magglutinating antibody N was detected at levels ranging from 0.015 γ to 0.007 γ, whereas PCA detected antibody N at levels ranging from 0.011 to 0.018 γ.

Discussion. In routine experiments, antibody to chemical allergens is usually determined by the method of PCA, and this method has been considered to be adequately sensitive. In addition to expense, it should be noted that there is some variation in susceptibility of individual animals to PCA. The method described above is relatively inexpensive, sensitive, and readily reproducible. Demonstration of the capacity of erythrocytes to be directly conjugated to chemical allergens suggests that this method may be employed for tracing antibodies to certain allergenic drugs used in treatment of human disease. The ability to use formalinized cells affords the opportunity of applying varied chemical procedures to effect conjugation without disturbing the physical integrity of the cells. Further, a constant and standard source of erythrocytes is available when these procedures are employed. Despite the apparent sensitivity of this *in vitro* method, it is obvious that extensive comparative studies with PCA should be carried out. Animals are currently being immunized to produce a sufficient quantity of hyperimmune sera for this investigation.

Summary. An *in vitro* method for demonstration of antibodies to chemical allergens, using formalinized erythrocytes is described. Results are reproducible and the test can be specifically inhibited. Except for chemicals of related structure, cross reactions were not observed.

The author gratefully acknowledges the technical assistance of Dianne Holland, Claudette Springle, and Claude Torrey.

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Received November 21, 1961. P.S.E.B.M., 1962, v109.

The Seminal Vesicle as the Source of the Spermatozoa-Coating Antigen of Seminal Plasma.* (27270)

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The seminal plasma of man and other mammals contains a strongly antigenic and highly organ and species specific compound (1,2; for additional references see 3). In man and rabbit, this antigen coats the spermatozoa during their passage through the adnexal structures of the male genital tract. This coat dominates the antigenic behavior of seminal spermatozoa in contradistinction

* Aided by a grant of Nat. Inst. Health, U.S.P.H.S.

to that of the testicular ones(4,5). Isoantigenicity of the spermatozoa-coating antigen (SCA) has been demonstrated in the rabbit (6).

As to the point of origin of SCA, previous studies revealed that vesicle fluid and aqueous extracts of seminal vesicles and of prostate react with anti-SCA sera in complement fixation tests(1,2). The current report presents evidence obtained by means of immunofluorescence showing that SCA originates in the seminal vesicles.

Materials and methods. Preparation of immune sera and their use *in vitro* has been described(1-6). Human semen and "testicular" spermatozoa(4,7) were collected for this work in the offices of Dr. John R. Herman and Dr. Leo Wilson.

Organs from rabbits sacrificed for study were quick frozen and cut into 5 μ slices in the Cryostat (Harris, Cambridge 38, Mass.) according to the method of Coons(8,9). Although indirect staining can be used, best results were obtained by direct staining with globulin solutions conjugated with fluorescein isothiocyanate(10). The only deviation from the generally used technic consisted in dipping the sections fixed on slides for 10 minutes in 1:10 diluted neutral commercial formalin, followed by washing with phosphate buffered saline (0.01 mol., pH 7.2), before staining with the fluorescent globulin solution. This eliminates non-specific fluorescence which otherwise tends to interfere with the specific reaction. Formalin in the concentrations used does not affect the immunological activity of SCA. Thus exposing seminal spermatozoa for one hour to 1 or 10% concentrations of formalin or adding 1% formalin to seminal plasma did not change their *in vitro* reactivity with anti-SCA immune serum.

Specificity of the staining reactions was controlled by use of fluorescein-conjugated globulins from normal sera and anti-human and anti-rabbit serum immune sera (produced in rabbit and guinea pig, respectively). Furthermore, fluorescein-conjugated anti-rabbit seminal plasma immune serum was absorbed with rabbit seminal plasma and tested for loss of its specific staining activity.

A Reichert Fluorex microscope was used with an Osram HBO 200 Mercury lamp and filters BG12, C-5113, C-8078 and excluding filter GG9 or, for photographic purposes, OG-1. Agfa Isopan Record film was employed for black-and-white and Super Anscochrome Daylight film for colored photography with a 35 mm Reichert camera.

Results. Seminal spermatozoa of man and rabbit show a delicate coating of apple green fluorescence encompassing both head and tail. The neck is often, but not always, spared on staining with the specific immune globulin conjugate (Fig. 1). No staining is seen with the control preparations. Spermatozoa from the epididymis of the rabbit and human spermatozoa from spermatoceles do not accept the stain.

The seminal vesicle of the rabbit is chambered. Sections stained with anti-SCA immune globulin conjugate show apple-green fluorescence localized as follows: The lumen of the chambers is heavily outlined by a continuous layer of fluorescent material. The epithelial cells contain numerous fluorescent granules (Fig. 2). Glands within the muscularis harbor very numerous, small, intracellular fluorescent granules, and their collecting ducts are filled with fluorescent material (Fig. 4). The muscularis itself and the stroma show no staining. Anti-SCA conjugate that had been absorbed with seminal plasma lacks the ability of specific staining (Fig. 3).

Testicle, epididymis, and prostate do not accept staining with anti-SCA immune conjugate. When preparations of the prostate included cross-sections of the seminal duct, this structure was seen to contain fluorescent material within its lumen. However, the epithelial lining does not harbor fluorescent granules. This shows the ejaculatory duct in its role as an outlet for the SCA containing fluid, but lacking indications that the duct's epithelium actively participates in production of SCA.

Discussion. Human and rabbit seminal spermatozoa show a delicate coating with antigen if stained by fluorescein-conjugated anti-seminal plasma immune globulin. This coat is lacking in epididymal rabbit sperma-

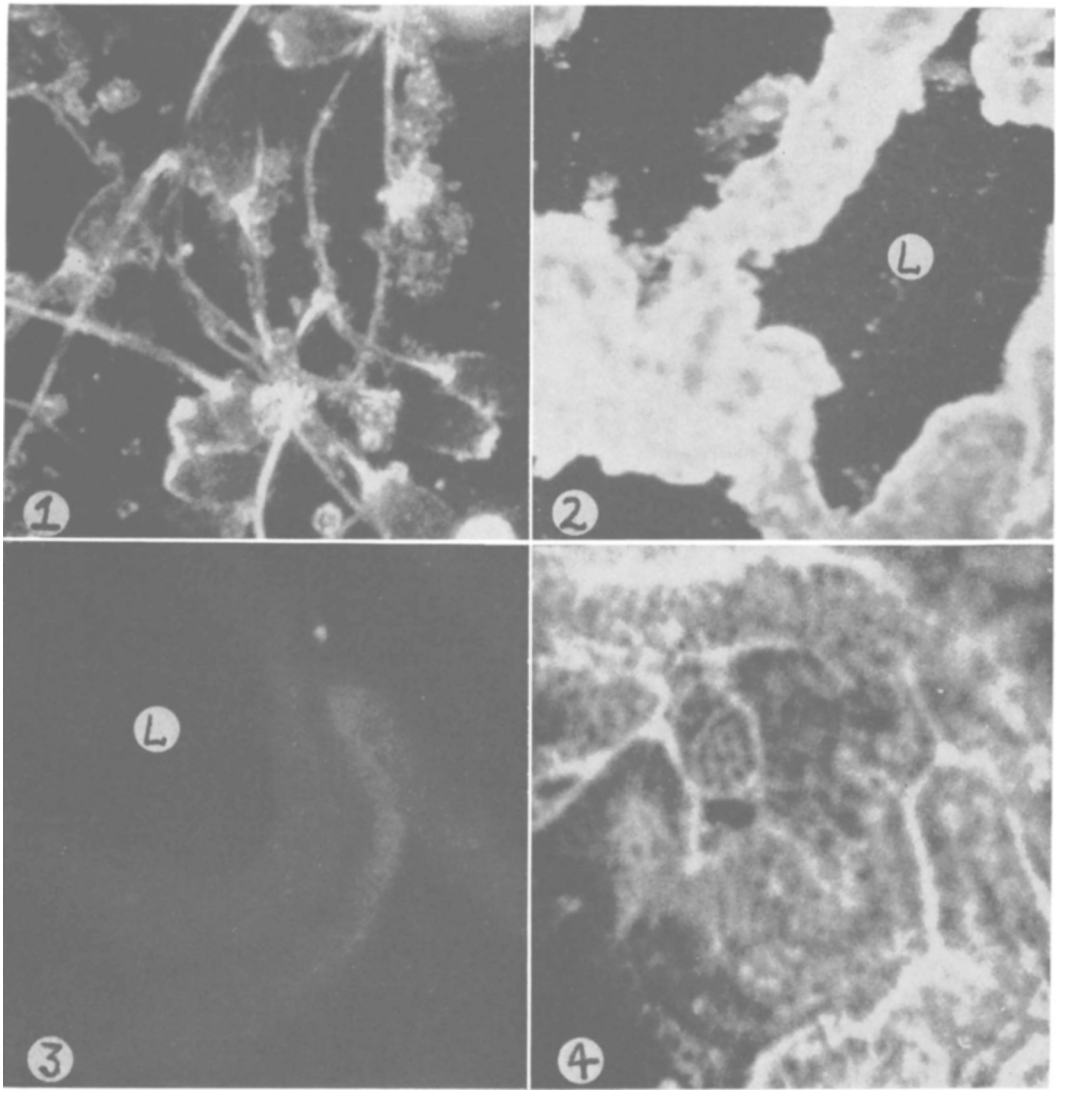

FIG. 1. Seminal spermatozoa of the rabbit stained with fluorescein-conjugated anti-rabbit seminal plasma globulin. (Small globules represent granular material of unknown nature characteristically present in rabbit semen. Their fluorescence is pale blue in contrast to the apple green of coating of spermatozoa.)

FIG. 2. Seminal vesicle stained with globulin as in (1).

FIG. 3. Seminal vesicle stained with anti-SCA fluorescein-conjugated globulin absorbed with seminal plasma. L = lumen of vesicle.

FIG. 4. Intramural gland of seminal vesicle stained as in (1).

Figures magnified *ca* 2½ times from originals taken at 1000× (Fig. 1) or 200× (Fig. 2 to 4).

tozoa and those obtained from human spermatoceles. This is in keeping with the results of previous tests(4,5).

Within the male genital tract of the rabbit, only the seminal vesicle yields fluorescent staining with anti-SCA immune globulin conjugate. The epithelial cells, both those lining the lumen and those of the glands within the muscularis, contain numerous fine, fluorescing granules and are, therefore, believed to be the site of production of SCA. The staining of the collecting ducts of the latter structures and the layer of fluorescent material along the border of the lumen dem-

onstrate the secretion of SCA into the fluid content of the organ. These findings are in keeping with the data reported previously (1,2).

SCA was also found to be present within the ejaculatory ducts. This explains the reactivity of prostate extracts with anti-SCA sera(1,2), because the ejaculatory ducts necessarily are included in these extracts.

Summary. Fluorescein-conjugated anti-seminal plasma immune globulin stains specifically human and rabbit seminal spermatozoa. Testicular spermatozoa lack reactivity with this reagent for SCA. The study of the components of the male genital tract of the rabbit indicates that SCA is produced in the seminal vesicle.

We gratefully acknowledge the opportunity of learning much about the technical aspects of these procedures during visits to the laboratories of Drs. E. Witebsky, and E. H. Beutner, Univ. of Buffalo School of Medicine, and Drs. B. Seegal and K. C. Hsu, College of Physicians and Surgeons, New York.

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Received November 29, 1961. P.S.E.B.M., 1962, v109.

The Kinetics of Heat Inactivation of Bovine Serum Agglutinins for Brucella.* (27271)

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Hess and Roepke(1), by a chromatographic method, demonstrated the presence of agglutinins for Brucella in bovine sera with low titers from herds apparently free of brucellosis. The method distinguished these apparently nonspecific agglutinins from the classical type of agglutinin in sera from Brucella-infected cows.

Differential tests to distinguish between nonspecific and specific agglutinins based on heat inactivation of the nonspecific agglutinin were devised by Hoerlein(2), Morse *et al.*(3) and Amerault *et al.*(4). The acidified plate antigen (APA) test of Rose and Roepke (5) also differentiated the nonspecific from the specific agglutinins.

Field studies have shown that the 18-hour tube agglutination test at 56°C(3) could not consistently distinguish the serum of Brucella-infected cows from the serum of cows which contained the nonspecific agglutinin; however, the 15-minute tube agglutination test at 65°C(4) gave results in better agreement with epidemiological, bacteriological, and clinical information. These data suggested that nonspecific agglutination reactions might be caused by several nonspecific agglutinins of differing heat labilities.

This report provides quantitative data on studies of heat lability of 3 types of agglu-

* Taken in part from a thesis submitted by the senior author to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Ph.D. degree.

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‡ These studies were supported in part by a grant from the Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture. Submitted as paper 4311, scientific journal series, Minnesota Agricultural Experiment Station, University of Minnesota.