Changes in Cardiac Lymph of Dogs during and after Anoxia

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CARDIAC lymph is a product of the interstitial fluid pool in the myocardium. Its composition, therefore, reflects that of the intercellular spaces, in health and in disease. Changes in the heart caused by any form of injury are related to the exchange of fluid and particles across the membranes of capillaries and myocardial cells. Alterations in this exchange will also be reflected in the volume and composition of lymph collected from the heart. Such changes have previously been demonstrated in lymph from other organs [8, 16, 17, 18, 24] and the findings support the logical assumption that lymph reflects the degree of tissue injury more accurately than blood.[2]

Very little is known about the behavior of cardiac lymph during and after injuries to the myocardium. Except for brief reports of the effects of anoxia on cardiac lymph flow in one dog examined by Maurer [18] and in two by Miller *et al*,[19] there is no reference to this subject.

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The present paper reports a series of experiments in 15 dogs, designed to demonstrate some of the effects of hypoxia and anoxia upon the flow and composition of cardiac lymph.

Materials and Methods

Fifteen adult mongrel dogs were anesthetized with sodium pentobarbital (30 mg./Kg.) and ventilated with a mixture of room air and oxygen (5 l./min.). A slow, constant infusion of Ringer's solution (10 drops/min.) was given through the femoral vein. The blood pressure was continuously monitored via a cannula inserted into the femoral artery. EKG was also monitored throughout the experiment.

The heart was exposed through a left anterolateral thoracotomy in the fourth intercostal space. The pericardium was opened and the cardiac lymphatic vessels entering the cardiac node were demonstrated by injection of T1824 dye. The afferent lymph vessel to the node was cannulated and lymph collected as described by us elsewhere.[23] The first hour's collection of lymph was used to determine the baseline of flow, pH, electrolyte, protein and lactate concentrations. Coronary sinus blood for corresponding estimations of electrolytes, proteins and lactates was obtained by direct puncture. Arterial blood gases were determined repeatedly during all stages of the experiments.

General anoxia was produced in ten dogs by stopping the ventilator and clamping

TABLE 1. *Parameters in Cardiac Lymph and Coronary Sinus Blood before and after Generalized Anoxia*

	Before Anoxia		After Anoxia					
			1st Hour		2nd Hour		3rd Hour	
	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood
Flow, cc./hr.	2.6		4.6		4.2		4.0	
(range)	(1.8–4)		(3.0–6.0)		(2.5–7.0)		(2.0–6.0)	
Na, mEq./l.	144	142	148	141	150	142	149	
(range)	(132–150)		(144–158)		(140–164)		(140–169)	
Cl, mEq./l.	109	107	112	106	111	107	108	
(range)	(96–119)		(98–121)		(98–117)			
K, mEq./l.	2.8	3.3	2.8	2.8	2.6	2.9	2.6	
(range)	(2.3–3.4)		(1.3–3.7)		(2.3–3.1)		(2.2–3.0)	
Total Protein (Gm./100 ml.)	3.2	5.5	3.4	5.5	3.3	5.4		
Albumin/globulin ratio	1.12		0.99		0.85			
Lactates (mg./100 ml.)	16.5	.417	25.7	20.7	25.1	16.6	22.7	15.6

the endotracheal tube. This resulted in an initial rise in blood pressure followed by a rapid drop about 3 to 4 minutes later. As soon as cardiac arrest had occurred, the dog was resuscitated with cardiac massage and ventilation with 100% oxygen. At the same time 80 mEq. of $NaHCO_3$ was given intravenously. These measures were sufficient to restore myocardial activity in nine animals, whereas one dog also required electric defibrillation of the heart. Arterial blood gases were determined and metabolic acidosis corrected by further administration of bicarbonate as indicated. The animals were then maintained on 96% O_2 and 4% CO_2 for the rest of the experiment. This series of experiments will in the following pages be referred to as *generalized anoxia.*

Cardiac lymph was collected continuously for the next 2 to 3 hours, and each ½ hour specimen was analyzed for pH, electrolytes, proteins and lactate concentrations. Parallel analyses were carried out in samples of coronary sinus blood.

In two dogs, generalized hypoxia was produced, using a breathing mixture of 10% O_2 and 90% N_2. Cardiac arrest did not occur in either of these animals. The experimental situation is termed *generalized hypoxia.*

In three animals, ischemic anoxia of a portion of the myocardium was produced by occluding the left anterior descending coronary artery for 1 hour. Lymph and coronary sinus blood specimens were collected during this period. The occlusion was then released and samples collected for another 2 hours. This situation will be referred to as *local anoxia.*

The lymph color in the thoracic duct in the posterior mediastinum was inspected in all animals. In four dogs of the first group, thoracic duct lymph was collected for comparative analyses.

Results

A. Lymph Flow and Color. Generalized hypoxia and anoxia both caused a sharp rise in the flow rate of cardiac lymph. The increase was noticeable within 5 minutes of the anoxia episode and continued for the next 1½ hours, when it started to decrease slowly. Even 3 hours after anoxia, however, the flow rate was still higher than the preanoxia level (Table 1). The cardiac lymph also became uniformly blood stained in all animals subjected to generalized anoxia, and numerous erythrocytes were present in smear preparations of the lymph.

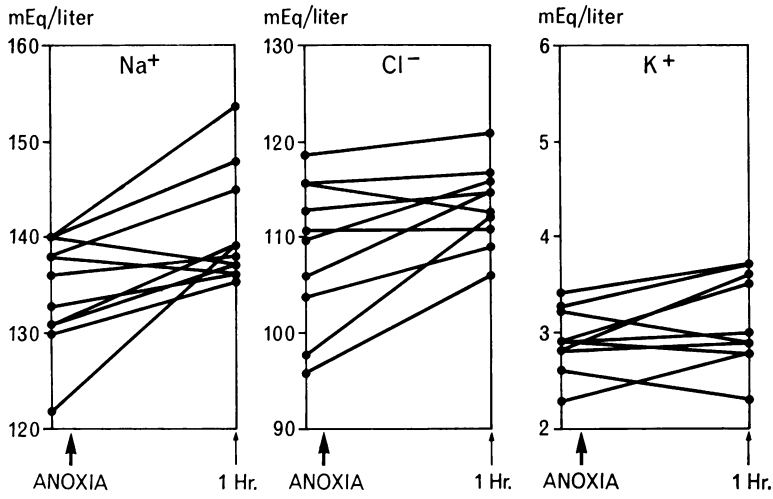

FIG. 1. Demonstrating the influence of anoxia on electrolyte concentrations in cardiac lymph.

Staining was noticed from 5 to 15 minutes after resuscitation and was present until termination of the experiment in all animals.

An identical increase in lymph flow was recorded in the two dogs where generalized hypoxia was induced by breathing the low-oxygen mixture. In contrast, however, the lymph remained clear throughout the experiment.

In the animals subjected to local anoxia, lymph flow increased slightly during the period of occlusion, and the lymph remained clear: Immediately after release of the occlusion, the flow rate increased considerably and remained high during the rest of the experiment. The lymph also became blood-stained within 2 to 3 minutes after re-establishment of coronary circulation to the previously ischemic myocardium.

The thoracic duct lymph also increased in volume following generalized hypoxia/anoxia, but the flow returned to pre-anoxic levels within 1 hour. Blood staining of this lymph was not noticed in any of the animals.

B. Electrolytes. The mean and individual changes in electrolyte concentrations are presented in Table 1 and Figure 1. In general there was a moderate increase in the sodium and chloride concentrations in cardiac lymph following arrest and resuscitation. The elevation usually persisted for 2 hours before values began to decline. Corresponding analyses of coronary sinus blood showed no significant changes. The potassium concentration was unchanged or slightly below preanoxic levels during the first hour and then decreased over the next two hours.

C. Proteins. The results of protein analyses are given in Table 1 and Figure 2. In the majority of animals there was an appreciable rise in total protein concentration of cardiac lymph following anoxia. Electrophoresis of cardiac lymph in six animals with generalized anoxia showed an increase both in albumin and globulin fractions as compared to the pre-anoxia levels. The increase in globulin fractions, however, was proportionately higher, thus giving a decreasing A/G ratio in all animals (Fig. 2). This rise in protein concentration and declin of the A/G ratio persisted for at least 2 hours after correction of the anoxia.

Two animals showed no significant change in the protein analyses, and two others had a decrease in total protein concentration.

D. Lactates. The preanoxia levels of lactate in cardiac lymph were generally higher than in the coronary sinus blood, corresponding to our earlier observations.[23] Following generalized anoxia and resuscitation, the lactate concentration increased both in cardiac lymph and in coronary sinus blood during the first hour. Thereafter, the coronary sinus levels invariably decreased and reached preanoxia values within 2 to 3 hours of the anoxic episode. The lymph values of lactate showed a noticeably different pattern. The rise in lactate usually continued for 1½ to 2 hours, after which it started a slow decline. In all animals, the lactate concentration in cardiac lymph remained significantly elevated throughout the experiment, while the value for coronary sinus blood usually reverted to preanoxia levels (Fig. 3).

In the experiments on local anoxia the lactate elevation, although present, was not marked in either lymph or coronary sinus blood during occlusion. Once the blood flow was reestablished, the lactate concentration continued to increase and followed the same pattern as describe above.

E. pH. The pH of cardiac lymph was uniformly above 8 (off instrument scale) at the beginning of all experiments. The actual values during anoxia could not be established since the amount of lymph collected during this short period was insufficient for analyses. Immediately after resuscitation from generalized anoxia, a decrease in pH took place; the lowest recorded value being 7.50. Within 15–30 minutes after resuscitation, the lymph pH climbed to preanoxia levels and remained steady for the remaining period.

Discussion

Impaired oxygenation has been shown to produce an increase in flow and protein transfer in cervical,[17, 18] pulmonary [24] and thoracic duct lymph.[8] It has been suggested that the raised flow is due to increased capillary permeability caused by endothelial damage from lack of oxygen.[11, 14, 20] Other authors claim that the cause of increased flow is a rise in capillary filtration pressure due to complex cardiovascular changes occurring in response to hypoxia or anoxia.[7, 13] The main argument in support of the latter concept is that in most experiments on tissue lymph and hypoxia, the flow rise is transitory with rapid re-

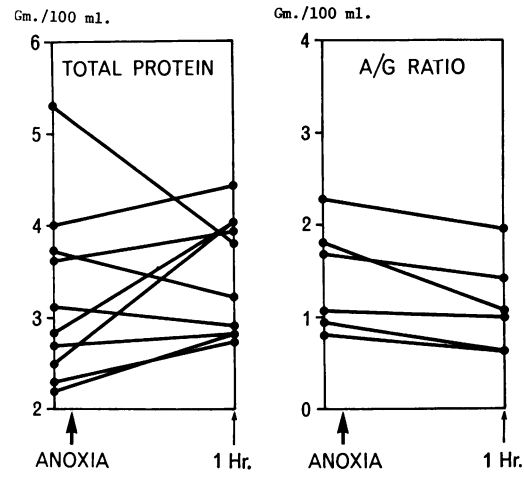

FIG. 2. Showing the changes in total protein concentrations and albumin/globulin ratio of cardiac lymph following anoxia.

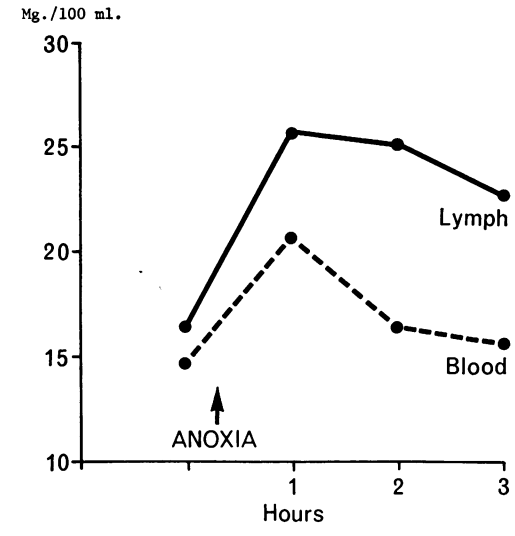

FIG. 3. Trend in changes of mean lactate concentrations in cardiac lymph and coronary sinus blood following anoxia.

versal to normal values. It would be expected that real endothelial damage would be followed by flow changes for prolonged periods of time. Recent work by Adamson *et al.*[1] on pulmonary lymph also tends to support the theory of increased capillary filtration pressure.

Our present results seem to indicate that in the myocardial capillaries, both mechanisms are operating, dependent on the degree and duration of tissue hypoxia. The increase in lymph flow following anoxia persisted for up to 2 to 3 hours without reversion to preanoxia levels. This pattern was present in spite of adequate circulation, ventilation and correction of acid-base-disturbances. Furthermore, the immediate and persistent blood staining of the lymph also is indicative of an increased capillary permeability.

Lymph collected from various organs has been shown to contain a few red cells [4] and erythrocytes have reportedly been observed passing through capillary walls into lymphatics.[6] To obtain macroscopic blood staining of lymph, however, whole blood must escape into the interstitial tissue. It thus seems very unlikely that pronounced blood staining of cardiac lymph can take place without a considerable increase in capillary permeability, most likely associated with reversible or irreversible endothelial damage.

A review of studies on asphyxia and capillary permeability concludes that a severe degree of hypoxia may be needed to produce a demonstrable effect, and that different tissues may differ in their sensitivity to lack of oxygen.[15] This view is supported by two experiments in our series where only mild generalized hypoxia without cardiac arrest was induced. Both these animals showed an increase in cardiac lymph flow but without blood staining. It appears possible that in these animals, the effect might be due to increased filtration pressure alone, as caused by the hypertension and other cardiovascular phenomena in response to hypoxia. Increased permeability of the capillary wall, possibly associated with endothelial damage, may well be absent in moderate hypoxia. In generalized anoxia, however, our observations strongly support the concept of increased endothelial permeability.

This point is not only of theoretical interest but also carries a clinical significance. As pointed out by Maurer [18] "It seems possible that the decreased oxygen supply has a more damaging influence on the capillaries of the heart than on those of the region drained by the cervical ducts." The extreme sensitivity of the cardiac muscle to lack of oxygen is well appreciated. Attention is drawn, however, to the present observations on cardiac lymph which indicate that even after adequate resuscitation from a very brief cardiac arrest, status quo is not restored for a considerable period of time.

Our results from the protein analyses differ greatly from those reported by previous investigators. Maurer [18] reported a fall in protein concentration after anoxia in one single dog. Considering the increased flow of lymph, however, he concluded that the total leakage of protein into the lymphatics was increased. Miller *et al.*[19] found the protein concentration to remain stable following anoxia and drew a similar conclusion. Our results, on the contrary, show an average increase in protein concentrations; most pronounced for the globulin fractions as expressed by a decrease of the A/G ratio. This observation also favors the above mentioned concept of increased capillary permeability.

Similar changes have been reported to occur in lymph from other organs after tissue injury.[8, 21, 22] The increased proteins were shown to be derived mainly from the circulating plasma.[21, 22] Further studies on permeability of myocardial capillaries under various circumstances are certainly warranted. A rewarding approach would seem to be the combination of lymph analysis

and studies of ultrastructure using electron-dense tracer proteins such as in the peroxidase method.

Anoxia affects not only the capillary circulation and permeability but also the myocardial muscle cell. There is evidence from physiologic and morphologic studies of human myocardium that anoxia; a) affects the function of the mitochondria; b) disrupts the structural integrity of the inotropic unit and; c) interferes with the transport of fluids and ions across the cell membrane.[5] Excessive lactic acid accumulation during anaerobic metabolism is believed to overcome the intracellular buffer capacity, thus releasing intracellular enzymes which destroy the cellular metabolic systems.[9, 10, 25] An increase in lactates and lysosomal enzymes with a concomitant fall in pH has been demonstrated in thoracic duct lymph of dogs following hemorrhagic or endotoxin shock.[2]

In the present experiments, the lactate concentration of cardiac lymph increased following anoxia and stayed elevated for several hours. Furthermore, the lymph analyses showed more pronounced and longer-lasting changes than corresponding samples of coronary sinus blood. These observations seem to support our previous theory that cardiac lymph is a more sensitive parameter of myocardial metabolism. They also indicate that myocardial damage from anoxic arrest may be more profound than previously believed after studies of coronary venous return only.

Further investigations of this question, including enzyme and lipid analyses, warrant attention because of their considerable clinical significance.

The normal pH value in our experiments was consistently around or above 8, and decreased sharply during anoxia. This drop is probably related to the concomitant increase in lactic acid production. With resuscitation and restored circulation, however, the pH returned to normal levels in spite of increasing lactate concentration in the lymph. The explanation is probably that plasma bicarbonate again was available in the interstitial fluid as soon as the capillary and para-capillary circulation was restored.

Our experiments showed minor electrolyte alterations in the cardiac lymph after anoxia, with an increase in the mean values for sodium and chloride and a decrease of potassium concentration. The explanation for these changes also must be sought in alterations of ion transfer across the capillary and myocardial cell membranes. The relative importance of these two factors to the changes in electrolyte composition cannot be established from the present experiments.

Summary

The effects of hypoxia and anoxia upon the flow and composition of cardiac lymph were investigated in 15 dogs. Both experimental conditions produced a considerable increase of lymph flow. Following generalized and local anoxia the lymph also became blood-stained. Analyses of proteins in the lymph showed increased concentrations of albumin as well as globulins, but particularly of the high-molecular fractions. These observations suggest that anoxia produces a considerably increased exchange across the capillary membrane and also alters the permeability of the capillary endothelium.

Lactate concentrations in cardiac lymph showed a pronounced increase following myocardial anoxia. The lactate alterations in the lymph specimens were more marked and long-lasting than changes in venous blood from the coronary sinus. This pattern supports the view that lymph is a more sensitive index of changes occurring in the myocardium, and that even short periods of myocardial anoxia have severe effects upon myocardial metabolism.

The physiological and clinical significance of these observations is discussed.

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References

1. Adamson, T. M., Boyd, R. H. D., Hill, J. R., Normand, I. C. S., Reynolds, E. O. R. and Stang, L. B.: Effect of Asphyxia Due to Umbilical Cord Occlusion in the Fetal Lamb on Leakage of Liquid from the Circulation and on Permeability of Lung Capillaries to Albumin. J. Physiol. (London), 207:493, 1970.
2. Berman, I. R., Moseley, R. V., Lamborn P. B. and Sleeman, H. K.:Thoracic Duct Lymph in Shock: Gas Exchange, Acid Base Balance and Lysosomal Enzymes in Hemorrhagic and Endotoxin Shock. Ann. Surg., 169:202, 1969.
3. Beznak, A. B. L. and Liljestrand, G.: The Effect of Variations in Oxygen Tension on the Lymph Flow. Acta Physiol. Scand., 19:170, 1949.
4. Bierman, H. R., Byron, R. L., Kelly, K. H., Gilfillan, R. S., White, L. P., Freeman, N. E. and Petrakis, N. L.: The Characteristics of Thoracic Duct Lymph in Man. J. Clin. Invest., 32:637, 1953.
5. Burdette, W. J. and Ashford, T. P.: Structural Changes in the Human Myocardium Following Hypoxia. J. Thorac. Cardiovasc. Surg., 50:210, 1965.
6. Clark, E. R. and Clark, E. L.: Observations on Living Mammalian Lymphatic Capillaries; Their Relation to the Blood Vessels. Am. J. Anat., 60:253, 1937.
7. Courtice, F. C. and Korner, P. I.: The Effect of Anoxia on Pulmonary Edema Produced by Massive Intravenous Transfusions. Aust. J. Exp. Biol. Med. Sci., 30:511, 1952.
8. Courtice, F. C.: The Transfer of Proteins and Lipids from Plasma to Lymph in the Leg of the Normal and Hypercholesterolemic Rabbit. J. Physiol. (London), 155:456, 1961.
9. De Haan, R. L. and Field, J.: Mechanism of Cardiac Damage in Anoxia. Am. J. Physiol., 197:449, 1959.
10. Dumont, A. E. and Weissmann, G.: Lymphatic Transport of Beta-glucuronidase during Hemorrhagic Shock. Nature (London), 201:1231, 1964.
11. Hendley, E. D. and Schiller, A. A.: Changes in Capillary Permeability during Hypoxemic Perfusion of Rat Hind Legs. Am. J. Physiol., 179:216, 1954.
12. Katzenstein, R., Mylon, E. and Wintermitz, M. C.: The Toxicity of Thoracic Duct Fluid after Release of Tourniquets Applied to the Hind Legs of Dogs for the Production of Shock. Am. J. Physiol., 139:307, 1943.
13. Korner, P. I. and Courtice, F. C.: The Effects of Acute Anoxia and Noradrenaline Vasoconstriction on Lymph Flow and Protein Dynamics Following Transfusions of Ringer-Locke Solution. Aust. J. Exp. Biol. Med. Sci., 32:321, 1954.
14. Landis, E. M.: Microinjection Studies of Capillary Permeability. III; The Effect of Lack of Oxygen on the Permeability of the Capillary Wall to Fluid and to the Plasma Proteins. Am. J. Physiol., 83:528, 1927–1928.
15. Landis, E. M. and Pappenheimer, J. R.: Exchange of Substances Through Capillary Walls. *In* Handbook of Physiology, Section II: Circulation. Ed. Hamilton, W. F. and Dow, P. Vol. II, Chapter 29, Washington, American Physiological Society.
16. Lewis, G. P.: Intracellular Enzymes in Local Lymph as a Measure of Cellular Inquiry. J. Physiol., 191:591, 1967.
17. Maurer, F. W.: The Effects of Carbon Monoxide Anoxemia on the Flow and Composition of Cervical Lymph. Am. J. Physiol., 133: 170, 1941.
18. Maurer, F. W.: The Effects of Decreased Blood Oxygen and Increased Blood Carbon Dioxide on the Flow and Composition of Cervical and Cardiac Lymph. Am. J. Physiol., 131:331, 1940–1941.
19. Miller, A. J., Ellis, A. and Katz, L. N.: Cardiac Lymph Flow Rates and Composition in Dogs. Am. J. Physiol., 206:63, 1964.
20. Pochin, E. E.: Edema Following Ischemia in the Rabbit's Ear. Clin. Sci., 4:341, 1942.
21. Roberts, J. C. and Courtice, F. C.: Measurements of Protein Leakage in the Acute and Recovery Stages of a Thermal Injury. Aust. J. Exp. Biol. Med. Sci., 47:421, 1969a.
22. Roberts, J. C. and Courtice, F. C.: Immuno Electrophoretic Analysis of Proteins in Lymph from the Leg before and after Thermal Injury. Aust. J. Exp. Biol. Med. Sci., 47:435, 1969b.
23. Ullal, S. R., Kluge, T. H., Kerth, W. J. and Gerbode, F.: Flow and Composition of Cardiac Lymph in Dogs. (Pending in Press.)
24. Warren, M. F. and Drinker, C. K.: The Flow of Lymph from the Lungs of the Dog. Am. J. Physiol., 136:207, 1942.
25. Weissmann, G.: Lysosomes. N. Engl. J. Med., 273:1084, 1143, 1965.
26. Woolley, G. and Courtice, F. C.: The Origin of Albumin in Hepatic Lymph. Aust. J. Exp. Biol. Med. Sci., 40:121, 1962.