

VITAMIN C AND WOUND HEALING*

II. Ascorbic Acid Content and Tensile Strength of Healing Wounds in Human Beings

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IN recent years, a considerable amount of evidence has established the efficacy of vitamin C in wound healing in animals. Lanman and Ingalls¹ first made direct measurements of the tensile strength of healing wounds in guinea pigs and found it decreased in the presence of scurvy. In the preceding paper,² we reported the results

a high ascorbic acid content was found to be much greater than that in those with low vitamin C values.

Elaborating on the work of Lanman and Ingalls, Taffel and Harvey³ studied the effect of partial as well as absolute vitamin C deficiency on the tensile strength of healing wounds in guinea

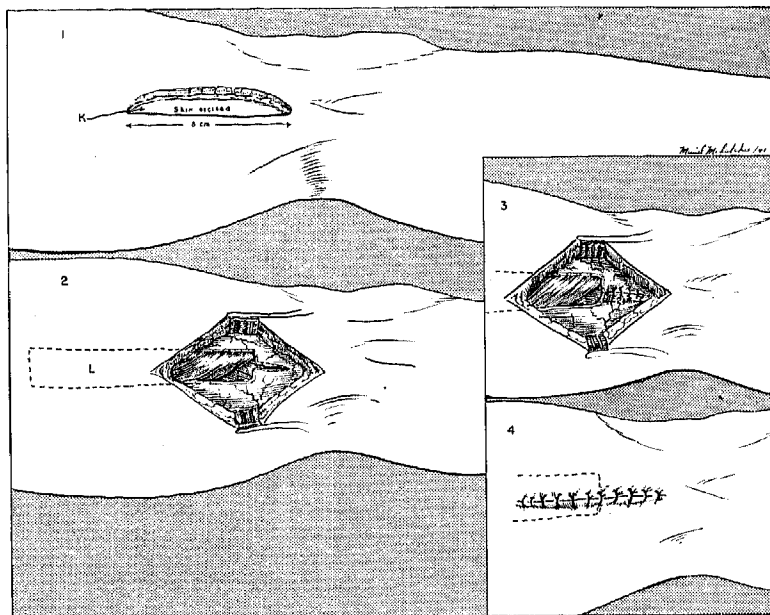


FIGURE 1. Method of Obtaining Tissue for Ascorbic Acid Assay and Tensile-Strength Determinations.

1—incision through skin and subcutaneous tissue with excision of a strip of skin for ascorbic acid assay (K); 2—fascia stripped out in the usual manner for repair of the hernia, and for ascorbic acid assay (L), with an incision made in the fascia lata for 2 cm. distal to the area stripped out; 3—incision in fascia closed with fine silk stitches; 4—skin closed in the usual manner.

of studies on the tissue ascorbic acid content of similar wounds in guinea pigs and correlated this with the tensile strength of these wounds. A much higher concentration of vitamin C occurs in the wounds of animals on a high ascorbic acid intake than in those of animals on a scorbutic diet, and the tensile strength of wounds showing

pigs. There was a slightly greater tensile strength at four days after operation in animals with absolute scurvy than in the normal controls. At six days, the tensile strength was markedly inferior to that in normal animals. None of the scorbutic animals survived for a longer postoperative period. In their experiments, a state of partial scurvy was established as follows: after a week on the scorbutic diet supplemented by 5 mg. of ascorbic acid daily, all vitamin C was withheld for two weeks, and then 0.2 mg. of ascorbic acid was given on alter-

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nate days. Abdominal incisions were made after ten days on this low vitamin C intake. It is extremely interesting that, although all the animals showed unmistakable gross evidence of scurvy at autopsy, tests for tensile strength on the healing wounds showed a significant decrease below that of normal controls only on the eighth and tenth days after operation. Tests on the fourth and sixth days showed no appreciable variation from the controls, and those on the twelfth and fourteenth days again approached normal.

The effect of prolonged reduced intake of vitamin C on wound healing in an otherwise normal adult human subject has been studied by Crandon, Lund and Dill⁴ and by Hunt.⁵ In both studies, the histologic appearance of the wound

the same patient, followed by the administration of large doses of ascorbic acid, showed no macroscopic or microscopic difference from the first wound.

The present studies were undertaken in an attempt to obtain further information regarding the value of vitamin C in wound healing in human beings, by means of direct observations on the tensile strength of healing wounds and a correlation of these with the tissue content of ascorbic acid and plasma ascorbic acid levels.

METHODS

Male patients admitted to the hospital for the repair of inguinal hernias who were good surgical risks and on whom the use of fascia lata in

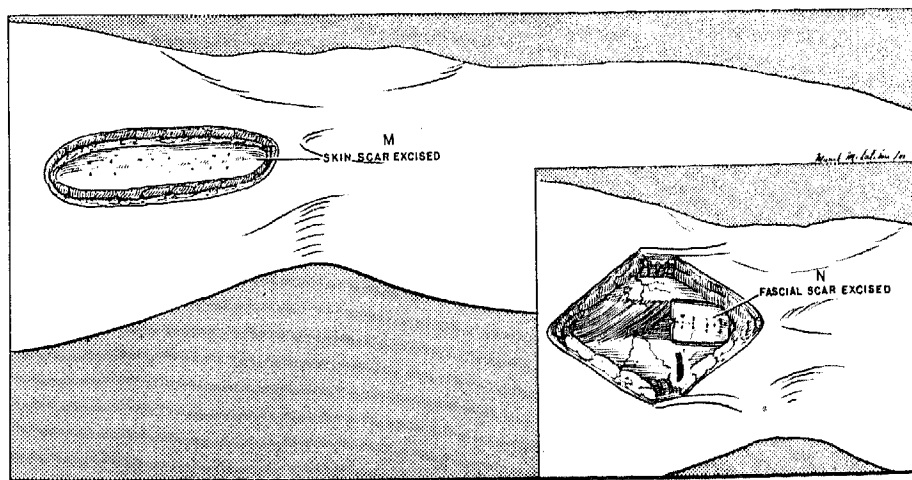


FIGURE 2. Method of Subsequently Obtaining Tissue for Ascorbic Acid Assay and Tensile-Strength Determinations.

The skin scar is excised (M), exposing the healing fascial scar (N), which is also excised. The skin is then closed in the usual manner.

was used as an index of abnormal healing. Crandon, Lund and Dill found that adequate wound healing occurred after the plasma ascorbic acid had been zero for forty-four days and when the white-cell and platelet ascorbic acid level was 4 mg. per 100 cc. A second wound, made when the plasma ascorbic acid had been zero for one hundred and forty-one days and the white-cell and platelet ascorbic acid content had been at zero for sixty-one days, failed to heal in a normal manner after ten days, and showed lack of intercellular substance and capillary formation on histologic examination. The intravenous administration of 1000 mg. of ascorbic acid daily for ten days brought about microscopic evidence of good healing. Hunt⁵ reported histologic evidence of healing of an experimental wound in a subject who had been on a reduced vitamin C intake for three months and who had a blood ascorbic acid content of 0.34 mg. per 100 cc. A second experimental wound in

the repair was contemplated were selected for these studies.

During the preliminary period of two to four days, the plasma ascorbic acid was determined by the method of Mindlin and Butler,⁶ and the daily urinary output measured by Bessey's⁷ method. Throughout the hospital stay, these patients were maintained on a diet containing 100 mg. of vitamin C daily. Supplementary ascorbic acid was given to some patients before and after operation, as described in the case reports.

Studies were carried out on a total of 6 patients. Of these, 5 had unilateral hernias, and 1 had bilateral hernias.

The operations were performed under spinal anesthesia, and the usual hernia repair was done. Fascia lata was obtained through a longitudinal skin incision about 8 cm. in length just above the knee on the lateral aspect of the thigh. A strip of skin, weighing about 2 gm., was excised

from the edge of this wound for ascorbic acid assay as a control biopsy. The fascia was removed by means of a stripper, and in addition to that necessary for the hernia repair, enough was removed for ascorbic acid assay. The tissue ascorbic acid content was determined on the ex-

skin was available to allow duplicate determinations. The tensile strength was measured by the application of a direct pull on the healing scar, as illustrated in Figure 3. The apparatus consisted of a 5-cc. glass syringe, with collars mounted on the barrel and plunger (Aa and Bb). On each collar a bar is attached, pivoted at its center (A and B). Two pins (D), spaced 1 cm. apart, are mounted on each bar, and the tissue to be tested (M or N) is placed on these four pins. Tension is applied by means of weights placed on the platform.

As a result of duplicate determinations on portions of the same skin scars, it became evident that there was considerable variation by this method, and we believe that only gross differences should be considered significant.

CASE REPORTS

CASE 1. A. A., a 65-year-old man, was admitted for repair of a right inguinal hernia. The plasma ascorbic acid was 0.60 and 0.66 mg. per 100 cc. on two occasions be-

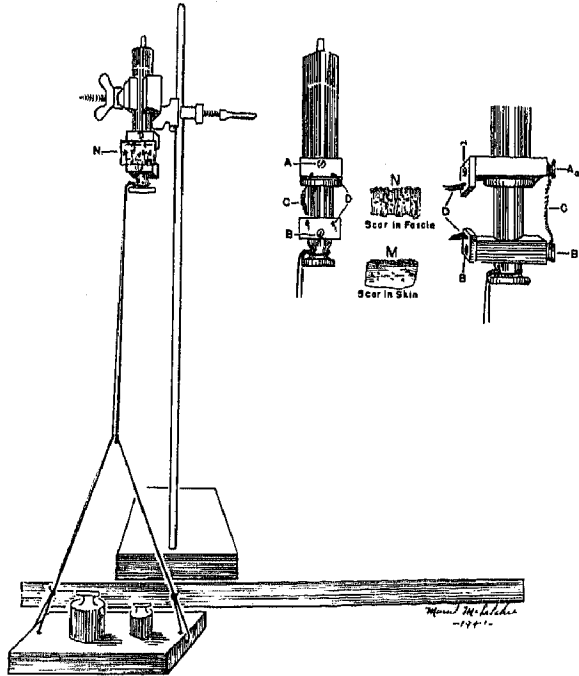


FIGURE 3. Method for Determining the Tensile Strength of Healing Scars in Skin and Fascia.

The apparatus consists of a 5-cc. glass syringe, with collars mounted on the barrel and plunger (Aa and Bb). On each collar a bar is attached, pivoted at its center (A and B). Two pins (D), spaced 1 cm. apart, are mounted on each bar, and the tissue to be tested (M or N) is placed on these four pins. Tension is applied by weights on the platform. The pull necessary to rupture the scar is recorded in grams.

cised skin and fascia by the method described by Bessey⁷ and previously employed in our animal experiments. Distal to the area of excised fascia, a longitudinal incision 2 cm. in length was made in the fascia lata, parallel to its fibers, and this was closed with interrupted sutures of fine silk. The skin was then closed in the usual manner with interrupted silk (Fig. 1).

The wound was allowed to heal for ten days, the skin sutures being removed on the eighth day. At the end of ten days, under novocain block anesthesia, the skin incision in the thigh was excised with a margin of slightly less than 1 cm. of skin on each side of the scar; the incision in the fascia was excised with the same margin of tissue (Fig. 2).

The tensile strength of the healing wound and the tissue ascorbic acid content were determined on the skin and fascia. In some cases, enough

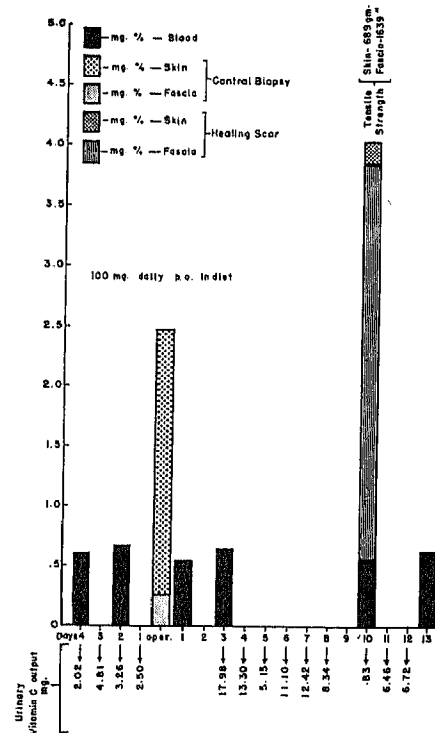


FIGURE 4. Case 1.

Determinations of ascorbic acid in the blood plasma, in control biopsies of skin and fascia and in healing skin and fascial scars are shown. Urinary excretion of vitamin C is recorded in twenty-four-hour amounts, and the tensile strengths of the skin and fascial scars are listed. Where columns are superimposed, the actual amount of each component is the maximum height of the column above the abscissa.

fore operation, and no significant variation from this level occurred following operation.

Control biopsies of skin and fascia from the left leg were obtained at the time of herniorrhaphy. The skin

showed an ascorbic acid content of 2.49 mg., and the fascia one of 0.29 mg. per 100 gm. At the end of 10 days, the skin and fascia scars were excised. The skin showed a tensile strength of 700 gm. and a vitamin C content of 4.04 mg. per 100 gm. The fascia scar broke at a tension of 1600 gm. and contained 3.88 mg. of ascorbic acid per 100 gm. The complete data on this patient appear in Figure 4.

CASE 2. E. J. L., a 36-year-old man, was admitted for repair of a left inguinal hernia. The preoperative plasma ascorbic acid was 0.20 mg. per 100 cc. on two occasions. Control biopsies of skin and fascia lata showed a vitamin C

After healing for 10 days, the skin and fascia scars were excised from the leg. The skin scar had a tensile strength of 1500 gm. and an ascorbic acid content of 5.23 mg. per 100 gm. The healing fascia broke under a tension of 1200 gm., with a vitamin C tissue level of 7.65 mg. per 100 gm. The complete data on this patient appear in Figure 6.

CASE 4. F. W., a 62-year-old man, proved to be the most interesting patient studied. He had bilateral inguinal hernias, which were repaired separately. He also had the lowest preoperative plasma ascorbic acid level in the group—0.09 and 0.08 mg. per 100 cc. on two determinations. There was no clinical evidence of scurvy.

At the first operation, the right hernia was repaired, and control biopsies of skin and fascia were obtained from the left leg. These tissues both showed a tissue ascorbic acid level of zero. No ascorbic acid, other than the 100 mg. daily in the diet, was given after operation, and the plasma ascorbic acid remained at the same low level.

At the end of 10 days, the left hernia was repaired, fascia from the right leg being used, and control specimens of skin and fascia were obtained from the new incision. They also showed a zero level of ascorbic acid. At the same

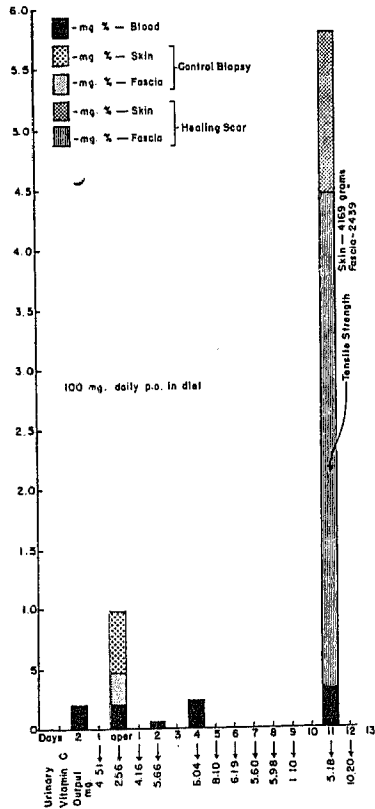


FIGURE 5. Case 2.

content of 0.99 mg. and 0.48 mg. per 100 gm., respectively. After healing for 10 days, the skin scar had a tensile strength of 4200 gm. and an ascorbic acid content of 5.82 mg. per 100 gm. The fascia scar ruptured at 1400 gm. with a vitamin C content of 4.48 mg. per 100 gm.

In this patient, except for a transient drop after operation, the plasma ascorbic acid showed no significant variation during the postoperative interval. This transient fall has been previously noted by various workers. The studies on this patient are shown in Figure 5.

CASE 3. J. S., a 24-year-old man, entered the hospital because of a left inguinal hernia. Two preoperative plasma ascorbic acid determinations showed 0.31 and 0.26 mg. per 100 cc. Control biopsies of the skin and fascia lata were taken. The skin contained 0.84 mg. and the fascia 0.11 mg. of ascorbic acid per 100 gm. From the day of operation, 1000 mg. of vitamin C was given daily, in addition to the 100 mg. contained in the regular diet. The plasma ascorbic acid level rose promptly and remained between 0.68 and 1.01 mg. per 100 cc. during the postoperative interval. A corresponding rise in urinary output of vitamin C occurred.

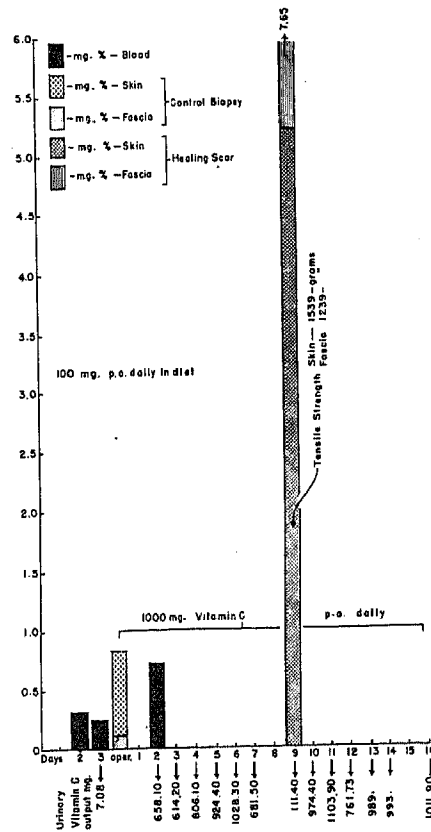


FIGURE 6. Case 3.

time, the 10-day-old scars in the skin and fascia of the left leg were excised. The skin scar had a vitamin C level of 0.67 mg. per 100 gm. and a tensile strength of 400 gm. (average of two determinations). The healing fascia had a tissue level of ascorbic acid of zero and ruptured at a tension of 300 gm.

The patient was then given 1000 mg. of ascorbic acid by mouth daily, in addition to that in the regular diet, and the blood ascorbic acid promptly rose to above 1.0 mg. per 100 cc. and remained at this level, with a corresponding increase in the urinary output of ascorbic acid.

Ten days after the second operation, the healing skin and fascia scars were excised from the right leg. An ascorbic acid assay on the skin gave a result of 6.77 mg.

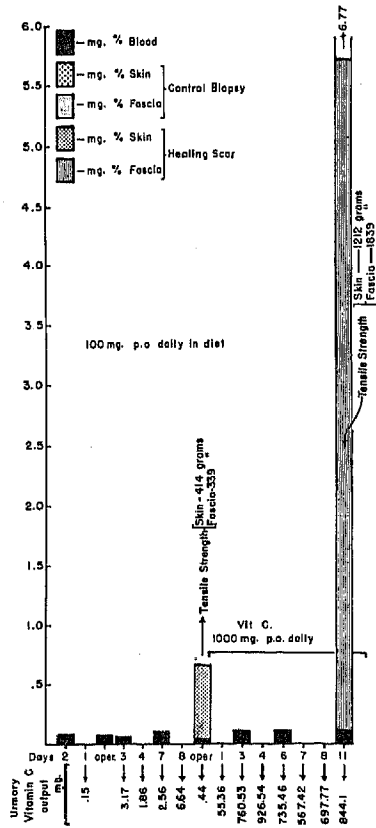


FIGURE 7. Case 4.

Tissue assay of vitamin C on the control biopsies of the skin and fascia at both operations and of the healing fascia excised at the second operation were all zero, and so do not appear.

per 100 gm., and the scar separated with a pull of 1200 gm. (average of three determinations). The fascia contained 5.73 mg. of vitamin C per 100 gm., and the scar had a tensile strength of 1800 gm. Figure 7 shows the results of these studies in graphic form.

CASE 5. A. D., a 26-year-old man, was recommended for admission to the hospital because of a left inguinal hernia. The plasma ascorbic acid was found to be 0.26 mg. per 100 cc., and he was given 200 mg. of vitamin C daily for 2 weeks prior to admission. After a week, the plasma ascorbic acid was 0.98 mg. per 100 cc., and on admission it was 0.81 mg. The patient was given the same dose (200 mg.) of ascorbic acid daily in addition to 100 mg. in the diet, and on the day of operation the plasma ascorbic acid was 0.87 mg. per 100 cc. The hernia was repaired, and skin and fascia were obtained from the right leg for vitamin C assay. The skin contained 0.78 mg. and the fascia 0.17 mg. of ascorbic acid per 100 gm.

Except for the day of operation, when no ascorbic acid was given, the daily intake was maintained at a total of 300 mg. of vitamin C daily. Ten days after operation, the healing skin and fascia scars were excised from the leg. In addition, a small pigmented nevus was removed

from the back, together with a margin of surrounding normal skin.

The healing skin showed 5.54 mg. of ascorbic acid per 100 gm., and the scar ruptured with a pull of 600 gm. (average of three determinations). The fascia had a vitamin C level of 7.24 mg. per 100 gm. and a tensile strength of 1100 gm. The pigmented nevus was discarded, and the surrounding skin was assayed for vitamin C. A level of 1.88 mg. per 100 gm. was obtained. These studies appear in Figure 8.

CASE 6. T. D., a 48-year-old man, was admitted to the hospital because of a large left inguinal hernia. A plasma ascorbic acid determination 2 weeks before admission showed a level of 0.73 mg. per 100 cc. The patient was given 600 mg. of vitamin C daily, and this was continued, together with the 100 mg. in the regular diet, until 10 days after operation, except for the day of operation, when he received none. The plasma ascorbic acid was 1.26 mg. per 100 cc. on admission, and except for a transient

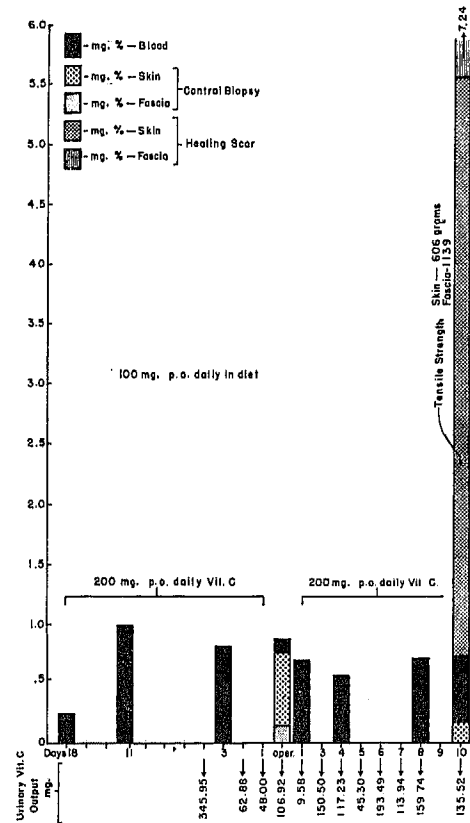


FIGURE 8. Case 5.

drop after operation, it remained above 1.0 mg. per 100 cc. until the supplementary dose of vitamin C was withdrawn.

At operation, the hernia was repaired with fascia from the right thigh, and control biopsies of the skin and fascia lata were obtained. The skin contained 1.63 mg. and the fascia 0.20 mg. of ascorbic acid per 100 gm.

After 10 days, the healing skin and fascia scars were excised from the leg. A pigmented nevus on the same leg, but at some distance from the healing area, was also excised, with a margin of normal skin. The healing skin

corbic acid level remained unchanged, and at the end of this time the excised fascia scar still showed an ascorbic acid value of zero and a tensile strength of only 300 gm., or about one fifth of normal (1600 gm.). The control biopsy of fascia from the second leg wound also showed a zero value for ascorbic acid. This wound was allowed to heal for ten days, 1000 mg. of ascorbic acid being given orally each day in addition to the regular diet. The plasma ascorbic acid promptly rose to over 1.0 mg. per 100 cc. At the end of ten days, the healing fascia scar was excised. Its ascorbic acid content was 5.73 mg. per 100 gm., which is slightly lower than the average of 6.01 mg. per 100 gm. noted in the other cases. The tensile strength of this fascia scar was 1800 gm., a figure slightly higher than the average of 1600 gm., for the other cases, and six times as high as that (300 gm.) for the scar in the wound in the other leg, which healed without benefit of vitamin C therapy.

The results of the studies of vitamin C content and tensile strength of skin healing appear in Table 2. These figures followed essentially the

TABLE 2. *Studies on Healing of Human Skin.*

CASE NO.	PRE- OPERATIVE PLASMA ASCORBIC ACID	CONTROL BIOPSY TISSUE ASCORBIC ACID	TEN-DAY-OLD SCAR TISSUE	
	mg./100 cc.	mg./100 gm.	ASCOR- BIG ACID	TENSILE STRENGTH gm.
1	0.63	2.49	4.04	700
2	0.20	0.99	5.82	4200
3	0.29	0.84	5.23	1500
5	0.84	0.78	5.54	600
6	1.19	1.63	4.64	1600
Average	0.63	1.35	5.05	1700
4 (first operation)....	0.09	0.0	0.67	400
(second operation)...	0.07	0.0	6.77	1200

same pattern as those on the healing fascial scars, although there was much more variation in the tensile-strength determinations.

If we exclude Case 4 and accept the averages of the other 5 cases as normal values, we find an ascorbic acid content of the control skin biopsy of 1.35 mg. per 100 gm., which rose to 5.05 mg. in the ten-day-old scar. The average tensile strength of these scars was 1700 gm.

Case 4 showed an ascorbic acid level of zero in the control specimen of skin, which rose only to 0.67 mg. per 100 gm. after ten days of healing without vitamin C therapy other than the 100 mg. daily in the diet. The tensile strength of this scar was 400 gm., one quarter of the normal value. The second leg wound showed no vitamin C in the control skin biopsy. After ten days of intensive vitamin C therapy, the scar had an ascorbic acid content of 6.77 mg. per 100 gm., which is

somewhat higher than the normal (5.05 mg.). Its tensile strength was 1200 gm., as against 400 gm. for the untreated scar in the same patient, and 1700 gm. for the average of the other cases.

We believe that Case 4 demonstrates conclusively two important points: that a sufficient depletion of vitamin C, reflected in a very low plasma ascorbic acid, interferes with normal wound healing, as measured by tissue ascorbic acid content and tensile strength; and that, in spite of a very low plasma ascorbic acid at the time of operation, normal wound healing can be brought about by adequate vitamin C therapy during the healing period.

There seems to be no evidence from these studies that a plasma ascorbic acid level of 0.20 mg. per 100 cc. indicates a sufficient depletion of vitamin C to interfere with normal wound healing, and this suggests that the generally accepted concept that plasma ascorbic acid levels below 0.50 mg. per 100 cc. indicate significant vitamin C depletion must be revised downward.

It was found in our work on guinea pigs that, with an adequate vitamin C intake, not only the tissues of the healing wound show a higher level of ascorbic acid than the control biopsy, but a similar though smaller rise is found in the abdominal wall at some distance from the wound. In 2 patients (Cases 5 and 6), specimens of skin were obtained at a distance from the healing wound at the time that the wound biopsy was done. As shown in Figures 8 and 9, one of these specimens showed a level of 1.88 mg. per 100 gm. in contrast to an original value of 0.78 mg. in the control biopsy, whereas the other showed a decrease from 1.63 mg. in the control biopsy to 1.10 mg. per 100 gm., in the normal skin excised ten days later.

CONCLUSIONS

A sufficient depletion of vitamin C produces a decreased ascorbic acid content and tensile strength in healing wounds in the skin and fascia of human beings.

A fasting plasma ascorbic acid level below 0.20 mg. per 100 cc. must be reached before these changes appear.

In the presence of adequate vitamin C, the magnitude of the rise in ascorbic acid content in healing fascial scars, compared with that of control biopsies, is much greater than that shown by healing skin scars.

In spite of a low plasma ascorbic acid level at the time of operation, normal wound healing may be produced by adequate vitamin C therapy during the postoperative period.

REFERENCES

1. Lanman, T. H., and Ingalls, T. H. Vitamin C deficiency and wound healing: experimental and clinical study. *Ann. Surg.* 105:616-625, 1937.
2. Bartlett, M. K., Jones, C. M., and Ryan, A. E. Vitamin C and wound healing. 1. Experimental wounds in guinea pigs. *New Eng. J. Med.* 226:469-473, 1942.
3. Taffel, M., and Harvey, S. C. Effect of absolute and partial vitamin C deficiency on healing of wounds. *Proc. Soc. Exper. Biol. & Med.* 38:518-525, 1938.
4. Crandon, J. H., Lund, C. C., and Dill, D. B. Experimental human scurvy. *New Eng. J. Med.* 223:353-369, 1940.
5. Hunt, A. H. Role of vitamin C in wound healing. *Brit. J. Surg.* 28:436-461, 1941.
6. Mindlin, R. L., and Butler, A. M. Determination of ascorbic acid in plasma: a macromethod and micromethod. *J. Biol. Chem.* 122:673-686, 1938.
7. Bessey, O. A. Method for the determination of small quantities of ascorbic acid and dehydroascorbic acid in turbid and colored solutions in the presence of other reducing substances. *J. Biol. Chem.* 126:771-784, 1938.

THE SO-CALLED "COAGULATION DEFECT" IN MENSTRUAL BLOOD*

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METHODS

THE fluidity of menstrual blood and its apparent failure to clot have been the subject of numerous investigations.¹⁻³ Although all observers agree on the existence of the phenomenon, there is little agreement concerning its cause. It has been with equal enthusiasm ascribed to an anticoagulant present in uterine cervical or vaginal secretions, to the removal or absence of one or more of the factors concerned with blood coagulation and to changes in the circulating blood. The existence of this form of incoagulable blood is of interest to the investigators of blood coagulation, since it represents a phenomenon that might possibly be explained in the light of the newer knowledge of the blood-coagulation reaction.

From a consideration of the theory of blood coagulation at present accepted in the United States, it appears that incoagulable blood may result from a deficiency of one or more of the plasma constituents concerned with coagulation,—calcium, prothrombin and fibrinogen,—or in a lack of the clot-promoting activity associated with the euglobulin fraction of plasma, which has been found to be markedly reduced in the blood of patients with hemophilia.⁴ Incoagulability of menstrual blood might also result from the presence of some specific anticoagulant. This communication presents the results of investigations undertaken to determine which of these various factors are concerned with fluidity of menstrual blood.

*Presented in part before the American Federation for Clinical Research, Atlantic City, New Jersey, May 5, 1941.

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, the Department of Medicine, Harvard Medical School, and the New England Hospital for Women and Children.

This study was aided in part by a grant, "In recognition of Dr. Francis W. Peabody's services to the Foundation," from the Ella Sachs Plotz Foundation.

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At the suggestion of Dr. George Van S. Smith, of the Fearing Research Laboratory, Free Hospital for Women, the collection of menstrual blood was made by the insertion of a rubber cup,[¶] which acted as an occlusive pessary, high in the vagina. Collections were usually made for twelve-hour periods, after which the contents of the cups were transferred to blood bottles, examined for the presence of clots, and placed in a refrigerator. For certain purposes, collections were made occasionally for shorter periods. Ten samples of menstrual blood were collected in this manner from 5 healthy women.

The methods for studying the effect of menstrual blood on normal human and hemophilic blood were those previously described from this laboratory. The rabbit-brain thromboplastin used in the investigation was prepared by our modification of the method of Quick.⁵ Thrombin was obtained from prothrombin, prepared by the method of Seegers et al.,⁶ by the action of rabbit-brain thromboplastin.

For control experiments against menstrual blood, two well-known laboratory forms of incoagulable blood were used: citrated and defibrinated blood. In the experimental work reported below, the observations were carried out on the supernatant fluids after centrifuging, at 1500 r.p.m. for ten minutes, samples of citrated, defibrinated and menstrual blood. All observations were made in a constant-temperature water bath at 37.5°C. For the sake of brevity, one typical experiment on each phase of the investigation is given. The results in all were entirely similar.

EXPERIMENTAL RESULTS

The presence of an anticoagulant in menstrual blood was investigated by the placing of 0.1 cc.

¶Commercially marketed under the trade name, Hycup.