# Ascorbic Acid and the Hyaluronidase Hyaluronic Acid Reaction. (18764)

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The histologic studies of Wolbach and Howe(1) showed the antiscorbutic value of vitamin C in scurvy to be due to its effect upon the formation of intercellular cement substance, and it has been generally assumed that a similar action explains its effects in cases of increased capillary fragility corrected by vitamin C. However, the recent work of Hines and Parker(2) showed that large doses of vitamin C given intravenously could correct increased capillary fragility in some patients within a period of 10-30 minutes, indicates an additional action of the vitamin. The rapidity of this action suggests a direct chemical or enzymatic response and since the spreading factor, hyaluronidase, has been shown to increase capillary permeability(3), the present studies were carried out to investigate possible relationship of ascorbic acid to the hyaluronidase-hyaluronic acid system. Three methods were used for the study:

*Experiment 1.* Experiments were first made to study the *in vitro* effect of vit. C on the hyaluronidase-hyaluronic acid reaction. For this purpose an impure solution of hyaluronic acid was obtained by filtering fresh vitreous humor from bovine eyes. One cc of this solution was mixed with an equal volume of serum and the amount of hyaluronidase* which would prevent precipitation of the hyaluronic acid by dilute acetic acid was determined(4), (the reaction was carried out at 37°C for 20 minutes with pH adjusted to 7.5). This amount of hyaluronidase was then mixed with the substrate which consisted of a serum mixture containing varying dilutions of buffered vit. C. (Simultaneous controls were run using normal saline in place of vit. C). The effects on turbidity were measured in a photolometer.

*Experiment 2.* A standard quantity of hyaluronidase colored with phyloxine dye, and brought to constant volume (0.5 cc) with isotonic sodium chloride was injected into the skin of the shaved abdomen of a rabbit and the amount of spread at the end of 20 minutes measured. The area of spread was calculated according to the formula for the area of an ellipse ($A = \frac{Dxd}{4}$, where D= greatest diameter of spread and d= smallest diameter of spread)(5). Having determined the area of spread of the control injections of hyaluronidase, the same quantity of enzyme was then injected with varying amounts of ascorbic acid while a constant volume of each injection was maintained by varying amounts of sodium chloride. The area of spread in 20 minutes was calculated by the above formula.

*Experiment 3.* In this set of experiments, the area of spread of the control solution of hyaluronidase was measured before, and at 10-minute intervals after, the intravenous injection of 500 mg of vit. C. Blood vit. C levels were also determined at varying intervals.

*Results.* The *in vitro* tests showed that slight inhibition of the hyaluronidase action was produced by ascorbic acid. The greatest

TABLE I. Effect of Mixtures of Ascorbic Acid and Hyaluronidase on Area of Spread.*

| | D | d | Avg area of spread |
|---|---|---|---|
| Control tests | 3.18 cm | 2.36 cm | 5.82 cm² |
| Tests with ascorbic acid | 1.25 | .85 | .79 |

* Three rabbits were each tested 3 times in the absence of ascorbic acid and 5 times with ascorbic acid.

1. Wolbach, S. B., and Howe, P. R., *Arch. Path. and Lab. Med.*, 1926, v1, 1.

2. Hines, L. E., and Parker R. J., *Quart. Bull., Northwestern Univ. Med. School*, 1949, v23, 424.

3. Duran-Reynals, F., *Yale J. Biol. and Med.*, 1939, v11, 601.

* The hyaluronidase used in this study was obtained through the courtesy of Wyeth, Inc.

4. McClean, *Biochem. J.*, 1943, v37, 169.

5. McClean, *Biochem. J.*, 1941, v35, 159.

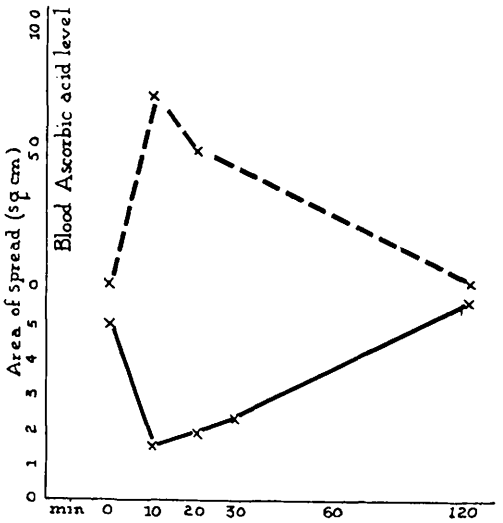


FIG. 1. Effect of intravenous ascorbic acid on capillary permeability "hyaluronidase spread."

inhibition was noted with 0.01 mg of ascorbic acid, but such inhibition was only 7% complete.

The local inhibitory effect of ascorbic acid on "hyaluronidase spread" in the rabbits' skin was much more marked. The area of spread of the control injections averaged† 5.82 sq. cm, but when ascorbic acid in amounts varying from 5 to 50 mg was added the area of spread averaged only 0.79 sq. cm (Table I).

The inhibitory effect of ascorbic acid on "hyaluronidase spread" was also quite marked when it was given intravenously to the animal. The average area of spread of hyaluronidase before injection of ascorbic acid was 5.43 sq. cm; following the injection of ascorbic acid the average area of spread was reduced to a low of 1.51, 10 minutes after the injection. This effect and the ascorbic acid blood levels are shown in Fig. 1.

*Discussion.* The structure of the capillary wall has been discussed by Chambers and Zweifach(4), who showed that there are 3 important components. The capillary endothelium is apparently not a factor in capillary permeability. However, the endothelial cells probably do secrete the intercellular cement, and alterations in this cement will result in permeability changes. The second important component of the capillary is the endocapillary layer, which is considered to be a coating of the endothelial cells, formed by absorption of blood proteins. Changes in the endocapillary layer may also result in increased capillary permeability. A third component of the capillary is the pericapillary sheath, composed of connective tissue, which surrounds the capillary vessels.

It is apparent that alteration in any one component of the capillary wall could result in increased permeability. The location of the defect corrected by ascorbic acid has not been determined. Extensive studies by Wolbach(1,7,8) have shown that in the absence of ascorbic acid normal cement substance is not produced. This has been confirmed by Dalldorf(9) who showed that scorbutic animals, when treated by vit. C, will produce normal cement substance in approximately 18-24 hours. However, in the capillaries, no morphologic changes have been seen, and whether the deficiency in capillary permeability is in the cement substance or in the pericapillary sheath has not been determined.

The exact composition of the cement substance is also not known and it is unknown whether or not it contains hyaluronic acid. Chambers and Zweifach(6) claim that hyaluronidase has no effect upon the cement substance and this indicates that it probably is not hyaluronic acid. Meyer(10), also contends that hyaluronic acid is not present in the cement substance. The pericapillary sheath, however, is composed of connective tissue and as such contains hyaluronic acid. It is probably this component of the capillary wall which is broken down when hyaluronidase is added, and causes increased permeability. It would seem, therefore, from the present studies, that the rapid action of ascorbic acid in some cases of capillary fragility is due to

† In this and the following set of experiments, 3 rabbits with 8-10 injections each were used and the results averaged.

7. Wolbach, S. B., *Am. J. Path.*, 1933, v9, Supplement No. 689.

8. Menkin, V., Wolbach, S. B., and Menkin, M. F., *Am. J. Path.*, 1934, v10, 569.

9. Dalldorf, G., *J. A. M. A.*, 1938, v114, 1376.

6. Chambers, R. and Zweifach, B. W., *Physiol. Reviews*, 1947, v27, 436.

10. Meyer, K., *Physiol. Rev.*, 1947, v27, 335.

its effect upon the hyaluronidase-hyaluronic acid reaction in the pericapillary sheath rather than to an effect upon the intercellular cement substance. This is suggested because the action is rapid and does not take place in the 18- to 24-hour period characteristic of action on the cement substance. Ascorbic acid either inhibits hyaluronidase directly, or it alters the hyaluronic acid in the sheath so as to make it more resistant to hyaluronidase.

*Summary.* Both *in vitro* and *in vivo* experiments show that ascorbic acid exerts an inhibitory effect upon the hyaluronidase-hyaluronic acid reaction. The inhibition is more marked in the animal than in the test tube.

The components of the capillary wall and their relation to capillary permeability have been discussed.

These studies suggest that in some cases where capillary fragility is corrected by ascorbic acid, the effect could be due to the inhibitory effect of ascorbic acid upon a hyaluronidase-hyaluronic acid system, rather than to an effect of vit. C on the intercellular cement.

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## Some Hormones Involved in Elaboration or Activation of the Mammary Spreading Factor.* (18765)

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In a previous paper(1) observations were presented indicating the presence of a spreading factor in the mammary glands of pregnant albino rats. This factor, which increased in potency during the first half of pregnancy, is thought to make possible the rapid advance of the mammary gland into the subcutaneous tissue. The close relation of mammary gland growth to the amount of active spreading factor suggested that the hormones regulating mammary gland growth might also regulate elaboration or activation of the spreading factor.

Hormones involved in mammary gland growth have been adequately reviewed by Turner(2), Riddle(3), Folley and Mallpress (4), and Trentin and Turner(5), so no attempt at review will be made.

*Methods and materials.* Castrate male and female albino rats weighing from 150 to 250 g were employed. If only castrate animals were to be used treatment was started on the eleventh day postoperatively, however, some castrate-adrenalectomized rats were used. In these cases the castrate animals were adrenalectomized on the eleventh day and treatment was started about 5 days later. The castrate-adrenalectomized animals were maintained on 1% sodium chloride drinking water. The estradiol benzoate and progesterone used in the treatment were dissolved in olive oil, the testosterone was dissolved in 60% alcohol, while the anterior pituitary extract† was made up as a suspension in distilled water. The treatment involved subcutaneous injections daily for 10 days. On the eleventh day the animals were killed, the mammary glands re-

* Contribution from the Department of Dairy Husbandry, Mo. Agric. Exp. Station, Journal Series No. 1257 Approved by Director of Missouri Agric. Exp. Station.

1. Elliott, J. R. and Turner, C W., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 384.

2. Turner, C. W., *The Mammary Glands.* In Allen, E., *Sex and Internal Secretions,* 1939, The Williams and Wilkins Co., Baltimore.

3. Riddle, O., *Ann. Rev. Physiol.,* 1941, v3, 573.

4. Folley, S. J. and Mallpress, F. H., Chap. IV in Pincus and Thimann. *The Hormones,* vI, 1948, Academic Press, Inc., New York.

5. Trentin, J. J., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.* 418, 1948.

† The preparation employed was an ant. pituitary extr. rich in lactogenic hormone. This extract was generously supplied by United Research Laboratories, Philadelphia, Pa.