STUDIES ON THE CAPILLARIOMOTOR MECHANISM.

I. The reaction to stimuli and the innervation of the blood vessels in the tongue of the frog. By AUGUST KROGH.

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THE series of papers of which the present one is the first has as its object a revision of current conceptions concerning vasomotor regulation and the mechanism of vasomotor changes. The prevalent line of thought and argument identifies vasomotor changes with arteriomotor. It considers the smaller arteries and arterioles as being generally capable of contraction and dilatation and assumes openly or tacitly that all other changes in calibre taking place in peripheral vessels and especially the state of filling of the capillary system follow as physical consequences from the general blood-pressure and the state of contraction of the arteries and arterioles.

In my paper on the supply of oxygen to the tissues (1) I have given several reasons against this view and quoted researches by other workers showing like my own that the calibre of capillaries is not simply a function of the pressure of the blood coming from the arterioles, but that the capillaries show independent reactions and may dilate and contract individually and independently of the blood-pressure within their walls. A special reference should here be made to the important work of Dale and Richards (2) and of Dale and Laidlaw (3) published after my paper had been written. The researches of Dale and Richards show conclusively that histamine and minimal doses of adrenaline have a dilator effect on the capillaries of certain animals which is independent of the nerve supply, while the effect of these substances upon arteries is that of constriction. Dale and Laidlaw have shown further that large doses of histamine produce an excessive dilatation of the capillary vessels followed by transudation of plasma.

In the paper mentioned (1) my chief object was the study of the supply of oxygen to the fibres of striated muscles, and the vasomotor problems involved could only be treated briefly. The experiments then made could only show the inadequacy of the orthodox conception, but nothing definite could be put in its place. I resolved therefore to follow up the problem, to investigate for a number of suitable tissues the reactions of capillaries and other small vessels to various stimuli and to find out as much as possible about the mechanisms—nervous or other—controlling their reactions.

Methods. The general method employed has been to observe the tissue directly under a binocular microscope with erecting prisms (Zeiss stand X *b*) at a suitable magnification. I have in nearly all experiments used the objective a_3 with the oculars I, III or V, giving magnification of 30, 50 and 80 diameters and fields of vision of 3·0, 2·5 and 2 mm. diameter. Transparent tissues like the tongue and web of the frog are observed by transmitted light. Others like muscles, skin and intestines of cold-blooded and warm-blooded animals are studied by reflected light from a Nernst or arc lamp provided with a suitable set of condenser lenses and a light filter for minimising the heat effect and increasing the contrast between the blood and the tissue.

By this method it is seen directly if the vessels react to a stimulus, in what manner and generally in what order. If by a local stimulus the sausage-shaped corpuscles in a narrow capillary become free and flow backwards from the venous towards the arterial end of the vessel there can be no doubt that the capillary has widened and that the widening is a process with which the arterial pressure can have nothing to do. On the other hand the method is qualitative and perhaps not very sensitive. The change in calibre of a vessel must be fairly large to be observed with certainty, and small changes occurring simultaneously over a larger area cannot be observed at all, unless another, similar but unstimulated, area is available for comparison. The method has the further drawback that the results cannot be recorded—except perhaps cinematographically.

Mechanical stimulation is usually performed by means of glass needles drawn out to a point of suitable fineness—straight or bent as requisite. These needles are manipulated under the microscope; the effect of stimulation is watched and the stimulating process repeated, altered or discontinued according to the results observed. In cases where the stimulus should be very sharply localised I have lately used hairs of the cactus Opuntia (amyclæa). When sealed on to a small glass rod these hairs can if necessary be pushed right through the wall of an artery of less than $\frac{1}{20}$ mm. diameter. For mechanical stimulation of approximately known strength I use various mammalian hairs sealed on to glass rods. The pressure which will bend such a hair is ascertained by means of a balance. The hairs which I have found suitable will bend at pressures of about 1, 5, 15 and 50 mg. respectively.

Reagents for chemical stimulation are applied by means of glass needles provided with a small knob at the end. Such a needle when just dipped in the reagent will deliver a nearly uniform quantity of water or a dilute solution when applied to a moist surface. I usually employ two different needles: one with a knob of 1 mm. diameter which will deliver about 0·1–0·2 c.mm. and another with a knob of 0·2 mm. which will deliver from 0·0005 to 0·001 c.mm. The first quantity will form a drop covering a large part of the field of vision. By the second a sharply localised effect can be obtained.

For a prolonged treatment with fluid reagents I use small paraffined brass rings which are put on to the tissue under investigation and filled with the fluid. In the following such rings are mentioned as reagent basins.

By means of the general methods described and a few special devices which will be mentioned in the following I have studied the reactions to various stimuli of the smallest blood vessels in different tissues and animals, primarily to find out whether the capillaries are able to react independently of the arterioles, whether there is evidence—conclusive or otherwise—of a special capillariomotor mechanism. In anticipation of the results to be given in detail in the present and subsequent papers I think it right to state at once that the question cannot be answered in a general way but must be solved for each tissue separately. The reactions of capillaries not less than of arterioles differ greatly from one tissue to another.

For the experiments on the blood vessels of the tongue I have used almost exclusively good sized *Rana esculenta*. The animals have generally been narcotised with a suitable dose of urethane injected into the dorsal lymph space in the vicinity of the anus. In summer frogs I found a dose of about 0·1 c.c., 25 p.c. solution for every 10 g. of the weight, suitable, but winter frogs require a much larger dose—at least 0·2 c.c. per 10 g. In some cases I have immobilised by means of curari. With the exception of a single reaction, mentioned below, the vessels of urethanised and curarised frogs behave in the same manner. A very deep narcosis or a too large dose of curari should be avoided as it affects the heart and renders the circulation slow and irregular.

I have in practically all cases filled the lungs of the frogs experimented on with oxygen from a cylinder to prevent lack of oxygen during the observation. The pressure in the lungs must not however become so high that the frog appears inflated, since that will obstruct the circulation.

During the experiments the frog rests upon a small bench with glass top and the tongue is spread out over a glass block 2 cm. broad and 3 cm. long for a 50–70 g. frog. The glass block is surrounded by a cork frame to which the tongue is pinned down by means of six small needles. When the ventral surface of the tongue is spread out a glass block of about 5 mm. thickness is most suitable. For examination of the dorsal surface a glass block of 10–15 mm. thickness is required.

The larger vessels of the tongue lie close to the ventral surface and are easily accessible from this side. Both the veins and the nerves follow the main ramifications of the arteries. There is a large anastomosis between the two principal veins at a distance of $\frac{1}{3}$ from the tip of the snout, but the arteries anastomose only slightly. Many of the capillaries are very long and therefore excellently adapted for experimental purposes. Near the edges the network of capillaries is much closer, and most of the vessels run parallel to the edge. Several of the muscles of the tongue can be observed through the ventral mucous membrane, but definite experiments upon their vessels cannot generally be made unless the mucous membrane is removed.

From the dorsal surface, which is covered with papillæ, the larger vessels cannot conveniently be reached, but the capillary clue found in the large papillæ is well suited for local stimulation experiments.

So long as the tongue remains untouched in the mouth of the frog it is usually very pale in colour, and during the pinning out even the largest vessels are often barely visible. Pinning out generally produces considerable hyperæmia with opening up of a large number of small arteries and capillaries and filling up of the veins. When left to itself in a moist atmosphere or under a cover-glass the tongue usually becomes rather pale after a quarter of an hour, but open arteries will go to the neighbourhood of the pins and some capillary hyperæmia will persist about these. When the general hyperæmia has subsided the tongue is suitable for experiments.

The independent reactions of capillaries. Among the numerous observations and experiments made I shall first select those which demonstrate the main point of my argument: that the reactions of capillaries can be independent of the simultaneous reactions of the arterioles and are always practically independent of the arterial pressure.

1. Mechanical stimulation—scratching of a small area on the ventral side of the tongue with a glass needle or a hair requiring a pressure of

15–50 mg. to bend—causes dilatation of capillaries and arterioles after a latent period of 10–15 seconds, usually over an area greater than that which has been stimulated. If this surface is fairly anæmic before the stimulation a number of hitherto invisible capillaries are opened up and filled with blood which generally passes through them in a rapid current.

Since the stimulus affects both arteries and capillaries the effect on the latter might be considered as secondary and due to the increase in pressure caused by the dilatation of the arterioles. To test this point a small adjustable clip was arranged on the right lingual artery and the vessel compressed until the current of blood became very slow and the pressure therefore low. Mechanical stimulation of an area on the right side had just the same effect as before: a number of capillaries were opened, blood flowed *slowly* into them and they became gradually so much dilated that several corpuscles could pass side by side. When the clip was opened the current through the open capillaries became rapid, but no further dilatation could be observed. Several capillaries became injected, however, which had not been visible before.

This experiment of producing capillary dilatation in spite of a greatly reduced blood-pressure has been repeated several times with identical results. The mechanical stimulation has been replaced by chemical with 1 p.c. acetic acid or 1 p.c. iodine. In one case in which a small drop of iodine was applied only a few capillaries became very strongly dilated and corpuscles were seen to flow into them from neighbouring capillaries.

In a special experiment the fall in pressure produced by the clip was measured by means of the apparatus described by Roy and Graham Brown(4). The tongue was pinned out over an open frame of cork and the manometric apparatus put in. In an arteriole a pressure of 22 cm. water was measured, and by compression of the main artery, until the current became visibly slower, the pressure was reduced to 9 cm. When therefore several kinds of stimuli will cause dilatation of the capillaries after the artery has been almost completely blocked it cannot be the increased blood-pressure which opens the capillaries.

When the artery is completely blocked so as to stop the circulation no visible dilatation of the open vessels takes place after stimulation and no new capillaries appear, but when the block is removed 15 seconds after the stimulation or later the arterioles and capillaries are filled at once which shows that the stimulus produces a relaxation of the walls, while a supply of blood is of course necessary to bring about a dilatation.

2. In a few cases (more or less accidentally observed) the dilatation after mechanical stimulation has affected only a short length of the

arteriole supplying a small group of capillaries. The dilated portion of the arteriole and the dilated capillaries then exhibit a slow rhythmic current of blood.

When capillaries are forced open by an increased blood-pressure the rate of flow through them must also become increased, and it can be concluded conversely when the diameter of capillaries is increased while the current becomes slower, that the dilatation is independent of the pressure, provided of course that the blood can flow off freely through the veins.

On the dorsal surface of the tongue it is easy to produce by stimulation of a single papilla a slow current through a much dilated capillary, but in this case the arteriole cannot be directly observed. By gentle mechanical stimulation of a very restricted area on the ventral surface the reaction can be confined to a single capillary or even part of a capillary without involving any arteries at all. In one case a local dilatation was produced in the arterial end of a capillary. The blood flowed in but stasis occurred, because the venous end remained contracted. By continued stimulation over the venous end this too was opened up and a current through the open capillary produced.

On a single very long capillary, through which blood was passing at a slow rate with the corpuscles squeezed out to sausage shape, two distinct dilatations were produced by mechanical stimulation, one near the venous end and one near the arterial end of the capillary. Through these the red corpuscles were flowing freely but with extreme slowness, the dilatation being from a diameter of 4–5μ to about 20μ.

In several cases the venous end of a completely closed capillary has been detected by gentle scratching along open capillaries or small veins. The blood will flow into the opening offered by relaxation of the wall of the closed capillary and fill up part of it, and by repeated application of weak stimuli in front of the visible column of blood the filling is extended backwards until another open vessel with higher pressure is reached and a current through the newly opened capillary produced.

3. Several substances have a dilator effect both on arteries and capillaries, but some act more strongly on capillaries than on arteries and a single one—among those so far examined—acts almost exclusively on capillaries. When a drop of 25 p.c. urethane is applied to a capillary a complete relaxation occurs. The capillary may become filled quite gradually from an arteriole so narrow that the corpuscles are squeezed through one by one. The relaxed capillary may reach a diameter finally of 50μ and show a peculiar varicose appearance. In such a capillary

complete stasis develops and even after several days the vessel may remain filled with a mass of corpuscles. Application of 25 p.c. urethane in a reagent basin produces a maximum dilatation also of the muscle capillaries below the basin. The arterioles are practically unaffected though in a few cases a slight dilatation has been observed.

The experiments detailed in the above paragraphs show—conclusively so far as I am able to judge—that the reactions of the capillaries are independent of the blood-pressure. The capillary wall is normally in a state of tonic contraction, but the tonus is relaxed after local stimuli of various kinds, and the relaxed capillaries can then become filled with blood at a very low pressure. The normal arterial pressure is much too low to overcome the resistance of contracted capillaries, though the venous pressure is often high enough to fill them when relaxed.

I do not doubt of course that an increase in pressure will dilate capillaries as it will dilate any elastic vessel, but the variations in diameter due to mere pressure changes are too slight to be observed under the microscope at the magnifications which I have found it convenient to apply.

The demonstration of the existence of primary vasomotor reactions in capillaries raises several important problems. The most urgent is perhaps the histological about the contractile elements in the capillary wall. Scarcely less important is the physiological problem about the mechanism—nervous or chemical—of the capillary reactions and the origin of the tonus exhibited by the unstimulated capillary wall. Before I proceed to consider the experimental evidence directly bearing on these problems I think it convenient to give a description of the reactions observed after the application of various stimuli.

The reactions of the blood vessels of the tongue to mechanical, electrical, thermal and chemical stimulation. In the preceding section I have mentioned certain reactions to mechanical stimulation, weak acid, iodine and urethane, but of these too a more detailed description must be given.

1. Mechanical stimulation in whatever way applied always causes active relaxation of capillaries and generally of arteries, while veins are apparently only passively affected. The response which follows after a latent period of several seconds is determined by the extensity and intensity of the stimulus. Dilatation localised to a short portion of either an artery or a capillary can be produced by local and gentle scratching or rubbing with a needle or a soft hair (1 mg.). A strong but sharply localised stimulus may cause dilatation of capillaries over a

considerable area and of arteries way back in the tongue. The area which can be made hyperæmic from a single point has never been observed to exceed a diameter of 2–4 mm., however. If a glass needle is drawn sharply across an anæmic tongue so as to actually injure the mucous membrane and produce a contraction of the underlying muscles, a hyperæmic zone 2–3 mm. broad will appear after the usual latent period.

More or less dilated arteries can almost regularly be observed directed towards the needles with which the tongue is pinned down, and a small area around each needle is hyperæmic with dilated capillaries.

Mechanical stimulation may lead to stasis in some of the capillaries. Two kinds of stasis can be distinguished. In the first—which is comparatively unimportant—the capillary in question is only partially opened; the blood flows in but cannot get out at the closed end. In the second kind of stasis the capillary is opened throughout and generally there is at first a more or less rapid current of blood through it, but this current becomes gradually slower and at last the capillary appears to be densely packed with red corpuscles which block the circulation. This kind of stasis occurs regularly when capillaries are mechanically injured by the stimulus; for instance, when the free edge of the tongue is nipped with a pair of forceps, but it may also occur after mechanical stimulation in capillaries which have not been touched. The mechanism of this stasis, which is a common phenomenon after several kinds of chemical stimulation, will be discussed in a separate paper.

In the tongue of *Rana platyrrhina* a reaction very different from that described has been repeatedly observed. Prickings with a cactus hair or a glass needle directly on the wall of arteries often causes a local contraction which develops slowly (generally during ½ to 1 minute) and affects a length of artery of 0·2–0·5 mm. The contraction spreads both proximally and distally from the point stimulated, but usually over a longer distance distally than proximally. The contraction seldom becomes complete, but it will remain, generally, for a period of 10–15 minutes. Before the artery contracts the usual dilatation of the capillaries about the point stimulated will have taken place. Many prickings are ineffective in causing arterial contraction, even when it can be observed that the artery was actually hit, and I have gained the impression that the receptors for this reaction are confined to the arterial wall and are few in number even here.

In the tongue of *R. esculenta* I have not succeeded in evoking this kind of response from urethanised animals, but on two curarised frogs I have obtained it from several arterioles and small arteries. It appears to be absent from the largest arteries and from a number of the smaller ones also. Small arteries often become mechanically obstructed by the pricking, but this effect is strictly local, shows no latent period and can generally be removed by gentle rubbing.

2. Local electric stimulation has been performed by means of a metal needle forming one electrode while the other was a lead plate on which the frog rested. The effect on arteries was inconstant. In several cases contraction was produced by tetanic stimulation, while single induction shocks caused dilatation. It was very doubtful whether any effect upon capillaries took place. The slight dilatation observed in several cases was probably due to the unavoidable mechanical stimulation.

3. Heating of a small area on the ventral surface of the tongue by means of a loop of 1 mm. silver tube, heated to 35° by a current of water, produces after a few seconds a pronounced hyperæmia affecting both arteries and capillaries. The current of blood becomes very rapid. When the area is allowed to cool to the temperature of the room (15–16°) a slow contraction of the vessels can be observed. It will take 10 minutes or more for the vessels to return to their normal state. Heating to 25° caused also in one experiment some hyperæmia and increase of the blood flow, but the dilatation took place very slowly.

Cooling to about 2° causes a slow dilatation of capillaries and arteries with a comparatively rapid return (2 m.) to the normal state when the cooling is discontinued.

4. Acids have a powerful dilator effect on both capillaries and arteries, and the reaction can be observed a certain distance beyond the area directly affected. I have generally used 1 p.c. acetic acid, but I have made a few experiments also with carbonic acid.

To study the influence of gases I have used a modification shown in Fig. 1 of the apparatus constructed by Roy and Graham Brown (4) for measuring capillary pressure. The gas is led into the lower chamber the top of which is covered with a thin peritoneal membrane and thence through the tube to the upper chamber. From the exit tube of this it is carried to a vertical glass tube which can be moved up and down in a cylinder filled with water and graduated in cm. When the gas is bubbling slowly through, the pressure necessary to empty a

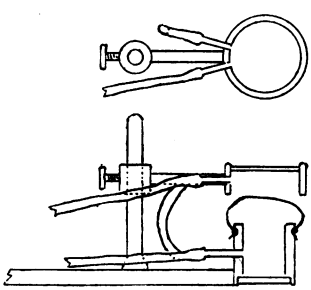

Fig. 1.

certain vessel under observation can be read off. For simple treatment with a gas a low pressure of 1 to 2 cm. water is maintained.

Pure CO_2 gas produces after about a minute a considerable hyperæmia with dilatation of both arteries and capillaries and a rapid current of blood. 10 p.c. CO_2 in air produced in one experiment after a couple of minutes a distinct increase in the circulation through a few capillaries selected for observation, but the general increase over the whole field was too slight to be definitely ascertained.

In experiments with pure nitrogen which should induce the production of acid metabolites only a slight and perhaps doubtful increase in the current of blood through selected capillaries could be observed. Pure oxygen has apparently no effect upon the circulation, as was to be expected, since the oxygen pressure in air must be ample to supply the mucous membrane with oxygen by diffusion.

5. 1 p.c. iodine in potassium iodide has a very powerful influence. The muscles just below the drop of iodine contract violently, the capillaries become strongly dilated and the dilatation spreads to a considerable distance. Iodine affects also the arteries but their dilatation is not generally so pronounced as that of the capillaries. In the capillaries directly affected stasis will usually develop in a minute or less.

6. *Urethane.* The action of 25 p.c. urethane has been described above. Application of 5 p.c. urethane in a reaction basin shows the same effect on the capillaries of the mucous membrane as the 25 p.c. solution, but the dilatation and especially the stasis develop more slowly. The arterioles are practically unaffected; in one case a slight dilatation and subsequent contraction of an arteriole has been observed, but similar spontaneous movements are by no means rare in the unstimulated tongue. 1 p.c. urethane in a reaction basin produces after several minutes a moderate dilatation of capillaries.

7. *Adrenaline.* A 0·1 p.c. solution of adrenaline applied in a basin produces hyperæmia with a rapid current of blood and dilatation both of capillaries and arterioles. In the numerous tests made by means of the drop method with different adrenaline preparations I have never seen any effect on larger arteries but generally dilatation of the smaller and always of capillaries. A few small arteries have been observed to contract somewhat for a brief period and in one of these cases a simultaneous dilatation of the corresponding capillaries was distinctly seen. In the tongue of *Rana platyrrhina* numerous arteries are susceptible to the action of adrenaline and contract strongly, but several, and especially the largest, arteries are not affected. In subsequent

papers a number of similar abnormalities in the behaviour of arteries towards adrenaline will be recorded.

8. *Cocaine.* A 5 or 2 p.c. solution applied to the mucous membrane produces considerable dilatation of capillaries and arterioles. A 0·5 p.c. solution applied in a reagent basin also produces some hyperæmia which develops slowly during 5–10 minutes.

9. *Nicotine.* A drop of 0·5 p.c. solution produces excessive hyperæmia and a 0·1 p.c. solution in a reaction basin has the same effect. Even by dilution of this solution to about one-thousandth the dilator effect is not completely abolished.

Amylnitrite produces capillary dilatation and probably also arterial. Mustard oil has no visible effect. Barium chloride which was tested in the hope that it might produce contraction of dilated capillaries shows no visible effect in 10 p.c. or 1 p.c. doses neither by application on the mucous membrane nor by injection into the lymph spaces of the tongue.

It follows from the descriptions given that almost all the substances tested as also local mechanical and thermal stimulation produce the same effect on the capillary wall, viz. relaxation of its powerful tone which it acquires again after a period of at least several minutes when left undisturbed. Most of the stimulants also produce relaxation of the arterial tone but in several cases, notably iodine and urethane, the effect on arteries is distinctly less pronounced or even practically absent.

The mechanism of the action of stimulants has been investigated in two series of experiments. In one of these I have studied the effect of cocainisation with the idea of anæsthetising the sensitive nerve endings and to find out whether the reactions were due to direct stimulation of the vessels or were effected through the nervous system. The observed spreading of the effect to a certain distance from the point stimulated points of course strongly towards the latter alternative, though combined effects are probably present in several cases.

In another series I have cut on one side the nerves to the tongue and studied the reactions both immediately afterwards and later at suitable intervals to follow the effects of degeneration.

The effects of cocainisation. As mentioned above cocaine causes dilatation of arteries and capillaries, but when a weak solution (0·2–0·5 p.c.) is applied in a reagent basin the hyperæmic effect can as a rule be reduced so far that the reaction to other stimuli can be investigated.

Mechanical stimulation applied through the layer of cocaine solution shows at first its usual effects, but after about 20–30 minutes the reactions usually become slight and strictly localised to the point stimulated.

Later (usually after about 1 hour) the effect of mechanical stimulation may become so completely abolished that even a powerful stimulus fails to evoke any reaction whatever[1].

Heating of the cocainised area by means of the silver tube with water of 35° has only been tried twice on one frog. As far as I was able to ascertain no dilatation of arteries or capillaries occurred, but the current of blood through the vessels observed was distinctly accelerated. I ascribe this effect to the greatly diminished viscosity of the heated blood, but I do not venture to lay much stress upon the observation.

Large drops of 1 p.c. acetic acid show their normal dilator effect on cocainised areas after the abolition of responses to mechanical stimulation, but small drops sometimes fail to elicit any dilatation whatever though they are effective on the normal mucous membrane.

A drop of 1 p.c. iodine applied to the mucous membrane after complete cocainisation shows no effect whatever upon the vessels and the contraction of the underlying muscles is also abolished. In one experiment a point about 1·5 mm. from the cocainised area was tested with iodine and the reaction was observed to spread into the area, but appeared to be much weaker here than at a similar distance in other directions.

The effect of urethane is rather curious. Touching with a small drop of urethane shows no effect whatever, but if the reagent basin is filled with 5 p.c. urethane capillary dilatation will develop in 2–10 minutes. The dilatation does not become maximal, no stasis occurs as in the normal tissue and the hyperæmia subsides again some minutes later.

Section and degeneration of nerves. The tongue of the frog is supplied with two pairs of nerves, viz. the glossopharyngeus and the hypoglossus. In a number of experiments I have cut both nerves on the right side of the body. The immediate effect is sometimes a distinct hyperæmia of the right side of the tongue with somewhat dilated capillaries and a rapid current of blood. The difference in colour can in such cases usually be observed directly on opening the mouth of the frog. In most cases, however, the right side of the tongue does not become visibly hyperæmic after section of the nerves. The effect of local stimulation both mechanical and chemical immediately and 24 hours after the nerve section does not differ from the normal though it is of course less apparent on a surface which is hyperæmic beforehand than in anæmic tissues.

[1] In a single case the cocainisation of an area near the root of the tongue proceeded much more slowly and even after an hour mechanical stimulation visibly affected the vessels at a distance of half a millimetre.

In one experiment on a curarised frog I convinced myself that the contraction shown after mechanical stimulation of some of the arteries also can be evoked after the section of both nerves to the tongue.

Instead of actually cutting the nerves I have in several experiments blocked the conduction of nervous impulses by freezing a short length of the nerve, a process which can be repeated several times without injury to the nerve. I have failed to observe any effect of freezing the N. glossopharyngeus, but in experiments on N. hypoglossus the freezing has repeatedly caused a distinct dilatation of arterioles and generally also a slight dilatation of capillaries. After cessation of the cooling a contraction of the vessels took place after $\frac{1}{2}$–5 minutes. In one case I observed when the nerve was being frozen that the blood in a venule flowed backwards towards the capillaries, but I do not venture to affirm that the freezing causes any relaxation of capillary tone. A distinct hyperæmia has never been produced by cooling of a nerve, and I think, therefore, that the hyperæmia sometimes produced by section of the nerves with a pair of scissors is due mainly to mechanical stimulation of fibres and not to inhibition of a flow of tonic impulses. This is borne out by the results of stimulation experiments described below.

In one frog, in which the right side of the tongue became hyperæmic after section of the nerves, the circulation and the reactions to stimuli were studied eight days later. The right side muscles were abnormally sensitive to mechanical stimulation. A distinct hyperæmia with dilated arteries and capillaries still persisted. The reactions of the vessels to mechanical stimulation showed the difference that the dilatation spread to a distinctly larger area on the left side than on the right. About the needles pinning out the tongue a very pronounced hyperæmia with dilated capillaries was observed on the left side reaching (as usual) to a distance of about 2 mm., but on the right side the reaction was very slight. On the application of a drop of iodine to the right side the muscles affected contracted violently, but the reactions of the vessels were confined to the capillaries in contact with the iodine and their immediate neighbourhood.

When the same frog was examined 18 days after the operation the abnormal sensitivity of the muscles was much diminished and the hyperæmia had somewhat subsided, though numerous capillaries were still open. Mechanical stimulation of capillaries produced in some cases a sharply localised dilatation, but in no case did the effect spread to the arteries. When small arteries were directly stimulated a local dilatation was also observed in several cases. 1 p.c. acetic acid showed a distinct dilator effect on arteries while the effect on capillaries was doubtful. 1 p.c. iodine gave no contraction of the muscles, a moderate dilatation and subsequent stasis in the capillaries directly affected. No spreading whatever of the reaction. 25 p.c. urethane produced a considerable dilatation of capillaries and stasis. In the dorsal surface of the tongue the papillary capillaries showed a distinct dilatation and in some cases stasis after mechanical stimulation of the papilla.

After 26 days there was no distinct change in appearance or reactions except in some arterioles. Several arteries showed no reaction whatever upon mechanical stimulation, others showed a distinct local dilatation, and a few of the arterioles showed a local dilatation and a contraction at the point where the branching from a slightly larger vessel took place.

In another frog operated in the same way no hyperæmia of the right side was produced. The reactions when tested after 24 hours were absolutely normal.

After 10 days the tongue was equally pale on both sides, and the pinning out produced a uniform hyperæmia. Mechanical stimulation of capillaries produced on the right a distinct effect which spread over a short distance. By stimulation of small arteries spreading of the effect to a distance of about 0·7 mm. towards the periphery was observed. By the application of iodine and acetic acid no distinct effect beyond the limits of the drops could be made out. After 18 days no change could be observed, and the distance effect of local mechanical stimulation was distinctly more pronounced than in the first frog after a period of corresponding length.

After 100 days the frog, which had obtained no food in the intervening period, was distinctly anæmic with a poor circulation. A number of capillaries had apparently disappeared altogether from both sides of the ventral surface of the tongue. The reactions to local stimulation (mechanical, acid) proved to be normal on the left side. On the right no visible effect was produced by mechanical stimulation of the capillaries in the ventral surface, but in a few of the dorsal papillæ a local dilatation was produced which in one case even led to stasis. Acid had a very slight local effect. Stimulation of arteries produced in some cases no effect whatever, in others an enormous but strictly local dilatation. The muscles on the right side showed normal contractility when directly stimulated by induction currents. The right hypoglossus was found to be divided. The glossopharyngeus was continuous and probably regenerating.

In frogs which had their lingual nerves cut in winter the degeneration proceeded so slowly that the reactions to local stimuli could still spread to some distance at the time of writing, 2½ months after the operation.

The conclusions which I think it possible to draw from the preceding series of experiments are the following:

1. The reactions to local stimuli have nothing to do with true reflexes, since they are in no way altered by simple section or blocking of the lingual nerves.

2. So far as spreading of the effect over a larger area than that directly stimulated occurs this spreading must be due to local axon reflexes, since the spreading is abolished by the action of cocaine and by the degeneration of the nerves of the tongue.

3. Certain substances have a dilator effect both through their action on the nervous elements and directly on the walls of the vessels. This is the case especially with acids and with urethane.

When attempting to form a picture of the nervous mechanism involved one is reminded at once of the antidromic reflexes so admirably studied and described by Bayliss(5). Bayliss showed that nerve fibres belonging to the posterior roots were connected with the vessels (arterioles) of the limbs and that their excitation produced dilatation. It has since been shown by Bruce(6) in experiments on the conjunctiva of the eye that the inflammatory reaction produced by mustard oil could be abolished by anæsthetisation with cocaine and by degeneration, though not by simple section of the corresponding nerve, and Bruce concluded that he had to do with antidromic local reflexes in the sense of Bayliss.

Bruce's experiments have been repeated and amplified by Bardy(7) who found that the inflammation could also be prevented by suitable doses of nicotine intravenously or locally applied or by a general narcosis with ether. Bardy considers that an axon reflex is set up in the sensory nerve endings and that the reflected impulse passes through a special branch of the sensory fibre connected with an autonomic ganglion cell in the conjunctiva which in its turn causes the arterioles innervated by it to dilate. According to Bardy nicotine will block the reflex at the ganglion cell, and general narcosis will abolish the conduction along the whole of the reflex arc.

As far as I am able to see my results can best be brought in harmony with the original conception of Bayliss (and Bruce). According to this we must assume that sensory nerve fibres which follow the arteries divide freely and give off branches, first to the arteries and arterioles and next to the capillaries and the mucous membrane. The ends (end organs) of some (or all) of these branches must have a very low threshold value for mechanical stimulation, and I think it natural to suppose that they are the organs of the ordinary cutaneous senses—especially the sense of pressure[1]. It must be assumed further that the nerve endings in the walls of capillaries and arterioles have a double function, being at the same time sensory and dilatory—that is inhibitory with regard to the vascular tone[2]. The stimulation of the capillary or arterial wall itself is certainly more effective and appears to spread further than stimulation of an intervascular space.

In order to explain the observation, which has been made again and again, that the effects of a stimulus will spread to an area which depends

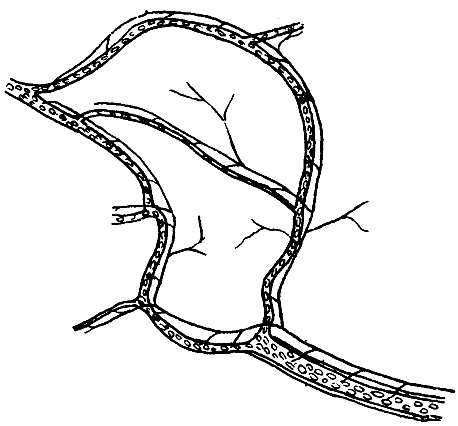

Fig. 2.

at least approximately upon the strength of the stimulus, it is necessary to assume that the nervous process set up by a stimulus is weakened at the points of branching by being diverted into two channels instead of one. This is precisely what happens in the primitive nerve nets of Medusæ and other forms.

The conception to which I arrive is therefore that illustrated diagrammatically in Fig. 2. The nerve fibre accompanying an artery divides and innervates both the capillaries and the intercapillary spaces. It must also give off branches to the artery, since a stimulus of

[1] Special nerves and nerve endings must be taken to be responsible for the contraction reaction on strong mechanical stimulation shown by some of the arteries.

[2] This assumption was made also by Bayliss though he took the nerve endings to be confined to the arterioles.

sufficient strength will cause the artery supplying the stimulated area to dilate even at a considerable distance.

It is quite possible of course that the branches of the nerve fibre anastomose and form a network, but it appears to be improbable that there is anastomosis between the fibrils belonging to different fibres, since the capillary reactions after stimulation of a single point are not observed to spread beyond a distance of a couple of mm.

Although in my experiments there is no definite proof that the vaso-dilator fibres have their nerve cells in the spinal ganglia, there are strong reasons in favour of this view. Before enumerating these I may give the results obtained by stimulation of the lingual nerves.

Bayliss showed that mechanical stimulation of a posterior root is a very effective means of causing vaso-dilation. The numerous experiments which have been made upon the sciatic show that in the presence of many vaso-constrictor fibres it is only mechanical stimulation that can be relied on to cause dilatation of the blood vessels. I have made the following experiments.

1. Electrical stimulation of the glossopharyngeal and hypoglossal nerves has been tried repeatedly. I have applied make and break of a constant current, rapid interruptions and inversions of a constant current by means of v. Kries' rheonome, single induction shocks repeated at varying intervals and tetanisation with very varied strengths of current.

I have never been able to elicit any response from the vessels by stimulation of the N. hypoglossus, except in some curarised frogs in which the muscles contract; the contraction is accompanied by dilatation of the vessels.

By stimulation of the N. glossopharyngeus with a short series of weak induction shocks or weak tetanising currents I have sometimes obtained contraction of small arteries and arterioles, but no effect on the capillaries has ever been observed.

2. Mechanical stimulation by pinching almost constantly produces a distinct hyperæmia with dilatation both of capillaries and arteries and consequently a rapid current of blood. In a few cases I have seen the dilatation of capillaries preponderate in so far that the current through them became visibly slower. The dilator effect develops after a latent period of several seconds or even $\frac{1}{4}$–$\frac{1}{2}$ minute. It lasts usually about as long as the dilatation produced by local mechanical stimulation. When it has subsided a repeated dilatation can be evoked by pinching the same nerve nearer the periphery. After complete crushing of both

nerves a very strong hyperæmia of the corresponding side of the tongue is produced which may take a day or two to subside.

In some experiments I have spread the ventral surface of the tongue and pinched the nerve branches which are distinctly visible accompanying the arteries with the invariable result that the arterioles and capillaries in the corresponding area became dilated. In other experiments I have spread the dorsal surface and isolated the nerves in the floor of the mouth. In one case I pinched first the N. hypoglossus, but did not obtain a general hyperæmia of the corresponding (right) side of the tongue. In certain restricted areas, however, the papillæ became so strongly injected that the hyperæmia was distinctly visible with the naked eye. This reaction was evoked several times. Subsequent pinching of the N. glossopharyngeus produced a general hyperæmia of the right papillary surface. The dilatation appeared to be most pronounced in those areas which did not respond to the stimulation of the hypoglossal. In another experiment I began by pinching the N. glossopharyngeus and observed a considerable hyperæmia of almost the whole of the corresponding half of the tongue. In two small areas, however, the effect was absent or very slight. Subsequent pinching of the hypoglossal produced dilatation of the papillary capillaries in one of these while the other failed to respond.

In one experiment the circulation was very feeble. A slow movement of the blood was just visible in the largest vessels, but the capillaries in most of the papillæ were empty and the surface of the tongue very pale. Repeated pinching of the glossopharyngeal nerve had no directly visible effect, but very slowly the capillaries in considerable areas of the corresponding half of the tongue became filled with blood and considerably dilated so that it appeared distinctly hyperæmic to the naked eye, though no circulation could be observed by means of the microscope. The other half of the tongue remained unaltered and there were no signs of venous stasis.

According to the experimental results detailed in preceding pages we have the following positive reasons for the assumption that the vasodilator fibres are sensory.

1. Their end organs respond readily to very weak mechanical stimulation—scratching with a hair bending at 1 mg. pressure.

2. The end organs become paralysed by the action of cocaine.

3. The nerve fibres respond to strong mechanical stimulation but not to the ordinary forms of electrical stimulation.

As reasons against the assumption that the fibres in question may be autonomic the following facts can be adduced.

4. Adrenaline in weak concentrations has no effect upon any of the vessels in the tongue. It is improbable therefore that the nerves belong to the dorsal sympathetic.

5. If they were cranial autonomic the ganglion cells would probably lie in the tongue, but in that case the postganglionic fibres would not degenerate after section of the nerves containing the preganglionic fibres.

The origin of the capillary tonus. According to the results of the nerve section and degeneration experiments the capillary tonus is only to a very slight extent—if at all—of a nervous nature, since it is generally maintained after section and also after complete degeneration of the lingual nerves[1]. It can be shown that the tonus is abolished by cutting off the blood supply.

I have in several experiments tied branches of the lingual artery[2] and kept them closed for 3–14 hours. The invariable result when the circulation was again opened has been a hyperæmia with some dilatation of arteries and generally an even more pronounced dilatation of capillaries. The degree of capillary hyperæmia has been more or less proportional to the duration of the ischæmia, and in an experiment which lasted 14 hours an excessive hyperæmia with almost complete stasis and exudation of plasma developed when the blood was again admitted. Similar effects of artificially produced ischæmia are well known from experiments on mammals, but they are generally ascribed to the accumulation of acid metabolites due to the lack of oxygen produced. In the case of the spread tongue of a frog the supply of oxygen by diffusion from the atmosphere to the mucous membrane, the larger vessels and most of the muscles, will be sufficient for their normal metabolism, though a calculation shows that there possibly is oxygen lack in the papillary surface. To make quite sure that lack of oxygen has nothing to do with the dilatation of the vessels after ischæmia, I have repeated these experiments on frogs, which were kept in an oxygen atmosphere during the whole period while the vessels were ligated. The

[1] In the degeneration experiment given in detail on p. 412 the tonus was distinctly weakened, however, for so long a time after section of both nerves that it is difficult to assume a persisting state of excitation from the cut fibres.

[2] Care has to be exercised in order to avoid damaging the nerves along the arteries. After arranging the ligatures but before the current of blood was interrupted I have made observations to make sure that the operation had only produced a local hyperæmia and showed no effect on the area irrigated by the artery.

results were practically the same as in air, though the hyperæmia produced after 14 hours' ischæmia in oxygen was somewhat less excessive than in the corresponding experiment in air, and no complete stasis occurred.

In another 14 hour experiment it was observed after the 14 hours, but before the circulation was opened, that the left lingual artery experimented on was not completely blocked. A movement of the blood could just be discerned in the largest arteries and veins, though it was much too slow to be seen in smaller vessels. It is very significant that in this case the admittance of the blood at full pressure produced only a very slight hyperæmia with a few more open capillaries than on the control side and perhaps a slightly more rapid current.

When these observations are applied to the normal resting condition, in which a large number of capillaries are closed, they lead to the conclusion that a capillary cannot remain completely closed indefinitely. When it gets no blood its tonus will diminish, and it must finally become relaxed and admit a current of blood which will allow it to regain its tonus. Every capillary must therefore alternately open and close, and in the resting tissue, which is very poorly supplied with blood, the positions of open capillaries must be continuously changing, with the result that the whole tissue is uniformly irrigated when considered over a period of sufficient length. Observations which seem to confirm this deduction have been made on muscles and will be recorded in a later paper.

Summary.

The reactions to various, mainly local stimuli of capillaries and small arteries in the tongue of the frog have been studied microscopically.

Experiments are described which show that the arterial pressure is unable to dilate the capillaries to any appreciable extent or open them up when they are tonically contracted, while the venous pressure may be sufficient to fill them, when relaxed in response to weak mechanical or chemical stimulation locally applied. The state of filling of the capillary vessels does not depend therefore upon the arterial blood-pressure but upon the degree of tonus or relaxation of the capillary wall.

Local mechanical or thermal stimulation or the application of minute drops of weak acid, cocaine, adrenaline, etc., may produce dilatation both of capillaries and arteries. When the stimulus is sufficiently strong the effect spreads to an area greater than that stimulated. Urethane causes dilatation of capillaries without affecting arteries.

The reactions of the vessels to local stimulation are abolished by cocaine. They are not affected by simple section of the nerves but disappear when sufficient time is allowed for the nerves to degenerate. It is concluded that the reactions are due to local axon reflexes of the antidromic type along the fibres of sensory nerves which are supposed to give off numerous branches to capillaries and small arteries.

Electrical stimulation of the lingual nerves was found to be without effect on capillaries, but strong mechanical stimulation may cause very considerable dilatation of capillaries and arteries in the area innervated.

The capillary tonus is not—at least not principally—of nervous origin, but is shown to depend upon the supply of blood. A vessel which has been closed by tonical contraction for a certain period will relax in consequence and admit a current of blood which will in turn allow it to regain its tonus.

The substance responsible for the tonic action of the blood is unknown, but it is shown that it cannot be oxygen.

REFERENCES.

(1) Krogh. Journ. of Physiol. **52**, p. 457. 1919.
(2) Dale and Richards. Ibid. p. 110.
(3) Dale and Laidlaw. Ibid. p. 355.
(4) Roy and Graham Brown. Ibid. **2**, p. 323. 1880.
(5) Bayliss. Ibid. **26**, p. 173. 1901.
(6) Bruce. Arch. f. exp. Path. **63**, 424. 1910.
(7) Bardy. Skand. Arch. Physiol. **32**, 198. 1915.