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STUDIES ON GANGRENE FOLLOWING COLD INJURY: VI. CAPILLARY BLOOD FLOW AFTER COLD INJURY, THE EFFECTS OF RAPID WARMING, AND SYMPATHETIC BLOCK¹

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Indirect evidence, based upon measurements of skin temperature and observations of skin color in the feet and ears of rabbits subjected to severe cold injury, indicates that complete arrest of blood flow does not occur until more than 50 hours after injury (1). Tests of the local circulation in cold-injured regions with intravenously injected fluorescein show that the exchange of this dye between blood and interstitial fluid is impaired during the interval when the minute volume blood flow is greater than that in comparable uninjured regions (2, 3). Early arrest of blood flow in regions injured by cold has been variously ascribed to "conglutination" of red-cells (4), to capillary stasis (5), and to intravascular clotting (2). While all of the above phenomena may be observed in frostbitten tissues, none has provided an explanation of the nature of local changes in blood flow adequate to account for the maintenance of high peripheral tissue temperature at a time following cold injury when exchanges of oxygen, nutrients, and metabolites appear to be impaired.

This report presents the results of microscopic study of blood flow in the small vessels of the ears of rabbits during the first hour after freezing. Blood flow was also studied in the ears of animals which were treated by rapid thawing of the ear in warm water and by procaine block of the stellate ganglion on the injured side.

METHODS

Cold injury was produced by immersing the distal one-half to one-third of the ear for 60 seconds in a mixture of water, ethylene glycol, and alcohol cooled to -55° C. with solid carbon dioxide. Details of this method for the production of controlled cold injury and the characteristic effects on the tissues are given elsewhere (6).

¹ The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Stanford University.

Both normal and frostbitten ears of a uniform strain of New Zealand white rabbits were observed through a binocular dissecting microscope having 9X oculars and 4.8X objectives. The light source was a 100-watt Spencer microscope lamp from which the light was conducted to the ear by means of a polished rod of $\frac{3}{4}$ -inch methyl methacrylate (Lucite) 2 feet long. The rod was tapered to a cone having a rounded tip 7 mm. in diameter. The distal 8 inches of rod were bent into a curve which presented the brilliantly illuminated tip at a right angle to the original path of light. With the rod in an adjustable clamp, its flattened end in contact with the blue glass of the microscope lamp, the ear was placed under the objectives of the microscope in direct contact with the methacrylate rod. Liquid petrolatum was applied to the shaved ear in order to clear the keratinized epithelium. Photographic recording of the changes observed proved to be unsatisfactory because of the thickness of tissue and the changing optical plane.

OBSERVATIONS

Blood flow in the capillaries of the normal ear. Movement of blood could not be seen in the larger vessels. In the smaller arterioles and venules, rapid flow was easily made out. Numerous channels affording relatively direct communication between arterioles and venules were seen. These channels were usually of smaller diameter than either their supplying arterioles or the receiving venules, and many of them were small enough to force the corpuscles to traverse them in single file. Reversal of flow was not observed in these vessels, and the flow was uniformly rapid. The relatively few true capillaries which were open at any one time could be identified as richly anastomosing branches containing corpuscles either singly or in small clumps interrupted by clear areas. Often the location of true capillaries could be identified only when an occasional corpuscle was seen to wander off through an area apparently free of vessels and then finally to enter a collecting capillary and venule.

Hyperemia induced by the application of heat caused opening of vessels of all types, and many

regions, which had been entirely free of visible channels, were seen to be richly supplied with capillaries.

Blood flow in the capillaries of the ear after frostbite. Observations of blood flow were made on the normal and the frostbitten regions of ears of 15 rabbits. The changes which were observed in the local circulation were consistently reproducible in terms of their time relations and nature. The following description applies to the sequence of changes observed in the blood flow within the frostbitten area after the distal half of the ear had been immersed in the freezing mixture at -55°C . for 1 minute and then allowed to thaw in air at room temperature without additional treatment. Blood flow began in the largest channels of the frostbitten portion of the ear within 3 minutes and before the ear was completely thawed. Flow in those channels was rapid and at that early stage only a limited number of capillaries in the immediate vicinity of large vessels became filled with blood. At the completion of thawing, the ears became markedly hyperemic. Progressive opening of capillaries was observed, and brisk blood flow could be detected for a few minutes after the capillaries became visible, but over a period of about 10 minutes slowing and final cessation of flow occurred. The capillaries contained packed red blood cells interrupted by clear regions along the length of the vessel. Within the interval, 10 to 20 minutes after frostbite, these vessels became completely filled with cells, their course was grossly distorted, and the walls were roughened and irregular.

The period of onset of stasis was accompanied by increasing edema. A homogeneous brownish substance began to accumulate in the areas adjacent to the capillaries and was found in largest amounts near the larger blood vessels in which flow persisted longest. Although no actual movement of blood could be observed, sluggish flow could be demonstrated by observing the refilling of vessels after they had been emptied of blood by pressure upon an overlying coverglass. Such persistence of flow was demonstrated in the larger arterioles and venules and also in some small, relatively straight vessels. The latter were bright pink in color and had smooth walls; their diameter was slightly smaller than that of the distended capillaries which contained packed red cells.

Vessels in the area proximal to the frostbitten region were widely dilated for the first 20 to 50 minutes after thawing. Usually within 2 hours these vessels became markedly reduced in diameter. The central artery and its branches were more noticeably affected than the veins, and there appeared to be a region of especially marked narrowing of the artery at the junction of the normal with the injured area. At the same time it was noticed that the large vessels within the frostbitten part of the ear remained widely dilated.

Study of the circulation after frostbite with the aid of intravenous injections of India ink. Intravenous injections of particulate carbon in the form of India ink were used to assist in the visualization of blood vessels in both normal and frostbitten ears. The ink was administered in doses of 5 ml. of 50 per cent India ink in saline both immediately before frostbite and at various intervals from 2.5 minutes to 49 minutes after the ear was removed from the freezing mixture. The circulation in the ears of 10 animals was observed after the injection of carbon.

By the selection of the appropriate time interval for the injection of carbon in relation to the time of frostbite it was possible to demonstrate the following: (a) carbon particles present in the circulating blood either just before frostbite or within 6 minutes afterward entered the capillaries of the frostbitten region; (b) varying amounts of carbon became trapped in the capillaries of both the normal and the frostbitten parts of the ear; (c) carbon trapped in capillaries of the normal part of the ear was removed by the increased blood flow when hyperemia was induced by heat, but particles caught in the dilated capillaries of the frostbitten region during the period of stasis could not be removed by this means; (d) up to 6 minutes after frostbite, more carbon was trapped in the capillaries of the frostbitten region when carbon injections were made late in this 6-minute interval than after pre-frostbite injection or very early post-frostbite injection of ink; (e) when the injection of ink was delayed as long as 20 minutes after frostbite, only occasional particles of carbon could be seen in the vessels of the frostbitten region,—when these did appear, they were seen only in the relatively large channels and not in the true capillaries.

Thus, the interval during which the entrance

and trapping of carbon particles could occur was brief and was terminated by stasis. The channels which did remain open contained flowing blood, were relatively large in diameter, and hence probably permitted any carbon which entered them to be carried on through.

The junction of the normal part of the ear with the frostbitten region exhibited some differences from both regions distal and proximal to it. When India ink was injected after stasis had become complete in the true capillaries, *i.e.*, more than 20 minutes after frostbite, carbon accumulated and became trapped at the junction when none or very little could be found in either the frostbitten region or the normal part of the ear. This observation suggests that the stasis which occurred here was relatively late in appearing, since it was not sufficiently advanced to prevent the entrance of carbon particles but later did progress to the point where large amounts of ink were trapped.

While no positive explanation of the above observation is justified on the basis of our present information, it is suggested that the tissues of the junction may have been less severely injured by reason of their failure to reach temperatures as low as those in the more distal portion of the ear. Since swelling progressed from the injured region toward the base of the ear, distortion and occlusion of capillaries may account for late appearance of stasis in the region of the junction even though the direct injury by cold was not severe enough alone to induce stasis.

Local blood flow in frostbitten ears after rapid thawing in warm water. Frostbite produced in the ears of rabbits by immersing the part in the freezing mixture at -55°C . for 1 minute resulted invariably in the development of gangrene and the ultimate loss of all of the ear up to the line of immersion, if the ears were allowed to thaw (in air at 25°C .) without treatment. In confirmation of the work of Arieu (7), we have found that the same exposure to cold followed by immediate warming of the ear in water at $+42^{\circ}\text{C}$. for 2 minutes prevents the development of gangrene and loss of the injured tissue (8). In spite of the favorable end result of rapid thawing, the tissues underwent a series of changes which were grossly similar to the pre-gangrenous changes in the frozen, untreated ears. The stages of hyperemia, swelling and later exudation of serous fluid

from the surface of the injured part of the ears were, if anything, more marked than in the untreated ears, but in spite of these alarming manifestations, only the surface epithelium was lost.

Observations were carried out in the manner described previously, and injections of India ink were used to test for the appearance of stasis. The ears of 3 animals were studied after subjecting both ears to frostbite (1 minute at -55°C .) and thawing one of them rapidly in warm water. The injections of ink were made at 20 to 25 minutes after frostbite and were thus administered during the period when capillary stasis is complete in similarly injured, untreated ears.

Before the intravenous injection of carbon, the changes of blood flow observed in the rapidly warmed ear were not strikingly different from those in the contralateral, frostbitten but untreated ear. Refilling of vessels emptied by external pressure persisted longer in the treated ear and larger amounts of brown extravascular material appeared. When particulate carbon was introduced into the blood stream, it was seen to enter the treated ear more readily than the untreated one in 2 out of 3 rabbits studied. In the third, the amounts of carbon were about the same in both ears (not more than 3 to 4 particles per field). For 1 or 2 hours after the injection of ink, the particles were either observed to move spontaneously in the vessels of the treated ear or could be made to move by mechanical manipulation. In one experiment, hyperemia was induced in the treated ear by infiltrating the tissues about the base of the ear with 1 per cent procaine 77 minutes after the injection of ink. The increased blood flow removed some of the carbon which previously had been trapped. Hyperemia induced by this means or by warming was shown to be ineffective in removing trapped carbon from frostbitten ears which had not been treated by rapid thawing.

The observations showed that rapid thawing of frostbitten ears had the effect of delaying complete stasis in the true capillaries. When stasis did develop, it coincided in time with the period of maximal swelling (approximately 2 hours after injury) and with the reduced blood flow brought about by the constriction of arterial supply in the uninjured portion of the ear. Thus, when perfusion pressure within the capillaries was dimin-

ished, and distortion of the tissues by the presence of large amounts of extravascular fluid occurred, cessation of flow in the true capillaries was an accompanying feature. However, flow of blood in the true capillaries could be made to proceed by changing the pressure relationships within those vessels either by increasing the flow of blood through the injured tissue or by mechanical pressure applied externally and released.

The local circulation in frostbitten ears following procaine block of the stellate ganglion. In order to determine whether the effects observed after rapid warming of frostbitten ears were the result of the changes in blood supply alone or whether some other effect of rapid warming was responsible for the delay of capillary stasis, 6 rabbits were subjected to frostbite of the distal half of both ears in the same manner as those described in the previous sections, and as soon as possible after the ears were removed from the freezing mixture, 1 to 2 ml. of 2 per cent procaine with epinephrine 1:30,000 were injected into the region of the stellate ganglion on one side. All of the animals in this group demonstrated satisfactory paralysis of the vasomotor supply of the ear as judged from the presence of the more readily palpable pulse in the central artery and skin temperatures 4° to 6° C. higher on the blocked side.

When carbon was injected into the blood stream within 5 minutes after frostbite, large amounts of it were observed in the capillaries of the frostbitten, untreated ear, but relatively little of it was found in the ear subjected to vasomotor paralysis. Stasis in the untreated ear resulted in the immobilization of large amounts of carbon, but in the capillaries of the ear on the side blocked by procaine, blood flow and movement of carbon particles persisted for more than 2 hours. Two hours and 10 minutes after the injection of India ink some stasis was noted in capillaries close to the surface; these vessels could not be emptied by external pressure, but in spite of this late stasis there was no trapping of carbon except at the junction of normal and frostbitten and normal tissue. In this case failure of trapping may have been due to removal of carbon from the circulation before stasis developed. When the injection of carbon was delayed until 22 minutes after frostbite, the usual exclusion of carbon particles from the capillaries of the injured but untreated ear was ob-

served. In the ear which was treated by the production of vasomotor paralysis, the capillaries were seen to contain moving carbon particles, but within an additional 30 minutes stasis occurred with immobilization of carbon granules. In this animal the duration of increased blood flow was rather brief, and both ears were cool and about the same temperature by the time that stasis was observed in the treated ear. The vessels did not refill after being emptied by external pressure.

These observations indicate that the increased blood flow which followed stellate ganglion block was associated with a delay of stasis in the true capillaries of frostbitten ears. While stasis was not postponed for a period as long as that following rapid thawing of the ear in warm water, it was qualitatively similar. This suggests that at least a part of the improvement in capillary blood flow noted after rapid thawing may be the result of increased intravascular pressure. However, that rapid thawing produced other changes in the response of the tissues was evident from the difference in final result: preservation of the frostbitten region after rapid thawing as compared with merely the delay for 24 hours of the wet and dry stages of gangrene and loss of the injured portions of the ear, which was the uniform experience after the use of procaine block.

Lymph flow and the spread of colloidal dye in frostbitten skin. Glenn, Gilbert and Drinker (9) reported an augmented lymph flow for at least 2 hours following severe hot water burns of the foot in dogs. Massive swelling was a constant feature of the response to injury in their experiments. The high rate of lymph flow points to a dynamic rather than a static alteration of fluid distribution in the injured tissues. In view of the many similarities between the vascular disturbance in burns and frostbite (10) the demonstration of augmented lymph flow in the frostbitten extremities of rabbits might throw some light on the nature of fluid exchanges following severe injury by cold. It has been pointed out that the edematous tissues after frostbite are to some extent organized (1), in that only limited quantities of edema fluid flow from the incised swelling. Organization of this fluid either in the form of a fibrin clot or in a gel stabilized by hyaluronic acid (11) might offer some mechanical barrier to free movement of fluid and plasma protein in the extravascular compart-

ment and also hinder the movement of fluid into capillaries even though adequate filtration gradients existed.

A small number of experiments designed to demonstrate lymph flow by the use of the dye Patent Blue V as employed by McMaster (12) and the state of the interstitial fluid by the spread of T-1824 by the method of McMaster and Parsons (13) were carried out on the frostbitten and normal ears of rabbits. Two rabbits were used for the studies with Patent Blue V. One ear of each animal was frostbitten by immersion in the freezing mixture for 1 minute at -55°C . The contralateral ear of each animal was used for control observations. Patent Blue V (0.01 ml. of an 11 per cent solution in water) was injected intradermally through a G-26 hypodermic needle. In one animal, the dye was introduced into both ears midway between the central artery and the marginal vein 4.5 hours after frostbite. In the frostbitten ear diffuse, blue streamers were seen to extend proximally within 5 seconds. After 10 seconds, a poorly defined blue band extended from the point of injection to the marginal vein. These bands blended into the neighboring skin areas with no clear, fine lines. The color became diffuse and faded completely within a few minutes. In the normal ear of the same animal, narrow, sharply defined blue lines radiated from the point of injection. Within 60 seconds after injection, a small blue streamer reached the marginal vein; and, after 4 to 5 minutes, fine blue lines extended proximally along the central artery.

A second rabbit was subjected to the same degree of injury as the first, but the dye was injected 30 minutes after frostbite. Rapid and diffuse spread of dye in the frostbitten ear toward the central artery and laterally toward the marginal vein was observed. Two hours after injection of dye, diffuse, blue streamers extended proximally along the central artery and near the marginal vein. After 5.5 hours all dye had disappeared from the frostbitten ear. In the normal ear, fine lines extended proximally along the central artery, but these moved at a much slower rate than those in the frostbitten ear. After 2 hours no streamers were seen. A large amount of dye remained at the original site of injection at the end of 5.5 hours.

These observations indicate that lymph flow in

the frostbitten ears of rabbits persisted after injury and that it was more rapid than that observed in normal ears.

The spread of T-1824 in normal and frostbitten skin of rabbits' ears was studied after injection of 0.01 ml. of dye (1 per cent T-1824 in isotonic sodium chloride solution) intradermally into both ears after one of them had been frostbitten in the usual manner. The size of the spot of dye was measured at intervals over periods of 17 to 21 hours. Seven rabbits were studied in the manner described. The results in all 7 animals were essentially similar. Spread of dye occurred in both normal and frostbitten ears but was more extensive in the frostbitten ears than in the normal ones. A single example will suffice to indicate the relative magnitude and qualitative aspects of changes observed. The size of the spot of dye immediately after injection was 4×4 mm. in each ear. At 170 minutes after injection of dye the spot had increased to 6×11 mm. in the normal ear while that in the frostbitten ear was 15×60 mm. After 21 hours the dimensions were 11×13 mm. in the normal ear while no dye was detectable in the frostbitten ear at this time.

The results of similar measurements on an animal in which the frostbitten ear was thawed rapidly in warm water (3 minutes at $+42^{\circ}\text{C}$.) did not differ strikingly from those obtained on untreated, frostbitten ears. A series of 5 animals studied after marked edema had been induced in one ear by wetting it with xylene showed differences between the edematous and the normal ears which were similar to those observed when edema was produced by frostbite.

As judged from the observations made with intradermally injected dye in frostbitten ears, there appears to be no evidence of physical organization of the edema in such a way as to impede the spread of colloiddally dispersed material within it.

COMMENT

Direct observations of blood flow changes in the minute vessels of normal and frostbitten ears of rabbits, both with and without intravascular India ink injections, are in substantial agreement with those reported by Tittel (14) and by Rotnes and Kreyberg (15). The following points are brought out:

(1) Cold injury leads to stasis in the true capillaries in proportion to the severity of injury as it is determined by time of exposure and temperature.

(2) Following a standard cold injury of 1 minute immersion of the distal half of the ear in the freezing mixture at $-55^{\circ}\text{C}.$:

(a) Blood flows into all vessels of the ear in the early period after thawing.

(b) Stasis appears first in the true capillaries, begins with the return of blood flow, and is complete in the true capillaries within 10 minutes after the first reappearance of blood in the injured region.

(c) Stasis is accompanied by loss of fluid from the true capillaries into the surrounding tissues and by dense packing of erythrocytes within the capillaries.

(d) Carbon particles (India ink), injected into the blood stream before stasis is complete, become trapped in the true capillaries during the development of stasis, but if the injection of carbon is delayed until after stasis is complete, none of it enters and none is trapped.

(e) Blood flow in the cold-injured region, after stasis has closed the true capillaries, occurs in arteriovenous anastomoses and in arteriolar-venular capillaries, described as "thoroughfare channels" by Chambers and Zweifach (16).

It is evident from the above summary that during the hyperemic phase after injury the total cross sectional area of the vascular bed between the arterioles and venules within the frostbitten region becomes sharply reduced to include only a-v anastomoses and arteriolar-venular capillaries. This response to injury by cold has been noted by other investigators (17 to 22). An important consequence of such a local redistribution of blood flow in the presence of a total increase in minute volume flow, as indicated by the changes of skin temperature, would be a marked rise in effective filtration pressure. Thus, in spite of the fact that the true capillaries may lose large amounts of fluid up to the time that stasis is complete, the later loss of fluid into the extravascular compartment must occur mainly as the result of the high filtration pressure within the "thoroughfare channels." The measurement of changes in volume and subcutaneous tissue pressure in frostbitten feet of rabbits

(23) showed that maximum volume is reached in about 2 hours after injury and that the pressure of fluid in the interstitial compartment reaches its maximum of 25 to 30 cm. of water at about the same time. In the period between thawing and the attainment of maximum swelling a new filtration equilibrium is established at high levels of capillary pressure, interstitial fluid pressure, and rate of fluid exchange. That fluid continues to be lost from the blood in the injured regions at rates which overwhelm the local routes of removal is illustrated by 2 observations: (a) the movement of edema fluid in the subcutaneous tissues far into the uninjured regions from the area injured by cold (23); and (b) the rapid lymph flow from the injured region as demonstrated with the aid of dyes.

The "flux" of plasma ultrafiltrate in normal tissues has been described by Zweifach (24) as filtration from the plasma in the capillaries with high internal pressure (the arteriolar-venular capillaries) and the re-entry of plasma ultrafiltrate into the blood stream by seepage into true capillaries, especially in the region close to their junction with an a-v capillary as it enters a collecting venule. In frostbitten ears of rabbits, the closure of true capillaries and the rapid inflow of blood during the hyperemic stage favor the vigorous formation of plasma ultrafiltrate and perhaps even the loss of some protein through the walls of a-v capillaries; but the stasis, effective in diverting inflowing blood into "thoroughfare channels" at high pressure, at the same time renders the true capillaries useless as routes of re-entry of fluid into the blood stream. In addition to closure by primary stasis, obstruction of some capillaries may be accomplished as a result of the spatial distortion and kinking which occur during swelling.

The effect of closure of true capillaries upon exchanges between the blood and the extravascular compartment is that of sharply limiting the available surface across which diffusion may take place and at the same time increasing the rate of filtration. Danielli and Stock (25) and Zweifach (24) have pointed out the separate nature of exchanges by diffusion and exchanges which occur largely by filtration. Resting tissues characteristically depend upon the flux of plasma ultrafiltrate; minute volume blood flow is relatively small and only a few true capillaries are open at a time. Active tissues depend upon a considerable increase in the area

available for diffusion as well as upon increased filtration. Thus, in active tissues the minute volume blood flow is large, many true capillaries are open, and an appreciable volume of filtrate is formed. In frostbitten tissues an anomalous and probably harmful situation exists: blood flow and local temperature are high at a time when the total area available for diffusion exchange is reduced. In spite of the high temperatures, which might be expected to increase the metabolism of tissues in the injured region, measurements of arterio-venous oxygen differences in the blood perfusing these tissues indicate that the rate of oxygen consumption is extremely low (23).

SUMMARY

1. Paralysis of vasomotor activity, both tonic contraction and phasic responses, was produced in the injured regions when the distal halves of rabbits' ears were exposed to -55°C . for 1 minute in a liquid freezing mixture. Vasomotor activity was preserved in vessels proximal to the line of immersion.

2. After thawing of the frozen ears in air at 25°C ., blood flow was re-established and all vessels became markedly dilated. Stasis began in the true capillaries with the return of blood flow and was complete in 10 minutes.

3. Particulate carbon, injected intravenously, became trapped in the true capillaries when injection was made within the first 10 minutes but failed to enter true capillaries when injection was delayed for longer intervals after injury.

4. Blood flow persisted in arterio-venous anastomoses and in arteriolar-venular capillaries for about 24 hours but declined virtually to zero over the ensuing 24 to 30 hours.

5. Rapid thawing of frozen ears in warm water (1 to 3 minutes at $+42^{\circ}\text{C}$.) delayed the development of stasis in the true capillaries until maximal swelling was reached at about 2 hours after injury.

6. Procaine block of the stellate ganglion augmented the hyperemia after injury and delayed the onset of stasis for 50 to 60 minutes.

7. Persistence of rapid lymph flow and rapid formation of interstitial fluid in frostbitten ears up to 21 hours after injury was demonstrated with the aid of intradermal injections of the dyes, Patent Blue V and T-1824.

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