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Research Article

THE FATE OF CIRCULATING LACTIC ACID IN THE HUMAN LUNG[1]

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The evidence of oxidative processes in the lung is conflicting. As early as 1897, Bohr and Henriques (1) observed in animals that the volume of oxygen transported through the lungs was sometimes less than the oxygen uptake and concluded that some oxygen must be utilized in the lungs. Evans and Starling (2) studying metabolism in heart-lung preparations on the other hand found no evidence of oxidation in the lungs under normal oxygenation or during extreme oxygen lack.

In connection with pulmonary metabolism attention has been directed to lactic acid, because it is oxidized only under aerobic conditions. If the disappearance or the reduction of the circulating lactic acid during passage through the pulmonary circuit were established, it would seem that a fraction of the oxygen uptake must be used in the lung to this effect.

Eppinger and Wagner (3) perfused lungs with blood-saline mixtures and found that lactic acid disappeared. Evans, Hsu, and Kosaka (4) however, found that the lung actually contributed lactate to the blood in heart-lung preparations. Alpern, Simonson, Sirkina, and Tutkiewitsch (5) observed in dogs that the lactic acid and glucose content of the right heart blood were markedly higher than the levels in femoral artery blood after exercise. Rosenbaum (6) found that venous blood of anesthetized dogs contained more lactic acid than arterial blood, and that the difference increased during periods of oxygen deficiency. Rein (7) showed in dogs that intrapulmonary disappearance of lactate occurred less frequently and to a lesser extent when the liver was shunted out of the vascular system. Beatty (8) found in dogs that venous blood averaged three mgm. per cent more lactic acid than arterial blood, and the difference rose to 24 to 36 mgm. per cent following severe hemorrhage. Goodale, Lubin, Eckenhoff, Hafkenschiel, and Banfield (9) demonstrated no difference in the lactic acid content of arterial and venous blood in anesthetized dogs with normal oxygenation. Bücherl and Schwab (10) maintained dogs in shock by phlebotomy and found that during the subsequent hypoxia lactic acid usually disappeared in passage through the lungs, though under normal oxygenation no such difference was demonstrable. Halmágyi, Reinbold, Felkai, and Iványi (11) studied 19 humans with various diseases at rest under basal metabolic conditions and found some disappearance of lactic acid in the lungs in the majority. In summary, the evidence in favor of a metabolic action of the lung upon circulating lactic acid is still open to question.

In this paper are presented data indicating that in man at rest, during exercise, and in a state of acute induced hypoxia or of pathologically caused chronic hypoxia, there is no significant difference in the lactic acid content of blood entering and leaving the lungs. Since the accuracy of the method used for the determination of lactic acid in whole blood is of primary importance in arriving at a correct conclusion concerning this problem, considerable attention has been given to details of the method employed.

TECHNIQUES

A. *Determination of Lactic Acid in Whole Blood*

(a) *Discussion of the method of Barker and Summerson*

Barker and Summerson (12) described a method for the determination of lactic acid which is now in wide use. By their procedure, a sample of blood is added to cold trichloroacetic acid for precipitation of blood proteins. Carbohydrates and other interfering substances are next removed with cupric and calcium hydroxides, and the

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lactic acid oxidized to acetaldehyde by heating with concentrated sulfuric acid for five minutes in a boiling water bath. The p-phenylphenol reagent is added to produce a violet color the intensity of which is measured.

Early in our studies when whole blood was analyzed according to this procedure, duplicate samples checked poorly and results were between 0.5 and 2.0 mgm. per cent, whereas the accepted normal values for whole blood of resting humans are in the range of 5 to 15 mgm. per cent. Two sources of difficulty were found which helped to explain the low results.

The first occurred in the removal of blood proteins. Whole blood forms a copious sticky brown precipitate with cold trichloroacetic acid. When 5 to 15 mgm. per cent of lactate was placed in 10 per cent trichloroacetic acid and carried through the procedure without the addition of blood, the recovery was 100 per cent, duplicate samples checked well, and similar values were obtained on repeated determinations. But when 4 mgm. per cent of lactate standards was placed in trichloroacetic acid and blood added, recovery of lactate was only 60 to 75 per cent at a ratio of one part of blood to four of precipitant, and about 85 per cent when one volume of blood was added to 10 volumes of trichloroacetic acid. Barker and Summerson, however, obtained recoveries of 95 to 105 per cent with a blood-trichloroacetic acid ratio of one to ten. Hemolysis of blood in distilled water containing 4 mgm. per cent of lactate standard before the precipitation of proteins did not improve the recovery, nor did the dropwise addition of trichloroacetic acid to blood instead of the addition of blood to trichloroacetic acid. The inability to recover lactic acid quantitatively from whole blood was considered probably due to inclusion and adsorption of the lactic acid by the precipitating blood proteins, since results were good if the protein precipitation was eliminated and because a greater ratio of precipitating agent to blood resulted in improved recoveries.

The second difficulty arose in the oxidation of lactic acid to acetaldehyde with sulfuric acid. The amount of heating in the boiling water bath after addition of sulfuric acid was found to be a critical factor. The maximal depth of color occurred when samples were heated 2.0 ± 0.5 minutes. A shorter period of heating resulted in a fainter color, presumably due to incomplete oxidation of lactic acid to acetaldehyde. Longer heating resulted in a progressively paler color, and this fading occurred regardless of whether or not the test tubes were covered, so that volatilization of acetaldehyde was apparently not the cause of the loss. It seems likely that part of the acetaldehyde is further oxidized upon prolonged heating, gives no color with p-phenylphenol, and therefore, results are low. Standards analyzed along with blood samples were affected in the same way, so that by comparison of unknown with standards the values obtained were suitably corrected. It is desirable, nevertheless, to measure the maximum intensity of color because sensitivity is greater and an additional source of error is thereby eliminated. However, Barker and Summerson state that the period of heating is not critical and may vary between three and ten minutes. Mendel and Goldscheider (13) who earlier made some observations on the oxidation of lactic acid with sulfuric acid, found that four minutes were required to achieve maximum aldehyde formation which was then unchanged on heating as long as eight minutes. Russell (14) reported that some lots of concentrated sulfuric acid gave too little color formation in the Barker-Summerson procedure, and these were found to contain traces of nitrate or nitrite ions by the ring test with ferrous ammonium sulfate. Our sulfuric acid and copper sulfate reagents did not give a positive ring test. The conditions for oxidation of lactic acid with concentrated sulfuric acid have long been recognized as critical (13). It is difficult to know whether the difference in results may be due to contaminants or to slight variations in the conditions or reagents. It is suggested that before any determinations are done, samples containing identical amounts of lactic acid be heated with sulfuric acid in boiling water for varying lengths of time between one and eight minutes. In this way the range and optimal time of heating can be determined for any given set of reagents and conditions. The same test should be done each time a new lot of reagent, especially sulfuric acid, is introduced. Whatever method is used, the efficiency of recovery of lactic acid added to blood samples also should be checked before unknown samples are analyzed.

(b) *Description of modified method*

The following procedure incorporates changes in the Barker-Summerson method to avoid the difficulties mentioned. Whole blood is first hemolyzed in water, then cupric sulfate and calcium hydroxide are added which remove most of the blood proteins as well as carbohydrates and other interfering substances. The remaining protein is precipitated with trichloroacetic acid. The rest of the procedure is unchanged except that the period of heating of lactic acid with sulfuric acid is shortened. Recovery of 4 mgm. per cent of added lactic acid is 100 $\pm$ 5 per cent, which is the error of the method.

Handling of samples. Duplicate blanks, duplicate standards, and duplicate or triplicate samples are done. Whole blood is collected in a syringe and 5 ml. is delivered immediately through a No. 20 needle into a 250 ml. Erlenmeyer flask containing 95 ml. of cold distilled water.

Separation of lactic acid. A 4 ml. aliquot of the aqueous blood mixture is immediately placed in a clean dry 18 by 150 mm. Pyrex test tube, and one ml. of 10 per cent copper sulfate added from a pipette. A brown precipitate forms slowly. The test tube is shaken and 0.5 Gm. of calcium hydroxide is added with a porcelain spatula. The tube is agitated vigorously, then allowed to stand at least 30 minutes with occasional shaking to assure thorough mixing of the olive-green precipitate. It may stand indefinitely at this point if stoppered.

The sample is centrifuged at 1,500 to 2,000 r.p.m. for five minutes, the supernatant carefully poured into an-

other dry test tube and again centrifuged for five minutes. A 2 ml. pipette is half-filled with supernatant and rotated for two minutes to rinse. A measured 2 ml. aliquot is taken up in the same pipette and transferred to another dry test tube. The precipitate from the surface film that remains after centrifuging must not be included. One-half ml. of 50 per cent trichloroacetic acid is added from a pipette and the test tube shaken thoroughly. After centrifuging for five minutes, a one ml. pipette is filled with supernatant and rotated for two minutes to rinse. A measured 1 ml. aliquot is taken in the same pipette and then transferred to a tube suitable for spectrophotometric measurements.

Oxidation of lactic acid. The test tube is cooled in an ice bath, and 6 ml. of concentrated sulfuric acid is added slowly dropwise from a pipette or fine-tipped burette with vigorous shaking. The tube is placed in a water bath at approximately 25° C. for five minutes, heated in a boiling water bath for exactly two minutes, then cooled in the 25° C. water bath for five minutes.

Color development and measurement. Two drops each of 4 per cent copper sulfate and p-phenylphenol reagent are added and the test tube immediately shaken. It is kept in a water bath at approximately 25° C. for at least 30 minutes with occasional agitation, but without shaking during the final five minutes. The tube is placed in a boiling water bath for exactly 90 seconds, then cooled in the bath at 25° C. for five minutes. Samples are measured at a wave length of 560 mμ. In comparison with distilled water, the transmittancy of the blank should be 80 per cent or more, and duplicate samples should check within 1.5 to 2 per cent.

(c) *Miscellaneous information on the procedure*

1. Syringes calibrated to deliver 5 ml. of blood are lubricated with one drop of parafin oil and stored in a refrigerator. No anticoagulant is used.

2. Stoppered Erlenmeyer flasks are stored in a refrigerator.

3. Samples stored in a refrigerator after blood is added to cold distilled water increase in lactate content, presumably due to conversion of blood glucose to lactate. A sample analyzed immediately after collection was found to contain 5.3 mgm. per cent of lactic acid and when re-analyzed 48 hours later had risen to 10.1 mgm. per cent. It is essential, therefore, to complete an analysis promptly.

4. The trichloroacetic acid is freshly prepared each week and stored in a refrigerator. An old solution tends to give a higher blank.

By a comparative set of analyses the results were 20 per cent higher when the addition of trichloroacetic acid was omitted, presumably due to interference by the protein remaining in solution.

5. P-phenylphenol precipitates when added to concentrated sulfuric acid and slowly dissolves with standing and agitation. To prepare the reagent, Eastman chemical No. 2174 was ground in a clear glass mortar; 1.5 Gm. was placed in a 100 ml. volumetric flask with 0.5 per cent sodium hydroxide and dissolved in a boiling water bath, cooled, and refrigerated in a brown bottle. There may be slight turbidity and a pink color.

6. Pfanstiehl zinc lactate, $Zn(CH_3CHOHCOO)_2 \cdot 2H_2O$, was heated four hours at 110° C. to remove the water of crystallization, stored in a desiccator with calcium chloride, and weighed as a standard when needed. The stock solution is stored in a refrigerator and freshly prepared every two months.

A calibration curve is constructed from data obtained by carrying suitable dilutions of the stock solution through the procedure. In the range under consideration, 0 to 12 mgm. per cent of lactic acid in whole blood, a straight line is obtained if lactic acid concentration is plotted against log per cent transmittancy of the colored solution. At greater concentrations the intensity of the color is not strictly proportional to lactic acid concentration, but samples may be measured after dilution with concentrated sulfuric acid.

7. Baker reagent grade chemicals were used except as noted, and color measurements made with a Coleman Model 6A Junior spectrophotometer.

8. Cleansing tissues are used for wiping pipettes, and Dreft for cleaning glassware. Dirty test tubes are first cleaned with a brush, then let stand with hydrochloric acid to dissolve adherent reagents.

9. Dust, dirt, chromic acid cleaning solution, perspiration, skin surfaces, and fingerprints are reported to interfere in the method.

10. Meticulous attention to detail is essential.

B. *Collection of Samples*

Cardiac catheterization was performed on 17 fasting patients under basal conditions. Blood samples were collected simultaneously from the pulmonary artery or right ventricle and the brachial artery during determinations of cardiac output, while the patient was in a steady state. Measurements were made at rest, during mild exercise with foot pedals which increased the oxygen uptake by 100 to 200 per cent after a steady state had been reached, and under conditions of acute hypoxia produced by breathing nitrogen-oxygen mixtures containing 11 to 16 per cent of oxygen, during a period not shorter than 20 minutes.

RESULTS

Tables I and II give data from 12 patients with normal arterial oxygen saturation, some of whom had cardiopulmonary disease and others had no disease. Table III lists the results of similar observations on five patients with moderate or severe chronic arterial oxygen unsaturation secondary to cardiopulmonary disease. In every instance the lactic acid content of the simultaneous mixed venous and arterial blood samples was the same within the limits of error of the analytical method, ± 5 per cent.

TABLE I

Determination of lactic acid in simultaneous arterial and mixed venous blood samples in four subjects without hypoxia

Pt.	Diagnosis	Lactic acid at rest					Lactic acid after exercise				
		Art. O_2 sat. %	Mixed ven. bl. mgm. %	Art. bl. mgm. %	Difference mgm. %	%	Art. O_2 sat. %	Mixed ven. bl. mgm. %	Art. bl. mgm. %	Difference mgm. %	%
1	Hypertensive H.D.	94	11.9	11.4	−0.5	4.3	96	19.6	20.0	+0.4	2.0
2	Idiopathic myocarditis, C.H.F.	98	9.9	10.1	+0.2	2.0	96	12.4	11.8	−0.6	5.0
3	Hypertensive H.D.	96	8.6	9.5	+0.9	9.9	94	9.3	10.3	+1.0	10.2
4	Minimal pulm. tbc.	98	14.3	13.4	−0.9	6.5	99	15.3	15.3	0	0
	Average	97	11.2	11.1	−0.1	5.7	96	14.2	14.4	+0.2	4.3

TABLE II

Determination of lactic acid in simultaneous arterial and mixed venous blood samples in eight subjects during induced hypoxia

Pt.	Diagnosis	Lactic acid at rest					Lactic acid during induced hypoxia				
		Art. O_2 sat. %	Mixed ven. bl. mgm. %	Art. bl. mgm. %	Difference mgm. %	%	Art. O_2 sat. %	Mixed ven. bl. mgm. %	Art. bl. mgm. %	Difference mgm. %	%
1	Chronic pulm. emphysema	95	7.4	7.6	+0.2	2.6	91	9.7	9.7	0	0
2	Chronic pulm. emphysema	95	9.4	9.7	+0.3	3.1	81	9.2	9.8	+0.6	6.2
3	Post-pneumonectomy	95	8.9	10.0	+1.1	11.6	74	12.8	12.8	0	0
		99	6.9	6.9	0	0	71	8.4	8.4	0	0
4	Normal	97	5.1	5.5	+0.4	7.5	70	6.4	6.5	+0.1	1.5
5	Minimal pulm. tbc.	95	8.6	9.5	+0.9	10.0	86	15.0	13.8	−1.2	8.3
6	Chronic pulm. emphysema	95	11.3	11.1	−0.2	1.9	81	9.5	9.9	+0.4	4.1
7	Normal	95	4.9	4.6	−0.3	6.9	76	6.0	6.3	+0.3	4.8
8	Normal	98	8.0	8.1	+0.1	1.2	84	15.3	15.4	+0.1	0.6
	Average	96	7.8	8.1	+0.3	5.0	79	10.3	10.3	0	2.8

TABLE III

Determination of lactic acid in simultaneous arterial and mixed venous blood samples in five subjects with chronic hypoxia

Pt.	Diagnosis	Lactic acid at rest					Lactic acid during induced hypoxia				
		Art. O_2 sat. %	Mixed ven. bl. mgm. %	Art. bl. mgm. %	Difference mgm. %	%	Art. O_2 sat. %	Mixed ven. bl. mgm. %	Art. bl. mgm. %	Difference mgm. %	%
1	Chron. pulm. emphys. Cor pulmonale	83	5.8	5.8	0	0					
2	Chron. pulm. emphys.	93	6.6	6.7	+0.1	1.5					
3	Art. scl. H.D., C.H.F.	92	6.8	6.8	0	0					
4	Silicosis; chron. pulm. emphys.	86	12.5	13.1	+0.6	4.7	66	12.5	13.8	+1.3	9.9
5	Chron. fibrotic tuberculosis	93	8.6	8.8	+0.2	2.3	71	10.0	10.0	0	0
	Average	89	8.1	8.2	+0.1	1.7	69	11.3	11.9	+0.6	5.0

DISCUSSION

The question of intrapulmonary metabolism is significant, because it is directly related to the measurement of cardiac output. The best present-day method for the determination of cardiac output in the steady state is by the Fick principle from oxygen uptake and arteriovenous oxygen difference during cardiac catheterization. However, the disappearance of lactic acid during passage through the lungs, reported by other investigators both in the resting state and under conditions of hypoxia or after exercise would suggest oxidation of this metabolite there. If the oxygen uptake in the lung of a subject is partly consumed in the pulmonary oxidation of lactic acid, calculated cardiac outputs would then be too high because according to the Fick principle, it is assumed that all the oxygen uptake is transported to the blood, and is identical to the oxygen consumption in the tissues. If it were not so, a large error in computing figures of cardiac output would obtain. For example, let us suppose that a patient has an oxygen consumption of 300 ml. per minute, an arteriovenous oxygen difference of five volumes per cent, and that 3 mgm. per cent of lactic acid disappears during passage through the lungs and is completely oxidized there.

$$CH_3CHOHCOOH + 3O_2 \rightarrow 3CO_2 + 3H_2O$$

Then 3 mgm. per cent × 41.66 (the real blood flow, see below) or approximately 120 mgm. of lactic acid disappears per minute, which is equivalent to 128 mgm. or 90.7 ml. of oxygen.

$$\text{Calculated cardiac output} = \frac{O_2 \text{ used}}{\text{A-V diff.}} = \frac{300}{5/100} = 6{,}000 \text{ ml. per min.}$$

$$\text{Real cardiac output} = \frac{300 - 90.7}{5/100} = 4{,}166 \text{ ml. per min.}$$

The calculated cardiac output would then be approximately 50 per cent too high in that instance.

Since our data show no evidence of the disappearance of lactate in the lung, no doubt is cast concerning the validity of measurements of cardiac output under these conditions. Although Bolt (15) found the arterial and venous content of pyruvate to be identical in 15 humans with and without cardiopulmonary disease, as did Goodale and his co-workers (9) in dogs, the possibility remains nevertheless that other metabolites may be oxidized in the lung, and any pulmonary oxidative process would vitiate cardiac output measured according to the Fick principle. To date no such evidence is available.

SUMMARY

1. The Barker-Summerson procedure for the determination of lactic acid has been modified for use with whole blood.

2. The sample is hemolyzed in water, and carbohydrates and most of the blood proteins are removed with cupric and calcium hydroxides. The remainder of the protein is precipitated with trichloroacetic acid. The period of heating with sulfuric acid in a boiling water bath for oxidation of lactate to acetaldehyde is shortened from five to two minutes.

3. Seventeen patients with and without cardiopulmonary disease were studied at rest, during mild exercise, and in acute hypoxia. Simultaneous blood samples from the right heart and the brachial artery invariably contained the same quantity of lactic acid. There was no evidence of intrapulmonary oxidation, and no doubt is cast on the validity of cardiac output by the Fick principle under these conditions.

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