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DISTRIBUTION OF LACTATE BETWEEN THE CORPUSCLES AND THE PLASMA IN BLOOD. By S. C. DEVADATTA.

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THE to-and-fro migration of anions between corpuscles and plasma during the respiratory cycle is not confined to the anions chloride and bicarbonate, but in the blood of resting individuals these two are present in considerably greater concentration than any other. In the blood of individuals who, as a result of exercise, have contracted a considerable oxygen debt, the amount of lactate in the blood becomes comparable with the amounts of chloride and bicarbonate. Table I. contains representative figures for the concentration of these three ions in the blood of resting and fatigued subjects. Any "lactate shift" during the respiratory cycle might have importance in the blood of individuals during exercise, although negligible in individuals at rest.

TABLE I.—CONCENTRATION (IN TERMS OF NORMALITY) OF CHLORIDE, BICARBONATE, AND LACTATE IN THE BLOOD OF RESTING AND FATIGUED INDIVIDUALS.

	Resting.	Exercise.
Chloride	0·082	0·082
Bicarbonate	0·021	0·011
Lactate	0·0022	0·016

The present work was undertaken to determine the conditions affecting the distribution of lactate between corpuscles and plasma. The conditions examined were:

1. Temperature.
2. pH.
3. Carbon dioxide pressure.
4. Oxygen pressure.
5. Overall lactate concentration.

Experimental Methods.

The concentration of lactate was determined by Clausen's method [1922] as modified by Friedemann, Cotonio, and Shaffer [1927], the distillation stage being affected without aeration. The plasma or corpuscles were freed from proteins by dilution with water and treatment with a twentieth of their volume of 80 per cent. trichloracetic acid. Reducing sugars were removed from the protein-free filtrate by the method of Van Slyke [1917]. When known quantities of pure zinc lactate were added to such a filtrate the method gave a recovery of 97–100 per cent. (mean 98·5), and subsequent estimations were corrected by the factor 100/98·5. Neither fluoride nor oxalate, in three times the amount subsequently used to preserve blood specimens, had any effect on the recovery of lactate added to blood. The possibility of adsorption of lactate on the protein precipitate [Long, 1924] was tested by repeated extraction of the protein precipitate with 4 per cent. trichloracetic acid, but the results showed no measurable adsorption. The efficiency of the quantity of oxalate-fluoride mixture (lithium oxalate 10 parts, sodium fluoride 1 part, 0·1 g./100 c.c. blood) used to inhibit glycolysis was also tested with considerable care, since glycolysis would be a serious error, but the amount used was found to be quite adequate.

Since the experiments recorded in this paper were all performed at room temperature on blood from warm-blooded animals, the effect of temperature on lactate distribution between corpuscles and plasma was tested. Blood samples, from horse and resting and fatigued subjects, were analysed at 0°, 15°, 23°, and 40° C.; it was found that the temperature had no significant effect on the distribution of lactate between corpuscles and plasma.

In all the experiments hæmatocrit measurements were taken to make sure that the percentage of corpuscles in the blood was kept constant, in spite of the addition of lactic acid, sodium lactate, or saline.

The expression "C/P ratio" is used throughout the text to indicate the ratio of the concentration of lactate in the corpuscles to that in the plasma, both concentrations being expressed in mg./100 c.c. of medium.

The C/P Ratio in the Blood of Resting and Fatigued Animals.

Blood from different animals, in resting and fatigued states, was drawn aseptically and the concentration of lactate estimated directly in the blood, in the plasma, and in the corpuscles. The table given below (Table II.) shows the relation of the C/P ratio to the lactate concentration in the whole blood. The ratio is low when the concentration of lactate in the whole blood is high, confirming the results of Hill, Long, and Lupton [1924]. Noshi [1929–30] has recorded a similar change, from 0·8 to 0·43. In human subjects, therefore, as well as in cats, the C/P ratio of approximately 0·7, characterising the resting state, drops to 0·55 in the condition of fatigue.

TABLE II.—THE DISTRIBUTION OF LACTATE BETWEEN CORPUSCLES AND PLASMA (C/P RATIO) IN BLOOD FROM RESTING AND FATIGUED ANIMALS.

Animal.	No. of experiments.	Lactate concentration in whole blood (mg /100 c.c.).	C/P ratio.
Cat resting	3	10–20	0·84–0·90
Horse resting . . .	7	10–17	0·62–0·73
Man resting	18	16–22	0·70–0·80
Man resting before breakfast .	12	10–12	0·70–0·89
Rabbit excited . . .	25	70–90	0·53–0·60
Cat fatigued	3	80–120	0·50–0·59
Man fatigued . . .	7	40–140	0·50–0·60

It was considered of interest to study the C/P ratio during a progressive fall in the concentration of lactate in the blood *in vivo*. Exercise was taken by human subjects and blood drawn for analysis from one of the veins in the arm at intervals of 3, 7, 20, and 40 minutes later. The C/P ratio immediately after exercise was 0·50, and as the concentration of lactate in the blood decreased the C/P ratio increased, attaining the normal value of 0·8 as the normal resting level of lactate in the blood was reached (fig. 1).

The value of the C/P ratio in the blood of resting animals does not differ consistently from that which would be expected on a purely osmotic basis. If the cells contain 65 per cent. of water and the plasma 90 per cent., the ratio to be expected is in the neighbourhood of 0·7. There are several possible explanations of the reduced C/P ratio observed in the blood of fatigued animals. In the first place, it is possible, although unlikely, that the equilibration between corpuscles and plasma in respect of lactate requires several minutes. To test this point a small quantity of 7 per cent. lactic acid solution was mixed with 50 c.c. of blood in a tonometer; 10 c.c. samples of blood were transferred immediately, and at 3, 5, 10, and 30 minutes, to centrifuge tubes for separation of plasma and corpuscles. Both direct and indirect estimations of the concentration of lactate in the corpuscles agreed satisfactorily. There was no progressive alteration in the ratio of lactate in the corpuscles to that in the plasma.

Another possibility is that of the presence in either corpuscles or plasma of indiffusible substances estimated as lactate by the method used. Both possibilities were explored by examining the behaviour of corpuscles suspended in saline. In one experiment 10 c.c. of blood from a resting animal were diluted with 100 c.c. of Ringer-Locke solution. The concentration of lactate in the corpuscles so treated was 2–3 mg./100 c.c., approximately equal to that in the saline. Hence lactate from the corpuscles can diffuse into saline, and if indiffusible "pseudo" lactate is present it can be only in very small traces. In other experiments all the plasma from 10 c.c. samples of blood was replaced by exactly the same amount of Ringer-Locke lactate solution, containing varying amounts of lactate; in some cases the corpuscles were repeatedly washed with saline to remove all traces of plasma before the addition of Ringer-Locke lactate solution. The percentage of corpuscles, the osmotic pressure, and the pH of the system, in both the cases, were kept the same as in blood. Over the range of concentration studied the concentration ratio between corpuscles and saline was 0·25–0·30, and was not significantly altered by repeated washing of the corpuscles to remove traces of plasma (Table III.). The ratio to be expected if a simple osmotic equilibrium had been reached is approximately 0·65. It seems that the departure of the corpuscle-plasma system from a simple osmotic equilibrium in respect of lactate is due to a property of the corpuscular membrane or contents, and not to any constituent of the plasma.

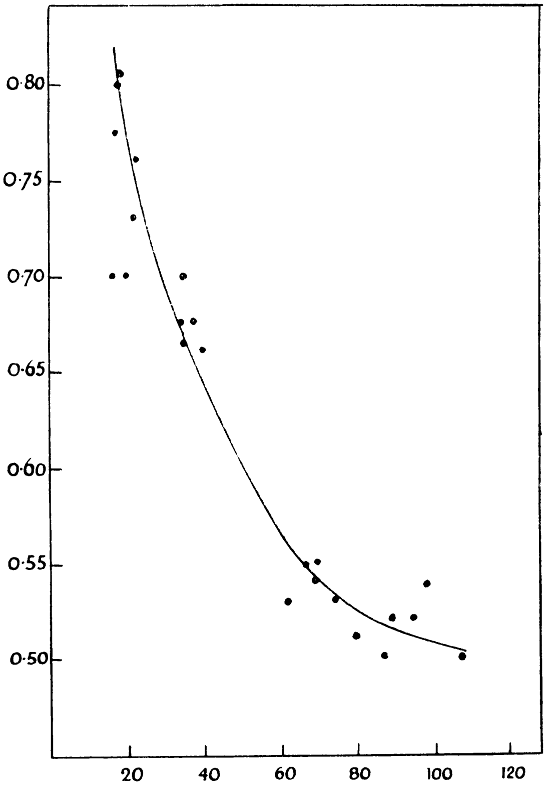

FIG. 1.—The effect of exercise on the distribution of lactate between corpuscles and plasma in blood. Venous blood samples were taken at different periods after severe exercise (standing-running). Ordinates: lactate concentration in corpuscles/lactate concentration in plasma. Abscissæ: lactate concentration in whole blood (mg./100 c.c.).

TABLE III.—THE DISTRIBUTION OF LACTATE BETWEEN CORPUSCLES AND SALINE.

Lactate in mg./100 c.c.

Saline.	Corpuscles.	Corp./Saline.
33·0	16·0	0·50
150·0	35·0	0·24
178·9	48·3	0·27
193·0	49·3	0·25
*120·0	34·0	0·29
*209·0	56·0	0·26
*238·5	69·7	0·29
*456·7	121·6	0·26

* Corpuscles repeatedly washed with saline to remove traces of plasma.

The Effect of the Partial Pressures of Carbon Dioxide and Oxygen on the Lactate Distribution in Blood.

One of the major changes in the composition of venous blood introduced by exercise is an increase in the carbon dioxide partial pressure and a decrease in the oxygen partial pressure, and it was felt that the influence of these factors should be examined. In all the preceding experiments venous blood was used, but the samples were not equilibrated with standard pressures of carbon dioxide and oxygen, and this fact might account for the rather large variation in the values found for the blood of resting animals.

Samples of blood were equilibrated with different mixtures of carbon dioxide and hydrogen in a tonometer, and the blood then centrifuged in stoppered tubes under a layer of liquid paraffin. There was a progressive increase in the concentration of lactate in the corpuscles as the pressure of carbon dioxide was increased (fig. 2). The C/P ratio rose from a value of 0·58 in the absence of carbon dioxide to one of 0·85 when the blood was exposed to 17·5 per cent. of an atmosphere of carbon dioxide. The experiments were then repeated with air-carbon-dioxide mixtures, to determine the response of oxygenated blood to changes of carbon dioxide pressure. The C/P ratio ranged in these experiments from 0·50 in a carbon dioxide-free atmosphere to 0·86 under a carbon dioxide pressure of one-fifth of an atmosphere. In both series of experiments the lactate concentration in the blood samples was about 50 mg. per 100 c.c., corresponding to a condition of mild fatigue.

Possible Significance in the Respiratory Cycle.

The lines in fig. 2 show that in blood containing 50–60 mg. of lactate/100 c.c. (that is, blood from exercised individuals with oxygen debts of the order of six litres) there must be a "lactate shift" during each respiratory cycle of the blood similar in direction to the "chloride shift." Increase of oxygen pressure and decrease of carbon dioxide pressure each have the effect of causing some lactate to migrate from the corpuscles to the plasma. If the venous blood in these conditions is supposed to be about 40 per cent. saturated with oxygen and under a carbon dioxide partial pressure of about 55 mm. Hg, the C/P ratio should change at each half-cycle approximately between the limits of 0·63 (arterial) and 0·7 (venous). If this effect is still present in blood containing higher lactate concentrations it may become a significant factor in the buffering of the blood in the respiratory cycle in conditions of severe oxygen debt, although quantitatively negligible in the blood of resting individuals.

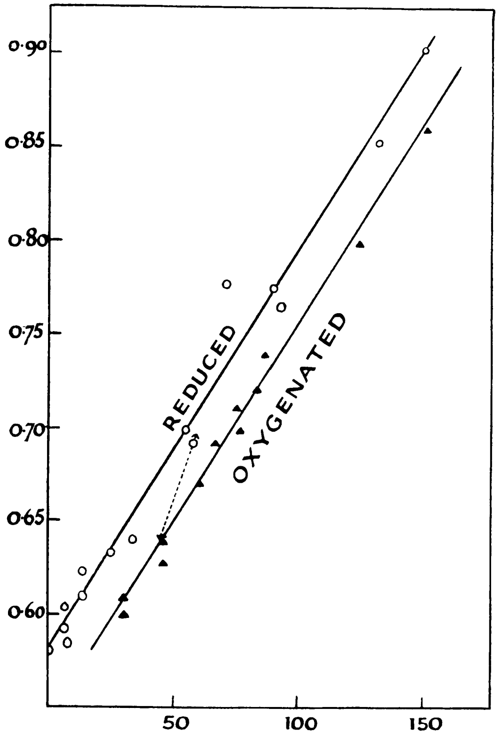

Fig. 2.—Effect of partial pressures of oxygen and carbon dioxide on the distribution of lactate between corpuscles and plasma in blood containing 50–60 mg. of lactate/100 c.c. The C/P ratio (ordinates) is plotted against the carbon dioxide partial pressure (abscissæ) for (*a*) fully reduced, and (*b*) fully oxygenated blood. The dotted line indicates approximately the changes occurring at each respiratory cycle.

Influence of Concentration of Lactate in Whole Blood.

The effect of an increase in the carbon dioxide pressure is to cause a shift of lactate from plasma to corpuscles. Exercise produces a rise in the carbon dioxide pressure of the blood, and this should result in a similar lactate shift—that is to say, one would expect to find in the blood of fatigued subjects a C/P ratio greater than is found in the subjects at rest. The opposite is, in fact, observed; the C/P ratio falls during fatigue to little over half the value characteristic of the resting condition. The position is, however, obviously complicated by the change in the total quantity of lactate present in the whole blood; there may be a tenfold increase in the amount of lactate per 100 c.c. of blood as a result of exercise.

The effect of increased concentration of lactic acid in the blood on the C/P ratio was studied by adding minute quantities (up to 0·2 c.c.) of 5, 10, and 15 per cent. solutions of lactic acid respectively, to 10 c.c. samples of blood in the tonometer; this did not alter appreciably the percentage of corpuscles in blood. The results of analyses of the lactate concentration in blood, in plasma, and in corpuscles are illustrated by the lactic acid curve in fig. 3. The C/P ratio fell from a value of 0·70 when the concentration of lactate in the whole blood was 45 mg./100 c.c. to a value of 0·32 when this concentration was 160 mg./100 c.c.

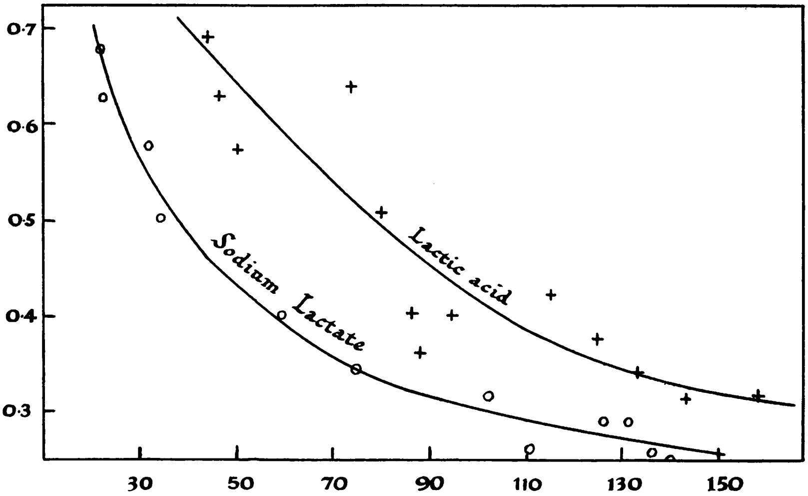

FIG. 3.—The effect of addition of (*a*) lactic acid, (*b*) sodium lactate, on the distribution of lactate between corpuscles and plasma in blood. The C/P ratio is plotted against the final concentration in the whole blood produced by the addition. The blood when drawn contained 20 mg. of lactate/100 c.c.

In order to distinguish between the properties of lactic acid and of lactate a neutral solution of sodium lactate was added to blood; the percentage of corpuscles in the blood, the osmotic pressure in the system, and the pH of the blood remained unaltered. This was accomplished by replacing 1 c.c. of plasma with 1 c.c. of Ringer-Locke lactate solution in 10 c.c. samples of blood. (In the Ringer-Locke lactate solution some of the sodium chloride was replaced by an equivalent amount of sodium lactate, as required.) The results of the analyses are shown in the sodium lactate curve in fig. 3. Here again the effect of increased lactate concentration in the whole blood is to depress the C/P ratio; it drops from 0·70 to 0·30 as the concentration of lactate in the whole blood is raised from 25 to 160 mg./100 c.c.

The Influence of H-ion Concentration.

A comparison of the two curves in fig. 3 clearly gives information as to the influence of H-ion concentration on the C/P ratio. The addition of sodium lactate produces a greater depression of this ratio than the equivalent amount of lactic acid over the range of concentrations studied; the effect of increased acidity is therefore to raise the C/P ratio—that is, to cause lactate to migrate into the corpuscles. The effect becomes smaller as the lactate concentration increases. The effect of increased carbon dioxide partial pressure is also to raise the C/P ratio, and it is therefore possible that this gas also produces its effect by increasing the acidity of the blood.

The effect on the C/P ratio of adding lactic acid to blood *in vitro* (fig. 3) is considerably greater than the effect of the addition of an equal quantity of lactic acid in the body during exercise (fig. 1). The latter case is, however, complicated by a simultaneous reduction in the partial pressure of oxygen and increase in the partial pressure of carbon dioxide. Both these changes produce effects opposed to that of increased lactate concentration and might therefore account for the difference between the two sets of results under consideration. A few experiments (Table IV.) in which lactic acid was added to blood under a carbon dioxide partial pressure of 150 mm. Hg gave results indicating that the carbon dioxide effect and the lactic acid effect can be superimposed, but it must be left undecided whether the change in the C/P ratio in exercise is sufficiently accounted for by these three variables.

TABLE IV.—THE C/P RATIO IN BLOOD EXPOSED TO CARBON DIOXIDE AFTER ADDITION OF LACTIC ACID.

Lactate in mg./100 c.c.			
Blood.	Plasma.	Corpuscles.	Corp./Plasma.
41·2	46·3	28·7	0·62
52·1	60·5	36·6	0·61
62·8	71·5	43·2	0·62
77·5	94·8	45·5	0·48
119·6	146·3	70·0	0·47
137·0	166·4	75·0	0·45
181·4	220·9	103·2	0·45

SUMMARY.

1. The ratio of the concentration of lactate in the corpuscles to that in the plasma (C/P ratio) is 0·6–0·9 in the blood of resting animals (cat, horse, man).

2. In the blood of fatigued animals (cat, rabbit, man) it is reduced to 0·5–0·6.

3. This change in the C/P ratio is not due to a delay in the diffusion of lactate from the plasma into the corpuscles, nor to the presence, either in the corpuscles or plasma, of some indiffusible substance estimated as lactate.

4. The C/P ratio is raised by

(*a*) increased partial pressure of carbon dioxide;
(*b*) decreased partial pressure of oxygen;
(*c*) increased H-ion concentration.

These facts lead to the conclusion that a "lactate shift" analogous to the "chloride shift" occurs during each respiratory cycle of the blood, which may become of importance in connexion with the buffering of the blood in conditions of severe oxygen debt.

5. The C/P ratio is reduced by an increased concentration of lactate in the whole blood.

6. The increased lactate content of the blood occurring in conditions of oxygen debt is shown to be more than sufficient to account for the fall observed in the C/P ratio. The discrepancy is at least partly explained by the opposing effects of the simultaneous changes in carbon dioxide and oxygen partial pressures.

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