

THE PERIPHERAL VASCULAR SYSTEM AND ITS REACTIONS IN SCURVY: AN EXPERIMENTAL STUDY¹

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The morphological pathology of scurvy has been well established in supporting tissues and in various organ systems (1, 2, 3), but there is a disagreement with regard to the status of the capillary bed. Although characteristic lesions have not been described in the capillaries (4), several studies of experimental and of clinical vitamin C deficiency have reported an "increased capillary fragility" in this condition (5, 6, 7, 8). Increased vascular fragility has been suggested, with certain limitations, as an early criterion of ascorbic acid deficiency, since it appears prior to the onset of other characteristic signs of the scorbutic state (6). Other workers, in studies chiefly of human vitamin C deficiency, have found no significant correlation between blood levels of ascorbic acid and the tendency to petechiae formation (9, 10, 11, 12, 13).

In view of this lack of agreement, and the absence of information concerning the physiology of the peripheral vascular bed in scurvy, it was decided to make direct microscopic observations of the reactions of the smallest blood vessels in living scorbutic animals. Within the past few years, methods for the study of the capillary bed in living mammals have been considerably improved and found suitable for the establishment of peripheral vascular phenomena in several different experimental conditions (14, 15, 16, 17). In the present study, these methods have been slightly modified and adapted to the examination of the terminal vessels in living unanesthetized control and scorbutic guinea pigs.

METHODS. The procedure involves observation with the microscope of the mesenteric capillary bed in guinea pigs which are not under a general anesthesia.

1. *Preparation of the Mesentery.* Animals were prepared for examination by a paravertebral block of the lower thoracic and upper lumbar nerves, using a total of 0.4 cc. of 1.0 per cent Procaine solution. In six animals, these nerves were sectioned surgically two weeks prior to study. For the microscopic observations, the animals were fastened into a suitable device which prevented them from moving about, and the abdominal wall was incised across the denervated or blocked area. Respiratory movements of the abdomen slowly extruded a gut loop which was gently guided with its mesentery over a movable glass ring on the microscope stage. The tissue was warmed by irrigation with a

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Ringer-gelatin solution at 37.5°C. and completely covered except for the region studied by cotton strips soaked with the warmed drip solution. Observations and readings were taken and checked. This loop of mesentery and gut were then pushed aside. The slow extrusion of gut loops through the incision by respiratory movements afforded new fields for study. The mesentery was never stretched prior to observation.

At no time during these studies did the animals exhibit any evidence of pain. The avoidance of general anesthesia, and the absence even of a local anesthetic in six animals, made possible the observation of the splanchnic vascular bed in a living, fully conscious mammal. This ruled out the possibility of an increased sensitivity in scurvy to the vaso-inhibited state which has been demonstrated following the use of some of the common anesthetic agents (17).

2. *Vascular Criteria.* In all animals, the following data were recorded: the presence or absence of spontaneous vasomotor activity, the diameters of the capillaries and the nature of their wall, the general state of capillary blood flow, the presence or absence of petechiae, and the presence or absence of any vascular phenomena following violent struggling by the animal. In addition, the threshold concentrations of epinephrin were determined which, when applied topically to the mesentery, produced complete closure of pre-capillary sphincters and contraction of arterioles to approximately one-half their diameter. This offered a quantitative measure of the reactivity of these muscular elements to an external stimulus. In 4 controls, and 3 scorbutic animals, studies were also made of the epinephrin reactivity of the small venules.

3. *Experimental Procedure.* A. Series A. *Controls.* Thirteen animals were used; ten were prepared by a Procaine nerve block and three by operative denervation of the flank two weeks prior to study. Nine animals were given a diet (diet A) known deficient in ascorbic acid (18) with greens *ad-libitum* to insure adequate vitamin intake. Four were given a purified ascorbic acid deficient diet (diet B³) with additional feedings of this vitamin, 15.0 mgm./day. Animals on either diet, supplemented by greens or by oral vitamin C, have been maintained for varying periods of time up to six months or more without evidence of any deficiency state, and have bred to the second generation (19).

Scorbutic. Sixteen animals were used; thirteen were prepared for observation by Procaine nerve block, and three by denervation. Twelve received diet B and four received diet A, without supplemental feedings. Studies on both the controls and the scorbutic animals were carried out during the fourth week on their respective diets. At this time, those guinea pigs on the scorbutogenic diets all showed noticeable evidence of scurvy, and their weights had fallen to levels present at the beginning of the diet (200-250 grams). The control animals had no evidence of scurvy or other disease state.

Irrespective of the type of diet or the use of Procaine or surgical nerve block,

³ Diet B contained: casein 18 per cent, dextrin 45 per cent, salts (S.M.A. Co.) 4 per cent, lard 8 per cent, sucrose 15 per cent, agar 2 per cent, C.L.O. 1 per cent, W.G.O. 2 per cent, yeast 5 per cent. The diet was made in bulk weekly and refrigerated. Adequate daily portions were fed *ad-libitum*. Control animals received an additional daily feeding of ascorbic acid, 15 mgm./100 grams body weight.

the peripheral vascular findings in the control animals were essentially the same. Similarly, regardless of the type of scorbutogenic diet received, or method used to permit exposure of the mesentery in unanesthetized animals, the vessels of the scorbutic animals approached an equal degree of dysfunction in all cases.

B. Series B. After completion of the first series of observations, the following experiments were made. Seven animals on diet A with abundant greens for one week were examined as controls, and the abdominal wound closed following the study. After a two week healing period, these same animals were then placed on scorbutogenic diet B until symptoms of scurvy appeared, when they were examined a second time. The results of this series were completely in accord with those of the first, and offer comparison of both control and scorbutic findings made on the same animals.

RESULTS. *The control animals.* The general topography of the capillary bed in the guinea-pig mesentery agrees closely with that observed in the rat and dog (13) (fig. 1). The arterioles were tonic and showed a threshold response to epinephrin with average concentrations of 1:1,000,000. Their walls were generally thickened, smooth muscle cells stood out sharply in partial contraction, and the vessels showed the intermittent vasomotor activity previously described in the dog and rat (13).

The pre-capillaries reacted to 1:2,000,000 concentration of epinephrin, on the average, but the variability at this region was extreme. In two animals, not included in the data because of their obvious hyper-excitability, complete pre-capillary closure occurred at 1:35,000,000 epinephrin. Vasomotion of the pre-capillary sphincters was usually active, and predominantly in the "closed" or constricted phase. Capillary blood flow was intermittent.

The true capillaries had an average diameter of 7.5-10.5 micra, with the distal third frequently 1.0-1.5 micra wider than the proximal portion. At no time were active contractions found in the capillaries, other than at the pre-capillary region where smooth muscle cells occur.

The small collecting venules appeared partially narrowed, and blood flow through them was rapid. Their epinephrin reactivity was generally comparable to that of the arterioles, although their contracted state was of a more varicose nature in contrast to the more uniform contractions of the arterioles.

The absence of a general anesthetic afforded study of the changes in the small blood vessels of the mesentery and serosa during occasional periods of excitement with violent muscular activity. Such vascular phenomena were seen in six of the twenty control animals. Briefly, when marked, these changes consisted of narrowing of arterioles and a complete closure of pre-capillary sphincters, with restriction of blood flow only to the direct A-V channels. Flow in all of the capillaries and in many of the small arterioles was stagnant. This sudden cessation of flow in many of the larger arterioles implied a contraction of vessels in the arterial tree located more proximally than those vascular beds examined in this study. When muscular activity ceased, the arterial flow gradually resumed; but it was frequently 30 to 60 seconds before capillary blood flow was restored to its previous level, in the mesentery and serosa.

Prominent lymphatics accompanied the larger arterioles and small arteries,

and frequently showed peristaltic contractions at a rate of 1 to 3 per minute. The addition of washed red cells to the Ringer-gelatin drip solution bathing the mesentery was followed by the appearance of red cells inside these lymphatics within 2 to 4 minutes. Forward movement of fluid containing these cells occurred only during the contractions of the lymphatic vessel. Topically applied epinephrin, 1:500,000, was followed by a spastic constriction of the lymphatics to approximately one-third their initial diameter, with a complete cessation of lymph flow. Weaker dilutions produced a speeding of the rate of periodic contraction to as many as 10 to 20 per minute, with varying degrees of constriction observed in the same lymphatic vessel.

The scorbutic animals. The peripheral vascular system was generally dilated, and blood flow was markedly slowed. The largest arterioles, of a diameter

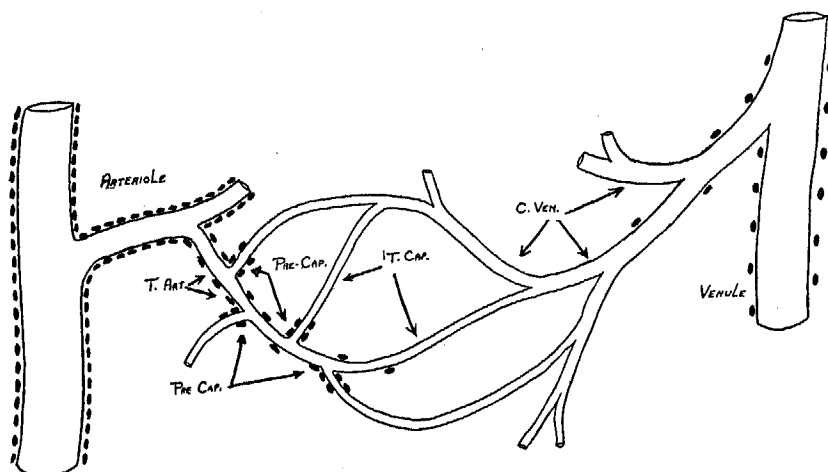


Fig. 1. Diagram of a functional unit in the peripheral vascular bed of the guinea pig mesentery. *T. Art.* = terminal arteriole; *Pre-Cap.* = pre-capillary; *T. Cap.* = true capillary; *C. ven.* = collecting venule. Smooth muscle cells are indicated by the black dots.

greater than 100 micra, showed an undiminished response to topical epinephrin (table 1). In contrast, the direct branches of such vessels, of approximately 30 to 60 micra in diameter, and the smaller arterioles supplied by them, were completely unresponsive to epinephrin, even when relatively strong concentrations were used (table 1). During the notable contraction of the larger parent arteriole in the epinephrin test, the rate of flow through these dilated side branches and the vascular beds supplied by them was greatly reduced, becoming sluggish and frequently stagnant.

The pre-capillary sphincters likewise did not respond to the usual concentrations of epinephrin used. They remained opened (7.0 to 9.0 micra wide) in approximately 90 per cent of the vessels observed (figs. 2 and 3). Spontaneous vasomotor activity was absent; the capillary flow was therefore continuous, but slow. We observed no abnormalities of the capillary wall; and capillary

diameters in scorbutic animals were the same as those found in the controls (table 1).

An especially prominent feature of the scorbutic state was the dilatation and sluggish flow observed in the small collecting venules. The dilatation often involved the venular end of the true capillary before it joined with a venule. The possible importance of this atonic state of the small venules with regard

TABLE 1

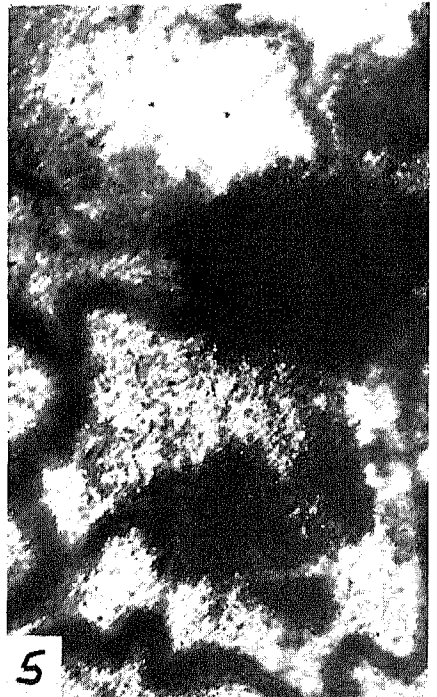
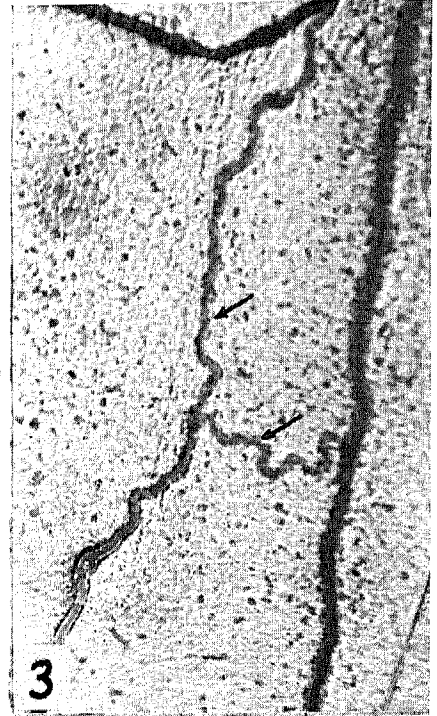
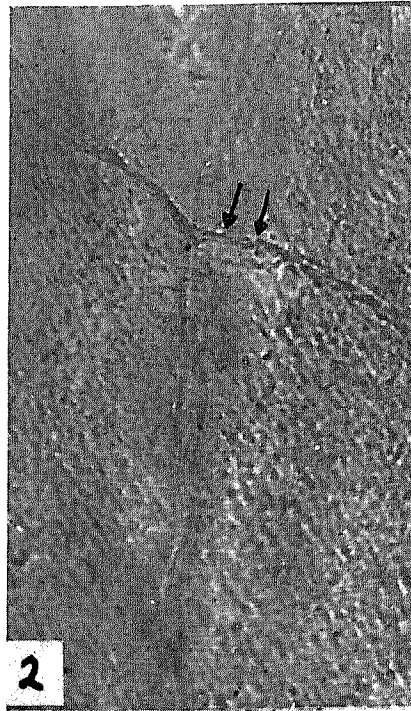
	CONTROL ANIMALS	SCORBUTIC ANIMALS
Epinephrin sensitivity of larger arterioles,* >100 μ in diam.	1:500,000 (1:100,000-1:5,000,000)	1:450,000 (1:100,000-1:4,000,000)
Epinephrin sensitivity of smaller arterioles,* <75 μ in diam.	1:1,000,000 (1:300,000-1:5,000,000)	No responses ever noted using 1:100,000
Epinephrin sensitivity of precapillary region**	1:2,000,000 (1:500,000-1:35,000,000)	No responses ever noted using 1:100,000
Epinephrin sensitivity of small venules,* <75 μ in diam.	1:500,000 (4 animals)	No responses noted using 1:100,000 (3 animals)
Capillary diameter (external), in micra	7.0-10.5	7.0-11.0
Presence of vasomotor activity in arterioles and precapillaries	+ - + + + +, Usually in the "closed" phase	None observed; precapillaries usually opened widely
General nature of blood flow in the arterioles, capillaries, and venules	Rapid, varying with vasomotion; vessels "tonic"	Sluggish, vessels usually dilated, especially in small collecting venules
Presence of petechiae in small venules following trauma	Three in 2 of 20 animals	Present in 11 of 23 animals; numerous

* Epinephrin concentration necessary to produce narrowing to approximately 50 per cent of internal diameter.

** Epinephrin concentration necessary to produce complete closure of the vessel at this site.

to petechiae formation will be mentioned in the discussion. The epinephrin reactivity of the venules was found to be greatly diminished in the three animals so tested.

The pronounced vascular response to violent struggling found in six control animals was not observed in any of the scorbutic cases, although they likewise struggled and attempted to free themselves as frequently and as actively as did the controls. In two instances, however, the struggling episodes occurred



Figs. 2-5.

fortuitously as epinephrin, 1:200,000, was applied to the mesentery. Within two seconds after the onset of muscular activity, all of the arterioles and pre-capillaries in the field under study showed marked constrictions with a complete stagnation of flow. This exaggerated constrictor response faded soon after struggling had ceased. The heightened responsiveness to epinephrin during these episodes of activity probably represents a vascular response to a summation of intrinsic stimuli (increased concentration of epinephrin in the blood?) with the topically applied vasoconstrictor agent, indicating that in marked scurvy the vascular bed is capable of maximum constriction provided that the stimuli are of adequate magnitude.

Peripheral vascular hemorrhages in scurvy. Petechiae rarely appeared spontaneously in the small vessels of the control or the scorbutic animals. In the latter, however, brushing the area observed with a small camel hair brush for ten strokes with a force just ample to stretch the mesentery slightly, or purposefully stretching the mesentery with rubber-tipped forceps, produced numerous small petechiae in eleven of the twenty-three scorbutic animals so treated. The same procedure produced three petechiae in only two control animals. Although the method is not sufficiently quantitative, it suggests that in scurvy there is an increased tendency of the dilated vessels to rupture following trauma.

The petechiae produced in this manner occurred chiefly in the venular portion of the peripheral vascular bed, where vasodilatation and the slowed rate of blood flow were most marked (figs. 4 and 5). For example, of eleven observed in one area, nine were in the small collecting venules which directly drain the capillary bed, one was in a true capillary, and one was in a small arteriole at a point of dichotomy. Similar distributions of petechiae were noted in the remaining ten scorbutic animals in which they were found.

DISCUSSION. The disfunction of the peripheral vascular apparatus in scurvy, as found with these methods, displays at least two prominent features. These are:

1. A decreased responsiveness of the contractile elements, particularly of the arterial portion beyond the pulsatile small arteries, to physiological concen-

Fig. 2. Normal animal: an arteriole, giving off a capillary (arrows); in an environment of epinephrin, 1/5,000,000. Note that the lumen of the capillary is empty, due to contraction of the precapillary sphincters in this concentration of epinephrin.

Fig. 3. Scorbutic animal: a terminal arteriole giving off two capillaries; in an environment of epinephrin, 1/150,000. Note that both capillaries are filled with a moving stream of blood, due to failure of the precapillaries to contract in response to this concentration of epinephrin.

Fig. 4. Scorbutic animal: the venular ends of capillaries are visible in the top of the picture, and are intact, without petechiae. In the center of the figure, the small venules draining the capillaries are partly surrounded by small hemorrhages, as dark masses. At the bottom of the picture, two larger masses of blood are seen outside the main collecting venule.

Fig. 5. Scorbutic animal: larger petechiae are shown here, along the tributary branches of the collecting venules.

(All magnification is approximately 120X)

trations of epinephrin, with dilatation of these muscular vessels and a relatively sluggish flow of blood.

2. A tendency of the terminal collecting venules, which drain the capillary bed, to become dilated and engorged, and to rupture at trauma.

Although these phenomena are probably closely related, they will be discussed separately.

1. The means by which peripheral vascular hypotonia develops in scurvy is obscure. It may be a direct impairment of the ability of the vascular smooth muscle cells to respond to stimuli. However, when epinephrin was applied to the mesentery of an active animal, or was used in very strong concentrations, all vessels constricted maximally. This indicates that the inherent contractile ability of these cells is not notably disturbed. The hypotonia may therefore represent an indirect result of ascorbic acid deficiency, from the dysfunction of some vasotonic mechanism(s), such as the adrenal gland. Adrenal vitamin C is markedly lowered in scurvy (3). The studies of Sayers and his collaborators indicate that this vitamin may prove instrumental in adrenal cortical function (20, 21). Lowenstein has recently isolated an ascorbic acid-cholesterol complex from aqueous adrenal extracts which has definite cortical activity (22). Giroud has concluded that the elaboration of adrenal cortical hormones is dependent on the presence of vitamin C (23, 24). These findings suggest that during ascorbic acid insufficiency, certain aspects of adrenal function may be depressed.

It would be possible to explain the vascular hypotonia of scurvy, in part at least, on the basis of such a scorbutic impairment of adrenal cortical activity. Swingle has shown that the cortex is necessary to maintain a normal vaso-compensatory resistance to hemorrhage (25). After numerous recent experiments, Zweifach and his co-workers have concluded; "Vascular tone, and specifically that of the terminal arterioles, is directly dependent on the presence of cortical hormones. In adrenalectomized rats, the tone and reactivity of these muscular components are markedly depressed. The administration of cortical extracts to such animals results in the restoration of normal vascular reactions" (26). Therefore, the hypotonic state of the vascular bed in scurvy may result from an inadequate function of the adrenal cortex, or perhaps other vasotonic mechanisms.

Scorbutic guinea pigs are killed by a degree of Noble-Collip drum trauma which is readily survived by control animals (18). In addition, the development of resistance to such injury which appears in normal animals after repeated sub-lethal damage, cannot be produced in the vitamin C deficient guinea pigs (18). It has been shown previously that death produced by the Noble-Collip drum is preceded by a hypo-reactive, atonic peripheral vascular system (27). The development of resistance to such trauma, on the other hand, is accompanied by a greatly heightened vasomotor tone. Epinephrin reactivity is markedly elevated, and spontaneous vasomotion is increased in occurrence and in rate (28). This hyper-reactive vascular state is in direct contrast to that obtaining in ascorbic acid insufficiency. Therefore, it is conceivable that the failure of scorbutic animals to withstand trauma or to develop resistance to it is inti-

mately related to their observed decrease in peripheral vascular reactivity, and a consequent impotence of vaso-compensatory mechanisms. Both conditions, however, may result independently from the widespread pathological changes which occur in scurvy (3).

Recent studies have offered evidence that pretreatment with ascorbic acid is of value in protecting guinea pigs against trauma (29, 30) and against hemorrhagic shock (31).

2. Vascular hemorrhages in scurvy, in this study, were found to result chiefly from traumatic rupture of the proximal collecting venules which drain the capillary bed directly (figs. 4 and 5). Dilatation and a sluggish rate of blood flow were most marked in these vessels. It is of interest to note that aggregations of collagen fibers first become notably apparent about the vessels in this region where the true capillaries empty into the venules. Smooth muscle cells are usually absent at this site in the guinea pig, and also in the frog (32). It has been suggested that hemorrhages in scurvy result from a weakness of either the endothelial cement or the collagen substance immediately adjacent to the capillaries (33). Our observation that the great preponderance of hemorrhages occur in the small venules about which collagen bundles are present, rather than in the true capillary endothelial tubes where such collagen aggregations are least prominent, tends to support a weakening of the collagen as a causative factor here, rather than any fault in the capillary endothelium or its cement substance. Chambers has concluded that in certain epithelial tissues ascorbic acid is not essential for the maintenance of the cement material (34). The defect in the perivascular supporting structures of the venules may be closely related in some manner to the greatly slowed flow of blood through these vessels.

An increased tendency of small vessels to rupture at trauma is not a specific response to ascorbic acid deficiency (13). Formation of petechiae was noted in the dilated vessels of the dog's omentum during the depressed terminal stage of "irreversible" hemorrhagic shock (15). Here they were readily produced by light trauma, and occasionally developed when trauma did not occur. They were not found in the capillary bed during its tonic stage prior to hemorrhage or during the early "hyper-reactive" stage of shock. Therefore, it is possible that the increased tendency of small vessels, especially venules, to rupture may be a function of their atonic dilated condition and the relatively slowed flow of blood through them.

The absence of spontaneous petechiae in these experimental studies agrees with the findings of Farmer and his collaborators in a recent clinical study of experimental human ascorbic acid insufficiency (11). These workers report small hemorrhages about wounds, which were probably produced by light surgical trauma. Petechiae were not found in other areas.

SUMMARY

1. A technique was devised which permitted study of the small vessels in the mesentery of unanesthetized guinea pigs.
2. The primary pathological condition in the peripheral vascular system of

scorbutic animals was found to be a hyporeactivity of the contractile vessels with dilatation, and a sluggishness of blood flow. This state was marked in the small terminal venules.

3. These conditions developed only in the small vessels distal to the pulsatile arteries and arterioles of approximately 100 to 150 micra in diameter. The responses of vessels larger than this range to topical epinephrin tests were within normal limits.

4. Following trauma, petechial hemorrhages were found in eleven of twenty-three scorbutic animals. They were present to a slight degree in two of twenty controls.

5. At least 85 per cent of the petechiae were located in the small collecting venules which drain the capillary bed directly.

6. In the scorbutic animals, the capillaries were of the same diameter as those of the controls. No abnormalities of the capillary wall were observed.

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