Aspermatogenesis Induced by Testicular Antigen Uncombined with Adjuvant. (26552)

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Immunologically induced aspermatogenesis in the guinea pig follows intracutaneous injection of testicular antigen combined with complete adjuvant of the Freund type(1,2,3). Previous investigations have indicated that one or a limited number of injections of testicular homogenate or of sperm alone fails to induce germinal lesions(1,2) as does administration of antigen and adjuvant into separate sites on different sides of the animal (1). Considerable emphasis has thus been attributed to the role of adjuvant in the reaction(4,5,6), and at least one theory has been proposed which requires previous combination and injection of antigen and adjuvant together into the same sites(7, *cf.* 8,9).

Evidence from other systems involving delayed-type hypersensitivity, however, suggests that incorporation of antigen into paraffin oil adjuvants may not be essential for ultimate elicitation of specific germinal responses. The early demonstration, for example, of induced disseminated encephalomyelitis in monkeys was accomplished by many injections of brain antigen without adjuvant (10). Stone and Freund (11) have recently shown that the delayed-type skin-hemorrhage sensitivity of guinea pigs, developed in response to separate injections of ovalbumin and complete adjuvant, is different depending on whether injections are made on the same or on different sides of the nuchal mid-line. Similar analyses have been applied to the aspermatogenic syndrome; the present report concerns results of 2 types of experimental procedure: (a) frequent injections of minute amounts of testicular homogenate and (b) administration of antigen and adjuvant in separate sites on the same dorsal quadrant of the guinea pig. A preliminary note concerning the latter study has been published(12).

Material and methods. Adult male guinea pigs of Hartley and mixed outbred strains were intracutaneously injected in the nuchal region as scheduled in Tables I and II. Homologous testicular homogenate in saline (1:1) was prepared from normal adult gonads as previously described(2). Adjuvant was of the Freund type which includes Bayol F paraffin oil, Arlacel A emulsifying agent, and mycobacteria (Difco Laboratories, Detroit, Mich.).

In the first, low-dose series, testicular homogenate was repeatedly injected in amounts and at rates shown in Table I. At sacrifice, 21 to 157 days after first injection, body weights indicated normal growth. Testicular tissue was fixed in formol-Zenker, sectioned at 5 μ, and stained in haematoxylin-eosin or Himes-Moriber trichrome stain(13). Three animals were unilaterally castrated at day 63 and sacrificed one month later. Scoring of germinal lesions follows the procedure previously adopted(2) and indicates increasing severity from 1 to 4.

Each guinea pig in the second, separate-site series received 0.5 ml testicular homogenate in saline and 0.5 ml complete adjuvant in locations separated by distances 2.0 cm or greater (Table II). In some animals, antigen was injected adjacent to the dorsal midline while adjuvant was administered laterally; in others, the positions were reversed. In 4 controls (Nos. 20-23), antigen was injected on the right side and adjuvant on the left of the mid-line. At sacrifice, 60 days after sensitization, animals were weighed and testes fixed and stained as above. Scoring of histological lesions includes minimal and maximal damage to both testes in accordance with the more complete index used by Bishop *et al.*(3). Sera, collected by cardiac puncture just prior to sacrifice, were tested for humoral antibody by passive cutaneous anaphylaxis (PCA)(14). PCA challenge was effected by testicular extract prepared according to the acetic acid-ammonium sulfate procedure of Freund *et al.*(4).

TABLE I. Germinal Lesions in Male Guinea Pigs Repetitively Injected with Testicular Antigen without Adjuvant.

Group	No. of animals	Strain	Testicular homogenate* ml/inj.	Inj./wk	Total inj.	Duration (days)	Germinal damage†
A	5	Mixed	.1	3	27-63	63-157	0, 3, 0, 4, 3, 4
B	6	"	.2	2	18-42	63-157	1, 3, 2, 4, 1, 2, 3, 3
C	5	Hartley	.1	3	9-47	21-109	2, 2, 3, 3, 3
D	6	"	.05	6	27-95	31-109	3, 3, 4, 4, 3, 3

* Approximately 250 mg (wet wt) testes/ml.
† One guinea pig in group A and 2 in B unilaterally orchiectomized at day 63.

Results. Repeated injections without adjuvant. Data for 22 guinea pigs injected repetitively with testicular homogenate alone are summarized in Table I. The most severe testicular responses occurred after 100 days in guinea pigs which had received 38, 54, 63, and 95 injections at rates of 3 or 6 times per week (Fig. 1, 2). Damage was discernible, however, within one month in some animals injected with 0.1 or 0.05 ml, 3 or 6 times per week, respectively. While not conclusive, the lower dosage (0.05 ml), frequently administered (6 inj/wk), tended to evoke the greatest response. In no case was germinal damage complete, such as that which frequently occurs when antigen is combined with adjuvant; on the contrary, lesions tended to be patchy and involved only one or several tubules. During the course of injections, it was observed that reinjection into the same area evoked a marked localized erythematous reaction which persisted for 1 to 2 days.

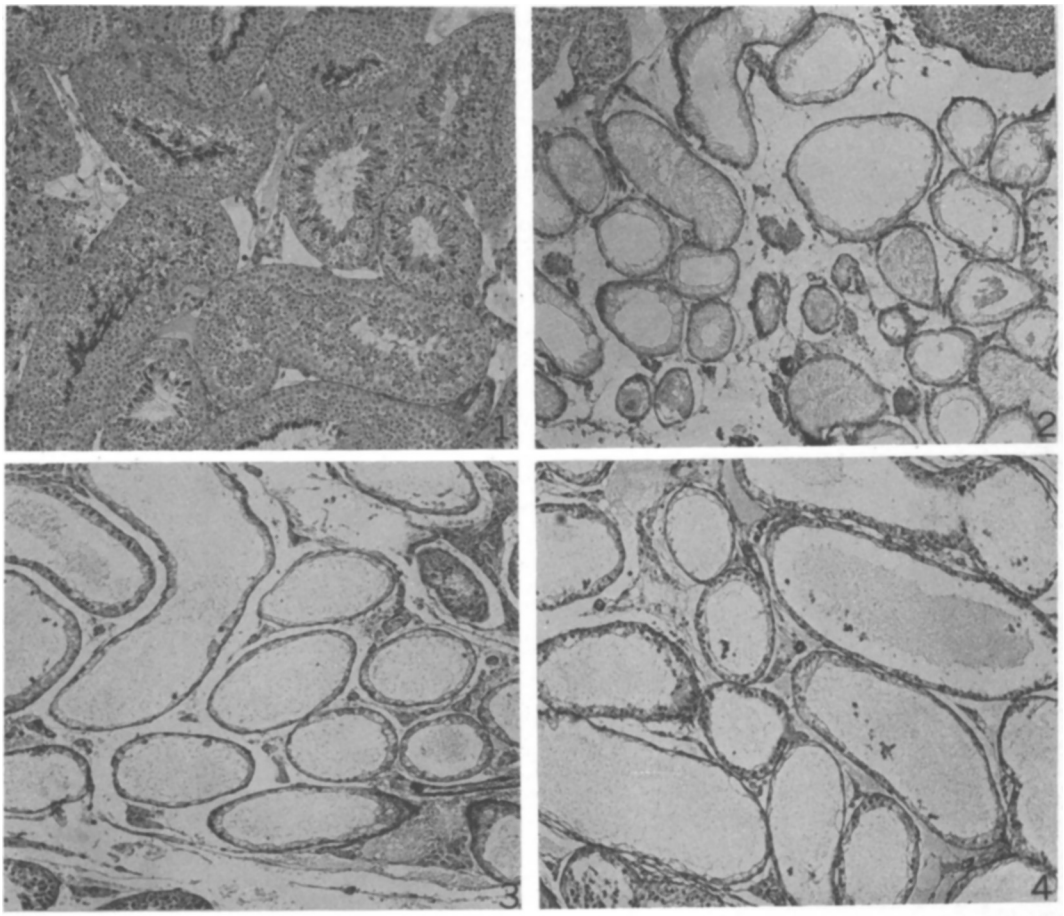
Antigen and adjuvant uncombined. Separate injections of testicular antigen and complete adjuvant quite readily elicited germinal responses, so long as both components were administered on the same side of the nuchal mid-line (Table II). Severe and almost complete destruction of germinal epithelium of both testes occurred in guinea pigs in which

TABLE II. Aspermatogenesis Induced in Guinea Pigs Injected with Testicular Antigen (0.5 ml) and Complete Adjuvant (0.5 ml) in Separate Sites on Dorsal Quadrant.

No.	Strain	Sites of inj. Antigen	Sites of inj. Adjuvant	Distance between inj. sites (cm)	Germinal damage Minimum	Germinal damage Maximum	PCA*
1	Mixed	Lateral	Median	3.5	0	3	
2	"	"	"	3.0	0	3	
3	"	"	"	3.0	0	3	
4	"	"	"	4.0	0	2	
5	"	Median	Lateral	3.5	0	3	
6	"	"	"	3.5	0	4	
7	"	"	"	4.0	0	3	
8	Hartley	"	"	2.5	0	4	—, —
9	"	"	"	3.0	1	4	+, +
10	"	"	"	2.5	2	4	+, —
11	"	"	"	2.8	2	4	+, +
12	"	"	"	2.7	3	4	+, +
13	"	"	"	2.5	0	3	+, +
14	"	Lateral	Median	2.5	0	3	+, +
15	"	"	"	2.5	0	3	—, —
16	"	"	"	2.5	0	4	+, +
17	"	"	"	2.0	2	4	+, +
18	"	"	"	2.4	3	4	+, +
19	"	"	"	2.8	3	4	+, +
20	"	Right	Left	6.0	0	1	—, —
21	"	"	"	6.0	0	2	+, —
22	"	"	"	6.0	0	1	—, —
23	"	"	"	6.0	0	1	+, —

* PCA tests run in duplicate: +, positive; —, negative response.

a minimal distance of 2.4 to 2.8 cm separated the sites (Fig. 3, 4). Aspermatogenesis was induced regardless of which component was medially injected; a slight edge seems to fa-



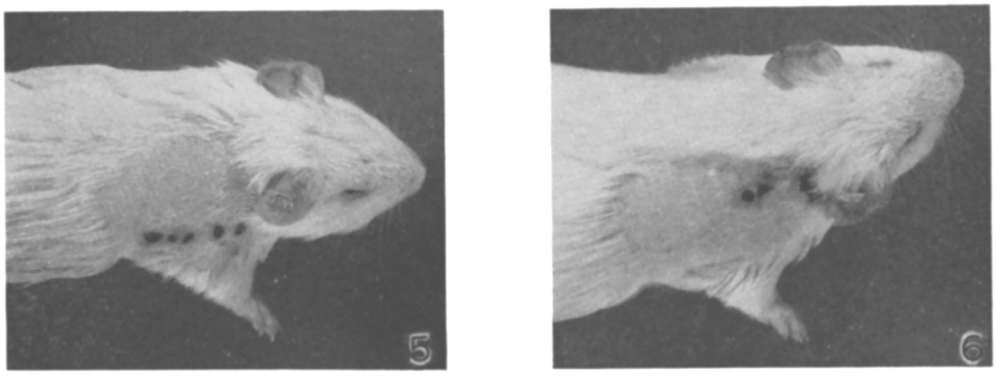


FIG. 1. Normal adult testis of donor guinea pig. × 65.

FIG. 2. Aspermatogenesis induced by testicular homogenate without adjuvant; 54 injections, 0.1 ml/inj, 3/wk. × 65.

FIG. 3-4. Severe germinal damage after separate-site injection of antigen and complete adjuvant; nos. 9 and 18, Table II. × 65.

FIG. 5-6. Granulomata at lateral and median sites of adjuvant injection in animals nos. 10 and 14, respectively (Table II).

vor those with medially administered antigen and lateral adjuvant. No histological abnormalities were detected in the testicular interstitium. The sensitizing injections were followed by granulomata at sites of adjuvant administration (Fig. 5, 6), reactions which are typical of animals treated with combined antigen-adjuvant mixtures(2).

Four control guinea pigs injected with antigen on the right and adjuvant on the left side of the mid-line were relatively free from germinal change (Table II). Depletion of sperm and sloughing of spermatocytes in the seminiferous tubules of one, however, indicated an induced response but the significance of this case is obscure.

PCA reactivity of sera of separate-site animals was generally positive (Table II). Two samples failed, by this method, to show circulating antibody. At the same time, sera of two other guinea pigs (Nos. 21, 23), injected on both sides of the mid-line, indicated the presence of antibody in one of 2 duplicate tests.

Discussion. These experiments show that aspermatogenesis can be induced in the guinea pig by testicular antigen without prior incorporation into adjuvant. When testis is frequently injected over a long period of time without adjuvant, the immune mechanism may be different from that when adjuvant is employed, but the ultimate tissue response appears to be similar. Although incomplete in these experiments, it is possible that total germinal destruction might occur if sufficient injections were made over an adequate period. The further possibility exists that in the absence of adjuvant, a constituent of the skin itself may play a role in mediation of the immune response(8,15). Aspermatogenesis, like experimental allergic encephalomyelitis(10), can thus be induced by maintenance of an adequate source of tissue antigen in appropriate sites.

Excellent germinal responses resulted from introduction of antigen and adjuvant in separate sites on the same side of the mid-line. This suggests that the lymphatic drainage system is involved, and that if there need be association of the 2 components, it must necessarily occur in, or be mediated by, the lymph nodes(11). The slight tendency for more severe responses to occur when adjuvant was injected laterally (Table II), where gross lymph nodes are more abundant, favors such an interpretation. Further attempts to test this experimentally have as yet proved unfruitful owing to the diffuse pattern of the guinea pig lymphatic system(16) and difficulties of ablation. The correlation of induced lesions in the 2 testes of a responsive animal confirms the phenomenon discussed more fully elsewhere(3).

The serological data are merely suggestive but support the contention that positive PCA reactions indicate an active immune state significantly related to the aspermatogenic response(3). Systemic anaphylaxis, as determined by the Schultz-Dale test, has also been associated with the aspermatogenetic syndrome(7), but for certain types of immune reactions, at least, this method is regarded as less sensitive than the PCA procedure(17). Active cutaneous sensitization can be induced in guinea pigs by injection of testicular antigen without mycobacterial adjuvant (4,5).

Summary. Aspermatogenesis was induced in adult guinea pigs (1) by repeated intracutaneous injections of homologous testicular homogenate without adjuvant, and (2) by injection of testicular antigen and complete adjuvant in separate sites on the same side of the nuchal mid-line. With testicular tissue alone, severe germinal lesions were induced after 100 days in animals injected 3 or 6 times weekly with 0.1 or 0.05 ml, respectively. Degree of germinal damage resulting from separate-site injection of uncombined antigen and adjuvant was comparable to that when testis is incorporated into complete paraffin oil adjuvants prior to administration. Results of separate-site injections suggest dependency on a common lymphatic drainage system.

1. Freund, J., Lipton, M. M., Thompson, G. E., *J. Exp. Med.,* 1953, v97, 711.

2. Katsh, S., Bishop, D. W., *J. Embryol. Exp. Morphol.,* 1958, v6, 94.

3. Bishop, D. W., Narbaitz, R., Lessof, M., *Develop. Biol.,* 1961, in press.

4. Freund, J., Thompson, G. E., Lipton, M. M.,

J. Exp. Med., 1955, v101, 591.

5. Freund, J., *Adv. Tuberc. Res.*, 1956, v7, 130.

6. ———, *J. Allergy*, 1957, v28, 18.

7. Katsh, S., *Internat. Arch. Allergy Appl. Immunol.*, 1959, v15, 172.

8. Raffel, S., *Immunity, Hypersensitivity, Serology*, Appleton-Century-Crofts, Inc., 1953, New York.

9. Eisler, M., *Z. f. Immunitätsforsch.*, 1959, v117, 294.

10. Rivers, T. M., Sprunt, D. H., Berry, G. P., *J. Exp. Med.*, 1933, v58, 39.

11. Stone, S. H., Freund, J., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 140.

12. Bishop, D. W., *Anat. Rec.*, 1960, v137, 340.

13. Himes, M., Moriber, L., *Stain Technol.*, 1956, v31, 67.

14. Ovary, Z., *Progr. Allergy*, 1958, v5, 459.

15. Chase, M., *J. Allergy*, 1957, v28, 30.

16. Gyllensten, L., *Acta anat.*, 1950, v10, 130.

17. Bier, O. G., Siqueira, M., Beraldo, W. T., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 29.

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Effectiveness of DTPA in Removing Plutonium from the Pig.* (26553)

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The superiority of diethylenetriaminepentaacetate (DTPA) over chelating agents previously employed for removal of internally deposited plutonium is attested by a number of reports of its effectiveness in small animals (1-5). Its effectiveness in the human has been reported by Norwood(6). To help fill the gap between the rather extensive data on the rat and the very limited experience with man experiments were performed employing miniature swine. These animals have a weight comparable to man, and appear from preliminary studies to metabolize plutonium in a manner similar to man(7). The experiments were designed to measure the effectiveness of DTPA when administered promptly following plutonium injection, and when administered after a long interval following plutonium injection.

Methods. The miniature swine employed were 13-15 month males of the Pitman-Moore strain, confined to metabolism cages throughout the experiment. In the prompt treatment experiment 6 animals weighing from 40 to 60 kg were injected intravenously with 25 μc of Pu^{239}, administered as the tetravalent citrate complex in pH 5 solution. One hour later, 3 of the animals were injected intravenously with 40 ml of a pH 7 solution containing 9 g of the calcium trisodium salt of DTPA. In the delayed treatment experiment two 15 month males weighing 62 and 75 kg, injected 2 months previously with approximately 175 μc of plutonium IV citrate, were given 5 consecutive daily intravenous injections of $CaNa_3DTPA$, one gram the first day and 2 g on each of the succeeding 4 days. Excreta were collected daily in both experiments; daily blood samples were obtained only in the delayed treatment experiment. All animals were sacrificed 6 or 7 days following treatment. Complete tissue analyses were performed on animals from the prompt treatment experiment.

Results of prompt treatment. Table I

TABLE I. Effect of Single Prompt Dose of DTPA on Excretion of Plutonium by Pigs.

	Excretion (% dose/day)			
	Urine		Feces	
Day post inj.	Control*	DTPA treated*	Control	DTPA treated*
1	.6	45.	.1	1.8
2	.1	29.	.3	1.3
3	.2	6.8	.4	1.7
4	.2	.5	.4	.4
5	.1	1.0	.2	.3
6	.1	.3	.1	.1
Total	1.3 (.9-1.9)	83 (78-88)	1.5 (1.2-2.0)	5.6 (3.5-8.9)

* Avg of results from 3 pigs (range in parentheses).

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