HYPERLACTATEMIA OF HYPERVENTILATION

by

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PART I

A : INTRODUCTION

It has been known since 1916 (1) that hyperventilation in humans as well as in experimental animals produces an increase in blood lactic-acid concentration. This lactatemia of hyperventilation has not been regarded as a pathological condition that could endanger a patient's life. It is inferred that the condition in man usually is benign and passes off when the cause of hyperventilation is controlled. Huckabee (2) states that this is a form of lactatemia in which "excess lactate" does not occur, i.e., the lactate/pyruvate ratio remains normal, consequently the $NADH_2/NAD$ ratio also is not altered. In other words, this form of lactatemia is not related to an oxygen deficit.

Primary hyperventilation giving rise to an initial respiratory alkalosis occurs in man in a number of disease states: a) voluntary or hysterical hyperventilation (3,4); b) CNS lesions, e.g., cerebrovascular accidents; c) anoxemia, especially that of altitude (5); d) hepatic coma (6); e) salicylate poisoning (7); f) encephalitis (8); and g) excessive ventilation by artificial means on a respirator or under anesthesia.

The nature of the mechanism whereby hyperventilation causes an elevation in blood lactate concentration remains obscure. The fact that several observers have noticed that hyperlactatemia

occurs with hyperventilation of the passive type refutes the theory that this mechanism is due to the muscular work involved in hyperventilation per se.

As early as 1916 it was suggested that: "The production of lactic acid assists in the neutralization of the relatively increased base which results from blowing off of CO_2 from the blood or addition of alkalies"; MacLeod also called attention to the effect of alkalinity in increasing blood glycolysis. In 1922, Dale and Evans (9) suggested: "Probably the combination of blood alkalinity and deficient oxygenation due to low arterial pressure (in hyperventilation) will promote the formation of lactic acid in the tissues". Later, in 1958, Huckabee (2) wrote: "Lactic acid accumulation during hyperventilation represents a mechanism for quickly reducing body bicarbonate as an adjustment to alkalosis". Tobin (10), in 1964, studying the effect of pH changes on lactic and pyruvic acid in blood, concluded: "Some other influence of pH must be responsible for the changes noted", and postulated the existence of vascular changes affecting oxidation potential of tissues and, or some other oxidation--reduction system in the total electron transport chain that may be more susceptible to hydrogen-ion concentration than the lactate pyruvate pair.

The purpose of our study was to reproduce and attempt to explain in an experimental animal (dog) a pathological condition previously documented in two patients in our hospital (11).

The patients had severe, uncrontrollable hyperventilation secondary to damage of the central nervous system; they had a severe disturbance in acid--base balance, characterized by an alkaline arterial pH, very low arterial pCO_2, low serum standard bicarbonate, and a tenfold or more increase in blood lactic-acid level, and increase in blood pyruvate, and elevated L/P (lactate--pyruvate) ratio. Both patients showed clinical improvement, as well as correction of the acid--base homeostasis, after treatment by inhalation of 5% CO_2 in air periodically for a period of several hours.

Our animal studies were designed to study not only the total body changes in hyperventilation as represented by the arterial blood, but also regional changes occurring in the liver, muscle, and gut. Also included are the effects of CO_2 on the cardiovascular system and tonometric studies on lactate production in blood in vitro. Our experimental design was based on experiments by Eichenholz et al. (12). They showed that hyperventilation induced in dogs causes a rise in lactic and pyruvic acid concentration, and addition of 5% CO_2 to the hyperventilating animal produces a decrease in both acids. They measured both acids separately, but presented their results as the sum of both and did not report the lactate/pyruvate ratio.

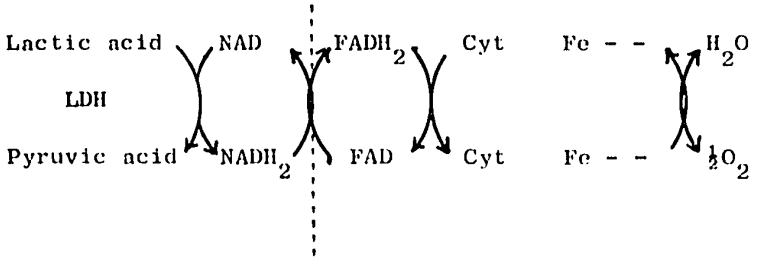
B : HISTORICAL AND PHYSIOLOGICAL REVIEW

1) Pathway of electron transport in oxidation of lactate to pyruvate

Lactic acid is the end product of anaerobic metabolism. Its precursor, pyruvic acid, is of primary importance, since lactic acid has to be reconverted to pyruvic acid to re-enter the metabolic pathways of the Kreb cycle for its utilization. Pyruvic and lactic acid, in resting normal circumstances, are in equilibrium. The reversible reaction

$$\text{Pyruvate} + NADH_2 \overset{\text{LDH}}{\rightleftharpoons} \text{LACTATE} + NAD$$

requires the presence of lactic dehydrogenase as a catalyst. The dehydrogenase systems are anaerobic, unable to transfer H ion directly to oxygen; consequently, the presence of another system is required to deal with molecular oxygen. Lactic dehydrogenase requires the presence of a coenzyme, nicotinamide adenine nucleotide (NAD), as an activator; this acts as a hydrogen- and electron-transfer agent by a reversible oxidation reduction system.



The link between the reduced NAD and its oxidized form is provided by the flavoproteins which act as a hydrogen-transfer agent to the cytochrome system.

All of these enzymes are located in the mitochondria of the intact cells.

The oxidation of reduced NAD by the flavoproteins located in the mitochondria is not a direct reaction. There is substantial evidence that direct mitochondrial oxidation of cytoplasmic NADH is slow or non existent (13,14). Systems of shuttling reducing equivalents from cytoplasmic NADH to the mitochondrial system and then to the flavoprotein system are described below. In the anaerobic glycolytic chain a single oxidative reaction occurs: the oxidation of glycerolaldehyde-3-phosphate to 1,3-diphosphoglyceric acid. NAD is the obligatory acceptor of electrons in this reaction. NAD is present in only catalytic amounts in the normal cell, and the presence of a mechanism for the continuous oxidation of the reduced NAD is essential for maintenance of glycolysis. The reduction of pyruvic acid to lactic acid is one such mechanism. The normal cell has other systems that compete with lactic dehydrogenase for oxidation of the extramitochondrial NADH in order to maintain the pyruvate-lactate equilibrium (14) and prevent lactate accumulation. These systems include the catalytic reduction and oxidation of 1) oxaloacetate and malate, and 2) dihydroxyacetone phosphate and glycerophosphate (15): both of these systems can react through the respective mitochondrial enzymes with the components of the respiratory chain. In this way hydrogen ion can be channeled toward its terminal oxidation in the mitochondria without actual reaction of lactate itself with the respiratory chain.

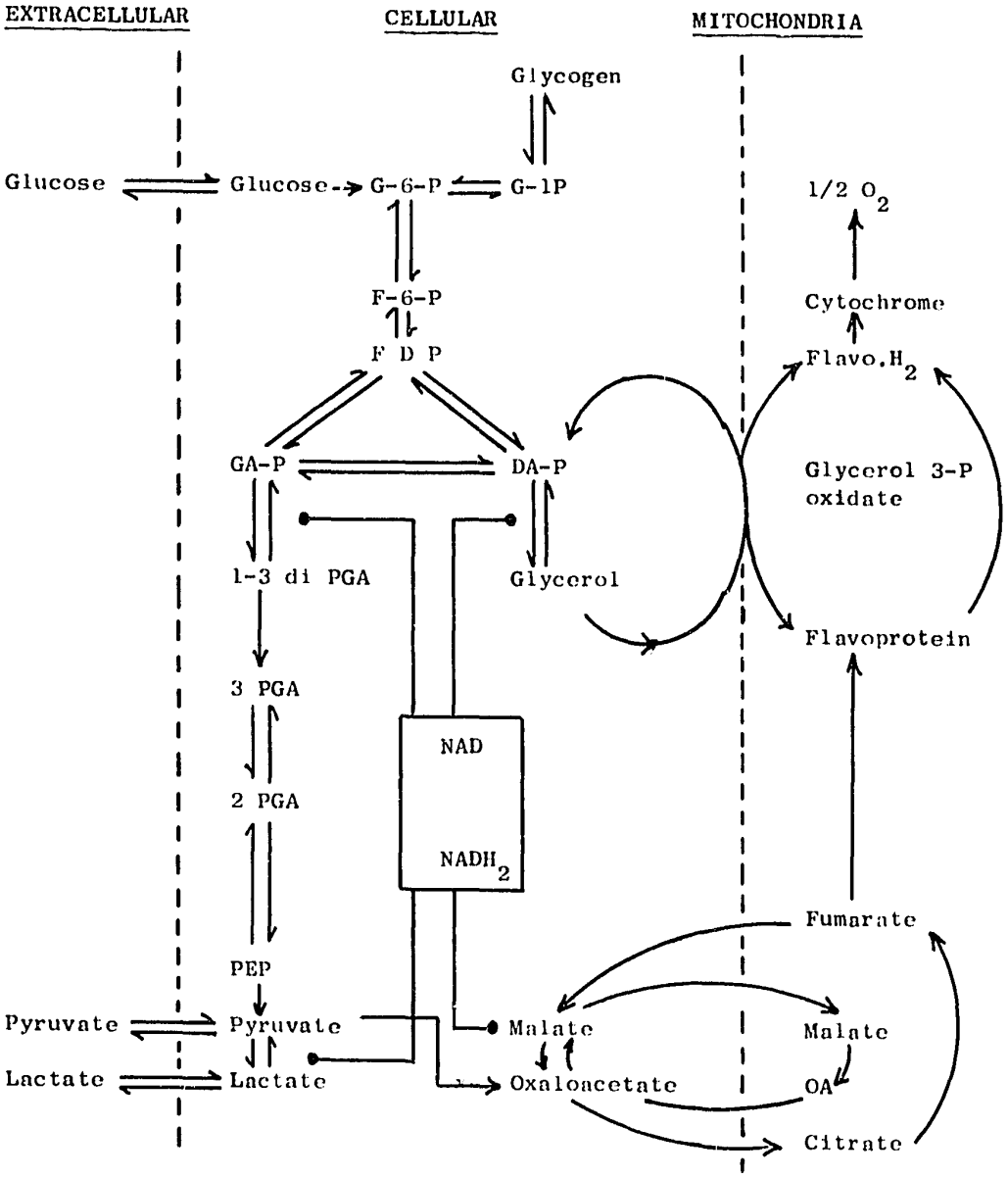


Figure 1: Schematic presentation of the catalytic reduction and oxidation of oxaloacetate/malate and dihydroxyacetone-phosphate/glycerophosphate shuttling systems (15).

2) Pyruvic acid: aerobic and anaerobic cycle

Pyruvic acid formed from the metabolism of glucose or from amino acids (e.g., alanine and aspartic acid) follows three main pathways: a) Conversion to lactic acid by lactic dehydrogenase and the NAD/NADH system; b) entrance into the Kreb's cycle by conversion to active acetate (e.g., acetyl coenzyme A) by oxidative decarboxylation, catalyzed by a decarboxylase requiring thiamine diphosphate, lipoic acid, and coenzyme A; and c) entrance into the dicarboxylic-acid shuttle by conversion to malic acid by direct carboxylation and reduction through the action of malic enzyme. Oxidation of malic acid to oxaloacetate follows and, through decarboxylation to enolphosphopyruvic acid, the reaction continues to the formation of glucose or glycogen.

If anaerobic conditions prevail, or if the reaction occurs in tissues without mitochondria (red blood cells), pyruvic acid is reduced to lactic acid by dehydrogenation of the reduced NAD which was produced by oxidation of glycerolaldehyde phosphate. The reduction of pyruvic acid to lactic acid then serves to regenerate NAD, which participates again as a hydrogen acceptor with oxidation of glycerolaldehyde phosphate to glyceric acid. This metabolic pathway is of special interest in the red cell, which is able to utilize glucose by the phosphogluconic pathway and the Embden--Meyerhof pathway, 90% of glucose being utilized by the latter (16).

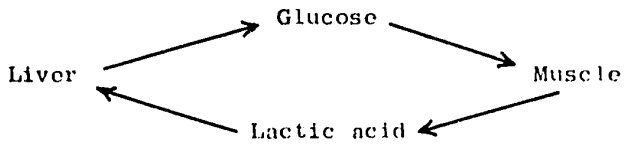
3) Physiological disposition of lactate and sites of production

Since the time of Claude Bernard it has been known that the liver supplies glucose to the body. Mann and Magath (17) have shown that the liver is the source of glucose for the maintenance of the blood levels of glucose. Cori and Cori (18) were the first to observe glycogen formation by the liver, and Hill, Long and Lupton (19) concluded from their studies in humans that the greater part of lactic acid produced during exercise was reconverted to glycogen.

Fletcher and Hopkins in 1907 (20) were the first to describe accumulation of lactic acid in the muscle during anaerobic contraction and its disappearance in the presence of oxygen. Meyerhof (21) and Embden et al. (22) studied the metabolism of carbohydrate and its by-products and delineated the anaerobic pathways in the metabolism of glucose: the Embden--Meyerhof pathway. Also Meyerhof (23) and, especially, Hill (24), made extensive independent studies on muscle metabolism, showing a relationship of lactic-acid production to contraction.

Himwich, Koskoff and Nahum (25) demonstrated the glucose--lactic-acid cycle existing between muscle and liver. They studied the regional concentration of blood lactate. The concentration of lactic acid in the blood leaving the muscle in the femoral vein is greater than that of the femoral artery.

The blood lactate coming from the liver in the hepatic vein is lower than that of the artery or portal vein entering the liver. Blood glucose concentration in the blood returning from the muscle, in the femoral vein, is less than that in the femoral artery, whereas the concentration of glucose in the blood leaving the liver, in the hepatic vein, is greater than that of the artery or portal blood. They proposed the classical scheme, also known as the Cori--Cori cycle:



Since the publication of the Cori--Cori cycle it has been accepted that lactic acid is produced by the muscle and utilized by the liver, but this interpretation of lactic-acid metabolism is far too simple in the light of present knowledge. The main limitation of this cycle is that it does not take into consideration the lactic-acid precussor, pyruvic acid. No such cycle exists for pyruvic acid; therefore, the Cori--Cori cycle is more an indicator of the difference in equilibrium of the tissue lactic dehydrogenase. Also, measurements of lactate and pyruvate content of different tissues of the body show great variability, as well as in their relative proportion to each other (15). The highest concentration of lactate is found in the blood, especially in the plasma. These findings point to another limitation of the Cori--Cori cycle, namely, that the blood itself was not considered as a metabolic organ and the metabolic activity of erythrocytes and leucocytes was ignored.

Guest et al., (26) have shown that blood utilizes 3.0 g. glucose in 24 hours; and Murphy (16) demonstrated that red blood cells utilize glucose mainly by the Embden--Meyerhof pathway, lactic acid being the final metabolic product (as the mature red cells do not have mitochondria and consequently no tricarboxylic-acid cycle).

4) The lactate--pyruvate ratio

With progressive understanding of carbohydrate metabolism and the discovery of different intermediary by-products, increasing attention has been paid not only to concentrations but also to the ratio of the concentrations of these two acids. The aerobic utilization of pyruvate in the presence of NAD and lactic oxidase, both necessary to maintain the lactate/pyruvate ratio, caused Friedeman (27), as early as 1941, to state: "The lactic/pyruvic ratio is an indicator of the oxidative condition of the tissues". He pointed out also that pyruvic and lactic acid accumulate in blood and tissues when carbohydrate is being metabolized at an increased rate in anoxia and during exercise (28). He gave the value of the L/P ratio as 11.3 ± 2. After attention had been called to the lactate/pyruvate relationship, various pathological and physiological states were described in which variation of L/P ratio was measured. These are considered below.

In the light of present knowledge concerning intracellular oxidation--reduction systems, a decrease in oxygen supply to the living

cell will shift the redox systems towards a more reduced state; cytochromes and their dependent systems (flavoproteins and NAD) will be shifted to their reduced forms. The lactic-dehydrogenase system is unique, in that lactic acid is a metabolic "cul de sac", unable to participate in any other reaction except for its reconversation to pyruvic acid.

$$\text{Pyruvate} + NADH_2 \xrightarrow{LDH} NAD + \text{Lactate}$$

Huckabee (2), in 1958, believed that lactate and pyruvate diffuses so readily out of cells that the lactate/pyruvate blood-concentration ratio will reflect the NAD/NADH equilibrium in the cells (67). He proposed a mathematical formula, based on the L/P ratio and NAD/NADH equilibrium, to express the intracellular state of oxygenation; the expression "excess lactate" indicated and index of the magnitude of the anaerobic glycolytic process.

XL = (Ln-Lo)-)Pn-Po) L/P

Where XL is excess lactate and

Ln and Lo represent experimental and control lactate, and

Pn and Po, experimental and control pytuvate, respectively.

Thus, Friedeman's concept (27) that the L/P ratio is an indicator of the oxidative condition of tissues now can be expressed in semiquantitative terms that take account of the ratio as well as the absolute concentration.

The presence of excess lactate, according to Huckabee, presupposes the presence of oxygen deficit that could be physiological or pathological. The only physiological state where excess lactate is present is during exercise (29). He showed close correlation between, removal of excess lactate and the oxygen debt acquired in exercise. Also in studies of severe induced hypoxia (at half alterial-blood saturation, with a PO_2 of 26 to 32 mm Hg.) excess lactate also correlated with oxygen deficit (30). The concept that whole-blood excess lactate is an indicator of the state of tissue oxygenation has been disputed lately by several authors. Hohorst et al., (15) called attention to the variability of lactate and pyruvate content as well as the L/P ratio in the different tissues. They pointed out that the highest content of both metabolites is found in the plasma and the resting skeletal muscle has a L/P ratio of 21.2. The heart has the lowest total amount of lactate and pyruvate. Alpert (13), in studies with exercised dogs, questioned the relationship between the excess lactate removed and oxygen debt (23). When hepatectomized dogs were exercised, the oxygen debt contracted was the same as in normal animals but the removal of excess lactate did not occur as it was prevented by exclusion of the liver.

The concept of excess lactate implies that lactate and pyruvate in the blood are in simple equilibrium with cytoplasmic lactate and pyruvate; it also implies that there is a simple relationship between cytoplasmic and mitochondrial NAD and $NADH_2$.

Finally, the content of mitochondrial NAD and $NADH_2$ determines cellular oxygen consumption. These three assumptions are questionable. Glaviano et al. (31) have shown in heart muscle a lack of equilibrium between plasma and tissue lactate. Thus, when plasma lactate was increased tenfold, the tissue lactate and pyruvate remained constant. Oxidation of cytoplasmic NADH by mitochondria is dependent on several metabolic reactions cited above, and may have an equilibrium different from the NAD/$NADH_2$ of mitochondria (15).

Nevertheless, Huckabee's major contribution was to call attention to the pathological states associated with hyperlactatemia in man (32). Before 1958, alterations in lactic-acid concentration were considered to be mainly of physiological interest and were studied in exercise, after exogenous epinephrine, and after glucose infusion. No attention was paid to the role of lactate in human disease. Hyperlactatemia in humans, according to Huckabee, can be classified into two groups:

Group 1: Various physiological stresses:

Hyperventilation with decreased pCO_2 and increased pH, treatment with epinephrine, glucose, insulin, or bicarbonate. In these patients both acids, lactate and pyruvate, increase proportionately, so no excess lactate occurs.

Group 2: Pathological states:

A) Hypoxia of recent onset, or cicurlatory collapse, in such excess lactate is present and pH is acid.

B) No obvious cause for lactatemia--patients with idiopathic excess lactate--also called "spontaneous lactic acidosis" (33).

The pathological significance of the lactatemia of hypoxic origin has been well documented (34,35). Hyperlactatemia of hyperventilation (Group 1) has not been considered a pathological situation likely to endanger a patient's life, a statemtn with which the group at the Royal Victoria Hospital cannot agree. To them, these patients are of special interest as, in their experience, hyperlactatemia of hyperventilation is a severe pathological condition that can be fatal. Also, it has been found to be a state, in man as well as in the dog, in which excess lactate and elevated L/P ratios do occur.

5) Experiments in which hyperventilation or alkalinisation cause hyperlactatemia

MacLeod (36,37), in 1917, reported in humans and dogs that an increase in blood lactic-acid levels occurred with addition of alkalis, together with a decrease in blood sugar concentration. He also observed increase in lactate in hyperventilation. His explanation for the lactatemia implied that it was a compensatory mechanism for restoration of the reduced free hydrogen-ion concentration.

Dale and Evans (9), studying the effects of hyperventilation on systemic blood pressure in cats, demonstrated a decrease in blood pressure as long as hyperventilation was maintained. The effect was the same if the animal breathed air, oxygen, or 7% oxygen and nitrogen mixture. When hyperventilation was discontinued the blood pressure returned to control levels. If the cats were hyperventilated with 4% or 5% CO_2 mixture, no drop in blood pressure occurred. Bicarbonate or hydrochloric-acid infusion had no effect on blood pressure. During hyperventilation they noted an increase in lactic-acid concentration, but it continued to rise despite the decrease in blood pH when hyperventilation was maintained for 1½ hours. They concluded that the rise in lactate was caused not only by the initial alkalinity but also by the deficient tissue oxygenation secondary to the decreased blood pressure.

Anrep and Cannan (38) studied the effect of hyperventilation on blood lactic-acid levels, using a heart--lung by-pass preparation in which they could maintain O_2 saturation and CO_2 tension at any desired level. They found that, even at oxygen saturation of 40%, blood lactic-acid concentration would decrease in the presence of 8% CO_2. Also, if oxygen content of blood increased to normal levels and CO_2 was removed, lactic acid increased fourfold. They concluded that the increase lactic in acid was determined primarily not by the oxygen or carbon-dioxide

tension but by the reaction of the blood, and that lactic-acid concentration in the blood was a buffering system against changes in pH. Long, in 1923 (39), breathing different gas mixtures, showed that pure oxygen has no effect on blood lactic-acid concentration but, if CO_2 is added to the inspired gas, blood lactate decreases. He also concluded that the changes in pH were the casual factor in determining blood lactate levels.

Bock, Dill and Edwards (40) found the normal level of lactic acid in humans to be 6 to 14 mg./100 ml., with an average of 10 mg./100 ml. In alkalosis induced by ingestion of high doses of bicarbonate, they find no significant increase in lactate; the same applied to ingestion of ammonium chloride. In hyperventilation induced by CO_2 inhalation, in spite of decreasing pH there was no increase in lactic acid. Anoxemia, induced by breathing low oxygen mixtures, caused lactic acid to rise only when extreme blood oxygen desaturation was produced. They concluded: "The lactatemia is not related simply to shifts of hydrogen ion concentration and the respiratory muscle production of lactic acid in hyperventilation cannot account for the rise in blood level concentration when the subject is hyperventilating voluntarily as opposed to CO_2 induced overbreathing".

Eichenholtz et al. (12) showed that sustained hypocapnia in dogs produced a bicarbonate deficit that is progressive as long as hyperventilation is maintained and can be accounted for by the rise in lactate and pyruvate. Addition of CO_2 with continuation of hyperventilation restores the bicarbonate level partially; again, the rise in bicarbonate was accounted for by decrease in lactate and pyruvate. In experiments where pH was controlled and pCO_2 varied, increases in lactic acid and pyruvic acid were related only to reduced CO_2. No rise in lactate and pyruvate occurred in hypoxia unless associated with hypocapnia. The authors concluded that the rise in lactate and pyruvate in hyperventilation is a homeostatic compensatory mechanism to pH changes but may become a pathological state later and may even become extensive enough to cause metabolic acidosis. By the administration of CO_2 the metabolic changes could be corrected. Unfortunately, they did not report separate values for lactate and pyruvate.

PART II

A : CLINICAL PRESENTATION

Case I. Hyperlactatemia in association with alcoholism and Wernicke's encephalopathy

The first patient, T. C., was a 55-year-old man, an alcoholic who was malnourished. For some months before admission he had an ataxic gait. His admission to hospital was precipitated by a confusional state of subacute onse associated with shortness of breath. He was hypotensive on admission and in acute congestive heart failure. On neurological examination he was noted to be confused and stuporous, hyperventilating, with a coarse nystagmus to lateral gaze, sluggish pupil responses, and absent reflexes at knees and ankles. He had no ophthalmoplegia. These changes were compatible with the diagncsis of Werniske's encephalopathy. Later, Korsakoff syndrome was diagnosed because of amnesia for recent events, confabulation, peripheral neuritis of the glove--stokcing type, and peripheral areflexia. Hepatic pre-coma was considered, but the consensus of opinion was against this view.

The subsequent changes in this patient can best be described by referring to Figure 2, in which arterial pH, standard bicarbonate, pCO_2 and blood lactate are plotted, as well as alveolar ventilation (in litres per minute) and urine volume, during the first four days of his stay in hospital. The ordinate of hydrogen-ion

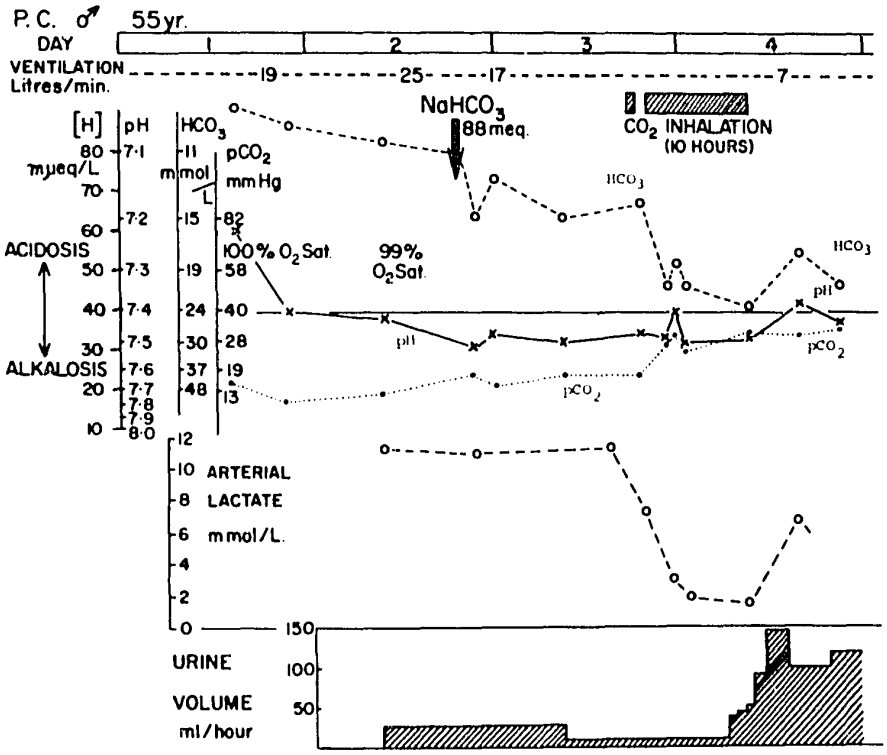


Figure 2: Arterial pH, standard bicarbonate, arterial pCO_2, alveolar ventilation and urine volume on patient P.C.

See explanation in the text.

concentration is expressed as m/mols/litre with equivalent pH values alongside. Bicarbonate concentration, in mMols/litre, is the standard bicarbonate of Astrup: this ordinate represents bicarbonate values present if the pCO_2 had been 40 mm. Hg in each sample. The pCO_2 ordinate represents the values at a given pH if the standard bicarbonate has been 24 mMols/litre in each sample. The degree of displacement of the bicarbonate line above normal range denotes the extent to which the serum bicarbonate is depressed. Similarly, the degree of displacement of pCO_2 below normal range indicates the degree of depression of arterial pCO_2. The blood lactate level, in mMol/litre, is indicated by the same symbol as serum bicarbonate. It can be seen that there is a parallel change between the degree of depression of serum bicarbonate and the degree of elevation of arterial lactate, eventhough the scale of the bicarbonate ordinate is not a simple arithmetic one.

The initial pH was 7.22, with a pCO_2 of 14 mm. Hg and a bicarbonate of 7 mMols/litre. The patient was treated for heart failure and later on, on the same day, the arterial pH had risen to 7.40 with a pCO_2 of 11 mm. Hg and bicarbonate of 9 mMols/litre. The ventilation rate was high and the total minute ventilation was measured by a Wright spirometer at 19 l./min. An arterial-blood lactate was drawn the following morning, at which time pH was 7.42, pCO_2 was 13.5 mm. Hg, and standard bicarbonate was 10.4 mMol/litre. During the second day the alveolar

ventilation was 25 litres/minute and later that day he was infused with 88 mMol sodium bicarbonate. This did not affect the degree of the hyperventilation significantly, and the pH was raised to 7.50 with a pCO_2 of 18 mm Hg and standard bicarbonate 15 mMol/litre. Throughout this period of time the arterial blood pressure was 100--110 mm Hg systolic and 65--80 diastolic. The patient was breathless and clammy, with cold extremities and peripheral cyanosis. The arterial oxygen saturation was measured twice and was 99--100%. Later, on the third day, the patient's general condition appeared to be deteriorating rapidly, blood pressure was lower, and the urine volume diminished. For this reason it was decided to try the effects of inhalation of 5% CO_2 in oxygen. As the initial 30-minute period of CO_2 inhalation was not associated with further or immediate deterioration, CO_2 was administered for 15 minutes in every 20 minutes over the subsequent 10 hours. The arterial pH was checked four times during this period of administration and it can be seen (Fig. 2) that, although the arterial pH remained on the alkaline side, there was a progressive rise in standard bicarbonate, to 23 mMols/litre and arterial pCO_2 was raised to 28 mm Hg. This period of forced CO_2 breathing was associated with considerable clinical improvement, especially noticed by increased body warmth, disappearance of peripheral cyanosis, and return to consciousness. The urine volume increased markedly.

During the fourth day hyperventilation continued but was much diminished. Throughout these four days the patient received a large amount of intravenous vitamin B_1, riboflavin and hypertonic glucose, on the basis that he had an acute vitamin-B deficiency. He gradually improved thereafter and returned to a semblance of normal health, though with Korsakoff syndrome, and was sent home.

Case II. A patient with diabetes mellitus, severe cerebrovascular disease, and lactatemia.

The second patient, M. S., was a 55-year-old female with a 35-year history of diabetes mellitus. Hyperglycemia and glycosuria had been difficult to control for many years. The major complication had been a cerebrovascular accident (with hemiplegia) 4 years previously and another episode of the same sort one year previously. She had residual motor aphasia but was fully conscious up to the time of her past admission, which was to re-establish control over her hyperglycemia.

A disturbance of acid--base homeostasis, with lactatemia, occurred during the last 5 days of her life; she had been placed on an oral hypoglycemic agent (DBI, 50 mg, b.i.d.) two days previously. Although this agent has been incriminated as a cause of lactic acidosis (65,66) we are not sure whether it played any role in the events that preceded her death. These events are shown in Figure 3.

It may be stressed that the blood glucose levels were considered "safe" throughout this 5-day period, and at no time were ketone bodies detected in the blood, nor was there oxygen desaturation of the arterial blood. Figure 3 records the respiratory rate per minute, blood pressure, and pertinent facts about the condition. Also shown are the arterial pH, pCO_2, standard bicarbonate, and lactate. The scales of the ordinate are the same as those on Figure 2.

As indicated already, the patient was placed on DBI on the 25th day; she remained well until the evening of the 27th day, when she was noted to be confused and hypotensive. During the night she became clammy and comatose and was noticed to be hyperventilating. As blood-streaked mucus was aspirated from her throat, gastro-intestinal bleeding was diagnosed and 3 units of blood were given on the 28th morning. However, hematocrit value just before the transfusion was unchanged. The blood pressure improved to a 95 systolic and 70 mm Hg diastolic, and attention then was drawn to the increased respiratory rate. By the early part of the afternoon of the 28th day the arterial-blood pH was 7.42, with arterial standard bicarbonate of 13 mMols/litre and pCO_2 of 18 mm Hg. Later that day she received blood and 88 mMols sodium becarbonate, with resultant decrease in the respiratory rate. Blood lactate was raised to 15.5 mEq/litre.

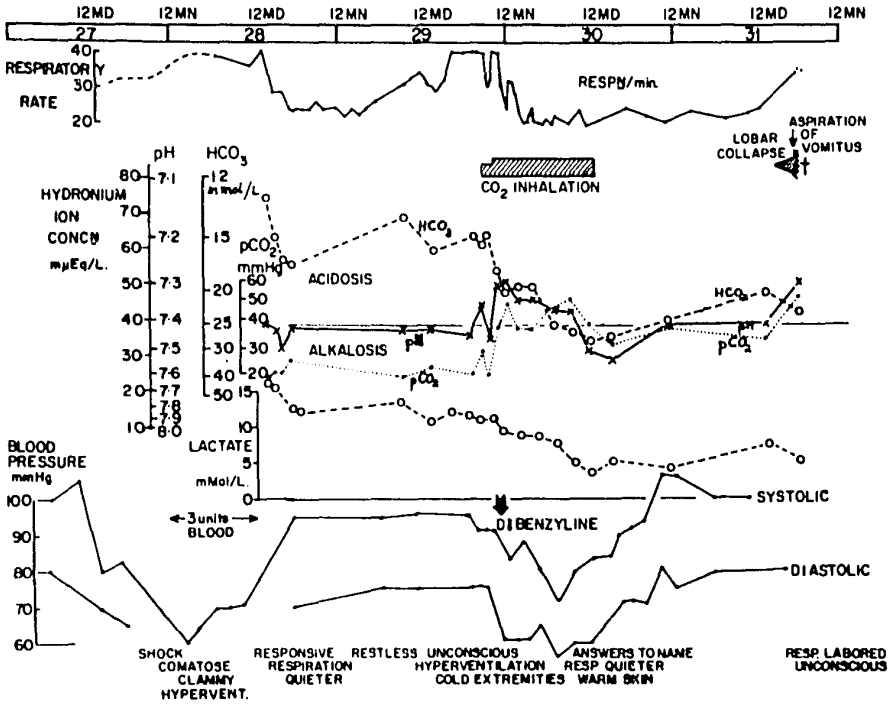


Figure 3: Arterial pH, pCO_2, standard bicarbonate, lactate, respiratory rate and blood pressure on patient M.S.

By the morning of the 29th day the hyperventilation was worse, with a respiratory rate of 30 to 34 per minute, pCO_2 19 mm Hg, and standard bicarbonate 14 mMol/litre; however, the pH was 7.45 (i.e., she was in mild alkalosis). Her condition deteriorated during the 29th day; blood pressure remained low but she was not in shock, although still unconscious and hyperventilating; her extremities were cold. There were few changes in the parameters of arterial pH, pCO_2 and standard bicarbonate during the rest of the day. It was decided late in the evening of the 29th day that she was moribund and that an attempt should be made to revive her by the administration of 5% CO_2 in oxygen for 15 minutes in every 20 minutes. It may be repeated here that the arterial oxygen saturation was above 96% on each occasion that the blood was sampled. After two periods of administration of CO_2 there was no apparent improvement and peripheral circulation was still very poor. For this reason it was decided to add the effects of peripheral ganglion blockage to any beneficial effects that might be occurring from CO_2 administration. Dibenzyline was administered in normal saline in a dose of 1 mg/kg/body-weight over 30 minutes. This, combined with CO_2, was associated with a fall in blood pressure, but an increase in warmth of the extremities and improved peripheral circulation was detected clinically. At the same time, the three parameters of acid--base homeostasis reverted to normal range, as shown in Figure 2, by the convergence of pH, pCO_2, and bicarbonate.

Arterial blood was drawn at the end of periods of CO_2 administration so that the pH would be at its lowest value and pCO_2 at its highest. There was a tendency for pCO_2 to fall again after the CO_2 was discontinued and the pH remained slightly more alkaline than normal. However, after the CO_2 and dibenzyline therapy, the respiratory rate was approximately 22 per minute; she was able to respond to spoken word, her skin was warm, and she appeared much better. Coincident with the return of standard bicarbonate toward normal values there was a decrease in the arterial blood lactate which reached its minimal value of 3.5 mMol/litre at the end of the period of CO_2 breathing, though subsequently rising again to 7.4 mMol/litre on the 31st day when the respiratory rate began to increase once more. On the 31st evening she vomited, aspirated, and died within a few minutes. On post-mortem examination, the medulla oblongata showed evidence of numerous, scattered, fresh hemorrhages.

SUMMARY

Case I: The first patient showed: a) lactatemia due to primary hyperventilation, b) regression of the lactatemia with forced CO_2 breathing, c) improvement in the peripheral circulation and general condition with CO_2 inhalation, and d) cessation of the hyperventilation when the primary mechanism of acute Wernicke's encephalopathy was treated with vitamin-B complex in massive doses.

Case II: This patient had an acid--base disorder as a result of primary hyperventilation caused by brain hemorrhage. As in the first patient, the lactatemia regressed with forced CO_2 breathing and ganglion blockade and the peripheral circulation and state of consciousness improved.

Both patients presented with alkaline pH, despite the high concentration of blood lactic acid.

A) DISCUSSION:

It is not known how respiratory alkalosis causes accumulation of lactic acid. In the past, it has been postulated that the lactatemia of hyperventilation is a homeostatic compensatory mechanism to pH changes (1,2). Others have suggested a combination of factors, mainly the blood alkalinity associated with low tissue oxygenation caused by the vasospastic effects of low arterial pCO_2 (9). Low tissue oxygen concentration will affect the oxidation reduction systems in the total electron-transport chain, not only of the lactate--pyruvate pair but perhaps also of some other redox system more susceptible to pH changes (10).

Blood pytuvate was not measured in the first patient; in the second case, blood pyruvate was elevated, although not proportional to the blood lactate increase, with consequent elevation of L/P ratio and "excess lactate"(Figure 4 and Table 1).

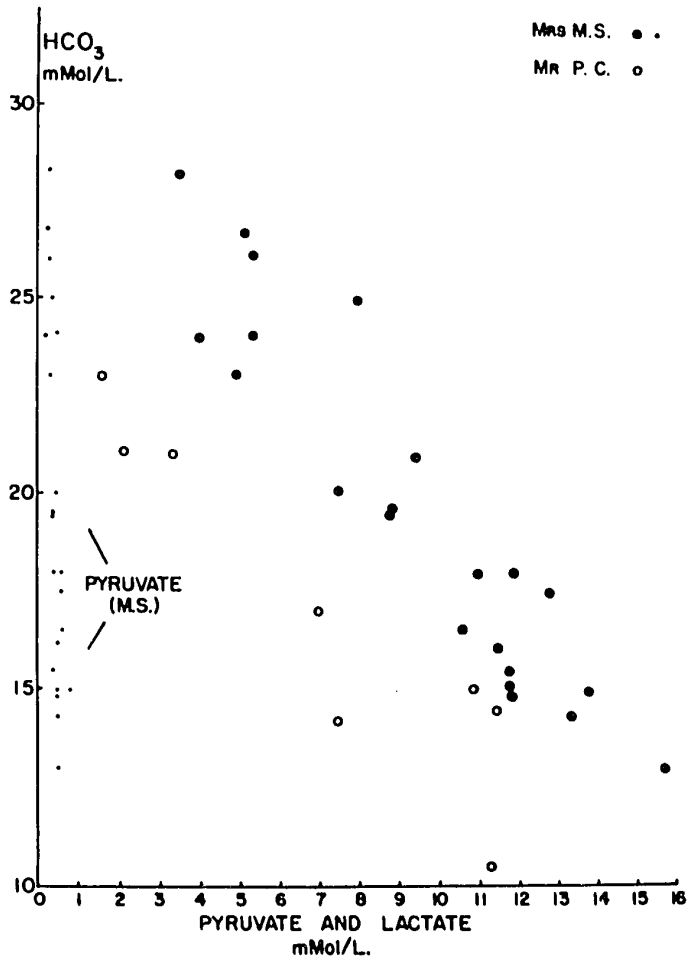


Figure 4: Blood pyruvate and lactate concentration in mM/l on the abcissa and standard bicarbonate in mEq/l on the ordinate.

St. HCO_3^- mEq/l.	Lactate mEq/l.	Pyruvate mEq/l.	Lactate / Pyruvate ratio
13.0	15.7	0.52	30.2
15.0	13.8	0.83	16.6
17.5	12.8	0.61	21.0
18.0	11.9	0.60	19.8
14.3	13.3	0.55	24.0
16.5	10.6	0.60	17.7
15.5	11.7	0.45	26.0
15.0	11.7	0.51	23.2
16.2	11.5	0.49	23.2
14.8	11.8	0.51	23.2
18.0	11.0	0.46	23.8
21.0	9.45	0.49	19.3
19.5	8.7	0.46	18.7
19.5	8.8	0.45	19.3
25.0	8.0	0.36	22.0
26.2	5.3	0.35	15.1
28.4	3.45	0.26	13.2
26.8	5.15	0.32	16.1
24.2	4.0	0.25	8.7
24.2	5.35	0.46	11.6
20.2	7.55	0.45	16.5
23.0	4.9	0.34	14.4

Table 1: Standard bicarbonate, lactate and pyruvate concentration in mM/l, and the L/P ratio on patient M.S.

This finding is opposed to Huckabee's statement (2) that the L/P ratio in hyperventilation is not altered and that no "excess lactate" occurs. It is of historical interest that, as early as 1923, Harrop and Loeb (41) presented a patient with epidemic lethargic encephalitis with severe uncontrollable hyperventilation and blood pH 7.59. No treatment was given to this patient, but the authors commented: "Alkali therapy which might be suggested by reason of the low plasma bicarbonate, would be contraindicated, although breathing of air mixtures containing carbon dioxide might conceivably be useful". Since 1938 attention has been called to hyperventilation in hysterical patients (42--44), and the beneficial effects have been known of rebreathing expired air in controlling the hyperventilation as well as in relieving neurological symptoms associate with overbreathing. It is doubtful if hysterical overbreathing would ever be severe enough to cause such a severe degree of lactatemia as that suffered by the present patients.

It is believed by the Royal Victoria Hospital group that the accumulation of blood lactic acid is caused by the low pCO_2 rather than by the pH changes *per se*. Low pCO_2 of the arterial blood is known to cause peripheral vascular changes (45,46) as well as vasoconstriction of the brain vessels (47), and McGregor and Donevan (48,49) have reported an increase in cardiac output, related to the CO_2 content in the inspired air, during hyperventilation.

Their experiments lasted a total of 3 to 4 minutes. (No references can be traced in the literature that related to cardiac output and sytemic blood pressure during long-lasting hyperventilation.) Also, the dissociation curve of oxyhemoglobin is affected by low pCO_2, causing hemoglbin to cling more to oxygen. It has been suggested that hyperventilation may actually impair release of oxygen from hemoglobin to the tissues (50) even when hemoglobin is well saturated. This is not probable as an explanation of hyperlactatemia, as it has been shown that only when oxygen saturation is reduced to 50% is hypoxia the causal factor in lactatemia: This will postulate a pO_2 in the range of 20--30 mm Hg at pH range 7.2--7.6 (29).

The vascular changes caused by low pCO_2, together with a decrease in O_2, supply would be a stimulating factor for increased production of lactic acid by the tissues, especially the muscles. Also, the effect of low pCO_2 on the hepatic blood flow must be considered: impairment of hepatic blood flow might cause impairment in hepatic utilization of lactic acid. Glucose metabolism and its by-products is of special interest in those circumstances. Glycolysis is stimulated by alkaline pH and low pCO_2 in liver, kidney and muscle in vitro (51) as well as in the red cells (16). On the other hand, glycogen synthesis is inhibited by alkaline pH and by low pCO_2 (52). As there is no simple explanation for the mechanism(s) of the hyperlactatemia of hyperventilation, an attempt was made to reproduce the hyperventilation syndrome in a laboratory animal, to try to elucidate this complex problem.

PART III

ANIMAL EXPERIMENTS IN VIVO

A : METHODS

(1) Ten mongrel dogs, 10 to 15 kg. wt., were anesthetized lightly with pentobarbital sodium 6%, 30 mg./kg./body weight, intravenously, while intubated with an endotracheal cuffed tube and connected to a positive-pressure Harvard piston-pump respirator, and breathing room air. A small-lumen catheter was inserted into the trachea through the endotracheal tube and was connected to a water manometer to monitor the endotracheal pressure. Respiratory rate and volume were calculated from the Harvard ventilation graph for laboratory mammals in resting state. The inspired and expired volumes were checked periodically with a Wright spirometer. Succinocholine (Anectin), 10 to 20 mg., was injected intravenously to achieve muscular paralysis; this was repeated periodically as necessary, especially after the animals recovered from baseline anesthetic. The right femoral vein and artery were exposed and cannulated with polyethylene tubing. The artery was connected to a mercury manometer and blood pressure was recorded. The femoral vein was used for whole-blood transfusion at a preset speed by a Sigmamotor pump to maintain steady blood volume during the whole experiment.

The left femoral vein was exposed and catheterized with a small-lumen polyethylene tube, against venous flow, deep into muscle, for sampling of venous blood that drained from the muscle. The right external jugular vein was catheterized with a radiopaque catheter, and the tip was placed into the pulmonary artery for measurements of cardiac output by the Fick method. Another catheter was guided into the hepatic vein. An abdominal midline incision was made and the position of the hepatic catheter was checked. The portal vein was exposed and a small polyethylene catheter was inserted directly into the portal vein, as close to the liver as possible, under manual guidance (Fig. 5). The abdomen was closed, and a period of 30 to 60 minutes was allowed to reach a steady state. Also, as a rule, at this time the animals had regained consciousness; paralysis was maintained by periodical injections of succinocholine.

(2) In two experiments, the internal jugular vein and the femoral artery were cannulated with polyethylene catheters. In these experiments, abdominal surgery was omitted.

(3) In one experiment, a porto-caval shunt (end to side) was performed and the hepatic artery was ligated, excluding the liver from the circulation.

(4) In three experiments, liver blood flow was measured. The hilum was exposed through an incision in the 11th left inter-

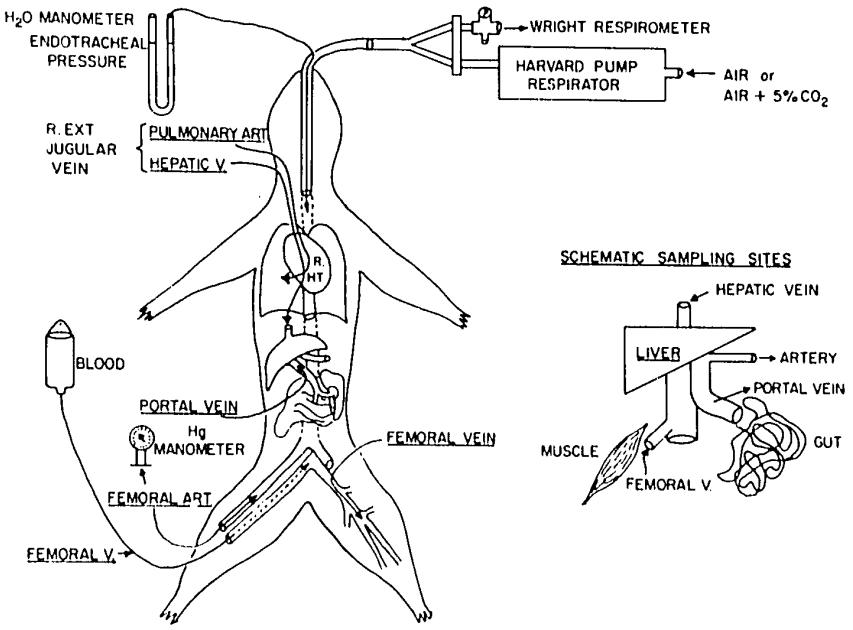


Figure 5: Schematic presentation of the experimental design and sampling sites.

costal space and incision of the left diaphragm. After exposing the hepatic hilum an electromagnetic flow-meter probe was carefully placed around the hepatic artery and the portal vein (53-55).

(5) In three experiments, a catheter was guided to the right renal vein and the femoral artery also was cannulated.

B : EXPERIMENTAL PROCEDURE

The design of all experiments was the same; after steady state was achieved at the end of the surgical procedure (usually 30--60 minutes from the end of surgery), base-line blood samples were collected, as well as cardiac output or liver flow, depending on the type of the experiment. After sampling for base-line values, the animals were hyperventilated with room air for a period of 3 hours, and 1 hour with 5% CO_2 in air. During the hyperventilation period with room air or 5% CO_2, special care was taken to maintain, steady respiratory volume and rate, as well as steady endotracheal pressure, as high positive pressure is known to decrease cardiac output (56). Hourly samples of blood were collected throughout the whole experimental procedure. Arterial blood samples were collected in heparinized syringes for pH, pCO_2, standard bicarbonate and O_2 saturation, and in 4 experiments for glucose. Samples for lactic and pyruvic acid were pipetted

directly into 2-ml. volumetric pipettes which were connected to the tip of the catheter and drained immediately into ice-cold (0°C) test tubes containing 8 ml. 11% trichloroacetic acid. Venous blood samples were collected for lactic and pyruvic acid as well as for oxygen saturation, and in some experiments for glucose.

In all experiments an arterial blood sample was obtained 15 minutes after the start of hyperventilation, for pCO_2, pH, and standard bicarbonate. This blood sample was used as a guide of the degree of hyperventilation achieved, aiming at a pCO_2 below 15 mm Hg with maintenance of this value for the next three hours.

C : LABORATORY PROCEDURE

Cardiac output was measured by the Fick method in five experiments. Simultaneous Blood samples were collected from the pulmonary artery and femoral artery simultaneously with determination of two-minutes expired gas volume. Blood oxygen saturation was measured on a Beckman DU spectrophotometer using Nahas cuvettes. The expired gas was collected into a meteorological balloon (made of Neoprene) and the oxygen content was analysed by a Beckman F3 paramagnetic O_2 analyser. CO_2 was measured by a Cambridge thermoconductivity CO_2 analyser. Arterial pH, pCO_2

and standard bicarbonate were measured on a macro-Astrup apparatus attached to a pH 22 Radiometer pH meter. Lactic acid was measured by the Barker and Summerson (57) method (S.E. $\pm$ 0.008 mMol/litre in this laboratory). Pyruvic acid was measured by the Friedman and Haugen (58) method, with S.E. $\pm$ 0.005 mMol/litre in this laboratory.

Glucose was measured by Hoffman's method (59) adapted for use in an AutoAnalyzer (Technicon Instrument Corp., Clauncey, N.Y.).

D : RESULTS

(1) Blood pH, pCO_2, and standard bicarbonate (Fig. 6)

In all hyperventilation experiments the blood pH rose 15 minutes after the start of hyperventilation by a mean of 0.290 pH units from control value (pH 7.3) and thereafter gradually fell toward control though remaining alkaline. When 5% CO_2 was added to the inspired gas, pH became acid, with a mean decrease to 0.170 pH units below baseline (Fig. 6 A).

The arterial blood pCO_2 decreased an average of 26 mm Hg after 15 minutes from the start of hyperventilation (pCO_2, 38.5) and remained steady thereafter. When the animal breathed 5% CO_2, arterial pCO_2 rose an average of 18 mm. Hg above the control value (Fig. 6 B).

Standard bicarbonate decreased an average of 5 mM/litre after 15 minutes of hyperventilation, and continued to decrease, but at a slower rate, during the next three hours, to a mean of 10 mM/litre. When 5% CO_2 was added to the inspired air, bicarbonate rose by a mean of 6 mM/litre. In no instance did it reach the control level (Fig. 6 C).

(2) Arterial blood pressure and cardiac output (Fig. 7)

The mean systemic blood pressure, initially 134 mm. Hg,

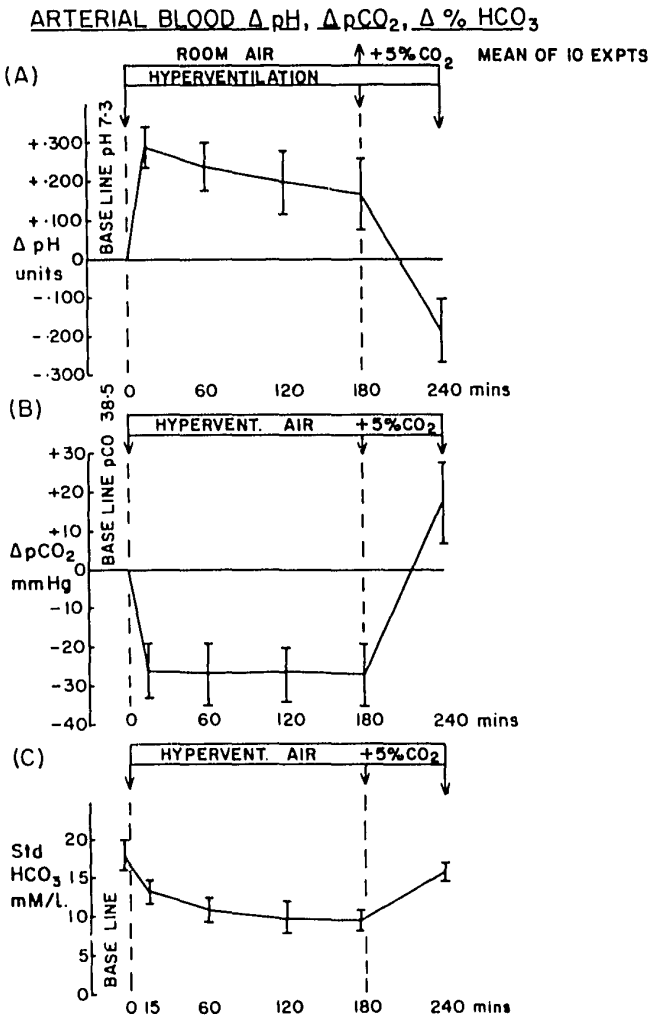


Figure 6: Changes in pCO_2, standard bicarbonate and pH of arterial blood during hyperventilation. Time in minutes in the abcissa.

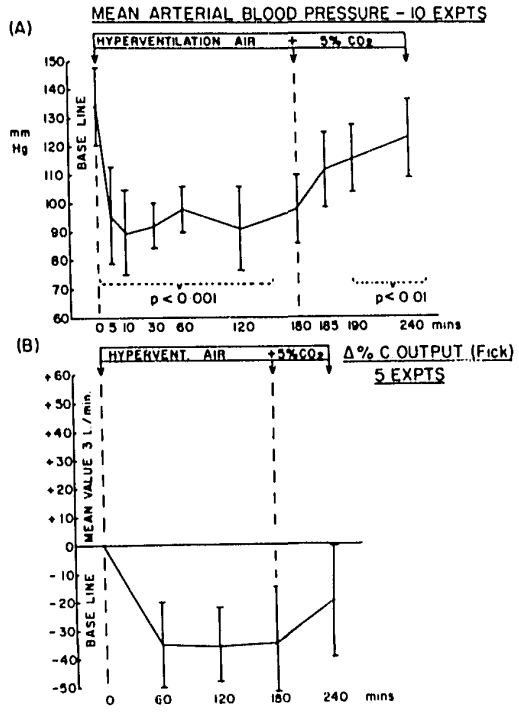


Figure 7: Mean arterial blood pressure and changes in cardiac output during hyperventilation.

dropped rapidly in the first 5 minutes of hyperventilation to a mean of 95 mm. Hg and remained low until, when 5% CO_2 was added to the inspired gas mixture, it gradually increased to a mean of 124 mm. Hg (Fig. 7 A).

Cardiac output was measured in 5 experiments. From a mean base-line value of 3 litres/minute, there was a drop of 35% during hyperventilation with room air. With the addition of 5% CO_2, cardiac output increased slightly but only to 20% below the control level (Fig. 7 B). It can be seen in Fig. 8 that the changes in cardiac output followed closely those in systemic blood pressure.

(3) Oxygen saturation (Fig. 8)

Oxygen saturation was measured in four different sites: arterial blood, hepatic, and portal and femoral venous blood. The arterial-blood oxygen saturation did not show statistically significant change throughout the experiment, nor did that in the hepatic or portal venous blood. There was a tendency for hepatic vein and portal vein oxygen saturation to drop after 3-hours' hyperventilation of room air (Fig. 9), returning toward but not achieving control level after 5% CO_2 was added: this decrease was not statistically significant. (The changes in portal vein follow closely the decrease in portal blood flow as measured by the magnetic flow meter.)

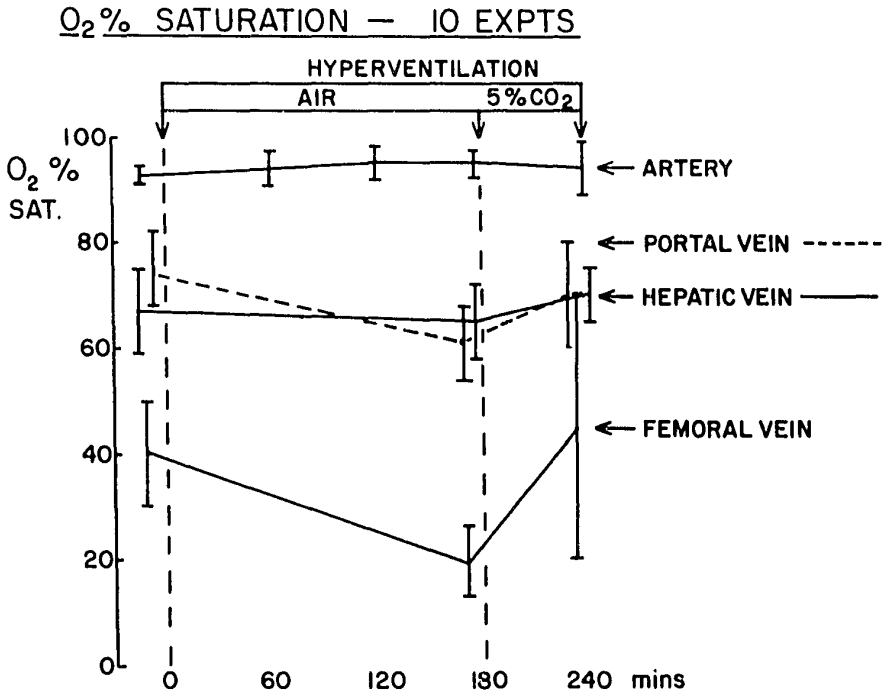


Figure 8: Changes in oxygen saturation in arterial and venous blood during hyperventilation.

Femoral-vein oxygen saturation decreased significantly after 3 hours' hyperventilation; when 5% CO_2 was added, in some animals the oxygen saturation remained low and in others it increased above control value (Fig. 8).

(4) Oxygen uptake (Fig. 9)

Oxygen uptake was measured in five experiments. No significant change occurred throughout room-air hyperventilation, but a significant increase occurred when 5% CO_2 was added to the inspired-air line (Fig. 9).

(5) Blood lactic-acid and pyruvic-acid concentration (Fig. 10)

Blood lactic acid and pyruvic acid were measured at four different sites: artery, hepatic vein, portal vein, and femoral vein. In all four sites the blood lactate increased linearly during 3 hours' room-air hyperventilation. When 5% CO_2 was added to the inspired gas, blood lactic acid returned to, or below, control values (Fig. 10 A--D).

Blood pytuvic-acid concentration also behaved similarly in all four sites, although the increase in pyruvate was lenear only during the first hour of hyperventilation; thereafter it levelled off as a plateau. When 5% CO_2 was added, pyruvate concentration decreased, but at no time did it return to the base-line value.

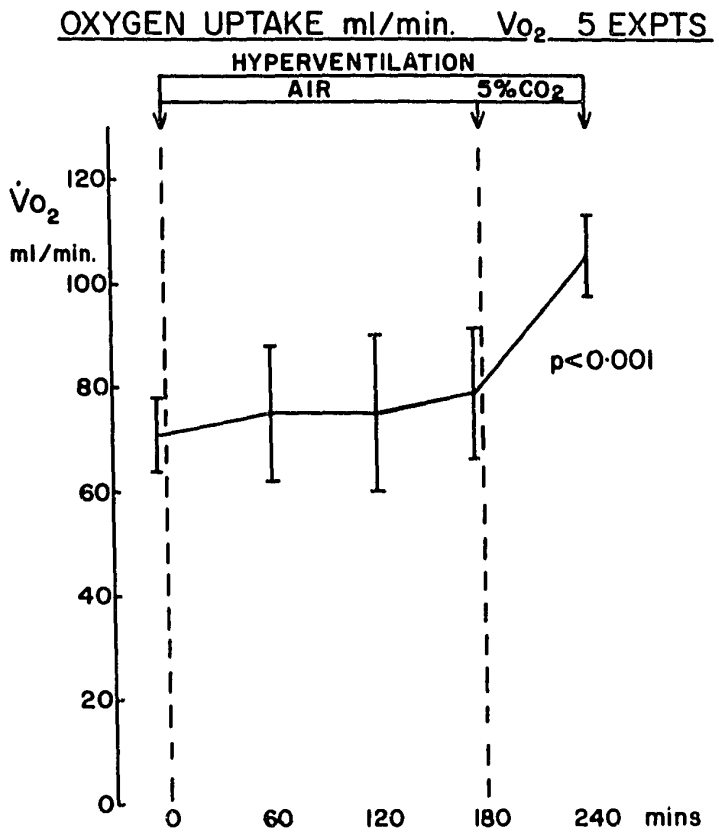


Figure 9: Oxygen uptake in ml./minute during hyperventilation.

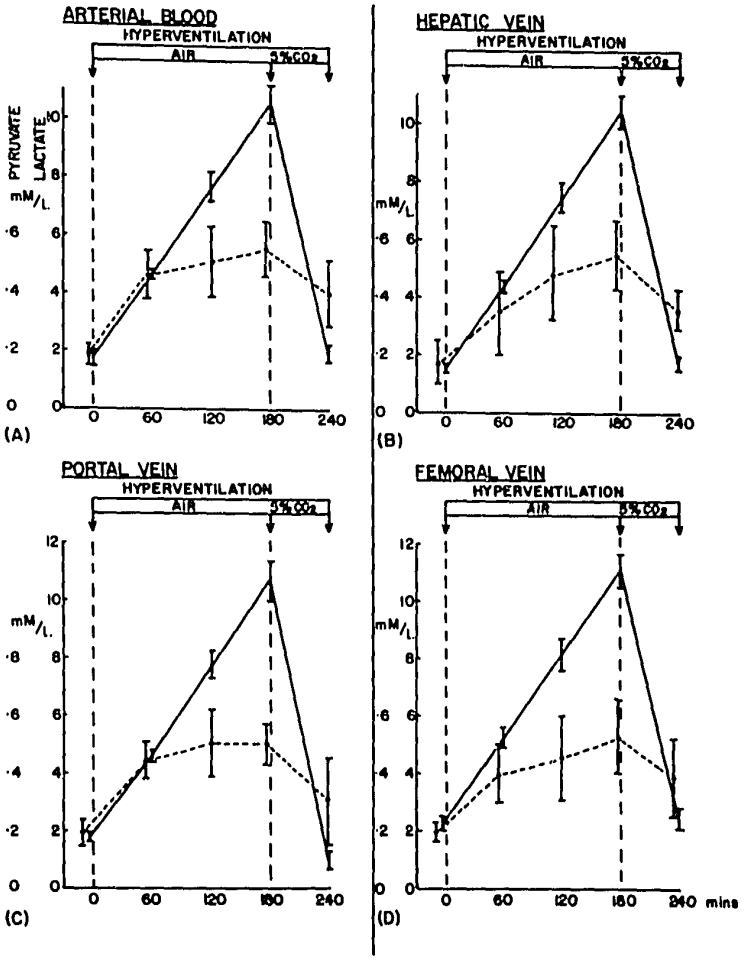


Figure 10: Blood lactate and pyruvate concentration in hyperventilation
Lactate is plotted as a solid line; pyruvate as a dotted line.

Fig. 10 shows that lactate and pyruvate rose in parallel fashion in the first 60 minutes and the lactate/pyruvate ratio remained unchanged; after the first hour the L/P ratio increased gradually until when 5% CO_2 was added, it decreased below the control value, since lactate returned rapidly to base-line levels and pyruvate values remained elevated.

(6) Correlation between arterial pCO_2 and bicarbonate with lactate and pyruvate (Fig. 11 and 12)

During hyperventilation, close correlation was noted between the blood pCO_2 content above 15 mm. Hg and lactic-acid concentration, with a correlation coefficient of 0.845 ($P > 0.001$). When the pCO_2 value was less than 15 mm. Hg, lactic-acid levels continued to rise, despite the steady arterial pCO_2. Similar correlation can be seen with arterial-blood standard bicarbonate. There is a poor correlation between blood pCO_2 and blood pyruvate and between standard bicarbonate and pyruvic acid.

(7) Blood lactate arterial--venous differences (Fig. 13)

Data from three organs will be presented: liver, muscle, and gut. It can be seen in Fig. 13 A and B that at all times during the experiment the liver utilized lactate with a steady A--V concentration difference of 0.20 to 0.30 mM/litre. The gut showed minimal production at all times, with A--V-concentration difference 0.03 to 0.07 mM/litre. Muscle produced lactate, with an average concentration

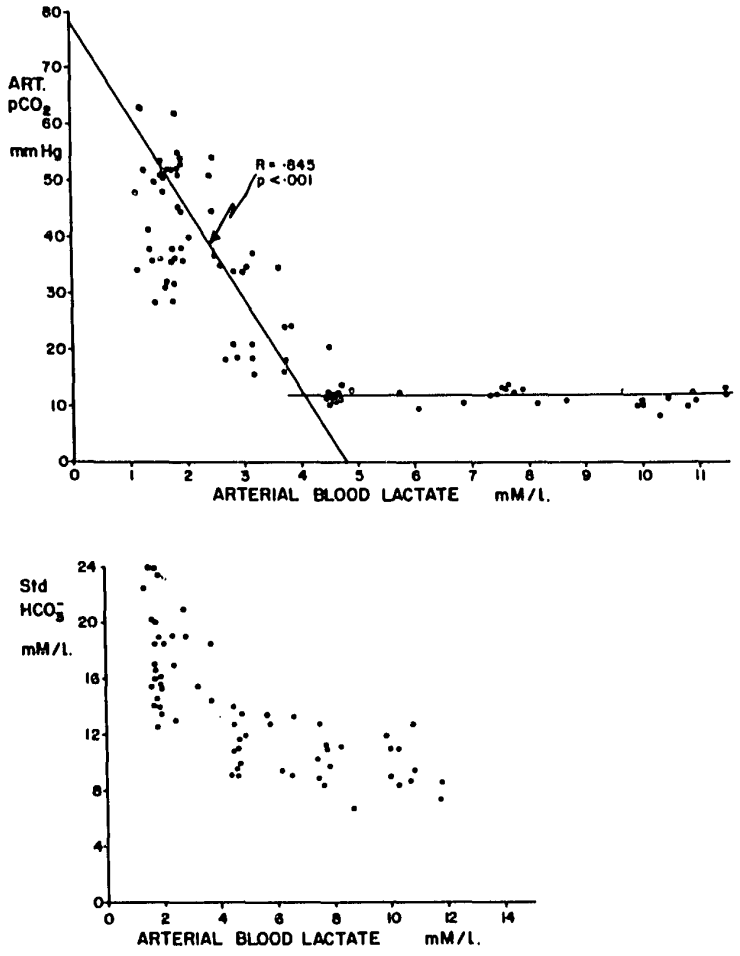


Figure 11: Correlation between arterial pCO_2 and arterial blood lactate (upper half). Arterial standard bicarbonate and arterial blood lacatate (lower half).

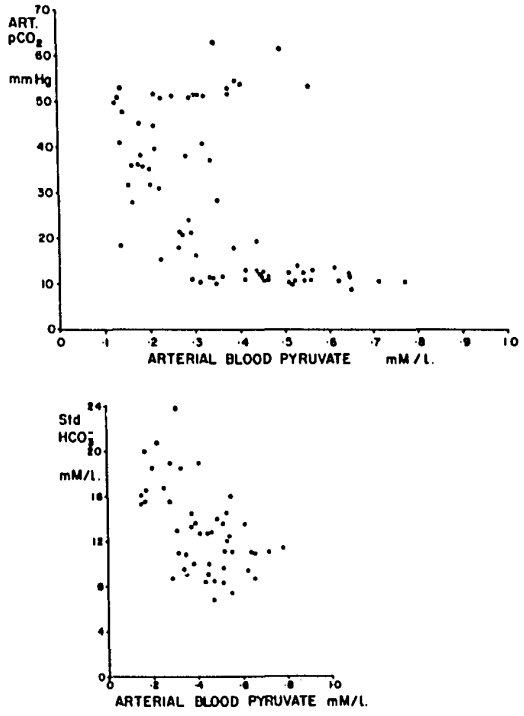


Figure 12: Correlation between arterial blood pCO_2 and arterial blood pyruvate (upper half). Arterial blood standard bicarbonate and arterial blood pyruvate (lower half).

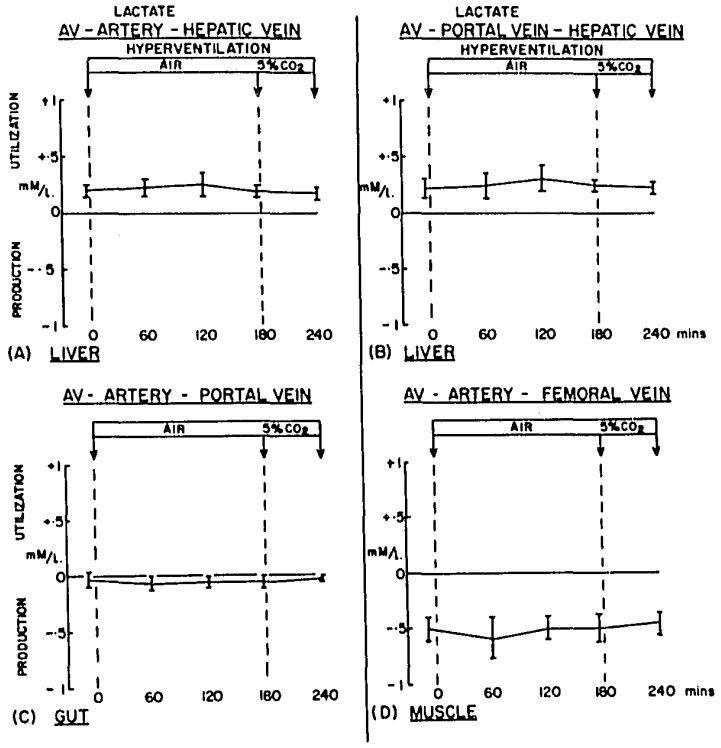


Figure 13: Blood lactate arterio-venous (A-V) differences.

difference of 0.47 to 0.59 mM/litre; but, again, this concentration difference was unaffected by hyperventilation. Hyperventilation with room air or with 5% CO_2 did not alter the metabolic balance between utilization and production across the liver, gut, or muscle, as detected by A--V-concentration differences (Fig. 13 A--D).

(8) Blood pyruvate arterio--venous differences (Fig. 14)

The liver, as seen in Fig. 14 A and B, showed net pyruvate utilization. At 60 and 120 minutes' hyperventilation this utilization was significantly increased above control values, but at the end of the 3rd hour, the liver started to add pyruvate to the circulation (Fig. 14 B). When 5% CO_2 was added, no statistical changes were observed, compared with values as the preceding hour. The gut (Fig. 14 C), judging by the mean values, did not utilize or produce pyruvate in the control period nor in the first two hours' hyperventilation. At three hours, and when CO_2 was added, a statistically significant utilization of pyruvate occurred. The muscle (Fig. 14 D) showed statistically significant increase in pyruvate utilization after one hour's room-air hyperventilation, decreasing thereafter and returning to control values with 5% CO_2. The standard deviations for pyruvate were greater than for lactate, a fact that has been observed by other authors (60).

(9) The $\frac{\text{lactate/pyruvate artery}}{\text{lactate/pyruvate vein}}$ ratio (Fig. 15)

The $\frac{\text{L/P A}}{\text{L/P V}}$ ratio across the liver, gut and muscle should

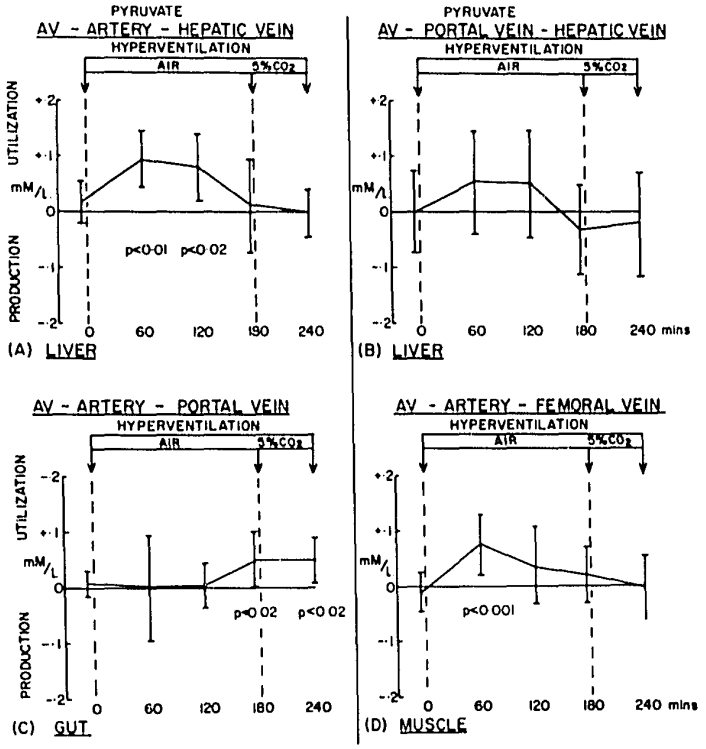


Figure 14: Blood pyruvate arterio-venous differences.

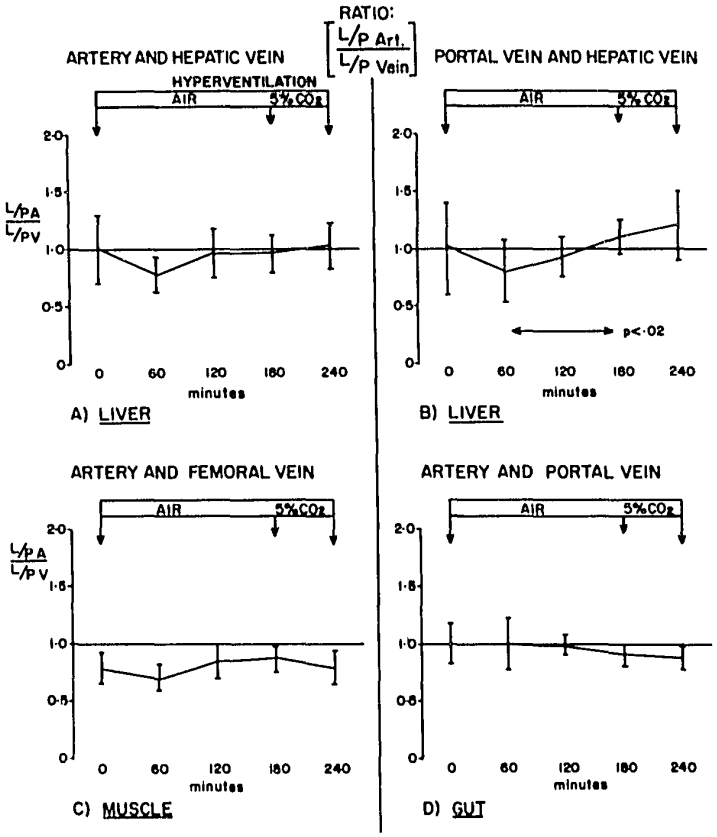


Figure 15: The $\frac{\text{L/P Artery}}{\text{L/P Vein}}$ ratio

theoretically portray the state of oxygenation of these tissues. It can be seen in Fig. 15 A and B that the $\frac{L/P\ A}{L/P\ V}$ ratio across the liver decreased slightly by the end of one hour's hyperventilation with room-air, but these changes were not statistically significant. At the end of 3rd and 4th hour the $\frac{L/P\ A}{L/P\ V}$ ratio increased significantly as compared with the one-hour value, indicating a better state of oxygenation or an increased proportion of pyruvate released into venous effluent blood (Fig. 15 B). The $\frac{L/P\ A}{L/P\ V}$ ratio across the gut (Fig. 15 D) remained unaltered for the first two hours' room-air hyperventilation. After three hours, there was a slight decrease in ratio, but this was not significant. In muscle (Fig. 15 C), the $\frac{L/P\ A}{L/P\ V}$ ratio remained unaltered throughout the experiment.

(10) Glucose arterial--venous difference and arterial-blood glucose concentration (Fig. 16)

Arterial-blood glucose concentration was measured in four hyperventilation experiments, and in two the arterial--venous-concentration differences across liver, gut, and muscle. It can be seen (Fig. 16 A,B) that the liver added glucose to the circulation in the control period. At the end of one hour's hyperventilation the liver increased its production of glucose, returning toward control values thereafter but continuing to produce glucose at all times. The muscle, as portrayed in Fig. 16 C, utilized glucose at a higher rate at one hour's hyperventilation and decreasing thereafter. When 5% CO_2 was added, no statistically significant changes occurred. It can be

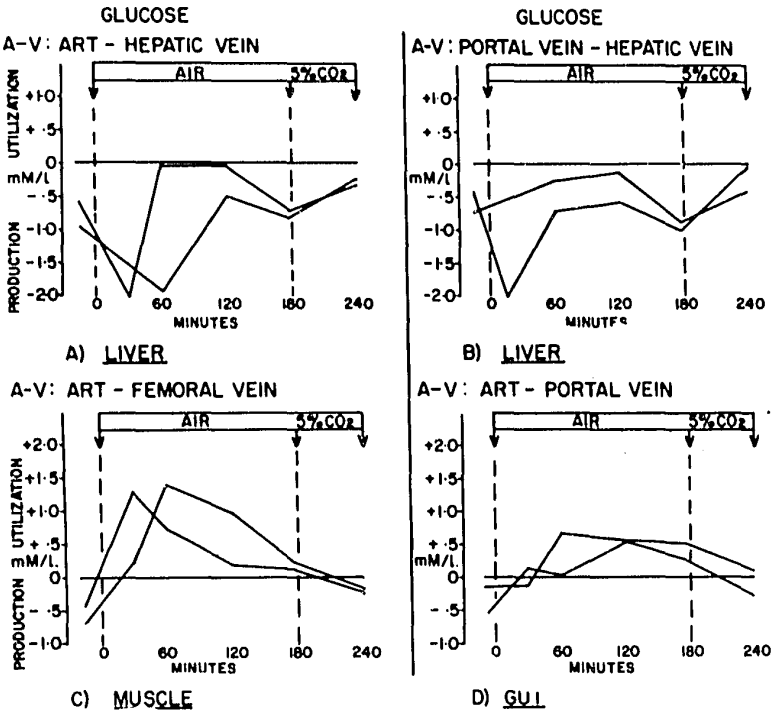


Figure 16: Blood glucose arterio-venous differences.

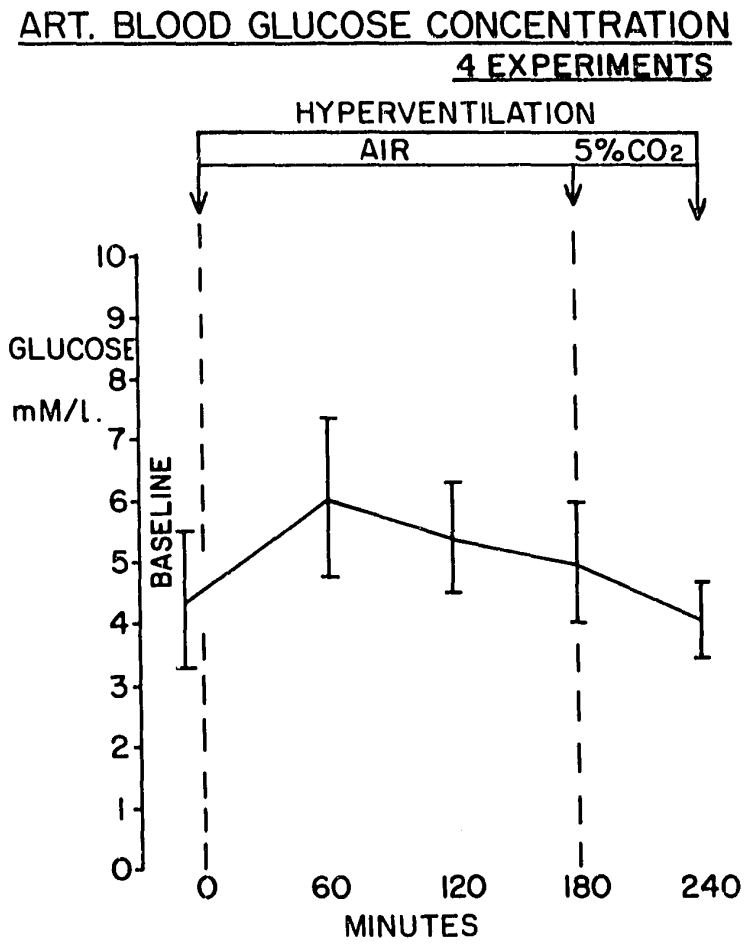


Figure 17: Arterial blood glucose concentration during hyperventilation.

seen (Fig. 17) that at the end of one hour's hyperventilation, arterial-blood glucose concentration increased significantly from control values, decreasing thereafter as long as hyperventilation persisted.

(11) Liver exclusion from the circulation (Fig. 18)

In one experiment, the liver was excluded from the circulation by means of a porto-caval shunt and ligation of the hepatic artery. When hyperventilation was instituted for one hour after "removal" of the liver, an increase in arterial blood lactate occurred; after one hour this elevation was proportionately the same as that which occurred in the intact animal (Fig. 18 A). Blood pyruvate changes were slight, indicating rapid and more complete conversion to lactate and suggesting a high NADH/NAD ratio in the tissues and red cells. When 5% CO_2 was added, lactate decrease occurred; again, no changes in pyruvate were noted.

It can be seen by the progressive drop in glucose concentration that the liver was in fact excluded from the circulation. After one hour's hyperventilation with room air the decrease in glucose concentration was more pronounced than during the hour preceding hyperventilation.

(12) Liver blood flow measured by magnetic flow-meter probes (Fig. 19)

Fig. 19 shows simultaneous recording of arterial blood pressure,

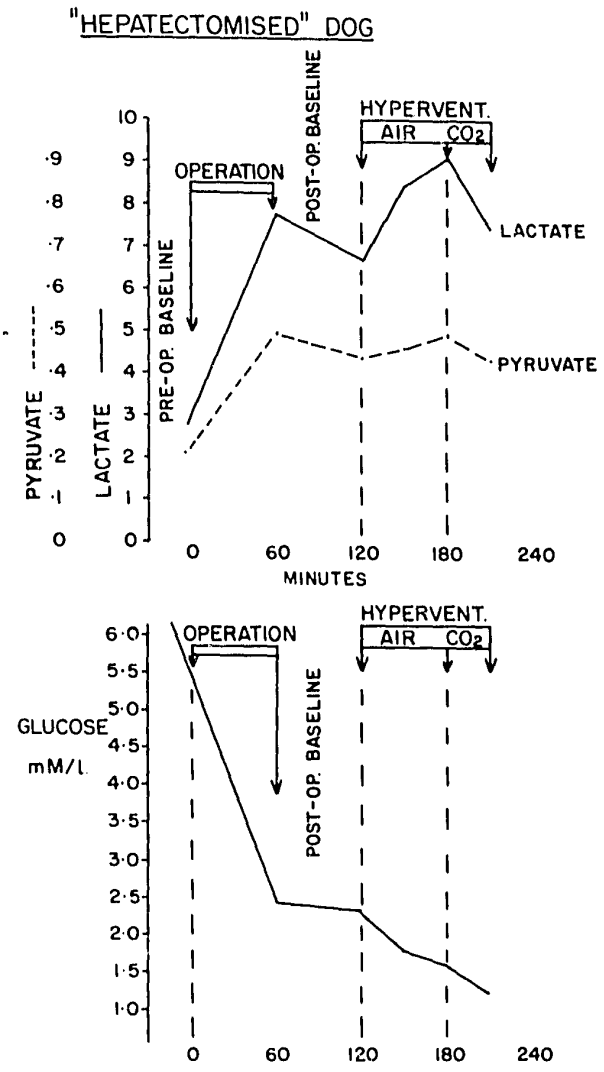


Figure 18: Blood lactate, pyruvate and glucose concentration in a "hepatectomised" dog.

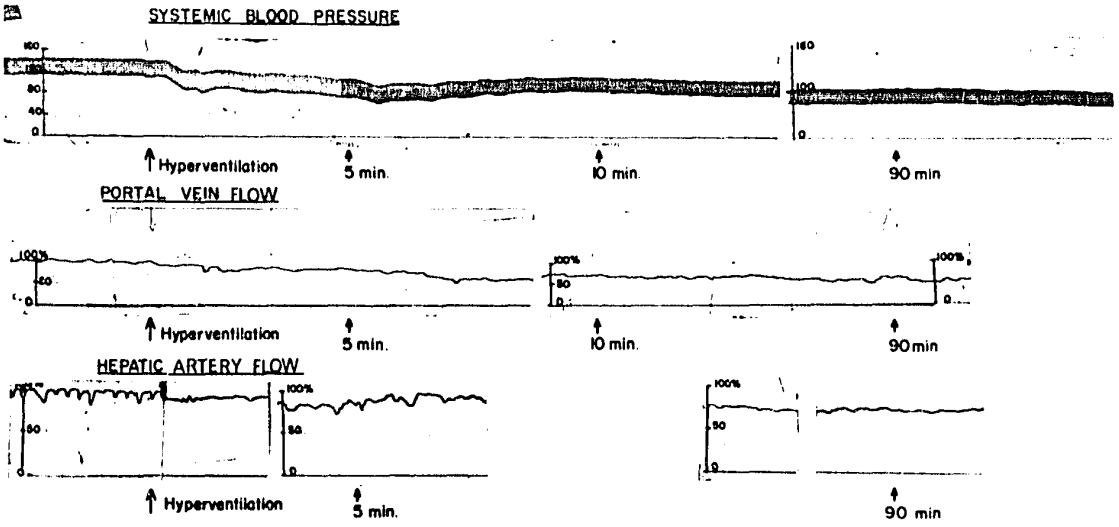


Figure 19A: Arterial blood pressure, hepatic artery flow and portal vein flow by magnetic flow meter technique.
Hyperventilation with room air.

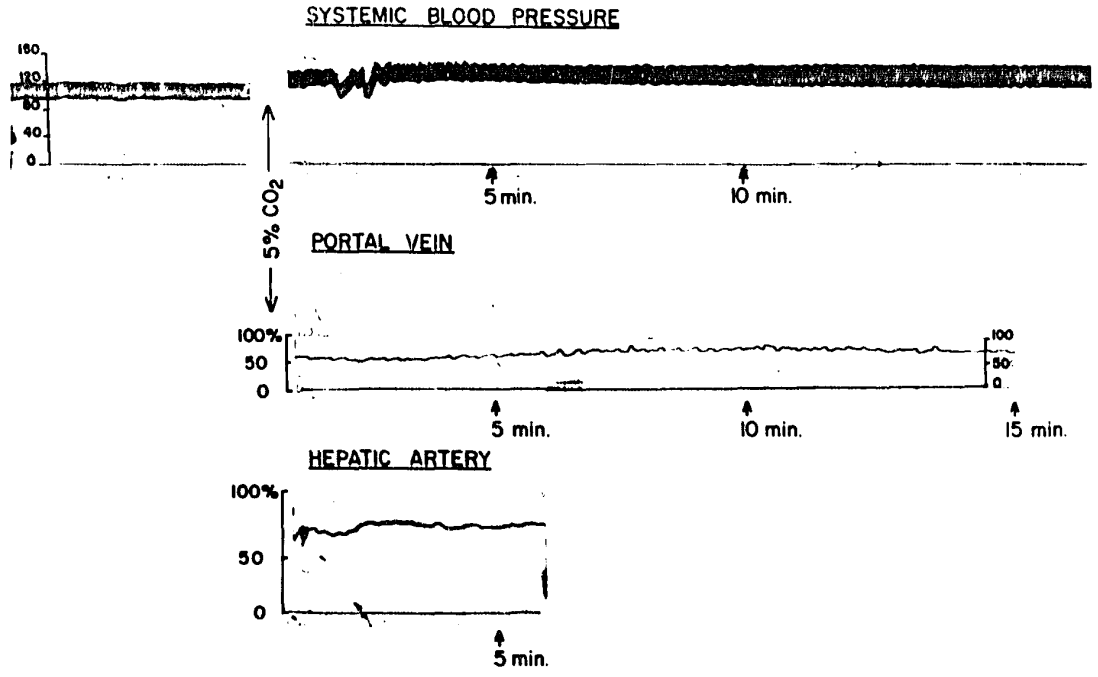


Figure 19B: Hyperventilation with 5% CO_2 and room air.

portal blood flow, and hepatic artery flow. Changes in blood pressure have been discussed already. Portal blood flow decreased 25% after 10 minutes' hyperventilation and gradually dropped further, so that at 90 minutes it was 50% of the original value. Hepatic-artery flow also decreased, but by a lesser degree, to 25% at 90 minutes. When 5% CO_2 was added to the inspired gas mixture, both portal vein and hepatic showed increases in blood flow, but only to 75% of initial (control) flow rate, similar to the depression in cardiac output, as depicted in Fig. 7).

(13) The brain and kidney arterial--venous differences

The A--V differences were measured, in one experiment across the brain and in three across the kidney. The brain added lactate throughout the experiment by a mean of 0.5 mM/litre, and the kidney behaved in similar fashion, adding 0.25 mM/litre lactate at a steady rate. Also, both brain and kidney utilize pyruvate at a steady rate: this is similar to muscles and dissimilar to utilisation liver. Again, no change in the A--V-concentration differences was detected for lactate or pyruvate throughout the experiment. These results are not portrayed or reported in further detail.

PART IV

ANIMAL EXPERIMENTS IN VITRO

A : METHODS

Seven tonometric experiments were performed, using whole arterial blood.

A 3-flask (200 ml. capacity) blood tonometer was used, with swirling motion, and attached to a controlled-temperature-bath set at 37° C (Sage Instruments). Two tonometry flasks were equilibrated with a gas mixture of 1.5% and 5% CO_2 in air, at an average gas flow of 300 ml./min. The 1.5% CO_2 gas mixture was prepared in the laboratory and the CO_2 content of all gas mixtures used was analysed previously by the Scholander technique. In one experiment, 5% and 8% CO_2 gas mixtures were used.

Arterial blood samples were collected anaerobically into three 100-ml. heparinised syringes from the femoral artery of lightly anesthetised dogs. An average of 60 ml. blood was collected into each syringe. After the initial aliquot of blood was taken for base-line values, 50 ml. of blood was placed in each tonometric flask and was equilibrated with 1.5% and 5% CO_2 in air at 37° C for a period of 30 minutes.

B : EXPERIMENTAL PROCEDURE

A base-line sample of blood was taken for lactate and pyruvate analysis as well as for pCO_2, pH, and standard bicarbonate, in all experiments. After the start of equilibrium, samples were taken at 10, 15, 20, 25, and 30 minutes. All samples were analysed for lactate, pyruvate, and pH. The last was analysed for pCO_2 and standard bicarbonate also.

In two experiments, three flasks were equilibrated simultaneoudly with the same gas mixture, 1.5% or 5% CO_2. The control flask contained 50 ml. whole blood alone; in the second flask, 19 mM/glucose/litre was added; and into the third flask, 35 mM/pyruvate/litre was added. All flasks were equilibrated for 30 minutes; samples were taken at five-minute interval, as described above. In one experiment, blood from a hyperventilated dog was collected anaerobically and equilibrated with 5% and 8% CO_2 for one hour. Samples for lactate and pyruvate were taken at 15, 30, and 60 minutes.

All laboratory analytical procedures were the same as described in the in vivo experiments.

C : RESULTS

Blood equilibrated with 5% and 1.5% CO_2 in air (Fig. 20)

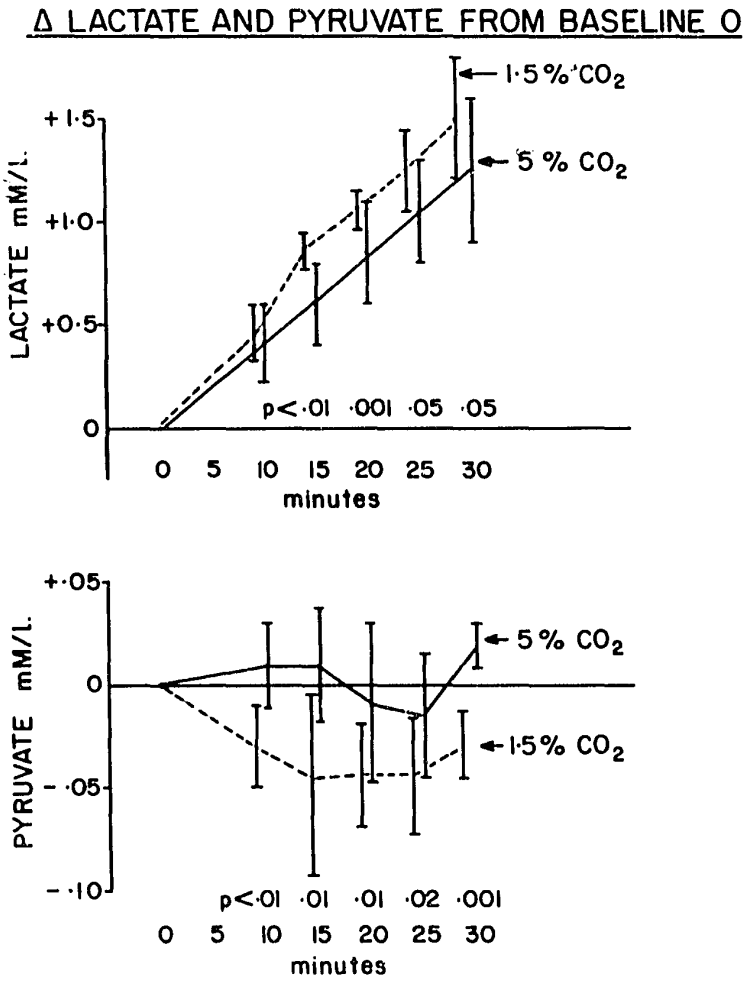


Figure 20: Whole blood equilibrated with 5% and 1.5% CO_2. Lactate in the upper part, pyruvate in the lower part of the graph.

Glycolysis as reflected by lactate production continued in the blood *in vitro* at a steady rate (Fig. 20). When equilibrated with 1.5% CO_2, as compared with 5% CO_2, the blood produced a statistically significant greater amount of lactate. The average increase in concentration was 0.25 mM/litre after 10 minutes' equilibration. Pyruvate utilization was evident by 10 minutes; this was more marked with 1.5% than with 5% CO_2.

Blood pH and pCO_2 are not shown in Fig. 20. Although the initial pH (mean, 7.38) remained constant throughout equilibration experiments with 5% CO_2, there was an increase to an average of pH 7.721 by 10 minutes in blood equilibrated with 1.5% CO_2. This increase was followed by a slow decrease in pH to a mean of 7.70 at 30 minutes.

Blood equilibrated with 5% and 1.5% CO_2 with added glucose and pyruvate (Fig. 21)

No significant changes in lactate production occurred when extra glucose was added to either the 1.5% or 5% CO_2 equilibration incubations. When pyruvate was added to the blood, an increase in lactate production occurred at both gas tensions but was greater in the blood equilibrated with 1.5% CO_2 than in that equilibrated with 5% CO_2 (Fig. 21).

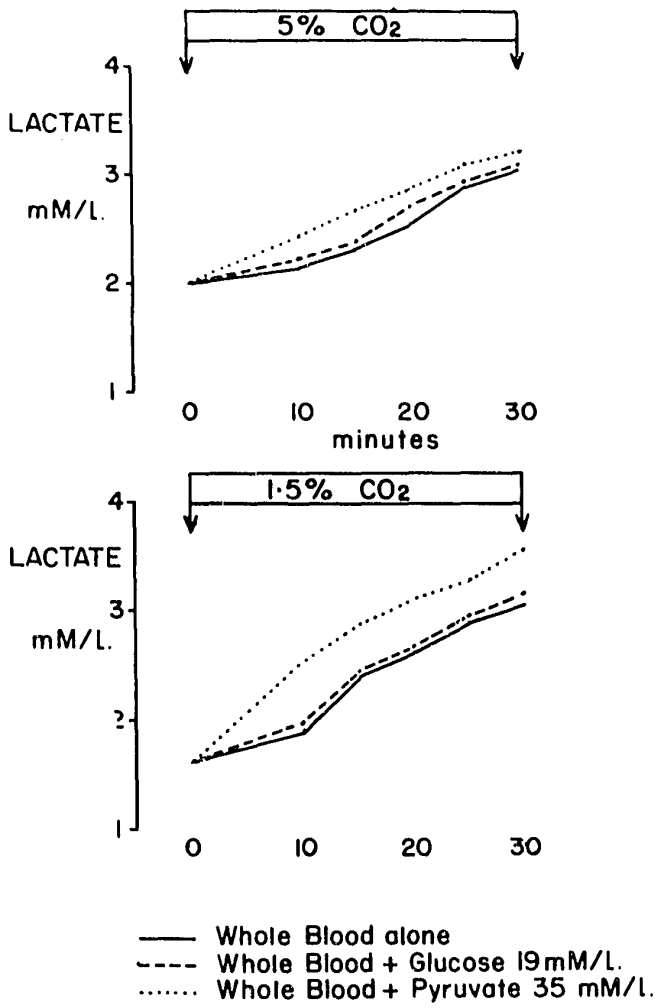


Figure 21: Whole blood equilibrated with 5% and 1.5% CO_2 with addition of glucose and pyruvate.

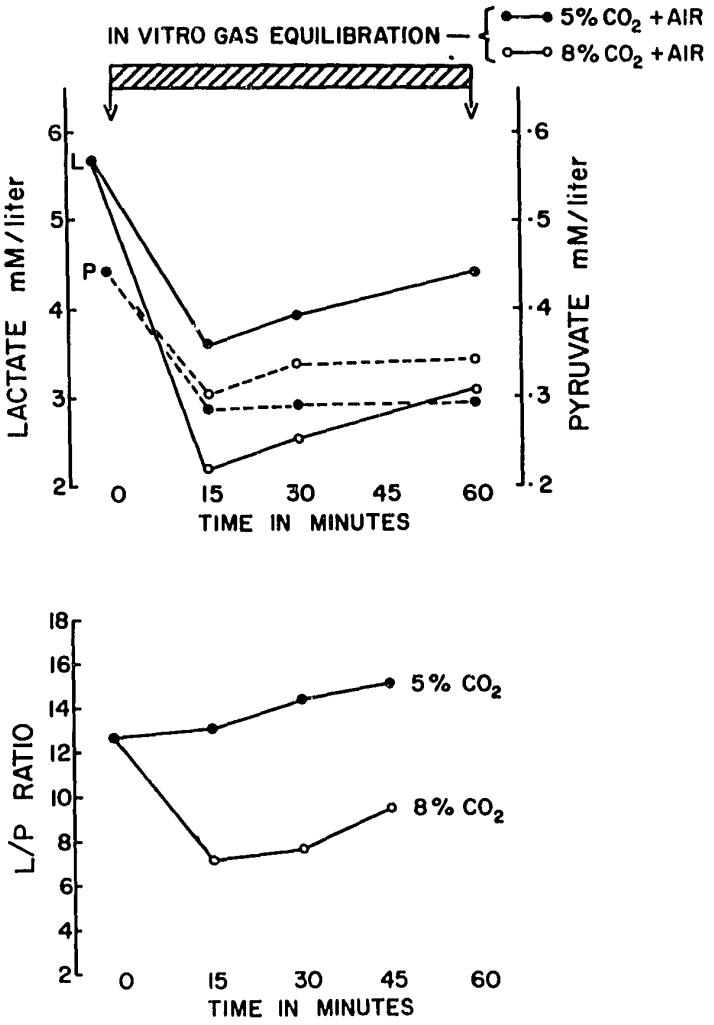


Figure 22: Hyperlactatemic blood equilibrated with 5% and 8% CO_2. Upper portion of the graph represents lactate (solid line) and pyruvate (dotted line) concentration. Lower portion of the graph represents the L/P ratio.

Blood with initially high lactate concentration equilibrated with 5% and 8% CO_2 (Fig. 22)

Blood with initially high lactate and pyruvate concentrations (5.70 and 0.445 mM/litre, respectively) was equilibrated with 5% and 8% CO_2. Lactate concentration decreased rapidly at the end of 15 minutes with both gas mixtures, but 8% CO_2 caused a significantly greater decrease than 5% CO_2. After 15 minutes' equilibration, lactate concentration started to increase slowly in both tonometric flasks, at a rate similar to that portrayed in Fig. 21. Pyruvate concentration also decreased rapidly during the first 15 minutes in both samples, but less so with 8% CO_2.

The difference between the effect of 5% CO_2 and 8% CO_2 gas mixtur on whole blood is better portrayed by the L/P ratio in those experiments. The L/P ratio in the 5% CO_2 flask did not change significantly in the first 15 minutes of equilibration but increased slowly thereafter. In the flask equilibrated with 8% CO_2 the L/P ratio dropped significantly and remained below the control value at all times, indicating a rapid decrease in lactate and a relative increase in pyruvate as compared with the sample equilibrated with 5% CO_2.

PART V

DISCUSSION

In discussing the mechanism of the lactatemia of hyperventilation one must consider three points: first, whether lactate is produced in increased amount or is utilized less rapidly; second, where the changes occur; and third, whether tissue hypoxia (the cause of other lactatemias) is involved.

A hypothesis will be presented that increased production does occur; that it is due to stimulated glycolysis, especially in the blood; and that tissue hypoxia may occur, but is not the cause of lactatemia although it may play a part in the changes in L/P ratio. The experimental data have been summarized in Fig. 23 and will be discussed by considering the changes that have occurred in each hour of the experiment.

At the end of the first hour of hyperventilation the following are apparent:

1. Decreased pCO_2.
2. Blood pH in the alkaline range (pH 7.6).
3. Increased blood lactate and pyruvate values, with a normal L/P ratio.
4. Increased blood glucose concentration, due in large part to increased release by the liver.
5. Unchanged oxygen uptake by the whole animal.

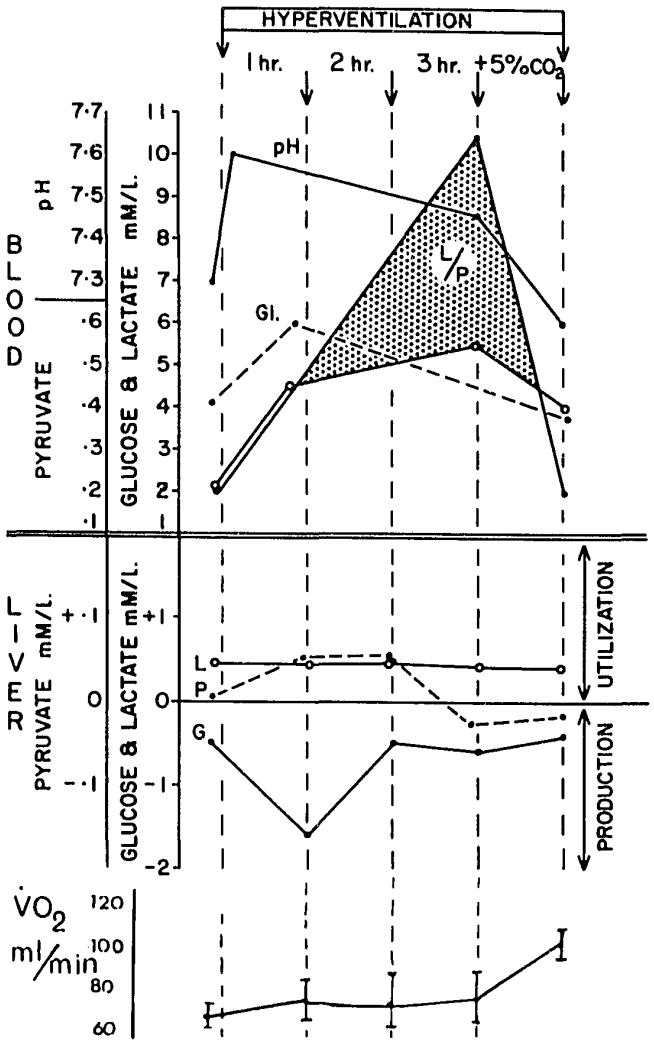


Figure 23: Summary of hyperventilation experiments at hourly intervals.

6. Steady utilization of lactate by the liver.

7. Steady lactate production in muscle.

8. Increased uptake of pyruvate by the liver and muscle, though possibly without increase in total pyruvate.

9. Decreased cardiac output, blood pressure, hepatic blood flow, and peripheral-muscle perfusion.

Gevers (51) and Katzman et al. (61) have shown that anaerobic glycolysis, as measured by lactate production, is stimulated by alkaline pH and low pCO_2 when liver and muscle slices are incubated in vitro. Murphy (16) demonstrated 50% increase in lactate production by red cells in alkaline pH achieved by low pCO_2. The in vitro experiments in the present study also have shown increased lactate production by whole blood incubated with low pCO_2. It is postulated that the lactatemia of the first hour of hyperventilation is due to stimulated glycolysis in whole blood. Increased glycolysis did not occur at other sites, as reflected in measurements of A--V differences, and this is made more significant by the fact that blood flow to the muscle is known to be reduced. The quantitative aspects of reduced liver lactate uptake, due to decreased live blood flow, will be discussed later. Simultaneously with the stimulated glycolysis, the liver releases glucose: the mechanism of this is not known. Concurrent with the increase in blood glucose concentration, lactate and pyruvate rise proportionately, so that the L/P ratio, and presumably the NAD/NADH ratio, remain constant in the blood.

In the presence of a normal L/P ratio and a steady oxygen uptake, the oxidative pathways of glucose metabolism may be presumed to remain unaltered. There is no reason to suppose that the lactatemia that occurs during the first hour of hyperventilation is caused by tissue hypoxia as, in addition to the normal L/P ratio, there is no change in A--V lactate and no statistically significant changes in the L/P ratio across the muscle mass of the legs, even though femoral venous blood shows a significant decrease in oxygen saturation at the end of three hours (Fig. 9).

The decrease in cardiac output during hyperventilation has been described previously by Dale and Evans (9) and more recently by Kontos et al. (62). The latter authors believe that the decrease in cardiac output is due to intermittent positive-pressure breathing alone, rather than to effects of low pCO_2. This does not seem feasible, as the blood pressure and cardiac output increase when 5% CO_2 is added to the inspired air, despite the persistence of positive-pressure breathing. The mechanism by which low pCO_2 decreases the cardiac output is not understood.

The present experiments have shown a decrease in liver blood flow during hyperventilation. In the presence of a steady A--V difference in lactate across the liver, and a decreased flow, liver lactate utilization will be altered. Lactate uptake by the liver can be calculated by the formula:

$$Q_L = A—V \times \text{ml. flow/minute}$$

If it is assumed that the liver blood flow for the dog is 500 ml./min. (63,64), then, using the recorded A--V lactate differences of 0.28 mM/litre (or 0.00028 mM/litre), net uptake of lactate will be:

$$Q_L = 0.00028 \times 500 \text{ ml./min.}$$

$$Q_L = 0.14 \text{ mM/min. (approximately)}$$

If this amount (0.14 mM/min.) is utilized under control conditions, and if flow decreases by 50% with hyperventilation, then:

$$Q_L = \mathbf{0.07} \text{ mM/minute}$$

Uptake of 0.07 mM/minute would lead to a lactate accumulation of 12.6 mM/ in 3 hours. Assuming total body water distribution (65% body-weight) of 8 litres, the net increase in lactate in a three-hour period will be 1.6 mM/litre. This figure is too small to explain the rise of 8 mM/lactic acid per litre observed in the present experiments, even assuming only extracellular water distribution the net increase in lactate would be 3.3 mM/litre.

Further evidence that decreased liver uptake is not the cause of the lactatemia of hyperventilation is obtained from the experiment in which the liver was isolated from the main circulation by a proto-caval shunt and ligation of the hepatic artery. After one hour of hyperventilation, lactic acid concentration rose by the same amount and at the same rate as when the dog was intact, and not at a faster rate.

The muscles are excluded as the site of excessive lactate production in hyperventilation since, although they produce lactate

at all time, the A--V difference is unchanged. If the muscle blood flow decreases in hyperventilation then the actual production of lactate by the muscle must be decreased. Kontos et al. (62) and Fleishman et al. (46) showed the vasoconstrictive effect of low pCO_2 in the vessels of the dog limb; and, in the present experiments, the decreased O_2 saturation in the femoral vein at a time when total body oxygen uptake remained constant, yielded circumstantial evidence for peripheral vasoconstriction.

If these three mechanisms of lactatemia are excluded, namely, a decrease in liver utilization of lactate, an increase in lactate production by the muscle, brain, or kidney, and hypoxia (30), it still remains to be considered whether glycolysis by blood cells can produce the large amount of lactate that accumulates during hyperventilation. If glycolysis in vivo is as active as in vitro (Fig. 21), then 1.5 - 2 mM/lactate per litre can be produced in 30 minutes, or 9-12 mM/l. in three hours. Even after diffusion throughout the extracellular fluid, and to a lesser extent into the cells, it seems reasonable to believe that red-cell glycolysis could account for the most of the observed increase in lactate in this form of lactatemia.

The present findings at the end of one hour's hyperventilation agree with Huckabee's (2) observation in dogs that there is a proportional rise in lactate and pyruvate without change in oxygen uptake. In the present experiments as well as in those of Eichenholz et al. (12) and Tobin (10), lactic acid continues to increase at the

same rate even when blood pH returns to normal range. Indeed, when hyperventilation is maintained for a longer period the blood pH shifts into acidosis. These findings are contrary to the explanation put forward by Huckabee (2) and others (1,9), who state that hyperventilation lactatemia is a "compensatory or homeostatic" mechanism for body pH.

At the end of the second and third hour of hyperventilation the following were observed:

1. Blood pH decreased toward normal range (pH 7.4).
2. pCO_2 remained at 10 mm. Hg.
3. Lactic acid continued to increase at the same rate.
4. Pyruvic-acid concentration reached a plateau, although the constantly rising lactate indicated undiminished glycolysis.
5. The liver appeared to add pyruvate to the circulation.
6. The L/P ratio began to rise from 10:1 to 20:1 (approximately).
7. Blood-glucose concentration decreased.
8. Oxygen uptake remained steady.
9. Cardiac output, hepatic flow and blood pressure remained depressed.

As the arterial pCO_2 remained low and the blood pH returned to normal range, it is necessary to postulate the low pCO_2 rather than pH, as the stimulus to glycolysis.

The oxygen uptake remains unaltered, reflecting a steady oxidative metabolic rate, but the L/P ratio is significantly increased.

As the A--V differences for lactate as well as the $\frac{\text{L/P artery}}{\text{L/P vein}}$ ratio across the liver and muscle remain constant, there is no obvious evidence for hypoxia to explain the rising L/P ratio. It appears that NAD is reduced at a faster rate than NADH can be oxidized via the oxidative electron-transport scheme, and that pyruvate produced by the stimulated anaerobic glycolysis is utilized as a hydrogen acceptor, with release of NAD and formation of lactate. In such circumstances pyruvate will be expected to level off or to rise only slightly, which is consistent with the present observations. The supply of NAD is adequate for glycolysis, as shown by the undiminished rate of lactate accumulation, but the dynamic equilibrium has been changed. However, this postulated change in NADH/NAD ratio in the blood does not mean that the same changes are present in the mitochondrial NADH/NAD in the tissues.

Alpert (13) and Hohorst et al. (15) have thrown doubt on the validity of the assumption that the L/P ratio in blood, or excess lactate, are indicators of the state of mitochondrial oxygenation. Thus, Alpert (13) has shown that the oxygen debt acquired during exercise does not correlate with the removal of formed excess lactate (30). Also, Hohorst et al. (15) have stressed the variability in total amounts of lactate and pyruvate in various tissues of the body, as well as their relative proportion to each other; therefore, the direct relationship between the L/P ratio in the blood and the state of reduction of NADH/NAD system, or the state of mitochondrial oxidation in the cells of the body, has to be

viewed with reservation. The increased L/P ratio in the blood in the second and third hour of the experiments is indicative of an altered NADH/NAD equilibrium in the blood.

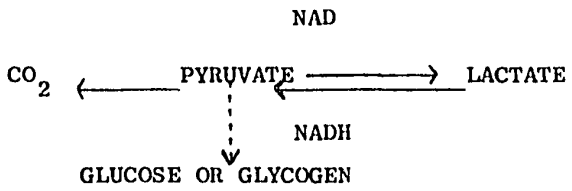
When 5% CO_2 was added to the inspired air and hyperventilation continued, the changes observed one hour later (the fourth hour of the experiment) were:

1. Decrease in blood pH, toward acid range.
2. Increase in pCO_2, to 60 mm. Hg.
3. Rapid decrease in lactic-acid concentration, to control levels or lower.
4. Decrease in blood pyruvate, but proportionately less than in lactate.
5. Decrease in L/P ratio, to below control value.
6. Significant increase in oxygen uptake.
7. Increase in blood pressure, cardiac output and liver blood flow toward normal but remaining depressed.

With the acid pH and high pCO_2, glycolysis presumably reverts to its resting rate and, consequently, lactate and pyruvate production by the blood cells is decreased. As oxygen uptake increases, the oxidative pathways probably are stimulated and lactate is rapidly oxidized to pyruvate. The increase in oxygen uptake in the presence of CO_2 is difficult to explain. It may be postulated that the low pCO_2 (below 15 mm. Hg) is sufficient to maintain the pCO_2-dependent reaction in the Kreb's cycle at a base-line rate. When the pCO_2 is

restored to its normal or highest values, the limiting factor (low pCO_2) is removed and stimulation of the Kreb's cycle occurs. With increase in pCO_2, lactate decreases at a faster rate than pyruvate; however, the data from A--V-concentration differences do not show where that occurs. It is of such large magnitude that much of the lactate must be converted back to pyruvate by the blood cells themselves. Results of the in vitro experiments in which blood with high lactate and pyruvate levels was equilibrated with 5% and 8% CO_2 (Fig. 22) support such a hypothesis.

The reactions below are shifter to the left:



The fast diappearance of lactate in the presence of a still-depressed cardiac output when 5% CO_2 is added once again strongly suggests that the lactatemia of hyperventilation is not caused by hypoxia.

It has been stressed by Boxer and Devlin (14) and by Chance and Hess (68,69) that there may be barriers that prevent the mitochondrial oxidation of extramitochondrkal NADH (i.e., cytoplasmic NADH). Mechanisms that link cytoplasmic NADH to

mitochondrial flavoproteins and cytochromes are (i) the cytoplasmic oxidation by dihydroxyacetone phosphate (which forms glycerophosphate), and (ii) the cytoplasmic reaction whereby acetoacetate is reduced to beta-hydroxybutyric acid. The reduced compounds of those two reactions, α-glycerophosphate and β-hydroxybutyrate, are able to pass on electrons into the mitochondria.

In tumour tissue, the "aerobic glycolysis" of Warburg is believed to be due to a relative deficiency of one or both of these "shuttle" systems that link cytoplasmic electron acceptor to mitochondrial electron transport. Also, the permeability barrier of the mitochondria is a major regulatory factor in cell metabolism, as the enzymes and co-factors involved in glycolysis are primarily located in the cell sap, i.e., the soluble extramitochondrial portion of the cytoplasm of mammalian cells (14). The mechanism of hyperventilation lactatemia proposed in this thesis is also a form of "aerobic glycolysis" in which there are perhaps two components" (i) stimulated glycolysis in mitochondrial free red blood cells, and (ii) failure to transfer the electrons accepted by NAD in the red cell to the mitochondrial systems of cells of other tissues, thus obligating pyruvate as a hydrogen acceptor and the formation of lactic acid if glycolysis is to continue. Such a hypothesis is compatible with an unaltered mitochondrial oxygen uptake in other tissues, since the oxidation reactions remain in operation. No changes in total body oxygen uptake will be expected. When CO_2 is added to a hyper-

ventilating animal, glycolysis is no longer stimulated and presumably other electron acceptors take over cytoplasmic pyruvate and allow both pyruvate and lactate to become oxidized. In such circumstances the oxygen uptake will be expected to increase. It is more speculation to suggest that low pCO_2 prevents the coupling between cytoplasmic NADH and mitochondrial electron acceptor by interfering directly with the "shuttle" systems or indirectly by altering the permeability barrier of the mitochondria.

PART VI

SUMMARY

1) Two patients diagnosed as having hyperlactatemia of hyperventilation are presented and discussed.

2) Hyperlactatemia was induced experimentally in dogs hyperventilated for three hours with room air and then for one hour with 5% CO_2.

3) Arterial-blood pCO_2 was maintained below 15 mm. Hg, as it was observed that only below this value did severe lactatemia occur. Above 15 mm. Hg a close correlation existed between pCO_2 and lactate concentration ($R = 0.85$ and $p\ 0.001$).

4) Arterial blood pressure, cardiac output and liver blood flow decreased during hyperventilation with room air and were partially restored with 5% CO_2 despite continuing hyperventilation at a steady rate.

5) Blood lactate and pyruvate concentration increased linearly in the first hour of room-air hyperventilation. In the second and third hour, lactate continued to rise linearly and pyruvate increased more slowly. When 5% CO_2 was added to the inspired air and hyperventilation continued, lactate decreased to or below control value. Pyruvate also decreased, but remained above control.

6) The L/P ratio remained unaltered in the first hour of hyperventilation and increased thereafter until 5% CO_2 was added; then the L/P ratio decreased below control values.

7) The arterial--venous-concentration differences across liver, muscle and gut were measured for lactate, pyruvate and, in two experiments, for glucose. The A--V differences for lactate remained constant across all organs studied throughout hyperventilation with room air or 5% CO_2. The A--V pyruvate showed increased utilization by the liver in the first two hours of hyperventilation and production in the third and fourth hour.

8) Oxygen uptake remained constant throughout room-air hyperventilation; with 5% CO_2 a significant increase in oxygen uptake was observed.

9) Lactatemia of hyperventilation was not derived from release from muscle, live, gut kidney, or brain. No evidence for hypoxia was observed of a degree capable of causing lactatemia.

10) Whole blood, equilibrated in vitro with varying gas mixtures, showed the blood to be a metabolic source of lactic acid, especially so in experiments in which pyruvate was added.

11) Whole blood, contained from a previously hyperventilated animal (elevated lactate), when equlibrated with high CO_2 gas mixtures was able to metabolise lactate back to pyruvate during in vitro experiments.

12) A hypothesis is presented and discussed explaining the lactatemia of hyperventilation on stimulated glycolysis of the whole blood, especially red blood cells, when pCO_2 is reduced below 15 mm. Hg in the experimental animal.

PART VII: BIBLIOGRAPHY

1- MacLeod J.J.R. and D.H. Hoover. Studies in experimental glycosuria. Lactic acid production in the blood following the injection of alkaline solutions of dextrose or of alkaline solution alone. Amer. J. physiol. 42: 460, 1916-17

2- HUCKABEE W.E.. Relationship of Pyruvate and Lactate during anaerobic metabolism. I-Effect of infusion of pyruvate or glucose and hyperventilation.
J.Cl. Invest. 37: 244, 1958

3- White P.D. and R. G. Hahn. The symptoms of sighing in cardiovascular diagnosis with spirographic observations.
Am. J.M.Sc.. 177:179, 1929

4- Talbott J.H., Cobb S., Coombs F.S., Cohen M.E. and W. V. Consolazio. Acid base balance of the blood in a patient with hysterical hyperventilation.
Acta Neurologica Psych. 39: 973, 1938

5- Fenn W.O., Rahn H., Otis A. B. and L. E. Chadwick. Physiological observations on hyperventilation at altitude with intermittent pressure breathing and pneumolator.
J. Appl. Physiol. 1:773, 1949

6- Vanamee P., Poppell J.W., Glicksman A.S., Randall H.T and K.E. Robert. Respiratory alkalosis in hepatic coma.
Arch. Int, Med. 97: 762, 1956

7- Eichenholz A., Mulhausen R.O. and P.E. Redleaf. Nature of Acid-Base Disturbance in Salicytate Intoxication.
Metab. 12: 164, 1963

8- Harpor G.A. and R.F. Loeb. Uncompensated alkalosis in encephalitis.
J.A.M.A. 81: 452, 1923

9- Dale H.H. and C. L. Evans.
Effect on the circulation of changes in the carbon dioxide content of the blood.
J. of Physiol. 56: 125, 1922

10- Tobin R.B.. In vivo influences of hydrogen ions on lactate and pyruvate of blood.
Am. J. Physiol. 207:601, 1964

11- Dossetor J.B., Zborowski D., Dixon H.B., and J.A.P. Pare.
"Hyperlactatemia due to Hyperventilation: Use of CO_2 inhalation".
An. N.Y. Ac. Sc. 119: 1153, 1965

12- Eichenholz A., Mulhausen R.O., Anderson W.E. and F.M. MacDonald.
Primary hypocapnia: a cause of metabolic acidosis.
J. Appl. Physiol. 17: 283, 1962

13- Alpert, Norman R., Lactate production and removal and the regulation of Metabolism.
An. N.Y. Ac. Sc. 119:995, 1965

14- Boxer G. E. and T.M. Devlin. Pathways of Intracellular Hydrogen Transport.
Science 134: 1495, 1961

15- Hohorst H.J., Krese P., Bartels H., Stratmann B. and H. Talke.
L (+) Lactic Acid and the steady state of cellular red/ox- systems.
An. N.Y. Ac. Sc. 119: 974, 1965

16- Murphy J.R.. Erythrocyte Metabolism- II Glucose Metabolism and Pathways.
J. of Lab. Cl. Med. 55:286, 1960

17- Mann F.C. and T.B. Magath. Studies on physiology of liver; effect of administration of glucose in condition following total extirpation of liver.
Arch. Int. Med. 30: 171, 1922

18- Cori C.F. and G.T. Cori. Glycogen formation in the liver from d - and l - Lactic Acid.
J. Biol. Chem. 81: 389, 1929

19- Hill A.V., Long C.N.H. and H. Lupton. Muscular Exercise, Lactic Acid, and the Supply and Utilisation of Oxygen - Parts IV - VI.
Proc. Roy. Soc. London, Series B 97: 84, 1925

20- Fletcher W.M. and F.G. Hopkins. Lactic acid in amphybian muscle.
J. Physiol. 35: 247, 1907

21- Meyerhof O.. Recent investigations on aerobic and anaerobic metabolism of carbohydrates.
J. Gen. Physiol. 8: 531, 1927

22- Embden G., Lehnartz E. and H. Hentschel. Der Zeitliche Verlauf der Milchsäurebildung bei der Muskelkontraktion.
Ztschr. F. Physiol. Chem. 165: 255, 1927

23- Meyerhof O.. Zur Verbrennung der Milchsaure in der Erholungsperiode des Muskels.
Pfluger's Arch. ges. Physiol. 88: 175, 1919

24- Hill A.V.. The oxidative removal of lactic acid.
J. Physiol. 10: 48, 1914

25- Himwich H.E., Koskoff Y.D. and L. H. Nahum. Studies in carbohydrate metabolism. I. A glucose lactic acid cycle involving muscle and liver.
J. Biol. Chem. 85: 571, 1929-30

26- Guest G.M., Mackler B., Graubarth H. and P. A. Ammentorp. Rates of Utilization of Glucose in Erythrocytes and Leucocytes.
Am. J. Physiol. 172: 295, 1953

27- Friedeman T.E. and C.J. Barborka. The significance of the ratio of lactic to pyruvic acid in blood after exercise.
J. Biol. Chem. 141: 993, 1941

28- Friedeman T.F., Haugen G.E. and T.C. Kmieciak. The level of pyruvic and lactic acids, and the lactic pyruvic ratio, in the blood of human subjects. The effect of food, light muscular activity, and anoxia at high altitude.
J. Biol. Chem. 157: 673, 1945

29- Huckabee W.E.. Relationship of pyruvate and lactate during anaerobic metabolism.
II Exercise and formation of O_2 debt.
J. Cl. Inv. 37: 255, 1958

30- Huckabee W.E.. Relationship of pyruvate and lactate during anaerobic metabolism. III. Effect of breathing low oxygen gases.
J. Cl. Inv. 37: 264, 1958

31- Glaviano V.V., Apel A. and J. Doerschling. Distribution and gradient of lactate between blood and heart muscle.
Physiologist 7: 143, 1964

32- Huckabee W.E.. Abnormal resting blood lactate I. The significance of hyperlactatemia in hospitalized patients.
Am. J. Med. 30: 833, 1961

33- Waters C.W.III, M.D., Hall Y. Denny. and W.B. Schwartz.
Sponaneous Lactic Acidosis.
Am. J. Med. 35: 781, 1963

34- Peretz D.I., MacGregor M. and J.B. Dossetor. Lactic acidosis: a clinically significant aspect of shock.
Can. Med. Assn. J. 90: 673, 1964

35- Peretz D.I.. Metabolic acidosis in shock.
M. Sc. Thesis, McGill University, 1964

36- MacLeod J.U.R.. Further observations on the production of lactic acid following alkaline injections.
Amer. J. Physiol. 45: 539, 1917-1918

37- MacLeod J.J.R.. Concentration of lactic acid in blood in anoxemia and shock.
Amer. J. Physiol. 55:184, 1921

38- Anrep G.V. and R.K. Cannan. The concentration of lactic acid in the blood in experimental alkaelemia and acidaemia.
J. Physiol. 58: 244, 1923

39- Long C.N.H.. Lactic acid in blood of resting Man.
J. Physiol. 58: 455, 1924

40- Bock A.V., Dill D.B. and H. T. Edwards. Lactic acid in blood of resting man.
J. Clin. Invest. 11: 775, 1932

41- Harrop G. and R. F. Loeb. Uncompensated alkalosis in encephalitis.
J.A.M.A. 81: 452, 1923

42- Talbott J.H., Cobb S., Coombs F. S., Cohen M.E. and W.V. Consolazio Acid base balance of the blood in patient with hysterical hyperventilation.
Arch. Neurol. and Psychiat. 39: 973, 1938

43- Carryer H.M.. Syndrome of hyperventilation with Tetany: Report of a case.
Proc. of Staff meeting Mayo Clinic 18: 522, 1943

44- Carryer H.M.. The hyperventilation syndrome.
Med. Clin. N. America 31: 845, 1947

45- McDowall R.J.S.. The effect of carbon dioxide on the circulation.
J. Physiol. 70: 301, 1930

46- Fleishman M., Scott J. and F.J. Haddy. Effect of pH changes upon systemic large and small vessels resistance.
Circulation Res. 5: 602, 1957

47- Wasserman A.J. and J.L. Patterson, Jr.. The cerebral vascular response to reduction in arterial carbon dioxide tension.
J. Cl. Invest. 40: 1297, 1961

48- Donevan R.N., Anderson N.M., Sekelj P., Papp O. and M. McGregor. Influence of voluntary hyperventilation on cardiac output.
J. Appl. Physiol. 17: 487, 1962

49- McGregor M., Donevan R.E. and N.M. Anderson. Influence of carbon dioxide and hyperventilation in man.
J. Appl. Physiol. 17: 933, 1962

50- Carryer M.N.. Tissue anoxia resulting from respiratory alkalosis.
Staff meeting of the Mayo Clinic 22: 456, 1947

51- Gevers W. and E.B. Dowdle. The effect of pH on glycolysis in vitro.
Cl. Sc. 25: 343, 1963

52- Hastings A.B. and E.B. Dowdle. Effect of CO_2 tension on synthesis of liver glycogen in vitro.
Trans. Ass. Amer. Physiol. 73: 240, 1960

53- Greenfield J.C., Jr., Patel D.J., Mallos A.J. and D.L. Fry. Evaluation of Kolin type Electromagnetic flowmeter and the pressure gradient technique.
J. Appl. Physiol. 17: 372, 1962

54- Spencer M.P.,and A.B. Denison, Jr.. Squarewave electromagnetic flowmeter for surgical and experimental application.
Meth. Med. Res. 8: 321, 1960

55- Galindo A.. Hepatic circulation and hepatic function during anaesthesia and surgery.
Can. Anaes. Soc. J. 12: 262, 1965

56- Holt J.P.. The effects of positive and negative intrathoracic pressure on cardiac output and venous pressure in dog.
Am. J. Physiol. 142: 594, 1944

57- Barker S.B. and W.H. Summerson. The colorimetric determination of lactic acid in biological material.
J. Biol. Chem. 138: 535, 1941

58- Friedemann T.E. and G.E. Haugen. Pyruvic Acid.II. The determination of keto acids in blood and urine.
J. Biol. Chem. 147: 415, 1943

59- Hoffman W.S.. A rapid photoelectric method for the determination of glucose in blood and urine.
J. Biol. Chem. 120: 51, 1937

60- Harris P., Bateman M. and J. Gloster. The metabolism of glucose during exercise in patients with rheumatic heart disease.
Cl. Sci. 23: 561, 1962
The regional metabolism of lactate and pyruvate during exercise in patients with rheumatic heart disease.
Cl. Sci. 23, 545, 1962

61- Katzman R., Vilee C.A. and H.K. Beecher. Effect of Increased Carbon Dioxide concentration on Fixed Acid Production in Vitro.
Am. J. Physiol. 172: 317, 1953

62- Kontos A.H., Mauck P.H.Jr., Richardson D.W. and J.L. Patterson, Jr.. Circulatory responses to hypocapnea in the anesthetized dog.
Am. J. Physiol. 208: 139, 1965

63- Drapans T., Kluge D.N. and W.G. Schenk. Measurements of hepatic blood flow by Bromosulphalein and by the electro-magnetic flowmeter.
Surgery 48: 1017, 1960

64- Werner, Attie Yvonne and Steven M. Horvath. Measurement of Hepatic Blood Flow in dog by the Bromosulphalein method.
J. Cl. Inv. 31: 433, 1952

65- Tranquada R.E., Bernstein S. and H.E. Martin. Irreversible lactic acidosis associated with phenformin therapy.
Report of three cases.
J.A.M.A. 184: 37, 1963

66- Bernier G.M., Miller M. and C.S. Springate. Lactic acidosis and phenformin hydrochloride.
J.A.M.A. 184: 43, 1963

67- Huckabee W. E... Control of Concentration Gradients of Pyruvate and Lactate across Cell Membranes in Blood.
J. Appl. Physiol. 9: 163, 1956

68- Chance B. and B. Hess. Spectroscopic Evidence of Metabolic Control.
Science 129: 700, 1959

69- Chance B. and B. Hess. Metabolic Control Mechanisms IV. The affect of glucose upon the steady state of respiratory enzymes in the ascitic cell.
J. Biol. Chem. 234: 2421, 1956