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# The effects of cortisone acetate on tissue regeneration in the rabbit's ear

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## INTRODUCTION

The regeneration of the tissues observed after punching a hole 1 $cm^2$ in size in the rabbit's ear was described by Joseph & Dyson (1966). The effects of androgens (Dyson & Joseph, 1968) and female sex hormones (Dyson & Joseph, 1971) on the regeneration suggested that this technique could be used for studying some aspects of the way in which cortisone acts on regenerating tissues although, in view of the well-known effect of corticosteroids on fibroblasts (Dougherty & Schneebeli, 1950; Berliner & Nabors, 1967), it could be assumed that cortisone would slow or stop the growth of the blastema. There has been no report dealing either with a quantitative measurement of this type of tissue replacement, or with the question whether or not regeneration would continue after the cessation of the administration of the cortisone. Histological studies of the blastema after varying intervals of cortisone administration would provide information on the functioning of the fibroblasts and the formation of collagen. The effects of cortisone on wound healing (Ragan *et al.* 1949; Baker & Whitaker, 1950; Creditor *et al.* 1950; Alrich, Carter & Lehman, 1951; Bangham, 1951; Bukhonova, 1960; Ehrlich & Hunt, 1968), and the quantitative replacement of the mobile skin of the rabbit (Hunt, Ehrlich, Garcia & Dunphy, 1969) have been studied, but the regeneration seen in the rabbit's ear is very different from the healing of incised wounds and the closure of defects in mobile skin, as has been confirmed by Braun & Efimov (1971) and Goss & Grimes (1972).

## MATERIAL AND METHODS

Male rabbits more than 6 months old, of mixed stock, and weighing $2{\cdot}78 \pm 0{\cdot}22$ kg, were used. Males were chosen because regeneration of the tissues is more rapid than in females (Joseph & Dyson, 1965).

The animals were anaesthetized with pentobarbitone administered intravenously, and the ears were cleaned with a 1% solution of Cetrimide. A square punch which excised 1 $cm^2$ of the whole thickness of the ear was used to produce a standard lesion, and tattoo marks were made in the ventral skin around the edges of the hole with a needle dipped in India ink. Bleeding was stopped by inserting a plug of sterilized gauze in the hole. The ears were photographed with a ruler graduated in centimetres lying adjacent to the hole immediately after excision, and subsequently at intervals

of 7 days, so that a photographic record of the regenerated tissue was obtained. The animals were weighed once a week. The photographs were used for measuring the surface area of the regenerated tissue and the method involved projecting the transparencies on to graph paper as described by Joseph & Dyson (1966). The areas were recorded as percentages of the initial lesion.

The animals were divided into four groups. Three groups were given cortisone acetate (Boots) for 6 consecutive days each week by subcutaneous injection in the following doses: (1) 10 mg/kg body weight for 28 days; (2) 2·5 mg/kg for 28 days; (3) 2·5 mg/kg for 49 days. The fourth group received no cortisone and acted as a control.

The administration of 10 mg/kg proved to be very toxic and was stopped after 28 days. These animals showed a weight loss of $0{\cdot}42 \pm 0{\cdot}08$ kg. The animals receiving the smaller dose showed a weight loss of only $0{\cdot}04 \pm 0{\cdot}04$ kg after 28 days and appeared to remain reasonably healthy throughout the experiment.

The means and standard errors of the means of the regenerated areas were calculated for each group of rabbits at 7 day intervals from 14 to 49 days. The means of the treated groups were compared with the means of the control group at each interval and Student's *t*-test was applied according to the method of Simpson, Roe & Lewontin (1960) for calculating the differences between means. Two-sided tests were used. In groups 1, 2 and 3 means and standard errors of means were calculated at 70 and 98 days, and these means were compared with the mean at 49 days of the control group.

For histology, regenerated tissue was removed from additional animals 14, 21, 28, 35, 42 and 49 days after treatment as in groups 1, 2 and 3. For light microscopy the tissue was fixed in 10% neutral formalin at 4 °C for 24 hours. It was then dehydrated, cleared and embedded in Fibrowax (paraffin wax and plastic polymers) at 56 °C. Sections at 10 $\mu$m were cut and stained with: (1) haematoxylin and eosin, (2) Alcian blue and periodic acid Schiff, (3) Weigert and Van Gieson. For electron microscopy the tissue was cut into pieces approximately 1 $mm^3$ in size and fixed in 4% glutaraldehyde in pH 7·4 phosphate buffer for 2 hours. It was postfixed in 2% osmium tetroxide for 1 hour, dehydrated with alcohols, transferred to a 'link' reagent (propylene oxide) and embedded in TAAB epoxy-resin (TAAB Laboratories, Reading). Ultrathin sections were cut with an LKB ultramicrotome, stained with saturated uranyl acetate and 0·4% lead citrate, and examined with the RCA EMU3 electron microscope.

In addition, sections of the ears in control and cortisone-treated rabbits were examined for acid phosphatase at 7 and 14 days. The tissues were fixed in 10% neutral formalin for 2 hours at 4 °C and sections at 15 $\mu$m were cut in the cryostat. The sections were treated by a modification of the naphthol AS and hexazotized pararosaniline technique of Lojda and Barka and Anderson (Pearse, 1968).

## RESULTS

### *Regeneration*

Fig. 1 summarizes the results obtained for the four groups of rabbits. There was a marked decrease in regeneration at all intervals up to 49 days in the three groups of

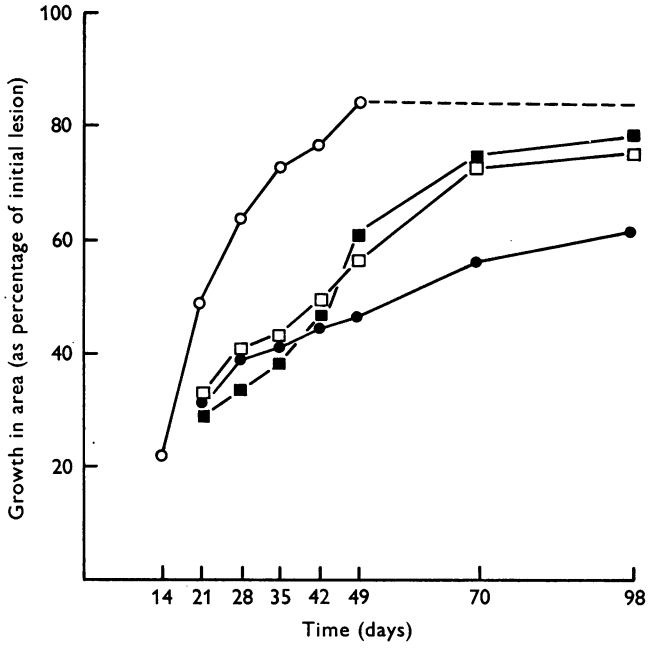


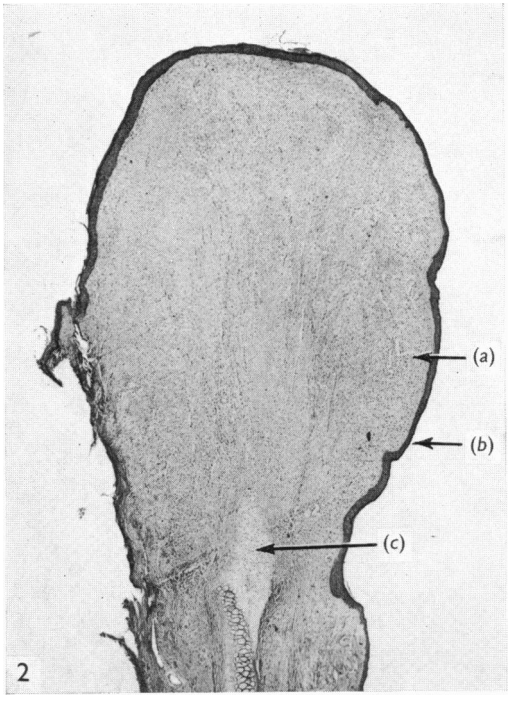

Fig. 1. Regeneration of ear tissue in male rabbits: ○—○, controls; ■—■, 10 mg/kg cortisone acetate for 28 days; □—□, 2·5 mg/kg for 28 days; ●—●, 2·5 mg/kg for 49 days.

rabbits treated with cortisone as compared with the control group. These differences were highly significant (at 21 days $P < 0{\cdot}005$, at 28 days $P < 0{\cdot}005$, at 49 days $P < 0{\cdot}005$). Subsequent to 49 days, however, regeneration in the ears of group 1 (10 mg/kg for 28 days) continued, with the result that the difference between this group at 70 days and the controls at 49 days was no longer significant ($P > 0{\cdot}1$). (It should be noted that in almost all the control rabbits the hole in the ear was completely filled at 49 days, as a result of a combination of new growth and contraction. This explains why comparison was made between the 70 and 98 day cortisone-treated groups and the 49 day controls.)

Similarly, the rabbits of group 2 (2·5 mg/kg for 28 days) showed continued regeneration, so that at 70 days the difference between this group and the controls at 49 days was no longer significant ($P > 0{\cdot}1$). On the other hand, the rabbits of group 3 (2·5 mg/kg for 49 days), although continuing to show regeneration, did so very slowly. The difference at 70 days as compared with the control group at 49 days was significant ($P < 0{\cdot}005$). At 98 days group 3 still showed a significant reduction in regeneration as compared with the control group at 49 days ($P < 0{\cdot}005$).

### *Histology*

The regenerated tissue in the control group has been fully described in a previous paper (Joseph & Dyson, 1966). The blastema consisted of epidermis, dermis and elastic cartilage, although none of these was the same as those tissues found in the



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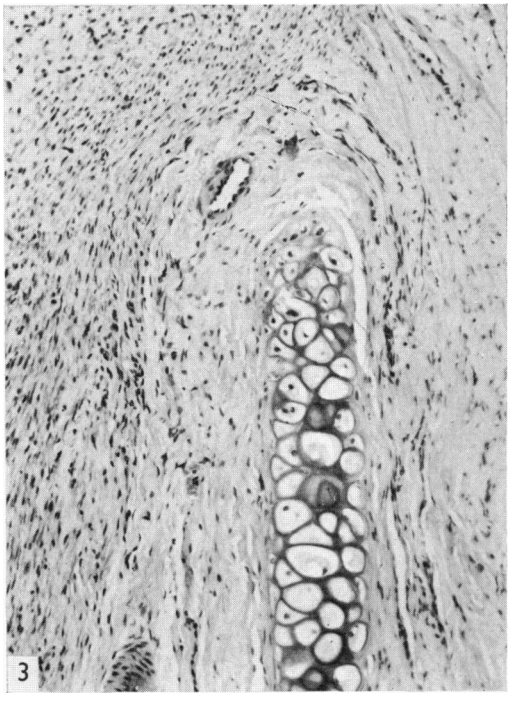
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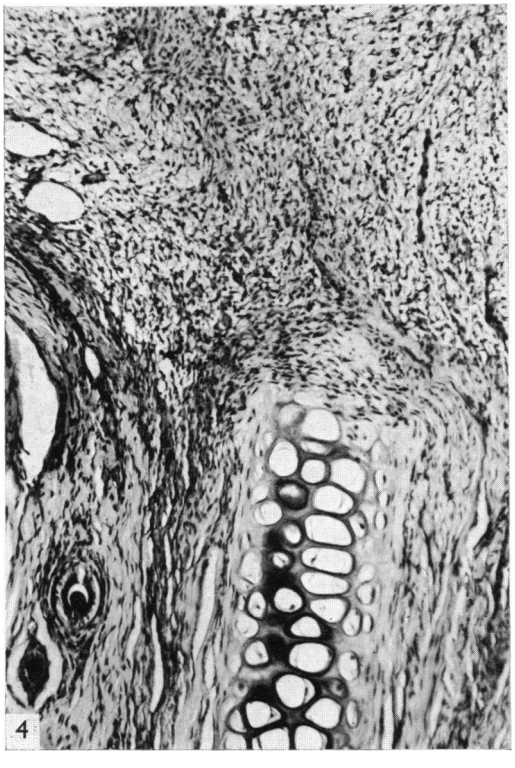
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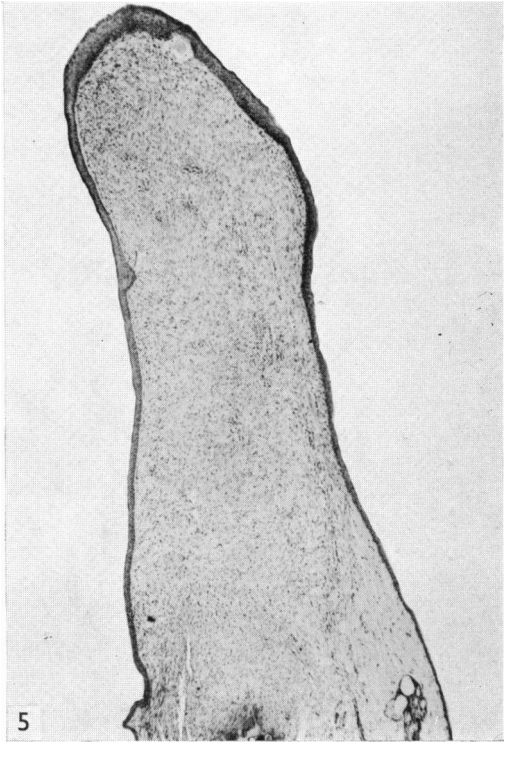
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normal ear, the epidermis being thicker, the dermis much more cellular, and the cartilage more cellular with denser ground substance and smaller lacunae (Fig. 2).

In at least half the cortisone-treated animals at 14 days the scab had not yet separated, in contrast to the control group, in which it had invariably dropped off by 10 days. In the experimental animals the edge of the wound was not completely covered by epidermis. The blastema was much smaller than that seen in the controls, and consisted of fibroblasts in large numbers, and collagen. Fewer histiocytes were seen than in the control sections. One of the most marked differences was the appearance of the cut end of the cartilage (Fig. 3). The cells of the perichondrium showed little or no evidence of the proliferation which was so striking a feature of the cartilage in the controls (Fig. 4). The sections stained with Van Gieson were pink and not red. This indicated that the collagen of the blastema was either relatively immature or less plentiful than normal, a finding similar to that observed in the controls.

At 21 days and at succeeding stages of regeneration (28, 35, 42 and 49 days), those animals which continued with cortisone injections showed a variable amount of regeneration consisting of an epithelium-covered mass of granulation tissue. This contained large numbers of fibroblasts and collagen fibres. If there was a considerable amount of granulation tissue, many blood vessels were present. The collagen appeared to be immature or less plentiful than normal, and did not form the typical large bundles seen in the normal dermis. The blastema was narrow when compared with the bulbous appearance seen in the control group (Fig. 5). At all intervals there was little or no evidence of regeneration of cartilage.

In groups 1 and 2 (10 and 2·5 mg/kg for 28 days) histological examination of the blastema 7 days after cessation of cortisone administration showed a marked regeneration of cartilage, which consisted not only of the proliferation of chondroblasts, but also of the formation of more mature cartilage with cells in lacunae (Fig. 6). The matrix of the cartilage showed a positive Alcian blue reaction for acid mucopolysaccharides (Fig. 7). This was absent in all the ears which were examined for acid mucopolysaccharides while cortisone was being administered (Fig. 8).

### *Electron microscopy*

At 14 days in the control ears the fibroblasts of the blastema showed evidence of marked activity. The cisternae of the rough endoplasmic reticulum, which were abundant, were filled with granular material, and the Golgi complex was well marked. There were many small vesicles and filaments in the cytoplasm. Many

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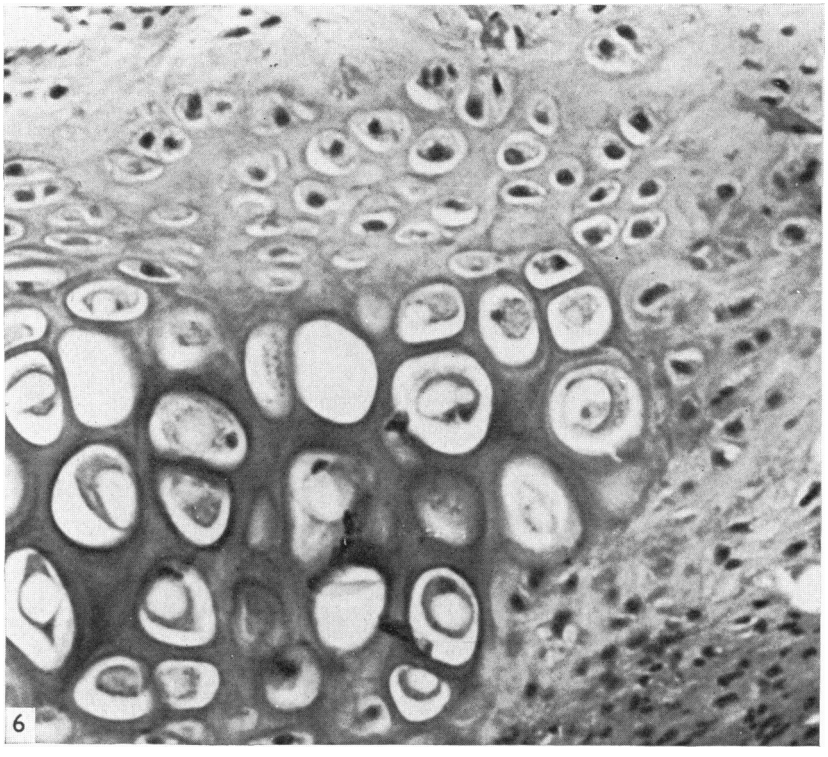
Fig. 2. Section of ear showing regenerated tissue of control consisting of (*a*) epidermis, (*b*) dermis, (*c*) cartilage. H. & E. × 16.

Fig. 3. Section of ear of cortisone-treated rabbit at 14 days showing absence of proliferation of cartilage. H. & E. × 110.

Fig. 4. Section of ear of control rabbit at 14 days showing cellular proliferation at the cut end of the cartilage. H. & E. × 110.

Fig. 5. Section of ear of cortisone-treated rabbit at 21 days showing relatively long narrow blastema without any new cartilage. H. & E. × 35.



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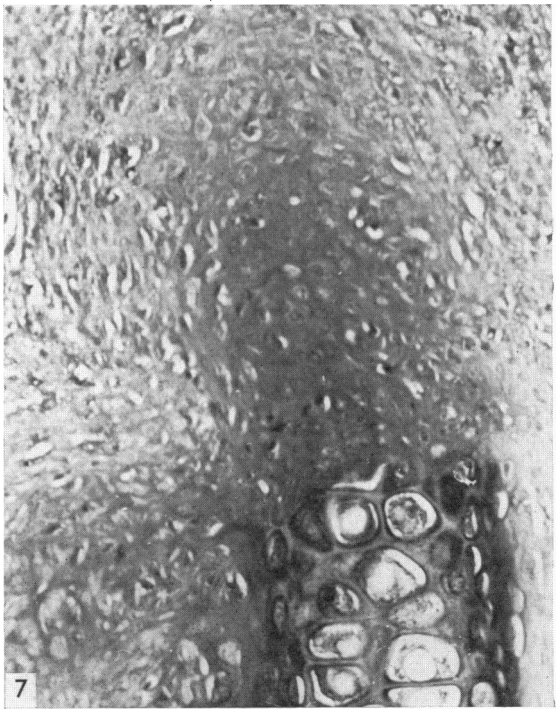

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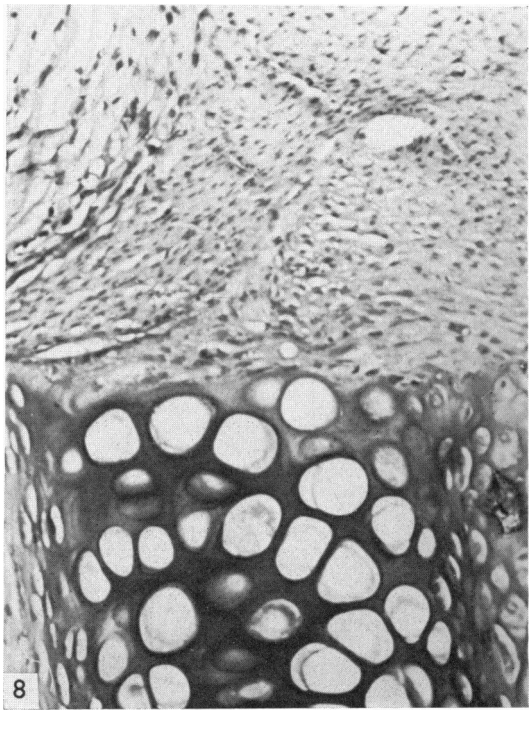

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macrophages were seen containing ingested red blood corpuscles, and in the intercellular substance there were many maturing collagen fibres (Fig. 9). At 14 days the blastema of the ears of the cortisone-treated rabbits contained fibroblasts which were smaller and more rounded, and which showed much less dilated rough endoplasmic reticulum and a small Golgi complex. The vesicles and cytoplasmic filaments were much less evident. There were fewer macrophages, and they did not contain any of the red blood corpuscles which were seen in the surrounding intercellular substance. This substance showed a reduced number of collagen fibres as compared with the control, and many large spaces (Fig. 10).

At 21 days the control ears showed few if any changes with regard to the fibroblasts and macrophages. The collagen fibres, however, were more mature; they showed typical banding and were thicker. The ears of the cortisone-treated rabbits at 21 days had the same appearance as at 14 days.

At 28 days the control ears showed active fibroblasts with large numbers of closely packed, mature, well orientated bundles of collagen fibres in the ground substance (Fig. 11). Macrophages were still present and mast cells were seen. In the ears of the cortisone-treated rabbits there were many degenerating fibroblasts, as was evidenced by the grossly dilated cisternae of the endoplasmic reticulum and the condensation of the chromatin of the nucleus. Collagen fibres were seen, and many of them were much more densely staining than usual (Fig. 12).

One week after the cortisone injections were discontinued the blastema contained fibroblasts which were obviously active, as was evidenced by the presence of granular material in the cisternae of the endoplasmic reticulum and a well-marked Golgi complex. In one animal the collagen fibres showed abnormally dense regions along their length, and in another the maturing fibres were abnormally large (about 250 nm diameter) as compared with the maximum diameter of about 100 nm in normal animals (Fig. 13).

Two weeks after the cessation of cortisone administration the fibroblasts appeared to be active, and the collagen was much more regularly arranged and of normal diameter (Fig. 14). In the fibroblasts there were also cytoplasmic filaments which were concentrated at the periphery of the cells.

Three weeks after stopping the cortisone the main changes appeared to be an increase in the number of collagen fibres and in the regularity of their arrangement in bundles. The blastema was less cellular and there appeared to be a higher proportion of macrophages.

There was much more acid phosphatase in the blastemata of the 7 and 14 day controls than in the 7 and 14 day cortisone-treated rabbits.

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Fig. 6. Section showing maturing regenerated cartilage in the ear of rabbit at 35 days after receiving cortisone for 28 days and no treatment for 7 days. H. & E. ×400.

Fig. 7. Section of ear of rabbit at 35 days after receiving cortisone for 28 days and no treatment for 7 days. Alcian blue/PAS. ×210.

Fig. 8. Section of ear at 28 days after receiving cortisone for 28 days. Alcian blue/PAS. ×210 (cf. Fig. 7).

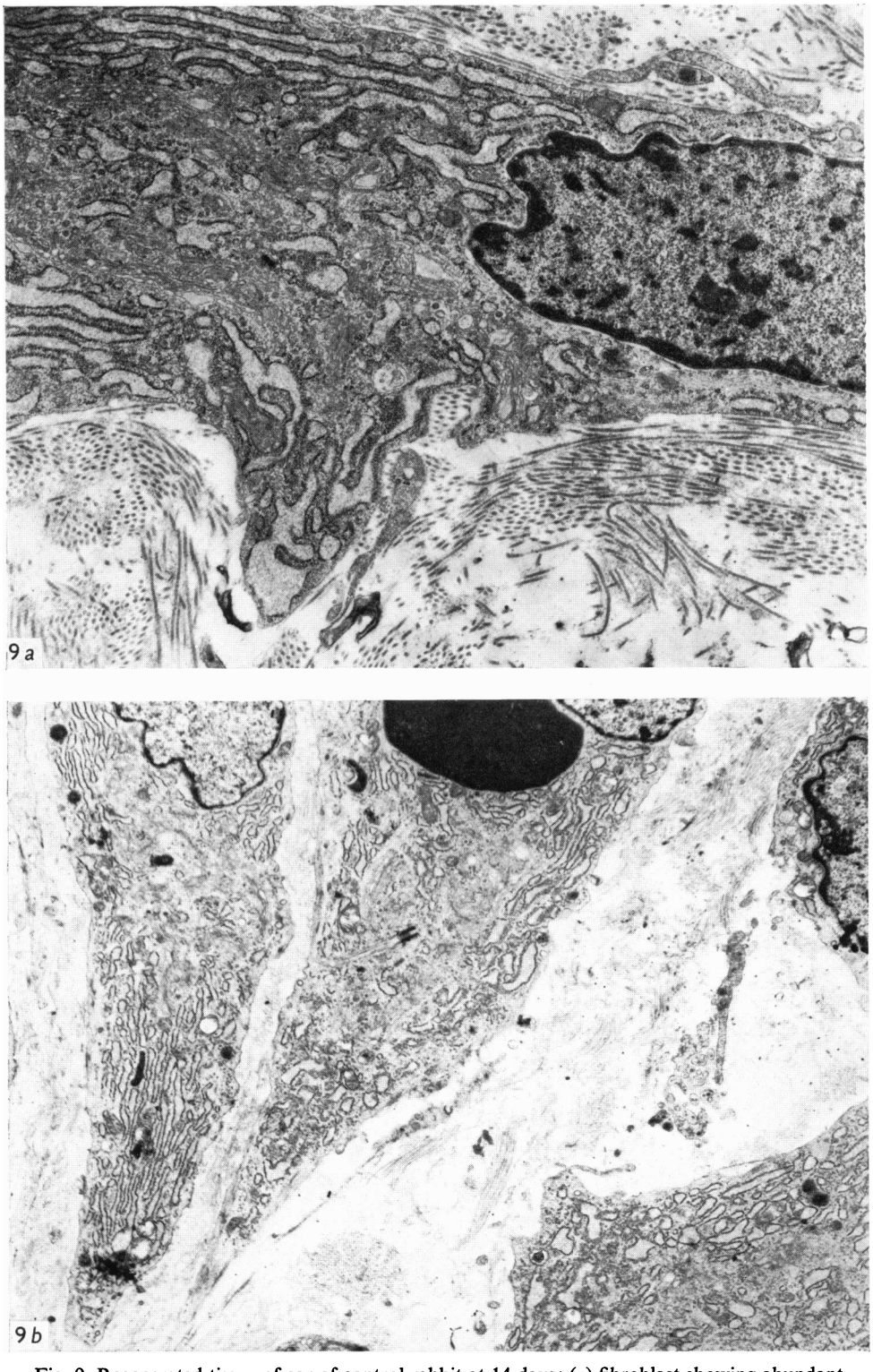
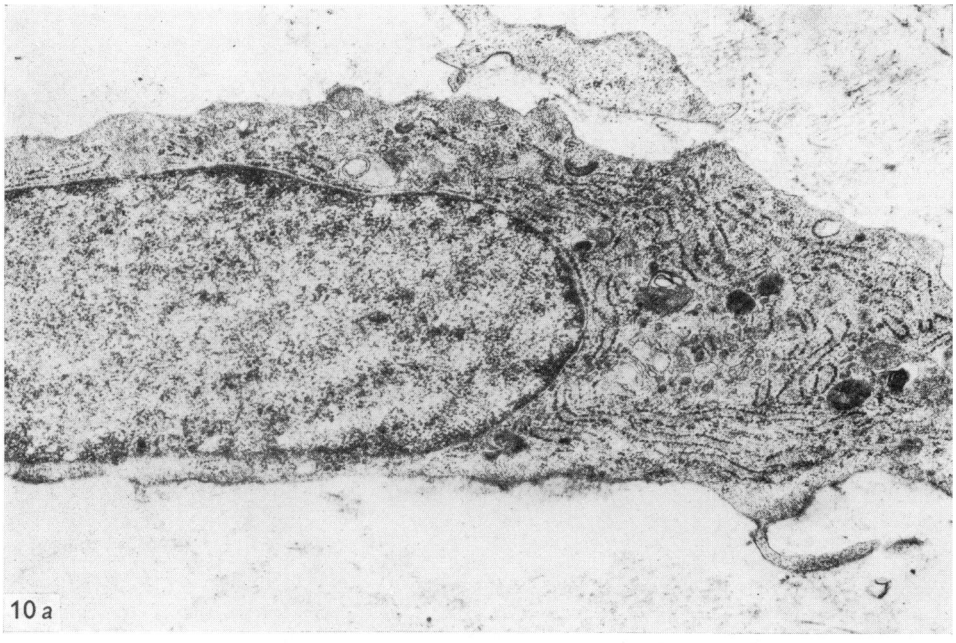



Fig. 9. Regenerated tissue of ear of control rabbit at 14 days: (*a*) fibroblast showing abundant dilated cisternae of rough endoplasmic reticulum filled with granular material, and an extensive Golgi complex: ×11000; (*b*) three active fibroblasts, one of which contains an ingested red blood corpuscle: ×3500.



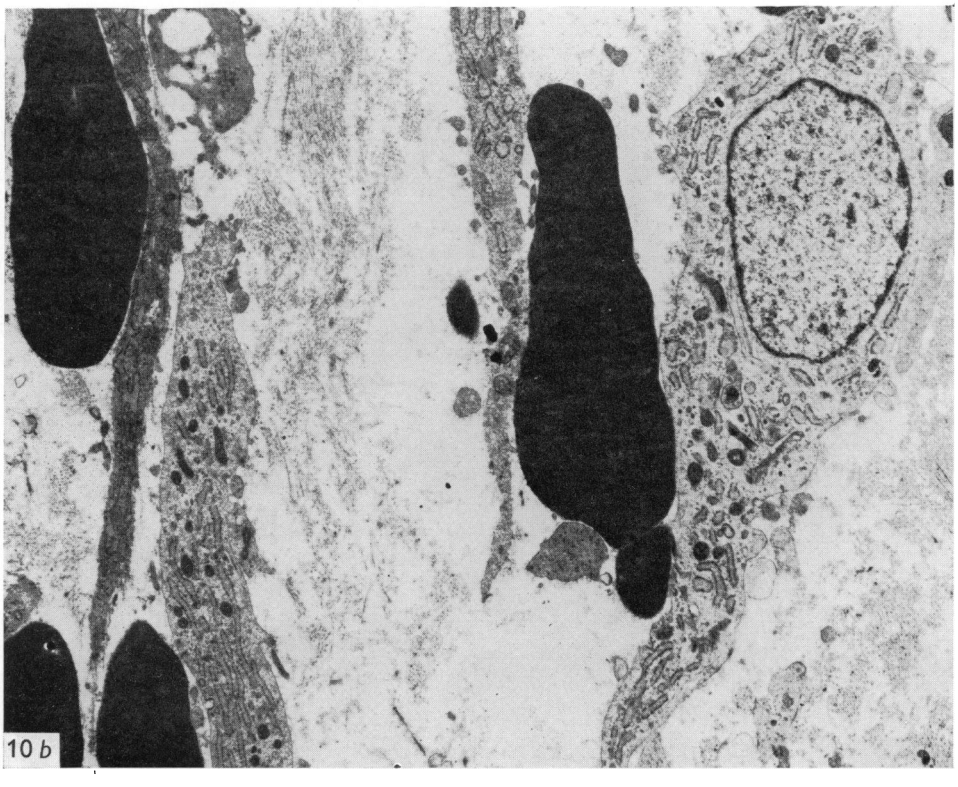
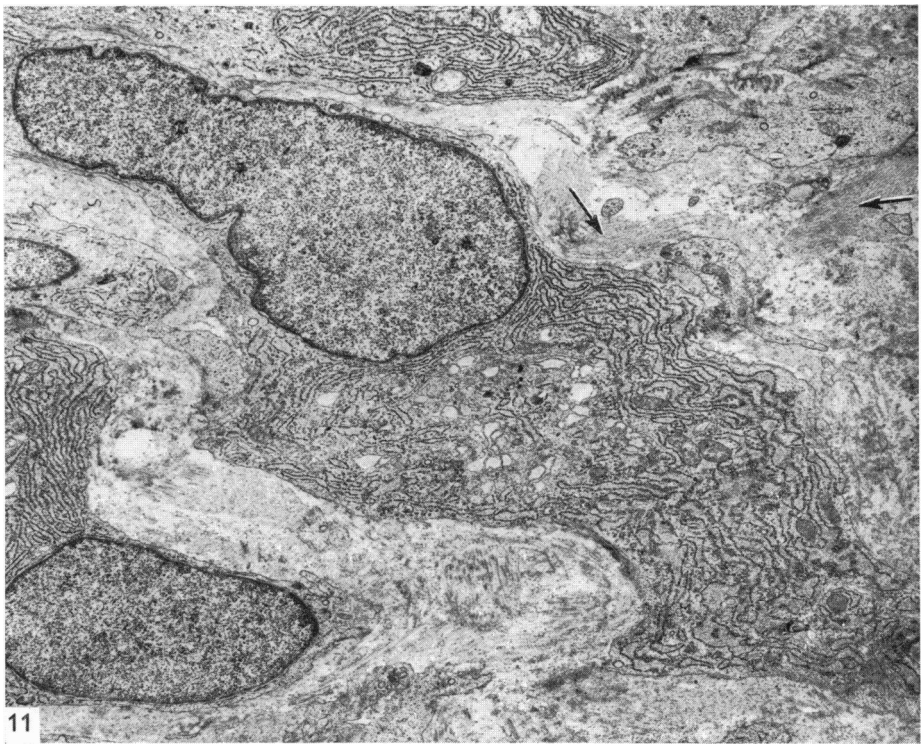




Fig. 10. Regenerated tissue of ear of cortisone-treated rabbit at 14 days: (*a*) as compared with Fig. 9 the rough endoplasmic reticulum has fewer dilated cisternae: ×8000; (*b*) shows red blood corpuscles free in the ground substance: ×4500.



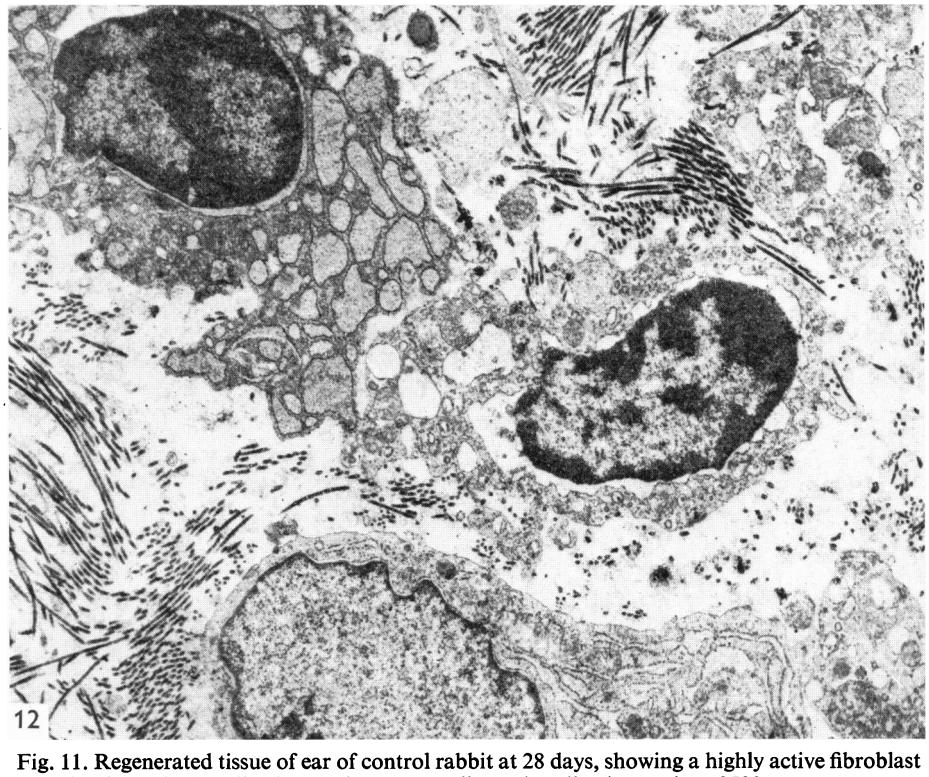


Fig. 11. Regenerated tissue of ear of control rabbit at 28 days, showing a highly active fibroblast and closely packed, well orientated, mature collagen bundles (arrows). × 3500.

Fig. 12. Regenerated tissue of cortisone-treated rabbit at 28 days showing some degenerating fibroblasts and densely staining collagen fibres. × 6800.

## DISCUSSION

The results of this investigation showed that cortisone significantly decreased the rate of regeneration of new tissue in the rabbit's ear. They also showed that if the administration of cortisone was stopped after 4 weeks, regeneration continued, so that after a further period of 10 weeks, it was not significantly different from that of control animals after 7 weeks of regeneration. If, however, the cortisone was given for a continuous period of 7 weeks, the subsequent regeneration was very much slowed, so that 7 weeks later the regeneration was significantly less than in the 7 weeks controls.

Cortisone affected the regeneration of both the dermis (fibroblasts and collagen) and the cartilage. Its effect on the epidermis was almost certainly indirect, insofar as the edges of the wound were covered by proliferating cells, and further epidermal growth depended entirely on growth of the underlying tissue. It is difficult to explain the way in which cortisone depresses or prevents the regeneration of the tissues, since the results obtained could fit in with different theories regarding regeneration and wound healing. Abercrombie (1964) discussed the possibility that the products of cell damage in a wound produce a substance which acts as a 'wound hormone'. Similarly, the inflammatory changes around a wound are caused by substances produced by cell damage (Wilhelm, 1962). Cortisone may act by preventing both cell damage and the initial inflammatory reaction because of its ability to strengthen lysosomal membranes (Weissmann & Thomas, 1963). In this investigation the amount of acid phosphatase in the blastemata of the 7 and 14 day controls was very much greater than that seen in the 7 and 14 day cortisone-treated rabbits. Acid phosphatase can be an indication of cell breakdown (Weiss & Rosenbaum, 1967) and these results can be interpreted as reflecting the cell damage which occurred in the controls, but was prevented in the experimental rabbits because of the strengthening of the lysosomal membranes by the cortisone. Cortisone has been found to prevent damage to fibroblasts after wounding (Dougherty & Berliner, 1959; Weiss & Dingle, 1964) and also to chondroblasts (Weissmann & Thomas, 1964). The rounded appearance of the fibroblasts described in this investigation suggests that the fibroblasts are more resistant to destruction (Dougherty & Berliner, 1959). It should be added that the sections also showed evidence of reduced inflammatory reaction.

The role of cartilage in regeneration in the ear has been emphasized by Goss & Grimes (1972) and earlier workers have found that cartilage itself, and extracts of cartilage, stimulated healing (Nageotte, 1918; Prudden, Nishihara & Baker, 1957; Inoué, 1961; Houck & Vickers, 1962; Sabo, Oberlander & Enquist, 1965). Lattes, Martin, Meyer & Ragan (1956) and Prudden & Wollarsky (1967) found that cartilage reduced the cortisone-induced inhibition of wound healing. The striking lack of cartilage proliferation in the cortisone-treated animals in the present study is possibly the main effect of the cortisone and the inhibition of the fibroblasts may be secondary. However, this is unlikely, since cortisone can affect fibroblasts in the absence of cartilage, and some animals in this investigation showed a relatively large regenerate without cartilage proliferation (Fig. 5). Probably cortisone affects both the fibroblasts and the cartilage cells simultaneously by its effect on mitochondrial membranes, so that ATP production is reduced (Whitehouse & Bostrom, 1961).

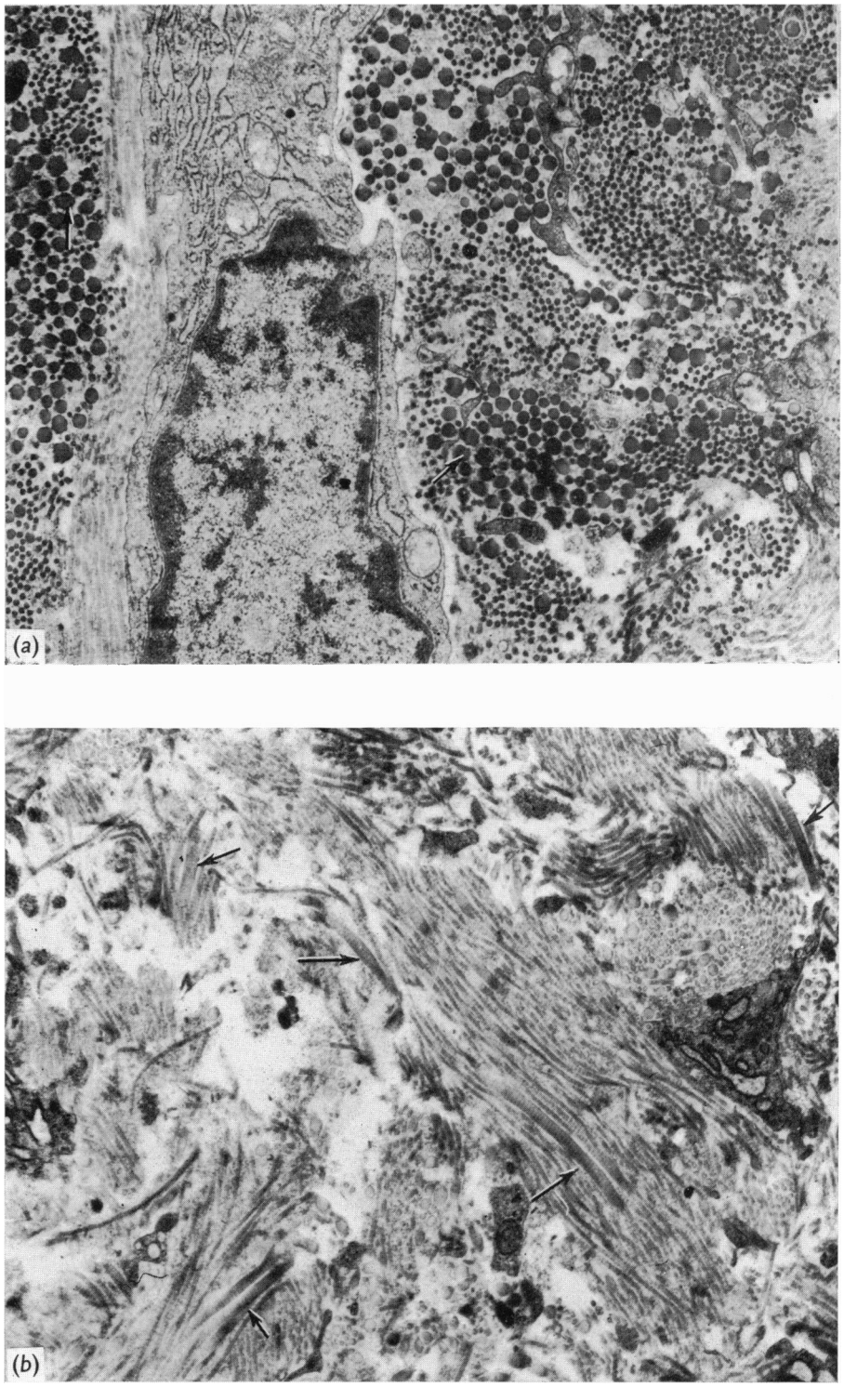



Fig. 13. Regenerated tissue of ear of cortisone-treated rabbit at 35 days after receiving cortisone for 28 days and no treatment for 7 days. Many abnormally large collagen fibres can be seen (arrows). (*a*) T.S. ×11000; (*b*) L.S. ×13000.

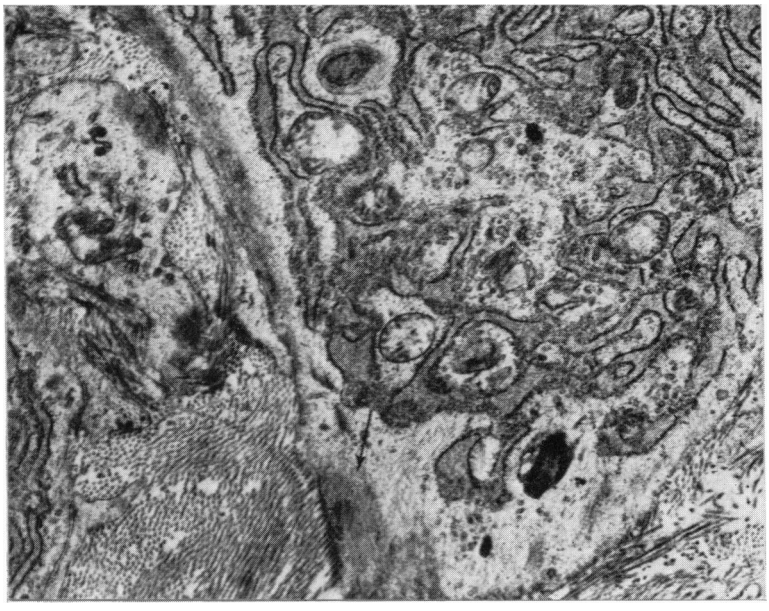


Fig. 14. Regenerated tissue of ear of cortisone-treated rabbit at 42 days after receiving cortisone for 28 days and no treatment for 14 days, showing part of cytoplasm of an active fibroblast with microfilaments at the periphery (arrow); the extracellular collagen fibres are regularly arranged and of normal diameter. × 12000.

Cartilage regeneration can, however, be of considerable importance in stimulating regeneration. Prudden, Gabriel & Allen (1963) suggested that this stimulus is due to a protein associated with an acid mucopolysaccharide and it has been shown in the present experiments that one week after cessation of cortisone administration there is abundant acid mucopolysaccharide in the region of the regenerating cartilage.

Whatever the stimulus for regeneration may be, it appears that prolonged cortisone administration (for 7 weeks) can prevent subsequent regeneration after withdrawal of the cortisone. Earlier withdrawal (after 4 weeks) does not have this effect. Perhaps the growth-promoting factor released by injured cells, as described by Menkin (1960), persists for a certain length of time but is no longer present after an interval of 7 weeks.

With regard to the collagen in the blastema of the cortisone-treated rabbits, the reduced number of fibres at 14 and 21 days can be related to the effect of the cortisone on the fibroblasts, rather than to a direct effect on the process of formation of collagen. At a later stage, at 28 days, cortisone may affect the polymerization of the collagen by inhibiting the sulphation of mucopolysaccharides and thus the formation of acid mucopolysaccharides (Kodicek & Loewi, 1955; Whitehouse & Lash, 1961). This could give rise to the dense staining of the collagen fibres and to the lack of banding, since acid mucopolysaccharides are concerned with the precipitation and orientation of collagen fibres (Lowther & Toole, 1968; Myers, Highton & Rayns,

1969). This is supported by the observations in the present investigation. There were few or no acid mucopolysaccharides in the blastema of the cortisone-treated animals. In the control animals the sections stained with Alcian blue showed the presence of acid mucopolysaccharides. These were seen in the whole of the blastema at 14 days but were largely limited to the regenerating cartilage at later times (21–49 days). The abnormally large fibres (Fig. 13) may also result from the decreased concentrations of mucopolysaccharides in the matrix, as suggested by Silberberg, Silberberg & Hasler (1966).

## SUMMARY

A study was made of the effects of subcutaneous injections of cortisone acetate, administered for 6 consecutive days each week, on the regeneration of tissue following full-thickness removal of 1 $cm^2$ of the rabbit's ear.

Large doses (10 mg/kg for 4 weeks) and smaller doses (2·5 mg/kg for 4 weeks and 2·5 mg/kg for 7 weeks) slowed regeneration significantly. This appeared to be due to the depressed activity of the fibroblasts and the failure of regeneration of the cartilage, both of which might have been caused by the suppression of the inflammatory reaction by the cortisone. This was confirmed by light and electron microscope studies and by the decreased amount of acid phosphatase in the blastema of the cortisone-treated rabbits.

If the cortisone was stopped after 4 weeks, regeneration continued to such an extent that, after 10 weeks, there was no difference in the amount of regeneration as compared with the controls after 7 weeks. On the other hand, if the cortisone was given for 7 weeks and then discontinued, regeneration was significantly less than that of 7 weeks in the controls, even after another 7 weeks. This was related to the appearance of acid mucopolysaccharides in the blastema after the cessation of the administration of cortisone.

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## REFERENCES

ABERCROMBIE, M. (1964). Behaviour of cells towards one another. In *Advances in the Biology of Skin* (Ed. W. Montagna and R. E. Billingham), pp. 95–112. New York: Macmillan.

ALRICH, E. M., CARTER, J. P. & LEHMAN, E. P. (1951). The effect of ACTH and cortisone on wound healing. *Annals of Surgery* **133**, 783–789.

BAKER, B. L. & WHITAKER, W. L. (1950). Interference with wound healing by local action of adrenocortical steroids. *Endocrinology* **46**, 544–551.

BANGHAM, A. D. (1951). The effect of cortisone and ACTH on wound healing. *British Journal of Experimental Pathology* **32**, 77–84.

BERLINER, D. L. & NABORS, C. J. Jr. (1967). Effects of corticosteroids on fibroblast functions. *Journal of the Reticuloendothelial Society* **4**, 284–313.

BRAUN, A. A. & EFIMOV, E. A. (1971). Completeness of post traumatic regeneration of the skin of warm blooded animals. *Bulletin of Experimental Biology and Medicine* **71**, 82–86.

BUKHONOVA, A. I. (1960). Histofunctional changes in a wound at different concentrations of the hormones of the adrenal and thyroid glands. *Proceedings of the Academy of Science, USSR* **134**, 1256–1259.

CREDITOR, M. C., BEVANS, M., MUNDY, W. L. & RAGAN, C. (1950). Effect of ACTH on wound healing in humans. *Proceedings of the Society for Experimental Biology and Medicine* **74**, 245–247.

DOUGHERTY, T. F. & BERLINER, D. L. (1959). Some ways by which ACTH and cortisol influence functions of connective tissue. In *Connective Tissue, Thrombosis and Atherosclerosis* (Ed. I. A. Page), pp. 143–166. New York: Academic Press.

DOUGHERTY, T. F. & SCHNEEBELI, G. L. (1950). Role of cortisone in regulation of inflammation. *Proceedings of the Society for Experimental Biology and Medicine* **75**, 854–859.

DYSON, M. & JOSEPH, J. (1968). The effects of androgens on tissue regeneration. *Journal of Anatomy* **103**, 491–505.

DYSON, M. & JOSEPH, J. (1971). The effect of female sex hormones on regeneration in the rabbit's ear. *Journal of Endocrinology* **51**, 685–698.

EHRLICH, H. P. & HUNT, T. K. (1968). Effects of cortisone and vitamin A on wound healing. *Annals of Surgery* **167**, 324–328.

GOSS, R. I. & GRIMES, L. N. (1972). Tissue interactions in the regeneration of rabbit ear holes. *American Zoologist* **12**, 151–157.

HOUCK, J. C. & VICKERS, K. (1962). Inhibition of inflammation and acceleration of tissue repair by cartilage powder. *Surgery* **51**, 632–638.

HUNT, T. K., EHRLICH, H. P., GARCIA, J. A. & DUNPHY, J. E. (1969). Effect of vitamin A on reversing the inhibitory effect of cortisone on healing of open wounds in animals and man. *Annals of Surgery* **169**, 633–641.

INOUÉ, T. (1961). The 'cartilage effect' on healing wounds. *Archives of Surgery* **82**, 432–434.

JOSEPH, J. & DYSON, M. (1965). Sex differences in the rate of tissue regeneration in the rabbit's ear. *Nature, London* **208**, 599–600.

JOSEPH, J. & DYSON, M. (1966). Tissue replacement in the rabbit's ear. *British Journal of Surgery* **53**, 372–380.

KODICEK, E. & LOEWI, G. (1955). The uptake of $S^{35}$ sulphate by mucopolysaccharides of granulation tissue. *Proceedings of the Royal Society of London*, Series B **144**, 100–115.

LATTES, R., MARTIN, J. R., MEYER, K. & RAGAN, C. (1956). Effect of cartilage and other tissue suspensions on reparative processes of cortisone-treated animals. *American Journal of Pathology* **32**, 979–991.

LOWTHER, D. A. & TOOLE, B. P. (1968). The interaction between acid mucopolysaccharide-protein complexes and tropocollagen. In *Symposium on Fibrous Proteins* (Ed. W. G. Crewther), pp. 229–232. Australia: Butterworth.

MENKIN, V. (1960). Role of inflammation in carcinogenesis. *British Medical Journal* i, 1585–1594.

MYERS, D. B., HIGHTON, T. C. & RAYNS, D. G. (1969). Acid mucopolysaccharides closely associated with collagen fibrils in normal human synovium. *Journal of Ultrastructural Research* **28**, 203–213.

NAGEOTTE, J. (1918). Formation de pièces squelletiques surnumeraires provoquée par la présence de greffons morts dans l'oreille du lapin adulte. *Compte Rendu des Séances de la Société de Biologie, Paris* **81**, 113–118.

PEARSE, A. G. E. (1968). *Histochemistry Theoretical and Applied* (3rd ed.), vol. 1, p. 732. London: Churchill.

PRUDDEN, J. F., GABRIEL, O. & ALLEN, B. (1963). Acceleration of wound healing. *Archives of Surgery* **86**, 157–161.

PRUDDEN, J. F., NISHIHARA, G. & BAKER, L. (1957). Acceleration of wound healing with cartilage. *Surgery, Gynecology and Obstetrics* **105**, 283–286.

PRUDDEN, J. F. & WOLLARSKY, E. (1967). Reversal by cartilage of the steroid induced inhibition of wound healing. *Surgery, Gynecology and Obstetrics* **125**, 109–113.

RAGAN, C., HOWES, E. L., PLOTZ, C. M., MEYER, K. & BLUNT, J. W. (1949). Effect of cortisone on the production of granulation tissue in the rabbit. *Proceedings of the Society for Experimental Biology and Medicine* **72**, 718–721.

SABO, J. C., OBERLANDER, L. K. & ENQUIST, I. F. (1965). Acceleration of open wound healing with cartilage. *Archives of Surgery* **90**, 414–417.

SILBERBERG, M., SILBERBERG, R. & HASLER, M. (1966). Fine structure of articular cartilage of mice receiving cortisone acetate. *Archives of Pathology* **82**, 569–582.

SIMPSON, G. G., ROE, A. & LEWONTIN, R. C. (1960). *Quantitative Zoology*. New York: Harcourt, Brace.

WEISS, C. & ROSENBAUM, R. M. (1967). Histochemical studies on cell death and histolysis during regeneration. I. Distribution of acid phosphomonoesterase activity in the normal, the regenerating and the resorbing forelimb of the larval spotted salamander, *Amblystoma maculatum*. *Journal of Morphology* **122**, 203–230.

WEISS, L. & DINGLE, J. T. (1964). Lysosomal activation in relation to connective tissue disease. *Annals of Rheumatic Diseases* **23**, 57–63.

WEISSMANN, G. & THOMAS, L. (1963). Studies on lysosomes. II. The effect of cortisone on the release of acid hydrolases from a large granule fraction of rabbit liver induced by an excess of vitamin A. *Journal of Clinical Investigation* **42**, 661–669.

WEISSMANN, G. & THOMAS, L. (1964). The effects of corticosteroids upon connective tissue and lysosomes. *Recent Progress in Hormone Research* **20**, 215–245.

WHITEHOUSE, M. W. & BOSTROM, H. (1961). Studies on the action of some anti-inflammatory agents in inhibiting the biosynthesis of mucopolysaccharide sulphates. *Biochemical Pharmacology* **7**, 135–150.

WHITEHOUSE, M. W. & LASH, J. W. (1961). Effect of cortisone and related compounds on biogenesis of cartilage. *Nature, London* **189**, 37–39.

WILHELM, D. L. (1962). The mediation of increased vascular permeability in inflammation. *Pharmacological Reviews* **14**, 251–280.