Neisseria gonorrhoeae: Experimental Infection of Laboratory Animals

Abstract. *Experimental* Neisseria gonorrhoeae *infections were established in five species of small laboratory animals (rabbits, guinea pigs, hamsters, mice, and rats) after subcutaneously implanted chambers were inoculated with gonococci. The chamber fluid was easily available for study or culture. A systemic immune response was indicated by hemagglutination assay.*

A procedure has been developed for infecting rabbits, guinea pigs, hamsters, mice, and rats with *Neisseria gonorrhoeae*. The gonococci in these infections remain easily accessible for in vivo study. Research on gonorrhea, currently the most frequently reported infectious disease in the United States, has been handicapped for decades by lack of experimental animal infections. Miller (*1*) isolated a strain of *N. gonorrhoeae* which was lethal for mice inoculated intraperitoneally, and he succeeded in infecting the rabbit eye by inoculating gonococci into the anterior chamber. Unfortunately, neither animal infection was ever exploited in studies of the immunology or pathogenesis of gonococcal infection. Experimental gonococcal urethritis and cervicitis in chimpanzees (*2*) have great potential for study, but the need remains for a model infection in a small laboratory animal that is more widely available, less expensive, and easier to handle.

Rabbits were infected by a modification of a technique used by Tosi *et al.* (*3*) to produce antibody to viral antigen. Hollow polyethylene practice golf balls with holes in their walls were sterilized and surgically implanted in the subcutaneous tissue of the dorsolumbar region of each animal. Either one or two chambers were implanted on each side, and at least 30 days elapsed before they were inoculated with *N. gonorrhoeae*. During this period the outer incision healed completely, with no visible signs of irritation or discomfort. Also, the chambers became encapsulated by a thin layer of fibrous connective tissue containing small blood vessels. "Papillae" protruded slightly inward through the holes in the surface of the polyethylene sphere, and small blood vessels were prominent in these protrusions (Fig. 1). As the chamber became encapsulated within the subcutaneous tissues, it gradually filled with about 20 ml of light-colored transudate, which could be easily sampled with a hypodermic needle and syringe.

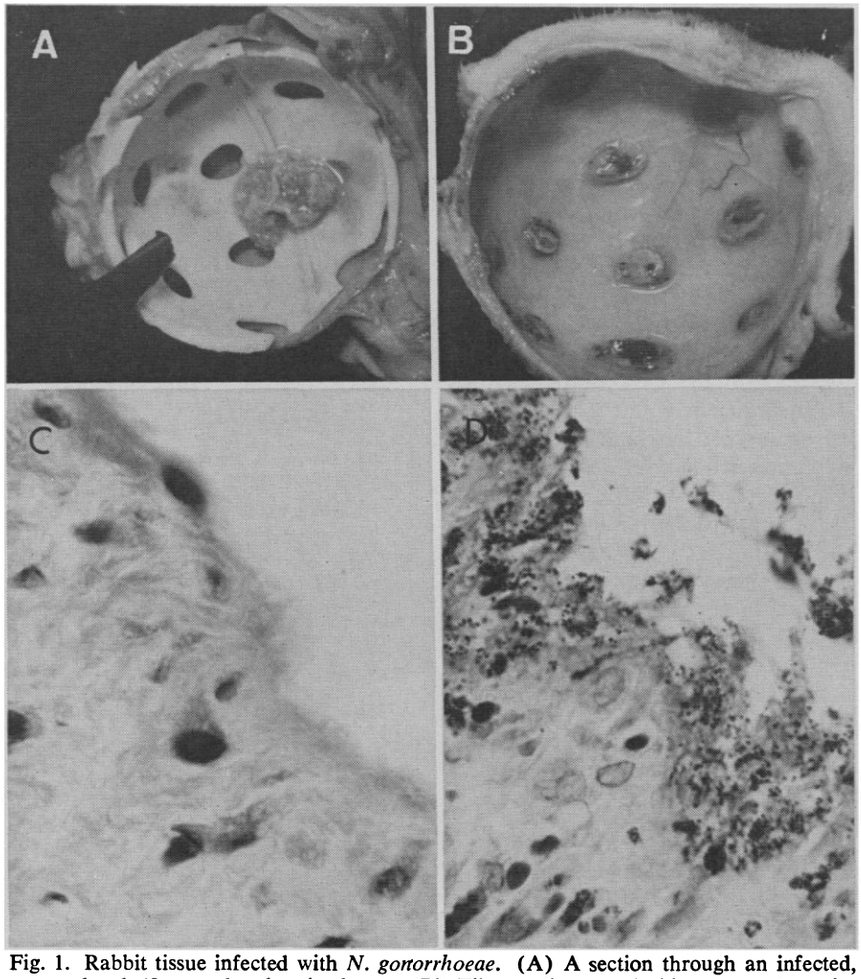

Fig. 1. Rabbit tissue infected with *N. gonorrhoeae*. (A) A section through an infected, encapsulated 42-mm chamber is shown. (B) Fibrous tissue and skin surround an infected chamber where "papillae" with petechial hemorrhages are seen. (C and D) Microscopic sections of tissue surrounding (C) noninfected and (D) infected chambers are shown (×700).

The inoculum used for infecting the first rabbit consisted of an 18-hour culture of *N. gonorrhoeae*, Veneral Disease Research Laboratory (VDRL) strain 2686, grown on Difco G.C. base medium enriched with isovitalex; at least 90 percent of the inoculum was of colony type 1, as classified by Kellogg *et al.* (*4*). A 4-ml portion of liquid culture medium (*5*), containing approximately 10^8 colony-forming units of *N. gonorrhoeae* per milliliter, was injected through a 25-gauge needle into the encapsulated sphere along with 2 mg of dexamethasone (Azium Schering).

Afer inoculation, fluid was removed from the chambers at 2- to 3-day intervals for bacteriological culture and microscopic examination. In addition to being classified by morphology and colony type, cultures obtained from the infected chambers were identified by oxidase reaction, Gram stain, and sugar fermentation tests. Identification of the organism as *N. gonorrhoeae* was also confirmed independently by W. J. Brown and J. Lewis, VDRL staff members.

Fluid from the initially inoculated chamber was first found to be culture-positive for gonococci 3 days after inoculation; cultures of fluid from this chamber have remained positive for *N. gonorrhoeae* for longer than 9 months. Repeated primary cultures of fluid from the initially infected chamber served as the inocula for infecting nine other rabbits. All rabbits except one received dexamethasone at the time of inoculation. Cultures of blood, pharynx, urethra, and rectum, obtained 2 weeks after infection, were all negative for *N. gonorrhoeae*.

Serum antibody to *N. gonorrhoeae* was detected by a passive hemagglu-

tination test (*6*) in all ten rabbits and first appeared 6 to 30 days after inoculation. Titers for individual rabbits varied from 1:20 to 1:640 during infection; all animals were nonreactive before inoculation. Antibody was also detected in fluid from both the infected and the noninoculated chambers of the same animal.

A biopsy taken 3 weeks after infection revealed massive intracellular and extracellular infiltration of gonococci into the tissue and blood vessels surrounding the infected chamber (Fig. 1). A blood culture taken at the same time was negative for *N. gonorrhoeae*.

A modification of the technique for rabbits was used to infect guinea pigs, hamsters, rats, and mice. Coil-shaped subcutaneous culture chambers were prepared from 0.5-mm stainless steel wire. After implantation the chambers were inoculated with 0.2 ml of liquid growth media containing *N. gonorrhoeae* (VDRL strain 2686). Both dexamethasone-treated and nontreated animals were infected, and although dexamethasone may promote establishment of infection, it is not required (Table 1).

The results presented here extend the principle of Miller's research (*1*) concerning gonococcal infection of the rabbit eye. The gonococci in our experiments persisted within the trunk of the rabbit, in a place easily accessible for withdrawing specimens for culture and microscopic examination. The ability to repeatedly sample gonococci growing in vivo opens the possibility of studying the in vivo interaction of bacteria with antibody, complement, leucocytes, and other host factors. These studies will facilitate research on serologic tests, strain typing, and vaccine development. Finally, the techniques reported here should also permit in vivo study of the antigonococcal effects of antibiotics and other chemotherapeutic and prophylactic substances.

Table 1. Experimental *N. gonorrhoeae* infection in guinea pigs, hamsters, rats, and mice; CFU, colony-forming units of *N. gonorrhoeae* inoculated per animal; Dex., dexamethasone.

Inoculum (CFU)	Dex. (1 mg)	Number of animals: Inoculated	Number of animals: Positive cultures 5 days after inoculation
	Guinea pigs		
10^7	+	4	4
10^7	−	4	0
	Hamsters		
10^7	+	4	3
10^7	−	7	6
	Rats		
10^7	+	5	1
10^7	−	5	0
	Mice		
10^8	−	4	4
10^6	−	4	4
10^4	−	4	3
10^2	−	4	3
10^1	−	4	0

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Variation in Tyrosine Aminotransferase Induction in HTC Cell Clones

Abstract. *Examination of tyrosine aminotransferase induction in many HTC cell subclones revealed a wide and unstable distribution of inducibility. The instability of this phenotype cannot easily be explained by classical mutation rates. These observations may be important in interpreting certain cell fusion experiments.*

Understanding gene expression and its control in higher organisms will require the development of a model that can explain the high rates of "mutation" often described in eukaryotes (*1*). We examined the inducibility of the enzyme tyrosine aminotransferase (TAT) in several clones obtained from the HTC cell line [originally cultured from Morris hepatoma 7288C (*2*)] and we found considerable variation in the extent of inducibility, with increases over basal TAT ranging from less than 100 percent to greater than 3000 percent. On recloning two widely differing clones, we found that the subclones again showed considerable variation in inducibility. In contrast, inducibility in the wild type HTC (HTC+) has been relatively stable for more than 7 years. There is no known selective pressure for or against TAT expression, nor have we been able to devise one. Therefore, to explain the apparent paradox between the two sets of observations we have to assume similar rates of change from high to low inducibility and from low to high inducibility.

The phenotypic variation described here, while not readily explained in terms of classic genetics, cannot be overlooked. It may also be occurring in other cell lines in culture. This possibility should be taken into account when quasigenetic studies are done in tissue culture systems. Simply establishing a clonal cell line does not guarantee uniformity of expression of a particular enzyme within that line, as the data in this report demonstrate.

Two clones from HTC+, one with low inducibility and one with high, were first selected. Each was recloned by plating a single-cell suspension in sterile plastic trays containing 96 wells, with an average of 0.2 cell per well. Poisson analysis indicated that not more than 10 percent of the subsequent colonies arose from two cells. Forty colonies were isolated and analyzed for TAT inducibility, and a clone with high inducibility [C3-30(H)] and a clone with low inducibility [C2-10(L)] were picked and recloned as before. For comparison, HTC+ cells were freshly cloned as well. To minimize physiologic variability in enzyme content, studies for TAT induction in the final clones were done as follows. An identical number of cells of each clone from each parent were simultaneously plated, grown for 24 hours (log phase), and induced for 24 hours with $2 \times 10^{-7} M$ dexamethasone phosphate. Then the cells were collected, broken with a probe sonicator, and assayed for TAT by a slight modification of the Diamondstone method (*3*). To further insure that the enzyme activities seen were comparable between groups, the parental lines (high and low inducibility and wild type) were identically analyzed along with each group.

The distribution of clones with respect to TAT inducibility (Fig. 1) shows that although there is a clustering