EXPERIMENTAL IMMUNOLOGIC ADRENAL INJURY*, ‡

A Response to Injections of Autologous and Homologous Adrenal Antigens in Adjuvant

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Plates 10 to 12

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The morphology of the adrenal lesions of the idiopathic form of Addison's disease (cytotoxic contraction) (Fig. 1.) has been repeatedly reviewed (3, 9, 12, 17, 20, 21, 27). The etiology of this condition still remains obscure.

The concept of an auto-immune nature of the pathologic process has been based upon the following evidence:

(*a*) The finding of lymphoid infiltrations of the thyroid gland resembling Hashimoto's disease in association with idiopathic Addison's disease (20, 22, 27). Struma lymphomatosa is now known to have an auto-immune etiology (7, 18, 19, 25, 26).

(*b*) The demonstration of circulating complement-fixing auto-antibodies to thyroid and adrenal extracts in a patient with lymphadenoid goitre and Addison's disease and in another Addisonian patient without overt thyroid disease (1).

(*c*) The production of iso-immune adrenal lesions in 9 guinea pigs injected with pooled adrenal tissue in Freund's adjuvant (5).

The purpose of the work reported in this paper was the production and study of auto-immune adrenal lesions in guinea pigs.

Materials and Methods

Pilot Experiments.—Six adult rabbits of mixed New Zealand White and Giant White stock, weighing approximately 2.5 to 3.0 kg. were injected with pooled isologous rabbit adrenal homogenates in buffered saline, each animal receiving a total of 900 mg. of this material in 11 daily intracutaneous injections in divided doses. All the animals were sacrificed on the 40th day. No lesions were found in any of these animals in either the adrenal or other organs.

A second group of 14 rabbits received 4 injections of autologous and homologous pooled adrenal tissue respectively with Freund's adjuvant. A control group of animals was injected with Freund's adjuvant alone. The animals were sacrificed serially between the 56th and 70th day following the first injection. Lesions were found in the adrenals of both the control and

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experimental groups. Since the lesions caused by the adjuvant alone could not be separated from those produced by the mixture of adjuvant and antigen, the experiments were considered to be unsatisfactory. These lesions will be reported separately (24).

Main Experiments.—Young adult guinea pigs of both sexes and mixed stock, each weighing 250 to 450 gm. were used and kept under identical conditions of nutrition and maintenance. Twenty-nine animals were unilaterally adrenalectomized 3 weeks prior to receiving their first injection. There was a considerable mortality following the operations and adrenalectomies were performed in the same way on substitutes.

The unilaterally adrenalectomized animals were divided into three groups:

Group I, consisting of 11 animals, received 4 weekly intramuscular injections of 1 ml. of homologous adrenal tissue homogenate in adjuvant.

Group II, consisting of 12 guinea pigs, received 4 weekly intramuscular injections of 1 ml. of autologous adrenal tissue homogenate and adjuvant.

Group III, consisting of 6 animals, received 4 weekly intramuscular injections of 1 ml. of Freund's adjuvant alone (Chart 1).

Animals dying in the course of the experiment were replaced and were not included in the final assessment. This was necessitated by rapid postmortem autolytic changes in the tissues which made histologic evaluation impossible.

The animals in group I were sacrificed serially between the 40th and 112th day, in group II between the 30th and 105th day, and in the control group III between the 34th and 84th day (see Chart 1).

Preparation of Antigens.—The average weight of the paired adrenals in young adult guinea pigs was found to be 506.2 mg., based on the weight of the suprarenals of 50 normal animals. An amount of 120 mg. of this tissue was injected into each animal, representing approximately 50 per cent of the weight of the residual adrenal tissue after unilateral adrenalectomy. This amount was chosen since the weight of individual adrenals was found to range from 138 to 420 mg.

The appropriate quantities of adrenal tissue were weighed and washed repeatedly in cold (4°C.) phosphate buffered saline (pH 7.2). Each antigen was finally suspended in an equal volume of buffered saline. The pooled homologous antigen obtained from 50 randomly selected guinea pigs was homogenized in a Waring blendor for approximately 5 minutes with repeated interruptions to avoid overheating. The auto-antigen was homogenized in separate individual batches with a hand-operated blender. The smooth homogeneous suspensions were exposed to ultraviolet radiation in Petri dishes for a period of 4 hours and 24-hour aerobic and anaerobic cultures were taken from each sample. These were uniformly sterile. After addition of an aqueous solution of merthiolate in a final concentration of 1:10,000 the homogenates were stored at −20°C. The adjuvant mixture was added to each lot and mixed on a rotary shaker for ½ hour immediately prior to injection.

Preparation of Adjuvant.—A stock culture of *Mycobacterium butyricum* No. 362 (American Type Culture Collection, Washington, D. C.) was grown in Long's synthetic medium, harvested by centrifugation, killed by mixing with 0.5 per cent formalin, and dried at 100°C. in an oven. A mixture of bayol F (Esso Standard Oil Company, Toronto) and arlacel A (Atlas Powder Company, Toronto) was sterilized by autoclaving and the dried acid-fast bacilli, previously ground in a mortar, were added and thoroughly mixed. Each milliliter of the adjuvant mixture contained 0.425 ml. of bayol F, 0.075 ml. of arlacel A, 2.0 mg. of mycobacterial residues and 0.500 ml. of phosphate buffered saline (pH 7.2).

Injections and Dosage.—All injections were administered into the muscles of the dorsal nuchal region. The injections in groups I and II consisted each of:

Adrenal antigen—30 mg.

Buffered saline—0.5 ml.

CHART I

Distribution of Systemic Lesions

Group	Antigen	Code No.	Sacrifice*	Adrenal‡	Liver§	Kidney	Other‖
I	Homologous	75	40	—	N	—	—
	"	81	43	—	—	+	—
	"	52	50	—	N	—	—
	"	78	53	+	—	+	Thyroid
	"	82	56	—	N	—	—
	"	59	63	+	N	+	—
	"	84	70	+	N	+	—
	"	63	77	+	N	+	—
	"	85	84	++	N	+	—
	"	86	105	+	N	+	—
	"	80	112	—	—	—	—
II	Autologous	57	30	+	N	—	—
	"	53	40	—	N	+	—
	"	68	43	++	—	+	—
	"	56	50	+	—	+	Thymus
	"	66	53	++	N	+	Thymus
	"	61	56	+++	N	—	—
	"	73	63	++	N	+	—
	"	62	70	+	—	—	—
	"	83	77	++	N	—	Thyroid
	"	65	84	+++	N	+	Pericardium Amyloid spleen
	"	94	91	+++	N	+	—
	"	69	105	++	—	+	Amyloid spleen and liver
III	Control adjuvant only	64	34	—	—	+	—
	"	40	40	—	N	—	—
	"	49	42	—	—	—	—
	"	41	43	—	—	+	—
	"	43	56	—	—	+	Thyroid
	"	37	84	—	—	+	—

* Days following first injection.

‡ Grading based on extent of lesions—0 to 3+.

§ Eosinophilic hepatic necrosis—N.

‖ All animals showed changes in portal triads, spleen, lymph nodes and lung.

Complete adjuvant—0.5 ml. (0.212 ml. bayol F; 0.038 ml. arlacel A; 0.250 ml. buffered saline and 1 mg. mycobacterial residues)

The injections in the control group III consisted of:

Buffered saline—0.5 ml.

Complete adjuvant—0.5 ml. (as above)

Histologic Techniques.—At autopsy, tissues were obtained from adrenal, heart, lung, skeletal muscle, lymph nodes, thyroid, thymus, gonads, mesentery and omentum, spleen, kidney, and liver. The specimens were fixed in buffered formalin (pH 7.0) and the fixation procedure outlined by Hoerr (11) was followed in the case of adrenal tissues. All sections were stained with Lilly's azure eosin and when appropriate, with methyl violet and Congo red respectively for amyloid. Serial sections of the adrenals were stained with hematoxylin and eosin, Lilly's azure eosin, the periodic acid–Schiff stain, Unna-Pappenheim's methyl green–pyronin stain, the Ziehl-Neelsen and Masson's trichrome stain, and with Laidlaw's method for reticulin fibers.

RESULTS

Adrenal Lesions

I. Histologic Considerations.—

The literature on the histology of the guinea pig adrenal was partly reviewed by Hoerr (11). The structural arrangement is basically identical with that of the human suprarenal gland. Several points need be stressed.

The lipid-containing cells of the zona fasciculata and reticularis referred to as "spongiocytes" (15) become less numerous when progressing from the outer zones of the cortex towards the deepest portion of the reticularis (Fig. 2). In sections stained with the periodic acid–Schiff (PAS) stain, the decreasing lipid content is accompanied by an increasing aggregation of deeply PAS-positive granular material in perinuclear locations in the cortical cells (Fig. 4). In the deep reticularis in particular, cells are found, in which the granules are so numerous and dense as to make the cells uniformly red with the PAS stain. These cells are considered to correspond to the "dark" cells described by others (6, 10, 15). The nuclei of these and occasional other cells in the deeper layers of the cortex are found to be pyknotic even under normal circumstances. The rather deeply acidophilic cytoplasm of these cells when seen in tissues stained with Lilly's azure eosin is quite distinct from the pigmented "chromatophore" cells of the reticularis. The nuclear hyperchromatism and the cytoplasmic acidophilia have led Hoerr (11) to remark that degenerating cells are *always* found in the zona reticularis.

It has been stated (25) that the radially arranged *capillaries* between the cords of cortical cells change to small veins in the zona reticularis; *i.e.*, the location of the principal lesions in "iso-immune adrenalitis". However, Maximow and Bloom (15) and others have stated repeatedly that there is no venous system in the cortex, but merely a sinusoidal system which drains into the medullary veins. The *sinusoidal structure* of the cortex has been compared with that of the liver (9). In the guinea pig a difference can be discerned. The sinusoidal lining cells, the littoral cells of the reticulo-endothelial system, unlike their Kupffer cell counterpart in the liver lobules, are not evenly distributed throughout the cortex. They are considerably more numerous in the reticularis and deep fasciculata than in the outer fasciculata and they are practically

non-existent in the glomerulosa. Their presence is easily identified because of of their elongated shape and in some guinea pigs by the presence of a coarse, granular, green pigment in their cytoplasm in azure eosin—stained sections. This material is strongly PAS-positive (Fig. 4).

II. Pathologic Considerations.—

Cortical Lesions.—

Incidence and grading: Microscopic lesions were present in 6 out of 11 animals receiving the pooled antigen (group I) and in 10 out of 12 animals given their own antigen (group II). Gross lesions could not be identified in any group, nor were any microscopic lesions found in the control group (III).

Grading of the lesions from 0 to 3+ was adopted on the basis of the extent rather than the severity of the lesions. In this way the incidence of extensive (2 and 3+) lesions was greater in the auto-immune than in the homo-immune group in a statistically significant number of cases at the 0.002 per cent and 0.01 per cent levels (Chart 1).

Duration of lesions: The earliest lesion in group I was found on the 53rd day and group II on the 30th day following the first injection, 32 and 9 days respectively after the last injection. In general there was no direct relationship between duration and extent, severity and progression of the lesions (Chart 1).

Localization of lesions: The location of the lesions coincided with the presence of littoral cells in the sinusoids and partly with the presence of PAS-positive granules in the cortical cells (Fig. 4). In this way most lesions were found in the deep fasciculata and in the reticularis (Figs. 2 and 3), being most extensive in juxtaposition to the cortico-medullary junction. No lesions were found in either the glomerulosa or outer fasciculata.

Morphology of the lesions: The changes could be divided into early focal, perisinusoidal lesions and later, confluent, extensive cellular infiltrates aggregating at the expense of cortical cells.

A. The *early lesions* consisted of proliferating cells immediately outside the basement membrane of sinusoids, though occasionally they appeared to be within vascular lumina (Figs. 3 and 4). They were often aligned in a bead-like fashion along the extrasinusoidal aspect of the reticulum fibers. Such cells were mostly lymphocytes intermingled with histiocytes. Plasmoblasts and plasma cells and pyroninophilic cells were rare in these lesions. Reticulum stains of these areas showed as yet no new fiber formation. Littoral cells could be clearly identified as separate intrasinusoidal entities (Fig. 4).

B. The *late lesions* developed by confluence of the focal early lesions (Figs. 3 and 5). This was achieved by increasing cellular aggregations in adjoining perisinusoidal locations until the intervening parenchyma became bridged and later still replaced by cells of mesenchymal origin. The spread of the early lesions also occurred centripetally, thereby producing narrowing and occlusion

of sinusoids. Vessels approaching such foci were dilated in a lacunar fashion on the upstream side of the lesion. The proliferating cells produced total disorganization of the affected area so that littoral cells were often found in the midst of cellular infiltrates apparently outside the confines of the sinusoids. They could occasionally still be identified in the azure eosin–stained section because of their content of green-staining pigment.

It has been stated that very little congestion was needed in the loosely reticulated cell cords to cause injury to adrenal cortical cells (11). The *loss of cells* as a result of displacement and perhaps ischemic atrophy was obvious (Fig. 5), but the massive parenchymal destruction described by others (25) could not be seen. Occasional cells with pyknotic nuclei, increased cytoplasmic acidophilia or reduced eosinophilia could be found, but lysis of nuclei (5) could not be identified in the midst of such a closely packed infiltrate. In general, the numbers of degenerating parenchymatous cells were slightly more numerous than under normal conditions. Occasional normal cortical cells, some surrounded by a clear halo, were found surviving in the centre of a mesenchymal cell aggregate.

Mitotic activity, the most tangible evidence of guinea pig cortical cell destruction (11), was seen very rarely. Parenchymatous giant cells or regenerative nodules, hallmarks of the human lesion (3), were not seen.

The *cellular infiltrates* were markedly pleomorphic (Fig. 5), consisting predominantly of histiocytes with reticulum cells and occasional reticular cells and plasmoblasts, identifiable by their prominent nucleoli. Large multinucleated giant cells were seen rarely in the midst of the granulomatous infiltrate. Lymphocytes were less common in these late lesions as plasma cells were increasing in number. Differentiation in the direction of fibroblasts was not seen. Pyroninophilic cells constituted as much as 30 per cent of the infiltrate. Cytoplasmic PAS-positivity in plasma cells was seen only extremely rarely as compared with its prominence in these cells in the spleen.

The normal *reticulum fibers* of the cortex are ordinarily rather coarse and are orientated in parallel with the radially arranged sinusoids. Occasional branches are projecting in a lateral manner between cortical cells in the reticularis where branching of sinusoids is common. In the late lesions there was evidence of new reticulin fiber formation. They were identifiable by their delicate appearance and by their haphazard distribution as well as by their tendency to surround individual cells of the infiltrate. Collapse and condensation of reticulin fibers, which are seen regularly in the late stages of human cytotoxic contraction (3) were not found.

Medullary Lesions.—

Since ectopic cortical cells are usually found in the adrenal medulla, a clear separation of cortical and medullary lesions was not always possible (Fig. 6).

Medullary lesions were found in all instances when cortical lesions were present and their severity roughly paralleled the latter. Medullary lesions as an isolated phenomenon were not found, nor were any present in the animals of the control group (III).

The *cellular make-up* was similar to that of the cortical lesions (Fig. 7). The pheochromocytes were frequently displaced by the infiltrate but there was no evidence of parenchymatous cell necrosis. Ganglion cells were often found surviving in the midst of areas of cell proliferation (Fig. 7). Loss of these cells was difficult to assess because even in normal circumstances they are found only in small numbers.

The *location* of the lesions in the medulla was in no way related to veins. They were usually found some distance away from the large venous channels and compression of vessels was not noted.

The pheochromocytes are normally arranged in "Zellballen," each group being surrounded by a delicate *reticulum* framework. In the area of the lesions there was evidence of deposition of new fibers leading to a marked disorganization of the local architecture (Fig. 8).

Sympathetic Ganglia.—No changes were demonstrated in these structures which are frequently found within or in the immediate vicinity of the adrenal capsule.

Liver.—All animals in all three groups were found to have hepatic lesions on microscopic examination. "Eosinophilic" necrosis was present in 8 of 11 animals in group I, in 8 of 12 animals in group II and a small focal area in 1 of 6 animals in group III. These necrotizing lesions were present as early as 30 days following the first injection and as late as 105 days. No definite time relationship could be established between the severity and the duration of these lesions. The necrosis involved either individual or small groups of cells in the center of focal granulomata distributed in random fashion through the lobules. In other instances large map-like areas of lobules showed progressive degenerative changes culminating in an acidophilic homogenization of the cytoplasm with, at first, preservation of nuclei, and later, total coagulative necrosis (Figs. 9 and 10). Though distributed at random, they were seen most frequently in midzonal locations. The cellular reaction to the presence of such areas of necrosis was usually slight, though occasional plasmoblast, plasma cells, and histiocytes were found in the margins of the lesions. Hypertrophied Kupffer cells were found surviving in the midst of some areas which had undergone necrosis. Reparative or regenerative changes were not seen in any animal.

In all guinea pigs there was an intense mesenchymal cell infiltration of the portal triads often associated with disruption of liver plates. In addition to the small intralobular granulomata in association with focal "eosinophilic" necroses, discrete sarcoid-like epithelioid granulomata were also present in random loca-

tions within the lobules. In general, these changes were identical with those previously described as being a "specific" manifestation of an iso-immune hepatic injury in guinea pigs (2).

Amyloid, staining typically with both methyl violet and Congo red, was found in the liver of one animal (surviving 105 days, group II).

Kidney.—Diffuse or focal cortical cellular infiltrates were found in 7 of 11 animals in group I, in 8 of 12 animals in group II and in 4 of 6 animals in group III. The cells were mostly lymphocytes with occasional histiocytes and plasma cells. There was no destruction or involvement of either tubules or glomeruli. Although Gram-stained sections did not reveal the presence of bacteria in these lesions, it was considered possible that they represented an activation of interstitial lesions frequently found in adult guinea pigs, possibly as a result of potentiation of latent infections by the Freund's adjuvant.

Lung.—Pulmonary lesions were found in all animals of all groups. They consisted of diffuse, interstitial, cellular reactions occasionally accompanied by the formation of focal epithelioid cell granulomata. In some animals the lesions were so severe as to produce widespread atelectasis. Intense plasma cellular proliferations were found in the walls of both bronchial and pulmonary arterioles. Unlike Freund's adjuvant lesions in the lungs of rabbits (24), lipid vacuoles were not demonstrable in the pulmonary lesions of guinea pigs.

Thymus and Thyroid.—Thymic lesions were found in 2 animals of group II and thyroid lesions in one animal of each group. As in the lesions of cervical lymph nodes, lipid vacuoles were prominent in these areas. Extensive granulomatous infiltrates were present mainly in the cortex of the thymus and around and between the acini of the thyroid. Displacement of the latter structures was seen frequently (Fig. 12). Occasional discrete sarcoid-like granulomata were also found in the midst of an intact thyroid parenchyma.

Lymph Nodes.—Cervical, mediastinal or abdominal, and inguinal nodes were obtained from all animals and all were found to be involved in granulomatous inflammatory processes. In the case of cervical nodes, lipid vacuoles were conspicuous in the afferent lymphatics of the cortex. In the cortex, and less commonly in the medulla, there were large numbers of discrete, sarcoid-like, epithelioid cell granulomata, often with prominent Langhans' giant cells in the centers of the lesions. Confluence of such foci was seen fairly frequently (Fig. 11).

Spleen.—Changes, present in all animals, consisted of an intense histiocytic reaction in the red pulp and of a massive proliferation of pyroninophilic cells in the centers and mantle zones of the lymphoid follicles of the white pulp. Occasional follicles were found in which there were no residual lymphocytic elements, all cells being either plasmoblasts or mature plasma cells. Many of these cells showed varying degrees of PAS-positivity culminating in the ap-

pearance of large numbers of randomly distributed Russell bodies. Discrete granulomata or lipid vacuoles were not found in the spleen.

Typically staining amyloid was found in the periphery of lymphoid follicles of 2 animals in group II surviving 84 and 105 days respectively.

Pericardium.—A granulomatous pericarditis was present in one animal of group II. The pericardial cavity was filled with an amorphous, eosinophilic exudate, the material failing to stain with the usual methods for amyloid. The visceral pericardium was involved in a granulomatous process with a marked plasma cellular component and an intense fibroblastic reaction with early organization of the exudate.

DISCUSSION

The essence of auto-immune adrenal lesions in this experiment is a focal and later diffuse proliferation of mesenchymal cell derivatives which differentiate in the direction of immunologically competent cells. This process is accompanied by a progressive disorganization of the architecture of the deep cortex and medulla of the gland (Fig. 3). Colover and Glynn (5) suggested that there were infiltration and destruction of parenchyma in their iso-immune adrenal lesion. Waksman (25), in his review of this experiment, described a massive parenchymal destruction. In the present experiment there is evidence of loss of cortical cells (Fig. 5). There are also parenchymal cells in the involved areas which show nuclear pyknosis and an increase or decrease of cytoplasmic eosinophilia. These changes are seen more often than is the case under normal circumstances in the deep fasciculata and reticularis. There is no evidence of necrosis or of massive cell destruction. The changes are attributable to displacement of the parenchyma by the infiltrate and to injury resulting from ischemia. The situation is somewhat analogous to the conditions prevailing in the central nervous system in some cases of experimental allergic encephalomyelitis. Paterson (18), in a review of this condition, suggests that demyelination, the destructive component of the lesion, may be secondary to vessel injury.

It has been pointed out that various forms of adrenal cortical destruction, both experimental (11) and human (3, 9, 12, 17, 20, 21, 27) are accompanied by evidence of active parenchymatous regeneration, and collapse of the reticulum framework. Absence of these phenomena in the present experiment suggests that destruction is not occurring or that the process is so gradual and slight that evidence of regeneration has not as yet appeared at the time of the conclusion of the experiment.

It has been suggested (25, 26) that the experimental adrenal lesion may provide a possible model for cytotoxic contraction of the adrenal gland in the human (Fig. 1). Apart from the differences already noted, namely the absence

of cellular regeneration and collapse of the reticulum framework (3, 12, 17) in the experimental lesion, there may also be noted the limited distribution of the latter lesions in the guinea pig adrenal (Fig. 2) in contrast to the diffuse cortical involvement in the human process.

Three factors may determine the development of the lesions in the deep fasciculata, reticularis and in the medulla of the guinea pig adrenal:

(a) The antigenicity of the cells of the various layers may play a role in the localization. The medullary involvement could also be explained in this fashion, since reticularis cells are found frequently in ectopic locations in the medulla (Fig. 6).

(b) The histologic differences between the cells of the outer layers and those of the deeper layers of the cortex may be related to their secretory function. If antiphlogistic adrenocortical hormones were elaborated in the outer layers of the guinea pig adrenal cortex, then their presence may have a restraining effect on the development of the lesion. It is known that such hormones inhibit the delayed or tuberculin reaction and it is thought that they may interfere with the mechanism of sensitization or with the liberation of antigens (16).

(c) The presence of littoral cells in the deeper layers of the guinea pig cortex and their scarcity or total absence in the outer layers may have a part to play in this connection. In the conditions prevailing in these experiments it is these fixed phagocytic cells which presumably would convey to the precursors of immunologically competent cells of the adrenal the information relating to the presence of a circulating autologous or homologous antigen.

The origin of the immunologically competent cells is not clear. The situation is analogous to the development of extramedullary hematopoiesis in the adrenal. It has been suggested that hemopoietic cells in the adrenal originated from endothelial (littoral) cells acting as mesenchymal precursors (23). Others have concluded that these cells, although phagocytosing carmine (13, 15), trypan blue (6), and red blood cells (13), did not become transformed into other cells of the mesenchymal series. Lang (13) suggested that cellular proliferations in the adrenal resulted either from migration of blood cells or by differentiation of perivascular mesenchymal cells.

The adrenal lesions developing in the course of these experiments formed part of a widespread systemic disease. Extra-adrenal lesions, apart from some enlargement of lymph nodes, were not described in the study of iso-immune adrenalitis by Colover and Glynn (5). In their experiment guinea pigs received 4 intramuscular injections of the antigen with a total of 2 mg. of mycobacterial residues in the adjuvant, using *Mycobacterium tuberculosis*. Changes in adrenals were not demonstrated in large numbers of guinea pigs injected with nervous tissue components in Freund's adjuvant (5). The *Mycobacterium butyricum* residues contained in the adjuvant used in the present experiment amounted

to 4 mg. divided amongst 4 injections. Despite widespread systemic lesions, *no changes were demonstrated in the adrenals of the control group of animals* (group III) injected with the adjuvant alone.

The systemic lesions described in this study, with the exception of those in the adrenal and the "eosinophilic" hepatic necrosis, have been noted previously in guinea pigs receiving Freund's adjuvant containing 7.5 mg. *Mycobacterium tuberculosis* residues, sacrificed 5 months after single injection (14). Similar hepatic lesions were produced in guinea pigs by injections of whole liver homogenates with adjuvant (2) and were considered to be a specific antigen-antibody reaction with injected liver substances acting as an iso-antigen. The finding of "eosinophilic" necrosis in the present experiment (Figs. 9 and 10) suggests that adrenal and liver tissue components may share common or closely related antigenic determinants responsible for the cross-reactivity.

The pathogenesis of the various systemic changes found in this experiment may be attributed to: (*a*) the direct extension of the injected material along muscular and fascial planes from the initial focus of granulomatous inflammation at the site of the injection, *e.g.*, thyroid, thymus, mediastinum, and pericardium; (*b*) the lymphatic and hematogenous dissemination of the injected material with consequent establishment of metastatic granulomatous lesions, *e.g.*, lung, liver, lymph nodes, and kidney; (*c*) the non-specific stimulus of the adrenal homogenates with adjuvant or of the adjuvant alone on the reticulo-endothelial system, *e.g.*, spleen and liver; (*d*) the specific stimulus of the adrenal homogenate in Freund's adjuvant on the immunologically competent cells of the reticulo-endothelial system of the adrenal.

Based upon such considerations, the adrenal lesions are thought to be a specific manifestation of an immunologic process induced and potentiated by the injections of homogenates of adrenal antigens in complete Freund's adjuvant (8). Immunologic process is here defined as the proliferation of immunologically competent cells in the specific target organ. It does not imply any *prior* injury of the adrenal as part of such a process. Consequently the suffix "itis" in the designation of such lesions would seem unjustifiable, since it would imply that the aggregation of cells of mesenchymal origin forms part of a reaction to an antecedent tissue injury.

Grading of the extent of the lesions in guinea pig adrenals was facilitated by the ease with which the entire organ could be surveyed in a single low-power microscopic field. Serial sections were, however, required to achieve a reasonably valid quantitative result.

Autologous and homologous, rather than isologous, antigens were used in the present experiment to allow for as wide a variation of antigens without transgressing the limits of species specificity. Widespread (2 and 3+) lesions were found in a statistically significant higher number of animals injected with

auto-antigen than with pooled homologous antigen. This finding suggests that the former possesses a greater capacity to induce proliferation of immunologically competent cells in the specific target organ than does the latter. Considering this from an egocentric point of view, it would be anticipated that homologous tissue would be more foreign and consequently more antigenic than autologous tissue which is truly "self" and therefore should be non-antigenic or at best poorly antigenic.

To explain this apparent paradox the following hypothesis has been formulated. The proliferation of mesenchymal cells in the adrenal may be explained as a defense reaction to the presence of a circulating adrenal antigen, the antigenicity of which has been potentiated and enhanced by Freund's adjuvant. The latter substance is known to stimulate the proliferation of mesenchymal cells in various organs even in the absence of a complementary antigen (8, 24). The antigen may then be envisaged as possessing qualities which determine the specific localization of the reaction. The response to the presence of "free" adrenal antigen may be the normal physiological function of certain mesenchymal cells in the adrenal. They may proliferate under normal circumstances in response to a release of adrenal antigens *within* the gland from whatever cause. This reaction may be defensive since it would tend to prevent by means of production of "binding" globulins the escape of such antigens into the circulation where they may not be recognized as "self" by the other components of the reticulo-endothelial system. This concept would then confer upon the reticulo-endothelial cells of an organ (adrenal) the specific "self" recognition capacity within the framework of the reticulo-endothelial system of the entire organism.

In the present experiment the specific antigenic stimulus reaches the adrenal *from without*, but the reaction, mediated by the littoral cells of the adrenal cortex, once initiated, progresses in the same manner as if the antigen had been released within the gland itself. The mechanism of "organ-self" recognition could be so delicately adjusted that the response to an auto-antigen would be more intense than that resulting from injection of more remote, though cross-reacting, homologous antigen.

The absence of an adrenal reaction after injection of the antigen without adjuvant, which was the case in the pilot experiment in this study and in some control animals of Colover and Glynn's experiment (5), could be explained by the lack of a sufficient antigenic stimulus in the absence of the synergistic effect of an adjuvant. An alternative explanation, offered by Burnet (4), would be that the release of large quantities of antigen into the circulation over a relatively short period of time may lead to the inhibition of differentiation and/or destruction of immunologically competent cells.

SUMMARY AND CONCLUSIONS

Twenty-three unilaterally adrenalectomized guinea pigs were injected with autologous and homologous adrenal tissue homogenates respectively, in Freund's adjuvant. Widespread adrenal lesions were found in 10 of 12 animals receiving auto-antigen and to a lesser extent in 6 of 11 animals injected with homologous pooled antigen. Widespread systemic lesions were present in both these and in control animals receiving Freund's adjuvant alone. These latter animals showed no adrenal involvement.

The early changes within the adrenal consisted of perisinusoidal cellular proliferations in the deeper layers of the cortex. Focal granulomata developing at a later stage tended to become confluent and to displace cortical cells. Some loss of these cells was attributable to ischemic injury.

The localization in the deep fasciculata and reticularis was thought to depend (*a*) on the varying antigenicity of adrenal cortical components, (*b*) the possible inhibitory effect of antiphlogistic adrenal cortical hormones on the development of lesions in the outer cortex, and (*c*) the presence of littoral cells in the deep cortex. These cells are thought to be involved in the mediation of the stimulus initiating differentiation of primitive mesenchymal cells in response to a circulating auto-antigen. The medullary lesions may be related to the presence of ectopic reticularis cells in this location.

It was suggested that the cellular response in the target organ to injections of adrenal homogenates may denote a specific "organ-self" recognition mechanism involving an immune (*i.e.* defensive) reaction. It was postulated that this may be an accentuation of the physiological function of immunologically competent cells. Their proliferation, under normal circumstances, would prevent by means of production of "binding" globulins, the escape and dissemination of endogenous freed adrenal antigens into the circulation. Although the experimental stimulus arose from without the gland, by virtue of the presence of a circulating adjuvant-bound antigen, the adrenal reaction followed the same pattern as would obtain if the antigen was liberated within the suprarenal cortex.

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EXPLANATION OF PLATES

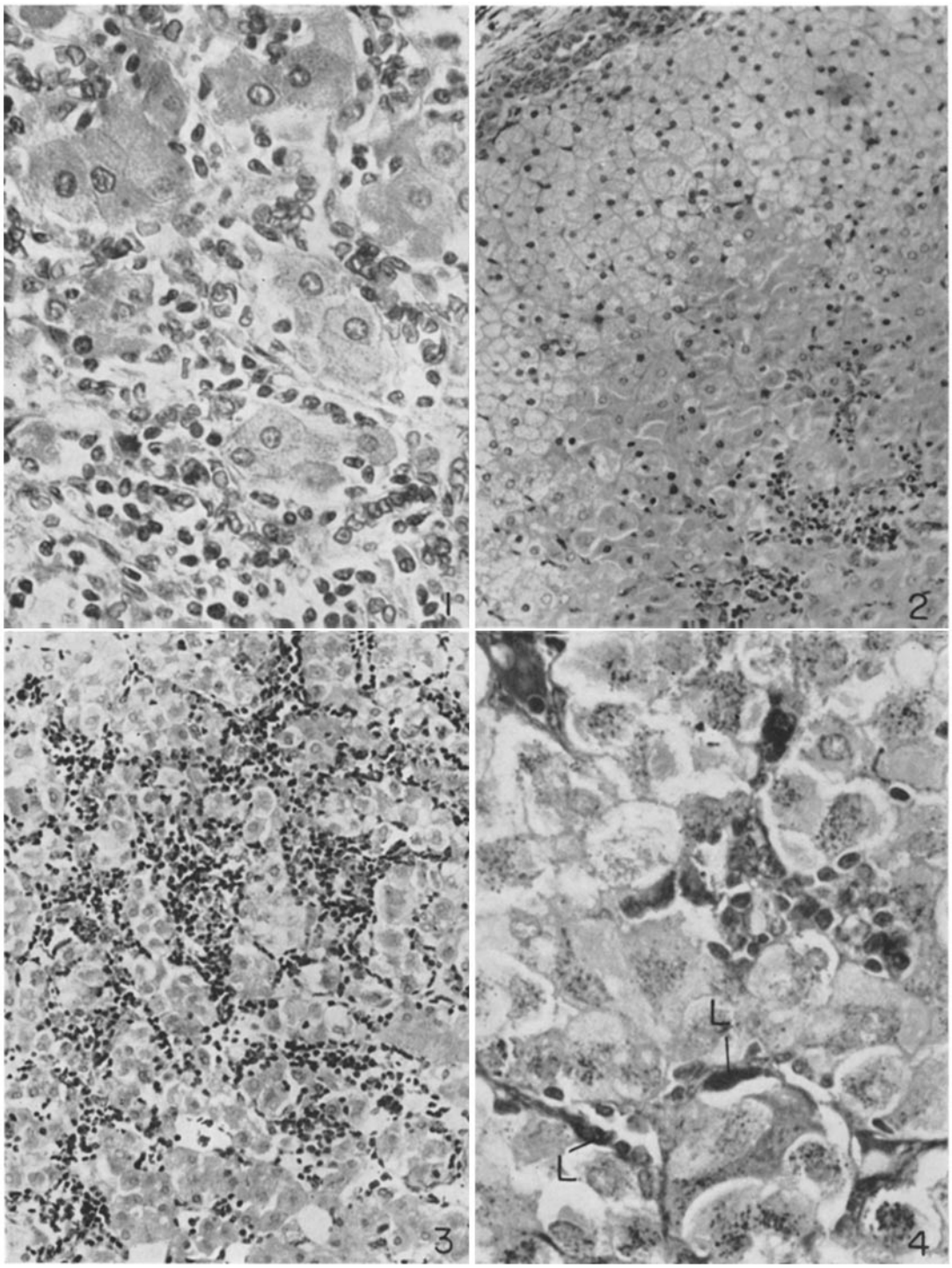
PLATE 10

FIG. 1. Human adrenal gland from a case of cytotoxic contraction (idiopathic Addison's disease). The hypertrophied parenchymal cells are separated from one another by an infiltrate of lymphocytes, histiocytes, and plasma cells. Hematoxylin and eosin stain. × 284.

FIG. 2. Guinea pig (63). Proliferation of immunologically competent cells in deep fasciculata of adrenal cortex. Note absence of reaction in glomerulosa and outer fasciculata. Azure eosin stain. × 132.

FIG. 3. Guinea pig (65). Early, focal, and beginning confluent lesions in reticularis. Note distribution of cellular infiltrates alongside sinusoids and isolation of groups of cortical cells. Azure eosin stain. × 132.

FIG. 4. Guinea pig (68). Strongly PAS-positive littoral cells (*L*) line sinusoids. Early proliferation of mesenchymal cells in perisinusoidal areas with displacement of cortical cells. Note PAS-positive granules in cytoplasm of cells of reticularis. Periodic acid–Schiff stain. × 396.



(Steiner *et al.*: Immunologic adrenal injury)

PLATE 11

FIG. 5. Guinea pig 61. Confluent granuloma in deep fasciculata displacing cortical cells between two sinusoids. Note pleomorphic character of infiltrate and persistence of some cortical cells in its midst. The perisinusoidal distribution of the early reaction can still be seen in the margin of the granuloma. Azure eosin stain. × 284.

FIG. 6. Normal guinea pig adrenal medulla. Large venous sinusoids separate reticularis from medulla. Syncytial masses of pheochromocytes surround ganglion cells. Deeply staining, ectopic cortical cells can be seen around two capillaries in center of picture. Masson's trichrome stain. × 132.

FIG. 7. Guinea pig 85. Pleomorphic cellular infiltrate in adrenal medulla. Note displacement of parenchyma and intact ganglion cells in midst of proliferated cells (right center). Azure eosin stain. × 284.

FIG. 8. Guinea pig 94. Focal granuloma in adrenal medulla. The normal architecture of the pheochromocytic "Zellballen" is disturbed by displacement parenchymatous elements. Note formation of new reticulin fibers by the infiltrating cells. Laidlaw's stain for reticulin fibers. × 284.

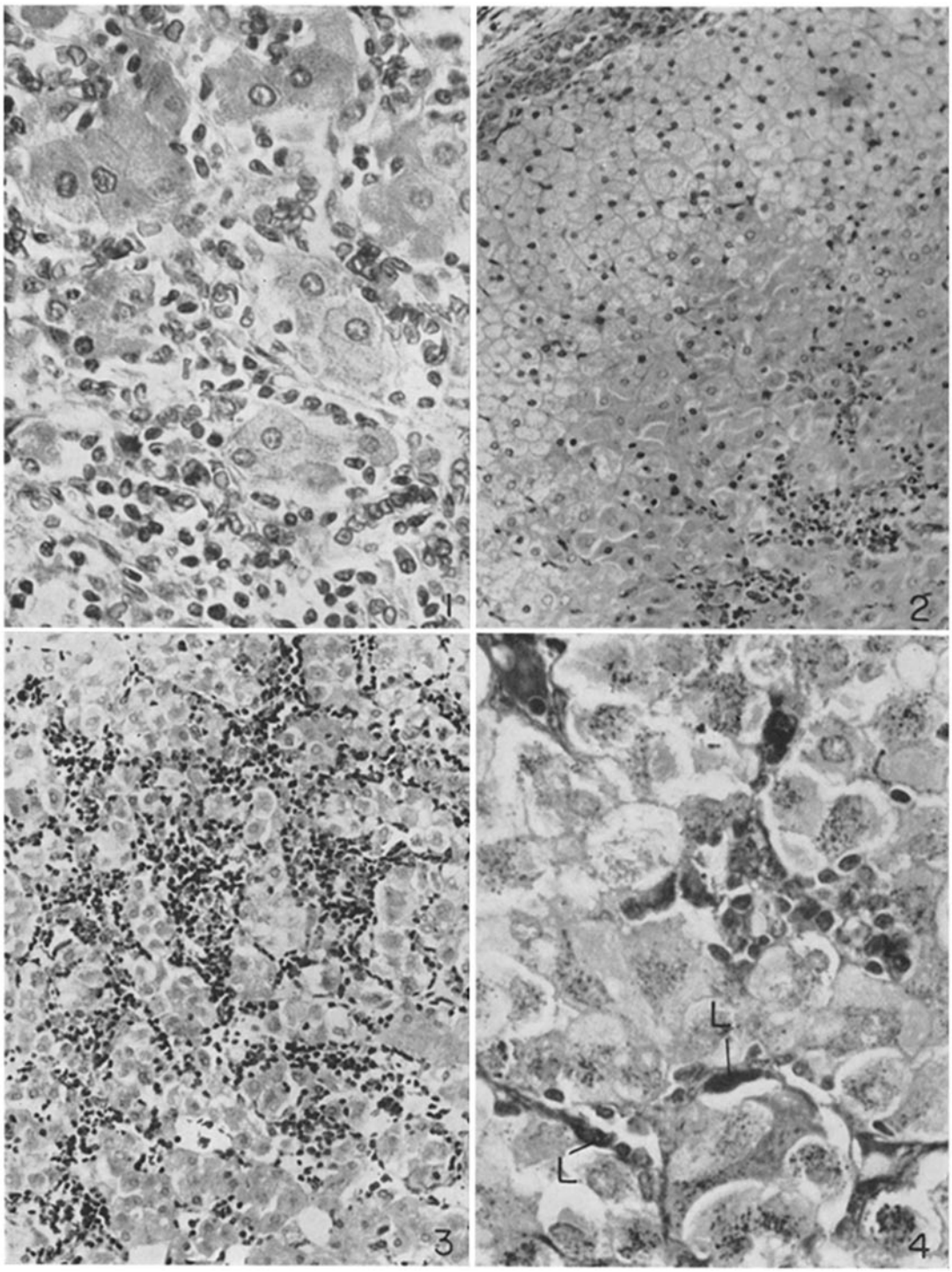


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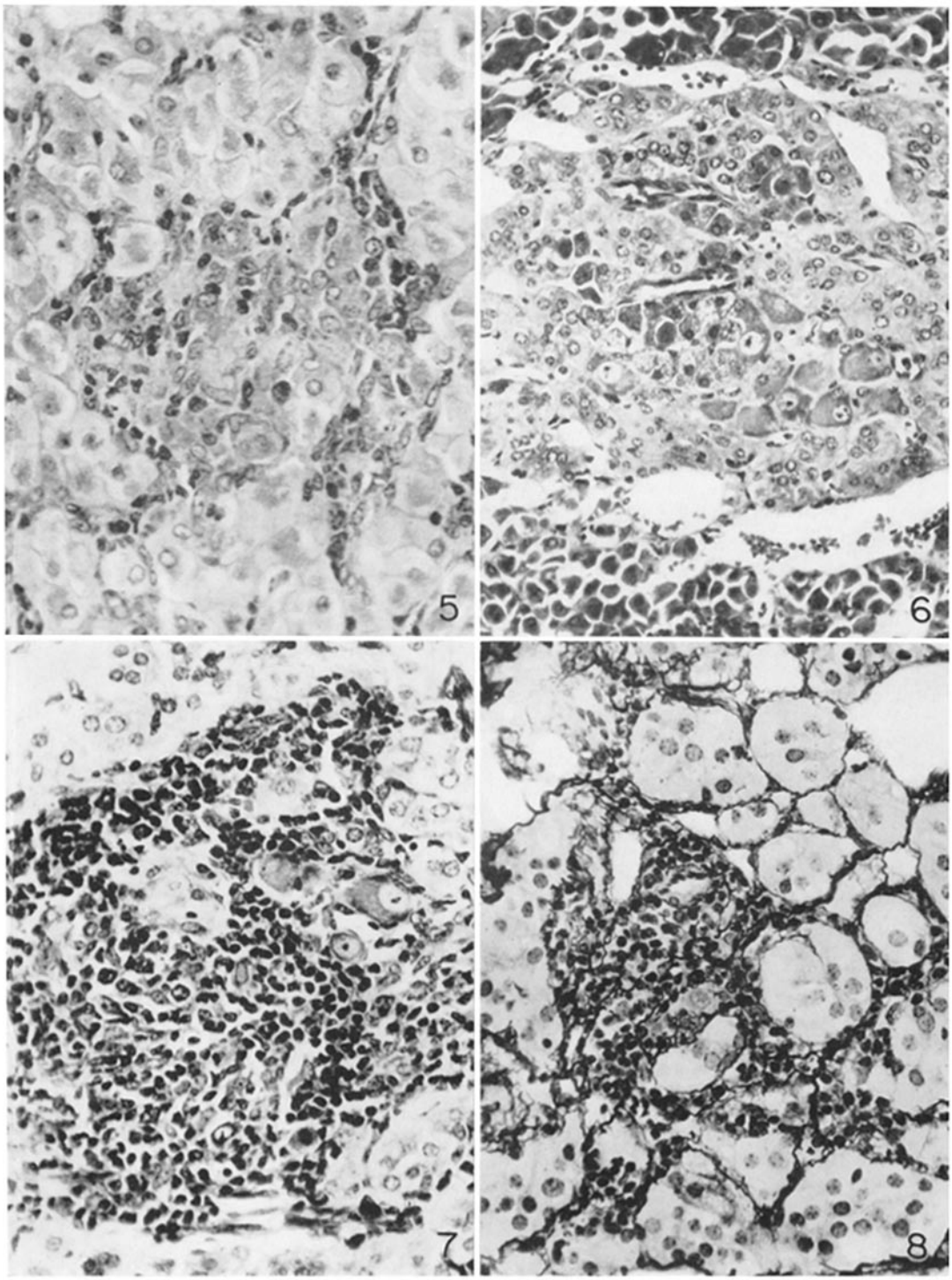
PLATE 12

FIG. 9. Guinea pig 57. Midzonal "eosinophilic" necrosis of liver in lobules on either side of a portal triad. Note intact liver plates and slight mesenchymal cell reaction in margin of triad. Azure eosin stain. × 153.

FIG. 10. Guinea pig 66. Severe degenerative changes in centrilobular liver cells culminating in early "eosinophilic" necrosis (right center). Azure eosin stain. × 306.

FIG. 11. Guinea pig 69. Cortex of cervical lymph node. A large lipid vacuole is present beneath capsule. Focal and partly confluent epithelioid cell granulomata are replacing almost entire architecture. Note large multinucleated giant cell in margin of central granuloma. Azure eosin stain. × 153.

FIG. 12. Guinea pig 78. Granulomatous thyroiditis surrounding and isolating acini containing pale-staining colloid. Large lipid vacuoles are seen in upper right corner. Azure eosin stain. × 153.



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