

Immunologic and testicular response in guinea pigs after unilateral thermal orchitis

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Unilateral thermal orchitis was induced in adult guinea pigs by intratesticular infiltration with boiling saline. The immunologic response and lesions which developed in the contralateral gland were studied. It was found that: (1) With the exception of the sperm-immobilizing test, which was positive in some animals, no detectable anti-spermatic antibodies of complement-fixing or precipitating types appeared. (2) Delayed hypersensitivity, as revealed by skin test, was present. (3) Lesions similar to those of allergic orchitis appeared in the contralateral gonad and corresponding epididymis. (4) No evident difference was observable in this immunologic response when injured animals were in addition injected with complete Freund's adjuvant.

A report describing the circulating antibodies and lesions which developed in the contralateral testis after unilateral thermal orchitis in guinea pigs has recently been published.¹ In this publication, no attention was paid to delayed hypersensitivity, epididymal lesions, or the effect of added Freund's adjuvant. Therefore it was considered of interest to carry out an investigation concerning these points.

MATERIAL AND METHODS

Experimental animals

Three groups of outbred male adult guinea pigs were used, weighing between 400 and 600 grams and maintained on a standard diet. In the animals of the first group, comprising 18 specimens, testicular damage was induced in the left testis as follows: Under light anesthesia with sulphuric ether, 1 ml. of boiling saline solution was injected through the skin into the equatorial zone of the testis with the use of sterile precautions and a 20/5 needle. Care was taken not to harm the right testis or the septum which separates these glands. In the second group, comprising 28 animals, a similar procedure to damage the left testis was applied, followed by intradermal injection of 0.5 ml. of complete Freund's adjuvant in the left thigh. In the third group, comprising 10 animals and used as a control group, a similar volume of saline solution at room temperature was injected in the left testis. At various intervals between 3 and 8, 13 and 23, and 28 and 65 days, all animals of the first and second groups were bled, and both testicles were dissected out and fixed in Bouin's fluid. The equatorial zone and both poles as well as the epididymis were embedded in paraffin, serially cut, and stained with hematoxylin and eosin for histologic examination. Animals of the third group were bled between 5 and 59 days and the testicles similarly processed (Table I).

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TABLE 1. Immunologic response and lesions developed in the contralateral gonad after

Group No.	Days after testicular injury	No. of animals	Injured left testis
1, Without adjuvant	3-7	4	Necrosis + inflammation
	13-21	8	Necrosis + inflammation + sclerosis
	28-65	6	Sclerosis
2, With adjuvant	5-8	7	Necrosis + inflammation
	14-23	9	Necrosis + inflammation + sclerosis
	28-59	12	Sclerosis
3, Control	5-23	5	Mild inflammation
	27-59	5	No lesion

In order to compare the intensity of the injury provoked in the injected testis with the histologic alteration developed in the contralateral gland, lesions in the injected testis were graded according to the proportion of tubules damaged by necrosis or inflammation as follows: 60 to 100 per cent was considered a grade 3 lesion, 30 to 59 per cent grade 2, and less than 30 per cent a grade 1 lesion.

Skin tests

Before the animals were killed, a skin test was performed in each on one part of the dorsum by intradermal injection of 0.1 ml. of testicular homogenate prepared from adult normal glands diluted in saline 1/400. As a control and in only half of the animals of all groups, a skin test with the use of a similarly prepared kidney homogenate was performed on the other part of the dorsum. Readings were made at 24 and 48 hours, and the skin reaction was considered positive when the papulae had a diameter of at least 5 to 7 mm. Then the papula was excised and fixed in Bouin's fluid for histologic study.

Immunological tests

The serum samples obtained were used for: (1) complement-fixing tests (50 per cent hemolysis) employing as antigen a testicular homogenate prepared from adult homologous testis in varied concentrations, as previously reported,^{2, 3} and (2) immobilization tests with the use of homologous motile epididymal spermatozoa added with complement and the experimental sera in an *in vitro* system, following procedures previously published.²

RESULTS

The following results are detailed in Table I.

Immunologic reactions

Complement-fixing antibodies could not be detected in any animals of all groups tested at various periods of time. The immobilization test was positive in a very few animals of the first and second groups, especially in those killed within the first week. In the control group no immobilization was seen.

Skin tests

No cutaneous reaction was noted in the two groups of experimental animals during the first period. A weak one, consisting of a small papulae measuring 2 to 4 mm. in diameter, was observed at 48 hours in some animals of these groups during the second period. Only in the last period (28 to 65 days) and more frequently in the second group of animals (with added adjuvant) was

unilateral thermal orchitis

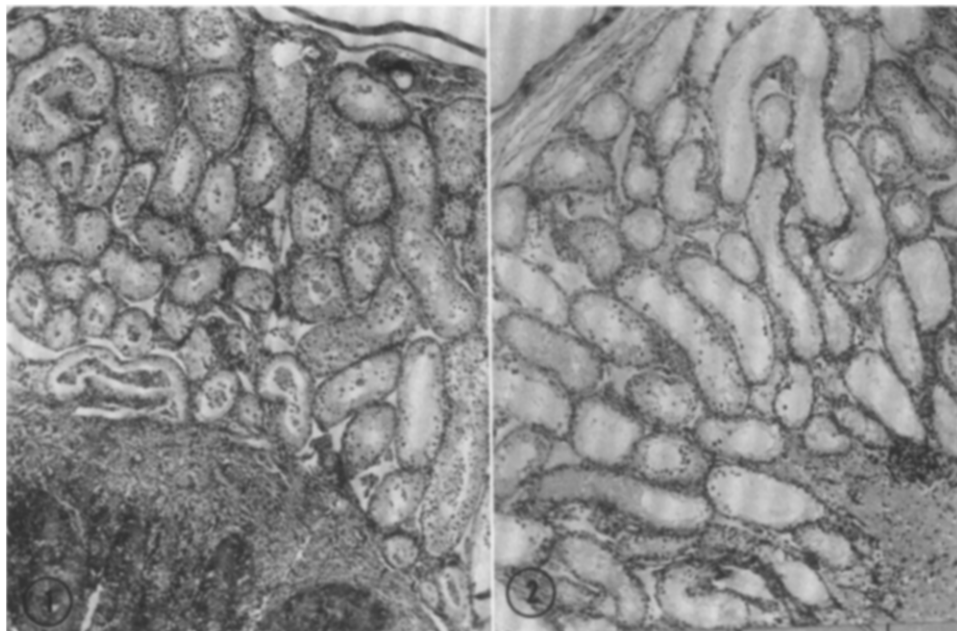
Lesion in the left epididymis	Lesion in the contralateral testis	Lesion in the contralateral epididymis	Skin test	Immobilizing test	Complement fixation test
0/4	0/4	0/4	0/4	2/4	0/4
2/8	5/8	0/8	0/8	2/8	0/8
5/6	5/6	3/6	3/6	0/6	0/5
3/4	0/7	0/4	0/7	2/7	0/7
6/7	6/9	2/5	0/9	1/9	0/9
7/8	8/12	5/8	9/12	1/12	0/10
No lesion	No lesion	No lesion	Neg.	Neg.	Neg.
No lesion	No lesion	No lesion	Neg.	Neg.	Neg.

a clearly evident papule measuring 6 to 12 mm. in diameter detected at 48 hours. These reactions were observed in response to testing with testicular homogenate, whereas none or only a very slight one could be observed following intradermal injection with kidney homogenate. Microscopically the lesion developed in the upper and middle layers of the dermal tissue as histiocytic proliferation and round mononuclear cell infiltration predominantly located around small size vessels. No skin reactions to either antigen were observed in the control group of animals.

Histology of testis and epididymis

In all animals of the first and second groups the thermally injured left testis showed similar lesions. During the first period after injury, in the equatorial zone and with less intensity in both poles, there was a central necrotic area of tissue which included seminiferous tubules and structures of the intertubular spaces. A second area surrounding the preceding one included all elements of an inflammatory reaction, i.e., congested vessels, polymorphonuclear and mononuclear cells, and infiltration of leukocytes into the lumen of tubules. Finally, a peripheral area near the albuginea exhibited mild signs of inflammation, and the tubules showed varying degrees of sloughing of germinal cells (Fig. 1). During the second period after injury, all the inflammatory features gradually subsided, and a fibrotic reaction appeared in the central area. In the third period, this zone had been completely replaced by sclerosis, no identifiable seminiferous tubules being seen, but small foci of inflammatory cells remained in the intertubular spaces (Fig. 2). In the peripheral area, most of the tubules remained with partially atrophied germinal epithelium, but some of them began to show signs of spermatogenic regeneration. The adjacent epididymis showed canaliculi without spermatozoa, and only a few immature germinal cells were present. In addition, a slight mononuclear cell accumulation was detected in the spaces between the canaliculi or behind the capsule. This epididymal lesion was present in the first group during the last two periods and more frequently in all periods of the second group.

In the contralateral right testis, lesions appeared as one or multiple foci separated by areas of normal tissue. They consisted of seminiferous tubules

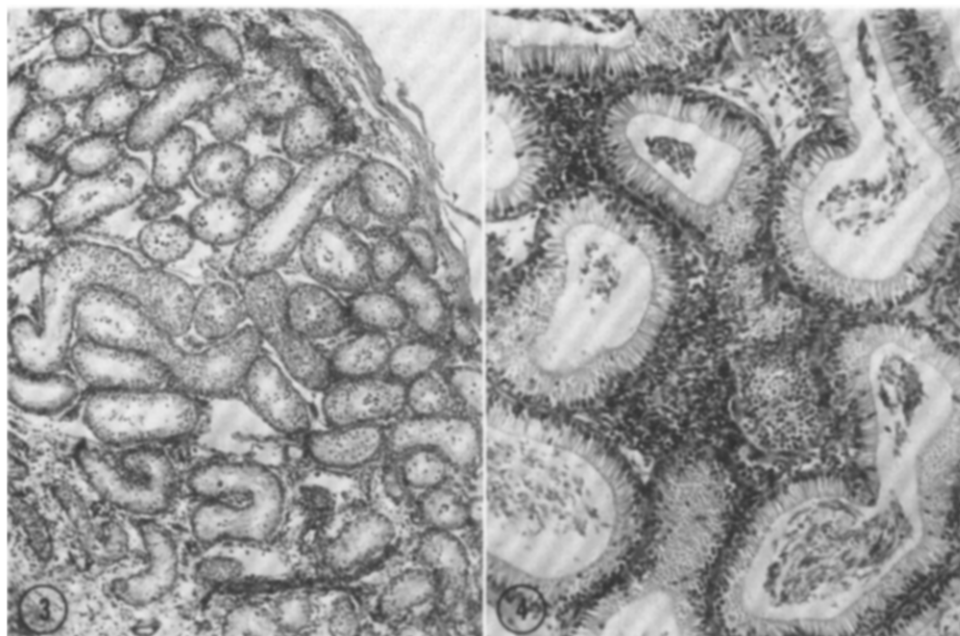


FIGS. 1 and 2. 1, Guinea pig left testis 3 days after thermal damage. A central necrotic area, surrounded by an inflammatory zone, and cortical seminiferous tubules with sloughed germinal cells are seen. (Original magnification $\times 55$.) 2, Guinea pig left testis 30 days after thermal injury. A central sclerotic zone and a cortical area with tubules containing largely atrophied germinal epithelium are seen. (Original magnification $\times 55$.)

with sloughing of germinal cells, vacuolization of Sertoli cells, and mononuclear cell infiltration in the intertubular spaces; no alteration in Leydig cells was observable (Fig. 3). In no case were the lesions confluent in the testicular sections. These lesions were absent during the first period in both groups of animals and appeared in the second one to persist until the end of the experiment. Frequency of lesions did not differ substantially between the first and second groups of animals. The epididymis of same side showed a similar mononuclear cell infiltration as described above; these lesions were found more frequently in the second than in the first group (Fig. 4).

Correlation between the degree of injury in the injected testis with the occurrence of lesions in the contralateral gland showed that grade 3 lesions were accompanied by lesions in the contralateral gland in the majority of animals; grade 2 lesions were associated with lesions in the contralateral gland in about half of the animals; no abnormality was present in the latter gland when grade 1 lesions developed in the injected testis.

In the control group, the left testis which had been injected with saline solution at room temperature, showed only disrupted seminiferous tubules and a mild leukocytic infiltration in a small central area of the gland. This picture, seen during the first period, tended to disappear in the second one, and no pathologic signs were observable at the end of the experiment. No morphologic alterations were detected at any period in the right testis or in either epididymis.



FIGS. 3 and 4. 3, Contralateral right testis of guinea pig shown in Fig. 1, 21 days after thermal injury of the left testis. The majority of the seminiferous tubules show sloughing and cytolysis of germinal cells. An accumulation of mononuclear round cells is seen in the intertubular spaces and behind the tunica albuginea. (Original magnification $\times 55$.) 4, Epididymis of contralateral testis showing infiltration of mononuclear cells in the intercanalicular spaces. Spermatozoa and immature germinal cells are present in the lumen. (Original magnification $\times 55$.)

DISCUSSION

Results here presented indicate that severe heat damage in one testis may be followed by lesions typical of so-called allergic orchitis in the contralateral gland, accompanied by an immunologic response of the delayed type as revealed by skin test. These findings corroborate and amplify those recently reported on the same subject¹; lack of demonstration of circulating antispermatic antibodies of a complement-fixing type also is in agreement with this report. We are unable to assess the immunologic significance of the sperm-immobilizing properties of the sera of some experimental animals in our studies, and neither do we know whether they are related to organ- and species-specific antispermatic antibodies as described previously.² Our findings on the positive skin tests, particularly in the last period of experiments, recall similar ones encountered in so-called allergic orchitis.^{4, 5} The lesions which developed in the contralateral testis, such as destroyed germinal epithelium and mononuclear cell infiltration, also are very similar to those found in allergic orchitis, although the latter are more diffused over the gland and lead the animal to aspermatogenesis and azoospermia. This similarity appears also corroborated by the occurrence of mononuclear round cell accumulation in

the intercanalicular spaces of the epididymis of the same side, which is also a common feature in allergic orchitis.⁶

As these lesions developed during the second and third periods after injury, a higher correlation with delayed hypersensitivity than with circulating antibodies seems likely. This was also believed to be the case in allergic orchitis, in which testicular lesions were accompanied by immediate and delayed skin reactions and a variety of humoral antitesticular antibodies.⁷ Although pathogenic significance has been attributed to both types of immune response as being causally related to testicular damage,⁸ homologous passive transference of gonadal lesions seems to be effective with lymph node cells of sensitized animals and not with immune serum.⁷

It is difficult to explain so far the absence of notable differences in our experiments between the lot of animals injected with Freund's adjuvant and the other one without. However, the higher frequency of sensitization of dermal tissue to testicular antigens observed in the lot of animals injected with adjuvant suggests that the latter may potentiate the skin test. This indicates that thermal injury to guinea pig testis, at variance with induced allergic orchitis, does not need the addition of adjuvant and is capable per se of evoking a similar response. On the other hand, the need for the simultaneous presence of destroyed germinal epithelium and Freund's adjuvant in the production of allergic orchitis has been substantiated by the induction of a local inflammation in the contralateral testis in response to an intradermal injection of the adjuvant⁹ or by the injection into the contralateral testis of the adjuvant alone.¹⁰

We would like to emphasize that in our experiments only extensive lesions in the injected testis are concomitant with the development of lesions in the contralateral gland. Since the only possible source of spermatogenic "autoantigens" in this phenomenon seems to be the thermally injured testis, the question arises as to whether the necrotic, inflammatory, or cortical subnormal areas might liberate or produce abnormal proteins capable of behaving as antispermatic agents. Current studies in our laboratory favor the idea that heat-denatured testicular proteins might be associated with this apparent antitesticular autoimmunization phenomenon.

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