612.741.61

THE BLOOD FLOW THROUGH THE SKELETAL MUSCLE IN RELATION TO ITS CONTRACTION.

By G. V. ANREP and E. von SAALFELD.

(*From the Physiological Laboratory, University of Cairo.*)

(*Received July* 25, 1935.)

The changes which take place in the arterial blood flow in the skeletal muscle during and immediately following its contraction have been described in two previous communications, where the literature relating to the subject has also been fully discussed [Anrep, Blalock and Samaan, 1934; Anrep, Cerqua and Samaan, 1934]. It was found that the inflow of the arterial blood into the muscle, as measured with a hot-wire anemometer shows, during muscular contraction, four distinct events which rapidly succeed one another. At the beginning of a strong contraction, evoked by direct stimulation of the muscle or of its nerve, a certain amount of blood is thrown back into the muscle artery against the high arterial blood-pressure. This back thrust is followed by a diminution or arrest of the arterial blood flow. The arrest continues for the whole time that the maximal strength of contraction is maintained. On relaxation of the muscle the intramuscular blood vessels present a somewhat diminished resistance to the blood flow, so that at first the arterial blood rushes into them with a considerably increased velocity. This stage is obviously very short. It lasts only 0·1–0·2 sec. and it terminates as soon as the blood vessels are refilled. This is followed by a period of hyperæmia. The four phases therefore are: (1) the back thrust, (2) the diminution or arrest of the inflow, (3) the overshoot during relaxation, and (4) the hyperæmic after effect. Under different conditions these separate phases may become less or more distinct or may be superimposed one upon another. The hyperæmia reaches its maximum a short time after the relaxation of the muscle; it continues for a variable length of time depending on the strength and length of the preceding muscular contraction and on the general condition of the muscle. In a fresh warm

muscle which is kept in a good condition the hyperæmia is considerably more conspicuous, but at the same time it disappears much more rapidly than in a fatigued or cold muscle. The first three changes in the arterial blood flow are due to a compression of the blood vessels by the contracting muscle fibres. This compression has also been observed in the heart, skeletal muscles, diaphragm and in the smooth muscles of the small intestine [Anrep, 1935]. The degree of compression of the blood vessels depends on the strength of the muscular contraction, that is, on the number of muscle fibres participating in it and not on the nature of the contraction whether isometric or isotonic. The fourth phase—the period of hyperæmia, has been explained since Gaskell's time by the peripheral vaso-dilator action of locally produced metabolites.

The object of the experiments described in this communication was to study the effect of muscular contraction upon the venous outflow of blood from the muscle, to correlate the changes of the outflow with those of the arterial inflow, to provide, if possible, a direct proof of the chemical mechanism of the hyperæmia and to study the time relations between the muscular contraction and the liberation of the chemical vaso-dilator substances responsible for the hyperæmia.

Experimental procedure and method.

The experiments were made on the gastrocnemius muscle of dogs. The animals were anæsthetized with chloroform and ether followed by chloralose (0·07 g. per kg. body weight). The animal's temperature was controlled at about 37·5° C. by warming in the winter and cooling in the summer, and most stringent precautions were taken to avoid drying of the tissues. The blood vessels of the gastrocnemius were dissected as described in the paper referred to above [1934]. The dissection was made with the utmost care, and it was not considered satisfactory unless the venous outflow was within $\frac{1}{2}$ c.c. per min. equal to the arterial inflow into the muscle. Only under these conditions could one be reasonably certain that no blood was drained by some accessory veins, and that the muscle was not receiving some additional blood supply from collateral arteries. The dissection of the blood vessels was verified after the end of each experiment by the injection of a fine suspension of Indian ink into the muscle artery. After the end of the dissection the muscle was left to rest for about 1 hour. In some experiments the gastrocnemius muscle was perfused at a constant pressure with blood freshly drawn from the experimental animal. The periods of such perfusions were always short, they

were interposed between long periods of a normal blood supply from the animal's own circulatory system (autoperfusion). The blood of the whole animal was rendered uncoagulable by a mixture of chlorazol fast pink and heparin, half the necessary dose of each being used. This mixture serves as a very satisfactory anticoagulant. It does not lead to the toxic effects which sometimes occur when the dye is used alone, and it is less prohibitive in price than pure heparin. Either American heparin, or heparin from Messrs Schering, was used. The latter was the weaker and had to be used in larger doses. In between the observations the blood of the gastrocnemius vein was allowed to flow back into the animal. During the observations it was diverted to a hot wire anemometer of the usual construction [Anrep and Downing, 1926]. In many experiments a simplified Bayliss blood-pressure compensator was employed. This consisted of a large separating funnel containing about 200–300 c.c. of heparinized blood. The compensator was connected to a T-piece tube, one branch of which led to a mercury manometer and the other to the central end of the carotid artery. The surface of the blood in the funnel was exposed to a constant pressure approximately equal to the mean arterial blood-pressure of the animal. The top opening of the separating funnel was connected to a Woulffe's bottle below the animal. The Woulffe's bottle was in turn connected with a pressure bottle filled with water and placed at any desired height. This simple compensator was found to answer the purpose. The muscle was made to contract by stimuli applied to the cut or uncut gastrocnemius nerve, and the following methods were used for this purpose: a faradic current from an induction coil (40–70 per sec.), condenser discharges the frequency of which was regulated by a rotating commutator (50–6000 per sec.) [Hill, 1934] or an oscillating valve circuit (150–600 per sec.). In some experiments the muscle was stimulated directly by means of specially constructed electrodes, one of which was placed on the tendon while the other surrounded the fleshy upper attachments of the muscle.

The effect of tetanic contractions upon the venous outflow from the skeletal muscles.

Sadler [1869] and later Gaskell [1877] have shown that the venous outflow from a muscle is greatly increased at the beginning of a tetanic contraction. As the tetanus continues the outflow rapidly diminishes or even stops. After the end of the tetanus the blood flow increases considerably above normal. The large initial increase in the outflow and its

following diminution were considered to be caused by a compression of the intramuscular blood vessels. The increase of the blood flow observed as an after effect of contraction was attributed to the potent chemical vaso-dilator substances which were released in the muscle during contraction. Burton-Opitz [1903] made somewhat more exact measurements of the venous outflow, and confirmed the findings of the previous observers, except that he failed to observe the prolonged increase of the blood flow following tetanic contractions.

In striking contrast with these conclusions stand the observations of Keller, Loeser and Rein [1930] who measured the blood flow in the femoral artery and vein by means of the thermostromuhr. Rein finds that the first effect of the tetanic contraction is an abrupt increase in the arterial inflow into the muscle. This is followed by a period, extending throughout the whole contraction, in which the flow is either equal to the resting blood flow or gradually increases above normal. The termination of the tetanic contraction is immediately followed by hyperæmia. Rein considers that the contraction of the muscle produces no compression of the intramuscular blood vessels. In this he follows the theoretical conclusion of Spalteholz [1924] based on the anatomical distribution of the blood vessels in the muscles, in which the finer vessels and the capillaries run parallel to the cylindrical muscle fibres. Since on contracting the fibres become thicker the inter-spaces between them should become wider, and therefore the blood vessels should offer a smaller resistance. The contraction of the muscle is therefore regarded by Rein as favouring the blood flow by reducing the resistance in the intramuscular blood vessels. The initial increase in the arterial inflow is considered by Rein to be due to an abrupt diminution of the resistance to the blood flow, and he compares it with the concurrent increase in the venous outflow described by the previous authors. He thus differs from previous observers in that he looks upon the outrush of the venous blood from the contracting muscle as a real increase in the circulation of the blood through the muscle, and not as a result of the compression of the blood vessels. Certain difficulties in accepting this view have been pointed out by one of us in a previous communication [1934], but a real proof for or against it can be obtained only through a comparison of records of the inflow and outflow of blood in the contracting muscle. Fig. 1 shows such records. The gastrocnemius nerve was stimulated for 0·15 sec. with condenser discharges of a frequency of 400 per sec. The inflow and outflow of blood from the muscle were recorded by two hot-wire anemometers of an equal sensitivity. The inflow was recorded before and after the introduction of a sensitive valve

into the arterial stream so as to abolish the first phase in the change in the blood supply, which was referred to above as the "back thrust". Owing

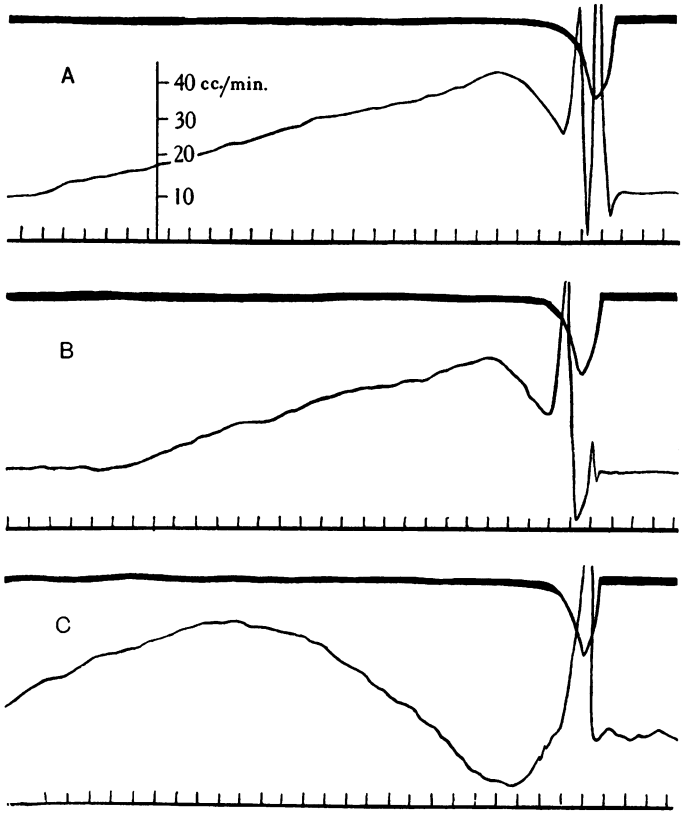

Fig. 1. Bottom line is base line for blood flow. Time units, 0·1 sec. Middle line is hot-wire record of blood flow. Top line shows contraction of gastrocnemius. *A*, The inflow of blood showing the back thrust, the succeeding diminution of the blood flow, the overshoot and the following hyperæmia. *B*, A similar record with a valve in the artery to prevent the back thrust. The small upstroke is caused by the closure of the valve. The record shows an almost complete arrest of the blood flow during contraction. *C*, The outflow of blood. This is increased during contraction; on relaxation of the muscle it becomes diminished below the resting level and the hyperæmic reaction is considerably delayed. Read from right to left in all figures.

to the nature of the recording system this backflow appears as a deflection of the record in the same direction as that due to an increase in the blood

[1] The tracings reproduced in this paper are original records which, in order to facilitate their publication, have been retraced with waterproof Indian ink and bleached with potassium ferricyanide. A comparison of an unbleached and a treated record is published in the *Proc. Roy. Soc.* B, **114**, 234.

flow to the muscle. The second phase, the overshoot and the following hyperæmia all being changes of the blood flow in the forward direction, are unaffected by the valve. It is seen, on comparing the arterial inflow curve with that of the venous outflow, that during the period when the inflow is diminished the outflow is greatly increased. This increase lasts throughout the short period of contraction. The blood flow then rapidly declines to below normal after which it again increases during the period of the hyperæmic after effect. The hyperæmia develops, however, very much slower on the venous side than on the arterial side.

The effect of a prolonged tetanus is fundamentally the same. Fig. 2 shows a simultaneous record of the inflow and outflow during and following a maximal tetanus of about 5·5 sec. A valve was introduced into the arterial system. At the onset of the contraction the arterial inflow

Fig. 2. Simultaneous registration of inflow and outflow of gastrocnemius. Two hot-wire anemometers were used. Time units, 0·1 sec. Valve used in arterial stream to prevent back thrust of blood. Arterial inflow, dotted line; venous outflow, continuous line. The thick uppermost line records the muscular contraction; maximum tension developed, 2·7 kg.

rapidly diminishes and stays at zero until the end of the tetanus, after which an abrupt overshoot and a rapidly developing hyperæmia follow one another. The venous outflow, on the other hand, is greatly increased at the beginning of the tetanus. This increase rapidly subsides during the early part of the tetanus, and ultimately the blood flow stops completely, so that during the latter part of the tetanus there is no circulation through the muscle at all. On relaxation the inflow gradually increases; during the period of the hyperæmia the venous outflow becomes again equal to the arterial inflow.

The records obtained during short and prolonged tetani support the conclusions reached by the previous authors and by ourselves; they do not conform with the deductions made by Rein. The venous outflow is increased during a simultaneous diminution of the arterial inflow. Such conditions cannot arise as a result of diminished intravascular resistance, they can only be explained by a vaso-compression.

It is well known that the average blood flow through a rhythmically contracting muscle is considerably increased above normal. This is not incompatible with the fact that the muscle produces by its contraction a compression of the blood vessels. The blood flow is increased not during but in between the contractions. After each period of activity the blood flow rapidly increases. If a second contraction of the muscle is evoked during the hyperæmia caused by the first contraction, it leads in its after effect to a still further increase in the blood flow. The after effects of a series of contractions summate with each other. The ultimate extent of the increase in the blood flow between the contractions depends on the duration of the contractions and on the length of the pause between them. If the contractions rapidly succeed each other, the vascular dilatation has no time to become fully effective. Very characteristic changes are

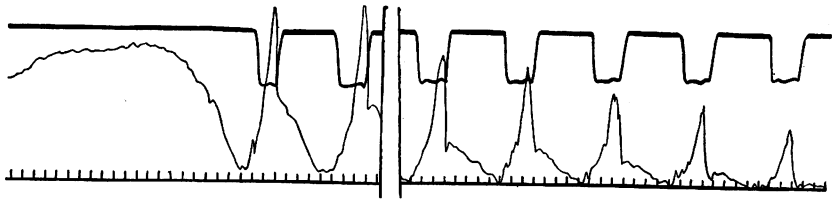

Fig. 3. Outflow of blood during rhythmical contractions of gastrocnemius. Right hand side of figure shows effect of first five contractions. Six contractions then omitted. The following two contractions as well as the hyperæmic after-effect are reproduced on the left hand side of the figure. Time units, 0·2 sec.

observed in the venous outflow during the period of rhythmic contractions of the muscle (Fig. 3). The resting muscle contains a relatively small amount of blood, therefore the first contraction leads only to an insignificant expulsion of blood. In between the contractions the emptied blood vessels are refilled with blood and as the hyperæmic after effect makes its appearance the muscle contains a progressively larger amount of blood at the beginning of each succeeding period of contraction. The expulsion of blood becomes more and more conspicuous and the refilling more rapid, so that a considerable increase in the outflow of blood can be observed in between the contractions. After the last contraction the hyperæmic reaction develops without being broken up by the interference from a further compression by the muscle. The blood flow thus becomes markedly increased during the period of a rhythmic activity of the muscle, and this increase remains as a more or less prolonged after effect.

Characteristics of the hyperæmic reaction following muscular contraction.

All other conditions being equal the vascular dilatation which follows muscular contraction depends on the strength of the preceding period of contraction. The amount of work done by the muscle and the nature of it,

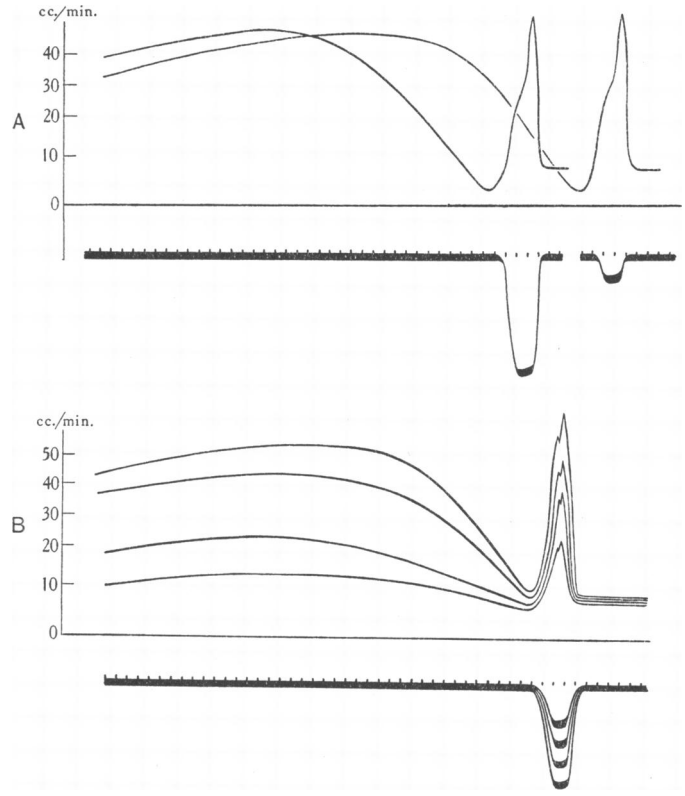

Fig. 4. Venous outflow from gastrocnemius. Effect of contraction and of the following hyperæmia. *A*, In the first record the tension was 4·5 kg., in the second it was 200 gm. Both effects on outflow unaltered by the change of tension. *B*, Strength of contraction progressively increased by strengthening stimulus to nerve. Both effects increase as the stimulus increases. Time units, 0·2 sec.; the base line of the blood flow is shown above the time.

that is, whether it consists in lifting a small weight to a considerable height or a large weight to a small height, does not influence the degree of after dilatation. Both the diminution of the blood flow through the muscle during its contraction, and the dilatation which follows the

contraction depend only on the number of muscle fibres involved, and not on whether the contraction proceeds in isotonic or, as far as possible, isometric conditions. This holds true, of course, only when the state of the muscles remains unchanged during the experiment. In other words, in experiments with electrical stimulation of the muscle, the hyperæmic after effect depends on the strength of the stimulus and not on the work performed. Fig. 4*A* shows two superimposed records taken from an experiment in which the muscle was made to lift gradually increasing weights. The increase in the blood flow is the same in all cases. In contrast with this Fig. 4*B* shows four superimposed tracings which were obtained from the same muscle when it was stimulated with different strengths of current. With each increment of the contraction the hyperæmia becomes more conspicuous and lasts for a longer time. This continues until the maximum strength of contraction is reached, after which the hyperæmia is not increased any further so long as the duration of the contraction remains the same.

The independence of the hyperæmia on the work performed by the muscle has already been suggested by Rein. The experiments described here completely confirm his conclusions. Since it is reasonably certain that the strength of contraction of the muscle, under constant conditions, depends on the number of fibres participating in this contraction, it must be concluded that each muscular unit produces on its contraction a definite quantum of vaso-dilator influence. These vaso-dilator influences easily undergo summation so that, while a short and weak contraction may not be followed by a period of increased blood flow, a series of such weak contractions may gradually lead to a fairly appreciable hyperæmia.

The chemical nature of the hyperæmia.

It is a matter of surprise that, although the chemical nature of the hyperæmia following the muscular contraction has always been taken for granted, there are no direct experiments proving it. The researches of Krogh [1922] and chiefly of Lewis [1927] and his co-workers make it more than likely that strong vaso-dilator substances are produced in the tissues during a temporary arrest of the blood supply to them, and that most probably the same substances are responsible for the hyperæmia which follows muscular contraction. This conclusion is chiefly based on the analogy of the effect of temporary occlusion of the circulation and of the action of various known vaso-dilator substances, as well as on the fact that the hyperæmic reaction is obtained also after the degeneration

of the nerve supply to the tissues. Most of these experiments, however, were made on the skin and not on the skeletal muscles.

In order to obtain a direct proof of the chemical nature of the vasodilator effect, we designed experiments the technique of which is shown

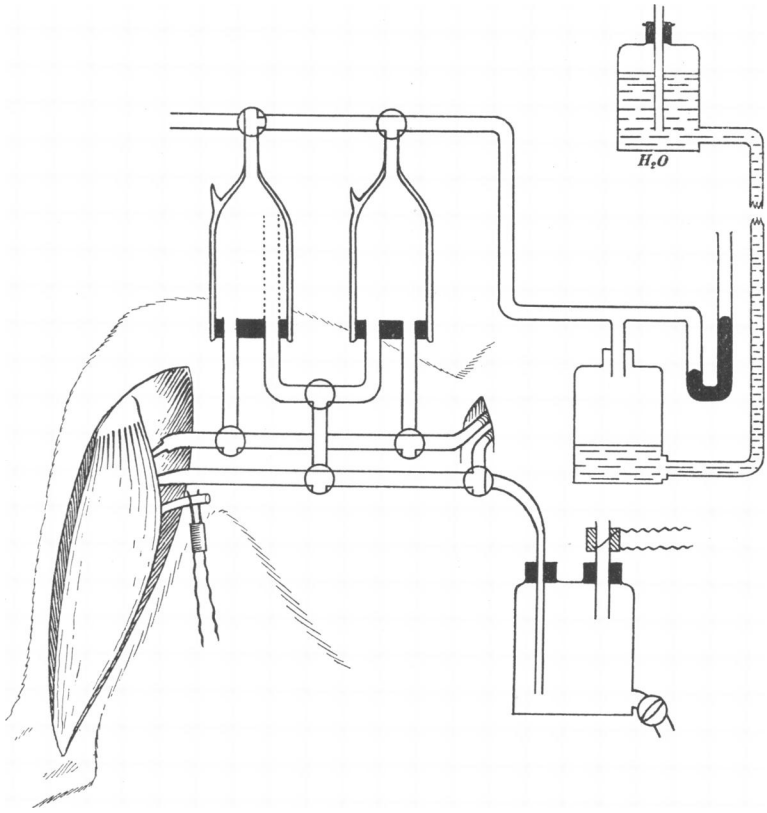

Fig. 5. The method used for reperfusion. The two Dewar flasks can be filled with arterial blood or with the venous blood emerging from the muscle. The venous blood can be diverted, to return to the animal or towards the hot-wire anemometer. In some experiments specially constructed electrodes were applied directly to the muscle. The extension of the glass tube shown by dotted lines in the left-hand vacuum flask was used only in the experiments on oxygenation. The pressure system shown on the right side of the figure was used for reperfusion of the blood through the muscle.

in Fig. 5. A cannula was introduced into the femoral artery pointing towards the heart. The blood emerging from the artery could be diverted to either of the 30 c.c. Dewar flasks, shown on the figure, without interrupting the blood supply to the muscle. The blood emerging from the

muscle vein could be returned to the animal *via* the femoral vein, diverted to the hot-wire anemometer or collected into either or both of the vacuum flasks. During the collection of the venous blood the taps at the top of the vacuum flasks were opened to the atmospheric air, and a gentle suction was applied so as to prevent the venous pressure from rising during the gradual filling of the flasks. In this manner the vacuum flasks could be filled with arterial blood or with venous blood emerging from the muscles during periods of rest or during periods of contraction. The blood stored in the vacuum flasks was then reperfused through the muscle. For this purpose the tap on the top of the vacuum flask was opened towards the constant-pressure bottle shown on the right-hand side of the figure. The pressure necessary for the reperfusion could be varied within a wide range. Usually the perfusion pressure was maintained at such a height that the blood flow through the muscle was equal to that observed during the autoperfusion by the animal's own arterial pressure. This was obtained when the perfusion pressure was kept approximately at the height of the mean arterial pressure of the animal (recorded by a Hürthle manometer). A constant hot-wire registration of the blood flow greatly helps to set the perfusion pressure correctly. The effect of perfusion of the muscle with arterial blood was then compared with that of reperfusion with venous blood. In some experiments one large vacuum flask was used instead of two small ones.

Freund [1920], Phemister and Handy [1927] and others have shown that any mechanical disturbance, even an insignificant agitation, of the blood imparts to it strong vaso-dilator properties. Zipf [1931] has advanced evidence that this is due to an increase in the concentration of adenylic acid in the shaken blood. A more severe agitation leads to an appearance of some vaso-constrictor substances which are probably identical with the "Spät-gift". In view of these changes which appear on shaking, the blood must be collected in the flasks with avoidance of rough mechanical disturbances. This is not difficult, since the degree of agitation which is unavoidably caused by the inrush of the blood into the flask is insufficient to produce any of these changes even when the blood is allowed to fill the flask with a full jet from a pulsating artery. Besides the vaso-dilator effect which shaken blood has on the blood vessels of the muscle, it also temporarily diminishes the hyperæmic reaction which follows muscular contraction. For example, the normal blood flow through the gastrocnemius was 7·5 c.c. per min., two successive tetanic concentrations of the muscle of 0·2 sec. each, were followed by a hyperæmia with a maximal blood flow of 37 c.c. per min. and lasting nearly a minute.

The same reaction was obtained four times in succession. The muscle was then perfused with 25 c.c. of arterial blood gently shaken for 2 min. This caused a considerable increase in the blood flow which, however, rapidly returned to normal as soon as the muscle was reconnected with the animal's femoral artery. The two short tetani were then repeated, but they were followed by almost no hyperæmia at all. The hyperæmic response remained subnormal for some time and the succeeding contractions of the muscle evoked at intervals of 90 sec. after perfusion with the "traumatized" blood caused a maximal hyperæmia of 17 and 32 c.c. per min. Only on the fourth application of the stimuli was the normal hyperæmic response of 38 c.c. per min. again obtained.

This noxious effect of traumatized blood explains the reason why it is more difficult to obtain constant and definite hyperæmic responses in muscles which are perfused with defibrinated blood or, generally speaking, with blood which is agitated by a perfusion apparatus outside the organism. During such perfusion the hyperæmia following muscular contraction gradually becomes less conspicuous and finally disappears. This may happen long before the resting blood flow undergoes a noticeable diminution.

The venous blood which is collected with the above elementary precautions during muscular rest causes, on reperfusion, no change in the blood flow through the muscle. The resting venous blood like the arterial blood can be kept in the Dewar flask for many minutes, and so long as its temperature remains constant it does not acquire any vasomotor properties. On the other hand, the vaso-dilator properties of the venous blood which is collected during rhythmic contractions of the muscle are quite definite. On reperfusion through the muscle this "active" venous blood produces a distinct hyperæmia which rapidly disappears after the return to the perfusion with arterial blood. The vaso-dilation is, however, much smaller than that observed during the period of rhythmical contractions, that is, during the period of collection of the "active" venous blood. The vaso-dilator effect of the blood collected during muscular activity varies a great deal from one experiment to the other. In some it is quite conspicuous, while in others it is negligible. The cause of this variability is not difficult to find. If we deal with the production of some definite quantity of vaso-dilator substances, then their concentration in the venous blood must decrease when the hyperæmia caused by them increases. So that while the vaso-dilator substances may be produced in considerable amounts, the increase in the blood flow may be so large that they are washed out of the muscle and

diluted to an extent which makes their determination difficult. It was hoped that the following procedure may diminish the effect of such a dilution. The arterial blood supply of the muscle was restricted by means of a finely adjustable screw clamp; the clamp was screwed down to such an extent that it would just fail to diminish the resting blood flow, but would greatly diminish the magnitude of the hyperæmia. By diminishing the large inrush of blood into the muscle we expected to obtain a higher concentration of the vaso-dilator substances in the venous blood. Reperfusion of the venous blood collected under these conditions evoked a most conspicuous hyperæmia (Fig. 6).

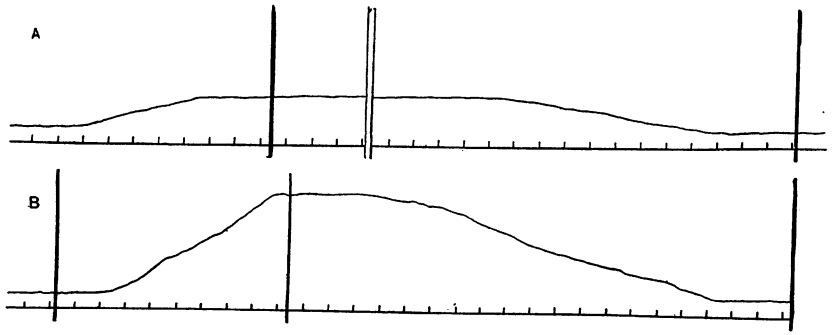

Fig. 6. *A*, Effect on venous outflow of reperfusion of active venous blood collected during rhythmical contractions of the gastrocnemius. Blood flow not controlled. Resting blood flow, 5 c.c. per min., maximum blood flow during contractions, 37 c.c. per min. At the first black line on the right perfusion with active venous blood started; at the second line, the muscle was reconnected with the femoral artery. Interval equal to 20 sec. of record omitted. *B*, The same experiment except that the blood flow had been controlled during the collection of venous blood. The maximum blood flow increased during the rhythmical contractions from 5 to about 15 c.c. per min. First black line—beginning of reperfusion; second line—perfusion with arterial blood from the second Dewar flask; third line—re-establishment of normal circulation. Time units, 4 sec.

The hyperæmia which is obtained on reperfusion of the active venous blood collected under these controlled conditions is of the same order of magnitude as that which normally follows rhythmical contractions of the muscle of the same duration. For example, in one experiment the rhythmical stimulation of the gastrocnemius produced an increase in the blood flow from 7 to 49 c.c. per min. The blood which was collected during another similar period of rhythmical contractions, with a controlled blood flow, produced on perfusion through the muscle an increase in the blood flow from 7 to 46 c.c. per min. In order to demonstrate the presence of vaso-dilator substances in the blood it is not necessary

to have a perfectly constant blood flow during the collection of the blood from the rhythmically contracting muscles. The blood flow may be allowed to double or even to treble during the period of collection, the vaso-dilator action of the active venous blood still remaining considerable. The blood which is collected at the time when the normal hyperæmia is in its decline has a very much less pronounced vaso-dilator effect, and the blood collected after the end of the hyperæmic period has none.

Stability of the vaso-dilator substances.

The hyperæmic properties of the active venous blood are extremely stable. At least they were not found to diminish on keeping the blood in the vacuum flask for about 20–30 min. The vaso-dilator properties of the active venous blood seem to undergo no appreciable deterioration even when the blood is allowed to remain for some time in contact with the tissues. At any rate, under these conditions the destruction of the vaso-dilator substances is not a rapid one. This can be seen from the following experiments. The artery to the muscle is clamped for some definite length of time, and the muscle is stimulated either at the beginning or in the middle or just before the end of the clamping. The artery is then opened and the hyperæmia is recorded. Had a certain destruction of the vaso-dilator substances taken place, the hyperæmic effect of the contractions evoked early within the period of clamping would be somewhat smaller than that of the late contractions. The result of the experiments is, however, just the reverse. The contractions which are evoked in the beginning of the period of clamping invariably have a considerably greater hyperæmic after effect than those evoked at the end of clamping. The following table gives some examples of this.

Table I. The hyperæmic effect of two short tetani of 0·2 sec. each evoked during the suppression of the blood flow through the muscle.

The stimuli were applied at different intervals of time after clamping the muscle artery. The hyperæmia is recorded after the release of the artery. The figures give the maximal increase of the blood flow above the resting blood flow in cubic centimetres per min. The total period of clamping was equal to 20 sec. in each case. The resting blood flow was 6 c.c. per min.

Clamping of artery for 20 sec.	5
Two contractions without clamping	12
„ „ at beginning of clamping	42
„ „ at 5th second of clamping	35
„ „ at 12th second of clamping	29
„ „ at 18th second of clamping	23
„ „ at the beginning of clamping repeated	44

A large number of similar experiments was made and the result was always the same. The duration of the clamping of the artery was varied

from a few seconds to 3 min. So long as the preparation of the blood vessels of the muscle has been properly carried out, all muscular contractions occurring towards the end of the arterial occlusion are followed by a somewhat less conspicuous hyperæmia than that which follows the early contractions. If, however, there is some circulation in the muscle through collateral channels, during the period of arterial clamping, then the hyperæmia caused by the early contractions is distinctly smaller than that which follows the contraction occurring towards the end of clamping. Most probably a certain amount of the hyperæmic substances which are, so to speak, trapped inside the muscle are washed out by the collateral blood flow. With this exception the results are conclusive in showing that the vaso-dilator properties are not rapidly destroyed when in contact with the tissues.

This conclusion does not explain why the hyperæmia is so much larger if the contractions are evoked during the early period of the arterial occlusion. We should like to advance the following as a most likely explanation of this curious phenomenon. Since the blood vessels are normally in the state of a partial tonic contraction, some definite time is necessary for them to lose this tone and to relax under the action of the vaso-dilator substances. Moreover, these substances must also take some time to diffuse from the muscle fibres to the blood vessels. In the condition when the artery is occluded, enough time is given for the development of the maximal vaso-dilator effect corresponding to the concentration of the hyperæmic substances. If, however, fresh blood is allowed to rush into the dilating blood vessels, a certain amount of the vaso-dilator substances is washed out of them before the dilatation becomes complete. In consequence of this the hyperæmic response becomes less conspicuous. A contraction occurring during the latter part of the period of arterial occlusion causes comparatively little vaso-dilatation because the substances are washed away before they have produced their full effect.

On applying two or three stimuli to the muscle at various moments of the arterial clamping, it is possible to determine the length of time necessary for the development of the maximal vaso-dilator effect. For instance, the artery is occluded for 3 min. each time, two short tetani are evoked at different moments of the occlusion and the vaso-dilator effects which follow the release of the artery are measured. All the contractions which occur more than about 20 sec. before the release of the artery gave approximately the same hyperæmic effect. The hyperæmia following the contractions occurring within the period 20 sec. before the release of the artery, is the smaller the nearer the contractions are

towards the moment of re-establishment of the circulation. On the average of all experiments 15–20 sec. can be taken as the time necessary for a complete development of the maximal vaso-dilation in the contracting muscle. In some experiments this period was as long as 30 sec. It must be again emphasized that these relations cannot be observed in the presence of a collateral circulation in the muscle. If the collateral blood flow is large, the hyperæmic effect of the early contraction is invariably greatly reduced as compared with that of the contraction occurring towards the end of the occlusion.

Comparison of the hyperæmia following the rhythmic and tetanic contractions.

There is no reason to suppose that the vaso-dilatation following a prolonged tetanus of a skeletal muscle should differ in any way from that which follows rhythmical contractions of an equal duration. If, however, a comparison of the two is made in a muscle the circulation of which is free, it is invariably found that the tetanus produces a considerably greater hyperæmia. This must again be explained by a certain amount of the vaso-dilator principle being washed out of the muscle in between the rhythmical contractions, while in the case of the tetanus it is retained owing to the vaso-compression lasting throughout the whole period of tetanus. The correctness of this explanation can be shown if the rhythmical and the prolonged tetanic contractions are evoked during a temporary suppression of the circulation through the muscle. For example, in one experiment the resting blood flow through the gastrocnemius muscle was equal to 7 c.c. per min.; the artery was clamped and five short tetanic stimuli 0·4 sec. each were applied to the muscle. Five seconds after the last contraction the artery was released—the maximal hyperæmia equalled 18 c.c. per min. The experiment was now repeated with a tetanic stimulus lasting for 2 sec. The hyperæmia observed on release of the artery was exactly the same as in the first case. Fifteen rhythmic stimuli led, under these conditions, to a hyperæmia of 45 c.c. per min., and a tetanic contraction of 6 sec. in duration increased the blood flow to 43·5 c.c. per min. In the above experiment a compression of an artery lasting for 10–15 sec. without any contractions of the muscles was followed by a negligible hyperæmia of a few cubic centimetres.

THE MOMENT OF APPEARANCE OF THE VASO-DILATOR SUBSTANCES IN RELATION TO THE MUSCULAR CONTRACTION.

It is suggested by Rein that the production of the vaso-dilator substance occurs during the contraction of the muscle, but that they are not liberated from the muscle fibres until the moment of relaxation. This suggestion is not based on any experimental evidence, but is advanced in order to explain certain contradictions arising between Rein's theory and Rein's experiments. Like most previous observers, Rein explains the hyperæmia by a liberation of the vaso-dilator substances. Since, however, he considers that muscular contraction does not lead to a compression of the blood vessels, but on the contrary is accompanied by some diminution of their resistance, it becomes difficult to explain why the vaso-dilator substances fail to show their effect during the period of a tetanus, but do so after the relaxation of the muscle. It is in order to overcome this difficulty that the theory was evolved about the liberation of the vaso-dilator substances only after the onset of the relaxation of the muscle. This does not conform with the opinion expressed by other observers who consider that the vaso-dilator substances are liberated during the contraction of the muscle but prevented from exerting their effect on the blood flow because of the compression of the blood vessels by the surrounding contracting muscle fibres. Our own experiments which were specially made for the purpose of testing Rein's theory also fail to support his point of view. We believe that the production and liberation of the vaso-dilator substances both take place during the period of muscular contraction. This suggestion rests on the following three groups of experiments.

THE SIMILARITY OF THE HYPERÆMIC RESPONSES WHICH FOLLOW RHYTHMICAL CONTRACTIONS AND PROLONGED TETANI.

If the vaso-dilator principles are not released before the relaxation of the muscle, then the rate at which the hyperæmia develops must be more rapid after a number of rhythmic contractions than after a tetanic contraction of the same duration. For instance, five rhythmic contractions would be followed by five relaxations, each of which is supposed to contribute a certain amount of vaso-dilator material so that at the end of the last contraction the blood vessels would be to a considerable extent relaxed. At the end of a tetanic contraction of the same duration the whole amount of the vaso-dilator material would be released at once. It

was seen from the experiments described before that the amount of the vaso-dilator substances produced in both cases is the same. However, in view of the different timing, as suggested by Rein, one would expect a considerably more rapid development of the hyperæmia following rhythmic contraction as compared with a single tetanic contraction of the same duration. Such comparisons cannot, of course, be made unless the blood flow through the muscle is stopped while the muscle contracts, so that none of the vaso-dilator substances can be washed away. Extreme precautions must also be taken to avoid any collateral communications with the muscle under observation.

Under these conditions it could be seen in every experiment that not only the extent of the vaso-dilatation but also the rate of its development and disappearance are exactly alike in both cases. For example, five short contractions of 0·3 sec. each occurring at the beginning of a 20 sec. clamping of the artery caused, on the release of the artery, a hyperæmia of 34 c.c. above the resting blood flow. Exactly the same maximal hyperæmia was observed when a tetanus of 1·5 sec. was evoked instead of the five short contractions. At intervals of 15 sec., following the release of the artery after the rhythmic contractions, the blood flow was 19, 9 and 2 c.c. above normal. All other experiments gave a similar result.

The hyperæmia during partial tetanus.

The previous experiments have shown that the blood flow through a strongly contracted muscle is completely stopped, while during a weak contraction it is only diminished. There is, however, no fundamental difference between weak and strong contractions. It is generally admitted that the difference is not a qualitive but a quantitative one. So long as the condition of the muscle is unchanged, a weak contraction of it should be looked upon as nothing else but a partial contraction, some muscle fibres contracting to their full extent and others not at all. There is no reason to suppose that what is true for a whole muscle should not also be true for a part of it. It should therefore be assumed that the circulation in a weakly contracting muscle mainly proceeds around the relaxed muscle fibres, while it is arrested in the blood vessels surrounded by the contracted fibres. This is suggested by experiments in which a fractional contraction of the gastrocnemius muscle was produced by stimulating a part of its motor nerve. For example, the muscle nerve was split longitudinally into two parts, either of which could be stimulated separately. The blood flow through the muscle was 13 c.c. per min. A strong stimulation of one part of the nerve reduced it to 8 c.c., a similar stimulation

of the other part reduced it to 5 c.c., and a stimulation of the whole nerve stopped the blood flow completely. Such relations can be observed only during the early period of strong tetanic contractions. As the tetani continue the relations between their effect on the blood flow become strikingly different. So long as the tetanus of the whole muscle does not diminish in strength, the circulation remains arrested to the end of the stimulation and hyperæmia appears only after relaxation of the muscle. If, however, the strong tetanic contraction is evoked in a fraction of the muscle by stimulation of a part of its nerve, the blood flow begins to increase already during the period of stimulation although the strength of the partial tetanus remains unchanged to the end. The blood flow may increase to a considerable extent. If the contracting fraction of the muscle is not very large in comparison with the resting fraction, the blood flow may increase well above the original blood flow through the whole muscle. On relaxation of the muscle the blood flow rapidly increases still further.

These experiments suggest that, since the strength of the maximal partial tetanus did not change during the period of stimulation, the dilatation is taking place in the blood vessels around the resting muscle fibres, that the vaso-dilator substances responsible for this dilatation are produced in the contracting fibres throughout the period of stimulation, and that from there they rapidly diffuse into the whole muscle. Exactly the same relations are observed when the whole nerve is stimulated with a comparatively weak current. Under these conditions the contraction of the muscle is also a partial tetanus. The blood flow at first abruptly diminishes, then gradually it begins to increase, reaches its resting value and continues to increase well above it. The maximal hyperæmia occurs immediately after the muscle relaxes.

Denny-Brown [1929], by direct microscopical observation of the surface of the soleus muscle during a stretch reflex, noticed that even a moderate amount of pull on the tendon opens up numerous capillaries and hastens the blood flow in them to a remarkable extent. As a result of this observation Denny-Brown believes that the capillaries are not compressed by the muscle fibres when these contract reflexly. Evidence has been advanced elsewhere [Anrep, Cerqua and Samaan, 1934] that there is no such fundamental difference between the reflex and the motor tetanus. The observation of Denny-Brown is nevertheless correct as we could satisfy ourselves on many occasions. The most likely explanation of this is that a stretch response of a muscle is nothing but a partial contraction, and that therefore the blood flow becomes considerably increased

in those blood vessels which lie around the non-contracting muscle fibres because of the diffusion of the vaso-dilator principles out of the contracting fibres.

LIBERATION OF VASO-DILATOR SUBSTANCES DURING TETANUS.

The following direct observation can be taken as finally deciding the question in favour of the theory that the vaso-dilator substances are produced and liberated during the whole period of contraction. The experiments were made with the help of our reperfusion method. A large Dewar flask was filled with arterial blood from the animal's artery. The

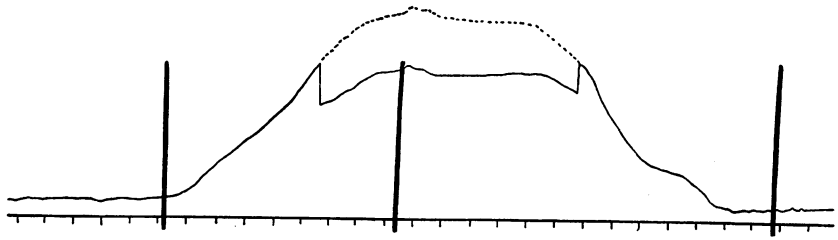

Fig. 7. Reperfusion of venous blood collected during a maximal tetanus. During the tetanus the blood flow was maintained by raising the perfusion pressure to 160 mm. Hg. The tetanus was begun a few seconds before the beginning and finished some time after the end of the collection of the venous sample. The active venous blood was perfused at the mean arterial blood pressure of the dog (110 mm. Hg.). First black line on the right—admission of the active venous blood; second line—perfusion with arterial blood; third line—reconnection with the femoral artery. In the middle of the tracing the string of the galvanometer went out of the slot, the galvanometer was therefore shunted through a resistance of 400 ohms. The dotted line represents the deflection as it would have been without the shunt being used. Time unit, 4 sec.

blood was then exposed to such a high pressure that a certain blood flow was maintained in spite of the compressing force of a maximal tetanus. The venous blood from the muscle was collected during the tetanus, the muscle being allowed to relax only after the end of the collection of the venous sample. The blood vessels of the muscle were then again connected with the femoral artery of the animal. A few minutes later, after the hyperæmia caused by the tetanus had subsided, the venous blood was reperfused through the muscle at a pressure equal to the mean arterial pressure of the animal. It caused a most conspicuous vaso-dilator effect. (Fig. 7.)

All these considerations lead to the same conclusion, namely, that the vaso-dilator principles are produced and released by the muscle during its contraction, but prevented from exhibiting an effect because of the compression of the blood vessels.

SOME PROPERTIES OF THE VASO-DILATOR SUBSTANCES RELEASED DURING MUSCULAR CONTRACTION.

It would be as yet premature to speculate as regards the nature of the vaso-dilator substances produced during muscular contraction. It is, however, possible to indicate some of their characteristics. Gaskell was the first to advance the theory of locally produced metabolites, and since acid products, especially lactic acid and carbonic acid, are the usual results of cell metabolism, it was natural to look for direct evidence of increased hydrogen-ion concentration. Gaskell indeed showed that lactic acid reduces the tone of the blood vessels, Bayliss [1901] demonstrated the same for carbonic acid, and Hooker [1912–13] and Fleisch and Sibul [1933] confirmed these results. Oxygen lack has also been shown to cause vaso-dilatation. We thus have a whole array of factors which, besides the more recently discovered specific vaso-dilator substances: histamine, acetylcholine, adenosine and the vaso-dilator effects of adrenaline, may play a role. It is difficult to see how any one of these specific vaso-dilator substances may account for the capillary as well as the arterial vaso-dilation observed as a result of muscular contraction. At present there is no clear evidence that any of the specific vaso-dilator substances are formed in physiologically active tissues. Rigler [1932] showed the liberation of some such substance during activity, but his conclusion that this substance was an adenylic compound was made without any direct evidence. It is, however, difficult to believe that such potent substances could be present in the tissues and yet fail to play a part in the local adaptation of the blood vessels. On the other hand Rous and Drury [1929] have demonstrated by direct observation a considerable increase in the hydrogen-ion concentration in the ischæmic tissues. The increase was so pronounced that there is every reason to suppose that acidity may under certain conditions control the blood flow. The acid metabolites may either act directly or indirectly through the release of some more specific substances.

The present evidence is rather of a negative character. It is very unlikely that acetylcholine plays any role, since eserine and atropine have no effect on the hyperæmia. It is also unlikely that the vaso-dilatation is produced by the same agent which appears in the blood when it is exposed to mechanical disturbance and which Zipf suggests is adenylic acid. The reactive hyperæmia following muscular contractions which are evoked after perfusion with shaken blood is distinctly smaller than that following contractions which are evoked after perfusion with venous

blood collected during activity. If the mechanical disturbance of the blood is considerable, as is frequent in artificially perfused organs, the reactive hyperæmia disappears altogether. There is another distinction. Shaken blood perfused through the muscle rapidly loses its vaso-dilator properties, active venous blood does not. In some experiments active venous blood was reperfused through the muscle four times in succession, recollected and tested for its hyperæmic effect; it was not found to be diminished. It has been mentioned already

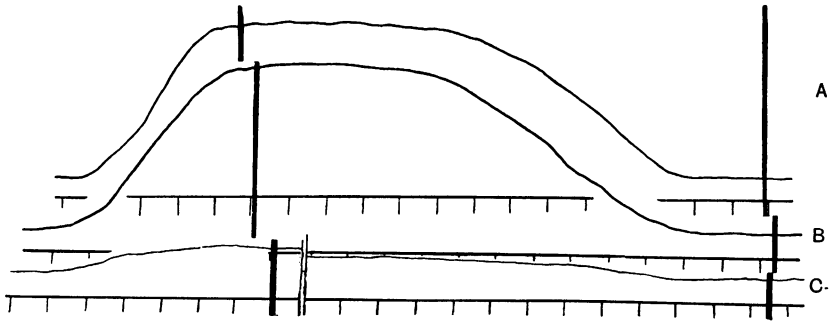

Fig. 8. *A*, Reperfusion of the active venous blood from a rhythmically contracting gastrocnemius with controlled blood flow. *B*, The effect of the third perfusion of the muscle with the same active blood. *C*, The effect of the fourth reperfusion with the resting venous blood. The black line on the right side—connection with the reservoir; second black line—re-establishment of normal circulation. This time line is in every case also the base line for the corresponding records from above downwards. Time unit, 4 sec. In tracing *C*, 32 sec. of record omitted.

that resting venous blood when reperfused leads to no hyperæmia. A certain hyperæmia is observed, however, when the same resting venous blood is reperfused through the muscle three to four times, but in no way is this hyperæmia comparable to that caused by the active venous blood (Fig. 8). There is, however, one similarity between the active venous blood and the resting blood which has been reperfused through the muscle for several times. Neither leads to a diminution of the hyperæmic after effect of muscular contraction. Some of these relations can be seen from Table II.

The question may be raised as to whether the contraction of the muscle fibres is wholly responsible for the production of the vaso-dilator substances appearing in the venous blood, or whether possibly some of these substances are produced in the various nerve endings situated in the muscle. Experiments with curare provide a conclusive answer. After curarization of the muscle, no diminution of the blood flow is observed

TABLE II. Showing the after effect of the active venous blood and of the shaken arterial blood upon the hyperæmia evoked by two short tetani of 0·2 sec. each.

The interval between the two tetani was equal to 0·4 sec. The figures give the maximal increase of the blood flow in cubic centimetres per min. The stimuli were applied in every case only after the blood flow had returned to the resting value. The resting blood flow was 6 c.c. per min.

Interval between the application of the stimuli	Maximal increase of blood flow above normal in c.c. per min
Two control stimuli	32
Repeated after 90 sec.	32
40 c.c. of active venous blood perfused through the muscle; the maximal hyperæmia during the perfusion was equal to	27
Two stimuli 72 sec. after return to arterial perfusion	35
Repeated 90 sec. later	34
40 c.c. of shaken arterial blood perfused through the muscle; maximal hyperæmia during the perfusion was equal to	30
Two stimuli 100 sec. after return to arterial perfusion	13
Two stimuli repeated after 90 sec.	26
Again repeated after 90 sec.	33

during the stimulation of the muscle nerve, no hyperæmia follows the stimulation, and the venous blood which is collected during the stimulation possesses no vaso-dilator properties. The direct stimulation of the curarized muscle is, on the other hand, accompained and followed by the usual changes in its blood flow. The above statement must be qualified in view of the fact that stimulation of the muscle nerve may lead to various vaso-motor changes; these, however, develop slowly, never bear any strict time relations to the moment of stimulation and cannot be confused with the vaso-compression and the following hyperæmia caused by muscular contraction. A comparison of the blood flow through normal and curarized muscle will form the subject of a further communication.

In order to determine how far the oxygenation of the blood plays a role, we introduced the following modification in the reperfusion apparatus shown in Fig. 5. The venous blood returning from the active muscle was collected in the right-hand Dewar flask, while a stream of oxygen was being passed through it. When this was filled the blood was slowly transferred into the left-hand flask along the tube which is represented in the figure by dotted lines. During this whole time oxygen was passed through the flask but not through the blood, so as to avoid any mechanical disturbance. The blood was transferred into this second flask so slowly that a considerable degree of oxygenation of it was affected. It did not lose, however, its vaso-dilator properties. It is possible that a de-oxygenation

of the blood or a shift of its hydrogen-ion concentration towards the acid side may be the cause of the liberation of some other vaso-dilator substances of a more specific nature, but it seems unlikely that the oxygen lack itself causes the hyperæmia. This is especially so since it is of frequent occurrence that the venous blood collected during rhythmical contractions of the muscle has a higher oxygen saturation than the resting venous blood. This is so obvious in some experiments that it can be seen with the naked eye.

Conclusions.

1. A comparison of the venous outflow and of the arterial inflow of blood into a contracting skeletal muscle shows that muscular contraction is accompanied by a compression of the intramuscular blood vessels. The suggestion made by Rein that the blood vessels of a muscle present during contraction a diminished resistance to the blood flow cannot be confirmed.

2. The compression of the blood vessels by the contracting muscle depends on the strength of its contraction and not on the character of its contraction—isometric or isotonic.

3. The vaso-dilatation following the contraction also depends on its strength, that is, on the number of muscle fibres participating in the contraction. In other words, it depends on the stimulus evoking the contraction, and not on the type of work which the muscle is allowed to perform.

4. Potent vaso-dilator substances appear in the venous blood emerging from a contracting muscle. These substances are stable in blood at least up to half an hour. A most conspicuous hyperæmia is observed when the venous blood collected during activity of the muscle is reperfused through it. This is not observed on reperfusion of the venous blood which is collected during rest. Even when the resting venous blood is reperfused through the muscle four times in succession its hyperæmic effect is small.

5. The theory of Rein, that the vaso-dilator substances are not released from the muscle fibres until the moment of their relaxation, finds no support in our experiments. The vaso-dilator substances are produced and released from the muscle during its contraction. The liberation of these substances continues for some time following the relaxation; how far their production also outlasts the period of contraction cannot as yet be ascertained.

6. Indirect evidence is provided to show that the vaso-dilatation following muscular contraction is not directly due to changes in the gaseous content of the blood.

REFERENCES.

Anrep, G. V. (1935). *Lane Medical Lectures.* Stanford University Publications. (In the Press.)

Anrep, G. V., Blalock, A. and Samaan, A. (1934). *Proc. Roy. Soc.* B, **114**, 223.

Anrep, G. V., Cerqua, S. and Samaan, A. (1934). *Ibid.* **114**, 245.

Anrep, G. V. and Downing, A. C. (1926). *J. sci. Instr.* **3**, 221.

Bayliss, W. M. (1901). *J. Physiol.* **26**, 32*P*.

Burton-Opitz, R. (1903). *Amer. J. Physiol.* **9**, 161.

Denny-Brown, D. (1929). *Proc. Roy. Soc.* B, **104**, 252.

Fleisch, A. F. and Sibul, I. (1933). *Pflügers Arch.* **231**, 787.

Freund, H. (1920). *Arch. exp. Path. Pharmak.* **86**, 266; **88**, 39.

Gaskell, W. H. (1877). *Arb. physiol. Inst.*, Lpz. **12**, 45.

Hill, A. V. (1934). *J. Physiol.* **82**, 423.

Hooker, D. R. (1912–13). *Amer. J. Physiol.* **31**, 47.

Keller, C. J., Loeser, A. and Rein, H. (1930). *Z. Biol.* **90**, 260.

Krogh, A. (1922). *The Anatomy and Physiology of Capillaries.* New Haven.

Lewis, T. (1927). *Blood Vessels of the Human Skin and their Responses.* London.

Phemister, D. B. and Handy, J. (1927). *J. Physiol.* **64**, 155.

Rigler, R. (1932). *Arch. exp. Path. Pharmak.* **167**, 54.

Rous, R. and Drury, D. R. (1929). *J. exp. Med.* **49**, 435.

Sadler, W. (1869). *Arb. physiol. Inst.*, Lpz. **12**, 45.

Spalteholz, K. W. (1924). *Die Arterien der Herzwand.* Leipzig.

Zipf, K. (1931). *Arch. exp. Path. Pharmak.* **160**, 579.