DESOXYCORTICOSTERONE ACETATE AND WOUND HEALING*

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PLATES 8 TO 11

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According to Selye and coworkers (1-3) overdosage of desoxycorticosterone acetate (DCA) in the rat may induce changes characterized by nephrosclerosis with hypertension and by cardiovascular and articular lesions of the "rheumatic type." On the basis of his experiments Selye (4, 5) theorized that certain chronic diseases, such as rheumatic fever, periarteritis nodosa, arthritis, and hypertension, are the result of an abnormal response or of a perverted adaptation to stress mediated through the endocrine system, particularly an overproduction of adrenocortical hormones or hypercorticism. These conditions, which are all known to involve mainly tissues of mesenchymal origin, were therefore classified by Selye as "diseases of adaptation."

Recently Taubenhaus and Amromin (6) reported that DCA stimulates the proliferation of fibroblasts and encourages the deposition of a homogeneous groundwork of collagen. They obtained these results by studying the effect of the hormone on the granulation tissue around subcutaneous turpentine abscesses in rats. On the basis of more recent studies (7) these investigators advanced the opinion that the stimulating effect of DCA may take place through mechanisms other than direct influence upon granulation tissue. The possibility that the action of DCA is mediated through an enzymatic mechanism must be considered especially in view of observations (8, 9) pointing to the important role of alkaline phosphatase in the proliferation of tissues of mesenchymal origin, and of other studies (10) indicating that DCA increases the activity of alkaline phosphatase in the epiphyses of rats' femora. The effect of DCA administration on other factors which influence mesenchymal cell proliferation, such as ascorbic acid metabolism and adrenocortical function, must also be considered in this respect.

Previous unpublished studies from our laboratory (11) on the effect of large doses of DCA in intact guinea pigs showed that, as in the rat (4, 5), the main lesions attributable to this hormone are localized in the cardiovascular-renal system and in the adrenals. These changes consist of moderate to severe nephro-

* The opinions expressed in this paper are those of the authors, and do not necessarily represent the official views of any governmental agency.
sclerosis, accompanied by tubular hypertrophy, cardiac hypertrophy, and in small arteries thickening of the media and endothelial hyperplasia. Occasionally small perivascular foci of mononuclear cells and of histiocytes were seen in the heart. No changes were observed in the articulations. The adrenals showed marked atrophy of the zona glomerulosa and moderate atrophy of the fasciculata as described by Greep and Deane (12). The cardiovascular-renal lesions observed in these animals were considered to be for the most part secondary to the DCA-induced hypertension and not to a direct action of this hormone on mesenchymal structures (13). Although no lesions resembling those usually seen in the so called "diffuse collagen diseases" were obtained, it was decided to extend this investigation with a study of the effect of large doses of DCA on granulation tissue of healing wounds. The guinea pig was chosen as experimental animal because of its inability to synthesize ascorbic acid, resembling man in this metabolic character. Since ascorbic acid is essential for normal connective tissue proliferation (14, 15) on the one hand, and is most probably related to adrenocortical metabolism (16-18) on the other, it seemed logical to choose an experimental animal in which the important factor of ascorbic acid intake could be controlled.

The purpose of this study then was to see firstly whether or not DCA actually induces changes in proliferating connective tissue; and secondly, if such changes are present, to analyze them in detail and with the help of other observations to attempt to gain a better understanding of the mechanism by which they are produced.

**Methods**

Young male guinea pigs, obtained from a local dealer, were used. The individual animals were placed in separate wire screen raised-bottom cages and offered tap water and a pellet ration ad lib. Rockland guinea pig diet without vitamin C was used, and 2.5 mg. of ascorbic acid per day was administered orally. This quantity of ascorbic acid, although not an optimum, is adequate under normal conditions for growth and reproduction (19, 20). It was chosen to avoid the possibility that some of the effects induced by the administration of DCA could be masked by excessive amounts of available ascorbic acid. After a week of adjustment to this regimen under laboratory conditions 60 healthy animals weighing between 300 and 325 gm. were selected for the experiment and divided into two groups.

In the first group of 48 guinea pigs, 24 animals were injected subcutaneously with 3 mg. per day of DCA suspended in 0.5 cc. of sesame oil for the duration of the experiment and 24, which served as controls, only with sesame oil. On the 5th day of DCA administration a linear midline laparotomy wound measuring 3.5 cm. in length was performed in each animal under nembutal anesthesia (3 mg. in saline per 100 gm. body weight intraperitoneally). No efforts were made to obtain complete sterile conditions. The wounds were then closed by means of 3 or 4, steel wire, through and through sutures. The animals were weighed three times weekly and closely watched for abnormal signs and wound changes. Groups of animals were killed by exsanguination under nembutal anesthesia at days 1, 2, 3, 5, 7, 10, and 14.

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1. This diet was especially prepared by the Arcady Farms Milling Company of Chicago through the courtesy of Mr. F. J. Rech.
after operation. The 5th and 10th postoperative days (10th and 15th respectively of DCA administration) were considered as "critical," a larger number of guinea pigs being killed on these days. The wounds were excised, blocks were taken from the center of the wound, a block being fixed in 10 per cent neutral formalin and then embedded in paraffin and a block frozen-dried with the Altman-Gersh technique (21). Sections were stained with hematoxylin-eosin, by the Van Gieson and Foot (reticulum) methods, with toluidine blue, by the Hotchkiss periodic acid routine, and with the Gomori technique for alkaline phosphatase (22-26). Details and modifications of these and other histological techniques used in this study are given elsewhere (27). The adrenals were removed immediately after death, the left one being used for determination of total and free cholesterol (28) and half of the right gland for the determination of ascorbic acid (29). The remaining half adrenal was fixed in 10 per cent formalin and frozen sections were stained with hematoxylin and eosin, oil-red-O for lipids, Schultz's technique for sterols, or examined unstained under polarized light for birefringent material. Whole blood was analyzed for ascorbic acid (30) and blood serum for total protein and albumin-globulin ratio (31, 32).

In the second group, 12 guinea pigs were handled in an identical fashion except that in a subgroup of 6 animals DCA administration was begun 5 days after operation, and in the other subgroup 10 days after operation. DCA was then continued for 10 days to study its effect on both partially and well developed wound granulation tissue. In each subgroup 3 guinea pigs received the hormone and 3 were controls. 5 animals which either died after the operation or had developed infection of the wound were discarded, reducing the number of animals studied in the entire experiment to 55.

The wound sections were examined in a uniform manner without previous knowledge of the origin of the slide. The wound area was arbitrarily defined as that limited by the epidermis and the peritoneal surface in one direction and by the width of two low power (X 35) fields on the other. Each histological detail being investigated was then graded in arbitrary units (1+ to 4+) and tabulated in an attempt to obtain a quantitative evaluation of the possible changes.

RESULTS

There was no significant difference between the behavior of the DCA-treated guinea pigs and that of the controls. Food consumption was approximately the same in the two groups while water consumption appeared slightly increased after 2 weeks of DCA administration.

Effect of DCA on Wound Healing

No gross changes of note were observed in the wounds. There were, however, significant microscopic differences in the two groups (Text-fig. 1):

Control Animals.—Epithelization of the wound began on the 2nd and was completed on the 7th postoperative day. In the first 2 days the wound space was partially filled with serous fluid, a small amount of extravasated blood, and a small number of polymorphonuclear leucocytes. Fibroblasts were first seen on the 2nd day and rapidly increased in number to reach a maximum between the 5th and 7th postoperative days. Fibroblasts then decreased with the maturation of part of them to fibrocytes, but were still present in small to moderate number at day 14. Capillarization of the granulation tissue was first apparent at day 3, reaching a maximum at day 7 to 10, to become less promi-
TEXT-FIG. 1. The effect of desoxycorticosterone acetate (DCA) on healing wounds. A, intensity of the fibroblastic proliferation; B, amount of metachromatic intercellular material (toluidine blue); C, rate of precollagen (reticulum) and collagen formation.
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Ground substance as revealed by metachromatic stain with toluidine blue was first seen at day 3, reached a maximum at day 10, and then declined considerably to day 14. The intensity of the metachromatic staining followed a similar pattern, the maximum being reached at day 5. The formation of collagen fibers, as judged by the transition from yellow to pink to red with the Van Gieson stain, began on the 5th day and increased progressively afterwards up to day 14. Even at day 14, however, the scar tissue had not taken the brilliant red color of normal connective tissue. Precollagen fibers or reticulum was already present in considerable quantity on day 3 to increase slightly to day 7, and then almost completely disappear at day 14.

DCA-Treated Animals.—Epithelization of the wound was not affected. The amount of granulation tissue was considerably greater than in the controls, this difference being more pronounced at day 10 but also noticeable at days 7 and 14 (Figs. 1a and 1b). The greater amount of granulation tissue present was due not only to an actual increase in the number of fibroblasts but also in the amount of intercellular metachromatic material. Maturation of fibroblasts to fibrocytes was somewhat delayed (Figs. 2a and 2b). Similarly the metachromasia of the intercellular material did not diminish after having reached a maximum at day 10, indicating a delayed maturation. The immaturity of granulation tissue was also apparent by the failure of collagen fibers to stain as intensely red with Van Gieson's stain as in the controls especially at days 10 and 14. Further confirmation of this lag in maturation was given by the persistence of an excessive number of argentaffin reticulum fibers particularly at days 10 and 14 (Figs. 3a and 3b).

Periodic acid routine (Hotchkiss') disclosed leucofuchsin-reacting material in small amounts irregularly distributed throughout the wound granulation tissue especially around capillaries. A positive reaction first appeared at day 3, the greatest amount being noted at day 5 and slowly declining afterwards. No significant differences could be detected in the amount or staining intensity of glycoproteins, as shown by the Hotchkiss technique, between controls and DCA-treated guinea pigs except at day 3 when positively reacting ground substance seemed relatively more abundant in the latter group of animals.

In general there was a close correlation between the findings in formalin-fixed tissue previously described and those obtained in frozen-dried material. The lesser shrinkage produced by freezing-drying afforded a more accurate observation of cellular and intercellular detail. In addition a more reliable evaluation of histochemical changes was possible with this technique. However, no abnormalities in size and shape of fibroblasts were observed. Similarly the Van Gieson, toluidine blue, and Hotchkiss' stains failed to yield additional information, while Foot's stain for reticulum could not be applied to this material.

The alkaline phosphatase method of Gomori gave technically satisfactory results in frozen-dried sections. Enzymatic activity could be demonstrated in
varying amounts in the wound granulation tissue particularly in leucocytes, young fibroblasts, in the wall of capillaries, and at times focally in the ground substance. It was also present in hair follicles and in much smaller amounts near the basal cell layer of the epidermis. Alkaline phosphatase was relatively more abundant at days 5 and 7 than at days 10 and 14 postoperatively. It appeared to be slightly less abundant at corresponding days in the DCA-treated guinea pigs (Figs. 4a and 4b). Individual variation, however, was consider-

![Graphs showing effects of DCA administration.](image)

**Text-Fig. 2.** Some effects of desoxycorticosterone acetate administration.

able, even in the animals belonging to the same group, limiting the value of the findings.

**Other Effects of DCA**

The adrenal glands were slightly but not significantly reduced in weight in the DCA-treated guinea pigs as compared to the controls. No changes could be detected in the gross but histologically the thickness of the cortex was distinctly reduced in the experimental animals. This reduction was due to a marked atrophy of the zona glomerulosa, whose cells were smaller and less numerous, while their nuclei appeared spindle-shaped and hyperchromatic. The zona
fasciculata exhibited a less obvious atrophy (Figs. 5a and 5b). There was a marked depletion of sudanophilic material particularly in the glomerulosa and in the inner and middle fasciculata. The outer fasciculata presented only a moderately reduced sudanophilia (Figs. 6a and 6b). Diminution of birefringent material (Figs. 7a and 7b) was particularly severe after 15 days of DCA administration. Similar results were obtained with Schultz's technique for sterols. The sudanophilic lipids and birefringent material of the zona reticularis were also somewhat reduced while the medulla did not appear to be affected.

The ascorbic acid and cholesterol content of the adrenals was considerably less on the average in the DCA-treated guinea pigs than in the controls especially on the 10th and 15th days of treatment (Text-fig. 2). This confirmed a definite trend toward cholesterol depletion of the adrenal cortex which had already been indicated by the histochemical findings. The reduction in adrenal ascorbic acid content after DCA, although not as consistent as that of cholesterol, can also be considered significant, in our opinion, in view of the observations of Greep and Deane (12) in the rat and of other experiments (16, 18), in which it was shown that adrenal ascorbic acid and cholesterol behave in a parallel fashion and diminution of one substance is always accompanied by diminution of the other. Whole blood ascorbic acid levels were approximately the same in the experimental and in the control animals, with values ranging toward the lower values of normal and showing a decrease from the 10th to the 15th days. Total serum proteins and the A/G ratio showed no significant changes (Text-fig. 2).

In the second group of animals microscopic study of the wound disclosed, when DCA administration was started on the 5th postoperative day, an increased amount of granulation tissue accompanied by a moderate lag in healing. No significant changes, however, were observed when treatment was begun on the 10th postoperative day. The histochemical and chemical findings in the adrenals were fundamentally similar to those described for the previous group although less marked. The difference was probably due to the fact that in this group DCA was administered for only 10 days and also that the animals were sacrificed at a longer period following the operation.

**DISCUSSION**

From the results obtained in this study it appears that DCA stimulates the proliferation of fibroblasts and the formation of intercellular material in the granulation tissue of healing abdominal wounds in guinea pigs. The production of a larger amount of granulation tissue is accompanied by a delayed maturation of fibroblasts, collagen fibers, and ground substance. It is probable in fact that the increased number of fibroblasts is due not only to an actual augmentation of proliferative activity but in part also to a delayed maturation with a resulting temporary numerical increase of these cells. The fact that the
lag in maturation was less obvious on the 14th than on the 10th or 7th postoperative day seems to indicate that possibly no significant changes induced by DCA would have been found if the experiment had been prolonged for 1 or 2 weeks more. Similarly the ground substance appears to have been increased not only because a larger amount was produced but also because it had become more reactive to some of the connective tissue stains, for example, toluidine blue. The latter phenomenon can be better understood in the light of the recent work of Gersh and Catchpole (33) which indicates that a depolymerization of complex substances, e.g. mucoproteins, may take place in connective tissue in a number of conditions including infections and ascorbic acid deficiency. According to these investigators depolymerized mucoproteins show a greater reactivity to a number of stains.

Our findings, therefore, are only in partial agreement with those obtained by Taubenhaus and Amromin (6, 7) in the rat. Their method to produce granulation tissue, however, was different from ours, mainly because of the use of turpentine whose possible action on connective tissue is not well known and because of the presence of a large number of polymorphonuclear leucocytes near the granulation tissue. It seems likely, in our opinion, that proteolytic or other enzymes liberated from the leucocytes may modify to some extent the morphologic appearance and staining reactivity of the intercellular material. For this reason we discarded from our series those animals which showed either gross or microscopic infection of the wound.

The tendency to a diminution of histochemically demonstrable alkaline phosphatase in the wounds as a result of DCA administration was not sufficiently marked to allow definite conclusions, especially in the absence of quantitative determinations. In addition, the role of this enzyme in connective tissue proliferation is by no means clear and does not permit at this moment an accurate evaluation of this finding. Alkaline phosphatase has been shown to be produced by osteoblasts, fibroblasts, and in general, by proliferating mesenchymal cells (8, 26). Probably its activity is related not only to cell proliferation but also to maturation of these cells and also of intercellular material. The production of alkaline phosphatase in bone and in granulation tissue has been found to be reduced in scurvy (9, 34, 35), a condition characterized, amongst other features, by an excessive proliferation of immature mesenchymal cells and by formation of a reduced amount of intercellular material which fails to mature properly. It is interesting to note in this respect that in the DCA-treated guinea pigs some of the changes observed in the wounds were similar to those found in scorbutive guinea pigs in the absence of any detectable signs of scurvy or vitamin C deficiency. Failure to find an increased amount of alkaline phosphatase is not in agreement with the findings of Williams and Watson (10) but is not surprising in view of the difference in tissue examined and in techniques used.
The less marked changes or lack of them observed in the wounds when DCA administration was begun on the 5th and 10th postoperative days, respectively, indicate that DCA does not have the same effect on granulation tissue which is already well developed at the beginning of hormonal treatment. Connective tissue in the earlier stages of proliferation is more reactive to DCA probably on the basis of greater metabolic requirements and therefore of a greater sensitivity to change from normal conditions.

The mechanism by which DCA induces the previously described changes in connective tissue of healing wounds can be discussed in the light of other data obtained in the course of this study. Since some nutritional factors are known to affect connective tissue proliferation, the nutritional state of the experimental animals was carefully checked. No significant variations from the controls were, however, observed in body weights, in gross food consumption, and in serum proteins and whole blood ascorbic acid levels. The relatively low blood ascorbic acid values which occurred to the same extent in both experimental and control guinea pigs, may be an indication of increased requirements for this vitamin in wounded animals. The possibility that a selective tissue depletion of ascorbic acid, not reflected in the blood levels, had taken place in these animals cannot be ruled out, however, in the absence of more complete metabolic studies.

The slight diminution in adrenal weight observed after administration of DCA was accompanied by a distinct reduction of cholesterol and ascorbic acid concentration and histochemically by a marked depletion of stainable lipids and of birefringent material in the adrenal cortex. As expected (12), atrophic changes were particularly severe in the zona glomerulosa and less marked in the zona fasciculata. The atrophy of the zona glomerulosa can be satisfactorily explained on the basis of disuse atrophy induced by parenteral administration of DCA which replaces the hormone or hormones produced by this zone. The atrophy of the zona fasciculata is probably secondary to the inhibitory action of DCA on the anterior pituitary (36–38). The finding of a moderate reduction of cholesterol and ascorbic acid concentration in adrenals which were either normal or slightly reduced in weight is compatible, in our opinion, with the concept that in these animals the adrenocorticotrophic stimulating action of the pituitary was diminished. It would appear therefore that possibly some of the connective tissue changes observed are not due necessarily to a direct action of DCA but rather to an indirect one mediated through the adrenal cortex. Our interpretation is in agreement with the view expressed by Sayers and his coworkers that administration of DCA to experimental animals induces a state of hormonal imbalance characterized by an excess of mineral corticoids (DCA-like) and a deficiency of glucocorticoids (cortisone-like).

This view seems also to be compatible with the known action of cortisone on connective tissue. This hormone when administered in large amounts inhibits
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mesenchymal cell proliferation and production of intercellular material (39, 40, 7). It would seem possible that a lack or diminished output of cortisone may be followed by the opposite phenomenon, i.e., excessive mesenchymal cell proliferation and production of intercellular material. This problem has been recently reevaluated by Selye (5, 41) on the basis of new experimental data and critically reviewed by Sayers (42).

The recent controversial reports (43, 44) on the beneficial effect of the simultaneous administration of DCA and ascorbic acid in rheumatoid arthritis add interest to the problem of the interrelationship between adrenocortical hormones and vitamin C on the one hand, and the so-called collagen diseases on the other. Although our investigation was not primarily directed to the study of this particular problem, its results may be useful as a base line for future studies in this field.

SUMMARY

The effect of desoxycorticosterone acetate (DCA) on the granulation tissue of healing and healed linear laparotomy wounds was studied in young adult male guinea pigs maintained on a complete diet and on a known intake of ascorbic acid.

DCA induces the production of an excessive amount of granulation tissue, as evidenced by a relatively great number of fibroblasts and by a larger amount of ground substance. This effect was accompanied by a slight to moderate lag in the maturation process of both cellular and intercellular elements. These changes were observed when DCA administration was begun 5 days prior to operation, but were less obvious or absent if DCA was injected, beginning on the 5th or 10th postoperative day. The results indicate that the action of DCA on immature, proliferating connective tissue is marked, and is considerably less or absent when connective tissue elements have reached partial or almost complete maturity.

The effect of DCA on connective tissue does not appear to rest on the basis of an altered nutritional status. Chemical and histochemical studies of the adrenals suggest that the action of DCA on connective tissue is probably mediated through a disturbance of adrenocortical function, namely an imbalance between hormones of the zona glomerulosa (excess of DCA) and those of the zona fasciculata (deficiency of glucocorticoids).

The presence of changes in granulation tissue and the lack of them in mature resting connective tissue of DCA-treated guinea pigs confirm the view that a profound difference in the response mechanism exists between resting and actively proliferating connective tissue.

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EXPLANATION OF PLATES

PLATE 8

Fig. 1 a. Wound of a control guinea pig on the 10th postoperative day. Hematoxylin and eosin stain. × 19.

Fig. 1 b. Wound of a DCA-treated guinea pig on the same day. Note the considerably greater amount of granulation tissue. Hematoxylin and eosin stain. × 19.
(Pirani, Stepto, and Sutherland: Desoxycorticosterone acetate and wound healing)
PLATE 9

FIG. 2 a. Detail of Fig. 1 a. Note the mature appearance of the fibroblasts which are regularly arranged parallel to the wound surface. Hematoxylin and eosin stain. X 148.

FIG. 2 b. Detail of Fig. 1 b. Note the large number of irregularly arranged fibroblasts in different stages of maturation. Numerous poorly developed capillaries are also present. Hematoxylin and eosin stain. X 148.

FIG. 3 a. Control wound on the 10th postoperative day. Foot's stain for reticulum. Numerous collagen fibers and few reticulum fibers are present. X 178.

FIG. 3 b. Wound of a DCA-treated guinea pig on the same day. A large number of reticulum fibers are present. Foot's stain. X 178.
(Pirani, Stepto, and Sutherland: Desoxycorticosterone acetate and wound healing)
PLATE 10

Fig. 4 a. Control wound on the 10th postoperative day. Gomori’s technique for alkaline phosphatase. There is considerable intra- and extracellular enzymatic activity near the edge of the wound. × 147. Armed Forces Institute of Pathology Neg. No. 218971–16.

Fig. 4 b. There is very little extracellular alkaline phosphatase in the granulation tissue after DCA administration. 10th postoperative day. × 147. Armed Forces Institute of Pathology Neg. No. 218971–1.

Fig. 5 a. The adrenal cortex of a control guinea pig on the 14th postoperative day. Note the well developed zona glomerulosa. Hematoxylin and eosin. × 90. Armed Forces Institute of Pathology Neg. No. 218971–19.

Fig. 5 b. Adrenal cortex of a guinea pig after 19 days of DCA administration (14th postoperative day). The zona glomerulosa is very markedly atrophied. Only a slight atrophy can be seen in the zona fasciculata. Hematoxylin and eosin stain. × 90. Armed Forces Institute of Pathology Neg. No. 218971–9.
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FIG. 6 a. Adrenal cortex of a control guinea pig on the 10th postoperative day. There is abundant sudanophilic material throughout the entire thickness of the cortex. Oil-red-O stain for lipids, frozen section. × 95.

FIG. 6 b. Adrenal cortex of a guinea pig after 15 days of DCA administration (10th postoperative day). Sudanophilic material is almost completely absent in the inner two thirds of the zona fasciculata and in the zona reticularis. Oil-red-O stain, frozen section. × 95.

FIG. 7 a. Adrenal cortex of a control guinea pig on the 7th postoperative day. There is abundant birefringent material in the outer two-thirds of the zona fasciculata. Frozen section. × 95.

FIG. 7 b. Adrenal cortex of a guinea pig after 12 days of DCA administration (7th postoperative day). There is a considerable reduction both in the size and number of birefringent particles. Frozen section. × 95.
(Pirani, Stepto, and Sutherland: Desoxycorticosterone acetate and wound healing)