STUDIES ON THE PHYSIOLOGY OF CAPILLARIES.

II. The reactions to local stimuli of the blood-vessels in the skin and web of the frog. By AUGUST KROGH.

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THE experiments dealt with in the present paper are a continuation of the corresponding studies on the tongue of the frog (1), and the tissue chosen has been selected, partly because some preliminary comparisons showed that the vessels here behaved very differently from those in the tongue, partly also because the anatomy of the nervous system rendered possible a detailed study of the innervation of the vessels of the hind leg. The innervation problems will, however, be reserved for discussion in a separate paper and only incidentally be touched upon in the following pages.

The blood vessels in the skin of the frog, which can be easily observed by reflected light when the skin is strongly illuminated and a part is chosen which is not too strongly pigmented, present a picture very different from that seen in the tongue. The larger vessels, especially the arteries, run on the interior surface of the skin and are only dimly seen. In a large part of the surface the arteries carry venous blood and show up darkly against the white pigment of the ventral surface. These subcutaneous arteries give off numerous branches which pierce the skin, run for a short distance just below the epithelial layer and divide into a very close meshed network of capillaries which are usually open and show a more or less regular current of blood. The veins usually run for a considerable distance at the surface and collect the blood from a large number of capillaries before they pierce the skin. In some places and especially at the ventral surface of the tibia superficial arteries of greater length and diameter can also be observed.

The web consists of two layers of skin which is very thin and, in *R. temp.*, often so slightly pigmented that it can be conveniently observed by transmitted light. Comparatively large arteries and veins run out from the toes between the two layers, but each epithelial surface possesses its own network of capillaries, very similar to that of other cutaneous areas. The capillaries nearest the angle between the middle toes are usually most suitable for detailed observations on account of their comparatively large distance from those of the opposite surface, and also because the black pigment cells are usually fewer in number than further out.

It has been asserted, especially for the web, that certain capillaries leave the arteries under acute angles and form short connections with diminished resistance between arterioles and venules (W. Jacobj (2), Stromkapillaren), while the larger number, which leave the arteries under more obtuse angles, combine into a network which is only occasionally provided with blood (W. Jacobj, Netzkapillaren, Cohnstein und Zuntz (3), Vasa serosa). Though there are undoubtedly large differences in the resistance of different capillary paths I have been unable to observe any definite distinction between them, and the main point is, as will be shown below, that the resistance and perfusion of any single capillary is extremely variable.

The *methods* employed require very few words since they are essentially the same as those described in the first paper. Medium-sized or small *R. temp.* have been used. They have generally been narcotized with urethane, but most observations have been controlled on curarized or quite normal frogs. Generally the web has been spread by means of small needles on glass plates of suitable size. In some cases I have used glass triangles pushed in between the toes. The two methods have given identical results. The magnifications employed have usually been between 40 and 60 diameters.

1. *The independent reactions of capillaries.* That the capillaries are able to react to local (or general) stimulation independently of the arterial pressure is much less apparent in the case of the web or skin than in the case of the tongue. A local mechanical stimulation at most elicits a response over a very limited area and very often does not elicit any visible response whatever, and the response to most of the chemical substances, which have such a powerful dilator effect on the capillaries of the tongue, is very feeble or altogether absent. Nevertheless, a number of facts can be adduced which show clearly enough that the skin and web capillaries do possess independent tonicity, can contract actively and become dilated by relaxation of their own tonus. A very striking reaction, which I have observed repeatedly on frogs in which the spread web is allowed to dry up somewhat, has been described by Ebbecke (4) (p. 52): "Just after the spreading, the circulation is poor, the arteries are contracted, and many capillaries do not take part at all in the circulation, while others are so far contracted that a corpuscle passes only occasion-

ally. In about half an hour the circulation increases and a number of new, hitherto unobserved, capillaries become visible. The arteries are now very dilated and the flow of blood in the capillaries so rapid that the corpuscles cannot be distinguished. Later on, the arteries contract more and more, while the capillaries dilate and become wider than many of the arteries. The number of visible capillaries is increased three to fourfold and the diameter of the veins is increased in similar proportion. Instead of the rapid continuous flow a distinct rhythm develops, in which the corpuscles are driven forward only during the cardiac systole.... Finally a complete stasis is produced over large areas, and the web appears as a very close network of very dilated capillaries injected with a bright zinnober red dyestuff." Ebbecke's paper contains a large number of valuable observations and is the first in which the significance of independent capillary reactions has been emphasised.

Spontaneous variations in the diameter of single capillaries are of very common occurrence, and it is easy to observe that they are quite independent of the arterial pressure, a dilatation of a capillary often taking place at the same time as a contraction of the artery supplying it. I give as a specially instructive illustration the details of observation made on an arteriole and a small group of capillaries in the web of a frog the sciatic nerve of which had been cut four weeks earlier. Quite similar observations have been made time and again, however, on frogs with intact nerves.

At the beginning of the observation the artery (A, Fig. 1) is narrow, the capillary C_1 allows the corpuscles to pass with some change of form, C_2 is dilated, filled with corpuscles, stasis. Somewhat later C_2 is narrow and shows a feeble current, C_1 varies repeatedly from a large diameter allowing the corpuscles to pass freely down almost to obliteration. During these variations C_2 remains for some time closed at x, but lower down a single corpuscle can be seen which is not compressed by the capillary wall. A flow through C_2 sets in suddenly, but the resistance at x remains considerable, allowing the corpuscles to pass in an elongated shape only. A dilatation at x takes place slowly, and after some minutes corpuscles can pass freely along the whole length of C_2. During this process C_1 remains narrow with a feeble current and allows only a small amount of blood to pass through the dilated C_2. A little later C_2 con-

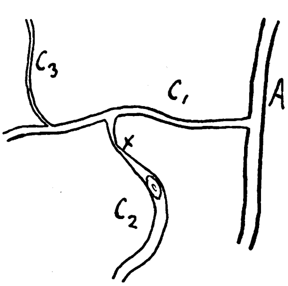

Fig. 1.

tracts and C_1 opens up and shows a rapid current. After weak mechanical stimulation (scratching with a hair) both C_1 and C_2 become somewhat dilated while the artery remains unaffected. After a strong mechanical stimulus applied on the middle of C_2, both C_1 and C_2 contract and become closed and some seconds later the current in A becomes slow and ceases, without the artery being closed within the field of view. After about one minute the current in A is resumed and becomes rapid, and afterwards C_1 and part of C_2 are opened and dilated, while the part directly stimulated, which opens somewhat later, remains so narrow that corpuscles can pass only in a very elongated shape. A little later the peripheral end of C_1 closes up and a capillary C_3, which had hitherto remained closed, opens up. This vessel closes again a minute or two afterwards and the current through C_1 and C_2 becomes rapid.

Weak mechanical stimulation—scratching with a 1–5 mg. hair—has no influence upon wide capillaries, but narrow or closed capillaries are often seen to dilate, though the effect is never considerable. It is only the capillaries directly stimulated or at most at a distance of a few tenths of a mm. which react to this stimulus.

Somewhat stronger stimulation—pressure with a hair of 15–50 mg. or pricking with a fine cactus needle—sometimes causes contraction of the capillary stimulated and one or two neighbouring capillaries. This response can be elicited both from wide and from rather narrow capillaries, but in many cases the effect is local only and in such it may be impossible to decide whether there is active contraction or simple mechanical compression. A very strong stimulus may cause contraction also of the corresponding artery but the time of latency for this reaction is distinctly longer than for the capillary contraction. The response both to weak and to stronger mechanical stimulation takes place after a latent period of 1–5 secs. and lasts usually one to a few minutes.

These observations show very clearly that the cutaneous capillaries can contract and dilate independently of variations in the arterial blood-pressure[1].

An important supplement to this result is furnished by the fact that even considerable variations in pressure have no visible influence upon the diameter of capillaries. Spontaneous variations in the diameter of arteries are of common occurrence in the web, but even when an artery

[1] It is not surprising that the reaction to mechanical stimulation is often difficult to obtain when it is remembered that any stimulus probably affects two distinct sets of receptors, one causing contraction and one dilatation, while the final result must depend both upon the relative sensitivity and upon the local distribution of the receptors.

becomes strongly dilated or contracted the capillaries supplied are not affected except in so far as the velocity of the blood flow through them is altered. When an artery is completely contracted (spontaneously or as a result of stimulation) it can often be observed that the blood flows through a group of its capillaries from a small vein towards another larger vein thus showing that an extremely small pressure is sufficient to keep the relaxed capillaries open. When an artery is very strongly dilated by application of a drop of 0·005 p.c. acetylcholine the dilatation of the corresponding capillaries is scarcely visible though the velocity of the blood flow through them is greatly increased. Not infrequently, however, the application of acetylcholine may *appear* to open up capillaries through which no blood was flowing and which contained only plasma (Zuntz's Vasa serosa). This phenomenon, however, does not necessarily involve capillary dilatation and will find its explanation later in the present paper.

The action of drugs upon the capillaries in the skin and web is much less pronounced than in the tongue. I have never been able to find any distinct effect of 1 p.c. acetic acid which acts so strongly on the vessels of the tongue, but 1 p.c. hydrochloric acid applied in small drops causes dilatation of the capillary or capillaries affected and usually, but somewhat later, of the corresponding artery. 0·5 p.c. nicotine and 1 p.c. iodine produce some dilatation of capillaries. 5 to 25 p.c. urethane produces distinct capillary dilatation and sometimes contraction of the corresponding artery, but the effects obtained are much less striking than in the tongue. When 25 p.c. urethane is applied to the skin the effect can often be observed with the naked eye, the dilated capillaries giving the skin a distinctly red hue showing very nicely against the stratum of white pigment.

In the case of histamine which has been tested in concentrations up to about 5 p.c. no distinctly visible effect upon the capillaries has been found. In a recent paper it has been shown by W. Jacobj (5) that the cuticle of the frog's skin, without being impermeable to such substances as strychnine and adrenaline, offers so much resistance to their passage that their effect is considerably delayed and diminished. W. Jacobj has found further that by treating the skin with alkali or especially with veronal sodium the permeability of the skin is greatly increased. Alkalis and veronal sodium have, themselves, a powerful dilator effect on both capillaries and arteries of the web, but when they are applied in sufficiently weak concentrations the increase in permeability can be demonstrated independently of their dilator effect. I can confirm the observations of Jacobj which I have utilized to see whether any histamine effect could be found when the permeability of the skin was increased. A drop of 1 p.c. Na_2CO_3, which has no visible dilator effect on the capillaries, was placed on the web and histamine was added until its concentration was at least above 1 p.c. No effect on the underlying capillaries could be detected. Injection of histamine into the web has also been tried with on the whole negative results[1], and although in view of Doi's measurements I do not venture to deny that histamine may show some dilator effect upon the capillaries of the frog's skin I consider this effect to be slight only.

The most powerful dilator stimulus for the capillaries of the web and skin is cutting off the supply of blood. By clamping the femoral artery the current of blood in the web is usually diminished so as to be barely visible in the largest arteries, and when such clamping is maintained for 10–15 min. both arteries and capillaries become slowly dilated and filled with blood. The diameter of capillaries can be observed to increase from about 5 to about 20μ. When the clamp is removed a rapid current of blood will pass through the dilated vessels which after 5 min. are again distinctly contracted and may return to the normal state in 10 min. or less. Other experiments have been made by compressing part of the web in Roy and Brown's apparatus (1) until the circulation was interrupted. On opening after 15 min. the capillaries became strongly dilated while the effect on the arteries was inconsiderable. Cutting off the blood supply in this way for $1\frac{1}{2}$ hours caused the capillaries to relax completely and made their walls permeable so that stasis developed shortly after the opening of the circulation.

As in the case of the tongue it can easily be shown that lack of oxygen has very little to do with this reaction. When the web is exposed to the atmosphere it remains well supplied with oxygen, and the colour of the stagnant blood in the vessels remains arterial throughout. When the web is shut off from the atmosphere by a cover glass the stagnant blood rapidly becomes venous in colour but otherwise the reaction proceeds as in the exposed web[2]. Lack of oxygen does, however, cause some

[1] When a strong solution of histamine is injected a considerable dilatation of arteries may take place and in such cases some dilatation of capillaries is observed also.

[2] It is worthy of note that in these conditions the stagnant corpuscles in the capillaries become practically invisible, because the colour of the reduced hæmoglobin is very faint compared with oxyhæmoglobin. The web therefore appears very pale, and the capillaries may easily be taken to be contracted and empty. When the cover glass is removed the corpuscles become arterialized usually in less than half a minute and the capillaries are seen to be dilated and filled with blood.

dilatation of skin vessels. This can be shown when a cannula is arranged so as to ventilate the lungs artificially. I find that ventilation with hydrogen, which makes the arterial blood in the web turn distinctly blue, produces some dilatation of arteries and also a slight dilatation of capillaries with a distinct increase in flow.

2. *The local reactions of arteries.* While in the tongue of the frog mechanical stimulation of any point will cause dilatation of the artery supplying the point and its surroundings, such a reaction is practically absent in the case of the skin (and web). The arteries themselves are, however, sensitive to mechanical stimulation, and their reactions are on the whole similar to those of the capillaries.

A weak mechanical stimulus such as scratching with a hair of 1–15 mg. or pressing the artery so as to obstruct the passage for a few seconds will generally produce dilatation of the artery at the point stimulated and for some distance both proximally and distally. The reaction usually reaches farther up stream than down stream and may involve a total length of several mm. The time of latency is some 5 to 20 secs., the dilatation begins practically simultaneously over the whole length affected and will last for a very variable period from a few seconds to several minutes. This response is generally easy to obtain when the artery stimulated is in a contracted condition, but very difficult or impossible to elicit when it is dilated. Any part of the superficial arteries in the skin and web can react in this way, but the reaction cannot be obtained from any point along an artery and sometimes a number of trials are required before the vessel reacts.

Contraction of arteries is generally brought about by strong stimuli such as pressing the skin just over an artery with a 50 mg. hair or pricking it with a cactus needle. This latter stimulus will often evoke contraction even when applied at a distance up to 0·2 or sometimes even 0·5 mm. from an artery. The contraction takes place with a latent period of 5–15 secs. and usually almost simultaneously over a more or less considerable distance down stream and especially up stream. Occasionally the portion of the artery to the nearest proximal branch contracts first and a further portion a little later. The contraction often becomes quite complete, and this is the case especially when the artery has been hit and pierced. The contraction usually lasts for a long time up to 10 or even 15 min. When contraction takes place in separate steps relaxation proceeds by the same steps, and the point directly stimulated may often remain contracted for some time after the relaxation of the rest.

As far as my experience goes any part of the superficial arteries can show the contraction reaction, but it cannot be obtained from all points though the sensitive points are, perhaps, more numerous than those responsible for dilatation. When a highly sensitive point has been found it can be observed that a comparatively weak stimulus may cause contraction over a short distance only while a strong one may act upon the whole visible length of the artery up to 3 or 5 mm. As was to be expected it is generally easier to induce contraction on wide arteries than on narrow, but I have found that it is always possible by powerful stimulation to obtain further contraction so long as an artery is at all open.

The biological significance of this contraction reaction is obvious and it is beautifully illustrated when a cutaneous artery is hit and pierced by a needle. A small drop of blood only is lost before the artery is completely closed, and when it opens again after several minutes this blood has generally formed a clot which prevents further bleeding.

Several drugs, which in the tongue induce a pronounced dilatation of arteries, have a slight and inconstant effect when applied to the skin. Acid (1 p.c. acetic and 1 p.c. HCl) and iodine may sometimes cause dilatation and sometimes contraction, but often these drugs like several others have no effect whatever.

Acetylcholine in ·005 p.c. concentration shows a powerful dilator effect on arteries when applied in drops on the skin, and the effect is equally pronounced on all branches from the largest visible down to the arterioles.

Adrenaline shows a powerful constrictor effect on the larger arteries but does not act upon arterioles and most of the smaller arterial branches. This interesting phenomenon has been studied by placing small drops (·001 c.mm.) of 0·1 p.c. adrenaline on the skin or web just over the superficial branches of arteries. When such a drop is placed over an arteriole it shows no constrictor effect. By following up the artery putting drop after drop over its course a point is finally reached where the action begins. When the drops are placed a short distance apart and half a minute or more allowed to elapse between the single tests it is often observed that the artery begins to contract at a certain distance proximally from the last drop and the contraction spreads slowly in a proximal direction while the artery just below the drop remains open. In the web of medium-sized frogs I have found most of the arteries of less than 0·1 mm. diameter when dilated insensitive to adrenaline, but occasionally even much smaller arteries are distinctly influenced though they will seldom contract completely. In the skin the arterial branches which penetrate from below are usually sensitive to adrenaline, while the superficial arteries branching from them are not. Exceptions to these rules are not very rare, however.

Jacobj (2) has found that the arteries of the web react to adrenaline only when the substance is applied to the skin in high concentrations (about 0·1 p.c.). After bathing the web with 10 p.c. veronal sodium for some minutes he found the susceptibility of the arteries greatly increased: adrenaline down to ·0002 p.c. being now able to evoke a distinct contraction. I have tested these results and can confirm that the arteries which normally react only to 0·1 p.c. adrenaline will react distinctly to 0·01 p.c. after the web has been bathed for a few minutes with veronal, but all those branches which did not respond to 0·1 p.c. adrenaline before were also completely refractory after the treatment with veronal. It must be concluded therefore that the wall of most smaller arteries and arterioles does not contain the elements acting as "acceptors" to adrenaline.

In connection with the adrenaline experiments a special observation must be mentioned which has been made very often and which is of some importance in judging local capillary reactions both in frogs and higher animals. When part of a small artery branching from a larger vessel is made to contract, without the contraction being complete, as indicated in the diagram Fig. 2, the current of blood through it may seem to cease altogether, and at the same time the corpuscles disappear from the capillaries perfused from the artery. The impression can very easily be produced that the capillaries themselves are obliterated by contraction. A closer inspection shows, however, that the corpuscles are washed out by a current of plasma. The explanation of this phenomenon is to be found in the distribution of the elements in the larger artery, which shows the well-known axial current of corpuscles with a marginal zone of almost clear plasma. When the current in the branch artery is sufficiently reduced by the contraction, the plasma from the marginal zone is simply skimmed off by it. In favourable circumstances it can be observed how at each pulse the column of corpuscles bulges into the mouth of the branch artery and retreats again immediately afterwards leaving only a few corpuscles which become detached and pass swiftly along through the contracted portion of the artery. This plasma skimming is usually very pronounced when adrenaline is applied in small drops to muscle arteries of which all branches, even the smallest, react and show a contraction which in

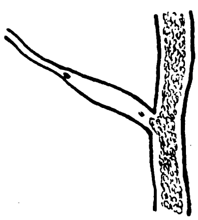

Fig. 2.

a short time may become complete. The portion of the muscle supplied by the contracting artery becomes blanched and the capillaries often disappear completely from view, while application of adrenaline to capillaries and venules alone shows that these vessels do not react visibly to the substance. Plasma skimming may of course take place to any intermediate extent between giving clear plasma and blood with a normal number of corpuscles, and in the artery skimmed the corpuscle count must rise to a corresponding extent. This phenomenon is probably responsible for many irregularities in the number of red corpuscles observed in counts from capillary blood.

SUMMARY.

The capillaries in the skin and web are contractile and can react independently of the arterial pressure. They show frequent spontaneous, but generally localised, variations in diameter, and they react to various local stimuli. Their reactions, however, differ in several respects from those occurring in the lingual capillaries, and it is desirable to emphasise the quantitative and even qualitative difference in the behaviour of vessels supplying different organs.

The contractile tonus of the cutaneous capillaries is much weaker than that of the lingual capillaries and depends much more upon the blood supply. While the cutaneous capillaries dilate a few minutes after the cessation of the blood flow, the lingual capillaries require a minimum of blood only and remain actively contracted for hours after complete blocking of the circulation.

The cutaneous capillaries may respond to certain stimuli by contraction, to others by dilatation, the lingual capillaries respond solely by dilatation. Localised stimuli have a local effect on the cutaneous capillaries, a more or less widespread effect on lingual capillaries.

The cutaneous *arteries*, when contracted, respond to weak mechanical stimulation by dilatation, sometimes over a considerable length, but a strong stimulus, especially a mechanical insult; evokes a contraction which may also extend over a long distance.

Adrenaline generally affects only the larger arteries, viz. those about ·1 mm. and upwards. Adrenaline causes contraction of all the arteries in frog's muscles, but not of the capillaries.

The blood in a partially contracted arterial branch and the capillaries supplied by it may become anæmic even to the complete disappearance of all corpuscles by skimming off the plasma from the marginal zone of the main artery.

I am indebted to my collaborators, Dr G. A. Harrop and Dr P. v. Liebermann for checking by independent observation most of the results given in the preceding pages.

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