Immunological and Testicular Response of Guinea Pigs Sensitized with Homogenate from Homologous Thermal Injured Testis[1] (36282)

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The unilateral thermal injury to guinea pig testis induced the formation of organ and species specific antibodies against testicular antigens and damage of germinal epithelium (1). In addition, similar experiments also demonstrated lesions in the epididymis and no ostensible variation in the incidence of this response when complete Freund adjuvant was added (2). Therefore, it was considered of interest to verify the antigenic potency of homogenates from heat damaged guinea pig testis in homologous sensitization without the use of adjuvants.

Materials and Methods. Antigen material. The testicular homogenate was prepared using decapsulated testes from healthy outbred guinea pigs, 400–600 g following a technique already recommended (3). The animals were previously subjected to the following procedures: (A) *Termal orchitis* was induced by intratesticular injection of 1 ml of boiling solution through the skin under sterile precautions. After 3 days the animals were bled and castrated. The same procedure was used in another lot of guinea pigs which were castrated after 1 month. (B) *Testis infiltrated* in vitro. To avoid the inflammatory reaction, glands obtained by castration from intact animals were treated *in vitro* in the same manner as above described *in vivo*. (C) *Testes heated* in vitro. In order to get an acute necrosis by coagulation, gonads obtained by castration from intact animals were divided into several pieces and subjected to boiling for 10 min in saline solution. (D) As controls two other lots of adult guinea pigs were used. In the first, the homogenate was prepared with testes from normal intact adult guinea pigs. In the second, animals were subjected to thermal nephritis *in vivo* by injection of 2 ml of boiling saline solution in the lower pole of both kidneys. After 3 days the animals were bled, the kidneys were dissected out and homogenate was prepared as described above (3).

Sensitization of animals. A total of 79 male adult guinea pigs were divided into six different lots (Table I). In each lot, animals were intradermally inoculated in various sites of the dorsum without using adjuvants, with a single dose of one of the five types of testicular or kidney homogenates already described. The individual dose administered was 650 mg/wet wt of normal or injured testis or kidney. The animals were bled and sacrificed at 15 and 30 days after sensitization. Testis and epididymis as well as kidney and liver, were fixed in Bouin's fluid and sections stained with hematoxylin–eosin for histological examination. One of the dermal sites of sensitization was also removed at 15 and 30 days and similarly processed for histological study.

Immunological Tests. Appearance after sensitization of antispermatic circulating antibodies was explored applying the complement-fixation test (50% hemolysis), double agar diffusion technique, immobilization, and PCA tests (ovary), following procedures reported in previous papers (4, 5). In all immunological techniques 0.1 to 0.5 ml of an extract of normal testis (1) in a protein concentration of 25.5 mg/ml (6) was used as antigen. As regards the detection of delayed hypersensitivity, the skin test and the inhibition of the macrophage migration (IMM) *in*

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TABLE I. Immunological Response and Testicular Lesions Developed in Guinea Pigs After Sensitization With Different Testicular Homogenates Without Adjuvants.

Lot number	Animal sensitized with homogenate from	No. of animals	Days after sensitization	Delayed skin test (mm)			Inhibition of macrophage migration (%)			Circulating antibodies		Testicular lesion
				No. of positive	Mean	Range	No. of positive	Mean	Range	PCA–C. fix. precipit. No. of positive	Immobil. test No. of positive	No. of positive
1	3 days thermal orchitis	8	15	6/6	9.17	(7.5–11)	5/6	64	(51–77)	0/8	5/8	5/8
		9	30	9/9	8.5	(5.5–11)	6/6	64.5	(59–70)	0/9	0/9	7/9
2	1 month thermal orchitis	5	15	0/5	2	(1.5– 3)	0/4	11	(5–18)	0/5	0/5	0/5
		6	30	0/6	1.7	(0.5– 3)	0/4	8.7	(2–17)	0/6	0/6	0/6
3	Testis infiltrated *in vitro*	10	15	2/8	10.5	(10 –11)	2/6	59.5	(56–63)	0/10	5/8	5/10
	with boiling saline	8	30	6/8	9.3	(7 –11)	5/5	67	(59–77)	0/8	0/6	5/8
4	Testis heated *in vitro*	6	15	0/6	2	(0.5– 3.5)	0/6	6	(1–12)	0/6	2/6	3/6
	with boiling saline	7	30	0/7	1.7	(0.5– 3.5)	0/7	9.1	(5–17)	0/7	0/7	3/7
5	3 days thermal nephritis	5	15	0/5	1.8	(0.5– 2)	0/4	8.5	(5–12)	0/5	0/5	0/5
		5	30	0/5	1.7	(0.5– 2.5)	0/4	10.2	(4–18)	0/5	0/5	0/5
6	Normal testis	10	30	0/10	1.8	(0.5– 3)	0/10	7.3	(3–17)	0/10	0/10	0/10

vitro (7) were used. The skin test was made by intradermal inoculation into the shaved flank of 0.1 ml of testicular homogenate in the protein concentration mentioned above. Readings were made at 12, 24, 48, and 72 hr. Diameter of papulae was measured and only those above 5 mm were considered positive; mean size and range in each group of animals are detailed in Table I. The validity of this test was assessed by histological study of the excised skin at the site of reaction; those showing the typical perivascular infiltration of histiocytes and mononuclear cells were taken into account. Specificity of this test was checked by intradermal injection of thyroid and kidney homogenate prepared as in the case of testis.

The IMM was performed and evaluated as detailed in a previous publication where this test was applied to the study of experimentally induced allergic orchitis in guinea pigs. (8). The average from several experiments per animal (8–10 tubes) was calculated and expressed as the percentage inhibition of macrophage migration. Inhibition levels of 25% or more were found to be significant and thus considered as positive; mean and range in each group of animals are detailed in Table I. As a control, supernatant of thyroid gland homogenate was also used.

In some animals of Lot 1, which were sacrificed at 15 days, and in some others which were sacrificed at 30 days, hemicastration and blood samples were also performed at 7 and at 20 days, respectively.

Results. Immunological response. (See Table I). Circulating antibodies, using different concentrations of the antigen, were not demonstrated with the immunological techniques applied. This lack of response was evident not only in the control animals but also in the experimental ones. Only the immobilization test in the presence of complement appeared positive with undiluted serum in a high percentage of animals immunized with homogenate from 3 days thermal orchitis (Lot 1), or with homogenate from testis infiltrated *in vitro* with boiling saline (Lot 3), and with less frequency in those injected with glands heated *in vitro* (Lot 4). In these cases positive results were detected at 15 days and in some of them even at day 7, but were negative at 30 days after sensitization. In the remaining experimental and control animals results were always negative. On the other hand, delayed hypersensitivity was positive in a number of guinea pigs of the experimental lots as reflected by the skin test and the IMM technique. Both tests developed approximately in parallel at 15 and 30 days after inoculation. Controls for both tests using other antigens instead of testicular homogenate, such as kidney or thyroid gland, were consistently negative. Both tests appeared positive at 15 and 30 days in the majority of animals sensitized with homogenate from 3 days thermal orchitis (Lot 1), but not in those injected with similar material from one month thermal orchitis (Lot 2). This delayed immunological response was much less frequent, particularly at 15 days in animals sensitized with testis homogenate infiltrated *in vitro* (Lot 3) and negative in those inoculated with similar material from testes directly heated in boiling saline (Lot 4). Specificity was assessed by negative results provided by guinea pigs injected with normal testes (Lot 6) or with homogenate from thermal nephritis (Lot 5).

Microscopy of skin at sites of sensitization. The histology of these areas at 15 days after inoculation of antigen showed a characteristic inflammatory reaction occupying the superficial and deep layers of the dermis. Surrounding a central zone where coagulated eosinophilic material is observable, a thick area of hypertrophied histiocytes, dilated small vessels, and moderate accumulation of polymorphonuclear and mononuclear leukocytes were present. At 30 days the cellular reaction and leukocyte infiltration had already disappeared and a fibrotic collagenous tissue tended to replace the primitive nodule. Except for the smaller size and less intense inflammatory response induced by normal testicular homogenate, no ostensible qualitative differences were appreciable among the nodules of animals injected with different types of injured testicular or kidney homogenates.

Histology of testis and epididymis. A typical picture of the so-called allergic orchitis of guinea pigs was detected in some lots of

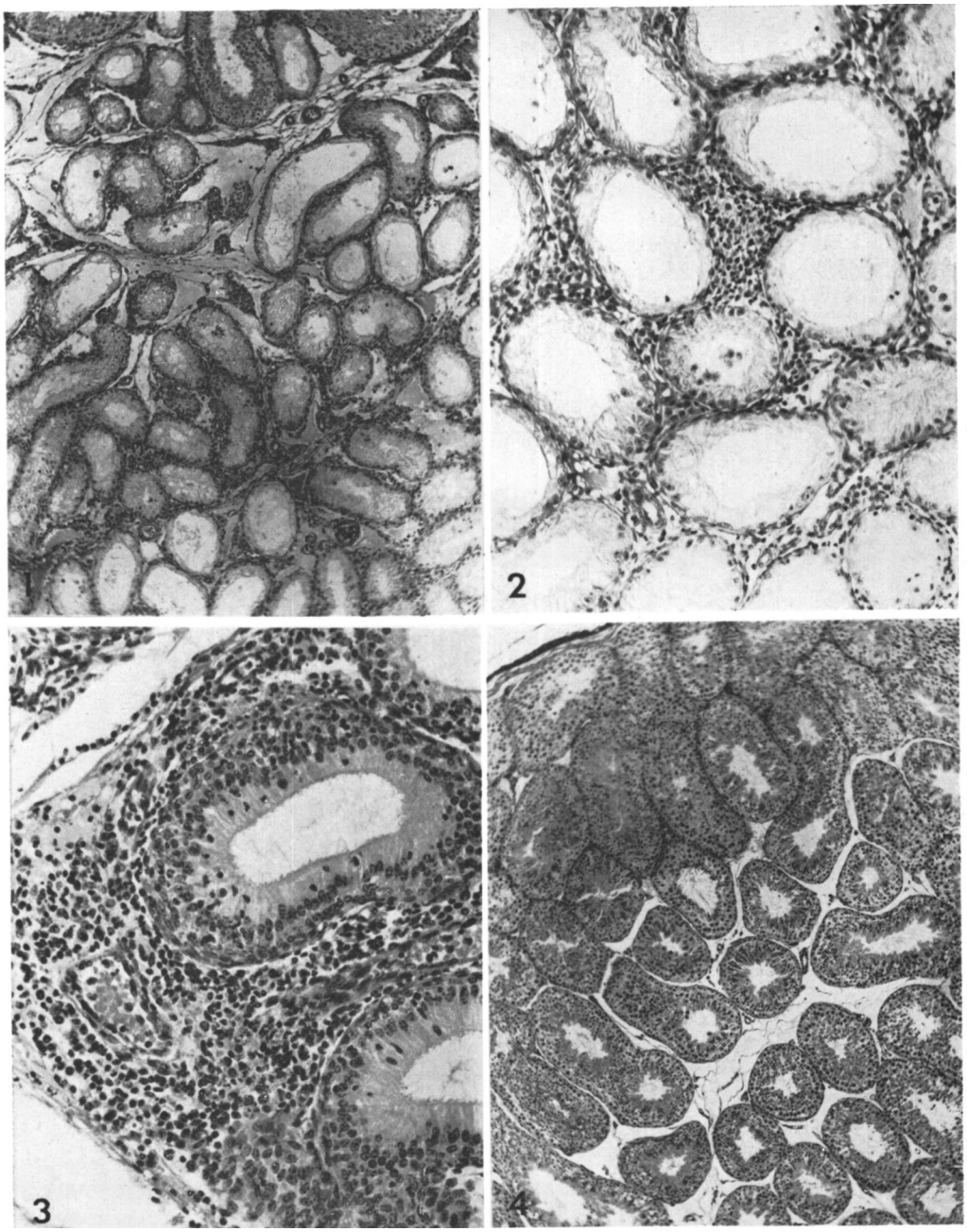

FIG. 1. Guinea pig testis 30 days after sensitization with homogenate prepared from 3 days thermal orchitis without adjuvant. Atrophied germinal epithelium in the majority of seminiferous tubules. Intertubular infiltration of mononuclear cells. Some tubules remained undamaged (bottom). Hematoxylin–eosin stain. ×46.75.

FIG. 2. Same at higher magnification showing absent germinal cells but Sertoli cells remaining inside the tubules; mononuclear cells in the intertubular spaces. ×119.

FIG. 3. Epididymis adjacent to the testis showed in Fig. 1. Perivascular and diffuse mononuclear cells accumulation in the intercanalicular spaces. No alteration is evident in the epithelium.

experimental animals (Table I). Beginning at 7 but more evident at 15 days, the testis showed peripheral and/or central foci of seminiferous tubules showing vacuolated Sertoli cells, dissociation and cytolysis of germinal cells, intertubular edema, dilated vessels with hypertrophied endothelium, and perivascular accumulation of mononuclear cells. Polymorphonuclear leukocytes or other foreign cells were never observed in the intertubular spaces or inside the tubules of the testis and epididymis (Figs. 1, 2). Some relationship could be established at 30 days between damage of testis and the presence of mild mononuclear cell infiltration in the intercanalicular spaces of the epididymis (Fig. 3); however no alteration was noticeable in the epithelium of the canaliculi but numerous exfoliated immature germinal cells were seen in the lumen. This testicular and epididymal abnormal response was induced with higher frequency in animals sensitized with homogenate from 3 days thermal orchitis (Lot 1) but was absent in those injected with one month thermal orchitis (Lot 2) (Fig. 4). On the other hand the incidence was lower in animals inoculated with homogenate from testis infiltrated *in vitro* (Lot 3) and in those injected with testis directly boiled in saline (Lot 4). No lesions in the testis or epididymis were present in animals inoculated with homogenate from normal testis (Lot 6) or homogenate from thermal nephritis (Lot 5).

It is of interest to point out that at 15 days after immunization, a correlation could be established among testicular lesions, delayed hypersensitivity, and sperm immobilizing effect of serum; at 30 days only a correlation between the first two parameters was observable (Lots 1, 3, and 4).

Discussion. Present results demonstrate the possibility of inducing allergic orchitis in guinea pigs without the addition of complete Freund adjuvant. As it has been long admitted, adjuvants mixed with normal testicular homogenate are necessary to get such a response (3, 9, 10). However, it has been claimed that repeated injection during several months of homogenate prepared from normal testis is able to elicit germinal cell damage and a positive PCA test in guinea pigs (11). It has also been postulated that under those conditions the adjuvant may be provided by the chronic inflammation induced at sites of injection in the skin. In our experiments a single subcutaneous administration of the antigen was capable of provoking testicular alteration, delayed hypersensitivity, and complement dependent immobilization of sperm, but not detectable circulating antispermatic antibodies of anaphylactic (PCA), complement-fixing or precipitating types were observed. As the dermal inflammatory nodule developed at sites of sensitization did not have specific features as those present when complete Freund adjuvant is added (3), the question arises as to whether or not the heat denatured testicular homogenate used as antigenic stimulant is entirely responsible for the immunological and testicular response obtained. Concerning this point, it seems likely that only the area of necrotic testicular tissue caused by thermal injury, and not the surrounding inflammatory zone of the subnormal peripheral one (2), contains the denatured protein substances which may behave as antigenic agents. This appears backed by comparable results obtained both in guinea pigs sensitized with homogenates from thermal orchitis (Lot 1) or with homogenate from testis infiltrated with or directly boiled in saline *in vitro* (Lots 3 and 4, respectively). Specificity is supported by the absence of such immune response if injections are performed with testes lacking acute heat damage, such as those carrying only a chronic inflammatory area (Lot 2), or normal testicular tissue (Lot 6), or kidney with thermal nephritis (Lot 5). Our findings evoke the circulating antibody and delayed reaction induced in rabbits by injection of heterologous denatured serum

Hematoxylin–eosin stain. ×102.

FIG. 4. Guinea pig testis 30 days after sensitization with homogenate prepared from 30 day thermal orchitis without adjuvant. Normal appearance of seminiferous tubules and intertubular spaces. Hematoxylin–eosin stain. ×46.75.

proteins (12). The absence of demonstrable circulating antibodies in our experiments is in contrast with the presence of delayed hypersensitivity. As this last finding appears in correlation with damage of seminiferous epithelium and the remarkable mononuclear cell infiltration, attention is called to the predominant delayed reaction obtained even in the absence of added adjuvant. Besides, the mononuclear cell infiltration in the epididymis also evokes that encountered in the Freund phenomena (13). Our results are probably more related to those reported in unilaterally induced thermal orchitis, where more restricted lesions in the contralateral gland and precipitating antibodies develop without adjuvant (1); it also recalls our previous studies in a similar system in which gonadal lesion, delayed skin reaction, and sperm immobilizing factor were present, but addition of complete Freund adjuvant did not modify the incidence of the immunological and testis response (2). More work is needed to clarify the nature of the testicular antigen induced by heat and capable of provoking testicular injury, and a consistent cell-mediated antibody response without using any adjuvant. Incidentally, it was assumed that thermostable brain and adrenal antigens are active in hetero- but not in isoimmunization procedure (14), while an autoclaved material extracted from homologous testis is antigenic in guinea pigs in the presence of adjuvants (13).

Summary. Adult outbred guinea pigs were sensitized, without added adjuvants, with a single dose of homogenates prepared from homologous testis previously subjected to thermal injury *in vivo* or *in vitro*. It was observed: (A) With exception of positive results obtained with the sperm immobilizing test, no antisperm antibodies of anaphylactic, precipitating, and complement-fixing types were induced. (B) Delayed hypersensitivity, as revealed by skin test and inhibition of macrophage migration, appeared positive in parallel with testicular and epididymal damage similar to the so-called allergic orchitis. (C) Only homogenate prepared with acute thermal damaged testis was able to provoke such a response but not that prepared with testis having thermal chronic lesions. (D) Homogenates from normal adult testis or prepared with kidney having acute thermal nephritis gave negative results.

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