ASPERMATOGENESIS IN THE GUINEA PIG INDUCED BY TESTICULAR TISSUE AND ADJUVANTS

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Rabbits injected with rabbit lens protein produce precipitins against this antigen, but the lenses of the immunized animals show no histological changes (for literature, see reference 1). It has also been shown that the injection of homologous spermia into guinea pigs results in antibody formation against these cells; again, no changes were seen in the testes of the immunized animals (2). But by often repeated injections of material from the central nervous system (CNS) into monkeys Rivers and his associates (3) succeeded in inducing the formation of circulating antibodies and, in addition, a small proportion of the animals developed sterile disseminated encephalomyelitis. By the use of adjuvants, furthermore, similar effects were readily accomplished in monkeys, guinea pigs, and other species of animals (4). Again, the same disproportion between the production of circulating antibodies and the incidence of organic lesions was observed (4 *c*, 5).

Witebsky and Steinfeld (6) found an alcohol-soluble organ-specific antigen in the brains of different species of animals. Later, Lewis (7) observed that the testes and brain possess a common alcohol-soluble antigen; sera of rabbits immunized with brain extracts fixed complement in the presence of extracts from testes as well as from brain. From these observations it would seem possible that pathological changes might be found in the testes of guinea pigs which have been injected with CNS tissue and adjuvants; indeed those which develop encephalitis thereby would merit special attention in this regard. Lumsden (8), however, has reported that in the guinea pig the testes remain normal following the injection of CNS tissue and adjuvants and that, conversely, the CNS is not affected by the injection of testicular material combined with adjuvants. The observations of Lumsden were based on subcutaneous injections, whereas it has been found recently (9) that the intracutaneous route is far more effective than the subcutaneous for the production of allergic encephalitis. It seemed desirable to repeat the experiments of Lumsden using intracutaneous injections, particularly, because his work left unanswered the interesting question,

whether the introduction of testicular tissue and adjuvants into the skin of guinea pigs results in injury to the gonad. This problem was studied recently by Voisin, Delaunay, and Barber (10). These authors found injury to the germinal cells, orchitis, and epididymitis in guinea pigs injected with homologous testicular material and adjuvants. Their efforts to demonstrate specific circulating antibodies (immobilizing antibodies) failed. Furthermore testicular injury was found not only in the guinea pigs injected with testicular tissue but also in others receiving suspensions of liver or kidney combined with adjuvants. For these reasons and because their animals became emaciated or died shortly after injection, Voisin and associates concluded that the observed testicular damage was not an immunological phenomenon but the result of "stress."

The experiments to be presented show that the injection of a suspension of homologous or autologous testicular material combined with paraffin oil and killed myobacteria into guinea pigs produces aspermatogenesis without inflammation and also the formation of specific antibodies. Control experiments of various kinds seem to indicate that the aspermatogenesis so induced is organ- and species-specific; thus it is of immunological nature.

Materials and Methods

Several types of testicular antigens[1] were used, including whole tissue suspension, "mitochondrial" fraction, alcoholic extract from testis, and spermia. The whole tissue suspension was prepared by freeing the testis from the tunica albuginea and suspending the finely minced organ in an equal volume of distilled water containing 0.25 per cent phenol using a TenBroeck grinder or Waring blendor. The fraction of tissue designated as "mitochondrial" was prepared by differential centrifugation of the aqueous suspension of testis (11). The alcoholic extract was made by repeated extraction of testis with 95 per cent ethanol as described by Lewis (7). To obtain spermia, saline solution was injected into the ligated vas deferens and the epididymis incised (12). The suspension of cells was spun and taken up in phenolized distilled water.

Suspensions of CNS tissue, liver, and kidney were also used. Brain or spinal cord was suspended in phenolized distilled water in concentrations ranging from 5 to 100 mg. per ml. Liver and kidney tissues were mixed with equal amounts of phenolized water. All tissues were from the guinea pig save spinal cord which was obtained from the rabbit.

Unless stated otherwise, the antigenic material was combined with paraffin oil and killed mycobacteria. For this purpose, water-in-oil emulsions were made in the following way. Arlacel A (an emulsifying agent) and paraffin oil were mixed in the proportion of 1.5 to 8.5 and autoclaved. A weighed amount of killed tubercle bacilli (strain Jamaica 22) or *Mycobacterium butyricum* was suspended in the mixture. The aqueous suspension of antigen was emulsified in the oily phase with the aid of a syringe. Equal volumes of each were employed (for other information regarding the material and technique see reference 13).

In order to detect and measure the immune response of the injected guinea pigs, several serological tests were used: complement fixation, immobilization tests, and a test for antibodies against hyaluronidase. The blood samples were taken, as a rule, when the animals were killed to obtain the organs for histological observations. The sera were stored at −10°C. For the

[1] A large number of guinea pig testes were obtained through the generosity of Mr. C. N. Wentworth Cumming of Carworth Farms, New City, New York.

immobilization and complement fixation tests they were inactivated at 56°C. for 30 minutes: for antihyaluronidase tests the sera were not heated.

In the *complement fixation tests*, as a rule, freshly obtained and washed spermia were used. To 0.2 ml. of twofold serial dilutions of inactivated sera were added a suspension of spermia (2.4×10^7 spermia) and 2 units of complement. The mixtures were kept for 1 hour at 4°C. and for 1 hour at 37°C. Then, a 2 per cent sheep red blood cell suspension and 2 units of hemolysin were added. The volume of each reagent was 0.2 ml. The tubes were incubated at 37°C. for 30 minutes and then read. *Immobilization tests* were made by mixing 0.1 ml. of serum dilution, 0.1 ml. of guinea pig complement, and 0.1 ml. of a saline or 5 per cent glucose suspension of spermia. After 10, 20, and 30 minutes' incubation at 37°C., the percentage of active spermia was estimated. *Antihyaluronidase titrations* were made employing guinea pig testicular hyaluronidase partially purified by ammonium sulfate precipitation (14). The hyaluronic acid used was of mammalian origin.[2] Sera, hyaluronidase, and hyaluronic acid were diluted with 0.1 M acetate buffer at pH 6 in 0.15 M sodium chloride solution. A mixture of 0.1 ml. of enzyme solution (the minimal amount hydrolyzing 0.125 mg. of substrate) and 0.2 ml. of twofold serial dilutions of sera were incubated at 37°C. for 30 minutes. Then, 0.125 mg. of hyaluronic acid in 0.25 ml. buffer solution was added to each tube. After further incubation at 37° C. for 30 minutes, 1.5 ml. of horse serum reagent (1:40 dilution of normal horse serum in 0.5 M acetate buffer at pH 3.4) was added to detect the presence of unhydrolyzed hyaluronic acid. After an additional incubation at 37°C. for 30 minutes, the degree of turbidity was noted. The immune sera did not neutralize bovine hyaluronidase.[2]

Effort was made to ascertain whether the aspermatogenesis induced affects the hyaluronidase content of the testis. Since the histological findings were almost identical in the two testes of any given animal, usually one of the gonads was sectioned and the other used for the estimation of enzymic activity. The hyaluronidase content was measured in the following way. A 50 per cent suspension of testis in 0.1 M acetic acid was prepared with a TenBroeck grinder and cleared by centrifugation. Constant amounts of hyaluronic acid were mixed with 0.2 ml. of a series of twofold dilutions (in 0.1 M acetate buffer at pH 6) of the supernate. After incubation at 37°C. for 30 minutes, 1.5 ml. of horse serum reagent was added to each tube. The highest dilution of testis supernate causing hydrolysis to such a degree that no precipitate was obtained on addition of horse serum was taken as the end-point.

The guinea pigs used weighed from 400 to 600 gm. The antigenic material was injected into the skin in five to seven sites (0.1 ml. per site) on both sides and about 2 cm. from the spine, unless otherwise stated. The animals were observed frequently and weighed weekly. They were killed at varying intervals of time after injection. In some pigs, one testis was removed prior to sacrificing the animal. The gonads and seminal vesicles were weighed and put into Bouin's fixative, and the organs into 10 per cent formalin. All animals gained weight normally, except those injected with CNS tissue.

EXPERIMENTAL

1. Production of Aspermatogenesis by the Injection of Testicular Material

The first attempt to determine whether the intracutaneous injection of testicular material plus oil and mycobacteria results in injury to the gonads was made with whole testis suspension. Six animals were injected once with 125 mg. of testis and 1 mg. of mycobacteria in water-in-oil emulsion. The testes were removed from 14 to 153 days after injection. All the gonads exhibited

[2] Bovine hyaluronidase and mammalian hyaluronic acid were received through the courtesy of Schering Corporation, Bloomfield, New Jersey.

aspermatogenesis. There were no histological changes either in the seminal vesicles or in the prostate. The secretion in the vesicles was abundant. Histological examination of other organs including the CNS, spleen, liver, kidney, and lungs revealed no pathological changes. The sites of injections and their regional lymph nodes showed the characteristic morphological response (15).

The pathological findings in the testes of these guinea pigs and of others to be mentioned later may be described as follows. The essential changes are confined to the germinal cells in the seminiferous tubules of the testis and may be reflected by the cells found within the lumina of the tubules of the epididymis. In far advanced cases, the Sertoli cells may undergo hyperplasia and later necrobiosis followed by destruction of the basement membrane of the seminiferous tubules with subsequent fibrosis.

When damage is mild or moderate its distribution is patchy, the degree of injury varying considerably in the tubules even within lobules.

The pathological process ending in aspermatogenesis usually begins with the diminution of the number of mature spermia and the appearance of numerous immature spermia in the lumen of the seminiferous tubules; as a rule, there are a few swollen exfoliated cells in some tubules. In the epididymis there are many immature spermia whose spherical non-pyknotic heads are in rouleau formation mingled with a few mature spermia and exfoliated spermatogenic cells. The moderately advanced stage is characterized by the absence of spermia, conspicuous degeneration, and exfoliation of the spermatogenic cells involving the spermatogonia last. The cells may be swollen, vacuolated, or pyknotic and large multinucleated cells with acidophilic cytoplasm appear. There are usually "mucoid," eosinophilic wavy masses in the seminiferous tubules extending from the periphery toward the lumen. The epididymis usually exhibits the same kind of exfoliated cells.

As the process advances the spermatogonia become scant and later they disappear altogether, while the Sertoli cells may undergo hyperplasia. Conspicuous by their elongated nuclei, they may become numerous, even forming incomplete rows separated from the basement membrane. At this stage, or even before, the tubules of the epididymis may be free of cells but contain some eosinophilic amorphous material. Throughout these stages, the interstitial space between the tubules remains unchanged. The connective tissue cells do not multiply, nor do inflammatory cells appear.

In extreme stages, apparently the number of seminiferous tubules may be diminished, their size reduced, and atrophied testes may show conspicuous fibrosis. Occasionally, one may find focal accumulations of mononuclear cells, lymphocytes, and plasma cells.

It is noteworthy that in all these stages the Leydig cells are unaffected (they may *appear* hyperplastic), the tubular epithelium of the epididymis and that of the seminal vesicles remain normal with abundant and coagulable fluid in the vesicles.

Damage was graded as follows:—

1+, Very few mature spermia present; immature spermia in the lumen; swelling and vacuolization of a few of the spermatogenic cells, some of these being exfoliated.

2+, May or may not show a few mature spermia; swelling, vacuolization, and exfoliation of fairly large number of spermatogenic cells.

3+, No mature spermia present; swelling, vacuolization, and exfoliation of a large number of spermatogenic cells.

4+, Damage ranging from partial to complete devastation of the tubules, with only Sertoli cells and the basement membrane remaining (Figs. 1 to 3).

To complement the morphological observations, the hyaluronidase content of a few normal and severely injured testes was compared. The supernates of 50 per cent suspensions of unaffected gonads hydrolyzed hyaluronic acid in dilutions up to 1:64 or 1:128 while those with 4^+ damage were inactive in a dilution of 1:2.

In order to ascertain whether the mycobacteria are essential to produce the described effect, a second group of seven guinea pigs was injected with whole testis suspension emulsified in paraffin oil omitting tubercle bacilli. Although three of the animals received a second injection 47 days after the first one, the testes of all of them were normal when removed 21 to 83 days after the first injection.

It has been shown that the "mitochondrial" fraction of testicular suspension (11) and the alcoholic extract of testis (7) are antigenic. These fractions were combined with adjuvants and injected into groups of guinea pigs to learn whether they induce aspermatogenesis. Each of a group of guinea pigs was injected with 1.88 mg. (dry weight) of *"mitochondrial"* fraction derived from 260 mg. (wet weight) of testis and 1 mg. of mycobacteria in 0.5 ml. of emulsion. Aspermatogenesis was observed in all animals killed between 23 and 105 days after injection. *Alcoholic extract* derived from 340 mg. of testis combined with paraffin oil and mycobacteria was injected into each of eight guinea pigs. Some of the animals received a second and some a third injection. No testicular damage occurred.

The use of *spermia* rather than whole testis suspension or its fractions has the advantage of employing a single type of cell. Therefore, spermia were substituted for testicular suspension in several further experiments. Groups of guinea pigs were injected with: (1) spermia emulsified in oil and mycobacteria; (2) the same material with no bacteria; (3) spermia in aqueous suspension. Each animal received 4×10^7 spermia except for five animals in group 1 that were given ten times fewer cells. In group 1, the testes of seven animals were removed from the 3rd through the 9th day and found to be normal. From the 10th to the 20th day, moderate damage was seen in the testes of five of nine animals. All of fourteen animals killed from 21 to 161 days after injection showed damage, severe in nine animals and moderate in five. Of the five injected with the smaller number of cells only one had severe injury. In group 2,

all twelve animals killed from the 18th to the 124th day were without damage save one killed on the 124th day which showed slight damage. During the same interval of time, the testes of five animals of group 3 were found to be normal.

It was of considerable interest to know whether testes of sexually immature guinea pigs would induce aspermatogenesis when injected into adult animals. Testes of 2-week-old guinea pigs containing no spermia were suspended in distilled water. Each animal was given 0.6 ml. of water-in-oil emulsion containing 150 mg. of tissue and 1.2 mg. of *Myco. butyricum*. Of three animals injected with this material, two had moderate (2^+) and one slight testicular damage (1^+).

It has been shown that spermia parenterally administered to guinea pigs from which they were obtained will stimulate antibody production against the guinea pig's own spermia (immobilizing antibodies), but without the occurrence of testicular damage (2). In order to see whether aspermatogenesis can be caused by the use of autologous tissue, each of three guinea pigs was injected with a suspension of one of its own testes, emulsified with paraffin oil, containing 1.4 mg. of *Myco. butyricum*. Three others were injected in a similar manner, but with their own spermia and adjuvants. In both groups the remaining testis was removed from 27 to 77 days later, and found to be severely damaged (3 to 4^+).

To ascertain whether mycobacteria need be in intimate association with the antigen to exert the adjuvant effect, guinea pigs were injected intracutaneously in three sites on the left side with a water-in-oil emulsion containing spermia (4.3×10^7 spermia in 0.3 ml.), and on the right side in three sites with a suspension of 0.3 mg. of *Myco. butyricum* in 0.3 ml. of paraffin oil. No testicular injury was seen in any of the six animals so injected. One animal receiving 0.3 ml. of the same batches of material, but combined as usual, reacted with characteristic changes in the testes.

2. Production of Aspermatogenesis by Injection of Tubercle Bacilli and Oil in the Neighborhood of the Testes

It has been found (16) that when killed tubercle bacilli in paraffin oil are introduced in the subcutaneous tissue, tubercles may appear not only at the site of injection and draining lymph nodes but also in the lungs, namely, in subpleural situations. Furthermore, we have observed that killed mycobacteria in paraffin oil injected directly into the testis of the guinea pig cause aspermatogenesis.

Prompted by these observations, we injected 3 groups of guinea pigs with killed tubercle bacilli in oil in three different sites. These sites were not used in the main experiments. The 1st group of six animals was injected with either 0.4 mg. tubercle bacilli in 1 ml. or 0.2 mg. of organisms in 0.5 ml. of paraffin oil *subcutaneously over the abdomen*; the 2nd group of guinea pigs was injected with the same material *intraperitoneally*; a 3rd group was injected with 0.8 mg. tubercle bacilli in 0.4 ml. oil *intracutaneously into four sites* (0.1 ml. per site) *of the inguinal regions*. They were killed from the 15th to the 49th day after injection.

In group 1, two of the six animals had a moderate degree of testicular damage. In group 2, with intraperitoneal injection, all the guinea pigs showed sterile peritonitis. The tunica albuginea was also involved; there the changes ranged from diffuse edema to focal accumulation of large numbers of mononuclear cells, epitheloid cells, a few giant cells, and oil vacuoles. Polymorphonuclear leukocytes were not seen. In three of the animals the testicular injury was advanced, in two moderate, and in one slight. It is of interest that intertubular inflammatory reaction in the testes was absent or inconspicuous. In group 3, two of the animals had a considerable inhibition and three others a slight inhibition of spermatogenesis. The tunica albuginea was edematous in three animals. Thus, the *regional* injection of tubercle bacilli and paraffin oil either into the skin or the subcutaneous tissue or the abdominal cavity resulted in an inhibition of spermatogenesis.

We have avoided the gonadal regions and chosen sites near the spine and above the lumbar region for the injection of tissue suspensions and adjuvants. That killed mycobacteria in oil injected into the selected sites are free from aspermatogenic effect is amply shown in our control experiments including those with suspension of liver or kidney incorporated in oil containing mycobacteria.

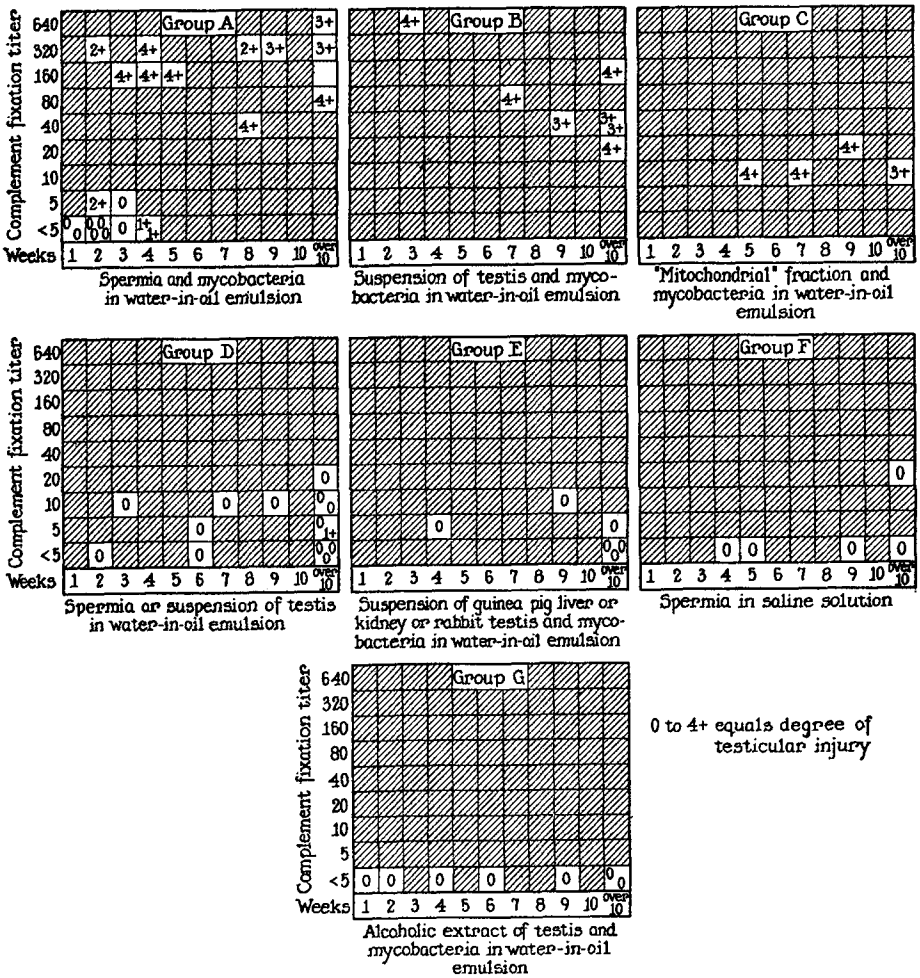
3. Antibody Formation after the Injection of Testicular Antigen

Antibodies fixing complement in the presence of spermia were demonstrated in the sera of guinea pigs injected with spermia (group A) or a suspension of testicular tissue (group B) combined with adjuvants. It can be seen from Text-fig. 1 that there is an apparent correlation between testicular injury and complement-fixing antibody titer after the injection of either testis or spermia with mycobacteria, and that when mycobacteria were omitted (group D) the testes were normal and the complement-fixing titers were low. However, the relationship between pathological changes and circulating antibody may not be a causal one since with "mitochondria" plus adjuvants (group C) the degree of testicular injury is high yet the complement-fixing antibody titers are low, not higher than in group D with no testicular injury.

Other groups of animals with no testicular injury were also tested for complement-fixing antibodies. They were injected with either kidney or liver or rabbit testis plus adjuvants (group E) or guinea pig spermia in salt solution (group F) or alcoholic extract of guinea pig testis plus adjuvants (group G). In these animals, which may be considered as controls, the antibodies were either not demonstrable or the titers were very low. Not included in the text-figure are the tests on sera of guinea pigs with testicular injury following the injection of CNS tissue. These sera did not fix complement in the presence of spermia.

Immobilizing antibodies in a titer of 1:20 to 1:80 were found in the sera

of guinea pigs injected with homologous testis or spermia in adjuvants with or without mycobacteria. There was no apparent correlation between immobilizing antibody titer and degree of testicular injury.



TEXT-FIG. 1. The relationship of complement-fixing antibody titer, degree of testicular injury, and time. (By mistake the hatching was omitted from the blank square in the upper left hand block.)

Antihyaluronidase titers ranged in the group with testicular suspension and adjuvants from 1:64 to 1:1024, most of them being 1:256 or higher; in the group with spermia and adjuvants the titers ranged from less than 1:8 to 1:512, the majority being 1:32 or lower. Sera of normal guinea pigs did not inactivate hyaluronidase when diluted beyond 1:8. There was no correlation between testicular injury and antihyaluronidase titer.

4. The Effect on Spermatogenesis of Tissue Antigens Other Than Homologous Testis

Since the observations on antibody formation suggest that the aspermatogenesis is induced by an immunological process, it seemed desirable to test its specificity. For this reason, guinea pigs were injected with various tissue materials such as suspensions of CNS tissue or kidney or liver or whole suspensions of rabbit testis; the suspensions were combined with paraffin oil and mycobacteria. After various intervals of time, the animals were killed, their organs sectioned, and the sera tested for the presence of antibodies.

TABLE I

Incidence of Aspermatogenesis in Guinea Pigs after the Injection of CNS Tissue and Adjuvants

Tissue injected	Total No. of guinea pigs	No. with encephalitis	No. without encephalitis	No. of guinea pigs with degree of aspermatogenesis indicated
Guinea pig brain	10	7	3	
		2	1	0
		2	2	1^+
		2	0	2^+
		1	0	3^+
		0	0	4^+
Rabbit spinal cord	44	37	7	
		29	6	0
		2	1	1^+
		2	0	2^+
		2	0	3^+
		2	0	4^+

The possible effect of injection of CNS tissue and adjuvants on the testis is of especial interest because, as mentioned, these two organs are reported to have a common antigen (7). Furthermore, animals so treated might serve as controls for the organ-specific nature of this phenomenon. During the course of this study, there was opportunity to examine the testes of a large number of animals injected with guinea pig brain or rabbit spinal cord and adjuvants (Table I). Of ten guinea pigs given a subcutaneous injection of guinea pig brain combined with paraffin oil and mycobacteria, seven developed encephalomyelitis in 15 to 31 days. The others were killed 34 days after injection. Of the seven with encephalomyelitis, two had essentially normal testes; three showed a mild injury (1^+) and two had moderate to severe injury (2 to 3^+). Three animals did not develop encephalomyelitis although they failed to gain weight. One of these had normal testes, two had a mild degree of inhibition of spermatogenesis (1^+).

Of 44 guinea pigs injected intracutaneously with rabbit spinal cord (from 0.125 to 1 mg.) combined with adjuvants, 37 developed encephalomyelitis with a considerable loss of weight. Most of them were killed when the symptoms were pronounced. Of these 37 animals killed from 2 to 6 weeks after injection, 29 had normal testes. In eight guinea pigs, aspermatogenesis was found; the degrees of injury (from 1^+ through 4^+) were equally distributed among them. Severe injury (3^+ or 4^+) occurred in four animals killed 4 weeks after injection. Of the seven guinea pigs which failed to develop symptoms and histological lesions of encephalitis, one exhibited mild (1^+) inhibition of spermatogenesis. This animal weighed 620 gm. when injected and lost 70 gm. in 28 days.

Five guinea pigs were injected with 0.5 ml. of emulsion containing 125 mg. of *kidney*, paraffin oil, and mycobacteria, and five other animals received 125 mg. of *liver* instead of kidney. Three animals in each group were reinjected 60 days after the first injection. No testicular injury was seen in any of the animals.

As mentioned above, the alcoholic extract of testes of various species of animals has been found to contain an antigen that is capable of fixing complement with sera of rabbits immunized with the alcoholic extract of rabbit testicle (7). Mudd and Mudd (17) and Henle (12) found that the spermia from different species of animals had some antigens in common. The question arose whether rabbit testis could be substituted for homologous gonad in producing testicular damage in the guinea pig. Rabbit testicular tissue combined with adjuvants was injected into six guinea pigs. Four animals received one injection and were killed after 3 to 8 weeks. Two animals were reinjected after 72 days and killed 102 and 266 days after the first injection. The testes were normal.

DISCUSSION

The experiments show that the intracutaneous injection of homologous testicular tissue or spermia combined with paraffin oil and killed mycobacteria results in aspermatogenesis in guinea pigs: degeneration and necrobiosis of germinal cells, and their elimination through the epididymis. The columnar cells of the epithelium of the seminal vesicles remain high and contain granules. The fluid in the vesicles is abundant and coagulable, indicating the presence of male hormone that stimulates this accessory organ of reproduction. Similar results can be obtained when the individual guinea pig's own cells are injected. The testicular injury occurs without inflammation although in some animals fibrotic changes may develop several months after injection. The fibrosis seems to follow the atrophy of germinal cells and may be a replacement phenomenon. The production of aspermatogenesis appears to be both organ- and species-specific since neither the liver or kidney of guinea pig nor testes from rabbits induce it. Among animals receiving CNS tissue and adjuvants, the occurrence of a mild degree of inhibition of spermatogenesis might be ascribed to an immunological cause based upon the presence of a common antigen. It is more

probable, however, that this injury is secondary to the physiopathological changes associated with meningo-encephalomyelitis (loss of body weight and other symptoms) rather than that it is of immunological nature. It may be added that the sera of these animals did not fix complement in the presence of spermia.

The introduction of antigen in water-in-oil emulsion alone was not sufficient; the testicular changes did not occur even if mycobacteria in oil and oil emulsion of testicular cells were injected at separate sites (remote from the testes). These observations do not exclude the possibility that frequently repeated injections of antigenic material in the absence of mycobacteria would cause aspermatogenesis.

Antibody formation against spermia has long been investigated. Indeed, more than 50 years ago Landsteiner (18), Metchnikoff (19), and Metalnikoff (20) demonstrated immobilizing antibodies even by autoimmunization. Agglutinins and complement-fixing antibodies have been found against several distinct antigens in the head and tail, some of which are species-specific (Henle *et al.* (21)).

In the present experiments, immobilizing antibodies, complement-fixing antibodies, and those which inhibit hyaluronidase have been shown to develop after the injection of testicular material, the highest titers occurring in animals injected with mycobacteria. Under the conditions of these experiments, there was a good correlation between the complement-fixing antibody titers and aspermatogenesis, particularly when testis suspension was used as an immunizing agent. This relationship, however, may not be a causal one. The injection of 15 ml. of serum from actively sensitized guinea pigs into normal guinea pigs failed to produce aspermatogenesis.

In this connection, the effect of mycobacteria in various types of experiments from the standpoint of sensitization merits consideration. While Lewis and Loomis (22) found that tuberculous guinea pigs injected with diverse antigens form antibodies more vigorously than non-tuberculous guinea pigs, Dienes (23) has shown that the injection of egg albumin into a tuberculous focus is followed by the development of both (evanescent) anaphylactic and also (delayed) tuberculin types of skin sensitization to egg albumin. The latter type bears no relationship to circulating antibodies and, like sensitization to tuberculin, does not seem to be transferable with serum. Sensitization of the skin to picryl chloride is strikingly promoted by killed tubercle bacilli in paraffin oil and is again not transferable with serum but rather with cells from an exudate or from lymphatic organs (24). The role of mycobacteria in paraffin oil is conspicuous in experimental "allergic" encephalomyelitis. It is possible to produce this malady by the injection of CNS tissue alone; however, at least 30 injections are necessary to accomplish it and it occurs irregularly. With the addition of mycobacteria in oil to CNS tissue, a single injection is sufficient.

Efforts to transfer allergic encephalomyelitis by means of the serum have failed (4 *c*, 25), although antibodies fixing complement in the presence of a suspension of brain are present in the serum. Skin sensitization to the suspension of CNS tissue from the rabbit has been demonstrated (4 *c*) in guinea pigs previously injected with rabbit CNS tissue in water-in-oil emulsion containing mycobacteria. Although it is not unlikely that guinea pigs become hypersensitive to germinal cells after the injection of these cells combined with adjuvants, thus far we have not been able to bring forth convincing evidence for skin sensitization.

The specific nature of the production of the aspermatogenesis described and the role of mycobacteria suggest that this phenomenon may be an immunological one and perhaps allergic in nature. However, it is remarkable that the injury to the germinal cells is not associated with inflammatory changes such as occur conspicuously in disseminated experimental meningo-encephalomyelitis. The lack of inflammation may be related to the topographical relationship of germinal cells to the blood vessels or to the elimination of injured cells through the epididymis or to their decomposition by enzymes other than by those of inflammatory cells. When aspermatogenesis and degeneration of germinal cells occur as a result of local injury, such as by x-radiation, elevation of temperature, diminution of blood supply, or in association with vitamin E deficiency or cachexia, the elimination of germinal cells usually proceeds without inflammation. In the present experiments, however, infiltration of the intertubular connective tissue by mononuclear cells, lymphocytes, and occasional plasma cells has been seen in a few guinea pigs which had intercurrent infections, such as pneumonia and streptococcal infection of lymph nodes (Fig. 4).

A possible source of complication to be avoided in experimental production of aspermatogenesis is the occurrence of testicular damage in stock animals and in those having pneumonia or streptococcal lymphadenitis. We have frequently seen testicular atrophy in guinea pigs which had been used for testing batches of diphtheria toxoid or titration of diphtheria antitoxin preparation. Voisin, Delaunay, and Barber (10) reported damage to the germinal cells of the seminiferous tubules associated with inflammatory changes in the intertubular connective tissue and epididymis in guinea pigs following the subcutaneous injection of testicular or kidney or liver tissue suspension combined with paraffin oil and killed tubercle bacilli. The pathological alterations were similar in kind and degree regardless of the tissue injected. Less severe but similar changes occurred when the tissues were omitted and water-in-oil emulsions with or without tubercle bacilli were introduced into the subcutaneous tissue. The site of injection was over the abdomen which might have favored the production of regional lesions. Their guinea pigs, half of them discarded animals previously used mostly for testing toxoid preparations, lost weight rap-

idly and about half of them died within a few weeks after injection. The extratubular changes in the gonad of their animals were remarkable. As early as 1 day after the first injection (most of the animals were given three injections 1 week apart) the blood vessels were engorged and the intertubular connective tissue was edematous. Later there was perivascular cuffing "resembling periarteritis nodosa;" the intertubular spaces were infiltrated by large mononuclear cells and lymphocytes which mingled diffusely with the multiplying fixed cells. Similar infiltrative lesions were seen in the connective tissue beneath the tunica albuginea and also in the epididymis. The seminal vesicles were not examined. The authors concluded that the injection of testicular material did not induce an immunological process and the pathological changes were the result of "stress." The several discrepancies between their results and ours appear to us to be attributable to intercurrent infections or excitation of latent infection or subcutaneous injection over the abdomen, particularly when the injections are repeated. Thus, in the experiments of Voisin and his associates the basic phenomenon, namely the production of aspermatogenesis by the injection of testicular tissue and adjuvants, was present but its significance was obscured.

SUMMARY

The injection into the dorsal skin of a suspension of guinea pig testis or spermia incorporated in a water-in-oil emulsion containing killed mycobacteria induces aspermatogenesis in guinea pigs. The injury begins with the inhibition of the maturation of spermia and proceeds through the degeneration and exfoliation of spermatids, spermatocytes, and finally spermatogonia. These germinal cells pass from the seminiferous tubules into the epididymis. The process is not associated with inflammation. No significant changes occur in the intertubular spaces and the Leydig cells do not seem to be affected. The seminal vesicles and the prostate remain normal. The aspermatogenesis may begin in 10 days and it lasts for more than 5 months. The process may lead to atrophy of the seminiferous tubules and fibrosis. Guinea pigs which receive a suspension of their own testis or spermia and adjuvants develop a similar injury.

The "mitochondrial" fraction of the testis of guinea pig is effective while repeated injections of alcoholic extract of testis emulsified with paraffin oil containing mycobacteria do not cause aspermatogenesis. The presence of acid-fast bacilli in the water-in-oil emulsion containing testis or spermia seems to be essential for the production of testicular lesions; the injection of antigen and mycobacteria into different sites is ineffective.

When guinea pig testis is replaced by guinea pig liver or kidney or rabbit testis no testicular damage occurs.

The injection of rabbit spinal cord combined with adjuvants results in allergic encephalomyelitis in a large proportion of guinea pigs, accompanied by a great loss of weight. The testes of a few of these animals show a varying degree

of aspermatogenesis. When guinea pig brain is combined with adjuvants and administered subcutaneously the incidence of testicular injury is high, although the damage is, in general, mild. From the standpoint of mechanism, the inhibition of spermatogenesis which occurs in these animals may be unrelated to the injury which follows the injection of germinal cells.

Aspermatogenesis follows the injection of killed mycobacteria in paraffin oil into the testis as well as into certain sites related to the gonad: the abdominal cavity, the subcutaneous tissue over the abdomen, and the skin of the inguinal region.

Antibodies fixing complement in the presence of spermia are demonstrable in the sera of guinea pigs injected with testis or spermia and adjuvants. When the mycobacteria are omitted the titers are low and no testicular injury occurs. Although there seems to be a correlation between testicular damage and complement-fixing titer, this may not be a causal relationship.

Antibodies which neutralize guinea pig hyaluronidase and those which immobilize spermia have also been demonstrated in the sera of these guinea pigs.

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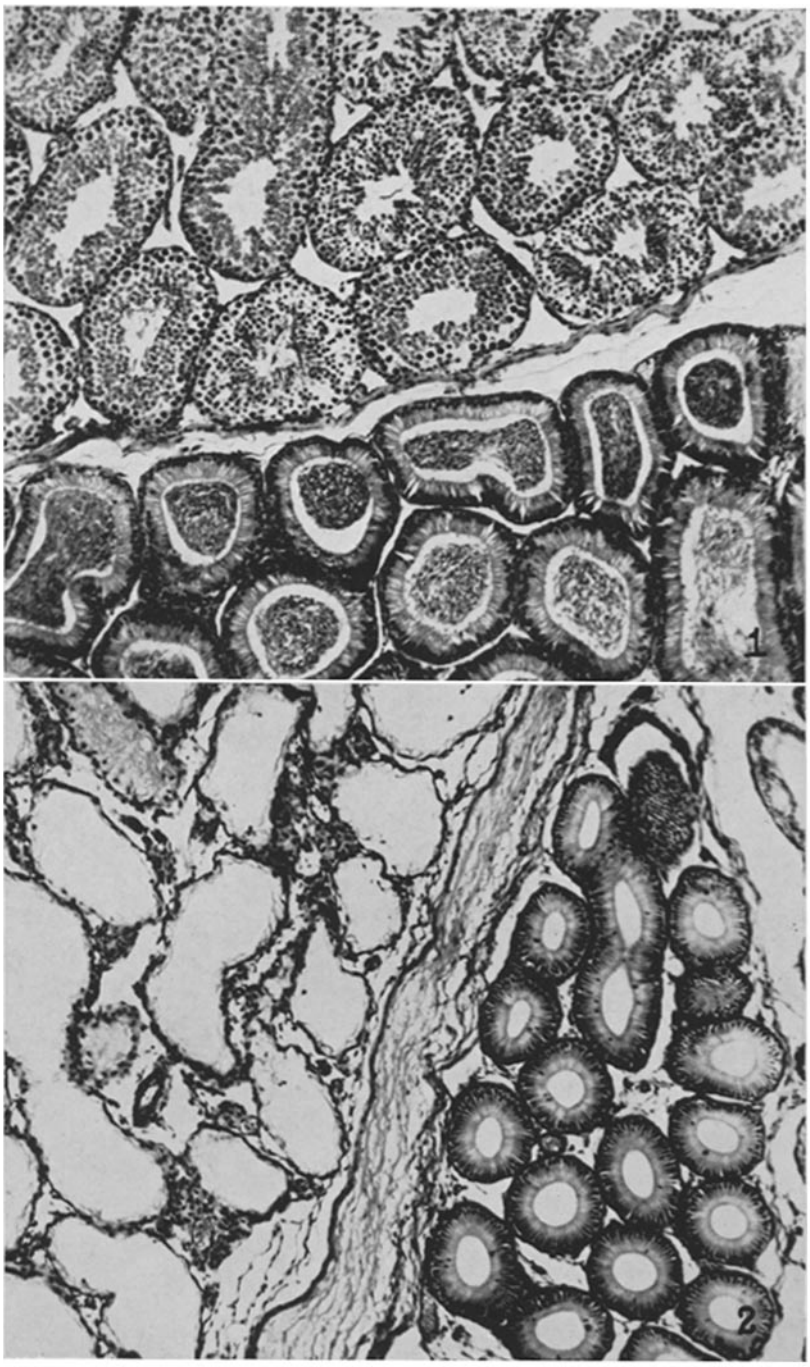
EXPLANATION OF PLATES

PLATE 42

FIG. 1. Normal guinea pig testis and epididymis. × 86.

FIG. 2. Testis and epididymis of guinea pig injected with testicular suspension and adjuvants. 4^+ injury. × 86.

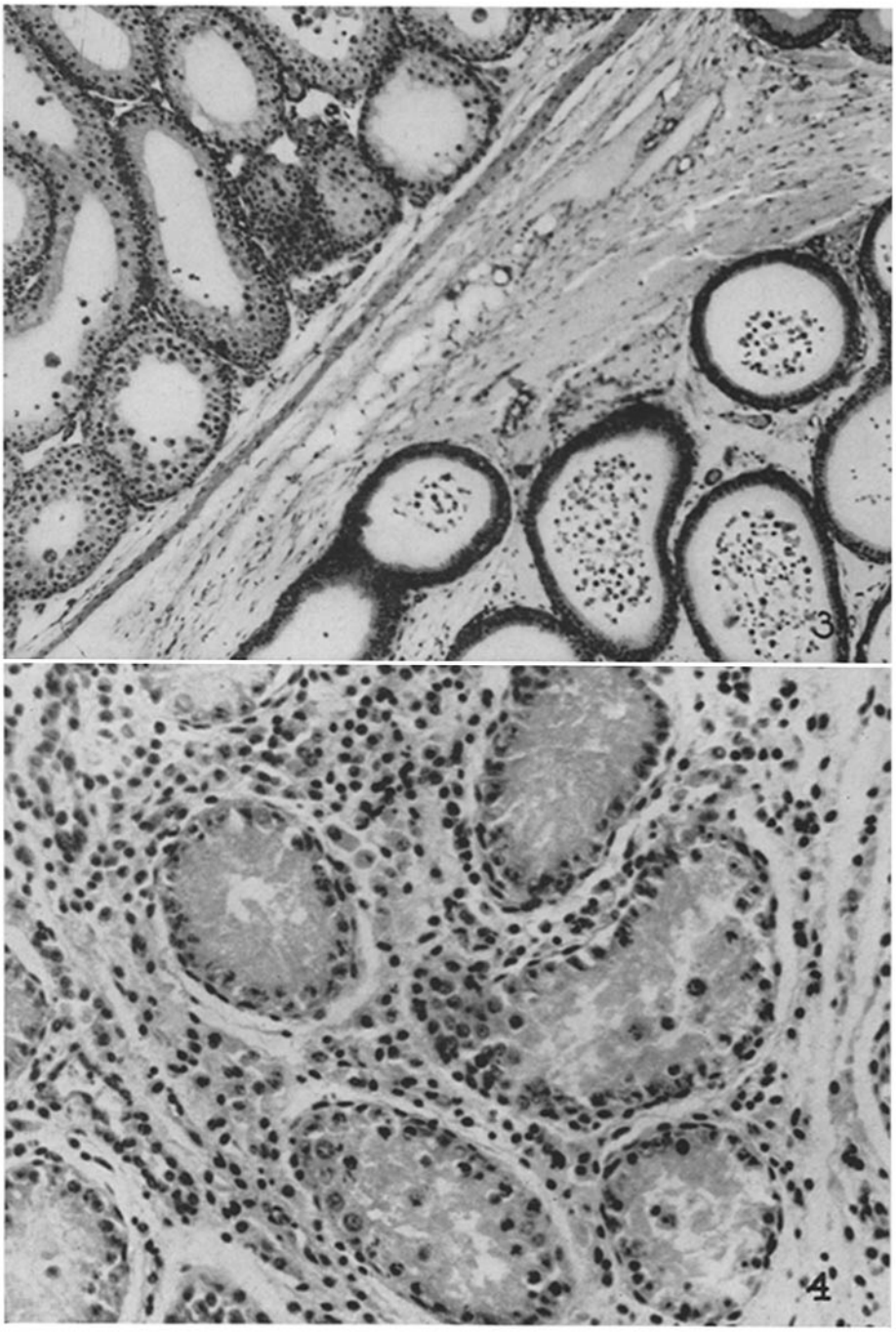


(Freund *et al.*: Aspermatogenesis induced by testicular tissue)

PLATE 43

FIG. 3. Testis and epididymis of guinea pig injected with spermia and adjuvants. 3^+ injury. × 85.

FIG. 4. Testis of guinea pig 250 injected with spermia and adjuvants and having intercurrent infection (streptococcal lymphadenitis). × 240.



(Freund *et al.*: Aspermatogenesis induced by testicular tissue)