NOTE TO USERS

This reproduction is the best copy available.

UNIVERSITY OF CINCINNATI

May 15, 1939.

I hereby recommend that the thesis prepared under my supervision by Nilkanth Manohar Phatak

entitled Carbohydrate Metabolism in Ether Anesthesia.

I. Fate of Injected d-Lactic Acid in the Dog, the Rabbit, and the Rat.

be accepted as fulfilling this part of the requirements for the degree of DOCTOR OF PHILOSOPHY

Approved by:

Albert P. Mathews

CARBOHYDRATE METABOLISM IN ETHER ANESTHESIA

I. FATE OF INJECTED D-LACTIC ACID IN THE DOG, THE RABBIT, AND THE RAT

A dissertation submitted to the

Graduate School

of the University of Cincinnati

in partial fulfillment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

1939

by

Nilkanth Manohar Phatak

B. S. North Pacific College 1924

A. B. University of California 1931

M. S. University of California 1935

ACKNOWLEDGEMENT

Dr. P. K. Knoefel suggested the problem. Some of the early work was done with Mr. B. E. Abreu and Dr. G. A. Emerson at the pharmacology laboratory of West Virginia University. Grateful acknowledgement is also due to my teachers and fellow workers of the Biochemistry department, University of Cincinnati, for without their cooperation and helpful guidance this work would not have been possible.

TABLE OF CONTENTS

INTRODUCTION

Since the successful demonstration of ether anesthesia by Morton in 1846, many new anesthetic agents have come into use. The search for such continues, not because the older ones are less powerful or inadequate but because their administration produces many undesirable functional and metabolic disturbances.

Experimental work undertaken to elucidate the mechanism of the undesirable side-effects of anesthetic agents is therefore very essential in advancing our understanding of the fundamental basis of the physiology of anesthesia.

One of the undesirable effects of ether anesthesia thus investigated is the profound disturbance of carbohydrate metabloism resulting from its use. Ether causes a rise of blood sugar, blood lactic acid and blood acetone bodies. Knoefel (1936) advanced the so-called "adrenin-release" theory relating ether anesthesia and hyperglycemia, from a critical appraisal of the then available literature. He pointed out that an anesthetic agent like ether, brings about a release of adrenin from the adrenal glands by sympathetic stimulation; and that this released adrenin in turn accelerates the breakdown of liver glycogen to blood glucose.

Evidence in support of Knoefel's hypothesis was obtained by Emerson (1935, 1936, 1938), and by Emerson and co-workers (1937).

Since ether anesthesia produces an increase of blood lactic acid, a further increase of it in the blood stream by intravenous injection of sodium d-lactate might indicate the nature and scope of the mechanisms involved in its utilization. The present investigation studies the fate of injected sodium d-lactate into experimental animals such as the dog, the rabbit and the rat, under normal conditions as well as when anesthetized with ether.

HISTORICAL

General Metabolic Disturbances Produced by Ether Anesthesia

The production of the state of anesthesia is associated with the loss of pain perception and the loss of consciousness. The inhibition of the controlling influence of the central nervous system over normal body functions results in a variety of metabolic disturbances, which are reflected by the marked variations in the composition and properties of the blood.

Among the metabolic disturbances produced by anesthetic agents like ether or chloroform can be mentioned a rise of blood fat; noticed by Bloor (1914). He found that blood fat level rose about 40 to 100 percent during ether anesthesia in the dog. This rise was remarkably high during the first hour.

An increased blood cholesterol was reported by Mahler (1926), while investigating the relationship between the lipemia and the increase of blood sugar, noticed after ether anesthesia in man. The rise of "total blood cholesterol" was roughly proportional to the rise of blood glucose. If at a proper interval before anesthesia, insulin was administered to the patients, these changes in blood cholesterol and blood glucose were prevented. This was interpreted as indicating a partial suppression of the internal secretion of the pancreas by ether.

Bolliger (1926) noticed a depression of the blood inorganic phosphates of the dog during uncomplicated anesthesia. Drugs like morphine or atropine injected previous to the induction of anesthesia masked the effects of the anesthetic agents. These so-called pre-medication drugs

were shown to be capable of producing changes in the blood inorganic phosphates.

Magee, Anderson and Glennie (1928) reported an increase of the blood inorganic phosphates in rabbits under ether anesthesia. Marenzi and Gerschman (1933, 1934) also reported an increase of the phosphates in the blood plasma of etherized dogs.

The same authors (1933) reported a fall in the blood potassium of the dogs under ether anesthesia. Robbins and Pratt (1936) confirmed these findings. In dogs deeply anesthetized for half an hour with ether they noticed a fall in serum potassium of about 15% below the normal. This decreased level was maintained during the first 30 minutes of the recovery period, but at the end of four to five hours of recovery the serum potassium level was back to normal. The same type of change was found in guinea pig blood.

Van Slyke, Austin and Cullen (1922) showed that ether anesthesia in the dog produces a disturbance of the blood acid-base balance tending towards an acidemia. They noticed a consistent fall of blood pH shortly after the anesthesia was started and the alkaline reserve also continued falling. They suggested that this true acidosis may be due either to introduction of acid into the blood or to withdrawal of base from it. Leake, Leake, and Koehler (1923) confirmed these findings in the dog. They were unable to observe an increase in the blood acetone bodies to account for the early ether acidosis. But in the light of the findings by Stehle, Bourne and Barbour (1922) that sodium and potassium excretion was diminished during ether anesthesia but was increased remarkably during

the postanesthetic period, they concluded that the alternative explanation of Van Slyke et al (1921) i.e., withdrawal of base from blood, was the proper explanation.

Barbour and Bourne (1923) studied the influence of ether on the heat regulation and water exchange in the dog. They confirmed earlier observations of Hamburger and Ewing (1908) that ether anesthesia produces a hemoconcentration. Under ether anesthesia the dog is unable to regulate its body temperature unless the environmental temperature happens to be near 31° C. At this environmental temperature the dog's temperature remains constant.

Lastly the hyperglycemic effect of ether anesthesia has attracted the attention of numerous workers and a great deal of experimental work has been devoted to elucidation of its mechanism.

As far back as 1853 Reynoso, among earlier workers, is reported to have observed glycosuria following etherization. Hawk (1903) investigated the influence of ether anesthesia on the dog. He observed an unfailing glycosuria after ether anesthesia of from one-half to four and a half hours. Incidentally he noticed some diuresis and a small increase in the nitrogen output in the urine. Seelig (1905) is quoted as having reported ether glycosuria during anesthesia. MacLeod and Pearce (1915) reported a fall in the blood sugar level during the first half hour of etherization. Ross and McGuigan (1915) and many other observers such as Mahler (1926), MacKay (1928), Minnitt (1932) and Hospers (1933), all recorded considerable increases in the blood glucose level caused by ether anesthesia.

Mechanism of Ether Hyperglycemia

A number of mechanisms were suggested to explain the hyperglycemic effect of anesthetic agents like ether, chloroform and others. They might exert a direct enfluence on the liver or might affect it indirectly thru the general stimulation associated with the excitement stage of anesthesia. They might produce hyperglycemia as a result of asphyxia and passive congestion accompanying anesthetization; or perhaps by the changes in the H-ion concentration of the blood passing thru the liver. A reflex secretion of epinephrin produced by the state of anesthesia could also bring about an increased breakdown of liver glycogen to blood glucose. Thus the exact mechanism of the hyperglycemia was not definitely known.

The regulation of blood glucose level is one of the important functions of the liver and naturally attention was directed to this organ and its relationship to the hyperglycemia of ether.

King et al. (1911) noticed a hyperglycemia and glycosuria in the dog following etherization. The reducing substance in the urine was identified as glucose. The source of this increased blood glucose was shown to be the liver; for when the liver was removed from circulation by Eck fistula, hyperglycemia and glycosuria did not appear on etherization.

Shaffer (1914) attributed the hyperglycemic effect of ether to the general excitement and partial asphyxia commonly produced in the process of etherization. Later Zwemer and Newton (1928) successfully demonstrated that the denervated adrenal glands respond directly to severe asphyxia.

In preparations with denervated adrenals they induced asphyxia by compressing the aorta near the diaphragm, for 10 seconds. The presence of the discharged adrenin was demonstrated by a large increase in the rate of the denervated heart in surviving animals.

Keeton and Ross (1919) had shown that ether hyperglycemia was prevented to a great extent by inactivating the adrenals. Ether hyperglycemia in the dog is persistant, but if the splanchnic nerves are severed it becomes only transient. If the liver is denervated and the nerve supply to the adrenals is kept intact, ether produces a persistent hyperglycemia, but of a lower grade than in the normal animal. The mechanism of the hyperglycemia produced by ether and that produced by electrical stimulation of the splanchnic nerves was claimed to be identical. That stimuli such as fear, excitement, cold, asphyxia, loss of blood and the anesthetic agents are quite effective in raising the blood glucose level by increasing the blood adrenin, is evident from the remarkable work of Cannon and his co-workers. Bulatao and Cannon (1925) had, however, indicated that adrenin, rather than the sympathetic impulses, forms the potent factor in the liberation of glucose from the liver.

Phillips and Freeman (1933) worked with sympathectomized or adrenalectomized cats and found that the rise in blood glucose after ether was reduced considerably by inactivation of the adrenals and by cutting the nerve supply to the liver. They also found that in the completely sympathectomized cat, a rise in blood glucose persisted after etherization. "Sympathin" production was, therefore, not responsible for this effect.

Relation of Epinephrin to Metabolic Disturbances Produced by Etherization

Following an injection of ephinephrin there is observed a rise in blood glucose, a rise in blood lactic acid, an increased excretion of ketone bodies in the urine, a fall in the blood alkaline reserve and an immediate fall in liver glycogen (Cori 1931).

The same effects are known to be produced by anesthetic agents like ether and chloroform. That ether may bring about a release of adrenin from the adrenal glands into the blood stream at an augmented rate, was suggested by Elliott (1912)

Reid (1932) used a triple experimental procedure to evaluate the relation of the adrenals to the anesthetic hyperglycemia. He experimented with normal rabbits, with rabbits in which the adrenal gland on one side was removed and a third group in which the remaining gland was removed or inactivated at a second operation. Thus he found that during a surgical anesthesia produced by ether for half an hour, the "glycemic" response was greatest in normal rabbits, less marked in singly-operated group and least in the doubly-operated group. He further corroborated the findings of Evans and his co-workers (1931) that amytal prevented the marked hyperglycemia of ether. Since the barbiturates are supposed to act primarily on the bulbar centers, he attributed this hyperglycemia-preventing effect of amytal as due to the depression of the bulbar portion, controlling the adrenal medullary secretion.

The soundness of such an "adrenin-release" hypothesis, explaining

a major part of ether hyperglycemia is amply supported by the extensive researches of Evans and his co-workers. Particularly the works of Brown and Evans (1933); Vidal (1933); and Banerji and Reid (1933) show conclusively that inactivation of the adrenals greatly reduces the fall in the liver glycogen and prevents the rise of blood glucose produced by etherization.

Knoefel's Hypothesis

Knoefel (1936) gave a critical review of the role of the sympathetic nervous system in anesthesia. He presented available evidence pointing out the parallelism between the cardiac, vascular and intestinal effects of anesthesia and epinephrin. He stated that "many of the undesirable disturbances of function present in anesthesia with ether and chloroform appear to be due to over activity of the sympathetic nervous system including the hypersecretion of adrenin. The effects are most marked on carbohydrate metabolism and the function of the cardiovascular and digestive systems".

Experimental Work in Support of Knoefel's Hypothesis

Emerson and co-workers, (1937) have noted further analogies from a biochemical biewpoint. Ether and epinephrin produce the same qualitative changes in many blood constituents such as lactic acid, glucose, cholesterol, phosphates, iodine and blood gases. Both produce ketonuria,

reduce the alkaline reserve of the blood and decrease its pH. Direct experimental data regarding the effect of epinephrin on blood pH is however not available. Both decrease the circulating blood volume and shorten the coagulation time.

Both agents reduce the glycogen content of the liver. Further similarities of effect on a variety of other body functions are also known.

Emerson (1936) studied the effect of ether on intestinal motility of the rat. He fed a group of rats an emulsion of charcoal in gum acacia. He then etherized part of that group for one hour. At the end of this period he sacrificed all the rats and measured the progress of the charcoal meal in their intestinal tract between the pylorus and anus. He found that during the period of etherization there was almost complete inhitition of intestinal motility as compared with the control group. A rapid recovery was observed during the first hour after termination of of anesthesia. Intestinal activity reached approximately normal level by the end of the second hour of recovery. Epinephrin also causes intestinal stasis.

Emerson (1935) has reported the effects of various anesthetic agents on the "autoxidation" rate of surviving brain tissue of the rat. For comparative purposes he used a group of rats receiving subcutaneously 0.5 mg./Kg. of epinephrine hydrochloride. Using Warburg manometric technic he found decreased "autoxidative" rates in brains of rats anesthetized with glycogenolytic narcotics such as ether or treated with epinephrine. Similar observations have been made by many others, and the theory that anesthetics check respiration has long been maintained.

Further evidence to substantiate the general concept that anesthetic agents like ether, mediate many biochemical processes thru stimulation of adrenin output, was obtained by Emerson and co-workers, (1937). They studied the ketonemic response and the hyperglycemia produced by ether and epinephrin in rabbits. They confirmed the usual hyperglycemic effect of etherization and of epinephrine injections. There was a latency in the rise of blood ketone bodies. Epinephrine produced ketonemia, reaching a peak between the eighth and twelfth hour after the injection. Ketonemia after etherization in also reported to appear some hours after the anesthesia (Killian, 1934), and to persist for a long time afterwards. (Emerson, 1935).

Finally Emerson (1938) procured direct evidence showing that after 30 minutes of deep ether anesthesia the adrenin content of the adrenal glands of the cat was significantly depleted. Premedication with barbiturates prevented this decrease. Further, anesthesia produced by another anesthetic agent for example divinyl oxide, showed no such effect.

Metabolism of Lactic Acid

Having established the general correctness of the hypothesis -- which states that practically all of the effects of ether anesthesia on carbohydrate metabolism are related to sympathetic stimulation and an increased adrenin output -- it was decided to investigate the effects of ether anesthesia on the intermediary carbohydrate metabolism, particularly the lactic acid phase of the Cori cycle.

Blood glucose level is a measure of the composite changes in the cycle of carbohydrate metabolism as postulated by Cori (1931). He graphically represented this cycle as follows:

Liver glycogen -----→ blood glucose -----→ muscle glycogen -----→ hexose phosphate -----→ blood lactic acid -----→ liver glycogen.

Lactic acid is an important link in this chain of events as the source of liver glycogen. Knoefel (1936) has stated that the reactions -- liver glycogen -----→ blood glucose, muscle glycogen -----→ hexose-phosphate, hexosephosphate -----→ blood lactic acid are accelerated; while the reactions -- blood glucose -----→ muscle glycogen, and blood lactic acid -----→ liver glycogen are inhibited in ether anesthesia. The accumulation of lactic acid in the blood under ether anesthesia is thus a natural consequence of the kinetic changes in this cycle.

Parnas (1912) studied the fate of injected lactic acid isomers in rabbits. He injected the optically active isomers subcutaneously into rabbits and searched for their appearance in the urine. The urines were

extracted with ether and the optically active forms were identified by their zinc salts. Thus after an injection of 4.5 grams of l-lactic acid 2.17 grams were recovered in the urine. After a similar amount of d-lactic acid injection, only 0.1 gram was found to be excreted. When he injected 9 grams of dl-lactic acid he was able to recover 0.2 gram of l-lactic acid and 0.3 gram of dl-lactic acid. The d- component was stated to have been almost completely burnt in the body; but the levo component was more completely oxidized when given in the recemic mixture than when injected by itself..

Meyerhof and Lohmann (1926) demonstrated that isolated hepatic tissue of the rat was able to synthesize carbohydrate from d-lactic acid but hardly any from l-lactic acid. Cori and Cori (1929) have also shown that sodium d-lactate fed or injected subcutaneously is completely utilized and retained as liver glycogen. They found that by injecting about 0.1 gm./Kg. of sodium-d-lactate per hour into rats no rise in blood lactic acid was produced and no appreciable amount was excreted in the urine. The same experiment repeated with sodium dl-lactate produced considerable urinary excretion of the injected lactate. In contrast to sodium d-lactate, it was noted that about 30 percent of the amount of injected sodium l-lactate was excreted in the urine and failed to give rise to liver glycogen. Janssen and Jost (1925) had pointed out that intravenous injection of sodium d-lactate and sodium dl-lactate into intact dogs fails to produce muscle glycogen. Since it disappears from the blood stream, and is not excreted by the kidney it was assumed to give rise to liver glycogen.

Hartmann and Senn (1932) investigated the fate of intravenously injected sodium dl-lactate in children. They injected intravenously 4 to 7 cc. of a molar solution per kilogram body weight. About 18 percent of the injected lactate appeared in the urine, the rest was utilized within two hours. They concluded that since the racemic compound is a mixture of both the d and l forms, about 80 percent of the d- form was converted into glycogen, while the l-lactate was oxidized. Recently Soffer and his co-workers (1937) studied the utilization of intravenously injected sodium d-lactate by normal persons. The quantities of sodium d-lactate injected ranged from 50 to 75 mg. per kilogram of body weight in a 14 percent solution. Blood glucose, carbon dioxide content of the serum and urinary lactic acid were determined for a period of 2 hours following the injection. Since injected sodium d-lactate disappeared rapidly from the blood stream reaching the control level within one-half hour, and since there was no excretion of lactic acid in the urine, they concluded that it must have been utilized as a source of liver glycogen. The same group of workers (1937) found a delayed disappearance of injected sodium d-lactate from the blood stream in patients with acute diffuse parenchymal injury of the liver. This they thought gave added, but indirect support to the concept that the lactic acid disappearing from the blood stream must be taken up by the liver for conversion to glycogen.

EXPERIMENTAL

(A) Preparation of d-lactic acid

Since there is such a marked difference between the end results or metabolic fate of the two optically active isomers of lactic acid and since it is d-lactic acid (sarcolactic acid) which represents the intermediary stage between the reaction liver glycogen ⇄ muscle glycogen, it was decided to prepare some d-lactic acid for use in this experimental work.

Difficulties were encountered in preparing sarcolactic acid by any of the accepted methods. The bacterial fermentation with Lactobacillus delbruckii, (a special strain from the Collection of Professor W. H. Peterson, University of Wisconsin, secured thru his generosity), was not quite successful. In spite of the special strain and all usual precautions against bacterial contamination, the fermentation product was optically inactive. Besides the yields were poor. Ether extraction of the liberated lactic acid after decomposition of the calcium lactate precipitated from the fermentation media, gave such scanty yields that it was not possible to determine the proportionality of the two optically active lactic acids in the optically inactive product obtained.

Resolution of the racemic lactic acid into its two optically active isomers with cinchonine, quinine, or morphine was not feasible as the processes are too time consuming and too expensive to enable the production of large amounts of d-lactic acid needed for animal experiments.

While working with these time consuming procedures some work was started using the racemic lactic acid (Merck, U.S.P.). However it became possible to secure enough of d-lactic acid for our experimental needs.+ This acid, obtained by fermentation methods on a large, commercial scale represented a 50% solution of lactic acid. Of this lactic acid 92 percent was d-lactic acid component. What other impurities were present, such as the amino acids and pigment substances is not known. The acid is a pale straw-yellow, clear liquid, with an acid fermentation odor. Lactic acid content by titration (U.S.P. method) or by a colorimetric procedure, as later used in the animal experiments, checked to give the figure of 50% $\pm$ 1. In 3 dogs this d-lactic acid was used while an attempt was made to further concentrate it by continuous isopropyl ether extraction at room temperature.

This extraction procedure is a modification of the method devised by Jenemann (1933 -- U.S. Patent No. 1,906,068 Lactic Acid). In this procedure the heavy lactic acid is introduced drop-wise into a large glass extraction cylinder, containing glass beads, from the top. Isopropyl ether is introduced in a slow, gentle flow from the bottom of the same extraction cylinder. The slowly rising isopropyl ether extracts the slowly falling lactic acid. The extracted lactic acid is drained off from the tap at the bottom of the extraction cylinder and reintroduced at the top for repeated extractions. The isopropyl ether, collecting at the top

---

+ Dr. Geo. T. Peckham, Jr. Research Supervisor, Clinton Company, Clinton, Iowa kindly supplied sufficient material for these experiments.

in the extraction vessels, above the glass beads, and enriched in lactic acid, is then made to pass thru another small, narrow bead tower from the bottom. This bead tower receives distilled water in drops from the top.

As the isopropyl ether rises in this bead tower the distilled water coming in contact with it on its way down takes up the extracted lactic acid from it due to the favorable partition coefficient of lactic acid in water as compared with isopropyl ether. Thus gradually lactic acid gets concentrated in the water layer at the bottom in the bead tower and can be further concentrated under vacuum. The exhausted isopropyl ether is again reintroduced at the bottom of the large extraction vessel as before.

The 50% d-lactic acid obtained from the commercial fermentation, was thus concentrated to a strength of 68-72%. The dextrorotatory component was identified by formation of its zinc salt as outlined in the procedure described by Phelphs and Palmer (1917). This concentrated d-lactic acid was used in all subsequent animal experiments.

Generally the d-lactic acid was titrated with 0.1 NaOH, using phenolphthalein as indicator, and its total strength calculated. Of this 92% was d-lactic acid. Before injecion 0.5 gm./Kg. of d-lactic acid, calculated as above, was neutralized with 20% NaOH to just short of a change in color to alkaline side of phenol red indicator. This slightly acid sodium d-lactate was then diluted to a convenient volume and injected into the experimental animals. For convenience of expression whenever d-lactic acid is said to have been injected it is to be understood that this almost neutralized acid is meant.

(B) Animal Experimental Work

In the present investigation the problem of the utilization of injected d-lactate by both normal and ether anesthetized animals is divided into two parts. The first deals with observations on the blood changes in 12 dogs and 21 rabbits after ether anesthesia, after ether anesthesia accompanied by d-lactate injection and after sodium d-lactate injection alone. The same dogs were used in following the blood changes under the three experimental conditions mentioned above. The rabbits were divided into three groups of six animals each; and separate groups were used for studying the effects of etherization, of lactate injection, and of etherization with lactate injection. All animals were fasted 24 hours before use. A uniform dose of 500 mg. per kilo was injected in all animals when necessary. Ether was administered by open drops method from a wire mask covered with gauze. When indicated etherization was maintained for one hour only. When lactate was injected in accompaniment with etherization, the injection was started within first two minutes of anesthesia and completed rapidly within five minutes. Blood chemical changes were followed for four to eight hours. Blood glucose was determined by Hagedorn-Jensen micro method (1923). Blood lactic acid by the method of Rappaport and Reifer (1937), which is a modified Mendel-Goldscheider procedure. Plasma inorganic phosphates were determined by the Bodansky modification (1932) of the Kuttner and Lichtenstein colorimetric procedure. Plasma carbon dioxide combining power was determined by the Van Slyke and Cullen method. (1917)

Results of these observations are given in tables 1 to 10.

The second part of the investigation deals with observations on liver glycogen in rats after etherization, after lactate injection, and after etherization with lactate injection. A total of 56 rats were used. Of these 36 were divided into four groups of 10, 10, 8 and 8 respectively. All rats were fasted 48 hours before use. Group I (10 rats) was used as normal control; group II (10rats) was simply anesthetized with ether. Group III (8 rats) was injected intravenously with 500 mg. per kilo of sodium d-lactate; and group IV (8 rats) received the same dose of d-lactate as group III just before etherization. An hour after the various treatments, the rats were quickly decapitated, their livers exposed and pieces of livers weighing approximately 0.3 to 0.5 gram were removed into tared centrifuge tubes containing 60% KOH. Liver glycogen in these was then determined following the method of Sahyun (1931, 1933).

The remaining 20 rats were similarly divided into four more groups of five each and treated exactly as the groups mentioned above except that instead of the intravenous injedtions these received sodium d-lactate intraperitoneally.

Results of these experiments are shown in tables 11, 12, and 13. For convenience the results of all experimental findings are averaged and shown in composite tables 14, 15, and 16. Fig. I and II are drawn from tables 14, and 15 respectively and show graphically the average trend of blood changes in the dogs and in the rabbits.

RESULTS.

A. Effects of d-lactate injection in dogs and rabbits. In the dogs blood glucose changes after lactate injection were variable. Sometimes the values decreased a little, sometimes they increased. On the whole the changes were not very marked or significant.

In the rabbits, taking the average values for the group of six, blood glucose showed a rise of 10 mg. per 100 cc. over the resting value of 108 mg. fifteen minutes from the lactate injection. Blood glucose then fell rapidly to 73 mg. per 100 cc. at the end of one hour, a drop of 35 mg. from the control. It then rose slowly, reaching normal level six hours after the lactate injection (see table 14).

In the dogs, with two exceptions, sodium d-lactate injection raised blood lactic acid from an average control of 17.9 mg. per 100 cc. to an average high of 63.8 mg. per 100 cc. in fifteen minutes. It then fell rapidly to normal level at 90 to 120 minutes, from the beginning of the experiment. Dog No. I gave the maximum value of 110 mg. per 100 cc., after lactate injection, while the minimum rise was noted in the case of dog No. 4 in which the blood lactic acid reached only 31 mg. %.

Urinary excretion of lactic acid following lactate injection was not studied as a rule. However, on two or three occasions, dogs were kept in metabolism cages during some experiments and pooled urine samples were collected up to four hours from the beginning of lactate injection. Lactic acid values in these specimens did not show any significant rise over the normal control value of 7 to 18 mg. per 100 cc.

In rabbits, taking averages for the group, blood lactate showed a peak of 71.4 mg. per 100 cc. at fifteen minutes after lactate injection.

The control was 21.3 mg. per 100 cc. at fifteen minutes after lactate injection. The drop following this, was gradual and blood lactic acid did not reach normal level till the end of the experiment (see table 15).

In dogs, simultaneously with the rise of blood lactic acid there was observed a fall of plasma inorganic phosphate after sodium d-lactate injection. This fall continued with slight fluctuations reaching a maximum average dip of 1.02 mg. below the control during the first and second hour after lactate injection. Plasma inorganic phosphate then returned to normal level or rose higher to the end of the experiment.

Plasma CO_2 combining power showed a consistant rise after the injection of sodium d-lactate. CO_2 rise in plasma was evident within 15 minutes. This rise then continued still higher or remained at a high plateau of 17 to 20 vols. % above the control level. Plasma CO_2 returned to normal six hours after lactate injection.

Plasma inorganic phosphate and plasma CO_2 combining power were not determined in the rabbits. However varying amounts of sodium d-lactate were injected into rabbits to determine the relative safety limits of such injections. Very large doses, for example 2 to 4 grams per kilo, produced a marked decrease of blood glucose in rabbits, often to convulsive levels; and as a result some of these animals died within 18 to 24 hours after lactate injection. Intravenously rabbits tolerate 500 to 600 mg. per kilo of sodium d-lactate without any harm (see table 10).

B. Effects of ether anesthesia in dogs and rabbits. Ether anesthesia in both dogs and rabbits produced an increase of blood glucose and blood lactic acid (see tables 14 and 15). In dogs, blood glucose rose to a maximum between 60 and 75 minutes, i.e., during the first 15 minutes of recovery from anesthesia. Six hours after etherization blood glucose was generally below the control value.

In rabbits, blood glucose rose from 121 mg. per 100 cc., the control, to 211 mg. per cent during etherization period. It reached a peak of 237 mg. per 100 cc. at the end of first hour of recovery and then gradually dropped to 141 mg. per 100 cc. at the end of the experiment. Thus it failed to return to normal even after five hours of recovery from ether anesthesia.

Blood lactic acid in the etherized dogs rose from a control of 17.9 mg. per 100 cc. to 28.0 mg. per 100 cc. at the termination of anesthesia. It then rose to a peak of 39.7 mg. per 100 cc. during first half hour of recovery and then fell down rapidly to normal during the subsequent hour. Blood lactate remained within normal limits thereafter to the end.

In rabbits, blood lactic acid rose from an average control of 18.1 to 26.3 mg. per 100 cc. at the end of etherization period. It then dropped gradually to normal during next two hours and remained so to the end.

Ether anesthesia in dogs produced an initial rise of plasma inorganic phosphate. From a control of 4.58 mg. per 100 cc. phosphate level rose to 5.38 mg. per 100 cc. during first thirty minutes of etherization. When ether was discontinued plasma inorganic phosphate had decreased to 4.89 mg. per cent. It then dropped to a low value of 3.41 mg. per cent at the end of the first hour of recovery and then rose gradually to normal

or even above it, at the end of the experiment.

In dogs, ether anesthesia produced a marked lowering of blood alkali reserve. Plasma CO_2 combining power dropped from an average control of 57.3 vols. % to 26.0 vols. percent, fifteen minutes after the end of ether anesthesia. It remained low at this level for another 45 minuted and returned to normal from the end of the third hour of recovery to the close of the experiment.

Plasma inorganic phosphate and plasma CO_2 combining power after etherization were not determined in rabbits.

C. Effects of sodium d-lactate injection accompanying ether anesthesia. In dogs, sodium d-lactate injectuon did not prevent eth er the rise of blood glucose or of blood lactic acid produced by etherization. Plasma inorganic phosphorus showed an average increase of 1.07 mg. per 100 cc. over the control of 4.41 mg. per 100 cc. during first 15 minutes; but it dropped to a very low level of 2.5 mg. per 100 cc. at 150 minutes from the beginning of the experiment. It then approached normal level during next 30 minutes and then rose to above normal towards the end of the experiment.

In rabbits, injection of sodium d-lactate accompanying ether anesthesia showed a decided inhibitory effect on blood glucose (see table 15). From a control of 103 mg. per 100 cc. blood glucose rose to only 133 mg per 100 cc. at the end of etherization. It then decreased to normal or even subnormal levels at the end of six hours from the beginning of ether anesthesia.

Blood lactic acid showed the same trend as after lactate injectuon alone. The values were slightly higher. From an average control of 20.7 mg. per 100 cc. blood lactate reached a peak of 75.7 mg. per 100 cc. at

15 minutes. It then fell down gradually to normal by the end of the experiment.

D. Effect of ether, lactate, and of ether and lactate on liver glycogen in rats. Ether anesthesia caused a decrease of the liver glycogen content of the rat. Liver glycogen after etherization diminished from an average control of 0.074% to an average of 0.061%, a decrease of 17.5% below normal. After injection of lactate, liver glycogen rose to 0.092%, a rise of 25% above control. After lactate injection just prior to etherization, liver glycogen showed a level of 0.066%, a decrease of 6.7% below control.

In 20 rats, receiving lactate intraperitoneally, the average control value of liver glycogen was 0.09%. After ether anesthesia it decreased to 0.072%. a lowering of 20% below normal. After lactate injection, liver glycogen was at 0.11%, an increase of 22% above control; while after ether and lactate together, liver glycogen dropped to 0.066%, a decrease of 26%.

DISCUSSION

Table 14 shows that intravenously injected sodium d-lactate does not disappear from the blood stream of normal dogs and rabbits as rapidly as is reported in the literature for dl-lactate. Riegel (1927) reported that of the dl-lactate injected intravenously in normal dogs about two-thirds disappeared from the blood in 5 to 10 minutes; and the rate of removal slowed down about 30 minutes after the injection. Hartmann and Senn (1932) reported similar rapid disappearance of injected sodium dl-lactate from normal subjects. They calculated that about 90% of the injected lactate left the blood stream during the first quarter hour after the lactate injection. Soffer et al. (1937) also reported that after sodium d-lactate injection in normal persons a peak rise of blood lactate occured at 5 minutes, and following a major drop within 20 minutes, blood lactate returned to approximately control level within 30 minutes. Results from bothethble 14 and table 15 show that after an intravenous injection of sodium d-lactate, blood lactic acid in both dogs and rabbits, reaches a peak within 15 minutes. Following a comparatively rapid fall during the first hour blood lactic acid disappears slowly during the second hour before reaching to normal.

The early rapid fall of blood lactic acid after intravenous lactate injection in dogs and rabbits can be explained by the rapid diffusion of lactate into the tissues of bidy fluids. This simple diffusion process however, decreases as blood lactate concentration approaches that in the tissues. The later more gradual fall would then depend upon the slow disposal of the lactate by the tissues by metabolic processes such as oxidation, convertion into glucose or glycogen, or by excretion.

That the lactate ion was being gradually metabolized is evident from the changes in the plasma CO_2 combining power noticed after sodium d-lactate injection. Table 14 shows that plasma CO_2 begins to rise within 15 minutes after the lactate injection, and the rise is sustained from 30 minutes thereafter to the end of the experiment. As the injected lactate ion is oxidized or utilized for carbohydrate synthesis, the liberated base is retained in the blood stream as reserve alkali, as the carbonate, until excreted in the urine.

The state of the blood after sodium d-lactate injection would be somewhat comparable to that after exercise; and it would be a reasonable assumption that part of the injected lactate is removed by oxidation. Hill Long, and Lupton (1924) were able to show that during recovery period after exercise, when lactic acid accumulated in the blood is being removed, there is an increased oxygen consumption.

Lastly the injected d-lactate leaving the blood stream, might be converted into liver glycogen. This possibility is well supported by the results of liver glycogen determinations in rats after sodium d- lactate injection. (See table 16.) After either intravenous or intraperitoneal injection of sodium d-lactate in rats, liver glycogen shows a decided increase of 25% and 22% respectively, above the average controls.

Indirect evidence that injected lactate was being utilized for carbohydrate synthesis can also be had from plasma inorganic phosphate changes observed after sodium d-lactate injection in the dog. Table 14 shows that plasma inorganic phosphorus dropped an average of 1.02 mg. per cent below the control of 4.26 mg. per 100 cc.. Simultaneous with the decrease

of plasma inorganic phosphate, the blood lactic acid concentration is also dropping from its peak value. About 75 minutes after lactate injection, blood lactic acid approaches very near normal when plasma inorganic phosphate is at its lowest. The latter however begins to rise and soon rises above normal to the end of the experiment. Apparently as the lactate leaves the blood stream, the plasma inorganic phosphate also migrates into the tissues where it is needed for the synthesis of carbohydrate from the injected sodium d-lactate. As the process of the gradual convertion of lactate into glucose or glycogen proceeds in the tissues, the reserves of tissue inorganic phosphate are depleted and more of plasma inorganic phosphate diffuses into the tissues. Thus the drop in plasma inorganic phosphate.

Ether anesthesia in dogs produces the usual increase of blood glucose and blood lactic acid, and a decrease of plasma inorganic phosphorus and plasma CO_2 combining power. (See table 14.) Ronzoni et al. (1927) found an increased blood lactic acid content of dogs proportional to the amount of ether used in anesthetizing them. They concluded that lactic acid accumulation in the blood could account for a large part of ether acidosis. They indicated that the chief source of this increased blood lactic acid was muscle tissue. Results from table 14 or table 15 show this to be partially true. The discrepancy can however be explained by the difference of etherization technic and the duration of anesthesia maintained by Ronzoni et al.

The work of Cori (1931) and of Chidsey (1935) has shown that the element of time plays a prominent role when analyzing the effects of epinephrin injected into experimental animals. Failure to consider the time

factor results in a conflict of data. For example, epinephrin injection brings about an early breakdown of both the muscle and the liver glycogen. But several hours following such an injection liver glycogen is found to be high. Though the primary effect of epinephirin injection is tissue glycogenolysis, resulting in an increase of blood glucose from liver glycogen, and an increase of blood lactic acid from muscle glycogen, the end result is a shift of muscle glycogen to liver glycogen. Further Cori and Cori (1930) have shown that epinephrin injection causes a temporary increase of hexose monophosphate in the muscle and that this hexose monophosphate is the precursor of the increased blood lactic acid produced by epinephrin injection.

Since the effects of ether anesthesia are shown to be correlated to adrenin-release the action of epinephrin in beinging about degredation of both muscle and liver glycogen must evidently hold in explaining the present findings after ether anesthesia alone of after ether anesthesia accompanied by sodium d-lactate injection.

Holmes and Holmes (1925) and later Holmes and Schriff (1932) showed that the brain does not possess much carbohydrate reserve as do other tissues but depends on the blood glucose for its carbohydrate needs. Using mice and fixing the brains rapidly in liquid air, these authors found that the amount of lactic acid formed on anaerobic incubation depends on the blood glucose level at death. Kerr and Ghantus (1937) have improved the liquid air fixation technic and have confirmed the above findings. They, however, found more of the brain carbohydrate as glycogen than was previously supposed. Kerr (1935) had also demonstrated the presence

of creatine phosphate in the brain.

Uchida (1926) demonstrated that ether and other narcotic agents diminish the free carbohydrate and the total glycogen of the rat brain. Liver glycogen is certainly depleted during anesthesia (Bollman, 1929). Ether also decreases the muscle glycogen (Major and Bollman, 1932).

There is thus good evidence that ether and some other anesthetic agents, produce glycogenolysis in the brain, the liver, the muscles and probably in all the tissues of the body. The exact mechanism which accelerates this universal glycogenolysis is not completely understood but it stands to reason that integrity of function of the central nervous system is involved. Tashiro and Adams (1914) in their studies on narcosis found a diminished CO_2 production in the narcotized nerve of the spider crab. When they used weaker concentrations of the fixed narcotic agents, for example, ethyl urethane or cholral hydrate, the CO_2 output increased. They then suggested that the primary effect of the narcotic on the protoplasm may be to produce a change in its chemical instability, from which a change in the metabolic rate and loss of irritability result. Such an effect on the chemical instability might be brought about by rendering the protoplasm less oxidizable thru a union of protoplasm and narcotic as suggested by Professor Mathews.

Administration of sodium d-lactate to dogs anesthetized with ether results in a more or less summation of effects of the procedures used singly. Blood glucose and blood lactic acid changes in dogs and rabbits after ether alone and after lactate injection alone could be nearly superimposed, they are not quantitatively additive(see table 14). Administration of ether shows greatest rise of blood lactic acid at 90

to 120 minutes while lactate injection causes a rapid increase of blood lactic acid. Thus blood lactic acid shows two maxima after ether anesthesia accompanied by lactate injection, one at 15 minutes and another at 90 to 120 minutes. Further a return to normal of blood lactic acid is delayed when ether alone or ether and lactate together are used. The implication to be drawn from this is that ether anesthesia interfers with the utilization of lactic acid whether it is of endogenous or of exogenour origin.

Table 15 shows that injection of sodium d-lactate decreases the blood glucose of normal rabbits and inhibits the hyperglycemia of ether anesthesia in these animals. A probable explanation of this latter finding is that acidosis is prevented by large doses of sodium d- lactate, and normal insulin activity can then proceed. A depression of insulin activity has been suggested to be present under ether anesthesia, but a clear cut evidence to that effect is lacking. Blood glucose studies after ether and after ether with injected lactate also indicate that the interference of ether anesthesia with the utilization of lactic acid is not due to any structural liver damage. The disturbance is temporary and functional. This agrees with Knoefels theory that the effects of ether anesthesia on carbohydrate metabolism are functional and not structural.

Changes in plasma inorganic phosphate after lactate injection alone or after ether anesthesia accompanied by lactate injection are essentially similar. However, lactate fails to prevent the initial rise of plasma inorganic phosphate observed in ether anesthesia. During recovery period when blood glucose level falls, plasma inorganic phosphorus also falls to its lowest. When blood glucose is either normal or subnormal as re-

covery from anesthesia is more or less complete, plasma inorganic phosphate returns to normal or higher (see table 14). These changes in plasma inorganic phosphate probably indicate that phosphate is being utilized for glucose or glycogen synthesis from the injected lactate.

Results of plasma CO_2 combining power (table 14) show that sodium d-lactate offers an agent of potential usefulness in combating anesthetic acidosis. At the same time it serves to increase the liver glycogen during post-anesthetic period (see table 16). The greater safety of sodium lactate over bicarbonate is recognized in its other clinical uses where acidosis is treated. The more rapid buffer effects of sodium d-lactate make it preferable in most cases to sodium dl-lactate.

SUMMARY

Disturbances of carbohydrate metabolism in ether anesthesia were related by Knoefel to adrenin-release from the adrenal glands mediated by ether anesthesia. Since ether anesthesia produces an increase of blood lactic acid, a further increase of it in the blood stream by intravenous injection of sodium d-lactate should indicate the nature and scope of the mechanism involved in the utilization of injected lactate Such a study of lactate injection is reported. It indicates that:

1. Intravenlusly injected sodium d-lactate at a dose of 0.5 gm./Kg. body weight leaves the blood stream of normal dogs and rabbits at a rate slower than is reported for sodium dl-lactate.

2. The injected lactate is not excreted in significant amounts in the urine.

3. Three possible factors are suggested in the mechanism of the disappearance of the injected lactate from the blood stream. (I) The process of simple diffusion into the tissues explaining the early rapid rate of disappearance. (II) Oxidation in the tissues.an(III) Glucose or glycogen formation in the tissues explaining the later more gradual rate of disappearance from the blood stream.

4. Sodium d-lactate appears to be better utilized in both normah and etherized dogs, rabbits and rats than sodium dl-lactate. Evidence for glycogen formation from injected d-lactate in rat liver is indicated by liver glycogen determinations after intravenous and intraperitoneal injection of sodium d-lactate in rats.

5. Ether anesthesia interfers with the proper utilization of injected d-lactate; but during the recovery period lactate furnishes a ready source for replenishing the depleted stores of liver glycogen.

6. Incidently sodium d-lactate injection counteracts the severe acidosis of ether anesthesia, and due to its rapid buffer effect may serve better that sodium dl-lactate for that purpose.

TABLE I

Blood glucose changes in dogs produced by etherization (E), by etherization and injection of sodium d-lactate (E+L), and by injection of sodium d-lactate alone (L).

Dog No.	Sex	Wt.		Time - Minutes: Control	15	30	45	60+	75	90	120	150	180	240	300	360
				Mg. per 100 cc. blood												
			E	110	114	116	117	134	141	139	139	143	132	102	102	88
1	M	13 Kg.	E+L	104	143	137	148	155	155	135	108	101	98	87	91	94
			L	78	122	122	119	124	117	121	100	102	91	98	97	100
			E	96	124	135	140	153	161	140	109	105	93	95	92	87
2	M	8 Kg.	E+L	96	128	117	144	153	159	161	112	94	93	100	95	102
			L	102	114	98	112	107	112	110	108	101	105	103	103	98
			E	85	114	127	145	139	-	-	-	-	-	-	-	-
3	M	8.6 Kg.	E+L	89	117	108	103	101	108	116	107	110	105	97	93	95
			L	84	91	77	87	77	-	79	75	73	61	73	-	-
			E	84	104	127	139	145	136	129	107	107	100	92	82	81
4	M	11 Kg.	E+L	79	85	107	125	143	123	135	102	88	75	73	76	71
			L	107	101	98	89	93	103	91	96	101	93	78	-	93
			E	98	102	113	135	149	135	109	103	100	92	82	88	87
5	F	9.6 Kg.	E+L	97	120	118	139	163	139	141	121	109	93	101	98	91
			L	100	101	81	83	79	93	100	113	109	91	93	81	88
			E	79	100	103	117	138	151	130	109	119	95	82	77	70
6	F	8.4 Kg.	E+L	81	108	128	136	128	123	95	82	94	93	79	75	80
			L	103	113	109	118	120	113	119	101	97	97	83	81	91
			E	86	112	122	114	121	115	114	105	-	114	87	75	76
7	M	6.8 Kg.	E+L	82	125	123	107	107	-	111	116	105	93	91	-	-
			L	124	120	113	108	104	100	-	101	-	108	117	115	105
			E	-	-	-	-	-	-	-	-	-	-	-	-	-
8	F	10.2 Kg.	E+L	80	117	108	110	112	110	117	96	-	77	82	77	82
			L	87	86	73	81	76	75	76	78	-	75	80	77	75

+ Administration of ether terminated here.

TABLE II

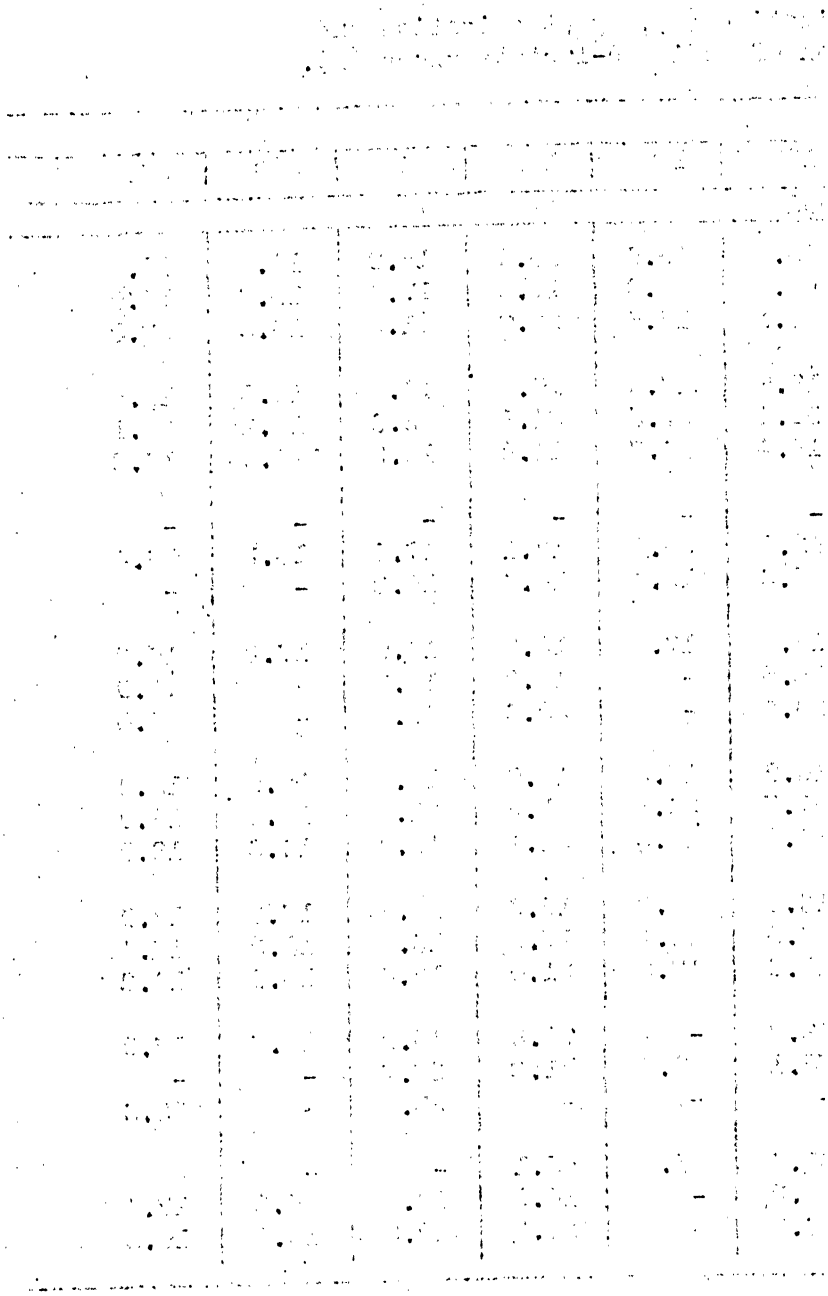


TABLE II

Blood lactic acid changes in dogs produced by etherization (E), by etherization and injection of sodium d-lactate (E+L), and by injection of sodium d-lactate alone (L).

Dog				Time -- Minutes												
				Control	15	30	45	60+	75	90	120	150	180	240	300	360
No.	Sex	Wt.		Mg. per 100 cc. blood												
1	M	13 Kg.	E	17.4	17.1	17.3	18.3	21.3	53.8	53.4	45.2	19.6	15.9	15.0	15.2	15.2
			E+L	17.8	76.2	78.0	53.5	49.0	52.1	48.8	40.0	23.0	17.8	17.4	15.7	14.9
			L	18.8	95.7	110.5	87.9	31.4	20.0	19.3	18.1	15.5	23.0	21.3	16.8	22.0
2	m	8 Kg.	E	15.3	16.8	17.2	25.2	29.8	29.0	28.6	18.9	17.0	19.8	16.8	18.5	15.8
			E+L	21.0	69.0	63.8	60.0	71.5	72.1	56.8	51.4	37.3	24.8	22.6	19.2	20.1
			L	16.8	41.1	39.1	43.6	44.8	40.6	35.4	18.4	16.7	14.8	15.3	16.0	16.2
3	M	8.6 Kg.	E	20.1	27.8	24.2	30.6	22.8	-	-	-	-	-	-	-	-
			E+L	22.2	75.5	69.2	53.0	62.6	62.0	56.5	52.0	39.0	18.1	17.4	15.2	16.4
			L	17.2	59.8	50.0	29.8	18.9	-	18.3	15.6	15.4	14.6	15.2	-	-
4	M	11.0 Kg.	E	17.3	17.1	16.9	28.2	26.8	28.8	29.2	18.2	17.0	18.0	16.9	17.2	16.2
			E+L	15.9	96.0	88.8	93.6	71.2	67.8	74.8	25.5	-	15.9	17.8	-	14.9
			L	19.5	29.7	31.2	28.6	27.3	20.3	23.4	20.4	-	30.6	40.8	-	35.0
5	F	9.6 Kg.	E	18.0	16.8	16.9	20.6	28.9	27.9	28.8	16.9	17.0	18.9	16.8	15.9	15.8
			E+L	19.6	72.5	68.3	69.2	68.2	75.2	58.6	51.3	36.8	21.3	21.2	18.8	17.9
			L	20.1	56.8	35.2	38.3	31.4	19.8	19.2	16.9	15.3	21.1	19.8	16.6	18.9
6	F	8.4 Kg.	E	17.9	17.6	15.9	21.8	27.6	29.8	31.2	16.9	21.2	19.3	17.8	17.3	18.9
			E+L	18.8	51.3	43.5	42.6	52.6	45.8	48.2	36.5	31.1	17.9	16.8	15.2	17.4
			L	15.9	45.9	38.7	29.7	17.9	20.0	18.3	18.6	17.5	21.0	15.6	15.9	16.2
7	M	6.8 Kg.	E	17.0	29.9	24.2	27.9	25.8	45.6	52.8	42.7	-	21.8	15.9	18.7	17.9
			E+L	25.8	116.9	91.8	81.1	73.9	-	77.9	70.1	38.8	41.2	27.7	-	-
			L	19.8	82.7	61.9	59.9	42.5	-	48.3	-	-	-	23.9	-	23.6
8	F	10.2 Kg.	E	17.0	33.7	47.8	57.3	41.1	-	54.8	65.6	43.8	45.1	-	-	-
			E+L	14.8	130.3	89.5	71.0	63.9	59.8	71.8	40.2	-	14.9	18.3	19.2	17.4
			L	15.3	98.9	51.9	51.0	37.3	38.1	37.3	26.8	-	24.3	19.3	18.9	19.2

+ Administration of ether terminated at this point.

TABLE III

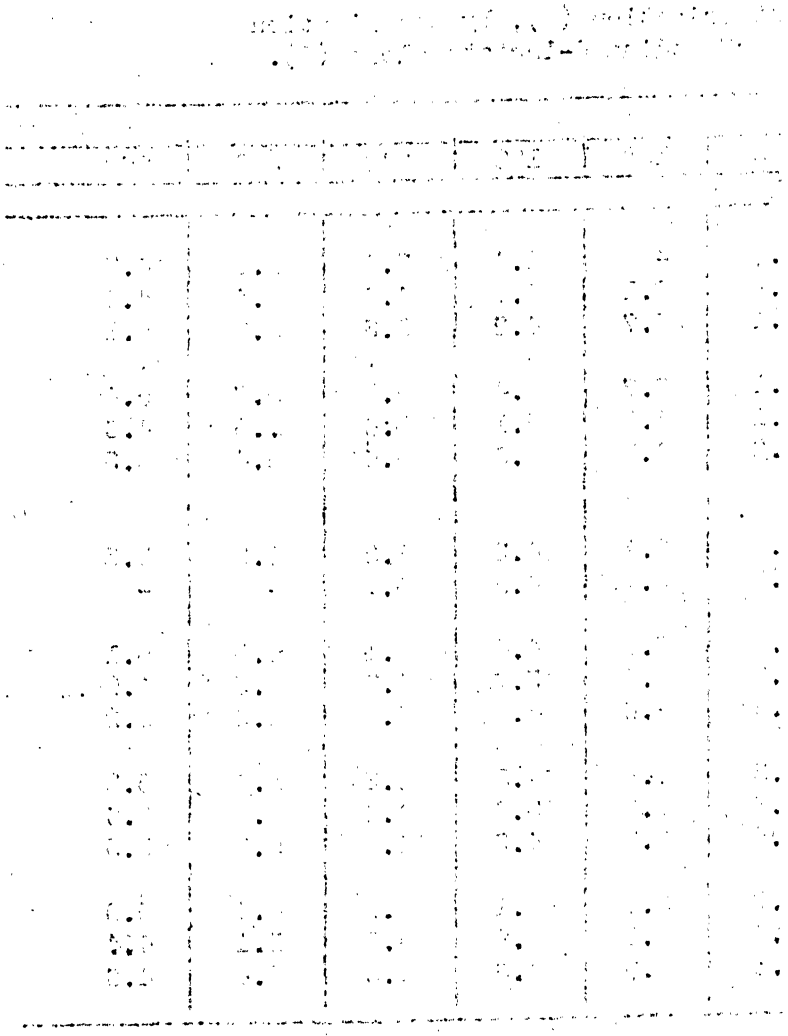


TABLE III

Plasma inorganic phosphorus changes in dogs produced by etherization (E), by etherization and injection of sodium d-lactate (E+L), and by injection of sodium d-lactate alone (L).

Dog No.	Sex	Wt.		Time - Minutes. Control	15	30	45	60+	75	90	120	150	180	240	300	360
				Mg. per 100 cc. plasma												
1	M	13.0 Kg.	E	4.3	5.5	-	4.5	4.4	3.7	3.9	3.6	-	4.4	3.8	5.9	4.3
			E+L	3.7	5.9	5.3	4.7	5.0	3.3	2.5	2.8	2.1	3.7	4.8	5.4	4.4
			L	3.4	3.3	3.2	2.7	2.7	2.5	2.3	3.1	3.7	4.8	5.3	5.2	5.1
2	M	8.0 Kg.	E	5.6	5.7	5.2	4.2	4.7	3.9	3.8	3.1	3.7	4.4	5.0	5.7	5.4
			E+L	6.4	6.0	5.2	3.8	2.5	2.4	2.4	3.1	3.9	5.1	5.6	5.5	4.8
			L	5.6	4.9	4.6	4.6	5.3	5.2	5.3	5.2	4.8	5.6	5.6	5.4	5.3
3	M	8.6 Kg.	E	3.8	5.1	6.3	7.0	6.8	Dog Died							
			E+L	4.5	4.1	3.9	3.7	3.9	-	2.3	2.3	2.4	4.2	3.2	5.3	5.5
			L	3.8	3.2	3.5	2.8	2.6	-	2.5	2.9	3.7	4.5	4.7	-	-
4	M	11.0 Kg.	E	4.3	5.4	5.1	4.3	4.4	3.7	3.7	3.4	3.3	4.0	4.1	4.3	5.1
			E+L	4.0	5.7	5.2	4.3	5.2	3.7	2.6	2.3	2.3	3.7	4.3	5.3	4.9
			L	3.9	3.3	3.1	2.7	2.6	2.3	2.5	3.2	3.6	4.3	5.4	5.3	5.2
5	F	9.6 Kg.	E	5.1	5.3	5.2	5.0	4.7	3.8	3.6	3.2	3.9	4.3	5.3	5.6	5.2
			E+L	5.0	5.8	5.4	4.6	5.0	3.4	2.5	2.3	2.2	3.6	4.6	5.4	5.6
			L	5.2	4.6	4.4	3.2	3.0	2.8	3.2	3.5	3.7	4.3	4.6	5.3	5.5
6	F	8.4 Kg.	E	4.3	5.2	5.1	4.8	4.4	3.9	3.8	3.7	3.5	4.1	3.9	4.4	5.0
			E+L	4.0	5.5	5.7	4.8	3.7	3.3	3.1	3.4	3.5	4.8	4.8	5.1	4.4
			L	3.7	3.5	3.5	3.3	3.3	3.4	3.5	4.1	4.3	4.6	4.6	4.4	4.6

+ Etherization terminated at this point.

TABLE IV

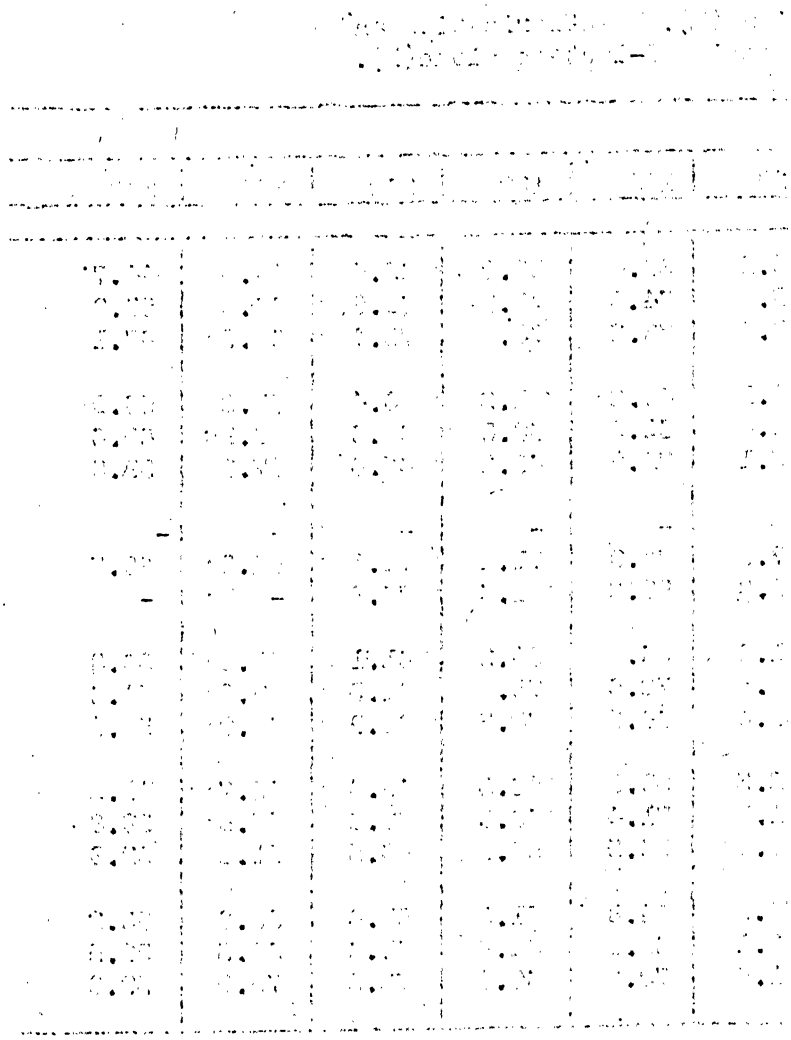


TABLE IV

Plasma CO_2-combining power in dogs produced by etherization (E), by etherization and injection of sodium d-lactate (E+L), and by injection of sodium d-lactate alone(L).

Dog				Time - Minutes												
				Control	15	30	45	60+	75	90	120	150	180	240	300	360
No.	Sex	Wt		Mg. per 100 cc. plasma												
1	M	13.0 Kg.	E	65.5	37.2	33.4	33.4	25.8	22.1	25.8	15.5	39.0	52.2	65.5	48.5	44.7
			E+L	54.7	46.7	61.6	75.4	62.5	58.8	56.0	69.0	74.5	71.8	69.9	66.2	58.8
			L	48.6	68.0	74.5	75.5	77.3	70.8	67.1	63.4	78.2	70.8	75.4	69.9	67.1
2	M	8.0 Kg.	E	61.6	46.7	40.3	42.1	38.4	36.5	31.9	42.1	44.2	41.2	50.4	53.2	56.9
			E+L	59.3	43.0	41.2	43.0	37.4	42.1	46.7	50.4	62.5	69.0	62.6	57.9	60.6
			L	56.0	53.2	59.8	62.6	61.7	63.6	63.6	72.1	75.8	69.2	63.6	57.9	59.8
3	M	8.6 Kg.	E	54.1	42.8	39.0	41.9	43.0	-	-	-	-	-	-	-	-
			E+L	53.4	40.9	57.0	50.4	55.1	41.0	-	57.9	74.9	71.1	71.1	56.0	68.3
			L	52,3	69.0	75.4	82.0	78.2	-	83.8	89.8	88.2	91.6	76.4	-	-
4	M	11.0 KG	E	58.8	39.0	33.6	32.5	25.9	24.1	21.4	21.6	36.9	50.2	61.1	51.5	56.8
			E+L	52.3	43.1	48.1	52.8	62.9	59.8	66.4	69.8	72.6	78.5	61.3	59.8	54.6
			L	55.4	66.5	74.2	71.2	68.9	67.8	61.9	59.8	66.3	70.3	69.9	65.9	58.8
5	F	9.6 KG.	E	49.4	36.6	32.2	33.4	28.3	21.9	26.9	22.3	38.8	46.5	48.6	45.7	49.2
			E+L	53.5	41.6	55.3	66.8	62.9	71.3	68.5	71.5	71.4	66.8	62.6	57.8	52.8
			L	56.6	51.5	61.6	68.6	59.9	60.2	60.1	58.8	56.6	50.9	56.5	51.8	52.8
6	F	8.4 Kg.	E	54.1	39.2	33.6	23.3	28.9	25.6	28.6	32.9	34.8	51.3	58.6	55.6	58.2
			E+L	52.3	51.5	56.8	72.2	68.3	59.5	55.4	62.1	68.9	64.6	61.6	63.3	59.5
			L	49.6	60.1	69.9	75.5	76.1	71.3	68.6	61.6	70.2	69.8	72.5	70.0	59.8

+Administration of ether terminated at this point.

TABLE V

Blood Lactic acid changes in dogs produced by intravenous injection of sodium lactate (d- or dl-) alone (L.A.), or accompanied by ether anesthesia (E+LA)

Dog Number		Control	15	30	45	60*	120	240	360
		Mg. per 100 cc. Blood							
No. 9 Male 10.0 Kg. (d-lactate)	LA	20.1	83.3	62.3	60.1	43.1	24.4	21.2	22.6
	E+LA	21.0	62.1	60.3	59.7	52.3	41.2	19.8	20.7
No. 10 Female 8.2 Kg. (dl-lactate)	LA	21.0	59.7	52.2	49.5	33.6	29.8	21.7	20.9
	E+LA	19.2	51.7	39.7	36.3	33.2	49.8	30.7	20.2
No. 11 Female 7.3 Kg. (d-lactate)	LA	19.6	72.1	59.7	52.5	39.3	2o.6	19.5	18.7
	E+LA	19.9	79.3	62.7	59.6	50.2	39.4	18.9	20.3
No. 12 Male 9.2 Kg. (dl-lactate)	LA	22.6	69.1	56.1	48.4	30.7	28.7	23.1	20.4
	E+LA	21.5	71.2	50.3	42.7	39.7	38.7	24.2	20.9

LA Lactic Acid
E+LA Etherization + Lactic Acid
* Ether Anesthesia Terminated Here.

TABLE VI

Blood glucose changes in dogs produced by intravenous injection of sodium lactate (d- or dl-) alone (L) or accompanying ether anesthesia (E + L).

Dog Number		Time. Minutes							
		Control	15	30	45	60+	120	240	360
		Mgm. per 100 cc. blood							
No. 9 10.0 Kg. Male (d-lactate)	L	121	116	113	103	98	101	115	120
	E+L	119	125	108	132	149	98	103	117
No. 10 8.2 Kg. Female (dl-lactate)	L	92	94	87	81	88	76	72	86
	E+L	102	113	116	129	133	117	98	101
No. 111 7.3 Kg. Female (d-lactate)	L	119	107	105	98	92	94	99	109
	E+L	130	119	103	123	145	126	138	116
No. 12 9.2 Kg. Male (dl-lactate)	L	117	106	111	109	121	118	98	107
	E+L	106	121	141	139	136	129	113	105

L Lactate alone
E+L Etherization and Lactate Injection
\+ Anesthesia terminated here.

TABLE VII

Blood Glucose and Lactic Acid Changes in 24 hour fasted rabbits, given 0.5 Gm./Kg. of sodium d-Lactate Intravenously.

Rabbit Number		Time. Minutes.							
		Control	15	30	45	60	120	240	360
		Mgm. per 100 cc. blood							
No. 11 Female 2.8 Kg.	G.+	129	109	92	71	68	78	89	106
	L.A.+	19.8	71.1	58.2	46.1	45.3	41.1	29.6	21.1
No. 12 Male 2.66 Kg.	G.	99	121	101	92	87	80	101	110
	L.A.	21.3	63.4	52.3	43.3	32.1	29.5	26.8	19.8
No. 13 Female 2.59 Kg.	G.	115	119	96	87	79	81	93	99
	L.A.	23.7	73.3	51.2	47.4	37.3	33.6	24.7	20.9
No. 14 Male 2.54 Kg.	G.	88	112	87	68	70	82	86	102
	L.A.	19.9	67.6	50.8	42.6	34.4	31.9	22.9	18.0
No. 15 Male 2.62 Kg.	G.	120	131	99	72	64	74	94	118
	L.A.	23.1	81.2	69.9	50.1	42.3	36.2	29.8	19.2
No. 16 Male 2.66 Kg.	G.	101	116	82	76	70	66	79	103
	L.A.	20.2	71.8	54.3	39.6	37.5	30.6	29.5	22.9

G. Blood Glucose
L. A. Blood Lactic Acid

TABLE VIII

Blood Glucose and Lactic Acid Changes in 24 Hour Fasted Rabbits, Given 0.5 Gm./Kg. sodium d-lactate Intravenously and Anesthetized with Ether.

Rabbit Number		Time. Minutes.							
		Control	15	30	45	60++	120	240	360
		Mgm. per 100 cc. blood							
No. 17 Male 2.51 Kg.	G.+	118	130	134	148	153	141	129	116
	L.A.#	20.1	102	91.1	68.2	44.3	32.6	28.7	22.1
No. 18 Female 2.36 Kg.	G.	109	121	117	107	136	130	116	101
	L.A.	19.8	76.9	50.7	43.3	39.8	35.3	22.7	21.9
No. 19 Male 2.72 Kg.	G.	121	137	129	137	150	139	120	112
	L.A.	23.4	98.7	73.2	59.6	42.7	40.4	26.6	20.5
No. 20 Female 2.42 Kg.	G.	100	113	120	122	129	139	101	107
	L.A.	22.7	97.8	70.2	49.9	51.2	38.7	23.9	24.2
No. 21 Male 2.59 Kg.	G.	97	100	131	143	115	92	81	95
	L.A.	19.9	92.1	64.6	50.1	43.7	30.3	26.1	19.6
No. 22 Male 2.31 Kg.	G.	89	92	117	130	112	116	82	78
	L.A.	18.6	78.4	50.7	46.1	39.9	36.2	24.1	19.3

++ Ether Administration Terminated Here
+ Glucose
# Lactic Acid

TABLE IX

Blood Sugar and Lactic Acid changes in 24 hour fasted rabbits, anesthetized with ether.

Rabbit Number		Time. Minutes.							
		Control	15	30	45	60++	120	240	360
		Mgm. per 100 cc. blood							
No. 5 male 2.39 Kg.	G.+	113	137	159	175	220	244	168	137
	L.A.#	21.7	26.1	36.4	32.9	20.3	19.8	20.1	19.2
No. 6 Female 2.38 Kg.	G.	129	149	173	184	199	211	159	143
	L.A.	18.9	16.3	29.7	18.4	27.9	21.1	16.9	17.3
No. 7 Male 2.40Kg	G.	131	164	191	199	218	259	188	148
	L.A.	16.8	14.9	30.2	26.3	22.2	19.6	15.9	16.1
No. 8 Male 2.48 Kg.	G.	119	161	184	185	213	223	146	128
	L.A.	15.3	16.7	21.7	22.2	31.1	28.6	20.5	19.2
No. 9 Female 2.39 Kg.	G.	130	166	189	200	225	266	178	151
	L.A.	18.7	16.5	21.2	23.9	36.2	29.7	24.3	19.8
No. 10 Male 2.34 Kg.	G.	120	151	172	179	191	219	157	139
	L.A.	19.3	21.1	26.7	24.5	20.7	23.2	19.8	19.6

++ Ether administration terminated here
+ Glucose
# Lactic Acid

TABLE X

Variable response to different amounts of sodium d-lactate injected intravenously in 24 hour fasted rabbits:

Rabbit Number	Time. Minutes							
	Control	15	30	45	60	120	240	360
	Mgm. per 100 cc. blood							
No. 1 Male 2.38 Kg. 4.5 Gm./Kg. sodium d-lactate G.+	121	117	88	81	50	17##		
L.A.#	22.7	115	58.2	120.6	147	22.7		
No. 2 Male 2.58 Kg. 0.8 Gm./Kg. sodium d-lactate G.	129	111	77	61	34	52	95	120
L.A.	28.6	79.5	71.5	51.4	40.6	39.3	21.9	--
No. 3 Female 2.58 Kg. 0.5 Gm./Kg. sodium d-lactate G.	97	119	101	92	83	94	100	108
L.A.	21.4	94.8	61.8	44.2	33.3	29.2	21.2	19.4
No. 4 Male 2.61 Kg. 0.6 Gm./Kg. sodium d-lactate G.	130	125	117	108	89	101	119	121
L.A.	23.9	95.5	64.2	50.7	43.0	28.6	26.8	22.8

In bad shape. Dead next day.
\+ Blood Glucose
\# Blood Lactic Acid

TABLE XI

Liver Glycogen Content of 48 hour Fasting Rats

	No.	Body Wt.	Wt. of liver tissue used for analysis.	Liver Glycogen	Remarks
		Gm.	Gm.	Percent	
Group I					
	1	110	0.386	0.09	
Normal	2	108	0.330	0.10	
Control	3	118	0.296	0.05	
	4	102	0.354	0.06	
	5	115	0.432	0.11	
	6	118	0.496	0.08	
	7	106	0.379	0.06	
	8	112	0.297	0.05	
	9	110	0.398	0.08	
	10	116	0.412	0.06	
	Av. wt. 110 ± 8			Av. 0.074%	
Group II	1	112	0.377	0.07	Etherized in a large 12-liter glass jar, containing soda lime. Air in jar replaced by oxygen. Ether 2.5 mM/Liber. Liver Glycogen determinations done one hour after terminating anesthesia.
	2	112	0.352	0.06	
Etherized	3	118	0.488	0.11	
	4	106	0.296	0.04	
	5	108	0.310	0.06	
	6	109	0.412	0.04	
	7	116	0.355	0.05	
	8	101	0.331	0.04	
	9	104	0.298	0.04	
	10	118	0.354	0.06	
	Av. Wt. 110 ± 9			Av. 0.061%	

TABLE XII

Liver Glycogen Content of 48 hour fasting rats

	No.	Body Wt.	Wt. of liver tissue analyzed	Liver Glycogen	Remarks
Group III		Gm.	Gm.	Percent	
Intravenous injection 0.5 Gm./Kg. sodium d-lactate	1	120	0.402	0.09	Liver Glycogen determined an hour following the intravenous injection of sodium d-lactate.
	2	113	0.386	0.10	
	3	108	0.352	0.08	
	4	101	0.298	0.08	
	5	119	0.412	0.09	Liver Glycogen determined 2 hours after injection of sodium d-lactate.
	6	126	0.511	0.12	
	7	132	0.463	0.10	
	8	111	0.329	0.09	
	Av. Wt. 116 ± 15			Av. 0.92%	
Group IV					
Intravenous injection 0.5 Gm./Kg. sodium d-lactate and ether anesthesia (2.5 mM/Liter).	1	113	0.410	0.08	Rats individually etherized quickly in a 2 L. jar containing soda lime. Sodium d-lactate injected. All 4 then placed in the large 12 L. jar and etherization continued for 1 hr. An hr. after the lactate injection each rat decapitated for liver glycogen determination.
	2	128	0.378	0.06	
	3	112	0.299	0.05	
	4	101	0.442	0.06	
	5	130	0.394	0.08	Sodium d-lactate injection first. Then all together anesthetized with ether in 12 L. jar for 1 hr. After an hr. of recovery, i.e. 2 hr. after lactate injection, decapitation and liver glycogen determination.
	6	112	0.383	0.07	
	7	116	0.410	0.08	
	8	118	0.369	0.07	
	Av. Wt. 116 ± 15			Av. 0.069%	

TABLE XIII

Liver Glycogen Content of 48 hour fasting rats

	No.	Body Wt.	Wt. of liver tissue analyzed	Liver Glycogen	
		Gm.	Gm.	Percent	
Group V	1	130	0.479	0.10	
Normal	2	139	0.511	0.09	
Control	3	118	0.392	0.08	
	4	130	0.451	0.09	
	5	131	0.429	0.09	
	Av. Wt. 128 ± 12			Av. 0.09%	
Group VI	1	130	0.392	0.06	
Anesthetized	2	128	0.292	0.06	Treated
with ether	3	139	0.506	0.11	Same as Group II
2.5 mM/L.	4	119	0.468	0.07	
	5	116	0.398	0.06	
	Av. Wt. 127 ± 10			Av. 0.09%	
Group VII	1	126	0.438	0.09	
Intraperi-	2	138	0.490	0.17	Liver glycogen an
toneal in-	3	112	0.379	0.10	hour after intraperi-
jection 0.5	4	109	0.368	0.09	toneal injection.
Gm./Kg.	5	118	0.311	0.11	
sodium d-lactate					
	Av. Wt. 128 ± 10			Av. 0.11%	
Group VIII	1	120	0.393	0.08	Intraperitoneal lactate
Intraperi-	2	138	0.449	0.07	immediately preceding ether-
toneal in-	3	132	0.512	0.07	ization. All anesthetized
ection 0.5	4	110	0.291	0.06	together in 12 L. jar. Ether
gm./Kg. sod-	5	108	0.304	0.05	2.5 mM/Liter - 1 hour.
ium d-lactate					Recovery of 1 hour more
and ether anesthesia.					before decapitation.
	Av. Wt. 122±14			Av. 0.066%	

TABLE XIV

Average blood changes in 8 dogs after ether anesthesia (E), after sodium d-lactate injection (L), and after ether and sodium d-lactate injection (E+L).

Time Minutes	Blood Glucose E	E+L	L	Blood lactic acid E	E+L	L
	Mg. per 100 cc.					
C	91	88.5	97.2	17.5	19.5	17.9
15	110	118	106	19.6	86.0	63.8
30	120	118	95	22.5	74.1	57.0
45	130	123	99.6	28.7	64.4	42.4
60 x	139	133	97.5	28.0	64.1	31.4
75	140	131	102	35.5	62.1	26.5
90	127	126	99.4	39.7	61.7	27.4
120	112	106	97.	32.0	45.9	18.3
150	115	100	98.	22.6	34.3	16.1
180	103	91	90.	22.7	21.5	20.2
240	91	89	91.	16.5	19.9	20.8
300	86	86.4	92.3	17.1	17.2	16.8
360	82	88	93.	16.6	17.0	21.6

Time Minutes	Plasma Inorganic P. E	E+L	L	Pl. CO_2 Comb. Power E	E+L	L
	mg. per 100 cc.			Vols. per cent.		
C	4.58	4.41	4.26	57.3	54.3	53.1
15	5.37	5.48	3.80	40.2	44.5	61.4
30	5.38	5.11	3.72	35.4	53.3	69.2
45	4.86	4.32	3.23	35.9	60.1	72.6
60x	4.89	4.22	3.25	31.4	58.2	70.4
75	3.82	3.22	3.24	26.0	55.4	65.7
90	3.74	2.57	3.31	26.9	58.6	67.5
120	3.41	2.58	3.67	26.9	63.5	67.6
150	3.59	2.50	3.96	38.7	70.8	72.5
180	4.24	4.17	4.57	48.3	70.3	70.4
240	4.45	4.56	4.99	56.8	64.8	69.1
300	4.93	5.36	5.11	50.9	60.2	63.1
360	5.01	4.89	5.14	53.1	59.5	59.7

x Etherization terminated at this point.

TABLE XV

Average blood changes in 18 rabbits after ether anesthesia (E), after sodium d-lactate (L), and after ether and sodium d-lactate injection (E+L).

Time in Minutes	Blood Glucose			Blood Lactic Acid		
	E	E+L	L	E	E+L	L
	mg. per 100 cc.			mg. per 100 cc.		
Control	123	106	108	18.4	20.7	21.3
15	155	116	118	18.6	75.7	71.4
30	178	125	93	27.6	66.8	56.1
45	187	131	78	24.7	52.9	44.8
60♯	211	133	73	26.4	43.6	38.2
120	237	126	77	23:6	35.6	33.8
240	166	105	90	19.6	24.3	27.2
360	141	102	106	18.5	21.3	20.3

♯ Etherization terminated at this point.

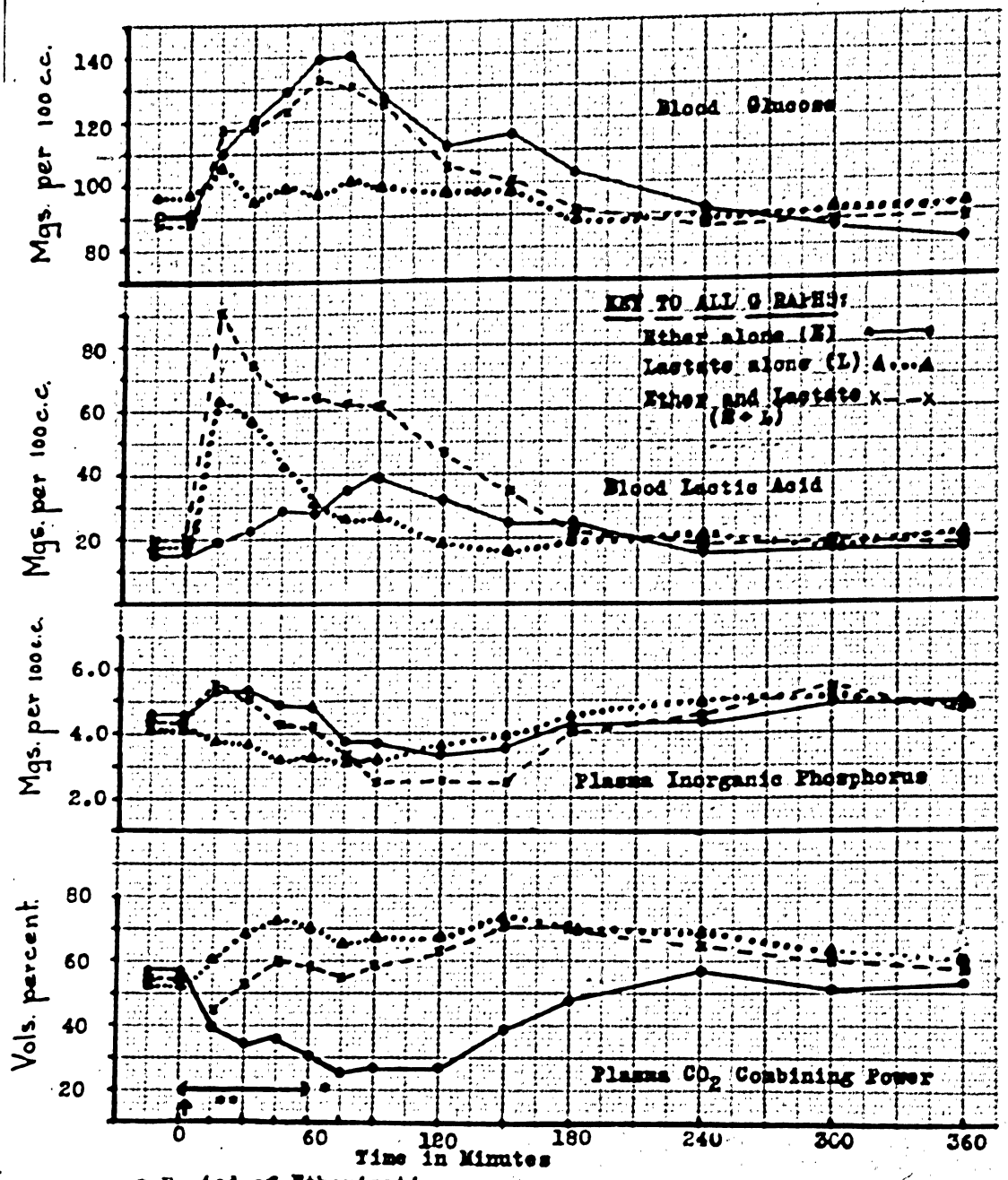
TABLE XVI

Liver glycogen changes in 48 hour fasted rats after ether (E), after sodium d-lactate injection (L), and after ether and sodium d-lactate injection (E+L).

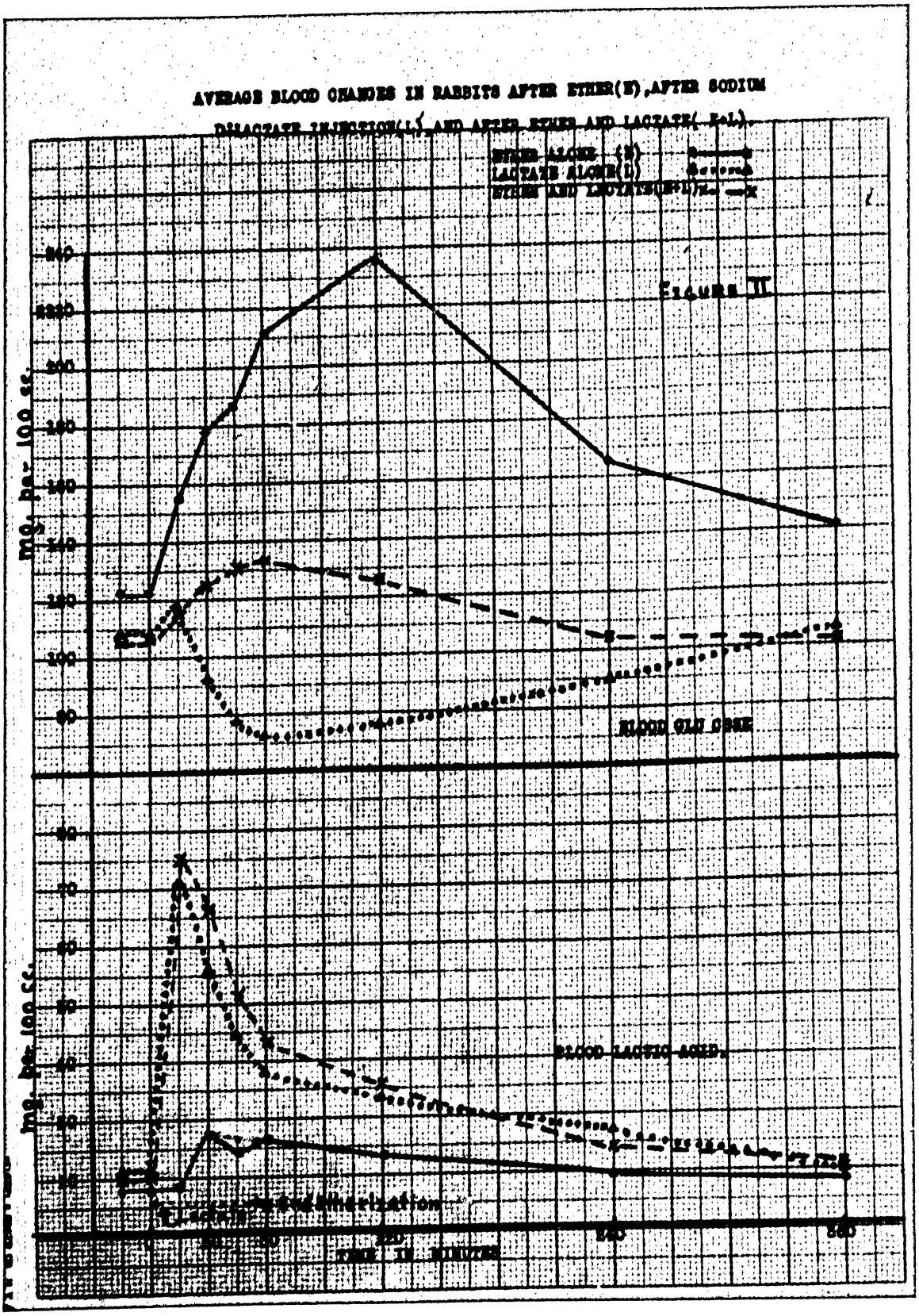
Group No.	No. of Rats	Av. Wt. Grams	Liver Glycogen (Per cent)	Indecrease or Decrease over Normal in %.	Remarks
1	10	110±8	0.074		Normal control
2	10	110±9	0.061	-17.5	2.5 mM/l. ether, 1 hour. Liver glycogen after 1 hour of recovery from anesthesia.
3	8	116±15	0.092	+25	0.5 gm./kg. sodium d-lactate intravenously. Liver glycogen 1 hour after injection.
4	8	118±15	0.069	-6.7	2.5 mM/l. ether, 1 hour, immediately after 0.5 gm./kg. sodium d-lactate intravenously. Liver glycogen after 1 hour of recovery from anesthesia.
5	5	128±10	0.090		Normal control
6	5	127±10	0.072	-20	Same as group 2 above.
7	5	120±10	0.110	+22	Sodium d-lactate intraperitoneally, otherwise same as group 3.
8	5	122±14	0.066	-26	Sodium d-lactate intraperitoneally, otherwise same as group 4.

FIGURE I

Average Blood Changes in 8 Dogs after Ether (E), after Injection of Na d-Lactate (L), and after Ether and Na d-Lactate (E L).



* Period of Etherization
** Lactate Injected


AVERAGE BLOOD CHANGES IN RABBITS AFTER ETHER(E), AFTER SODIUM
ETHER ALONE (E)
LACTATE ALONE(L)
ETHER AND LACTATE(E+L)
Figure II
mg. per 100 cc.
BLOOD SUGAR
BLOOD LACTIC ACID
TIME IN MINUTES

BIBLIOGRAPHY

Barbour, H. G. and Bourne, W. (1923) Am. J. Physiol., 67, 399.

Banerji, H. and Reid, C. (1933) J. Physiol., 78, 370.

Bloor, W. R. (1914) J. Biol. Chem., 19, 13.

Bodansky, A. (1933) J. Biol. Chem., 99, 197.

Bolliger, A. (1926) J. Biol. Chem., 69, 721.

Bollman, J. L. (1929) Proc. Staff Meet. Mayo Clin., 4,369.

Browne, J. S. L. and Evans, C. L. (1933) J. Physiol., 80, 1.

Butatao, E. and Cannon, W. B. (1925) Am. J. Physiol., 72, 295.

Cannon, W. B. and Hoskino, R. G. (1911) Am. J. Physiol., 29, 274.

Chidsey, J. L: and Dye, J. A. (1935) Am. J: Phusiol., 111, 223.

Cori, C. F. (1931) Physiol. Rev., 11, 143.

Cori, C. F. and Cori, G. T. (1929) J. Biol. Chem., 81, 389.

Cori, C.F. and Cori, G. T. (1930) Proc. Soc. Exptl. Biol. Med., 27,934.

Elliott, T. R. (1912) J. Physiol., 44, 374.

Emerson, G. A. (1935) Proc. Soc. Exptl. Biol. Med., 33, 171.

Emerson, G. A. (1936) Am. J. Dig. Dis. and Nutrition, 4, 255.

Emerson, G. A., Klyza, S. J., Abrau, B., and Phatak, N. M.
(1937) Anesthesia & Analgesia, 16, 3.

Emerson, G. A. (1938) Anesthesia & Analgesia, 17, 109.

Evans, C. L., Tsai, C., and Young, F. G. (1931) J. Physiol., 73, 67.

Guedel, A. (1936) Anesthesia & Analgesia, 14, 1, 55.

Hagedorn, H. C. and Jensen, B. N. (1923) Biochem, Z., 135, 46.

Hamburger, W. W. and Ewing, F. E. (1908) J. A. M. A., 51, 1586.

Hartmann, A. F. and Senn, M. J. E. (1932) J. Clin. Investigation 11, 327, 337, and 345.

Hawk, P. B. (1903) Am. J. Physiol., 10, xxxvii.

Hill, A. V., Long, C. N. H. and Lupton, H. (1924, 1925) Proc. Roy. Soc. London, Series B., 96, 435; 97, 84.

Holmes, B. E. and Holmes, E. G. (1925) Biochem. J. 19, 492, 836.

Holmes E. G. and Sheriff, M. A. F. (1932) Biochem. J., 26, 381.

Hospers, C. A. (1933) Arch. Surg., 26, 909.

Janssen, S. and Jost, H. (1925) Z. Physiol. Chem. 148, 41.

Jenemann (1933) U. S. Pat. No. 1,906,068.

Keeton, R. W. and Ross, E. L. (1919) Am. J. Physiol., 48, 146.

Kerr, S. E. and Ghantus, M. (1937) J. Biol. Chem., 117, 217.

Killian, H. (1934) Die Narkose, Berlin.

King, J. H., Chaffee, B. S., Anderson, D. B. and Redelings, L. H. (1911) Johns Hopkins Hosp. Bul., 22, 388.

Knoefel, P. K. (1936) Anesthesia & Analgesia, 15, 137.

Leake, D.D., Leake, E. W. and Koehler, A. E. (1923) J. Biol. Chem., 56, 319.

Mackay, R. L. and Dyke, S. C. (1928) Brit. J. Anesth., 6, 61.

Macleod, J. J. R. and Pearce, R. G. (1910) Am. J. Physiol. 27, 341.

Magee, H. E., Anderson, W. and Glennie, A. E. (1928) Brit. J. Exper. Path., 9, 119.

Mahler, A. (1926) J. Biol. Chem., 69, 653.

Major, S. G. and Bollman, J. L. (1932) Proc. Soc. Exptl. Biol. Med. 29, 1109.

Marenzi, A. D. and Gershman, R. (1933) Compt. rend. soc. biol., 114, 1226.

Marenzi, A. D. and Gerschman, R. (1934) Compt. rend. soc. biol., 116, 891.

Meyerhoff, O. and Lohmann, K. (1926) Biochem. Z., 171, 421.

Minnitt, R. J. (1932) Proc. Roy. Soc. Med., 26, 347.

Parnas, J. K. (1912) Biochem. Z., 38, 53.

Phelps, I. K. and Palmer H. E. (1917) J. Am. Chem. Soc., 39, 136.

Phillips, R. A. and Freeman, N. E. (1933) Proc. Soc. Exptl. Biol. Med. 31, 286.

Rappaport, F. and Reifer, I. (1937) Mikrochim. Acta, 2, 62.

Reid, C. (1932) J. Physiol. 75, 40 P.

Reynoso, A. (1853) Compt. rend. soc. biol., 5, 116.
Quoted by Phillips and Freeman.

Riegel, C. (1927) J. Biol. Chem., 74, 135.

Robbins, B. H. and Pratt, H. A. (1936) J. Pharm. Exptl. Therap., 56, 205.

Ronzoni, E., Koechig, I. and Eaton, E. P. (1924) J. Biol. Chem., 61, 465.

Ross, E. L. and McGuigan, H. (1915) J. Biol. Chem., 22, 407.

Sahyun, M. (1931) J. Biol. Chem., 93, 227.

Sahyun, M. (1933) J. Biol. Chem., 103, 203.

Seelig, A. (1905) Arch. f. exp. Path.Pharmakol., 52, 481.
Quoted by Phillips and Freeman.

Shaffer, P. A. (1914) J. Biol. Chem., 19, 297.

Soffer, L. J., Dantes, D. A., Newburger, R. and Sobotka, H. (1937)
Arch. Int. Med., 60, 876, 882.

Tashiro, S. and Adams, H. S. (1914) Internat. Ztschr. f. physik-chem. Biol., 1, 450.

Uchida, S. (1926) Biochem. Z., 167, 9.

Van Slyke, D. D. and Cullen, G. E. (1917) J. Biol. Chem., 30, 289.

Van Slyke, D. D., Austin, J. H. and Cullen G. E. (1922)
J. Biol. Chem. 53, 277.

Vidal, J. (1933) Compt. rend. soc. biol., 112, 760.

Zwemer, R. L. and Newton, H. F. (1928) Am. J. Physiol., 85, 507.