EFFECT OF CORTISONE ON HEALING OF CORNEAL WOUNDS

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The evidence that cortisone inhibits wound healing in animals and man through its depressant action upon fibroblastic activity has already been reviewed (Duke-Elder and Ashton, 1951), but this conclusion might not apply equally to repair-processes in the non-vascularized tissue of the cornea, in which the metabolism is peculiar and the structure unique. The following experiments were therefore designed to determine the effect of cortisone upon the healing of perforating wounds of the rabbit cornea.

Methods

EXPERIMENT 1.—Twenty rabbits were used. Under general anaesthesia, a horizontal perforating wound, approximately 5 mm. long, was made in the central portion of the cornea of each rabbit. One group of ten rabbits was used as a control. The second group of ten rabbits was given daily subconjunctival injections of 2.5 mg. cortisone, beginning 48 hrs before the infliction of the corneal wound. One control and one treated rabbit were killed together at 24-hr. intervals. The eyes were then removed, and placed in 10 per cent. formol saline, and celloidin sections were prepared for histological examination. Each injured eye was photographed immediately before death. The purpose of this experiment was to determine and compare the histological stage of healing on each day over a period of 10 days in treated and untreated corneal wounds.

EXPERIMENT 2.—A repeat series of eight rabbits was given larger doses of cortisone, viz., 15 mg. subconjunctivally every day beginning 48 hrs before the corneal wound was made. The animals were killed on the 1st, 2nd, 5th, 6th, 7th, and 10th days (these days were selected after examining the results of the first experiment, as having shown the most marked differences between the control and treated animals). The last two rabbits were killed on the 14th and 17th days. The purpose of this experiment was to confirm the findings in the first series and to ascertain whether the histological changes produced by cortisone in wound healing can be related to the quantity of hormone administered.

Results

EXPERIMENT 1.—By photographing each section a series of pictures showing the daily developments in the histological process of healing was obtained, but the individual reactions of each rabbit naturally resulted in a series which was not always progressive. Indeed, in a
few instances, the stage of healing was less well advanced at a later day in one animal than at an earlier day in another; in general, however, the gradation of healing was well shown. No attempt will be made to describe the minute histological developments in each of the sections examined, but the most important stages of healing in the normal control series will be described and will subsequently be compared with the cortisone treated series.

**Control Series.**—On the **first day**, 24 hrs after the perforation was made, the wound had filled with a fine fibrinous coagulum. Epithelial regeneration had already begun and proliferating epithelial cells extended inwards over the outer lips of the wound (Fig. 1). The ruptured Descemet's membrane retracted and curled forward accompanied by the severed endothelium. The stroma was lightly infiltrated with polymorphonuclears and macrophages, which by the **second day** had aggregated at the wound edges (Fig. 2). The healing process progressed slowly, but there was no very significant change until the **fifth day** when fibroblastic activity was evident immediately under the regenerated epithelium and to a lesser extent at the free margins of the wound. On this day also the endothelium showed considerable activity, and it had already advanced towards the centre of the wound, progressing along the inner aspect of the fibrinous coagulum (Fig. 4). By the **sixth and seventh day** the outer half of the wound was completely sealed with a mass of fibroblasts which had pushed the previously inverted epithelium outwards. The endothelium had completely healed and the continuity of the

![Fig. 1.—Control rabbit, first day. (24 hrs after infliction of wound). Epithelial regeneration has already begun, and proliferating epithelial cells extend inwards into the wound which contains a fine fibrinous coagulum. Descemet's membrane is retracted and curled forward. Haematoxylin and eosin. × 55.](http://bjo.bmj.com/)
Epithelial regeneration has progressed, the wound contains fibrin coagulum and the stroma is infiltrated with polymorphonuclears and macrophages which have aggregated at the wound edges. Haematoxylin and eosin. × 50.

Fibroblastic activity is beginning under the regenerated epithelium and the endothelium has already advanced towards the centre of the wound. Haematoxylin and eosin. × 50.

The outer half of the wound is now completely sealed with a mass of fibroblasts which have pushed the previously inverted epithelium outwards. The endothelium is completely healed and the continuity of the membrane restored. Note cellularity of the stroma. Haematoxylin and eosin. × 50.

Fibroblastic activity is impaired and inner half of wound gapes widely. Anteriorly the gap resulting from the inhibition of fibroblasts has been replaced by epithelial regeneration. There is very little evidence of endothelial regeneration (cf. Fig. 6). Haematoxylin and eosin. × 50.
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Fig. 8.—Control rabbit (Experiment 1), fourth day. Note marked opacity around central perforating wound (compare Fig. 9).

Fig. 9.—Cortisone-treated rabbit (Experiment 1), fourth day. Note greatly reduced opacity around central perforating wound as compared with the control (see Fig. 8).

membrane was restored (Figs 6 and 10). By the ninth day the corneal epithelium was of uniform thickness and the wound was healed by a solid mass of fibroblasts which were already beginning to align themselves into lamellar formation (Fig. 13). By the tenth day the normal corneal thickness was restored and the wound bridged by a mass of laminated fibrocytes (Fig. 15).

Cortisone Series.—On the first day the cortisone-treated rabbit showed a less exuberant epithelial regeneration and a deficient
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FIG. 10.—Control rabbit, seventh day. The anterior half of the wound is sealed with a mass of fibroblasts and the endothelium is completely healed. Haematoxylin and eosin. ×50.

Fig. 11.—Cortisone-treated rabbit (Experiment 1), seventh day. The fibroblastic activity is considerably less than in the control and the endothelial regeneration is markedly inhibited (compare Fig. 10). Haematoxylin and eosin. ×50.

Fig. 12.—Cortisone-treated rabbit (Experiment 2), seventh day. There is some impairment of epithelial regeneration. Fibroblastic activity is more severely affected than with smaller doses of cortisone (the cavity contains serous fluid), and endothelial regeneration is completely arrested (compare Figs 10 and 11). Haematoxylin and eosin. ×50.

fibrinous coagulum in the wound. A curious feature was a failure of Descemet's membrane to curl forward; as an isolated observation this fact would hardly be worth recording, but the tendency was noted in all the cortisone-treated eyes. The sections of the second day showed a remarkable acellularity of the wound edges as compared with the normal wound (Fig. 3), and the epithelial regeneration was again slightly less. On the fifth day the sections differed from the control in that epithelial regeneration was less, fibroblastic activity beneath the epithelium very slight, the wound edges much less cellular, and evidence of endothelial regeneration absent (Fig. 5). On the sixth day epithelial regeneration was more marked in the cortisone-treated eye than in the control, but fibroblastic activity was
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FIG. 13.—Control rabbit, ninth day. The corneal epithelium is of uniform thickness and the wound is now healed by a solid mass of fibroblasts which are beginning to align themselves into lamellae. The intact endothelium may be seen. Haematoxylin and eosin. × 80.

FIG. 14.—Cortisone-treated rabbit (Experiment 1), ninth day. The wound is firmly bound together with fibroblastic tissue, but there is considerably less than in the control and the anterior deficiency is replaced by epithelial proliferation. Endothelial regeneration is completely arrested (compare Fig. 13). Haematoxylin and eosin. × 80.

again less and the inner half of the wound gaped widely. There was practically no evidence of endothelial regeneration, although the corresponding control section showed complete endothelial regeneration by this time (Fig. 7). The cellular activity throughout the stroma and at the edges of the wound was considerably less than in the control specimen: this inhibition of infiltration around the wound was recognizable clinically throughout the experiment (Figs 8 and 9).
A similar difference was noted on the seventh day (Fig. 11). On the ninth day the epithelium was hyperplastic, whereas the control showed an epithelium of normal thickness. The wound was now firmly bound together with fibroblastic tissue but there was considerably less of this tissue than in the control, and it was particularly deficient anteriorly and posteriorly. Anteriorly the deficiency was compensated by the epithelial hyperplasia which had restored the surface level of the cornea. Endothelial regeneration was still completely arrested (Fig. 14). On the tenth day the epithelial regeneration over the wound was again markedly greater than normal and formed a triangular-shaped mass, but the wound itself showed only a delicate fibrous scar. The endothelium now exhibited a tardy and sluggish attempt at regeneration (Fig. 16).

Experiment 2.—The six corneae examined on the first, second, fifth, sixth, seventh, and tenth days, when compared with the controls of the first experiment, all showed inhibition of the healing process, but this was considerably more marked than in the first cortisone series in which smaller dosages had been used. There was but little formation of fibrinous coagulum in the early stages, polymorphonuclear and macrophage infiltration were severely inhibited, fibroblastic activity was practically arrested, and no endothelial regeneration occurred (Fig. 12). In the later stages of healing the findings varied to some extent, but in each section repair was grossly inadequate. On the tenth day the wound was joined with only a narrow attenuated strip of fibrous tissue, and the epithelium had not regenerated (Fig. 17). On the fourteenth and seventeenth days the sections showed a remarkable arrest of healing; in the section of the seventeenth day (Fig. 18) there was no evidence of fibroblastic activity or of endothelial regeneration, very few infiltrating cells could be seen in the stroma, and the wound was joined by a thin, delicate, fibrinous coagulum containing a few inflammatory cells. The epithelium had entirely desquamated, but this may well have been a post-mortem change. The stage of healing after 17 days with daily subconjunctival injections of 15 mg. cortisone was thus comparable to that of the first or second day in the control animal.

Discussion

The study of the effect of cortisone upon wound healing is not a simple one, for there are differences between species and individual variations, both in the response to the initial injury and in the reaction to the quantity of cortisone administered. One cannot, therefore, in this respect, lay down rigid criteria nor apply animal experiments to man without further evidence to support the analogy. It would seem from our experiments, however, that the inhibitory effect of cortisone upon the healing process in the cornea is exactly similar to that which has already been abundantly demonstrated in
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Fig. 15.—Control rabbit, tenth day. The normal corneal thickness is restored and the wound is bridged by a mass of laminated fibrocytes. Haematoxylin and eosin. × 80.

Fig. 16.—Cortisone-treated rabbit (Experiment 1), tenth day. The wound is healed by a delicate fibrous scar and there is a triangular mass of epithelial cells in the anterior part of the wound. The endothelium shows a sluggish attempt at regeneration (compare Fig. 15). Haematoxylin and eosin. × 80.

Fig. 17.—Cortisone-treated rabbit (Experiment 2), tenth day. The wound is joined with but a narrow attenuated strip of fibrous tissue and the epithelium has not regenerated. The larger doses of cortisone have greatly impaired the healing process (compare Figs 15 and 16). Haematoxylin and eosin. × 80.
FIG. 18.—Cortisone-treated rabbit (Experiment 2), seventeenth day. There is no evidence of fibroblastic activity nor of endothelial regeneration. Very few infiltrating cells can be seen in the stroma and the wound is joined by a thin, delicate fibrinous coagulum containing a few inflammatory cells. The epithelium is desquamated. Haematoxylin and eosin. × 135.

other tissues in both animals and man. It is justifiable, therefore, to assume that the following assessment of the action of cortisone upon perforating wounds of the rabbit cornea at least indicates the reactions to be expected in similar circumstances in man.

It has been shown that cortisone acetate administered subconjunctivally is able to modify the healing of perforating corneal wounds in the rabbit. The chief features of its action may be summarized as follows:

(1) **EPITHELIAL REGENERATION.**—In the series of animals in which the doses of cortisone given were small and corresponded to those which would normally be used therapeutically, there was no significant effect upon epithelial regeneration; indeed, in this series the epithelium was able to proliferate sufficiently to fill the anterior gap resulting from the inhibition of fibroblastic repair (Figs 7, 14, and 16). With larger doses however, epithelial repair may be retarded (Fig. 17). The inhibition of epithelial regeneration is, therefore, apparently related to the dose of cortisone exhibited, and this fact may to some extent explain the conflicting reports in the literature.

(2) **FIBRINOUS COAGULUM.**—This normally fills the wound cavity during the first few days after injury and provides the scaffolding for its subsequent
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reorganization; it was diminished quantitatively in the cortisone-treated cases (Fig. 3).

(3) CELLULAR INFILTRATION.—Polymorphonuclears and macrophages were considerably less in the cortisone-treated cases, and the relative acellularity of the wound margins was a striking feature in these sections.

(4) FIBROBLASTIC ACTIVITY.—This was inhibited to an appreciable extent even with small doses of cortisone; however, the eventual degree of healing was adequate, although less satisfactory than in the untreated controls. With larger doses healing by fibrosis was seriously impaired and either a shrunken inadequate scar (Fig. 17), or complete arrest of fibrous repair (Fig. 18), was the result.

(5) ENDOTHELIAL REGENERATION.—This was more markedly inhibited in the cornea than was any other component. In the normal animal the endothelium rapidly proliferated and had completely regenerated by the 6th day, whereas even small doses of cortisone greatly impaired its regenerative activity (Figs 7, 11, and 14), and larger doses completely arrested the process. It may be that cortisone acts similarly upon vascular endothelium; if so, the inhibition of new-vessel formation in cortisone-treated lesions may be attributable to this action.

It is interesting to note that all the above effects were related to the quantity of cortisone administered, being more severe with the greater dosage; this finding has already been suggested by the work of Leopold and others (1951). It is clear that when this hormone is indicated immediately after intra-ocular operations, the minimal effective dose should be used in order to avoid undesirable complications; it has been shown elsewhere (Duke-Elder, 1951) that, providing this principle is followed, ocular operations can safely be carried out while the patient is under treatment with cortisone.

Summary

The effect of cortisone upon the healing of perforating corneal wounds in the rabbit has been studied with small subconjunctival doses of cortisone corresponding to the usual therapeutic level, and with larger non-therapeutic doses.

Cortisone was found to inhibit the formation of the fibrinous coagulum, cellular infiltration, fibroblastic repair, and endothelial regeneration. The inhibition was related to the quantity of cortisone administered, being moderate with small doses (with which the final stage of healing was impaired but adequate), and severe with large doses (with which healing may be completely prevented).

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