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EFFECT OF GLUCOCORTICOID HORMONES ON EXPERIMENTALLY INDUCED ALLERGIC REACTIONS ON HUMAN SKIN

A Histologic and Histochemical Study

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EXPERIMENTALLY produced skin allergic reactions on human beings have a histologic basis similar to those seen in experimental animals.¹⁻⁶ Moreover, the macro- and microscopic similarity to the wheals obtained with the intradermic injection of histamine is also well known.⁷⁻¹¹

The inhibitory action of ACTH and corticoids in different allergic conditions have been proved,^{12, 13} but this is not so in regards to experimentally produced skin allergic reactions¹⁴ or wheals caused by histamine.¹⁵ To substantiate the apparent absence of hormonal effects in these latter conditions, it was considered that a careful study of the tissues at the microscopic level was necessary. For this reason we have carried out a histologic and histochemical study of the wheals produced by histamine and specific allergens before and after the administration of glucocorticoid hormones, either by mouth or by local treatment of the skin.

MATERIAL AND METHODS

Fifty allergic patients, divided into two different groups, were studied. Their only ostensible clinical manifestations were either seasonal rhinitis or asthma that had been proved to be of pollenic etiology. Only those showing constant and easily reproducible wheals were included in the group under study.

The first group was composed of 21 patients who were treated with prednisolone by mouth. Of these, 9 were males and 12 females, and their

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ages ranged between 27 and 52 years; the majority were from 32 to 44 years of age. They were given prednisolone for a period from 1 to 30 days (1 day—2 cases; 2 days—2 cases; 10 days—4 cases; 20 days—4 cases; 30 days—9 cases). Half of the patients in each period received a daily minimum dose of 10 mg. and the other half a maximum daily dose of 30 mg.

The second group, including 29 patients, was treated with topical application of ointments on a given area three times daily. Ten were males and 19 females whose ages ranged from 25 to 51 years, the majority were between 28 and 40 years of age. They were treated in the following way: (a) 10 patients with 0.5 per cent chloral hydrate of diethylaminoacetate of hydrocortisone from 1 to 3 weeks (first week—3 cases; second week—4 cases; third week—3 cases); (b) 11 patients with 1 per cent hydrocortisone acetate from 2 weeks to 9 months (2 weeks—1 case; 1 month—1 case; 2 months—1 case; 3 months—4 cases; 5 months—2 cases; 9 months—2 cases); (c) 8 patients with 1 per cent prednisolone from 2 to 6 months (2 months—1 case; 3 months—2 cases; 4 months—3 cases; 6 months—2 cases).

In both groups, before and after treatment, three separate intradermic wheals were made in the form of an arrow in each patient in the middle of the anteroexternal region of the thigh, about 6 to 8 cm. apart. Each wheal was provoked by using 0.10 c.c. of saline solution as a control in the first, 0.10 c.c. of histamine in the second, and 0.10 c.c. of a pollen solution in the third. The amount of histamine applied varied between 1 and 20 gammas, according to the reactivity of each patient. Pollen from maple and plane trees, gramineous and Compositae was used in quantities, varying from 2 to 200 Noon units, according to the patient's sensitivity. In patients treated with corticoid ointments the wheals were made in a restricted marked area, which had been previously topically treated.

Thirty minutes after the intradermic injection, the wheals were measured and, under ethylchloride local anesthesia, a biopsy of each one was made. The skin thus obtained was cut into two parts, one being placed in Bouin's solution and the other in 10 per cent buffered formol for 24 hours. Serial sections were obtained from each biopsy and the following histochemical and histologic techniques were applied: (1) for general morphology—hematoxylin-eosin and Heidenhain-azan; (2) Weigert's method for elastic fibers; (3) Hortege's silver impregnation method for collagenous and reticular fibers; (4) Hortege's panoptic silver technique for cellular morphology; (5) mucopolysaccharides were stained with 0.055 per cent toluidine blue at pH 3.5 and Alcian blue, controlled by incubation in hyaluronidase (30 TRU per c.c.) at 37° C. for 3 and 24 hours; (6) nucleoproteins with 0.055 per cent toluidine blue at pH 4.7 controlled by incubation in 0.1 per cent ribonuclease (Nutritional Biochemical) at 37° C. for 30 and 60 minutes; also, with the use of this technique, metachromatically stained mast cells were demonstrated; (7) PAS technique controlled with ptyaline and methanol-chloroform for polysaccharides, basement membranes, and reticular fibers.

RESULTS

Tolerance to the treatment was very good, there being no noticeable side effects; only moderate signs of artificial hypercorticalism developed in patients given the hormones by mouth.

No changes were observed in any case in the wheals induced before treatment when compared with the macroscopic aspect and measurement of those obtained in the same patient after treatment with prednisolone by mouth, whatever the period of administration or the dosage of corticoid used. In the second group, those treated with corticoid ointments in which chloral hydrate of diethylaminoacetate of hydrocortisone was used, the histamine and pollen wheals showed a less prominent and less congestive papule. Conversely, no appreciable changes were found in patients topically treated with hydrocortisone and prednisolone.

In spite of the absence of macroscopic changes, the major part of the biopsies showed variable degrees of histologic and histochemical modifications after the hormonal treatment. In the first group of cases, this was in direct relation to the different doses used and the length of time administered. Doses of 10 mg. daily for 10 to 20 days produced no changes, but these were noticeable at 30 days. Doses of 30 mg. during 1, 2, and 10 days produced no changes at all but at 20 and 30 days these were evident. In total, 8 patients showed no histologic changes, 8 showed moderate changes, and in 5 slight changes were present.

In the second group of patients, the histologic and histochemical changes were directly related to the type of corticoid used and the time during which it was administrated. With chloral hydrate of diethylaminoacetate of hydrocortisone ointment, no changes appeared when it was used for 1 or 2 weeks, but moderate changes were detected after 3 weeks. No changes whatsoever were noticed in those patients who had used hydrocortisone for 2 to 4 weeks, but some changes took place after 2 months, and this became quite evident after 5 months of treatment. The prednisolone ointment caused no changes during the first 2 or 3 months; however, some changes could be seen at 4 months and become noticeable after 6 months of treatment. In total, there were 7 cases with no modifications at all and 3 with moderate changes with the use of chloral hydrate of diethylaminoacetate of hydrocortisone. Where hydrocortisone was used, 4 patients showed no changes at all, 5 showed moderate changes, and evident changes could be seen in 2. With prednisolone, 4 patients remained the same, 2 showed moderate changes, and 2 intense changes.

To evaluate the already mentioned microscopic modification observed in the wheals after treatment, two main structural components of the allergic inflammation were taken into account: (1) the *vascular reaction* which includes congestion, hypertrophy of the endothelium, leukocytic (mainly eosinophils) and serous exudation; (2) the *changes in the neighboring connective tissue* which comprised intercellular edema, alterations of fibroblasts,

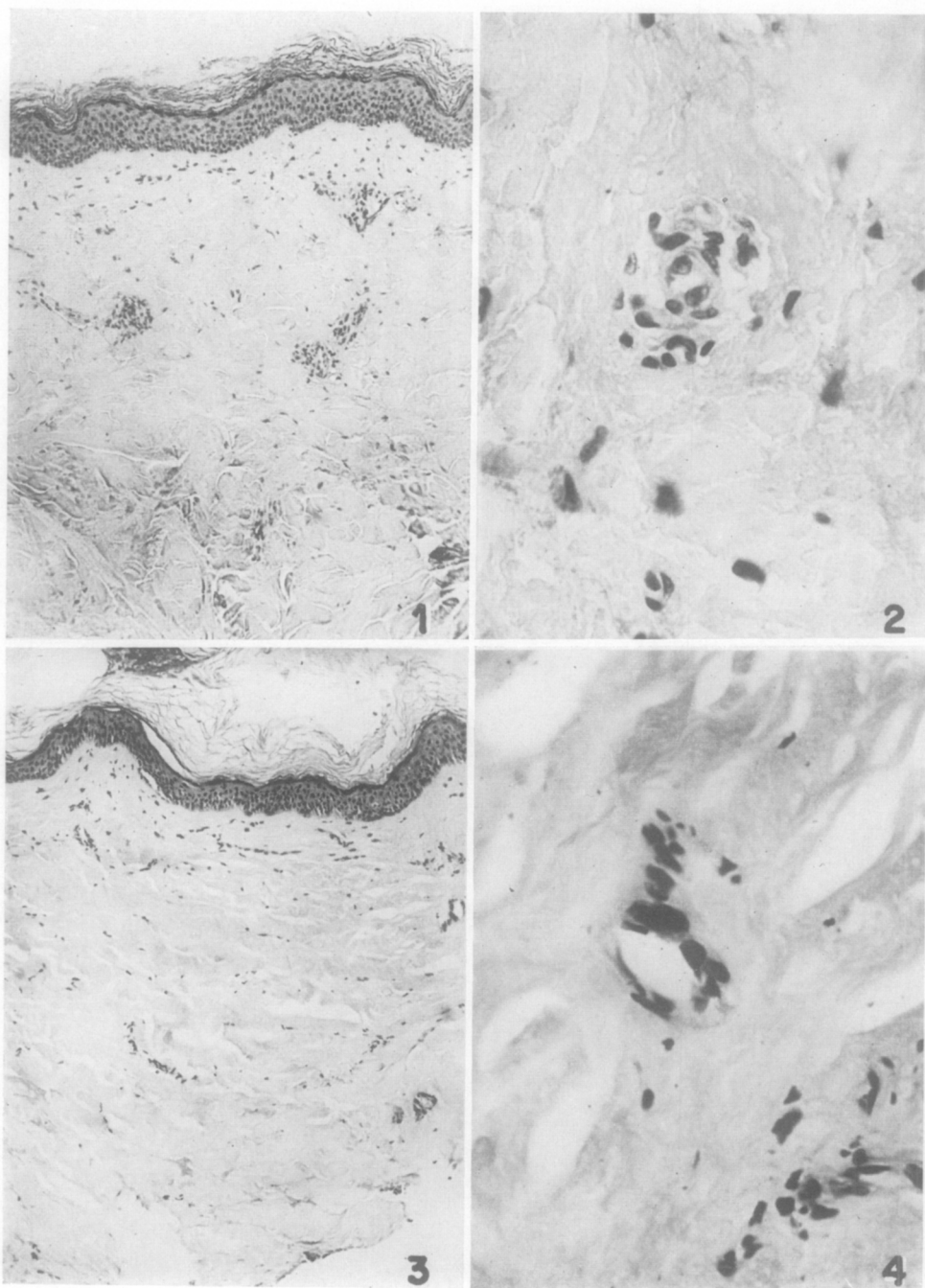
mast cells, collagenous and elastic fibers, mucopolysaccharides of the amorphous substance, and basement membrane of the adjacent epithelium. As there were no remarkable differences in the results obtained either in the histamine or pollen wheals, Table I shows detailed results in both types before and after treatment.

TABLE I. ACTION OF GLUCOCORTICOID HORMONES ON POLLEN AND HISTAMINE WHEALS

STRUCTURAL COMPONENTS OF WHEELS	BEFORE HORMONAL TREATMENT	AFTER HORMONAL TREATMENT
Vascular Reaction		
Congestion	Intense	Diminished
Hypertrophy of endothelium and edema of the wall	Intense	Diminished; dissociation of reticular fibers
Serous exudate	Intense	Diminished
Cellular exudate	Abundant neutrophils and eosinophils	Few cells found
Alterations of the Dermal Connective Tissue		
Edema	Intense and interfibrillar	Diminished
Fibroblasts	Normal appearance; in part hypertrophic	Smaller number; in part atrophied
Mast cells	Normal appearance; some are degranulated	Degranulation and vacuolization in most of them
Collagen fibers	Edematous due to serous imbibition	Less edematous; partial dissociation
Elastic fibers	Normal appearance, slightly separated	Thicker and fragmented
Mucopolysaccharides	Normal amount and distribution	Diminished
Basement membrane	Nitid and continuous	Attenuated and sometimes discontinuous

TABLE II. ACTION OF GLUCOCORTICOID HORMONES ON CONNECTIVE TISSUE OF SALINE-INDUCED WHEEL

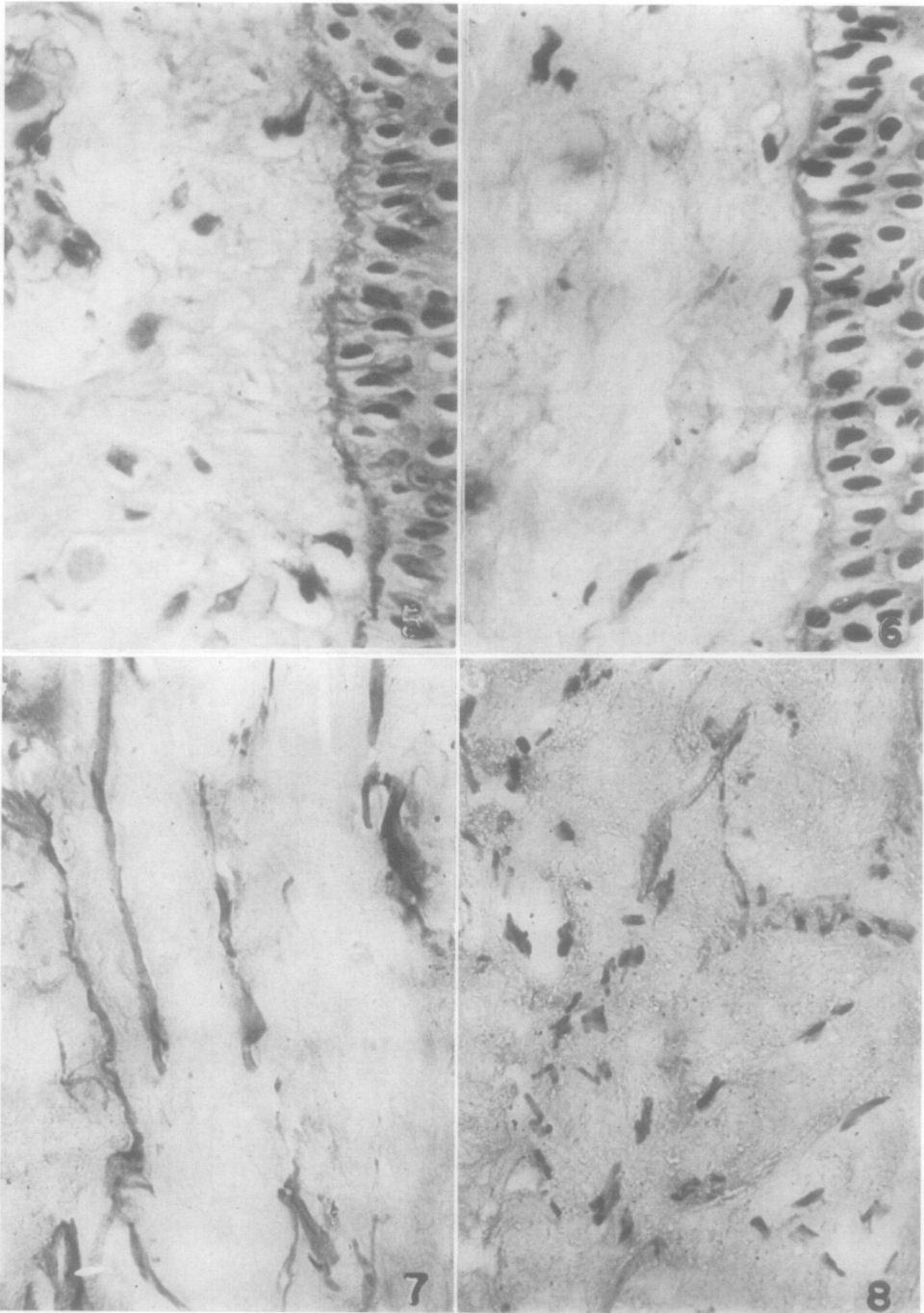
STRUCTURAL COMPONENTS OF CONNECTIVE TISSUE	BEFORE HORMONAL TREATMENT	AFTER HORMONAL TREATMENT
Fibroblasts	Normal appearance	In part atrophied and pyknotic
Mast cells	Normal appearance	Degranulation and vacuolization in some
Collagen fibers	Normal appearance	Partial dissociation
Elastic fibers	Normal appearance	Thicker and fragmented
Mucopolysaccharides	Normal amount and distribution	Diminished
Basement membrane	Nitid and continuous	Attenuated and sometimes discontinued
Blood vessels (capillaries and arterioles)	Normal appearance	Loosening of network of reticular fibers



Figs. 1-4.—Biopsies of saline-induced wheals before and after treatment with prednisolone for 4 weeks.

Figs. 1 and 2.—Before treatment. Note the normal aspect of dermal connective tissue and of an arteriole. (Hematoxylin and eosin; $\times 120$ and $\times 700$, respectively; reduced $\frac{1}{2}$.)

Figs. 3 and 4.—After treatment. Dissociation of collagen fibers and bundles and partial atrophy of fibroblasts. (Hematoxylin and eosin; $\times 120$ and $\times 700$, respectively; reduced $\frac{1}{2}$.)



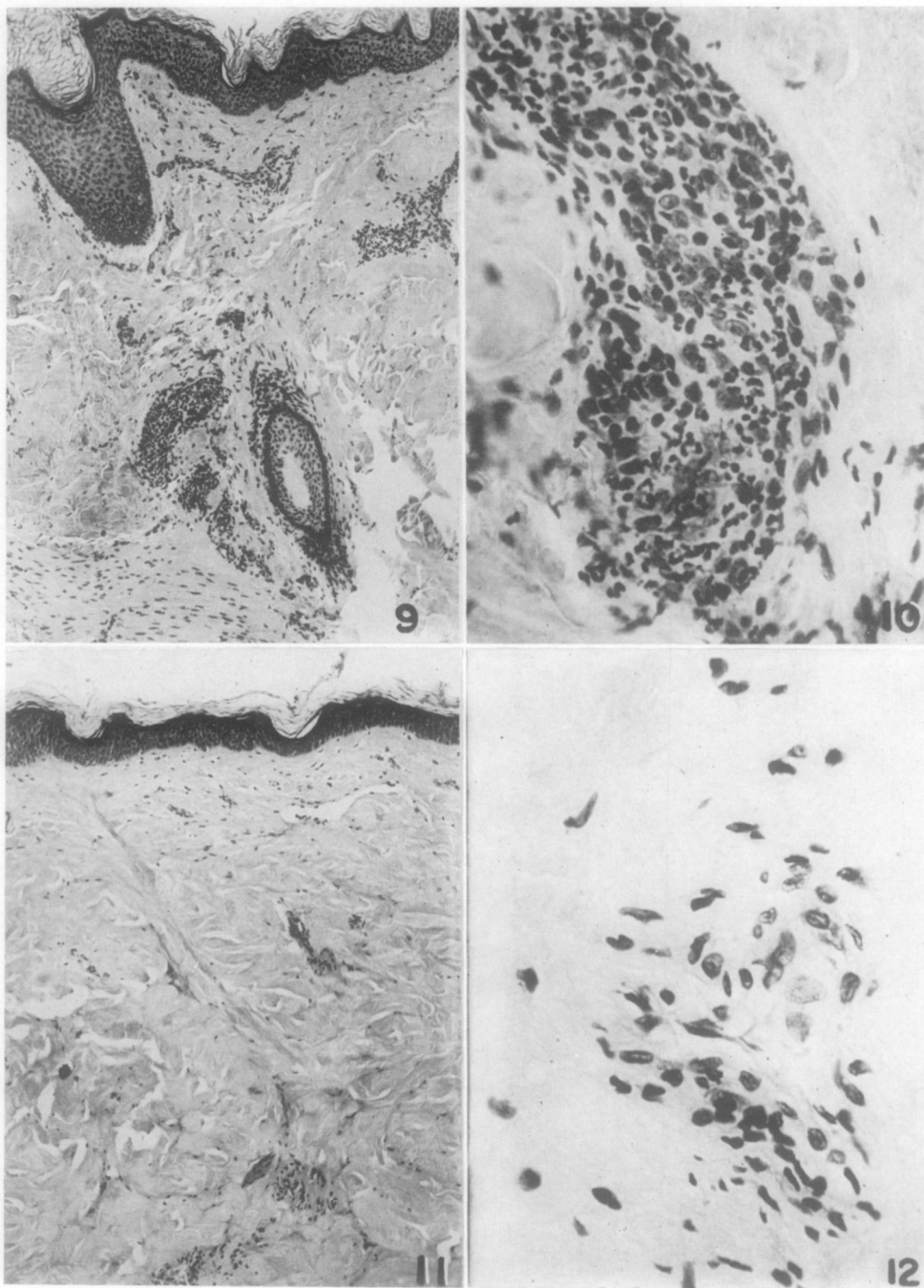
Figs. 5-8.—Biopsies of saline-induced wheals before and after treatment with prednisolone for 4 weeks.

Fig. 5.—Before treatment. Normal basement membrane and collagen fibers of papillary layer demonstrated with PAS technique; counterstaining with hematoxylin and eosin; $\times 700$, reduced $\frac{1}{2}$.

Fig. 6.—After treatment. Thinner basement membrane and loose connective tissue fibers of dermis. PAS technique, counterstaining with hematoxylin and eosin; $\times 700$, reduced $\frac{1}{2}$.

Fig. 7.—Before treatment. Normal elastic fibers of reticular layer of dermis. (Orcein; $\times 700$, reduced $\frac{1}{2}$.)

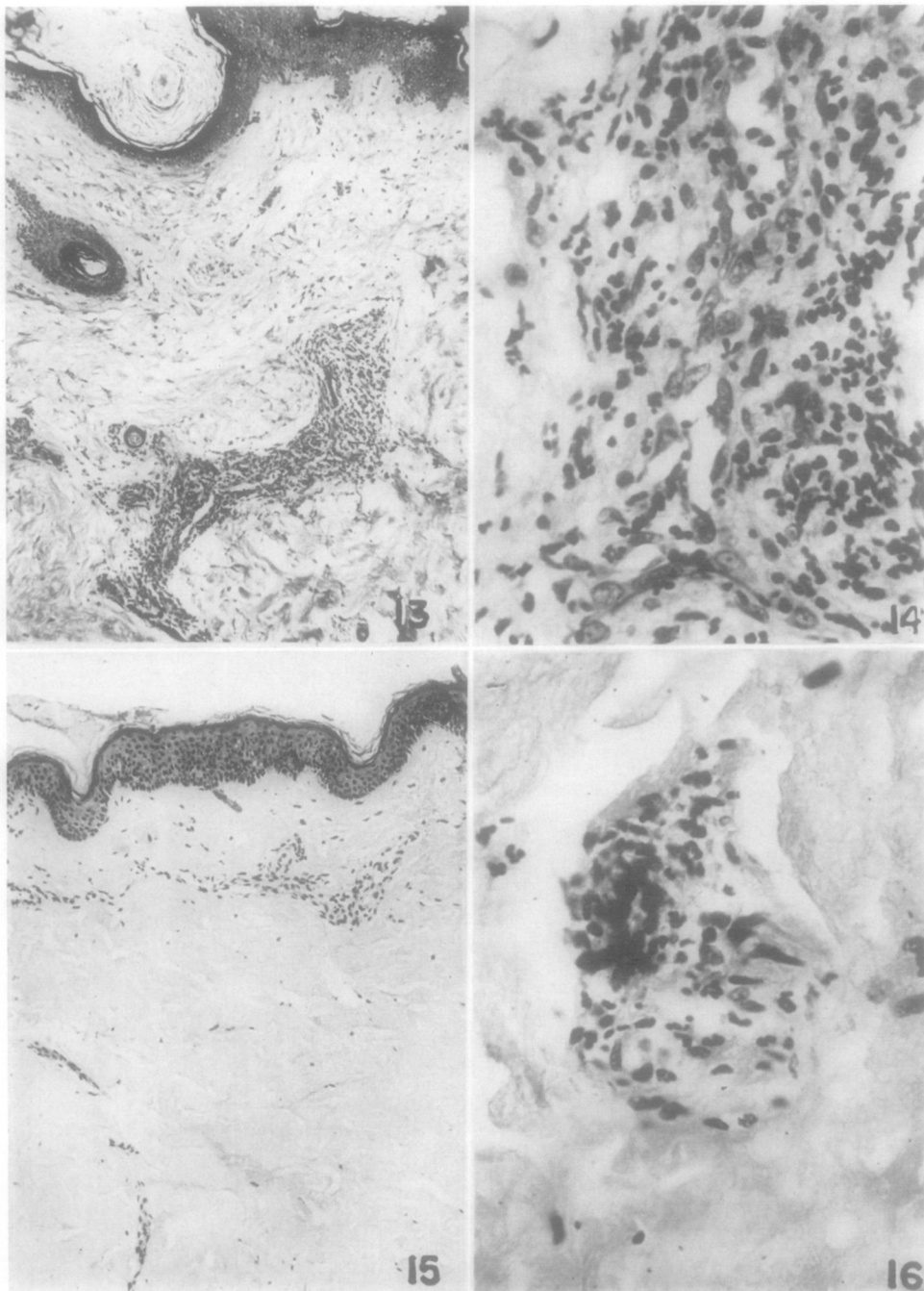
Fig. 8.—After treatment. Fragment elastic fibers of the dermis (Orcein; $\times 700$; reduced $\frac{1}{2}$.)



Figs. 9-12.—Biopsies of pollen-induced wheals before and after treatment.

Figs. 9 and 10.—Pollen-induced wheal before treatment. Congestion, exudation, and edema of the dermis. Cellular exudate around vessels. (Hematoxylin and eosin; $\times 120$ and $\times 700$, respectively; reduced $\frac{1}{2}$.)

Figs. 11 and 12.—After treatment. Less congestion, exudation, and edema of connective tissue. Reduced cellular exudate around vessels. (Hematoxylin and eosin; $\times 120$ and $\times 700$, respectively; reduced $\frac{1}{2}$.)



Figs. 13-16.—Biopsies of histamine-induced wheals before and after treatment.

Figs. 13 and 14.—Histamine-induced wheal before treatment. Congestion, exudation and intense edema of connective tissue. Tumefaction of the wall of the vessels and cellular exudate. (Hematoxylin and eosin; $\times 120$ and $\times 700$, respectively; reduced $\frac{1}{2}$.)

Figs. 15 and 16.—After treatment. Reduced vascular reaction, connective tissue edema, and cellular exudate. (Hematoxylin and eosin; $\times 120$ and $\times 700$, respectively; reduced $\frac{1}{2}$.)

In regard to the saline-induced wheals used as controls, the microscopic changes brought about by hormones on the structures of normal connective tissue of dermis were equally dependant on the dosage and length of time of administration; they are described in Table II. It should be borne in mind that obviously these changes were also developed by the dermal tissue of neighboring skin in which histamine or pollen wheals were induced at the end of the treatment.

DISCUSSION

Our studies mostly confirm other authors' statements that provoked pollen and histamine skin reactions are not ostensibly modified macroscopically after the administration of corticoids. Nevertheless, the present investigation demonstrates, in the major number of cases, an inhibitory action on the allergic reaction at the histologic level and, also, that a partial involution of normal connective tissue was observed in the control saline wheal.

These effects of the glucocorticoid hormones depend, in general, on the dosage and length of time of administration, either by mouth or topically applied. The higher the dose and the longer the treatment, the more intense the inhibitory action. Even with the higher doses, 4 weeks of treatment by mouth or several months topically applied, are needed. The exception was in favor of the ointment containing chloral hydrate of diethylaminoacetate of hydrocortisone,¹⁶ which proved to be more active than prednisolone and hydrocortisone acetate, even at a similar concentration. Changes in the wheals could be shown at the end of the third week. The modifications of the saline-induced wheals after both kinds of treatment were also paralleled by the amount and length of time of administration of hormones. Even in those patients where the allergic and histamine wheals were not modified, the corresponding saline-induced wheal invariably exhibited the complete picture of regressive changes. In no case could a separate effect on either of the wheals, whether allergic or caused by histamine, be observed; it was noted, when present, in both types of wheals.

In regard to the nature of the microscopic results, whether obtained by local or oral treatment, they were qualitatively similar in the three types of wheals. Therefore, the mechanism of action of the glucocorticoid hormones could be counted as direct.

The inhibitory effect, although always of a slight or moderate degree, consists essentially in a decrease in the intensity of congestion and edema of wall of minute vessels and of the serous and cellular exudate. In this respect not much difference was found between the wheals caused by the allergens and those caused by the histamine. There was also a marked attenuation of the edema in the surrounding connective tissue and a simultaneous regression of almost all its structures, such as, partial atrophy in most of the fibroblasts and mast cells, dissociation of collagen fibers, fragmentation of the elastic fibers, disappearance of the interfibrillar mucopoly-

saccharides, and loosening of the network of reticular fibers in the wall of small arterioles and in the basement membrane of the epithelium. On the other hand, the only remarkable changes noticeable in the saline-induced wheals were the purely involutive ones of the structures of the dermal connective tissue. This histologic picture, which agrees with previous findings on the effects of glucocorticoids on the skin of animals¹⁷ and of normal subjects,¹⁸ was present in the rest of the dermal skin. Therefore, the allergic and histamine wheals induced after treatment were developed on an abnormal anatomic substrate, which gives support to a possible explanation on the mechanism of action of glucocorticoids on the experimentally induced skin allergic reactions. As it was demonstrated, these hormones can affect the level of circulating antibodies but not that of reagins.^{19, 20} Although two other ways might be involved, one could be referred to some disturbance in the production and/or liberation of histamine,²¹⁻²⁵ and the other one to the relative inability of the vessels and connective tissue structures to react adequately to histamine-like substances. Both hypotheses could be related to the presence of degranulated mast cells, alteration of the walls of vessels, partial atrophy of the fibroblasts, and involution of intercellular substance of dermal skin, as a consequence of treatment with glucocorticoids.

Other reports on the effect of these hormones on the biology of connective tissue may have some connection with the results of our study, such as the inhibition of the fibroplasia of dermal skin,²⁶ the partial regression of the lesions in scleroderma,^{18, 27, 28} the increase in the capillary resistance,²⁹⁻³² the inhibition of provoked inflammation,^{33, 34} and the arrest in the development of normal connective tissue.³⁵⁻³⁸ On the basis of these facts, we may finally assume that the inhibitory effect of glucocorticoids on experimental allergic reactions could be due to a previous intense and long action of these hormones on the connective tissue, which as a consequence would modify its reactivity to the injury.

SUMMARY

Histologic and histochemical study of wheals induced by saline solution, pollen, and histamine, were made in allergic patients before and after the oral administration of prednisolone and topical application of different corticoids. Negative results were obtained with daily doses of 10 mg. of prednisolone for from 10 to 20 days, but moderate changes appeared after 30 days. They were noticeable when daily doses of 20 mg. were given for periods of 30 days and after 20 days with doses of 30 mg. Topically applied 0.5 per cent chloral hydrate of diethylaminoacetate of hydrocortisone showed microscopic modification after 3 weeks; with 1 per cent hydrocortisone ointment, these were noticed after 2 months of treatment, and only after 4 months when 1 per cent prednisolone ointment was used. As compared with the wheals induced before treatment, the changes were qualitatively similar in both histamine and pollen wheals and consist of (a) diminution of congestion and of the serous and cellular exudate, (b) dissociation of collagen fibers and

fragmentation of elastic fibers of dermal connective tissue, (c) atrophy in part of the fibroblast cells, degranulation, and partial vacuolization of the mast cells, reduction of the mucopolysaccharides, and discontinuity of the basement membrane of epidermis. In the saline-induced wheals, the only noticeable changes were those of partial regression of the cellular and extracellular constituents of dermal connective tissue.

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