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LACTIC ACID FORMATION AND REMOVAL WITH CHANGE OF BLOOD REACTION.

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It is well known that the rate of lactic acid formation in surviving muscle is accelerated when the muscle is placed in fluids of alkaline reaction, and retarded by acids. It is also known that the lactic acid content of the blood is influenced, among other things, by the H-ion concentration of the blood itself. Thus Macleod and Knapp [1918] showed that after administration of alkali the blood lactic acid was increased. Anrep and Cannan [1923] demonstrated that the lactic acid content of the blood circulating in the heart-lung preparation could be caused to rise and fall with the *p*H of the circulating blood.

In the case of the blood alone, it is generally admitted that a lowering of H-ion concentration, as by the addition of alkali, or by the withdrawal of CO_2, leads to an increased rate of glycolysis with accelerated lactic acid formation [Lovatt Evans, 1922].

It would seem, therefore, from these facts that the production of lactic acid in the blood itself, and perhaps in the tissues generally, is accelerated by an increase in alkalinity, while the reverse change can also be effected by certain tissues when the alkalinity is reduced. So far as the blood itself is concerned, a reduction of alkalinity has apparently no other effect than to retard the rate of glycolysis, and cannot lead to a reversal of the process with disappearance of lactic acid already formed.

In the course of some experiments in which we were engaged, it became necessary to know whether these changes in the lactic acid content of the blood, consequent on its changes in H-ion concentration, could also be effected by other tissues, particularly by the muscles. That mammalian muscle can remove lactic acid from the blood was shown by Bornstein and Schmutzler [1929], though they did not take into consideration the factor with which we are now concerned.

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Methods.

Some of the experiments were carried out on heart-lung preparations, one on the isolated perfused lung, and the remainder on isolated hind legs, perfused with oxygenated defibrinated blood at 37° C. by the Dale-Schuster pump [1928]. All the experiments were performed on the tissues of dogs. The leg preparation was perfused from the abdominal aorta, at pressures of 80–120 mm. Hg, the venous blood draining out from the inferior vena cava into a funnel leading into the venous reservoir. The samples taken for analysis were drawn from the venous reservoir. Lactic acid was estimated by the Friedemann-Cotonio-Shaffer modification [1927] of the Clausen method [1922], and the blood sugar by Hagedorn and Jensen's method [1923]. The limb preparation was kept warm by an electric bowl radiator placed at a suitable distance, and the exposed tissues of the lumbar region and pelvis were kept from drying by being covered with oiled silk. Aeration of the circulating blood was performed by passage of the venous blood by means of one of the Dale-Schuster pumps through an isolated dog's lung ventilated with a Starling "Ideal" respiration pump. The lungs were connected with the pump by way of a Palmer-Brodie air heater and a warm water vessel for moistening the air. The pump at "full" stroke delivered about 225 c.c. of air into the lungs per stroke and about 120 c.c. at "half" stroke. The reaction of the blood was altered by varying the extent of the pulmonary ventilation with air, by ventilation with mixtures of CO_2 and air or O_2 in varying proportions, or by addition of suitable amounts of dilute hydrochloric acid or sodium bicarbonate to the circulating blood.

As a check on these procedures the plasma bicarbonate was estimated in some of the experiments by van Slyke's method: in others the H-ion concentration of the blood was obtained by the dialysis method of Dale and Evans.

Results.

So far as the experiments with the heart-lung preparation are concerned, it is only necessary here to state that we were able fully to confirm the results previously obtained by Anrep and Cannan.

The experiment with isolated lungs further showed clearly that the lungs alone, even in the presence of a considerable concentration of CO_2, were not competent to reverse, or even completely to check, the accumulation of lactic acid, presumably due to glycolysis in the blood. In this experiment the initial lactic acid concentration in the blood was 78·5 mg. per 100 c.c., and after 40 min. ventilation of the lungs with air this had risen to 113 mg. per 100 c.c. The air was then replaced by O_2 containing 10 p.c. CO_2, and a further increase to 134 mg. per 100 c.c. was observed after a further 70 minutes of perfusion.

Perfusions of hind-limb-lung preparations, however, showed that these behaved in very much the same way as the heart-lung preparation, the lactic acid rising and falling with the *p*H of the blood. The experiment illustrated in Fig. 1 shows the changes which accompanied the administration during alternate periods of air and of a CO_2-rich mixture (18 p.c. CO_2: 47 p.c. O_2).

The preparation consisted of the hind limbs of a 10 kg. dog, with about 500 c.c. of blood in the circuit at the commencement of the experiment. The blood flow was about

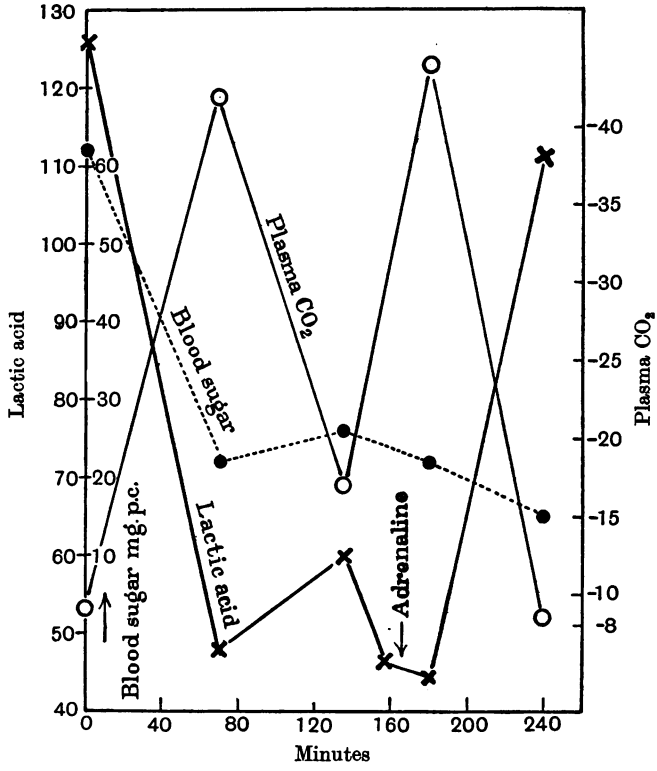

Fig. 1. Lactic acid, glucose, and plasma CO_2 contents of blood perfused through lung-limb preparation of dog. The lungs were ventilated with the following gas mixtures:

0—70	minutes	with	18 p.c. CO_2 + 47 p.c. O_2
70—138	,,	,,	air
138—180	,,	,,	18 p.c. CO_2 + 47 p.c. O_2
180—240	,,	,,	air

110 c.c. per minute, the venous blood being moderately de-oxygenated in appearance. "Pituitrin" (0·5 unit) was added in two portions, one an hour and one 40 minutes before the observations were begun, in order to maintain vascular tone, and adrenaline 1 : 10,000 solution was also added in small amounts (3 c.c. in all) during the last 1¼ hours of the experiment for the same reason, the blood-pressure on the arterial side being kept above 90 mm. In view of the results of Cori and Cori [1929] it may be that the rapid rise of lactic acid in the fourth hour of the experiment was to be attributed in part to the presence of adrenaline in the circulating blood.

In other experiments without addition of "pituitrin" or adrenaline similar results were obtained, so that we do not think that the somewhat complicated procedure described above had materially affected the results.

We have found in most of these experiments that, whereas it is relatively easy, by adding CO_2 to the air given, or by appropriately reducing the ventilation, to cause a decrease in the lactic acid concentration of the blood (see Fig. 2), it is less easy by over-ventilating the lungs

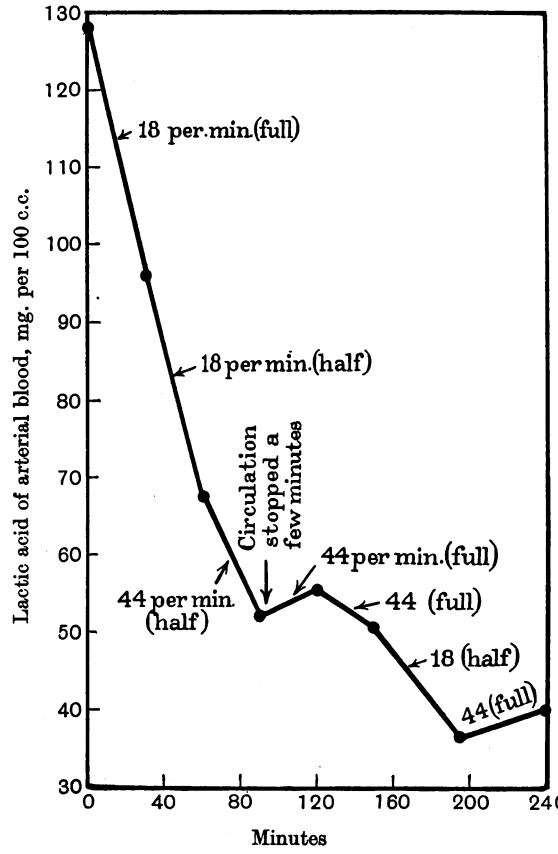

Fig. 2. Fall in blood lactate accelerated by reduction of ventilation, in lung-limb preparation of dog, and converted to slow increase by over-ventilation.

to cause the lactic acid to rise. For example, a respiration rate of 36 per min. at full stroke was needed in the experiment illustrated in Fig. 1 to give the observed rise in lactic acid content of the blood, whereas, in Fig. 2, a respiration rate of 18 per min. at half stroke (= about 2·2 l. per min.) led to a rapid fall in lactic acid concentration in the blood, which was only checked when the respiration rate was increased to 44 per min. at full stroke (= about 10 l. per min.). It may be because

the active tissue available in this preparation is so much greater than that in the heart-lung preparation, that it is less readily over-ventilated and becomes far less acapnic than a heart-lung preparation tends to become.

It has been suggested by Kilborn [1928] that the high R.Q. of the decapitated eviscerated cat is due to a washing out of CO_2 from the blood, owing to a relative over-ventilation, together with (and possibly producing) an increase in the lactic acid content of the blood. It would appear from his experiments to be easier to remove CO_2 from the blood than was the case in our own experiments. We think that this may possibly be explained by the relatively smaller blood flow through the muscles in our experiments. It is clear that the smaller the blood flow through the muscles relative to the ventilation, the greater will be the difference between the CO_2 pressures in the arterial and venous bloods, and it is evident that beyond a certain point, an increase in the extent of ventilation will produce little if any further effect on the composition of the venous blood. In Kilborn's experiments, on the other hand, we may presume that the circulation rate through both muscles and lungs

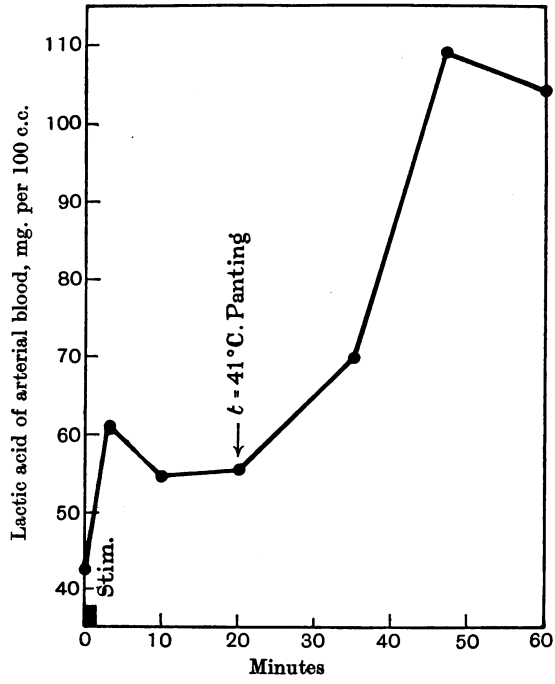

Fig. 3.

would be largely increased after evisceration, with the consequence that, unless the ventilation previous to evisceration had been minimal, there would be a much more rapid rate of transference of CO_2 away from the muscles, which indeed his experiments show to be the case. It should be noted that our analyses have been made on the venous blood returning from the muscles, and not on the arterial blood, since we think that the composition of the venous blood gives the clearer indication of the conditions in the muscle.

The necessity for avoidance of over-ventilation in artificial, or even natural respiration, in experiments in which changes in the lactate content of the blood are being followed is illustrated by the experiment shown in Fig. 3. The changes in lactate concentration in the blood during recovery from fatigue were being followed in a decerebrate dog breathing naturally. The temperature of the animal accidentally rose to 41° C., and for this or some undiscovered reason there was prolonged and vigorous panting. The very large change in the lactate concentration of the blood entirely masked the changes we were endeavouring to follow.

In some experiments we have tried altering the reaction of the blood

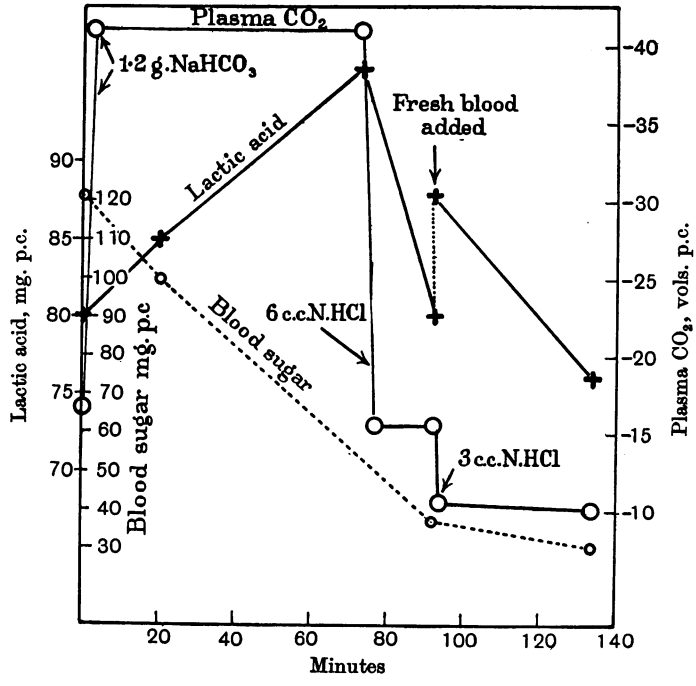

Fig. 4.

by addition of sodium bicarbonate or hydrochloric acid. The results were essentially similar (Fig. 4), increase of *p*H by addition of sodium bicarbonate causing an increase, and decrease of *p*H by addition of HCl a decrease in the lactic acid content. Since the increase in the *p*H of the blood by over-ventilation leads, by a Hamburger interchange, to a fall of plasma bicarbonate and *vice versa*, the effect, as regards plasma bicarbonate, of altering the blood reaction by addition of bicarbonate or HCl is the reverse of that produced by over- or under-ventilation.

The results of these experiments on the addition of acid or alkali therefore indicate that, so far as the effects on the tissues themselves are concerned, the important factor is not the bicarbonate concentration but the H-ion concentration, or what amounts to the same thing, the acid-base ratio.

As regards the origin of the lactic acid which is formed, and its fate when it disappears, there are several possibilities. In the first place, it is evident that some at all events of the lactate may be derived from the blood sugar, since it is known that blood sugar disappears and lactic acid takes its place when carbon dioxide is pumped off from the blood. In the present series of experiments also, we find that when blood is passed through a well-ventilated lung, lactic acid makes its appearance. Another source of the lactic acid would be muscle glycogen. That some at any rate of the lactic acid of the blood comes from this source is clear from experiments such as that illustrated in Fig. 1 which shows that lactic acid may make its appearance abundantly in the blood without appreciable change in the blood sugar level, as happened in the fourth hour of that experiment.

There are three possible ways in which the lactic acid which disappears from the blood may be disposed of: firstly, by combustion in the muscles; secondly, by glycogen synthesis in the muscles, and thirdly, by distribution and ultimate disposal in one or both ways in other tissues of the body, *e.g.* in this case in bone and skin.

Since there is no reason to suppose that the method of disposal of this lactic acid differs essentially from that produced in muscular exercise, we do not propose further to discuss its fate here, but merely conclude that the results of these experiments indicate that, under these conditions, mammalian muscular tissue can directly utilize lactic acid and remove it from the blood.

It is quite conceivable that other tissues than skeletal or heart muscle can produce or remove lactic acid in this way. In fact there is some attraction in regarding such formation and removal of lactic acid as

having some teleological significance by participating, as it must do, in the regulation of the H-ion concentration of the tissues themselves.

Conclusions.

1. The lactic acid content of the blood used to perfuse isolated mammalian muscle is increased by reduction of the hydrogen ion concentration. On increase of H-ion concentration, the lactic acid falls again, if the blood is perfused through muscle (properly oxygenated), but not if perfused through lung alone.

2. It is presumed that the lactic acid removed by the muscles is dealt with in the same manner as that removed after exercise.

3. The importance of avoiding over-ventilation in experiments in which variations in the lactic acid content of the blood are being studied is emphasized.

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