The permeability of blood capillary sprouts and newly formed blood capillaries as compared to that of older blood capillaries

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The literature dealing with the permeability of blood capillaries is voluminous. Nothing appears to have been published, however, concerning the permeability of blood capillary sprouts and newly formed blood capillaries as compared to that of older capillaries. Yet this would seem to be a matter of some importance, since there now appears to be a general agreement (1) that the growth of the entire vascular system, after a brief period of primary differentiation, is accomplished by the sending out of capillary sprouts from pre-existing endothelium, and their anastomosis to form new blood capillaries. Not only are large numbers of blood capillary sprouts and new blood capillaries formed during all stages of growth, but also during the repair of wounds. If blood capillary sprouts and newly formed blood capillaries are more permeable than older capillaries, this would suggest that there may be a freer exchange of materials between the vascular system and the surrounding tissue in all conditions in which sprouts and newly formed capillaries are present in large numbers, such as during growth and during the earlier stages of wound healing.

Material and Methods. In the present experiments the permeability of blood capillary sprouts and newly formed blood capillaries, as compared to that of older capillaries, was studied by injecting the blue dye T. 18241 intravenously into rabbits having transparent moat chambers in their ears, and determining the time elapsing between injection of the dye and its appearance in the tissue outside of the sprouts and newly formed capillaries, and the older capillaries.

The moat chambers (2) used in these experiments are made of glass and mica, and each encloses a shallow space called the “bay.” Following insertion of the chambers, vascularized tissue grows from the subcutaneous tissue of the ear into the bay through two entrance holes at one end. The bay has a glass bottom and a mica top and is only approximately 75 microns deep. Consequently the blood capillary sprouts, newly formed blood capillaries, and older capillaries in it can be seen distinctly with the microscope.

1 The author is indebted to Dr. Magnus I. Gregersen for the T. 1824 used in these experiments.
The injections of dye were made, in all instances, when the vessels had grown about \( \frac{3}{4} \) of the way across the bay. Since about three weeks are required for the tissue to grow this far, there were present in the bay at this time capillaries varying in ages from 1 day (at the growing periphery) to 21 days (in the region of the tissue first formed). The sprouts were in a state of active growth, and were forming new capillaries in the manner described by Clark and Clark (1), and at such a rate as to give rise, on the average, to a new plexus of capillaries approximately 100 microns in width every 24 hours. Capillary sprouts, newly formed capillaries, and older capillaries in tissue in a portion of the bay of a moat chamber are shown in figure 1.

The tissue was studied with the microscope before and after the injections at magnifications of 200 and 400 diameters, and was also photographed both before and after the injections. The film used in these experiments (panatomic X) did not show the distinct color differences produced by the dye. Therefore water color records were made to demonstrate the amounts of dye that passed through the walls of the sprouts, and newly formed capillaries, and the older capillaries, as indicated by the depth of color outside of these structures. The color of the dye in the tissues was observed directly with the microscope, and then matched as closely as possible with water color on drawing ocular tracings made just before injection of the dye. Such records were made one-half hour and four hours after injection of the dye.

Four rabbits were used, each with a chamber in one ear. In the case of rabbits 1, 2 and 3, 100 mgm./kgm. of body weight of dye were injected intravenously, via one of the lateral ear veins of the ear that did not contain the chamber. No dye was injected into rabbit 4. Thus the normal color of the tissue outside of the vessels in the chamber in this rabbit served as a control for changes in color of the tissues in the other chambers following injection of the dye.

The results secured with rabbit 1 will be described in detail; those secured with rabbits 2 and 3 were similar.

Since, as reported by Gregersen and Stewart (3), T. 1824 is not stable in saline unless some protein is also present, the dye was injected in 6 cc. of sterile distilled water in all instances.

Observations. Careful observations of the sprouts and capillaries were made before injection of the dye. In no instance was there any evidence of stickiness of the endothelium toward leukocytes, nor was there any indication that the permeability of the capillaries, or of the sprouts, was greater than normal.

Three minutes after injection of the dye the vessels were again examined with the microscope. At this time the dye was seen to be entirely confined within the vessels and sprouts. The color of the plasma was a clear blue, in contrast to the reddish-yellow color of the erythrocytes and the general pinkish hue of the tissue surrounding the sprouts and vessels.

The first traces of dye in the tissue were seen outside of the sprouts, adjacent to the youngest of the circulating capillaries, in the region marked zone 1 in figure 1. Within \( \frac{1}{2} \) hour the dye was clearly visible in this region.

Within \( 1\frac{1}{2} \) hours after injection, the dye began to be visible outside of the newly
Fig. 1. Drawing ocular record of tissue in a part of the bay of the chamber in rabbit 1, showing blood capillary sprouts, newly formed blood capillaries, and older capillaries. This record was made 4 hours after intravenous injection of 100 mgm./kgm. of body weight of the dye T. 1824. A, tip of blood capillary sprout; B, side of blood capillary sprout; C, region of newly formed blood capillaries (approximately 24 hrs. old); D, region of older blood capillaries (approximately 48 hrs. old or older). This figure was made from a drawing in which the color of the dye was recorded with water color. The dark color within the sprouts and capillaries represents the blue dye, which at this time was still present within these structures in higher concentration than in the tissue outside of them. The different depths of color outside of the sprouts, the young capillaries, and the older capillaries, is due to differences in amount of dye that has passed through the walls of these structures into the surrounding tissue during the 4 hour period following its injection. Thus, as shown in zone 1, more dye has passed through the walls of the sprouts and the most recently formed row of circulating blood capillaries than elsewhere. As shown in zone 2 more dye has passed through the walls of the young blood capillaries than through the walls of the older ones in zone 3. X200
formed blood capillaries (not older than 24 hrs.) in the region marked zone 2 in figure 1.

Between 2 and 3 hours after the injection, dye began to be visible in the tissue outside of capillaries which were 48 hours old or older, including those in the tissue 3 weeks old.

Three hours after the injection blue granules could be seen in macrophages and leukocytes outside of the sprouts and in the connective tissue close to the most recently formed capillaries. Between these cells the dye, for the most part, was still distributed diffusely.

Four hours after the injection, three zones, based upon depth of color of the dye outside of the sprouts and circulating blood capillaries could be observed. In zone 1 the blue color was deepest. This was the region of the sprouts, and was adjacent to the peripheral side of the most recently formed row of blood capillaries. In zone 2 the blue color was paler than in zone 1. This was the region of recently formed blood capillaries (not older than 24 hrs.). In zone 3 the blue color was palest. This was the region of capillaries 48 hours old and older. These zones are shown in figure 1.

Each of the color changes noted above was checked by comparison with the color of the tissue in the control chamber in rabbit 4, into which no dye was injected. No color changes such as seen in the experimental chambers could be detected in the control chamber.

DISCUSSION. The above observations demonstrate that in the present experiments the blue dye T. 1824 passed more rapidly through the walls of the blood capillary sprouts and the most recently formed of the circulating blood capillaries than through those of the older capillaries (48 hrs. old or older).

Following its injection the dye could be detected first in the region of the sprouts (in zone 1 of fig. 1). It is not felt, however, that this is conclusive evidence that the sprouts were more permeable than the most recently formed of the circulating capillaries, since some of the dye may have passed into this region from the adjacent row of most recently formed capillaries. Therefore it is concluded only that the sprouts and most recently formed capillaries are more permeable than the older capillaries.

The present observations are in accord with studies of the permeability of blood capillaries during wound healing reported by Lange (4). This investigator injected the dye fluorescein intravenously and observed the amount of dye that appeared per unit time in surgical wounds during the process of healing. Using the dermofluorometer (5) for recording the dye concentrations in the tissue, it was found that 4 to 5 times as much dye appeared in the growing tissue of healing wounds per unit of time as in other regions of the body surface. That this was not due to the dye contained within the blood capillaries, which are increased in number during the early stages of wound healing, but to dye in the tissue outside of the capillaries, was shown by the fact that pressure upon the tissue with a glass slide, which forced the blood out of the capillaries, did not appreciably alter the readings. Subsequent measurements showed that the amount of dye appearing in the area of the wound, per unit time, returned to normal within approximately 2 or 3 months.
A question that must be answered in regard to the present findings is whether or not in the chambers used in these experiments the tissue surrounding the older capillaries was supplied with lymphatics, whereas that surrounding the newly formed capillaries was not. If this were the case, the dye might have accumulated to a greater extent outside of the newly formed capillaries due to lack of lymphatic drainage rather than to these capillaries being more permeable than the older ones. There are three reasons for thinking that this was not the case. In the first place, lymphatics frequently fail to grow into moat chambers, and in one of the chambers used in these experiments, a careful microscopic examination failed to show that any were present. Yet in this chamber, as in the others, the color of the dye outside of the newly formed capillaries became deeper than that outside of the older ones. In the second place, even when lymphatics do grow into moat chambers, they are comparatively few in number, usually not more than 3 or 4, so that much of the tissue in the chamber (even the older tissue) is not supplied by them. Yet no differences in depth of color surrounding the older capillaries were seen in different parts of the chambers. In the third place, even when lymphatics are present in moat chambers there is very little flow of lymph through them. Thus, as in the case with lymphatics in round table chambers (6), when red blood cells or white blood cells get into the lymphatics, they can often be seen bobbing back and forth in the same place for hours. It is not to be expected that such an inadequate flow of lymph would be very effective in removing dye from the tissue.

In view of the above considerations, it is felt that the paler color of the dye outside of the older capillaries was not due to removal of the dye from the tissue by the lymphatics, but to less dye passing through the walls of the older capillaries than through those of the younger ones.

SUMMARY

The permeability of blood capillary sprouts and newly formed blood capillaries, as compared to that of older blood capillaries, was studied by direct microscopic observation, using transparent moat chambers in rabbits' ears. These studies show that in such chambers the blue dye T. 1824, injected intravenously, passes more rapidly through the walls of blood capillary sprouts and newly formed blood capillaries than through the walls of older blood capillaries. Thus the permeability of blood capillary endothelium was found to vary with age, the more recently formed endothelium being more permeable.

REFERENCES

(3) Gregeresen, M. I. and J. D. Stewart. This Journal 125: 142, 1939.
(4) Lange, K. Personal communication, January 2, 1946.