THE ROLE OF VITAMIN C IN WOUND HEALING

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THE biological factors of wound healing have received surprisingly little attention from surgeons, although great pains have been taken to give wounds every possible mechanical advantage in the form of expert surgical technique. Migration and proliferation of epidermal and mesodermal cells, the production of an intercellular matrix, and the formation of new blood-vessels are the essence of wound healing. This process, as Carrel has recently remarked, is the most fundamental in surgery, and yet most wounds are left to look after themselves. Clean sutured wounds will heal satisfactorily as a rule, but there are occasions when they inexplicably break down in spite of the most meticulous attention at operation. In these cases there is usually evidence of a nutritional deficiency affecting the proliferating powers of the cells or the capacity of the mesodermal cells to lay down intercellular substances.

Cellular Proliferation.—Cellular proliferation begins after a latent period of a few days[16, 22] and is accompanied by active phagocytosis of tissue which has been damaged or rendered functionless by the incision. The cells, fibroblasts in the case of soft-tissue scars, proliferate and migrate amongst the fibrinous strands which alone complete the sealing of the gap during the preliminary one to four days of wound healing, and they are accompanied in their growth by budding blood-vessels. The severed edges of the wound are thereby joined by a granulation tissue which has formed in response to the stimulus of damaged cells. A normal rate of cellular proliferation is essential to the formation of the scar. It varies with age and is dependent to a considerable extent upon the protein content of the blood. This was first shown by Clark[8] and confirmed by Thompson, Ravdin, and Frank,[40] who demonstrated that there was not only a delay in the appearance of new fibroblasts but also a slowing up of their multiplication in experimental hypoproteinæmia. Complementary experiments were carried out by Harvey and Howes,[17] who showed that the proliferation of fibroblasts could be speeded up by feeding with a high protein diet.

Little is known about the substances which initiate the proliferation and migration of epithelial cells and fibroblasts. Arey,[3] in reviewing the whole problem of wound healing, discusses the various theories and concludes that the most effective stimulants are embryonic juices, substances containing the sulphydril radical, and products of cell disintegration. It is possible to make practical use of these discoveries in the case of open wounds, for example, in the application of amniotic fluid or sulphydril compounds to stimulate epithelialization. But there is no known substance that can be administered internally or parenterally for the purpose of stimulating the proliferation of mesodermal cells in closed wounds. The correction of hypoproteinæmia can only be considered a healing stimulus in that an inhibition has been removed. That common substances may inhibit fibroblastic proliferation has been shown by Heaton[18, 19] and Medawar[31], who found that certain extracts of malt and a number of mammalian organs produce this effect even when taken by mouth.

Deposition of Collagen.—The strength of a scar is dependent on the intercellular substance laid down by the fibroblasts and the full maturation of this substance, precollagen, to collagen. Effective and rapid deposition of collagen is the essential process in scar formation, but it is well to recognize that this process is, in the first place, itself dependent on the proliferation of mesodermal cells. Callus is formed in the same way, the osteoblasts laying down osteoid tissue which matures to bone. In the past it had been supposed that collagen arose either by transformation of the fibrin or from the fibroglia of the cell-protoplasm itself, but it is now more generally accepted that it arises as a condensation of the extracellular material in immediate association with the fibroblasts and their processes. The production of this material and its maturation to collagen can be followed out histologically by a combination of two methods :—

1. In sections impregnated with silver; the intercellular substance laid down around the newly proliferating fibroblasts is at first clearly demonstrable as coarse irregular black fibres. This capacity to take up silver disappears rapidly during the process of maturation, the fibres becoming brown, and then, in about 10 to 14 days from the suture of the wound, a translucent yellow.

2. An affinity for van Gieson's stain develops while the capacity of the fibres to stain with silver is being lost. Thus, by the sixth day, some of the precollagenous fibres, brown to silver impregnation, are pale pink with van Gieson ; by the tenth day they will be almost indistinguishable in colour from mature fibrous tissue.

These reactions of normal healing may be expressed in graphic form, as in *Fig.* 261. This representation of the healing process is not intended to be mathematically accurate, since it is based on histological observation, supported by the conclusions of other workers such as Howes, Sooy, and Harvey.[22] However, the staining methods serve as a reasonably accurate estimate of the speed with which the fibroblasts are laying down intercellular material, the maturation of this material to collagen, and, therefore, of the development of the holding power of the scar.

THE RELATIONSHIP OF VITAMIN C TO COLLAGEN PRODUCTION

It has long been known that wounds heal poorly in scurvy. The classic account by Anson[1] in 1748 is reproduced in *Fig.* 259. James Lind,[27] in 1772, refers often to this aspect of scurvy. " Whereas, when one has been confined from exercise by having a fractured bone, or from a bruise or hurt, these weak and debilitated parts become almost always first scorbutic ". Describing the development of the disease, he says that " it is not unusual at this time, for such persons as have had ulcers formerly healed up to have them break out afresh ". A Dr. Grainger, writing to Lind concerning scurvy (" a subject of which I had read much, but knew little "), also describes how old ulcers broke out again. In 1919 Aschoff and Koch,[4] working on human material, showed that in scurvy there was always a deficiency in the formation of new intercellular substances and an inability to maintain established supporting structures. Höjer[21] demonstrated a general atrophy in collagen in scorbutic guinea-pigs. Wolbach and Howe,[41] Wolbach,[42, 43] and Menkin, Wolbach, and Menkin,[32] in a series of papers from 1926 onwards, have shown in guinea-pigs that reticulum and collagen are not formed in the absence of vitamin C or ascorbic acid. According to von Jeney and Törö,[24] ascorbic acid added to the culture medium

of explants of fibroblasts accelerated the deposition of intercellular substances, and they confirmed the observation of Doljanski and Roulet[10] that collagenous fibres could form in parts of the medium free from cells. Lanman and Ingalls,[26] in 1937, and Taffel and Harvey,[39] in 1938, have shown that the tensile strength of

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body, but more eſpecially the legs, were ſubject to ulcers of the worſt kind, attended with rotten bones, and ſuch a luxuriancy of funguous fleſh, as yielded to no remedy. But a moſt extraordinary circumſtance, and what would be ſcarcely credible upon any ſingle evidence, is, that the ſcars of wounds which had been for many years healed, were forced open again by this virulent diſtemper: Of this, there was a remarkable inſtance in one of the invalids on board the *Centurion*, who had been wounded above fifty years before at the battle of the *Boyne*; for though he was cured ſoon after, and had continued well for a great number of years paſt, yet on his being attacked by the ſcurvy, his wounds, in the progreſs of his diſeaſe, broke out afreſh, and appeared as if they had never been healed: Nay, what is ſtill more aſtoniſhing, the callous of a broken bone, which had been compleatly formed for a long time, was found to be hereby diſſolved, and the fracture ſeemed as if it had never been conſolidated. Indeed, the effects of this diſeaſe were in almoſt every inſtance wonderful; for many of our people, though con-

FIG. 259.—Photostat reproduction from p. 102 of *A Voyage Round the World*, by George Anson, compiled by Richard Walter, 1748.

healing wounds in guinea-pigs suffering from a partial deficiency of vitamin C is considerably less than in normal animals. It is supposed that ascorbic acid acts as an oxidation-reduction enzyme enabling the deposition of reticulum, collagen, bone, enamel, dentine, and possibly cartilage and elastin to take place. It has not yet been established whether the defect in vitamin-C deficiency is due to a failure of the process of gellation of the intercellular matrix[41] or to a degeneration of the specialized mesodermal cells of the supporting tissues.[12, 14, 29]

Vitamin-C Deficiency.—Scurvy is now a rare disease. Interest is centred on the state of partial deficiency know as 'sub-scurvy' or 'asymptomatic scurvy', though these terms should be accepted with reserve. The following figures for the ascorbic acid content of the blood given by Ingalls and Warren[23] roughly define this state :—

		Ascorbic Acid in mg. per 100 c.c. of Blood
Optimum :	Saturation	2·00–1·00
	Normal	1·00–0·70
	Low normal	0·70–0·50
Suboptimum		0·50–0·30
Deficiency :	Asymptomatic scurvy	0·30–0·15
	Scurvy	0·15–0·00

It has been shown in numerous papers[2, 5, 11, 13, 15, 23, 34, 37] that a state of partial deficiency exists in a high proportion of individuals, especially among the poorer classes. This is particularly so in patients suffering from diseases of the gastro-intestinal tract when the supply of vitamin C is inadequate from dietetic restrictions (e.g., in peptic ulceration) or from defective absorption of the vitamin (in carcinoma of the stomach or colon, or chronic intestinal obstruction from any cause). In acute or chronic infections, injuries (including operations), and in all malignant neoplastic diseases a higher intake of the vitamin is necessary to maintain a normal blood saturation than in the case of normal individuals.

INVESTIGATION

The present research was undertaken to elucidate further, by histological examination of wounds, the defects in the process of wound healing in guinea-pigs suffering from a partial deficiency of vitamin C. Bearing in mind the warning of A. F. Hess[20] that the guinea-pig is far more sensitive to scurvy than man and that " this prevents our carrying out delicate quantitive experiments and cautions against drawing too finely spun deductions ", an attempt was also made to discover whether these experimental findings could be applied to man.

The investigation was restricted to incisions of the abdomen and gastro-intestinal tract, because it is in gastro-intestinal surgery that dietetic deficiencies are most commonly encountered. Further, it is essential for these scars to heal rapidly and well so that they can withstand the strains and stresses to which they are often subjected. If they break down the consequences may be disastrous. They are, therefore, of particular interest.

A. ANIMAL EXPERIMENT

Twenty-four young virgin female guinea-pigs, weighing between 300 and 450 g., were given *ad libitum* a scurvy-producing diet[36] of whole crushed oats (59 per cent), full-cream milk powder heated to 110–115° C. for three hours (30 per cent), butter (10 per cent), and salt (1 per cent), to which was added iron and vitamins A and D. The animals were divided into two groups: 12, the controls, were given adequate doses of ascorbic acid (4 mg. on alternate days); and 12, the deficient group, were given only enough ascorbic acid to prevent the development of any objective sign of scurvy ($\frac{1}{2}$ mg. on alternate days, administration beginning on the tenth day of the experiment). The vitamin, freshly dissolved in $\frac{1}{2}$ c.c. of recently boiled distilled water, was given by pipette. The animals were operated on in pairs, one from each group, after they had been on the special diet for at least two weeks. The pairs were killed at intervals of 1, 2, 3, 5, 7, 10, 14, 17, and 21 days after operation.

The operation was done by a carefully standardized technique and with full aseptic precautions. The prepared abdominal wall was incised for 1 in. down from the xiphisternum and the peritoneal cavity opened. The stomach was partially delivered, clamped with a small rubber-covered clamp, and an incision half an inch long made into the lumen. This gastrotomy wound was closed with a continuous inverting suture of 00 plain catgut. The abdominal incision was closed in three layers, a continuous suture of 00 plain catgut for the peritoneum, interrupted finest silk sutures for the muscles and fascia, and a continuous finest silk suture for the skin. The wounds were covered with a temporary collodion gauze dressing. The animals were starved for 24 hours preceding operation except for the administration of

10 c.c. of 50 per cent glucose solution about 4 hours beforehand. They were allowed to feed immediately they had recovered from the anæsthetic, which was open ether.

When the animals were killed, the abdominal and gastric wounds were excised with as little surrounding tissue as possible, fixed in Bouin's fluid for 14 hours, blocked in wax, and sectioned. The sections were stained by four methods; hæmatoxylin and eosin, van Gieson's connective-tissue stain, Foot's modification of the Bielschowsky-Maresch silver impregnation method, and Mallory's connective-tissue stain. Section cutting and staining were done entirely according to a standardized routine so as to obtain exactly comparable pictures.

Macroscopical Findings.—All the wounds healed by first intention, in that there was no macroscopical infection. The wounds of the controls, in the earlier stages of healing, were less red, less thick, and showed less surrounding induration than those of the sub-scurvy animals, but the latter showed no greater tendency to bleed at operation. The scabs, in the case of the controls, came off earlier; the shrinking of their scars, both across and along the line of the incision, was greater; the final scars were almost invisible, pale, and projected slightly above the surrounding skin. In the sub-scurvy animals, on the other hand, they were puckered, stretched, sunken, and showed a mauvish discoloration (*Fig.* 260). The contrast

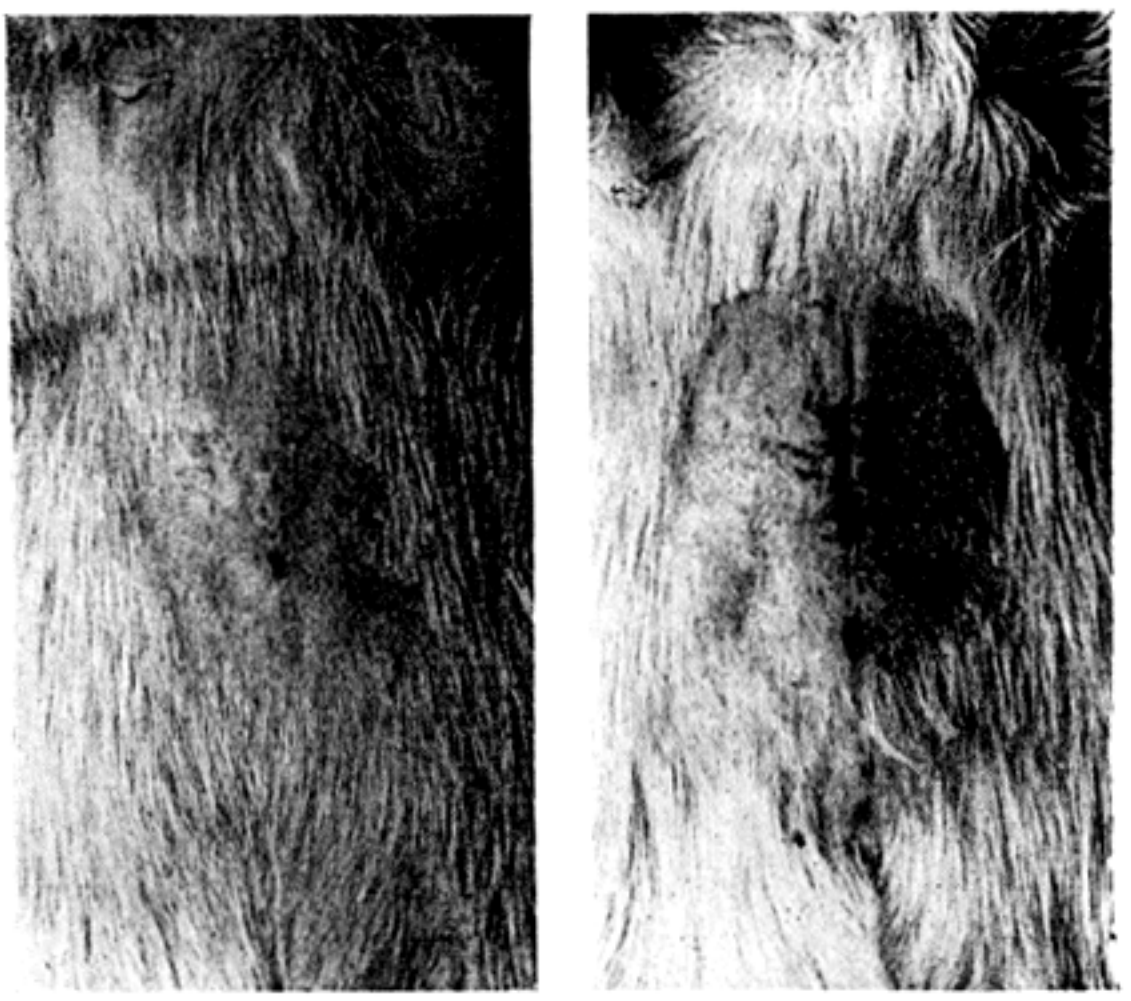


FIG. 260.—Abdominal incisions of guinea-pigs, 21 days after operation. Life size. Both healed by first intention. A, Control animal; B, Sub-scurvy animal.

between these gross appearances was striking, and, apart from anything else, sufficient to distinguish the two groups of animals from each other. To the casual observer, on the other hand, the scars of the sub-scurvy animals appeared to be well healed, until compared with those of the control group.

Microscopical Findings.—

1. *Cellular Proliferation.*—Cellular proliferation was found to be little affected by a deficiency of ascorbic acid during the earlier stages of healing. New fibroblasts began to appear on the third post-operative day in both groups and then multiplied to about the same extent. In the controls this proliferation ceased on

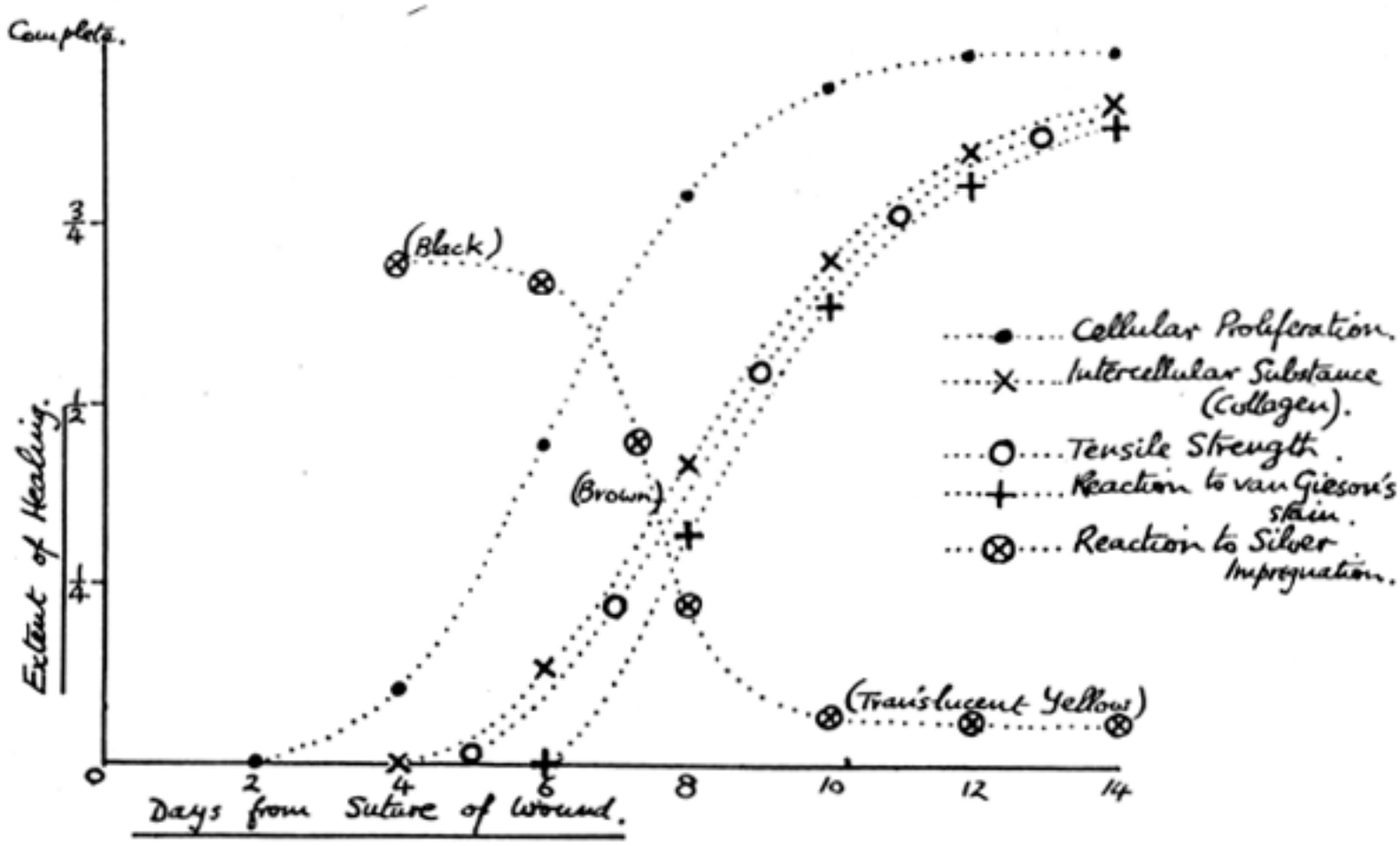


FIG. 261.—Graph to illustrate the approximate course of healing, with the staining reactions of the intercellular substance, of wounds in normal guinea-pigs.

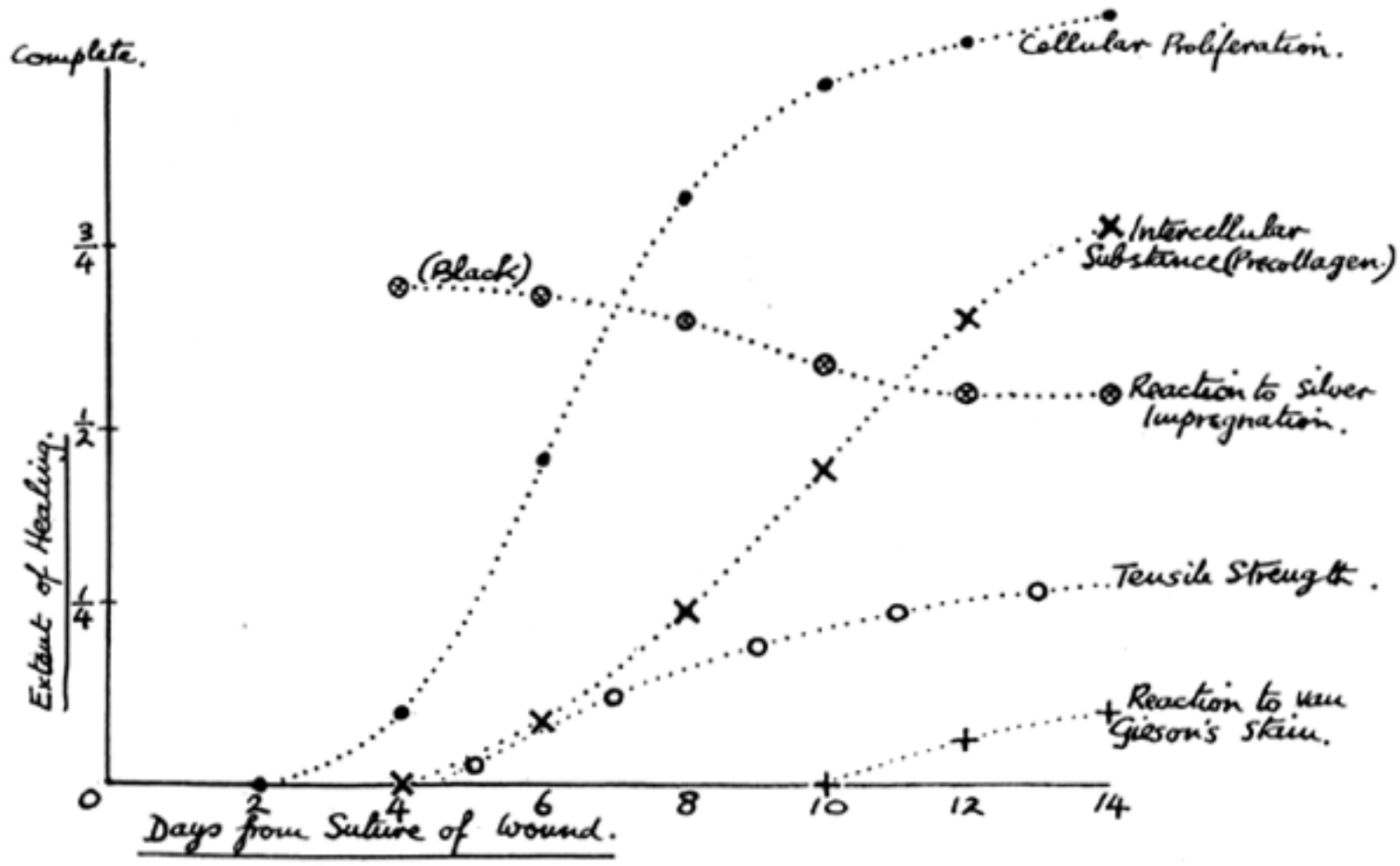
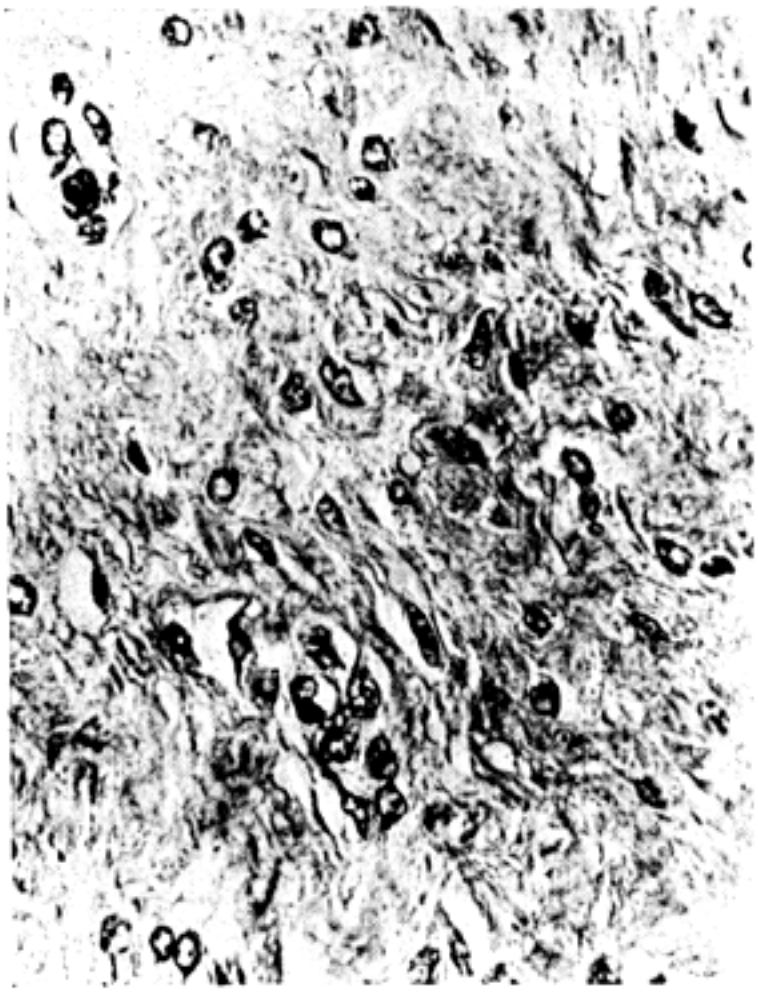
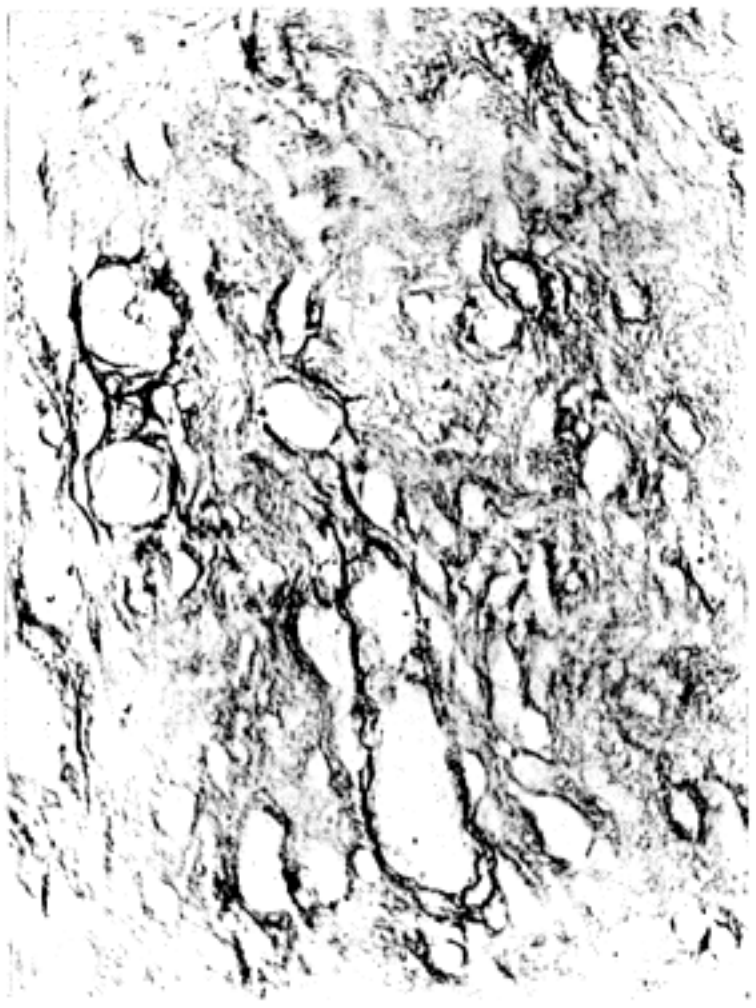


FIG. 262.—Graph to illustrate the approximate course of healing, with the staining reactions of the intercellular substance, of wounds in sub-scurvy guinea-pigs.



A1

B1



A2

B2

FIG. 263.—From the abdominal scars of guinea-pigs killed 21 days after operation. (× 400.) (Enlarged from *Fig.* 265.) A_1, A_2, Control animal; B_1, B_2, Sub-scurvy animal.

A_1, Fibrocytes. B_1, Fibroblasts (stain, hæmatoxylin and van Gieson).

A_2, Black, silver-staining reticulum fibres only around the blood-vessels. The intercellular substance is composed of mature collagen. B_2, Black, silver-staining precollagen forming the whole intercellular substance (stain, silver impregnation).

about the fourteenth day, when the scar was consolidating, whereas in the sub-scurvy animals it continued long beyond that time. This suggested that the lack of ascorbic acid actually stimulated the proliferation of cells. It is more likely, however, that fibroblasts will continue to multiply so long as there is need of the formation of scar tissue, and, if this formation is delayed, cellular proliferation will be prolonged (*Fig.* 262). The cells remain immature (*Fig.* 263). There was no difference in the production of 'foreign-body' giant cells around the sutures, but the mesodermal cells proliferating around damaged muscle-fibres produced more giant multinuclear forms in the sub-scurvy animals. The regeneration of epidermis and stomach epithelium was similar in the two groups, though more regular in the controls.

2. *Production of Intercellular Material.*—In the production of intercellular material there were great differences. In the controls, argyrophil precollagen was laid down in large quantities around the fibroblasts and was clearly demonstrable by the fifth day. Maturation to collagen began by the seventh day and progressed rapidly. In the sub-scurvy animals precollagen appeared a little later and in smaller quantities. It failed to mature and remained argyrophil in nature throughout the whole period of 21 days (*Fig.* 262). No van Gieson-staining intercellular substance could be seen in the 'scar' of the healed wound. *Fig.* 263 shows the difference between the intercellular substances in the two groups. In the control animal, A, black argyrophil fibres can be seen only around the capillaries. These fibrils are probably true reticulum forming the cement substance and basement membrane of the endothelial cells. The rest of the ground substance is dense collagen and appears a translucent yellow in the section. In contrast, the whole of the intercellular substance of the sub-scurvy animal, B, stains densely black with silver. It does not show the fine fibrillary nature of reticulum, but the coarse irregular strands of precollagen.

3. *Structure of the Wound as a Whole.*—In comparing the wounds of these two groups of animals, differences in the structure of the wound as a whole were quite as striking as the variations in the production of intercellular material.

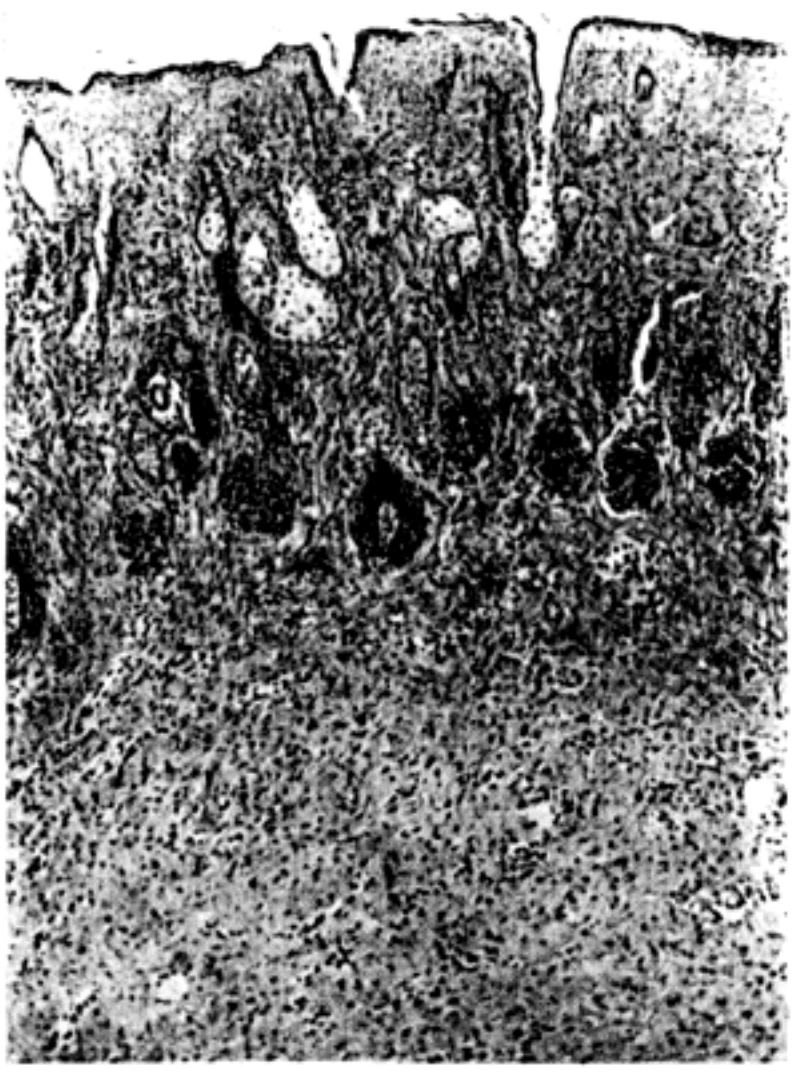
a. Dermis: Where an incision passes through skin in a normal animal and there is accurate approximation of the cut surfaces, secure fibrous healing takes place across the full width of the corium, with little, if any, reduction in the final thickness of the skin (*Fig.* 264, A). This was well shown in the control animals. In the sub-scurvy animals, on the other hand, a small hæmorrhagic effusion appeared after a few days and gradually extended between the opposed surfaces so that the corium opened out internally. The skin became greatly reduced at the site of the incision, and finally, on the twenty-first day, consisted of little more than epidermis overlying ill-formed granulation tissue (*Fig.* 264, B). The final scar, therefore, appeared sunken and discoloured. Possible technical variations cannot be impugned for producing this effect, because the stretching of the scar could be traced through the successive stages of wound healing.

b. Scar: The scar itself in the controls was compact, uniform, and vascular throughout, composed of mature fibrocytes in a matrix of collagen. There was no effusion (*Figs.* 264, A and 265, A). In the sub-scurvy animals it was loose, irregular, and almost avascular, composed of fibroblasts in a matrix of precollagen (*Figs.* 264, B and 265, B). It was far more cellular and split up by extravasations of blood-stained fluid. These effusions continued to appear throughout the healing

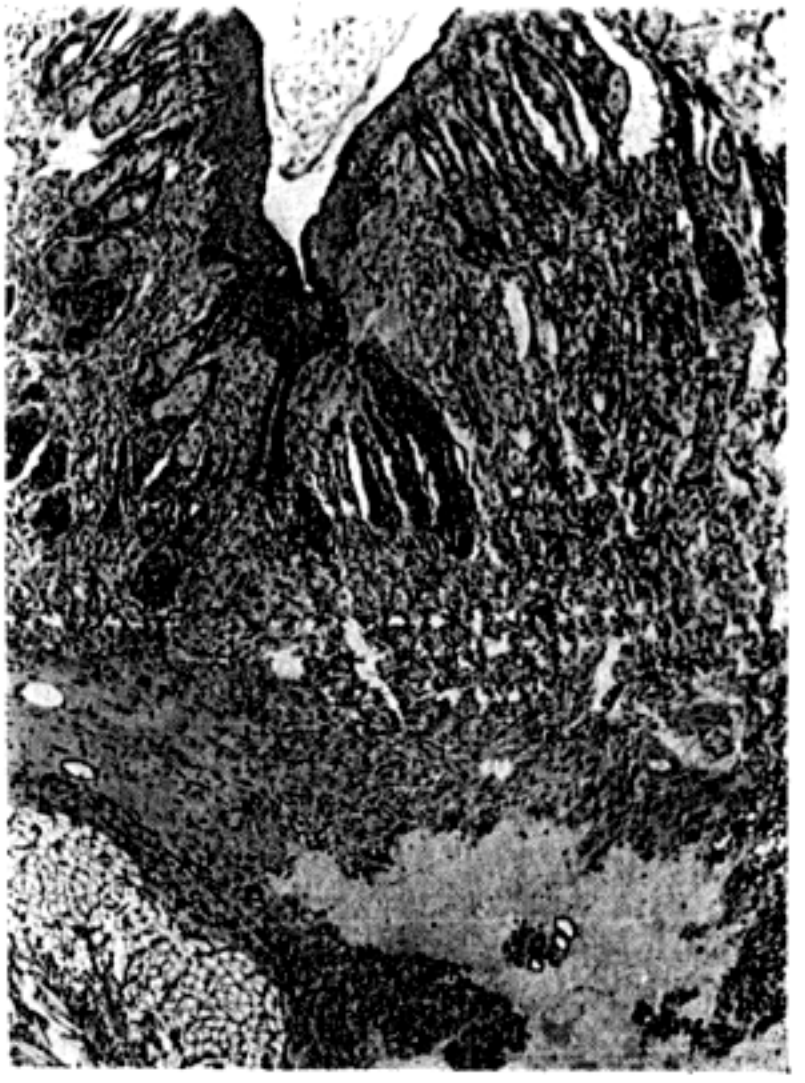
and were bordered by fibroblasts and not capillaries. The few microscopic hæmatomata that could be found in the wounds of the normal animals were organized in the usual way by the ingrowth of blood-vessels followed by fibroblasts.

A_1

B_1



A_2



B_2

FIG. 264.— Cross-sections of the abdominal incisions of guinea-pigs killed 21 days after operation (stain, hæmatoxylin and van Gieson). A, Control animal; B, Sub-scurvy animal.

A_1, Dense, homogeneous, collagenous scar with corium of good thickness and without effusions; B_1, Soft, thick, œdematous, poorly-formed 'scar' with puckered, thinned-out corium and many effusions. (× 7.)

A_2 and B_2, The same (× 56). (Silk ligature bottom left in B_2.) Cf. *Fig.* 260.

c. Phagocytosis and absorption of damaged tissues and removal of catgut ligatures: An essential part of wound healing is the phagocytosis and absorption of damaged cells and the 'knitting-in' of remnants of tissues bordering on the incision. In the controls, this whole process had been completed by the twenty-first day and there was then no trace of aponeurotic strata extending through the uniform scar from the adjacent muscle layers (*Fig.* 264, A). In animals deficient in ascorbic acid, this reorganization and fusion of old and newly-formed fibrous tissue was long delayed. Remnants of intermuscular strata could be traced through the forming scars for a long time. *Fig.* 267 illustrates the abdominal

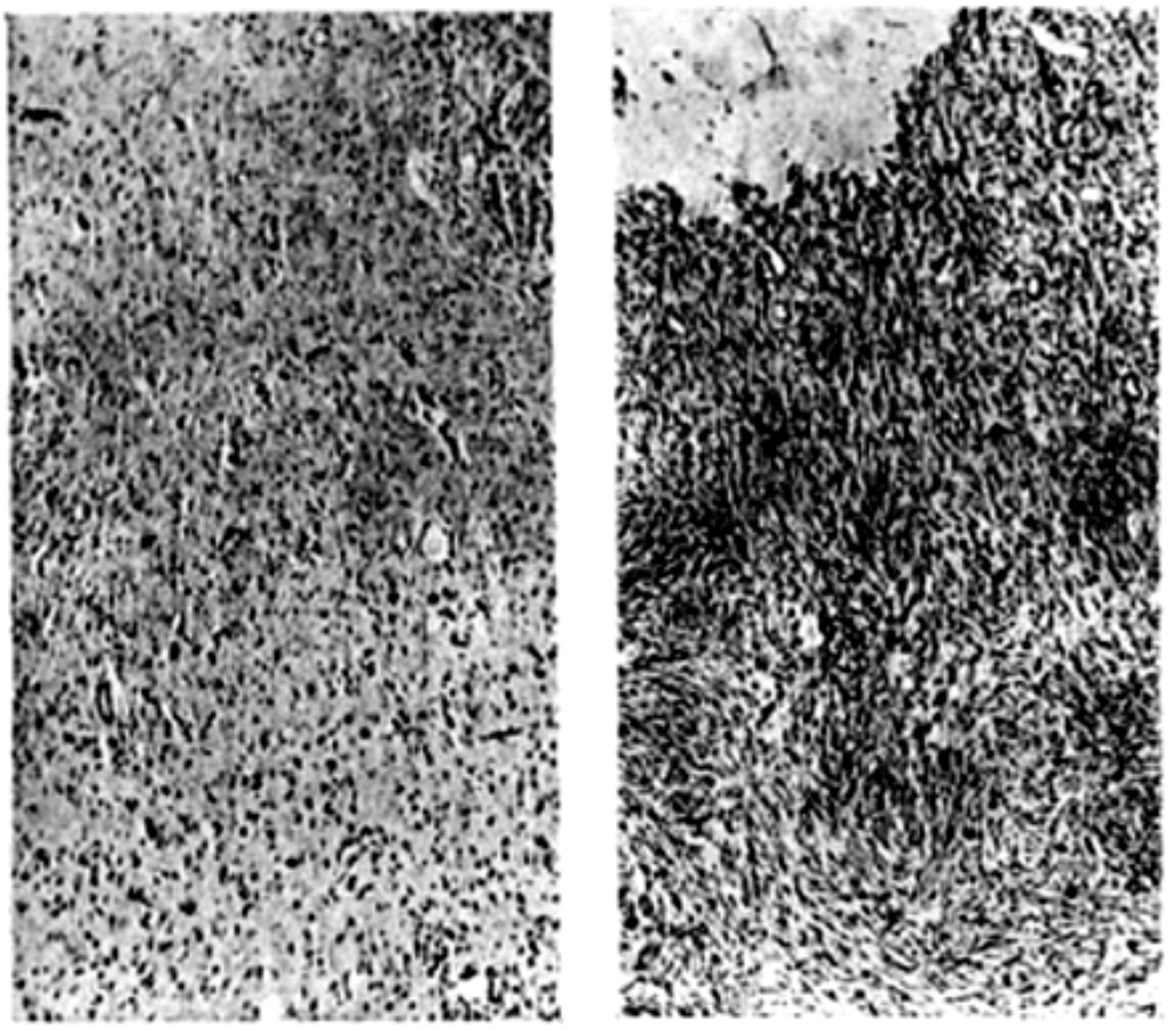


FIG. 265.—From the abdominal scars of guinea-pigs killed 21 days after operation. (× 60.) (Stain, hæmatoxylin and van Gieson.) A, Control animal; B, Sub-scurvy animal.
A, Homogenous, vascular scar composed of mature fibrocytes in a matrix of collagen; B, Extremely cellular scar composed of fibroblasts in a matrix of precollagen. (The edge of an effusion shows top left in B.) *See Fig.* 263.

incision of an animal which had been maintained in the sub-scurvy state for 20 days after operation and then allowed to develop full scurvy, dying on the thirty-fifth post-operative day. The original intermuscular septa can be seen passing right through the scar without being incorporated in it. There had been only partial removal of damaged muscle.

Examination of the sections of this series of animals also suggested that a deficiency of ascorbic acid delayed the removal of catgut ligatures, either by phagocytosis or extrusion. In the controls, the catgut of the gastrotomy wounds had disappeared by the seventeenth day. By the twenty-first day the scar was uniform and mature, with the epithelium well healed, so that the only evidence of where the incision had been was the fusion of the layers of the muscularis and substitution of the muscle by fibrous tissue. In the sub-scurvy animals the catgut ligatures remained and the incision was only partially healed (*Fig.* 266).

A

B

FIG. 266.—Cross-sections of the gastric incisions of guinea-pigs killed 21 days after operation. (× 13·5.) (Stain, silver impregnation.) A, Control animal; B, Sub-scurvy animal.
A, Uniform, neat healing; B, Irregular, delayed healing with a catgut ligature still not absorbed or extruded, and precollagen forming the intercellular substance in the subserous part of the scar.

Effects of Scurvy on Healed Wounds.—Three animals were observed for the effects of established scurvy on healed wounds. Two sub-scurvy and one control guinea-pig were deprived of all ascorbic acid from the twentieth day after operation and lived for a further 15, 19, and 25 days respectively. The first animal to die ruptured spontaneously the superficial parts of its abdominal incision on the day before death (*Fig.* 267). There was no evidence of infection. The 'scars' of this and the second animal to die, which was also from the sub-scurvy group, showed in an extreme form all the appearances already described for the partially deficient animals, except that in the centre of groups of fibroblasts the intercellular matrix was entirely structureless (*Fig.* 268). The last animal (20 days saturated with the vitamin and then 25 days without it) still showed a perfectly healed wound

at death. The new collagen of the scar had, however, reverted to an argyrophil precollagenous state (*Fig.* 269) very different from the comparable intercellular material in the scar of the control animal that had been killed on the twenty-first post-operative day. The cells of the scar appeared to be mature fibrocytes (cf. *Fig.* 263).

A

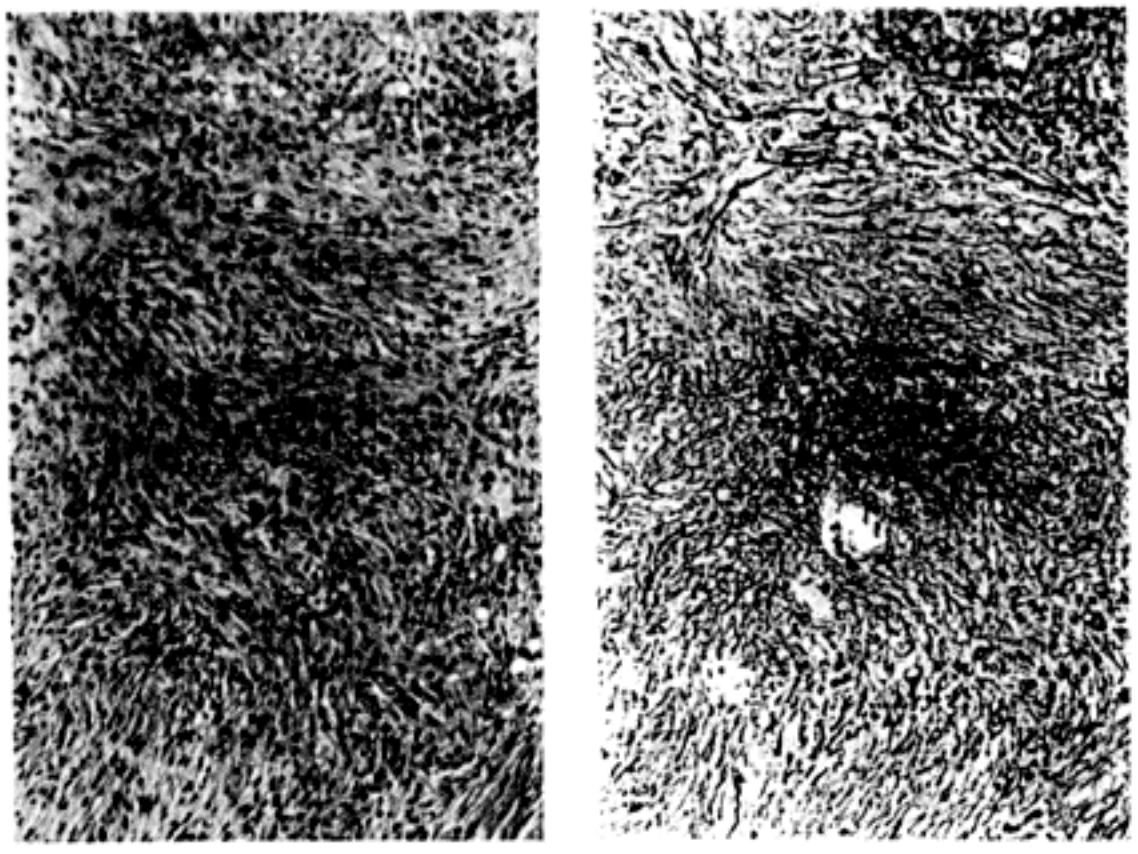
B

C

FIG. 267.—Cross-section of the abdominal incision of a guinea-pig dying 35 days after operation, 20 days sub-scorbutic and then 15 scorbutic. A, × 7 (stain, hæmatoxylin and van Gieson). B, × 7 (stain, silver impregnation). C, × 56 (stain, hæmatoxylin and van Gieson).

To show: (1) Partial abdominal disruption occurring spontaneously the day before death and in the absence of infection. The granulation tissue in C is free from inflammatory cells. (2) Failure of incorporation and fusion of the intermuscular septa bordering the incision with the newly-formed fibrous tissue. In B the fascial planes can be traced through the scar from one side to the other.

Assessing the Degree of Vitamin-C Deficiency.—The two most efficient methods of determining vitamin-C deficiency in man are the excretion of a test dose of ascorbic acid and the estimation of the ascorbic acid content of the blood. In guinea-pigs the former is impossible, and enough blood for the latter test is obtainable only by exsanguinating the animal at death. There is, therefore, no accurate standard of deficiency for comparison with human cases. The guinea-pig is known



A B

FIG. 268.—From the scar of the same scorbutic guinea-pig as *Fig.* 267. (× 88.) (Stain, A, hæmatoxylin and van Gieson; B, silver impregnation.) Consecutive sections, identical fields. To show fibroblasts with amorphous intercellular substance centrally and precollagen peripherally.

to be far more susceptible to scurvy than man, and therefore presumptive evidence, based on histological appearances, is not strictly valid: but it must be assumed, and with some degree of conviction since the histological changes in human and guinea-pig scurvy are identical, that the results of experiments on guinea-pigs are, to a considerable extent, applicable to human beings.

The following methods were used in the present experiments to assess the degree of deficiency or to exclude the presence of frank scurvy.

1. *Clinical Evidence.*—The sub-scurvy animals remained active, continued to consume a normal quantity of food, their gums and teeth remained healthy, and they had no hæmorrhages. They did not lose weight, in contrast to scorbutic guinea-pigs which were found to lose between one-third and one-quarter of their body-weight during the days preceding death (Graph, *Fig.* 270). (Höjer[21] found a comparable weight loss in a large number of scorbutic guinea-pigs.) Compared with the normal controls, on the other hand, their coats were not as shiny, they did not withstand operation so well, nor did they react to surprising external stimuli with quite the same alacrity. Hæmoglobin estimations and red blood-cell counts done on all guinea-pigs at operation and at death showed no appreciable difference between the two groups, and erythrocyte sedimentation-rates were similar in each case.

2. *Rotter's Intradermal Dye Test.*[35]—This revealed no relevant deficiency in the sub-scurvy group of animals. As a test it proved of no value, and as applied to human beings it is, in my opinion, of less value than a few questions about habits of diet.

3. *Szent-Györgyi's Test.*[38]—This gives a rough estimate of the reducing substances, particularly ascorbic acid, present in the suprarenals. Coarse sections of the suprarenal glands are cut as soon after death as possible (immediately in the case of the animal experiments), cleaned of blood and serum with blotting paper, and placed in 0·4 per cent neutral silver nitrate solution in the dark for 15 minutes.

The degree of reduction of the silver nitrate, shown by the blackening of the tissue, indicates the amount of reducing substance present in the tissues. The control group, according to this test, contained about three times as much ascorbic acid in

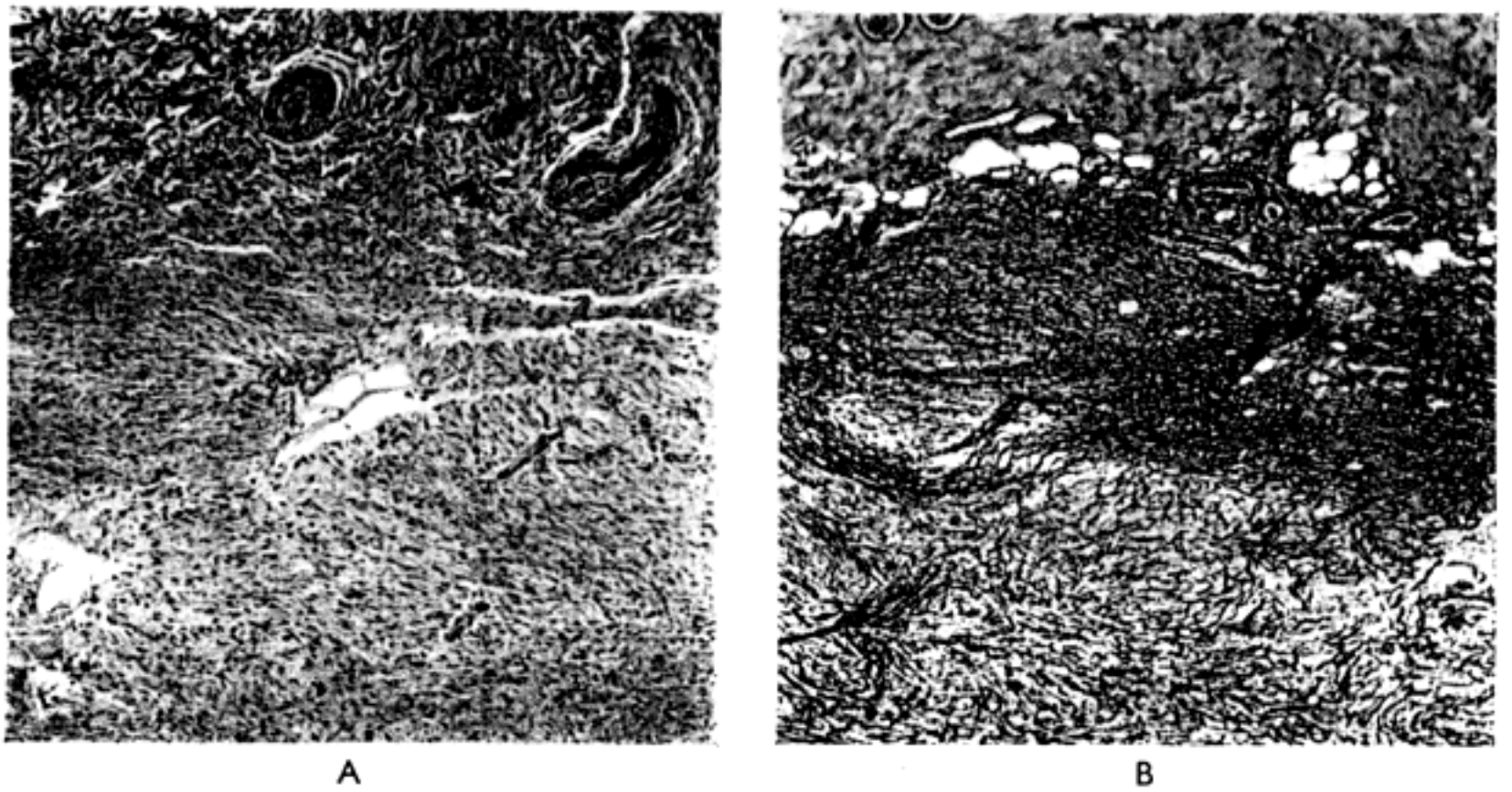


FIG. 269.—From the abdominal scar of a guinea-pig dying 45 days after operation, 20 days normal and then 25 scorbutic. A, × 56 (stain, hæmatoxylin and van Gieson); B, × 56 (stain, silver impregnation); C, × 400 (stain, hæmatoxylin and van Gieson); D, × 400 (stain, silver impregnation).

To show: (1) Reversion of newly-formed collagen to precollagen (B and D). Compare the wound of this animal, which healed normally for 20 days, with that illustrated in *Fig.* 263, A, 21 days of normal healing. (2) Fibrocytes in the scar (C), cf. *Fig.* 263, B. (3) Collagen remaining in the established fibrous tissue of the corium (A and B).

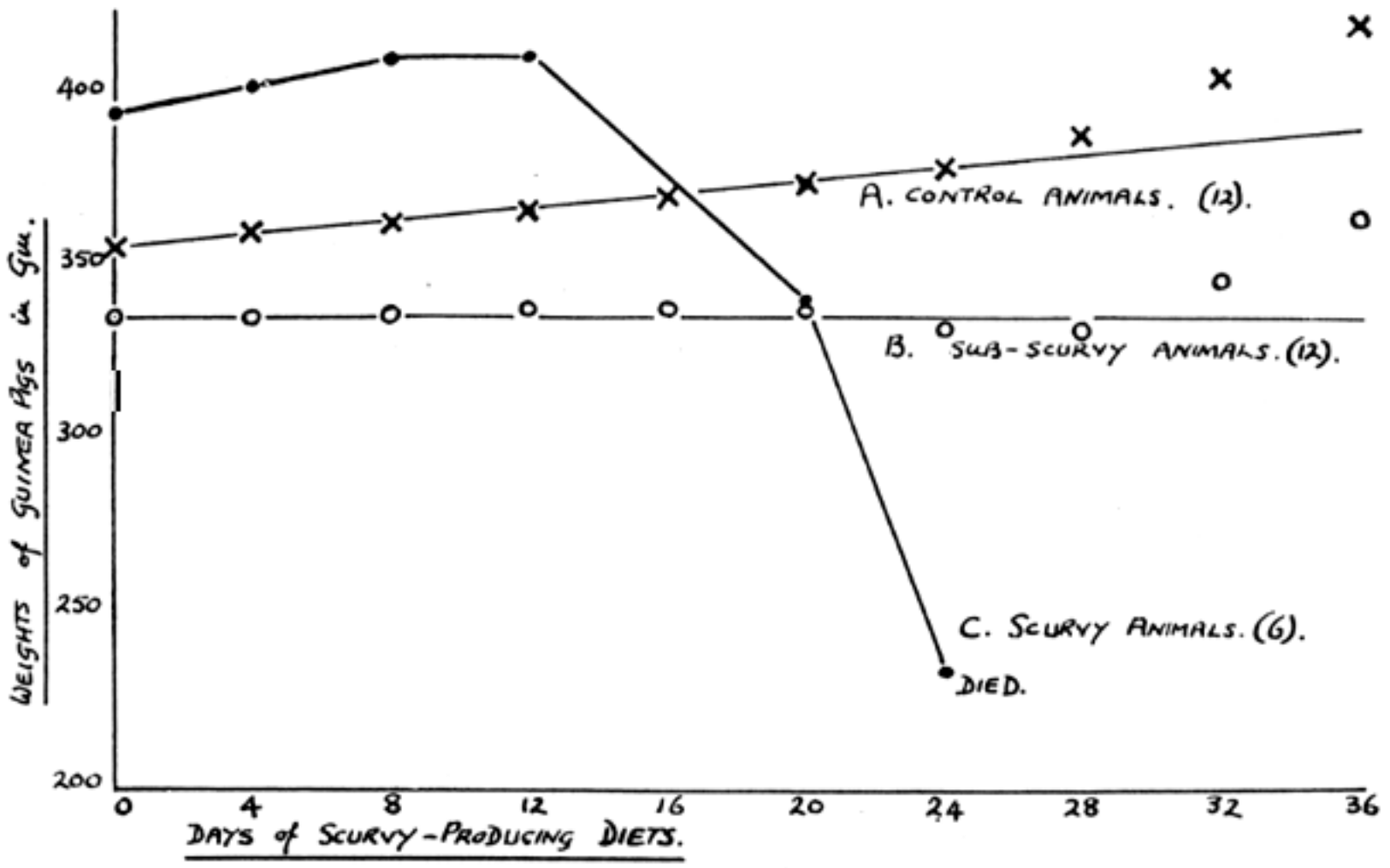


FIG. 270.—Graph showing the average weights of three groups of guinea-pigs.

their tissues as the sub-scurvy animals, yet all of the latter contained an appreciable amount. Scorbutic guinea-pigs were shown to contain none.

4. *Microscopical Evidence.*—Microscopical sections were made of the hearts, suprarenals, and costochondral junctions of all the guinea-pigs. The hearts and suprarenals of the sub-scurvy animals showed none of the degenerative changes found in scurvy,[20, 21] but the costochondral junctions showed changes intermediate between the normal and scorbutic (*Fig.* 271).

5. *Blood Estimations.*—Blood ascorbic acid estimations on 3 animals from each group showed an average of 0·46 mg. per 100 c.c. for the controls and 0·35 mg. for the sub-scurvy animals. These estimations are too few in number, yet the readings for the animals of each group were surprisingly constant.

Taking all the tests into consideration, it is evident that a state of severe deficiency was induced in the sub-scurvy animals, bordering, but not trespassing, on scorbutus major.

B. HUMAN EXPERIMENT

The subject of the experiment, a young adult male in the best of health, ate no fruit, preserves, jam, or uncooked vegetables, and consumed only one portion of cooked vegetable each day for 3 months. By this time the blood ascorbic acid content, estimated seven times, averaged 0·34 mg. per 100 c.c. Under local anæsthesia a left submammary incision 1½ in. long was made down to muscle. It was closed with a continuous 0 chromic catgut suture for the pectoral fascia and interrupted silk stitches for the skin. At the end of two weeks this incision was excised and a second identical incision made on the opposite side. 1000 mg. of ascorbic acid were then taken by mouth in divided doses for three days and then 400 mg.

daily till the end of the second fortnight, when the second wound was excised. The blood ascorbic acid rose to 0·5 mg. in three days and remained at that level. Twelve days after the second incision, a test dose of 400 mg. of ascorbic acid taken by mouth

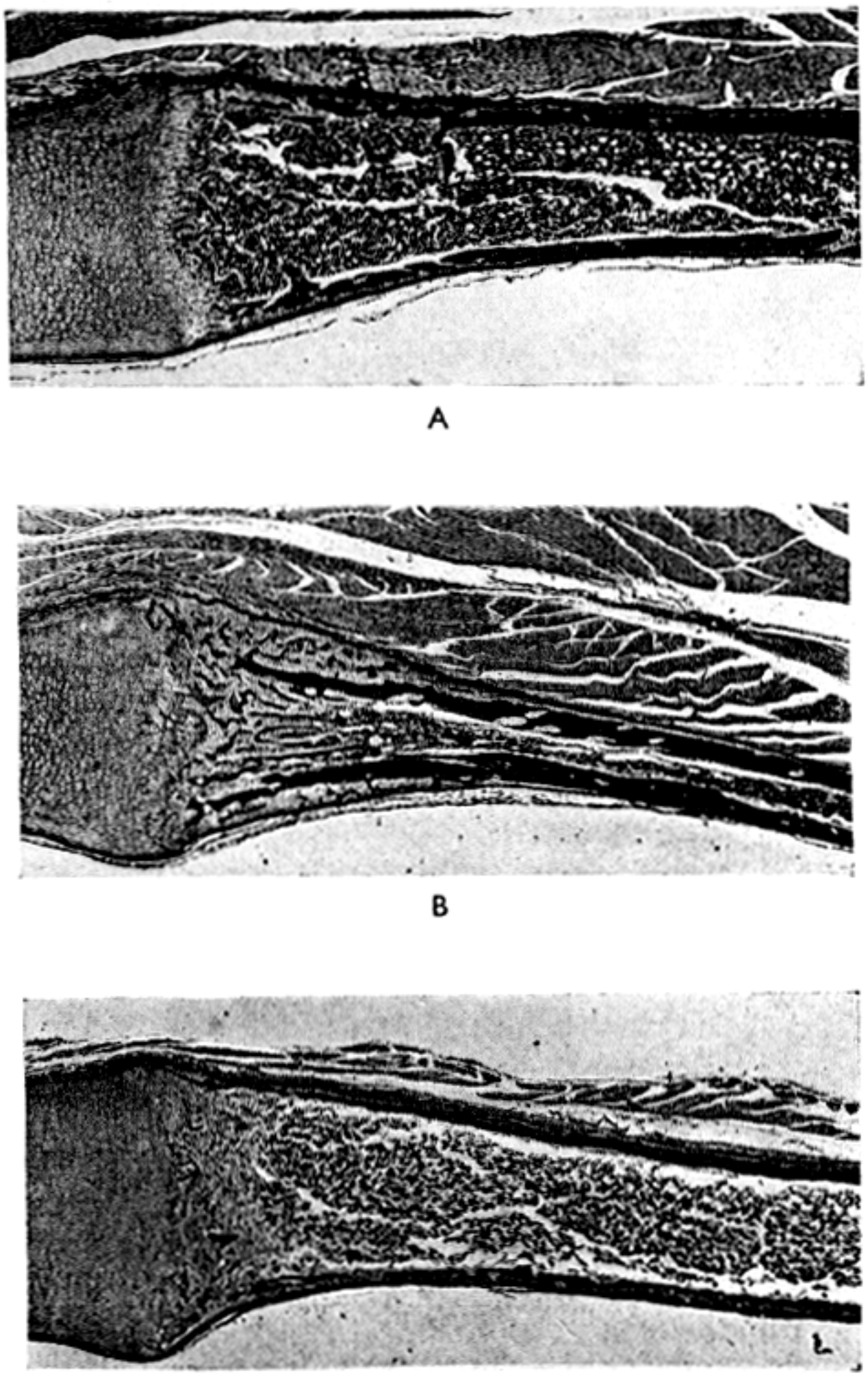


FIG. 271.—Costochondral junctions of growing guinea-pigs. (× 16.) (Stain, hæmatoxylin and van Gieson.) A, Control animal. B, Sub-scurvy; C, Scurvy.
Note: (1) In B, increase of cellular activity and calcification of cartilage columns. Cortex of good thickness and staining power. (2) In C, cessation of all calcification or ossification. Cortex thin and poorly staining. The 'framework marrow' (Gerüstmark) of proliferating mesodermal cells replaces the red bone-marrow near the cartilage.

showed a 61 per cent excretion in 6 hours, indicating a high degree of saturation. Both incisions were treated for histological examination in the same way as the guinea-pig scars. Both healed perfectly, with no macroscopical or microscopical difference and with identical reactions to the catgut ligature.

C. HUMAN NECROPSY MATERIAL (*Table I*)

Abdominal incisions, gastro-intestinal anastomoses and stomata, sutured duodenal ulcers, one repaired intestinal rupture, and some other specimens were obtained from an unselected consecutive series of 28 relevant cases. Each specimen was prepared for histological examination by the same methods as those used in the guinea-pig experiments. The routine administration of vitamin for all such cases had not been recognized so that some cases had received ascorbic acid and some had not. Blood ascorbic acid estimations were seldom available. Szent-Györgyi's test[38] was done in 17 cases as set out in *Table I*. The outbreak of war prevented the accumulation of a more complete series, but it was decided, as a result of a careful histological study of the material so far available, to place on record the findings in this present small selection. (Biopsies of healing wounds and granulation tissue taken during life were found to be too fragmentary to be of any value.)

Analysis of the Post-mortem Findings.—*Table I* is self-explanatory in placing certain aspects of wound healing side by side with the histological appearances of the wounds, the amount of supplementary ascorbic acid given, and, in 17 of the cases, a rough estimate of the ascorbic acid content of the tissues. Wound infection was common in certain groups of cases, particularly the perforated peptic ulcers,

Table I.—SUMMARY OF

CASE NO.	SURVIVAL AFTER OPERATION	NATURE OF DISEASE	NATURE OF OPERATION	CAUSE OF DEATH
1	Days 1	Acute gastric ulcer; hæmatemesis	Gastro-enterostomy	Heart failure
2	2	Carcinoma of stomach	Gastro-enterostomy and entero-enterostomy	Heart failure
3	2	Carcinoma of pancreas	Cholecyst-gastrostomy	Liver shock
4	3	Perforated duodenal ulcer	Suture with drainage	Diffuse peritonitis
5	3	Perforated duodenal ulcer	Suture without drainage	Diffuse peritonitis with *B. welchii* septicæmia
6	4	Gastric ulcer-cancer	Partial gastrectomy	Diffuse peritonitis
7	4	Strangulated umbilical hernia	Relief of obstruction with resection and anastomosis	Diffuse peritonitis
8	5	Chronic gastric ulcer	Partial gastrectomy	Diffuse peritonitis
9	6	Carcinoma of cardia	Œsophagogastrostomy	Diffuse peritonitis; pericarditis; pyelonephritis
10	7	Perforated duodenal ulcer; carcinoma of bladder	Suture without drainage	Bronchopneumonia
11	8	Carcinoma of stomach	Partial gastrectomy	Bronchopneumonia; subphrenic abscess

but it interfered with healing to the extent of causing a breakdown of the wound in 1 case only, *Case* 23. Infected sinuses are not included under abdominal disruptions. Complete disruption implies a rupture of all the layers of the abdominal wall, not necessarily extending for the whole length of the incision. Some of the cases which died during the first few days after the operation showed no ' reaction ' at the suture line. This proved on microscopical examination to be due to necrosis or failure of cellular proliferation or a combination of these two factors.

It has been shown that collagen, in normally healing wounds, becomes evident at the end of the first week and then steadily increases in amount. Of the 18 cases dying after the seventh day, eight (*Cases* 11, 13, 14, 15, 17, 22, 25, and 27) showed precollagen in place of collagen. In all these cases there was good indication that there had in the past been an insufficient intake of vitamin C. Only 1 had received supplementary ascorbic acid and that in inadequate doses (*Case* 25). In 4 cases (*Cases* 11, 13, 17, and 27) the Szent-Györgyi test indicated negligible amounts of ascorbic acid in the tissues, in 3 (*Cases* 14, 22, and 25) small amounts, and in *Case* 15 the test had not been done. This small group of 8 cases included all 5 in which abdominal disruption had occurred in the absence of gross infection. Three had also leaked at the suture line without local necrosis. Microscopically their wounds were

:8 Human Cases

Abdominal Disruption	Leakage at Suture Line	Necrosis at Suture Line	Cellular Proliferation	Intercellular Substance Formation	Ascorbic Acid given	Szent-Györgyi Test
No	No	Superficial	None	None	1000 mg.	$\frac{1}{5}$
No	No	No	None	None	0	$\frac{1}{5}$
No	No	Marked	Beginning	None	0	$\frac{1}{2}$ (0·4 mg. ascorbic acid /100 c.c. blood)
No	No	No	Present	None	0	$\frac{1}{5}$
No	No	No	Present	None	0	$\frac{1}{5}$
No	No, but incipient	Complete	Present	None	6200 mg.	—
No	Yes	Yes	Marked	Precollagen	0	$\frac{1}{5}$
No	No	No	Present	None	Inadequate doses	—
No	Yes	Complete	None	None	0	—
No	No	No	Moderate	Precollagen	0	—
Partial	Yes	No	Present	A little precollagen	0	0

Table I.—SUMMARY

CASE NO.	SURVIVAL AFTER OPERATION	NATURE OF DISEASE	NATURE OF OPERATION	CAUSE OF DEATH
12	Days 8	Duodenal ulcer with pyloric stenosis	Gastro-enterostomy	Heart failure; dilatation of stomach
13	8	Carcinoma of cardia	Witzel jejunostomy	Diffuse peritonitis; bronchopneumonia
14	12	Carcinoma of ampulla of Vater	Cholecystgastrostomy	Pyelonephritis; suppurative parotitis
15	12	Chronic gastric ulcer	1. Partial gastrectomy 2. Abdominal suture	Diffuse peritonitis
16	12	Perforated duodenal ulcer	Suture without drainage	Diffuse peritonitis; bronchopneumonia
17	13	Traumatic rupture of jejunum	1. Suture with drainage 2. Re-drainage	Diffuse peritonitis with abscesses
18	17	Gastric ulcer with hæmatemesis	1. Excision of ulcer 2. Gastro-enterostomy	Hypostatic pneumonia
19	17	Carcinoma of colon	Resection and anastomosis	Diffuse peritonitis
20	18	Duodenal ulcer with hæmatemesis	Partial gastrectomy	Agranulocytosis
21	20	Subacute gastric ulcers; hæmatemesis	Exploratory laparotomy	Bronchopneumonia; pyæmia
22	22	Carcinoma of rectum	1. Colostomy 2. Perineo-abdominal excision	Diffuse peritonitis
23	22	Perforated duodenal ulcer with pyloric stenosis	Suture and gastro-enterostomy without drainage	Tuberculous bronchopneumonia
24	23	Jejunal ulcer (10 years gastro-enterostomy)	Partial gastrectomy and entero-enterostomy	Diffuse peritonitis and empyema
25	28	Perforated duodenal ulcer	1. Suture with drainage 2. Drainage of subphrenic abscess	Subphrenic abscess and empyema
26	39	Intestinal obstruction from abdominal metastases of bronchial carcinoma	Gastro-enterostomy and entero-enterostomy	Multiple metastases
27	39	Perforated duodenal ulcer	1. Closure without drainage 2. Drainage of subhepatic abscess	Subphrenic and subhepatic abscesses
28	Years	Multiple injuries	Old healed gastro-enterostomy	Multiple injuries

HUMAN CASES—*continued*

ABDOMINAL DISRUPTION	LEAKAGE AT SUTURE LINE	NECROSIS AT SUTURE LINE	CELLULAR PROLIFERATION	INTERCELLULAR SUBSTANCE FORMATION	ASCORBIC ACID GIVEN	SZENT-GYORGYI TEST
No	No	Superficial	Marked	Precollagen and collagen	1800 mg.	$\frac{1}{3}$
Partial	No	No	Scanty	A little precollagen	0	Trace only
No	No	No	Present	Precollagen	0	$\frac{1}{3}$
Complete. 10th day, resutured	Yes	No	Marked	Scanty, precollagen only	0	—
No	No	No	Good	Good, mostly collagen	2600 mg.	Saturation
Complete	Yes	No	Good	Precollagen, with little collagen	0	Trace
Gastric fistula	At fistula only	No	Good	Collagen	100 mg. daily for 40 days	$\frac{2}{3}$
No	No	No	Good	Collagen	0	—
No	No	No	Good	Collagen	0	$\frac{2}{3}$
No	—	—	(Skin only) Poor	Collagen	6600 mg.	$\frac{1}{2}$
No (sloughing perineum)	No	No	Adequate	Collagen and precollagen	0	$\frac{1}{3}$
Complete. Transverse incision	No (omental closure)	No	Good	Collagen at gastroenterostomy, precollagen at abdominal wall	5000 mg.	$\frac{2}{3}$
No	No	No	Good	Collagen	0	—
Partial	No (omental closure)	No	Scanty	Precollagen	Inadequate dose for last few days	$\frac{1}{3}$
No	No	No	Good	Collagen	800 mg.	—
No	No (omental closure)	No	Poor	Precollagen	0	Trace
Healed anastomosis for comparison with other cases						

very similar to those of the sub-scurvy guinea-pigs. *Fig.* 273 shows the skin incision of *Case* 17, who died 13 days after operation. The corium is opened out internally and the gap is filled by proliferating fibroblasts producing precollagen. For comparison, *Fig.* 272 illustrates a similar wound in *Case* 16, who died 12 days after operation, but who had received plenty of vitamin C. Abdominal distension had been similar in the two cases.

ILLUSTRATIVE CASE RECORDS

Case 16.—W. H., male, aged 50. Admitted July 8, 1939, with a perforated duodenal ulcer. Operation for closure, without drainage of the peritoneal cavity, was done eight hours after the perforation had occurred. Peritonitis developed, with vomiting and abdominal distension from ileus. Treatment with morphia and intravenous fluids was begun immediately. The hæmoglobin remained at 80 per cent. On July 14 the patient developed bronchopneumonia which was treated with sulphapyridine. From July 16 onwards, ascorbic acid was also given, totalling 2600 mg. in divided doses, either by mouth or intravenously. On July 18, venesection of 800 c.c. of blood was done for left-sided heart failure, but the patient died on July 20, twelve days after operation.

Necropsy was done on July 21. The heart failure was evident and the lungs showed a diffuse *B. Pfeiffer* bronchopneumonia. The abdomen was distended, but the midline incision was well healed in accurate approximation. The peritoneum showed a diffuse purulent peritonitis (mixed infection) with, in addition, a small localized abscess around the duodenal ulcer. There was no leak through from the lumen. The Szent-Györgyi test indicated a saturation of the tissues with ascorbic acid and the histological findings agreed with this estimate.

Sections of the skin showed that the corium had healed in exact apposition without any reduction in thickness and with good collagen formation, which was almost indistinguishable in the van-Gieson-stained section from the adjacent established fibrous tissue. Silver impregnation showed little remaining precollagen (*Fig.* 272). The ulcer showed no sign of epithelial healing, but the perforation was well closed. There was great mesodermal proliferation with the formation of collagen as well as precollagen in the actual floor of the ulcer. (Usually the intercellular substance of granulation tissue exposed to an infected space is entirely precollagenous in nature, whether that space is an abscess or the internal or external surface of the body.)

Case 17.—J. B., an under-nourished boy of poor physique, aged 17. Admitted July 14, 1938, in a state of severe shock, having been crushed between two motor cars. On recovery

FIG. 272.—Cross-section of the sutured skin incision in *Case* 16. (× 16.) (Stain, silver impregnation.) There is exact apposition of the cut edges. Little precollagen remains. (Cf. *Fig.* 273.)

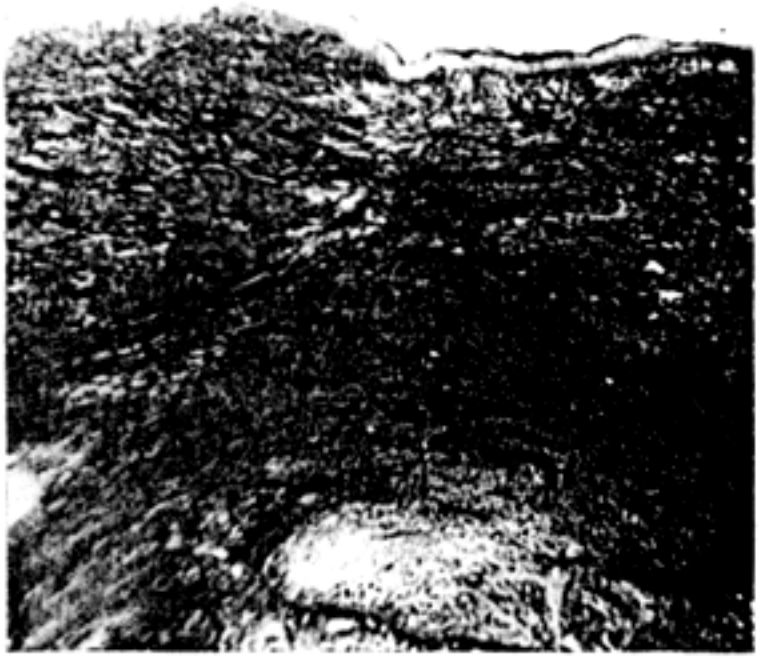


FIG. 273.—Cross-section of the sutured skin incision in *Case* 17. (× 16.) (Stain, silver impregnation.) The opposed edges of the corium are stretched apart and the gap filled by immature precollagenous scar. (Cf. *Fig.* 272.)

from the shock it became evident that a viscus had been ruptured. The abdomen was explored through a right paramedian incision and a circumferential tear, 2 in. long, was found on the antimesenteric border of the jejunum 6 in. from the duodenojejunal flexure. It was closed with thread sutures and oversown. The peritoneum was drained through a separate suprapubic stab incision. The peritoneal fluid grew both aerobic and anaerobic organisms, but the patient made good progress. Intravenous therapy was continued till July 19, and on July 22 the stitches were removed. The wound was partially infected and gradually gaped open, although the abdomen was only moderately distended. On July 27, 12½ days after the accident, there was sudden severe abdominal pain, more on the left side than on the right, suggestive of perforation of a viscus. A second drainage of the peritoneum was done through a left muscle-splitting incision. The patient died on July 28, 13 days after the first operation.

Necropsy was done on the same day within a few hours of death. The abdomen was moderately distended and the incision was gaping open for some of its length, with loops of adherent small intestine effectively closing the gap. At either end the incision was stretched without separation of the edges and without macroscopical infection. The peritoneum showed diffuse plastic peritonitis of recent origin, with localized abscesses of longer standing. The suture line itself formed a linear ulcer 1 cm. in width, traversed by thread sutures lying partly free in the lumen of the jejunum, with a perforation 1 mm. across communicating with the general peritoneal cavity.

The boy had been given no vitamin C and had received nothing but intravenous fluids for most of the time he had been in hospital. The Szent-Györgyi test indicated a mere trace of vitamin C in the tissues.

Microscopically the skin incision, where best healed, showed complete epithelialization, but the opposed surfaces of the corium were stretched apart and the gap filled, under the new epithelium, by a mass of proliferating fibroblasts producing precollagen only (*Fig.* 273). Deeper, this granulation tissue was very vascular and was producing small quantities of immature collagen as well as precollagen. There was no pus formation and but little infiltration by mononuclear cells. In other regions where the scar was even more stretched, the gap was filled by nothing more than loose-textured œdematous granulation tissue in which there was little precollagen and no collagen.

The intestinal suture line, from lumen to serosa, consisted of similar unhealthy granulation tissue broken up by collections of œdema fluid and microscopical abscesses. In no place was the healing satisfactory, except from the point of view of cellular proliferation, and it gave the appearance of imminent breakdown along its whole length. The picture, both in the abdominal incision and intestinal suture line, reproduced almost exactly the appearances in the sub-scurvy group of guinea-pigs.

DISCUSSION AND CONCLUSIONS

It has been shown experimentally on guinea-pigs that a partial deficiency of vitamin C produces a most profound disturbance in the healing of clean wounds. When there is sufficiency of the vitamin, the mesodermal cells build up a mature vascular scar, composed of fibrocytes, collagen, and small blood-vessels, within 14 days. In scurvy the only intercellular material produced is fluid and amorphous. In the intermediate state of sub-scurvy, when the deficiency is severe though not clinically evident, the scar matrix remains immature and of poor holding power, though it is produced in adequate amounts. (Cf. the two graphs, *Figs.* 261, 262.) The divergence between the tensile strength of the healing wound and the cellular proliferation becomes most marked on about the tenth day after operation, the time when wound disruption is commonest. The quantitive relationship between the vitamin-C content of the tissues and the maturation of the intercellular substance has not been worked out. It is not known whether a state of saturation is necessary for the most rapid and effective deposition of collagen, or whether a vitamin content far short of saturation is enough.

As a concomitant of this defect in collagen production the whole architecture of the healing wound is disturbed. The proliferation of fibroblasts, at first apparently normal in rate, continues so long as the scar production is abnormally retarded. The cells remain immature. Blood-vessels do not readily penetrate this ill-formed granulation tissue. Hæmatomata are not organized or absorbed, and the scar becomes split up by further extravasations of blood-stained œdema fluid. The phagocytosis of damaged tissues is delayed and the supporting structures bordering upon the incision are not satisfactorily incorporated in the newly-formed scar. These secondary effects may well be due to a disturbance of the blood-supply or vascular permeability, but this is itself directly or indirectly due to the deficiency of vitamin C. It is further suggested that the absorption of catgut ligatures may be delayed.

When scurvy supervenes on a recently healed wound, the newly-formed collagen of the scar has been shown to revert to precollagen. Yet the established fibrous tissue bordering the incision, from which the recently laid down scar is barely distinguishable before the advent of scurvy, does not revert in this manner (*Fig.* 269). This indicates that our present methods of demonstration are inadequate to show the finer differences between the various states of white-fibrous-tissue fibres and that scars do not fully mature for many months at least. The ready reversion of the collagen of scars to its immature and weaker form offers an explanation of the breaking down of healed wounds in scurvy (*Fig.* 269).

Studies of human wounds are of necessity less well controlled and lead to less definite conclusions than animal experiments. It should be remembered that when attention is focused on one aspect of the problem, greater care is bestowed on all the other factors concerned. It is, therefore, of no scientific value to state that wound disruption has been reduced by about 75 per cent since the routine administration of ascorbic acid has been adopted for all major abdominal operations. This, however, has been the case, and leakage from suture lines has occurred in but one of a large number of operations during the past 30 months. When wounds have thus failed to heal in spite of adequate supply of the vitamin, there always was gross local infection, with or without a hæmatoma, or ischæmic necrosis. This emphasizes the importance of ordinary studied operative technique and meticulous post-operative care. The mechanical strains of coughing and vomiting and of abdominal distension must be minimized until the wound is fully healed.

In the 28 human cases which were studied post mortem, 10 died within the first week when collagen production is not to be expected. Of the remaining 18, those most deficient in vitamin C showed poorest collagen production. In 5, the failure to form scar tissue was a contributary cause of death, by facilitating the breakdown of the abdominal wound, leakage from the suture line, or both. In 4 of these 5 cases the disruption occurred between the eighth and thirteenth days. It is significant that wounds most commonly break down on about the tenth day, and it is possible that, by saturating all patients with vitamin C, this major bloc of wound disruptions may be eliminated. Leaks and disruptions due to other causes were found to occur earlier or later than this. However, the cases are not numerous enough and the control of the vitamin-C content of the tissues not accurate enough for definite conclusions to be reached on this evidence alone. Taken in conjunction with the animal experiments, the results are presented as being very suggestive that a partial deficiency of vitamin C produces effects which are as important in the healing of human wounds as in those of guinea-pigs.

The human experiment showed that there was no difference between wounds made before and after the administration of ascorbic acid. Crandon and Lund[9] induced a marked deficiency in a healthy human subject in only three months and showed that a wound healed well. Isolated observations such as these suggest that the critical level for vitamin C in the formation of collagen is probably well below saturation point, though they should not be regarded as conclusive. Short-lived deprivation in an otherwise healthy individual does not compare with patients suffering from chronic illnesses with long-standing deficiencies.

The relationship between local infective processes and vitamin-C concentration has, unfortunately, not been investigated histologically. By analogy with the clean-wound healing experiments, it is reasonable to presume that all subacute and chronic infective lesions would be assisted in their healing by saturation with the vitamin. Evidence to support this contention from the clinical standpoint is to be found as far back as 1924, when Höjer[21] noted that the progress of cases of pulmonary tuberculosis was materially improved by the addition of one orange daily to the diet. Healing, after all, is a single process, whatever the tissue and whether infection is present or not. All patients, therefore, who suffer from localized chronic infective diseases, particularly tuberculosis and residual osteomyelitis, are advised to modify their diets, if they are not already satisfactory, so as to consume more fruit and vegetables and to cook these foods so as to conserve as much of the vitamin as possible. This change in diet should be permanent.

It is not the purpose of this paper to discuss wound healing apart from its relationship with vitamin C, but it is thought advisable that other factors should be briefly mentioned.

Hypoproteinaemia and the Administration of Parenteral Fluids.—Poor cellular proliferation occurs commonly, and was found in 5 out of the 28 human cases studied histologically. Epithelium and mesothelium are both concerned, so that the separated edges of the wound (even if it has broken open as late as in the third week) may appear as if the incision had just been made. Hypoproteinæmia is partly responsible for this lack of cellular response.

In such cases it is of little avail to saturate with vitamin if the lack of protein is not also attended to. When fluids are being administered entirely by the rectal or intravenous routes, there is a tendency to give blood only when the hæmoglobin falls below a certain arbitrary level. Such cases may not be deficient before operation, but may develop hypoproteinæmia after the intravenous therapy has begun and in the absence of anæmia. The blood-proteins should, therefore, be repeatedly estimated and maintained.

Œdema consequent on protein deficiency is of particular importance at gastric and intestinal anastomoses where trauma and infection already predispose to an inflammatory œdema. To aggravate the condition further will certainly interfere with the healing.

Ligature Material.—Ligature material also affects the appearance of wounds, though no instance suggestive of catgut sensitivity[25, 33] has been seen.

Local Applications.—The bombing of London has unfortunately provided opportunities for testing out the efficacy and effects of the sulphonamides in potentially infected wounds. The powder, sprinkled into the excised wound, indubitably delays healing. The experimental work of Bricker and Graham[6] supports this finding, whereas Mayo and Miller[30] consider that a saturated solution of sulphanilamide in normal saline stimulates healing.

Administration of Vitamin C Before and After Operation.—1000 mg. of ascorbic acid, given daily for three days, is enough to produce saturation even in a grossly deficient patient, and 100 mg. daily to maintain it. These doses are increased in cases of infection, new growth, chronic gastro-intestinal disease, and achlorhydria. The daily dose should be divided so as to minimize the rapid excretion due to the low renal threshold. It should be combined with dilute hydrochloric acid when achlorhydria is present or suspected, as in carcinoma of the stomach and ulcerative colitis. It should be given intravenously or intramuscularly when circumstances are such that it cannot be given by mouth. In intravenous drip infusions, a maximum of 200 mg. of sodium ascorbate (' redoxon ') is added to each pint. The saline should have been recently heat-sterilized to drive off the contained air. Ascorbic acid itself is more easily destroyed and is more irritating to the intima of the vein than the sodium salt. Both these substances produce considerable local irritation when given by intramuscular injection, but it has been suggested that the mono-ethanolamine salt can be given safely and effectively by this route without producing any immediate or delayed systemic or local reaction.[28]

The normal intake of vitamin C should be 50 mg. daily, though this will not produce saturation of the tissues. Few of the poorer classes of this country consume more than a fraction of this amount and yet the majority remain in the best of health. However, a good physique and a ruddy complexion is no criterion of the healing power of the tissues. Vitamin C has been shown to be of the greatest importance in wound healing, though it is not a panacea. The present paper has stressed its value particularly in the healing of soft-tissue scars, but it is of equal value in the laying down of other intercellular substances such as bone. It should be administered: (1) when clean and quick healing of the wound is particularly desirable, (2) in major abdominal operations, (3) when a hollow viscus has been opened, (4) when post-operative complications are anticipated, (5) where there is evidence of a nutritional deficiency, and (6) in all cases of serious injury. On the evidence at present available, these patients should be saturated, if possible before operation. In emergencies, saturation point should be reached before the seventh day.

SUMMARY

1. Collagen does not form in the healing wounds of guinea-pigs partially deficient in vitamin C. The intercellular substance remains immature and of poor holding power.

2. The proliferating mesodermal cells also fail to mature.

3. The healing of the wound as a whole is profoundly disturbed in this experimental sub-scurvy.

4. The collagen of normally healed wounds reverts to precollagen if scurvy supervenes.

5. A series of 28 human cases were studied post mortem. In 8 the wounds showed a deficiency in the formation of collagen resembling that in the sub-scurvy guinea-pigs.

6. In these 8 cases, there were 5 instances of abdominal disruption and 3 of leakage at gastro-intestinal suture lines.

7. Vitamin C, in good concentration, is of the greatest importance in wound healing.

I am indebted to the trustees of the Luther Holden Research Scholarship and the Medical Research Council for financial assistance; to Professor Hadfield for providing facilities in the Pathological Department of St. Bartholomew's Hospital; to Mr. A. M. Boyd for his co-operation in the human experiment; to Dr. T. D. Cuttle and Dr. Arthur Jordan for the blood ascorbic acid estimations; and to Miss Peiser for the admirable photomicrographs.

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