Observations on a Model of Proliferative Lung Disease

I. TRANSPULMONARY ARTERIOVENOUS DIFFERENCES OF LACTATE, PYRUVATE, AND GLUCOSE

B. STRAUSS, P. R. B. CALDWELL, and H. W. FRITTS, JR.

From the Department of Medicine, College of Physicians & Surgeons, Columbia University, New York, New York 10032

ABSTRACT Intravenous injections of complete Freund's adjuvant, used by others to stimulate the reticuloendothelial system of small laboratory animals, produced granulomas resembling sarcoid in the lung of the dog. At the height of the disease, when granulomas occupied more than half of the alveolar tissues, transpulmonary arteriovenous ($A\text{-}\bar{V}$) differences of lactate, pyruvate, and glucose were measured. When the diseased dogs breathed room air, the $A\text{-}\bar{V}$ differences of lactate and pyruvate were greater than normal; and when the dogs breathed an hypoxic mixture, the differences increased further. Hence the model affords the opportunity for studying the in vivo metabolism of diseased lungs. It may also prove useful for studying other aspects of granulomatous disease which cannot be easily approached in man.

INTRODUCTION

Compared to the information available on most aspects of proliferative pulmonary disorders, knowledge of the in vivo metabolism of the diseased lung is meager. Even in active tuberculosis, where the lung appears to have an enhanced energy consumption (1), the metabolic behavior of the lung is uncertain because transpulmonary arteriovenous ($A\text{-}\bar{V}$) differences of metabolites have not shown consistent patterns. One contributing factor has been the large ratio of blood flow to tissue mass in the lung, a circumstance tending to minimize the $A\text{-}\bar{V}$ difference of any metabolite. Another factor has been the inhomogeneous distribution of pulmonary lesions, a condition which may cause differences across involved regions to be diluted to unmeasurably small values by blood perfusing normal lobules. This latter effect was demonstrated in an earlier study of lactate metabolism in the lungs of patients undergoing thoracotomy for tuberculosis (2). Whereas the difference across each diseased lobe or lobule showed a definite production of lactate, the difference across both lungs was often near zero.

To circumvent the difficulties inherent in measuring $A\text{-}\bar{V}$ differences in man, we sought an experimental model in which the involvement of the lung would be so extensive that the diluting effect would be minimized. We therefore investigated the feasibility of utilizing in dogs a method employed by others to evoke a granulomatous response in smaller laboratory animals (3, 4). The procedure entailed injecting complete Freund's adjuvant[1] intravenously, then waiting several weeks for the development of lesions. Our results were similar to those obtained in the smaller animals (3, 5); namely, the lungs filled with nodules which resembled the lesions of sarcoid. Although the lungs were severely affected, the dogs appeared healthy and could be easily studied. Hence, the model not only resembled human disease but could be examined by the techniques commonly applied in man.

The purpose of this paper is to describe our initial experience with the model, and to present data on the following points: (*a*) assessment of the pathologic changes in the diseased lung; and (*b*) measurement of $A\text{-}\bar{V}$ differences of lactate, pyruvate, and glucose when the dogs breathed either 21% oxygen or an hypoxic mixture.

This work was presented as an abstract. Strauss, B., S. Kammerman, and A. Cournand. 1966. Study of pulmonary granulomatous disease in dogs. *Fed. Proc.* **25**: 694.

Received for publication 5 May 1969 and in revised form 3 March 1970.

[1] Complete Freund's adjuvant (Difco Labs, Detroit, Mich.) contains 5 mg of *Mycobacterium butyricum*, killed and dried in 8.5 ml of paraffin oil and 1.5 ml of emulsifier.

METHODS

To produce the pulmonary lesions, complete Freund's adjuvant (0.3 ml/kg) was slowly injected into the forepaw vein of the dog on each of two successive days. Thereafter the dog lived in standard animal quarters without special care or special diet. Between the 21st and 35th days after injection, when the pulmonary lesions were maximal, $A-\bar{V}$ differences of lactate, pyruvate, and glucose were measured and compared to those found in control dogs free of lung disease. For these studies each dog was fasted overnight, then anesthetized with intravenous pentobarbital sodium (25 mg/kg). The trachea was intubated, the femoral artery cannulated, and the pulmonary artery catheterized under fluoroscopic control. The dog was then placed supine on an animal cradle with protective blankets to prevent fluctuations in body temperature. When these preparations were completed, an intravenous dose of heparin (100 U/kg) was administered to prevent clotting of blood samples. As a means of insuring a stable level of anesthesia, an intravenous drip of sodium thyamylol (2 mg/ml) was adjusted so that the inner canthus reflex was present, while the outer canthus reflex was abolished. As a further check on the constancy of the anesthetic level, the minute volume of ventilation was measured every 3 min during each experimental period.

At the beginning of the period the tracheal tube was connected to an open breathing circuit comprising a tank of compressed gas, a one-way breathing valve, and a Benedict-Roth spirometer. Through this system the control dogs breathed either 21 or 14% oxygen. Between the 20th and 22nd min of the run, expired gas was collected for Micro-Scholander analysis of oxygen and carbon dioxide concentrations. At the same time, samples of blood were simultaneously drawn from the pulmonary and femoral arteries into acid-washed, heparinized glass syringes. Aliquots from the syringes were promptly transferred by volumetric pipettes to iced test tubes containing acid for denaturation of protein. The aliquots were then analyzed for lactate[2] and for pyruvate (6). A third sample was analyzed for glucose.[3] In addition, a separately drawn sample of arterial blood was analyzed for oxygen content, oxygen capacity, and carbon dioxide content by the method of Van Slyke and Neill (7). To calculate alveolar oxygen tension, the inspired oxygen concentration, the calculated respiratory quotient, and the arterial blood carbon dioxide tension were substituted in the alveolar air equation. A preliminary study showed that comparable levels of alveolar oxygen tension could be achieved by administering 13% oxygen to the control dogs and 14% oxygen to the diseased dogs.

Each of the eight control dogs breathed 21% oxygen during one study period, and five of them also breathed 13% oxygen during a second, identical period. In the latter five the sequence of breathing 21 or 13% oxygen was randomized. Similarly, the diseased dogs breathed 21 or 14% oxygen in random sequence. Both periods were successfully completed in four of the diseased dogs (Nos. 781, 844, 880, 1135), but clot formation in the blood of one (No. 1066) during hypoxia necessitated substituting another animal (No. 866) to complete the group.

At the end of the study each dog was exsanguinated by transecting the femoral arteries. As soon as respiratory movements ceased, the trachea was transected 1 inch above the carina, and the heart and mediastinal structures were cut away. After being filled with 10% formalin, the lungs were placed in a formalin bath for 72 hr before being processed for histologic and morphometric studies. Four samples of tissue, each measuring approximately 3.0 × 1.5 cm, were removed from the peripheral portion of the right lung, and four from the left. The eight pieces were imbedded in paraffin, then cut and stained with hematoxylin and eosin. From the eight specimens, four were chosen at random for analysis by the point-counting method of Chalkley (8). This method, adapted to the lung by Weibel and Gomez (9) and applied by Dunnill (10) and by Strauss (11), entailed examining sections of lung with a microscope equipped with a Zeiss integrating eyepiece holding a grid of 25 points. 11 fields were examined in each specimen, and the number of grid points touching the various structures were recorded. By this technique the lung was divided into normal and abnormal components. Since the grid contained 25 points, the analysis embraced 25 points × 11 fields × 4 specimens = 1100 points for each dog.

RESULTS

Gross and microscopic features of the diseased lung. The injection of adjuvant produced florid lesions in the lungs of the majority of dogs. While the time course of the development of lesions was variable, most dogs showed extensive pulmonary involvement by the beginning of the 3rd wk. From this time to the end of the 4th wk the lesions in the lung remained at a rather constant level, and thereafter regressed.

At the height of the disease the surface of the lung had a dark raspberry color. Palpable nodules, with diameters up to 5 mm, were diffusely distributed over the pleura. When examined under low-power magnification, the lungs appeared to be filled with both discreet and conglomerate lesions. Under high-power most of the lesions had structures characteristic of granulomas, with epithelioid cells surrounded by lymphocytes, polymorphonuclear leukocytes, and plasma cells. There were occasional eosinophils. Although cavitation was not seen, some fields contained small holes which lipid stains showed to be filled with fat. The droplets presumably came from the paraffin oil of the injected adjuvant.

The results of point-counting are presented in Table I. On the average, 56% of the lung was granulomatous

TABLE I

Composition of the Lung of Eight Control and Six Diseased Dogs

	Normal parenchyma	Granuloma	Micro-abscess	Necrotic area
Control dogs	95 (91–98)	0	5 (2–8)	0
Diseased dogs	40 (33–53)	56 (46–64)	2 (0–8)	2 (0–6)

Each component is expressed as an average percentage with a range.

[2] C. F. Boehringer and Soehne, Calbiochem, Los Angeles, Calif.

[3] Glucostat, Worthington Biochemical Corp., Freehold, N. J.

TABLE II

Measured Whole Blood Concentrations (mm/liter) of Lactate (L) and Pyruvate (P); Calculated Values of the Lactate-Pyruvate Ratio (L/P); and Calculated Transpulmonary A-V Differences of Lactate, Pyruvate, and Lactate-Pyruvate Ratio in Normal and Diseased Dogs. A Positive Difference Signifies a Higher Concentration in the Systemic Arterial than in the Mixed Venous Blood

Dog No.	Systemic arterial			Mixed venous			Difference		
	L	P	L/P	L	P	L/P	L	P	L/P
I Normal dogs breathing 21% O_2									
809	0.63	0.046	13.7	0.66	0.049	13.5	−0.03	−0.003	+0.2
837	1.20	0.113	10.6	1.25	0.107	11.7	−0.05	+0.006	−1.1
845	0.98	0.086	11.4	1.07	0.082	13.1	−0.09	+0.004	−1.7
853	1.14	0.106	10.8	1.26	0.098	12.9	−0.12	+0.008	−2.1
1136	0.94	0.091	10.3	0.91	0.085	10.7	+0.03	+0.006	−0.4
1029	0.40	0.051	7.8	0.36	0.045	8.0	+0.04	+0.006	−0.2
1098	1.03	0.086	12.0	0.91	0.082	11.1	+0.12	+0.004	+0.9
1173	2.14	0.148	14.5	2.03	0.141	14.4	+0.11	+0.007	+0.1
1223	1.73	0.168	10.3	1.80	0.159	11.3	−0.07	+0.009	−1.0
912	0.80	0.103	7.8	0.83	0.107	7.8	−0.03	−0.004	0
II Diseased dogs breathing 21% O_2									
781	0.80	0.067	11.9	0.76	0.061	12.4	+0.04	+0.006	−0.5
844	1.24	0.080	15.5	1.16	0.068	17.0	+0.08	+0.012	−1.5
880	2.58	0.227	11.4	2.49	0.220	11.3	+0.09	+0.007	+0.1
1135	3.03	0.155	19.6	2.89	0.144	20.1	+0.14	+0.011	−0.5
1066	1.27	0.107	11.9	1.16	0.086	13.5	+0.11	+0.021	−1.6
III Normal dogs breathing 13% O_2									
1029	0.46	0.052	8.8	0.56	0.048	11.7	−0.10	+0.004	−2.9
1098	1.32	0.094	14.0	1.37	0.092	14.9	−0.05	+0.002	−0.9
1173	2.29	0.148	15.5	2.34	0.142	16.5	−0.05	+0.006	−1.0
1223	1.69	0.140	12.1	1.70	0.135	12.6	−0.01	+0.005	−0.5
912	1.30	0.144	9.0	1.34	0.134	10.0	−0.04	+0.010	−1.0
IV Diseased dogs breathing 14% O_2									
781	1.26	0.110	11.4	1.09	0.093	11.7	+0.17	+0.017	−0.3
844	1.78	0.103	17.3	1.35	0.080	16.9	+0.43	+0.023	+0.4
880	3.51	0.292	12.0	3.29	0.268	12.3	+0.22	+0.024	−0.3
1135	2.37	0.135	17.6	2.10	0.120	17.5	+0.27	+0.015	+0.1
866	0.89	0.065	13.7	0.62	0.049	12.6	+0.27	+0.016	+1.1
Mean values									
Group I	1.10	0.100	10.9	1.11	0.096	11.4	−0.01	+0.004	−0.5
Group II	1.78	0.127	14.1	1.69	0.116	14.9	+0.09	+0.011	−0.8
Group III	1.41	0.116	11.9	1.46	0.110	13.1	−0.05	+0.005	−1.3
Group IV	1.96	0.141	14.4	1.69	0.122	14.2	+0.27	+0.019	+0.2

tissue, while 2% had the appearance of microabscesses. Necrosis was uncommon, being absent in three of the six diseased dogs, and present in the remaining three to the extent of 2, 5, and 6%, respectively. The lungs of the control dogs were normal, apart from a few microabscesses which, on the average, constituted 5% of the pulmonary tissue.

Measured concentrations of blood lactate, pyruvate, and glucose; calculated values of the lactate-pyruvate (L/P) ratio. To simplify the presentation of the metabolic data, the dogs have been divided into the following four groups: group I, control dogs breathing 21% oxygen; group II, diseased dogs breathing 21% oxygen; group III, control dogs breathing 13% oxygen; and group IV, diseased dogs breathing 14% oxygen. Table II lists the measured concentrations and calculated ratios, while Table III shows the statistical analyses.

As may be seen in Table II, the mean arterial blood concentrations of lactate and pyruvate in the control dogs breathing 21% oxygen (group I) were, respectively, 1.10 and 0.100 mm/liter. These concentrations are comparable to those reported by others for dogs un-

TABLE III

Probability Values Derived from Comparison of Means by t Test of Fisher

Comparison	Systemic arterial			Mixed venous			A-V difference		
	L	P	L/P	L	P	L/P	L	P	L/P
Diseased and normal eupoxic dogs (group II vs. group I)	NS	NS	<0.05	NS	NS	<0.05	<0.05	<0.05	NS
Diseased and normal hypoxic dogs (group IV vs. group III)	NS	NS	NS	NS	NS	NS	<0.001	<0.001	<0.02
Hypoxic and eupoxic normal dogs (group III vs. group I)	NS	NS	NS	NS	NS	NS	NS	NS	NS
Hypoxic and eupoxic diseased dogs (group IV vs. group II)	NS	NS	NS	NS	NS	NS	<0.01	<0.05	<0.05

der barbiturate anesthesia (12, 13). Neither disease (group II) nor hypoxia (group III) significantly altered these levels. The same was true for lactate and pyruvate concentrations in the mixed venous blood.

The mean lactate-pyruvate (L/P) ratio in the arterial blood for group I was 10.9, and in the mixed venous blood, 11.4. The corresponding values in the other groups were not significantly different, with the exception of the arterial and mixed venous L/P ratios in the diseased dogs breathing 21% oxygen (group II).

For the control dogs breathing 21% oxygen (group I), the mean arterial blood concentration of glucose was 3.83 mm/liter and the mean mixed venous concentration was 3.82 mm/liter. The corresponding concentrations in the other groups did not differ significantly.

Calculated A-$\bar{V}$ differences of lactate, pyruvate, glucose, and L/P ratio. The control dogs breathing 21% oxygen (group I) had a mean A-$\bar{V}$ difference for lactate of -0.01 mm/liter, and for pyruvate $+0.004$ mm/liter. Disease (group II) increased these values to $+0.09$ and $+0.011$ mm/liter, respectively. When compared by the *t* test of Fisher to the values in group I, each of these changes would have occurred by chance less frequently than 1 time in 20 ($P < 0.05$). A similar comparison between control hypoxic dogs (group III) and diseased hypoxic dogs (group IV) revealed that increments in the A-$\bar{V}$ differences of lactate and pyruvate were highly significant ($P < 0.001$). Further, diseased hypoxic dogs (group IV) had wider A-$\bar{V}$ differences than diseased dogs breathing 21% oxygen (group II). The increment for lactate would have occurred by chance less frequently than 1 time in 100 ($P < 0.01$) and for pyruvate less frequently than 1 time in 20 ($P < 0.05$). Since the increase in A-$\bar{V}$ lactate difference was greater than that for pyruvate, the L/P ratio increased ($P < 0.05$) in the diseased dogs during hypoxia.

Unlike the A-$\bar{V}$ difference of lactate and pyruvate, the A-$\bar{V}$ difference of glucose was unaffected by disease or hypoxia.

Important to all of these observations was the fact that the degree of hypoxia, as judged by the calculated alveolar oxygen tension, was comparable in the control (group III) and diseased (group IV) dogs. The range of alveolar oxygen tension for each group was 55–65 mm Hg.

DISCUSSION

In the past, complete Freund's adjuvant has been used to evoke a granulomatous response in small laboratory animals, including rabbits, mice, rats, and hamsters. One study, performed by Moore and Schoenberg in rabbits (5), traced the development of lesions in the lung. The granulomas appeared 3 days after the injection of adjuvant, increased in number to the 4th wk, and then slowly regressed. In the present study the pulmonary lesions in the dog followed a similar time course of development and regression.

When compared to lung diseases in man, the lesions in the dog resemble in morphology and distribution the noncaseating granulomatous pulmonary disorders, thereby providing a model of human disease. Moreover, the value of the model is enhanced by a number of advantageous features. First, the dog is not severely ill and can be studied by standard methods. Second, his blood volume is sufficiently large to permit withdrawal of several blood samples. Third, the disease does not produce adhesions, so the possibility that vessels connecting the chest wall and lungs will complicate the analysis of either

blood flow or A-$\bar{V}$ difference is eliminated. Fourth, the time-course of the disease affords an opportunity to study the process in the lung at different stages. And finally, the dispersion of the lesions throughout the pulmonary tissue minimizes the diluting effect of blood flow through normal regions.

A large number of reports have presented data on transpulmonary differences of lactate in men and animals. While the majority of the human studies have failed to find a significant difference, the study by Bucherl and Schwab (14) showed, on the average, an increase in lactate concentration of blood perfusing the lung in a large group of patients, many of whom had proliferative lung disease. In respect to pyruvate, neither Bolt, Schild, Valentin, and Venrath (15) nor Harris et al. (16) found a significant A-$\bar{V}$ difference across the normal human lung. None of these studies, however, is exactly comparable to that which we performed. In the first place, the lungs of our dogs were diffusely involved by granulomas, and in the second place, the dogs were made hypoxic.

The interpretation of the A-V differences of lactate and pyruvate remains to be considered. Although production of these metabolites by granulomata seems the most likely explanation, other possibilities exist. One is the formation of these metabolites by red cells during the seconds required for their transit between sampling sites. It can be estimated from *in vitro* measurements of the rate of lactate production (17) that the red cells would have had to remain in transit for several minutes to produce the increments in lactate and pyruvate which we found in the hypoxic diseased dogs.

A second possibility is that the widened A-$\bar{V}$ differences of lactate and pyruvate resulted from a reduced blood flow during disease or during disease plus hypoxia. Cited alone, A-$\bar{V}$ differences give no information about the total amounts of substances being metabolized and for this reason, the ideal study matches each A-$\bar{V}$ difference with a measurement of blood flow. Although aware of this fact, we intentionally designed the present study to measure only A-$\bar{V}$ differences. The point seemed important because Lochner, Piiper, Schurmeyer, and Bostroem (18) demonstrated through a series of cross perfusion experiments that hemorrhage can substantially affect the A-$\bar{V}$ difference of lactate in the dog. Hence, to determine whether disease or disease plus hypoxia changed the pulmonary blood flow, we measured flow by the dye dilution method under identical conditions in a second series of normal and diseased dogs. 19 measurements in five control dogs breathing 21% oxygen gave a mean value of 137/ml per min per kg; while 15 measurements in four disease dogs breathing 14% oxygen gave a mean flow of 141/ml per min per kg (19). Since both disease and hypoxia left blood flow virtually unchanged, it seems unlikely that the consistent increase in A-$\bar{V}$ differences of lactate and pyruvate reflected a change in perfusion of the diseased hypoxic lung.

A third alternative merits consideration. This concerns the question of reaching a steady state for lactate and pyruvate exchanges in the pulmonary circulation during an experimental period of 20 min. Our data do not answer the question, but measurements of the respiratory quotient, one of the indices of steadiness, showed that values were virtually the same in all four groups of dogs.

The influence of hypoxia on the metabolism of the diseased lung is difficult to evaluate. When the alveolar oxygen tension was reduced by breathing an hypoxic mixture, other variables, such as the blood carbon dioxide tension and pH, simultaneously changed. The possibility therefore arises that the changes in A-$\bar{V}$ differences seen are related to alkalosis in the diseased pulmonary tissues. Huckabee has shown that a threefold increase in ventilation produces a rise in arterial levels of lactate and pyruvate in anesthetized dogs (20). The contribution of the pulmonary tissues to these changes is not known, hence a comparison to the present data is not readily available. On the other hand, Zborowska-Sluis and Dosseter (21) measured arteriovenous differences of lactate across normal liver, muscle, brain, kidney, and gut in hyperventilated anesthetized dogs, but found no changes.

Regardless of the precise mechanism, the A-$\bar{V}$ differences in lactate and pyruvate probably reflect the metabolism of the diseased pulmonary tissues. Additional evidence that diseased lung may produce lactate comes from the earlier study performed during thoracotomy (2). Here differences measured directly across tuberculous regions invariably showed higher concentrations of lactate in blood draining the lobe.

The experiments have not settled the matter of substrate for the diseased lung since the A$\bar{V}$ differences of glucose did not show any measurable change. Assuming glucose were the substrate, one would expect its A-$\bar{V}$ differences to be half that measured for lactate, giving a mean value of 0.14/mm per liter of glucose for the diseased, hypoxic dogs (group IV). Based on the reproducibility of the glucose measurements in our hands, this difference would approach the error of the method. Hence the failure to see a glucose difference is not inconsistent with the role of glucose as substrate in the lung, a topic recently reviewed by Heinemann and Fishman (22).

ACKNOWLEDGMENTS

Dr. Strauss was a Career Investigator of the New York City Health Research Council. Dr. Caldwell was a Senior Research Fellow of the New York Heart Association and is

currently a Career Investigator of the New York City Health Research Council.

This work was supported by Grants HE-02001, HE-5443, and HE-05741 from the National Heart Institute, United States Public Health Service.

REFERENCES

1. Fritts, H. W., Jr., D. W. Richards, and A. Cournand. 1961. Oxygen consumption of tissues in the human lung. *Science (Washington).* **133**: 1070.
2. Fritts, H. W., Jr., B. Strauss, W. Wichern, Jr., and A. Cournand. 1963. Utilization of oxygen in the lungs of patients with diffuse, non-obstructive pulmonary disease. *Trans. Ass. Amer. Physicians Philadelphia.* **76**: 302.
3. Laufer, A., C. Tal, and A. J. Behar. 1959. Effect of adjuvant (Freund's type) and its components on the organs of various animal species. A comparative study. *Brit. J. Exp. Pathol.* **40**: 1.
4. Rupp, J. C., R. D. Moore, and M. D. Schoenberg. 1960. Stimulation of the reticuloendothelial system in the rabbit by Freund's adjuvant. *Arch. Pathol.* **70**: 43.
5. Moore, R. D., and M. D. Schoenberg. 1964. The response of the histiocytes and macrophages in the lungs of rabbits injected with Freund's adjuvant. *Brit. J. Exp. Pathol.* **45**: 488.
6. Friedemann, T. E., and G. E. Haugen. 1943. Pyruvic acid. II. The determination of keto acids in blood and urine. *J. Biol. Chem.* **147**: 415.
7. Van Slyke, D. D., and J. M. Neill. 1924. The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. *J. Biol. Chem.* **61**: 523.
8. Chalkley, H. W. 1943. Methods for the quantitative morphologic analysis of tissues. *J. Nat. Cancer Inst.* **4**: 47.
9. Weibel, E. R., and D. M. Gomez. 1962. A principle for counting tissue structures on random sections. *J. Appl. Physiol.* **17**: 343.
10. Dunnill, M. S. 1962. Quantitative methods in the study of pulmonary pathology. *Thorax.* **17**: 320.
11. Strauss, B. 1964. In vitro respiration of normal and pathologic human lung. *J. Appl. Physiol.* **19**: 20.
12. Swan, M. M. 1943. The effect of severe hemorrhage, seasonal temperature and diurnal variation on blood lactate in the dog. *Amer. J. Physiol.* **140**: 125.
13. Starzecki, B., and W. W. Spink. 1968. Hemodynamic effects of isoproterenol in canine endotoxin shock. *J. Clin. Invest.* **47**: 2193.
14. Bucherl, E., and M. Schwab. 1950. Zur Frage der intrapulmonalen oxydationen. *Klin. Wochenschr.* **28**: 321.
15. Bolt, W., K. T. Schild, H. Valentin, and H. Venrath. 1954. Zur Frage der intrapulmonalen Oxydation. *Z. Kreislaufforsch.* **43**: 840.
16. Harris, P., T. Bailey, M. Bateman, M. G. Fitzgerald, J. Gloster, E. A. Harris, and K. W. Donald. 1963. Lactate, pyruvate, glucose and free fatty acid in mixed venous and arterial blood. *J. Appl. Physiol.* **18**: 933.
17. Bird, R. M. 1947. Glycolysis in human blood. *J. Biol. Chem.* **169**: 493.
18. Lochner, W., J. Piiper, E. Schurmeyer, and B. Bostroem. 1957. Uber die Grosse eines Milchsaureschwundes in der Lunge narkotisierte Hunde. *Pfluegers Arch. Gesamte Physiol.* **264**: 549.
19. Caldwell, P. R. B., U. Echeverri, M. M. Kilcoyne, and H. W. Fritts, Jr. 1970. Observations on a model of proliferative lung disease. II. Description of pulmonary gas exchange and comparison of Fick and dye cardiac outputs. *J. Clin. Invest.* **49**: 1311.
20. Huckabee, W. E. 1958. Relationships of pyruvate and lactate during anaerobic metabolism. I. Effects of infusion of pyruvate or glucose and of hyperventilation. *J. Clin. Invest.* **37**: 244.
21. Zborowska-Sluis, D. T., and J. B. Dossetor. 1967. Hyperlactatemia of hyperventilation. *J. Appl. Physiol.* **22**: 746.
22. Heinemann, H. O., and A. P. Fishman. 1969. Nonrespiratory functions of mammalian lung. *Physiol. Rev.* **49**: 1.