patterns were observed.

The evidence would indicate that the administration of heparin, as well as a high fat meal, modifies the surface active substances in the plasma with the result that a marked change occurs in the protein pattern seen in the paper chromatograms. This is noted also in the effects due to administration of a surface active agent such as Tween 81. It has been shown recently(8) that heparin inhibits the action of hyaluronidase, possibly by competition with hyaluronic acid for the enzyme. Conceivably the change in the protein chromatogram of a plasma after heparin administration is due to some similar association with a protein fraction.

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Summary. 1. Changes in the plasma protein patterns of dogs after high fat meals, and after the intravenous administration of heparin or Tween 81, have been demonstrated, using the paper chromatographic technic of Franklin and Quastel. 2. Concurrent reductions in the hematocrit, and changes in the sedimentation rate were observed after these procedures. The chylomicron counts were decreased after intravenous heparin. 3. The evidence indicates that the surface active substances present in the blood are modified after the above administrations, thus affecting the protein patterns of the paper chromatograms.

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Role of Cortisone in Regulation of Inflammation.*† (18368)

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It is well established that certain generalized reactions such as lymphocytolysis(1), lymphocytopenia(2) and eosinopenia(3,4), which occur following the application of noxious stimuli, are mediated by the secretion of the adrenal cortex. However, the role of the secretion of the adrenal cortex in localized host reactions elicited by topically applied stimuli has not yet been evaluated. Outstanding among these local reactions is the process of inflammation. The term "inflammation" is used here to denote only the local reactions that occur when an injurious agent acts topically upon tissues. It is the object of this study to secure information on the influence of the secretions of the adrenal cortex upon each of the various component events composing the inflammatory response. A stimulating agent was chosen which was not only known from prior studies to produce typical inflammatory alterations, but which has also been implicated in the etiology of connective tissue disease. To eliminate the influence of endogenous hormone secretion, which is difficult to estimate, adrenalectomized animals were used. The involvement of secretion of the adrenal cortex was then studied by comparing the inflammatory reaction of untreated adrenalectomized animals with that of adrenalectomized animals treated with a standard dose of cortisone acetate (Cortone acetate, Merck & Co.).

Materials and methods. Approximately 150 CBA mice 16 weeks of age, of both sexes,

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were used in these experiments. All animals were kept at a constant external temperature and were fed *ad libitum* on dog chow (Purina), supplemented with calf meal (Larro), vitamins and water. The animals were sensitized with 0.7 cc and 0.5 cc of horse serum given intraperitoneally in an interval of 2 days. The local inflammation was then produced 19 days after the second sensitizing dose by topical subcutaneous injection of a challenging antigen dose into the subcutaneous tissue of the abdomen. All animals received a uniform challenging dose, namely 0.0005 cc horse serum per 20 g mouse given in a total volume of 0.25 cc saline. This dose was selected because it had previously been established as the anaphylactic LD_{50} in adrenalectomized animals(5), when injected intravenously. The volume was chosen to insure sufficient spread of the antigen to produce an area of response adequate for the subsequent biopsy. The mice were adrenalectomized in the customary fashion by the bilateral posterior approach. In all experiments, the challenging dose was injected 3 to 4 hours after adrenalectomy. The area injected was marked with wax pencil for exact identification of the site of topical treatment. The adrenalectomized animals were divided into 2 groups. To Group 1, 1 mg of cortisone acetate per 20 g mouse was given by the intraperitoneal route 2 hours after adrenalectomy and 2 hours before the administration of the challenging dose of horse serum. The second group received only the antigen dose at the same time interval after adrenalectomy. Five groups of control animals were used in these experiments: (1) non-sensitized intact animals. (2) sensitized intact animals. (3) sensitized adrenalectomized animals and (4) sensitized adrenalectomized animals given 1 mg of cortisone acetate by intraperitoneal injection 2 hours after adrenalectomy. These 4 groups received no local serum injection. The fifth control group was composed of intact sensitized animals receiving the challenging antigen dose 19 days after the second sensitizing dose. This control group was introduced in order to study the inflammatory response in sensitized non-adrenalectomized animals. In all instances at least 7 animals were studied at each of the time intervals at which subcutaneous spreads were taken, with the exception of the control Groups 2 and 5, which consisted of 5 animals.

The methods for studying the inflammatory reaction were essentially those described in detail previously(6). At varied intervals following administration of the antigen, the skin of the challenged area was opened and the subcutaneous connective tissue excised in thin sheets for the study of the local inflammatory response. Such spreads were taken 2, 4, 6, 8, 10, 12, and 24 hours after the injection of the antigen dose from all challenged animals, 6 hours after removal of the adrenals from the adrenalectomized, and 4 hours after cortisone from the intact controls. The excised sheets of areolar tissue were spread thinly on slides, air-dried and stained with May-Grünwald Giemsa. Six or 8 spreads were prepared from the area of inflammation and at the same time control spreads were taken from other areas far removed from the injected zone. Moreover, loose connective tissue spreads were prepared simultaneously from the site of incision for adrenalectomy. In those groups in which no topical inflammatory phenomenon was arranged, the spreads were taken from a comparable subcutaneous site. In each spread, in addition to an extensive qualitative cytological study, differential cell counts were made, ascertaining the percentage distribution of fibroblasts, polymorphonuclear leucocytes, macrophages, lymphocytes and eosinophils. A differential count of 800 cells was performed on each set of spreads originating from the site of horse serum injection.

Results. Comparison of the control groups revealed that sensitization alone or combined with adrenalectomy did not produce any marked alteration in either morphology or number of the cells of subcutaneous connective tissue. The eosinophils in areolar tissue were markedly decreased in all sensitized animals. The same holds true for sensitized intraperitoneally cortisone-treated controls.

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TABLE I. Changes in Percentage Distribution of Cells in Allergic Inflammation Produced By Locally Applied Horse Serum.

Group	Time,* hrs.	Fibroblasts Mean† % S.E.	PMNs Mean % S.E.	Macrophages Mean % S.E.	Lymphocytes Mean % S.E.	Eosinophils Mean % S.E.
1. Non-sensitized intact controls		88.3 ± 1.26	0	.7 ± .38	2.2 ± .33	8.2 ± 1.12
2. Sensitized intact controls. + .0005 cc H.S. s.c.	6	58.5 ± 5.70	31.2 ± 5.82	3.0 ± 1.00	2.3 ± .73	4.5 ± 1.00
3. Sensitized adrenect local injection of .0005 cc H.S. s.c.	2	69.7 ± 4.98	20.1 ± 5.88	1.6 ± .51	4.9 ± .71	3.1 ± .79
	4	40.4 ± 5.43	49.9 ± 5.46	1.6 ± .34	3.9 ± .52	3.5 ± .91
	8	29.5 ± 3.35	61.5 ± 4.20	2.5 ± .70	2.3 ± .44	3.5 ± 1.13
	12	30.8 ± 4.61	49.8 ± 4.62	11.9 ± 1.88	1.5 ± .40	5.3 ± .90
	24	29.7 ± 4.09	30.3 ± 2.03	30.9 ± 3.08	2.6 ± .47	6.0 ± 1.03
4. Sensitized adrenect + 1 mg Cortisone i.p. + .0005 cc H.S. s.c.	2	79.2 ± 4.98	6.9 ± 3.97	4.1 ± .85	5.2 ± .71	4.4 ± .92
	4	75.7 ± 4.40	17.2 ± 4.47	2.1 ± .41	3.3 ± .53	1.3 ± .33
	6	69.0 ± 4.73	18.5 ± 5.68	1.2 ± .37	4.0 ± .91	10.6 ± 1.86
	8	77.4 ± 3.55	17.1 ± 3.63	.9 ± .28	1.9 ± .61	2.7 ± .52
	12	79.6 ± 7.59	13.9 ± 3.39	1.4 ± .54	1.4 ± .52	2.9 ± .67
	24	80.9 ± 2.42	11.0 ± 2.83	1.5 ± .46	2.0 ± .30	4.7 ± 1.00

* After local application of H.S.

† All means and standard errors represent values obtained on 10 animals, with exception of Group 2 in which 5 animals were used.

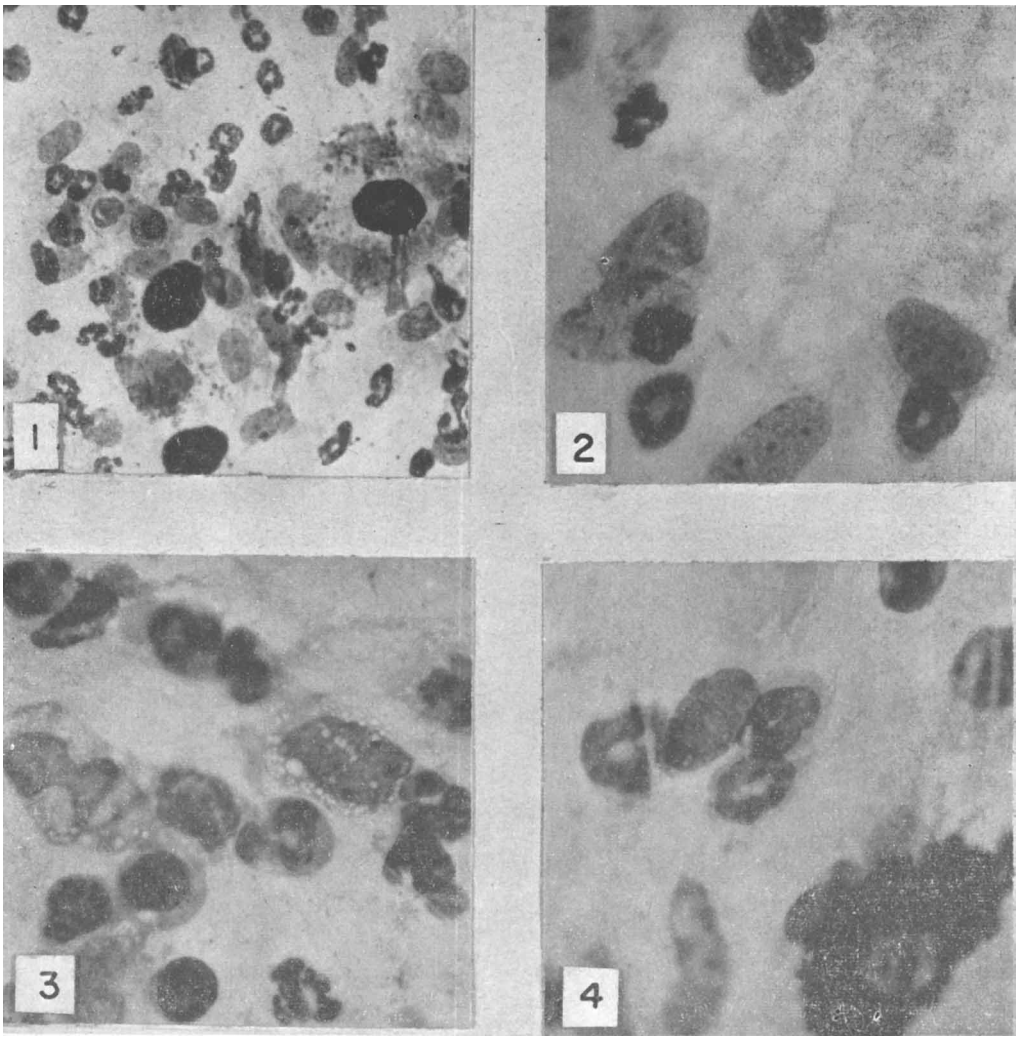
The local inflammatory response of sensitized animals to the challenging antigen injection was qualitatively the same in intact (Table I, Group 2) and in adrenalectomized animals (Table I, Group 3). Within the first 2 hours following administration of the challenging dose of antigen, the connective tissue at the site of injection exhibited localized edema. The fibroblasts underwent early degenerative changes such as plasmolysis, swelling of nuclei and, in many instances, actually karyorrhexis (Plate 1, Fig. 1). The inflammatory response was quite different when cortisone was administered 2 hours before the challenging dose (Table I, Group 4). Whereas the connective tissue became edematous, the destruction of fibroblasts and the invasion of polymorphonuclear leucocytes and macrophages were much less extensive.

The above described qualitative findings are supplemented by the quantitative data obtained from the differential cell counts in the inflamed areas.

The abnormal percentage distribution characteristic of the local inflammatory response exhibits the same trend in the intact group (Table I, Group 2), and in adrenalectomized animals (Table I, Group 3); the shifts in cell count after 6 hours appear more pronounced in the adrenalectomized animals, particularly the decrease in fibroblasts and the invasion of macrophages. In the cortisone-treated animals, the inflammatory response is quite different, as illustrated by a comparison of the data for percentage cell distribution in the areas of inflammation of cortisone-treated (Table I, Group 4), with those of non-cortisone-treated animals (Table I, Group 3). In the cortisone-treated group (Table I, Group 4) the inflammatory response was not completely suppressed but it was greatly diminished and shortened.

The traumatically induced inflammation in the adrenalectomy wounds paralleled in both groups the allergic inflammation in the serum-challenged area, *i.e.* exhibited a similar diminution by systemic cortisone treatment.

Comment. In the present study, a local antigen-antibody reaction was used as the method of producing acute focal inflammation. The local inflammatory phenomena here described demonstrate that the cytological changes and their temporal characteristics are similar in such allergen-produced inflammation to those inflammations produced by various other inflammatory stimuli, *e.g.* traumatic, chemical or bacterial(6,7). Technically, the procedure employed focuses the



All Preparations were Air-dried and Stained with May Brünwald Giemsa Solution.

FIG. 1. PMN-response in loose connective tissue 4 hr after local horse serum injection to sensitized adrenalectomized animals. Fibroblasts present in the injection area show cytoplasmic and nuclear destruction. ×540.

FIG. 2. Connective tissue of cortisone (1 mg i.p.) treated animals 4 hr after local horse serum injection to sensitized adrenalectomized mice. Few PMN's and eosinophils present. Few injured fibroblasts. ×760.

FIG. 3. Macrophage response in untreated animals 24 hr after local horse serum injection to sensitized adrenalectomized mice. Thirty per cent of cells present are macrophages, approximately 30% PMN's and another 30% fibroblasts. ×760.

FIG. 4. Connective tissue of cortisone treated animals 24 hr after local horse serum injection to sensitized adrenalectomized mice. Note absence of macrophages and slight increased number of locally developing eosinophils. Mast cell in the lower right corner. ×760.

observation to the study of the primary arena of those exacerbations of physiologically fluctuating processes, called inflammation, and to their major performers the cellular elements of connective tissue. Regarding the stimulus employed, the method is attractive because the inflammatory process is produced by allergy, which more and more emerges as an

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important factor in the production of connective tissue disease(8.9). Furthermore the method allows the detailed study of the changes in cellular morphology, the fluctuations in cell population and the alterations in tissue structure in the sequence in which they characterize the destructive, the defensive and the reparative stage of inflammation.

Adrenal cortical secretions unquestionably exert a marked antiphlogistic action. This is indicated by the results of adrenalectomy, which noticeably increases the inflammatory response and by cortisone treatment which profoundly depresses the cellular changes of inflammation.

This inhibitory effect of cortisone involves both the alterative process of fibroblast damage and the invasion of non-autochthonous cells. The influx of polymorphonuclears is markedly diminished by the adrenal hormone. Analysis of the data in Table I would seem to indicate the cortisone treatment does not influence the invasion of lymphocytes. This, however, is not the case. Since the lymphocytes entering the field of inflammation in non-cortisone treated adrenalectomized animals transform rapidly to macrophages, a low value for lymphocytes is maintained at the various intervals studied. The absence of a macrophage response to the phlogogenic stimulus in cortisone-treated animals could be a result of the capacity of this hormone to minimize lymphocyte invasion and thereby eliminate the cells which are known to be the precursors of macrophages in the inflamed area(6,10,11). It is possible that by this mechanism, administration of large amounts of cortisone or hypersecretion of adrenal cortical hormones can diminish the resistance of the organism to many noxious stimuli.

Adrenal cortical hormones could exert their antiphlogistic effect upon allergic inflammation through one or two major mechanisms. They could interfere either with the antigen-antibody reaction itself or with its sequels. Regarding the former mechanism, cortisone may increase the amount of circulating antibody, which would bind more antigen and leave less free for reaction with cellular antibody. It is possible also that cortisone might decrease the amount of cellular antibody or prevent its union with antigen. The literature is completely contradictory concerning the influence of adrenal cortical hormones on the level of circulating antibody. Some claims exist that adrenal cortical secretions enhance circulating antibody titer(12-15). On the other hand it has recently been suggested that adrenal cortical hypersecretion diminishes circulating antibody(16,17). The controversial reports on the producibility of an anamnestic response(13,18-20) by these hormones further increases the contradictory status. That cortisone does not prevent the *in vitro* Schultz Dale reaction(21) nor the tissue alterations of anaphylactic shock *in vivo*(5) indicates that the antiphlogistic effect of cortisone is not due to interference with antigen-antibody union.

It has been suggested(5) that cortisone may inhibit allergic inflammation not by some mechanism specifically related to the antigen-antibody union but rather through its general antiphlogistic action. Evidence which supports this view is that acute inflammations

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produced by trauma are inhibited as well as those originating from chemical stimuli(22). There is much evidence emphasizing that the substances released by the antigen-antibody reaction elicit the responses of the effector organs, tissues and cells. The influence of cortisone upon the release, transfer, distribution or action of these mediator substances will be of primary importance for future investigations designed to ascertain the mechanism of the antiphlogistic action of this hormone.

Summary. By investigation of experimentally induced allergic and traumatic inflammation in (a) adrenalectomized and (b) adrenalectomized and cortisone-treated animals, the role of the adrenals and of exogenous cortical hormone in the regulation of the cellular changes of the inflammatory process was evaluated. Adrenal cortical secretions and exogenous cortisone were demonstrated to inhibit allergic inflammation through an antiphlogistic action *sui generis*, rather than by interfering with the antigen-antibody union. It is suggested that this antiphlogistic action of cortisone accounts for the capacity of this hormone to minimize the consequences of a wide variety of unrelated inflammation producing stimuli.

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A Study of Elastic Arteries in Irradiated Mice of Different Ages.* (18369)

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In a recent paper(1), aging changes in the tunica media of the aorta of animals from newborn through old age were described. The present study is concerned with the effects of x-radiation on the media of elastic arteries of mice of different age groups. A review of previous work on the effects of irradiation on blood vessels has been presented by Rhoades (2). She reported that "the most constant and obvious effects of irradiation . . . were changes in collagen" and that these changes could be "considered characteristic of the treatment." Her discussion was concerned primarily with the tunica adventitia; however, since that portion of the arterial wall is poorly developed in the mouse, a study of the well developed media was considered more feasible.

Methods. Fifteen mice, 20 to 30 days of age, 15 mice, 80 to 100 days, and 15 as old as could be obtained from the Roscoe B. Jackson Memorial Laboratory were used in this investigation. X-ray of 250 kv filtered with 2 mm of copper and 1 mm of aluminum was used for total body external exposure. The animals were held in a plastic wire cage(3) and received single treatments of 410 r. The intensity of x-radiation was 31 r per minute. According to Murray(4), the dose lethal to 50% of the mice within 30 days has been determined by Hagen, Simmons and Sacher to be about 500 r. Mice from each age group were killed at 2,4,8,12,22,28,36,48,72,84,96, 117 hours and 6,7,8 days after x-radiation. The aortae of 58 mice studied for aging changes in the media from birth through 760 days of age were used as controls(1).

* This work was supported by a grant from the United States Public Health Service. The mice were irradiated through the courtesy of the Westfield State Sanatorium.

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