THE GRADIENT OF PERMEABILITY OF THE SKIN VESSELS AS INFLUENCED BY HEAT, COLD, AND LIGHT

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PLATE 23

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The object of the work here reported has been to determine whether the gradient of vascular permeability demonstrable in the skin of mammals (1) undergoes alteration during active hyperemia, and to learn the consequences to it of vascular injury by heat and cold. The ear of the mouse has been utilized because any alterations of the gradient of permeability during the distribution of vital dyes from the blood is almost diagrammatically visible in an altered color pattern.

General Method

Young mice of 18 to 20 gm. under luminal anesthesia have been employed throughout. After exposure of one of the ears to heat, cold, or light, a dye was injected into a tail vein, and soon thereafter both ears were lopped off with scissors to check the progress of the staining in them. They were at once arranged symmetrically in paraffin oil under the same large cover glass and studied over white porcelain in a combination of transmitted and reflected light. The exposed ear was always cut off first, since it tended to stain the more promptly. In the amputated organ the vascular network was not as sharply outlined as in the living animal, because of the loss of some of the contents of the vessels, but it could still be seen plainly enough for the relations of the staining to arterioles, capillaries, and venules, respectively, to be readily made out.

Pontamine sky blue, a dye that escapes but slowly, was employed as routine because it serves better than more rapidly escaping materials to disclose slight local differences in vascular permeability. For corroboratory tests Chicago blue 6B was injected. A study of photographs taken at once, after the amputation of ears into which pontamine blue had emerged, and 5 minutes later, disclosed no significant extravascular redistribution of the dye. Nevertheless, the comparison of the experimental and control ears was always carried out very rapidly. The technic of the lighting has been described in a previous paper (1).

Evidence of the Normal Gradient of Vascular Permeability

Ordinarily pontamine blue, Chicago blue, and other vital dyes escape with greatest ease through the walls of the small venules of the corium, and a little less readily through the wall of the adjoining portion of the capillary web, the permeability of the capillaries diminishing greatly in the direction of the arterioles. The influence of these local differences is manifest in the color pattern seen in the ear soon after the dye has been put in circulation. Brilliant patches develop in the region supplied from the venules and the further capillary region when as yet no staining has occurred anywhere else in the ear. The restricted distribution of the dye is not referable to specific affinities for certain sorts of tissue. The tissue indeed is not stained, the dye being contained in the interstitial fluid (2). After a greater or less time, depending on the diffusibility of the dye, the colored patches are lost in a general staining which is partly the result of dye escape from the proximal capillary region, and partly due to a secondary spread through the tissues from the region first stained. Any deviation from the general course of events implies an alteration in the opportunity for materials to pass out of the blood.

The Effects of Heat

The effects of heat on the color pattern were studied in detail.

The anesthetized mouse was placed on its back upon a cork platform beyond which its head extended over two converging test-tubes about 15 cm. long and 2.5 cm. in diameter into which the ears dipped. The edges of the tubes, which were not flared, came within 1 mm. of each other, and one tube had been filled brim full of water at room temperature, while the other contained water that was kept at the desired warmth by means of a small Bunsen flame. The ears were moistened with alcohol to facilitate immersion, and the hair between them on the top of the head was oiled. Under such conditions the tubes remained full after immersion of the ears, even to a bulging of the meniscus, whereas in lack of the oiling, water was drawn out by capillarity and a drip ensued. The head was held free of the tubes, by a clamp on the skin of the lower lip. When the apparatus had been properly arranged both ears were symmetrically immersed nearly to their bases and they did not touch the glass anywhere. The temperature was followed with a thermometer placed immediately next them. After one ear had been heated for a greater or less time, dye was injected into a tail vein of the mouse without altering its position. As routine, 0.1 cc. of isotonic half strength pontamine blue (a 21.6

per cent watery solution of our new preparation of the dye, mixed with an equal part of Locke's solution) was injected in the course of 1 minute. Isotonic half strength Chicago blue 6B (a 17.1 per cent watery solution mixed equally with Locke's solution) was sometimes substituted. The progress of the staining of the ears was followed through a lens, and when the moment for closer inspection seemed to have arrived the animal was lifted away by means of the mosquito forceps and the ears cut off.

Exposure to water at between 42° and 43°C. for 7 minutes caused a pronounced active hyperemia, the ear becoming bright pink; and staining occurred so rapidly as to necessitate amputation of the organ within less than a minute after the end of the dye injection. It was already thickly patched with dark blue on a paler blue ground (Fig. 1.). Very little dye had as yet left the vessels of the control ear. The patches in the heated specimen were far more numerous than ordinarily, as could be seen when the animal was not killed until patching had begun in the control ear; but they were localized to the tissue about the smallest venular trees, just as usual. Seen amidst the general staining they appeared smaller than ordinary. The capillaries were still visible in the amputated specimen because of dye-stained blood within them, as was not the case in the control.

When the heating at 44°C. had been kept up for 14 minutes prior to dye injection, stain escaped abundantly from all of the small vessels, but still in greatest amount from the venules. The tissue had become slightly edematous prior to the injection (Fig. 2). Exposure to 45°C. for 14 minutes caused a more considerable edema. There now developed a deep diffuse staining with but the slightest intensification in the perivenular region and this soon lost (Fig. 3). At 46°C. for 12 minutes the aural muscles contracted, crinkling the ear. Since a circulatory disturbance due to such cause could not be ruled out, the specimens were discarded.

For additional tests the ear was heated by blowing a fine jet of compressed air through the upper part of a Bunsen flame to reach the ear at a distance of 10 to 15 cm. The mouse was laid on its side upon a dais immediately behind a wooden block above which the outer two-thirds of the ear projected; and the head was so placed at the edge of the dais that the control ear and the region about it were not pressed upon. The temperature was taken with a thermometer moved here and there immediately about the ear, throughout the period of exposure.

The ear in the blast of air soon became brightly hyperemic. Heating at 46°C. for 3 minutes, with dye injection during another minute while the exposure was continued, yielded, in yet another minute, a diffuse staining that was deep and even near the edge of the ear, with an intense patching superimposed on somewhat lighter staining further in toward the head, where the tissue was thicker and the vascular disturbance not so great. Patching had just begun at this time in the control ear,—which was not wholly protected from the warm air,—but the patches were relatively few and pale.

Heating at 56°C. for 4 minutes caused an intense local hyperemia with edema and immediate diffuse staining. Toward the base of the ear, where the heating had been less, a brilliant, abundant patching on a background of diffuse color had developed at the time when the ear was lopped off, 3 minutes after the injection of pontamine blue. Lymphatics laden with dye-stained fluid, from the dark blue, edematous tissue further out, coursed through this region. The control ear showed no staining whatever.

A degree of heating which induces active hyperemia without edema brings about several important changes in the staining of the ear. Patching takes place far more quickly than in the control organ; and the patches are more numerous. Also in the case of pontamine blue diffuse coloration develops concurrently with the patching as never happens with this poorly diffusible pigment under normal conditions,—though with highly diffusible dyes it is a regular occurrence (1). Increased blood flow, heightened intracapillary pressure, and the capillary dilatation of active hyperemia are doubtless responsible for the phenomenon.

Heating that suffices to cause slight edema in the hyperemic ear renders the capillary web in the corium everywhere so permeable to pontamine blue and Chicago blue that an intense diffuse staining follows practically at once upon the injection into the blood stream; yet even under such circumstances, with the effective concentration of dye rapidly diminishing through loss to the tissues on the way to the venules, the escape is still greatest from these latter, the color about them being definitely more intense than elsewhere. Only when heat has so damaged the vessels as to cause fulminant edema is the distribution from the blood approximately an even one, as evidenced by the intense general staining.

The Effect of Cold

The cold to which the mouse ear was exposed ranged from that which induced only an active hyperemia to a temperature that caused prompt freezing.

For most of the tests the ear was placed in a current of cold carbon dioxide mixed with air. Care was taken that the organ should be dry, for wet mouse skin lets carbon dioxide through so readily that the tissue pH undergoes alteration in the direction of acidity (3). It was found that when a single large piece of solid carbon dioxide is placed in a funnel held vertically in a ring-stand, and inclosed by inverting upon it another slightly smaller funnel, a continuous jet of cool air emerges from the end of the lower funnel so forcibly as to make itself felt 4 to 5 cm. away. This only happens if the end of the upper funnel is open, for the carbon dioxide evaporates very slowly when no air current can pass through the apparatus.

The anesthetized mouse was kept warm while one ear was exposed to the jet. The width of the latter was conditioned, of course, by the funnel opening. It was wide enough for a thermometer to be put next the ear in the cold stream. Complete precision in the degree of cooling was not necessary to the work.

Ears chilled at 1–4°C. for 10 minutes became brightly hyperemic during this period; and on the injection of dye the characteristic patchy staining took place but much more rapidly than in the control, irrespective of whether cooling was continued or not. This happened even when the hyperemia had largely subsided after removal of the ear from the stream. There was also some diffuse staining at a time when none had taken place in the control.

When the ear was placed close to the end of the funnel, where the temperature of the jet was −2°C., it froze within 1 minute. Immediately that this happened it was taken out to thaw; and dye was injected during the subsequent period of intense hyperemia. The ear stained practically at once, with intense perivenular patchings on a ground of diffuse blue, being already deeply colored at a time when the control was practically unstained.

When the outer two-thirds of the ear was frozen at a low temperature and left in this condition for several minutes, the muscle of the affected part contracted on thawing; and though this contraction wore off and the tissue became bright pink, the circulation was imperfect, as appeared when pontamine blue was injected, the dye penetrating with difficulty or not at all into the region that had been frozen. Nearer the base of the ear was a marginal zone which had become markedly edematous and stained rapidly and diffusely, while still nearer the head,

where there was no edema, an intense, abundant patching of the usual distribution in relation to the vessels was to be seen. The staining took place before any occurred in the control ear.

To find whether carbon dioxide, as such, had any part in the results, the ears of some animals were chilled or frozen in cold alcohol or paraffin oil. The results were identical with those described. When the temperature was so chosen as to produce hyperemia merely, a patching with blue on a ground of lighter color developed.

The findings in ears rendered actively hyperemic by cold, without evident damage to the vessels, resembled those when the hyperemia had been caused by heat; and those when the ears had been sufficiently injured for the development of edema, were like those during heat edema.

The Effect of Light

The effect of light was tested by varying slightly the procedure used for heating the ears.

The mouse was placed on its back, the head held horizontally by a clamp attached to the lip, and the ears immersed in two Petri dishes brim full of water. Light from an arc lamp was cooled by passage through two filters containing Mohr's solution and concentrated to a disc about 6 mm. in diameter upon the submerged part of one of the ears. The other was completely protected by a strip of black cardboard placed between the Petri dishes. A thermometer bulb was submerged next the lighted ear. Throughout the period of experiment the temperature of the water did not rise.

The light, though intense, produced hyperemia but slowly and it was rather closely limited to the illumined part of the ear. When, after nearly an hour, dye was injected, there developed an abundant blue patching of the exposed tissue, with some diffuse staining as well, all this at a time when no coloration had taken place in the control ear. This result was obtained irrespective of whether the light struck on the upper or under surface of the organ.

The findings were like those after a heating or cooling sufficient to produce hyperemia of the ear without edema.

DISCUSSION

The staining phenomena observed in ears rendered hyperemic by heat, cold, and light were practically identical, as was to have been expected from the fact that "mechanical, electrical, thermal and chemical stimuli all produce essentially the same response of the vessels

of the skin" (4). Dye got out into the exposed tissue much sooner than into the control, and a generalized staining with the poorly diffusible pontamine blue, as result of an escape everywhere along the capillary web, took place simultaneously with a greater staining from the venules, not, as ordinarily, long after venous escape; but save in this respect, and in the unusually abundant patchings with color, the staining pattern was unaltered. Obvious reasons for the observed differences can be found in the quickened local circulation of blood, in the general capillary dilatation, in a circulation through regions where it is not maintained ordinarily, and in the increased pressure prevailing within capillaries and venules. Under normal circumstances there are many regions in the skin of the ear of the rabbit, cat, and dog through which blood flows almost not at all. The same has been found true of the mouse (1). After a dye injection into this animal one observes not a few venules in normal ears containing almost unstained blood, the reason being that none has got through most of the capillaries of the web that they drain; and needless to say, the tissue surrounding such venules remains unstained. Under the circumstances of general capillary relaxation induced by heat, the dye reaches these venules in abundance and staining occurs. Landis has found that the maximal relaxation of the cutaneous vessels of man, produced by heating, causes the pressure in the arteriolar capillaries to double and in the venous capillaries almost to quadruple (5).

To break down the gradient of distribution disclosed by the patchings with stain—a gradient referable in large part at least to a special permeability of the venules and further capillary meshes—it was necessary to produce such injury to the vessel wall as would result in rapid, abundant edema. Only under such circumstances did an approximately even staining of the tissue take place. In the persistence of the gradient despite lesser but still considerable degrees of vascular disturbance one can find a new reason besides those already advanced (1, 6–9) for the assumption that the gradient depends upon the structure of the vessel wall, not on functional conditions. Its practical disappearance when this wall is badly damaged proves that it cannot be the result of local differences in the extravascular fabric, a possibility brought up in a preceding paper.

The experiments disclosed incidentally some of the functional changes which precede and are doubtless responsible for the structural alterations characteristic of injury by heat and cold. One such change deserves special mention. The slightest freezing of the mouse ear, at the highest temperature at which this can soon be accomplished (−2°C. in our experience),[1] causes the walls of the large arteries to become readily permeable to dyes (pontamine blue, Chicago blue) which fail to pass through them at all under ordinary circumstances. A zone of deep color soon forms along the outside of the injured vessels. With longer freezing at lower temperatures the arterial leakage is accentuated. Most stuffs carried by the blood are greatly more diffusible than the dyes we used, and the damaged arterial wall must provide but a slight barrier to their escape. The fact is known that after freezing, blood platelets soon collect on the walls of the arteries of the affected part, and that arterial thrombosis is responsible for much of the late damage (11). The media of the arteries undergoes a degeneration. It is plain that a pathological seepage through the vessel wall precedes these changes. Probably it has much to do with them.

SUMMARY

The mounting gradient of permeability along the small vessels of the corium is essentially unaltered by active hyperemia produced by heat, cold, or light. Only when the vascular walls are so damaged that rapid leakage ensues, as shown by the development of edema, does the permeability of the capillary web as a whole approximate that of the venules. It is plain that the normal gradient of vascular permeability depends upon the integrity of the vessel wall.

The method of experiment described can be utilized for a study of the functional changes which result in the lesions due to burning and freezing.

[1] Mammalian tissues freeze at −0 56° to −0.97°C. in the absence of circulation (10). Lewis (4) found that a local temperature of −2.2°C. will freeze the skin of normal human beings.

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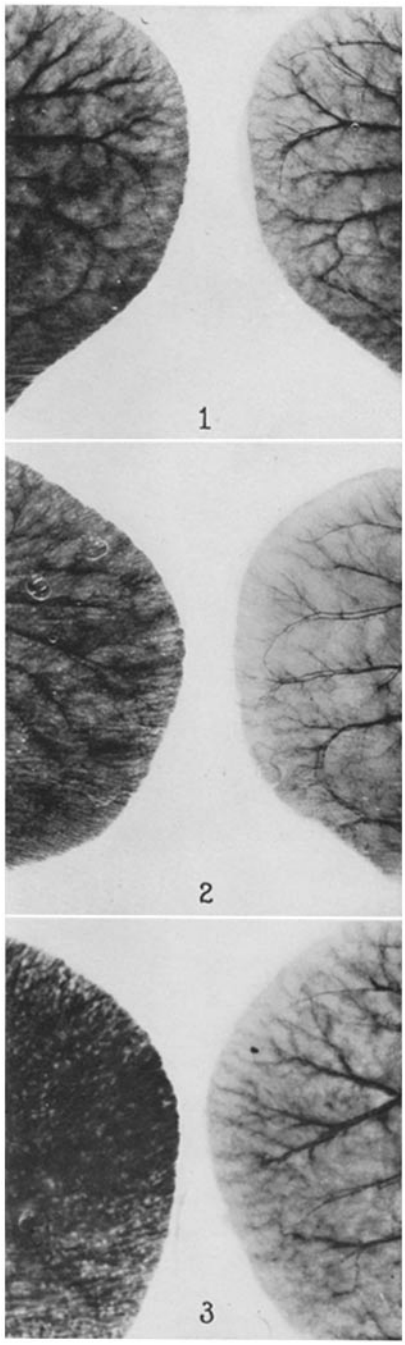
EXPLANATION OF PLATE 23

For purposes of comparison the ears have been transposed, so that the marginal regions lie next each other. All have been photographed from above.

FIG. 1. One ear of an 18 gm. female mouse under luminal anesthesia was submerged for 7 minutes in water between 42.0° and 42.6°C. During the next minute 0.1 cc. of half strength pontamine sky blue was injected intravenously. 52 seconds later the heated ear already showed a pronounced staining. Both ears were cut off, placed side by side on a porcelain plaque, and covered with paraffin oil. The picture was taken approximately 3 minutes after the amputation. The heated ear shows a pronounced patchy staining with some general coloration as well. Dye has just begun to escape from the venules of the control.

FIG. 2. Results of heating an ear at 42–44°C. for 14 minutes. Same general technic as in the experiment of Fig. 1. There are still local differences in staining indicative of a gradient of vascular permeability. No dye has escaped as yet into the control ear.

FIG. 3. Results of heating an ear at 45°C. for 14 minutes; technic as in the experiments of Figs. 1 and 2. The fine white dotting is caused by the dispersion of light by the sebaceous glands situated around the hair follicles.



Photographed by Louis Schmidt

(Hudack and McMaster: Gradient of permeability of skin vessels)