MORPHOLOGICAL STUDIES ON FIMBRIAE OF *PSEUDO-MONAS AERUGINOSA* AND *AEROMONAS HYDROPHILA* WITH SPECIAL REFERENCE TO THEIR BIOLOGICAL FUNCTIONS

BY

Masakazu Hashimoto, Sankichi Horiuchi, Jiro Kosugi, Wataru Koohara, Minoru Nakane and Kihachiro Uchikawa[*1]

Abstract

Electron microscopic studies were performed with a series of 36 strains of *Pseudomonas aeruginosa* and 2 strains of *Aeromonas hydrophila*. Most strains of *Ps. aeruginosa* were found to possess bipolar fimbriae. The fimbriae of this species varied greatly in length, the short ones being considered to retain their biologic activities and the long ones being devoid of their function. By contrast, electron micrographs of *A. hydrophila* revealed numerous peritrichous fimbriae besides the monopolar flagellum. The fimbriae were found to be constant in length and in width. While the two species are classified into the family *Pseudomonadaceae*, it may also serve as an effective clue to the characterization of these species that they differ in their pattern of fimbriation and in the number of fimbriae.

The fimriae of *Ps. aeruginosa* were observed to be capable of adhering onto the surface of the red blood cells but fail to agglutinate them. It appears that the ability of the fimbriae to adhere onto the surface of the red blood cell (more appropriately called haemadsorption) may be related to the rosette formation and also to colony dissociation displayed by this species.

The fimbriae of *A. hydrophila* actively agglutinate the carmine dye and yeast cells and produce only a slight agglutination of the red blood cells. These fimbriae are of the mannose-resistant type. Acid agglutination by this species occurs within the range of pH 3.4 to 3.8, and *A. hydrophila* remained capable of showing acid agglutination even after thermal treatment though a shift of the pH range to the acid side occurred.

Introduction

Since the numerous fine filamentous appendages, extending peritrichously from the cell surface of *Escherichia coli,* are clearly distinguishable from the flagella (Houwing and van Iterson 1950)[1], electron microscopic evidences have been accumulated showing the presence of such fimbriation

[*1] 橋本雅一, 堀内三吉, 小杉二朗, 香原　亘, 中根　実, 内川喜八郎: Department of Microbiology (Chief: Asst. Prof. M. Hashimoto), School of Medicine, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku).
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over the cell surface of the organisms of many of the other genera be-
longing to the family *Enterobacteriaceae* such as *Aerobacter, Shigella,*
Salmonella, Klebsiella, Proteus and *Serratia*. The fine filamentous appen-
dages were designated as "fimbriae" (Duguid, Smith, Dempster and Ed-
munds 1955)[2], as "pili" (Brinton 1959)[3] and as "sen-moh" in Japanese
(Hashimoto 1965)[4]. It was already described that these fine filamentous ap-
pendages were composed of a double helical structure consisting of a great
many of subunits, 20 to 25 Å in length (Thornley and Horne 1962[5]; Naka-
jima and Hashimoto 1966[6]).

The fimbriae with such structural characteristics showed the ability
to agglutinate animal or plant cells (Duguid et al. 1955[2]; Constable 1956[7];
Duguid and Gillies 1957[8] and 1958[9]; Duguid 1959[10]). any of the mem-
bers of the family *Enterobacteriaceae,* for example, agglutinate the red
blood cells because their fimbriae cause the so-called bacterial haemaggluti-
nation reaction. Furthermore, the fimbrial components, mechanically re-
moved from the bacterial cells, are also known to be capable of giving
rise to such a phenomenon (Duguid and Gillies 1957[8]).

In the year 1962, Marx and Heumann[11] reported on the fimbriation
of *Pseudomonas echinoides* and in 1964 Heumann and Marx[12] studied the
relationship between the fimbriae and the phenomenon called star-forma-
tion which had been observed with the cells of this species. Unlike the
members of the family *Enterobacteriaceae*, this species showed a pattern of
monopolar fimbriation, which, according to them, takes part in star-forma-
tion whereby the fimbriae may participate in the sexual cycle of this species.

There are many reports on the pathogenicity of the organisms of the
genus *Aeromonas* to fresh-water fish and amphibians. This group of or-
ganisms is also known as the pathogen responsible for the "black-germ"
disease of chicken eggs (Miles and Halnan 1937[13]; Caselitz und Buck
1958[14]). Recently, members of this group of bacteria were isolated from
patients with myositis, gastroenteritis, osteomyelitis and liver cirrhosis, thus
posing a problem concerning its pathogenicity to man (Rosner 1964[15];
Fukaya et al. 1962[16]; and Conn 1964[17]). Two strains of *Aeromonas hydro-*
phila, which the authors recently dealt with, had been identified upon
isolation from the blood and bile juice, respectively, of two patients in
shock due to infection with Gram-negative bacilli. These are considered
rare cases in that the organisms were isolated from the blood, whereas in
most of the human cases the organisms are demonstrated in the saliva,
stool and such organs as the liver and spleen (Kjems 1955[18]; Dean and
Post 1967[19]). As for the morphology of *Aeromonas,* the bacteria of this
group are short rods known to possess monopolar flagellation, and there
exists a report on fimbriation as well (Caselitz und Günther 1960[20]).

This communication deals with the electron microscopic observations

on the structure of the fimbriae of a series of 36 strains of *Pseudomonas aeruginosa* and 2 strains of *A. hydrophila,* comparing these with some species already known to be fimbriated and with the studies on their fimbrial functions including haemagglutination and other biological properties.

MATERIALS AND METHODS

Organisms: Thirty-six strains of *Ps. aeruginosa* and two strains of *A. hydrophila* (Yasuno and Moriyama strains) were used. Of the 36 *Ps. aeruginosa* strains, 25 strains were capable of producing a deep blue pigment, pyocyanine, 5 were characterized by the production of a yellowish green fluorescent pigment, fluorescein, 1 synthesized a brownish-red pigment, pyorubin, while the remaining 5 strains did not produce any pigment metabolites.

Techniques of haemagglutination test: Red blood cells from the guinea pig, sheep, rabbit, rat, horse, cattle, chicken, mouse and man (groups A and O) were used.

A broth culture of a strain, after incubation at 37°C for 24 hours, was dispensed in 0.25 ml aliquots in wells in a clear plastic plate, and to each of these wells was added an equal volume of red blood cells suspended in physiological saline to the amount of 1 or 3 per cent (The red blood cells have been previously washed with physiological saline by centrifugation at 3,000 r.p.m. for 10 minutes), and the mixtures in the wells were agitated for 3 or 5 minutes at room temperature on a rotator (120 r.p.m.) to determine whether the organisms produced red cell agglutination masses. For observation of haemadsorption, on the other hand, sheep erythrocytes were used. After washing with physiological saline, the red blood cell suspension was diluted with broth. Since the broth itself was hypotonic, the suspension in broth was made by adding glucose to a final concentration of 0.1 per cent. One ml of centrifuged sheep erythrocytes was then added to each 9 ml of the glucose-broth to make a 1 per cent suspension of sheep erythrocytes. After incubation of the mixtures at 37°C for a given length of time, electron microscopic examination was made of the suspensions to observe the adsorption of bacterial cells onto the surface of the red blood cells.

Techniques for acid agglutination test: Suspensions of the bacterial cells, of which the concentrations had been adjusted to the No. 3 tube of MacFarland's turbidimetric scale, were used. Two-tenth ml aliquots of the bacterial suspension were added to the sodium phosphate acetic acid buffer at various, progressively increasing pH's, and the resulting mixtures were allowed to stand at room temperature for 60 minutes. The mixtures were

then examined for agglutination of the bacterial cells to determine the optimal pH for acid agglutination.

Electron microscopy: For observation of the structure of the fimbriae, a heart infusion broth culture of a strain incubated at 37°C was diluted with sterile distilled water and the diluted bacterial suspension was then dried on a collodion film supported by a grid. The grid with the film loaded with specimen was then placed in an evaporator chamber and shadow-casting was done with chrome vapor. Wherever deemed necessary, specimens prepared by using sodium phosphotungstate as a negative stain were also examined under the electron microscope. The equipment employed in this study was the Hitachi electron microscope, Model HU11.

RESULTS

1. Observation of fimbriae

Morphological observation was made of the fimbriae of *Ps. aeruginosa* comparing these with the well studied fimbriated members of the family

Left   Right
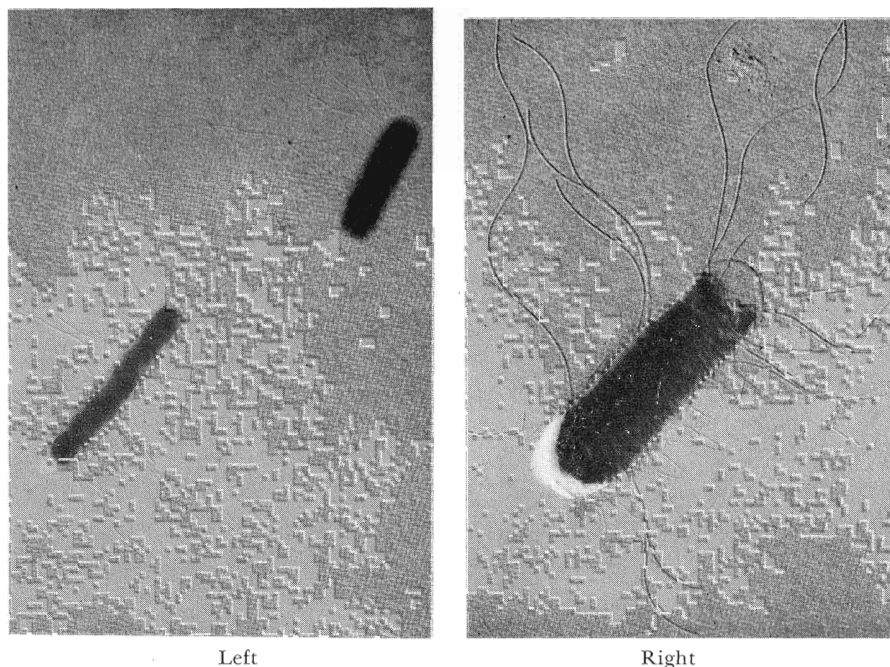
Fig. 1.   Left: Electron micrograph of *Shigella flexneri* (×15,000). Unflagellated cells have numerous peritrichous fimbriae.

Right: Electron micrograph of the peritrichous fimbriae of *Proteus vulgaris* which has peritrichous flagella (×20,000).

Enterobacteriaceae.   Presented in Fig. 1 are the electron micrographs of the fimbriae of *Shigella flexneri* and *Proteus vulgaris.*   As can be seen, numerous fimbriae exist on the cell surface of these bacterial species. Fig. 2 shows the electron micrographs with shadow-casting of *Ps. aeruginosa* revealing their pattern of fimbriation.   It is very interesting that these micrographs reveal a fairly interesting relationship between the site of the fimbrial projection and the location and number of the flagella. Namely, *Ps. aeruginosa,* in which the flagella show a polar pattern, was seen to have also a bipolar fimbriation, the fimbriae arising from both ends of the rods, whereas *Prot. vulgaris,* which has peritrichous flagella, was observed to possess fimbriae distributed at random all over the cell surface. Unflagellated *Sh. flexneri* was found to have a large number of peritrichous fimbriae.   Upon summing up, the electron microscopic findings on the morphology of fimbriae of many of the members of the family *Entero-bacteriaceae* and of *Ps. aeruginosa,* it can be pointed out there is an interesting relationship.   That is to say, the organisms of the family *Entero-bacteriaceae,* the flagellation of which is peritrichous or atrichous, display a peripherally distributed fimbriae, while *Ps. aeruginosa* which shows mono-trichous or lochotrichous flagellation has a polar pattern of fimbriation.

As evident from the electron micrographs of *Ps. aeruginosa* with the shadow-casting shown above, the fimbriae varied greatly in length.   The length, for example, as shown in Fig. 2, ranged from approximately 1 micron to more than 6 microns.   Figure 3 is an enlarged electron micrograph with a negative staining of the fimbriae of this species.   As can be seen, the calibers of the individual fimbriae show a helical structure, each consisting of several subunits, being approximately 20 Å in diameter.   Comparison with the well-known structure of the fimbriae of *Escherichia coli* and *Sh. flexneri* reveals a fairly strong similarity in the morphology of the fimbriae, particularly with respect to the pitch and angle of the helical structure (Brinton 1965[21]; Nakajima and Hashimoto 1966[6]).

An electron microscopic study on the morphology of the fimbriae of *A. hydrophila* grown in a nutrient broth at 37°C was also performed by the shadow-casting and negative staining techniques.   Most of the morphological studies of the organisms of the genus *Aeromonas* published were based on the findings obtained by photomicroscopic observations and the genus has been described to be a Gram-negative short rod which measures 1 to 2 microns in length and 0.6 micron in width and possessing one polar flagellum or, depending upon the condition of the cultivation, a few lateral flagella (Leifson and Hugh 1953)[22].   Electron micrographs of the organism, by a direct magnification of X5,000, revealed numerous fimbriae distributed over the cell surface besides the single polar flagellum.   As can be noted from Fig. 4 (electron micrograph with shadow-casting of *A. hydrophila*),
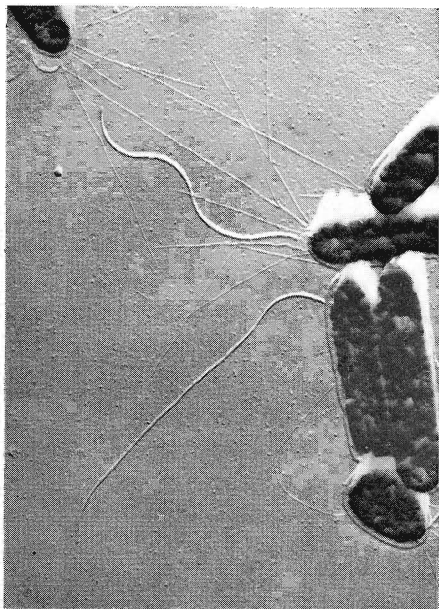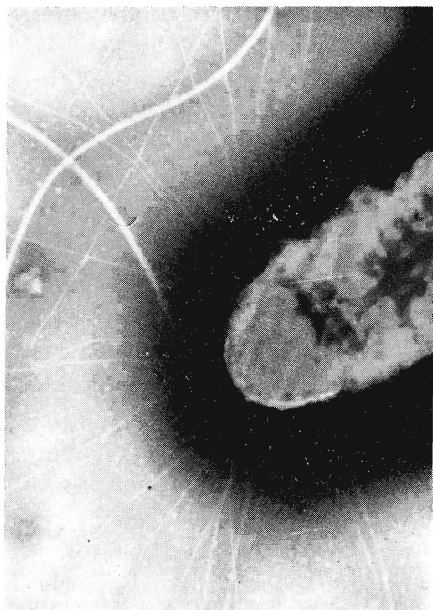
Fig. 2.   Fig. 3.

Fig. 2.   Electron micrograph of the polar fimbriae of *Pseudomonas aeruginosa* ($\times 20,000$). This micrograph also reveals the polar flagellation of this species. The fimbriae varies greatly in length.

Fig. 3.   Negative staining micrograph of the fimbriae of *Pseudomonas aeruginosa*, showing the helical structure of the fimbriae, each consisting of several subunits ($\times 40,000$).
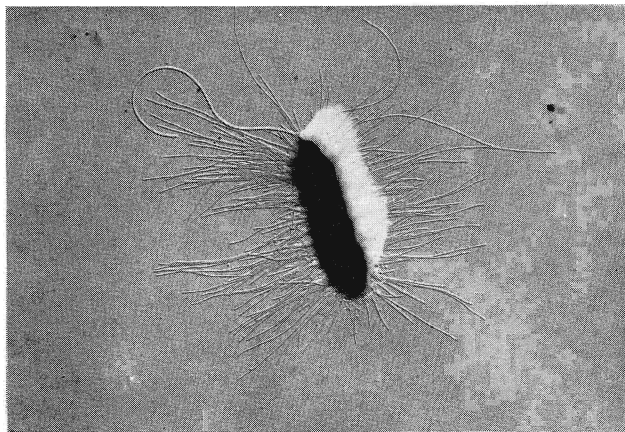
Fig. 4.   Electron micrograph of *Aeromonas hydrophila*. Peritrichous fimbriae arise upright all over the cell surface ($\times 5,000$).
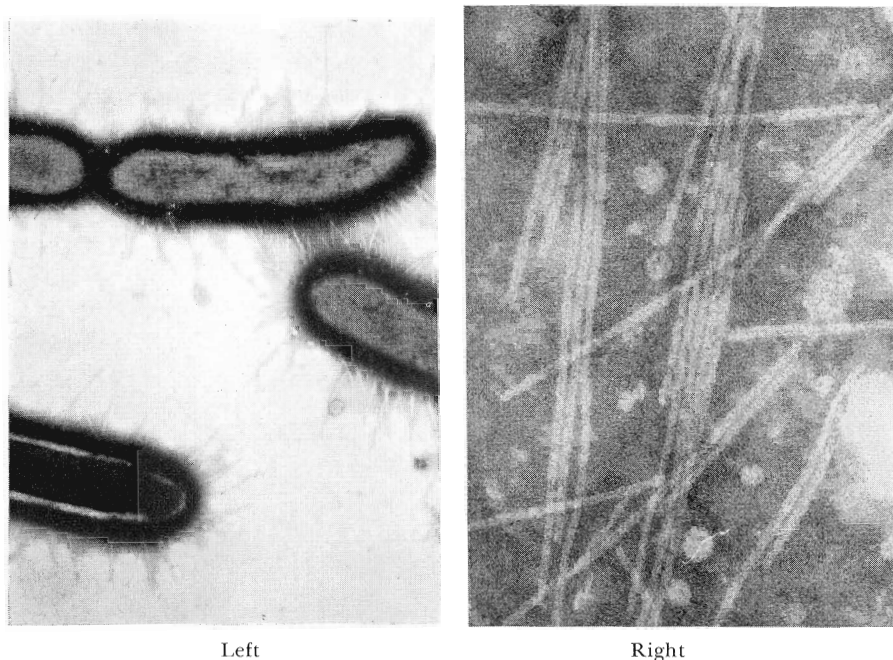
Left   Right

Fig. 5.   Left: Negative staining micrograph of *Aeromonas hydrophila* (×20,000).
Right: Negative staining micrograph of the purified fimbriae of this species
(×200,000). The fine filaments consist of many subunits, which show a
considerable resemblance to those of the fimbriae of *Shigella flexneri* with
respect to the pitch and angle of the helical structure.

fine, mostly straight filamentous appendages arise upright all over the cell
surface. They number approximately 200 to 300 and measure approximate-
ly 1.0 to 1.5 microns in length. Fig. 5 is an enlarged electron micro-
graph of *A. hydrophila* with negative staining, showing its fimbriae. As
may be seen, the fine filaments are virtually uniform in width, measuring
50 to 70 Å. In observing the fine structure of these fimbriae (Fig. 5, Right),
subunits are also seen arranged likewise to constitute the fimbriae of *A.
hydrophila,* showing also a considerable resemblance to those of *Sh. flexneri*
and *Ps. aeruginosa* with respect to the pitch and angle of the helical struc-
ture.

2.   Haemagglutination

Ps. aeruginosa was thus found to possess fimbriae as the external ap-
pendage besides the already known flagella, but our knowledge of the func-
tion of the fine filamentous structure of this species is practically nil. How-
ever, at least with the organisms of the family *Enterobacteriaceae,* it is
known that the fimbriae function as an organelle which takes part in bac-
terial adhesion, and also in the fimbriae, some F-pili facilitate the trans-

mission of the genetic materials (Brinton 1965)[21].   Table 1 summarizes the results of the haemagglutination tests performed with the representative strains of this species.   None of the strains studied was found, as can be seen, to demonstrate erythrocyte agglutination, when tested by the conventional glass slide method.   However, observation by electron microscopy

Table 1.   Haemagglutination by *Pseudomonas aeruginosa*

Strain reference number	Red blood cells								
	Guinea pig	Sheep	Rabbit	Rat	Horse	Cattle	Chicken	Mouse	Man
9	—	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—	—
35	—	—	—	—	—	—	—	—	—
36	—	—	—	—	—	—	—	—	—
38	—	—	—	—	—	—	—	—	—

of *Ps. aeruginosa,* grown for 18 hours at 37°C in a blood-containing nutrient broth, showed evidence of adsorption of the organisms onto the surface of the red blood cells although the species undoubtedly failed to produce macroscopic agglutination of the erythrocytes (Fig. 6, Left).   The authors would like to designate this property of this species as haemadsorption at the moment.   It is evident that *Ps. aeruginosa* ataches its fimbriae to the red blood cells by their ends, on the contrary to the lateral attachment of fimbriae of *Sh. flexneri* causing the bacterial haemagglutination (Fig. 6, Right).   Further study of this phenomenon by electron microscopy showed, as can be seen in Fig. 7, a feature appearing to correspond to the "star-formation" previously noted with *Ps. echinoides.*   This electron microscopic feature may be called rather more suitably "rosette formation".

Meanwhile, it was noted that some strains among the *Ps. aeruginosa* organisms examined demonstrated colony dissociation forming small and large colonies on the agar plates (Fig. 8).   Inasmuch as the rosette formation displayed by *Ps. aeruginosa* is characterized by the formation of numerous small colonies, the rosette formation by *Ps. aeruginosa* and consequently the presence of polar fimbriae may well be construed as representing a property pertaining to colony dissociation.   Similarly, tests conducted with *A. hydrophila* strains from broth cultures showed no agglutination of all the red blood cells of variously laboratory and domestic animals and group A and O human erythrocytes.   All the haemagglutination tests with the
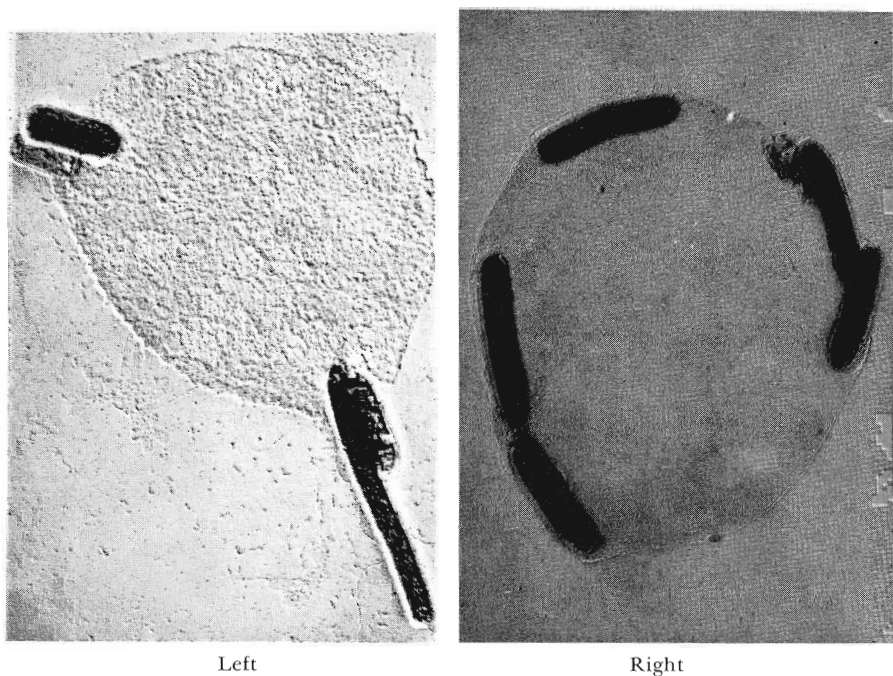
Left   Right

Fig. 6. Left: Haemadsorption of *Pseudomonas aeruginosa* (×10,000). The bacterial
cells attach their fimbriae to the red blood cells by their ends.
Right: Haemagglutination of *Shigella flexneri* (×11,000). The bacterial
cells attach the lateral sides to the red blood cells by their fimbriae.

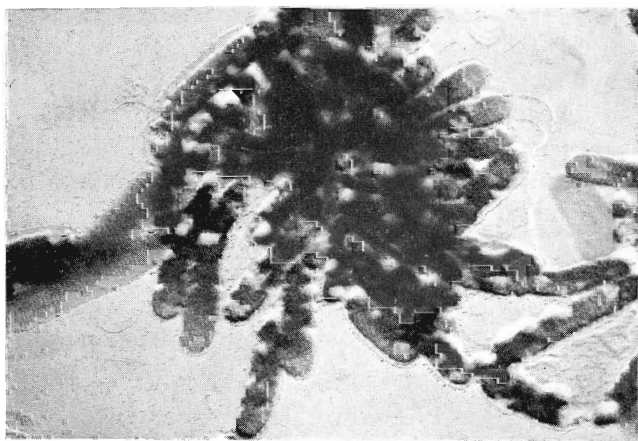Fig. 7. Rosette formation observed in a small colony of *Pseudo-
monas aeruginosa* No. 35 (×18,000).
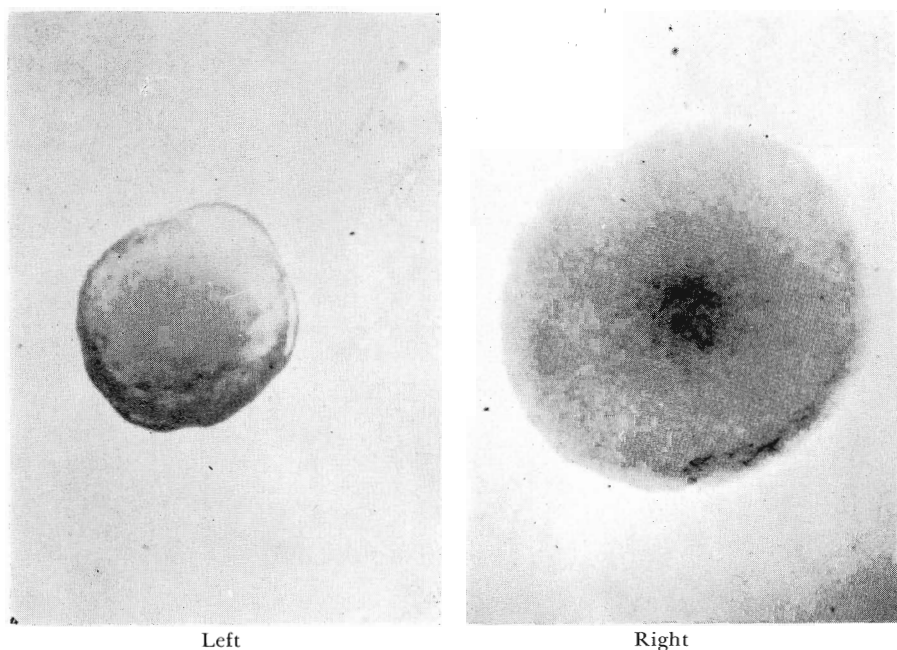
Left   Right

Fig. 8. Colonial dissociation demonstrated in *Pseudomonas aeruginosa*. The small colony in the left figure shows the rosette formation of the bacterial cell.

organisms prepared after the serial passages in nutrient broth, and hence assumed to be favorable for the development of fimbriae, were similarly negative. Furthermore, when the tests were carried out using suspensions of the organisms in a medium containing 5 per cent formalin, thereby being assumed to have had their fimbriae "fixed", no appreciable agglutination of the red blood cells occurred at all (Table 2). When the bacterial cells grown in the nutrient broth were suspended in physiological saline, washed and concentrated by centrifugation in physiological saline (adjusted approximately to tube No. 10 of the MacFarland's turbidimetric scale), and tested for haemagglutination, the Moriyama strain showed agglutination of the red blood cells of all animals used while the Yasuno strain was found to agglutinate the erythrocytes of the guinea pig, cattle, chicken and rabbit to the same extent as the Moriyama strain but showed only a weak agglutination of the red blood cells of the rat and mouse, and produced no agglutination with the red blood cells of the horse, sheep and man. In view of this, suspensions of the strains previously treated with 5 per cent formalin and then concentrated after washing with physiological saline were tested, but none of these suspensions proved capable of producing agglutination of the red blod cells of any of the species tested (Table 3).

Table 2.   Haemagglutinating activity of *Aeromonas hydrophila*

Red blood cells	Bouillon culture		Folmalin-fixed bouillon culture	
	Moriyama	Yasuno	Moriyama	Yasuno
Guinea pig	−	−	−	−
Sheep	−	−	−	−
Rabbit	−	−	−	−
Rat	−	−	−	−
Horse	−	−	−	−
Cattle	−	−	−	−
Chicken	−	−	−	−
Mouse	−	−	−	−
Human	−	−	−	−

Table 3.   Haemagglutination by concentrated cells of
Aeromonas hydrophila

Red blood cells	Concentrated cells by washing and centrifugation		Concentrated cells after formalin fixation	
	Moriyama	Yasuno	Moriyama	Yasuno
Guinea pig	╫	╫	−	−
Sheep	╫	−	−	−
Rabbit	╫	╫	−	−
Rat	╫	+	−	−
Horse	╫	−	−	−
Cattle	╫	╫	−	−
Chicken	╫	╫	−	−
Mouse	╫	+	−	−
Human	╫	−	−	−

Mannose sensitivity test was performed by adding a 0.05 M mannose solution to a suspension of washed and concentrated bacterial cells and observing the agglutination of the red blood cells.   However, no evidence of inhibition of haemagglutination by mannose or of disintegration of the agglutinating masses of the red blood cells once formed was obtained.

In parallel with this series of experiments, the *A. hydrophila* strains were also tested for the agglutination of carmine particles and *Saccharomyces cerevisiae* cells.   Suspensions of the organisms of both strains, grown simply in nutrient broth, were found to actively agglutinate carmine particles and *S. cerevisiae* cells although the agglutination was not affected at

all by the 0.05 M solutions of various sugars and especially by the same concentration of mannose solution (Table 4).

3. Acid agglutination

Suspensions in physiological saline of *A. hydrophila,* grown in a nutrient broth for 24 hours at 37°C. and adjusted to the concentration of tube No. 3 of MacFarland's turbidimetric scale, were observed to show acid agglutination at the pH values ranging from 3.0 to 4.2. The isoelectric point, at which particularly marked agglutination was noted to occur, extended from pH 3.4 to 3.8 (Table 5). The range of pH thus observed is practically consistent with that already reported as the isoelectric points of pH 3.5 to 4.2 for *E. coli* and *Sh. flexneri* (Hashimoto et al. 1963)[23].

Table 4. Agglutination of carmine particles and *Saccharomyces cerevisiae* by *Aeromonas hydrophila*

Strains	Agglutination of carmine particles		Agglutination of S. cerevisiae	
	Without mannose	With mannose	Without mannose	With mannose
Moriyama	⧻	⧻	⧻	⧻
Yasuno	⧻	⧻	⧻	⧻

Table 5. Acid agglutination of *Aeromonas hydrophila*

Strains		Range of pH								
		2.2	2.6	3.0	3.4	3.8	4.2	4.6	5.0	5.4
Alive	Moriyama	−	−	+	⧻	⧻	+	−	−	−
	Yasuno	−	−	+	⧻	⧻	+	−	−	−
Heat-killed	Moriyama	+	+	−	−	−	−	−	−	−
	Yasuno	+	+	−	−	−	−	−	−	−

These findings suggest some similarity in the structural components of fimbriae between the genus *Aeromonas* and the family *Enterobacteriaceae* despite the considerable differences in the agglutinating behavior. When suspensions of the *Aeromonas* cells, treated by heating at 120°C. for 10 minutes after growth in bouillon, were tested, a shift of pH range to 2.2–2.6, being the usual pH range of acid agglutination of the fimbriated *Serratia marcescens* cells, occurred wherein the acid agglutination reaction was noted to be substantially weakened (Table 5). The same thermal treatment of the fimbriated cell suspensions of *E. coli* and *Sh. flexneri* lead

to the disappearance of their ability to produce acid agglutination, possibly due to the destruction of their fimbrial constituents. By contrast, acid agglutination did occur with the *A. hydrophila* cells even after such thermal treatment, and the optimal pH range was observed to correspond to the isoelectric point of the fimbriated cells of *Ser. marcescens* of the mannose-resistant type. These findings are considered to be of extreme interest in studying the chemical properties of the fimbriae of the genus *Aeromonas.*

DISCUSSION

In about 90 per cent of all strains examined, the electron micrographs with shadow-casting of *Ps. aeruginosa* revealed two or five bipolar surface projections or fine filamentous appendages on each cell. They varied greatly in dimension from the flagella, and, even in the same cell, the length varied from 1 micron up to more than 6 microns. Furthermore, electron microscopy by the negative staining technique showed a fine spiral filamentous structure, which is comparable in appearance to that usually seen in *E. coli* and *Sh. flexneri,* and hence it can no doubt be called fimbriae (or pili). Negative staining at a high magnification also revealed individual spherical subunits. However, the observation that all the fimbriated strains of *Ps. aeruginosa* studied failed to demonstrate the haemagglutination suggested that with this species of bacteria the fimbriae represented a component incapable of producing erythrocytic agglutination.

Evidence has been obtained on the property of *Ps. aeruginosa* of forming characteristic aggregation of cells, called rosette formation, and electron micrographs of rosette-minus strains of this species disclosed the presence of polar fimbriae as in the cases of rosette-plus strain. While these findings, therefore, seem to indicate that the fimbriation on the surface of the *Ps. aeruginosa* cells is irrelevant to the property of rosette formation, both the electron microscopic findings and haemadsorbing property of this species appear to suggest that the lack of the ability of the rosette-minus strains to form rosette is derived from the loss of the rosette-forming function due to the excessive prolongation of the fimbriae. Namely, assuming that the fimbriae, i.e., fimbriae of rosette-plus cells, which are fully capable of discharging the functions, retain the ability to mutually adhere and to contact, they should be able to combine with red blood cells situated closely nearby and consequently to let the erythrocytes come into intimate contact with them by their contraction, and, by contrast, the fimbriae of the rosette-minus cells are considered no longer capable of congealing. Furthermore, these fimbriae appear to have their contractility disturbed and, in consequence, they frequently manifest themselves as completely out-stretched filaments, with their length being more than six microns surpassing the

length of the cells. The fimbriae, if fully stretched straight out, are suit-
able for making a morphological observation of their fine structure. The
fimbriae of the rosette-plus strains, on the contrary, arise invariably vis-a-vis
at the cellular poles and are apparently shorter and thicker.

Studies on the external structure of *A. hydrophila* in comparison with
the structure of the flagella and fimbriae of *Sh. flexneri, E. coli*. and *Prot.
vulgaris* have shown that the unflagellated *Sh. flexneri* has numerous peri-
trichous fimbriae and that *E. coli* and *Prot. vulgaris* with peritrichous flagel-
lation also have fimbriae distributed at random over their cell surface.
That is, at least the various species of the family *Enterobacteriaceae* have
a peritrichous fimbriation irrespective of the presence or absence of flagel-
lation. By contrast, as already described above, *Ps. aeruginosa,* the pattern
of flagellation of which is either monotrichous or lochotrichous, show a
polar fimbriation, although *A. hydrophila,* which essentially belongs to
the family *Pseudomonadaceae* and has a few flagella at one or both poles
as in the case of *Ps. aeruginosa,* was found to exhibit a peritrichous fimbria-
tion such as in *Vibrio cholerae.* Among the species belonging to the family
Pseudomonadaceae, the group of species belonging to the genus *Pseudo-
monas* shows a polar fimbriation consisting of two or five fimbriae whereas
the species of the genera *Vibrio* and *Aeromonas* have peritrichous fimbriae.
This fact stresses the importance of the differences in their pattern of
fimbriation when taking into account the taxonomy of this particular
family.

While it has already been described that the peritrichous fimbriae of
the organisms of the family *Enterobacteriaceae* serve as a factor contribut-
ing to the haemagglutinating ability of the fimbriated cells, several facts
have been reported indicating that haemagglutination by this group of
microorganisms is not necessarily based on a single uniform mechanisms.
In other words, the haemagglutination reaction differ according to the
species of microorganisms and the kind of red blood cells, and also of
particular interest is the difference in agglutination reaction involving
mannose. Namely, haemagglutination by certain species of microorganisms
is inhibited in the presence of mannose (mannose-sensitive type) and in other
species of microorganisms it is not inhibited in the presence of this sugar
(mannose-resistant type). Consistent with the difference in the reactivity,
the presence of several different patterns of antigenicity of the fimbriae has
also been demonstrated (Hashimoto 1965)[4]. It is considered worthy of
note that in this study both *Ps. aeruginosa* and *A. hydrophila* having the
fimbriae, which may be classified as the mannose-resistant type, demon-
strated a haemagglutinating property of a rather different kind from that
of the members of the *Enterobacteriaceae* which possess the peritrichous
fimbriae. That is to say, with none of the *Ps. aeruginosa* strains studied

was there any macroscopic or microscopic evidence of haemagglutination at all while cell-to-cell adhesions were observed with erythrocytes of certain animals. This suggests a considerably high affinity of the fimbriae of this species to the cellular membrane of the erythrocytes, and it may be said that there was some peculiar reason for the failure of cell-to-cell adhesion to progress into agglutination of the red blood cells. Inasmuch as, for example, the individual cells of *Ps. aeruginosa* invariably show a polar fimbriation, the cells rarely forms pairs even if they have combined with the red blood cells, thereby failing to give rise haemagglutination. As the fimbriae of each cell of this species were able to combine only one or two red blood cells, the reactivity may well be designated as haemadsorption rather than using the usual term haemagglutination. Consequently, when the bacterial cells are grown under conditions where the cells possessing active fimbriae have a relatively high possibility of attaching to the erythrocytes. for example, under the condition where the erythrocytes are in constant contact with such bacterial cells in a fresh broth, or more concretely, when the bacterial cells have been grown for 12 to 16 hours along with the erythrocytes in the same tubes, it can be seen by electron micrographs that the numerous bacterial cells are adsorbed onto the surface of red blood cells.

The same is true for *Aeromonas*. While broth cultures of this species failed completely to cause any agglutination of the erythrocytes of various animal species, haemagglutination was found to occur when the concentration of the *A. hydrophila* cells were increased by prewashing with physiological saline. The phenomenon, therefore, was derived probably from the fimbriae of *A. hydrophila* which had an extremely low affinity for the surface of the red blood cells. In addition the authors are more or less at a loss to make out the meaning of the phenomenon that *A. hydrophila* lost its ability to agglutinate the red blood cells by fimbriae when they had been fixed with formalin whereas the finding obtained heretofore had indicated augmentation of haemagglutinating activity of the fimbriae after such treatment. If, thereby, the failure is attributed totally to formalin fixation, the phenomenon may be that the formalin gave rise to some denaturation of the protein constituting the fimbriae of this species by its protein-coagulating action and, in consequence, the organisms lost its agglutinating activity which has been originally retained only to a small extent, However, despite the relatively poor ability of this species to agglutinate the red blood cells, *A. hydrophila* demonstrated a profound agglutinating activity toward carmine dye and toward yeast cells and the agglutination thus produced was of the mannose-resistant type. The pattern of haemagglutination produced by *A. hydrophila* differed from strain to strain, nevertheless, and, in this regard, evidence was obtained for the difference in the pattern of antigenicity between the two strains of *A. hydrophila*

used in these experiments.

Furthermore, interesting phenomena were observed with *Ps. aeruginosa* and *A. hydrophila* which belong to the same family, that is, the phenomenon of colony dissociation of *Ps. aeruginosa* and that of acid agglutination of *A. hydrophila* and their respective relations to fimbriation.

It has been long known that colony dissociation is observed fairly frequently with *Ps. aeruginosa*. Namely, Kramer (1935)[24] reported that the colony dissociation exhibited by this species under certain conditions of cultivation was related to the ability of the cells to utilize carbohydrates, protease activity, and to the variatio nof its H antigen. Gaby (1945)[25] studied the changes in the biochemical properties and antigenicity of this species associated with the colony dissociation and thereby drew a conclusion that colony dissociation had definitely no significant relation to the ability of the bacterial cells to utilize saccharides or to the protease activity of the cells but could serve as a factor responsible for the induction of variation in H antigen. Having made an observation that 66 per cent of all strains of *Ps. aeruginosa* studied showed colony dissociation, Zierdt and Schmidt (1964)[26] pointed out that alterations in bacterial susceptibility to bacteriophage were associated with colony dissociation.

On the other hand, *A. hydrophila* used in the present study showed a range of isoelectric points of acid agglutination from 3.0 to 4.2 which were virtually the same as those shown by *E. coli* and *Sh. flexneri,* thus indicating a similarity of these species in regard to the constituents of their fimbriae. While the latter species proved to lose the property of acid agglutination when treated by heating at 120°C for 10 minutes, *A. hydrophila* was found to retain its acid agglutinating ability at pH 2.2 to 2.6, though a shift of the optimal pH values to the acid side occurred. Moreover, the isoelectric points shown by *A. hydrophila* practically corresponded to those shown by *Ser. marcescens* which has mannose-resistant fimbriae. To sum up, the fimbriae of *A. hydrophila* and *Ps. aeruginosa* were found to bear some similarities to those of *Sh. flexneri, E. coli* and *Prot. vulgaris* with respect to the structural characteristics revealed by electron micrograph. However, the results of this study also indicate the characteristic difference among the species of the family *Pseudomonadaceae* and of the family *Enterobacteriaceae* regarding the pattern of flagellation and fimbriation, and the various aspects of the fimbrial functions. Hence, the differences in the fimbrial constituents among these bacterial species, and the diversity of the antigenic structures as well have been suggested.

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