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# THE LACTIC ACID CONTENT OF THE BLOOD AFTER MUSCULAR CONTRACTION UNDER EXPERIMENTAL CONDITIONS.

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Investigations into the chemical changes connected with muscular contraction have been mainly carried out on the muscles of cold-blooded animals. The theories constructed from the results of these researches have been usefully employed in application to the much more difficult problems which concern the changes associated with muscular contraction in mammals. However fundamental these results may be, we cannot reasonably expect that the changes which occur in isolated cold-blooded muscles in strange environments can be transferred without any modifications to the consideration of intact, innervated warm-blooded muscles, provided with a circulation, and forming part of a complex organism.

The study of the changes in muscular contraction in warm-blooded animals is evidently well worth while, and should be undertaken by methods as direct as the complicated conditions will allow. But in most cases, and especially in the case of muscular exercise in man, in which it has been principally pursued, and in which it has particular interest, the study must necessarily be undertaken by somewhat indirect methods.

Among these indirect methods is that involving the estimation of lactic acid in the blood as an index of its concentration in the tissues, including the active or recovering muscles.

In their investigations on this subject Hill and his collaborators [1924] drew blood from a superficial arm vein usually not less than 10 minutes after the completion of exercise, and an essential point in their argument was the following assumption: "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

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stationary or changing only slowly, or at a maximum value" [1924, p. 441].

Though it would be difficult in human experiments to obtain any nearer approximation than that obtained in this or similar researches, it seemed to us desirable directly to investigate this and other related points by carrying out observations on mammals under experimental laboratory conditions.

Among the questions which we have considered are the following:

1. To what extent does the lactic acid content of the venous blood from a superficial vein, which, as in some of the veins of the ante-cubital fossa in man, drains mainly superficial areas, represent that of the general arterial circulation? According to Barr and Himwich [1923], the differences in carbon dioxide capacity between arterial and superficial vein blood, which differences they attribute principally to differences in the lactic acid content, are so great that no conclusions from one can be transferred to the other.

2. When lactic acid is produced in the muscles, do these muscles, and also the other tissues of the body, come into diffusion equilibrium with the blood (arterial or venous) as regards their lactate content, and if so, how soon?

3. Which tissues are concerned in removing from the blood the lactic acid which enters it as a result of exercise?

4. To what extent, in experiments carried out under these conditions, can the physiological effects of the stimulation of muscles be regarded as comparable with those of exercise in the intact and conscious animal? For example, if anæsthesia is induced by amytal, are the conditions as regards the production and disposal of lactic acid in any way modified? Or if, on the other hand, decerebrate animals are employed, to what extent are the biochemical changes in muscle influenced by the preliminary administration of a general anæsthetic, or by any disturbances in the local conditions of circulation, muscular tonus, etc., consequent upon decerebration?

## METHODS.

The experiments were performed on dogs and cats, mostly the former. In the earlier experiments decerebration was believed not to be satisfactory, because the respiratory ventilation after the exercise did not always show sufficient augmentation to keep the blood properly arterialized. Further, the application of artificial respiration to decerebrate or spinal animals was out of the question, since, as shown in the previous paper, it is impossible entirely to exclude alterations of the lactic acid in either direction consequent upon inadequate adjustment of the ventilation. For many of the experiments, therefore,

we decided to use amytal, which enabled the animal to adjust its breathing naturally. When amytal was given intraperitoneally in doses of 0·07 g. per kg., definite hyperpnœa accompanied extensive muscular contraction and persisted for some while afterwards.

In later experiments, however, Schmidt's method of decerebration [1923] was used with success: little or no hæmorrhage occurs normally with this method, and the brain substance can be divided so far forward that no rigidity occurs. Respiration is never suspended, and the animal responds to exercise by well-marked hyperpnœa.

As we wished to have widespread muscular activity in most of our experiments, and found that stimulation of nerve trunks or of the spinal cord was not satisfactory, we had recourse to more generalized stimulation by the application of electrodes to the skin, previously moistened with salt solution. For example, if two electrodes in parallel were placed on the pads of the fore-paws and the other two on the hind-paw pads, very good contractions of most of the muscles could be obtained, though there was a liability for certain muscular groups to be missed out. We found it better to insert a metal plate (about 5 cm. square) actually under the skin in the "lower" cervical region or in the "upper" lumbar region, connected with one terminal of the secondary coil, and to grip the tail, moistened with salt solution, by a clip electrode several sq. cm. in area connected to the other terminal. The results indicated that, under these circumstances, much of the current was conducted down the spinal cord and the great nerve plexuses, as the contractions in both limbs and trunk were very generalized. With the "upper" electrode in the lumbar region the movements of the chest were not interfered with during short tetani; when the "upper" electrode was at the lower cervical level, the diaphragm usually still contracted normally, though the intercostal muscles and general trunk muscles were tetanized. Stimulation was sometimes by means of single twitches at 60 to 80 per min., but more often by short tetani, about $\frac{1}{4}$ to 2 sec. or even up to 10 sec., with 0·5 to 5 sec. intervals. The stimuli for tetani were given at the rate of 50 break shocks per sec. by a Palmer inductorium, 2 volts in primary, and with the secondary coil usually at about 5 cm.

Samples of arterial blood were drawn from the carotid artery. Where venous blood was required the sample was drawn from a collateral branch of the vein, thus avoiding interference with the blood flow in the main channel. All blood samples were treated with approximately 0·2 p.c. of their weight of a 9 : 1 mixture of solid potassium oxalate and sodium fluoride. Blood sugar was estimated by the Hagedorn-Jensen method [1923]. For the lactic acid estimation about 2 c.c. of blood was measured, or preferably an equal amount weighed, laked with about 8 times its volume of water, and 40 p.c. trichloracetic acid added in quantity sufficient to bring its concentration up to 4 p.c. After filtering off the precipitated proteins, and removing sugar from the filtrate by the van Slyke copper-lime method, the lactic acid was estimated by the Friedemann-Cotonio-Shaffer modification of Clausen's method [1927]. The results were calculated on the basis of our observed recovery factor of 97 p.c.

Muscle which was to be sampled for analysis was dissected free without interfering with its blood supply, and two stout ligatures passed under it: these were then tied simultaneously and the intervening piece of muscle (usually 1 to 2 g.) cut out and at once dropped into, and held under, liquid air until it was frozen solid. The time taken in thus excising and freezing the muscle was only a matter of seconds. For lactic acid estimations, the frozen muscle (1 to 2 g.) was later weighed into a weighing bottle containing 5 to 10 c.c. of 4 p.c. trichloracetic acid and then rapidly transferred with the acid to a mortar in which it was ground up with the aid of sand (acid washed). Details of the further procedures were as for blood.

For glycogen estimations, the frozen muscle (0·5 to 2 g.) was weighed into a pyrex test tube containing 1 to 2 c.c. of 60 p.c. potash. The tube was plunged into a boiling water bath

and shaken at intervals until complete disintegration had been effected. After 3 hours, the tube and contents were cooled, neutralized with glacial acetic acid, and filtered. To an aliquot part of the filtrate absolute alcohol was added to make 60 p.c. and the precipitate centrifuged off, stirred with a little absolute alcohol and re-centrifuged. It was then dissolved in 5 c.c. of 2·2 p.c. HCl, heated in the boiling water bath for 4 hours, cooled, neutralized with anhydrous $Na_2CO_3$ and, after making up to a suitable volume, the reducing power of 2 c.c. was estimated by the Shaffer-Hartmann method and the results expressed as glucose. A correction was made for loss of glycogen in the precipitation fluids, in accordance with the principles worked out by Kerly [1930]. By the use of this technique the following results for the glycogen content of the corresponding muscles of the opposite limbs were obtained:

TABLE I. The glycogen content of similar muscles in the two hind limbs of the cat.

| | Muscle | Right leg (g. per (100 g.) | Left leg (g. per 100 g.) | Difference | Difference (p.c.) |
|---|---|---|---|---|---|
| *Exp.* 1 | Adductor 1 | 0·72 | 0·78 | +0·06 | +7½ |
| | „ 2 | 0·77 | 0·755 | −0·015 | −2 |
| | Sartorius | 0·59 | 0·635 | +0·045 | +7 |
| *Exp.* 2 | Sartorius | 0·812 | 0·726 | −0·086 | −11½ |
| | Gastrocnemius | 0·94 | 0·997 | +0·057 | +5½ |
| | Vastus internus | 1·14 | 1·085 | −0·055 | −5 |
| | | Sum = 4·972 | 4·978 | +0·006 | +0·12 |

Best, Hoet and Marks [1926] and Elias and Schubert [1918] in anæsthetized animals had found the glycogen content of pairs of muscles to vary but slightly (from +5 to −5 p.c.), but these results have not infrequently been seriously questioned by investigators who found individual pairs of muscles varying to a considerable extent in their glycogen contents, although the mean values for each leg were approximately equal. Our own results on this point are entirely in accord with those of the earlier workers. We have frequently observed that any delay or increased damage occurring during the removal of the muscle does lead to a definite difference between the glycogen contents of the two muscles, and can only suggest that the liquid air technique which we habitually use is definitely safer for this type of work than mere removal of the muscle without instantaneous death.

## RESULTS.

### (1) *The lactic acid of the blood of superficial veins.*

We give below the results of three experiments in which blood samples were collected simultaneously from the carotoid artery and from a superficial vein (the median cubital), before and after the execution of the muscular contractions. In Exp. 3 these contractions involved only the hind limbs, so that the superficial blood was drawn from an unstimulated fore limb. In the other two experiments the attempt was made to cause contraction of the muscles of the whole body, and it is certain that the majority did participate in the contraction. Stimulation was made in the form of 10 sec. tetani with 5 sec. pauses in each case.

TABLE II. Comparison of arterial with superficial venous blood.

*Exp.* 3. Stimulation of hind limbs only.

| Time in minutes after end of stimulation | Lactic acid of arterial plasma (mg. per 100 g.) | Lactic acid of superficial forearm venous plasma (mg. per 100 g.) |
|---|---|---|
| 0 (Rest) | 23 | 15 |
| 13 | 82 | 64 |
| 19·5 | 58 | 55 |
| 30 | 34 | 34 |
| | Mean 49 | Mean 42 |

*Exp.* 4. Stimulation of whole body.

| | | Lactic acid of superficial leg venous plasma |
|---|---|---|
| 0 (Rest) | 29 | 27 |
| 2·5 | 151 | 152 |
| 14 | 120 | 101 |
| 30 | 49 | 59 |
| 90 | 17 | 23 |
| | Mean 73 | Mean 72 |

*Exp.* 5. Stimulation of whole body.

| | | |
|---|---|---|
| 0 (Rest) | 42 | 40 |
| 10·5 | 156 | 169 |
| 26 | 89 | 93 |
| 42 | 66 | 67 |
| 132 | 39 | 45 |
| | Mean 78 | Mean 83 |

These results show that previous to the exercise the lactic acid content of the blood plasma from the superficial vein was slightly lower than that of the arterial blood plasma. The mean results suggest that there was also very little difference between the arterial and venous blood after the exercise, though the individual determinations showed considerable variations. It will be seen that, in the first experiment, the lactic acid content of the superficial venous blood of an unexercised limb 13 minutes after the end of the exercise was considerably lower than that in the general arterial blood. This deficit became reduced in the subsequent periods. This we may interpret, as a similar finding in man was interpreted by Barr and Himwich [1923], as being due to an absorption of lactic acid by the superficial tissues of the rested limb. Janssen and Jost [1925] found a very similar retention of lactic acid by resting muscle immediately after injection of lactates into the blood stream. We have not enquired further into this difference, and therefore could not express an opinion as to whether the lactic acid which

disappeared in this way did so as a result of mere physical processes or was disposed of chemically.

In the next two experiments, in which the whole body was stimulated, it will be seen that, in several of the periods, the lactic acid in the superficial vein blood was definitely higher than that in the general arterial circulation. A similar relationship was observed by Barr and Himwich [1923] in man when the venous blood was drawn from an exercised limb. This might be due to several causes, acting separately or conjointly. In the first place, the superficial vein might be draining some muscles (or other tissues) which had been thrown into activity by the stimulation. Secondly, it was observed in the collection of these samples that the vein was very collapsed and the blood flow slow; we interpreted this at the time as a confirmation of the belief that the vein was draining only inactive tissues, and explained its slow filling as being due to diversion of the blood stream to the actively contracting, or recovering, muscles. If such were the case, however, it is conceivable that the superficial tissues were being inadequately supplied with blood, and that the excess of lactic acid which was present owed its origin to the existence of local asphyxial conditions. A third possibility is that lactic acid had been absorbed by the superficial tissues during the contraction or early period of recovery, and was then being slowly given back again to the blood stream in the later stages. This explanation was the one given by Janssen and Jost in their experiments, in which the lactic acid content of the venous blood from muscles, following the injection of lactates, was at first lower and afterwards higher than that in the arterial blood. A fourth possibility is that the venous blood which flowed so slowly from the superficial vein corresponded, not with arterial blood collected contemporaneously, but with that of higher lactate content which had left the arterial stream some time before collection of the sample. It has been our invariable practice to collect samples of arterial blood at about the mid-point during the time of the collection of the venous samples; we cannot suppose that the discrepancy due to the slow flow of blood along the veins would be great enough to introduce differences of the magnitude here seen, and must therefore suppose that the fluctuations observed are to be regarded as the algebraic sum of the other three factors mentioned.

Our conclusion on this point is that, while the average composition of superficial venous blood does run closely parallel with that of the average arterial blood, yet individual samples show variations, the direction or magnitude of which cannot be foreseen or readily explained.

Although it is not certain that the relations between the lactic acid content of arterial and venous blood in human subjects after exercise would be subject to similar arbitrary though small variations, these direct experiments certainly suggest that if, as is probable, such variations do occur, they would be very difficult to allow for, and the work of Barr and Himwich on man has given similar results to our own.

(2) *Lactic acid distribution between blood and tissues.*

The next question that arises is that concerning the partition of lactic acid between the blood and tissues during rest and activity. In the work on man it has generally been tacitly assumed that there is an equilibrium between blood and tissues which amounts to an equality in the lactic acid content of the plasma and tissues, and it may be conceded that, on *a priori* grounds, such an equilibrium is reasonable and likely. Before proceeding to discuss the matter, however, it is perhaps well to consider what is the state of affairs in a still simpler case, namely, that of the relative concentrations of lactic acid in plasma and corpuscles.

It was shown by Hill, Long, and Lupton [1924] that the lactic acid concentration of the plasma, both before and after the addition to the blood of lactic acid in known amounts, was not equal to that in the whole blood, but was greater; for they found that the lactic acid concentration of plasma to whole blood was 1·28 : 1. We have repeated these observations and found the same figure of 1·28 : 1 for lactic acid concentrations of plasma : whole blood. This would mean that, if the corpuscular volume was 50 p.c. of the whole blood, the lactic acid concentration of plasma to corpuscles would be 1·78 : 1.

We do not propose to speculate as to the cause of this distribution, other than to state that in our opinion it has nothing to do with the Donnan equilibrium ratio, since in the first place the distribution is quantitatively different from that which the Donnan equilibrium would require, and in the second place a few orientating experiments have shown us that the ratio remains the same when the $CO_2$ pressure of the blood is varied widely.

It was thought that perhaps the distribution might be related to the different amounts of free water present in the two phases. Calculations based on the usually accepted figures for the water content of corpuscles and plasma (68 and 90 p.c. respectively) would, however, lead us to a ratio of only 1·32 : 1 for plasma to corpuscles, instead of the actual ratio of 1·78 : 1.

A. V. Hill [1930] found that, when added to blood (and equally when added to corpuscles), lactic acid produced an abnormally *small* lowering of vapour pressure, as though it had been removed, *e.g.* by the corpuscles. His experiments, therefore, throw no light on the cause of the observed distribution of lactic acid between plasma and corpuscles.

If the distribution of lactic acid between plasma and corpuscles presents these unexplained features, it would seem unjustifiable to regard the relation between the lactic acid concentrations of plasma, on the one hand, and of tissues lying outside the capillaries on the other hand, as presenting a case which could be regarded as theoretically more simple, and we could not be surprised if a condition of equality corresponding to a simple diffusion equilibrium were not reached. Even in the apparently simple case of the equilibrium between blood and cerebro-spinal fluid, it has been found by several investigators [Nishimura, 1925; Glaser, 1926; Wittgenstein and Gadertz, 1927] that the lactic acid content of cerebro-spinal fluid is definitely below that of the plasma, and Barnett and McKenney [1926] find that it is similar with transudates.

(i) *Rest.* The following results were obtained for resting values of blood, muscle, and other tissues:

TABLE III. Resting tissues.

| Lactic acid (mg. per 100 g.) | | Arterial blood plasma lactic acid (mg. per 100 g.) | | Difference | |
|---|---|---|---|---|---|
| Muscle ... ... | 13 | | 20 | − 7 | |
| | 24 | | 20 | + 4 | |
| | 31 | | 29 | + 2 | |
| | 54 | | 27 | +27 | |
| | 68 | | 27 | +41 | |
| | 40 | | 23 | +17 | |
| | 37 | Average 37 | 25 | Average 28 | +12 Average +9 |
| | 42 | | 35 | + 7 | |
| | 21 | | 35 | −14 | |
| | 25 | | 30 | − 5 | |
| | 40 | | 30 | +10 | |
| | 43 | | 32 | +11 | |
| Uterus ... ... | 31 | Average 29 | 32 | Average 30·5 | − 1·5 |
| | 27 | | 29 | | |
| Thyroid ... ... | 21 | | 32 | | −11·0 |
| Submax. gland ... | 32 | Average 25 | 32 | Average 30·5 | − 5·5 |
| | 18 | | 29 | | |
| Conn. tissue (largely fat) | 11 | | 29 | | −18·0 |

These results for muscles are on the whole higher than those obtained by Davenport and Davenport [1928], who froze the resting muscles *in situ*, and obtained average values of 20 mg. p.c. They give only one result for blood lactate (8 mg. p.c., *i.e.* 10·5 mg. p.c. for plasma) and this corresponded with 11 mg. and 19 mg. p.c. in two muscle samples (average 15 mg. p.c.). The difference between resting muscle and plasma according to their results would therefore be +4·5 mg. p.c. Our own results show a difference of +9 mg. p.c., though for fatigued muscles there is reason to suppose that it would be considerably less than this.

It would be hazardous to suggest any actual figure as representing the real lactic acid content of the resting muscle, and for the following reasons. First, we may suppose that in muscle, more than in other tissues, there is a continual production of lactic acid, and also a continual

restitutive removal. There is also, no doubt, a balance of lactic acid diffusion into or away from the muscle. The lactic acid concentration in the muscle at any one moment will, therefore, be the net balance of these opposed processes, the tendency being for the lactic acid concentration in the muscle to rise during oxygen lack, whether local or general; during muscular activity, whether local or general; or during and consequent upon the infliction of damage, particularly local damage. Further, any method of killing the muscle cannot but result in an accelerated rate of lactic acid formation, though even only for a very short time. Though we are unable to advance any direct proofs in support of such a suggestion, collateral evidence leads us to suspect that it is by no means improbable that the normal lactic acid concentration in resting, well-oxygenated muscle may be slightly below that in the blood plasma. This, at all events, is always the case for the other tissues which we have examined, though the difference between the lactic acid concentration of tissue and plasma is not so great as between red corpuscle and plasma, perhaps in part because the differences between their water contents are less.

(ii) *Recovery.* Whatever the relative concentrations may be during rest, it is certain that, during strenuous muscular contraction, the lactic acid content of the muscle rises at first to a level high above that in the blood plasma. The lactic acid, once it is formed, may be disposed of either by diffusion into the blood stream or by oxidative restitution. If only the former happened we should anticipate an equilibrium at a high level between muscle and blood, if only the latter the discrepancy between tissue and blood would be followed by a fall of the lactic acid in muscle without an accompanying rise in the lactic acid of the blood, which we know is not what usually happens. We should expect from theoretical considerations that both processes would happen, and that at first the lactic acid content of the blood would rise, while that of the muscle fell, but that later both would continue to fall if it were assumed that the lactic acid of the blood could be removed in any way. Examination of the lactic acid content of the arterial blood at short intervals during recovery, as in Fig. 1, indicates that the lactic acid rises very rapidly, and that the maximum concentration in the blood is usually reached within a very few minutes, this being followed by a steady fall. The question before us is what relation the lactic acid content of the muscle bears to that of the blood plasma during this rise and subsidence.

Table IV shows typical lactic acid concentrations of muscle and of arterial blood plasma during the first 12 minutes of recovery, and the

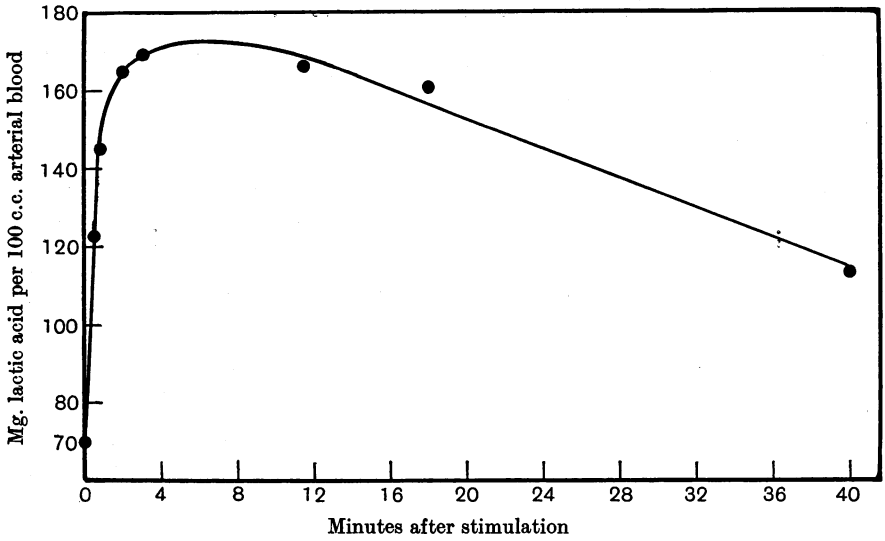


Fig. 1. Lactic acid concentration in arterial blood following stimulation of muscles. Dog 12 kg. Stimulation of whole body with three 10 sec. tetani with 5 sec. intervals. First blood sample 30 sec. after last stimulation. Others at 45 sec., 2, 3, 11·5, 18 and 40 min.

TABLE IV. Lactic acid concentration in muscle and arterial blood during first 12 minutes of recovery.

| Time of sample (minutes after end of stimulus) | Lactic acid content of muscle (mg. per 100 g.) | Time of sample (minutes after end of stimulus) | Lactic acid content of arterial blood plasma (mg. per 100 g.) | Difference (mg. p.c.) |
|---|---|---|---|---|
| 7 | 460 | 5 | 260 | +200 |
| 7 | 126 | 9 | 104 | + 22 |
| 2 | 313 | 4 | 237 | + 76 |
| 11 | 335 | 12 | 220 | +115 |
| | | | Mean | +103 |

TABLE V. Lactic acid concentration in muscle and arterial blood during 12–140 minutes of recovery.

| Time of sample (minutes after end of stimulus) | Lactic acid content of muscle (mg. per 100 g.) | Time of sample (minutes after end of stimulus) | Lactic acid content of arterial blood plasma (mg. per 100 g.) | Difference (mg. p.c.) |
|---|---|---|---|---|
| 12·5 | 98 | 12·5 | 143 | -45 |
| 18 | 87 | 18 | 105 | -18 |
| 14 | 133 | 17 | 160 | -27 |
| 30 | 156 | 30 | 175 | -19 |
| 71 | 57 | 72 | 67 | -10 |
| 124 | 90 | 124 | 78 | +12 |
| | | | Mean | -11·5 |

next table (Table V) the concentrations from 12 minutes onwards. (The results are quoted from experiments on different animals.)

Although it is probable that the production of lactic acid during the killing of the recovering muscle would be less than in a resting muscle, yet it is bound to occur to some extent, so that our experimental values for the lactate content of the recovering muscles will necessarily err on the high side.

If there were free diffusion between the muscle and the blood plasma it might have been expected from Fig. 1 that the lactic acid contents of plasma and muscle would have been equal rather earlier than Tables IV and V show to have been the case. It should be borne in mind that these figures are not all taken from the same animal, and are, therefore, not directly comparable one with another. The fact which emerges from these results, however, is that the lactic acid content of the muscle during recovery falls at a greater rate than that of the blood plasma.

This might be due to the occurrence of lactic acid removal in the muscle at a rate greater than that at which lactic acid can diffuse in again, or to the arrival at an equilibrium with a partition between plasma and muscle similar to that between plasma and corpuscles.

Experiments to be described in the next section will supply reasons for believing that, although the arterial lactate soon becomes higher than the muscle lactate, the passage of lactic acid continues for a considerable time during recovery outwards from the recovering muscle, and not in the reverse direction.

### (3) *Lactic acid content of blood and of tissues other than recovering muscles.*

In order to obtain some information regarding the distribution of lactic acid in the body generally, we have determined the lactic acid

TABLE VI.

| | Time after stimulation of muscles (min.) | Tissue | Lactic acid content of tissue (mg. per 100 g.) | Lactic acid content of arterial blood plasma (mg. per 100 g.) |
|---|---|---|---|---|
| *Exp.* 6. | 14 | Submaxillary gland | 77 | 138 |
| | 15 | Thyroid | 61 | 133 |
| | 16·5 | Uterus | 98 | 127 |
| *Exp.* 7. | 20·5 | Submaxillary gland | 53 | 98 |
| | 21·5 | Uterus | 86 | 91 |
| | 22 | Connective tissue | 21 | 89 |

content of various other tissues of the dog during recovery from general muscular contraction. The results are given in Table VI.

Similarly we have determined the lactic acid content of unstimulated muscles, *e.g.* those of the fore limbs, when only the hind limbs and lower part of the trunk had been stimulated. The results are given below (Table VII).

TABLE VII.

| | Time after stimulation (min.) | Lactic acid content of unstimulated muscle (mg. per 100 g.) | Lactic acid content of arterial blood plasma (mg. per 100 g.) |
|---|---|---|---|
| *Exp.* 8. | 8·5 | 56 | 91 |
| *Exp.* 9. | 15·5 | 15 | 73 |
| | 25·5 | 32 | 44 |

These results give support to the view that the equilibrium between the blood and the resting tissues is not one of equality, but one in which the lactic content of the tissues is often lower than that of the blood plasma.

This being so, it is of interest to compare the lactate content of the arterial blood during recovery with that from recovering and with that

TABLE VIII.

| | | | Lactic acid content of | | |
|---|---|---|---|---|---|
| *Exp.* | Remarks on stimulation | Time after end of stimulation (min.) | Venous blood plasma from stimulated limb (mg. per 100 g.) | Arterial blood plasma (mg. per 100 g.) | Venous blood plasma from resting limb (mg. per 100 g.) |
| 10 | 7 kg. dog. 1½ min. stimulation of both hind limbs by a series of six 10 sec. tetani with 5 sec. rests | 0 | 26 | 31 | 24 |
| | | 4–5½ | 110 | 95 | 56 |
| | | 36 | 48 | 41 | 39 |
| | | 76 | 26 | — | 25 |
| 11 | 12¾ kg. dog. 3 min. stimulation of both hind limbs by a series of twelve 10 sec. tetani with 5 sec. rests | 3–4 | 116 | 105 | 79 |
| | | 20–21 | 106 | 100 | 89 |
| | | 48–49 | 56 | 56 | 49 |
| | | 96–97 | 25 | 20 | 17 |
| 12 | 7 kg. dog. 1½ min. stimulation of both hind limbs by a series of six 10 sec. tetani with 5 sec. rests | 2½–4 | 151 | 106 | 79 |
| | | 13½–15 | 96 | 83 | 72 |
| | | 180 | 27 | 30 | 28 |
| 13 | 6·5 kg. dog. 7 min. stimulation of both fore limbs by a series of twenty-eight 10 sec. tetani with 5 sec. rests | 0 | — | 21 | — |
| | | 12 | 49 | 39 | 35 |
| | | 19 | 37 | 27 | 28 |
| | | 28 | 31 | 26 | 26 |
| | | 40 | 26 | 24 | 21 |
| 14 | 7 kg. dog. 5 min. intermittent tetani (70 per min. to fore limbs) | 0 | — | 20 | — |
| | | 10½ | 39 | 37 | 31 |
| | | 27½ | 25 | 25 | 24 |

from resting unstimulated muscle. The results of such experiments are given in Table VIII.

These experiments indicate that while the fatigued muscle is for some time giving up lactic acid during recovery, so that the venous blood leaving it contains more lactic acid than the arterial blood, the resting limb, particularly in the earlier stages of recovery, is taking up lactic acid from the blood, so that the venous blood coming from it contains considerably less lactic acid than the arterial blood. These results are very similar to those obtained in man by Barr and Himwich [1923].

A possibility which should be considered is that when the lactate content of the venous blood is higher than in the arterial blood this may be due to the transfer of water from the blood to the muscle. Whilst it must be acknowledged that the muscles do remove water from the circulating blood during exercise, so that the blood may be somewhat concentrated, yet even though a similar state of affairs exists in some of our experiments it does not seem likely that an appreciable degree of concentration would occur during the passage of blood once through a tissue, nor that movement of water into the muscle would continue throughout recovery.

(4) *The disappearance of lactic acid from the blood.*

(i) We have seen that the lactic acid content of the arterial blood rises rapidly during and immediately after severe muscular contraction, reaches its maximum shortly after its termination, and then sinks steadily during the next hour or two to reach the normal again, and we must now enquire into the causes of this subsidence in the lactic acid content. First, we must consider whether, at any time, there is any equality of lactic acid concentration over the body. So long as the contracting muscles form only a small fraction of the soft tissues of the body, it would be expected that, owing to the removal of lactic acid by resting muscle and other tissues, the average venous blood of the right heart, and hence the arterial blood, would always contain less lactic acid than that returning from the recently contracted muscles. This difference would be expected to become smaller as the mass of exercised muscle increased, but since there would always be a considerable mass of tissue which was not adding, but probably removing, lactic acid, we could expect that there would always be some difference.

Actually, when we cause widespread contraction, and compare the venous blood from different limbs with the arterial blood, we do find smaller differences, but apparently a state of equality is not reached.

If it were the case, indeed, that the major part of the venous return to the heart had a considerably higher lactate content than that of the arterial blood, we should be at a loss to explain the lower content of lactic acid in the arterial blood, and it would be necessary to examine the possibility that oxidation of lactic acid might be occurring in the lungs. The view that the execution or completion of oxidations might occur in the lungs, though revived by Bohr and Henriques in 1897, is now considered to have been definitely disproved, but we have nevertheless examined this point and found that such a hypothesis is definitely inadmissible, so far as lactic acid is concerned. In the first place, we have shown in the preceding communication that the isolated perfused lungs are unable to remove lactate from the blood under conditions in which the heart or muscle could do so. Further, direct determinations of the lactic acid content of the blood of the right and left hearts after

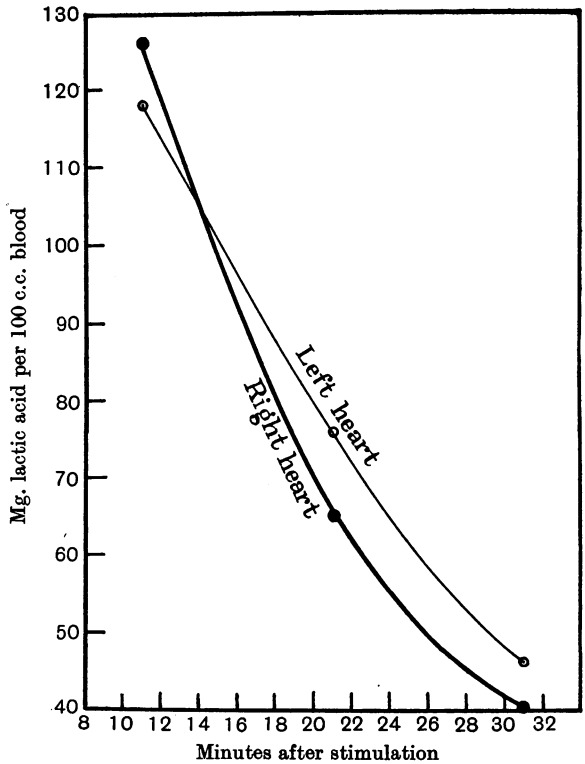


Fig. 2. Lactic acid contents of blood samples taken simultaneously from right and left hearts after exercise. Dog. 9 kg. Stimulation of whole body for 7 min. by series of short tetani 2 to 3 sec. with 2 to 3 sec. intervals.

stimulation of the general musculature showed that, at first, the lactic acid content of the arterial blood was actually slightly below that of the venous blood; we attribute this to temporary absorption of lactic acid by the lungs, acting as an inert tissue. This conclusion is supported by the fact that at a later stage the curves cross for a time, as this absorbed lactic acid is given out again into the arterial blood (Fig. 2).

(ii) *Removal of lactate by the liver.* In view of the generally admitted statements that lactic acid is excreted in large amount after hepatectomy or in phosphorus poisoning, and that the excised liver can remove lactic acid from perfusion blood, it seems natural to regard the liver as probably responsible for removing a good deal of the lactic acid from the blood during recovery, and this opinion has recently been definitely expressed by Himwich, Koskoff and Nahum [1930]. We therefore carried out experiments to examine this possibility, by collecting for comparison samples of general arterial blood and of hepatic vein blood.

The collection of hepatic vein blood was made as follows: A number 0 gum-elastic catheter provided with a stilette slightly curved at the end, both of them coated with liquid paraffin, was introduced into the external jugular vein, and was passed down, with suitable manipulations, until the end had entered the inferior vena cava just below the diaphragm. A small incision was previously made in the upper abdominal wall for the purpose of verifying the location of the curved extremity of the catheter. In the dog, the most orally placed hepatic vein on the left side of the vena cava enters at an oblique angle and its ventral aspect is easily discernible. By palpation from below, together with suitable manipulation of the catheter stilette from above, the catheter was directed into this large branch of the hepatic vein, which it easily entered, when the sample was at once drawn by a syringe. As an additional safeguard, in some experiments, a loop of thread was placed round the inferior vena cava just below the liver, and pulled during collection of the hepatic sample. This did not appear to give any different results, and was discontinued. The chest need not be opened in order to facilitate the passage of the catheter down the vena cava. We subsequently found that an almost identical method was used by Claude Bernard [1879].

The results of the first few experiments were equivocal, but when the extent of stimulation was altered so that the lower part of the trunk and hind limbs alone were stimulated, the results were at once, and in every case, clear cut.

The experiments recorded in Table IX show unmistakably that, in passing through the liver (and intestines), lactic acid is removed from the blood throughout the course of recovery. We would lay more stress on the lactic acid disappearance during later phases of the recovery than on the differences, even if greater, during the earlier minutes. Samples of venous blood returning from any inert tissue show a lower lactic acid content than does arterial blood during the first 10 minutes of recovery

TABLE IX. Lactic acid and blood sugar content of blood from hepatic vein and of arterial blood during recovery from muscular fatigue in dogs. Stimulation of hindquarters alone.

| *Exp.* | Wt. of dog (kg.) | Treatment | Stimulation series of tetani (min.) | Time after end of stimulus (min.) | Lactic acid content of: Arterial blood plasma (mg. per 100 g.) | Lactic acid content of: Blood plasma from hepatic vein (mg. per 100 g.) | Difference | Blood sugar content of: Arterial (mg. per 100 g.) | Blood sugar content of: Hepatic (mg. per 100 g.) | Difference |
|---|---|---|---|---|---|---|---|---|---|---|
| 15 | 8·5 | Amytal | 4 | 10 | 135 | 123 | +12 | 74 | 86 | −12 |
| | | | | 20 | 105 | 102 | + 3 | 79 | 77 | + 2 |
| | | | | 31 | 97 | 67 | +30 | 70 | 79 | − 9 |
| | | | | 46 | 46 | 36 | +10 | 83 | 83 | 0 |
| | | | | 62 | 31 | 23 | + 8 | 83 | 93 | −10 |
| 16 | 12·25 | Decerebrate | 4 | 13 | 169 | 151 | +18 | 147 | 167 | −20 |
| | | | | 20 | 141 | 120 | +21 | 149 | 161 | −11 |
| | | | | 33 | 116 | 97 | +19 | 145 | 154 | − 9 |
| | | | | 49·5 | 84 | 77 | + 7 | 154 | 158 | − 4 |
| | | | | 63·5 | 66 | 63 | + 3 | 156 | 165 | − 9 |
| 17 | 8·5 | Decerebrate | 7 | 11·5 | 170 | 156 | +14 | 170 | 173 | − 3 |
| | | | | 22 | 141 | 121 | +20 | 170 | 190 | −20 |
| | | | | 31·5 | 119 | 109 | +10 | 186 | 179 | + 7 |
| | | | | 44 | 91 | 74 | +17 | 192 | 200 | − 8 |

following stimulation of the musculature, as we have shown above, so that the results obtained at a 5-minute interval after the stimulation would be less significant. The results of Himwich *et al.* suffer from this drawback, most of their samples being drawn within a few minutes of the exercise.

Since no samples of portal blood were obtained, these experiments in themselves do not definitely prove that the liver alone was causing this removal of lactic acid, though there are other reasons which indicate that this was so: nor could any opinion be offered as to the fate of the lactic acid which is removed, and we have not attempted to attack this aspect of the matter. From the recent investigations of Cori and Cori [1929] it would appear that some of it is probably converted into glycogen: there is no indication whether or not some of it is oxidized.

In two of the samples recorded in Table IX (one in Exp. 15 and one in Exp. 17) the blood sugar of the hepatic vein is less than that of the arterial blood, which may perhaps be explained by assuming that the catheter in these cases was not completely in the hepatic vein: this would result in a mixture of hepatic vein and vena cava blood being withdrawn. The difference in lactic acid content of the two bloods is correspondingly less marked than that of other samples in the same experiment, so that in attempting to get an average figure for the amount

of lactic acid removed by the liver, these samples may be ignored. The average obtained from the remaining figures is roughly 15 mg. per 100 g. of plasma, or 12 mg. per 100 g. of blood, that is, from every 100 g. of blood passing through the intestines and liver 12 mg. of lactic acid are removed. This is a definitely larger difference than we have obtained between the arterial blood and the venous blood from resting muscles, and it would therefore seem probable that a not inconsiderable fraction of the lactate disappearing from the blood does so by removal by the liver.

We can obtain from our experiments an average figure for the rate of disappearance of lactic acid from the arterial blood after muscular contraction. These results are recorded in Table X, and show an average fall of 2·1 mg. p.c. per minute. (Actually, the rate of subsidence of lactic acid in the arterial blood is approximately linear during the early part of recovery, and the values taken for Table X all fall on this part of their curves.)

TABLE X. Rate of recovery.

| | | | | Lactic acid content of arterial blood | | | | |
|---|---|---|---|---|---|---|---|---|
| *Exp.* | Animal | Wt. (kg.) | Treatment | Initially (mg.p.c.) | Finally (mg. p.c.) | Fall (mg. p.c.) | Time (min.) | Fall per min. (mg. p.c.) |
| 18 | Dog | 8·5 | Decerebrate | 133 | 71 | 62 | 32 | 1·9 |
| 19 | „ | 8·2 | „ | 108 | 54 | 54 | 27 | 2·0 |
| 20 | „ | 12·2 | „ | 131 | 66 | 65 | 37 | 1·8 |
| 21 | „ | 9·0 | Amytal | 64 | 26 | 38 | 17·5 | 2·2 |
| 22 | „ | 9·0 | „ | 118 | 46 | 72 | 20 | 3·6 |
| 23 | „ | 6·5 | „ | 119 | 38 | 81 | 28 | 2·9 |
| 24 | „ | 9·7 | „ | 123 | 52 | 71 | 31·5 | 2·2 |
| 25 | „ | 7·5 | „ | 115 | 35 | 80 | 30 | 2·6 |
| | | | Injected Na lactate | 309 | 118 | 191 | 65 | 2·9 |
| 26 | „ | 11·0 | Amytal | 224 | 158 | 66 | 32 | 2·0 |
| 27 | „ | 12·7 | „ | 79 | 44 | 35 | 28 | 1·3 |
| 28 | „ | 8·5 | „ | 105 | 36 | 69 | 35·5 | 1·9 |
| 29 | Cat | 2·5 | „ | 180 | 65 | 115 | 56 | 2·0 |
| 30 | „ | 2·7 | „ | 123 | 52 | 71 | 56 | 1·3 |
| 31 | „ | 2·2 | „ | 155 | 61 | 60 | 91 | 1·5 |
| | | | | | | | Average | 2·1 |

Despite the lack of complete equilibrium between the lactic acid content of the tissues and blood under the conditions of these experiments, the disappearance of 2·1 mg. p.c. per minute in the blood probably represents a similar degree of disappearance in all the wet tissues of the body. Taking the average figure obtained by Burton-Opitz [1917] for the blood flow through the liver in the dog as 84 c.c. per 100 g. of liver per minute, and the average weight of the liver as 3·3 p.c. of the body weight, it would appear that the rate at which lactic acid dis-

appears from the body during recovery is about three to four times as great as it would be were the liver removing the whole of it. It is unlikely that this very large discrepancy could be due to errors in the average values taken at each stage: from these experiments alone, therefore, it can be concluded that the liver (with intestines) is certainly not the only organ in the body concerned with the removal of lactic acid after muscular contraction.

(iii) *Removal by other tissues.* Further evidence that the liver is not solely responsible for lactate removal is obtained by studying the rate of recovery after muscular contraction either in completely "eviscerated" animals, or in animals with the liver removed from circulation. Of the three experiments quoted below (Table XI) complete evisceration was performed in the cat, both portal vein and hepatic arteries being tied off. In the dogs the hepatic arteries were tied, and the portal vein anastomosed by means of Crile's tubes and a length of external jugular vein to the right renal vein, as described by Dale and Laidlaw [1919]; so that in both cases the only connection of the liver with the circulation was by the hepatic veins. Though there may be a slight ebb and flow of blood in and out of the hepatic veins during respiration or exercise, and perhaps even a very restricted flow owing to the existence of trivial anastomotic connections, it seems inconceivable that the liver so situated should be able to remove lactic acid from the circulating blood, though it might contribute a certain amount of reducing sugar or even lactic acid to it.

TABLE XI.

| *Exp.* | Animal | Wt. (kg.) | | Lactic acid content of arterial blood: Initially (mg. per 100 g.) | Finally (mg. per 100 g.) | Fall (mg. per 100 g.) | Time (min.) | Fall per min. (mg. p.c.) |
|---|---|---|---|---|---|---|---|---|
| | | | Complete evisceration. | | | | | |
| 32 | Cat | 2·7 | (Amytal) | 116 | 74 | 42 | 72 | 0·6 |
| | | | Portal short circuit. | | | | | |
| 33 | Dog | 12·5 | (Decerebrate) | 126 | 98 | 28 | 40 | 0·7 |
| 34 | Dog | 8·5 | (Amytal) | 91 | 66 | 25 | 30 | 0·8 |

The average fall of lactic acid in the general blood stream under these conditions was 0·7 mg. p.c. per minute, or roughly one-third the rate obtained in the intact animal, and seems to be unaffected by presence or absence of the intestines. No particular importance can of course be

attached to this particular figure, since the liver may have even been adding small amounts of lactic acid as a result of its anaerobic state.

It is obvious from these experiments that lactic acid can be removed from the circulating blood without the intervention of the liver. This has, however, been denied by workers recently (principally Himwich, *et al.*), who claim that lactic acid gradually accumulates in the blood of an eviscerated animal at rest. Such has not been our usual experience, as is seen from Table XII, which gives some typical results we have obtained.

TABLE XII.

| *Exp.* | Time after evisceration | Lactic acid content of arterial blood (mg. p.c.) | Remarks |
|---|---|---|---|
| 35 | 0 | 16 | Cirrhosis of liver found at autopsy |
| | 1 hr. | 11 | |
| | 2 ,, | 10 | |
| | 3 ,, | 7½ | |
| 36 | 1½ ,, | 41 | |
| 37 | 40 min. | 24 | |
| 38 | 4½ hr. | 47 | Sciatics stimulated 1 hr. after evisceration<br>Lactic acid just afterwards = 62 mg. p.c. |

It is difficult to explain precisely why other workers, carrying out apparently the same experiment, should have found the lactic acid of the blood invariably to rise. In the rare instances where this has happened in our own experiments, we have explained the occurrence as being due to the intervention of abnormal conditions. For example, a grossly improper degree of ventilation, in either direction, or a failing circulation, could cause this result. In any case, the fact that the blood lactate can, and, in our experience, usually does, remain low or even fall, after evisceration, is here of more importance than the fact that it can, in certain circumstances, show a rise.

(iv) *Removal by the muscles.* An obvious possibility, which was actually the starting point of our investigation, is that of restitution of lactic acid to glycogen in the muscles, and we therefore compared the rate of glycogen formation both in intact and in eviscerated animals (Table XIII). In these experiments the glycogen contents are always compared with those of the same muscle on the opposite side of the body.

The results show a gain in muscle glycogen in most cases and, though this gain is more clearly seen with intact than with eviscerated animals, its occurrence in the latter seems to be beyond doubt. The clearest rises were seen in cases where the initial glycogen was very low. As regards the source of this added glycogen, we have little evidence to offer. It

TABLE XIII. Glycogen re-synthesis in muscles of cats during recovery from muscular exercise.

A. Intact animals. Glycogen (g. p.c.).

| | | Time after end of exercise (hr.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Exp.* | | 0 | ½ | 1 | 2 | 3 | 4 | Increase (g. p.c.) | Increase (p.c.) |
| 39 | Amytal | Trace | — | — | — | — | 0·46 | +0·4 | Over 400 |
| 40 | ,, | 0·17 | 0·18 | — | — | — | — | +0·01 | 6 |
| | ,, | 0·20 | — | 0·29 | — | — | — | +0·09 | 45 |
| | ,, | 0·25 | — | — | 0·41 | — | — | +0·16 | 64 |
| 41 | ,, | 0·22 | — | 0·29 | — | — | — | +0·07 | 32 |
| | ,, | 0·23 | — | — | 0·27 | — | — | +0·04 | 17 |
| | ,, | 0·17 | — | — | — | 0·29 | — | +0·12 | 70 |
| 42 | ,, | 0·19 | — | 0·35 | — | — | — | +0·16 | 84 |
| | ,, | 0·39 | — | 0·44 | — | — | — | +0·05 | 13 |
| 43 | Decerebrate | 0·07 | — | — | 0·13 | — | — | +0·06 | 85 |
| | ,, | 0·212 | — | — | 0·21 | — | — | 0 | 0 |
| | ,, | 0·17 | — | — | 0·29 | — | — | +0·12 | 70 |

B. Eviscerated animals.

| | | Time after end of exercise (hr.) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Exp.* | | 0 | 1 | 2 | 3 | 3½ | 4 | Increase (g. p.c.) | Increase (p.c.) |
| 44 | Amytal | 0·11 | 0·15 | — | — | — | — | +0·04 | 36 |
| | ,, | — | 0·11 | 0·23 | — | — | — | +0·12 | 108 |
| | ,, | — | — | 0·3 | — | — | 0·3 | 0 | 0 |
| 45 | ,, | 0·27 | 0·40 | — | — | — | — | +0·13 | 48 |
| | ,, | 0·25 | — | 0·33 | — | — | — | +0·08 | 32 |
| | ,, | — | — | 0·55 | — | 0·56 | — | +0·01 | 2 |
| 46 | ,, | 0·13 | 0·23 | — | — | — | — | +0·10 | 77 |
| | ,, | 0·25 | — | 0·30 | — | — | — | +0·05 | 20 |
| | ,, | 0·47 | — | — | — | 0·48 | — | +0·01 | 2 |

might arise equally well from blood sugar, from blood lactate or from lactate which has never left the muscle.

The possibility of formation from blood sugar is reduced, though not completely eliminated in the eviscerated animals. Thus in one of the experiments quoted in Table XIII (Exp. 44) the arterial blood sugar was determined and the following results obtained:

TABLE XIV.

| Interval after evisceration (min.) | Arterial blood sugar (p.c.) |
|---|---|
| 40 | ·184 |
| Stimulation of both sciatic nerves at 47–90 min. | |
| 93 | ·112 |
| 165 | ·072 |
| 226 | ·061 |
| 342 | ·029 |

It is not impossible that an ebb and flow of blood in and out of the hepatic veins might have carried in a certain amount of sugar from the liver.

It seems uncertain, in view of these results, whether the lactic acid which is produced in exercise can actually be converted into glycogen in the muscles themselves, and it is possible that lactic acid once it has entered the blood stream is not convertible into demonstrable amounts of glycogen by the muscles, though our experiments indicate that some of it may be removed, since the venous blood leaving muscle contains in some circumstances less lactic acid than the arterial blood.

Various attempts have been made, by ourselves and by others, to find whether lactic acid administered to animals can be converted into muscle glycogen, but there seems no doubt that, apart from the possible intermediation of the liver [Cori and Cori, 1929], the administration of lactate, *e.g.* intra-arterially as was investigated by Elias and Schubert [1918], or intravenously, is not followed by deposition of muscular glycogen.

Our own experience is entirely in agreement with this. In experiments on cats, for instance, we have found no increase of muscle glycogen to follow the injection of large amounts of lactic acid into the blood stream. Nor, in our opinion, is the synthesis of glycogen from lactate, even in amphibian muscle, capable of quite such a clear-cut demonstration as is commonly supposed. We have tried to repeat some of Meyerhof, Lohmann and Meier's [1925] experiments by perfusing the hind limbs of frogs with 0·12 p.c. lactic acid solutions at *p*H 7·4 in Ringer or phosphate Ringer (8 mg. p.c. P) precisely as they recommended. The results given in the table on p. 290 (Table XV) indicate that only minimal quantities of glycogen made their appearance under these circumstances, while amounts of the same order were apparently produced by perfusion even with ordinary Ringer's solution.

We do not consider these results to be so unequivocal as to give very strong support to the belief that glycogen synthesis in the muscle can be effected from lactate perfused through the blood vessels. The process would, however, be expected to be slow in cold-blooded animals, so perhaps no great stress should be laid on these facts in relation to our own experiments on mammals.

(5) *Relation of experimental to normal conditions.*

It remains to be considered how far the results we have obtained under the conditions of these experiments can be transferred to the interpretation of problems of exercise in normal animals. The most significant differences lie in our method of inducing muscular contraction and in the procedures adopted to render the animal unconscious. As

TABLE XV. Glycogen content of the gastrocnemii of frogs.

| | Resting muscle (p.c.) | Muscle perfused with lactate-Ringer solution (p.c.) | Time | Increase (p.c.) |
|---|---|---|---|---|
| *Rana esculenta* | 0·635 | 0·635 | 2 hr. 40 min. | 0 |
| ,, | 0·92 | 1·05 | 2 hr. 40 min. | +14 |
| ,, | 0·895 | 0·905 | 2 hr. 45 min. | + 1 |
| ,, | 1·04 | 1·11 | 2 hr. 30 min. | + 7 |
| | | | Mean | +5½ |
| | Resting muscle (p.c.) | Muscle perfused with Ringer solution (p.c.) | | |
| *Rana esculenta* | 0·975 | 1·01 | 3 hr. | +3½ |
| ,, | 0·675 | 0·745 | 2 hr. 30 min. | +10 |
| | | | Mean | +6½ |
| | Fatigued muscle (mg.) | Fatigued muscle perfused with lactate-Ringer solution (mg.) | | |
| *Rana esculenta* (Hungarian) | 5·9* | 6·3 | 2 hr. | + 7 |
| ,, | 15·5* | 11·3 | 3 hr. | −26 |
| | | | Mean | −9 |
| | Muscle perfused with Ringer solution | Muscle perfused with lactate-Ringer solution | | |
| *Rana esculenta* | 0·86 p.c. | 1·1 p.c. | 2 hr. 45 min. | +27 |
| ,, | 1·16 p.c. | 1·15 p.c. | 3 hr. | 0 |
| (Hungarian) | 13·6 mg.* | 13·2 mg. | 2 hr. 30 min. | − 3 |
| ,, | 12·45 mg.* | 11·65 mg. | 3 hr. | − 7 |
| | | | Mean | + 4 |

* The weight of the muscles was unknown in these experiments, so that the figures given express the total glycogen content of the muscles concerned. In the other experiments, in order to eliminate the effects of œdema, we followed Meyerhof's procedure by assuming that the control muscle and the perfused muscle were of the same weight.

regards the first, provided that only the lower portion of the animal is stimulated, so that respiratory movements are not hindered, and that excessively prolonged tetani are avoided, we can see no reason to suppose that the results are essentially different from those of similar contractions produced voluntarily. The other difference is perhaps a more serious one, since Long [1928] found that amytal caused a disappearance of glycogen from muscles in the resting cat, and Best, Hoet and Marks mention

the same fact but give no figures. Further, Hines, Boyd and Leese [1926] observed that injection of glucose into a dog under amytal anæsthesia caused a greater hyperglycæmia and glycosuria than when an anæsthetic was absent. Hinsey and Davenport [1929], on the other hand, found no fall in glycogen during amytal anæsthesia in cats (2), guinea-pigs (2), or rats (2). Since we have obtained good recovery of glycogen in animals under amytal, it appears to us that this anæsthetic is satisfactory enough in this respect. That amytal has no evident inhibitory action on lactic acid removal, moreover, is indicated by the facts that (1) recovery from exercise (as evidenced by the rate of disappearance of lactic acid from the blood stream) is as rapid as, or more rapid than that occurring in the decerebrate animal (Table X), and (2) the uptake of lactic acid in the portal circuit during recovery is not impeded by amytal anæsthesia (Table IX). During amytal anæsthesia the blood sugar level and lactic acid content of the blood are those of a normal resting animal. Following decerebration in an animal both values are abnormally high, whether rigidity is present or not [*v.* also Hinsey and Davenport, 1929]. Nevertheless, the processes of recovery seem to occur at much the same rate in the decerebrate animal as in the animal under amytal. However, until both states (amytal anæsthesia and decerebration) have been more fully investigated, the question as to which approximates more nearly to the normal is in our opinion an open one. We see no reason to suppose, since both give the same results, that these could not be considered fairly to represent the course of events in the normal animal.

We have not up to the present considered the state of affairs in those instances in which with mild [Owles, 1930] or even, under exceptional circumstances, with severe exercise [Bock, van Caulaert, etc., 1928] the lactic acid of the arterial blood shows no appreciable rise. Since many instances have now been dealt with in the literature in which this state of affairs obtains, it seems that, under certain conditions, muscles do not add any lactic acid at all to the blood stream travelling through them, since if they did so, even allowing for rapid rates of removal of lactic acid by the liver, there could not fail to be a detectable increase in the lactic acid content of the arterial blood.

There are, it seems, only two possibilities by which these effects can be explained: either, under conditions of adequate oxygenation lactic acid is never formed in the muscles, or else the lactic acid is removed *in situ* as fast as it is formed. The existence of such examples shows clearly that an exit of lactic acid from the muscles is an accessory phenomenon not connected with the fundamental process of muscular contraction,

but rather associated with a rate of oxygen supply which is inadequate to enable removal to take place within the muscles.

That transfer of lactic acid to the blood stream does occur in many perfectly normal people when performing severe exercise is incontestible, and it is with individuals of this type that we are concerned in the present discussion. There is one difficulty in connection with the statement that in certain individuals muscular exercise is not associated with an increase in the arterial lactate, namely that it is surprising that in those individuals there should be under any circumstances any lactate at all in the circulating blood. Such lactate as there is would, according to these results, probably be produced from some source of lactate, other than the muscles, which is able to keep the blood lactate constant in spite of the various means by which lactate can continuously be removed from the blood.

## Summary.

1. While the average lactic acid content of superficial venous blood runs closely parallel to that of the arterial blood, yet individual samples show variations, the direction or magnitude of which cannot be foreseen or readily explained.

2. The lactic acid content of tissues in general is somewhat less than that of the blood plasma, both at rest and during recovery from exercise, this difference being less readily observable in muscle owing to production of lactic acid during the removal and killing of the tissue. Up to about 10 p.c. difference, this could be accounted for on the basis of the higher water content of the plasma.

3. The lactic acid content of muscles which have been given a short period of severe exercise does not come into equilibrium with that of the blood and other tissues until at least 10 minutes after the end of the exercise. In the equilibrium then reached the muscle lactate is lower than the arterial blood lactate.

4. The lactic acid content of the venous blood returning from muscles which have been contracting remains higher than that of the arterial blood throughout recovery, while that from unexercised muscles is lower.

5. The lungs play no part in the removal of lactic acid during recovery.

6. The blood returning from the liver contains less lactic acid (average 12 mg. per 100 c.c.) than the arterial blood, throughout recovery. Hence the liver removes lactic acid from the blood.

7. Under the conditions of experiment the average rate of dis-

appearance of lactic acid from the blood during recovery was about 2 mg. per 100 c.c. per minute. In the eviscerated animal this rate was only reduced to about one-third of that value; hence the liver is not solely responsible for lactic acid removal.

8. The lactic acid content of the blood of the resting eviscerated animal does not rise during the 4 hours following the evisceration.

9. Glycogen re-synthesis occurs to a considerable extent in muscles during recovery from exercise, using amytal as anæsthetic. The recovery is almost as great in the eviscerated as in the intact preparation. The source of the glycogen is uncertain.

10. Amytal anæsthesia as compared with decerebration has no depressing influence on the uptake of lactic acid by the liver during recovery from exercise, nor on the rate of disappearance of lactic acid from the blood stream in general. In the resting condition the blood sugar and lactic acid content of the blood are normal in the animal under amytal anæsthesia, but abnormally high in the decerebrate preparation.

11. Perfusion of the hind limbs of frogs with lactate led to no appreciable synthesis of glycogen by the muscles.

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