J. Physiol. (1964), **172**, *pp.* 123–137
With 1 plate and 2 text-figures
Printed in Great Britain

DIRECT OBSERVATION OF SKELETAL MUSCLE BLOOD VESSELS (RAT CREMASTER)

By R. T. GRANT

From the Department of Medicine, Guy's Hospital Medical School, London, S.E. 1

(*Received* 6 *January* 1964)

The recent description of the rat's cremaster muscle by Majno, Palade & Schoefl (1961) suggested that this muscle might provide a suitable site for direct observation of skeletal muscle blood vessels in the living animal. This proved to be so and the method developed allows the study of the reactions of the vessels to various stimuli applied topically and intravenously. It is thought that the vessels are seen in a state that, for a time at least, is nearly normal for resting skeletal muscle. The only comparable preparations that I can trace are those of Zweifach & Metz (1955*a*, *b*) and Hyman & Paldino (1962), but their observations do not cover the same ground as those now reported.

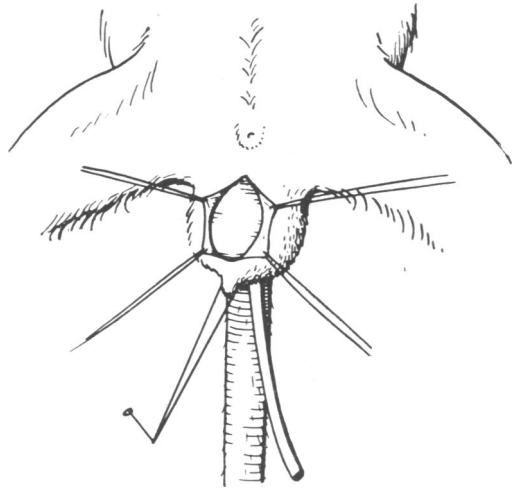
The observations are directed more to exploring the possibilities of the method than to solving particular problems. They show, however, that in general the vascular reactions, in terms of dilatation and constriction, are in keeping with those derived from blood flow studies in man and other animals. They also show that the dilatation resulting from the intravenous administration of adrenaline affects mainly the arteries, as with acetylcholine, rather than the minute vessels, as with histamine.

METHODS

Animals. A rat of about 100 g body weight is anaesthetized with urethane (150 mg) sometimes with pentobarbitone sodium (3·0 mg) injected intraperitoneally, and placed on its back on a cork mat with the hind legs widely splayed. Body temperature is regulated by an electric lamp placed above and moved nearer or further away as required. Temperatures are measured by a thermo-electric thermometer (copper-constantan junctions); body temperature from a polythene covered junction inserted into the colon about 5 cm beyond the anus, tail temperature (when required) by a junction attached near its tip with zinc oxide plaster; that of the fluid irrigating the exposed muscle by a junction attached to the tip of the irrigating tube (Text-fig. 2).

Blood pressure is measured (when required) from the carotid artery through a polythene tube, 0·58 mm internal diameter (PE 50, Clay Adams Inc., New York) and about 4 cm long, joined by a 3 mm (about) internal diameter tube (PE 330) to a miniature membrane manometer like that described by Sherrington (1919). Ringer's solution with heparin (10 u./ml.) perfused through the manometer at a rate of 0·5–1·0 ml./hr prevents clotting in the tube tip.

Intravenous injections are made into the external jugular vein through a fine polythene tube (0·28 mm internal and 0·60 mm external diameter, PE 10) connected through a three way Perspex tap (Armin & Grant, 1955) and a wider polythene tube (PE 50) to a motor driven syringe with variable gears (Armin, Grant & Wright, 1960) containing Ringer's solution. Infusion at the rate of about 0·5 ml./hr suffices to prevent clotting at the catheter tip. To avoid air embolism, the jugular vein is incised and the catheter inserted through a pool of Ringer's solution. Single doses of test substances are injected through the free nozzle of the tap in a volume of 0·05 or 0·1 ml. plus the small volume contained in the fine tube, usually about 0·01 ml. This small volume remains in the tube after the 0·1 ml. has entered the vein but, without visibly affecting the vessels, is slowly passed into the blood stream by the Ringer's solution perfused during the usual interval of at least 3–5 min between successive doses. It is therefore unnecessary to follow the injection with a washing-in dose. For the



Text-fig. 1. Shows the splayed thighs, tail and scrotum of a rat, the cable of a thermo-junction in the anus, the stitch with pin to extend the cremaster and the four stitches to retract the incised skin.

infusion of a vaso-active substance, the syringe containing it is substituted for that with the Ringer's solution alone and the pump gears adjusted to give the desired rate of infusion. Since the blood volume of a 100 g rat is only about 7 ml (Belcher & Harris, 1957), it is essential to keep the volume of fluid introduced into the vessels as low as possible to avoid overloading the circulation.

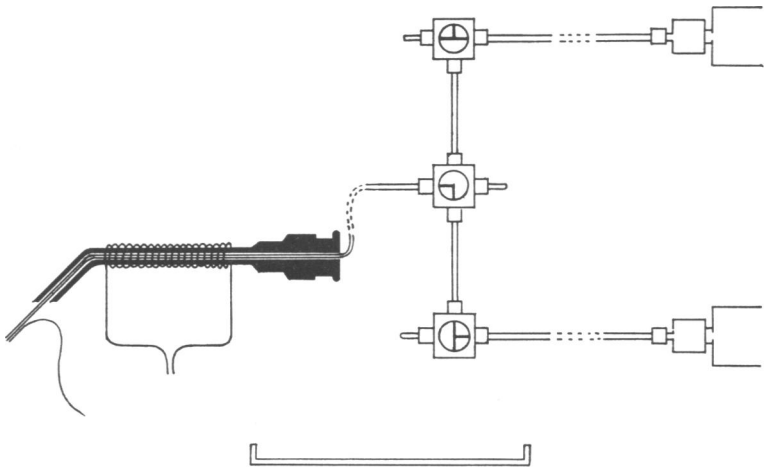
Cremaster exposure. A testis is pressed with the finger into the hind end of the scrotum while a stitch is passed through the scrotum and the hind pole of the testis. This stitch, fixed by a pin to the cork mat, prevents retraction of the testis and keeps the cremaster extended. The hair over the ventral surface of the scrotum is cut short. An incision about 1 cm long is made in the scrotum and four stitches are placed to retract the skin (Text-fig. 1). Irrigation of the exposed area is begun. The subcutaneous tissue is separated by gentle blunt dissection from the skin, so that the testis and its coverings sink beneath the level of the skin incision and lie in a shallow pool. Then, under a magnification of 25 diameters, the loose connective tissue covering the cremaster is picked up and gently pulled apart, taking care to avoid the larger vessels, so far as possible, for these may bleed for a time when torn through. The epimysium is left intact. The cremaster is now ready for examination by incident light;

a stereoscopic microscope mounted on a rack-work long arm stand and with a magnification of 50 diameters is suitable. Lighting is provided by a bench light with a condensing lens; a green filter serves both to cool the light and to increase the contrast between the tissues and the blood.

From the injection of the anaesthetic to completion of the exposure, including the insertion of the venous catheter, usually occupies no more than a half hour and can be carried out single handed. The insertion of the carotid catheter adds a few minutes.

Thigh muscle exposure. For comparison with the cremaster, the vessels of the inner aspect of the thigh are exposed and irrigated in the same way.

Irrigation of the exposed muscle. Text-figure 2 shows the arrangement of motor driven syringes, Perspex taps and connecting pieces and polythene tubes (Armin & Grant, 1955; Armin, *et al.* 1960) for conveying solutions to the muscle from either of two syringes without alteration of rate or significant interruption of flow. The rate of irrigation is adjusted to keep the muscle continuously immersed in a shallow pool and is usually from 10 to 20 ml./hr.



Text-fig. 2. Shows the arrangement of two syringes, Perspex connexions and three taps and polythene tubes, the hypodermic needle encircled by the heating coil and enclosing the irrigation tube with a thermo-junction attached to its tip. A drip tray is placed beneath the taps.

The time taken for a solution to pass from the centre tap to the pool is measured beforehand and is usually 15–30 sec. The terminal part of the tube leading to the muscle passes through a closely fitting hypodermic needle bent at its tip and surrounded by a heating coil of Nichrome wire. Heating is controlled by a variable resistance. The tip of the tube together with the attached thermal junction dips into the pool. The hypodermic needle is mounted in an easily adjustable clamp.

Solutions. Since the muscle vessels are very sensitive to introduced substances all apparatus must be clean. Solutions are freshly prepared for each experiment and are made from fresh double glass-distilled water. For irrigation, Ringer's solution with 1 % added gelatin is used (Chambers & Zweifach, 1944). Dilutions of stable substances to be tested, usually in a series of ten-fold dilutions, are made in this gelatin–Ringer's solution before the experiment. Dilutions of substances, such as adrenaline and noradrenaline, liable to break down quickly when in dilute solution in Ringer's solution, are made in NaCl solution (0·9 g/100 ml.) up to the penultimate ten-fold dilution. The final dilution is made in Ringer's solution just before

use. All solutions are kept in stoppered glass tubes in a Dewar flask with crushed ice until required. Doses withdrawn for intravenous injection are allowed to warm to room temperature before injection, so also are the larger quantities used for irrigation. The following are the substances mainly used; their concentrations stated in the text are in terms of the bases: adrenaline and adrenaline acid tartrate, noradrenaline bitartrate, histamine acid phosphate, 5-hydroxytryptamine creatine sulphate, acetylcholine perchlorate, succinylcholine chloride.

Minimum effective concentrations have been estimated approximately by applying successive ten-fold dilutions and approaching the threshold from either above or below. Each concentration is applied for 2–3 min and an interval of 3 min is allowed to elapse between the end of a reaction and the next application. To avoid confusion with a possible spontaneous change in vessel calibre, each concentration is repeated two or three times. For intravenous injection the volume has been kept constant in individual experiments at 0·1 or 0·05 ml. Changes of calibre have usually been judged subjectively, not only because of the interfering respiratory movement (see later) but also because with 50 times magnification, the smallest eyepiece micrometer subdivision represents 20 μ.

RESULTS

Exposure of the cremaster muscle and its blood vessels succeeds best in young rats of no more than about 120 g body weight. In them the scrotal subcutaneous tissue is thin and easily parted and has no or only an occasional capillary connexion with the epimysium and the muscle blood vessels. In older animals the connective tissue is thicker and tougher; the view of the vessels may not be so clear and connexions between the two blood supplies are liable to be more numerous and when torn across to give rise to petechial haemorrhages which interfere with the view.

Under the green filter, the muscle is pale and is clearly seen to be arranged in two thin sheets of parallel bundles, the deeper sheet at an angle to the superficial. Beneath the muscle the testis forms a pale background which acts as a reflector. Its superficial tortuous veins are indistinctly seen in the background (Pl. 1).

When first exposed, the muscle fibres sometimes show flickering, uncoordinated contractions like fasciculation. Half an hour or more may pass before these movements subside, but they are abolished in 5–10 min by adding succinylcholine chloride (100 mg/100 ml.) to the irrigating fluid. Usually when the muscle fibres are relaxed and quiet they remain so for several hours but, if the twitchings return, they may again be quietened by renewing the succinylcholine. This substance does not alter the calibre of the blood vessels, nor modify the vascular reaction to vaso-active substances, but it is inadvisable to continue its irrigation beyond about 10 min. On several occasions, but not always, when this was done, the rat developed respiratory failure, possibly the result of absorption of this substance into the general circulation. Though the muscle itself is quiet, the whole field moves slightly to and fro with respiration. I have not succeeded in abolishing this movement, but it is made minimal by adjusting the tethering of

the rat and the tension of the stitches. The eye soon accommodates itself to this slight movement, but it interferes with the use of a micrometer eyepiece and with photography (Pl. 1).

Also when the muscle is first exposed its blood vessels are more or less dilated. The larger ones lie between the two muscle sheets and their diameters range from 50 to 120 μ, the veins being wider than the arteries. Arteries are readily distinguishable by their straighter course, more uniform calibre, less profuse branching and the more rapid blood flow within them. Usually the arteries and veins do not run side by side in this part of the cremaster but follow separate courses not specially related to the direction of the muscle bundles. The arteries and their arteriolar branches anastomose freely with each other, as also do the veins, but arteriovenous communications larger than capillaries have not been seen, either in or between the muscle layers.

The minute vessels (terminal arterioles, capillaries and venules) lie between the muscle bundles in the sheets. Their diameters range from about 5 to 10 μ. Visibility is insufficient to recognize such detail at metarterioles and precapillary sphincters (Chambers & Zweifach, 1944) but easily seen is the branching of small arteries into arterioles, terminal arterioles and capillaries which emerge as collecting venules, arranged rather like candelabra. These features are shown, though rather indistinctly, in Pl 1. This also shows traces of the transversely placed minute vessels of the deeper muscle layer and behind that again a *U* of a testicular vein.

The initial dilatation soon begins to subside and, after the muscle is quiet, passes off. It passes sooner with urethane as the anaesthetic than with pentobarbitone. The larger arteries, usually the last to contract, are reduced to a half or a quarter of their initial diameters. Most of the smaller ones and the terminal arterioles disappear from view. The veins constrict but little and the leashes of collecting venules remain visible. Therefore, in the quiet resting state of the muscle, the obvious vessels are mainly veins, through which blood trickles slowly, with a few just visible small arteries and arterioles and a few minute vessels passing blood from one side of the circulation to the other. The open minute channels may be of considerable length, as much as 1–2 mm between points definitely recognizable as terminal arteriole on the one side and on the other as a collecting venule; these channels are no more than about 5–10 μ in diameter. An area injured during preparation shows as a patch of persistently dilated vessels.

Once this resting state is attained it may persist unchanged, or almost so, for several hours. Occasionally a small artery may show localized spindle shaped dilatations, which occur irregularly and change place, or a more uniform slight relaxation may allow a trickle of blood to pass through a group of minute vessels hitherto invisible. But an unstable field with

large and frequent change in arterial calibre is unusual in the early hours, though it may develop later, and is probably due to injury. Such preparations are unsuitable for observation. Stability of the field is promoted by avoiding mechanical stimulation and by securing steady and continuous irrigation.

The presence of the stitch extending the cremaster does not seem to alter the state of the muscle vessels. Without the stitch the vessels show the same features as with it, provided the muscle remains quiet and relaxed. It is, however, liable to contract more or less and so to throw the vessels into corkscrew-like folds. Occasionally the insertion of the stitch causes bleeding into the cremaster sac and the blood may extend headwards between the muscle and testis, obscuring the view of the vessels.

Even gentle *mechanical stimulation*, such as flushing the field with Ringer's solution from a Pasteur pipette, causes transient dilatation. A localized touch with a glass rod causes only a local dilatation. Stronger pressure on an artery may result in a local constriction followed by dilatation. The dilatation to a slight touch is occasionally useful for re-locating vessels that have become invisible. Rough or too often repeated gentle stimulation is liable to lead to persistent dilatation and haemorrhage from the minute vessels.

Exposure of the muscle surface to air soon leads to dilatation of all the vessels. Unless exposure has been prolonged, the dilatation subsides when the area is again covered with irrigating fluid. The exposed muscle surface seems to be water repellent; the returning fluid forms a raised edge and does not spread readily over the area. Exposure may happen unnoticed, but attention should be drawn to it by the general dilatation and by the appearance of light reflexes, seen in Pl. 1, from irregularities of the surface. Uncovering can be avoided by adjusting the skin edges to ensure sufficient depth of the pool and maintaining the rate of flow to counter the seepage of fluid from the subcutaneous space. It may be noted that substituting NaCl solution (0·9 %) for Ringer's solution also dilates the vessels; the mixture of one tenth NaCl solution and nine tenths Ringer's solution does not affect vessel calibre.

Local and body temperature. Scrotal subcutaneous temperature in the newly anaesthetized rat is around 31° C and colon temperature 37–38°. When the cremaster is exposed and irrigated with Ringer's solution at room temperature, the temperature of the pool of irrigating fluid is usually between 25 and 27° C. Raising this temperature to 31°, while keeping colon temperature steady, provokes no detectable change in the vessels; raising pool temperature to 38° causes only a slight dilatation which subsides as the temperature falls. Raising colon temperature from 31 to 40° C causes no detectable dilatation of the muscle vessels though it dilates those of

the tail (Grant, 1962). It seems, therefore, that close control of local and body temperature is unnecessary. Colon temperature, however, has usually been maintained between 37 and 38°, and pool temperature between 30 and 32° C.

I had hoped that the temperature of the sympathectomized tail might be used as an index of vasoconstrictor activity in the circulating blood. But the raised tail temperature following abdominal sympathectomy (Grant, 1963) soon declines. By about the fourth day it returns to and remains at room temperature whether the rat is anaesthetized or not or if colon temperature is raised to 40–41° C.

Effects of some vaso-active substances

Only a few such substances have been tested and attention has been directed mainly to change of vessel calibre and the minimum intravenous dose or local concentration required to provoke a distinct change. These concentrations and doses range by a factor of 10, and occasionally 100, for different animals.

Except in the case of adrenaline, intravenous injection and topical application have yielded the same kind of effect. The reactions now to be described can be obtained repeatedly in an individual animal over several hours.

Histamine dilates all classes of vessels but especially the minute vessels. It is striking to watch the pale field of the resting muscle become flushed with vessels appearing between the muscle fibres when histamine is applied. The minimum effective concentration is about 0·1–0·01 ng/ml. and the dose is less than 50 μg. With small doses and weak concentrations the dilatation is transient. For example, after 50 μg intravenously the dilatation is detectable within 2–3 sec, comes to its height in 10–15 sec, begins to subside within the first minute and by the end of the second has passed off. But with stronger concentrations and larger doses the dilatation may continue declining for half an hour.

5-dydroxytryptamine has the same effect as histamine but acts in a dilution about 100 times weaker, the minimum effective concentration being usually 0·001 ng/ml. It has not been given intravenously.

Acetylcholine dilates the arteries but has little effect on the minute vessels, the dilatation of which subsides before that of the arteries. The minimum effective concentration is 0·1–0·01 ng/ml.; the minimum dose has not been determined but is less than 0·5 μg. This dose gives a conspicuous arterial dilatation subsiding after 3–5 min.

Noradrenaline constricts vessels of all classes when applied topically and intravenously. The constriction is accompanied by a rise of blood pressure. For example, after 0·1 μg systolic blood pressure rose by about 20 mm Hg

and returned to the pre-injection level in 1¼ min. On two occasions the minimum effective concentration was estimated as 0·001 ng/ml.; the dose has not been determined.

Adrenaline given intravenously provokes a more or less conspicuous dilatation which may or may not be followed by an obvious constriction. In general, the smaller the dose the less likely is constriction to follow, but sometimes even with the minimum effective dose (0·1–0·01 μg) the reaction is biphasic. Some initial dilatation occurs even with a larger dose, for example, 5 μg. In a few instances in which it was measured, 0·1 μg lowered systolic blood pressure by 10–30 mm Hg when the vessels dilated and pressure returned to the pre-injection level as dilatation passed off. The fall of pressure was preceded by a momentary rise of 5–10 mm Hg and in this phase the cremaster vessels showed no change, a transient speeding of flow or a slight dilatation. The main dilatation affects chiefly the arteries (their diameters may be doubled) while the minute vessels, though dilated, are but little widened. The adrenaline dilatation thus resembles that due to acetylcholine and differs from that provoked by histamine. This is readily displayed by injecting these substances in succession using doses chosen to give so far as possible about the same degree of arterial dilatation with each; for example, 0·5 μg adrenaline and acetylcholine and 50 μg histamine.

In two instances adrenaline was infused intravenously for periods of 3 and 4 min (rate of fluid perfusion 2 ml./hr). An adrenaline rate of 1·0 μg/100 g rat/min caused a dilatation which subsided after about 1 min and was replaced by a constriction which persisted until the infusion ceased and for a minute or two thereafter. Infusion at the rate of 0·1 μg/100 g/min provoked a just detectable dilatation followed by little or no constriction.

It is to be emphasized that to obtain the adrenaline dilatation the cremaster vessels must have attained their resting state. If the arteries do not narrow considerably, as they may not in a poor preparation and sometimes when pentobarbitone is the anaesthetic, then only constriction is seen and is accompanied by a rise of blood pressure. Thus, in one rat, difficulty was experienced in inserting cannulae into the carotid artery and the femoral vein; a period of respiratory difficulty followed. The initial dilatation of the minute vessels largely subsided but the arteries remained more or less dilated. Three doses of 0·1 μg and three of 0·2 μg adrenaline each caused blood pressure to rise 15–20 mm Hg and a constriction of the cremaster vessels lasting 1–2 min. A dose of 0·05 μg had no effect.

Adrenaline applied locally has (in all but two of many instances) exerted only a constrictor effect, even in instances in which the intravenous injection, before and after the local irrigation, provoked dilatation. The

constriction affects all classes of vessels. The minimum effective concentration is 0·1–0·01 ng/ml. A concentration of 0·1 ng/ml. may provoke a constriction lasting about 1¼ min after the end of irrigation; with 10 ng/ml. a greater constriction lasts about 3 min while with 1 μg/ml. practically all the vessels disappear and at least a half hour elapses before they return to their initial state.

In the two exceptional instances, the adrenaline irrigation was made during the course of other observations. In one, two successive applications of a concentration of 0·01 ng/ml. caused a dilatation followed by a slight constriction; stronger concentrations caused constriction only. In the other, 0·1 ng/ml. caused a dilatation only. Nothing unusual was noted at the time to account for these results which could not be repeated in subsequent experiments.

Isopropylnoradrenaline, 0·5 μg given intravenously in two instances provoked a dilatation of the same kind as did adrenaline, though it lasted longer.

Adrenaline inhibitors. A few observations were made with three substances known to inhibit adrenaline effects under other circumstances (1) Rogitine (Ciba) (2-(N-3-hydroxyphenyl-4-toluidinomethyl)iminazoline methanesulphonate); (2) Pronethalol (I.C.I. 38174), 2-Isopropylamino-1-(2-naphthyl)ethanol, an inhibitor of β (dilator) receptors; (3) Dibenyline (Smith, Klein & French) Benzyl-2-chloroethyl(1-methyl-2-phenoxyethyl) amine hydrochloride, an inhibitor of α (constrictor) receptors.

Rogitine. On one occasion, Rogitine perfused intravenously at the rate of 2 μg/100 g rat/min for ten minutes did not alter the calibre of the cremaster vessels, but reduced the previously gross constriction to adrenaline 0·1 μg/ml. applied topically. The rate of Rogitine perfusion was then doubled and 10 min later the same adrenaline concentration provoked no constriction. The temperature of the sympathectomized tail did not rise, remaining at about 24° C.

On another occasion, Rogitine was perfused at the rate of 4 μg/100 g/min for 20 min. Perfusion was momentarily interrupted at 8, 12 and 15 min to allow the intravenous injection of 0·1 μg/adrenaline. These provoked no constriction though it had been gross before the Rogitine. In this instance also the temperature of the sympathectomized tail did not rise, remaining between 19 and 20° C.

On a third occasion, the cremaster was irrigated with Rogitine in a concentration of 0·1 mg/ml. for 20 min. This did not alter the calibre of the vessels but abolished for at least 1½ hr the constrictor response to noradrenaline applied topically in a concentration of 1 ng/ml. On a fourth occasion Rogitine applied in weaker concentration for a shorter time (1·0 μg/ml. for 10 min) abolished the constrictor response to noradrenaline

for at least the subsequent 40 min. At 50 min, ten times stronger noradrenaline yielded only a doubtful response but at 1½ hr a gross constriction.

Pronethalol was given intravenously in a dose of 0·1 mg on two occasions. On the first, the dilator element of the biphasic response to 0·1 μg adrenaline intravenously was abolished for at least 20 min, the constrictor element occurring as usual. On the second occasion, the dilator element to 0·2 μg adrenaline had partly returned at 20 min and was back to the initial degree by 30 min.

Dibenyline is unsuitable for local application because when added to Ringer's solution with or without gelatin the solution becomes cloudy. Dissolved in NaCl solution (0·9 g/100 ml.) dibenyline was injected intravenously in a dose of 0·2 mg on one occasion and of 0·4 mg on another. Neither injection altered the calibre of the cremaster vessels nor did they alter the biphasic reaction to repeated doses of adrenaline, on the first occasion of 1·0 μg and on the second of 0·1 μg over the subsequent 1½ hr. These observations were not pursued.

Vessels of the thigh muscles

A number of observations were made on these vessels. This site, however, is not so favourable as the cremaster because of the greater thickness of the muscles. Though the minute vessels and superficial veins can be seen, the arteries for the most part approach from the depth of the tissues, though some enter the side of the muscles from the subcutaneous tissue about the knee joint.

The vessels respond like those of the cremaster to touch, exposure, local and body heating and to the vaso-active substances tested. The minimum effective doses and concentrations are the same as for the cremaster. The adrenaline inhibitors were not tested on the thigh.

DISCUSSION

Since the cremaster vessels behave in the same way, so far as tested, as those of the thigh, it seems reasonable to accept their responses as representing those of limb skeletal muscles in general. Moreover, unpublished personal observations show that histologically the cremaster has the same structure as limb muscle; it is richly supplied with myelinated nerves and with motor end-plates (though spindles are wanting) and it contracts immediately and strongly to faradic stimulation of the genito-femoral nerve. Its vessels are supplied with sympathetic nerves.

It seems reasonable also to believe that the exposed vessels are so little disturbed that their state and responses may be accepted as normal for limb muscles, at least for several hours. The area exposed is small, usually no more than a square centimetre; only one surface is exposed, and that

surface is continuously bathed in Ringer's solution with added gelatin. The initial dilatation subsides and the vessels remain narrow for several hours and respond repeatedly in a reproducible manner to minute doses of vaso-active substances. It is evident that blood flow through the resting cremaster is small and remarkably stable. This is in keeping with what is known for resting muscle in man (Grant, 1937–8). In the cremaster, however, the veins do not shut down to nearly the same degree as the arteries and minute vessels. Whether or not this holds good for man and other animals is unknown. It is possible that the veins remain capacious to form a depot for blood displaced from other vessels. Another feature that the cremaster has in common with the human limb is the relative lack of effect of changes of body and local temperature (Grant, 1937–8; Barcroft & Edholm, 1943; Green & Kepchar, 1959).

The absence of arteriovenous anastomoses is in keeping with my unpublished histological findings in human forearm muscle and with more recent physiological experiments; for example, Piiper & Sune (1961) inject wax spheres and find no evidence of artereovenous shunts of any importance. So far as my observations go, they agree with those of Zweifach & Metz (1955*b*) on the existence of thoroughfare vessels that carry on the circulation when blood flow is reduced. These channels are arteriolar and venular and lie in the cleavage planes of the muscle while the interior of the muscle is ischaemic; an intermittent capillary circulation comparable to that in other tissues is readily apparent. In the cremaster preparation, however, the channels through which circulation persists in the resting state lie mainly parallel to the muscle bundles; they can be traced through from terminal arterioles to collecting venules and have a diameter little or not at all wider than that of neighbouring capillaries when these are dilated. From clearance studies in cat and rabbit muscle, Hyman & Lenthall (1962) conclude that in resting muscle nutritional blood flow is restricted to special preferential capillary channels permanently open.

In addition to the thoroughfare vessels, other capillaries and small arteries may open up from time to time, but whether or not this is due to metabolic requirements is unknown. In a good preparation there is but little vasomotion, and I suspect that much vasomotion is, at least in part, a response to injury during preparation. Whether or not it is in part due to circulating substances is unknown. As has been seen, the temperature of the rat's sympathectomized tail cannot be used in the same way as the rabbit's denervated ear as an indicator of circulating vaso-active substances (Armin & Grant, 1959). In passing, it may be noted that the failure of tail temperature to rise when the constrictor action of introduced adrenaline is abolished by Rogitine suggests that a circulating endogenous adrenaline-like substance is not responsible for the regain of tone in the tail vessels

after sympathectomy, as is thought to be the case in the rabbit ear (Grant, 1935–6).

That the muscle vessels dilate to mechanical stimulation suggests that the dilatation to exercise may in part be caused in this way. However, Professor Barcroft informs me that vigorous massage of the human forearm results in only a relatively small and transient increase of blood flow.

Several features of the vascular responses to vaso-active substances require comment. Majno *et al.* (1961) find that the increased permeability of the cremaster vessels in response to local injections of histamine and serotonin (5-hydroxytryptamine) is limited to venules of a certain diameter. The fact that these substances appear to dilate vessels of all classes is not contradictory, since the observations of Lewis & Grant (1924) show that, in human skin at least, dilatation and permeability are separable phenomena. Majno *et al.* (1961) obtain approximately equal responses from doses of serotonin about 100 times smaller than those for histamine. In my preparation also, the minimum effective concentrations of these substances differ by a factor of 100.

Not only histamine and serotonin, but also acetylcholine, adrenaline and noradrenaline act in very low concentration when applied topically. Zweifach & Metz (1955*a*) and Hyman & Paldino (1962) also applied adrenaline to their muscle preparations but do not state the concentrations used. The high dilutions topically effective on the cremaster recall those for the central artery of the rabbit's denervated ear (Armin & Grant, 1953). This, however, cannot be taken to indicate a comparable sensitivity because the methods of application differ. In the cremaster, the substance is applied to the surface for several minutes; in the ear, it is applied to the intima of the central artery for only 5–10 sec. This suggests a lower sensitivity in the cremaster vessels. Infused intravenously, adrenaline seems to be less effective on the cremaster than on the denervated ear artery. The minimum dose required to provoke a just appreciable constriction in the cremaster vessels is about 0·1 μg/100 g/min, whereas in the ear it is about 0·006 μg/kg/min (Armin & Grant, 1959). I have not tested the effect of denervating the cremaster vessels on their responses to vaso-active substances.

According to Zweifach & Metz (1955*a*) topical applications of adrenaline cause a constriction lasting only 10–20 sec followed by an appreciable dilatation of the terminal arterioles and venules persisting for 2 or 3 min. Topical application on the cremaster has almost always resulted in constriction without appreciable preceding dilatation. On the other hand, the intravenous application of adrenaline almost always results in a more or less conspicuous dilatation which may or may not be followed by constriction. A pre-requisite for this is subsidence of the initial dilatation

caused by the exposure and which may be delayed or prevented by various factors, such as injury during exposure and the anaesthetic. Lundholm (1956) comes to similar conclusions from blood flow studies on the cat's hind limb. According to Zanetti & Opdyke (1953) the response to intravenous adrenaline seems to be influenced by the vasomotor status of the vascular bed. If the bed is constricted, dilatation appears to predominate; if dilated, constriction prevails. These authors, however, find the same response to noradrenaline as to adrenaline, whereas in the cremaster, constriction alone results from noradrenaline. The same holds good for the human forearm (Barcroft & Konzett, 1949). Shipley & Tilden (1947) and Crawford & Outschoorn (1951) who use the rat's blood pressure to assay pressor substances, do not mention a depressor effect. Both preparations involve considerable anatomical disturbance. In the pithed rat, Shipley & Tilden (1947) obtain pressor responses usually with 0·05–0·1 μg adrenaline intravenously, doses that yield a depressor effect and dilatation in the cremaster preparation.

The class of vessel involved by the adrenaline dilatation has long been disputed. Dale & Richards (1918–19) suggest that the dilator action is an effect on the capillaries, the antagonistic vaso-constriction mainly on arterial muscle. In the cremaster, however, the dilatation is an effect mainly on the arteries, the minute vessels being much less affected. Whether or not this is due to the subsequent constrictor effect acting earlier on the minute vessels was not determined, since dibenyline, so far as tested, failed to block the constrictor element of the biphasic reaction.

I have no observations to account for the difference between the effects of local and intravenous adrenaline. If both are direct effects on the vessel wall, it might be explained by supposing that the dilator receptors lie nearer the intima and the constrictor nearer the adventitia of the vessels. But this and many other aspects of the muscle vessels require further investigation.

SUMMARY

1. A method is described for observing microscopically the blood vessels of the cremaster muscle in the living rat.

2. The vessels are seen in a state thought to be nearly normal for resting skeletal muscle.

3. The circulation in resting muscle is much restricted and is carried on through thoroughfare channels of capillary size. Larger arteriovenous anastomoses are lacking.

4. The responses of the vessels to various stimuli are described.

5. Various vaso-active substances have been applied topically and intravenously and except for adrenaline both methods of application produce

the same kind of effect. The effects are in keeping with those obtained by blood flow methods in man and other animals.

6. Given intravenously, adrenaline provokes dilatation followed by constriction; the dilatation affects mainly the arteries, as with acetylcholine, rather than the minute vessels, as with histamine. Applied topically adrenaline causes constriction only.

I wish to thank Miss M. A. Burton and Mr. Richard Piper for technical assistance and Mr Marvin Gilbert, student of Medicine at Columbia University, New York, for assistance with some of the observations; I am indebted to the Medical Research Council for a grant.

REFERENCES

ARMIN, J. & GRANT, R. T. (1953). The artery of the denervated rabbit's ear as a sensitive pharmacological test object. *J. Physiol.* **121**, 593–602.

ARMIN, J. & GRANT, R. T. (1955). Vasoconstrictor activity in the rabbit's blood and plasma. *J. Physiol.* **128**, 511–540.

ARMIN, J. & GRANT, R. T. (1959). Adrenaline release during insulin hypoglycaemia in the rabbit. *J. Physiol.* **149**, 228–249.

ARMIN, J., GRANT, R. T. & WRIGHT, P. H. (1960). Experimental diabetes in rats produced by parenteral administration of anti-insulin serum. *J. Physiol.* **153**, 146–165.

BARCROFT, H. & EDHOLM, O. (1943). The effect of temperature on blood flow and deep temperature in the human forearm. *J. Physiol.* **102**, 5–20.

BARCROFT, H. & KONZETT, H. (1949). On the action of noradrenaline, adrenaline and isopropyl noradrenaline on the arterial blood pressure, heart rate and muscle blood flow in man. *J. Physiol.* **110**, 194–204.

BELCHER, E. H. & HARRISS, E. B. (1957). Studies of plasma volume, red cell volume and total blood volume in young growing rats. *J. Physiol.* **139**, 64–78.

CHAMBERS, R. & ZWEIFACH, B. W. (1944). Topography and function of the mesenteric capillary circulation. *Amer. J. Anat.* **75**, 173—205.

CRAWFORD, T. B. B. & OUTSCHOORN, A. S. (1951). The quantitative separation of adrenaline and noradrenaline in biological fluids and tissue extracts. *Brit. J. Pharmacol.* **6**, 8–20.

DALE, H. H. & RICHARDS, A. N. (1918–19). The vasodilator action of histamine and some other substances. *J. Physiol.* **52**, 110–165.

GRANT, R. T. (1935–36). Further observations on the vessels and nerves of the rabbit's ear with special reference to the effects of denervation. *Clin. Sci.* **2**, 1–33.

GRANT, R. T. (1937–38). Observations on the blood circulation in voluntary muscle in man. *Clin. Sci.* **3**, 157–173.

GRANT, R. T. (1963). Vasodilatation and body warming in the rat. *J. Physiol.* **167**, 311–317.

GREEN, H. D. & KEPCHAR, J. H. (1959). Control of peripheral resistance in major systemic vascular beds. *Physiol. Rev.* **39**, 617–686.

HYMAN, C. & LENTHALL, J. (1962). Analysis of clearance of intra-arterially administered labels from skeletal muscle. *Amer. J. Physiol.* **203**, 1173–1178.

HYMAN, C. & PALDINO, R. L. (1962). Local temperature regulation of microtissue clearance from rat skeletal muscle. *Circulation Res.* **10**, 89–93.

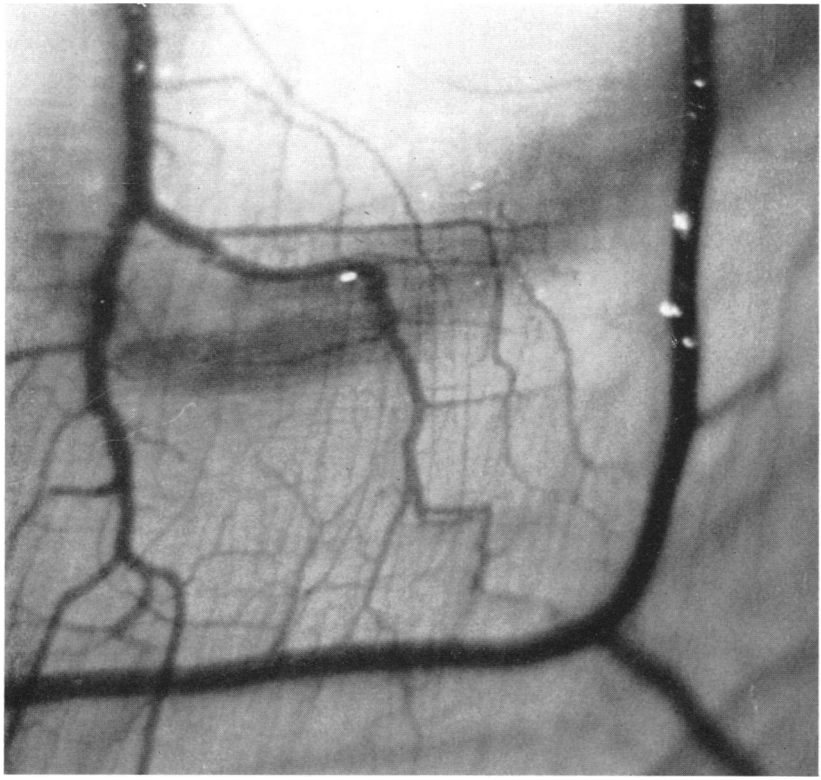
LEWIS, T. & GRANT, R. T. (1924). Vascular reaction of the skin to injury. *Heart*, **11**, 209–265.

LUNDHOLM, L. (1956). The mechanism of the vasodilator effect of adrenaline. *Acta physiol. scand.* **39**, suppl. 133, 1–52.

MAJNO, G., PALADE, G. E. & SCHOEFL, G. K. (1961). Studies on inflammation. *J. biophys. biochem. Cytol.* **11**, 607–626.

PIPER, J. & SUNE, R. (1961). Attempt to demonstrate large arteriovenous shunts in skeletal muscle during stimulation of sympathetic vasodilator nerves. *Acta physiol. scand.* **53**, 214–217.

SHERRINGTON, C. S. (1919). *Mammalian Physiology; a course of Practical Exercises.* Oxford: Clarendon Press.



R. T. GRANT

(*Facing. p.* 137)

SHIPLEY, R. E. & TILDEN, J. H. (1947). A pithed rat preparation suitable for assaying pressor substances. *Proc. Soc. exp. Biol., N.Y.*, **64**, 453–455.

ZANETTI, M. E. & OPDYKE, D. F. (1953). Similarity of acute haemodynamic response to L-epinephrine and L-arterenol. *J. Pharmacol.* **109**, 107–115.

ZWEIFACH, B. W. & METZ, D. B. (1955*a*). Regional differences in response of terminal vascular bed to vasoactive agents. *Amer. J. Physiol.* **182**, 155–165.

ZWEIFACH, B. W. & METZ, D. B. (1955*b*). Selective distribution of blood through the terminal vascular bed of mesenteric structures and skeletal muscle. *Angiology*, **6**, 282–289.

EXPLANATION OF PLATE

Photograph (× 50 diam.) of an area of cremaster blood vessels. An artery passes to the right at the bottom and curves up at the right; several branches arise from it. A vein passes down from the left above and branches freely. Numerous minute vessels lie parallel to the vertically disposed muscle fibres (invisible). The transverse minute vessels of the deep muscle layer are faintly seen. In the upper half is a faint broad U of a testicular vein. The vessels are dilated by exposure to air, hence the bright spots of light reflexes from the surface.