THE EFFECT OF CORTISONE ON WOUND HEALING.

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THE review of Arey (1936) covers the many and various factors influencing wound healing. In particular, he quotes the reported effects of adrenal extracts and of suprarenal gland stimulation as studied by several authors, and emphasizes the disagreement in their results. Voronoff and Bostwick (1918) and Aievoli (1923) agree that direct application of suprarenal gland extracts to wounds results in the formation of rich granulation tissue. Kosdoba (1934), on the other hand, found that any method that increased the amount of circulating adrenal hormones, e.g. homotransplants or stimulation of the glands, resulted in a prolongation of the healing period.

More recently, Ragan, Grokoest and Boots (1949) drew attention to the well-known fact that in patients exhibiting hyperadrenalism, wounds healed poorly. They also noticed that in those patients receiving ACTH, open wounds, incised abscesses and biopsy sites failed to show normal granulation tissue and were slow to heal. Ragan, Howes, Plotz, Meyer and Blunt (1949) in animals and Creditor, Bevans, Mundy and Ragan (1950) in man showed that the administration of ACTH depressed the formation of granulation tissue in experimentally produced wounds. On the assumption that ACTH stimulates the adrenals to produce cortisone or cortisone-like substances, Blunt, Plotz, Lattes, Howes, Meyer and Ragan (1950) and Ragan, Grokoest and Boots (1949) studied the effect of cortisone on experimentally produced wounds and fractures in animals. These authors found that the reparative processes were greatly depressed, and concluded that the response represented an " inhibition of reactivity."

Acute inflammatory changes are so essentially a part of the process of wound healing that it is of interest at this stage to review the effects of cortisone on these changes. Selye (1949), by injecting formalin subcutaneously into rats, showed that both the acute and self-maintaining chronic inflammatory responses were depressed by cortisone. Jones and Meyer (1950) showed that the inflammatory response to chemical burning of the rabbit's cornea was subdued when cortisone was injected locally into the conjunctiva. Allergic skin responses characterized by hyperaemia and local oedema have also been shown to be suppressed by prior administration of cortisone and ACTH (Long and Miles, 1950 ; Derbes, Dent, Weaver and Vaughan, 1950 ; Harris and Harris, 1950 ; Humphrey, unpublished).

The purpose of the present investigation was to find how cortisone suppressed the process of healing in experimental skin wounds. In Part I the effects of cortisone on wound healing in different animals is described, and in Part II some observations on the effects of cortisone on intracutaneous histamine and a solution of leucotactic peptides are described.

PART I.

A. *The Effect of Cortisone on the Healing of Skin Wounds in Rats.*

Male white rats weighing between 150–250 g. were divided into two groups of 14 each. Group I received 2·5 mg. (approx. 12·5 mg./kg. body weight) cortisone acetate (Merck) daily by intramuscular injection ; the controls, Group II, received injections of saline. The rats from both groups were anaesthetized 24 hours after the injections started and the wounds made. The skin was first shaved and then cleaned with soap, water and spirit. A fold of skin on the back was then pinched up and a leather punch crushed through the two thicknesses of skin. The result was two equally-sized wounds, 5·0 mm. in diameter, involving the skin, panniculus adiposus and panniculus muscularis. The rats were then returned to their cages without any dressing. Both groups of rats were fed liberally on Parkes No. 41 diet.

On days 1, 3, 5, 7, 9, 11 and 13 two animals from each group were killed and the wounds examined histologically. The material obtained was fixed in both 10 per cent formol-saline and 85 per cent alcohol. The formol-fixed material was stained with Ehrlich's acid haematoxylin-eosin, Weigert's elastica, van Gieson, Sylven's method for metachromasia, and by Gomori's method for reticulin fibres. The alcohol-fixed material was processed for alkaline phosphatase by a combination of the methods of Gomori (1939) and of Kabat and Furth (1941).

Results.—Ideally, in this sort of experiment, the results should be assessed on statistical data. This was not possible owing to a shortage of cortisone, but the results of this experiment made it clear that cortisone, in the doses used, had no dramatic effects either on the healing time or in the quality of the granulation tissue formed in the rat. There was a suggestion, however, that in some of the cortisone-treated animals the amount of granulation tissue was reduced (Fig. 1*a* and *b*).

The cortisone-treated rats also showed marked thymic involution, enlargement of the liver due to increased glycogen storage, and changes in the quality of the skin. The latter were noticeable in the living animal, the skin becoming looser and less elastic. Histologically the changes in the skin consisted of a

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EXPLANATION OF PLATES.

FIG. 1*a*.—Rat. Control. A 9-day-old wound showing maturing granulation tissue. Compare the thickness of this wound with that in Fig. 1*b*. H. and E. × 45.

FIG. 1*b*.—Rat. Cortisone-treated. A 9-day-old wound. The granulation tissue present is normal but less abundant. H. and E. ×45.

FIG. 2*a*.—Rat. Control. The panniculus adiposus is prominent, and the hair follicles are seen to extend almost down to the fatty layer. H. and E. × 45.

FIG. 2*b*.—Rat. Cortisone-treated. The appearance of the skin after 12 days' treatment. The panniculus adiposus is absent and the hair follicles do not penetrate very deeply. H. and E. × 45.

FIG. 3*a*.—Rabbit. Control. The appearance of a large full thickness skin wound on the 16th day.

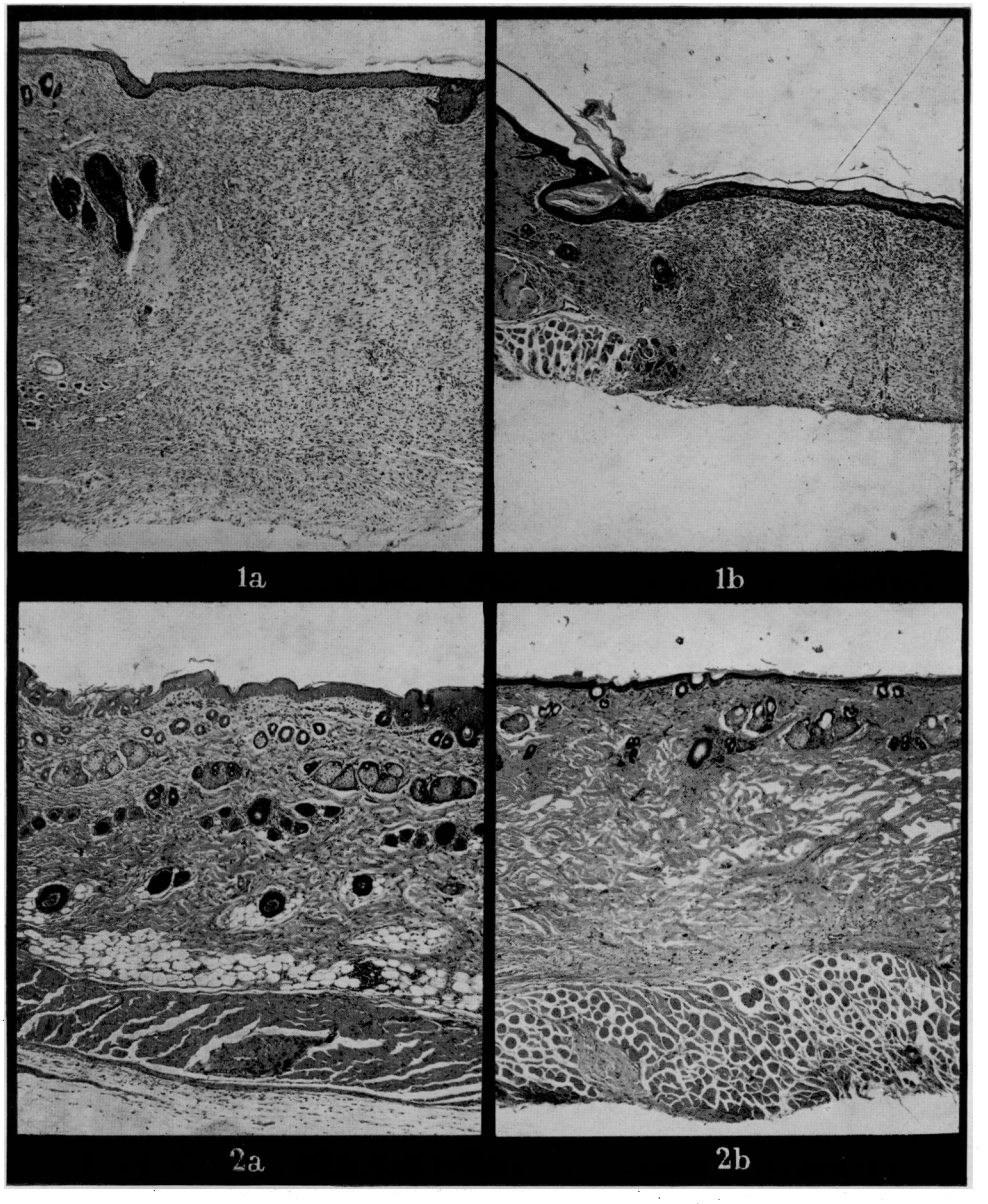
FIG. 3*b*.—Rabbit. Control. The same wound as in Fig. 3*a* photographed 10 days later.

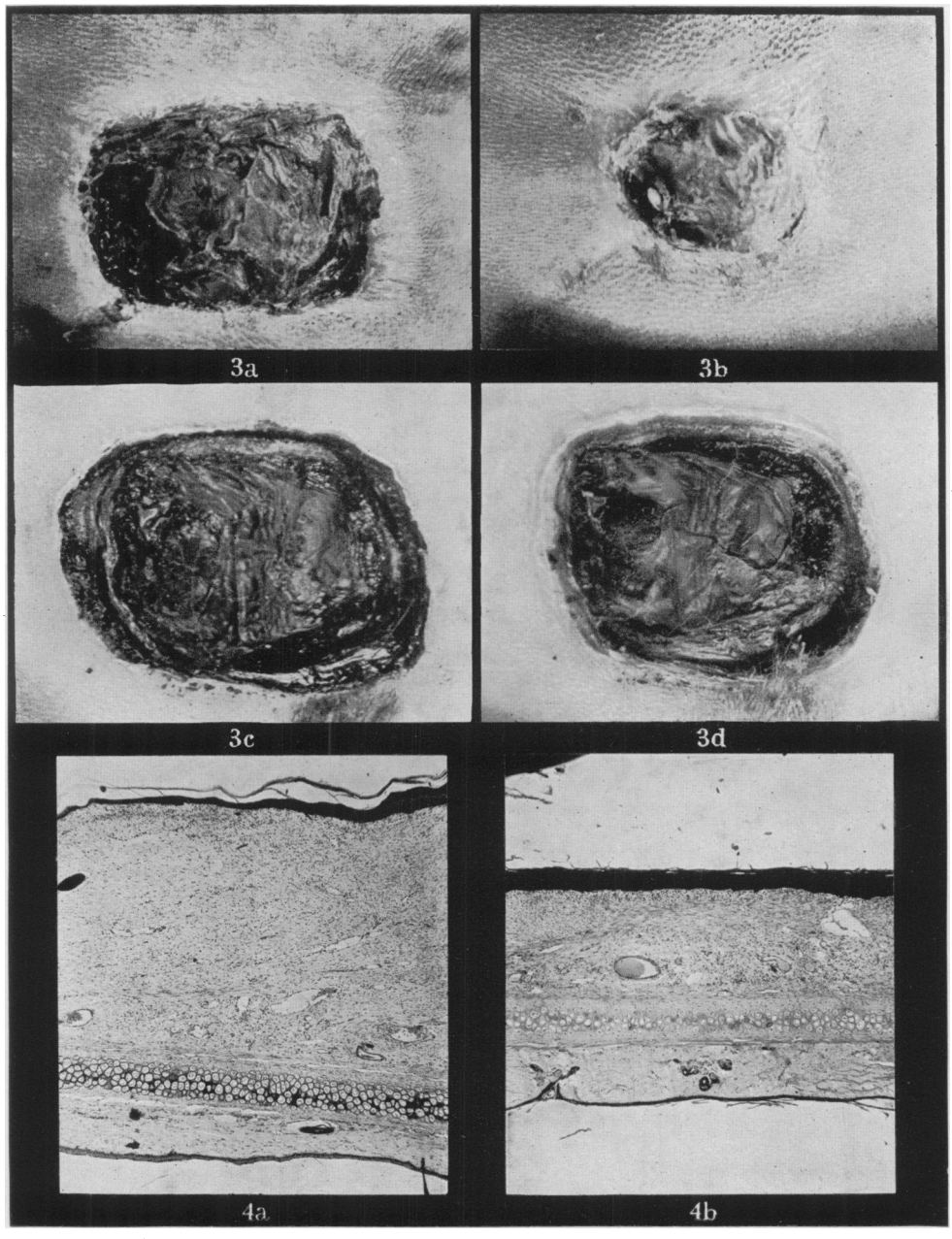
FIG. 3*c*.—Rabbit. Cortisone-treated. This rabbit was wounded on the same day as the control, but received cortisone. Appearance of wound on the 16th day.

FIG. 3*d*.—Rabbit. Cortisone-treated. The same wound as in Fig. 3*c* photographed 10 days later.

FIG. 4*a*.—Rabbit. Control. Transverse section of the ear 13 days after wounding. Note the abundant granulation tissue. H. and E. × 45.

FIG. 4*b*.—Rabbit. Cortisone-treated. Transverse section of the ear 13 days after wounding. Compare the thickness of granulation tissue with that in Fig. 4*a*. H. and E. × 45.


1a
1b
2a
2b


3a
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3c
3d
4a
4b

narrowing, and in some animals a loss of the panniculus adiposus and repression of hair-follicle growth. There was no evidence of any gross changes in the amount or distribution of elastic fibres, but the collagen fibres appeared more compact (Fig. 2*a* and *b*).

B. *The Effect of Cortisone on the Healing of Skin Wounds in Guinea-pigs.*

Young male guinea-pigs weighing 200–250 g. were divided into two groups of 8 each. Group I received 2·5 mg. of cortisone intramuscularly (approx. 12·5 mg./kg. body-weight) daily throughout the experiment. Group II received an equivalent volume of saline. The animals were anaesthetized 24 hours after the first injections, and 4 equally-sized wounds made in the skin, using the technique already described. Both groups of guinea-pigs were fed liberally on Parkes No. 41 diet with fresh cabbage daily.

On days 3, 5 and 9 two animals, and on days 11 and 13 one animal from each group were killed and the skin wounds examined histologically.

Results.—In this series the healing time of each wound was recorded, the criterion for a fully healed wound being an intact epithelial surface with no adherent scab. At the dose level of cortisone used, no difference in the healing time was observed between the two groups of animals. Histologically the wounds from the cortisone-treated animals were similar in all respects to those in the control animals.

C. *The Effect of Cortisone on the Healing of Wounds in Rabbits.*

Two groups of 3 rabbits each were used. Group I received cortisone (7·5 mg./kg. body-weight daily) by intramuscular injection throughout the experiment and the controls received injections of saline. Three days after the injections had started each rabbit was anaesthetized and wounded. The wounds consisted of 5-mm. punch wounds, a large resection of skin of approximately 4 sq. cm. and the removal of 1 sq. cm. of skin from the surface of the ear. One control and one experimental animal were killed on the 4th, 8th and 13th day after wounding and the lesions were examined histologically. The rabbits were fed on Parkes No. 18 diet supplemented with cabbage three times a week.

Results.—From a day-to-day examination of the wounds it was obvious that the cortisone-treated animals were healing differently from the controls. By the 16th day the large wounds in the control animals showed some contraction and filling with granulation tissue; the cortisone-treated animals showed complete absence of any sort of response (Fig. 3*a–d*). The ear wounds showed similar changes up to the 8th–9th day, but by the 13th day a small amount of granulation tissue had appeared and the epithelium had regenerated and covered the wound. Histologically the granulation tissue in the cortisone-treated animal did not differ qualitatively from that of the control, the most striking difference being quantitative. All the elements of normal granulation tissue were present in a quantity proportionate to the amount of new tissue that had formed. In other words, neither the inflammatory cellular elements nor the capillaries, fibroblasts and intercellular ground substances showed any gross abnormality (Fig. 4*a* and *b*). This finding is contrary to that of Ragan, who likened the granulation tissue in cortisone-treated animals to that found in animals rendered scorbutic.

DISCUSSION.

The changes in the skin of the rats treated parenterally with cortisone are very similar to those reported by Baker, Ingle, Li and Evans (1948). These authors administered ACTH to their rats, and found that after a period of some 10 days' treatment there occurred thinning of the skin, a reduced growth of hair and a reduction in the size of the panniculus adiposus. These changes were also accompanied by general loss of weight. ACTH has also been shown by Evans, Simpson and Li (1943) to inhibit general body growth, and even to antagonize the action of pituitary growth hormone. Marx, Simpson, Li and Evans (1943), Baker and Ingle, Li and Evans (1948), in discussing these findings, suggested that the existence of protein catabolism and an increase in the excretion of urinary nitrogen proportional to the loss in body weight was the most probable explanation for the inhibition of growth. Baker and Whitaker (1948), however, in a later paper were able to show that cortisone could produce involution of epidermal elements without catabolism of liver proteins, presumably without the existence of a negative nitrogen balance. In their experiments cortisone was applied locally to the skin and only the treated areas showed involutionary changes. These changes correspond well with the observations of Baker and Whitaker (1950), who delayed the healing of wounds and the formation of granulation tissue with a locally applied suprarenal extract. There is, therefore, a strong case for suspecting that cortisone inhibits wound healing either by antagonizing pituitary growth hormone or by retarding cell growth directly.

Another aspect of the problem is the effect of cortisone on the development of acute inflammatory responses. Selye (1949) injected formalin into the tissues, and thereby produced acute and subsequently chronic inflammatory changes at the site of inoculation. Cortisone reduced or abolished the inflammatory response, and so protected the animal from the development of a granuloma and its extensive scar. In this sort of experiment, cortisone may be reducing the amount of granulation tissue by subduing the initial inflammatory response. Carrel (1930) has indeed suggested that growth-activating substances are liberated by leucocytic and other ferments acting on cell débris and fibrin—in other words, just those substances that are known to be produced in inflamed tissues and which cortisone seems able to reduce.

There remain two further possibilities, both of which might reduce hyperaemia —altered capillary permeability and the formation of fibrin. Firstly, cortisone may be preventing the formation of chemical stimulators, e.g. leukotaxin and H substance or histamine, or secondly the surviving cells may be made insensitive to these substances by cortisone.

That suprarenal hormones play a significant role in the regulation of fluid exchange as manifested by capillary filtration has long been suspected. Menkin (1940), for instance, showed that the effect of inflammatory exudates or of leukotaxin in increasing capillary permeability was wholly or partly inhibited by the presence of an extract of suprarenal cortex.

In order to test this latter hypothesis an investigation was made to determine the influence of cortisone on the action of solutions of leucotactic peptides, and of histamine in increasing skin capillary permeability.

PART II.

The Effects of Cortisone on Histamine Skin Responses and on Intracutaneous Injection of Leucotactic Peptides.

The leucotactic peptides were prepared by Dr. W. G. Spector, working in this department. They are made by de-proteinizing a peptic digest of fibrin, and in the dry state remain active for many months.

The dilutions of leucotactic peptides were made up immediately before use, 10 mg. being dissolved in 1·5 ml. of 0·9 per cent NaCl and 10-fold dilutions made from the 1/100 stock. The histamine dilutions were also made up in 0·9 per cent NaCl.

The rabbits were anaesthetized with Nembutal and their abdomens carefully depilated with a depilatory paste. After a thorough washing with warm water the rabbits were placed on their backs on top of a warm water bath, where they remained throughout the experiment. Trypan blue (Gurr), 3·5 ml./kg. body-weight of a $2\frac{1}{2}$ per cent solution, was then injected intravenously, and after waiting about 20 min. to make sure that there were no inherent blue areas, the dilutions of luecotactic peptides and histamine were injected. The intracutaneous injections were limited to a volume of 0·1 ml., and were made with a tuberculin syringe and a fine needle.

Readings were made 30 minutes after injection, and the colour intensities of the control and experimental animals compared and awarded arbitrary units, thus: +++, ++, +, 0. In each experiment two rabbits were used, one receiving an intramuscular injection of cortisone (10 mg./kg. body-weight) 18–24 hours before testing, while the controls received an equivalent volume of saline. Three weeks later the same two rabbits were again tested, the original control now receiving the cortisone.

In each of these experiments it was noticed that the areas that had been manipulated by the left hand, the right holding the syringe, gradually assumed a blue colour due presumably to localized injury and increased capillary permeability and leakage of dye. The intensity of staining of these areas in the control and experimental animals was recorded and appears with the other results in Table I. These results show that cortisone given 18–24 hours before testing reduced the amount of dye leakage following the intracutaneous injections of histamine and of leucotactic peptides. Moreover, the staining that results from the manipulation of the skin was also reduced.

In an effort to overcome animal-to-animal variation and to find out how soon cortisone was effective, a further series of rabbits was anaesthetized, depilated and injected with leucotactic peptides and histamine. Cortisone was then injected and the substances were re-tested at intervals of 1, 2 and 3 hours. In this way each rabbit acted as its own control, the activity of the solutions being checked by including an untreated animal.

The results of this series of experiments appear in Table II. Here again, and within two hours of an intramuscular injection, cortisone prevented the leakage of dye into the tissues.

DISCUSSION.

It is tempting to correlate the subduing effects of cortisone on the formation of granulation tissue with its apparent ability to protect small blood vessels from

TABLE I.—*The Degree of Staining at the Site of Intracutaneous Injections of Histamine and Leucotactic Peptides in Rabbits Injected Intravenously with Trypan Blue.*

Experiment number.	Rabbit number.	Control. Leucotaxin. 1/100.	1/1000.	1/10,000.	Histamine. 1/1000.	1/10,000.	1/100,000.	Background.	Rabbit number.	Cortisone pre-treated. Leucotaxin. 1/100.	1/1000.	1/10,000.	Histamine. 1/1000.	1/10,000.	1/100,000.	Background.
1	1	+++	++	++	+++	+++	++	+++	2	0	0	0	0	0	0	0
2	3	++	++	+	+++	+++	++	+	4	+	+	0	0	+	0	+
3	5	+++	++	+	+++	++	+	++	6	+	+	0	0	0	0	0
4	7	++	++	+	++	+	0	++	8	++	0	0	0	..	..	0
						3 *weeks later.*										
1	2	+++	++	+	+++	+++	++	++	1	+	+	0	+	0	0	+
2	4	+++	++	+	++	++	+	++	3	++	0	0	0	+ 0		0
3	6	+++	+++	++	+++	+++	++	+	5	+	0	0	+	0	0	0
4	8	+++	++	+	+++	++	+	++	7	+	++	0	0	0	0	0

TABLE II.—*The Degree of Staining at the Site of Intracutaneous Injection of Histamine and Leucotactic Peptides in Rabbits Injected with Trypan Blue,* 1 *and* 3 *Hours after a Single Intramuscular Injection of Cortisone.*

Rabbit number.	Initial. Leucotaxin. 1/100.	1/1000.	1/10,000.	Histamine. 1/1000.	1/10,000.	1/100,000.	1 hour. Leucotaxin. 1/100.	1/1000.	1/10,000.	Histamine. 1/1000.	1/10,000.	1/100,000.	3 hours. Leucotaxin. 1/100.	1/1000.	1/10,000.	Histamine. 1/1000.	1/10,000.	1/100,000.
							Control.											
9	+++	++	+	++	++	++	+++	++	+	++	++	++	+++	++	+	++	++	++
10	+++	++	+	++	+	+	+++	++	+	++	+	+	No record.					
							Cortisone.											
11	+++	++	0	+++	++	0	++	++	0	0	0	+	+	0	0	0	0	0
12	+++	++	0	+++++		+	+++	+	0	++	0	0	+	0	0	0	0	0
13	+++	+	0	++	++	0	++	+	0	++	++	0	+	0	0	0	0	0

substances capable of increasing their permeability. Inflammation and repair can be regarded as a sequence of reactions, each reaction dependent both qualitatively and quantitatively upon its predecessor. If, as seems likely in the case of wound healing in rabbits, cortisone is merely depressing a normal process, its action is most probably that of controlling the degree of reactivity, rather than altering its actual pattern. Whether cortisone exerts its effect throughout the process of wound healing is not known, but it is evident from the results obtained that cortisone is active in its earliest stages, namely, the stages of hyperaemia and oedema formation. It is not surprising therefore to find that cortisone is capable of inhibiting a variety of allergic skin responses, all of which are thought to depend ultimately upon the local release of histamine or of active leucotactic peptides.

A hypothesis, therefore, that cortisone acts by reducing the sensitivity of surviving tissue to the prime movers of the inflammatory response lends itself to an interpretation of the known effects of cortisone in the treatment of rheumatic fever and rheumatoid arthritis, both conditions representing an exaggerated inflammatory response. Furthermore, the report by D'Arcy Hart and Rees (1950) that a quiescent form of tuberculosis in mice flares up when cortisone is given, supports the view that the inflammatory reaction is being subdued, in this instance to the detriment of the animal.

SUMMARY.

1. The effects of cortisone on the healing of skin wounds in various animals have been studied. Cortisone has no effect on wound healing in guinea-pigs ; it probably reduces the amount of granulation tissue formed by rats, and retards wound healing in rabbits.

2. Solutions of leucotactic peptides and of histamine have been injected into the skin of rabbits, and the effect of cortisone on the formation of the wheals has been observed.

3. These experiments confirm the results of other workers, and show that at a relatively high dose level in rabbits the process of wound healing may be retarded. The demonstration that cortisone may inhibit the skin reactions to histamine and leucotatic peptides suggests a mechanism whereby inflammatory and the subsequent reparative processes are subdued.

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REFERENCES.

Arey, L. B.—(1936) *Physiol. Reviews*, **16**, 327.
Aievoli, E.—(1923) *Ann. ital. di Chir.*, **2**, 436.
Baker, B. L., Ingle, D. J., Li, C. H., and Evans, H. M.—(1948) *Anat. Rec.*, **102**, 313.
Idem and Whitaker, W. L.—(1948) *Ibid.*, **102**, 333.
Iidem—(1950) *Endocrinology*, **46**, 544.

BLUNT, J. W., PLOTZ, C. M., LATTES, R., HOWES, E. L., MEYER, K., AND RAGAN, C.—(1950) *Proc. Soc. Exp. Biol and Med.*, **73**, 678.
CARREL, A.—(1930) *Proc. Inst. Med., Chicago*, **8**, 62.
CREDITOR, M. C., BEVANS, M., MUNDY, W. L., AND RAGAN, C.—(1950) *Proc. Soc. Exp. Biol. and Med.*, **74**, 245.
D'ARCY HART, P., AND REES, R. J. W.—(1950) *Lancet*, ii, 391.
DERBES, V. J., DENT, J. H., WEAVER, N. K., AND VAUGHAN, D. D.—(1950) *Proc. Soc. Exp. Biol. and Med.*, **75**, 423.
EVANS, H. M., SIMPSON, M. E., AND LI, C. H.—(1943) *Endocrinology*, **33**, 237.
GOMORI, G.—(1939) *Proc. Soc. Exp. Biol., N.Y.*, **42**, 23.
HARRIS, S., AND HARRIS, T. N.—(1950) *Proc. Soc. Exp. Biol. and Med.*, **74**, 186.
JONES, I. S., AND MEYER, K.—(1950) *Ibid.*, **74**, 102.
KABAT, E. A., AND FURTH, J.—(1941) *Amer. J. Path.*, **17**, 303.
KOSDOBA, A. S.—(1934) *Arch. f. klin. Chir.*, **179**, 435.
LONG, D. A., AND MILES, A. A.—(1950) *Lancet*, i, 492.
MARX, W., SIMPSON, M. E., LI, C. H., AND EVANS, H. M.—(1943) *Endocrinology*, **33**, 102.
MENKIN, V.—(1940) *Amer. J. Physiol.*, **129**, 691.
RAGAN, C., GROKOEST, A. W., AND BOOTS, R. H.—(1949) *Amer. J. Med.*, December, 1949.
Idem, HOWES, E. L., PLOTZ, C. M., MEYER, K., AND BLUNT, J. W.—(1949) *Proc. Soc. Exp. Biol. and Med.*, **72**, 718.
SELYE, H.—(1949) *Brit. Med. J.*, ii, 1129.
VORONOFF, S., AND BOSTWICK, E.—(1918) *Comp. rend. Acad. Sci.*, **167**, 385.