

Effect of Estrogens on Bacterial Adherence to HeLa Cells

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Incubating confluent cell culture HeLa cells for 18 h with increasing concentrations of estrogens progressively enhanced the subsequent attachment of a variety of radiolabeled bacteria to the HeLa cells. This effect was not caused by other hormones and was not produced by 1-h incubations of HeLa cells or bacteria with hormones. Estrogens did not similarly affect two other receptor cell lines studied. The addition of metabolic inhibitors showed that this effect of estrogens on HeLa cells was energy dependent and involved protein synthesis. Concurrent incubation of the HeLa cells and estrogens with the antiestrogen nafoxidine blocked the subsequent increase in adherence. These data suggest that estrogen receptors are present in HeLa cells and that hormonally-induced alterations in the synthesis of bacterial receptor sites may modify the capacity of certain cells to bind bacteria.

The attachment of bacteria to mammalian receptor cells seems, in many instances, to be an early step in the development of bacterial colonization and infection (6). Putative mediators of attachment, including lipoteichoic acids of staphylococci (1) and streptococci (5) and type I and other pili of gram-negative bacilli (10, 21, 26, 35), have been identified. The receptor sites on mammalian receptor cells are not well characterized and include albumin-like proteins for staphylococci and various glycoproteins (and perhaps glycolipids) for mycoplasma and many gram-negative bacteria (3, 6, 12, 16, 29, 34).

Other factors such as receptor cell damage, surrounding glycosaminoglycans (mucopolysaccharides), and biochemical environment have been reported to alter adherence characteristics (6, 9, 19, 20, 23-25). In vitro adherence of *Neisseria gonorrhoeae*, group B streptococci, *Escherichia coli*, and *Proteus mirabilis* to isolated human genitourinary tract epithelial cells from women has been reported to change with the stages of the menstrual cycle (7, 11, 27, 33) of the cell donors. In one report, the cyclic nature disappeared in subjects taking oral contraceptives (7). However, not all similar studies reported cyclic changes in adherence for all of these bacteria (28, 30, 33). Additionally, the carriage or dissemination of infection by certain bacteria in women has been correlated with menstrual cycle stage and pregnancy (2, 13). Hormonal changes may influence bacterial adherence by altering epithelial cell number and morphology, modifying receptor site synthesis on epithelial cells, changing the surrounding pH or mucus composition, etc. We decided to deter-

mine systematically which, if any, hormones act upon bacteria or receptor cells to modify subsequent adherence. To negate effects that may be caused by differences in cell numbers, viability, morphology and the like, confluent tissue-cultured cells were used for most of these studies. Tissue-cultured cells with known cytoplasmic estrogen receptors (T-47D) and others without established estrogen responsiveness (HeLa, L) were studied (15, 17).

MATERIALS AND METHODS

Bacteria. Clinical specimens passed twice or less were maintained on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, MD. Becton, Dickinson & Co., Detroit, Mich.) plates at room temperature. Members of the *Enterobacteriaceae* were identified by the API system (Analytab Products, Plainsville, N.J.), and staphylococci were identified by the Gram stain, colonial morphology on blood agar plates, and coagulase activity. Bacteria were radiolabeled by incubation at 37°C for 18 h in Eagle minimal essential medium with Earle salts (GIBCO Laboratories, Grand Island, N.Y.) for the *Enterobacteriaceae* or Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) for the staphylococci and streptococci. [*meth*-³H]thymidine (New England Nuclear Corp., Boston, Mass.) with a specific activity of 50.5 Ci/mmol was added at 5 µCi/ml. Organisms were collected and washed three times by centrifugation in 0.05 M phosphate-buffered saline (PBS; pH 7.3) without calcium or magnesium. Bacteria were finally suspended in PBS after being blended in a Vortex mixer to break clusters, and the concentration was adjusted spectrophotometrically. Concentrations were verified by quantitative cultures.

Hormones. Hormones, including porcine pancreas insulin, were crystalline pure preparations (Sigma

Chemical Co., St. Louis, Mo.) except for follicle-stimulating hormone and luteinizing hormone from porcine and equine pituitaries. Hormones and the antiestrogen nafoxidine HCl (The Upjohn Co., Kalamazoo, Mich.) were dissolved in PBS. Estrogens, nafoxidine, progesterone, and testosterone were dissolved in 100% USP ethanol initially, with final ethanol dilutions in PBS <0.1% (vol/vol).

Receptor cells. Cells were grown on cell culture petri dishes with 8-cm² surface areas (Falcon Plastics, Oxnard, Calif.) in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. HeLa cells from a human carcinoma of the cervix (fetal bovine serum adapted, number 03-117; Flow Laboratories Inc., McLean Va.) were grown to confluency in pH 7.2 McCoy modified 5A medium (GIBCO) with 6% (vol/vol) fetal calf serum (K. C. Biological, Lenexa, Kans.), 2 mM glutamine, 5.5 mM glucose, and antibiotics. T-47D cells from a human breast carcinoma were graciously provided by David Sirbasku of the University of Texas Medical School at Houston, Tex. The cells were cultivated in Eagle minimal essential medium with Earle salts and 10% (vol/vol) fetal calf serum, 25 mM glucose, 2 mM glutamine, and antibiotics. L cells (mouse fibroblasts) were propagated in Eagle minimal essential medium with Earle salts and 6% (vol/vol) fetal calf serum, 2 mM glutamine, 5.5 mM glucose, and antibiotics. The antibiotics used for these cell lines were penicillin (100 U/ml) and streptomycin (100 µg/ml). Receptor cells were quantitated by hemacytometry. The total cell protein was calculated after the medium was removed and the cells were washed by using a modification of the technique of Lowry et al. (4). Within 1 day of confluency (or no further proliferation) of tissue-cultured cells, hormones were added to yield concentrations (see Table 1) several times the normal maximum nonpregnant human serum concentration (unless otherwise indicated). Controls included the addition of PBS only. Cell culture plates were then incubated again at 37°C for 18 h (HeLa, L) or 48 h (T-47D), which approximately represents the population doubling time for proliferating cells. Cell viability was determined by trypan blue staining (22) and morphology with phase-contrast light microscopy.

Adherence assay. On at least 3 separate days the

medium was gently decanted from plates with adherent and viable tissue-cultured cells and was replaced with 2 ml of fresh medium without hormones, serum, or antibiotics. A 0.5-ml volume of PBS with 2×10^8 bacteria per ml was added, and the plates were gently agitated for 45 min at room temperature. Next, nonadherent bacteria were removed from the plates which contained attached receptor cells and adherent bacteria by four rapid rinsings with fresh medium (final washing contained counts not significantly above those of background). The remaining plate contents were solubilized with 0.1 N NaOH and counted in a liquid scintillation counter after neutralization to pH 7 and the addition of xylene-surfactant-based scintillation fluid; the calculated counting efficiency was 41%. The counts per minute of known quantities of each bacterial isolate were used to convert counts to the number of bacteria. Background counts of bacteria incubated with tissue-culture plates without receptor cells were subtracted for assays which used nonconfluent tissue-cultured cells. This assay is similar to one described previously (31).

Statistical methods. Unless otherwise indicated, all data are expressed as the mean \pm the standard deviation of the mean with $n > 3$. Differences were analyzed with probabilities determined by Fisher's *F* distribution analysis of variance.

RESULTS

Effect of hormones. With this in vitro system, several bacteria, on the average, attached to the exposed surface of each HeLa cell after 45 min of incubation (Table 1). Prior incubation of the HeLa cells for 18 h with each of the three dominant human estrogens significantly ($P < 0.05$) increased the subsequent attachment of bacteria (Table 1). The incubation of HeLa cells for 18 h with equivalent concentrations of ethanol used for hormone solubilization did not alter subsequent bacterial adherence. The group B streptococci and *Proteus* species tested displayed similar, although less-marked, characteristics (data not shown). No other hormones

TABLE 1. Effect of 18-h incubation of HeLa cells with various hormones on subsequent bacterial adherence^a

Hormone (amt)	Bacteria/HeLa cell		
	<i>E. coli</i> 1	<i>S. aureus</i> 1	<i>S. aureus</i> 2
None (PBS control)	1.1 \pm 0.1	2.1 \pm 0.2	0.8 \pm 0.1
17- β -Estradiol (1 ng/ml)	1.8 \pm 0.2 ^b	2.9 \pm 0.2 ^b	1.6 \pm 0.2 ^b
Estriol (1 ng/ml)	1.6 \pm 0.2 ^b	2.7 \pm 0.2 ^b	1.2 \pm 0.1 ^b
Estrone (1 ng/ml)	1.9 \pm 0.1 ^b	2.9 \pm 0.2 ^b	1.3 \pm 0.1 ^b
Follicle-stimulating hormone (60 mIU/ml)	1.1 \pm 0.1	2.3 \pm 0.3	0.6 \pm 0.1
Hydrocortisone (0.4 µg/ml)	1.1 \pm 0.1	2.3 \pm 0.3	0.6 \pm 0.1
Insulin (20 µIU/ml)	1.0 \pm 0.1	2.0 \pm 0.2	0.6 \pm 0.1
Luteinizing hormone (80 mIU/ml)	1.2 \pm 0.1	2.2 \pm 0.1	1.0 \pm 0.1
Progesterone (20 ng/ml)	1.3 \pm 0.2	1.9 \pm 0.1	0.9 \pm 0.1
Testosterone (20 ng/ml)	1.3 \pm 0.2	1.9 \pm 0.1	0.9 \pm 0.1
L-Thyroxine (0.2 µg/ml)	1.3 \pm 0.2	2.0 \pm 0.1	1.0 \pm 0.1

^a Data expressed as mean number of bacteria adherent per HeLa cell \pm 1 standard deviation.

^b Differences compared with control were statistically significant ($P < 0.05$).

TABLE 2. Effect of 1-h exposure of bacteria or HeLa cells to hormones on subsequent bacterial adherence

Hormone (amt)	Adherent bacteria/mm ^a		Adherent bacteria/HeLa cell ^b	
	<i>E. coli</i> 1 ^c	<i>S. aureus</i> 2 ^c	<i>E. coli</i> 1 ^d	<i>S. aureus</i> 2 ^d
None (PBS control)	100	100	100	100
17- β -Estradiol (1 ng/ml)	96	94	107	96
Estriol (1 ng/ml)	103	97	104	104
Estrone (1 ng/ml)	98	101	95	101
Follicle-stimulating hormone (60 mIU/ml)	112	104	107	108
Hydrocortisone (0.4 μ g/ml)	104	100	95	92
Insulin (20 μ IU/ml)	111	109	106	110
Luteinizing hormone (80 mIU/ml)	92	104	102	106
Progesterone (20 ng/ml)	105	91	103	97
Testosterone (20 ng/ml)	103	93	98	92
L-thyroxine (0.2 μ g/ml)	96	96	99	95

^a To completely negate potential receptor cell influence, bacterial adherence to 50-mm strands of 0.2-mm diameter silk sutures was determined (32) after incubating bacteria with hormones. Values represent number of adherent bacteria per millimeter of suture, expressed as percentage of PBS controls. All *P* values were >0.1.

^b After exposure to hormones, HeLa cells were gently rinsed and used as receptor cells for bacterial adherence. Values represent mean number of adherant bacteria per HeLa cell, represented as percentage of PBS controls. All *P* values were >0.1.

^c Bacteria exposed.

^d HeLa cells exposed.

tested altered subsequent bacterial adherence (Table 1). No alterations were noted in receptor cell morphology, total number, or viability after 18 h of exposure to any of the hormones.

The incubation of HeLa cells with each of the hormones for 1 h did not alter the subsequent attachment of bacteria to the HeLa cells after 45 additional min of incubation (Table 2). The adherence of bacteria to HeLa cells after 0 min of incubation was nominal (less than 10% of the 45 min of incubation, total) whether the HeLa cells had been previously exposed to hormones. The incubation of bacteria with hormones for 60 min, longer than the maximal exposure to hormones in any of the other experiments reported here, did not alter subsequent attachment ability (Table 2).

Estrogens did not alter significantly (*P* > 0.2) cultured HeLa cell total protein (mean, 683 μ g) or number (mean, 1.3×10^6) per 8-cm² plate (Table 3).

Effect of increasing estrogen concentrations. The dose-response curve in Fig. 1 shows that the 18-h incubation of HeLa cells with $\geq 10^{-1}$ -ng/ml amounts of estrogens was necessary for subsequent increased bacterial adherence. The effect

was maximal, with between 10 and 100 ng of estrogens per ml increasing to about double the control value for *E. coli* 2. Figure 1 shows the lack of effect of increasing the progesterone concentrations over a large range of concentrations. The lack of response of T-47D and L cells to estrogens and other hormones in regard to subsequent bacterial adherence is shown in Fig. 2.

Effect of antiestrogens. The incubation of HeLa cells with estradiol and the antiestrogen nafoxidine muted the increase in subsequent bacterial adherence (Fig. 3). The effect of nafoxidine increased with increasing dosage and completely negated the increase secondary to estradiol at a 1,000:1 antiestrogen/estrogen ratio (Fig. 3).

Effect of metabolic inhibitors. The incubation of HeLa cells with puromycin, cycloheximide, dinitrophenol, or sodium azide for 2 h was succeeded by 18 h of additional incubation with estradiol. This decreased the effect of the estrogen on subsequent bacterial binding, suggesting that the alterations already mentioned were energy requiring and involved protein synthesis (Table 4).

TABLE 3. Effect of hormones on cell culture total protein^a

Hormone (amt)	Protein/plate		
	HeLa	L	T-47D
None (PBS)	701 \pm 42	915 \pm 58	1,429 \pm 83
17- β -Estradiol (1 ng/ml)	711 \pm 38	924 \pm 61	1,388 \pm 89
Progesterone (20 ng/ml)	692 \pm 37	906 \pm 51	1,405 \pm 76

^a Cells were propagated in 8-cm² tissue culture petri dishes, and data are expressed as mean micrograms of total protein per plate \pm 1 standard deviation. All *P* values were >0.2 when comparing PBS with hormones.

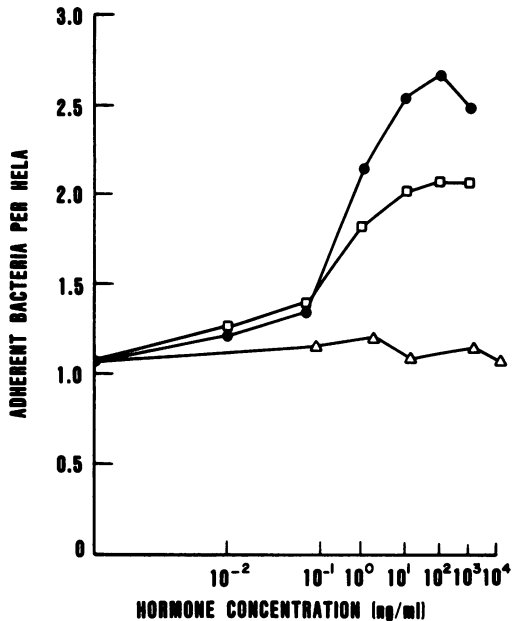


FIG. 1. Effect of various hormone concentrations on subsequent adherence of *E. coli* 2 to HeLa cells. HeLa cells were incubated for 18 h with estradiol (●), estrone (□), or progesterone (△) in the concentrations noted on the abscissa before being incubated with *E. coli* 2 to determine the number of bacteria adherent per HeLa cell (ordinate).

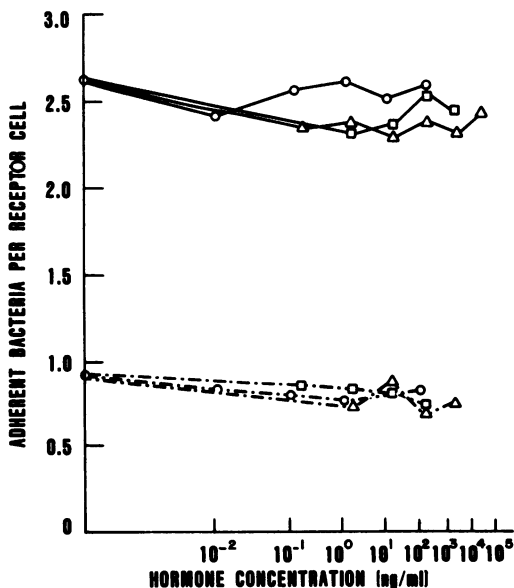


FIG. 2. Effect of various hormone concentrations on subsequent adherence of *E. coli* 2 to T-47D (—) and L (---) cells. Receptor cells were incubated for 18 h with estradiol (○), progesterone (□), or L-thyroxine (△) in the concentrations noted on the abscissa before being incubated with *E. coli* 2 to determine the number of bacteria adherent per receptor cell (ordinate).

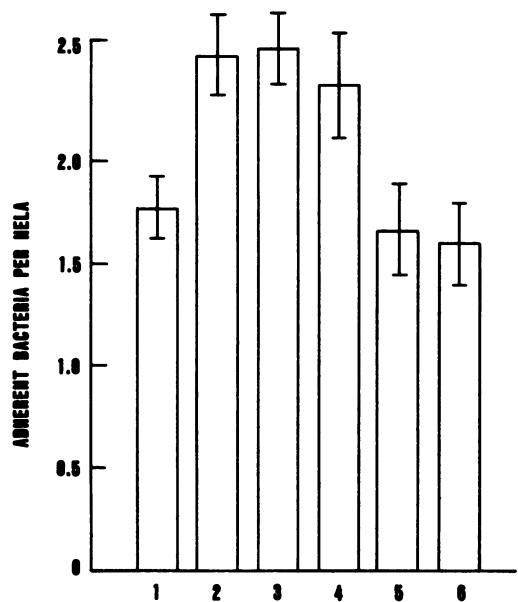


FIG. 3. Effect of estradiol and nafoxidine on subsequent bacterial adherence. HeLa cells were incubated for 18 h with mixtures 1 to 6 before incubation with *E. coli* 3 and determination of adherent bacteria per HeLa cell. Key: Mixture 1, PBS control; mixture 2, estradiol (1 ng/ml); mixture 3, estradiol (1 ng/ml) and nafoxidine (10 ng/ml); mixture 4, estradiol (1 ng/ml) and nafoxidine (100 ng/ml); 5, estradiol (1 ng/ml) and nafoxidine (1,000 ng/ml); and 6, no estradiol and nafoxidine (1,000 ng/ml). Vertical bar represents ± 1 standard deviation.

DISCUSSION

These data show that HeLa cells display estrogen responsiveness and suggest that subsequent modification of the outer HeLa layers mediated by nanogram-per-milliliter concentrations of estrogens over time alters subsequent bacterial attachment. We are not certain whether the effect of estrogens is a direct one at HeLa cell surfaces or a secondary effect to the altered metabolism of HeLa cells; however, the lack of effect with 1-h estrogen incubations suggests the presence of secondary effects. In vivo estrogens are known to cause additional cell responses, including epithelial cell proliferation (18), and it is also known that surface glycolipids can change during cellular maturation (14). Thus, estrogens may also act in other ways to alter bacterial adherence. The absence of estrogen effect on the subsequent adherence of bacteria to L cells without, and T-47D cells with, known cytoplasmic estrogen receptors (15) suggests that, in this regard, all cells do not respond in a similar manner to estrogens.

The epithelial receptor cells studied derived from the cervix (HeLa) altered their ability for subsequent bacterial adherence after 18 h of

TABLE 4. Effect of metabolic inhibitors on subsequent attachment of *E. coli* to HeLa cells^a

Inhibitor	Concn (mM)	<i>E. coli</i> 3 adherence ^b	
		PBS	Estradiol
None		100	142
Cycloheximide	0.007	95	108 ^c
2,4-Dinitrophenol	1.7	106	127
2-Deoxy-D-glucose	50	103	134
Puromycin	0.002	107	118 ^c
Sodium azide	5	111	119 ^c
Sodium cyanide	2.5	93	139
Sodium fluoride	3.7	94	145

^a Inhibitors were added for 2 h before the addition of PBS or 10 ng of Estradiol per ml and 18 h of further incubation. Concentrations of inhibitors were maximal ones which did not alter HeLa cell viability, morphology, the presence of confluent monolayer, or the pH of the medium when compared with PBS control plates incubated for more than 20 h.

^b Data are expressed as relative percentages compared with the PBS control.

^c *P* was <0.05 when compared with the corresponding control.

exposure to $\geq 10^{-1}$ ng of estrogens per ml. The exact concentrations of hormones used were no doubt slightly higher because of amounts present in the fetal calf serum. These data offer direct support for the hypothesis that hormone level alterations may influence bacterial attachment to genitourinary epithelial cells (7, 11, 27, 33), thereby effecting colonization and infection in these (2, 13) and other areas. The data also suggest that estrogens are the most active in this regard and can alter subsequent bacterial adherence in manners more subtle than the change of gross epithelial cellular morphology or number or by the indirect alteration of the *in vivo* surrounding environment.

The exact response to estrogens that mediates the alteration in subsequent bacterial adherence still needs to be elucidated. Part, if not all, of the effect probably occurs at the outer membranes of HeLa cells or other surrounding (proteinaceous) material because under the incubation conditions used, HeLa cells internalize *Enterobacteriaceae* marginally, if at all (8). Alterations in subsequent adherence of both the *Enterobacteriaceae* and staphylococci, which possess different adherence mediators (1, 10, 26), also suggest significant HeLa membrane changes. The biochemical characterization and *in vivo* relevance of the findings reported here remain to be determined.

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LITERATURE CITED

1. Aly, R., H. R. Shinefield, C. Litz, and H. I. Maibach. 1980. Role of teichoic acid in the binding of *Staphylococcus aureus* to nasal epithelial cells. *J. Infect. Dis.* 141:463-465.
2. Baker, C. J., D. K. Goroff, S. Alpert, V. A. Crockett, S. H. Zinner, J. R. Evrard, B. Rosner, and W. McCormick. 1977. Vaginal colonization with group B streptococcus: a study in college women. *J. Infect. Dis.* 135: 392-397.
3. Banai, M., S. Razin, W. Bredt, and I. Kahane. 1980. Isolation of binding sites to glycoprotein from *Mycoplasma pneumoniae* membranes. *Infect. Immun.* 30:628-634.
4. Barley, J. L. 1962. Techniques in protein chemistry. Elsevier/North-Holland Publishing Co., London. p. 293-294.
5. Beachey, E. H. 1976. Binding of group A streptococci to human oral mucosal cells by lipoteichoic acid. *Trans. Assoc. Am. Physicians* 88:285-292.
6. Beachey, E. H. 1981. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. *J. Infect. Dis.* 143:325-345.
7. Botta, G. A. 1979. Hormonal and type-dependent adhesion of group B streptococci to human vaginal cells. *Infect. Immun.* 25:1084-1086.
8. Byrne, G. I., and J. W. Moulder. 1978. Parasite-specified phagocytosis of *Chlamydia psittaci* and *Chlamydia trachomatis* by L and HeLa cells. *Infect. Immun.* 19:598-606.
9. Engelhardt, J. A., and M. G. Gabridge. 1977. Effect of squamous metaplasia on infection of hamster trachea organ cultures with *Mycoplasma pneumoniae*. *Infect. Immun.* 15:647-655.
10. Fader, R. C., A. E. Avots-Avotins, and C. P. Davis. 1979. Evidence for pili-mediated adherence of *Klebsiella pneumoniae* to rat bladder epithelial cells *in vitro*. *Infect. Immun.* 25:729-737.
11. Forslin, L., D. Danielsson, and V. Falk. 1979. Variations in attachment of *Neisseria gonorrhoeae* to vaginal epithelial cells during the menstrual cycle and early pregnancy. *Med. Microbiol. Immun.* 167:231-238.
12. Gabridge, M. G., and D. Taylor-Robinson. 1979. Interaction of *Mycoplasma pneumoniae* with human lung fibroblasts: role of receptor sites. *Infect. Immun.* 25:455-459.
13. Holmes, K. K., G. W. Counts, and H. N. Beaty. 1971. Disseminated gonococcal infection. *Ann. Intern. Med.* 74:979-993.
14. Karlson, K. A. 1976. Aspects on structure and function of sphingolipids in cell surface membranes, p. 245-274. *In* S. Abrahamsson and I. Pascher (ed.), *Structure of biological membranes*. Plenum Publishing Corp. New York.
15. Keydar, I., L. Chen, S. Karby, F. R. Weiss, J. Delarea, M. Radu, S. Chaitcik, and H. J. Brenner. 1979. Establishment and characterization of a cell line of human breast carcinoma origin. *Eur. J. Cancer* 15:659-670.
16. Myhre, E. B., and G. Kronvall. 1980. Demonstration of specific binding sites for human serum albumin in group C and G streptococci. *Infect. Immun.* 27:6-14.
17. Nelson-Rees, W. A. 1977. Three papers on hormones and breast cancer *in vitro*. *Cancer Res.* 37:3464-3465.
18. Papanicolaou, G. G. 1933. The sexual cycle in the human female as revealed by vaginal smears. *Am. J. Anat.* 52:519-532.
19. Parsons, C. L., S. G. Mulholland, and H. Anwar. 1979. Antibacterial activity of bladder surface mucin duplicated by exogenous glycosaminoglycan (heparin). *Infect. Immun.* 24:552-557.
20. Parsons, C. L., J. J. Pollen, H. Anwar, C. Stauffer, and J. D. Schmidt. 1980. Antibacterial activity of bladder surface mucin duplicated in the rabbit bladder by exogenous glycosaminoglycan (sodium pentosanpolysulfate). *Infect. Immun.* 27:876-881.
21. Pearce, W. A., and T. M. Buchanan. 1978. Attachment role of gonococcal pili. Optimum conditions and quantitation of adherence of isolated pili to human cells *in vitro*. *J.*

- Clin. Invest. 61:931-943.
22. Phillips, H. J. 1973. Dye exclusion tests for cell viability, P. 406-408. In P. R. Krause and M. K. Patterson, Jr. (ed.), Tissue culture methods and applications. Academic Press, Inc., New York.
 23. Ramirez-Ronda, C. H. 1978. Adherence of glucan-positive and glucan-negative streptococcal strains to normal and damaged heart valves. J. Clin. Invest. 62:805-814.
 24. Ramphal, R., M. T. McNiece, and F. M. Polack. 1981. Adherence of *Pseudomonas aeruginosa* to the injured cornea: a step in the pathogenesis of corneal infections. Ann. Ophthalmol. 13:421-425.
 25. Ramphal, R., P. M. Small, J. W. Shands, Jr., W. Fischlschweiger, and P. A. Small, Jr. 1980. Adherence of *Pseudomonas aeruginosa* to tracheal cells injured by influenza infection or by endotracheal intubation. Infect. Immun. 27:614-619.
 26. Salt, I. E., and E. C. Gotschlich. 1977. Type I *Escherichia coli* pili: characterization of binding to monkey kidney cells. J. Exp. Med. 146:1182-1193.
 27. Schaeffer, A. J., S. K. Amundsen, and L. N. Schmidt. 1979. Adherence of *Escherichia coli* to human urinary tract epithelial cells. Infect. Immun. 24:753-759.
 28. Schaeffer, A. J., J. M. Jones, and J. K. Dunn. 1981. Association of in vitro *Escherichia coli* adherence to vaginal and buccal epithelial cells with susceptibility of women to recurrent urinary-tract infections. New Engl. J. Med. 304:1062-1066.
 29. Simpson, W. A., I. Ofek, and E. H. Beachey. 1980. Fatty acid binding sites of serum albumin as membrane receptor analogs for streptococcal lipoteichoic acid. Infect. Immun. 29:119-122.
 30. Sobel, J. D., J. Schneider, D. Kaye, and M. E. Levison. 1981. Adherence of bacteria to vaginal epithelial cells at various times in the menstrual cycle. Infect. Immun. 32:194-197.
 31. Sugarman, B., and S. T. Donta. 1979. Specificity of attachment of certain Enterobacteriaceae to mammalian cells. J. Gen. Microbiol. 115:509-512.
 32. Sugarman, B., and D. Musher. 1981. Adherence of bacteria to suture materials. Proc. Soc. Exp. Biol. Med. 167:156-160.
 33. Svanborg Edén, C., P. Larsson, and H. Lomberg. 1980. Attachment of *Proteus mirabilis* to human urinary sediment epithelial cells in vitro is different from that of *Escherichia coli*. Infect. Immun. 27:804-807.
 34. Svanborg Edén, C., and H. Leffler. 1980. Glycosphingolipids of human urinary tract epithelial cells as possible receptors for adhering *Escherichia coli* bacteria. Scand. J. Infect. Dis. 24:144-147.
 35. Woods, D. E., D. C. Straus, W. G. Johanson, Jr., V. K. Berry, and J. A. Bass. 1980. Role of pili in adherence of *Pseudomonas aeruginosa* to mammalian buccal epithelial cells. Infect. Immun. 29:1146-1151.