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AN ENQUIRY INTO THE NATURE OF THE MEDIATOR OF THE VASODILATATION IN SKELETAL MUSCLE IN EXERCISE AND DURING CIRCULATORY ARREST

REVIEW LECTURE

Given at the joint meeting of the Physiological and Anatomical Societies at Nottingham University on 17 and 18 December 1971.

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I am indeed greatly honoured to have been invited by the Physiological Society to deliver this Review Lecture. I shall never forget that I delivered it at the first meeting of the Physiological Society to be held in the University of Nottingham, where my colleague and friend Professor Greenfield is Professor of Physiology and Dean of the new Medical School.

Many of you were at the International Congress of Physiological Sciences at Munich this summer and will remember the President of the Executive Committee, Professor Kurt Kramer. In the 1930's he and his colleagues did beautiful experiments on the effect of exercise on the blood flow through the isolated perfused dog's gastrocnemius muscle. One of the most important of their findings was that, in the steady state, the blood flow through a skeletal muscle is directly proportional to its rate of oxygen consumption (Kramer, Obal & Quensel, 1939). This is shown in Fig. 1. It has been confirmed by Welch & Stainsby (1967). That is to say we have in our muscles a local mechanism which decreases the resistance of the blood vessels as oxygen demand increases. When the demand for oxygen increases the vessels open up, so that with almost constant arterial blood pressure, that is perfusion pressure, muscle blood flow may increase from 1 to 20–30 l./min. How does the oxygen demand open up the vessels? That is the subject of this lecture.

You will remember that at the beginning of exercise muscles go into debt for oxygen, after a few minutes they reach the steady state, and when exercise is over the debt is paid back in the first few minutes. It will be convenient to note here that Welch & Stainsby (1967) found a direct proportionality between oxygen debt and oxygen consumption. This is seen in Fig. 2, so that blood flow is linearly related to oxygen consumption and to oxygen debt.

Why this oxygen debt? Margaria, Edwards & Dill (1933) suggested that it was probably for oxidative processes needed for the resynthesis of high

energy phosphates. Fig. 3 shows how phosphoryl creatine in the human quadriceps femoris muscle breaks down at the beginning of moderately severe exercise and reaches the steady state (Hultman, Bergström & Anderson, 1967). It is resynthesized immediately after exercise and oxidative energy is required for this. Margaria's idea has been confirmed by Piiper, di Prampero & Cerretelli (1968). Welch & Stainsby (1967) have calculated that the resynthesis of all the phosphoryl creatine in the dog's

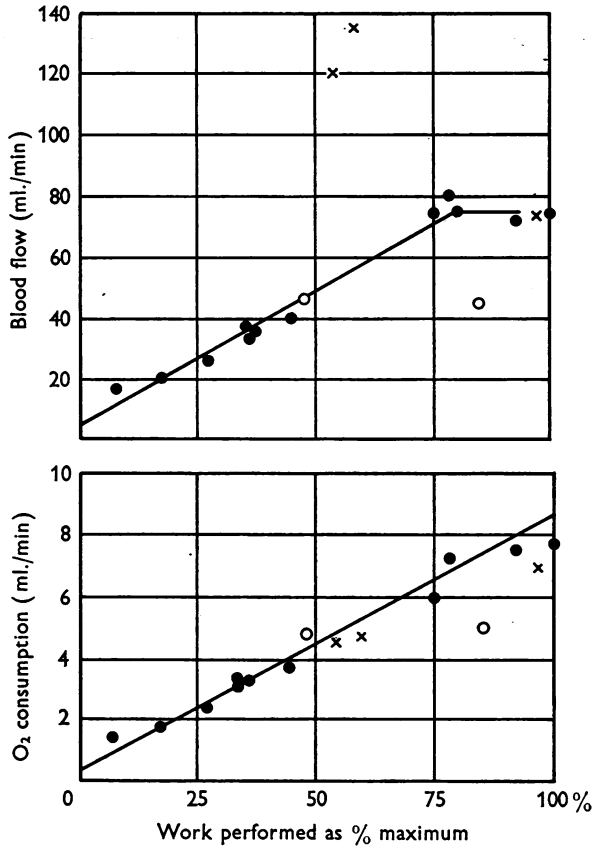

Fig. 1. Results showing that blood flow and oxygen consumption were proportional to the work done during rhythmic contraction of the isolated perfused dog's gastrocnemius muscle (Kramer, Obal & Quensel, 1939).

gastrocnemius muscle would take between 15 and 22 ml. oxygen per 100 ml. muscle, which agrees very well with the figure they obtained for the oxygen debt in this muscle after vigorous contractions.

I have stressed here the linear relation between blood flow, break-down of phosphoryl creatine, oxygen debt and oxygen consumption. Any hypo-

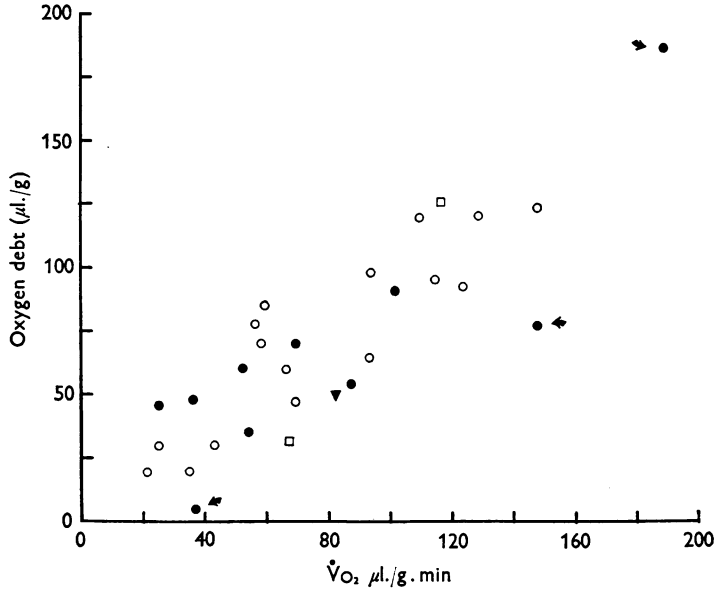

Fig. 2. Results showing that oxygen consumption was proportional to oxygen debt during rhythmic contraction of the gastrocnemius–plantaris muscle group of the dog (Welch & Stainsby, 1967).

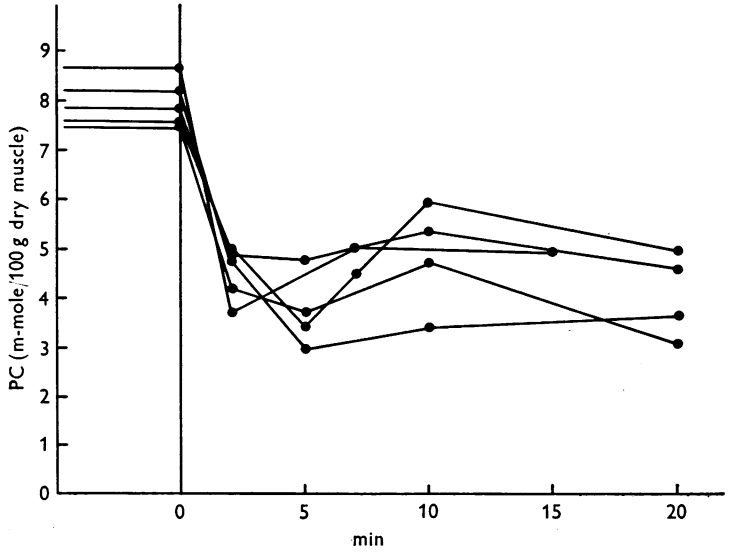

Fig. 3. Results showing the concentration of phosphoryl creatine (PC) in biopsy samples of the human quadriceps femoris muscle before and during 20 min exercise (Hultman, Bergström & Anderson, 1967).

thesis for the regulation of muscle blood vessels in exercise must take account of it.

There are various suggestions for the mediator(s) of hyperaemia in exercising muscle. Anoxia is believed to be the link between blood flow and metabolism by Guyton and his school (Guyton, Ross, Carrier & Walker, 1964); Kjellmar (1965) has suggested increase in potassium; Mellander, Johansson, Sarah Gray, Jonnson, Lundvall & Ljung (1967) have pressed the claim of increase in osmolarity; Khayutin (1968) has suggested that contraction lessens stretching of the arterioles so their smooth muscle relaxes; Forrester & Lind (1969) and Forrester (1972) have drawn our attention to ATP; inorganic phosphate is favoured by Hilton & Vrbová (1970) and Hilton & Hudlická (1972); and by Skinner & Costin (1970) a combination of anoxia, increase in potassium and increase in osmolarity. Mellander & Johansson (1968) have reviewed the literature.

In preparing this lecture I found my whole attention absorbed by the claims of anoxia. I believe it deserves rather more consideration. That being so, this lecture will be not so much a review as the submission of evidence that oxygen lack may play a part, and in the steady state, maybe a large part in the regulation of muscle blood flow.

I must begin at the beginning of the story. In point of fact it does not begin with the hyperaemia of exercise at all, but with another condition in which the tissues do not get all the oxygen that they want. In that respect a first cousin of exercise, namely the vasodilatation that takes place in a limb after its blood supply has been cut off. The cousins are active muscle wanting oxygen and resting muscle wanting oxygen. I propose to start with what happens when a resting limb is denied its oxygen.

The classical experiments on this were done by Lewis & Grant in 1925. 'Observations upon reactive hyperaemia in man' was the title of their paper.

They found that the longer the period of arrest of the circulation, (1) the brighter the flushing of the skin after the circulation had been released, and the longer it lasted, (2) the greater the increase in the forearm volume after release, (3) the greater the forearm blood flow after release. For these and other reasons they concluded that the vasodilatation took place during the period of arrest, and that it was due to the action of metabolites, continuously formed and accumulating in the tissues during the period of arrest. The longer the arrest, the greater the accumulation of metabolites, and the larger the vasodilatation during arrest and after release. As the flush was bright arterial red they concluded that reactive hyperaemia was not due to anoxia.

Therefore I was very surprised to see a paper by Fairchild, Ross & Guyton (1966) entitled 'Failure of recovery from reactive hyperemia in the

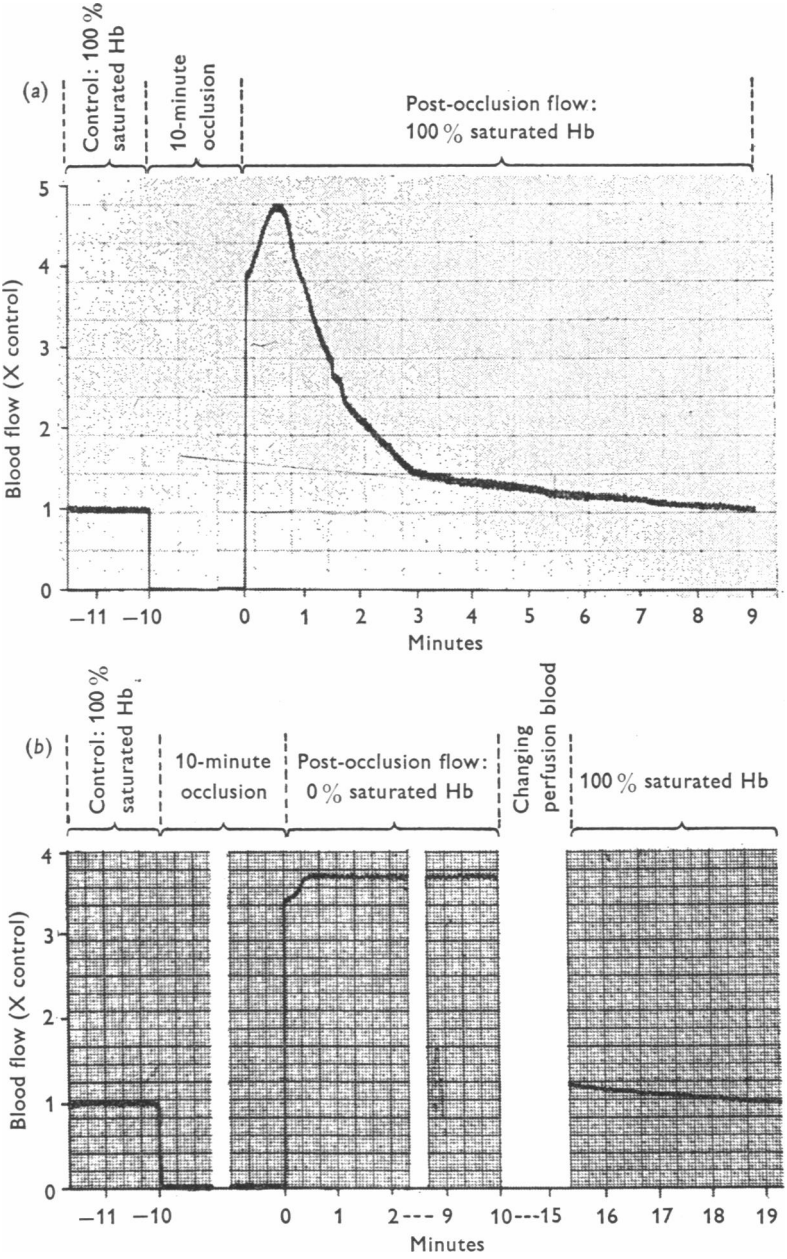

Fig. 4. Results showing (*a*) reactive hyperaemia, and (*b*) failure of recovery from reactive hyperaemia in the absence of oxygen.

absence of oxygen'. Fig. 4 shows results that they obtained. The record is of the rate of the blood flow through a dog's hind limbs. These were perfused at exactly 100 mm Hg pressure with blood which had been ventilated either with 100 % oxygen or 100 % nitrogen, the tension of CO_2 being always 35–45 mm Hg. The top record, (*a*), was recorded during oxygenated blood perfusion. The circulation was arrested for 10 min, flow was zero. Release of the circulation was followed by a reactive hyperaemia. There was a peak of between four- and fivefold the resting blood flow followed by an almost exponential return of the flow to the resting level. The lower

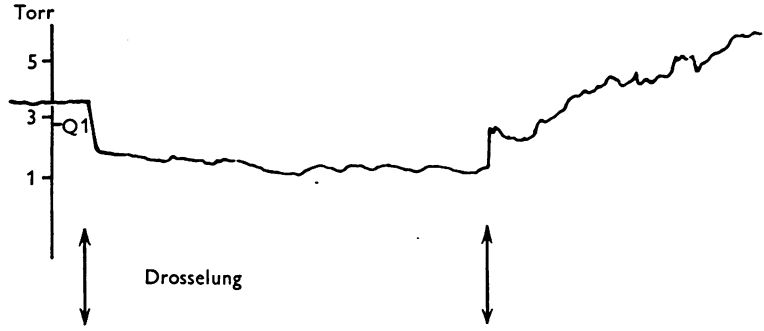

Fig. 5. Results showing decrease of oxygen tension to 1 mm Hg in the human tibialis anticus muscle during arrest of the circulation. Occlusion for 3 min between the arrows (Kunze, 1968).

record, (*b*), shows 10 min circulatory rest with zero flow. On release the hind limbs were perfused with blood ventilated with 100 % nitrogen. The rate of flow was about 3½ times the resting rate, and remained constant for some 10 min, with no recovery of the blood flow till oxygenated blood perfusion was resumed. It was indeed 'Failure of recovery from reactive hyperemia in the absence of oxygen.'

This suggested that vasodilatation in these hind limbs would go along with exhaustion of their oxygen stores. How soon after arresting the circulation would the stores be exhausted? One would have to divide the amount of oxygen in the store by the rate of the oxygen consumption.

Let us make the calculation. Farhi & Rahn (1955) reckon that human muscle myoglobin has a store of about 0·6 ml. oxygen/100 ml. muscle. Allowing for oxygen in physical solution and in haemoglobin in the blood vessels it might be 1 ml./100 ml. Mottram (1955) estimated oxygen consumption of the forearm muscles to be 0·24 ml./100 ml. min. He told me this should now be increased to 0·28 – make it 0·3 ml. We have then 1 ml./100 ml. stored being consumed at 0·3 ml./min. Time for all the oxygen to

be consumed is 1/0·3 equals 3 min approximately. This agrees fairly well with the results obtained by Millikan (1937) from his observation of the rate of reduction of the myohaemoglobin (i.e. 1 %/sec) in the cat's soleus muscle following clamping the thoracic aorta. 3 min agrees fairly well for the time taken for oxygen tension in the human tibialis anticus muscle to fall to less than 1 mm Hg after the arrest of its circulation, as may be seen in Fig. 5 (Kunze, 1968). We have no corresponding data for the amount of oxygen stored in the skin or the rate of cutaneous oxygen consumption, but this does not matter, for Evans & Naylor (1967) have shown that following circulatory arrest oxygen tension in the skin declines to zero in less than 2 min, as Fig. 6 shows. In summary the evidence from these diverse sources amounts to the fact that oxygen stores in the forearm would be exhausted in 2–3 min after arresting the circulation.

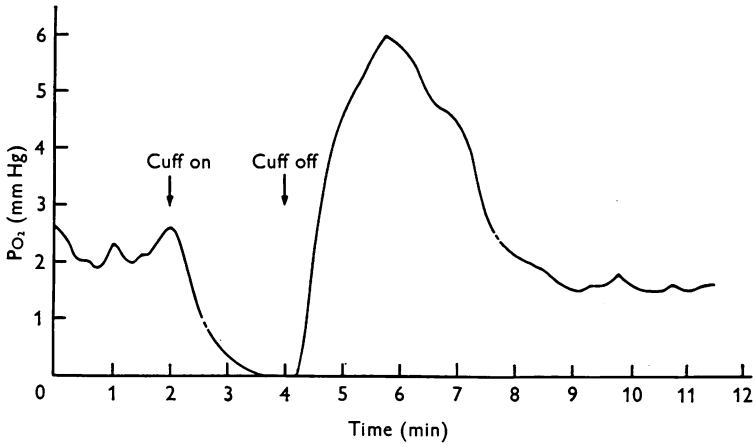

Fig. 6. Results showing that oxygen tension at the surface of the forearm skin decreased to zero in 2 min following occlusion of the circulation in the upper arm (Evans & Naylor, 1955).

Assuming that the time for the oxygen stores to be exhausted in the dog's hind limbs after arresting the circulation would be about the same it seemed likely that a simple switch from oxygenated blood perfusion to nitrogenated blood perfusion should be accompanied by vasodilatation in the first 2–3 min. I wrote to Dr Guyton asking if he had tried this, and if so, with what result. I have his permission to quote his reply which was as follows: 'The two minutes that you calculated is very close to the actual results we obtained in these experiments. That is, upon switching from control arterial blood to anoxic blood, there was a few seconds before any change took place, but as the anoxic blood entered the muscle, blood flow began to increase and reached its maximum level somewhere between 1 and

2 min. There is reason to believe that some of the oxygen diffuses backwards from the muscle into the anoxic blood, which could make the response somewhat more rapid than one would suspect from calculations of oxygen utilization.'

This striking relation between oxygen lack and vasodilatation in the dog's hind limbs prompted me to search the literature to see whether corresponding vasodilatation occurred in the forearm in the first few minutes after circulatory arrest, as oxygen lack made itself felt.

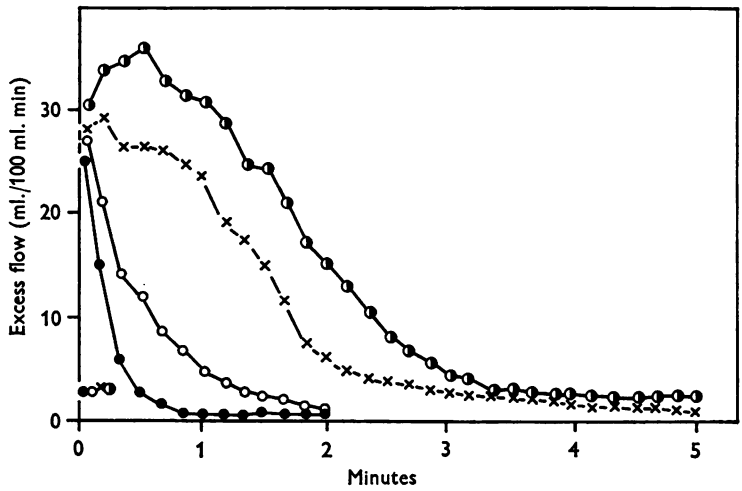

Fig. 7. Averaged results of six experiments showing reactive hyperaemia in the human forearm after periods of arrest of 3, 5, 10 and 15 min respectively (Patterson & Whelan, 1955).

Fig. 7 has been taken from the paper by Patterson & Whelan (1955). We see the averaged results of six experiments. Resting blood flow averaged 3 ml./100 ml. min. Peak blood flows averaging 25·5, 28, 28·5 and 30 ml./100 ml. forearm.min were recorded 4 sec after the release of occlusions lasting for 3, 5, 10 and 15 min respectively. That is to say the dilatation following arrest of the circulation was almost complete in 3 min, corresponding to the time at which oxygen stores would have been almost exhausted.

Fig. 8 has been taken from Sir Thomas Lewis's (1927) book, *Blood Vessels of the Human Skin and their Responses*. Resting blood flow in Sir Thomas Lewis's own forearm was 4 ml./100 ml. min. We see peak blood flows of 15, 34, 49 and 55 ml./100 ml. forearm.min recorded beginning immediately after intervals of occlusion of $\frac{1}{2}$, $1\frac{1}{2}$, 5 and 15 min respectively. Inspection of the results shows that the vasodilatation must have

been almost complete within 3 min of arresting the circulation. Similar results were obtained in five other sets of observations on Lewis's forearm and in two other sets on Grant's forearm (Lewis & Grant, 1925). (As we have seen already Lewis & Grant used these results as evidence that metabolites accumulated continuously during the period of arrest. In fact this was not very good evidence for continuous accumulation, as the vasodilation was rapid during the first 3 min and very slow from then to the 15th min.)

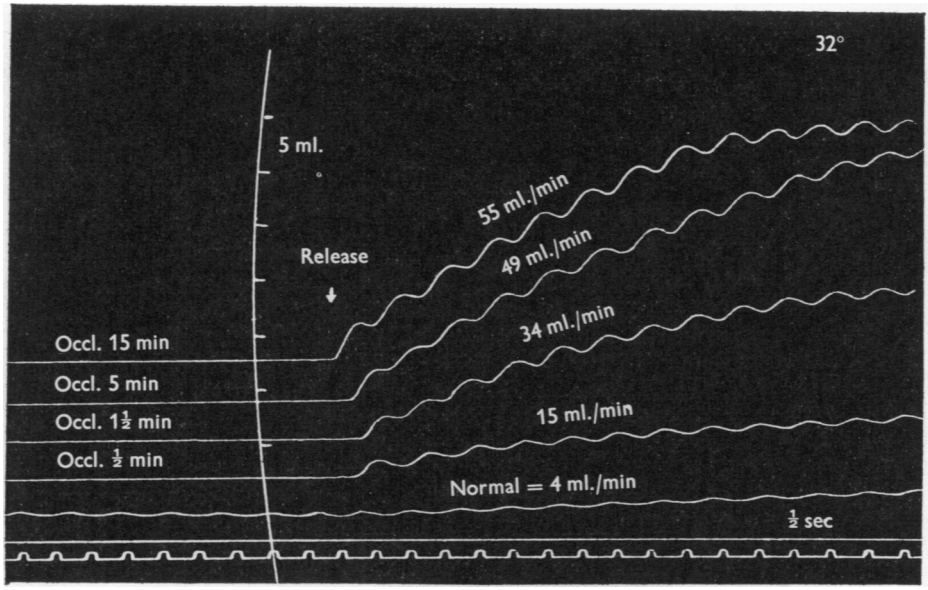

Fig. 8. Results showing the rates of the inflow of blood into Sir Thomas Lewis's forearm immediately after periods of arrest of ½, 1½, 5 and 15 min (Lewis & Grant, 1925; Lewis, 1927).

The data from Fairchild *et al.* (1966), from Patterson & Whelan (1955) and from Lewis & Grant (1925) have been brought together in Fig. 9. The Figure shows how rapidly cutting off the oxygen supply to the dog's hind legs, and to the human forearm was followed by vasodilatation. The smallest increase was in the dog's hind legs, the reason why it was only threefold is unknown, and is a little disquieting. Of the human results those of Patterson & Whelan, showing a tenfold increase, are probably the nearest to the truth. The reason why Lewis & Grant got a greater increase may have been because the blood flow through the hand was not arrested in their experiments. The thing that all three graphs have in common is that most of the vasodilatation takes place in the first 3 min, as the oxygen

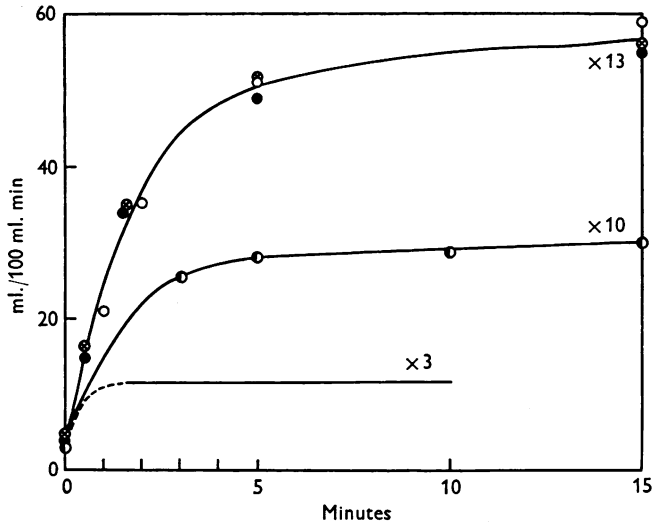

Fig. 9. Results showing: top, forearm blood flows immediately after different periods of arrest (Lewis, 1927; Lewis & Grant, 1925); middle, forearm blood flows immediately after different periods of arrest (Patterson & Whelan, 1955); bottom, blood flow through the hind limbs of the dog, perfusion with nitrogenated blood begun at time 0 (A. C. Guyton, personal communication, 1971; Fairchild, Ross & Guyton, 1966).

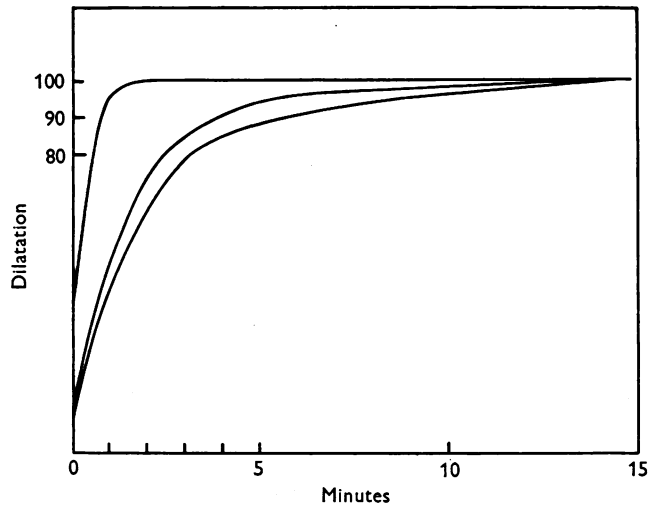

Fig. 10. Results showing that stopping the supply of oxygen to the limbs was followed by vasodilatation, reaching 80% of its final value in 3 min. The graphs have been obtained from the graphs in Fig. 9. The values on each graph have been expressed as percentages of a final value of 100. The graphs rise in the following order from left to right: Fairchild *et al.* (1966); Patterson & Whelan (1955); Lewis & Grant (1925).

stores run out. This is even better shown in Fig. 10 in which the final blood flows indicated by the three graphs have each been given a figure of 100, and the earlier figures on each graph have been expressed as percentages. The Figure shows very clearly that 80% of the vasodilatation in the forearm took place within 3 min of cutting off the oxygen supply. Obviously this vasodilatation could have been brought about by oxygen lack. Anaerobic respiration would have increased as the oxygen stores ran out, but as we shall soon see the vasodilatation could not have been due to the action of diffusible metabolites.

As Fig. 10 shows, scarcely any further vasodilatation took place between 5 and 15 min, although the concentration of anaerobic metabolites must have been rising continuously. In the presence of oxygen lack these did not manifest their vasodilator activity. This is confirmed by results shown in Fig. 4 of this paper. Referring to the Figure in question, and to its lower frame, (*b*), and to the 10 min circulatory arrest shown there, no doubt while the circulation was arrested, diffusible anaerobic metabolites would have accumulated continuously. If these had had a vasodilator action during the arrest, then, after the institution of nitrogenated blood, flow must have subsided somewhat from the initial high level, as excess of the diffusible metabolites escaped into the nitrogenated blood stream. The fact that the flow of nitrogenated blood rose abruptly to a constant level and never declined till oxygen was readmitted indicates that oxygen lack alone was the effective stimulus for the vasodilation during the period of the circulatory arrest.

The events following release of the circulation, that is during so called reactive hyperaemia proper are also very relevant to the mechanism of the hyperaemia of exercise. They are seen in Fig. 11 which is from results obtained by McNeill (1956). The data are typical of those recorded in six experiments. The graphs are: lowest, oxygen consumption; middle, forearm blood flow; top, oxygen saturation of blood drawn from the antebrachial vein. Following 5 min of circulatory arrest the circulation was released at time 0. Oxygen consumption, initially rapid, subsided to the resting rate in 1 min. A rough estimate of the excess oxygen consumed is 0·75 ml./100 ml. forearm . min which is about the amount of oxygen needed to refill the oxygen store, estimated earlier in this paper to be between 0·6 and 1·0 ml./100 ml. forearm muscle. In other words this is the repayment of an oxygen debt. Turning now to the middle graph, that of the reactive hyperaemia, we see that it subsides more slowly than the oxygen consumption. From the standpoint of oxygen utilization the rate of the stream is faster than it need be. This is probably the cause of the upward 'overshoot' of the venous blood oxygen saturation seen in the uppermost graph. These facts led McNeill (1956) to conclude that oxygen lack was not

an important factor in maintaining the high rate of the blood flow after release of circulatory arrest. It is generally accepted that this reactive hyperaemia is due to the vasodilator action of metabolites diffusing out of the tissues.

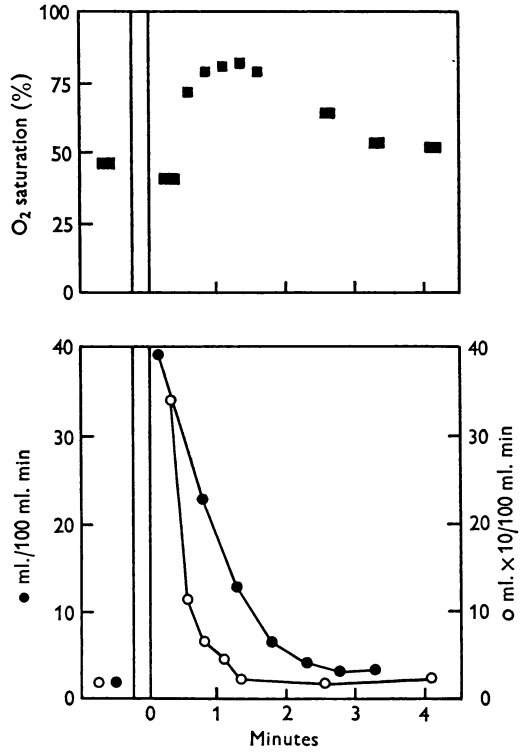

Fig. 11. Results showing the effect of 5 min circulatory arrest on the percentage saturation with oxygen of the venous blood, on the blood flow, and on the oxygen consumption of the forearm (McNeill, 1956).

As we have seen vasodilatation in the forearm in both skin and muscle takes place in the first few minutes after arrest of the circulation. According to Dornhorst (1963) most of the increase in forearm blood flow would be in the muscles.

Before returning to our topic, the hyperaemia of exercise, we must refer briefly to another explanation for the vasodilatation following circulatory arrest, put forward by Bayliss (1902). Namely, that it is due to collapse of the arterial tree, and lack of the stimulus of stretch acting upon the smooth muscle of the vessel walls. Evidence that this is not the cause of the vasodilatation is to be seen in Fig. 4 of this paper. Referring to this Figure, and to the lower frame, (*b*), we see that when, at the end of 10 min arrest of the

circulation in the hind legs of the dog, nitrogenated blood perfusion at 100 mm Hg pressure was begun, blood flow returned almost instantly to a level which remained quite constant. According to Bayliss's stretch hypothesis the vessels should have responded to this restoration of their stretch by constricting, which would have been manifested by a decrease in the rate of blood flow. Such a decrease was not seen in any of Fairchild, Ross & Guyton's experiments.

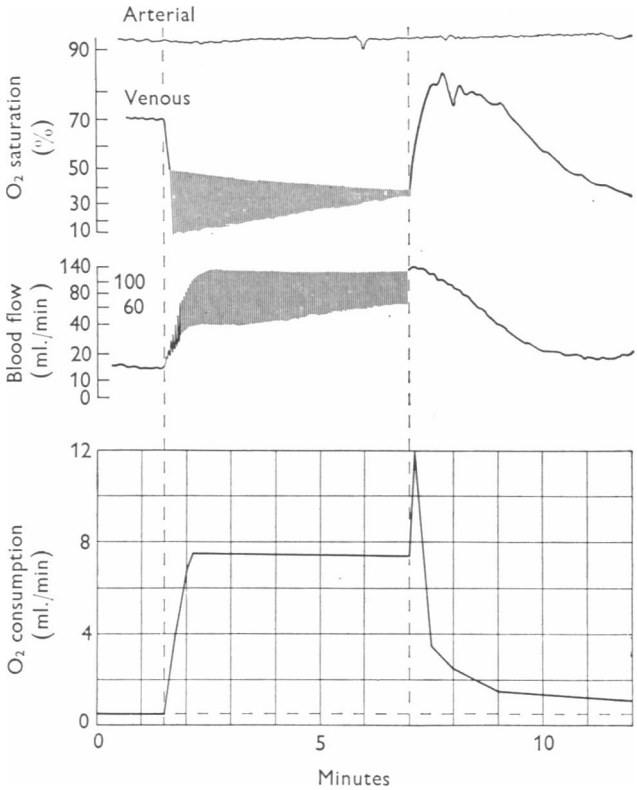

Fig. 12. Results showing the effect of rhythmic stimulation of the isolated perfused gastrocnemius muscle of the dog on the arterial blood pressure, on the oxygen saturation of the venous flow effluent, on the blood flow through the muscle, and on its oxygen consumption (Kramer, Obal & Quensel, 1939).

Further discussion of the papers by Bayliss and others suggesting that lack of stretch is the cause of the vasodilatation following circulatory arrest is to be found in Addendum I of this paper.

Let us compare certain changes which happen after arresting the circulation with those which happen in exercising muscle.

(1) Immediate decrease in the % saturation of myoglobin after occlusion, and at the beginning of exercise (Millikan, 1937).

(2) Decrease in oxygen saturation of the venous blood after occlusion (Pappenheimer, 1941) and at the beginning of exercise (Fig. 12).

(3) Oxygen stores run down after occlusion, oxygen debt builds up at the beginning of exercise.

(4) Simultaneously with 1–3 above, vasodilatation 1–3 min after occlusion (Figs. 7–10), and vasodilatation, often much more quickly, in exercise (Fig. 12).

(5) Then a steady state in which the vasodilatation is maintained (Figs. 7–10 and 12).

(6) Reactive hyperaemia, after release of the circulation (Fig. 11); post-exercise hyperaemia after ending exercise (Fig. 12).

(7) Replenishing of oxygen store after release of the circulation (Fig. 11); repayment of oxygen debt in 2–3 min after exercise (Fig. 12).

(8) Reactive (Fig. 11) and post-exercise hyperaemia (Fig. 12) subsiding more slowly than oxygen consumption.

(9) Overshoot of venous oxygen saturation during reactive (Fig. 11) and post-exercise hyperaemia (Fig. 12) (see also overshoot in oxygen tension in the skin, Fig. 6, and in the human tibialis anticus muscle, Fig. 5).

(10) Anoxia not a major factor in reactive hyperaemia (Lewis & Grant, 1925; McNeill, 1956) or in post-exercise hyperaemia (Love, 1955).

There are many likenesses here. We have seen that the vasodilatation in the first few minutes after arrest of the circulation is probably due to oxygen lack, thereafter it is probably maintained by oxygen lack till the circulation is released. After release the vasodilatation is mediated by metabolites. Is it not tempting to think that oxygen lack is the stimulus for vasodilatation at the beginning of exercise and is to a large extent responsible for the maintenance of this vasodilatation during the steady state? Is it not likely that post-exercise hyperaemia is mediated by metabolites diffusing from the muscle?

There are of course great differences in the magnitude of the parameters, and naturally because metabolism goes so much faster during exercise than at rest. In severe exercise muscle blood flow may be more than double that following circulatory arrest. Oxygen lack alone is probably not enough to explain the vasodilatation in severe exercise.

No doubt many readers will have wondered how oxygen lack could bring about vasodilatation. A very good question. We have seen that the vasodilatation following arrest of the circulation is not mediated by diffusible substances, and that it occurs simultaneously in both skin and muscle. In these tissues the wall of the blood vessels too must feel the general lack of oxygen. The simplest hypothesis is that the vasodilatation

is the result of not enough oxygen to maintain the contractile process in the smooth muscle of the arterioles and precapillary sphincters in the skin and muscle. In the case of exercise, where oxygen lack is manifested by an oxygen debt, venous blood oxygen saturation decreases, but rarely below 20 %. The luminal side of all patent vessels will have oxygen. Nevertheless, the regulation of the microcirculation is far from being understood, the recent review by Wittenburg (1970) does more than hint that its regulation by anoxia is far from being ruled out.

Several authors have concluded that oxygen lack is not the cause of the hyperaemia in exercise. A discussion of some of these papers will be found in Addendum II.

'Further experiments' are needed to discover the cause of the hyperaemia in exercise. I wonder if one day we shall see on the programme of a Physiological Society meeting a communication entitled 'Failure of recovery from post-exercise hyperaemia in the absence of oxygen'?

I will keep an eye open for it.

I am indebted to Professor A. C. Dornhorst for criticism, to Dr Richard Edwards for drawing my attention to many papers referred to in this lecture and to the Wellcome Trust for secretarial assistance. I am also grateful to authors and publishers for permission to reproduce many of the Figures.

ADDENDUM I

Bayliss (1902, 1924) recorded the volume of the hind leg of a dog. The vasomotor nerves had been cut. Compression of the abdominal aorta for 8 sec was accompanied by decrease in leg volume. After release of the aortic compression, leg volume rapidly increased to far above the initial volume. The increase to above the resting level after an occlusion of 8 sec was as great as that after an occlusion of 20 sec. Bayliss found it difficult to believe that deprivation of blood flow for 8 sec could cause an appreciable accumulation of metabolites in a resting curarized leg. The blood was fully oxygenated. The idea that the vasodilatation was due to metabolites being untenable, he considered that it was due to collapse of the vascular tree; lacking the stimulus of stretch the smooth muscle of the vessel walls had relaxed. The effect of the aortic occlusion on oxygen tension in the leg of an anaesthetized curarized dog, that had undergone surgery for acute denervation of the blood vessels, and for access to the abdominal aorta, is not known. It would be interesting to measure it.

Hilton (1953) arrested the venous outflow from the dog's gastrocnemius for several minutes, thus keeping the vessels on the stretch. After release of the circulation there was no reactive hyperaemia. No collapse of the vessels, therefore no removal of the stimulus of stretch and no sign of vasodilatation after removing the venous occlusion. The reason for this

may have been because, as he says in his paper, there may have been two or three other very fine veins which were not ligatured. Normally, of course, the flow through these fine veins would have been quite trivial but after occlusion of the main venous outflow venous pressure would have approached the arterial level, in such circumstances a significant stream might have been forced through the fine veins. If so the muscle might not have been denied much oxygen. Lewis & Grant (1925) found the venous occlusion of the circulation in the forearm was followed by reactive hyperaemia. Their reasons for rejecting Bayliss's theory of lack of stretch as an explanation will be found in their paper in great detail.

Patterson (1956) used another method to prevent the vessels from collapsing after arrest of the circulation. Before arresting the circulation through the forearm he packed it with blood by exposing it to sub-atmospheric pressure. Following release the 'excess flow above the resting level' was reduced in forearms whose blood volumes had been so increased. He considered that the results supported Bayliss's hypothesis. He argued thus. The greater the volume of blood left in the arterial tree, the greater the stimulus of stretch, the smaller the relaxation of the smooth muscle coats of the blood vessels and so after release of the circulation the smaller the 'excess flow above the resting level'. The results can be interpreted differently. According to Patterson's data exposing the forearm to sub-atmospheric pressures of -50, -100 and -150 mm Hg increased the volume of blood in it by 4·2, 6·0 and 8·5 ml./100 ml. forearm respectively. These volumes of blood would have contained 0·8, 1·2 and 1·5 ml. oxygen. Supposing that half of this oxygen was consumed at the rate of 0·3 ml./100 ml. forearm.min, it would enable normal metabolism to go on for $1\frac{1}{2}$, 2 and 3 min respectively. During occlusions of 5 min the standard duration, it would be $1\frac{1}{2}$, 2 and 3 min before the oxygen stores began to be drawn upon. The occlusions would have the effect of 5–$1\frac{1}{2}$, 5–2, 5–3 that is to say, $3\frac{1}{2}$, 3 and 2 min occlusions, and would have been followed by correspondingly diminished 'excess blood flows above the resting level'.

Wood, Litter & Wilkins (1955) stretched the forearm vessels by venous congestion before arresting the circulation. They too found that increasing the blood volume of the forearm before arrest decreased the subsequent 'excess flow above the resting level'. They concluded that the results supported Bayliss's stretch hypothesis. An alternative explanation is that venous congestion increased the amount of blood and oxygen in the forearm so that normal metabolism continued for longer before the oxygen stores began to run down.

Wood, Litter & Wilkins (1955) also did an experiment in which venous congestion was followed by arrest of the circulation and immediately followed arrest by raising the pressure in the plethysmograph to 100 mm

Hg. This procedure did *not* diminish 'excess blood flow above the resting level'. They considered that this result supported Bayliss's hypothesis. They argued that venous congestion by a pressure of 95 mm Hg would have increased the volume of blood in the forearm, but that then the application of an external pressure of 100 mm Hg would prevent the vessels from being stretched. This is difficult to understand, if stretching means increasing the length of the circumference of the vessels. It is also difficult to understand why the extra amount of blood and oxygen in the forearm did not delay exhaustion of the oxygen stores.

ADDENDUM II

Anrep & Saalfeld (1935) considered that the hyperaemia of exercise was not due to oxygen lack. Their experiments are very interesting. They worked on the dog's gastrocnemius muscle. The muscle could be perfused either from the dog's own circulation, or with its own venous blood collected while it was resting or contracting rhythmically. The rate of blood flow through the muscle was recorded.

Venous blood collected from the resting muscle and passed through it again had no vasodilator action.

Rhythmic stimulation was accompanied by marked hyperaemia. Blood collected during this hyperaemia of exercise and later re-perfused through the muscle at rest had no vasodilator action.

Blood collected during rhythmic stimulation, when the rate of flow was restricted by a screw clip on the artery to about the resting rate, and later re-perfused through the resting muscle caused marked vasodilatation, as great as that accompanying rhythmic stimulation with unrestricted blood flow. They called this 'active blood' since it was a powerful vasodilator.

Blood collected during stimulation with restricted flow, 'active blood', subsequently oxygenated, still had the vasodilator property. Since its vasodilator action was not abolished by oxygenation they concluded that it must have been due to metabolites and not to oxygen lack.

No doubt the vasodilator action of oxygenated 'active blood' was due to metabolites. But in the case of 'active blood' that had not been oxygenated the stimulus that caused the vasodilatation might have been oxygen lack. Venous effluent collected from active muscle during restriction of arterial flow has been shown to contain almost no oxygen (Donald, Wormald, Taylor & Bishop, 1957; Barcroft, Greenwood & Whelan, 1963). Perfusion of the muscle with such blood would, in effect, have been the same as perfusing it with nitrogenated blood as was done by Fairchild *et al.* (1966), and would be expected to cause vasodilatation, because the continued consumption of oxygen by the muscle would have exhausted the store of oxygen in 2–3 min.

At the end of their paper Anrep and Saalfeld say that the blood collected during rhythmic contractions had a higher oxygen saturation than that collected while the muscle was at rest. Surely they must have been referring to collections made without screw-clip restriction of the arterial blood flow. It would be interesting to repeat their experiment and to measure the oxygen saturation of the 'active blood'.

Holling & Verel (1957) recorded forearm blood flow before, during and after elevating the arm vertically above the subject. In the vertical position blood flow declined to about half its initial rate, owing to decrease in perfusion pressure. Oxygen tension in the muscle, measured polarigraphically, decreased. There was no compensatory vasodilatation during the period of reduced perfusion pressure, nor any reactive hyperaemia after the limb had been lowered. I cited this as evidence that oxygen lack in muscle did not cause hyperaemia (Barcroft, 1964). However my argument is no longer valid since Stainsby & Otis (1964) have shown that perfusion pressure of the arterial supply to the dog's gastrocnemius muscle can be reduced to 25 mm Hg before oxygen consumption decreases, and before peripheral resistance, calculated from their data, decreases appreciably.

Dornhorst & Whelan (1953) found that post-exercise hyperaemia in the calf of the leg was little altered when the subject breathed 8% oxygen. Stainsby & Otis (1964) studied the effect of progressive hypoxia on blood flow through the dog's gastrocnemius contracting once a sec. In the absence of hypoxia contractions caused an immediate rise in oxygen uptake and blood flow. When contractions and hypoxia were begun simultaneously, oxygen uptake and muscle blood flow followed the same pattern until a critical arterial P_{O_2}, averaging 40 mm Hg, was reached. Below this P_{O_2} oxygen uptake decrease was accompanied by a further large increase in blood flow. It follows that there may not have been any change in oxygen consumption or muscle blood flow in Dornhorst & Whelan's experiments when the subject changed from breathing air to breathing 8% oxygen. This might explain why post-exercise hyperaemia was almost unaffected.

Corcondilas, Koroxenidis & Shepherd (1964) thought it unlikely that oxygen lack was the stimulus for vasodilatation, because breathing oxygen did not reduce the hyperaemia accompanying a very brief contraction of the forearm. The contraction was over in 0·3 sec, blood flow increased within 1 sec of the end of the contraction, reaching a peak at once, and the result was the same whether the subject breathed air or 100% oxygen.

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