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Phase Transition of Gonococci in Mammalian Cell Cultures

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Neisseria gonorrhoeae was cultivated in mammalian cell cultures in an effort to determine if this environment will elicit a T4 → T1 transition. Of four avirulent (T4) isolates tested, only one, H4, yielded T1 colonies. This change was consistently obtained in HeLa, WI-38, and MK2 cells, even when the multiplicity of the gonococcal infection was less than 1 per culture. Growth of the gonococci took place primarily on the surface of the cells, as demonstrated by light and electron microscopy, but occasional bacteria were undoubtedly intracellular. T1 colonies were seen at 24 h and were the major population at 48 h. This shift was favored by the presence of viable cells, since smaller yields of T1 were obtained when the cells were irradiated or heat inactivated. It was also favored by low pH, since T1 recovery was reduced when the buffering capacity of the medium was increased. Although the results suggest that T1 gonococci derived from H4 have a selective advantage over T4 in cell cultures, this is not true of all T1 and T4 colony types. F62 T4, which does not undergo a T4 → T1 shift, propagated as well as T1 in HeLa cell cultures. The change in colony type of strain H4 to T1 was accompanied by formation of pili and by gain in capacity for deoxyribonucleic acid-mediated transformation. It is concluded that gonococci can undergo T4 → T1 phase transition in mammalian cell cultures, but this property is not retained by all strains.

The apparent association of virulence and avirulence of *Neisseria gonorrhoeae* with colonial morphology of bacterial isolates as observed and reported by Kellogg et al. (10, 11) has given new impetus to investigations on this important pathogen. In addition to forming distinctive colony types on agar medium, virulent gonococci (designated T1 and T2) possess pili (9, 23), resist phagocytosis by human leukocytes, are capable of attachment to various types of host cells (8, 16, 17, 22, 23, 25, 26, 28), and can act as recipients for genetic transformation (21). Examination of bacterial colonies derived from clinical specimens from both males and asymptomatic females generally reveal predominantly T1 and/or T2 morphologies.

Nonselective in vitro transfer of *N. gonorrhoeae* isolates results in a diminution of T1 and T2 colonies with increasing numbers of large amorphous colony types (T3 and T4) which were shown to be avirulent in male volunteers (10, 11). Continued transfer of the cultures resulted in bacterial populations exclusively of the avirulent (T4) type. Avirulent types of *N. gonorrhoeae* are not piliated (23), do not become competent for bacterial transformation (21), and are readily phagocytized by human leukocytes (17, 25).

In vitro maintenance of virulent gonococci can only be accomplished by selective transfer of colony types on agar medium. There is not, at the present time, a liquid growth medium that will permit cultivation of phenotypically virulent gonococci, although some strains tend to revert to avirulent types at a lower frequency than others (10). The inability to produce large populations of virulent organisms in broth cultures uncontaminated with the avirulent types presents a handicap for investigations on antigenic make-up, physiological properties, and genetics of *N. gonorrhoeae*.

Conditions favoring selection of virulent gonococci are obviously present in the host. Avirulent types are quickly phagocytized and removed from the inoculation site, whereas virulent types are present in exudates from experimentally infected urethras of males (10). In addition, T1 ⇆ T_2 alterations occur quite readily in the host, but reversion of T4 → T1 after prolonged subculturing of the T4 organisms does not appear to occur.

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An insight into host factors responsible for the maintenance of the virulent types may be derived from propagation of *N. gonorrhoeae* in mammalian cell cultures. Kenny and Aris (12) reported that HeLa and human embryonic cell lines inoculated with T3 or T4 gonococci yielded within 48 h only T1 organisms. If these results represent a general property of gonococci they would indicate that the genetic information governing the virulent colonial phenotypes had not been irretrievably lost.

A second possibility of maintaining virulent type gonococci by use of tissue cells is indicated by the results of Kenny and Sparkes (13), who reported the isolation of a "bacterial growth inhibitor" in used medium from cultured human cells (HeLa, embryonic kidney, and kidney). The inhibitor in the spent medium, identified as an α-keto-aldehyde attached to a carrier, prevented the growth of avirulent gonococci while having no effect on the virulent types. These results, if widely applicable to gonococci, may lead to the incorporation of the inhibitor into liquid media currently used for the cultivation of *N. gonorrhoeae*, thus permitting the formulation of a selective medium for virulent gonococci.

In this paper we describe the interactions of gonococci with various mammalian cell lines. We show that reversion of avirulent gonococci propagated in mammalian cell cultures to phenotypically virulent gonococci is not a generalized phenomenon.

MATERIALS AND METHODS

Bacterial strains. *N. gonorrhoeae* strains F62, RD-5, and "H" were obtained from D. Kellogg, Center for Disease Control, Atlanta, Ga. Strain "H" was a clinical isolate and arbitrarily assigned a designation in our laboratory. Strains ATCC 19424 and RD-1 were from departmental stocks. Virulent colony types (T1) of F62, 19424, RD-1, and "H" were maintained by selective transfer as described by Kellogg et al. (11). Avirulent colony types (T4) were isolated from these strains and repeatedly streaked on solid medium until free of other detectable colony types. RD-5, a laboratory strain, consisted of 100% T4 colony type (11).

Spontaneous mutants of F62 resistant to 1 mg of streptomycin sulfate (Sm-R) per ml or 10 μg of nalidixic acid (Na-R) per ml were isolated on appropriate solid media containing the corresponding antibacterial agents. Strain "H" was Sm-R when received in this laboratory.

All strains and types of *N. gonorrhoeae* were constantly monitored for purity by Gram stain, oxidase test, and production of acid from glucose but not from maltose or lactose. Maintenance of cultures, incubation conditions, enumeration, and differentiation of colony types have been previously described in detail (25).

Bacteriological media. GC medium base (Difco) supplemented with IsoVitaleX (BBL), or with supplements I and II (GSI) (21), was used throughout this study. Dilutions and washings of bacteria were done in saline-cysteine diluent (0.1% cysteine [free base] plus 0.86% NaCl, [7]). Sugar reactions were evaluated in cysteine Trypticase agar (Difco) containing 1% of the various substrates to be tested.

Bacterial inocula for tissue cultures. Inocula of gonococci were produced by either one of two methods. (i) Bacteria were harvested from 16- to 18-h GCI plates with saline-cysteine diluent, washed by centrifugation, and adjusted to the desired concentrations with the same diluent. (ii) Frozen cultures of known titer were thawed and diluted to the desired titer in tissue culture maintenance medium.

Mammalian cell cultures. Stock cultures of HeLa (CCL 2) and monkey kidney (LLC MK2) were obtained from departmental stocks. The diploid cell line WI-38 was obtained from the American Type Culture Collection. Experiments were performed with WI-38 cells in the 28th to 33rd passage. Stock cultures of the various cell lines were grown and maintained in 32-oz (about 960 ml) glass prescription bottles in antibiotic-free media specified by the ATCC (20) plus 2 mM added L-glutamine. All tissue culture media, supplements, and sera were obtained from Grand Island Biological Co., Grand Island, New York.

Irradiated MK2 cells were derived by exposing cultures to ^{60}Co (3,000 R) 6 days before use (6).

Nonirradiated or irradiated cells suspended in appropriate dilutions in the same medium were placed in 1-dram shell vials (15 by 45 mm), containing 12-mm circular cover slips, to provide confluent monolayers 48 h later.

All cell lines were periodically checked for pleuropneumonia-like organism contamination by the method of Rothblat (19).

Inoculation and examination of cultures. Before inoculation with gonococci, the growth medium was removed from the cell cultures and replaced with 1 ml of prewarmed maintenance medium (M199 or minimal essential medium plus 1% [final concentration] nonessential amino acids and 2 mM L-glutamine). Each vial was inoculated with 0.1-ml volumes of the bacterial suspension, stoppered, and centrifuged at 680 $\times$ *g* in a refrigerated horizontal centrifuge (International) at 18 C for 1 h (29). The cultures were placed at 37 C for 30 min, and the medium was withdrawn and replaced with 1 ml of prewarmed, fresh maintenance medium. This point represents zero time in our experimental results.

Uninoculated cell monolayers and maintenance medium receiving the bacterial inoculum were used as routine controls for all experiments.

At various time intervals after inoculation, the contents of the vials were examined as follows. (i) One-tenth milliliter of the maintenance medium was withdrawn, serially diluted, and plated on GCI medium. (ii) The remaining medium was withdrawn, and the tissue cells were released from the cover slip by incubation with 0.2 ml of 0.25% trypsin for 10 to 15 min at 37 C. The released cells were vigorously agitated on a Vortex mixer and 0.8-ml volumes of prewarmed maintenance medium containing 10% calf

serum were added. One-tenth milliliter of the cell suspension was then diluted and plated on GCI plates for enumeration of cell-associated gonococci. (iii) Companion monolayers were fixed with methyl alcohol and stained with Giemsa for microscope observations.

Genetic transformations of N. gonorrhoeae. Deoxyribonucleic acid (DNA) was extracted and purified from the various strains of *N. gonorrhoeae* by the method of Marmur (15). The various DNA isolates were dissolved in standard saline citrate (0.15 M NaCl + 0.015 M Na_3 citrate, pH 7.0), and stored at 4 C. The concentrations of the DNA preparations were determined by the Burton technique (2).

Transformations of *N. gonorrhoeae* recipients were conducted by the methods described by Sparling (21). Several loops of growth from a 16-h GCI plate or slant were suspended in 2 ml of GCI broth. After vigorous agitation on a Vortex mixer, the suspension was diluted until slightly turbid (about 10^7 colony-forming units [CFU]/ml) with GCI broth. A sample (0.85 ml) of this suspension was added to screw-cap tubes (100 by 13 mm) containing 0.1 ml of Sm-R or Na-R DNA (25 μg) and 0.05 ml of $CaCl_2$ (final concentration, 0.002 M). The reaction mixtures were incubated without shaking in a CO_2 incubator at 37 C for 30 min, after which 0.05 ml (50 μg) of deoxyribonuclease (once crystallized; Worthington Biochemical Corp., Freehold, N.J.) was added, and incubation was continued for 10 to 15 min. Cells untreated with DNA were used as controls in all experiments. Revertants were rarely observed.

Transformants to Sm-R or Na-R were diluted in a saline-cysteine diluent and plated on GCI medium (25 ml of medium per plate). After incubation at 37 C in a CO_2 incubator for 3 h to allow for expression, the agar was transferred to a large petri dish containing 25 ml of the medium plus sufficient streptomycin sulfate or nalidixic acid to provide 1 mg/ml or 10 μg/ml, respectively, after diffusion into the upper layer (1, 27). The plates were then incubated at 37 C in 7% CO_2 and the transformants were counted after 18 to 20 h with the aid of a dissecting microscope.

Electron microscopy. The methods of preparing specimens of *N. gonorrhoeae* for examination of piliation were as described by Swanson et al. (23).

Thin sections of HeLa cell monolayers infected with gonococci were prepared as follows. HeLa cells infected 16 h previously were fixed in situ by gently withdrawing the broth medium and adding 1 ml of 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2. After 1 h of fixation and two 5-min rinses in cacodylate buffer containing 7.5% sucrose, the specimens were treated for 1 h in 1% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.2. Before dehydration and embedding in Epon, they were rinsed twice for 5 min with cacodylate buffer.

The Epon embedding procedure of Chang (4) was used except that the cover slips were not treated with Teflon. The intact dehydrated cover slip cultures were flooded with Epon and inverted over capsules filled to capacity with Epon. After polymerization, the cover slips were separated from the embedded specimens by immersion in liquid nitrogen. The blocks were sectioned and the specimens were stained with uranyl acetate and counterstained by Reynolds' lead citrate (18). The preparations were examined and photographed with a Siemens Elmiskop 1A electron microscope.

RESULTS

Growth of N. gonorrhoeae in mammalian cell cultures. The data in Table 1 represent a summary of the types of *N. gonorrhoeae* recovered after incubation in cell cultures for 48 h. Although the various avirulent isolates propagated equally well in the mammalian cells, only strain "H" produced phenotypic virulent types after in vitro cultivation.

The following general observations were made on the interaction between gonococcal strains and mammalian cells. (i) None of the strains grew consistently in maintenance medium alone, minimal essential medium or M199, or when supplemented with 10% heat-inactivated calf serum. Occasionally, growth occurred in one vial of duplicate samples, but not in the other. (ii) All strains of gonococci propagated to titers of approximately 10^8 CFU/ml in the presence of mammalian cells, even from inocula as low as 10 CFU/ml. HeLa, WI-38, and MK2 cells were equally satisfactory. (iii) The growth of the gonococci reduced the pH of the fluid medium, with the larger inocula the culture medium becoming acid within 24 h. (iv) The monolayers were virtually destroyed after 48 h. (v) Spent tissue culture growth medium from the various cell lines did not support the growth of the different gonococcal strains and colony types used in this study.

TABLE 1. *Summary of N. gonorrhoeae colony type recovery after growth in cell cultures*[a]

Strain of *N. gonorrhoeae*	Inoculum type	Cell line	Colony type other than inoculum	
			Type	%
ATCC 19424	T4	HeLa		0
		WI-38		0
H4	T4	HeLa	T1	90
		MK2	T1	90
		IMK2	T1	49
		WI-38	T1	89
RD5	T4	HeLa		0
		WI-38		0
RD1	T4	HeLa		0
		WI-38		0

[a] Colony types were assessed by plating in triplicate appropriate dilutions of the cell culture medium on GCI medium after 48 h of incubation of the infected cell cultures. The plates were incubated at 37 C in a CO_2 incubator for 18 to 20 h and the colonies were enumerated with the aid of a dissecting microscope.

Because T4 gonococci of strain "H" appeared unique in that large populations of phenotypic virulent organisms (T1) were produced in cell cultures, the interactions of this strain with HeLa cells were studied in greater detail.

Cytological observations. Light microscopy of stained cover slip preparations at times 0, 24, and 48 h postinfection showed that a small number of gonococci gave rise to large numbers of bacterial aggregates on the monolayer within 24 h (Fig. 1A). It was not possible to clearly identify intracellular bacteria in these preparations. After 48 h, only few cells and extracellular gonococci were observed (Fig. 1B). During this period of incubation, the monolayers gradually sloughed from the cover slip and the pH of the medium became acid, but the medium did not become very turbid during the incubation period. The above observations suggested that the gonococci did not replicate primarily in the mammalian cells or free in the medium, but at the cell surface. We attempted to confirm our observations on the location of the gonococci by examining by electron microscopy thin sections of monolayers infected with "H" gonococci and incubated for 16 h. Occasional bacteria appeared to be intracellular as evidenced by a surrounding membrane and their close proximity to the nucleus of the cell (Fig. 2A). Most of the other bacteria observed were extracellular, some near the cell surface (Fig. 2B).

Alterations and growth of strain "H" propagated in HeLa cells. The data presented in Table 2 represent typical results when strain "H" was propagated for 48 h at 37 C in HeLa cell cultures. In the absence of HeLa cells, the gonococci did not survive in Eagle minimal essential medium. Similar observations were made when medium 199 was used as the maintenance medium. In the presence of HeLa cells T4 gonococci multiplied rapidly and were recovered in large numbers in the medium. Gonococci remaining in association with the HeLa cells (not shown) were more difficult to quantify, but they appeared to be smaller in numbers. T1 appeared at 24 h and, after 48 h of incubation, the population of gonococci in the cell cultures was predominantly T1.

Although we were unable to detect T1 colonies in our inocula for these experiments, the possibility existed that very small numbers were present and that they had a selective growth advantage in cell cultures. If this were the case, the data presented in Tables 1 and 2 did not necessarily represent phase transition. To test the possibility that T4 gonococci were contaminated with small numbers of T1, an end-point dilution experiment was performed (Table 3). The inoculum, containing no detectable T1, was diluted to the extent that only a fraction of the inoculated cultures were infected. It can therefore be assumed that the 10 infected cultures, of a total of 31, received only one or a very small number of T4 infective units. Still, at 48 h each one of the 10 infected cultures contained T1 gonococci and in all cases, except one, high proportions of this colony type (Table 3).

The data presented in Table 2 suggest that T1 gonococci have a selective growth advantage over T4 organisms in cell culture, but this is not necessarily true of strains which do not undergo change in colony type formation. T4 and T1 gonococci derived from strain F62 were grown in HeLa cell cultures independently or as a mixture. Growth and recovery of the individual types in the two cases was approximately the same (Fig. 3).

Effect of viability of cell cultures and pH on growth and phase transition of H4. H4 gonococci propagated on heat-inactivated monolayers of HeLa cells as well as on viable cells (Table 4). The transition from T4 colony types, however, did not occur as efficiently on the inactivated monolayers. These data are consistent with the lowered yield of T1 types from irradiated monkey kidney cells (Table 1).

As previously indicated, the growth of gonococci in HeLa cell cultures rapidly lowered the pH of the maintenance medium, and after 48 h the monolayers were virtually destroyed. Experiments were performed to determine if the H4 gonococci would propagate and shift to T1 types in a more highly buffered maintenance medium. The gonococci propagated, but phase transition was reduced (Table 5). Furthermore, microscope observations showed that the monolayers were approximately 50% intact.

Characteristics of T1-type gonococci from infected HeLa cultures. In addition to characteristic colony appearances, virulent gonococci are also capable of undergoing DNA-mediated transformation and possess pili. The phenotypically virulent colony types derived from cell cultures (end-point dilution experiment, Table 3) were examined for these characteristics. Only bacteria manifesting the virulent types transformed at appreciable levels (Table 6).

Examination of F62-T1 organisms served as controls for pili demonstration (Fig. 4A). Colony types that were phenotypically T1 when derived from HeLa cell cultures also possessed pili (Fig. 4B). We did not observe pili on F62-T4 or H4 gonococci.

DISCUSSION

Our results confirm those of Kenny and Aris (12) that phase transition of T4 to T1 gonococci

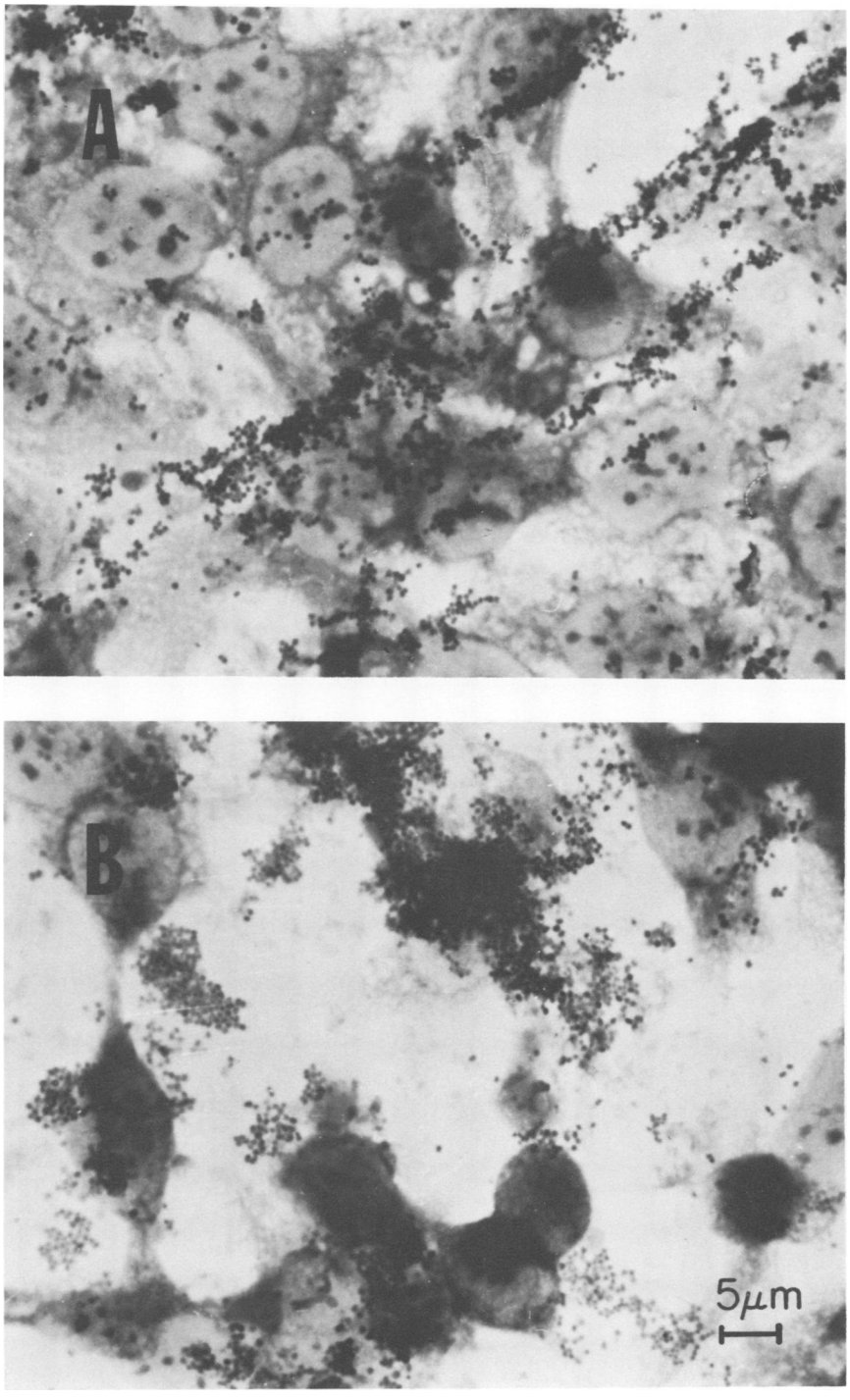

FIG. 1. *Growth of N. gonorrhoeae in HeLa cell cultures. (A) After 24 h of incubation at 37 C. (B) At 48 h.*

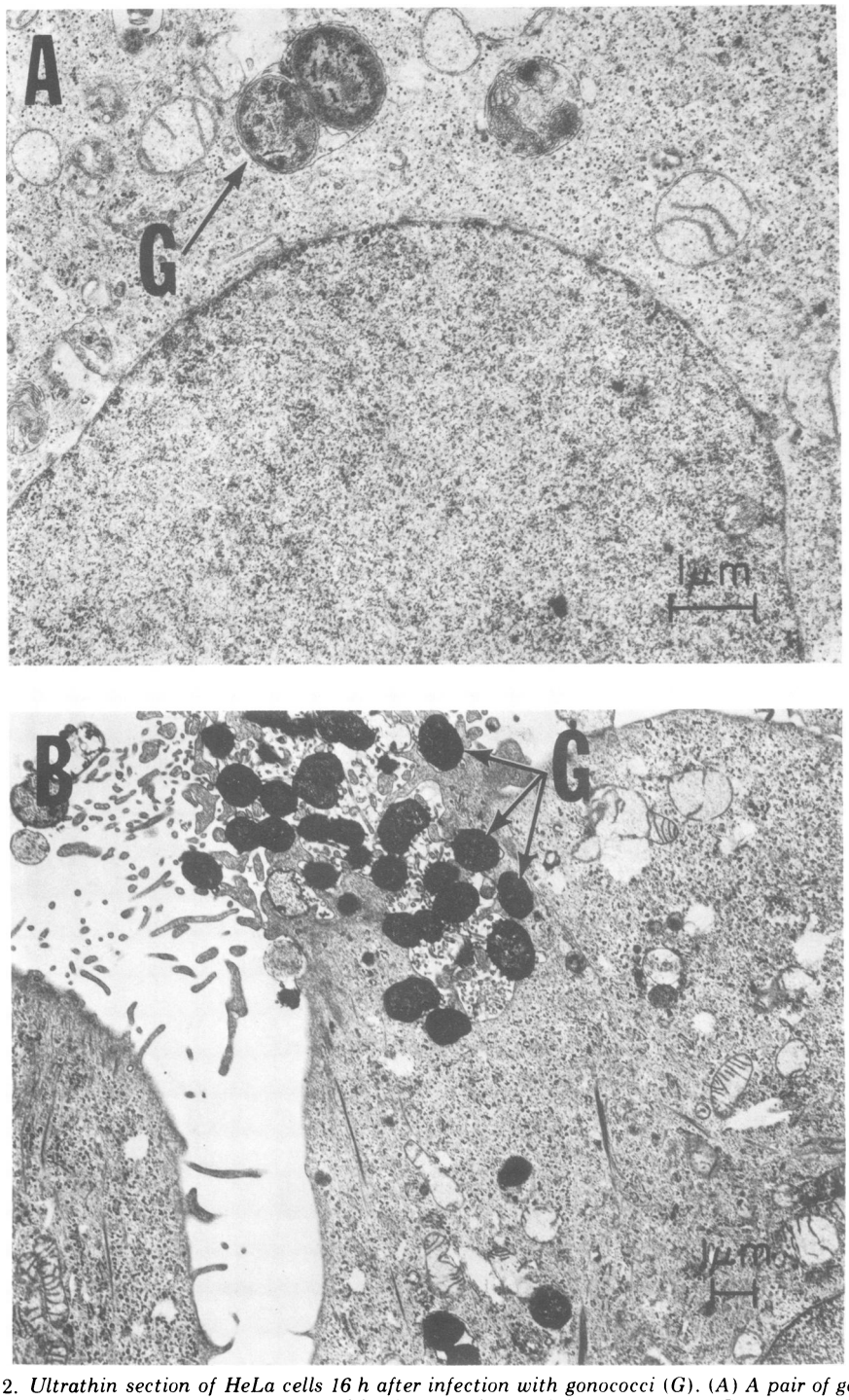

FIG. 2. *Ultrathin section of HeLa cells 16 h after infection with gonococci (G). (A) A pair of gonococci are enclosed within a membrane and are located in close proximity of the cell nucleus. (B) Aggregate of gonococci near cell surface. They do not appear to be intracellular.*

TABLE 2. *Growth and recovery of N. gonorrhoeae colony types from HeLa cell cultures inoculated with colony type T4 (strain H4)*[a]

Time (h)	CFU/ml				T1 type colonies from HeLa cell cultures (%)
	Medium[b] without HeLa cells		Medium from HeLa cell cultures		
	T1	T4	T1	T4	
0	<10	4.8×10^5	<10	6.5×10^4	
4	<10	3.0×10^5	<10	6.5×10^5	
24	<10	4.6×10^2	6.0×10^5	1.9×10^7	3
48	<10	<10	8.3×10^7	7.3×10^8	92

[a] The data presented were obtained from single specimens plated in triplicate. Entirely comparable results were obtained repeatedly with minimal essential medium or medium 199.

[b] The medium consisted of Eagle minimal essential medium with Hanks balanced salt solution, 0.29 mg of L-glutamine per ml, and the addition of 1% nonessential amino acids.

TABLE 3. *Dilution end-point infection of HeLa cells with N. gonorrhoeae T4 (strain H4)*

No. of cultures infected/no. inoculated	Calculated[a] infectious units/culture	CFU/infected culture ($\times 10^5$)[b]	T1-type colonies	
			Per culture ($\times 10^5$)[b]	(%)
7/15	0.63	25	24	96.0
		100	100	100.0
		178	124	69.7
		4.85	2.1	43.3
		17.5	13.1	74.9
		4.82	4.75	98.5
		143	143	100.0
3/16	0.21	311	309	99.4
		0.09	0.04	44.0
		28.9	0.10	0.3

[a] $P_{r>0} = 1 - e^{-s}$: $P_{r>0}$ = fraction of cultures infected; s = average no. of infectious units/culture (14). The inoculum contained no detectable T1.

[b] At 48 h.

TABLE 4. *Growth and recovery of N. gonorrhoeae colony types from viable and heat-inactivated HeLa cell cultures*[a]

Time (h)	HeLa cells heat inactivated[b]	CFU/ml	T1 colonies (%)
0	+	8.0×10^4	0
	−	2.0×10^4	0
24	+	8.7×10^7	25
	−	9.8×10^7	0
48	+	1.0×10^8	33
	−	2.5×10^8	84

[a] The medium was the same as described for Table 2, except that medium 199 was substituted for minimal essential medium.

[b] At 56 C for 15 min. Loss of viability was checked by the eosin Y dye exclusion test.

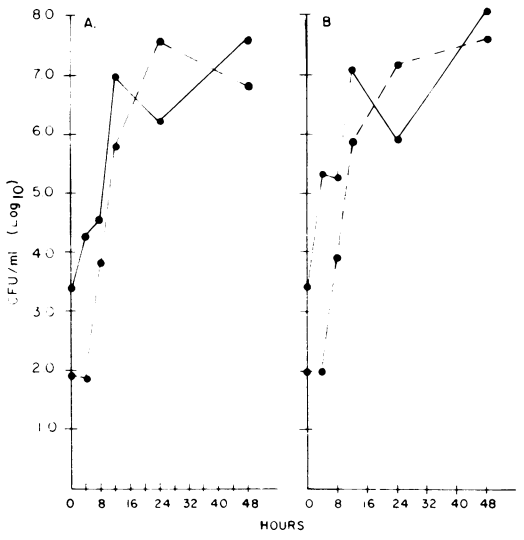

FIG. 3. *Recovery of N. gonorrhoeae F62T1 Na-R (dotted lines) and F62T4 Sm-R (continuous lines) from infected HeLa cell cultures. (A) Growth of the two types separately. (B) Growth of a mixture of the two types. The cultures were sampled at the times indicated and plated on selective media.*

TABLE 5. *Effect of 25 mM HEPES buffer on pH changes in HeLa cell cultures infected with N. gonorrhoeae H4*[a]

Time (h)	HEPES buffer present	pH	CFU/ml	T1 colonies (%)
0	+	7.5	6.0×10^5	0
	−	7.4	1.7×10^5	0
24	+	7.2	2.0×10^8	24
	−	<6.8	4.0×10^8	40
48	+	7.0	4.0×10^7	11
	−	<6.8	1.0×10^7	81

[a] Except for the inclusion of HEPES, the medium was the same as that described for Table 4. HEPES is *N*-2-hydroxyethyl-piperazine-*N*'-2'-ethanesulfonic acid (obtained from Grand Island Biological Co.).

TABLE 6. *Transformability of N. gonorrhoeae clonal types isolated from HeLa cell cultures to nalidixic acid resistance*[a]

Clonal type	Na-R isolated (CFU/ml)	Transformation (%)
T1	3.9×10^4	0.8
T1	2.9×10^4	0.5
T2	6.8×10^4	0.2
T4	5.5×10^1	0.0018
T4	3.8×10^2	0.0021
T4	1.5×10^1	0.00065

[a] With the DNA of strain $F62T_1$-Na-R.

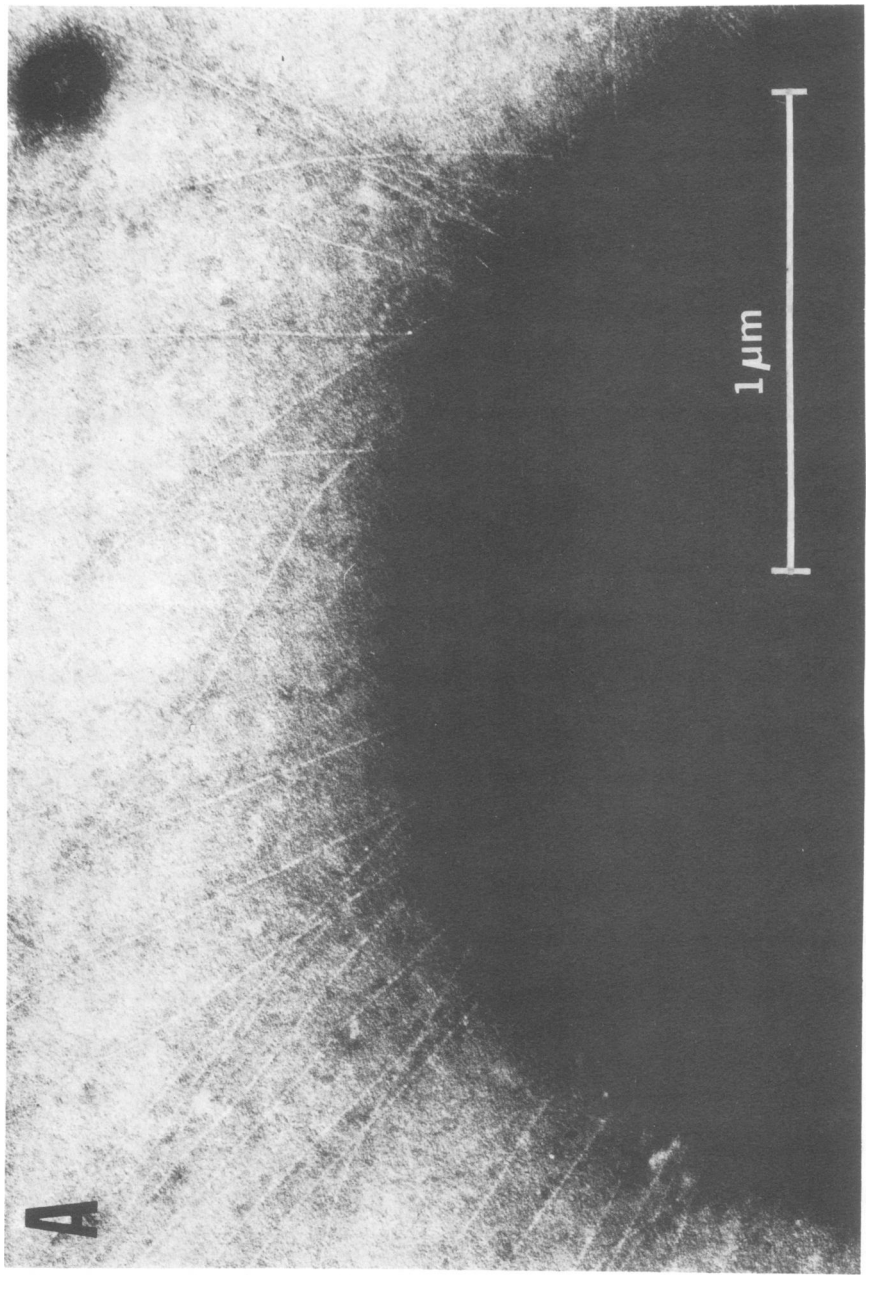

FIG. 4. *N. gonorrhoeae showing pili originating from the cells. Negatively stained with uranyl acetate. (A) Strain F62T1. (B) T1 isolate from HeLa cell culture (see Table 3).*

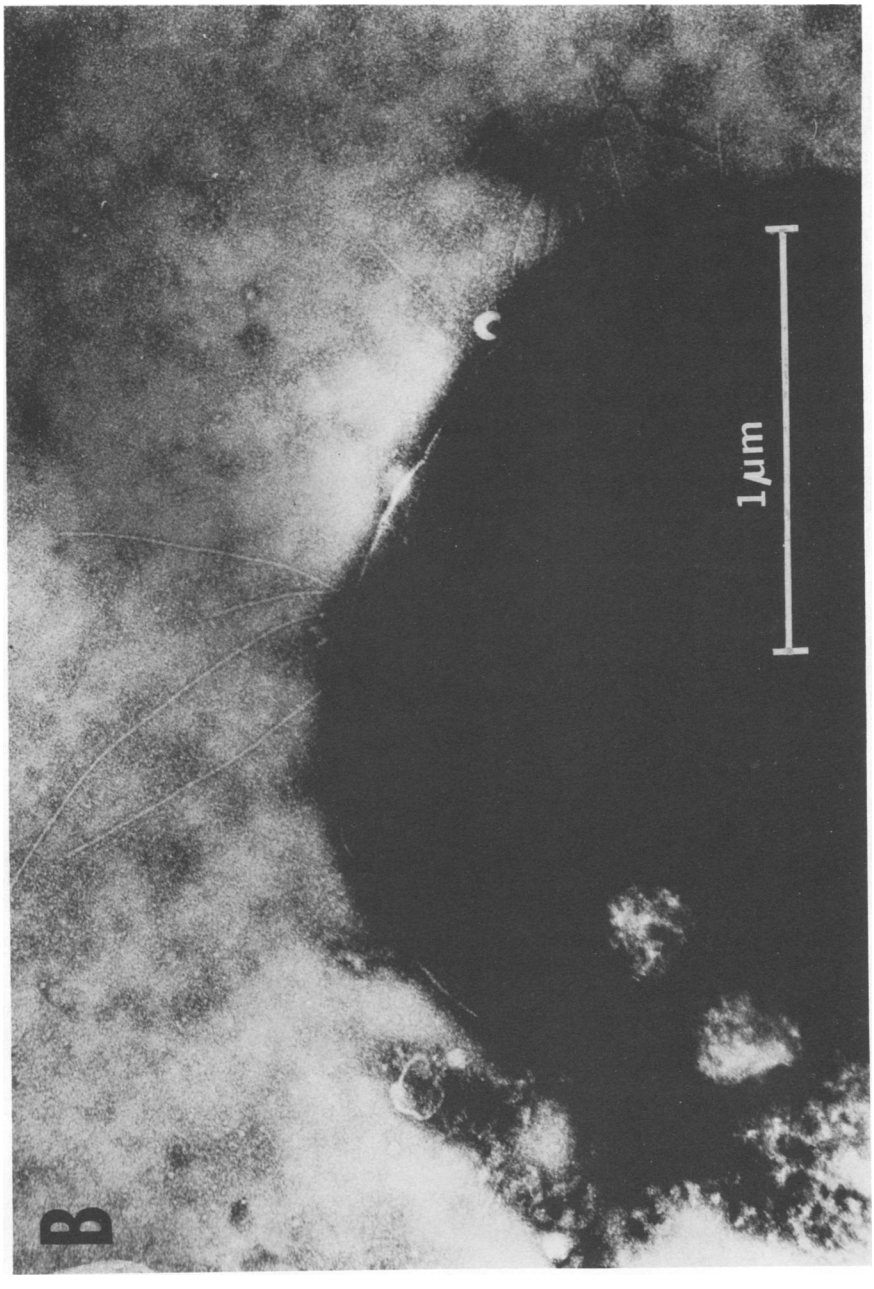

FIG. 4B.

can be obtained in mammalian cell cultures, but indicate that this happens only with certain strains. Of the four strains tested only H4 was shown to be capable of reversion to T1 and this reversion occurred quite consistently with three mammalian cell lines. The infrequency of T4 → T1 phase transition among gonococcal strains is possibly one of the reasons why the results of Kenny and Aris (12) have not been noted by other investigators. Most of the reported tissue culture experiments were short term and not true tests of phase transition. The experiments of Gavrilescu et al. (5) were carried out with an unidentified type. Carney and Taylor-Robinson (3) cultured a T4 strain for several days in fallopian tube or tracheal organ cultures, but made no mention of T1 isolations.

The factors in cell cultures responsible for phase transition have not been identified. The gonococci appeared to multiply in close association with the mammalian cells, but the majority were extracellular (Fig. 1 and 2). Some were clearly intracellular as indicated by Thayer et al. (24). It is not known if phase transition occurred in an extracellular or in an intracellular location. The time of first occurrence of the T1 type has not been determined, but this type could be easily detected at 24 h and constituted the predominant population at 48 h. This shift was favored by the viability of the cells, since the use of irradiated MK2 cells (Table 1) or heat-inactivated HeLa cells (Table 4) resulted in a lower proportional yield of T1. In one attempt in our laboratory at cultivation of the gonococci in tissue culture medium plus HeLa cell plasma membranes, the gonococci replicated but did not undergo phase transition. The T4 → T1 shift was also favored by an acid environment, since stabilization of the pH of the cultures with *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid buffer reduced the recovery of T1 (Table 5).

It is obvious that under the conditions of our experiments T1 gonococci derived from H4 had a selective advantage over T4. This advantage, however, does not seem to reflect properties of all T1 and T4 gonococci. In fact, T1 and T4 colony types derived from another strain (F62) appeared to grow equally well in HeLa cell cultures (Fig. 3).

The T1 gonococci isolated from H4 fulfill two major criteria for classification as T1 in addition to colony morphology (11): they possess pili (Fig. 4; 23) and can undergo DNA-mediated transformation (Table 6; 21). The results shown in Table 3 strongly suggest that T1 gonococci can be obtained from individual T4 cells. Thus, this change is a true phase transition and not just a population shift. The genetic basis for the T4 → T1 change remains obscure. Our observation that it was obtained with one strain but not with others offers an opportunity for further investigation. It is not known if this transition is a mutational event or involves a cytoplasmic or nuclear gene that is either lost or remains repressed in certain strains but not in others.

We were not able to reproduce with our strains the results of Kenny and Sparkes (13), who propagated certain bacteria in used tissue culture medium. The investigation of the effect of selective inhibitors, therefore, was not pursued.

In conclusion, our premise that the propagation of gonococci in mammalian cell cultures may lead to the formulation of a liquid medium which preserves the virulence of the organisms may be correct for certain strains, but further investigations of the nature of gonococcal phase transition is required. Our results have provided a broader basis for such studies.

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