Cryo-Immunology: A Method of Immunization to Autologous Tissue.* (31817)

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A number of reports have described the experimental production of autoantibodies to urogenital tissues in the rabbit, and in particular to antigens of the prostate gland and other male accessory tissues of reproduction (1-3). These were elicited by intensive isoimmunization procedures. In a typical series, crude saline extract of pooled accessory tissues was injected intradermally with fortified Freund adjuvant. This adjuvant contained 8 mg/ml of acid-fast bacilli in the first injection, and this quantity was decreased by 2 mg/ml in each succeeding injection. Approximately 10-15 mg of tissue protein was injected each time. Even with this intensive procedure, antibody was detected only after 4-6 weeks, involving 3-4 injections. Such lengthy delays in producing a significant antibody response to isoimmunization injections have also been characteristic of other systems, such as thyroid(4-6) and adrenal(7-9). Recent developments in procedure for surgical treatment of human prostatic disease have shown that cryosurgery can be applied as an effective and controllable method(10-13). Rabbits were therefore exposed to a cryosurgical treatment to explore the possibility that antibodies might be elicited by this procedure.

Materials and methods. A hard-frozen region in the coagulating gland and seminal vesicle area was engendered by contact with a thin probe, carrying liquid nitrogen into and out of the probe tip; various temperature and time data were collected by specially-constructed sensing and recording instrumentation. After 2 or 3 minutes, warming and thawing were permitted, the probe removed, and the appropriate sutures inserted. The operating field and the instrumentation involved can be partially seen in Fig. 1. Details of procedure have been published(14).

Several groups of rabbits have now been examined. These have also included a number of control animals, in which the surgical intervention and emplacement of the probe was identical to the others, but no flow of liquid nitrogen was permitted. In addition to these, there were also several control rabbits in which some other tissue, such as kidney, was frozen, instead of the coagulating gland-seminal vesicle complex.

The content of antibody in the various rabbit sera was evaluated by passive hemagglutination, using human tanned red blood cells, coated with an optimal dilution of extract of normal rabbit coagulating gland. To increasing dilutions of antiserum was added a 2% suspension of the coated cells, and readings were taken after 2-3 hours at room temperature. The patterns were graded as ++, +, ±, or −. Further details have been described(14).

Results and discussion. A number, though not all, of the earlier studied rabbits, in which there had been frozen (coagulating gland) tissue *in situ*, showed evidence of antibody development to this tissue, whereas the control animals did not. Several points were apparent in the positive responses among the first 20-25 rabbits: 1) a significant level of antibody was seen by 4 days and reached a maximum by about 7 days, 2) this maximum was however a modest one, with titers ranging from 1:16 to 1:256, 3) the 7-day titer was generally followed by a drop in antibody level, which was sometimes quite steep. Because there could be some uncertainty regarding the evaluation of positive sera with low titers and of negative sera with slight nonspecific agglutinating activity, a new group of rabbits was treated in order to perform a blind test. It was also believed that the tech-

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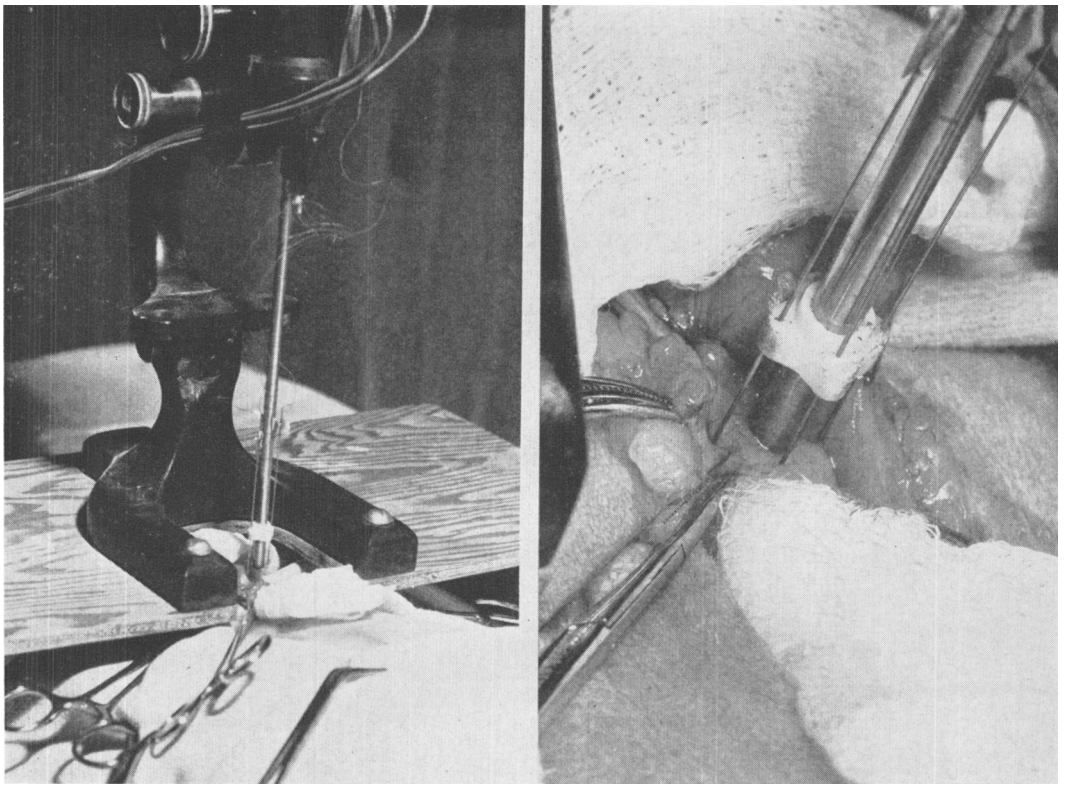

FIG. 1. Apparatus and procedure for cryosurgery of rabbit. At left is shown the rack and pinion assembly (a modified microscope stand) for placing the freezing probe in precise contact with the tissue exposed below. At right is shown the surgical field, with flaps of the cut seminal vesicle pulled back and the probe placed firmly against the surface of the coagulating gland. The attached thermocouple wires are also seen.

niques in surgery and freezing had been improved to the point where most animals should respond.

In this group, 7 rabbits were subjected to a freezing procedure in portions of the seminal vesicle and coagulating gland and, in some cases, part of the vas deferens. One of these animals (rabbit 437) had a coagulating gland which seemed rather atrophic when seen at surgery. Three additional rabbits were subjected to the freezing of a part of a kidney, as an alternative tissue. Two other rabbits were provided the same surgery as the first 7 animals, and the probe was inserted and positioned for the same 3-minute interval, but without freezing. All animals were treated similarly after the surgery. Three bleedings were taken from each animal. These included a pre-surgical sample (designated *p*) and samples on the 4th and 7th days after cryosurgery (designated *a* and *b*). These 36 samples were assembled and transferred, in a random fashion, into a new group of test tubes labeled only as 1, 2, 3, . . . , 36. These coded sera were then tested in tanned cell hemagglutination, using cells coated with extract of coagulating gland, and the readings throughout all the dilution series were agreed upon by several participants. After this, the labels were decoded to give the results shown in Table I. It was seen that not one of the 15 sera from the control animals gave any reaction whatsoever.‡ In contrast, 6 of the 7 other animals, all but the one with an atrophic tissue, gave positive results. Secondly, titers as

‡ This refers only to antibody activity against coagulating gland antigen. Sera from the "kidney" rabbits require evaluation in tests with kidney antigen, but this is not relevant to the central thesis of this report, which is to show unequivocally that a freezing treatment *in situ* can produce an antibody response.

TABLE I. Tanned Cell Hemagglutination.

Blind Test

Cryosurgery Rabbits Group 6

	Rabbits with frozen seminal vesicle and coagulating gland																				
Dilution of serum	R 431			R 432			R 434			R 435			R 437*			R 439			R 440		
	p	a	b	p	a	b	p	a	b	p	a	b	p	a	b	p	a	b	p	a	b
1:8	—	—	+	—	—	++	—	—	++	—	++	++	—	—	—	—	—	++	+	+	++
16	—	—	+	—	—	++	—	—	++	—	++	++	—	—	—	—	—	++	±	+	++
32	—	—	+	—	—	++	—	—	++	—	++	++	—	—	—	—	—	++	—	+	++
64	—	—	—	—	—	++	—	—	++	—	+	++	—	—	—	—	—	+	—	+	++
128	—	—	—	—	—	+	—	—	+	—	+	++	—	—	—	—	—	+	—	+	++
256	—	—	—	—	—	—	—	—	+	—	—	++	—	—	—	—	—	—	—	—	+
512	—	—	—	—	—	—	—	—	—	—	—	++	—	—	—	—	—	—	—	—	++
1024	—	—	—	—	—	—	—	—	—	—	—	++	—	—	—	—	—	—	—	—	++
2048	—	—	—	—	—	—	—	—	—	—	—	++	—	—	—	—	—	—	—	—	—
4096	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—
8192	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

	Rabbits with frozen kidney						Rabbits, non-frozen											
Dilution of serum	R 243			R 297			R 301			R 309			R 438					
	p	a	b	p	a	b	p	a	b	p	a	b	p	a	b			
1:8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			

* The coagulating gland in this rabbit had seemed atrophic before the cryosurgery.

high as 1:4096 and 1:1024 were seen. Thirdly, there was in all responding rabbits a progressive increase in titer with time. Fourthly, the highest titers shown here were achieved in only 7 days. Fifthly, only one of these 7 presurgery sera showed a slight activity,§ but even this was followed by progressive increases.

It is clear that antibodies can be produced in rabbits as a result of freezing procedures. These antibodies are tissue-specific and species-specific, and they are autoantibodies, as reported elsewhere. All previous experimental production of autoantibody has required addition of some kind of adjuvant. Here, no adjuvant has been necessary, the frozen tissue apparently fulfilling this role in some manner. Further studies are in progress to evaluate the molecular size of the predominant antibody at various stages after the freezing incident, and on the results of combining isoimmunization by injection with the process of freezing. Studies have been begun to compare other tissues as freezing immunogens. At this time, it seems appropriate to propose the term "cryo-immunology" to designate this system of phenomena and studies.

Summary. By means of a blind test, in which serum samples from a group of rabbits were relabeled with code designations, it was shown clearly that definite antibody production had occurred as a result of freezing of tissue in the animal. Freezing was done by means of a liquid nitrogen probe and the target tissue was the coagulating gland and seminal vesicle of the urogenital tract. Antibodies were detected by tanned cell hemagglutination and were found to be present within 7 days after cryosurgery. No antibody was detected in any of 15 sera from 5 control animals, whereas 6 out of 7 rabbits exposed to freezing showed positive results. Titers as high as 1:4096 were obtained, and there was a progressive increase with time.

§ This was probably an artifact, as judged by negative results obtained with this same serum in subsequent "open" titrations and comparisons.

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