Table I. Influence of actinomycin D on white blood count

White blood count (cells/mm³)	Day of blood taking 0	3	7
Leucocytes	6639 (5550–8050)	4232 (2500–6850)	9136 (3200–16,950)
Neutrophils	1899 (950–3320)	3242 (2480–4060)	5418 (640–14,750)
%	28.6	76.6	59.3
Lymphocytes	4687 (3340–6040)	986 (630–2800)	3691 (792–5650)
%	70.6	23.3	40.4
Other leucocytes	53	4	27
%	0.8	0.1	0.3

Animals were injected on day 0 with 30 µg of actinomycin D. The mean values of 7 animals are plotted, extreme values in parentheses.

Table II. Influence of actinomycin D on hemagglutinin production

Treatment	Immunization with 1 ml of SRBC	Day of blood taking 0	3	7	14	No. of animals
30 µg actinomycin D on day 0	1 pc.	0	0	4.9	4.9	12
	50 pc.	0	0	4.6	5.5	10
30 µg actinomycin D on day −2	1 pc.	0	0	3.8	4.7	6
	50 pc.	0	0	5.6	4.8	5
Control animals	1 pc.	0	0	3.7	3.3	10
	50 pc.	0	0.7	5.0	3.8	6

Animals were immunized on day 0 with sheep red blood cells (SRBC). Hemagglutinin titers were transformed to ^{2}log of $^1/_{10}$ of reciprocal titer + 1. Mean values of these transformed titers are plotted.

number of which actinomycin D exerts no further effect[18]. On the other hand, the cytotoxicity mentioned cannot explain the increasing neutrophilia. PHILIPS et al.[15] have produced in rats, treated with actinomycin D, an enhancement of neutrophils which was highest on day 4; he suggests that enteric toxins would penetrate the damaged intestinal walls and lead to an endotoxin-like shock. The steady rise of the neutrophils in our experiments, however, does not account for this suggestion; nor does it support a reactive regeneration of the depressed bone marrow, as it was concluded from serial investigations of alkylating agents[19]. In mice GELLER and SPEIRS[16] found no bone marrow leucocytosis which normally appeared after antigen injection, under treatment with actinomycin D.

Actinomycin D acts in a more pronounced way upon early immunologic processes, RNA then being unstable and short-lived[20]. Consequently, hemagglutinins[6], hemolysins[8] and antiglobulins[11] appear later than normal. However, though actinomycin D was given at the same time as antigen, our experiments showed no depression of the antibody titers, according to findings of WUST et al.[11], GELLER and SPEIRS[10] and DOBBS et al.[9]. We therefore injected the antigen at the time of lymphatic depletion, but no lowering of antibody titer could be observed either. Since increasing amounts of antigen are known to augment the effect of an immunosuppressive drug[20,21], a further attempt was made by varying the dose of sheep erythrocytes, but results remained unchanged.

Unchanged antibody response in combination with lymphopenia – as it has been observed under the conditions of these experiments – suggests a resemblance with the reaction of adult animals to thymectomy.

Zusammenfassung. Actinomycin D führt bei erwachsenen männlichen Hamstern in einer Dosis, die für 15% der Tiere letal ist, zu einem vorübergehenden Abfall der Lymphozyten und zu einem kontinuierlichen Anstieg der neutrophilen Granulozyten. Die Bildung agglutinierender Antikörper gegen Schaferythrozyten wird dagegen, unabhängig von der Antigendosis und vom Zeitpunkt der Antigenapplikation, durch Actinomycin D nicht beeinflusst.

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Fluorescent Studies of Antibodies to Rabbit Male Urogenital Tissue

Recent studies initiated in our laboratory employing the methods of tanned cell haemagglutination and gel diffusion precipitation have demonstrated that *multiple* in situ freezing of rabbit male urogenital tissue (coagulating gland and seminal vesicles) by means of a liquid nitrogen-cooled probe at intervals of 30 days elicits a more pronounced and consistent antibody response than that previously reported for a *single* freeze[1,2] analogously to the classical secondary or 'booster response'[3–6].

This report sets forth our initial observations employing the fluorescent antibody (FA) method for the possible histologic localization and identification of the antigen(s) involved in the immunologic response to freezing of rabbit male urogenital tissue.

Surgical methods and in situ freezing of male urogenital tissue of the rabbit by means of a liquid nitrogen-cooled

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probe, as well as the monitoring and control systems were employed as previously described[1,2]. Antisera employed for study by the FA method were selected on the basis of their activity as previously determined by tanned cell haemagglutination and gel diffusion precipitation[3-6].

FA staining for sensitivity and specificity of sera were carried out by established procedures[7-9]. Tissues were quick-frozen in liquid nitrogen, sectioned at 4 μ in a cryostat, treated (unfixed) with serial dilutions of rabbit sera and with a fluorescein conjugated goat anti-rabbit IgG preparation with an apparent F:P ratio of 3.56, used at $^1/_4$ unit of anti-IgG/ml. All titrations were tested with a constant dilution of conjugate. Sera were stored at -15 °C until used. Specifics with regard to methods of conjugate preparation and standardization have recently been reported[10].

Sera of 3 out of 8 male rabbits subjected to *multiple* in situ freezing of their urogenital tissue yielded characteristic fluorescent staining patterns when tested on sections of rabbit coagulating gland.

Figure 1 illustrates the appearance of a positive reaction obtained with the serum (1:20 dilution) from a male rabbit subjected to freezing of its urogenital tissue. A diffuse fluorescence appeared to be predominately localized to the cytoplasm of acinar epithelial cells and to material within the lumen. On occasion, non-specific staining of the cytoplasm was observed. However, this was readily distinguished from specific staining through utilization of appropriate controls included in each experiment. Figure 2 is a comparable photomicrograph of a negative control reaction obtained with a similar section of coagulating gland tested with a 1:20 dilution of normal rabbit serum.

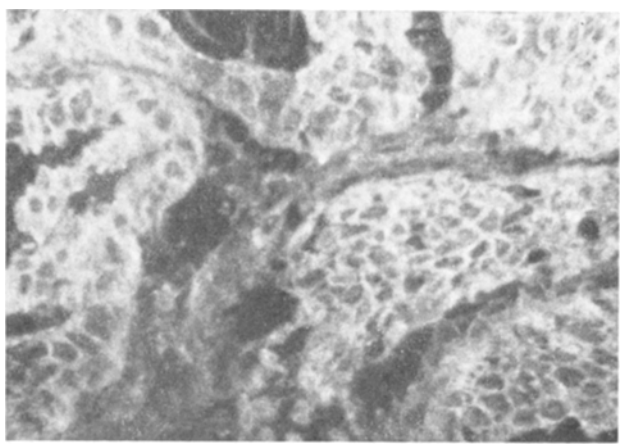

Fig. 1. Fluorescent antibody stain of a section of rabbit coagulating gland with a 1:20 dilution of serum from a rabbit subjected to *multiple* freezing of its urogenital tissues. Diffuse fluorescence appears to be predominately localized to the cytoplasm of the acinar epithelial cells and material within the lumen. × 600.

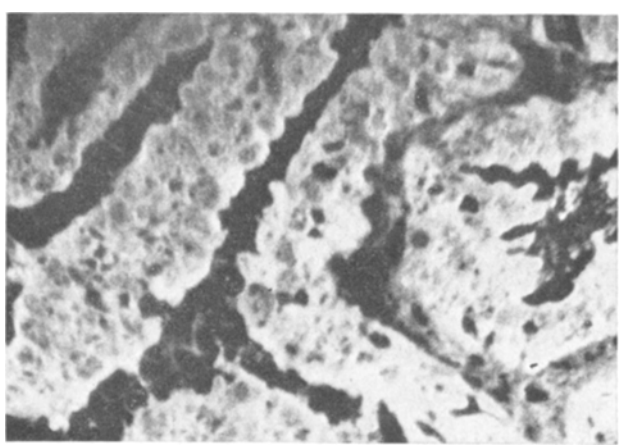

Fig. 2. A field comparable to the one shown in Figure 1, with a 1:20 dilution of normal rabbit serum. This is a negative reaction. × 600.

Preliminary fluorescent antibody studies of sera obtained from male rabbits subjected to *multiple* in situ freezing of their urogenital tissue suggests that these sera contain antibodies reactive with the cytoplasm of acinar epithelial cells and material, possibly secretory products, lying within the lumen of the acinus, of the coagulating gland. The latter finding lends support to the results of recent studies in which a normal secretory product of the coagulating and prostate glands-'coagulo-prostatic fluid' was found to possess comparable activity and specificity to that of male urogenital tissue when tested by haemagglutination and precipitation with antisera produced as a consequence of either isoimmunization with extracts of male urogenital tissue or *multiple* in situ freezing of male urogenital tissue[3,5,6].

Present results suggest that acinar epithelial cells of the coagulating gland and/or one of their secretory products, e.g., 'coagulo-prostatic fluid', are a major source of the antigen(s) responsible for the evocation of an immunologic response to either isoimmunization or *multiple* in situ freezing of male urogenital tissue. Further studies, presently in progress, are needed to evaluate the specificity of the present histologic localization and identification of the antigen(s) involved in the immunologic response to isoimmunization and/or to freezing of rabbit male urogenital tissue[11,12].

Zusammenfassung. Immunofluoreszenzoptische Vorversuche mit Seren männlicher Kaninchen, deren Koagulationsdrüsen Schädigung durch wiederholtes Tieffrieren in situ ausgesetzt waren, lassen vermuten, dass diese Sera Antikörper enthalten, die mit dem Zytoplasma von Azinusepithelzellen und anderen Anteilen der Koagulationsdrüsen reagieren.

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One of us (R.J.A.) expresses appreciation to Professor ERNST H. BEUTNER for his instructions in the principles and application of the fluorescent antibody method.
[12] Supported by a grant from the John A. Hartford Foundation, Inc.