A HISTOLOGIC STUDY OF THE AUTO-ALLERGIC TESTIS LESION IN THE GUINEA PIG*

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PLATES 36 TO 47

The studies of Voisin, Barber, and Delaunay (1–4) and of Freund, Lipton, and Thompson (5–7) have shown that a specific "auto-allergic" lesion of the testis is produced in guinea pigs and rats following injection of homologous testis or sperm plus the Freund adjuvant (killed tubercle bacilli and mineral oil). This disease seems comparable in its immunologic properties with experimental "auto-allergies" affecting the central nervous system, peripheral nervous system, lens, uvea, thyroid, and adrenal (see reference 8 for a review of these conditions). The testicular lesion appears to be due to sensitization of the delayed or tuberculin type, since the disease process is not correlated with anti-testicular antibody, measured by complement fixation, immobilization of sperm *in vitro*, or anti-hyaluronidase activity, and cannot be transferred passively to normal guinea pigs with serum from actively sensitized animals, since mycobacteria are essential in the technique of induction, and typical delayed skin reactions to purified testis antigen are elicited in animals developing the disease (5–7). Its histologic appearance might be expected to be closely similar to known delayed reactions, such as the tuberculin reaction (9), and to other "auto-allergies" (8) in which inflammation appears to be the primary change, inflammation and parenchymal destruction being found in close association.

Freund *et al.* described the principal change in the testis as aspermatogenesis of varying degree, leading in time to a decrease in the number and size of the seminiferous tubules (5, 6). Only in the most severe cases were occasional focal accumulations of monocytes, lymphocytes, and plasma cells observed between the seminiferous tubules or the tubules of the epididymis. In occasional advanced cases, they found destruction of the tubular basement membrane, fibrosis, and giant cells of the histiocytic type within empty tubules (one such cell, illustrated in their second paper, contained at least 22 nuclei), all findings suggestive of antecedent inflammation. In

311

their third paper (7), these authors laid greater stress on the magnitude of the cellular inflammation and its predominantly mononuclear character, especially in the testes of animals injected with purified antigenic fractions, but believed that this inflammation followed rather than preceded the aspermatogenesis. It is perhaps significant that animals were not sacrificed earlier than 14 days after intradermal inoculations in their first series of experiments with guinea pigs, 23 days in their study of rats, whereas the third paper included animals killed as early as the 7th day, lesions being found by the 9th day.

We undertook to reinvestigate the nature of the histologic lesion produced in guinea pigs by immunization with guinea pig testis plus adjuvants in the hope that such a study would permit a clear distinction between "delayed" and other forms of immunologic reaction as the possible basis for this "auto-allergic" lesion. Our findings justify the conclusion that the "auto-allergic" lesion of testis is primarily inflammatory. Aspermatogenesis appears to occur as a result of direct invasion of the seminiferous tubules by inflammatory cells but also, in young guinea pigs comparable to those used by Freund, as a non-specific event in the testis in which an inflammatory lesion is already present.

Methods

Albino guinea pigs of the Hartley strain, obtained from Tumblebrook Farm, Brant Lake, New York, and English short-haired guinea pigs of broken colors, obtained from Rockland Farms, New City, New York, were used. They were kept in roomy cages in groups of 5 and fed Purina rabbit pellets without antibiotics, a daily ration of lettuce, and water *ad lib.* With the exceptions noted in Table III, all gained weight steadily and remained in excellent health for the duration of the experiments. All received single injections of antigenic mixtures either intradermally (0.1 ml. over the sternum) or subcutaneously (0.5 ml. over the nape of the neck).

The tissues used in making antigenic mixtures were obtained from normal rabbits or guinea pigs and stored at −15°C. till needed. They were cleaned of fat and blood, gross connective tissue (*e.g.* capsule of testis) was removed, and the tissue was rinsed with sterile physiologic saline. A 30 to 50 per cent suspension in saline was incorporated in a water-in-oil emulsion consisting of: 40 per cent aqueous phase, 40 per cent light mineral oil (Bayol F), 20 per cent aquaphor, and heat-killed tubercle bacilli[1] 3 mg./ml. In the production of allergic encephalomyelitis, a saline suspension of various fractions of bovine white matter (10) similarly incorporated in adjuvant was used.

All animals were examined daily and autopsied at the times indicated in the tables. The testis and representative other tissues were fixed in formalin. Paraffin and celloidin sections stained with hematoxylin and eosin, occasionally by other appropriate techniques, were examined microscopically. Several groups of guinea pigs were skin-tested with 0.1 ml. of 25 per cent suspension of fresh guinea pig testis on the 15th day after inoculation and the reactions read at 24 and 48 hours. They were also bled at the time of sacrifice, and the sera tested for complement-fixing antibody against guinea pig testis, rat testis, and guinea pig salivary gland with the use of 0.01 per cent tissue suspensions as antigens (11).

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[1] Kindly provided by Dr. Jules Freund of the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.

RESULTS

The histologic findings in guinea pigs whose testes were examined in the present study are summarized in Tables I to IV. Animals injected with homologous testis plus adjuvant developed inflammatory testis and epididymal lesions as well as aspermatogenesis (Table I). Occasional minimal perivascular cuffing was found in the central nervous systems of these animals; this was not correlated with the presence of testis lesions. In confirmation of Freund and his coworkers, we found hypospermatogenesis in animals with allergic encephalomyelitis, its severity being correlated in some degree with that of the neurological disease (Table II). The testes of these animals, however, showed no signs of inflammation. Guinea pigs injected with other tissues and adjuvant or with adjuvant alone showed no testis lesions (Table III). However, those which died with intercurrent disease developed severe aspermatogenesis; and in certain instances small collections of lymphocytes were seen in the interstitial connective tissue, either of the testis itself or of the epididymis. Salivary glands were examined histologically in one series of guinea pigs given testis and adjuvant and in several of the groups of animals listed in Table III. In almost all of these, inclusion bodies were found of the type characterizing so called salivary gland virus of guinea pigs (12), similar to the cytomegalic inclusion virus of man (13). Of 4 animals dying during the control experiments, 2 showed evidence of extensive involvement of the salivary glands by this agent and of a generalized inflammatory process involving, in addition to the testes, the central nervous system and the pancreas (these and the adrenals were the only tissues examined in these animals). Production of a subcutaneous abscess by injection of turpentine (Table IV) resulted in mild to moderate aspermatogenesis in half of a small group of guinea pigs sacrificed at various intervals up to 5 days. Fig. 1 shows the essentially normal appearance of the testis and epididymis from an animal given adjuvants alone and Fig. 2 illustrates hypospermatogenesis and aspermatogenesis in guinea pigs with allergic encephalomyelitis or a turpentine abscess.

Closer inspection of Table I reveals a number of points of interest. The Hartley and English short haired guinea pigs appeared to behave essentially similarly. Inflammation, with or without aspermatogenesis, occurred much more frequently in smaller animals, in 13 of 20 guinea pigs weighing 500 gm. or less as against 3 of 13 weighing 800 or 1200 gm. The 1200 gm. animals did not show generalized aspermatogenesis at any time and presented at most quite limited foci of inflammation or aspermatogenesis. Subcutaneous injection of testis with adjuvant appeared to produce disease which was slower in its onset and course than that produced by intradermal inoculation. The earliest lesions observed appeared to consist exclusively of cellular inflammation with no accompanying hypospermatogenesis. Pure inflammatory lesions were seen

TABLE I

Relation of Histologic Changes to Route of Inoculation, Age of Animal, and Duration of Disease

Guinea pigs		Route of inocu-lation	Day of sacrifice	Inflammatory lesions				Aspermatogenesis		
Strain	Weight			Epidid-ymis	Rete	Tubules	Capsule and fat	Infiltr. tubules	Focal	Genl.
	gm.									
E	1200	id	12	0	0	0	0	—	0	0
			19	+	0	0	0	0	0	0
			26	0	0	0	0	—	0	0
			36	0	0	0	0	—	0	0
H	800	id	9	0	0	0	0	—	0	0
			11	0	0	0	0	—	0	0
			13	++	+++	++	+	+	−	++
			14	0	0	0	0	—	0	0
H	500	id	12	+++	+++	0	0	++	0	0
			19	+	0	+++	++	+++	−	++
			26	+	++	+	++	—	−	+++
			36	+*	0	+	++	—	−	+++
			36	0	0	0	0	—	0	0
E	400	id	9	+++	0	0	0	+++	0	0
			11	0	0	0	0	—	0	0
			13	+++	+	+	0	+	0	±
			14	0	+	±	0	+	0	0
H	300–400	id	7	++	0	0	+	0	0	++‡
			7	±	0	0	0	0	0	++‡
			9	0	0	0	0	0	0	+‡
			9	0	0	0	+	0	0	++‡
			11	++	+++	+++	+++	+	0	++
			11	++	+++	+++	+++	+	0	++
E	1200	sc	12	0	0	0	0	—	0	0
			19	0	0	0	+	—	0	0
			26	0	0	0	0	—	++	0
			36	0	0	0	0	—	0	0
			36	+	0	0	0	0	0	0
H	500	sc	12	0	0	0	0	—	0	0
			19	0	0	0	0	—	0	0
			26	+++	+	+++	+++	++	0	+
			36	+	+	+	0	+	0	0
			36	+++	0	0	+	0	0	++

Guinea pigs of the Hartley (H) or English short haired (E) strains inoculated with a single preparation of testis plus Freund adjuvants, either 0.1 ml. intradermally over the sternum (id) or 0.5 ml. subcutaneously over the nape of the neck (sc).

* Extensive fibrosis.

‡ Aspermatogenesis in epididymis only, attributed to immaturity of animals.

314

TABLE II

Production of Aspermatogenesis in Experimental Allergic Encephalomyelitis

Allergic encephalomyelitis, graded score*	Aspermatogenesis					Inflammation
	0	+	++	+++	++++	
0	2	—	—	—	—	None
1	1	3	2	—	—	"
2	—	1	—	—	1	"
3	1	—	1	1	—	"
4	—	1	1	2	1	"
5	—	—	—	1	3	"

Hartley guinea pigs, 400 to 500 gm., injected intradermally over sternum with various bovine white matter fractions (10) plus adjuvant.

* Score takes into account both day of onset and severity of encephalomyelitis as estimated histologically (10).

TABLE III

Aspermatogenesis in Guinea Pigs Injected with Other Tissues Plus Adjuvant

Antigen	Guinea pigs		Autopsied	Aspermatogenesis			Inflammation	
	Strain	Weight		No.	Epididymis	Tubules	No.	Degree
		gm.	days					
Guinea pig salivary gland	H	500	8–50	0/7	0	0	0/7	0
" " " "	H	300–400	7–32	9/10*	++- ++++	+-++	1/10‡	+
" " " "	E	300–400	8–50	2/5†	++++	++++	2/5†	+
Rabbit adrenal	H	400–500	9–30	0/5	0	0	0/5	0
" "	E	300	9–30	1/5§	++	++	0/5	0
Adjuvant alone	H	500	12–36	1/10‖	++++	++++	1/10	±

Guinea pigs of the Hartley (H) or English short haired (E) strains inoculated with tissue plus Freund's adjuvant intradermally, 0.1 ml., over the sternum, except that 4 of the group given adjuvant alone received 0.5 ml. subcutaneously over the nape of the neck.

* Aspermatogenesis, attributed to immaturity of animals.

‡ Two small cell collections in epididymis.

† These 2 animals died at 11 and 25 days with pneumonia. They showed evidence of generalized cytomegalic inclusion disease with meningitis, pancreatitis, and severe involvement of the submaxillary glands. They presented complete aspermatogenesis and a few interstitial collections of lymphocytes without invasion of the tubules.

§ Found dead at 21 days.

‖ Found dead at 20 days.

TABLE IV

Production of Aspermatogenesis with Turpentine Abscess

Subcutaneous dose of turpentine	Duration	Aspermatogenesis		Inflammation*
		Animals	Degree	
ml.	days			
0.5	1–5	1/4	+	1/4
2.0	1–5	3/4	+-++	0/4

Hartley guinea pigs weighing 400 to 500 gm.

* 5 of the 8 animals showed desquamated cells in the epididymis.

in guinea pigs of different ages sacrificed at 7, 9, and 12 days. In contrast, generalized hypo- or aspermatogenesis was not seen in the absence of inflammation.

The histologic changes in guinea pigs injected with testis and adjuvant are illustrated in detail in Figs. 3 to 12. In animals weighing between 400 and 800 gm. there appeared to be a characteristic and orderly sequence of events, which may be described as follows:—

The early lesion was a focal interstitial inflammation occurring most frequently in the epididymis, less often in the rete testis, and still less frequently in the body of the testis (Figs. 3A, 4, and 8). The inflammatory infiltrate consisted predominantly of lymphocytes and histocytes with a lesser and quite variable number of polymorphonuclear leukocytes. There was no arteritis but massive aggregations of these cells were found in and around the walls of small and medium-sized veins. There was direct invasion of tubules of all 3 types (epididymal, rete, and seminiferous) by histocytes alone or by histocytes and polymorphonuclears. Where invasion of seminiferous tubules occurred, there was beginning destruction of germinal epithelium, as evidenced by pyknotic nuclei and vacuolation of the involved cells (Figs. 4C and 4D). Eosinophilic remnants of degenerating spermatogenic cells and fragments of nuclei could be recognized adjacent to invading cells and indeed within macrophages in the inflammatory infiltrate. The Sertoli cells, however, maintained a normal appearance. The most severely involved tubules contained a central mass of necrotic spermatogenic cells, polymorphonuclear leukocytes, histocytes, and histiocytic giant cells. Except in the immediate vicinity of the invasive mass, spermatogenesis remained normal and the architecture of the tubules remained undisturbed. The basement membrane of the tubules appeared to be intact. In the rete testis, the large, thin-walled tubules were full of histocytes and a few polymorphonuclears (Fig. 11). The invasion of epididymal tubules (Figs. 4A and 4B) gave rise to a striking disparity between the many polymorphonuclear leukocytes within the tubules and the predominantly mononuclear infiltrate in the interstitial connective tissue. The invading cells tended to form a ring around the normal spermatozoa in the tubules rather than to be uniformly distributed as might have been expected had they come down the tubules from an inflamed site elsewhere. In support of this observation, it was noted that large numbers of polymorphonuclears appeared within the tubules only in areas where their number was also increased in the surrounding connective tissue. Lesions corresponding to this general description were found at 7 days in 300 to 400 gm. animals, and at 9 to 14 days in 400 and 500 gm. animals inoculated intradermally.

In more advanced lesions (Figs. 3B, 5, 9, and 10), the amount of inflammatory infiltrate remained essentially unchanged or increased. Papilloma-like invasive cell masses were prominent in many tubules, and few areas of the testis were completely free of inflammation. The interstitial infiltrate consisted principally of histiocytes, though polymorphonuclears, lymphocytes, and plasma cells were prominent in areas of loose connective tissue such as the epididymis. The cells in the lumen of the tubules could be characterized as syncytial histiocytic masses containing fragments of necrotic spermatogenic cells and polymorphonuclears. In a very few instances in which the inflammatory lesion was marked, there was frank necrosis of connective tissue elements, and in certain areas the epididymal tubule epithelium was altered and the tubular basement membrane destroyed (Fig. 9). However, in the great majority of instances, the tubular architecture remained essentially unaffected (Fig. 10). Eosinophilic fluid (fibrinous exudate) accompanied severe cellular inflammation; hemorrhage was not seen. In most of the experimental groups, exclusive of the 1200 gm. animals, there was now seen general hypospermatogenesis of varying degree (Fig. 5). This

change did not appear dependent on the invasion of tubules by inflammatory cells and, indeed, occurred in parts of the testis where there was no evidence of inflammation. It appeared as a simple arrest of maturation; in some tubules, spermatids and mature spermatozoa were still in evidence in the center of tubules showing arrest at the stage of spermatogonium or primary spermatocyte. Many tubules contained masses of cast off and abnormal-appearing cells, among them giant, vacuolated, and frequently multinucleate spermatids or spermatocytes, with occasional inflammatory cells among them. Hypospermatogenesis was not seen in testes showing no signs of inflammation. Lesions corresponding to this description were found at 11 days in 300 to 400 gm. animals and 13 to 19 days in 400 to 800 gm. animals inoculated intradermally. A comparable picture was observed in 500 gm. guinea pigs inoculated by the subcutaneous route at 26 and 36 days.

At a later stage in the evolution of the lesion, there was complete aspermatogenesis with relatively little inflammation (Figs. 3C and 6). An interesting finding was the presence of typical histiocytic giant cells or groups of epithelioid cells within tubules showing no other sign of inflammation (Fig. 6C). These cells must have originated in the part of the section in which they were found, since they appeared in a testis showing complete aspermatogenesis, in tubules arising in the same or an adjacent region of the testis. Their identification as being of histiocytic origin as distinct from degenerating and abnormal spermatocytes or spermatids, was based on their having a large number of nuclei (frequently more than 10) which were morphologically similar to histiocyte or macrophage nuclei. Nevertheless, the aspermatogenesis of the tubules in which these cells were found was not distinguishable from that in tubules showing no sign of antecedent inflammation. The testis as a whole showed some diminution in size at this stage. In certain areas groups of tubules were found which presented numerous mitoses; these may have represented proliferation of Sertoli cells or attempts at regeneration of the germinal epithelium.

The final stage observed was complete aspermatogenesis, atrophy of the testis as a whole, proliferation of Sertoli and Leydig cells, and in certain instances fibrosis suggesting severe earlier tissue damage (Figs. 3D and 7). A careful search revealed a few inflammatory cells scattered in the interstitium, and in one animal a chronic epididymitis was found together with extensive aspermatogenesis, but with no sign of inflammation in the testis itself. Aspermatogenesis dominated the histologic picture only in guinea pigs sacrificed at 26 to 36 days following intradermal inoculation.

Deviations from the sequence of events described were observed in extremely young and extremely old guinea pigs.

The earliest lesion we observed was an epididymitis at 7 days in a 300 to 400 gm. Hartley guinea pig. The inflammatory infiltrate here consisted exclusively of mononuclear cells and there was no invasion of the epididymal tubules. In a similar animal autopsied at 11 days, less than 5 per cent of the infiltrating cells were polymorphonuclears and there was still very limited invasion of the tubules. Since in the epididymal and testis lesions in older animals polymorphonuclear leukocytes appeared to respond primarily to destruction of the tubular contents, *i.e.* sperm or germinal epithelium, by invading cells, their absence here may have been due, on the one hand, to the fact that the cells making up the initial inflammatory infiltrate are primarily lymphocytes and histiocytes and, on the other hand, to the lack of sperm in the epididymal tubules of these very young animals. In accord with this interpretation, the epididymitis found at 9 days in a 400 gm. English short haired guinea pig (Fig. 8), which had a full complement of sperm in the epididymal tubules, was made up of massive interstitial infiltrations of lymphocytes and histiocytes and equally massive invasion of tubules by these cells and polymorphonuclears. It is possible, however, that in the young animals,

we merely caught the lesion in an early stage before invasion had begun. Certainly the interstitial inflammation was much more rapid and extensive at an early stage than in 400 to 800 gm. animals.

In guinea pigs weighing 1200 gm., very limited focal cellular collections were found at any time. In only one animal, sacrificed at 26 days, was aspermatogenesis observed. It too was focal, the remainder of the testis being entirely normal (Fig. 12). Adjacent to some of the patches of aspermatogenesis there was a zone of hypospermatogenesis varying in degree; and throughout the abnormal area there appeared to be hyperplasia and hypertrophy of the Leydig cells.

A finding which was difficult to interpret was the presence of inflammation in the connective tissue capsule of the testis and in the adjacent fat. Typical lesions of fat necrosis with collections of histiocytes and occasional giant cells were found here not only in a number of the test guinea pigs (Table I) but also in animals given control mixtures or adjuvants alone. The capsular lesions nevertheless showed some correlation with the presence of testicular inflammation. It appears probable that these lesions were in part specific and in part represented the effects of incidental traumata to this portion of the body. The histologic picture in both test and control animals was also complicated by the presence of certain changes identified as artefacts characteristic of formalin fixation and the usual embedding and staining techniques. Among these may be mentioned: a moth-eaten appearance of the peripheral seminiferous tubules in most testis sections—this effect appeared to depend on shrinkage particularly affecting Sertoli cells; pyknotic-appearing nuclei in the germinal epithelium, apparently a shrinkage artefact affecting young spermatogonia; and an over-all distortion of the morphology of Sertoli cells. These changes were taken into account in estimates of the presence and degree of hypospermatogenesis; they did not in general occasion any difficulty.

In none of the animals injected with testis and adjuvant and shown on microscopic study to have testicular lesions were there ante-mortem signs of disease. Specifically, we could not demonstrate local swelling or tenderness or retraction of the testis into the abdomen; nor did the animals show fever, anorexia, or obvious weight loss. Sera obtained at the time of sacrifice did not fix complement with a 0.01 per cent suspension of homologous or heterologous (rat) testis. However, there were very mild positive reactions of the delayed type of skin tests on the 15th day with a 25 per cent saline suspension of fresh homologous testis in specifically inoculated guinea pigs. The maximum reaction, read at 24 hours, was 11 mm. of + induration and 20 mm. of erythema and slight edema, as contrasted with values of 4 to 6 mm. of ± induration and 8 to 10 mm. of erythema seen in animals inoculated with adjuvants alone.

DISCUSSION

The morphologic data of the present study, taken together with the earlier findings of Voisin and his collaborators (1–4) and Freund et al. (5–7), permit unequivocal answers to questions regarding the detailed pathogenesis of the auto-allergic testis lesion and its relationship to delayed hypersensitivity and, more specifically, to the other auto-allergies.

Our control experiments agree with the earlier studies in showing that in-

jection of tissue and adjuvant does not of itself induce aspermatogenesis or inflammation in the testis. It may rarely activate latent infection, *e.g.* salivary gland virus (12), which is present in a large proportion of guinea pigs examined. Activation of infection appeared to have occurred in 4 of 72 control animals; there was no evidence that it occurred in any animals of our experimental groups. The presence of such infection or of non-infectious inflammatory lesions elsewhere in the body (such as allergic encephalomyelitis) may induce a non-specific depression of seminiferous tubular function, sometimes accompanied by a minimal degree of cellular "inflammation" in the testis.

In the testes of guinea pigs injected with homologous testis plus adjuvant, it is clear that inflammation and aspermatogenesis occur together. In determining which of these represents the primary lesion, the following experimental findings appear decisive. Hypospermatogenesis was never seen in our experimental animals in the absence of inflammation in the epididymis or the testis itself. Inflammation was seen in several animals in the complete absence of general hypospermatogenesis. The earliest change observed was inflammation alone. No histologic evidence of damage to cells within the seminiferous tubules could be found other than that occurring in tubules invaded by inflammatory cells or as part of a general diminution of tubular function. From these observations it seems justifiable to conclude that the primary change in this experimental disease is inflammation and that aspermatogenesis is a secondary consequence of the inflammatory process, either as a result of direct invasion of tubules by inflammatory cells, with destruction of germinal epithelium and mature sperm, or more remotely through a general non-specific depression of tubular function. This conclusion receives further support from the fact, already noted by Freund (5), that non-immunologic agencies which produce aspermatogenesis, *e.g.* x-radiation, elevation of temperature, ischemia, or vitamin deficiency, fail to produce an early inflammatory lesion of the type we have observed. The failure of the testis lesion to show any correlation with antitesticular antibody titers measured by various techniques and, more significantly, the failure to produce lesions in normal animals with sera from actively sensitized animals, among them hetero-antisera and sera shown *in vitro* to possess spermatoxicity, even when these are injected directly into the testis (5, 15), are telling arguments against the concept that damage of sperm or spermatogenic cells by antibody can be the primary lesion. The auto-allergic disease of testis should then be regarded as an *experimental allergic orchitis* rather than an immunologically induced aspermatogenesis.

Our data do not provide an unequivocal explanation of the hypospermatogenesis which is such a conspicuous part of the late picture of allergic orchitis in younger animals. This change could not be distinguished histologically from that seen in guinea pigs with allergic encephalomyelitis, turpentine abscess, or intercurrent infection. Hypospermatogenesis in the specific disease might

therefore be determined quite simply by the fact of illness. A widely held theory proposes that the aspermatogenesis of mumps orchitis is a consequence of increased local pressure due to inflammation with an organ having an un-yielding capsule (14). This hypothesis may be applicable to the process in the testes we have studied. Rats retract injured testes into their abdominal cavity, where the increased environmental temperature results in hypospermatogenesis; there was no evidence of such retraction in our guinea pigs. Finally, hypothetical effects of inflammation on spermatogenesis *via* the Leydig cells and the pitui-tary are not excluded. The focal aspermatogenesis seen in a 1200 gm. animal cannot be regarded as a sequel to general aspermatogenesis. It must have oc-curred as a consequence of antecedent focal inflammation.

Freund and his collaborators (7) concluded that the auto-allergic lesion of the testis was due to sensitivity of the delayed or tuberculin type. This con-clusion was based on the importance of mycobacteria in the antigenic mixtures used to induce sensitization, the effectiveness of the intradermal route of inocu-lation, the poor correlation of the disease process with circulating antibody, and the clearly established presence of delayed skin reactivity to testis antigen in animals developing allergic orchitis, demonstrated again in our study (see references 16 and 17 for the relevance of certain of these findings). Our obser-vations provide morphologic confirmation for this conclusion. Delayed re-actions are generally agreed to consist of foci of perivenous inflammation, lymphocytes and histiocytes being the principal reacting cells and parenchymal damage occurring only in intense reactions (9). The similarity between this histologic picture and that which we have described in the testis is obvious. It should be added that when tuberculin or tubercle bacilli are injected into the testis of a tuberculin-sensitive guinea pig, the resulting lesion is comparable in many of its features to that of allergic orchitis, differing mainly in its much greater intensity and acuteness (18–21).

These observations and interpretations align experimental allergic orchitis with the other experimental auto-allergies affecting the central and peripheral nervous systems, the lens and uvea of the eye, the thyroid, and the adrenal. The parallel may be summarized for one such process, allergic encephalomye-litis, by pointing out that it too is an irregularly disseminated focal inflammatory disease, in which the initial lesion appears to consist of perivenous collections of lymphocytes and histiocytes and in which parenchymal destruction (varying from demyelination to actual necrosis of all tissue elements) is associated with invasion of the nervous substance by the inflammatory cells. In it too the inflammatory lesion may resolve completely or may leave atrophy with or without glial scarring (22, 23). This type of encephalomyelitis is not correlated with the production of circulating antibody to nervous tissue nor has it been successfully transferred to normal animals with serum of sensitized animals. Non-immunologic destruction of myelin fails to produce inflammation com-

parable to that seen in the experimental disease. And finally there is convincing evidence for the presence of delayed sensitivity to nervous antigen in animals developing encephalomyelitis (24, 25). The same constellation of properties has been demonstrated in greater or less detail in each of the other auto-allergies (8). The one respect in which the testis lesion is distinct is the occurrence of the diffuse non-inflammatory hypo- or aspermatogenesis, which we have interpreted as incidental to the actual lesion. The testis is a much more satisfactory tissue for the study of the sequence of histologic events than many of the others, first, because there is such a sharp boundary between the interstitial connective tissue and the parenchyma (tubular contents), and second, because the spermatogenic cells are highly sensitive to any noxious agency. Thus there can be no question regarding the fact of invasion of the parenchyma at a given moment nor about the integrity of the germinal epithelium and its derivatives, both questions which are quite difficult to answer in relation to e.g. lesions in the white matter of the nervous system. Our findings imply that in the other auto-allergies, perivenous cellular inflammation may well be the primary lesion, a conclusion not well established hitherto.

The sequence of histologic changes in auto-allergic orchitis has several points of similarity to the development of the lesion of mumps orchitis in man, as described in a recent study of testicular biopsy material taken during the first 5 days of this illness (14).

The earliest specimens showed either no lesions, interstitial edema alone, or vaso-dilation, edema and perivascular collections of lymphocytes. In more advanced disease, the perivascular "lymphocyte" infiltrates were more extensive and a few poly-morphonuclears, irregularly scattered tiny hemorrhages, and some fibrin deposition were seen. The tubules were now invaded by inflammatory cells, predominantly polys, and there was progressive degeneration of the germinal epithelium, with pyknosis, cytoplasmic fragmentation, and arrest of spermatogenesis. The tubules contained a central mass of fragmented cells, debris, polymorphonuclears, and a few macrophages. The Leydig and Sertoli cells remained normal. There was inflammatory thickening of the tubular basement membrane. Still later, the inflammatory disease appeared diffuse, though still presenting a recognizable focal character. There was complete aspermatogenesis, but the tubular architecture remained intact. Perivascular "lympho-cyte" infiltration was also found in the testicular appendages and epididymis, with polymorphonuclears in the tubular lumen, the tubular epithelium remaining normal. This description agrees with that of earlier workers (reviewed in reference 14) except that the cells called "lymphocytes" were described by others as "endothelial leuko-cytes" and appear to correspond in part to cells we have designated as histiocytes.

It is pertinent to indicate that mumps orchitis bears the same relation to mumps, in terms of its relative infrequency and the usual delay which intervenes between the parotitis and its appearance, as post-infectious encephalitis bears to the measles, chicken pox, or rubella which may precede it. In both

cases the experimental auto-allergy appears to provide a convincing model, from the morphological point of view, for the human "post-infectious" complication. A comparable relationship may hold between other auto-allergies and "post-viral" inflammatory lesions affecting the peripheral nervous system, the uvea, and the thyroid. The morphologic similarity is not, however, grounds for regarding these human illnesses as allergies to tissue antigen. On the contrary, there are many reasons (8) for regarding them as probably being allergies to virus, their morphologic character being determined by the nature of the allergic response rather than the nature of the antigen.

The late picture of experimental allergic orchitis is characterized by general or focal aspermatogenesis in testes which may present little or no histologic evidence of foregoing inflammation. Certain cases of human infertility are described (26–28) as having "germinal cell aplasia," "adult seminiferous tubule failure," or more simply the absence of germinal cells, with no other abnormality of the testis, no sign of antecedent inflammation, and no endocrine abnormality to account for this finding. It is appropriate to ask whether some of these cases may have undergone an auto-allergic type of orchitis earlier in life. Whether cases described as "puberal seminiferous tubule failure" or "sclerosing tubular degeneration" could also result from a comparable inflammatory lesion is of course unknown.

SUMMARY

The auto-allergic lesion in guinea pigs inoculated with homologous testis plus the Freund adjuvant was investigated histologically. The lesion was found to consist of disseminated foci of perivenous inflammation, lymphocytes and histiocytes predominating in the cellular infiltrate, with invasion of epididymal, rete, and seminiferous tubules and destruction of tubular contents. Guinea pigs up to 800 gm. showed a rapidly progressing diffuse hypo- or aspermatogenesis, which appeared to be secondary to the inflammatory disease. In these animals, the process resolved leaving an atrophic testis with few or no indications of the preceding inflammation and fibrotic scarring only in the rare instances in which actual necrosis of connective tissue elements had occurred. In 1200 gm. animals there was no general hypospermatogenesis and the late findings were limited to foci of aspermatogenesis. This disease then is an experimental auto-allergic orchitis followed by testicular atrophy without scarring. Its morphologic similarity to mumps orchitis and to sterility with "germinal cell aplasia" in man is commented on.

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EXPLANATION OF PLATES

PLATE 36

FIGS. 1A to 1D. Normal testis from 500 gm. Hartley guinea pig given adjuvants alone intradermally over the sternum and sacrificed at 26 days. Fig. 1A. *Epididymis*, showing tubules filled with mature spermatozoa and scanty connective tissue. Fig. 1B. *Rete testis*, showing thin-walled, largely empty tubules and scanty interstitial connective tissue. Figs. 1C and 1D. *Seminiferous tubules*, showing closely packed tubules containing all stages of maturation of the germinal epithelium from spermatogonia to spermatozoa. Hematoxylin and eosin. Figs. 1A to 1C, × 80; Fig. 1D × 450.
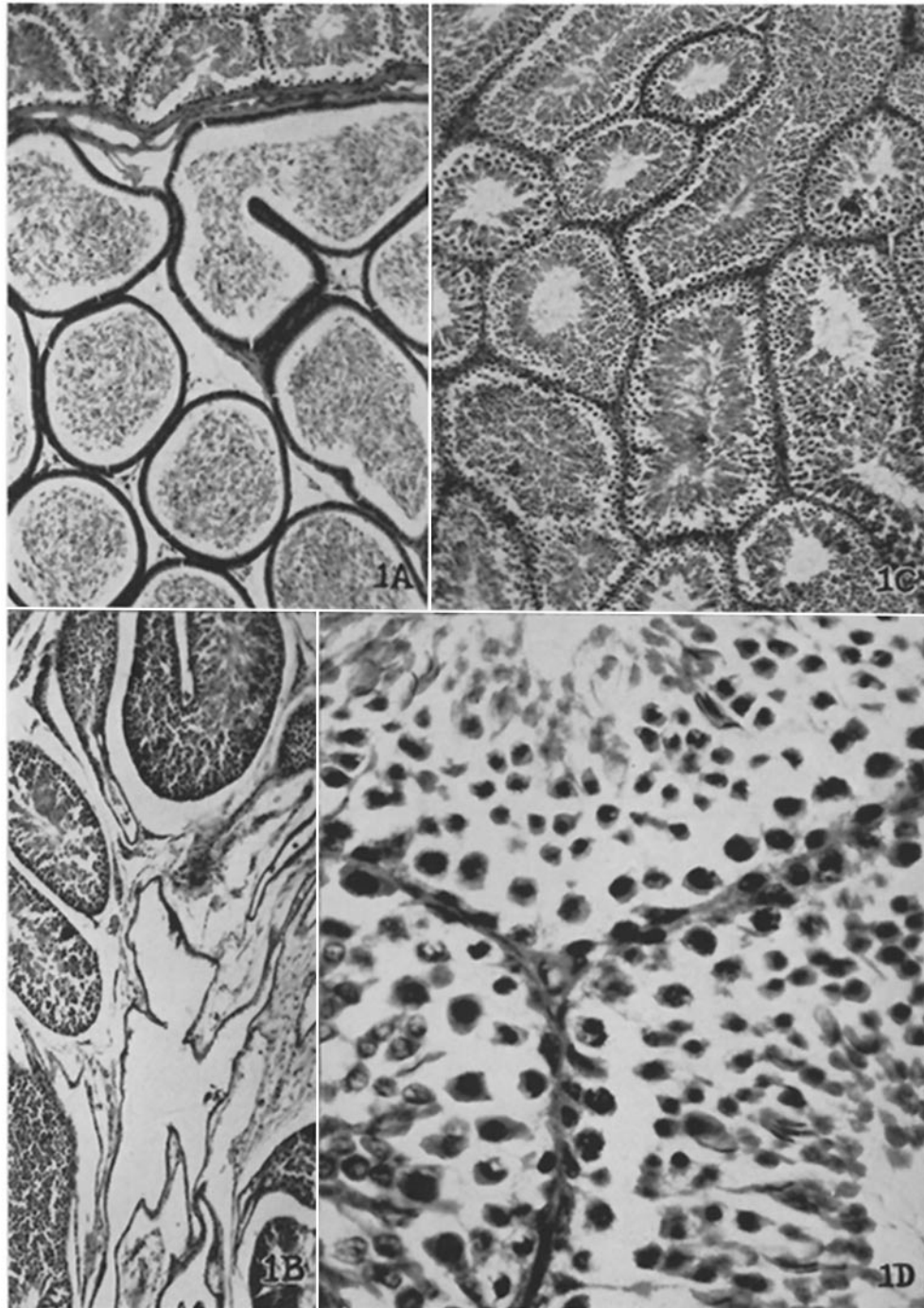
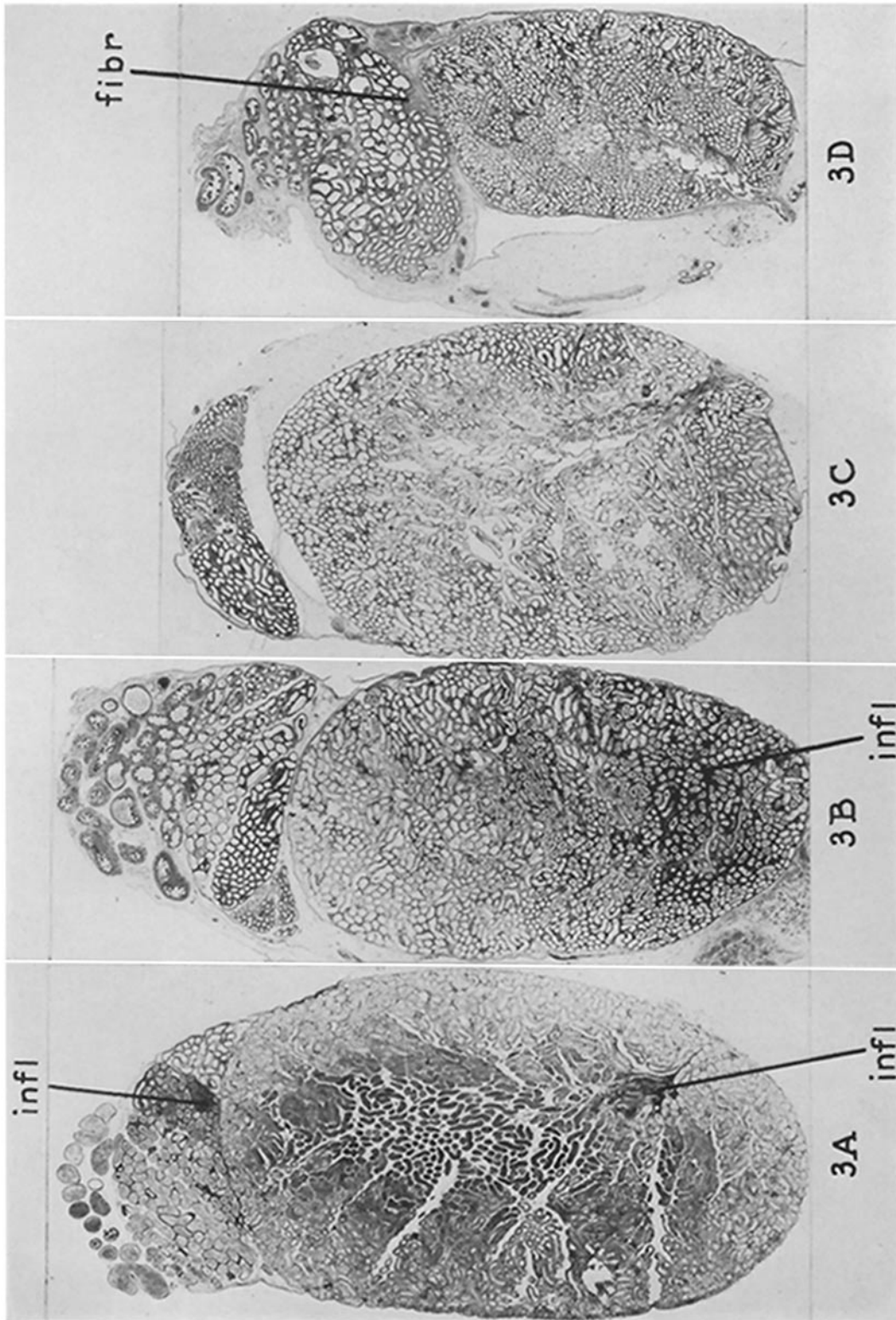(Waksman: Auto-allergic testis lesion)

PLATE 37

FIGS. 2A to 2D. Hypospermatogenesis with intercurrent disease in 400 to 500 gm. Hartley guinea pigs. Fig. 2A. ++-+++ allergic encephalomyelitis of 4 days' duration. Hypospermatogenesis graded as +++. Figs. 2B and 2C. ++ allergic encephalomyelitis of 2 to 3 weeks' duration. Complete aspermatogenesis. Note desquamated immature germinal cells in epididymis (Fig. 2C). Fig. 2D. Turpentine abscess (2 ml. of turpentine injected subcutaneously) of 2 days' duration. Rete testis and tubules showing ++ hypospermatogenesis. Hematoxylin and eosin. × 80.
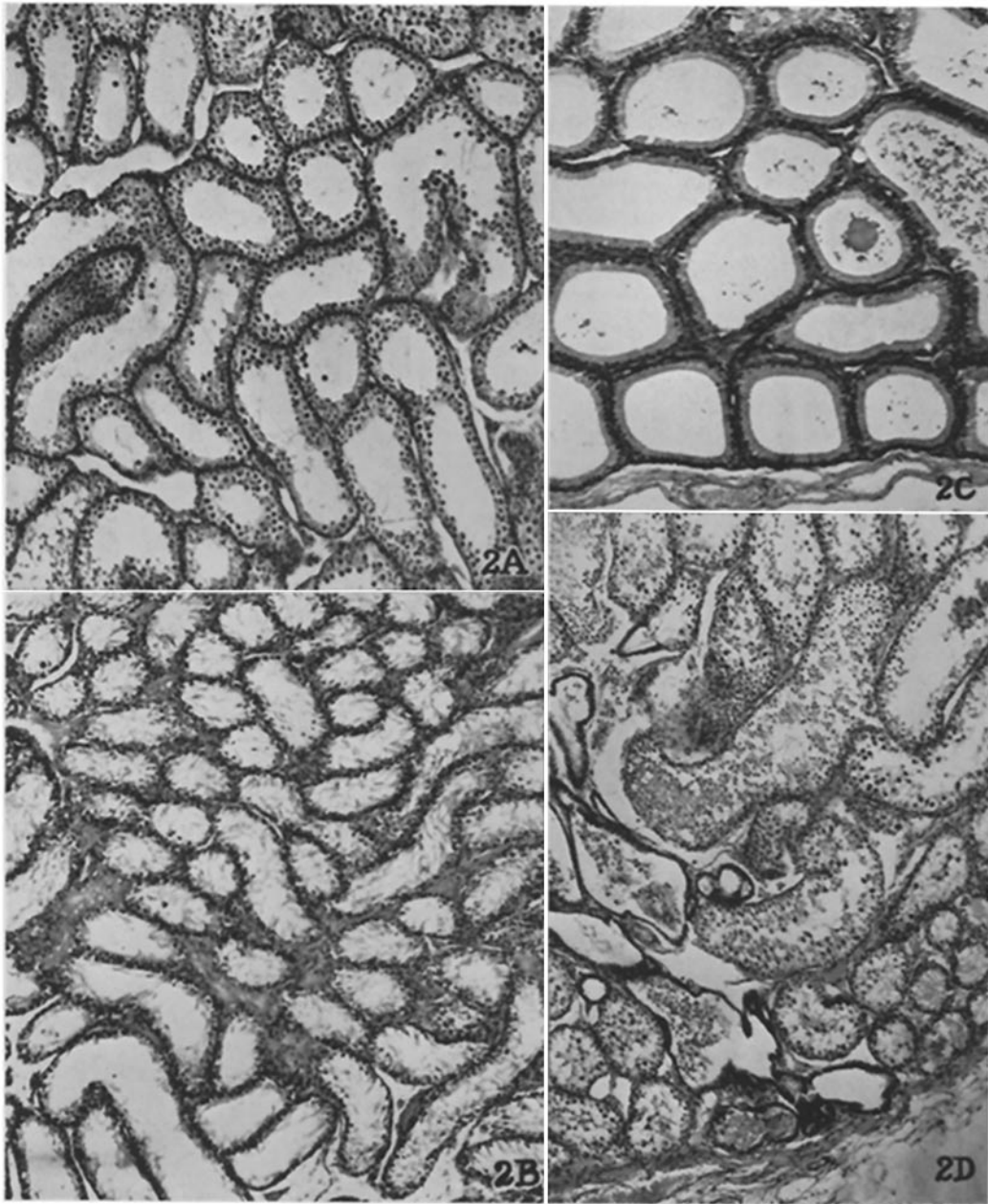
(Waksman: Auto-allergic testis lesion)

PLATE 38

FIGS. 3A to 3D. Experimental allergic orchitis in 500 gm. Hartley guinea pigs injected with guinea pig testis plus adjuvant intradermally over sternum and sacrificed at 12, 19, 26, and 36 days (see Table I). Massive inflammatory infiltrates (*infl*) in epididymis and rete of Fig. 3A and between seminiferous tubules in Fig. 3B. Mild inflammation present in Figs. 3C and 3D not seen at this power. Progressive hypo- and aspermatogenesis in Figs. 3B to 3D with atrophy of tubules and of testis as a whole, seen best Fig. 3D. Fibrosis (*fibr*), presumably at site of antecedent inflammation, in epididymis of Fig. 3D. High power views of details are shown in following plates. Hematoxylin and eosin. × 6½.

(Waksman: Auto-allergic testis lesion)

FIGS. 4A to 4D. Inflammatory lesions in epididymis (Figs. 4A and 4B) and rete (Figs. 4C and 4D) of testis shown in Fig. 3A. There is a massive interstitial infiltration, consisting predominantly of histiocytes and lymphocytes, with invasion of tubules and destruction of the tubular contents (spermatozoa in epididymal tubules, germinal epithelium in seminiferous tubules near rete). There are many polymorphonuclears in inflammatory mass within tubules. Note presence of completely normal tubules with normal tubular contents adjacent to involved area. Hematoxylin and eosin. Fig. 4A, $\times$ 77; Fig. 4C, $\times$ 36; Figs. 4B and 4D, $\times$ 436.
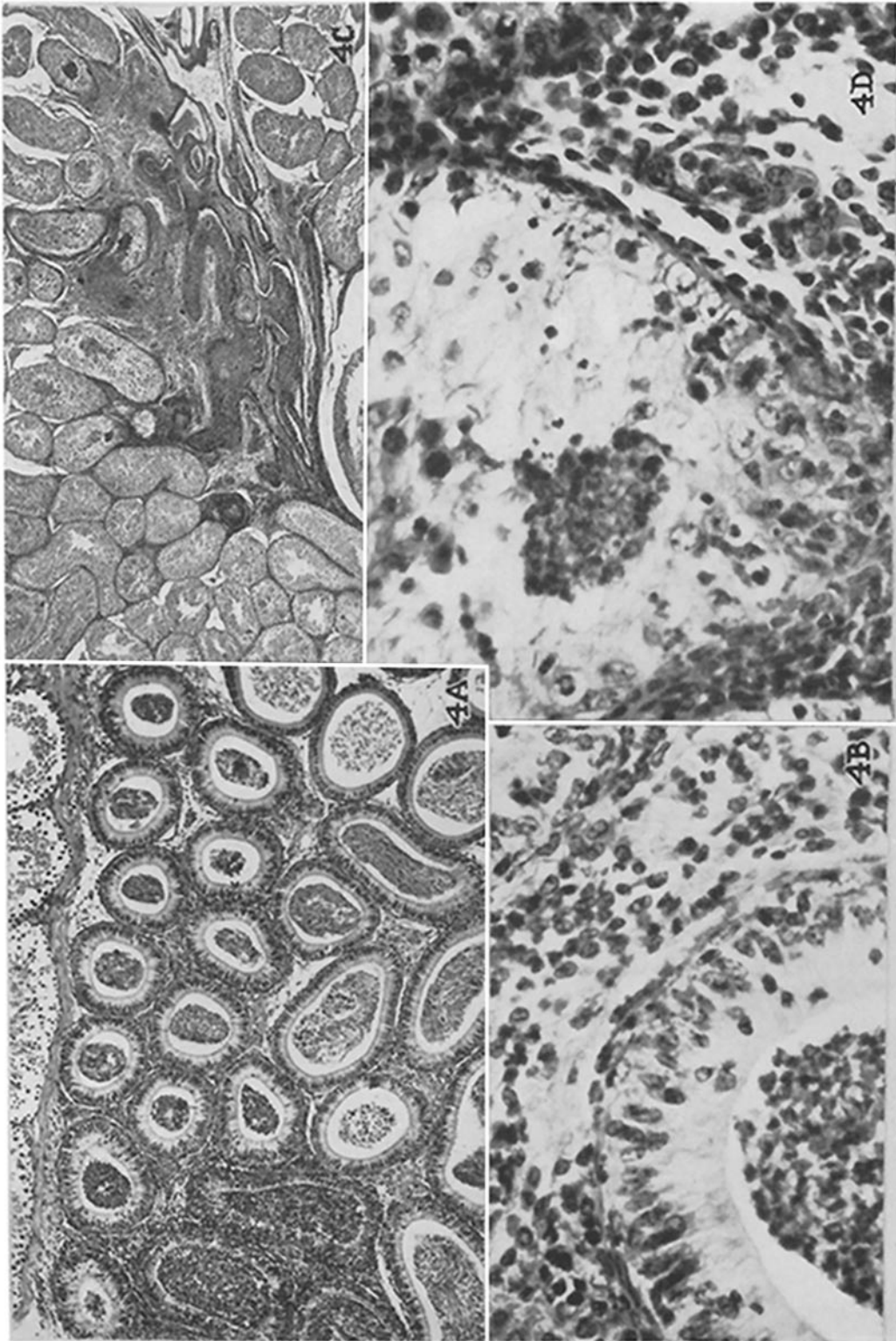
(Waksman: Auto-allergic testis lesion)

PLATE 40

FIGS. 5A and 5B. Inflammatory lesion in testis shown in Fig. 3B. Massive intersti-
tial inflammation with invasion of some tubules. Hypospermatogenesis graded as $++$.
Hemotoxylin and eosin. Fig. 5A, $\times$ 80; Fig. 5B, $\times$ 450.
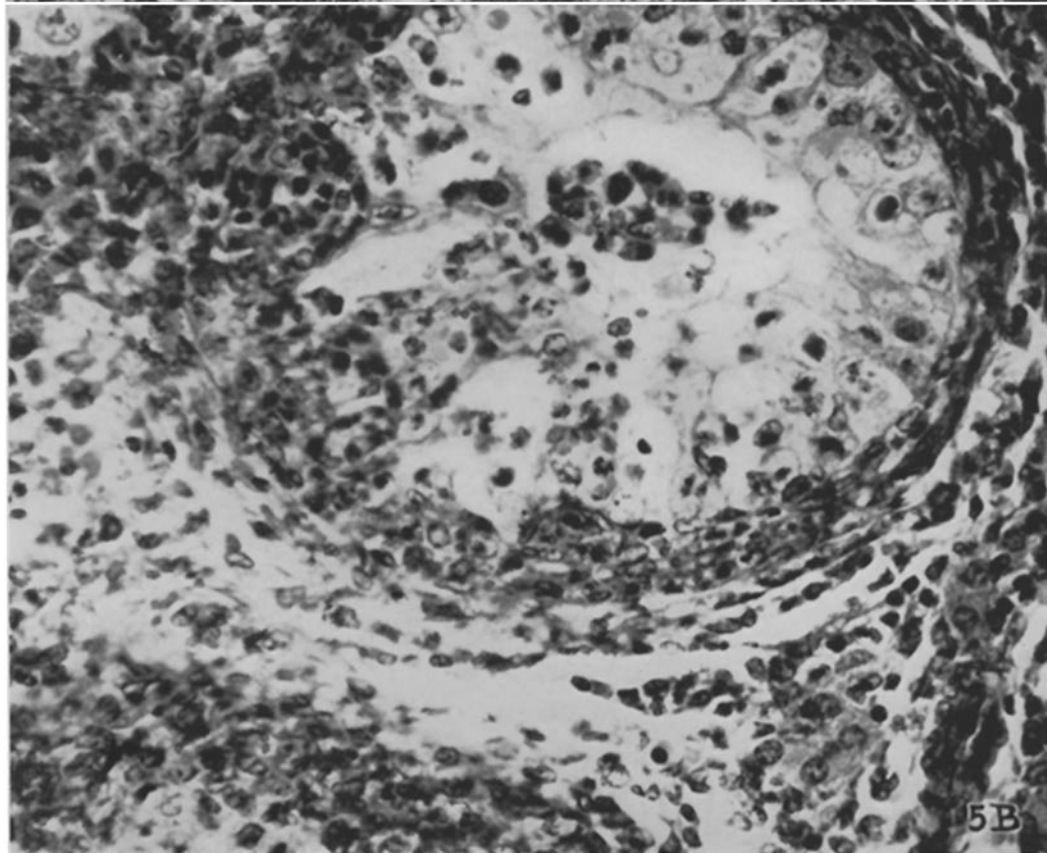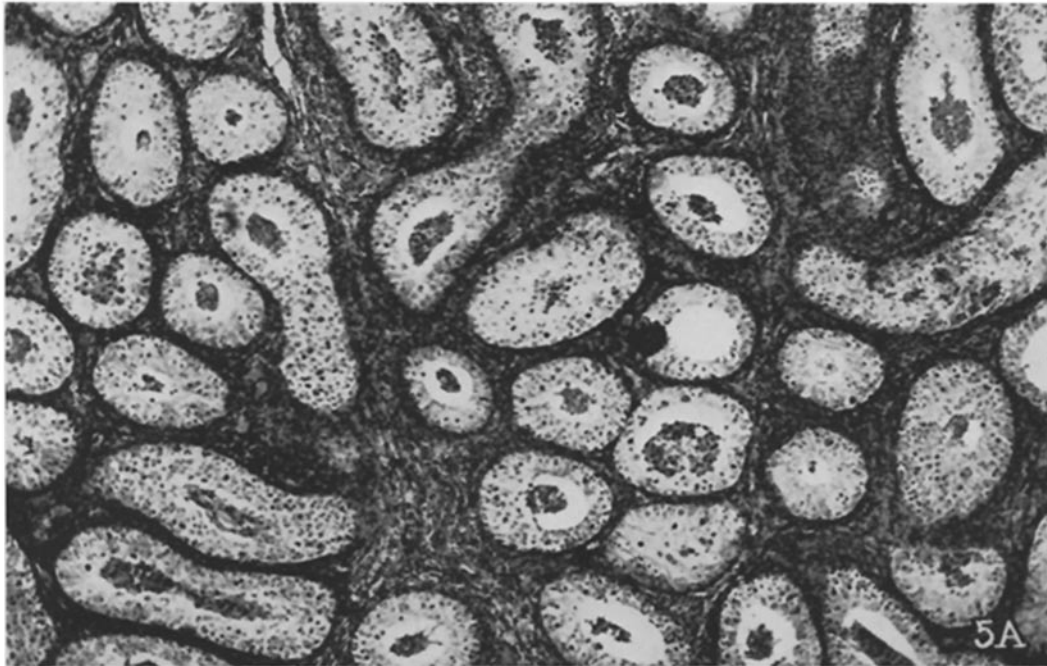
(Waksman: Auto-allergic testis lesion)

PLATE 41

FIGS. 6A to 6C. High power views of testis shown in Fig. 3C. Fig. 6A. Residual inflammatory lesion adjacent to rete tubules. Fig. 6B. Advanced general aspermatogenesis. Fig. 6C. Histiocytic giant cells found in empty tubules and interpreted as residues of antecedent inflammation with formation of histiocytic masses in lumina of tubules (illustrated in Fig. 10). Hematoxylin and eosin. Figs. 6A and 6C, × 225; Fig. 6B, × 80.
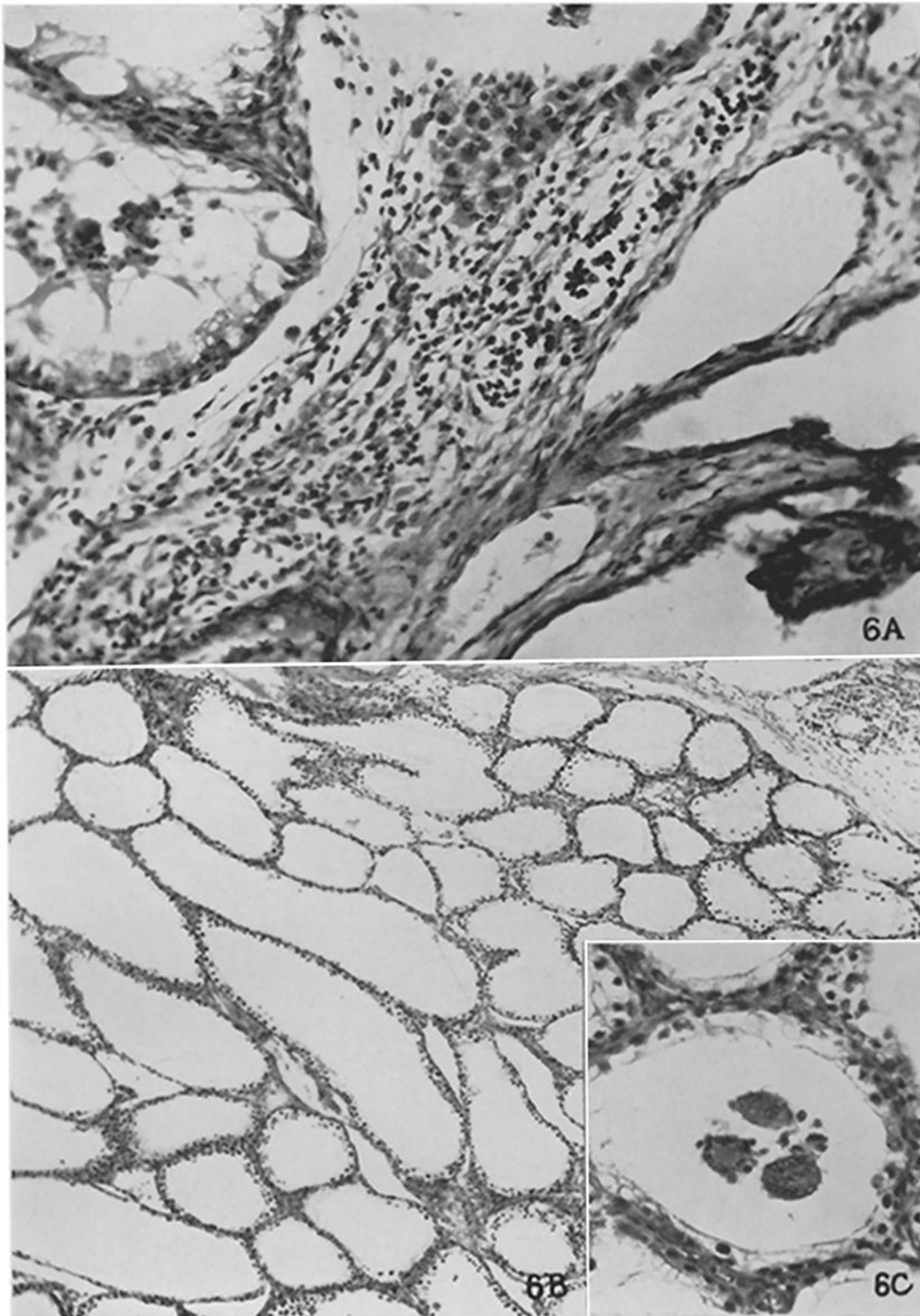
(Waksman: Auto-allergic testis lesion)

FIG. 7A to 7C. Higher power views of testis shown in Fig. 3D. Fig. 7A. Empty epididymal tubules with fibrosis, interpreted as residue of inflammation (illustrated in Figs. 4, 8, and 9). Figs. 7B and 7C. Rete and seminiferous tubules, showing complete aspermatogenesis, proliferation of Sertoli cells, and hypertrophy of Leydig cell masses. Hematoxylin and eosin. Figs. 7A and 7B, × 80; Fig. 7C, × 225.
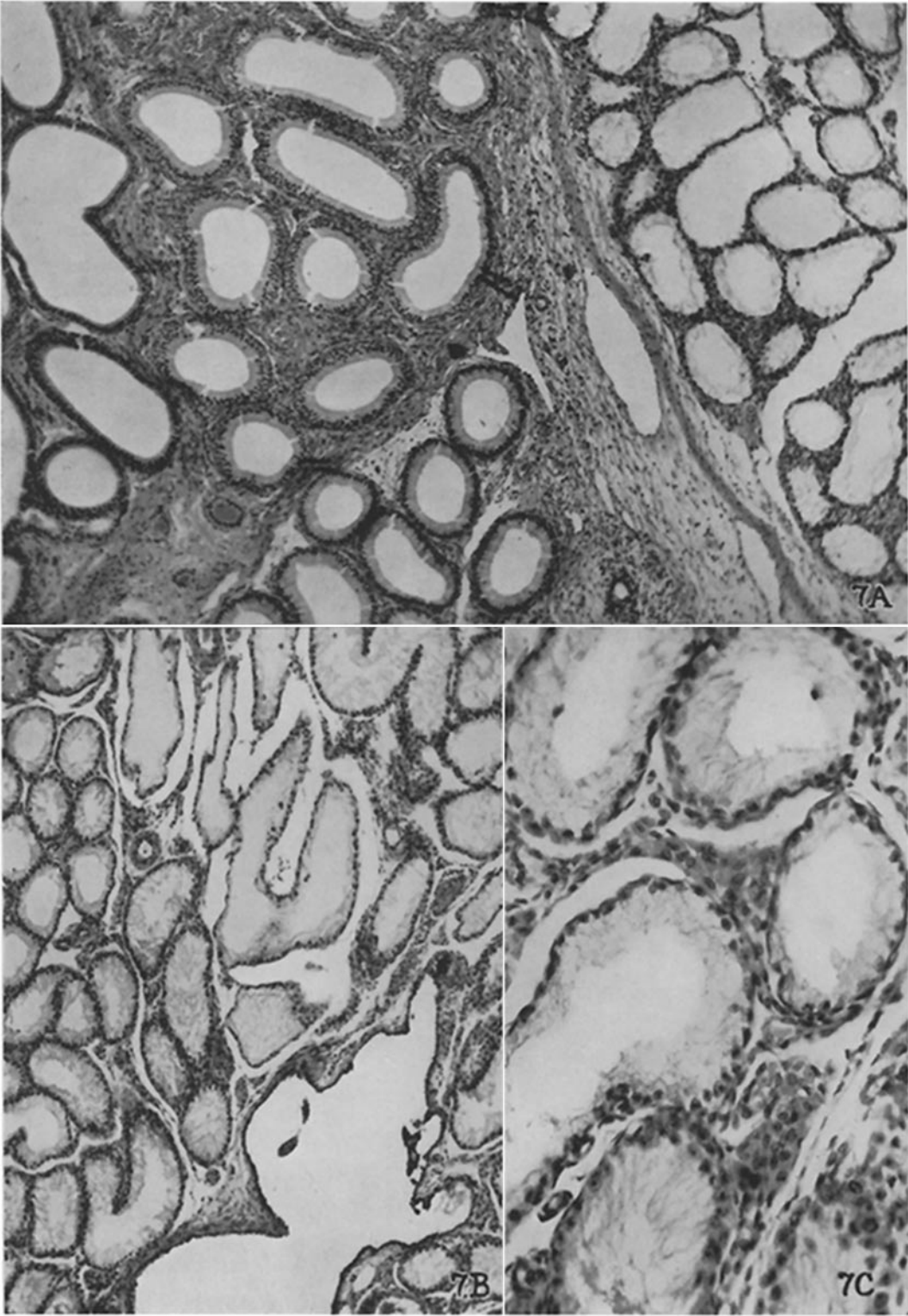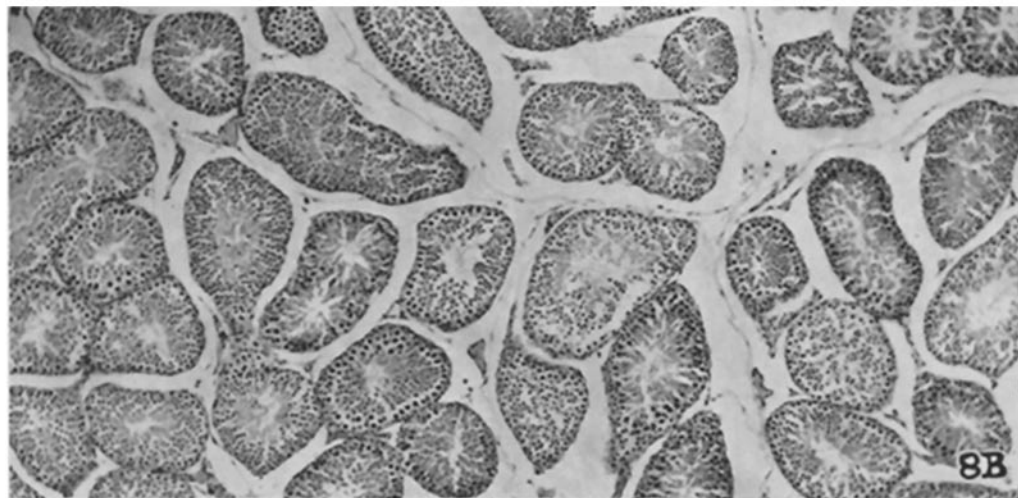
(Waksman: Auto-allergic testis lesion)

PLATE 43

FIGS. 8A and 8B. Severe epididymitis in 400 gm. English short haired guinea pig sacrificed 9 days after intradermal injection of testis and adjuvant.

FIG. 8A. Massive inflammatory infiltrate between tubules of epididymis, with invasion of tubules and destruction of tubular contents and tubular epithelium (in most severely affected tubules). Fig. 8B. Normal seminiferous tubules in same testis. Hematoxylin and eosin. Fig. 8A, × 35; Fig. 8B, × 80.

(Waksman: Auto-allergic testis lesion)

PLATE 44

FIGS. 9A to 9C. Destruction of epididymal tubules in 500 gm. Hartley guinea pig injected with guinea pig testis and adjuvant subcutaneously over nape of neck and sacrificed at 26 days. The tubular epithelium is stretched and flattened in Fig. 9A, has proliferated in Fig. 9B, and appears to have been completely destroyed, together with the tubular basement membrane, by the invading mass of leukocytes in Fig. 9C. Sweet's reticulum stain. × 225.
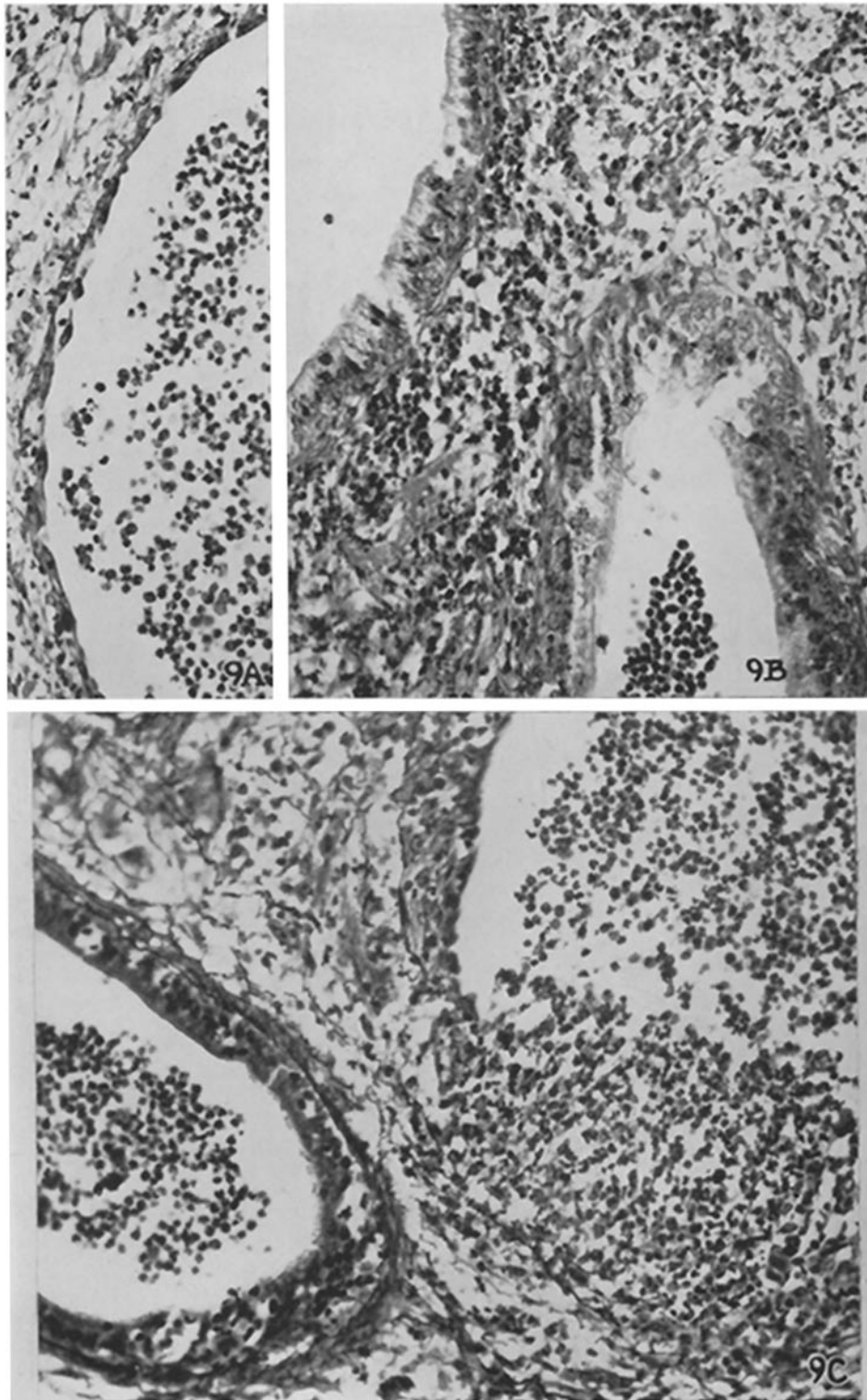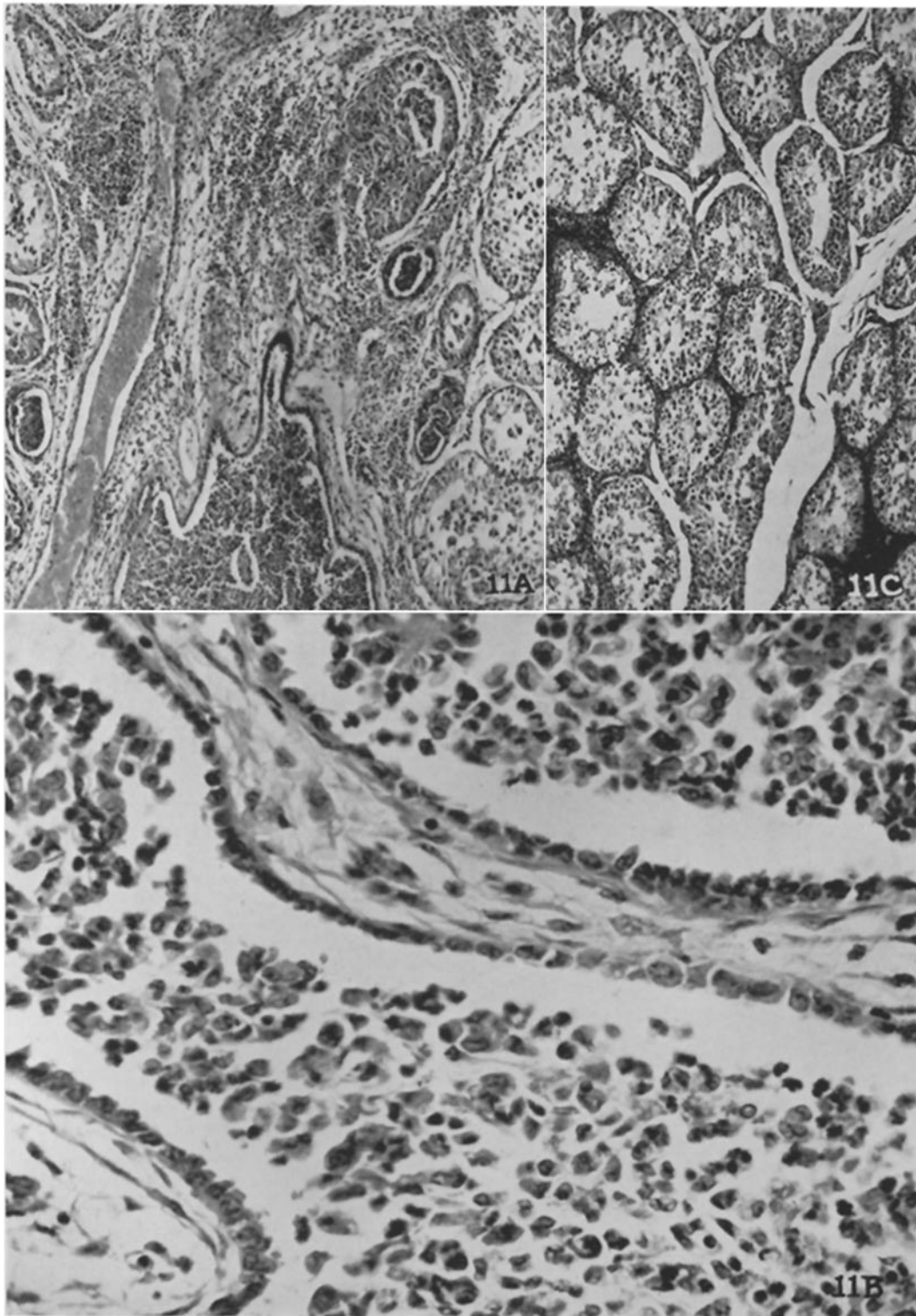
(Waksman: Auto-allergic testis lesion)

FIG. 10. Same guinea pig illustrated in Fig. 9. Formation of masses of inflammatory cells within the seminiferous tubules with preservation of tubular architecture. Except at one point, basement membrane of tubules appears intact. This testis showed early general hypospermatogenesis. Sweet's reticulum stain. × 225.
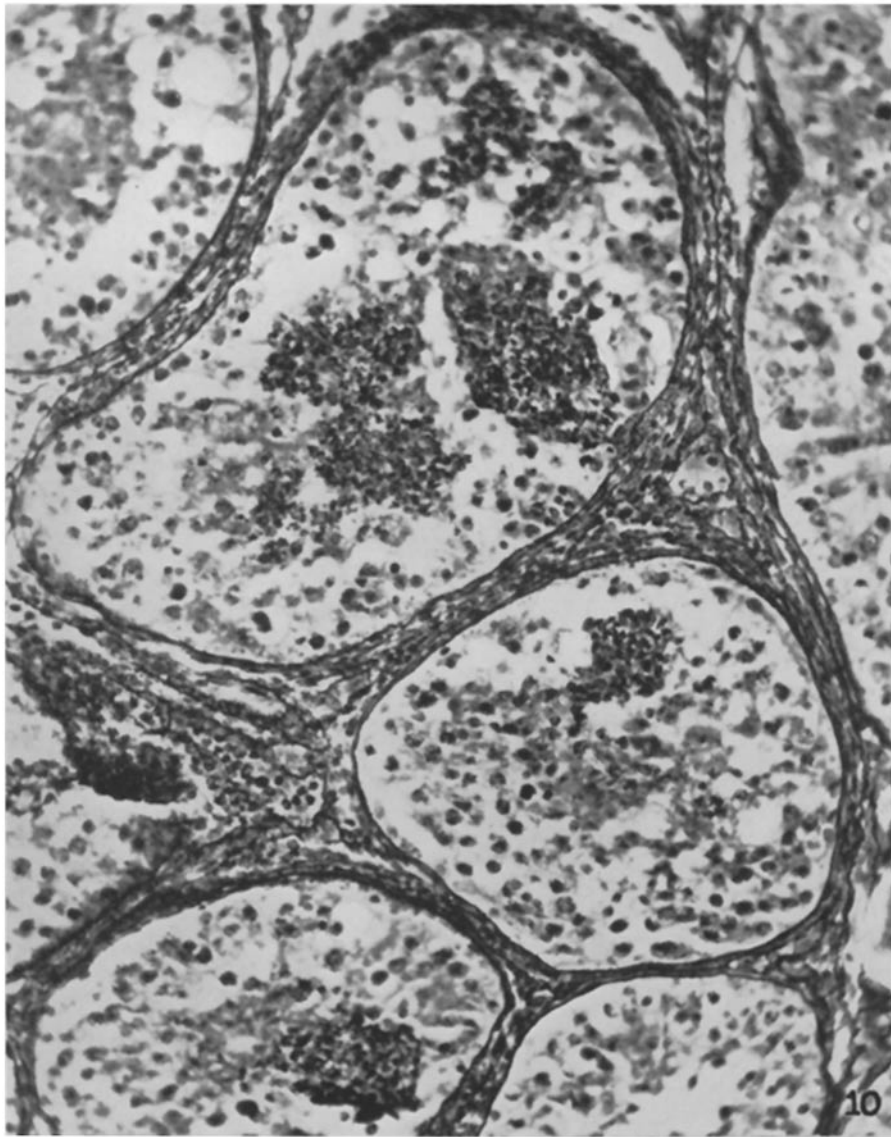
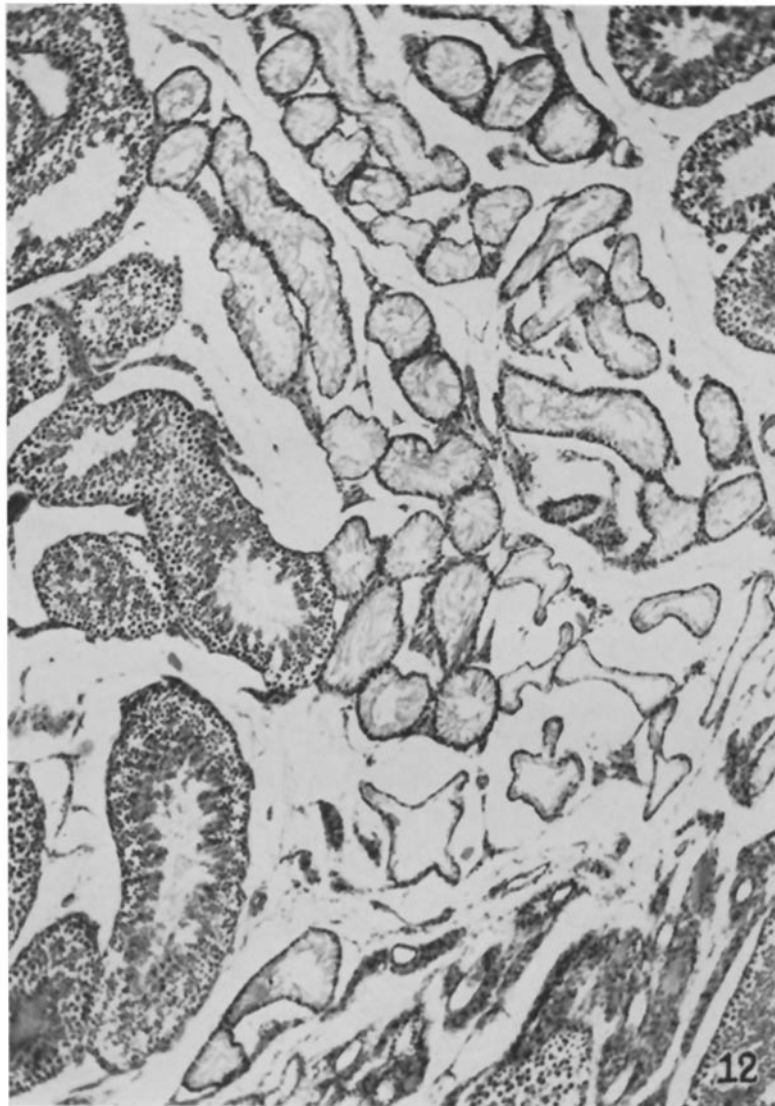(Waksman: Auto-allergic testis lesion)

PLATE 46

FIGS. 11A to 11C. Massive rete lesion (Figs. 11A and 11B) and small inflammatory foci (Fig. 11C) in testis of 800 gm. Hartley pig sacrificed 13 days after intradermal injection of testis and adjuvant. Note predominantly mononuclear character of exudate and early (+) hypospermatogenesis. Hematoxylin and eosin. Figs. 11A and 11C, × 80; Fig. 11B, × 450.

(Waksman: Auto-allergic testis lesion)

PLATE 47

FIG. 12. Lesion in 1200 gm. guinea pig 26 days after subcutaneous injection of testis and adjuvant. Island of complete aspermatogenesis adjacent to rete in otherwise normal testis. Hematoxylin and eosin. × 80.

(Waksman: Auto-allergic testis lesion)