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Muscular Exercise, Lactic Acid, and the Supply and Utilisation of Oxygen.—Parts I–III.

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(From the Physiological Laboratories, Manchester, and University College, London.)

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Part I.—Introduction.

By A. V. Hill.

In a recent paper under the general title of this series A. V. Hill and Lupton (1) gave a preliminary account of experiments made on man, in an attempt to press to its logical conclusion the physico-chemical view of muscular contraction arrived at by the investigation of the isolated muscle. The present series of papers contains a more adequate account of these experiments, which are still in progress.

(*a*) *The "Initial" Process.*

In the isolated muscle the complete cycle of contraction and relaxation, as distinguished from recovery, appears to be accompanied by no chemical change of any importance, other than the conversion of glycogen into lactic acid, and the subsequent neutralisation of the latter (2) (4). These "initial" phases of muscular activity are entirely non-oxidative in character (3), and the following "balance sheet" (Table I) shows that, if any other chemical changes do actually occur, they are negligible from the energy standpoint.

The lactic acid formation, therefore, is no secondary process, but the one involving the whole (or nearly the whole) of the energy liberated in the initial phases of contraction.

It will be noticed that no mention has been made of the energy involved in the mechanical response. We are considering the complete cycle of contraction

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Table I.

	Cals.	Cals.
Total energy in anaerobic muscular activity per 1 gram of lactic acid produced (Meyerhof (4))		370
Heat of combustion of dissolved glycogen* (Slater (5))	3,836	
Heat of combustion of dissolved lactic acid (Meyerhof (6))	3,601	
Heat of formation of lactic acid from glycogen	235	
Heat of neutralisation of lactic acid by sodium protein of muscle (Meyerhof (4))	135	
	370	370
Balance left for other chemical processes		Nil.

and relaxation, and any chemical change involved specifically in contraction is reversed in relaxation. It will be noticed also that the neutralisation of lactic acid has been attributed to the reaction

$$\underset{\text{(sodium-protein)}}{Na^+ + P^-} + \underset{\text{(lactic acid)}}{H^+ + L^-} \longrightarrow \underset{\text{(sodium lactate)}}{Na^+ + L^-} + \underset{\text{(undissociated protein)}}{HP}$$

An exactly similar reaction occurs when acid is neutralised in blood, the protein in that case being mainly hæmoglobin (7). In muscle, as in blood, in addition to the sodium-protein buffers there are alkaline salts Na_2HPO_4 and $NaHCO_3$. The total quantity, however, of these salts is inadequate to account for the amount of acid which can be taken up by muscle or blood, with but little increase in the hydrogen ion concentration (*c*H): actually the protein of muscle appears to be a weaker acid than carbonic, and it is probable that the primary process of neutralisation occurs largely, if not entirely, at the expense of the sodium-protein salt. The large excretion of carbon dioxide, and the high respiratory quotient, following sudden and severe exertion, must not be regarded as due directly to lactic acid turning out CO_2 from $NaHCO_3$, but rather as caused by the reaction of the respiratory centre to the increased *c*H resulting (even in the presence of very effective buffers) from the liberation of lactic acid. The importance of this we shall see in Part III and later.

Little is known about these buffers of living muscle. Their existence,

* Recent work, hitherto unpublished, by Meyerhof tends to make this value 30 to 50 cals. smaller.

† For convenience in these papers the term "sodium" is used, where more correctly it should be "sodium or potassium," and the symbol *c*H is used to denote the hydrogen ion concentration.

however, has been proved (2) (4), and their amount, in a healthy man, is adequate to neutralize more than 100 grams of lactic acid. The capacity of the body for severe short-lived effort probably depends upon their presence and efficiency. It will be realised that, apart from the actual ultimate mechanism of contraction, the lactic acid which appears in the body never occurs, to any appreciable extent, as such, but only as sodium, or potassium, lactate.

(*b*) *The "Recovery" Process.*

In the anaerobic processes of contraction and relaxation the following exothermic reaction occurs :—

Glycogen + sodium-protein → sodium lactate + protein + 370 cals. The heat is reckoned per 1 gram of glycogen $(C_6H_{12}O_6)_n$ broken down. In the oxidative process of recovery, in addition to certain oxidations, the converse endothermic reaction occurs :—

sodium lactate + protein → glycogen + sodium-protein — 370 cals.

Apart from the energy liberated by oxidation there is an *absorption* therefore of 370 cals. when 1 gram of glycogen is restored. Actually, however, in the recovery process there is a *liberation* (2) (8) of 370 cals. of heat ; thus the total energy involved in reversing the initial breakdown process is 370 cals. absorbed as chemical energy, plus 370 cals. liberated as heat, a total of 740 cals.

In the isolated muscle the substance oxidised in recovery is almost certainly carbohydrate (or lactic acid). The respiratory quotient is unity (Meyerhof (9)), and no change has ever been shown to occur in the fat contained in the muscle (Winfield (10)). The total energy liberated in recovery, 740 cals., must come, therefore, from the oxidation of carbohydrate, presumably of glycogen, so that in restoring 1 gram of glycogen to its original state a quantity $740/3836 = 1/5{\cdot}2$ grams of glycogen must be oxidised. In this sense the "efficiency" of recovery may be measured by the ratio $5{\cdot}2 : 1$. In the intact animal, as distinguished from the isolated muscle, it is not so certain that the substance oxidised in recovery is carbohydrate ; indeed, under some circumstances it almost certainly is not. All oxidation resulting from muscular exercise is "recovery" oxidation, and there is conclusive evidence that the true respiratory quotient during prolonged steady exercise may differ widely from unity. In that case the efficiency of recovery must be defined, not as the ratio of glycogen restored to glycogen oxidised, but as the ratio of the *energy of the glycogen restored* to *the energy liberated in oxidation in restoring it.*

It is possible that the recovery process is really of the nature of a "coupled

reaction," in which the restoration of lactic acid to glycogen occurs as part of a definite chain of chemical processes in which oxygen is used and foodstuff oxidised to provide the necessary energy. It is equally possible, however, that the restoration of lactic acid to glycogen occurs in a manner analogous to the recharging of an accumulator; there sulphuric acid is restored from the lead plates to the solution around them, by means of some mechanism (*e.g.*, a dynamo) entirely separate from the accumulator, driven by a separate supply of energy, and merely providing power to effect the restoration. Which of these views is the correct one cannot yet be stated; the decision is one of the important problems of muscle physiology.

The speed of the recovery process is dependent on various factors, the oxygen supply, the circulation, the temperature (8), the hydrogen ion concentration (11), possibly also on the presence of oxidative catalysts in the tissues. One might expect to find it increased in man, as the result of "training"; it would be difficult, however, in the intact animal to know which of these factors (or others) was responsible for the increase.

(*c*) *The Diffusion of Lactate Ions.*

The prompt appearance of lactate ions in the blood when muscular exercise is taken, is a sign that these can pass rapidly from muscle to circulating fluid; the disappearance of lactate ions from the blood during recovery implies the possibility of diffusion away from the blood, either back again to the muscles or into such organs as the liver. Barr, Himwich and Green (12) have shown that the venous blood from inactive muscles, during the vigorous activity of other muscles, may contain *less* lactic acid than the arterial blood. This proves that lactate ions can pass by diffusion from blood to muscle as well as from muscle to blood. We shall assume that diffusion of lactate ions is free in either direction. It does not follow that the concentrations in blood and muscle are equal, except on those occasions when they are stationary or changing only slowly, or at a maximum value.

(*d*) *The Respiratory Quotient.*

Provided that the hydrogen ion concentration of blood and tissue is remaining constant, and that no alteration is occurring in the amount of lactic acid present in them, the respiratory quotient is an indicator of the chemical substances undergoing oxidation in the body. If, however, these conditions be not fulfilled, as *e.g.*, during forced respiration, exercise, or the recovery process, the respiratory quotient may be affected, often very largely. During the

liberation of lactic acid, and the readjustment (mainly by the respiratory centre) of the acid-base equilibrium of blood and tissue to secure a return towards the normal cH, many litres of carbon dioxide may be eliminated and very high values of the respiratory quotient attained; during the subsequent recovery process, in which the acid is removed, a converse retention of carbon dioxide must occur, yielding low values of the respiratory quotient over long periods of time, values which on the ordinary view could result only from the transformation of fat into sugar in the body. A study of the respiratory quotient, if undertaken with sufficient caution, may throw light, not so much on the nature of the bodies being oxidised as on the acid-base changes occurring as a result of exercise and recovery.

(*e*) *The Steady State of Exercise.*

As Fletcher showed in 1902 (13) an isolated muscle left in oxygen and given a shock at regular short intervals soon attains a "steady state," the height of contraction being considerably reduced below the original level, but remaining constant for long periods. In the absence of oxygen complete inability to contract soon ensues. In the steady state, breakdown (the "initial" anaerobic process) is exactly balanced by restoration (the "recovery" oxidative process). The rate of breakdown depends upon the frequency of stimulation and the size of the response; that of recovery on the supply of oxygen (whether by diffusion in the isolated muscle, or through the circulation in the intact animal) and on the concentration of lactic acid from which recovery is necessary. A balance is attained, provided that the oxygen supply is adequate, after a few minutes of exercise, the oxygen intake, the carbon dioxide output and the lactic acid concentration in blood and muscle remaining constant thereafter till the exercise ends.

(*f*) *Oxygen Debt.*

If the exercise be so severe that its oxygen requirement cannot be met out of "income," *i.e.*, through the respiratory-circulatory system, then the steady state is never attained; the oxygen intake may indeed remain constant at its maximum value, but the lactic acid continues to accumulate in muscle and blood. In such a state the body has to "go into debt" for oxygen, to obtain its energy on the "security" of a concentration of lactic acid which it will require future "oxygen income" to eliminate. Enormous oxygen debts are sometimes found in man, up to nearly 19 litres; these imply a very high concentration of lactic acid in the active muscles. The conditions which produce

them are characterised by striking fluctuations in the respiratory quotient, and by a high degree of exhaustion.

(*g*) *Oxygen Requirement.*

In studying various types of muscular activity a knowledge of the oxygen *requirement* of the exercise, which is a measure of its vigour, is of value. When the exercise is mild the oxygen intake may be able to balance the oxygen requirement; the requirement may then be ascertained by measuring the intake during a steady state of exercise. When the exercise is severe the oxygen requirement is larger than any possible intake, the body must run into debt, and the only way to ascertain the requirement is to measure both the oxygen intake and the oxygen debt, in other words, to ascertain the oxygen used, as a result of the exercise, both during activity and also in complete recovery therefrom.

(*h*) *The Heart Muscle.*

The conceptions outlined above apply to the case of skeletal muscle; it is not known, however, how far they apply to that of the heart. Certain it is that the capacity of the body for muscular exercise depends largely, if not mainly, on the capacity and output of the heart. It would obviously be very dangerous for that organ to be able, as the skeletal muscle is able, to exhaust itself very completely and rapidly, to take exercise far in excess of its capacity for recovery; and there is no evidence at present that it is possible for the heart during activity, in the absence of adequate oxygen, to liberate lactic acid, and so, in effect, to draw on future, as well as on present oxygen supplies. The enormous output of the heart of an able-bodied man, maintained for considerable periods during vigorous exercise, requires a large contemporary supply of oxygen to meet the demand for energy. This oxygen supply to the heart muscle must be considerably in excess of that to any equal mass of skeletal muscle, implying a peculiarly effective coronary circulation in the athlete. When the oxygen supply becomes inadequate, it is probable that the heart rapidly begins to diminish its output, so avoiding exhaustion; the evidence for this, however, is indirect, and an important field of research lies open in the study of the recovery process in heart muscle, on the lines on which it has been developed in skeletal muscle.

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Part II.—The Estimation of Lactic Acid in Biological Fluids.

By C. N. H. Long.

Introduction.

A variety of methods have been suggested for the estimation of lactic acid in biological fluids and tissues. These fall under four main heads: (*a*) Oxidative methods, (*b*) colorimetric estimations, (*c*) measurement as zinc lactate, and (*d*) physical methods, such as the use of polarised light. The number and variety of methods which have been tried show that lactic acid, although a fairly simple organic substance, presents a number of difficulties to accurate and rapid analysis when present in small quantities. These difficulties are not entirely due to the small amounts present, but also to the fact that the acid is usually associated in living material with other chemically similar substances, which yield, under similar treatment, substances practically identical with those obtained from lactic acid itself. This is especially true when attempts are made to estimate lactic acid by oxidative methods.

The use of oxidising agents to obtain from lactic acid substances (usually acetaldehyde) which are more easily estimated than the acid itself has been made the basis of the greater number of lactic acid estimations. The chief oxidising agents used have been potassium permanganate, concentrated sulphuric acid, and chromic acid, and the methods are conveniently divided into two classes—(i) Those in which the product of the oxidation is acetaldehyde, and (ii) those in which some other oxidation product is estimated, or in which the amount of oxidising agent used is estimated by difference. The

estimation of the acetaldehyde obtained by oxidation with either permanganate or sulphuric acid has been made in several different ways. Boas (1) introduced dilute permanganate as an oxidising agent, caught the aldehyde in alkaline iodine solution, and found the amount of iodoform formed, either directly or by difference. This method was criticised by von Fürth and Charness (2) who showed that the alkaline iodine estimation was subject to gross errors. Jerusalem (3) also used the Boas method in his determinations. Some years previously Ripper (4) had used bisulphite solutions to catch the aldehyde, and had then measured the excess bisulphite by titration with iodine; this was incorporated by von Fürth and Charness into their method, and these workers, as Jerusalem had previously done, led the aldehyde by aeration into the bisulphite. Bellet (5) caught the aldehyde in ammoniacal silver nitrate solution and estimated the unreduced silver. Clausen (6) also used bilsulphite, but instead of estimating the excess of it with iodine and measuring the aldehyde found by difference, he decomposed the aldehyde-bisulphite compound with bicarbonate after removal of excess bisulphite, and then the bisulphite set free is estimated directly. Lately Scott and Flinn (7) have re-introduced the alkaline iodine method with certain precautions and modifications. Not many methods have been suggested in which other oxidation products of lactic acid are measured. Meissner (8) introduced concentrated sulphuric acid as an oxidising agent, and oxidised the lactic acid to aldehyde and carbon monoxide accordingly to the scheme $CH_3.CHOH.COOH - H_2O \longrightarrow CH_3.CHO + CO$. The CO evolved was measured. This method was also used by Schneyer (9) but has been adversely criticised by Mayer (10) since many other substances present in biological tissues and fluids yield carbon monoxide on such treatment even if not forming aldehyde. Paessler (11), instead of estimating the oxidation products of lactic acid, measures by difference the amount of oxidising material used, in this case potassium dichromate.

The colorimetric methods which have been used do not differ in principle from those which have been classed as oxidative methods, since in these also oxidising agents are used to break down the lactic acid into some substance which is more easily estimated colorimetrically. The first and best known is that of Ryffel (12) who used concentrated sulphuric acid as the oxidising agent and Schiff's reagent for the colorimetric estimation. Polonowski (13) used codeine instead of Schiff's reagent for estimations on urine, and Harrop (14) suggested guaiacol. These two authors also used sulphuric acid as the oxidising agent.

The estimation of lactic acid by obtaining and weighing the fairly insoluble zinc salt was the earliest one used. This method usually means a complicated separation of other substances before the salt can be obtained in a sufficiently pure condition, and it was not until the work of Fletcher and Hopkins (15) on lactic acid in muscle that this method was made sufficiently reliable for accurate work. Wolf (16) also has adopted this method for the estimation of lactic acid in blood.

The chief indirect methods for lactic acid estimation are the use of polarised light, and the measurement of the changes produced in the carbon dioxide capacity, or in the oxygen dissociation curve, of blood when lactic acid is added to it. The former method was used by Hoppe Seyler and Araki (17) and by Yoshikara (18); but the optical rotation of lactic acid (Zn salt) is only $[\alpha]_D^{20} \pm 8{\cdot}6$, so this method is hardly applicable to dilute solutions. Verzàr (19) estimated the amount of lactic acid appearing in the blood of an animal after stimulation of certain muscles by the changes produced in the O_2 dissociation curve of the blood. Barr, Himwich and Green (20) attempted to correlate the observed measurements of lactic acid with the fall in the carbon dioxide capacity of the blood, but found variations which were rather large and probably due to the presence of formed elements (corpuscles) in the blood, with consequent membrane equilibria.

Since both colorimetric methods and also methods where some breakdown product of lactic acid is measured, depend on a preliminary oxidation, it is likely that the errors due to this oxidation would affect the accuracy of both to the same extent. When lactic acid is oxidised by permanganate, pyruvic acid is stated to be formed (Aristoff (31), Schoorl (32)), but in dilute solution acetaldehyde is the chief product, although this does not preclude the possibility of a certain amount of the former body also being present. In methods where aeration is used to transfer the aldehyde to the receivers, any formation of pyruvic acid would cause an apparent loss of lactic acid. Again acetaldehyde itself is an easily oxidisable substance, and some loss seems bound to occur owing to its further oxidation. Furthermore, permanganate is such a powerful oxidising agent that it might easily split off volatile aldehydes or ketones from substances other than lactic acid which are present in biological tissues and fluids. The possibility of this last effect has been noted by Clausen (6) in a recent paper. Another point in connection with the oxidation with permanganate has been shown by Jerusalem (3). It is that the yield of lactic acid depends not only on the concentration of the permanganate used, but also on the rapidity of the air current and on the rate at which permanganate is

added to the solution. The fact that such precautions are necessary bears out the previous statement as to the possibility of further oxidation of acetaldehyde being an important factor in the poor yields of lactic acid which are often obtained by this method. Oxidation with sulphuric acid, while free from many of the objections to permanganate, presents difficulties of its own. This substance is the oxidising agent used in all the colorimetric determinations before noted. Usually 50 per cent. sulphuric acid is used and the mixture of lactic acid and sulphuric acid is steam-distilled. Clausen (6) also used this strength of acid in one of his methods, and found a temperature of 140° C. and aeration to be necessary in order to produce a good yield of aldehyde. From experiments on this method which I have recently carried out, and which are detailed later, it appears that even more drastic treatment is required, such as raising the temperature of the oxidation to 160° C. and using stronger acid.

For the estimation of lactic acid in muscle or other tissues, where fairly large amounts of material are available, extraction of the acid and conversion into zinc lactate is probably the best method, especially if the extraction technique of Fletcher and Hopkins (15) or of Meyerhof (21) be adopted. The latter author estimates the acid by oxidation after extraction. When attempts are made to apply this zinc lactate method to blood or urine the difficulties are twofold; firstly owing to the small quantities in which they are present large amounts of material are necessary (which often in the case of human subjects it is impossible to supply), secondly, these fluids contain other hydroxy-acids which are very similar to lactic acid and yield almost identical zinc compounds. The possibility of this last contingency was noted by Clausen recently (6). Wolf (16) describes a method for estimating lactic acid in blood by this means.

Method chosen.

The method finally chosen for the lactic acid estimations (chiefly in blood) required during our work on muscular exercise was that of Clausen (6). This method is the best one available at present for lactic acid estimation, and with practice satisfactory results are consistently obtained. Although it possesses the faults of oxidative methods, as noted above, yet if the conditions of oxidation be carefully controlled it yields results which, if not very accurate as far as absolute values are concerned, are yet sufficiently so for all purposes of comparative study.

Controls.

Controls on this method have been carried out, both on watery solutions of known strength of lactic acid and also on blood to which lactic acid had been added in known amount. Clausen carries out the oxidation of the lactic acid solution in a wide test-tube, dropping N/100 permanganate into the acidified solution, the oxidation tube being kept at 95° C. in a water-bath. It has been found an improvement to carry out the oxidation in a Claisen flask and to heat the flask directly with a micro-burner, the contents being left gently boiling while the permanganate was added. Although this does not greatly improve the percentage recovery of lactic acid it appreciably shortens the time required for a determination. If the flask is boiled it is necessary to place a condenser between the oxidation vessel and the bisulphite tubes. It has also been found better to drop permanganate acidified with sulphuric acid into the lactic acid solution, as this prevents the formation of manganese dioxide and enables a sharper end-point to be observed.

(*a*) *Percentage recovery in lactic acid solutions : zinc lactate solution used.*

Amount of Lactic Acid Taken (mgms.).	Amount Found (mgms.).	Percentage Recovery.
2·39	2·14	89·5
2·39	2·18	91·3
4·84	3·94	81·5
4·84	4·29	88·6
7·23	6·54	90·5
7·23	6·05	83·6
		Mean 87·5 per cent.

(*b*) *Percentage recovery of lactic acid from blood : zinc lactate added.*

Amount of Lactic Acid added to 100 c.c. of Blood : mgms.	Amount Present in Blood Originally : mgms. per 100 c.c.	Amount Found : mgms. per 100 c.c. blood.	Percentage Recovery.
47·8	50·0	93·2	83·1
161·3	28·0	162·8	83·6
161·3	48·0	214·3	102·0
161·3	48·0	194·0	90·5
161·3	39·0	196·0	97·2
161·3	50·0	168·6	73·1
161·3	50·2	174·6	75·9
			Mean 87·2 per cent.

In view of these figures (and others) it was decided to take the mean yield of lactic acid as being about 85 per cent. of that actually present.

Clausen's second method of oxidation is to heat the lactic acid with 50 per cent. sulphuric acid at 140° C. This method has been tried, and it has been found that 50 per cent. sulphuric acid is not strong enough to oxidise lactic acid at 140° C., and that oxidation occurs only when sufficient water has been distilled off to make the concentration of acid much greater. Even when the temperature is raised to 155° C. and the concentration of acid to 60 per cent., about one and a quarter hours is required to give a yield of 80 per cent. of the added lactic acid; since the permanganate method takes only half an hour the sulphuric acid method is not as suitable, especially when a number of determinations have to be made. Also aeration of the liquid in the oxidation vessel is not easy, owing to the increasing viscosity of the mixture as more and more water is distilled from it.

(*c*) *Lactic acid solutions. Oxidation with Sulphuric Acid.*

	Sulphuric Acid Added.	Temp. ° C.	Time of Oxidation: hours.	Lactic Acid Added: mgms.	Lactic Acid Recovered: mgms.	Percentage Recovery.
I	20 c.c. of 50 per cent. to 5 c.c. lactic acid solution	140	1	5	trace	—
II	Ditto	152	$\frac{3}{4}$	5	2·30	45
III	10 c.c. concentrated H_2SO_4 to 10 c.c. lactic acid solution	152	$1\frac{1}{4}$	5	3·93	84
IV	Ditto	152	$1\frac{1}{4}$	5	3·80	80
V	Ditto	155	$1\frac{1}{4}$	5·38	4·27	79·5
VI	Ditto	155	$1\frac{1}{4}$	5·38	4·27	79·5

Accuracy of the method: probable error of a single observation.

If a series of determinations be made of the same quantity of lactic acid the probable error of a single observation can be calculated from the formula

$$p_s = \pm 0{\cdot}67 \sqrt{\frac{d_1^2 + d_2^2 + \ldots d_n^2}{n-1}}$$

where d represents the deviation of an observation from the mean of the series and n the number of observations. Further the probable error of the mean (p_n) is given by the equation $p_n = \frac{p_s}{\sqrt{n}}$. These formulæ have been applied to the permanganate method for the estimation of lactic acid. Ox blood was taken

and the content of lactic acid determined. A known amount of lactic acid was then added, and ten determinations of the lactic acid content made. The above formulæ were then applied and gave the following figures.

Original blood, 32·5 mgms. lactic acid per 100 c.c. To this 286 mgms. lactic acid per 100 c.c. were added.

No. of Determination.	Amount Recovered, mgms. per 100 c.c. of Blood.	Error from the Mean.
I	222·5	−23·7
II	240·2	− 6·0
III	229·0	−17·2
IV	245·9	− 0·3
V	252·2	+ 6·0
VI	259·0	+12·8
VII	242·8	+ 0·6
VIII	247·9	+ 1·7
IX	260·3	+14·1
X	262·0	+15·8
	Mean 246·2 (86 per cent. Recovery.)	

The probable error of a single determination is calculated as, $p_s = \pm$ 8·9 mgms., *i.e.*, 3·6 per cent., and the probable error of the mean as, $P_n = \pm$ 2·5 mgms., *i.e.*, 1 per cent. In this series much larger amounts of lactic acid were present than are usually found in human blood. In a determination of this kind the error is likely to be a proportional one, so I repeated the experiment with blood to which had been added a total of 160·8 mgms. of lactic acid per 100 c.c.; p_s in this case was $\pm$ 5 mgms., *i.e.*, 3·7 per cent., and p_n about $\pm$ 1·5 mgms., *i.e.*, 1·1 per cent., which looks as though the error is really proportional to the amount of lactic acid being determined.

Sources of Loss of Lactic Acid.

A number of experiments have been carried out in an attempt to trace the source of loss of lactic acid when determined by the permanganate method of Clausen. Taking the case where an estimation of lactic acid in blood is being made the following steps are those where loss is most likely to occur: (*a*) In the removal of the proteins; (*b*) in the removal of glucose; (*c*) in the oxidation of the solution; (*d*) in the titration of the aldehyde.

It is well known that protein precipitates tend to carry down, adsorbed on them, any salts which are in solution, and indeed Mondschein (22) has shown that in the heat-coagulation of the blood proteins one-third of the lactic acid present may be carried down.

Glucose is removed from the blood filtrates by the method adopted by Van Slyke (23), which consists of treating the solution with 15 per cent. copper sulphate and a 10 per cent. suspension of lime $Ca(OH)_2$; this precipitates the glucose as an insoluble copper complex. To see if this formation of a precipitate in the blood solution caused any loss in lactic acid, equal amounts of a lactic acid solution were taken, and to one of them a small amount of glucose was added, nothing being added to the other. In the first solution the glucose was then removed by treatment with the copper sulphate-lime mixture, and then both were analysed for lactic acid. No difference could be found which was not within the experimental error of the method.

The titration with N/100 iodine solution of the bisulphite which is liberated from the aldehyde bisulphite compound by the addition of bicarbonate, gives (using starch as an indicator) a poor end-point. Not only does the blue colour appear in the solution and gradually fade again before the titration is complete, but usually it slowly changes from blue to purple before disappearing. The cause of this purple colour is unknown, but the only way to make sure of a consistent end-point is to add the iodine until a blue colour is obtained which persists for two minutes. Often it is found that the blue colour will disappear on the further addition of bicarbonate, although the bicarbonate has no effect on the iodine titre of a bisulphite solution.

Titre	With bicarbonate	Without bicarbonate
N/100 I_2 (c.c.)	2·50	2·50

Since the solutions of bisulphite which are titrated are very dilute, it was thought possible that dilution might have an effect on the iodine titre. To test this a known amount of bisulphite solution was diluted 10, 20, 30, 50 and 100 times with distilled water, and the solution was then titrated with N/100 iodine.

Dilution	10	20	30	50	100
Titre N/100 I_2 (c.c.)	2·40	2·50	2·40	2·60	2·50

From the figures it would appear that dilution has no effect on the iodine titre.

Assuming that the titration is so controlled that all the bisulphite liberated from the aldehyde compound is estimated, the source of loss in an estimation of lactic acid in blood lies in the protein precipitation and the actual oxidation. The loss due to any lactate that is carried down adsorbed on the protein coagulum might be minimised by washing of the latter, but this is hardly

advisable as the blood is already diluted ten times. Concentration of lactic acid solutions is liable to lead to serious losses of the acid, as has been shown by Wolf (16). In this connection it is interesting to note that McLeod (24) concentrates his solutions at temperatures below 40° C. by means of a special apparatus. The losses due to the oxidation have already been discussed, and it is here that the chief source of loss is to be found.

As can be seen from the figures given above, the actual yield of lactic acid in my experiments was about 85 per cent. of that actually present. Clausen (6) bases his calculations on a 90 per cent. yield, while Anrep and Cannan (25) found an 89–92 per cent. yield. All the figures given for lactic acid content of blood have been multiplied by 100/85 in accordance with the above results.

Estimation of Lactic Acid in Blood.

The method used throughout has been that detailed by Clausen (6). The proteins were first precipitated by the use of tungstic acid (Folin and Wu (26)). In practice it was found better to add the acid before the tungstate, and not *vice versa*, as stated by Folin and Wu, thus following out a suggestion of Haden (27). Glucose was removed by the method of Van Slyke (23) and the solutions then analysed.

A word is necessary as to the importance of preventing glycolysis in shed blood. Lovatt Evans (28) has shown how the carbon dioxide capacity of drawn blood diminishes owing to the formation of lactic acid, and how this glycolysis can be prevented by the addition of small amounts of sodium fluoride. Obviously, unless this precaution be taken the lactic acid content of the blood will be too high, and earlier observers have from time to time recorded very high values of the lactic acid content of blood even at rest (Fries (29)). The increase in lactic acid content in non-fluorided blood is shown below. Both samples were left several hours before analysis.

Lactic Acid Content of Ox-blood: mgms. per 100 c.c.

No fluoride	51·5	Mean of 9 determinations.
Fluoride added....	38·0	Mean of 7 ,,

The difference between these two is 13·5 mgms., and the production of this amount of acid in blood would diminish the carbon dioxide capacity by about 3·3 vols. per cent. Lovatt Evans found in human blood a diminution in carbon dioxide capacity of about 4 volumes per cent. as a result of this glycolysis.

A large number of determinations have been made on the lactic acid content

of human blood, both at rest and after all kinds of exercise. These will be considered in detail in Part III, but it is sufficient to note that the values found in resting human blood range usually from 10 to 20 mgms. per 100 c.c. The higher values in slaughter-house blood are due presumably to struggles or movements of the animal before death.

Acetone Bodies in Blood.

If determinations be made on the blood of diabetic patients, or on that of normal subjects who have completed a long bout of strenuous exercise, it is necessary to correct the value found for the lactic acid content by subtraction of the acetone bodies which appear under these conditions. Acetone bodies can be conveniently estimated by Shaffer's method (30) after the final titration with iodine. No controls as to the accuracy of this method have been made, but a few of the figures found to date are below.

Subject.	Sample.	Lactic acid in Blood : Mgms. per 100 c.c.	Acetone bodies in Blood : Mgms. per 100 c.c.
Diabetic S. : no insulin.	Rest	28·9	3·4
	2 mins. after exercise (standing running).	86·9	4·14
	110 mins. after exercise (standing running).	30·3	1·58
Diabetic T., with insulin.	Rest	18·6	2·56
	90 mins. after exercise (standing running).	19·7	4·48
Marathon runner, M.R.D.	Rest	21·0	None.
	After 1 hour's running	70·5	4·45
	After 6 mins. recovery from $1\frac{3}{4}$ hours' running.	61·6	8·90

Estimation in Urine.

The amount of lactic acid to be found in the urine when the subject is at rest is only small. Ishihara (33), using the von Fürth and Charness method, and ether extraction of the lactic acid, gives 8 mgms. per 100 c.c. as the normal amount. Dapper (34) gives 6 mgms. per 100 c.c. as the normal amount, and says it varies with the specific gravity of the urine. Feldman and Hill (35), using Ryffel's method, found fairly large amounts in urine immediately after exercise, but very little at rest. Clausen (6) gives 6 to 8 mgms. per 100 c.c. as the usual amount found in normal urine.

The chief difficulty in estimating lactic acid in urine by Clausen's method

is the extraction with ether. Clausen (6) details an apparatus for continuous extraction which I have tried, but which requires careful handling for its proper working. Another point to be noted is that the ether used for the extraction must be carefully purified: otherwise the impurities present yield bisulphite binding compounds when oxidised. So far no determinations have been made after exercise, but at rest the lactic acid content of urine varies in my observations from 3 to 14 mgms. per 100 c.c. The recovery of lactic acid added to urine is about the same as for blood, *i.e.*, about 85 per cent.

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Part III.—Lactic Acid in Blood and the Respiratory Quotient.

By A. V. Hill, C. N. H. Long, and H. Lupton.

(i) *Lactic acid in blood.*

The matter may be considered from two different aspects, according to the type of exercise undertaken :—

(*A.*) *Moderate exercise* in which the condition of the subject approximated to the steady state, and the final oxygen debt was small, and (*B.*) *severe exercise* in which the steady state was far surpassed, and the final oxygen debt was large.

(*A.*) *Moderate Exercise*—

Table I.

No. of Experiment.	Type of Exercise.	Subject.	Time of observation.	Lactic acid in Blood : mgr. per 100 c.c.	O_2 intake : c.c. per min. at rest or during exercise.	Respiratory. Quotient.
1	Standing running at 156 steps per min. for 55 mins.	C.N.H.L.	Rest (mean)	[20]	[287]	[0·85]
			After 18 mins. exercise.	58·1	2360	0·89
			After 37 mins. exercise.	52·5	2415	0·87
			After 55 mins. exercise. (Subject tiring and jumping less vigorously.)	32·9	2038	0·86
2	Walking at 4·1 miles per hour (110 metres per min.) for 33 mins.	C.N.H.L.	At rest before exercise.	21·4	282	0·85
			1 min. after exercise.	58·9	1241	0·89
3	Walking at 3·5 miles p.h. (93 metres per min.) for 28 mins.	C.N.H.L.	At rest before exercise.	20·9	292	0·85
			1 min. after exercise.	36·61	1155	0·84
4	Walking at 3·3 m.p.h. (89 metres per min.) for 25 mins.	J.C.H.	At rest before exercise.	31·5	285	0·85
			1 min. after exercise.	40·6	906	0·83
5	Running 9 miles in 1 hour (241 metres per min.) at constant speed.	A.A. (a practised runner).	At rest before exercise.	23·2	—	—
			1 min. after exercise.	54·6	—	—

The subjects of the experiments shown in Table I. are all very able-bodied men. It will be noted that the rise in the lactic acid concentration in the blood is comparatively small. From the point of view of the supply of oxygen the

subjects never passed the limit of the steady state of exercise. When fatigue was obvious, as in expts. (1) and (5), it was not due primarily to oxygen want but to other secondary or local causes. In less active subjects the limit of the steady state of exercise would of course be much lower, *e.g.*, most individuals could not run at 9 miles per hour without a rapid accumulation of acid in the blood and consequent exhaustion. The existence of a steady state of exercise is further demonstrated by the consideration that successive estimates of the lactic acid in expt. (1) tend rather to decrease than to increase. The fact that the amount of lactic acid in the body is not increasing is confirmed by the smallness of the respiratory quotient: this remains completely unaffected by a steady condition of exercise: if the lactic acid had been increasing in amount there would have been a driving off of carbon dioxide and a larger respiratory quotient.

We may conclude, therefore, that the rise of lactic acid concentration in the blood of man, during a steady state of exercise, though important, is comparatively small. This is confirmed by another line of reasoning which has been alluded to by one of us (Long (1)) in a recent paper and will be discussed more fully in a later Part. There it will be shown that lactic acid in the body may be regarded as a "governor" of oxidation, the oxygen intake being proportional (in the presence of adequate oxygen) approximately to the square of the concentration of lactic acid in the active tissues. Assuming that the oxygen intake can rise, at the most, 16 times from a state of complete rest to one of very strenuous continued exertion (as it may do in a healthy man), the lactic acid concentration should be able to rise about 4 times between the same states. Assuming a resting lactic acid concentration in the blood of 20 mgr. per 100 c.c., the maximum attainable during a steady state of exercise should be 80 mgr. per 100 c.c., a value higher than any given in Table I. Apparently in each experiment recorded there the subject was well within his limit of the steady state—as might be expected from the comparatively low oxygen intake in expts. (1) to (4).

It is interesting to discuss the physiological consequences of these lactic acid changes. Consider the following three cases, viz., increases in the lactic acid concentration of 15, 30 and 60 mgrs. per 100 c.c. of blood respectively. We will assume, moreover, that when the steady state of exercise has been fully attained the lactate ion concentration is the same in muscles as in blood plasma, *i.e.*, some 33 per cent. higher than in blood (see below, § (ii) Part III). Assuming some 40 litres of body volume to be so affected, there will then be approximately 8, 16, and 32 gms. of lactic acid at large in the body, in our

three cases respectively. If the hydrogen ion concentration of blood and tissues had been maintained constant by the activity of the respiratory centre, the equivalent amount of carbon dioxide would have been eliminated *via* the lungs, in excess of that produced by metabolism, viz., about 2, 4 and 8 litres respectively. This elimination of carbon dioxide by the activity of the respiratory centre should cause a temporary rise in the respiratory quotient of appreciable, though not large, extent. For example, if the true metabolism during exercise were, O_2 3 litres per minute, CO_2 2·4 litres per minute, respiratory quotient = 0·8, then 3 litres extra of carbon dioxide eliminated in 10 minutes would raise the apparent respiratory quotient to 0·9; even 6 litres extra of carbon dioxide in the same time would raise it only to 1·0. During the attainment, therefore, of the steady state there should be an extra elimination of carbon dioxide caused by a rise of hydrogen ion concentration affecting the respiratory centre, a certain degree of dyspnœa, and a rather high respiratory quotient: as soon, however, as the steady state is attained the respiratory quotient becomes normal again, the dyspnœa disappears, and the hydrogen ion concentration of the blood and tissues attains the value appropriate as a "governor" of the respiratory centre. We imagine this attainment of the steady state to be coincident with a phenomenon commonly (but not generally) experienced, the condition of "second wind."

The lactic acid accumulated during exercise is removed in recovery. Assuming an "efficiency" of recovery (2) of 5·2 : 1, *i.e.*, that 1 litre of oxygen causes the oxidative removal of 7 grams of lactic acid in recovery, the oxygen debts in our three cases should be 1·2, 2·3 and 4·5 litres respectively. Such oxygen debts are small compared with those attained after severe exercise; and the small calculated values agree well (as we shall see in a later Part) with actual observations of the oxygen debt at the end of exercise not exceeding the limit of the steady state. We are correct, therefore, in describing the steady state of exercise as being characterised by a small oxygen debt.

At a constant hydrogen ion concentration the introduction into blood of 15, 30 and 60 mgrs. of lactic acid per 100 c.c. in our three cases must necessitate the withdrawal* by increased ventilation of 3·7, 7·4 and 14·8 c.c. of carbon dioxide respectively per 100 c.c. The carbon dioxide dissociation curve of blood must be altered, therefore, from the normal to one appreciably below it, as, indeed, many observers have found. In a steady state of exercise, the

* This is not strictly true, owing to the non-uniformity of distribution of lactate ions between plasma and corpuscles.

amount of shift of the carbon dioxide dissociation curve, though appreciable, cannot be large; very extensive shifts, however, may be produced by severe short-lived exercise, as we shall see later.

The effect of steady exercise on the oxygen dissociation curve of blood in the body cannot be calculated from a knowledge of the lactic acid content alone, since the hydrogen ion concentration (which determines the form of the dissociation curve) is largely regulated by the respiratory centre; at a given carbon dioxide pressure, however, it is clear that the O_2 dissociation curve must be appreciably affected by the lactic acid accompanying a steady state of exercise, as Barcroft and his co-workers have continually found.

(*B.*) *Severe Exercise.*

During and after severe short-lived exercise the conditions are very different from those obtaining during the steady state. Indirect evidence, from the magnitude of the oxygen debt, shows that amounts of lactic acid of the order of 50 gms. or more may be liberated in the body in 30 secs. of exercise, and these massive productions of acid find a rapid expression in the blood.

Table II shows that immediately (1 min.) after exercise amounts of lactic acid of 110, 80 and 95 mgr. per 100 c.c. may be present in blood, even 16 mins. after exercise as much as 99 mgr. Experiment 11 is interesting as showing far greater values than any other. The subject was breathing 49 per cent. oxygen, which apparently relieved cerebral and cardiac distress to such a degree that a much higher degree of muscular exhaustion was attainable; the same fact is found in connection with the magnitude of the oxygen debt, as will be shown in a later Part, much higher values being attained while breathing oxygen-enriched air. The other experiment in oxygen, that on C.N.H.L. (No. 13), does not show such a high value, presumably because it was less prolonged. In experiment 11 the subject continued severe exercise for $9\frac{1}{2}$ minutes, so allowing the lactic acid plenty of time to escape into the blood; in experiment 13 the exercise lasted only 4 minutes, which, presumably, was not long enough to allow so much lactic acid to escape from the muscles.

The values given in this table are in good agreement with those found by Barr, Himwich and Green (3) and by Barr and Himwich (4), which are abstracted in the following table (Table III). In every case the subject took "vigorous" exercise on a Krogh bicycle ergometer for a comparatively short period.

Table II.

No. of Expt.	Type of Exercise.	Subject.	Time of Observation.	Lactic Acid in blood: mgr. per 100 c.c.	Remarks.
1	Running 8–9 m.p.h. for 18 mins.	D.	Rest before exercise	24	Subject could not maintain this speed.
			1 min. after exercise	110	
2	Running 8–9 m.p.h. for 8 mins.; then "standing running all out" for 1 min.	C.N.H.L.	13 mins. after exercise	57	
			140 mins. after exercise	12	
3	—	Miss D.A.	23 mins. after exercise	41	
			64 mins. after exercise	10	
4	—	A.V.H.	23 mins. after exercise	72	
			53 mins. after exercise	29	
5	—	E.C.S.	14 mins. after exercise	96	
			54 mins. after exercise	43	
6	—	E.W.	16 mins. after exercise	91	
			47 mins. after exercise	32	
7	—	D.S.	16 mins. after exercise	81	
			46 mins. after exercise	38	
8	—	H.S.	16 mins. after exercise	99	
			47 mins. after exercise	57	
9	—	L.W.	24 mins. after exercise	57	
			55 mins. after exercise	22	
10	—	H.L.	1 min. after exercise	80	*See* fig. 1.
			5 mins. after exercise	102	
			10 mins. after exercise	90	
			16 mins. after exercise	75	
			49 mins. after exercise	40	
11	Standing running for 9½ mins. very vigorously in 49 per cent. oxygen: 237 steps per min.	S.S.	Rest before exercise	8·5	Highest value recorded. *See* fig. 1.
			5 mins. after exercise	204	
			10 mins. after exercise	153	
			16 mins. after exercise	96	
			47 mins. after exercise	28	
12	Standing running for 10 min. not so vigorously in air: 237 steps per min.	S.S.	Rest before exercise	21	
			1 min. after exercise	95	
			6 mins. after exercise	90	*See* fig. 1.
			12 mins. after exercise	84	
			17 mins. after exercise	71	
			48 mins. after exercise	41	
13	Standing running at 300 steps per min. for 3 mins., then "all out" for 1 min.: breathing pure oxygen throughout.	C.N.H.L.	3 mins. after exercise	83·5	
			9 mins. after exercise	86·0	
			19 mins. after exercise	66·3	*See* fig. 1.
			34 mins. after exercise	42·4	
			64 mins. after exercise	25·0	

Table III.

No. of Expt.	Subject.	Exercise.	Blood.	Time of Observation.	Lactic Acid mgr. per 100 c.c.	Lactic Acid before Exercise.
1	D.P.B.	3,770 kg.m. in 3½ mins.	Venous	3 min. after exercise ...	101	15
2	D.P.B.	3,820 kg.m. in 3½ mins.	Arterial Venous	3 min. after exercise .... ,, ,, ...	117 89	— —
3	D.P.B.	3,504 kg.m. in 3⅓ min.	Venous	15 mins. after exercise	65	24
4	H.E.H.	3,400 kg.m. in 3¾ mins.	Venous	3 mins. after exercise....	46	14
5	H.E.H.	3,545 kg.m. in 3⅓ min.	Venous	During exercise ..... 3 mins. after exercise	48 47	14 —
6	H.E.H.	3,700 kg.m. in 3½ mins.	Arterial Venous	3 mins. after exercise ,, ,,	79 57	— —
7	H.E.H.	3,408 kg.m. in 3½ mins.	Venous	During second minute of exercise	45	18

Here we find, even during the second minute of exercise (exp. 7, Table III), a considerable increase in the lactic acid concentration resulting from quite short-lived vigorous effort. It is clear that the acid can pass readily and rapidly into the blood stream, a fact which is borne out by the striking experiments (2 and 6 of Table III) in which arterial blood was found by Barr and Himwich (4) to contain appreciably more acid than venous blood flowing from an inactive limb. In these two experiments a single passage through the blood capillaries of an inactive muscle was sufficient to diminish the lactic acid content of the blood by 28 and 18 mgr. per 100 c.c. respectively. Conversely, in an active limb (the arm raising a weight to the point of subjective fatigue) two or three minutes after exercise, the arterial blood contained *less* lactic acid (and not *more*) than the venous blood, to the extent of 27, 7 and 4 mgr. per 100 c.c. in three experiments. Thus lactate ions can freely travel from muscle to blood and *vice versa*.

In four of our experiments, given in fig. 1, the time course of the lactic acid concentration after exercise is shown (Table II, expts. 10, 11, 12 and 13). During severe exercise the lactic acid in the active muscles steadily increases in concentration, though at a decreasing rate as oxidative recovery gradually gathers way, and passes by diffusion into the blood stream and thence into other tissues

of the body. On the cessation of exercise the accumulation of acid in the muscles immediately ceases, and its concentration begins to decrease (*a*) by

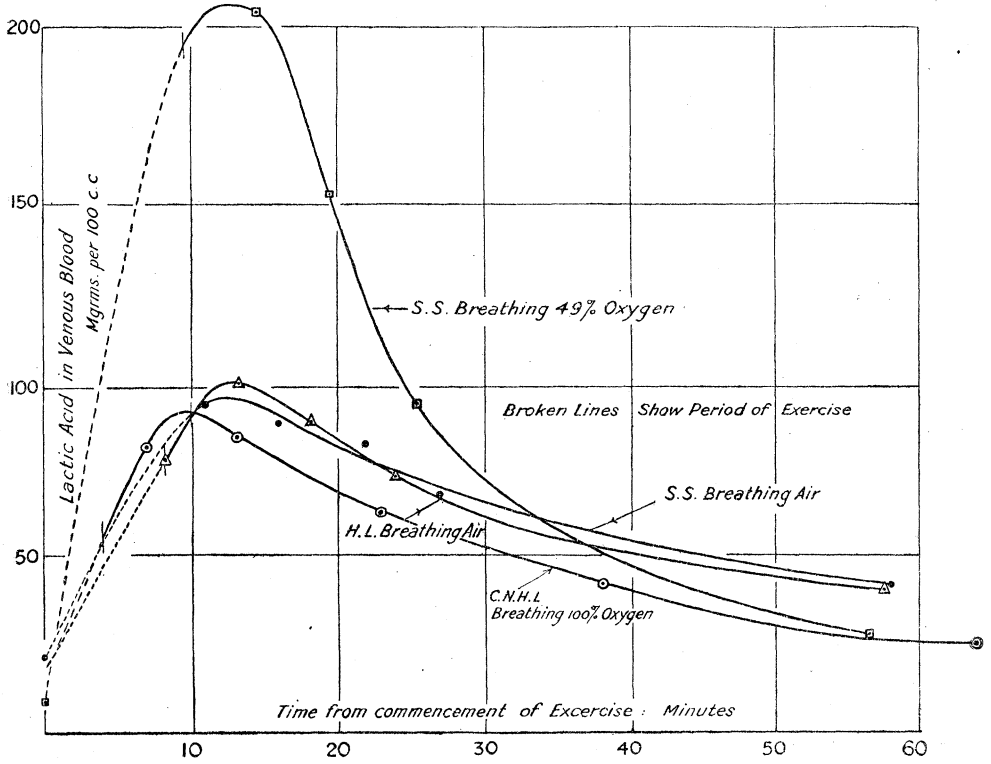

FIG. 1.—Lactic acid in human blood after severe muscular exercise; two experiments in air, one in 49 per cent. oxygen, one in 100 per cent. oxygen. Note that the recovery process is not quite complete at the end of the time shown in the diagram.

diffusion into the blood stream and thence to other tissues, and (*b*) by oxidative recovery. It is not yet known whether recovery (restoration of lactic acid to glycogen) can occur in tissues (*e.g.*, liver or muscle) other than those responsible for the initial breakdown. Barr and Himwich's results are sufficiently explained at present by the hypothesis that lactate ions diffuse from the capillaries into the "lactate-ion vacuum" of the inactive muscle without actually being "removed" there by oxidative recovery. Certain it is, however, that after severe and prolonged exercise recovery is comparatively slow. Recovery from moderate short-lived exercise is rapid, and it would seem probable that the slowness of recovery from violent effort is due to the gradual nature of the return, by diffusion, of the lactic acid from other tissues, to the localities where it can be dealt with by the recovery mechanism.

In short-lived violent effort the concentration of lactic acid in the active muscles may proceed so far that it continues, for some minutes after the exercise is over, to increase in the blood passing through the muscles. Consequently we see, in fig. 1, that the maximum concentration is attained in the blood, not immediately at the end of exercise but some minutes later. The maximum concentration in the muscles may be considerably greater than that attained in the blood. As recovery proceeds, however, the lactic acid must tend to reach approximately the same concentration in all the watery tissues in direct contact with the blood stream, and to diminish gradually by the recovery process to its initial resting value. Table II shows that the resting value (of about 20 mgr. per 100 c.c.) is attained again in the blood after rather more than an hour of recovery in normal individuals. A complete return may take longer than this, according to the nature and duration of the exercise, but even after the most severe effort there should be no appreciable divergence from resting conditions after $1\frac{1}{2}$ hours recovery.

§ (ii) *Distribution of Lactic Acid between Whole Blood and Plasma.*

Hitherto we have dealt only with the total lactic acid per 100 c.c. of whole blood. The lactate ions, however, are not evenly distributed between plasma and corpuscles, as the following table (Table IV) shows.

In animals' blood, therefore, to which lithium lactate has been added, the plasma attains a concentration about 50 per cent. greater than the blood regarded as a whole. Taking the blood as being plasma 60 per cent., corpuscles 40 per cent., this means that only a small fraction of the lactate is contained in the corpuscles. In human blood, in which lactic acid has been liberated inside the body by muscular exercise, the ratio is smaller, the concentration in the plasma being about 29 per cent. greater than in the blood regarded as a whole. This implies that the concentration in the corpuscles is only about 43 per cent. of that in the plasma. Apparently lactate ions do not distribute themselves evenly across the corpuscular wall, but tend to remain in the plasma. Whether this is due to a relative impermeability of the corpuscular boundary, or to a membrane equilibrium occurring there, cannot be discussed now. In our present work we are concerned rather with the fact that the concentration of lactate ions in the plasma is appreciably, some 30 per cent., larger than in the blood regarded as a whole. The muscles themselves tend to come into equilibrium with the plasma; hence, at a moment when there is a lactic acid concentration of 100 mgms. per 100 c.c. in the blood, the muscles in equilibrium with it must contain some 130 mgms. per 100 c.c. This fact must be borne

Table IV.

I. Animals' blood: lactic acid, calculated as $C_3H_6O_3$, added in the form of lithium lactate.

Description.	Lactic Acid added: mgm. per 100 c.c.	Lactic Acid found mgm. per 100 c.c.;		Ratio, Plasma: Blood.
		Whole Blood.	Plasma.	
Ox blood, oxalated, no fluoride: stood 1 hour after adding lithium lactate.	97	135	197	1·46
Ditto: but blood centrifuged immediately after adding lactate.	48·5	116·5	163	1·40
Dog's blood, defibrinated, no fluoride, centrifuged immediately after adding lactate.	45	80	129	1·62
II. Human blood, C.N.H.L.: oxalated but not fluorided: centrifuged immediately.				
Standing running for 2 mins., not very vigorously.	—	34·6	45·7	1·32
Standing running "all out" for 1 min., blood sample 3 mins. later.	—	75	100	1·33
Ditto	—	95	115	1·21

in mind in any discussion of the lactic acid concentration in tissues in contact with the blood: that concentration is some 30 per cent. higher than would be deduced for the analysis of the blood as a whole.

§ (iii) *The Apparent Respiratory Quotient during and after Exercise.*

It is well known that very stringent precautions must be taken if a true value of the respiratory quotient (*i.e.*, one representing the metabolism) is to be observed experimentally. Without such precautions, especially during a state of muscular exercise, or in recovery therefrom, the respiratory quotient may tell one practically nothing of the nature of the bodies undergoing oxidation. Even at rest, a nervous or unpractised subject may, by alterations in rate or depth of respiration, produce apparent values of the respiratory quotient which are well outside the realm of possibility. One of the subjects who volunteered for our experiments, a fair long-distance runner, we had to discard owing to the fact that he always gave such respiratory quotients at rest as 1·38 and 1·07,

even after a prolonged fore-period of breathing through pipes and mouth-piece. We imagine that this subject invariably and unconsciously quickened his respiration on being submitted to experimental conditions: only by taking a sample of the expired gases over a very long interval could such a peculiarity be neglected.

During a prolonged steady state of exercise the respiratory quotient is always relatively low, being greater, however, for more vigorous exercise. Thus, in walking steadily at 3·5 and at 4·1 miles per hour the respiratory quotient of C.N.H.L. was 0·84 and 0·89, that of J.C.H. at 3·3 miles per hour being 0·83: in C.N.H.L. standing running at 156 steps per minute it was 0·89 after 18 minutes exercise, 0·87 after 37 minutes, and 0·86 after 55 minutes: while in A.V.H. running at 239 metres per minute for 33 minutes it was 0·95 at 3 minutes, and 1·00 at 10 minutes, 18 minutes and 27 minutes. A representative collection of respiratory data is given in Table V. Experiments 1 to 5, and 7, 9, 10 and 13 are instructive in showing the absolute regularity with which the respiratory quotient rises as the vigour of the exercise is increased. If it had risen merely to unity we might (erroneously) have drawn conclusions as to the metabolism: its rise far above unity, *e.g.*, to 1·29 in experiments 7 and 9, and to 1·43 in experiment 12, shows that it depends upon other factors, presumably on the liberation of lactic acid and the effect of the rise of *c*H so produced on the respiratory centre.

The amount of lactic acid known to be liberated in the body during severe exercise is more than adequate to explain the observed changes in the respiratory quotient. Taking even the extreme case (expt. 12), of S.H. with a respiratory quotient of 1·43, and assuming the real respiratory quotient of his metabolism at the moment to have been 0·85, we may calculate that S.H. was eliminating about 1·4 litres of carbon dioxide *per minute*, in excess of that produced by combustion. This is the equivalent of only 5·7 grms. of lactic acid, and might have been turned out from combination with alkali in the tissues by that amount of acid produced per minute. When we realise that in very severe exercise (as will be shown in a later Part) a vigorous man may produce 2 to 3 gms. of lactic acid *per second*, it will be obvious that there is no difficulty in explaining even the most violent alterations of the respiratory quotient on the basis of lactic acid changes. Running into debt for oxygen to the extent of only 1 litre per minute—a very modest amount—implies the accumulation per minute of about 7 gms. of lactic acid. If the hydrogen ion concentration of blood and tissues were really maintained constant by the respiratory centre, such an accumulation of acid would require the elimination of an extra 1·7 litres of

Table V.

No.	Subject.	Type of Exercise.	Fore-period : mins.	Interval of collection.	CO_2/O_2 per min. } = R.Q.	Ventilation litres per min.
1	S.	Standing	—	—	242/296 = 0·82	6
		Running 170 metres per min.	3¾	2 mins.	2415/2635 = 0·92	39
		Do. 203 do.	3	100 secs.	3230/3365 = 0·96	52
		Do. 255 do.	2¾	80 secs.	4600/3985 = 1·15	86
2	W.	Standing	—	—	323/365 = 0·88	8·9
		Running 172 metres per min.	2	2¾ mins.	2540/2808 = 0·90	49
		Do. 203 do.	3½	2 mins.	2932/3140 = 0·93	58
		Do. 255 do.	2¾	72 secs.	4278/3995 = 1·07	86
3	J.	Running 203 metres per min.	6¾	2¼ mins.	3131/3325 = 0·94	58
		Do. 255 do.	3¼	83 secs.	4000/4010 = 1·00	72
		Do. 299 do.	1¾	22½ secs.	4420/4040 = 1·10	95
4	A.V.H.	Running 172 metres per min.	5½	2 mins.	2752/3080 = 0·89	52
		Do. 197 do.	4¾	1½ mins	3340/3491 = 0·96	65
		Do. 243 do.	3¾	1 m. 10 s.	4475/4175 = 1·07	90
		Do. 271 do.	3½	1 min.	4335/4055 = 1·07	114
		Do. 288 do.	2	51 secs.	4730/4080 = 1·16	117
5	H.L.	Rest, lying	—	—	181/217 = 0·83	—
		Rest, standing	—	—	222/256 = 0·87	—
		Walking 54 metres per min.	12	—	578/652 = 0·89	—
		Do. 94 do.	10	—	756/867 = 0·87	—
		Do. 134 do.	10	—	2365/2410 = 0·98	—
6	H.L.	Running	3½	76 secs.	3160/2550 = 1·24	80
7	H.L.	Rest	24	11 mins.	205/228 = 0·92	4·9
		Standing running 237 steps per min.	6¾	—	3018/2443 = 1·23	76
		Standing running 276 steps per min.	2	62 secs.	3080/2400 = 1·29	72
8	C.N.H.L.	Running fast till exhausted	0	2 m. 37 s.	8880/7860 = 1·13	204*
9	C.N.H.L.	Standing running 276 steps per min.	2	62 secs.	3670/3240 = 1·13	85
		Standing running "all out"	2½	60 secs.	4460/3466 = 1·29	109
10	A.V.H.	Standing running 237 steps per min.	3	61 secs.	4140/3415 = 1·21	115
		Standing running 264 steps per min.	3	62 secs.	4135/3592 = 1·15	121
11	S.S.	Standing running 276 steps per min.	2	62 secs.	4250/3465 = 1·23	107
12	S.H.	Standing running "all out"	2½	71 secs.	3435/2410 = 1·43	75
13	T.A.L.	Rest	40	10 mins.	236/265 = 0·89	5·4
		Standing running "all out"	2½	62 secs.	3345/2715 = 1·23	68

* Total quantities given, and not "per minute."

carbon dioxide per minute and a rise in the respiratory quotient at the least of 0·4 or 0·5. Actually the hydrogen ion concentration of blood and tissues cannot be maintained anywhere near constant: the ventilation rises, but to a degree quite inadequate to eliminate the whole of the carbon dioxide equivalent of the lactic acid set free: the acid combines mainly with the sodium-protein buffers of tissue and blood, and the hydrogen ion concentration rises, only a small part of the carbon dioxide equivalent of the lactic acid set free being driven off by the increased respiratory effort.

The highest respiratory quotients are not found during exercise itself, but, curiously enough, after it. In Table VI we find the maximum value of the respiratory quotient to occur in expt. 1, where during the second minute after very severe short-lived effort it reaches 2·03. A closer analysis of expts. 3, 4, 6 and 7 would undoubtedly have shown a similar value, since in expts. 3 and 4 in periods of 2½ and 3 minutes the respiratory quotients averaged 1·59 and 1·55 respectively, while in expt. 6 it averaged 1·59 for 10 minutes, and in expt. 7, 1·37 for 12 minutes. There is no question, therefore, that temporarily, after violent exercise, the apparent respiratory quotient may reach the value of 2, and may remain considerably above unity for long periods. Even after long-continued relatively moderate exercise (*e.g.*, expts. 2, 5 and 8), where we might perhaps have expected a return to the normal resting hydrogen ion concentration of the tissues to have occurred during the exercise itself, and consequently only a small elimination of carbon dioxide after it, we find a large rise in the respiratory quotient commencing on the cessation of exercise. In expt. 2, where the oxygen requirement of the exercise was definitely in excess of the subject's maximum intake (5,100 c.c. per minute instead of 3,700 c.c.) lactic acid was accumulating slowly during the exercise and the respiratory quotient was 1·12. On cessation of exercise it rose to 1·54 in 1¼ minutes, and then slowly fell again to unity in about 10 minutes. In expt. 5 the subject ran continuously at the same rate for 33 minutes, the speed being very slightly beyond the limit of the steady state of exercise, and the respiratory quotient constant at unity. On the cessation of exercise it rose to 1·09, and then fell rather rapidly to the low values generally assumed to be characteristic of the oxidation of fat. These low values we shall discuss shortly.

Fig. 2 gives two examples, expts. 1 and 2, from Table VI, which are characteristic of the effects of very severe and of moderate exercise on the respiratory quotient following it. The oxygen debt at the end of exercise, as also the presence of lactic acid in the blood, both show that very considerable quantities

Table VI.

No.	Subject.	Type of Exercise.	Interval after Exercise.	CO_2/O_2 in interval = R.Q.	Average Ventilation litres per min.
1.	C.N.H.L.	Standing running very violent for 36 secs. Shown in fig. 2.	0 to 34 secs.	1680/1250 = 1·34	74
			34 to 67 ,,	1450/894 = 1·63	59
			67 to 99 ,,	1100/587 = 1·87	50
			99 to 164 ,,	1670/820 = 2·03	40
			164 to 228 ,,	1095/630 = 1·73	28
			228 to 351 ,,	1630/1030 = 1·58	23
			351 to 593 ,,	1970/1600 = 1·22	15
			593 to 984 ,,	2160/2330 = 0·93	9·4
			984 to 1377 ,,	1810/2120 = 0·85	8·1
2.	C.N.H.L.	Fairly rapid running for 4½ mins. Shown in fig. 2.	2½ to 3½ mins. exercise	3670/3265 = 1·12	80
			0 to 31 secs. after	1650/1480 = 1·12	78
			31 to 61 secs.	1083/846 = 1·28	64
			61 to 94 ,,	980/639 = 1·54	56
			94 to 157 ,,	1400/1010 = 1·39	39
			158 to 279 ,,	1630/1310 = 1·25	23
			279 to 402 ,,	1210/1120 = 1·08	17
			402 to 585 ,,	1490/1460 = 1·02	14·6
3.	A.V.H.	Standing running very violent for 25 secs.	0 to 2 mins. 23 secs.	6570/4145 = 1·59	72
4.	A.V.H.	Standing running very violent for 20 secs.	0 to 2 mins. 57 secs.	6600/4260 = 1·55	51
			2 mins. 57 secs. to 10 mins. 46 secs.	4590/3925 = 1·17	15
			10 mins. 46 secs. to 14 mins. 13 secs.	1126/1304 = 0·86	9
5.	A.V.H.	Running 239 metres per min. for 33 mins.	0 to 3 mins. 23 secs.	5455/5025 = 1·09	45
			3 mins. 23 secs. to 12 mins. 37 secs.	3920/5015 = 0·78	13
			12 mins. 37 secs. to 27 mins. 34 secs.	3830/5560 = 0·69	9
6.	C.	Running round indoor track rapidly till exhausted.	0 to 10 mins. 4 secs.	15060/9475 = 1·59	43
7.	Y.	Running ¼ mile "all out"	0 to 12 mins. 9 secs.	13600/9900 = 1·37	32
8.	H.L.	Standing running 237 steps per min. for 6¾ mins.	0 to 5 mins. 53 secs.	5840/4380 = 1·33	25
			5 mins. 53 secs. to 11 mins. 45 secs.	1706/1900 = 0·90	8·1
			11 mins. 45 secs. to 16 mins. 48 secs.	1194/1604 = 0·75	6·6

of lactic acid may accumulate in the body during exercise. This lactic acid, when formed, combines immediately with the ionised sodium-protein ($Na^+ P^-$)

of the tissues to form sodium lactate ($Na^+ L^-$) and undissociated protein acid (HP). Consequently the hydrogen ion concentration does not rise far.

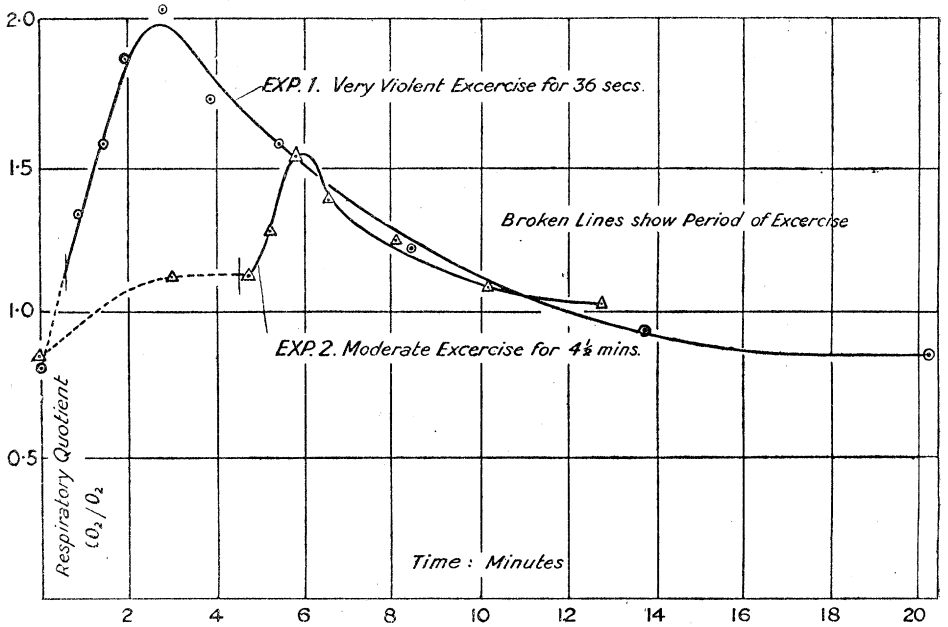

FIG. 2.—The respiratory quotient during and after muscular exercise. These figures show the initial phase of recovery only. The final phase is shown in fig. 3.

It must, however, rise to some extent; according to the equation for the ionisation of the protein acid,

$$HP \rightleftarrows H^+ + P^-$$

the hydrogen ion concentration is given by the formula, analogous to L. J. Henderson's formula for the case of carbon dioxide,

$$\text{hydrogen ion concentration} = k\frac{[HP]}{[P^-]} = k\frac{[\text{unionised protein acid}]}{[\text{ionised protein acid}]}.$$

Thus if the unionised protein acid has been increased at the expense of the ionised protein acid, by the liberation of lactic acid in the tissues, the hydrogen ion concentration must have risen, and it can only be brought back to its previous value by the action of the respiratory centre in eliminating carbon dioxide *via* the lungs. Lactic acid does not itself turn out carbon dioxide from bicarbonate; it merely deprives the ionised protein of its sodium and so forces it to "unionise." The consequent rise of hydrogen ion concentration, however, stimulates the respiratory centre to greater efforts, in which case the hydrogen ion concentration tends to revert to its previous value by the elimination

of carbon dioxide. It can return absolutely to that value only when the amount of carbon dioxide eliminated is the equivalent of the amount of lactic acid originally liberated; for the ratio [unionised protein acid] : [ionised protein acid] which is proportional to the hydrogen ion concentration must be made to return to its previous value, and this can occur only when the sodium originally taken away from the sodium-protein has been restored to it by sodium taken away from sodium bicarbonate.

This manner of regarding the mode of neutralisation of acid in muscle is very important and needs emphasis. *Lactic acid in muscle does not, to any serious extent, directly turn out carbon dioxide from bicarbonate.* It combines with sodium-protein and raises the hydrogen ion concentration; the elimination of carbon dioxide which results is the consequence of the induced activity of the respiratory system. The arguments in favour of this standpoint have been discussed elsewhere (2); they seem to be conclusive and their acceptance makes it possible, at once, to explain the large rise in the respiratory quotient following exercise.

If the *c*H of blood and tissues were maintained constant during exercise by the respiratory centre, then an amount of carbon dioxide rather more* than equivalent to the lactic acid set free would have to be driven off through the lungs by excessive ventilation. This certainly does not happen. The respiratory quotient does rise, as we have shown, during exercise, and to a higher value the more severe the effort. Quite moderate exercise however, exercise which could (in a healthy subject) be kept up for several minutes, may liberate in the body some 10 to 20 gms. of lactic acid per minute, and 15 gms. of lactic acid is the equivalent of 3·7 litres of carbon dioxide, an amount which, if actually eliminated, would double or more than double the respiratory quotient. Severe exercise may liberate 45 gms. of lactic acid in 20 seconds, which is the equivalent of 11·1 litres of carbon dioxide, a fantastic amount, which it is, of course, unthinkable that the body should be able to eliminate in the time. Clearly we must admit that the hydrogen ion concentration of blood and tissues can rise, and rise considerably, during muscular exercise; if so, the carbon dioxide driven off during exercise will be nowhere near the equivalent of the lactic acid set free, and when exercise ceases there will still be the stimulus

* *Rather more*, because not only must the exact equivalent be driven off from combination as $NaHCO_3$, to supply the equivalent of Na, but a small quantity must be driven off from simple solution in order to secure the constancy of the ratio (dissolved carbon dioxide) : (combined carbon dioxide) necessary if the hydrogen ion concentration is to be kept constant.

of a high hydrogen ion concentration to excite respiratory activity and to continue to drive off carbon dioxide. Now, as we shall see in a later Part, the oxygen intake falls rapidly on the cessation of exercise; this is due partly to a decreased blood flow, partly to a diminished rate of oxygen usage as recovery proceeds. Thus, in the expression R.Q. $= CO_2/O_2$, the denominator decreases owing to the rapid progress of recovery and the diminished blood flow, while the numerator is maintained at a high level by the activity of the respiratory centre. The amount of carbon dioxide eliminated varies more or less with the ventilation, while the oxygen intake is largely independent of the ventilation, so long as the alveolar oxygen pressure does not fall below about 16 per cent. Hence we can understand that the rapid and violent alterations in the respiratory quotient after violent exercise are due to the respiratory effort produced by the high hydrogen ion concentration of blood and tissue resulting from the exercise. The carbon dioxide elimination diminishes at first much more slowly than the oxygen intake, and consequently the respiratory quotient rises.

This enormous elimination of carbon dioxide, as the result of rapid ventilation during and after muscular activity, has certain interesting consequences in the later stages of recovery. For example, in Table VI, expt. 1, C.N.H.L. in about 10 minutes, after the end of 36 seconds only of exercise, eliminated some 3 litres of carbon dioxide in excess of that which might have been expected from the simultaneous oxygen intake and a real respiratory quotient of 0·85. In expt. 2, the same subject, after $4\frac{1}{2}$ minutes of moderate exercise eliminated more than 3 litres of carbon dioxide within five minutes from the end of exercise. In expt. 3, A.V.H. eliminated more than 3 litres extra of carbon dioxide within $2\frac{1}{2}$ minutes after 25 seconds of severe exercise, and in expt. 4 the same subject eliminated about 4 litres extra of carbon dioxide within 10 minutes after 20 seconds of severe exercise. As the lactic acid formed in exercise is removed in recovery, it is necessary for the body to retain the carbon dioxide which it previously eliminated, otherwise the tissues would become excessively alkaline. Consequently in the later stages of recovery there is a retention of carbon dioxide and a very low respiratory quotient. From fig. 1 it is obvious that for long periods the lactic acid formed in severe exercise continues to be removed. Long before the end of this process the ventilation and the hydrogen-ion concentration of blood and tissues have returned to normal; consequently for each molecule of lactic acid removed in the recovery process a molecule of carbon dioxide must be retained in order to prevent the body becoming too alkaline. This is clearly shown in Table VII,

and is the basis of a method, described in detail in a later Part, of determining the "efficiency" of recovery in man.

In each experiment the subject (post-absorptive) ran in a corridor at a fairly high speed for about 8 minutes, and usually finished with about 1 minute violent "standing running." The times given are from (after) the end of exercise; each collection of expired air occupied 10 to 15 minutes, of which the mid-point is that given as "time." The resting value was taken before exercise.

Table VII.

Experiment 1. *Subject T.A.L.* (*see* fig. 3).

Time (minutes)	Rest	5	15	27	42	57	72	87	102	117
R.Q.	0·81	1·38	0·82	0·66	0·65	0·73	0·77	0·79	0·79	0·81

Experiment 2. *Subject H.L.*

Time (mins.)	Rest	5	15	25	36	46	56	66	77	87	98	108
R.Q.	0·92	1·26	0·85	0·73	0·58	0·70	0·74	0·79	0·80	0·79	0·78	0·81

Experiment 3. *Subject H.L.*

Time (minutes)	Rest	5	16	26	37	47	57	67	77	87
R.Q.	0·84	1·35	0·77	0·72	0·74	0·71	0·77	0·77	0·78	0·80

Experiment 4. *Subject H.L.* (*see* fig. 3).

Time (mins.)	Rest	5	16	26	36	47	57	67	77	87	97
R.Q.	0·84	1·30	0·78	0·69	0·68	0·67	0·71	0·76	0·82	0·81	0·81

From Table VII and fig. 3 we see that after the initial high value of the respiratory quotient following severe exercise, there ensues a phase of low respiratory quotient, lower in some cases even than that (0·71) corresponding to the combustion of fat. The minimum value of the respiratory quotient occurs about 30 to 40 minutes after the end of exercise. It then rises gradually to its final resting value, attaining the latter in 70 to 80 minutes after the end of exercise. This sequence of events occurs with great regularity. In order to make it obvious, the effort in question must be a severe one; otherwise the changes involved, spread over a period of $1\frac{1}{2}$ hours, may be masked by chance experimental variations and become incapable of exact analysis. The experimental technique (that of the Douglas bag) must be very carefully applied in order to obtain smooth and consistent results. This will be described more fully in the next and succeeding Parts. With sufficient care, however, and after vigorous enough exercise, the sequence of events portrayed in fig. 3 invariably occurs, and it is obvious that we are dealing with some regular characteristic of recovery from muscular exercise.

In fig. 1 and Table II the decrease in the lactic acid concentration in blood during recovery is exhibited, and it is obvious that the lactic acid concentration

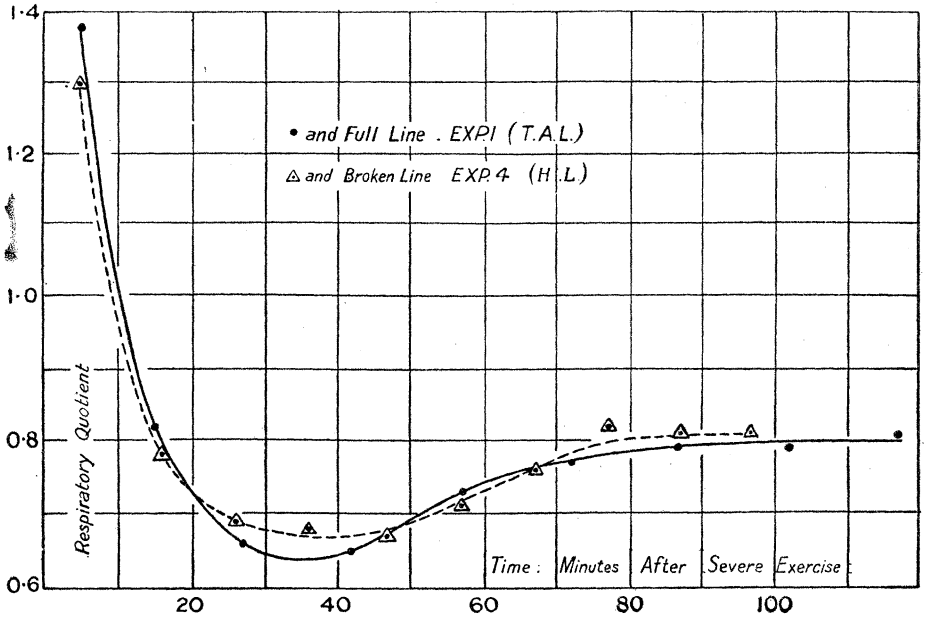

FIG. 3.—The respiratory quotient after severe muscular exercise. Note that in the later phases, while carbon dioxide is being retained to compensate for that initially driven off, the respiratory quotient falls to a very low level, returning to its final value at about 80 minutes.

has not returned completely to its resting value in 40 to 50 minutes from the end of exercise. It would seem likely, however, from an inspection of fig. 1, that the lactic acid concentration will have fallen to its resting value again at some time in the second hour after exercise. The lactic acid curves of fig. 1 are entirely consistent with the hypothesis that the respiratory quotient curves of fig. 3 are determined by the retention of carbon dioxide required to maintain the hydrogen-ion concentration during the removal of lactic acid in the recovery process. If this be so the curves of fig. 3 give us an admirable criterion of the duration of the complete recovery process, after severe exercise of this type, by healthy men in air.

It is noticeable that the final respiratory quotient attained 1½ to 2 hours after exercise is almost invariably rather less than that at rest initially. This may be due to a sparing of the carbohydrate stores, somewhat depleted by the exercise, and a greater utilisation of fat for basal purposes, or—what is essentially the same thing from the point of view of the whole balance of exchange in the body

—the re-formation of a certain amount of carbohydrate from fat, with no alteration in the basal oxidations. The phenomenon is shown in Table VII, and the following additional observations emphasise it :—

1. Subject T.A.L., post-absorptive. Respiratory quotient before 0·93, respiratory quotient after (60 minutes to 140 minutes) 0·90.

2. Subject T.A.L., post-absorptive. Respiratory quotient before 0·90, respiratory quotient after (85 to 205 minutes) 0·85.

3. Subject H.L. post-absorptive. Respiratory quotient before 0·84, respiratory quotient after (60 to 140 minutes) 0·82.

4. Subject C.N.H.L. Respiratory quotient before 0·85, respiratory quotient after (72 to 139 minutes) 0·81.

[N.B.—" Post-absorptive " in these cases implies no food during the preceding 15 or 16 hours.]

The difference is not large, but almost invariably present and in the same direction. There would seem to be little doubt that this—unlike all the other phenomena we have described—is a genuine metabolic effect, depending upon a slight change in the character of the oxidations supplying the basal heat-production. As to the precise cause of that change our data provide no evidence : probably it is no more than the change occurring with time in a fasting individual. The total oxygen used as a result of the exercise and recovery together, must have been of the order of 40 litres, an amount sufficient to maintain the basal metabolism for 3 hours. This, and the 2 to $2\frac{1}{2}$ hours elapsed between the two readings of the basal respiratory quotient, may well have lowered the real respiratory quotient by as much as 0·03 or 0·04. The change in question is of little interest except for the technical purposes of a prolonged experiment.

The following results obtained on a practised Marathon runner (M.R.D.), once a winner of the Polytechnic Marathon Race, during a training run of about 15 miles, at an average speed of about $8\frac{1}{2}$ miles per hour, may be of interest.

Before exercise at rest, 2 hours after a light lunch.

Lactic acid in blood, 21 mgr. per 100 c.c. Pulse rate, 42. Oxygen intake, 321 c.c./min.

After 60 minutes running.

Lactic acid in blood 70·5 mgr. per 100 c.c. (corrected for acetone bodies). Pulse rate after half-minute rest, 160.

After 80 minutes running.

CO_2/O_2 per minute, 3210/3660 c.c. ; respiratory quotient, 0·87. Ventilation, 77 litres per minute.

After 100 minutes running.

CO_2/O_2 per minute, 3025/3165 c.c. ; respiratory quotient, 0·95. Ventilation, 78 litres per minute.

After 6 minutes recovery.

Lactic acid in blood, 61·6 mgr. per 100 c.c.

Total oxygen debt at end about 7·5 litres.

Time after exercise (minutes)	11	29	54	80
Respiratory quotient	0·92	0·81	0·76	0·76

Hæmoglobin value, 85 per cent.; *R.B.C.*, 5,050,000.

Urine at end.—Acid to litmus, no sugar, acetone bodies strongly positive.

This experiment is striking as showing (1) the high oxygen intake maintained for nearly 2 hours; (2) the return to a low respiratory quotient after exercise [the exercise must have utilised something of the order of 550 litres of O_2, *i.e.*, about 24 gram molecules, and therefore about 720 gms. of sugar or the equivalent of fat; it is natural, therefore, that the respiratory quotient should have fallen]; (3) the high lactic acid content of the blood maintained for a long period; (4) the comparatively low respiratory quotient during the exercise, showing that the subject was running within the limit of the steady state; (5) the absence of high respiratory quotient immediately after exercise, showing that the blood and tissues were not excessively acid.

Summary of Part III.

1. The alterations in the lactic acid concentration of blood, during a steady state of moderate muscular exercise, are described. These, though important, are never very large.

2. The alterations in the lactic acid concentration of blood, during and after severe muscular exercise, are described. These are relatively large and can produce important effects on the dissociation curves of blood and its hydrogen ion concentration.

3. It is shown that the lactic acid concentration in the plasma of human blood after muscular exercise is some 30 per cent. greater than in the whole blood from which it was derived; the concentration in the corpuscles is considerably less than in the plasma. This is of importance because the muscles and other tissues come into contact and equilibrium with the plasma, in respect of lactate ion concentration.

4. The alterations in the lactic acid concentration of muscle, during and after severe exercise, are discussed. These produce the changes observed in the blood, and lead to wide variations in the respiratory quotient.

5. During severe exercise the respiratory quotient rises to a degree depending on the vigour of the exercise; after severe exercise it rises still further (up to a value of 2) during the first 10 to 15 minutes of recovery, and then falls to low values (*e.g.*, to 0·65) for a considerable interval, before returning finally to its normal resting value. All these changes depend merely upon the liberation of

lactic acid and its removal in recovery. The process of recovery appears to be complete, after the most strenuous exertion, in about 90 minutes.

6. The only genuine metabolic effect following exercise is a very slight displacement of the respiratory quotient, appearing after $1\frac{1}{2}$ hours' recovery. This displacement implies the oxidation of slightly less carbohydrate and more fat. It is probably analogous to the fall of respiratory quotient occurring during the early stages of fasting.

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The Influence of Initial Tension upon the Magnitude and Duration of the Mechanical Response in Skeletal Muscle.

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(Communicated by Sir Charles Sherrington, Pres. R.S.—Received May 22, 1924.)

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While investigating the reflex elicited in the semi-tendinosus muscle of frogs by a single break induction shock applied to one of the digits of the same side, it was found that a very slight increase in the initial passive stretch of the muscle greatly enhanced the magnitude and duration of the response recorded isometrically. Thus, as shown in fig. 1, at 1 gm. initial passive stretch the plateau was reached at 6 gms., while at 3 gms. the plateau height was 12 gms., and at 4·5 the plateau was approximately 15 gms. Above the reflex curves in this figure are shown the responses to single shocks (maximal) applied directly to the muscle, which incidentally show that the reflex response was repetitive in nature. Previously Liddell and Sherrington (1) had made the same observation upon the responses of mammalian muscle, and found that the enhanced response with increasing initial stretch occurred both in reflexes and in the response to direct motor stimulation. The effect would appear, therefore, to be peripheral in its origin. The experiments about to be described