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INTERACTION BETWEEN *YERSINIA PSEUDOTUBERCULOSIS* AND THE HeLa CELL SURFACE

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SUMMARY. The adherence of *Yersinia pseudotuberculosis* to the surface of HeLa cells at 4°C was studied. This temperature allows adhesion of bacteria but prevents engulfment. Adhesion between the bacteria and the cells was not dependent upon the presence of serum, Ca²⁺ or Mg²⁺ in the medium. Maximum adhesion was obtained at pH 6.5–7.9 and pretreatment of the cells with formaldehyde or glutaraldehyde inhibited the attachment of the bacteria. The interaction between the bacteria and the cell surface seems to involve cellular processes that are mostly microvilli. An intimate association between the bacteria and the cellular glycocalyx was found. Three virulent bacterial strains adhered more easily to the cell surface than five avirulent strains. Maximum adherence was obtained with bacteria from late logarithmic and early stationary phases of growth. The bacteria gradually lose their adhesive property when cultivated for several generations at 37°C in nutrient broth but not when cultivated at 20°C. Treatment of the bacteria with protease IV from *Streptomyces caespitosus* markedly reduced the efficiency of attachment.

INTRODUCTION

Selective adherence of pathogenic bacteria to the surface of epithelial cells of mucous membranes appears to be the first stage in the initiation of infection in the gastrointestinal tract (McNeish *et al.*, 1975), genitourinary tract (Ward and Watt, 1972; Stamey *et al.*, 1978), and respiratory tract (Ellen and Gibbons, 1974; LaForce *et al.*, 1976; Powell *et al.*, 1976; Sugarman and Donta, 1979). Subsequent penetration of bacteria through intestinal epithelial cells seems to be essential for the initiation of

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systemic infections with enteric bacteria and this has been demonstrated with *Shigella* and *Salmonella* spp. (Labrec *et al.*, 1964; Giannella *et al.*, 1973).

Yersinia pseudotuberculosis is pathogenic for birds, rodents, some other animals and man and is probably transmitted by the ingestion of the bacteria in infected food. Pseudotuberculosis may be an acute septicemia with a severe course; sometimes, however, the disease runs a chronic course with localisation of bacteria in small necrotic nodules in the mesenteric lymph nodes, liver, spleen and lung (Obwolo, 1976; Paff, Triplett and Saari, 1976; Böhm and Wybitul, 1978). Experiments in rabbits have shown that in the early stage of intestinal infection with virulent *Y. pseudotuberculosis*, organisms can be detected in mucosal epithelial cells and it is suggested that the bacteria may penetrate epithelial cells to reach the lamina propria and lymph follicles (Une, 1977b). *Y. pseudotuberculosis* also has the capacity to penetrate epithelial cells *in vitro*; HeLa cells rapidly ingest the bacteria by a phagocytosis-like procedure that has phases of attachment and engulfment (Bovallius and Nilsson, 1975; Brunius, 1980).

Because adherence to the epithelial cell surface may be an important prerequisite for systemic infection, we studied the adherence of *Y. pseudotuberculosis* to HeLa cells at a temperature (4°C) that allows adhesion but prevents subsequent engulfment of adsorbed bacteria.

MATERIALS AND METHODS

Yersinia pseudotuberculosis. The following strains of O-antigen groups I, III and IV were used: group I, strain 281; group III, strains 43, 702, 706, 707, 708; and group IV, strains 32, 324, 387. They were identified by the methods of Thal and Knapp (1971). All strains were grown in nutrient broth (Oxoid) overnight at 20°C or, in some experiments, at 37°C. After centrifugation, the bacteria were resuspended in phosphate-buffered saline (PBS) before use in adhesion tests. When strain 281 was grown for several passages at 37°C, 0.1 ml of an overnight culture was transferred to 100 ml of nutrient broth, prewarmed at 37°C.

Tests of virulence. Bacteria were grown for 2 days in nutrient broth at 30°C and injected intraperitoneally into guinea pigs; virulent strains caused death of the guinea pigs within a week and bacteria were demonstrated in internal organs. Toxic strains caused the death of mice within 24 h after intraperitoneal injection of 0.5 ml of a 5-day nutrient-broth culture.

Cell cultures. HeLa (laboratory strain), HeLa S3, HeLa Ohio, and HeLa 229 (Flow Laboratories, Irving, Scotland) cell strains were cultivated at 37°C in Leibovitz' medium (L15) with calf serum 10%, streptomycin 100 µg/ml and penicillin 100 IU/ml. The cells were trypsinised before use with 2.5 mg enzyme/ml of PBS and then cultivated on coverslips in new Petri dishes (17 cm²) for 24 h. After washing three times with 1 ml of PBS per Petri dish, the medium was changed to L15 without antibiotics. The cell density of the cultures was then $(1-2) \times 10^4$ cells/cm².

Assay of bacterial adherence. The non-confluent cell cultures were chilled for 90 min at 4°C and 1×10^7 , 3×10^8 or 3×10^9 bacteria in 0.1 ml of PBS were added to 5 ml of medium (without antibiotics) in separate cell cultures. The cell cultures were then incubated for 2-4 h at 4°C unless otherwise stated. They were washed three times with 1 ml of PBS (0°C) per Petri dish and fixed with formaldehyde 1% at 4°C overnight. Transmission electron microscopy showed that HeLa cells incubated for 4 h at 4°C with 3×10^9 cells of *Y. pseudotuberculosis* did not contain any intracellular bacteria. Bacteria bound to the cell surface were demonstrated by indirect immunofluorescence with OH-antisera prepared in rabbits (kindly provided by the National Veterinary Institute, Uppsala, Sweden) and fluorescein-conjugated sheep anti-rabbit globulin (The National Bacteriological Laboratory, Stockholm, Sweden). Usually, 100 HeLa cells in each culture were examined; the proportion of cells with bacteria attached to the surface and the number of bacteria per cell were counted. Bound bacteria were also demonstrated by scanning electron microscopy.

Assay of the invasive property of the bacteria. HeLa cells were washed in L15 medium without antibiotics and then left in this medium at 37°C; 1×10^7 cells of each test strain of *Y. pseudotuberculosis* were added to separate Petri dishes and the cultures incubated for 3 h at 37°C. The medium was changed to L15 with penicillin 100 IU/ml and gentamicin 10 µg/ml or streptomycin 100 µg/ml (for strain 281 only). The cells were incubated for 1 h in this medium and then washed three times in PBS (1 ml per Petri dish). Less than 0.01% of the bacteria remained in the last wash. The cells were detached from the Petri dishes by adding 1 ml of a 0.5% solution of sodium deoxycholate and homogenised by passing three times through a hypodermic needle. The number of viable intracellular bacteria per cell culture was measured by seeding dilutions of the homogenate on to blood agar plates and incubating for 3–4 days at room temperature (Bovallius and Nilsson, 1975).

Chemical treatment of bacteria. Suspensions of strain 281 containing 10^8 – 10^9 organisms were exposed to the following chemical agents in 5-ml volumes of PBS (pH 7.3) at 22°C: glutaraldehyde (0–0.1%) for 10 min., formaldehyde (0–1%) for 1 h, or sodium metaperiodate (10 mM) for 30 min. Similar suspensions were exposed at 37°C for 1 h to the following enzymes dissolved in 5-ml volumes of PBS; trypsin (Sigma, St Louis) 1 mg/ml; protease type IV from *Streptomyces caespitosus* (Sigma) 1 mg/ml; or neuraminidase (Boehringer, Mannheim, FRG) 1 mg/ml. After treatment the bacteria were washed three times with PBS, resuspended in 0.1 ml of PBS and added to separate cell cultures.

Chemical treatment of the cells. Cell cultures were incubated for 10 min at 22°C in 5 ml of PBS containing glutaraldehyde (0–0.1%), formaldehyde (0–1%) or sodium metaperiodate (10 mM) or at 37°C for 20 min in 5 ml of L15 containing trypsin (10 µg/ml), protease IV (10 µg/ml) or neuraminidase (1 mg/ml).

Assay of bacterial adherence in the presence of chemicals. The following agents were added to L15 medium before the bacteria were added to the cell cultures: heparin (Kabi, Sweden) 500 IU/ml; EDTA 0.005 M; dextran sulphate (mol. wt 500 000, Pharmacia, Sweden, or mol. wt 40 000, Sigma) 1 mg/ml; DEAE-dextran (Pharmacia, Sweden) 1 mg/ml; sialic acid 1 mg/ml; gastric mucin (Sigma) 0.5 mg/ml; or hyaluronic acid (Sigma) 1 mg/ml. D-galactose, D-glucose, D-maltose, D-mannose, rhamnose, glucosamine (Merck, Darmstadt, FRG) 25 mg/ml or D-fucose (Sigma) 10 mg/ml were added simultaneously with the bacteria to separate cell cultures.

Electron microscopy. For transmission electron microscopy (TEM) the cell cultures were fixed in glutaraldehyde 2% in 0.1 M cacodylate buffer, pH 7.2, and washed three times with 0.1 M cacodylate buffer. Cationic ferritin 0.35 mg/ml in the same buffer (Miller, Hay and Codington, 1977) was then added and left for 30 min at room temperature and the washing repeated. The cells were fixed in osmium tetroxide, dehydrated in ethanol and embedded in Epon. They were examined in a Philips 201 electron microscope at 60 kV.

For scanning electron microscopy (SEM) the cells were grown on coverslips and fixed in glutaraldehyde 2% in 0.1 M cacodylate buffer, pH 7.2; the preparations were dehydrated in a critical point apparatus (Polaron) and, after a gold evaporation step, were examined with a Cambridge S 4 Stereoscan scanning electron microscope at 20 kV.

RESULTS

Adsorption at 4°C. *Y. pseudotuberculosis* strain 281 became attached to non-synchronised HeLa cells (laboratory strain) at 4°C without being engulfed (fig. 1). By increasing the incubation temperature to 37°C after the adhesion step, phagocytosis of the attached bacteria was induced within 1.5 h. Adhesion and phagocytosis were observed by TEM. Fig. 2 shows that there were small quantitative differences between the variants of the HeLa cell line in their capacity to adsorb bacteria. This was most apparent when 3×10^8 bacteria/cell culture were added.

Table I shows the effect of different formulations of media on the adhesion of *Y. pseudotuberculosis* strain 281 to the laboratory strain of the HeLa cell line. Maximum

FIG. 1.—Scanning electronmicrographs of HeLa cells incubated for 2 h at 4°C with 3×10^8 cells of *Y. pseudotuberculosis* strain 281. (a) $\times 5120$, (b) $\times 10\,400$.

FIG. 2.—Adherence at 4°C of strain 281 to different HeLa cell lines. Number of bacteria added per cell culture: —○— 1×10^7 , —●— 3×10^8 and —△— 3×10^9 .

TABLE I

Effect of pH and media additives on the adhesion of Y. pseudotuberculosis to HeLa cells

Medium	Number of cells with adherent bacteria as percentage of figure for control culture*
L15 with calf serum 10%, pH 7.2 (control)	100
L15 without serum	99
L15 with 0.005 M EDTA	9
L15 with DEAE-dextran 1 mg/ml	60
L15 with heparin 500 IU/ml	66
L15 with dextran sulphate 1 mg/ml	96
L15, pH 6.5	100
L15, pH 7.9	94
L15, pH 8.2	87
PBS without Ca^{2+} or Mg^{2+}	97

* Inoculum of 3×10^8 bacteria added to each cell culture and incubated for 2 h at 4°C.

FIG. 3.—Scanning electronmicrograph of HeLa cells incubated for 30 min at 37°C with 3×10^8 bacteria of strain 281. $\times 9040$.

FIG. 4.—Electronmicrograph of a thin section of a HeLa cell after incubation for 3 h at 4°C with 3×10^8 bacteria of strain 281 and stained with cationic ferritin. $\times 27\,000$.

adhesion at 4°C was obtained in the pH-range 6·5–7·9; below pH 6·5 HeLa cells became detached from the coverslips. Adhesion was not dependent on the presence of serum in the medium. It appeared that divalent cations may participate in the interaction between bacteria and cells because addition of 0·005 M EDTA to the medium resulted in fewer cells (<10% of the number in control cultures) with adsorbed bacteria. However, a normal rate of adhesion was obtained in PBS without Ca²⁺ and Mg²⁺. The attachment of *Y. pseudotuberculosis* to the cell surface seemed to be irreversible; cells with bacteria attached at 4°C did not lose any bacteria after washing and then incubation for 24 h at 4°C in fresh medium.

Cell components involved in the adsorption of the bacteria. The interaction between the bacteria and the cell surface appeared to involve cellular processes, most of which were microvilli (figs. 1b and 3). Staining with cationic ferritin showed that these processes were intimately associated with the cellular glycocalyx (fig. 4). However, when DEAE-dextran or heparin was added to the medium before tests for adhesion, there was a 40% reduction in the number of cells with adherent bacteria. Dextran sulphate had no effect (table I).

Epithelial cells were pretreated with sodium metaperiodate, a reagent that preferentially cleaves vicinal hydroxyl groups in sugar residues to demonstrate whether or not the adhesive structures were carbohydrate. The capacity of cells to adsorb bacteria was only slightly affected by this treatment. Addition to the incubation medium of D-glucose, D-galactose, rhamnose, glucosamine, D-mannose, D-fucose or D-maltose did not interfere with the interaction between untreated cells and bacteria. Pretreatment of the cells with hyaluronidase or neuraminidase or addition to the

FIG. 5.—The effect on adhesion of exposure of *Y. pseudotuberculosis* strain 281 (—O—) or HeLa cells (●) to various temperatures for 10 min; 3×10^8 bacteria per cell culture were added and incubated for 2 h at 4°C.

FIG. 6.—The effect on adhesion of the treatment of *Y. pseudotuberculosis* (—▲—) or HeLa cells (—●—) with 0.1–1% formaldehyde; 3×10^8 bacteria per cell culture were added and incubated for 2 h at 4°C.

medium of hyaluronic acid, sialic acid (or gastric mucin, which is rich in sialic acid) did not reduce adhesion of the bacteria.

Heating the bacteria reduced their adhesive property (fig. 5) and the adhesion of unheated bacteria to HeLa cells held at $\geq 70^\circ\text{C}$ for 10 min was markedly reduced. Treatment of the cells at 50 and 60°C detached them from the surface of the cover slips. To investigate whether protein components on the cell surface were involved in the binding of bacteria, the cells were treated with protease IV from *Streptomyces*

TABLE II
Adhesion (at 4°C) and ingestion (at 37°C) of *Y. pseudotuberculosis* strains by HeLa cells

Strain* designation	Virulence	Percentage of† cells with adherent bacteria	Number of ingested‡ viable bacteria per cell culture (washed and homogenised)
281/I	Virulent	100	7×10^6
324/IV	Virulent	94	4.0×10^5
43/III	Virulent, toxic	40	3.4×10^5
32/IV	Avirulent	18	4.2×10^6
387/IV	Avirulent	0.1	1.6×10^3
702/III	Avirulent	2	6.2×10^4
706/III	Avirulent	4	8×10^4
707/III	Avirulent	...	7×10^3
708/III	Avirulent	14	4.4×10^4

* Thal and Knapp (1971).

† 1×10^8 bacteria were added to each cell culture and incubated for 1.5 h at 4°C.

‡ 1×10^7 bacteria were added to each cell culture and incubated for 3 h at 37°C.

caespitosus (Yokote and Noguchi, 1969), or with trypsin before tests for adhesion. Both treatments increased the adhesion of bacteria slightly. However pretreatment of the bacteria or HeLa cells with formaldehyde or glutaraldehyde, which react primarily with amino groups, resulted in a significant inhibition of attachment of bacteria (figs. 6 and 7).

Differences in adhesive capacity between strains. To investigate whether strains of *Y. pseudotuberculosis* differed in their affinity for the epithelial cell surface, 10^8 bacteria of each strain were incubated with HeLa cells at 4°C for 1·5 h. Virulent strains tended to adhere more frequently to the HeLa cell surface than strains classified as avirulent (table II). For comparison, the invasive property of each strain was determined at 37°C and there was a correlation between adhesion and invasiveness. However, one of the avirulent strains (32/IV) was ingested at a rate equivalent to that of the virulent strains.

The adhesive property of bacteria harvested at various stages of growth was studied with strain 281. The growth curve of strain 281 in nutrient broth at 20°C is shown in fig. 8. Maximum adherence was obtained with bacteria from late logarithmic and early stationary phases (fig. 9). The bacteria gradually lost their adhesive property at 37°C in nutrient broth (fig. 10a, b and c). Incubation for 3 h with 3×10^9 bacteria that had been passaged seven times resulted in 5% of the cells adsorbing bacteria. Bacteria were attached to 95% of cells when the organisms were grown at 20°C (fig. 10a). After transfer from 37°C to 20°C, however, the bacteria regained their adhesive property fully within one day (fig. 10a and d).

Bacterial components involved in adherence. Strains of *Y. pseudotuberculosis* were stained with ruthenium red and examined by light microscopy. A glycocalyx could not be demonstrated by this method, nor were bacterial pili observed by electronic-

FIG. 7.—The effect on adhesion of the treatment of *Y. pseudotuberculosis* (Δ) or HeLa cells (—O—) with 0·005–0·01% glutaraldehyde; 3×10^8 bacteria per cell culture were added and incubated for 2 h at 4°C.

FIG. 8.—Growth curve of *Y. pseudotuberculosis* strain 281 in nutrient broth at 20°C.

FIG. 9.—Adhesion of *Y. pseudotuberculosis* strain 281 harvested at different growth stages. Percentage of cells with attached bacteria (—Δ—) and mean number of bacteria per cell (—○—). Each cell culture was incubated for 2 h at 4°C with 3×10^8 bacteria.

FIG. 10.—(a) Adhesion of *Y. pseudotuberculosis* strain 281 cultivated in nutrient broth for several passages at 20°C (—O—) or 37°C (—●—). After seven passages (arrow) at 37°C the bacteria were transferred to 20°C and grown for one passage; 3×10^9 bacteria per cell culture were added and incubated for 3 h at 4°C. (b) Adhesion of *Y. pseudotuberculosis* strain 281 cultivated for one passage (I), three passages (II) and seven passages (III) at 37°C (□) or 20°C (■); 3×10^9 bacteria per cell culture were added and incubated for 3 h at 4°C. (c) Adhesion of *Y. pseudotuberculosis* strain 281 grown at 20°C in nutrient broth and then incubated in PBS for 1–4 h at 37°C; 3×10^8 bacteria per cell culture were added. (d) Adhesion of *Y. pseudotuberculosis* strain 281 grown for one passage at 37°C in nutrient broth and then incubated in PBS for 1–4 h at 20°C; 3×10^8 bacteria per cell culture were added.

roscopy of negatively stained cells. Moreover, *Y. pseudotuberculosis* did not agglutinate erythrocytes, a property associated with some bacterial pili.

Heating the bacteria, particularly at temperatures above 80°C, reduced their adhesive property (fig. 5). Pretreatment of *Y. pseudotuberculosis* with sodium metaperiodate affected the ability to adhere to HeLa cells at 4°C slightly; this indicated that carbohydrate adhesins were not present. Pretreatment of the bacteria with hyaluronidase, neuraminidase or trypsin did not reduce their ability to adhere to the cells. However, treatment of 3×10^8 bacteria for 1 h with protease IV 1 mg/ml in PBS reduced the attachment by 86%. Treatment of the bacteria with formaldehyde reduced their ability to adhere to cells but treatment with glutaraldehyde did not (figs. 6 and 7).

DISCUSSION

The HeLa cell line was derived from an adenocarcinoma of the cervix (Jones *et al.*, 1971). This type of carcinoma is derived from mucous glands, which indicates that HeLa cells may be related to microvilli-containing columnar cells of the endocervical canal. The HeLa cell has many microvilli on the cell surface but these possess some abnormal properties such as variations in diameter and cross-sectional form (Porter, Fonte and Weiss, 1974). The relevance of the HeLa cell as a model for the microvilli-containing columnar epithelial cells of the intestine is questionable, but several investigators have observed a correlation between the ability of certain pathogenic microorganisms to invade HeLa cells and their invasion into intestinal epithelium (Labrec *et al.*, 1964; Giannella *et al.*, 1973; Lee *et al.*, 1977).

The HeLa cell contains the polyanion heparan sulphate and probably also the polyanions chondroitin sulphate (Dietrich and De Oca, 1970; Kraemer, 1971) and dermatan sulphate (Suzuki, Kojima and Utsumi, 1970; Saito and Uzman, 1971) in the extracellular matrix. Hyaluronic acid is not present (Kraemer, 1979). The linear anionic polyelectrolyte heparan is a subgroup of the heparan sulphates that has a high content of sulphate groups. The compound may interfere competitively with any interaction between bacteria and heparan sulphate on the cell surface but addition of heparan to the medium in our studies had only a limited effect on the adhesion of bacteria. The polycation DEAE-dextran binds to polyanions of the cell surface (Kuroda, 1974) and may interact with potential cell surface-bound receptors but, again, the compound had only a limited effect on the adhesion of bacteria. These results suggest that charged groups or mucopolysaccharides may be of some importance, but the failure of sodium metaperiodate to affect adhesion and the absence of any effect when a wide variety of sugars were added to the medium suggests that cellular structures involved in adhesion are not carbohydrates.

As pre-treatment of HeLa cells at 70°C markedly reduced bacterial binding, it may be thought that the cell receptors concerned are protein. Binding was not reduced by pre-treatment of the cells with protease, but this often results in an increase in the number of microvilli (Follett and Goldman, 1970; Erickson and Trinkaus, 1976). The effect of heat may be attributed to non-specific morphological or conformational changes in the cells such that close association between the complementary regions of the bacteria and the cells becomes impossible. Electron microscopy demonstrated that *Y. pseudotuberculosis* is mainly attached to cellular microvilli. Treatment of the

cells with glutaraldehyde and formaldehyde, which react with amino groups inhibited the cells' capacity to absorb bacteria. This may be a secondary effect of an aldehyde-induced inhibition (fixation) of the mobility of the microvilli.

Pathogenic strains of *Y. enterocolitica*, a species closely related to *Y. pseudotuberculosis*, can penetrate HeLa cells. Non-pathogenic strains lack this ability (Lee *et al.*, 1977; Une, 1977a and b; Une *et al.*, 1977; Pedersen, Winblad and Bitsch 1979; Kapperud, 1980). The use of HeLa cell cultures to determine bacterial pathogenicity may therefore be of value in the examination of isolates from clinical cases. Une (1977b) suggested that the capacity of strains of *Y. pseudotuberculosis* to penetrate epithelial linings and survive within host cells is related to pathogenicity and demonstrated that three strains of *Y. pseudotuberculosis* classified as pathogenic by in-vivo tests could penetrate HeLa cells.

In the present study we showed that by incubating the cell system at 4°C adhesion could be separated from engulfment which needs a higher temperature. The three virulent strains studied adhered to the cell surface at 4°C more easily than the avirulent strains and at 37°C, infection with virulent strains resulted in more viable intracellular bacteria than infection with most of the avirulent strains. However, one avirulent strain was ingested at a rate equivalent to that of the virulent strains. Strains that adhere poorly at 4°C may adhere more effectively at 37°C and the rate of phagocytosis after the adhesion stage may vary between strains. Alternatively, strains may differ in their capacity to survive intracellularly during the periods studied.

The ability of virulent and avirulent strains of *Y. pseudotuberculosis* to invade human fibroblast cells *in vitro* has been assayed previously (Brunius *et al.*, 1980). No direct correlation between virulence and ability to invade the fibroblastic cells was observed. To establish a correlation between virulence and adhesion or invasion, further examination of avirulent and virulent strains is needed; adhesion and invasion may be only two of several factors necessary for the expression of virulence *in vivo*.

The difference between the adhesion of *Y. pseudotuberculosis* cells grown at 20°C and 37°C has a number of possible explanations. Adhesive chemical or physical structures on the bacterial surface may be present at 20°C but absent or masked at 37°C. Incubation at 37°C may alter the hydrophobic nature of the cell surface and its net charge. The different temperatures may select existing mutants with a greater or lesser ability to adhere to the cells. Finally, changes of incubation temperature may induce physiological changes in the bacteria. The rapid reversibility of the adhesive and non-adhesive properties indicates that physiological changes are the most likely explanation. The virulence-associated V and W antigens of *Y. pseudotuberculosis* are expressed at 37°C but not at 20°C (Carter, Zahorchak and Brubaker, 1980; Gemski *et al.*, 1980). The temperature-induced effects on *Y. pseudotuberculosis* adhesion may be mediated by the presence or absence of V and W antigens. However the V and W antigens are expressed at 37°C in medium lacking Ca²⁺ but containing Mg²⁺ (Yang and Brubaker, 1971) but we have shown that adhesion is more strongly expressed at 20°C and does not require Ca²⁺ or Mg²⁺.

Y. pseudotuberculosis is motile and has active flagella at 22°C but not at 37°C (Chen and Elberg, 1977). The adhesive capacity of the glutaraldehyde-fixed bacteria was as good as that of viable bacteria which indicates that motility is not necessary for adhesion and the thermostability of adhesion indicates that H-antigens are not involved.

Pili are involved in the attachment of certain bacterial pathogens, e.g., *Neisseria gonorrhoeae* and enteropathogenic strains of *Escherichia coli*, to epithelial cells (Pearce and Buchanan, 1980). Electron microscopy of negatively stained *Y. pseudotuberculosis* did not show any pili and the bacteria did not agglutinate erythrocytes. MacLagan and Old (1980) did not find mannose-sensitive or mannose-resistant haemagglutinins (pili) in 14 strains of *Y. pseudotuberculosis*.

The adhesion of virulent *Y. pseudotuberculosis* to cells is unlikely to involve carbohydrate components, because it was not affected by treatment with sodium metaperiodate. Formaldehyde and glutaraldehyde, that react primarily with amino groups, had little effect on adhesion. Treatment with protease IV, however, markedly reduced adhesion and this indicates the presence of protein adhesins. The absence of any effect of trypsin may be due to the specificity of trypsin for lysyl and arginyl bonds and its primary activity against denaturated polypeptides.

The early stages of the interaction between the HeLa cell surface and *Y. pseudotuberculosis* may be related to the different phases of receptor-mediated endocytosis of protein molecules. Minibeads coupled to proteins that bind to membrane receptors on non-professional phagocytic cells bind to microvilli in a process that is temperature independent. The beads are then ingested by a temperature dependent step (Takahashi, Tavassoli and Jacobsen, 1980). The receptor mediated endocytosis follows clustering of receptors, at 37°C, in specific areas or "coated pits" that invaginate and are then ingested (Goldstein, Anderson and Brown, 1979). The similarity between receptor mediated endocytosis and the early stages of the ingestion of *Y. pseudotuberculosis* by HeLa cells indicates that "coated pits" may participate in the ingestion of the bacteria.

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