VITAMIN C AND REPAIR OF INJURED TISSUES

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The evidence that adequate vitamin C is necessary for repairing damage to both soft and hard tissues is now considerable, though not widely known. Supplies of vitamin C from natural sources cannot be obtained as easily now as they could before the war, and since a deficiency of this vitamin seems likely to retard healing of wounds and it is important to secure rapid repair of war injuries it seems timely to present concisely the information available.

In the older medical writings there have been references to the breaking open of old wounds and the refracturing of old fractures in scorbutic persons. The latter finding has been criticised lately (Hertz 1936). Bleeding gums, loosened teeth, and petechial hæmorrhages are often mentioned in the early papers on scurvy. Though considerable clinical information about the disease has been available for many years it was not until the first twenty years of the twentieth century that the pathology of scurvy was extensively studied. Hölst and Fröhlich (1907) were the first to produce experimental scurvy in animals (guineapigs); they briefly described the pathology. This was followed by the work of Hart and Lessing (1913) who produced experimental scurvy in monkeys. In 1919 Aschoff and Koch published an account of the microscopic pathology of scurvy and in 1923 Höjer's comprehensive work on the histology of experimental scurvy appeared. These two papers showed that, as Hess noted in 1920, from a pathogenic point of view guineapig and human scurvy have many points in common.

These publications showed that there was a widespread degeneration of body tissues, the skeleton being severely affected. The cells of cartilage were degenerated and the proliferating cell columns disordered. Bones became deformed in shape and the marrow changed to a mucoid substance (Gerüstmark). The cortex of long bones became a thin shell as if absorption of calcium was still going on from the inside of the bone but that deposition on the outside had ceased. Microscopic changes in the teeth were first observed by Zilva and Wells (1919). There was fibroid degeneration of the pulp and disappearance of the cement substance. The teeth also became loose, no doubt owing to the degeneration of the collagenous fibres of the dental periosteum which hold the teeth in position in the jaw (for further details see Fish and Harris 1934).

COLLAGEN PRODUCTION

The most constant change in scurvy, noted by Höjer, was atrophy of the connective-tissue fibres in various parts of the body; this was confirmed by Wolbach and Howe (1926) and others and by the finding of Jeney and Törö (1936) that if vitamin C was added to the culture medium in which fibroblasts were being grown fibres were produced more rapidly. Mazoué (1937) showed by similar experiments that vitamin C was an active factor in the production of connective tissue fibres, and in 1939 he analysed the rate of production of collagen fibres around a mass of kieslguhr injected peritoneally into a guineapig and demonstrated a direct association between the dose of vitamin C and the time of the first appearance of fibroblasts and fibres.

Quérido and Gaillard (1939) demonstrated the relation between the vitamin and production of collagen fibres by culturing osteogenetic cells from the chick in three types of plasma: (1) from scorbutic guineapigs; (2) from scorbutic guineapigs but with crystalline vitamin C added; (3) from scorbutic guineapigs which had been treated with large doses of vitamin C for a few days before the experiment. Hardly any fibres were formed by the cells in the first plasma, but in the second and third fibres were formed as rapidly as in normal plasma. The cells in the scorbutic plasma showed fatty degeneration. Hunt (1941) has shown that although in scorbutic animals reticular (precollagen) fibres may be formed, in the continued absence of vitamin C maturation of these precollagen fibres is delayed; further, mature collagen may retrogress—for example, in a scar—if vitamin C is subsequently withdrawn from an animal's diet. Precollagen has a low tensile strength, and according to Hunt wounds containing mainly precollagen fibres have poor holding power.

Collagen fibres can be reduced by physical means to a condition resembling precollagen (Picken 1940). If for example collagen fibres are heated to 65° C. they contract and become reversibly extensible. When stretched and then cooled while in the stretched condition they do not contract again, and on further stretching they "flow" like unvulcanised rubber. This change in elasticity and in tensile strength therefore involves some change in the linkage of molecules to one another. According to Schmidt (1939) collagen fibres are made up of longitudinally orientated polypeptide chains bound together by side-chain linkages. It is on the length of these side-chains that the tensile strength of the fibre depends. That there is also some chemical difference between precollagen and collagen is suggested by Heringa and Weidinger (1940) who claim that reticular fibres in general contain more sulphur than collagen fibres (2–0·5%) and that this explains the affinity of reticular (precollagen) fibres for silver salts. It is difficult to imagine what part vitamin C plays in the transformation of precollagen to mature collagen. Presumably it has some effect on the attachment of side-chains; whether it does so indirectly, or directly by being involved in the structure of the fibre, it is difficult to say. If it is incorporated in the fibre it must lose its identity as a reducing substance, for connective tissue in general has a low vitamin-C content. The vitamin-C molecule has a structure resembling that of the hexose sugars and it is of interest that sugars have been found to be present in collagen. According to Beek (1941) the sugars may be the "*l*" isomers of *d*-glucose and *d*-galactose. Whatever the part played by vitamin C in the production and maturation of collagen fibres there seems little doubt that its presence is essential for both processes.

There are two principal views about the part played by vitamin C in the formation of intercellular fibres. The first, held by Wolbach and Howe (1926), was that "the failure of cells to produce intercellular substances in scorbutus is due to the absence of an agent common to all supporting tissues which is responsible for setting or jellying of a liquid product." This was supported by Dalldorf (1938). The second theory is that failure to form intercellular substances is due to an upsetting of the metabolism of the formative cells (Ham and Elliott 1938, Fish and Harris 1934). In the absence of vitamin C from a tissue there is certainly little development of collagen fibres, but we know little about the way collagen fibres are produced. Most workers appear to think that they are formed in association with cells—for example, fibroblasts. Dolianski and Roulet (1933) and Jeney and Törö (1936) claim, however, that in explants of fibroblasts collagenous fibres can form in parts of the medium free from cells, but there is no evidence that the fibres they observed were, in fact, collagen fibres. Danielli (1942) has pointed out that fibres which have an X ray structure similar to collagen can be spun from protein monolayers, and he suggests that tissue fibres may be formed by spinning from a layer of protein adsorbed on the surfaces of fibroblasts. If this view is accepted one must assume that in vitamin-C deficiency either the motility of the fibroblast is affected (possibly through some interference with its metabolic processes), so that it is unable to spin such fibres from its surface, or that there is a failure on the part of the fibroblast to adsorb protein, perhaps because of some alteration in its membrane or in the physical nature of the proteins which are normally absorbed there.

WOUND HEALING

One of the first experimental investigations into the effects of vitamin-C deficiency on wound healing was made in 1923 by Ishido, who found considerable delay in the healing of experimental wounds in scorbutic guineapigs. Saitta (1929) found delayed healing of wounds in guineapigs fed on a vitamin-C-deficient diet and showed that the application of vitamin-C extract to the surface of the wound increased the rate of healing whether the animal was kept on a normal or a scorbutic

diet. Lauber (1933) also found external application of the vitamin to wounds in mice accelerated healing. He found that, however, injections of vitamin C into normal guineapigs had no influence on the rate of healing of wounds. Against these results Proto (1936) found that injection or local application of vitamin C into normal pigeons did not affect wound healing. Mazoué (1937) found that vitamin-C deficiency had a profound effect on the organisation of a clot produced by severing the limb muscles of guineapigs. Lanman and Ingalls (1937) carried out direct measurements of the force required to break wounds in normal and scorbutic animals. Their measurements were made by distending the abdomen with air and noting the pressure, on a mercury manometer, at which the wound burst. Similar observations were made on wounds in the stomach wall. The pressure, in mm., at which rupturing took place was as follows:

	Animals receiving—	
	2 mg. vit. C	0·5 mg. vit. C
Abdominal wounds	160	65
Gastric wounds	70	30

Similar experiments have been carried out by Taffel and Harvey (1938).

Bartlett and others (1942a) found in guineapigs that the strength of the scar was proportional to its vitamin-C content. For example, the scars of animals receiving 33 mg. of vitamin C twice daily had on average 7·64 mg. of vitamin C. per 100 g. of tissue and burst at a pressure of 258 mm. of mercury. In guineapigs on a scorbutic diet the vitamin-C content of the scars averaged 0·31 mg. per 100 g. and they burst at 127 mm. of mercury.

I have also carried out tests on the tensile strength of wounds (measured by the maximum weight each excised scar could support) made by a complete incision through the skin over each femur of 15 guineapigs, a week after they had been on a scorbutic diet with daily supplements of vitamin C as shown below; there were 3 guineapigs in each group. The wounds were allowed to heal for a week while the animals were maintained on the scorbutic diet with the same vitamin-C supplements. The results were:

Doses of vitamin C injected subcutaneously (mg.)	2	1	0·5	0·25	0
Av. in g. at which scar broke	283	162	154	60	46

It may be seen that there are two dosage levels where there is a distinct drop in the tensile strength of the wound: between 2 and 1 mg., and between 0·5 and 0·25 mg. The strength of the wound at 0·5 mg. is about half that at 2 mg.; this result corresponds fairly well with those of Lanman and Ingalls. In the group receiving no vitamin C the figure represents the results obtained from only one animal. In the other two the scar was so loose that it broke across during excision. It would be worth while knowing whether the guineapig doses of vitamin C can be interpreted in terms of human requirements. Bourne (1942b) concluded that 2 mg. of vitamin C for a guineapig may be approximately equal to 40 mg. for man. These results therefore mean that in a person already saturated with vitamin C 40 mg. of the vitamin may be required each day to secure adequate healing of wounds and that less than 20 mg. may produce a scar of low tensile strength. Other investigations on the strength of scars resulting from skin wounds in guineapigs on normal and deficient amounts of vitamin C have been obtained by Hartzell and Stone (1942). On the 6th postoperative day they found the strength of the wound in sub-scurvy animals was only half the normal. From the 8th to 14th days it was a quarter normal.

Hunt (1941) has treated human surgical cases with vitamin C, giving 1000 mg. of ascorbic acid daily for 3 days to saturate the patient and 100 mg. a day to maintain it. Other workers have stressed the importance of adequate vitamin C for healing wounds; Lauber and Rosenfeld (1938) showed by silver nitrate staining the presence of vitamin C in healing wounds and its absence in the wounds of scorbutic animals; Wolfer and Hoebel (1940), Holman (1940) and Bartlett and his colleagues (1940) stressed the importance of vitamin C for surgical patients; and Lund and Crandon (1941b) in 58 patients having operations on the biliary tract found the incidence of postoperative hernia greatest in patients having low values of plasma vitamin C.

Crandon, Lund and Dill (1940) showed that in a man placed on a completely vitamin C free diet for 3 months a wound in the mid-back had healed quite well after 10 days, but that a similar wound made after 6 months on the diet showed no signs of healing after 10 days; there was a lack of intercellular substance and under the skin the wound was filled with an unorganised blood-clot. Thus after 3 months on a scorbutic diet a wound, judged by macroscopic and histological evidence, had apparently healed quite normally; but we do not know the holding power of such a wound. We have evidence however, from the work of Bartlett and others (1942b), that if the plasma vitamin-C level falls below 0·20 mg. per 100 c.cm. there is likely to be reduced tensile strength in healing wounds in human beings. As Hunt's work has made clear there may be plenty of intercellular fibres present in a healing wound, but if most of them are composed of immature collagen the scar will have poor holding power and that is the really relevant point as far as normal surgery is concerned. The same argument applies to the observations of Fox (1941). In addition, Crandon and his colleagues were reporting an acute experiment. What we need to know and what we do not know is the effect of suboptimal doses of vitamin C for periods of 30–40 years, and in particular in babyhood and childhood, on the reparative powers of tissues. Pfab (1927) has claimed that inferior nutrition during childhood will cause fractures incurred in later life to heal more slowly, but we have no real proof at present that this is so.

Not only is the growth and migration of fibroblasts affected in healing wounds in vitamin-C deficiency but phagocytosis is also inhibited. Hunt (1941) has stated that deficiency of the vitamin delayed the removal of catgut ligatures "either by phagocytosis or extrusion." Messina and Varga (1937) had previously shown that vitamin C increases phagocytosis in vitro and Tonutti and Matzner (1938) have shown that cells engaged in phagocytic activity in the body including those phagocytosing catgut sutures can be shown by silver nitrate staining (for specificity see Barnett and Bourne 1941) to contain relatively large quantities of vitamin C in their cytoplasm. I have found that in healing skin-wounds of rats these silver-nitrate staining cells are sometimes present in the scar tissue within 3 or 4 days of wounding.

REPAIR OF BONE

In the first stages of the repair of bone, cellular proliferation and production of fibres similar to collagen fibres are essential preliminary processes to calcification, and it is to be expected, therefore, that a deficiency of vitamin C would delay healing in bone. It has been shown (Bourne 1942b) that, in guineapigs with injured femurs taking a vitamin-C-deficient diet, the degree of healing was proportional to the amount of vitamin C given. In the completely deficient animals cells in the cambial layer of the periosteum failed to multiply and fibroblasts did not migrate into the fibrin clot surrounding the injury; in cases where fibroblasts were present fibre formation was reduced or absent and maturation of fibres (estimated by their ability to stain with van Gieson) was delayed. In general the normal inflammatory reaction of injured tissue was inhibited, and the change of fibroblasts into osteoblasts was also delayed. McLean and his colleagues (1939) also found delay in the differentiation of mesenchymal cells to osteoblasts. Hertz (1936) noted a deficient production of collagen and a delayed absorption of the hæmatoma at the site of a fracture (see also Ferraris and Lewi 1923, Watanabe 1924, Wolbach and Howe 1925, 1926). In partial deficiency of vitamin C there is a tendency for collagenous material to appear instead of osteoid (Dalldorf 1938).

These changes in scorbutic animals suggest that fracture healing would be delayed in scurvy and this has proved to be the case. Earlier writers were concerned with the fact that old healed fractures softened and became mobile in scurvy (Mead 1762, Marrigues 1783, Bell 1788, Callisen 1798, Hammick 1830, Budd 1840), though it seems that they were misled by fresh fractures or separated epiphyses into this belief. Moore in 1865 stated that fractured femurs would not consolidate while patients had scurvy. Bloch (1912) described scurvy as one of the causes of non-union of fractures. Lobmayer (1918) reported delay in fracture healing in scurvy.

Shinya (1922) found adequate vitamin C necessary for successful bone-grafting. Ferraris and Lewi (1923) found inhibition of the cellular activities involved in healing a fracture. They observed lack of absorption of dead material, lack of formation of osteoid trabeculæ and of cartilage. Watanabe (1924) found delay in the healing of fractures in scorbutic guineapigs but not in rats (rats synthesise their own vitamin C). Other workers (Wolbach and Howe, 1925, 1926, Israel and Frankel, 1926, Schilozew 1928, Roegholt 1930, Jeney and Korpàssy 1934, Hanke 1935, Hertz 1936, Lauber et al. 1937, Ham and Elliott 1938, McLean et al. 1939, Lexer 1939, Bourne 1942) have obtained similar results. In general they have shown that there is delayed absorption of the fracture hæmatoma, reduced fibroblast activity and deficient production of collagen. Bourne (1942b) has shown that optimum formation of bony trabeculæ in injured femurs of guineapigs is brought about by the administration of 2 mg. daily of vitamin C (probably equivalent to about 40 mg. for a human being) and that anything less than 1 mg. (human equivalent about 20 mg.) seriously retards the formation of bony trabeculæ.

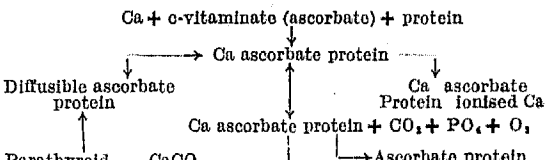
It is impossible then to resist the weight of evidence that vitamin C is essential for the formation of the fibrous ground substance of bone and it is now necessary to consider whether an amount of vitamin C in excess of that required to saturate an animal would further accelerate bone healing. Halàsz and Marx (1932) have shown that guineapigs given an amount of vitamin C far in excess of their normal requirements did not regenerate bone any faster than they did when it was given in normal amounts. Studies to decide whether rabbits regenerate bone more rapidly on a diet containing ample vitamin C has given some unexpected results. Rabbits are believed to synthesise their own vitamin C and yet Hanke (1935) found that if rabbits were placed on a scorbutic diet although they regenerated bone completely, the rate of regeneration was much slowed. Lauber and his colleagues (1937) stated that once the normal requirements of a rabbit were satisfied extra vitamin C had no further effect on the rate of regeneration of injured bone. It is difficult to appreciate that a rabbit has a normal requirement for vitamin C if it synthesises the vitamin. Giangrasso (1939) and Giangrasso and Gangitano (1939) were able to secure more rapid regeneration of injured bone in rabbits by giving vitamin C. The only obvious explanation of these facts is that a normal rabbit can synthesise its own vitamin C but that the healing of a fractured bone calls for more vitamin C than the animal is able to manufacture.

In 1 mm. holes bored in the femurs of rats I found (Bourne 1942a) that vitamin C did not have any accelerating effect on regeneration of bony tissue. Nor in similar holes in guineapigs (Bourne 1942b) was further acceleration of healing observed when amounts of vitamin C in excess (4 mg.) of the optimum requirement (2 mg.) were injected. The general conclusion is that there is an optimum amount of vitamin C required for the regeneration of injured bones and that possibly more extensive injuries require greater amounts of the vitamin (e.g., the rabbit work was performed with fractures and extra vitamin C accelerated healing, my work was performed on rats with small 1 mm. holes in the bones and extra vitamin did not accelerate healing) and that vitamin C in excess is of no value in further accelerating healing.

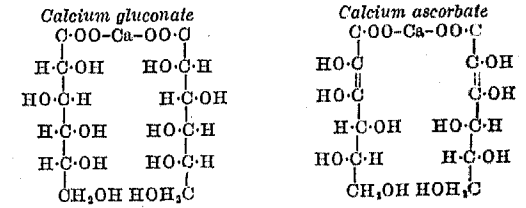
THE PROCESS OF CALCIFICATION

There is thus evidence that vitamin C plays an important part in the production of an inflammatory reaction to injury, to organisation of the clot and to formation of the ground structure of the trabeculæ after injury to the bone. The next question to be considered is whether the vitamin plays any part in the process of calcification either directly or through its association with phosphatase activity. Calcium has been found to be deposited in various tissues in scurvy but it is deposited in an amorphous form. This is probably due to the absence of an adequate fibrous matrix. Fish and Harris (1934) point out that in the teeth in scurvy calcium salts may be deposited but that there is no matrix available for their reception. Bone salt in normal bone is laid down in a crystalline form, in other words the molecules have a specific orientation which is absent from the amorphous masses produced in scurvy. Schmidt (1934) suggests that when collagen fibres are first formed they adsorb precipitated inorganic salts and he points out that such fibres possess the ability to orientate particles precipitated in their presence. It seems fairly certain from published work that there is a positive calcium balance in scurvy (Hess's (1920) summary of the work of Baumann and Howard). The same finding has been made for adult human scurvy (Lust and Klocman 1912, Moll 1919), infantile (Moll 1919) and monkey scurvy (Howard and Ingvaldsen 1917). Lust and Klocman found a positive balance in the scurvy stage and a negative balance in the healing stage. This suggests that in scurvy the calcium may not be deposited on the bones but is being excreted instead, and that in the healing stage the calcium is being used up for deposition on bones, so that excretion falls (Salter and Aub 1931). Barht and Edelstein (1913) found a diminished calcium content of the bones of a scorbutic infant, but Kapp and Schetty (1937) found that the mineral content of bones remained normal in scurvy. Höjer (1923) stated that his histological investigations gave no reason to suppose that the calcium metabolism is primarily disarranged in scurvy. He stated that dying tissue generally has a greater affinity for calcium and that this explains the calcification in late scurvy.

Humphreys and Zilva (1931) found that calcium and phosphorus retention by guineapigs was lowered in scurvy but only in the last stages of the disease when the whole metabolism was affected. Matricardi (1938) found that blood phosphate fell in scurvy and Lucké and Wolf (1938) claimed that extra vitamin C administered to an animal led to a greater retention of calcium and phosphorus but had no effect on the serum calcium and phosphorus. If one adrenal of an animal is removed there is an upset of the calcium and phosphorus balance which is restored by the administration of vitamin C (Lucké and Heckman 1938). Lanford (1939) has claimed that orange juice added to the diet of rats caused increased calcium retention. Against these observations Henry and Kon (1939) have found that the addition of 2 mg. of vitamin C daily to rats had no influence on the retention of calcium. Ruskin (1938) and Ruskin and Jonnard (1938) hold that there is an intimate association between vitamin C and calcium, believing that calcium is absorbed from the intestine as calcium ascorbate. This compound they find to be highly ionised, non-toxic by injection, and to be adsorbed on to the blood proteins much more strongly than other salts of calcium. Below is shown a presumptive scheme drawn up by Ruskin for a calcium metabolism which involves vitamin C.



I have found (Bourne 1942a) that if calcium ascorbate is injected into rats it accelerates healing in holes previously bored in their femurs. Calcium gluconate, even in the form of the glucono-galacto-gluconate, was ineffective. This is surprising in view of the chemical similarity between calcium ascorbate and calcium gluconate; but to some extent it can probably be explained by the greater degree of ionisation of the ascorbate, which presumably secures more effective absorption of the calcium from the subcutaneous tissues and possibly to its greater protein-combining power. It is of interest that the double-bond structure of the ascorbic acid is retained in calcium ascorbate and that this substance is therefore an antiscorbutic as well as a source of calcium.



That there is a connexion between vitamin C and calcification of bone is suggested by the work of Salter and Aub (1931). They showed that in guineapigs on a scorbutic diet and injected with sodium alizarin sulphonate (which forms a coloured lake with calcium ions so that freshly deposited calcium is stained) the bones of the animals were stained red indicating that calcium deposition was proceeding normally. In scorbutic guineapigs they found the bones unstained. Deficiency of vitamin C therefore causes inhibition of the mechanism which results in the deposition of calcium in bones. I have been able to support this result and have found also (Bourne 1942c) that in guineapigs receiving injections of sodium alizarin sulphonate and with small holes bored in the femurs, the repair tissue stained strongly in those receiving injections of 2 mg. daily of vitamin C for 2 weeks, less intensely in those receiving 0·5 mg. and not at all in those receiving no vitamin C.

The work of Gould and Shwachman (1942) suggests that vitamin C may play some part in the deposition of calcium by means of its effect on the phosphatase system of bone. They found a lowered phosphatase content of the bones of guineapigs in scurvy; and I have found (Bourne 1942c) that in scurvy the bones of guineapigs stain less intensely with Gomori's phosphatase technique than those of normal animals and that the repair tissue filling a small hole in the femur of a scorbutic guineapig also stains much less intensely than similar tissue in normal guineapigs. It seems, therefore, that vitamin C may play three important rôles in the regeneration of bone: it may promote activity of fibroblast cells and possibly their differentiation into osteoblasts; it may favour the attachment to each other of the polypeptide chains which make up the constituent collagen fibres of the bone matrix, and by promotion of phosphatase activity it may aid in the precipitation of bone salts.

SUMMARY

From the results presented in this paper it appears that vitamin C plays a fundamental part in the regeneration of tissues and for this reason it seems that the administration of vitamin C should become a routine in the treatment of any injury. Preliminary results with animals (Bourne 1942a) suggest that calcium ascorbate may prove a valuable therapeutic substance for the treatment of fractures, but clinical trials with this substance have not yet been carried out.

I wish to thank Roche Products for the vitamin C 'Redoxon') used in the wound healing and other experiments carried out by the present author quoted in this paper.

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INFECTED BURNS IN NAVAL PERSONNEL

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WE wish to report on a single series of 30 cases of severe multiple burns selected from a larger series of cases, since they occurred at the same time and were subjected to similar transportation. All the cases were severely burned, mainly as the result of cordite and other high temperature flash; the face, including the eyelids, and the hands were particularly affected. The eyes themselves were only slightly damaged in a few instances, protection being achieved by the very rapid, involuntary reflex closing of the lids and the added shielding by the hands. The ears also suffered. The legs, thighs and trunk were involved in varying degree.

The cases fall naturally into two groups in an experiment devised unwittingly by the medical officers concerned with early treatment. In one group (20 cases), tannic acid was employed throughout; in the other group (10 cases), tannic acid (5% aqueous solution, one application in compresses) having proved inadequate, dressings of cod-liver oil were used after cleansing. On admission to hospital, 8 of the 20 cases treated with tannic acid were not infected, 4 were moderately infected at the margins, and 8 were so infected as to require cleansing and retanning; of these last, 2 later became reinfected. All 10 treated with cod-liver oil were infected, 5 moderately so; the other 5 were cleansed and retanned (tannic acid), and of these 3 became reinfected, only 2 not becoming septic again.

ONSET OF INFECTION

The face and eyes became infected early, usually within the first four days and in all cases within the first week; the limbs were usually involved slightly

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