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# Pathological Features of Experimental Gonococcal Infection in Mice and Guinea Pigs

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The histopathological and immunofluorescent findings in tissues within and surrounding artificially created subcutaneous tissue cavities infected with *Neisseria gonorrhoeae* for 1 to 30 days were studied in mice and guinea pigs. Findings in the tissue cavities of the animal models were similar to the findings of disseminated gonococcal infection in humans. These similarities included an intense persistent polymorphonuclear leukocytic response with tissue necrosis, hemorrhage into the early lesion, a perivascular leukocytic response in adjacent tissue, difficulty in detecting large numbers of discrete morphologically typical gonococci by the tissue Gram stain and direct fluorescent antibody techniques, a decrease in the number of identifiable gonococci with duration of the infection, and moderate amounts of extracellular and intracellular immunofluorescent gonococcal debris. Studies into the pathogenesis of the animal infections may enhance our understanding of the pathogenic mechanism(s) associated with gonococcal infection in humans.

There have been several recent reports on the survival of *Neisseria gonorrhoeae* in artificially created tissue cavities in the subcutis of small laboratory animals (1, 2, 5). These animal models are now being used extensively to study the virulence and immunology of gonococcal infections (1-3, 5, 13). However, the pathological features of experimental infection have received little attention (3). For this reason, we report the histopathological and immunofluorescent findings in mouse and guinea pig tissues within and surrounding subcutaneous tissue cavities infected with gonococci for 1 to 30 days.

## MATERIALS AND METHODS

**Animals. (i) Guinea pigs.** Adult Hartley strain guinea pigs *(Cavia porcellus)* weighing at least 750 g were used. Plastic 2-ml droptainer bottles without tops (Dougherty Bros. Co., Buena, N.J.) were surgically implanted into the subcutis along the dorsolateral flank of each animal. Prior to implantation, four elliptical openings were cut into the sides and a round opening was made in the bottom of each bottle (Fig. 1). At least 2 weeks was allowed between surgical implantation and bacterial challenge for the purpose of creating a connective tissue-lined cavity for the accumulation of tissue fluids.

**(ii) Mice.** Adult, pathogen-free, ICR/Cds Swiss mice *(Mus musculus)* were used. Silicone rubber tubing (2-cm lengths, 3.2-mm inner diameter) (New Brunswick Scientific Co., New Brunswick, N.J.) was surgically implanted into the subcutis along the dorsolateral aspect of the back. At least 2 weeks was allowed between surgical implantation and bacterial challenge.

**N. gonorrhoeae.** Isolate B, colony type 1 (7) *N. gonorrhoeae*, was initially obtained from a patient with gonococcal urethritis. This gonococcus was grown on GC base medium supplemented with IsoVitaleX (Baltimore Biological Laboratories, Baltimore, Md.) (GCB medium) in a candle extinction jar for 18 h at 36.5 C. Cells were removed from GCB medium with sterile, cotton-tipped applicators and were suspended in Eagle minimal essential medium containing Earle balanced salt solution. The suspension was adjusted to an optical density of 0.3 at 530 nm (approximately $10^8$ colony-forming units/ml).

**Animal infection.** A 0.1-ml aliquot of the gonococcal suspension was injected percutaneously into the subcutaneous tissue cavity. Infection was documented when the tissue cavities were excised for histopathological examination by inoculating 0.05 ml of the chamber fluid onto GCB medium, as described above. Gonococci were identified by colony morphology, oxidase reaction (1% solution of $N_1$-$N$-dimethyl-$p$-phenylenediaminemonohydrochloride), Gram stain, and fermentation of glucose but not maltose. Cystine tryptic agar (Baltimore Biological Laboratories) containing 1% of the test sugar was used for fermentation studies.

**Experimental design.** Two animals of each genus were randomly selected and killed at time intervals of 1, 2, 7, 21, and 30 days after injection of the gonococcal suspension. The subcutaneous tissue cavities and surrounding tissues, including the overlying skin and underlying skeletal muscle, were excised in situ for histopathological examination. To serve as a control, one animal of each genus with an

uninoculated tissue cavity was killed at 1, 7, and 21 days along with the test animals.

**Histopathology.** After specimens were fixed in 10% neutral buffered formalin, longitudinal cuts were made through the subcutaneous tissue cavities and surrounding tissues, including the skin when possible. The plastic pieces of the implants were gently separated from the tissues and discarded. Tissues were then embedded in paraffin, sectioned at 6 $\mu$m, and stained with hematoxylin and eosin, Masson trichrome connective tissue stain, and Brown-Brenn tissue Gram stain (9). Sections of tissue containing known gram-negative bacteria were included as positive staining controls.

**Immunofluorescence.** Recently collected tissues were prepared for fluorescent antibody (FA) examination by embedding them in O.C.T. compound (Ames Co., Division of Miles Laboratories, Inc., Elkhart, Ind.) and then cryostat sectioning them at 4 $\mu$m. Fluorescein isothiocyanate-conjugated rabbit antigonococcal globulin, prepared in this laboratory by a modification of Peacocks procedure (11, 12), was then applied to each tissue cryosection. Smears of the tissue cavity fluid, removed with a needle and syringe just prior to tissue cavity excision, were also examined by the direct-staining FA method. Positive staining controls consisted of smears of *N. gonorrhoeae* that had been grown on GCB medium for 20 h. Negative controls consisted of cryosections of tissue surrounding noninfected tissue cavities and smears of mouse and guinea pig leukocytes (buffy coat) from peripheral blood. These controls were included with each run of slides. All slides were examined using an AO vertical fluorescence microscope, model 2070, with a BG12 exciter filter and an OG515 barrier filter (American Optical Corp., Buffalo, N.Y.).

## RESULTS

**Tissue cavity culture.** Gonococci from all inoculated tissue cavities were isolated on GCB culture medium at the time of animal sacrifice. Although the culture technique did not facilitate exact quantitation, the number of gonococci recovered after 30 days of infection was less than that recovered during week 1 of infection.

**Histopathology.** The histological changes in tissues from mice and guinea pigs were similar; hence, they will be considered together.

A narrow zone of dense, mature, fibrous connective tissue surrounded all noninoculated control tissue cavities (Fig. 2). Occasional groups of hemosiderin-laden macrophages were present within the fibrous tissue, which indicated areas of remote hemorrhage. Otherwise, cellular inflammatory response was minimal or absent. A clear to amber luminal transudate was consistently present on gross examination.

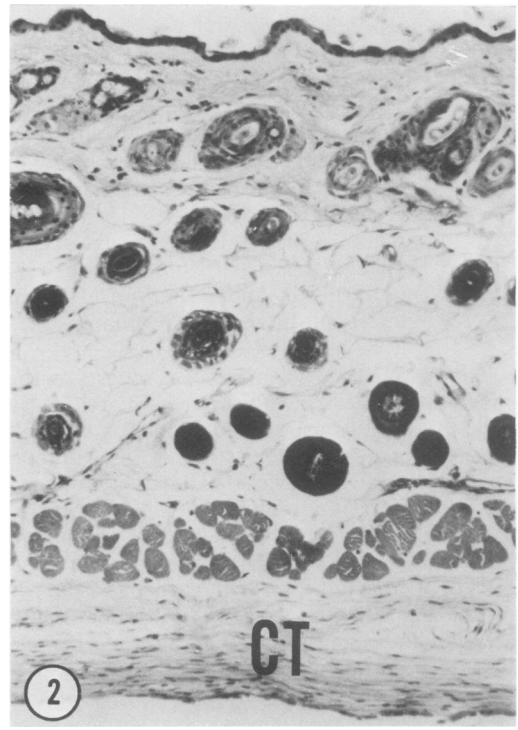


FIG. 2. *Photomicrograph of mouse skin. The narrow zone of well-differentiated fibrous connective tissue (CT) surrounded a noninoculated plastic implant that was surgically inserted in the subcutis (hematoxylin and eosin, ×150).*

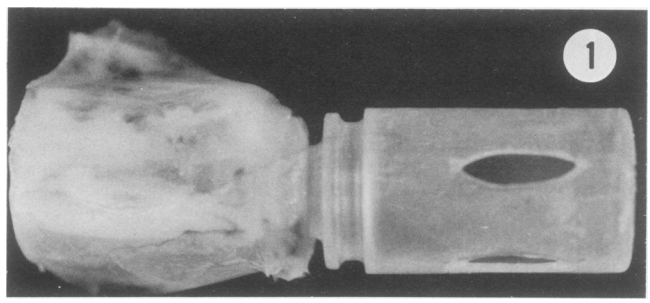


FIG. 1. *Guinea pig control implant. Surrounding fibrous capsule has been incised and reflected over the plastic implant to illustrate elliptical openings. ×2.*

A marked inflammatory response surrounded the infected tissue cavities. Polymorphonuclear and mononuclear leukocytes suffused a wide, highly vascular zone of proliferating granulation tissue (Fig. 3). The components of the inflammatory response were similar, regardless of time postinoculation (p.i.). However, the number of leukocytes decreased and the amount of granulation tissue progressively increased with time p.i. Inflammation was most severe in tissues adjacent to and protruding through the holes in the plastic implants (Fig. 1), i.e., in tissues directly exposed to the gonococcal inoculum. Highly vascular and inflamed granulation tissue completely filled each hole and projected well into the tissue cavity lumen (Fig. 4). The leukocytic infiltrate was most severe in these invaginations of granulation tissue, which were in direct contact with lumen contents and were commonly covered by fibrin, erythrocytes, and necrotic debris. Inflammatory cells, chiefly polymorphonuclear leukocytes, partially extended along the internal wall of the plastic implants and often connected one inflamed granulation tissue invagination with another. Granulation tissue containing scattered inflammatory cells extended externally into the adjacent subcutis and a short distance down the external sides of the implant before changing to a wide zone of fibrous connective tissue similar to that surrounding noninoculated control tissue cavities (Fig. 4). The contiguous dermis contained variable numbers of lymphocytes and plasma cells that were especially prominent in perivascular locations at 21 and 30 days p.i. (Fig. 5). No microscopic changes were observed in the epidermis or skin adnexa.

Lumens of infected tissue cavities were variably filled with polymorphonuclear leukocytes, fibrin, erythrocytes, macrophages, and cellular debris. The amount of each component present varied with time p.i. (Fig. 6 and 7). The degenerating polymorphonuclear leukocyte was always the major component of the lumen exudate at each interval p.i. Hemorrhage in the tissue cavity lumens at 1, 2, and 7 days p.i. was minimal to severe. By 21 days p.i., the luminal exudate was much less cellular, and early organization with partial filling of the lumen by large invaginations of granulation tissue was evident. However, the lumen was never completely filled by organized exudate, even at 30 days p.i.

Occasional intracellular and extracellular gram-negative cocci and diplococci whose size was compatible with that of *N. gonorrhoeae* were found within tissue cavity lumens and surrounding inflammatory tissues at each interval p.i. Organisms were never demonstrated elsewhere, and they were more readily found within the lumen exudate and in tissues infected for 1 to 7 days. In no instance were gram-negative cocci found within blood or lymphatic vessels of surrounding tissues. Most organisms appeared to be within degenerating polymorphonuclear leukocytes and rarely within macrophages. Typical gonococci were never found in fibroblasts. More organisms may have been present, but it was very difficult and often impossible to differentiate between necrotic debris and distorted or fragmented gonococci, both of which stained similarly with the Brown-Brenn tissue Gram stain.

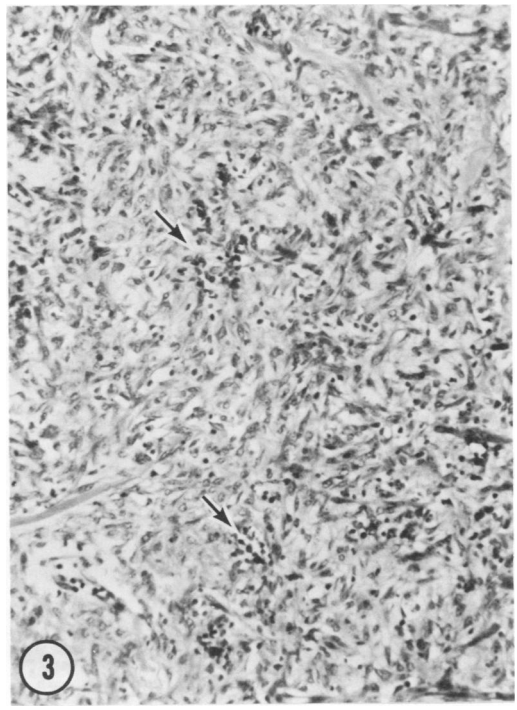


FIG. 3. *Exceptionally dense zone of granulation tissue contains scattered inflammatory cells (arrows) in an area that was directly exposed to the internal contents of a guinea pig subcutaneous tissue cavity infected with N. gonorrhoeae for 30 days (hematoxylin and eosin, ×150).*

**Immunofluorescence.** Small numbers of green, brightly fluorescent, intracellular and extracellular gonococci were demonstrated in smears of lumen exudate and cryosections of tissue immediately surrounding infected tissue cavities at each interval p.i. (Fig. 8). More commonly, myriads of intracellular and extracellular pleomorphic fragments that were brightly

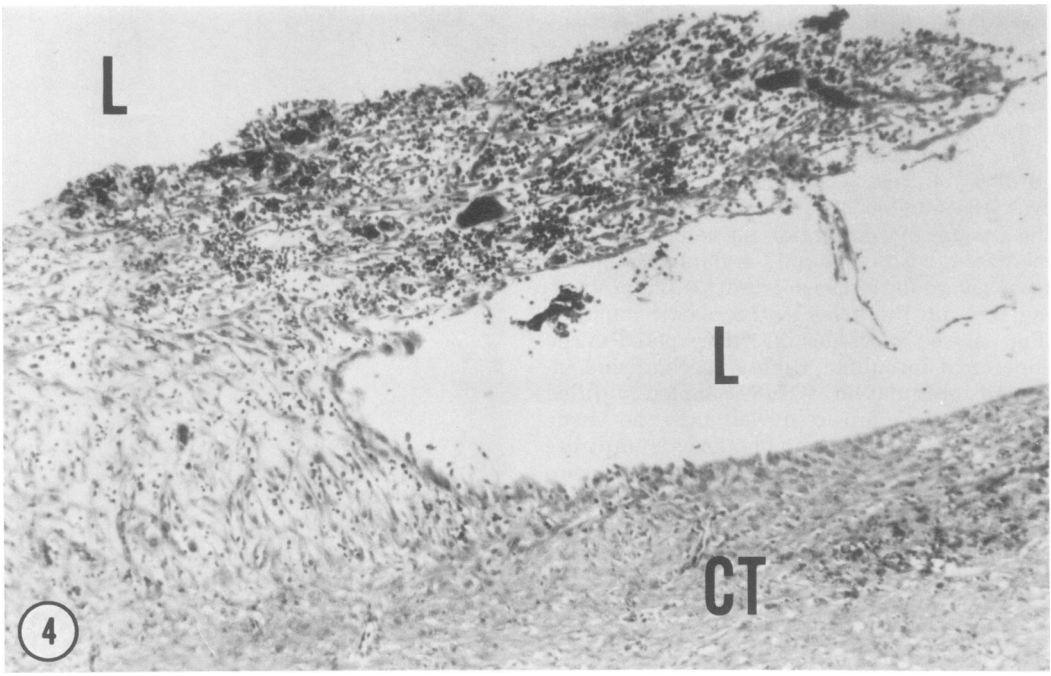


FIG. 4. *Invagination of inflamed and hemorrhagic granulation tissue into lumen (L) of guinea pig tissue cavity with gonococcal infection of 21-days duration. Note gradual transition of granulation tissue into dense fibrous connective tissue (CT) that surrounded plastic implant (hematoxylin and eosin, ×100).*

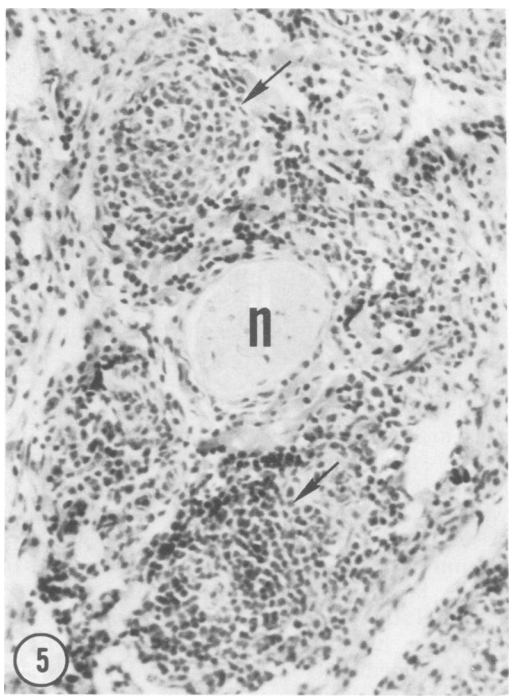


FIG. 5. *Perivascular accumulations of lymphocytes and plasma cells (arrows) in the dermis adjacent to granulation tissue that surrounded a mouse tissue cavity infected with N. gonorrhoeae for 21 days. Dermal nerve (n) (hematoxylin and eosin, ×250).*

fluorescent were seen (Fig. 8). Some leukocytes were engorged with this material, whereas others contained only one or two fragments. These fragments were considered to be antigenic material of distorted and disintegrated gonococci. Fibroblasts did not appear to contain fluorescent material, but it was difficult to determine this since leukocytes that contained FA-positive material were scattered throughout dense granulation tissue surrounding infected tissue cavities. The number of intact gonococci and gonococcal fragments demonstrated by FA progressively decreased with time p.i.

In tissue sections, nonspecific fluorescence was minimal and solid tissue appeared as a light yellow-brown background. In smears of lumen exudates, there was little or no background fluorescence. Control smears of gonococci that had been grown in vitro were uniformly and strongly FA positive each time slides were processed.

## DISCUSSION

Our observations clearly show that a single inoculation of *N. gonorrhoeae* into artificially created subcutaneous tissue cavities of mice

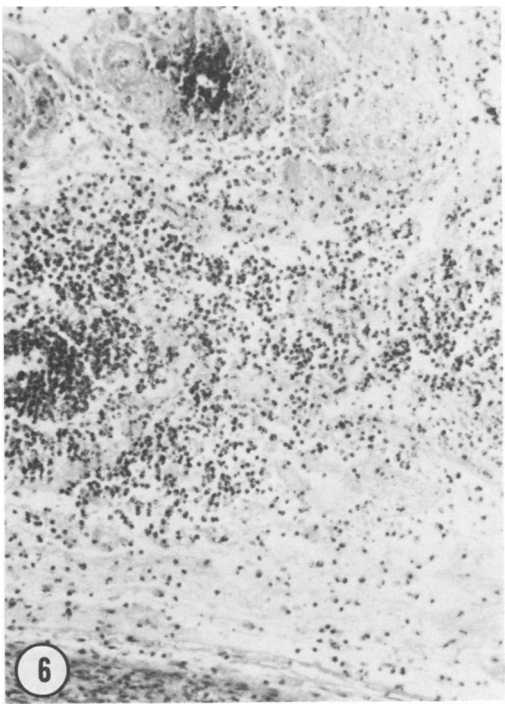


FIG. 6. *Scattered polymorphonuclear leukocytes, erythrocytes, and necrotic debris in lumen of mouse tissue cavity 1 day after gonococcal inoculation (hematoxylin and eosin, ×150).*

and guinea pigs can stimulate a continuous inflammatory process for at least 30 days. Occasional intact gonococci were found within polymorphonuclear leukocytes and macrophages, even at the longer intervals after gonococcal inoculation. In humans, chronicity is a common characteristic of untreated gonorrhea (10), but the mechanism(s) by which gonococci survive is poorly understood.

Infrequent demonstration of intact gonococci in tissue surrounding the tissue cavity and in smears of lumen exudate, despite initial inoculation with large numbers of gonococci, suggests that most bacteria are rapidly destroyed. This suggestion is further strengthened by the finding on FA examination of what appeared to be myriads of intracellular and extracellular fragments of gonococcal antigen. These fragments were strongly FA positive, and leukocytes were occasionally engorged with the brightly fluorescent material. Even at 1 day p.i., only small numbers of intact gonococci were demonstrated by FA examination, and the tissue Gram stain was even less helpful in identification of gonococci. In studying two cases of septic gonococcal dermatitis in humans, Kahn and Danielsson (6) reported similar results in their efforts to demonstrate the gonococcus. They demonstrated rare intact gonococci in skin lesions by an FA technique but not by the tissue Gram stain or by culture. With their FA method, many brilliantly fluorescent fragments of gonococcal antigen were located around blood vessels. In another study using FA techniques, Danielsson and Michaelsson (4) demonstrated small numbers of intact gonococci in smears from early vesiculopustular lesions of gonococcal dermatitis in two out of three patients.

Our interest in an animal model of gonococcal infection is that its study might lead to a better understanding of gonococcal infection in humans. Although the animal model represents an active gonococcal infection, it is not entirely analogous to nondisseminated gonorrhea in humans since the tissue cavity infections are not mucosal surface infections and since gonococcal mucosal surface virulence correlates, such as colony type (7) and pili (15), may not be virulence determinants in guinea pigs (3). On the other hand, the histological picture of disseminated gonococcal infection in humans is similar to the picture we describe in

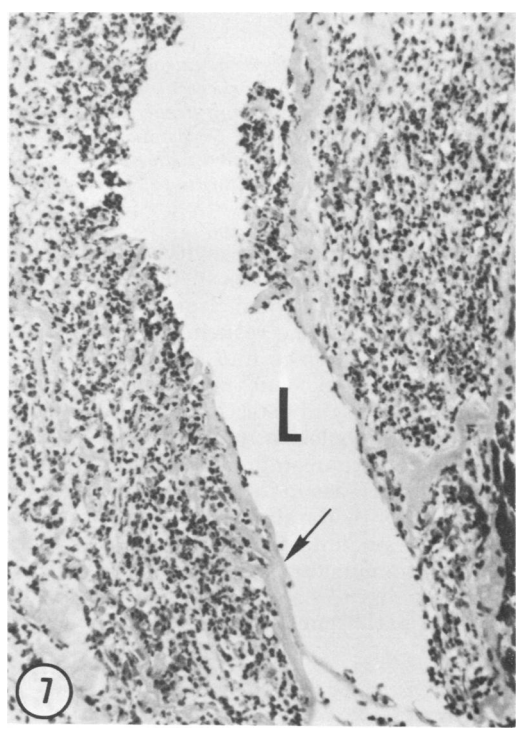


FIG. 7. *A tissue cavity lumen (L) is partially filled by immature granulation tissue that contains many polymorphonuclear leukocytes and is partially covered by condensed fibrin on its luminal surface (arrow). Guinea pig, 7 days after gonococcal inoculation (hematoxylin and eosin, ×150).*


FIG. 8. *Brightly fluorescent extracellular and intracellular fragments of gonococcal antigen in exudate from lumen of guinea pig tissue cavity 21 days after gonococcal inoculation. Occasional intact gonococci are seen (arrow). Stained with fluorescein isothiocyanate-conjugated antiserum to N. gonorrhoeae (×540).*

the subcutaneous tissue cavity animal model (8, 14). These similarities include an intense, persistent polymorphonuclear leukocytic response with tissue necrosis in the center of the reaction, hemorrhage into the early lesion, a perivascular leukocytic response in adjacent tissue, difficulty in detecting large numbers of discrete morphologically typical gonococci by the tissue Gram stain and direct FA techniques, a decrease in the number of identifiable gonococci with duration of the infection, and moderate amounts of intracellular and extracellular immunofluorescent gonococcal debris. These similarities suggest that further studies into the pathogenesis of the animal infections might lead to a better understanding of gonococcal infection in humans, particularly in its disseminated phase.

## ACKNOWLEDGMENTS

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