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Ascorbic Acid and Epithelial Regeneration

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In animals suffering from deficiency of ascorbic acid the healing of incised wounds is delayed and imperfect because of the defective formation of collagen (Wolbach & Howe, 1926; Crandon, Lund & Dill, 1940; Hunt, 1941; Bourne, 1944).

But not all wounds are incised; there may be also loss of epithelium. So far no attention seems to have been paid to the effect of lack of ascorbic acid on epithelial repair.

EXPERIMENTAL

Animals

Most of the work was carried out on guinea-pigs. To find if consumption of an excess of ascorbic acid affected wound healing, rats were also used, although rats cannot be made to suffer from deficiency of ascorbic acid.

Diets

All animals received a basal diet of rat-cake nuts (Thomson, 1936). These nuts are free from ascorbic acid. Control guinea-pigs received daily 5 mg. of ascorbic acid in solution by mouth. This dose kept the guinea-pigs in perfect health; immature animals grew normally on this diet. The guinea-pigs deficient in ascorbic acid received 0·5 mg. ascorbic acid every 2nd day. In these animals signs of deficiency appeared in about 14 days, but the dose was sufficient to keep them alive and fit to bear the operative interference.

Wounding

Removal of corneal epithelium. This operation was carried out only in guinea-pigs. Ether anaesthesia was used and Holocaine (Bayer Products Ltd.) dropped into the conjunctival sac. A 2% solution of fluorescein was also placed in the conjunctival sac

and excess washed off with a stream of Ringer's fluid. In daylight, but more especially in ultraviolet light, injured areas of the corneal epithelium then showed a brilliant green fluorescence.

A size 10 dental flat-ended fissure bur, driven by a dental engine, was used to make the lesion. The flat cutting end was applied with firm, steady pressure just sufficient to 'dimple' the cornea. Such pressure, as subsequent histological examination showed, removed only the epithelium, leaving Bowman's membrane intact.

Gum wounds. These also were made in guinea-pigs only and under full anaesthesia. A trephine, 3 mm. diam., without serrated edge, and driven by a dental engine, was used to cut a cylinder of muco-periosteum right down to bone. Fine forceps were used to pluck out the cylinder. The wounds were made in the diastema of the upper jaw or in the gum below the lower incisor teeth. Bleeding was slight and sepsis did not occur.

Skin wounds on the lateral aspect of the thighs. With minor modifications, the technique described by Cuthbertson, Shaw & Young (1941) for rats was used. Under ether anaesthesia the hair was removed by a hair clipper from the lateral aspect of each thigh. A cork borer, 18 mm. diam., was used to mark out a circle on the skin surface. The cork borer was not used to incise the skin. The circle of skin, epidermis and *cutis vera*, was then removed with scissors exposing the fascia covering the underlying muscles. There was rarely any bleeding. No dressing was applied, yet the wounds never showed sepsis. Both thighs were wounded.

In rats a smaller cork borer of 12 mm. diam. was used.

Wounds to the pinna of the ear. These again were made only in guinea-pigs and under full anaesthesia. A marginal strip, roughly 1 cm. long, was cut from the edge of each pinna.

RESULTS

Repair of corneal epithelium. Healing was very rapid. The values for the 'end-point' of healing showed rather a wide scatter, possibly partly on account of the difficulty of detecting the exact time of epithelial restoration. Fluorescein was used at each examination to show signs of persisting injury. No significant difference was found between the two groups of guinea-pigs (Table 1).

Healing of gum wounds. No significant difference was observed between the deficient animals and the controls (Table 1).

Healing of thigh wounds in guinea-pigs. In the deficient guinea-pigs the thigh wounds took much longer to heal (Table 1). Statistically the result was highly significant.

Daily direct application of a 3% sodium ascorbate solution to the thigh wounds in deficient guinea-pigs also accelerated the healing noticeably and significantly. The same strength of sodium ascorbate solution, when applied to thigh wounds in guinea-pigs already receiving 5 mg. daily of ascorbic acid by mouth, was without action.

Healing of thigh wounds in rats. Large doses of ascorbic solution by mouth did not accelerate the rate of healing of the thigh wounds, neither did direct application of a 3% solution of sodium ascorbate to the wounds.

Dusting of thigh wounds with ascorbic acid powder retarded healing considerably, presumably because of irritation from acidity.

Healing of pinna wounds in guinea-pigs. There was significant delay in repair of the wounds in the deficient animals (Table 1).

Proliferation of epithelium took place quickly in the controls, and, even on the first day after operation, the edges of the ears were rounded and healing well. In contrast, a thick scab remained on the ears of the deficient animals for some time and haemorrhage was apt to occur.

Table 1. *Effect of oral administration of ascorbic acid on healing of corneal epithelium, muco-periosteum of the gum, skin wounds and ear wounds in normal and vitamin C-deficient guinea-pigs*

Guinea-pigs		Daily dose of ascorbic acid	Site of wounds	No. of wounds	Mean period required for healing*	Difference in time of healing between control and deficient animals	
Type	No.	(mg.)			(hr.)	Value (hr.)	Statistical significance (P†)
Control	25	5	Corneal epithelium	50	45 ± 9·0	1	Not significant ($P > 0·9$)
Deficient	25	0·25‡		50	46 ± 10·2		
					(days)	(days)	
Control	15	5	Muco-periosteum of gum	30	6·2 ± 0·32	0·2	Not significant ($P > 0·8$)
Deficient	15	0·25‡		30	6·0 ± 0·80		
Control	12	5	Skin	24	16 ± 0·81	13	Highly significant ($P < 0·001$)
Deficient	12	0·25‡		24	29 ± 1·15		
Control	20	5	Ear	40	8 ± 0·6	6	Highly significant ($P < 0·001$)
Deficient	20	0·25‡		40	14 ± 0·7		

* Values with their standard errors.

† P = probability that a mean difference at least as great as the observed mean difference would have arisen by random sampling from a homogeneous population ('Student's' (1908, 1925) t-test).

‡ 0·5 mg. on alternate days.

DISCUSSION

A defect of the corneal epithelium is said to be repaired by the epithelial cells 'spilling over' into the denuded area without any increase in number of cells. Subsequently there is evidence of increased mitotic activity in the basal layers of the epithelium all over the cornea (Duke-Elder, 1946).

A corneal epithelial injury is probably as good an example of a pure epithelial lesion as can be obtained in the body. Ascorbic acid does not appear to be necessary for repair of corneal epithelium, at least when the wound is of the size here described.

On the other hand, the excised disks of skin contained both epithelium (epidermis) and collagenous tissue (*cutis vera*), and, from histological examination, there seemed to be delay in new formation of this collagenous tissue in the deficient guinea-pigs. It does not seem to be too far-fetched to suggest that a certain degree of fibrous tissue organization is a prerequisite for overgrowth by the epidermis which is normally so intimately associated with its underlying *cutis vera*. Hartwell (1929), from a study of skin wounds, says definitely: 'One of the chief factors determining the rate of advance of an epithelial membrane is the type of base over which the membrane must progress.' The absence of a proper base for the regenerating epidermis would then explain the

delay in closure of the skin wounds in the deficient guinea-pigs without the need to postulate a direct effect of ascorbic acid on epidermal regeneration, a postulate which would be at odds with the findings in the corneal epithelial wounds. The corneal epithelium, it should be remembered, had an undamaged Bowman's membrane over which to spread.

The delay in repair of wounds in the pinna could be similarly explained, for here too there was removal of subepidermal collagenous tissue.

The findings in the gum wounds came as a surprise, especially in view of the observations of Campbell & Cook (1942) on the effect of ascorbic acid in promoting healing of tooth-extraction wounds in man. In tooth-extraction wounds, however, collagenous organization of a fairly large blood clot is required before epithelial repair can take place. Furthermore, in the sockets, after multiple tooth extractions, contraction of collagen fibres plays a part during healing in drawing together not only the edges of the wounds but also, to some extent, even the inner and outer alveolar plates.

Although, in our experiments, epithelium and collagenous tissue of the mucoperiosteum were both removed, the epithelium may have managed to bridge the small gap even in the absence of proper new collagen formation beneath. Had the wounds been larger there might have been interference with regeneration of the overlying epithelium.

The alternative to this hypothesis is the postulate that the epithelium of the cornea and of the gum is insensitive to lack of ascorbic acid whereas the epidermis is sensitive. The present experiments cannot decide finally one way or the other.

Rats do not require an extraneous source of ascorbic acid, and a vitamin C-free diet serves to keep them in a state of permanent saturation. The giving of ascorbic acid to rats resulted, therefore, in excess consumption. The painting of sodium ascorbate solution on skin wounds of guinea-pigs, already receiving a sufficiency of ascorbic acid by mouth, led similarly to a form of excess consumption. The absence of effect on the rate of healing of skin wounds in such cases probably illustrates the dictum 'Enough is as good as a feast'.

SUMMARY

1. Guinea-pigs were placed on a diet free from ascorbic acid. Half of them received daily ample ascorbic acid by mouth, while the others had just sufficient ascorbic acid to keep them alive in a subscorbutic state.

2. Four types of injury were inflicted under full anaesthesia. Either an area of corneal epithelium was removed by a dental bur, or a small cylinder of mucoperiosteum cut out of the gum, or disks of skin were removed from the lateral aspects of both thighs, or a strip was cut from the edge of the ear pinna.

3. Lack of ascorbic acid delayed healing of skin wounds in the thigh and in the ear pinna but had no effect on the rate of repair of corneal epithelium or of gum mucoperiosteum.

4. It is suggested that regeneration of epithelium alone can take place with normal speed in guinea-pigs suffering from a deficiency of ascorbic acid. When, however, healing of the wound also demands new formation of collagenous tissue, lack of

ascorbic acid delays epithelialization because of the tardy formation of a satisfactory collagenous base for the regenerating epithelium.

One of us, N. M. G., carried out this work while in receipt of a Roche Scholarship tenable at University College, Dundee, and we are all indebted to Roche Products Ltd. for generous supplies of various preparations of ascorbic acid.

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Comparison of Nutrient Values of Individual Diets Found by Calculation from Food Tables and by Chemical Analysis

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Comparison of the results obtained by different methods of individual survey (Bransby, Daubney & King, 1948) showed considerable differences between the nutrient values of diets obtained by calculation from food tables and by chemical analysis. It was suggested that this may have arisen because the inquiry was made in a single children's home. This paper gives the results of a comparison between the nutrient values of a number of individual diets eaten by persons living at home, found by calculation from food tables and by chemical analysis. One of us (E. R. B.) was responsible for the planning and execution of the inquiry, while two of us (C. G. D., J. K.) were responsible for the chemical analysis of the diets.

EXPERIMENTAL

Plan of experiment. Records of the weights of food eaten in 3 days were obtained from thirty-three adults living at home in Cambridge, Reading, London and Surrey. Duplicates of the same diets were collected for chemical analysis. Those co-operating