarbital Alone

Number	Ten Minutes	Thirty Minutes	Sixty Minutes	At Recovery
1	127 mcg./Gm.	240 mcg./Gm.	193 mcg./Gm.	5 mcg./Gm.
2	110 "	272 "	190 "	40 "
3	158 "	147 "	167 "	49 "
4	157 "	259 "	185 "	81 "
5	236 "	196 "	226 "	63 "
6	200 "	175 "	123 "	59 "
7	280 "	161 "	146 "	14 "
8	181 "	138 "	162 "	30 "
9	290 "	126 "	174 "	19 "
10	257 "	227 "	136 "	49 "
11	197 "	291 "	230 "	123 "
12	190 "	264 "	193 "	109 "
Mean	199 "	208 "	177 "	53 "
S.E.	16.9 "	17.0 "	9.6 "	10.6 "

The average sleeping time of those animals allowed to recover was one hundred and thirty-three minutes.

Table 35

Levels of Pentobarbital in the Brain During Anesthesia
Induced by Pentobarbital and Glucose

Number	Ten Minutes	Thirty Minutes	Sixty Minutes	At Recovery
1	143 mcg./Gm.	183 mcg./Gm.	105 mcg./Gm.	83 mcg./Gm.
2	146 "	359 "	189 "	133 "
3	136 "	254 "	239 "	115 "
4	170 "	468 "	242 "	167 "
5	219 "	136 "	207 "	78 "
6	193 "	156 "	238 "	118 "
7	272 "	88 "	235 "	102 "
8	201 "	222 "	186 "	121 "
9	205 "	94 "	70 "	55 "
10	218 "	269 "	123 "	80 "
11	256 "	116 "	136 "	56 "
12	243 "	143 "	187 "	66 "
Mean	200 "	207 "	180 "	98 "
S.E.	13.2 "	33.8 "	17.2 "	10.0 "

The average sleeping time of those animals allowed to recover was one hundred and seventy-seven minutes.

Effect of Added Glucose upon Brain Pentobarbital Levels
in Rats Anesthetized with Pentobarbital

FIGURE 7

Legend

Pentobarbital Alone _____

Pentobarbital and Glucose - - - - -

THE INFLUENCE OF HIGH BLOOD GLUCOSE LEVELS
UPON ANESTHESIA INDUCTION TIME.

EXPERIMENT 20

In earlier experiments it was found that the time required for anesthesia to occur was four and six-tenths minutes if pentobarbital and glucose were given simultaneously to rats. If pentobarbital were given alone, the induction time was four and one-tenth minutes. Although the difference was slight, it seemed possible that the increasing blood glucose level might be able to delay the onset of anesthesia. If this were so, it follows logically that, if the level of blood glucose could be raised prior to the administration of the anesthetic, the time required for anesthesia to begin could be prolonged significantly. To test the validity of this assumption, the following experiment was carried out.

Procedure:- Starved rats were given 1 c.c. of 50 percent glucose solution per 100 Gm. of rat. From Table 29, it can be seen that the blood glucose level should rise to about 400 mg. per 100 c.c., in ten minutes. Ten minutes after the glucose injection, the rats were given 3 mg. of pentobarbital per 100 Gm. of rat, intraperitoneally, and the time required for anesthesia to occur was recorded.

Results:- The results obtained in this experiment are shown in Table 36.

Table 36

The Influence of High Blood Glucose Levels

Upon Anesthesia Induction Time.

<u>Number and Sex</u>	<u>Induction Time</u>
1 F.	6 minutes
2 F.	5 "
3 F.	6 "
4 F.	4 "
5 F.	5 "
6 F.	6 "
7 F.	5 "
8 F.	5 "
9 F.	4 "
10 F.	7 "
11 F.	5 "
12 F.	5 "
Mean	5.3 "
S.E.	0.25"

THE EFFECT OF GLUCOSE UPON THE UPTAKE OF
PENTOBARBITAL BY LIVER HOMOGENATE.

EXPERIMENT 21

Figures 5 and 6 indicate a relationship between glucose utilization and pentobarbital uptake by the liver. This relationship warranted closer investigation, and led to the following experiment.

Procedure:- (i) Starved rats were killed by a blow on the head, and the livers removed and sliced.

(ii) Ten grams of liver slices were added to 40 c.c. of

0.9 percent sodium chloride solution in a Waring Blendor, and homogenized for two minutes.

- (iii) The volume was made up to 50 c.c.
- (iv) A 30 c.c. aliquot of the homogenate was placed in a beaker, and 6 c.c. of a solution containing 60 mg. of pentobarbital in each c.c. were added, and the solutions mixed thoroughly.
- (v) The beaker was covered, placed in an incubator, and incubated for thirty minutes at 37°C. The beaker was shaken occasionally during the period of incubation to ensure maximum reaction.
- (vi) After thirty minutes of incubation, the beaker was removed from the incubator, and 24 c.c. of 0.9 percent sodium chloride were added.
- (vii) The mixture was again incubated at 37°C., this time for a period of fifteen minutes.
- (viii) The contents of the beaker were filtered through Celite, and the volume of filtrate measured.
- (ix) Samples, each of 0.1 c.c. volume, were taken for glucose content determination.
- (x) The filtrate was injected into starved rats in a dosage of 0.75 c.c. per 100 Gm. of rat, and both the time required for anesthesia to begin, and the sleeping times, were recorded.

The experiment was then repeated, but, this time, a solution containing 125 mg. of glucose per c.c. was added

instead of 0.9 percent sodium chloride, prior to the second incubation period.

Results:- Results are given in Tables 37 and 38.

Table 37

Induction Time, and Sleeping Time, in Rats, following Injection of Pentobarbital Incubated with Liver Homogenate

Number and Sex	Induction Time	Sleeping Time
1 F.	11 minutes	63 minutes
2 F.	8 "	79 "
3 F.	7 "	80 "
4 F.	8 "	76 "
5 F.	6 "	76 "
6 F.	6 "	163 "
7 F.	4 "	152 "
8 F.	4 "	110 "
9 F.	4 "	123 "
10 F.	6 "	141 "
11 F.	5 "	148 "
12 F.	5 "	157 "
13 F.	5 "	156 "
14 F.	4 "	126 "
15 F.	4 "	163 "
Mean	5.8 "	121 "
S.E.	0.53 "	9.7 "
16 M.	7 "	25 "
17 M.	6 "	27 "
18 M.	6 "	25 "
19 M.	6 "	33 "
20 M.	8 "	30 "
21 M.	4 "	80 "
22 M.	6 "	28 "
23 M.	4 "	60 "
24 M.	5 "	75 "
25 M.	5 "	50 "
26 M.	4 "	48 "
27 M.	4 "	71 "
28 M.	5 "	69 "
29 M.	4 "	44 "
30 M.	5 "	41 "
Mean	5.1 "	47 "
S.E.	0.32 "	5.0 "

Table 38

Induction Time, and Sleeping Time, in
Rats, following Injection of Pentobarbital
Incubated with Liver Homogenate and Glucose

Number and Sex	Induction Time		Sleeping Time	
1 F.	10	minutes	85	minutes
2 F.	8	"	100	"
3 F.	5	"	60	"
4 F.	8	"	75	"
5 F.	8	"	93	"
6 F.	5	"	87	"
7 F.	10	"	72	"
8 F.	6	"	152	"
9 F.	5	"	157	"
10 F.	5	"	144	"
11 F.	4	"	182	"
12 F.	3	"	156	"
13 F.	4	"	186	"
14 F.	5	"	191	"
15 F.	6	"	167	"
Mean	6.1	"	127	"
S.E.	0.55	"	13.9	"
16 M.	10	"	24	"
17 M.	8	"	28	"
18 M.	7	"	42	"
19 M.	10	"	35	"
20 M.	5	"	40	"
21 M.	4	"	74	"
22 M.	3	"	98	"
23 M.	5	"	48	"
24 M.	4	"	71	"
25 M.	4	"	73	"
26 M.	3	"	33	"
27 M.	5	"	48	"
28 M.	4	"	82	"
29 M.	4	"	68	"
30 M.	4	"	47	"
Mean	5.3	"	54	"
S.E.	0.61	"	5.8	"

The uptake of glucose by the liver which was incubated with pentobarbital and glucose was 90 mg. of glucose per Gm. of liver.

Statistical evaluation of the results shown in Tables 37 and 38 yielded the following values for t .:

Induction time of female rats, $t = 0.623$

Induction time of male rats, $t = 0.129$

Sleeping time of female rats, $t = 0.544$

Sleeping time of male rats, $t = 1.121$

Hence the differences in neither induction time, nor in sleeping time, following incubated pentobarbital, and incubated pentobarbital and glucose, can be considered significant. The slight differences could have arisen purely by chance.

However, the results in Figures 5 and 6 showed the greatest differences in pentobarbital levels occurred at the ten minute points. In the experiment described here, incubation was continued for a period of forty-five minutes, by which time the pentobarbital differences in the liver were not so great. In addition, it has not been possible to adjust pentobarbital dosages to yield constant sleeping times in rats, that is, the sleeping times shown in Tables 37 and 38 might very well be somewhat different at another time, following the same dose of the drug.

Because of these factors, it was decided to incubate the mixtures only for fifteen minutes henceforth. To obtain

greater accuracy, pentobarbital levels were to be determined by Goldbaum's assay procedure (64), rather than by tests on rats. This was carried out in the next experiment.

EXPERIMENT 22

Procedure:- (i) Starved rats were killed by a blow on the head, and the livers removed and sliced.

(ii) Ten grams of liver slices were added to 40 c.c. of 0.9 percent sodium chloride solution in a Waring Blendor, and homogenized two minutes.

(iii) The volume was made up to 50 c.c.

(iv) A 30 c.c. aliquot of liver homogenate was placed in a beaker, and 3 c.c. of a solution containing 60 mg. of pentobarbital per c.c. were added. The total volume was then made up to 60 c.c. with sodium chloride solution.

(v) The beaker was covered, placed in an incubator, and incubated for fifteen minutes at 37°C.

(vi) Following incubation, the mixture was filtered through Celite, and the volume of filtrate was recorded.

(vii) Aliquots, each of 0.1 c.c. volume, were used for glucose content determination.

(viii) Five cubic centimetre portions were taken and assayed for pentobarbital content.

(ix) Glucose and pentobarbital values were corrected

to show the amount of each per Gm. of liver.

The experiment was then repeated, but 27 c.c. of a 20 percent glucose solution were added to the liver homogenate and pentobarbital prior to incubation. Glucose and pentobarbital determinations were made as before.

Table 39

Pentobarbital Uptake by the Liver, During
Incubation for Fifteen Minutes

<u>Number</u>	<u>Pentobarbital Uptake</u> <u>per Gm. of Liver</u>
1	3.99 mg.
2	1.33 "
3	0.24 "
4	1.64 "
5	1.54 "
6	0.73 "
7	1.17 "
8	1.59 "
Mean	1.53 "
S.E.	0.39 "

Table 40

Effect of Glucose Upon Pentobarbital Uptake
During Incubation for Fifteen Minutes

Number	Pentobarbital Uptake per Gm. of Liver
1	2.22 mg.
2	1.02 "
3	0.58 "
4	0.53 "
5	0.36 "
6	1.02 "
7	0.17 "
8	0.36 "
Mean	0.78 "
S.E.	0.24 "

The glucose uptake was 122.7 mg. per Gm. of liver.

Here, $t = 2.744$, indicating that the difference in pentobarbital uptake is significant.

DISCUSSION

There can be little doubt that glucose exerts a potentiating effect upon pentobarbital anesthesia, in rats. Despite the failure of some workers to produce the "glucose effect", the results of experiments 1 and 2 provide ample evidence of the effect. On the basis of this evidence, rats are suitable experimental animals for the study of barbiturate potentiation.

The mechanism by which the potentiation is brought about is not immediately evident. In the introduction, a number of suggested mechanisms have been listed. Some of these now seem to be questionable. For example, Lamson (56) suggested that all the glucose metabolites are potentiating agents of pentobarbital, and that they all act through glucose. The inference would seem to be that all of the metabolites are able to increase blood glucose sufficiently to bring about potentiation. The studies on a number of metabolites made in experiment 3 indicate that all of them do, indeed, cause an increase in the level of blood sugar. However, none of them cause an increase of more than 16.5 percent. The injection of 50 percent glucose into rabbits, on the other hand, evokes a mean increase of over 37 percent, and a maximum increase of over 46 percent, in the level of blood glucose. In previous studies (20), it was shown that identical glucose injections, in rabbits, caused a return to anesthesia in only 50 percent of the

animals injected. These results indicate that an increase in blood glucose of 37 percent, or more, is necessary to cause potentiation in rabbits. None of the metabolites was able to cause an increase of this magnitude, so it is questionable whether they could potentiate by this mechanism. In addition, in order to produce a substantial increase in blood glucose, the glucose metabolites would probably have to reverse the Kreb's cycle. Under suitable conditions, such a reversion is possible, but does not occur readily. This is particularly true in the case of the conversion of pyruvate to acetate, which is a difficult reaction to reverse.

The results of subsequent tests on the glucose metabolites increase the doubt as to their potentiating ability. Regardless of concentration or dose, a return to anesthesia was not evident in any of the animals. It was possible, by using graded doses, to produce results ranging from no effect to death. In those animals effected by the injection of the salts, the symptoms observed were those of alkalosis. In view of the report by Sollmann (59), and the observations made in the course of this study, it appears much more likely that the sodium salts of the glucose metabolites produce alkalosis, accompanied by general depression and malaise, rather than a return to anesthesia.

The fact that the pH of the blood remains relatively

constant does not rule out the presence of alkalosis. Best and Taylor (65) state that the condition may or may not be associated with a rise in blood pH. They discuss four situations giving rise to alkalosis, yet in two of these there is not a pH change, and the latter are referred to as "compensated alkalosis". The mechanism by which the symptoms occur is also discussed by Best and Taylor (66). The excess sodium results in a decrease in ionic serum calcium, without changing the total serum calcium level. Tetany results from the decrease in ionic serum calcium. An upset in the acid-base balance of the body is accompanied by diuresis, a principle which is utilized therapeutically when increased diuresis is desired. Sollmann (67) states that alkalosis causes the hemoglobin to hold its oxygen more firmly, resulting in anoxia and cyanosis of the tissues. He also states that compensation of alkalosis is fairly efficient, being accomplished by the excretion of alkaline urine, increasing the ratio of Na_2HPO_4 to NaH_2PO_4 . Hence it is quite possible to have alkalosis, and its attendant symptoms of cyanosis, tremors, convulsions, and excessive urination, with little or no change in the pH of the blood.

Unless subsequent investigations provide more conclusive evidence, it must be assumed that the glucose metabolites probably do not potentiate pentobarbital anesthesia.

The opinion expressed by Krantz and Fassel (35) that potentiation of barbiturates is a property of substances of high osmotic activity, requires elaboration. They reached this conclusion following experiments using an organic iodide, and sodium iodide, as potentiating agents for phenobarbital. The intraperitoneal injection of these substances, according to their report, causes a withdrawal of fluids from the blood into the peritoneal cavity. As a result, there is an increase in concentration of the blood, with an accompanying increase in the concentration of anesthetic in the blood. The potentiation shown by glucose cannot be explained on this basis. While the intraperitoneal injection of glucose does result in potentiation of anesthesia, the same result is produced by the intravenous injection of solutions of glucose. Following the intravenous injection of such a highly hypertonic solution of glucose, fluids would be drawn from the tissues into the bloodstream, resulting in dilution of the blood, and a decrease in the blood content of the anesthetic agent.

It appears that a closer study of the involvement of the liver in barbiturate metabolism is more likely to indicate the mechanism involved in the "glucose effect".

It now seems quite evident that pentobarbital and similar barbiturates are detoxified by the liver. In addition, there is ample evidence to indicate that the

process of detoxification is carried out by oxidation of the barbiturate. Brodie and his associates (68) isolated a degradation product of Pentothal in the urine. The compound was physiologically inert. On the basis of spectrophotometric and infra-red studies, determination of molecular weight by potentiometric titration, and analysis of its composition, they concluded that this metabolite of Pentothal was changed from the natural compound only in that the terminal carbon in one of the side chains was replaced by a carboxyl group. In short, they feel that Pentothal is detoxified by oxidation in the side chain. Similar conclusions were reached by Maynert and Van Dyke (69). They reported detoxification of pentobarbital, containing N¹⁵, to be brought about by oxidation in the side chain. However, it is their opinion that oxidation occurs at the beta carbon of the 1-methylbutyl group, resulting in the addition of an hydroxyl group at this position. It should be pointed out here that this metabolite apparently does not interfere with barbiturate determinations made by Goldbaum's technique. Mark et al (70) reported that their method of determining pentobarbital was not invalidated by the presence of the metabolite, and it was learned that their method is essentially the same as Goldbaum's (71).

Williams (72), in a recent publication concerning drug metabolism, discusses the observations of both Brodie

and his group, and of Maynert and Van Dyke. He concurs with their conclusion that barbiturates of the pentobarbital type are detoxified by oxidation, but feels that this is only one step in the processes involved in detoxification. It is his opinion that the drugs are broken down still further, and are eventually degraded to urea, ammonia, and carbon dioxide.

This information led to another possible explanation of the mechanism involved in barbiturate potentiation. Since pentobarbital is destroyed in the body by oxidation, would it not be possible for glucose, a reducing sugar, to reduce it back to the active form? A number of other reducing substances have also been reported to potentiate the barbiturates (38, 39, 61). Consultation with several qualified chemists led to the belief that this was not a likely explanation. All were very definite in expressing the view that it was very improbable, if not impossible, for the reducing substances mentioned to reduce a carboxyl group. Apparently such a group is reduced only after quite drastic treatment; treatment not possible in living cells. Unless some enzyme system, as yet unknown, is able to cause such a reduction, it does not seem that reactivation of the oxidized barbiturate is the answer being sought.

It has been suggested by a number of workers that pH changes are involved in barbiturate anesthesia (49, 50).

Of the two investigators quoted, one of them (De Boer), believes that acidosis increases sleeping times in thio-pental anesthesia. On the other hand, Whitehead and his group are of the opinion that respiratory acidosis is responsible for a decrease in the plasma level of thio-pental. The administration of the sodium salts of numerous glucose metabolites produced all the symptoms of alkalosis, without causing a return to anesthesia, so it seems improbable that the potentiation is due to alkalosis. Further, glucose potentiation cannot be explained readily on this basis. Even though the ingestion of a carbohydrate meal results in an increase in the alkali reserve of the body, there is little reason to expect that result from the injection of glucose. The increase in alkali reserve following a meal is due to a relative depletion of hydrogen ion from the blood brought about by the secretion of hydrochloric acid into the stomach. However, following injection, there is no such action, so the alkali reserve should be comparatively unchanged. In addition, the blood buffers are usually quite efficient, and should be able to compensate for any slight change occurring.

There is considerable indication that the liver, in addition to being the site of barbiturate detoxification, is also the locale of the potentiating mechanism. Results obtained in the studies of the effect of glucose upon pentobarbital levels in the blood, brain, and liver,

indicate that this might be true. The incubation studies on liver homogenate tend to show that the liver does not take up pentobarbital as rapidly when glucose is present in excess, as it does when glucose is absent. This was also indicated by the fact that, in experiments 16, 17 and 18, it was found that liver pentobarbital levels were lower when glucose was injected into rats along with the barbiturate. However, the level of liver glycogen increased. Failure of the liver to remove the barbiturate from the blood would naturally result in higher blood levels, which, in turn, would be reflected in the brain. The graphs in Figures 3 to 7 indicate that this is so.

The hypothesis that liver glycogen increase is accompanied by a decreased pentobarbital destruction is not without foundation. Booker (73) found that Pentothal anesthesia severely taxed liver function, causing an inability to polymerize glucose in starved animals. He expressed the view that Pentothal probably disrupts carbohydrate enzyme systems in the liver, and markedly decreases liver glycogen. Kun and Abood (74) found that phenobarbital inhibits glycogen formation by 58 percent, due to the ability of the drug to inhibit phosphorylation. Ratcliff et al (26) observed increased glycogenolysis during Pentothal anesthesia. Booker and his associates (27) share the opinion of Kun and Abood. They believe that Pentothal "embarrasses" the liver, disrupting enzyme systems, with

resulting glycogen depletion. Gould and Shideman (75) made the interesting observation that sodium cyanide completely inhibits the disappearance of thiopental from a rat liver preparation. In addition, they found that the presence of high energy phosphate bound compounds was required for the liver to be able to detoxify thiopental, because the omission of adenosine triphosphate from the system diminished activity by 25 percent. Addition of 2,4-dinitrophenol inhibits thiopental destruction by 30 percent. In the same concentration as that used, 2,4-dinitrophenol prevents phosphorylation mechanisms. It is known that glucose must be phosphorylated prior to polymerization to glycogen. From the work of Gould and Shideman, it appears that phosphorylating enzymes are involved in thiopental detoxification. A logical conclusion would seem to be that, at some stage in their respective processes, glycogen formation and barbiturate destruction involve a common enzyme system.

In the presence of excessive amounts of glucose, the body restores normality, in part, by the process of glycogenesis. It seems logical to propose that, upon the injection of highly concentrated glucose solutions, such as those employed in these studies, increased glycogenesis surely follows. This glycogenesis would necessitate phosphorylation, thus hindering barbiturate detoxification. On the basis of available evidence, this seems to be the

most satisfactory explanation of the potentiating mechanism of glucose.

There is, however, another observation, which requires additional explanation. It was found that, at the onset of anesthesia, the blood level of pentobarbital was higher when glucose was administered simultaneously with the anesthetic, than when the anesthetic agent was given alone. However, it is known that glucose is the required metabolite of nervous tissue (76). Following the injection of glucose in large quantity, blood sugar rises, and, most likely the amount of glucose entering the brain also rises considerably. This would result in accelerated metabolism of nervous tissue. It appears not illogical that a greater amount of barbiturate would be required to inhibit this accelerated metabolism, and cause depression of the brain to the point of anesthesia. As a result, the level of pentobarbital could be expected to be higher before being able to induce anesthesia.

This situation also seems to obtain at recovery. From Figure 7 it can be seen that the level of pentobarbital in the brain at recovery from anesthesia is higher in those animals receiving glucose than in those deprived of glucose.

Even though the barbiturates are distributed to all parts of the body, it must be assumed that the degree of anesthesia is dependent upon the level of the drug in the brain. Any procedure which causes prolongation of elevated

blood levels would ultimately result in an increased level in the brain. That, apparently, is one result of glucose administration.

An interesting, but by no means original, observation made during the course of this investigation, is worthy of mention. That is the fact that female animals sleep much longer than do males from comparable doses of pentobarbital. This observation has been reported previously by a number of workers (20, 77). Adams (19) reported that the rat is the only experimental animal in which this difference in response between the sexes occurs. However, others have observed it in rabbits (20). In addition, Adams expresses the view that this difference in response depends upon the drug, and occurs where there is one short, and one long, straight or forked, chain, a cyclohexenyl or a methylated nitrogen group. In all cases, the male is more resistant than the female.

The series of experiments described in this dissertation provide a plausible, and possible, explanation of the mechanism involved in glucose potentiation of pentobarbital anesthesia. They do not, however, end the possibilities for study. On the contrary, new and interesting problems have arisen during the course of the investigation. Surely it would be of great interest to study the effects of various isomers of glucose as possible potentiating agents. The need for further study of the

processes involved in barbiturate metabolism also became evident. This study could probably be carried out by synthesizing the suggested oxidation products of pentobarbital. These could then be compared chemically and physiologically with the barbiturate metabolite isolated from the urine.

It is hoped that the possibilities suggested in this discussion will stimulate interest in their investigation. If that result is achieved, the time and effort devoted to these studies will have been well spent.

CONCLUSIONS

1. Glucose prolongs the sleeping time induced by pentobarbital, in rats.
2. Rats, upon recovery from pentobarbital anesthesia, are returned to sleep by the injection of concentrated solutions of glucose.
3. Sodium citrate, sodium lactate, sodium acetate, sodium succinate, and sodium pyruvate, cause only a slight increase in the level of blood glucose, in rabbits.
4. These same salts, in suitable dosage, produce the symptoms of alkalosis, but do not return rats to anesthesia.
5. Phosphate buffers are incapable of preventing the symptoms of alkalosis resulting from the administration of sodium lactate.
6. The blood level of pentobarbital becomes higher if glucose is given with the drug, than if pentobarbital is given alone.
7. The liver does not remove pentobarbital from the blood as rapidly as normal, if glucose is present in excess.
8. The level of pentobarbital in the brain increases slightly above normal levels if glucose is given along with the barbiturate.
9. The male rat is more resistant to pentobarbital than is the female.

10. It is suggested that the liver is unable to polymerize glycogen and detoxify pentobarbital simultaneously as rapidly as it can do either separately.
11. Pentobarbital injection results in an immediate slight rise in blood sugar level, followed by a gradual decrease.
12. Glycogenolysis, followed by glycogenesis, occur when pentobarbital is injected into rats.
13. In the presence of excess glucose, there is an apparent need for a higher pentobarbital level in the blood to produce anesthesia, than that required in the absence of glucose.
14. If glucose is present in excess, rats recover from anesthesia at a higher brain level of pentobarbital than that normally present at recovery.

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AUTOBIOGRAPHY

I, John Francis Bester, was born in Cargill, Ontario, Canada, April 17, 1922. I received my primary school education in the public schools of Cargill, Ontario, and my secondary school education in the Walkerton, Ontario, high school. Upon graduation from high school, I began apprenticeship in Pharmacy, but this phase of my training was interrupted by my entry into the Royal Canadian Air Force. Following service overseas, and attaining the rank of Flying Officer, I was honorably discharged in 1945, and began my undergraduate training at the University of Saskatchewan, College of Pharmacy, the same year. While studying at the University of Saskatchewan, I was the recipient of the Charles E. Frosst Proficiency Award in 1946, and again in 1948, and, in 1949, received the degree Bachelor of Science in Pharmacy, from that institution. From the Ohio State University, I received the degree Master of Science, in 1950. In 1950, I was appointed a Fellow of the American Foundation for Pharmaceutical Education, a position I held while completing the requirements for the degree Doctor of Philosophy.