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Chicken Embryo as an Animal Model for Gonorrhea

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Parameters of infection of the chicken embryo with *Neisseria gonorrhoeae* were defined in order to standardize infectious and lethal doses. Virulent (T1) and avirulent (T3) gonococci from two strains were used to infect 7- to 12-day-old White Leghorn chicken embryos via the yolk sac (YS) or chorioallantoic membrane (CAM) route. Infection of embryos was established following YS inoculation of 1 to 10 viable gonococci. Although 8- to 10-day-old embryos were the most susceptible, an inoculum of less than 100 gonococci was sufficient to kill any age embryo via this route. Embryos were less susceptible to infection via the CAM, where an inoculum of from 10^5 to 10^6 colony-forming units was lethal by 42 h. Strain and morphological type had a variable influence on the ability of the gonococcus to infect and kill the chicken embryo by either route; however, agar-grown and broth-grown organisms produced consistently similar mean lethal dose (LD_{50}) and mean infective dose (ID_{50}) values. LD_{50} and ID_{50} differences between T1 and T3 gonococci from strain 72H641 were not apparent after either YS or CAM inoculation of 8- or 10-day chicken embryos, respectively. YS and CAM LD_{50} values for strain 72H641 T1 and T3 and CDC 9 T3 were also similar; however, these values were slightly lower for CDC 9 T1. In terms of infectivity or colonization, CDC 9 T1 and T3 had higher ID_{50} values via the YS and lower ID_{50} values via the CAM than 72H641. CDC 9 T1 was slightly more infective via the YS and less infective via the CAM than its T3 counterpart. Although the gonococcal strain used will influence interpretation of results, infection of both YS and CAM was highly reproducible in terms of gross pathology and of LD_{50} and ID_{50} data for a particular strain and colony type.

Although infection with *Neisseria gonorrhoeae* has been accomplished in chimpanzees (8), in the anterior chamber of a rabbit's eye (8), and by surgical implants in rodents (1), these models have proved difficult to manipulate and standardize. The developing chicken embryo has been used successfully for the study of gonococcal pathogenesis since 1936, when Bradford and Tittsler (3) grew the organism in the yolk sac. Two years later, Hill and Pitts (6), as well as Morrow and Berry (10), infected the chorioallantoic membrane (CAM) with the gonococcus. Progress of infection of the CAM was described in detail by Bang (2), who noted its resemblance to that observed in gonococcal infection of the urethra of human male volunteers. In 1963, Walsh et al. (14) maintained the virulence of freshly isolated gonococcal strains and restored virulence for human beings to a strain that had become avirulent by serial passage in the allantoic cavity of the chicken embryo.

Interest in the development of the chicken embryo as an animal model for study of gonococcal pathogenesis heightened with the discovery by Kellogg et al. (7) that gonococci grow as several morphological types associated with virulence. Types 1 and 2 produced typical gonococcal urethritis in human males, whereas type 3 was isolated upon in vitro subculture of types 1 and 2. Types 3 and 4, which do not infect humans, are believed to represent laboratory-derived mutants.

The most recent applications of the chicken embryo technique for the study of gonococcal pathogenesis have used this colony-type difference. Buchanan and Gotschlich (4) reported a slight but significant difference in infectivity of gonococcal types 1 and 3 for 10-day embryos inoculated via the CAM. Bumgarner and Finkelstein (5) did not find such a difference between colony types on CAM inoculation, but found types 1 and 2 to be highly virulent as compared with types 3 and 4 when inoculated intravenously into 11-day embryos.

We have attempted to define more precisely

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the parameters of infection of the chicken embryo by *N. gonorrhoeae*. This paper presents results of gonococcal infection following two routes of inoculation, the yolk sac (YS) and the CAM. Factors examined included effects of the embryo age, source of inoculum, and gonococcal colony type.

MATERIALS AND METHODS

Strains of *N. gonorrhoeae*. Strain CDC 9, a laboratory-passed organism widely used in gonococcal studies, was provided by D. S. Kellogg, Center for Disease Control, Atlanta, Ga., and strain 72H641, a fresh isolate from joint fluid of a patient with gonococcal arthritis, was furnished by E. S. Murray of this department. Both strains are the wild type (*cys*$^-$). Colony types 1 and 3 (7) were isolated from each strain and transferred daily by selective subculture on agar medium described below. Periodically, T1 and T3 colonies were examined by electron microscopy for presence and absence of pili. Viable counts demonstrated that all inocula used were at least 98% pure with respect to colony type.

Cultivation of *N. gonorrhoeae*. Both solid and liquid media were used. The solid medium (GCA), consisting of GC agar (Difco) supplemented with 1% (final concentration) glucose and 1% IsoVitaleX (Baltimore Biological Laboratories), was used for daily subculture, for preparation and titration of inocula, and for culturing the chicken embryo and extraembryonic fluids. Cultures were incubated for 18 to 24 h at 37°C in an atmosphere of 5% CO_2.

The liquid medium (GCB), prepared according to the procedure of Morse and Bartenstein (11), consisted of proteose peptone no. 3 (Difco), 1.5% (wt/vol); K_2HPO_4, 0.4%; KH_2PO_4, 0.1%; NaCl, 0.5%; and soluble starch (Difco), 0.1%. After autoclaving and immediately prior to use, 1% (wt/vol) (final concentration) glucose, 0.042% $NaHCO_3$, and 1% IsoVitaleX (BBL) were added. Broth cultures were grown at 37°C in a gyratory water bath shaker (New Brunswick Scientific Co.; model G-76).

Preparation of inocula. Inocula consisted of gonococci propagated either on GCA or in GCB, hereafter referred to as agar-grown and broth-grown inocula (or microorganisms). Preliminary studies showed that an 18- to 24-h colony of *N. gonorrhoeae* on GCA contained about 5×10^5 viable microorganisms. In preparing an agar-grown inoculum, colonies of the appropriate morphological type were scraped with a platinum loop from 18-h cultures into GCB, and sufficient colonies were harvested to provide a concentration of about 5×10^9 gonococci per ml. Microorganisms were dispersed by vortexing the suspension in a vial containing glass beads.

In preparing the broth-grown inoculum, colonies of the desired morphological type from an 18-h culture on GCA were scraped into GCB. The gonococci were dispersed by vortexing, and the suspension, containing approximately 10^9 viable microorganisms per ml, was inoculated at approximately 2% into GCB in a Klett flask. The culture was grown from mid to late log phase (optical density of 0.2 at 540 nm). Experience showed that under these conditions the broth contained about 10^9 colony-forming units (CFU) per ml.

Serial 10-fold dilutions of inocula from either source were made in GCB from 10^{-1} through 10^{-10}, vortexing the tubes at each dilution. The number of CFU in the inocula was obtained by titrating, in triplicate, 0.1 ml of dilutions 10^{-4} through 10^{-10} on GCA. Colonies were counted after 18 to 24 h of incubation. Dilutions 10^0 to 10^{10} were immediately inoculated into chicken embryos, as described below.

Infection of chicken embryos. Embryonated eggs of various ages from White Leghorn chickens were bought (Spafas, Inc., Norwich, Conn.) 1 week in advance of use and incubated at 36°C at 20% relative humidity. Embryonated eggs from 6 to 12 days old were tested for infection via the YS route. From preliminary trials, we considered the 10-day-old embryo most suitable for inoculation onto the CAM. Inoculations into the YS and onto the dropped CAM followed standard procedures. Portions (0.2 ml) of dilutions 10^0 to 10^{-10} of the gonococcal suspension were inoculated into the YS of each of at least five embryos. The same series of dilutions in a volume of 0.1 ml was inoculated onto the CAM, with at least five embryos per dilution. Controls included five eggs inoculated with GCB onto both the CAM and YS and also five uninoculated eggs. The CAM eggs were rotated to disperse the inoculum, and incubation of all eggs was continued at 36°C. Eggs were candled to detect hemorrhage 1 h after inoculation and to determine embryo death 24 and 42 h after inoculation. Experiments were terminated 42 h after inoculation, since it was found that all embryos that died did so during this time span. Mean lethal doses (LD_{50}) were calculated by the method of Reed and Muench (12) from the data recorded at both 24 and 42 h.

Infection of the embryo or extraembryonic fluids and membranes was determined 42 h after inoculation. Yolk from embryos inoculated by the YS route was streaked on GCA. Infection after inoculation on the CAM was determined by streaking scrapings from the CAM, as well as allantoic fluid and embryo blood, on GCA. Mean infective doses (ID_{50}) were also calculated from infection data by the Reed and Muench method (12).

LD_{50} values determined at less than 42 h postinoculation proved inconclusive, since embryos died of massive infectious doses in the lower dilutions. More accurate LD_{50} and ID_{50} values could be determined from those dilutions in which gonococci could be directly counted; therefore, only 42-h data are presented in this paper.

An attempt was made to determine the number of viable gonococci in YS, allantoic fluid, and the embryo at time of death after YS inoculation. During the period when embryos were expected to die, eggs were candled hourly. Samples (0.1 ml) of yolk from newly dead embryos were pooled, as were 0.1-ml portions of allantoic fluid. Both were mixed by vortexing in vials containing glass beads. Embryos were washed twice in sterile saline, weighed, and

homogenized in an Omnimix with a measured volume of GCB. The numbers of CFU in all suspensions were determined by titration of serial 10-fold dilutions on GCA.

RESULTS

Infection via the YS. Several parameters of YS infection were investigated, including age of embryo at time of inoculation, source of inocula, and gonococcal colony type. Data presented were obtained from over 100 separate tests, with 60 to 70 embryos for each test.

Age of embryo. The YS of chicken embryos of different ages were inoculated with type 1 (T1) of CDC 9. Seven-, 8-, 10-, and 12-day embryos were tested, 15, 20, 14, and 12 times, respectively. When the LD_{50} was calculated 42 h after inoculation, approximately 5 to 100 CFU were found sufficient to kill embryos (Fig. 1, closed circles). A significant difference ($P < 0.001$) was found in numbers of gonococci required to kill 7- and 8-day-old embryos. A similar difference was also found in susceptibility between 10- and 12-day-old embryos. The mean ID_{50} (open circles) did not vary among 7-, 8-, and 10-day-old embryos, but it varied slightly between 12-day-old and younger embryos. Only 1 to 10 CFU were needed to infect embryos of all ages via the YS. On the basis of the results shown in Fig. 1, 8-day-old embryos were selected for use in the remaining experiments involving YS inoculation.

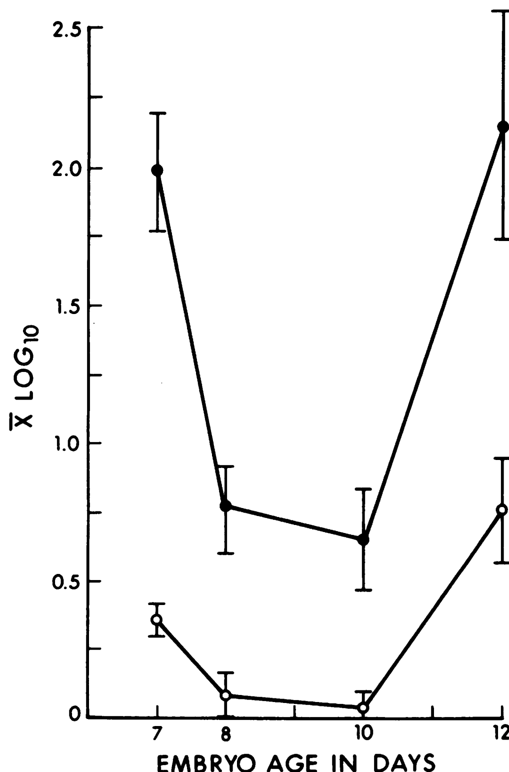

FIG. 1. *Influence of embryo age on infection via the YS with type 1 N. gonorrhoeae strain CDC 9. Embryos were tested at 7, 8, 10, and 12 days of age, and 15, 26, 14, and 12 experiments were performed, respectively. Symbols: (●) LD_{50} at 42 h; (○) ID_{50}. Limits represent SE, where SE = $SD/\sqrt{n}$.*

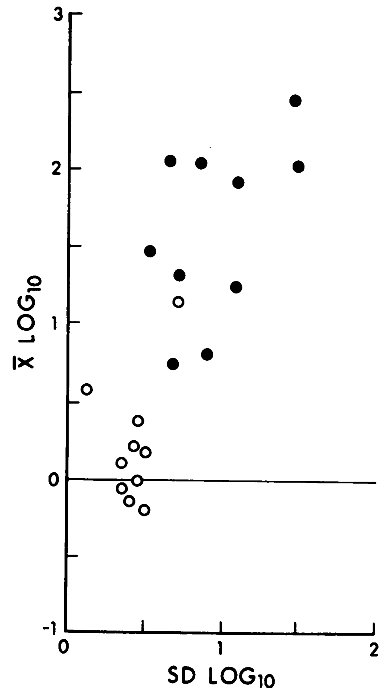

FIG. 2. *Infection of chicken embryos with N. gonorrhoeae via the YS. Comparison of mean ($\bar{x}$) and SD of the mean. Each point represents a given strain and colony type from either a broth- or agar-grown inoculum. Symbols: (●) LD_{50} at 42 h; (○) ID_{50}. Each point represents greater than 6 but less than 16 experiments.*

In Fig. 1, the vertical lines represent the standard error (SE) of the mean. The standard deviation (SD) of the mean of the titers is presented in Fig. 2. The SD were consistent at all LD_{50} titers and never exceeded 2 logs for any experiment. SE = $SD/\sqrt{n}$; therefore, large limits of SE simply reflect a smaller number of tests from which the LD_{50} was calculated.

Inoculum source. An inoculum composed of gonococci grown on solid medium for 18 to 24 h was estimated to contain as many as 95% dead microorganisms. On the other hand, inocula prepared from gonococci cultivated to late log phase in broth were considered to contain only negligible numbers of dead organisms. Each

was titrated in the YS of 8-day-old embryos to determine if the two types of inocula resulted in differences in infection and lethality.

The mean LD_{50} and ID_{50} calculated at 42 h after inoculation did not vary significantly between the broth-grown and the agar-grown inocula (Table 1) for any strain or type. A comparison of the SD of the mean LD_{50} and ID_{50} of the broth-grown inoculum verses agar-grown inoculum revealed that both inocula gave consistent results and were not influenced by technique.

Colony type. Table 1 also shows a comparison of LD_{50} and ID_{50} data produced by colony T1 and T3. Strain and inoculum source may be grouped as CDC 9 agar grown, CDC 9 broth grown, 72H641 agar grown, and 72H641 broth grown with 16, 18, 19, and 15 experiments, respectively. The mean LD_{50} and ID_{50} values were similar in all cases. Negative values indicate that not every embryo in a five- to six-egg set received a viable gonococcus. However, those that did were infected, and the effective infectious dose was 1 CFU via the YS route. Infection always occurred with from 1 to 5 CFU per embryo for each type, regardless of strain or inoculum source.

Comparison of the SD of the mean showed that the T3 results were slightly less variable than were those of T1, and, even in the latter case, the SD was not large. In this regard, it was noted that T3 grow and disperse more readily than do T1, and this may explain the greater consistency of the data.

Since the comparison of inoculum source demonstrated no significant difference in LD_{50} or ID_{50} (see Table 1), data were pooled in Fig. 3 to allow a greater sample size for determination of the effect of colony type on YS infection of 8-day embryos. Comparison of the two strains, CDC 9 and 72H641, showed that the LD_{50} was slightly lower for CDC 9 T1 than for CDC 9 T3, 72H641 T1, and 72H641 T3 (Fig. 3). CDC 9 T3 was less infective via this route than was CDC 9 T1, and both CDC 9 colony types were less infective than 72H641 T1 or T3. The colony types of 72H641 strain gave similar ID_{50} values.

Gonococcal titers at time of death. A determination was made of the number of gonococci present in the embryo and extraembryonic fluids at the time of death. Embryos were infected via the YS with an inoculum sufficient to kill the embryo at 20 to 24 h. YS, allantoic fluid, and embryos were tested after either pooling these materials from embryos dying at the same time (in three experiments with from two to four embryos per pool), or individually testing each embryo as it expired (one experiment). Average values of 1.2×10^8 CFU/ml, 9.88×10^7 CFU/ml, and 3.18×10^5 CFU/g were obtained for gonococcal titers in the YS, allantoic cavity, and the embryo, respectively.

Infection of the CAM: inoculum source. LD_{50} values obtained by inoculation of the dropped CAM of 10-day embryos occurred at very high titers and did not differ significantly with regard to agar-grown or broth-grown inoculum (Table 2). Comparison of SD for CAM experiments to those shown for the YS (see Table 1) shows that more variability occurred with the CAM. There was a slight bias to a broth-grown inoculum source, but really no systematic difference in the SD occurred compared with results of agar-grown inoculum.

Colony type. LD_{50} and ID_{50} values for CAM inoculations were similar when compared for colony type (Table 2). Results from experiments with both types 1 and 3 showed that from 5- to 6-log CFU were required to kill an embryo at 42 h after inoculation. Also, the SD of the mean did not vary significantly at any titer for either agar-grown or broth-grown inoculum experiments; therefore, the inoculum source data may be pooled to increase sample size in order to compare the effect of colony type on infection of the CAM (Fig. 4).

Comparison of the two gonococcal strains, CDC 9 and 72H641 (Fig. 4), showed a difference of infection via the CAM. When different colony types were compared, a disparity in the 42-h LD_{50} appeared between CDC 9 T1 and the CDC 9 T3, 72H641 T1, and 72H641 T3 (Fig. 4). There was also a difference in ID_{50}, with less of the CDC 9 T1 and T3 required to maintain

TABLE 1. *Infection of 8-day chicken embryos via the YS by N. gonorrhoeae*

Strain	Colony type	LD_{50}[a]		ID_{50}[a]	
		Agar grown	Broth grown	Agar grown	Broth grown
CDC 9	T1	0.80 ± 0.90 (12)[b]	0.73 ± 0.67 (14)	−0.03 ± 0.45	0.17 ± 0.48
CDC 9	T3	1.52 ± 0.53 (4)	1.28 ± 0.95 (4)	−0.07 ± 0.13	−0.49 ± 0.17
72H641	T1	1.23 ± 1.09 (15)	1.31 ± 0.70 (10)	−0.20 ± 0.47	0.35 ± 0.45
72H641	T3	0.33 ± 0.18 (4)	1.46 ± 0.53 (5)	−0.16 ± 0.90	−0.09 ± 0.35

[a] Expressed as $\log_{10}$.
[b] Mean ± SD (number of experiments).

infection of the CAM at 42 h. Vertical-line limits represent SE. The lowest ID_{50} value on CAM inoculation was achieved with CDC 9 T3 at 2.5-log CFU.

General. Although detailed autopsy of embryos was not performed in these experiments, it was obvious that *N. gonorrhoeae* produced a consistent gross pathology in the embryo whether infection occurred via the YS or the CAM. After YS inoculation, hemorrhage began in the cranium and along the spine. This progressed to generalized hemorrhage with loss of tissue integrity. Infection of the CAM proceeded as described by Bang (2). There was an initial growth of the gonococci with local hemorrhage along blood vessels. An infiltration of bacteria began along the vessels, and either it proceeded to kill the embryo, as described for the YS infection, or the gonococci were cleared by leukocytes. Successful clearance resulted in the thickening of the CAM at the initial foci of infection and a return to a normal appearance in all other respects. After 42 h, the surviving embryo (now 12 days old) became remarkably resistant to infection.

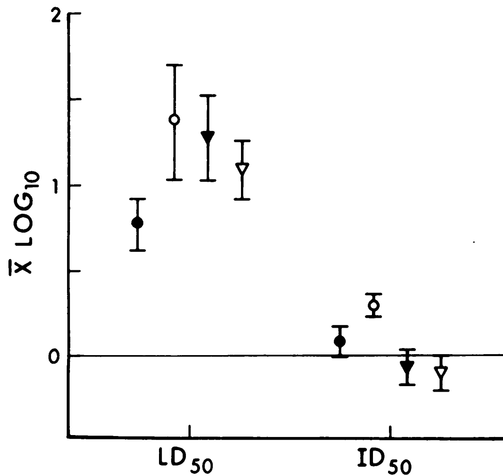

FIG. 3. *Influence of colony type on infection of 8-day chicken embryos via the YS. Symbols:* (●, ○, ▼, *and* ▽) *CDC 9 T1, CDC 9 T3, 72H641 T1, 72H641 T3 and, also, 26, 8, 25, and 9 experiments, respectively.* LD_{50} *and* ID_{50} *values are calculated from data collected at 42 h postinoculation. Limits are in terms of SE.*

DISCUSSION

The developing chicken embryo, susceptible to gonococcal infection, has proved to be a suitable model for studying bacterial pathogenesis. *N. gonorrhoeae* produced a typically consistent pathology in the embryo whether infection occurred via the YS or the CAM. In YS inoculation, an embryo is easily infected, since only 1 to 10 CFU were required to initiate infection by the gonococcus. Eight- to 10-day embryos were killed within 42 h by an initial inoculum of as few as 5 to 100 CFU. Other results showed that approximately 10^5 CFU/ml were present in the yolk at the time of death of an 8-day embryo. Therefore, the 42-h LD_{50} must reflect the time required for 5 to 100 organisms to become established and to multiply to a critically lethal number. At 24 h this number was not attained, and only those embryos initially inoculated with lower dilutions (10^3 or more gonococci) were killed. Since the higher dilutions can be counted directly in all of these experiments at the time of their use as inocula, the results are more accurate in the 0- to 300-CFU LD_{50} and ID_{50} ranges. In YS experiments, this was consistently the range of the 42-h LD_{50} and the ID_{50}. The data for 24-h LD_{50} lie in the range of lower dilutions, which must be calculated (or extrapolated) from more easily counted dilutions. The 24-h data, therefore, are subject to more error, and the LD_{50} values tend to be more variable.

Different LD_{50} and ID_{50} values were obtained for strain CDC 9 types 1 and 3 after inoculation via the YS (see Fig. 3). Piliated T1 gonococci were able to colonize and kill the embryo at slightly lower titers than their T3 counterparts. This difference, however, was not seen in 72H641 T1 and T3. For CDC 9 T1 and T3, the mean LD_{50} was 8 and 40 CFU, and the mean ID_{50} was 1 and 4 gonococci, respectively. Mean

TABLE 2. *Infection of 10-day chicken embryos via the CAM by N. gonorrhoeae*

Strain	Colony type	LD_{50}[a] Agar grown	LD_{50}[a] Broth grown	ID_{50}[a] Agar grown	ID_{50}[a] Broth grown
CDC 9	T1	5.08 ± 1.14 (6)[b]	4.39 ± 1.45 (5)	3.29 ± 0.30	2.63 ± 0.79
CDC 9	T3	5.61 ± 0.67 (7)	5.36 ± 1.31 (12)	2.32 ± 0.43	2.47 ± 0.72
72H641	T1	5.62 ± 1.13 (11)	6.34 ± 0.82 (5)	4.25 ± 1.87	3.94 ± 1.14
72H641	T3	5.77 ± 0.99 (8)	5.46 ± 1.22 (5)	4.58 ± 1.12	3.68 ± 1.03

[a] Expressed as log_{10}.
[b] Mean ± SD (number of experiments).

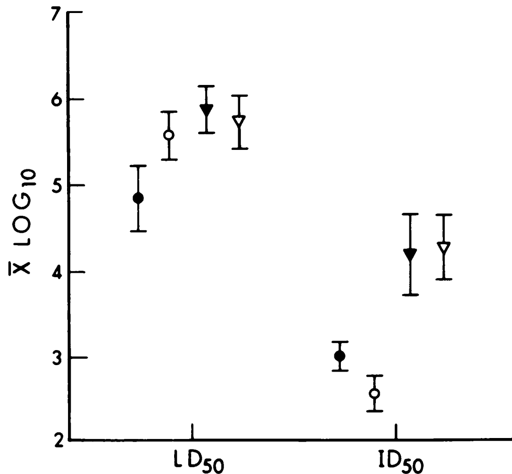

FIG. 4. *Influence of gonococcal type on infection of 10-day chicken embryos via the CAM. Symbols: (●, ○, ▼, and ▽) CDC 9 T1, CDC 9 T3, 72H641 T1, 72H641 T3 and, also, 11, 19, 16, and 13 experiments, respectively. LD_{50} and ID_{50} values are calculated from data collected at 42 h postinoculations. Limits are in terms of SE.*

LD_{50} values for CDC 9 T3 and mean ID_{50} values for CDC 9 T1 were similar to both values for 72H641 T1 and T3. SE was not large at about 1 CFU; however, at such low numbers, ascertaining virulence differences at either the strain or colony-type level is difficult.

Inoculation of the CAM produced hemorrhagic death of the 10-day chicken embryo. Regardless of the inoculum source or colony type employed, 10^5 to 10^6 CFU were required to kill an embryo by 42 h. Infection of the CAM was obtained at 42 h by the initial inoculation of 10^3 to 10^4 CFU. The 10-day embryo was resistant to infection, with less than 10^3 CFU on the CAM; therefore, all LD_{50} and ID_{50} values obtained in these experiments must also be calculated and are subject to the same variability as the 24-h "extrapolated" LD_{50} values for the YS. This larger SD is seen in Table 2.

The SD in extrapolated titers appeared to be more variable; however, even in the 1.5- to 2-log SD range, there was really no large difference in LD_{50} regardless of the inoculum source or colony type for either YS or CAM inoculation. There is also no indication that a larger number of dead, presumably autolyzed, gonococci in the agar-grown inoculum contributes to the death of the embryo. For both sources of inocula, only viable organisms are included in LD_{50} and ID_{50} calculations, and, since LD_{50} and ID_{50} results are similar in both cases, only the growth and invasion of the gonococcus is important.

Only slight differences in LD_{50} and ID_{50} values were obtained with colony types 1 and 3 from strain CDC 9 after CAM inoculation. The mean LD_{50} was slightly less and the mean ID_{50} was slightly greater for CDC 9 T1 than for CDC 9 T3 (Fig. 4). Strain 72H641 T1 and T3 did not differ in either LD_{50} or ID_{50} and were similar to CDC 9 T3 for LD_{50} but not for ID_{50}. The two strains differed significantly in ID_{50} for the CAM from 2.5- to 3-log CFU for CDC 9 to about 2.4-log CFU for 72H641. Apparently, CDC 9, and especially CDC 9 T3, is better able to colonize and grow on the CAM. Colonization at lower titers, however, did not effect lethality, since CDC 9 T1, which required more gonococci for colonization, killed embryos at lower initial titers than its T3 counterpart.

For a particular strain no great differences in virulence were seen between types 1 and 3 gonococci in either YS or CAM inoculation experiments, and therefore the conclusions of Bumgarner and Finkelstein (5) are supported. However, these differences may be greater or less depending upon the strain being used, and significant strain differences may occur as seen in the ID_{50} results for CAM experiments. Nevertheless, the chicken embryo, although a consistent model of gonococcal infection and pathology, is not, by the criteria of strain 72H641, a consistently sensitive indicator of virulence differences between gonococcal morphological types.

Susceptibility of the embryo to infection via the YS is maximal at 8 through 10 days. The slight resistance that occurs prior to 8 days is inexplicable, but it may be due to the lack of a confluent bridge between the YS and the embryo at this time. After 12 days, perhaps undetermined defense mechanisms of the embryo are able to ward off any but an overwhelming inoculum. A similar type of age-related resistance pattern has been noted for the chicken embryo in its response to an endotoxin from *N. meningitidis* inoculated onto the CAM (13). Presumably, the deficiency of the vascular system of the CAM prior to 8 days accounts for the early resistance to this material.

These results do not indicate how gonococci kill chicken embryos. Obviously, there is a critical number of organisms that must be present to cause death. Whether nutritional competition or the action of some active gonococcal metabolite (or other virulence factor) is responsible is not known at this time. The reproducibility of infection by the gonococcus by both YS and CAM routes, even though virulence differences are not apparent between morphological types, may prove useful for studying neutralization by specific antibody or the influence of

subcellular components as mediators of the hemorrhagic death produced in the chicken embryo by *N. gonorrhoeae*.

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LITERATURE CITED

1. **Arko, R. J.** 1972. *Neisseria gonorrhoeae*: experimental infection of laboratory animals. Science 177:1200-1201.
2. **Bang, R.** 1941. Experimental gonococcus infection of the chick embryo. J. Exp. Med. 74:387-396.
3. **Bradford, W. L., and R. Tittsler.** 1936. Experimental gonococcal infection in the chick embryo. Proc. Soc. Exp. Biol. Med. 34:241-242.
4. **Buchanan, T. M., and E. C. Gotschlich.** 1973. Studies on gonococcus infection. III. Correlation of gonococcal colony morphology with infectivity for the chick embryo. J. Exp. Med. 137:196-200.
5. **Bumgarner, L. R., and R. A. Finkelstein.** 1973. Pathogenesis and immunology of experimental gonococcal infection: virulence of colony types of *Neisseria gonorrhoeae* for chicken embryos. Infect. Immun. 8:919-924.
6. **Hill, J. H., and A. C. Pitts.** 1939. The growth of *Neisseria gonorrhoeae* on the chorioallantoic membrane of the chick embryo. J. Urol. 41:81-83.
7. **Kellogg, D. S., Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and C. I. Pirkle.** 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. J. Bacteriol. 85:1275-1279.
8. **Lucas, C. T., F. Chandler, Jr., J. E. Martin, Jr., and J. D. Schmale.** 1971. Transfer of gonococcal urethritis from man to chimpanzee. An animal model for gonorrhoeae. J. Am. Med. Assoc. 216:1612-1614.
9. **Miller, D. P.** 1948. Experimental gonococcal infection of the rabbit's eye. Am. J. Syph. 32:437-444.
10. **Morrow, G., and G. P. Berry.** 1938. The culturation of *Neisseria gonorrhoeae* on the chorioallantoic membrane of the chick embryo and the use of the technique for the study of sulfamilamide. J. Bacteriol. 36:280-281.
11. **Morse, S. A., and L. Bartenstein.** 1974. Factors affecting autolysis of *Neisseria gonorrhoeae*. Proc. Soc. Exp. Biol. Med. 145:1418-1421.
12. **Reed, L. J., and H. Muench.** 1939. A simple method of estimating fifty per cent end points. Am. J. Hyg. 27:493-497.
13. **Smith, R. T., and L. Thomas.** 1956. The lethal effect of endotoxins on the chick embryo. J. Exp. Med. 104:217-231.
14. **Walsh, M. J., B. C. Brown, L. Brown, and C. I. Pirkle.** 1963. Use of the chick embryo in maintaining and restoring virulence of *Neisseria gonorrhoeae*. J. Bacteriol. 86:478-481.