STUDIES OF TISSUE MAINTENANCE

I. The Changes with Diminished Blood Bulk

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Plate 8

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The present paper is the first of a series dealing with the service rendered to the tissues by the blood under various conditions. As indices to such service we have utilized the extravascular spread of easily recognizable, innocuous materials thrown into the blood stream. For the purpose of the work here reported certain highly diffusible vital dyes have been employed.

It goes without saying that the multifarious activities of the blood in relation to the tissues cannot be adequately comprehended through observations on the passage from and into the vessels of any single substance or set of substances. But one can at least obtain in this way a knowledge of the general problem in some of its quantitative aspects. Most of the information thus far accumulated concerning it is inferential in nature, being the outcome of observations on rates of blood and lymph flow, on the relative abundance of capillaries in different organs, on capillary pressures, and the state of the local circulation as determined by direct observation. Hooker, Krogh, Richards and others have made studies of the small blood-vessels which illumine the general field; and some investigators have followed directly the diffusion from or into individual capillaries. Our aim has been to determine what the blood does under pathological conditions for the various organs of the body as a whole.

The Choice of Materials

The peculiarities of living cells as manifested in what they reject or accept, and accepting, utilize, secrete, or store, renders the problem of tissue maintenance highly diverse. But it should be possible to find out in a general way whether the circulation is adequately serving the cells in bringing materials to them and away from them, irrespective of what the cells do with these materials. Susbstances that are let pass by the capillaries must, of course, be employed. True, these will be let pass in varying degrees. But it would seem to be a general law that diffusible, non-toxic "acid" stains penetrate the capillary wall at approximately the same relative rates as when they spread through gelatin (1). If a number that differ widely in this latter respect are selected for animal test it should be possible to gain an insight into how the tissues are served with materials normal to them. At first thought one would say that normal substances should be used. But not only are many of these subjected to change or removal through cell activities, with result that the gradient of permeability is altered locally, but their situation and quantity cannot be gauged with the eye as it can in the case of vital stains of intense color. The best of these stains have special affinities and are stored or excreted in ways that alter interpretations. But with the recognition of such peculiarities, errors due to them can be ruled out. The monographs of Schulemann (1) and of von Möllendorff (2) give one access to a large series of vital dyes. From amongst them and from other sources it should be possible to select test-substances covering the diffusion range of most materials normally purveyed to the cells, with exception of the gases.

The recent history of vital staining is an instructive one, illustrating as it does how rapidly the uses of a scientific tool of wide applicability can become stereotyped as result of success with it in a special field. Ehrlich brought vital staining to modern attention, making significant observations on the nervous system with the aid of methylene blue about thirty years ago (3). But with the subsequent discovery that certain of the poorly diffusible dyes are taken up and stored within living cells, interest turned almost wholly in the direction of the disclosures thus made possible. Not very often since, despite the immense gamut of available dyes, has diffuse vital staining been employed, and then, with a few noteworthy exceptions, for highly specialized purposes. Furthermore the range of dyes utilized in the study of cell-storage has of late narrowed instead of broadening, the mass of investigations nowadays being conducted with one or another of but a few slightly diffusible stains. An observation of the earlier investigators should be mentioned because of its bearing on our own problem, namely that dye storage is especially abundant in the eye muscles, diaphragm, and heart,—whence these workers inferred the existence of an especially great fluid interchange in the organs mentioned (4). Recently Okuneff (5) and Kusnetzowski (6), using the same criterion, have concluded that much more dye reaches the cells of regions that are inflamed or heated than is the normal case.

For the present work highly diffusible stains devoid of confusing tissue affinities have been selected—Patent Blue V, brom phenol blue, phenol red and sodium indigotate. In enlargement of some of the observations india ink has been pressed into service. Ordinarily we have followed only the distribution of the dyes from blood to tissues, not their subsequent removal from the latter.

Patent Blue V is an intense stain of great diffusibility (7).* Within a few seconds after it has entered the blood stream white animals become brilliantly blue; and by pressing out the blood from the tissues the extravascular situation of the dye can readily be demonstrated. To obtain data on its rate of diffusion into the various organs is difficult, so quickly does it enter most of them. One can always tell, though, where it is and where it is not; and it has the great advantage of rendering visible the walls of the arterioles. Unanesthetized rabbits injected with 1½ cc. per kilo of a warmed, unbuffered 8 per cent solution of Patent Blue V (Hoechst), of pH 6. approximately, manifest no symptoms whatever; and etherized ones show no disturbances of heart beat or blood pressure that are observable with the kymograph, other than those fleeting ones produced by a similar quantity of salt solution. The dye retains its color within the organism. More than three-fourths of it can be recovered as such from the urine of the first twenty-four hours, and most of the rest later on.

Brom phenol blue, somewhat less diffusible and leaving the blood not quite so rapidly, is almost as intense a vital stain. Its range as indicator lies too far to the acid side for the blood and tissue reactions to affect its hue. For our purposes it has proved well-nigh ideal, being devoid, when properly purified†, of action on blood vessels or heart, and diffusing at such rate that the stages in its distribution

* A correlation of the diffusibilities of the dyes *in vivo* and *in vitro* is being carried out in our laboratory by Dr. Frederick Smith who will report upon them later. It will suffice here to state that a watery solution of Patent Blue V diffuses through a porous glass disc of the sort employed for diffusion measurements by Northrop and Anson (*J. Gen. Physiol.*, 1929, **12**, 541) about as quickly as does dextrose. When the pores of the disc have been filled with gelatin it passes only about one fourth as rapidly as dextrose.

† As ordinarily sold for pH determinations brom phenol blue often contains contaminants affecting the blood pressure. Hynson, Westcott and Dunning have most kindly made for us a purification of their product, which has proved innocuous and devoid of cardiac or vascular effects in cats and rabbits.

can be followed in animals killed at brief intervals after injection.* Furthermore its color so dominates over that intrinsic to the tissues that direct comparisons of degrees of staining become possible. It is best employed in 4 per cent solution, and for intense staining a somewhat greater fluid bulk than of Patent Blue V must be injected intravenously, 3 cc. per kilo of cat or rabbit.† Our usual technic, as in the case of other stains, has been to inject the warmed dye solution during the course of one minute. By the end of this time the body surface is already an even, intense blue; and the failure of the tissues to change color when the blood is driven from them by pressure shows that the dye has already largely passed from the vessels. Neither then nor later are symptoms evident. Rabbits suddenly stained deep blue and placed on the floor sniff about inquiringly and when food is placed before them at once fall to. Three minutes after the dye injection the staining is nearly at a maximum throughout the animal, though accurate color comparisons show that it deepens slightly within another five minutes, remaining constant then for approximately 4 hours and gradually fading later. By next day the animal is practically decolorized, the dye lingering only where the circulation is poor, as in the cartilage of the ear which is still light blue, the tendons (paler blue), the sclerotics (faintly blue); or about a locus of special retention as in the case of the blue gall-bladder wall through which resorption is still taking place secondarily from bile blue-black with the dye excreted with it. At this time the blood plasma no longer is colored. The phthalein leaves the body chiefly by way of the kidney though a little escapes in the feces. As much as 94 per cent of the amount injected has been recovered in the urine of the first 48 hours. The liver takes much out of the blood at an early period, secreting it into the bile, but, as happens with so many other stains, it passes into the circulation again from the gall bladder and intestinal tract, and ultimately escapes in the urine after all. No evidence has been obtained of the least decolorization of the dye within the organism. If any occurs it is negligible.

Phenol red diffuses twice as fast as does brom phenol blue‡ and somewhat more rapidly than Patent Blue V. Much of what has just been said applies to it. Because of the current utilization of it in renal tests it is readily available in pure

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* Dr. Smith has found it to pass through the porous disc of Northrop and Anson at a speed slightly more than half that of Patent Blue V irrespective of whether the disc has been filled with gelatin or not.

† We are indebeted to Dr. MacInnes for freezing point determinations which show that the solution we have employed is isotonic with 0.92 per cent NaCl, and to Dr. Mirsky for observations with the glass electrode which show the pH to be approximately 7.24 at 37°C. The method of preparing the solution has been given in a previous paper.

‡ Unpublished observations of Dr. Smith.

form; and the injection of a freshly made, warmed, isotonic 4 per cent solution of it at pH 7.4, to the amount of 3¾ cc. per kilo, the optimal quantity for staining, causes no symptoms or cardiac or vascular manifestations that a corresponding amount of Ringer's solution would not elicit. It can be used to study blood service to the connective tissue and the other relatively alkaline matrix tissues (cartilage, fascia and tendon(8)) where it appears ruddy, but not for observations on the muscles and viscera, since in them it assumes various shades of orange and yellow that are not readily perceived and evaluated when only a small amount of the dye is present.

Sodium indigotate is poorly soluble at best, only to 2 per cent in water at body temperature; and as much as 7½ cc. per kilo must be injected if the color native to the organs is to be drowned in blue. It is reduced to indigo white in many of the tissues, though it turns blue again on exposure to air; and it is rapidly excreted by liver and kidneys. For all these reasons it has proved unsatisfactory in the study of blood service, although it can be used in confirmation of certain phenomena.

General Procedure

Rabbits and cats have been used for most of the work, and light ether as the anesthetic when one has been necessary. Many of the rabbits have been unanesthetized. To begin with, the normal distribution to the tissues of brom phenol blue was ascertained, and a routine method of examination was worked out. In order that the spread of the dye to the superficies might be studied the fur was in many instances removed from a large part of the trunk and thighs some days prior to the observations, the animal being guarded against chilling thereafter. Barium sulphide proved better for the purpose than shaving. Areas accidentally inflamed were rapidly discriminated by the special intensity of the staining. Ordinarily the animals were fasted 24 hours in order to avoid a digestive hyperemia of the gastro-intestinal tract (Bier); but they were allowed water. Etherized ones were kept on an electrically warmed pad, and were not stretched out but laid on the back or side, without ties. Tracheal cannulation ensured a more even anesthesia. The dye was injected into an ear vein of rabbits and into the basilic vein or internal saphenous of cats, these vessels frequently being cannulated for the purpose. Oiling the body surface brought out brilliantly the surface hues. Since the abrupt introduction of even as little as 1 cc. of fluid into the circulation of a large rabbit brings about compensatory readjustments (Tigerstedt) the dye solution was given gradually in the course of one minute, as already stated. Ordinarily three further minutes were let elapse and then the animal was killed, by cutting both carotids, or,—when no anesthetic had been used,—by decapitation at a blow. The complication of stained blood within the tissues was minimized by the rapid exsanguination. The autopsy was carried out at once and very rapidly, by two workers, with the animal on a slanting board, head down. The organs of special interest were first looked at, the order of inspection being purposely varied to rule out the possibility of errors due to post-mortem diffusion of the dye. The intensity of the staining as viewed in the gross, was frequently recorded in terms of Ridgway's color standards (9) according to the method used in previous investigations (10). Save in special instances we have not concerned ourselves with the precise location of the dye within the tissues, the main point being that it should have left the circulation, have been served up to the cells, so to speak, irrespective of acceptance or rejection by them.

Not infrequently an amount of blood equivalent to that of dye was removed just prior to injection of the latter. But needless to say this proceeding merely complicated the issue. For not only must some vasoconstriction have been invoked by it, but the salt solution containing the dye must practically at once have been removed from circulation. Whatever the importance of these various factors they did not suffice to bring about differences in the picture. The animals as a group yielded consistent findings, irrespective of the stain employed.

The Staining in Normal Animals

The phenomena observed after the injection of brom phenol blue were essentially the same in rabbits and cats, and were unaffected by etherization. By the time the injection was completed the *hairless tip of the nose*, the *gums*, *conjunctivae* and *fauces* had stained deeply and evenly. The pads of the feet became blue only a little more slowly and less markedly. The *general body surface* took on color progressively and evenly except for intensifications where vessels were abundant, as over the heels and about the mammae. After three minutes the surface hue was brilliant (between "cadet blue" and "diva blue"—Ridgway), the stain lying in the connective tissue. The thin sheet of *voluntary muscle* coming away with the pelt was but palely blue, much paler than the external and internal oblique and the pectoral and leg muscles. These in turn were not nearly so well stained as the *diaphragm*, *intercostals* and *lingual muscles*, the differences being especially plain when muscle layers of the same thickness were compared. To the unaided eye the staining of *subcutaneous tissue* and muscles appeared diffuse, and this still held true when the animals were killed only a few seconds after the injection. For example the tissues of a rabbit killed within 15 seconds after an injection lasting 30 seconds appeared diffusely stained, as did those of a cat killed 15 seconds after an injection which had itself required 30 seconds. But in certain noteworthy instances of animals kept for the routine period of 3 minutes there were indications in the muscles of a latticing or transverse banding with blue, a phenomenon later found to be pronouncedly present when staining was done after the blood bulk had been reduced by bleeding or through the production of anhydremia (11).

The *connective tissue* and *fascia* were everywhere of a medium blue, the *cartilage*,—except for the rib cartilages which fail to stain,—a lighter blue, and so too with the *tendons* and the newer portions of *bone*. Old bone remained uncolored.

Though the supporting framework of the *adipose tissue* was well colored the fat itself did not stain, nor did the white matter of the *central nervous system*, the gray matter being dubiously tinged. The *nerves* to the muscles were beautifully visible in grayish blue to their finest ramifications. The *red bone marrow* appeared deeply colored, but the dye was localized to the blood content. There was intense staining of the media of the larger *arteries* suggesting a special affinity, though the subsequent decolorization took place nearly if not quite as rapidly as elsewhere save in the aorta where it noticeably lagged. The latticing and banding in the muscles mentioned above was not due to this vascular staining. The walls of the *veins* stained poorly. The fibrin of post-mortem blood clots stood out in deep blue. Embolism and thrombosis were sought for but never found.

The tissues thus far described were only moderately blue as compared with most of the abdominal viscera. These were so suffused with the dye as to afford a startling contrast. The *intestines*, large and small, were a deep purplish blue, and so too with the *oesophagus* and *gall-bladder*. The bladder bile of rabbits was already definitely blue after three minutes, but that of cats only later. The peritoneal surface of the *stomach* appeared rather light blue, though finely stippled with darker blue points in the case of the cat; but when the organ had been slit open the mucosa and submucosa proved to be deeply stained, like the gut further down. It was the gastric muscularis into which relatively little of the dye had gone. Occasionally there were to be noted in it areas of local contraction which had not stained at all though the inner and outer layers of these areas had stained as well as ordinary. In this connection the fact deserves mention that the segments of large intestine distended with fecal masses were as excellently stained as the empty, contracted regions between. A notable example was furnished by the rabbit colon wherein fecal pellets are usually distributed at nearly regular intervals with a thinned wall over them and contracted gut between, like coarse beads on a thick, gristly string. Such a colon when slit longitudinally, emptied and inspected between glass plates had the same color intensity everywhere.

So rapidly did stain pass into the *mesenteric lymphatics* that they were distended with deep purply-blue fluid within 15 seconds after the dye injection. The glands at the root of the mesentery contained a similar fluid. In view of the extremely rapid diffusion the viscera were ordinarily inspected first of all, often before the heart had stopped.

In the gross the *liver* appeared deeply and evenly colored, but Valentine knife sections disclosed minor variations in hue, the periphery of the lobules being more intensely colored than the center, and both appearing greenish as compared with the clear blue of the interlobular connective tissue. In rabbits killed after three minutes some stain had already reached the bile. The *spleen* was a more or less deep blue,—less when the organ was somewhat contracted. The *kidney* cortex was dark blue, the medulla lighter, and brom phenol blue was present in the cortical tubules. The *urinary bladder* was medium blue, irrespective of whether it was full or empty, the hue approximating that of the superficial connective tissue. The *omentum* of the cat was lighter. That of the rabbit proved too filmy for useful observations. Both in omentum and *retroperitoneal* fat the stain was localized to the connective tissue framework. The *pancreas* appeared evenly and rather lightly stained. Here too the color was principally in the connective tissue.

The *ovaries* stained rather intensely, and so too with the medulla of the adrenals, the cortex staining scarcely at all. The *pregnant uterus* was deeply blue, but into amniotic fluid and well-developed fetuses the dye had not penetrated during the few minutes following the injection.

The *lungs* were evenly and lightly blue, and the *thymus* too. The *heart wall* appeared deep purple blue. We have not attempted to determine how much of the color was due to contained blood.

In significant contrast to the mesenteric *lymphatics and glands*, those of the limbs contained fluid that was at most but palely blue.

Substantially identical findings were obtained with Patent Blue V, though no banding or lattice work was disclosed in the muscles, so rapidly did the dye diffuse. With sodium indigotate, the far greater intensity of the staining in the viscera was readily demonstrated, but minor differences were not easily to be apprehended owing to the reduction of the dye to a colorless form.

A number of the organs considered above will not be referred to again in the course of the present paper, notably the heart, kidneys, thymus, ovaries and adrenals. We have described them merely to round out the picture.

In summary one can say that certain organs or tissues (brain, lamellated bone) are entered practically not at all by the dyes of our experiments, that others receive but little of them (cartilage, tendon, new bone, nerves), others show them in considerable quantity (connective tissue, urinary bladder, muscle, pancreas), while others yet become so suffused as to constitute a group apart (liver, gall-bladder, intestines, stomach). The deep color of the spleen and red bone marrow is deceptive, the dyes lying for the most part still in the blood contained in these organs. In not a few others they are localized almost entirely to connective tissue scaffolding and interstitial fluid. With the binocular microscope one can readily make out that the epithelium of the gut is stained only faintly if at all, and that in voluntary muscle the color lies in general between the fibres. But, as has already been stated, our object has been merely to determine whether the dyes are purveyed to the cells, not whether they are taken up. The observations just recounted yielded a norm for the distribution from the circulation.

Certain affinities of brom phenol blue require recognition at this time. It has some affinity for the media of arteries, as also for medullated nerves. In common with many other dyes it is rapidly removed from the blood by liver and kidneys. Möllendorff has correlated the rate of excretion of such dyes into the bile with their physical properties (12); yet the activities of the liver cells in secretion of them are still not wholly understood. The failure of the phthalein to penetrate old lamellated bone is evidently due to the constitution of the latter. The failure of the brain to stain with vital dyes in general has never been satisfactorily explained. The intense color of the diaphragm, intercostals and tongue as compared with the other skeletal muscles is understandable on the basis of their more abundant circulation (13).

The Technique of Depletion

When the blood bulk has been suddenly and considerably reduced, by bleedings or procedures causing anhydremia, profound alterations take place in the service rendered to the various organs by the circulation. Some of the changes have been briefly described in a report on the local, extravascular acidoses arising out of the state of affairs (14). We shall here consider them more fully.

To deplete the cats and rabbits used in the present observations successive bleedings have been employed. Since the circulatory alterations disclosed by the staining method are seen in pronounced form only when the blood bulk is not restored through readjustments within the body, the animals were fasted from 18 to 24 hours prior to experiment in order to lessen the utilization of fluid from the gut, as further, to avoid digestive hyperemia. Usually they had access to water; but under the circumstances they drank little. Those that were etherized and connected with the kymograph by way of the carotid were bled, either from this carotid by puncture of the rubber tubing just above the cannula, or preferably from an axillary artery cannulated for the purpose. Rabbits were bled with the aid of local anesthesia (novocain) by a method which involves a preliminary operation under general anesthesia to bring the carotid to the surface, cannulate it, and place about it an elastic clamp. The clamp was made from a short piece of rubber tubing about 3 mm. in outside diameter which was bent upon itself and tied so tightly at the bend that its limbs did not lie in parallel but sprung somewhat apart. The contrivance was slipped about the artery, and the free ends of the tubing were pulled through a constricting ring of rubber formed from the segment of a larger, thick-walled tube. To shut off the vessel the ring was rolled toward it along the pieces of tubing, the pressure of these latter upon each other sufficing for the purpose. For bleeding the ring was rolled away. There was an optimal position of the latter at which the vessel was held gently shut, to be opened merely by pressing the ends of the contrivance toward each other, thus springing its sides apart. Successive bleedings were readily carried out with the animal on its feet.

It was early found that a large depletion, especially in the absence of general anesthesia, was required to bring about deviations in blood service so pronounced that certain regions which ordinarily color well failed to stain. No effort was made to determine the least loss of blood that would suffice for this purpose. When it was accomplished by a progressive anhydremia the blood pressure often varied little from the normal (15); but when bleeding was employed it usually fell to about 100 mm. Hg in cats, and 60 mm. Hg in rabbits, in the absence of any efforts to conserve it. After a first hemorrhage, as is well known, fluid from the tissues enters the vessels; but this readjustment is ordinarily completed within a few minutes, and takes place to but a slight extent after later bleedings (16). To rule out its influence upon the findings, as also to permit conditions to become relatively stable, three or four bleedings were done in all, at ten to twenty minute intervals, and the dye injection was ordinarily deferred until twenty minutes after the last one. Great care was taken that the depilated, depleted animals should not grow cold during the experiments. The unanesthetized ones were kept in warm rooms, and those under ether were in addition placed on electrically warmed pads. With the successive bleedings the skin and mucous membranes became pallid, and the superficial veins more or less collapsed. Very occasionally an ill-defined surface mottling could be made out.

Ordinarily almost half, if not quite half, of the calculated blood volume (which is approximately 7.5 per cent in the cat, 5.5 per cent in the rabbit) was removed. The rabbits depleted with local anesthesia still kept their feet but the respirations were exaggerated. That the alterations in the staining were not traceable to low blood pressure, as such, had been disclosed by the observations on anhydremic animals (17), and was now further shown by the fact that the longer the interval elapsing between the final bleeding and the injection the more pronounced were the deviations from the ordinary staining, although in the interval the pressure often tended to recover. The same amount of dye per kilo was given as under normal conditions, and in the same way. It sometimes caused a partial recovery of the blood pressure, but no symptoms. As in the case of the controls, the animals were killed three minutes after the injection.

The Alterations after Reduction of the Blood Bulk

The phenomena occurring in the *superficial tissues*, in the pelt that is to say, of animals receiving brom phenol blue have been briefly described in a preceding paper. The cat or rabbit turned blue more slowly than usual, the rate depending on the degree of depletion and how long after it the dye was injected. In extreme instances only a faint blue staining developed and this was limited to the regions where large vessels entered the skin. In the majority of instances the staining was patchy, unstained areas being everywhere interspersed amidst others that became brightly and diffusely blue. This patching proved in the cat to be essentially similar to that already described for the rabbit and rat (18) but the white areas tended to be larger. They were irregular in outline, with serpiginous margins, frequently confluent, and varied in size according to the depletion conditions, the arrangement being sometimes of blue on a white ground and again of white on blue. In poorly marked instances there was merely a scattered sprinkling of small white spots on the blue expanse. The patching was especially well seen in well-nourished white cats with an underlying panniculus. In extreme instances, in which phenol red had been used instead of a blue dye, there took place a very gradual orange staining,—the color indicative of acidosis,—about the largest arteries entering the skin, while elsewhere the surface was unstained. The orange-red hue of the conjunctiva in such cases pointed to a blood acidosis. In less severely depleted animals the injection of the phthalein was followed by a brilliant mottling of red on white or white on red. Where the dye crept in later, at the edges of the unstained regions, it was seen to be orange.

Slight differences in local pressure sometimes exerted a great influence to determine the situation of patches in the depleted animal (19). Crouching rabbits often showed a broad, unstained strip along the ridge of the backbone and other large unstained areas over the bulge of the knees. It was necessary to allow for such localizations, as further for the influence of isolated masses of fat to make an overlying, thin, translucent skin appear poorly stained. Where errors of interpretation from these causes could be ruled out one saw that the patches were not only highly irregular in contour but without trace of symmetry. On reflecting the pelt the relation of them to the blood vessels could be made out; and the arrangement of the patching was found to be wholly independent of the vascular pattern visible in the gross, save in the extreme instances above mentioned in which only the tissue immediately about the largest vessels was stained. This independence was especially well to be seen where a number of arteries supplied the subcutaneous tissue in parallel, the lumbar vessels of the cat, for example. Here some of the arterial twigs ran to patches that were white, other corresponding ones to areas that were blue, and yet others to areas that were irregularly blue and white (Fig. 2).

The patching was not essentially dependent upon cooling of the skin, though frequently developing when it was cool. It appeared pronouncedly in one of our animals which was accidentally overheated on the pad; and it can be regularly elicited in rats submerged in oil at body temperature (20). If nothing occurred to relieve the depleted organism the white areas persisted for long periods, though tending gradually to diminish in size by a peripheral encroachment of the dye. When they were very large dye sometimes appeared secondarily at spots here and there within them. Obliteration of them occurred in both ways when the blood volume was restored by reinjection of the portion removed.

The *gums*, *nose tip*, *conjunctivae* and *mucous membranes of the mouth* colored promptly and deeply even when they had appeared absolutely bloodless prior to injection of the dye. They showed no patching, nor did the pads of the feet, which also stained rapidly but somewhat less well.

The *skeletal muscles* exhibited a remarkable reticulation or transverse banding with blue as has already been briefly recorded. And the pattern, unlike that of the skin, was regularly ordered, and did not vary in its dimensions with the degree of depletion. It could be studied directly under the binocular dissecting microscope in certain of the flat muscles (tibialis anticus, gracilis, pectorals, extensor longus digitorum) wherein it took the form of a transverse banding, and in others (as e.g. those of the abdominal wall) where a blue reticulum separated oval, unstained areas. To obtain specimens glass plates were slipped over and under the muscle which had been loosened from the tissues sufficiently for this purpose, and the attachments were then cut. The banding was now seen with the unaided eye to be discontinuous, consisting of many short blue segments displaced regularly a little to this side or that of the main axis of the band. When the muscle was thick (quadratus lumborum) the segments were frequently superimposed at different levels. The blue reticulum was likewise discontinuous. For the present it will suffice to say that the arrangement of the staining was determined by that of the vascular tree, and that it occurred about vessels of a special magnitude, several times larger than capillaries. The average length of the latter in rabbit muscle is 0.69 mm. (21), while the unstained regions between the blue bands (or blue reticulum) were more than 2.0 mm. across in this species, and even broader in cats.

Greater differences were evident in the staining of the various muscles than under normal circumstances. The cutaneous muscle layer did not color at all after severe depletion, and the sheet muscles of the abdomen but slightly as compared with the brilliantly banded quadratus lumborum and gracilis. Even these were pale in comparison with the diaphragm, intercostals and tongue muscles which were diffusely stained and almost if not quite as deep blue as ordinary. Sometimes the intercostals showed a slight relative pallor midway between the ribs; but the blue was uniformly distributed in diaphragm and tongue even when the animal had been sacrificed only a few seconds after injection.

The findings thus far described bear witness to pronounced and peculiar empoverishments of blood service. Not so with those in the *gastro-intestinal tract.* Here the staining appeared to have the same intensity as ordinary, and only on recording the hues in terms of Ridgway's book did one perceive it to be slightly less. As result of the fasting the duodenum and jejunum were wholly collapsed as a rule. The *intestines*, large and small, were always a diffuse, even, purply blue, and so too with the *gall-bladder* wall, *esophagus* and the mucosa of the *stomach.* In extreme instances the muscularis of this last organ showed an ill-defined, slight, pallid blotching but as a rule the coloration was diffuse. The staining was as good where the intestines were stretched over fecal masses as in the contracted lengths between. All this was true even when the animal was killed practically at once after the dye injection. Already the *lymphatics* of the mesentery and of the gall-bladder wall were distended with deep blue lymph.

The *liver* was, as in the normal animal, diffusely and deeply blue. The *pancreas* was stained about as well as ordinary and showed no patching. The *urinary bladder*, on the other hand, was decidedly paler than in controls, often indeed almost unstained, but without any patching. And the condition of the spleen showed frequently that it had been shut off *in toto* from the circulation, even in etherized animals. Contracted and small the organ stood forth in vivid red against deep blue surroundings. When depletion had not been severe small spreading areas of blue were found scattered amidst the red of the surface exposed on section.

The *omentum* of cats presented a singular picture, being splotched with blue and white wherever it was thick enough for the presence or absence of staining to be made out. The distribution of the stain was irregular, as in the case of the skin, and independent of the vascular patterning visible in the gross. It was confined to the connective tissue. The unstained patches were often several centimeters in greatest diameter. In the retroperitoneal fat a similar splotching or marbling with blue was brilliantly evident. The omentum of the rabbit proved too filmy for satisfactory study.

A contrast to these evidences of ischemia was furnished by the *uterus* of cats far pregnant. The organ was found markedly and evenly stained despite the severest depletion, and the veins coming away from it contained much deeply colored blood although there was little elsewhere owing to the exsanguination at death. In this connection mention may be made of the fact that inflamed areas in the skin of depleted animals stained excellently.

The *kidneys* were diffusely blue, to the naked eye at least. *Tendon* and *cartilage* (ear, knee joint) colored somewhat less well than in controls, but there was no patching. The deep hue of the *red bone marrow* (legs, ribs) on the other hand showed that the dye had reached this tissue abundantly. The *lungs* stained lightly, as in controls, save at their wedge edges which were often wholly uncolored. The tissue at the apices was no paler than that elsewhere.

The lymphatics and glands of axilla and groin, in contrast to those draining the intestines, contained a fluid only faintly tinged with the dye.

These various findings were readily confirmed with Patent Blue V, and some of them with phenol red. The patching of the skin could be demonstrated with sodium indigotate, as also the profound staining of the gastro-intestinal tract, gall-bladder included.

In summary of the observations it can be said that under the circumstances of a markedly reduced blood volume certain of the organs continue to be well and evenly served by the circulation, whereas in others a pronounced patchy ischemia occurs. The conjunctiva, lips, gums, pharynx, oesophagus, stomach, intestines, gall-bladder and liver all become rapidly and deeply stained; and the dye appears in quantity in the lymph from the gut and gall-bladder, even when the carotid pressure has been greatly lowered by the bleedings. Liver lymph was not studied. The red bone marrow continues to be excellently served by the blood in animals at the extreme of depletion, and so too does the pregnant uterus. The skin on the other hand and the voluntary muscles, with certain notable exceptions, are largely deprived of effective circulation. The deprivation takes a singular form, regions showing it being interspersed amidst others in comparison excellently served by the blood as evidenced by the staining. The size and number of the patches in the skin varies directly with the degree of depletion and the length of time it endures. Their distribution is entirely unsymmetrical and they seem unrelated to the vascular patterning visible to the eye. In the muscles on the other hand there is a regular disposition of unstained and stained regions, the latter situate about vessels of a special, and not inconsiderable magnitude. The unstained regions have a diameter several times greater than the length of the individual capillary. Not all of the muscles suffer this neglect. The diaphragm, intercostals and tongue muscles continue to be excellently supplied by the blood, as the depth and evenness of the staining attest.

Though blood service is remarkably well sustained in most of the abdominal organs there are certain significant exceptions. The spleen is largely, sometimes entirely, deprived of circulation, as shown by the failure of dye to enter it, and the urinary bladder, lightly colored in the controls, is often practically unstained in the bled animals. In the omentum pallid, ischemic regions are scattered irregularly amidst others well supplied from the blood as shown by their brilliant blue color.

Observations with India Ink

It has seemed important to determine the precise relation of the blood vessels to the ischemic patching just described. For this purpose we have resorted to india ink injections. A principal result of the work has been the recognition of how far the method falls short of demonstrating circulatory conditions within the body.

The distribution of india ink by the blood stream has been followed by a host of workers. Krogh has made large use of it in his studies of capillary regulation. Our own observations with ink of the sort he employed (Pelikan Perl Tusch, Günther Wagner), dialyzed against Ringer's solution and filtered in the way he describes (22), would seem to indicate that his findings were obtained in the face of serious technical draw-backs. When the amount of ink that Krogh employed is injected intravenously into a rabbit or a guinea-pig, and the vessels of the ear are watched under a microscope, one can perceive that the foreign particles tend to agglomerate into lumps as they are carried along. These lumps soon lodge here and there within or at the entrance to capillaries, effectually blocking them. Using such material one can be certain only that where it passes the vessels are open. There is, fortunately, no difficulty in demonstrating with it that many more such vessels are open in the diaphragm of the normal animal than in most of the other skeletal muscles, a phenomenon emphasized by Krogh. But to assure oneself that where the ink does not penetrate no circulation had existed is quite another matter. And this holds true even when an ink far better for the purpose is used, Higgins' American Drawing Ink (non-waterproof), dialyzed against Ringer's solution, filtered and centrifuged—the last two processes being unnecessary in our experience. On injection of this the particles can be seen to circulate separately during the brief period before they are removed from the blood by the sessile phagocytes.

Normal white animals receiving either Higgins' ink, or Pelikan Perl Tusch, become transiently gray. We had supposed that in depleted ones patchily but intensely colored with brom phenol blue, injected ink particles would pass into the stained regions of skin and muscle in sufficient quantity for histological recognition if the animals were killed while they still circulated. But this did not prove to be the case. To all intents and purposes the particulate matter was shut off from the organs mentioned, and this proved to be the case as well in animals that had been merely depleted, not stained. The skin did not turn gray anywhere. The abdominal viscera on the other hand were dark with ink, except for the spleen which contained practically none. The liver was black with that which had been taken up by the Kupffer cells. In contrast to the pallid state of the skin generally, the tip of the nose, and the pads were gray with ink, lying doubtless within the arterio-venous anastomoses there known to exist (23, 24). The quantity injected was only 2½ cc. per kilo, because of the need to avoid large increases of the blood bulk. Further findings will be detailed in a succeeding paper.

DISCUSSION

The method of the present work would seem to be validated by the disclosures it has yielded. The alterations we have observed in the service rendered by the blood to the skin, muscles, and certain other organs of the depleted animal cannot be apprehended by ordinary laboratory procedures; nor are they demonstrable with india ink. In animals depleted by large hemorrhages a rapid and deep staining occurs in some regions (lips, gums, tip of the nose) which under such conditions in unstained animals appear wholly bloodless; and furthermore staining occurs in skin regions to which india ink particles are not carried by the blood. It seems probable that in some of these situations only a stained plasma may have circulated, red cells being removed by the "skimming" that Krogh first described. The great diffusibility of the dyes we employed proved in some ways a disadvantage; for they were so readily distributed that only drastic reductions in service to the tissues were recognizable with their aid. To appreciate less considerable changes it will be necessary to employ vital stains that do not leave the blood so rapidly.

Can it be said that where brom phenol blue failed to go all interchange between the blood and tissues had ceased? Scarcely. For Patent Blue V gradually penetrated where brom phenol blue did not. And carbon dioxide, which passes through the tissues with unexampled ease (25), reaches situations inaccessible to other substances. To all intents and purposes nevertheless the circulation had ceased to be effective in the skin areas which brom phenol blue or phenol red failed to penetrate. For an acidosis developed in such areas, one referable to the local accumulation of acid metabolites (26).

In appraising our results a first question is, how far they were conditioned by special affinities of the dyes employed? By employing several of highly different constitution we have tried to minimize this factor; but in another and better way it has been proven unimportant, namely by following the process of decolorization. Those organs which stain most rapidly and deeply with brom phenol blue are, with exception of the liver and kidneys,—which actively excrete the dye,—precisely those which lose color soonest, as could scarcely happen were it fixed upon them as result of a special affinity. The gastro-intestinal tract and the diaphragm become colorless long before the skin does. Brom phenol blue stains the media of arteries with a special intensity; but even here decolorization does not lag notably.

A principal alteration in blood service after hemorrhage is a peripheral vascular shut-down, the blood supply to the viscera being maintained at the expense of that to the superficial tissues and the muscles. This readjustment has long been recognized as one of the means whereby the vital forces are conserved in individuals "bled white." Other changes disclosed by the staining method impress one with their purposefulness. Not all of the abdominal organs continue to be well served by the blood. Those which are essential, and which can be safely neglected,—the omentum, urinary bladder and spleen,—are neglected. By contrast the whole gastrointestinal tract —from which alone help can come to the organism under natural conditions,—continues to be well served. Special mention may be made of the maintenance of the circulation to the gall-bladder, since the realization is recent that a very active resorption takes place through the walls of the organ into the blood and lymph. Even the esophagus and fauces are well served, and the gums and lips are much better maintained than is the skin. The red bone marrow continues to be excellently supplied with blood, though situated within limbs that are for the rest largely deprived of it.

The amount of blood which would be conserved to the organism were there complete ischemia of the skin is but slight (2 to 3 per cent) (27), but the saving of heat is far more considerable. And the cooling of the neglected tissues lessens the formation of waste products within them. In the voluntary muscles, large, enduringly bloodless patches do not develop as in the skin, but there is a regular arrangement of smaller ones into which a certain amount of diffusion gradually occurs, as shown by the findings with Patent Blue V. But not all of the muscles suffer in this way. Those which are essential to respiration (diaphragm, intercostals) and to swallowing (tongue muscles) continue to be well served by the blood. The question whether this is true because these muscles go on working need not be taken up at the moment, though the fact may be mentioned that the muscles of the tongue continue to be well served from the blood, as shown by a diffuse, deep staining, even when the organ lies flaccid in animals anesthetized through a tracheal cannula.

Under normal circumstances much more stain has been found to pass from the blood into the tissues of the gastro-intestinal tract than into the skin and muscles. Numerous reasons for this can be thought of. The amount of blood passing in a unit of time through the skin and resting muscles is many times less than through the portal circulation. Capillaries are far more numerous in the viscera while furthermore a great proportion of those existing in the skin and resting muscles are ordinarily shut. There is an active flow of lymph from the blood into the mesenteric lymphatics, but practically none into the lymphatics of resting muscles (28, 29), whence it follows that a dye circulating in the blood penetrates into the muscles only by diffusion, whereas the process of distribution to the tissues of the gut is actively aided by a streaming of fluid out of the vessels. Furthermore the distribution to the gut is aided by a high capillary blood pressure, and the barrier offered by the capillary wall itself is imperfect, as shown by the presence of blood proteins in lymph collected from the mesenteric channels (30). Considering all this one cannot wonder that the stomach and intestines showed a specially intense and rapid staining with vital dyes.

From what is known of the physiological readjustments which take place in the bled animal one might expect the staining in the gastrointestinal tract to disclose wide deviations from the normal. The capillaries of the region are known to be actively contractile; and a narrowing of the portal channels through vaso-constriction is deemed one of the most important compensatory changes occurring when the blood volume is diminished (31, 32). Even losses of blood which do not suffice to lower the arterial pressure cause some blanching of the intestines (Starling); and when such a lowering has taken place the rate of formation of lymph in the gut and liver is markedly reduced (33). Not a few functional conditions have been described in which blanching of the intestines was so great that the tissue seemed to all intents and purposes bloodless. For every *a priori* reason, then, except the teleologic, one might suppose that blood service to the gut after hemorrhage would be greatly lessened, perhaps to the extent of ceasing in some regions. But the dye experiments showed quite another state of affairs to prevail. Staining of the gut, gall-bladder and liver was always deep, though the animal was at the extreme of depletion and was killed but a few seconds after the dye injection; and the staining was diffuse save occasionally in the muscularis of the stomach where an ill-defined blotching could be made out. The mesenteric lymphatics were always distended with deep blue fluid. The pancreas likewise was colored as usual. One must conclude that the compensatory constriction was never so great as to interfere seriously with blood service to the digestive organs.

Mention has been made of the fact that the staining of the intestine where it was stretched over fecal masses was of precisely the same intensity as in the empty, contracted segments lying between. The pressure condition where feces distend the gut is probably much like that in the full bladder, of which Sherrington remarks (34) that it "enfolds its contents in the same light grip whether these contents be ample or little." Owings, McIntosh, Stone and Weinberg (35) have ascertained that in normal dogs the greatest intraintestinal pressure is equivalent to only 2–4 cm. of water.

The amount of dye injected into the depleted animals was the same as in normal ones. Since the blood volume had been reduced by nearly half in most instances, it follows that the dye circulated in unusually great concentration. The intensity with which the abdominal viscera stained despite the untoward conditions, must be attributed in considerable part to this cause. But it will not explain the patching of the omentum, the more or less complete failure of the urinary bladder to stain, or the neglected state of the skin and muscles, which, so far as they received blood at all, received the same sort as did the viscera.

Starling has pointed out that normally absorption goes on from the digestive tract irrespective of whether there is a body need for the materials absorbed, the sole recourse of the organism being a regulation through the excretory organs which remove at an appropriate pace that which has been taken into the body willy-nilly (36). It is plain from our findings that even when the blood bulk has been diminished to the limit of tolerance, adequate circulatory conditions are maintained for absorption from the gut, a process which frequently acts to sustain life. Robertson and Bock have proved that salt solution introduced into the intestine is far more effective in permanently restoring the blood pressure of human beings after hemorrhage than when it is thrown directly into the circulation or injected into the tissues (37). The reason for this is not yet clear.

The dyes we have used are rapidly excreted into the bile; and this of course makes for a deep staining of the liver. Even within so brief a period as three minutes after the injection of brom phenol blue or Patent Blue V, much dye had reached the finer bile ducts. The dyes yielded no evidence that after hemorrhage some hepatic regions were better off than others. Yet the vaso-motor regulation within the liver is far from negligible (38); and a "stroking reaction" can be elicited on the surface of the organ, like the cutaneous one so much studied (39). In view of all this one of us has made a special study of the character of the hepatic blood service after hemorrhage. The results are detailed in the paper which follows.

The mackerel-sky or lattice work staining in the muscles of bled animals was obviously related to the arrangement of the vascular tree. That it was largely dependent upon contraction of the vessels, was shown by experiments in which vaso-constriction was prevented from occurring in the muscles of a leg by cutting the nerves to it just prior to injection of the dye. In the muscles of a limb so treated staining took place diffusely whereas in those of the control leg the usual mackerel-sky patterning was found. Our many experiments of the sort will be described in detail on another occasion. Not infrequently normal, stained cats and rabbits exhibited traces of the patterning here or there, more especially in the gracilis and quadratus lumborum. It may with good reason be attributed to that partial vaso-constriction on which maintenance of the normal blood pressure depends, but there are other important conditioning factors as will be shown subsequently. In not a few cases, just before the introduction of the dye an equivalent amount of blood was removed from the circulation. There was no more pronounced patterning in such instances.

The factors responsible for the patching of the skin are less readily to be explained. They too are dealt with in a subsequent paper. The possibility has already been ruled out (40) that the patching depends on a differing intrinsic permeability of vessels of like magnitude supplying tissue of the same general sort, a difference becoming effective only when the blood flow has been cut down by vaso-constriction.

Some foreshadowings of the changes we have observed in the service to the tissues after depletion can be found in previous work. Meek and Eyster, watching directly the circulation in the dog's ear, noted that after a considerable loss of blood there suddenly occurs an active contraction of the capillaries and small venules (41). They suggest that possibly "when the circulation is at the breaking point as it is when the bleeding equals 2 per cent of the body weight the venules and capillaries are constricted in widespread areas." The pronounced restriction of blood service occurring in the skin and muscles under such conditions is not uniform, as our experiments show, some regions being still fairly served while neighboring ones are wholly passed by. Langley (42) noted that the circulation continues through a few arterioles in the muscles of the frog after hemorrhages severe enough to stop it in the generality. He believed that a similar state of affairs would be found to exist in mammals. Gesell and Moyle (43), who ascertained the volume flow through the muscles of dogs repeatedly bled, found that at late stages of the gradual depletion it was reduced to an extent out of all proportion to the drop in blood pressure.

The vascular shut down in the spleen after hemorrhage is no new phenomenon (44). One may contrast therewith the state of affairs in the red bone marrow as disclosed by our experiments. Not only does dye still reach this tissue in quantity but india ink does as well.

The conditions as concerns blood service to the kidney under pathological conditions are complicated and we have made no attempts to study the organ. Richards states that relatively few kidney glomeruli are open to the circulation in frogs that have lost blood but that the number can be greatly increased by restoring the blood bulk (45).

SUMMARY

The spread through the living animal of various highly diffusible dyes has been utilized as an indicator of the ability of the circulation to serve the tissues under various conditions. The method is direct and searching. Blood service to the viscera, as demonstrated by it, is normally far more profuse than to the skin and muscles, for evident physiological reasons. After hemorrhages which greatly reduce the blood bulk service to the viscera is in general still well maintained even though the animal be *in extremis*. However great the compensatory contraction of the splanchnic vessels may be,—and physiologists have long supposed it to be very great,—it certainly does not suffice to

hinder blood service anywhere in the digestive tract. On the other hand the service to certain unessential abdominal organs (spleen, omentum, urinary bladder) is cut off in large part or wholly; and in comparison with the essential viscera, the skin and most of the skeletal muscles of the bled animal are largely deprived of circulation. This neglect takes a curious form, some regions being still fairly served by the blood while others next them are no longer ministered to. In the skin the areas served, or not served, are highly irregular but are to some extent determined in situation by local pressure factors. Within the muscles the neglect is orderly in arrangement and is largely referable to compensatory vaso-constriction. Certain of the muscles, those used in respiration and in swallowing, furnish significant exceptions to the general rule, being excellently served despite the serious general state. The red bone marrow of the depleted organism continues to be well served by the blood even though situated in limbs that are, for the rest, almost devoid of a circulation. The pregnant uterus also is excellently maintained despite the serious general state.

The changes are such as would tend to conserve the forces of the depleted organism and to contribute to its recovery.

BIBLIOGRAPHY

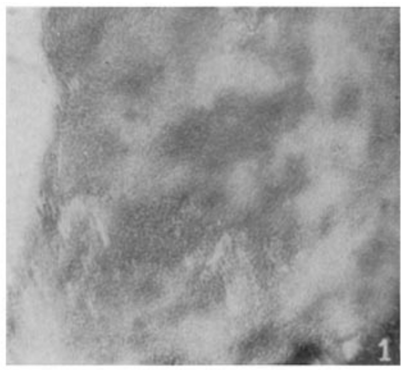
1. Schulemann, W., *Biochem. Z.*, 1917, **80**, 1.
2. v. Möllendorff, W., *Ergen d. Physiol.*, 1920, **18**, 141.
3. Ehrlich, P., *Vortr. Ver. f. inn. Med.*, 1898, **12**, 12.
4. Ehrlich, P., *Vortr. Ver. f. inn. Med.*, 1898, **12**, 12; Goldmann, E., Die äussere und innere Sekretion des Organismus im Lichte der "vitalen" Färbung, Tübingen, Laupp, 1909.
5. Okuneff, N., *Pflüger's Arch. ges. Physiol.*, 1924, **204**, 261.
6. Kusnetzowsky, N., *Z. ges. exp. Med.*, 1925, **44**, 646.
7. v. Möllendorff, W., *Ergebn. d. Physiol.*, 1920, **18**, 141.
8. Rous, P., *J. Exp. Med.*, 1925, **41**, 451, 739.
9. Ridgway, R., Color Standards and Color Nomenclature, Washington, D. C., 1912, published by the author.
10. Rous, P., *J. Exp. Med.*, 1926, **44**, 815.
11. Rous P., and Drury, D. R., *J. Exp. Med.*, 1929, **49**, 435.
12. v. Möllendorff, W., *Z. allg. Physiol.*, 1918, **17**, 125.
13. Krogh, A., *J. Physiol.*, 1918–19, **52**, 457.
14. Rous, P., and Drury, D. R., *J. Exp. Med.*, 1929, **49**, 435.
15. Rous, P., and Drury, D. R., *J. Exp. Med.*, 1929, **49**, 435.
16. Starling, E. H., The Fluids of the Body, 1909, London, Constable and Co.
17. Rous, P., and Drury, D. R., *J. Exp. Med.*, 1929, **49**, 435.

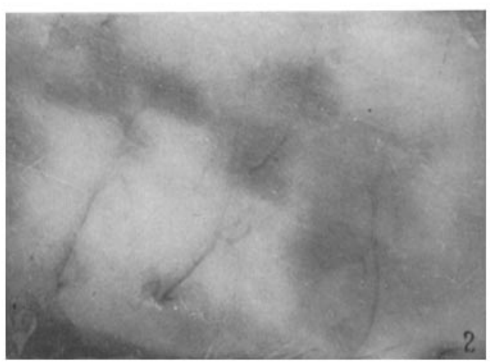
18. Rous, P., and Drury, D. R., *J. Exp. Med.*, 1929, **49**, 435.
19. Rous, P., and Drury, D. R., *J. Exp. Med.*, 1929, **49**, 435.
20. Rous, P., and Drury, D. R., *J. Exp. Med.*, 1929, **49**, 435.
21. Spalteholz, W., *Abhd. d. sächs. Ges. d. Wiss. M.-Ph. Cl.*, 1888, **14**, 509.
22. Krogh, A., *J. Physiol.*, 1918–19, **52**, 457.
23. Hoyer, H., *Arch. mikr. Anat.*, 1877, **13**, 603.
24. Clara, M., *Ergebn. Anat. u. Entwicklungsgesch.*, 1927, **27**, 259.
25. Krogh, A., *J. Physiol.*, 1919, **52**, 405.
26. Rous, P., and Drury, D. R., *J. Exp. Med.*, 1929, **49**, 435.
27. Meek, W. J., and Eyster, J. A. E., *Am. J. Physiol.*, 1921, **56**, 1.
28. Starling, E. H., *J. Physiol.*, 1896, **16**, 224.
29. Barcroft, J., and Kato, T., *Philosoph. Trans. Royal Soc. London*, series B, 1916, **207**, 149.
30. Starling, E. H., *J. Physiol.*, 1896, **16**, 224.
31. Bayliss, W. M., and Starling, E. H., *J. Physiol.*, 1894, **16**, 159.
32. Krogh, A., *Skand. Arch. Physiol.*, 1912, **27**, 227.
33. Starling, E. H., The Fluids of the Body, 1909, London, Constable and Co.
34. Sherrington, C. E., *Brain*, 1915, **38**, 191.
35. Owings, J. C., McIntosh, C. A. Stone, H. B., and Weinberg, J. A., *Arch. Surg.*, 1928, **17**, 507.
36. Starling, E. H., The Fluids of the Body, 1909, London, Constable and Co.
37. Robertson, O. H., and Bock, A. V., Reports of the Special Investigation Committee on Surgical Shock and Allied Conditions, Medical Research Committee, Great Britain, 1919, Special Report Series, No. 25, p. 213.
38. Mall, F., *Arch. Anat. u. Physiol., Physiol. Abt.*, 1892, 409.
39. Ebbecke, U., *Arch. ges. Physiol.*, 1917, **169**, 1.
40. Rous, P., and Drury, D. R., *J. Exp. Med.*, 1929, **49**, 435.
41. Meek, W. J., and Eyster, J. A. E., *Am. J. Physiol.*, 1921, **56**, 1.
42. Langley, J. N., *J. Physiol.*, 1911, **61**, 483.
43. Gesell, R. A., and Moyle, C. A., *Am. J. Physiol.*, 1922, **61**, 412.
44. Barcroft, J., *Lancet*, 1925, **1**, 319.
45. Richards, A. N., personal communication.

EXPLANATION OF PLATE 8

Fig. 1. Skin of the side of a white cat injected with brom phenol blue after reduction of the blood volume by repeated bleedings under ether. The brilliant blue and white mottling is only moderately well shown in the photograph; yet the contrast is sufficiently great to suggest that the white patches were raised above the blue, as was not the real case. The hair had been removed by shaving.

Fig. 2. Reflected skin of the same cat showing three parallel distributions to the subcutaneous tissue from the series of lumbar vessels. Some of the blue patches in this tissue occupy the regions supplied by one or another of the secondary arterial branchings; but of others this is not true. Three branches that correspond in situation are indicated by arrows. Two of them run to colorless patches whereas the third enters tissue that is heavily stained.





(Rous and Gilding: Studies of tissue maintenance. I)