
ASCORBIC ACID AND THE PERIPHERAL VASCULAR SYSTEM*

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Introduction

Biochemical progress on the investigation of vitamin C has moved with speed since the isolation and identification of this vitamin by King and Waugh.¹ Knowledge concerning the basic physiology of its vascular manifestations, however, has failed to keep pace with the progress of chemical information. Even today we hear the phrases "capillary fragility," "decreased resistance to trauma," and "increased capillary permeability," used in the same relation to the role of vitamin C as they were used nearly 100 years ago, and with little if any more knowledge of meaning or mechanism. Because of this void, some years ago I began an evaluation of the possible role that certain nutritional factors played in the economy of the peripheral vascular system. It was natural to begin with ascorbic acid as an important substance whose role needed exploration. Concurrently, the vascular physiology of certain flavonoid materials has been evaluated.

Methods

The basic approach in my investigations has been to observe the capillary bed through the microscope while the animal is either totally anesthetized or held immobile with local anesthesia, preventing pain, and to measure the reactivity of vascular elements to certain stimuli as applied in solution or by micromanipulative methods.²⁻³ The mesentery of small or large bowel of guinea pigs or rats with suitable anesthesia was supported by a movable glass stage and bathed with constantly flowing warmed Ringer-gelatin solution (FIGURE 1). Notes were made on the presence of spontaneous vasomotor activity, the general nature of blood flow, the presence or absence of petechiae, reactivity to epinephrine in known concentrations and, in certain instances, the reactivity to stimulation with known millivolt strengths of stimuli with a suitably prepared microelectrode of silver wire.

Direct trauma was applied by single strokes with a fine camel's hair brush to vessels under direct microscopic observation. Selected arterioles, capillaries, and venules were transected with a tiny scalpel affixed to the arm of the micromanipulator. Bleeding time of such severed vessels was measured with a fast stop watch from the time of bleeding initiated by the incision to cessation of bleeding by plugging of the severed ends with platelets. Intravascular thrombosis was induced by stimulation of the vessel wall in arterioles and venules with known microvolt stimuli, and the strength of stimulation necessary to produce such thrombosis was recorded. In certain studies on rats, increased bleeding of the peripheral vessels was induced by feeding Dicumarol (50 mg./100 gm. of body weight) to rats at 24 hours prior to observation.

* The work reported in this paper was supported in part by the Nutrition Foundation, Inc., New York, N.Y., and the A. H. Robins Company, Richmond, Va.

The guinea pigs were on three different diets: (1) a modified Sherman-type diet; (2) a diet comparable to that used by Reid and Briggs;⁴ (3) a standard guinea pig chow laboratory diet with 15 mg. supplements of vitamin C per 100 gm. of body weight.

Animals given hesperidin or rutin were fed 10 mg./100 gm. of body weight for an average period of 16 days prior to observation. It was found that on the various vitamin C-deficiency diets, the blood level of vitamin C in the guinea pigs fell to zero in about 14 or 15 days.³

In certain experiments relating to the ability to withstand hemorrhagic shock, control guinea pigs and animals, having been on the deficiency diet for

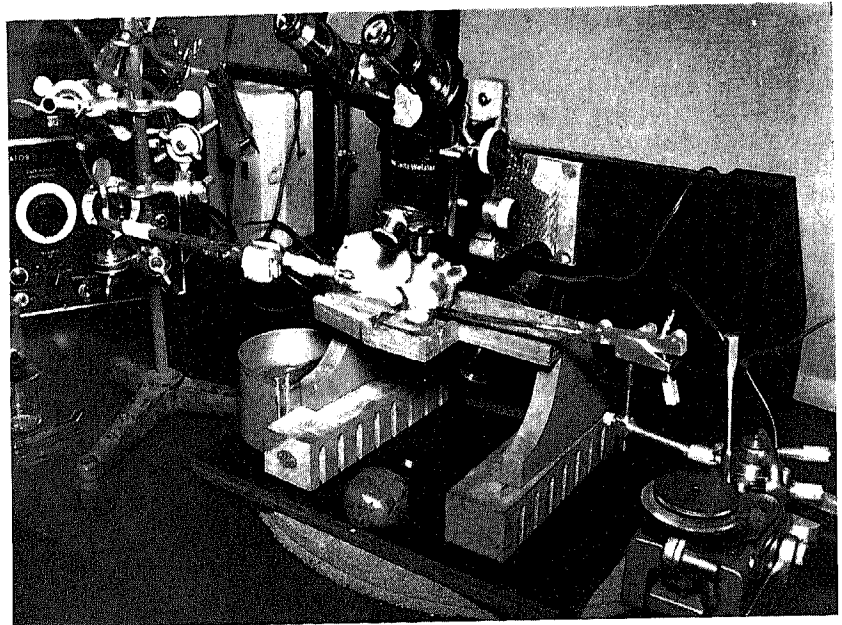


FIGURE 1. Micromanipulative apparatus for studying small vessels in laboratory animals.

15 days, were bled according to a standard procedure.⁵ The relationship between blood loss and the duration of hypotension at which the animal survived before exitus was measured in the deficient and in the supplemented guinea pigs.

Results

The general apparatus is shown in FIGURE 1. The animals so prepared could be observed continually under the microscope for from 2 to 3 hours, and observations were made repeatedly on vessels with regard to the parameter measured. It was found that the end points of reactivity to drugs and voltage stimulation were reproducible.

In TABLE 1 the findings with regard to the reactivity and other features of

the capillary bed are shown in the control guinea pigs and in guinea pigs after 14 to 16 days on vitamin C deficiency. It is clear that scurvy per se in the animal is featured by a loss of reactivity to epinephrine stimulation in the small arterioles, by increased spontaneous fragility of the blood vessels in the capillary bed, and—especially—the location of the petechiae is almost completely in or about the collecting venular system.

FIGURE 2 displays the relationship between the total volume of blood removed from vitamin C-deficient and vitamin C-supplemented guinea pigs and

TABLE 1

| | Control animals | Scorbutic animals |
|--------------------------------------------------------------------------|-------------------------------------------------|---------------------------------------------------------------------------|
| Epinephrine 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) |
| Epinephrine 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 |
| Epinephrine sensitivity of precapillary region† | 1:2,000,000 (1:500,000-1:35,000,000) | No response ever noted using 1:100,000 |
| Epinephrine sensitivity of small venules,* 75 μ in diam. | 1:500,000 (4 animals) | No response noted using 1:100,000 (3 animals) |
| Capillary external diameter (μ) | 7.0-10.5 | 7.0-11.0 |
| Presence of vasomotor activity in arterioles and precapillaries | ++++, Usually in "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 |

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

† Epinephrine concentration necessary to produce complete closure of the vessel at this site.

the duration of the hypotension they are able to withstand before exitus. It is evident that an avitaminosis C is associated with a prominent reduction in the ability of the animal to withstand blood loss or to survive hypotension in a manner that is encountered in the control animal.

TABLES 2, 3, and 4 outline our findings in rats with regard to: the possible influence of the flavonoids hesperidin and rutin on spontaneous petechiae (TABLE 2); the clotting tendency of intravascular blood after electrical stimulation (TABLE 3); and reactivity to epinephrine (TABLE 4). Supplementation with either of these flavonoids did not influence any of the features examined. The number of spontaneous and induced petechiae, the clotting tendency in the various vessels as measured, and the reactivity to epinephrine did not

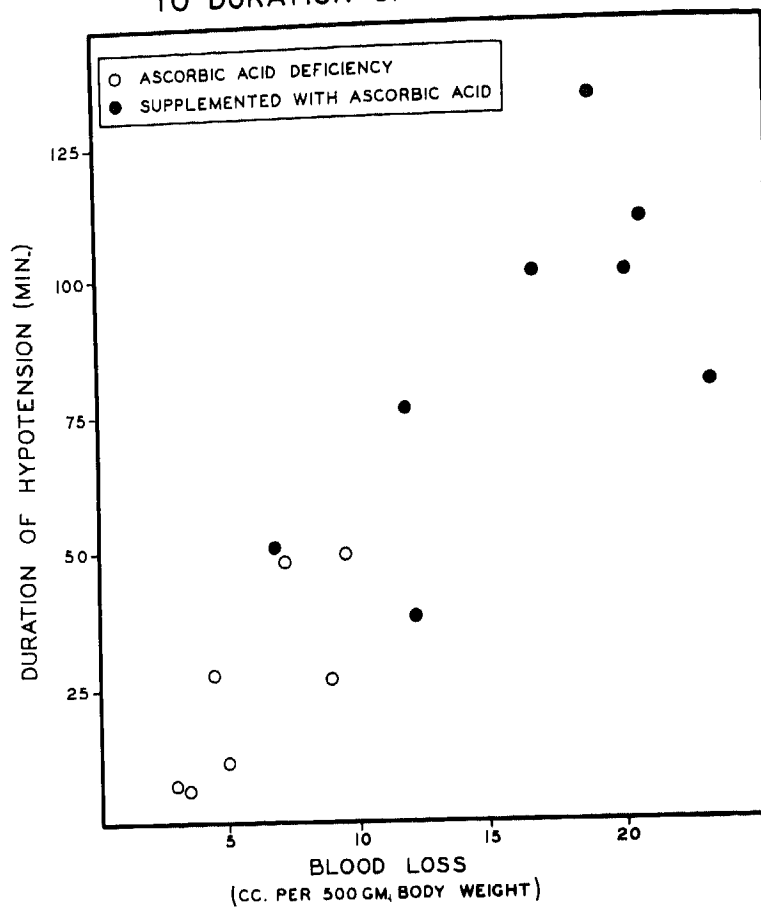
RELATION OF BLOOD LOSS
TO DURATION OF HYPOTENSION

FIGURE 2. The relationship between blood loss and survival during hypotension of normal and vitamin C-deficient guinea pigs.

TABLE 2
THE APPEARANCE OF PETECHIAE IN RATS FED BIOFLAVONOIDS*

| Dietary treatment | No. of animals | Spontaneous petechiae (No./field) | Fresh petechiae after brushing |
|-------------------------|----------------|-----------------------------------|--------------------------------|
| Normal diet | 10 | 7 (2-13)† | 2 (0-5)† |
| Hesperidin-supplemented | 20 | 9 (3-15)† | 2 (0-4)† |
| Rutin-supplemented | 20 | 6 (3-12)† | 4 (0-6)† |

* Rats received 20 to 100 mg. of dicoumarol 24 hours previous to observation.

† Numbers in parentheses indicate range of petechiae.

change. Spontaneous vasomotor activity seemed to be somewhat heightened, however, in the rutin-supplemented animals.

In TABLE 5 the bleeding time of guinea pigs on various diet classes is determined after the vessel is completely transected with a microscalpel. In general,

TABLE 3
THE EFFECT OF ELECTRICAL STIMULATION (VM)* UPON CLOTTING
TENDENCY IN THE VASCULAR BED OF THE RAT

| Dietary treatment | No. of animals | Arterioles | | Capillaries | Venules | |
|-------------------------|----------------|-------------|----------|-------------|-------------|----------|
| | | 20-50 μ | 20 μ | | 20-50 μ | 20 μ |
| Normal diet | 9 | 107 | 70 | 0 | 82 | 28 |
| Hesperidin-supplemented | 20 | 98 | 87 | 0 | 63 | 46 |
| Rutin-supplemented | 20 | 110 | 106 | 0 | 78 | 34 |

* VM indicates volts \times milliseconds.

TABLE 4
TOPICAL APPLICATION OF EPINEPHRINE TO VESSELS OF RATS FED BIOFLAVONOIDS*

| Dietary treatment | No. of animals | Epinephrine threshold | Vasomotor activity |
|-------------------------|----------------|-----------------------|--------------------|
| Normal diet | 9 | 1.6×10^{-7} | + |
| Hesperidin-supplemented | 20 | 1.6×10^{-7} | ++ |
| Rutin-supplemented | 20 | 1.6×10^{-7} | +++ |

* No effect was observed from application of hesperidin complex, 1 per cent, hesperidin methyl chalcone, homovanillic acid, dihydroxyphenylacetic acid, calcium flavonglucoside, or hydroxyphenylacetic acid.

TABLE 5
BLEEDING TIME OF GUINEA PIGS (IN SECONDS) AFTER DIRECT MICROTRANSECTION*

| Dietary treatment | No. of animals | Arterioles | | Capillaries | Venules | |
|----------------------------------|----------------|-------------|----------|-------------|-------------|----------|
| | | 20-50 μ | 20 μ | | 20-50 μ | 20 μ |
| Normal diet | 18 | 170 | 209 | 21 | 210 | 160 |
| Normal diet + rutin | 20 | 182 | 210 | 22 | 219 | 178 |
| Normal diet + hesperidin | 20 | 190 | 198 | 20 | 206 | 180 |
| Vitamin C-deficient | 24 | 206 | 220 | 23 | 218 | 174 |
| Vitamin C-deficient + hesperidin | 22 | 184 | 198 | 19 | 204 | 168 |
| Vitamin C-deficient + rutin | 20 | 197 | 188 | 22 | 216 | 157 |

* Data in TABLES 4, 5, and 6 are from studies on those guinea pigs given the "semisynthetic" basal diet (Reid and Briggs, 1953). Findings in approximately equal numbers of control animals fed laboratory chow alone or supplemented with rutin or hesperidin are comparable.

vitamin C deficiency is not associated with an increase in the bleeding time, and the addition of hesperidin or rutin to normal diets or to vitamin C-deficient diets did not alter the findings in any way.

TABLE 6 illustrates the clotting tendency in guinea pigs on various types of diets. Blood clotting as induced by the microelectrode method was influenced

neither by vitamin C deficiency nor by the addition of rutin or hesperidin to either a normal or ascorbic acid-deficient diet.

TABLE 7 lists the data with regard to the effect of direct trauma (as applied with a fine camel's hair brush) to the capillary vessels of guinea pigs. The increase in hemorrhages so induced in vitamin C deficiency is apparent. This occurs in all vessel classes; as in the previous studies on spontaneous and induced petechiae, however, these ruptures are almost entirely in the venular side of the capillary bed. Rutin and hesperidin, added either to a complete or

TABLE 6
CLOTTING TENDENCY IN GUINEA PIGS*

| Dietary treatment | No. of animals | Arterioles | | Capillaries | Venules | |
|----------------------------------|----------------|-------------|----------|-------------|-------------|----------|
| | | 20-50 μ | 20 μ | | 20-50 μ | 20 μ |
| Normal diet | 20 | 55 | 48 | 0 | 60 | 35 |
| Normal diet + rutin | 30 | 62 | 60 | 0 | 54 | 44 |
| Normal diet + hesperidin | 28 | 58 | 66 | 0 | 72 | 36 |
| Vitamin C-deficient | 32 | 48 | 56 | 0 | 76 | 26 |
| Vitamin C-deficient + hesperidin | 24 | 54 | 62 | 0 | 54 | 41 |
| Vitamin C-deficient + rutin | 24 | 42 | 51 | 0 | 41 | 52 |

* Measured in volts \times milliseconds.

TABLE 7
EFFECT OF DIRECT TRAUMA TO CAPILLARY BED OF GUINEA PIGS*

| Dietary treatment | No. of animals | Arterioles | | Capillaries | Venules | |
|----------------------------------|----------------|-------------|----------|-------------|-------------|----------|
| | | 20-50 μ | 20 μ | | 20-50 μ | 20 μ |
| Normal diet | 42 | 0 | 0 | 0-2 | 0-2 | 0-2 |
| Normal diet + rutin | 29 | 0 | 0 | 0-4 | 0-3 | 0-2 |
| Normal diet + hesperidin | 40 | 0 | 0 | 0-3 | 0-2 | 0-4 |
| Vitamin C-deficient | 34 | 0 | 1-8 | 2-5 | 3-18 | 5-12 |
| Vitamin C-deficient + hesperidin | 28 | 0 | 1-9 | 2-6 | 4-16 | 7-11 |
| Vitamin C-deficient + rutin | 28 | 0 | 0-8 | 2-4 | 4-20 | 6-11 |

* Petechiae per low power field.

an ascorbic acid-deficient diet, did not reduce the number or the size of the petechiae.

Discussion

The foregoing data illustrates that vitamin C's role in the peripheral vascular system is one of a dynamic nature, with maintenance of peripheral vascular tone and reactivity a prime feature. The poor responsiveness to epinephrine stimulation and the moderate vasodilatation accompanying vitamin C deficiency are probably related directly to the impairment encountered in the ability of the animal to withstand hemorrhage. As blood is lost in the normal animal, the peripheral vascular system "shrinks down" by vasoconstriction, maintaining an elevated blood pressure in spite of reduced volume of circulating fluid. The reduced ability to constrict in response to stimulation can be

considered a prime defect in the peripheral vascular system's role in responding to a stress situation.

The perivenular location of petechiae associated with vitamin C deficiency is a matter of interest. In this portion of the peripheral vascular bed, the smooth muscle cells about the vessel are discontinuous with relatively large areas of vessel wall consisting only of endothelium, ground substance, and collagen fibrils. With reduced tone in the smooth muscle cell, it is conceivable that the entire venular wall is weakened, the most likely site of rupture being the intermuscular cell regions; this has not yet been examined by appropriate cytological and histological methods. The dilated venule in vitamin C deficiency is probably not an exception to the concept that the dilated vessel is particularly liable to rupture solely from the distended state rather than from any mechanical structural defect in the mural elements.

The two flavonoid materials, hesperidin and rutin, examined thus far with these techniques were completely incapable of even partially relieving the disturbed physiology of avitaminosis C. The status of ascorbic acid is therefore unique in maintaining those features of the capillary bed and its component parts that have been examined with the methods outlined. With regard both to the rats and the guinea pigs, neither genus developed any symptoms on the synthetic diets that could either be explained or relieved by these two flavonoid materials. Although both hesperidin and rutin may exert certain physiological influences on the animal, it seems clear from this study that they are not essential nutrients.

Summary and Conclusions

With synthetic diets and the use of micromanipulative techniques previously developed, the role of vitamin C in peripheral vascular physiology has been examined. This substance has been found necessary to maintain responsiveness to stimulation with epinephrine, to maintain the animal's total vasocompensatory responsiveness and resistance to vascular stress, such as that produced by hemorrhage, and to maintain intact the tonic state of the venules. Hemorrhages occurring in vitamin C deficiency are primarily perivenular. With the methods used, neither rutin nor hesperidin was found to correct the vascular abnormalities of vitamin C deficiency to any degree whatever. The functional pathology scurvy is relieved completely by ascorbic acid feedings but by neither of the two flavonoids mentioned. It is clear from these studies that the peripheral vascular role of ascorbic acid is primarily that of a "tonic," its absence featured by disturbed function of a widespread symptomatology of great magnitude.

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