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THE ROLE OF INTESTINAL BACTERIA IN ACUTE DIARRHEAL DISEASES

Annual Report

Sherwood L. Gorbach, M.D.

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ST enterotoxin	Enterotoxigens	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report describes investigations in these four major areas of research:

(1) Reassessment of the infant rabbit model of E. coli colonization.

(2) Testing of E. coli strains isolated from humans with diarrheal disease for enterotoxin production and presence of colonization-specific surface antigens.

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3. a. Methodology for isolation of specific pili (i.e. surface antigens which function in colonization).
   b. Characterization of pili.
   c. Preparation of pili-specific antisera.

4. In vitro adhesion assays specific for recognition of E. coli strains which are potentially pathogenic for humans.

The investigation during the first year of this Contract covered four major areas:

1. Reassessment of the infant rabbit model of E. coli colonization

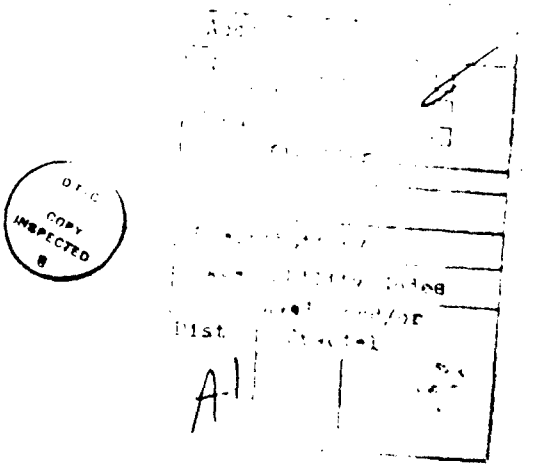
2. Testing of E. coli strains isolated from humans with diarrheal disease for enterotoxin production and presence of colonization-specific surface antigens

3. a. Methodology for isolation of specific pili (i.e. surface antigens which function in colonization).

   b. Characterization of pili

   c. Preparation of pili-specific antisera

4. In vitro adhesion assays specific for recognition of E. coli strains which are potentially pathogenic for humans.



SECTION 1.

REASSESSMENT OF INFANT RABBIT COLONIZATION MODEL:

Our efforts to demonstrate bacterial colonization ability (adherence) and growth using an infant rabbit model involved a number of different approaches.

a. Intubation or Surgical Implantation of Relatively Numbers of Organisms (10^5 - 10^6 cfu/ml): After 6 hours, animals are sacrificed and a significant increase in numbers (2 to 3 logs) is taken as indicative of adherence and growth in the small bowel. The results shown on Table 1 indicate that all three strains tested could "colonize" under these conditions.

b. Intubation or Surgical Implantation of a Large Inoculum (5×10^8 - 10^9cfu/ml). Following six hours of incubation,the animals small bowel is examined for maintenance of a large number of organisms which should be indicative of enhanced "sticking ability" (Table 2). Under these test conditions we could not discriminate between human strains with and without colonizing ability. Alternatively, all strains examined have colonizing ability.

c. Direct Inoculation into Ligated Loops of Small Bowel: While this method is more artificial than those described above, personal communication has suggested other laboratories use this method. The results,as shown on Table 3, revealed that the ligated loop tends to support growth of 334, 1111A and the plasmid-free derivative 334LL. Strain $TD427_{C2}$ appears not to grow under these conditions.

d. 18 hr. Assays Employing Relatively Low Inoculum (5×10^6cfu/ml): The results shown on Table 4 indicate that both toxigenic and non-toxigenic control strains were able to maintain themselves at approximatley input

levels. The rough E. coli K12, strain J5,was consistently found to undergo a dramatic loss while strain RDEC-1, a rabbit pathogen recently described by Cantey & Blake (J.I.D. 135:454-462, 1977) increased in numbers by approximately 1 log during the majority of these assays.

e. Suckling mice 2day and 7 day-old) and suckling rats were also tested and found to support growth of both control and human pathogenic strains. We presume this growth is intraluminal as apposed to the human case where multiplication occurs specifically on the mucosal surface.

Conclusions:

After exploring this model under a variety of procedural changes, we strongly feel that the infant rabbit small bowel is not physiologically suited for use in discriminating between colonizing and non-colonizing strains of toxigenic E. coli of human origen. Therefore, we have turned our attention to the utilization of human buccal mucosal cells for an adherence assay.

SECTION II.

ENTEROTOXIN AND SPECIFIC HA (sHA) TESTING OF E. COLI STRAINS

During the contract period we have continued to screen Escherichia coli isolated from humans and food with diarrhea for their ability to produce LT and/or ST enterotoxin and for the presence of a surface antigen (analogous to the K88 antigen of porcine enteropathic E. coli) which can cause specific hemagglutination (sHA) of guinea pig red blood cells at 4°C in the presence of mannose. Seventy strains were tested (Tables 5,6). The Y1 mouse adrenal cell assay and/or the 18 hour rabbit loop was used to screen for LT enterotoxin production and the suckling mouse assay was used to detect ST enterotoxin. The specific HA reaction was performed as previously described and,to date, we have found only 2 strains, 334, 193-4 which have this ability. Due to the probable involvement of plasmids in expression of this characteristic , we are making every effort to obtain fresh isolates. Dr. R. B. Sack has kindly offered to send us recently isolated toxigenic E. coli from his studies of travelers diarrhea in Peace Corp volunteers. It is also possible that the colonization by E. coli strains capable of causing disease in man is not sHA associated, unlike the K88 in strains isolated from pigs. We will continue to screen from sHA reactions while concomintantly screening for "sticking ability" using the human buccal cell adhesion assay.

SECTION 3.

PILI AND COLONIZATION FACTOR

a. Purification of specific pili: Pili and fimbriae are surface appendages which may be removed and isolated by purely physical means, i.e. ultracentrifugation, precipitation and chromatography. However, to make antisera specific for the colonization factor(s) it is important to isolate the specific pili involved. We have isolated the E. coli 334 receptor which is responsible for the agglutination of guinea pig red blood cells in the presence of mannose and at 0C. Thus, we have used a specific adsorption reaction based on the following observations: the hemagglutination of guinea pig red blood cells by E. coli 334 could be reversed by increasing the temperature of the reaction from 0C (ice bath) to 37C. Upon recooling, the HA reaction was again observed, suggesting a temperature-dependant reversable equilibrium rather than an irreversable process occurred.

For the preparation of specific pili E. coli 334 was grown overnight on peptone agar and was harvested and washed twice in saline. The washed cells were treated in a blender for 3 minutes using short bursts and cooling with ice to prevent heat denaturation. The intact cells and cellular debris were removed by centrifugation (10,000 RPM for 10 min) and the supernatant containing pili which had been sheared off was mixed with washed guinea pig erythrocytes in PBS containing 1% mannose and incubated 15 minutes in cold (ice bath). The red cells which should have attached specific pili, but not common fimbriae, were washed with PBS + mannose, incubating 5 minutes in ice between each centrifugation. The supernatants were termed PBS-mannose 1 through 3 and their protein concentrations were determined (OD_{280}) which is shown in Table 7. The specific pili were then eluted from the red blood cell surface by raising the temperature. The first

two PBS washes were each incubated for 5 minutes at 37C, then the temperature of the 5 minute incubation was increased to 45C for PBS fraction 3 and to 55C for PBS fraction 4. At the higher temperatures some hemolysis was noted. This procedure is summarized in Figure 1.

These preparations, containing specific pili, were then used to establish purification conditions. Ultracentrifugation techniques (Table 8) took the form of gradually increasing the centrifugation time while quantitating the amount of sedimented protein. The 50Ti rotor operating at 45,000 RPM (max force 183,000 xg) was used throughout these experiments. In Table 8 the OD_{280} of the sedimented material (resuspended in 2.0 ml distilled water) and of the final supernatants are shown. The hours of centrifugation shown are the number of hours in that run, rather than the cumulative total. The increase in centrifugation time of the most gently dissociated fraction (PBS 1) from 2 to 6 hours did not increase sedimented material. This rapid sedimentation implies that the receptor involved in the HA of guinea pig RBC in the cold and in the presence of mannose is a relatively large macromolecule. The ability to isolate a specific pili will allow us to make a specific antiserum.

b. Characterization of specific pili: The availability of this purified E. coli HA receptor has allowed us to begin physio-chemical characterization. The molecular weights of the isolated pili preparations are being determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis.

As isolated by the methods described above the purified pili or fraction PBS 1 contains two polypeptide chains,both with molecular weights less than15,000. Other material staining with Coomassie Blue are not present. Polypeptide chains of equivalent molecular weight are also found in other fractions including the final supernatant fraction

which has been ultracentrifuged (183,00 xg) for 2,4 and 6 hours consecutively. Thus,indicating that ultracentrifugation was not sedimenting all of the specific pili protein which has been eluted/desorbed from the red cell surface.

c. Antisera: We have used this pili preparation from E. coli 334 as well as the intact organism to prepair specific rabbit antisera. This specific antisera has then been used to examine the antigenic surface structures of various toxigenic E. coli strains and their derivatives. These data are summerized in Table 9. As can be seen strain 193-4 which shows binding to the buccal cells also gives a positive reaction (agglutination) with either antisera against 334 or 334 pili. This strain also agglutinates guinea pig red blood cells in the cold and in the presence of mannose, which suggests that a common antigenic site is present in both these strains and is responsible for the sHA of guinea pig red blood cells. Initial results with the buccal cell adhesion assay indicate that these strains also share the characteristic of binding to human epithelial cells. The anti pili antiserum shows the HA reaction. We feel that the presence of an antigenic determinant(s), common to a number of strains, which are responsible for colonization, is of great significance in screening potentially toxigenic E. coli isolated from food and cases of human diarrhea.

We have been able to find a derivative of 334 ST^+/LT^+, called 334LL $ST^-LT^-HA^-$ which is missing all six of the plasmid species present in 334 (Figure 2). The lack of reaction of 334LL, and 334-37 with both 334 and pili specific anterisera suggest either that;1) the pili have been a major antigen; or 2) the loss of plasmids produces major changes in the surface structure. This will be clarified when our stock O, H, and K typing sera is complete and we can clarify the role of these antigens in the original

strains and derivatives.

SECTION 4.

BUCCAL CELL ASSAY

We have continued to evaluate various assay methods to measure the ability of E. coli isolates to attach to intestinal mucosal epithelium. We have tested this in tissues of guinea pigs, rats, and rabbits without consistant positive results. However, using human buccal epithelial tissue we have been able to measure the attachment and consistently discern significant differences in binding ability between certain of the E. coli strains in our collection.

The adherence system is attractive since it uses human cells against human pathogens - the homologous system. Also, the buccal cells are easy to obtain, and have certain similarities with the gastrointestinal mucosa. The major part of this work has involved human buccal mucosa obtained from volunteers. We also plan to develop assays similar to those described below using human fetal intestinal cells maintained in tissue culture. Strain FHS 74 Int described in the Journal of the National Cancer Institute, April 1976, by Owens, Smith, Nelson-Rees, and Springer was recently received from the N.C.I.

Our first assay method is direct microscopy following indirect fluorescent antibody staining. In this method, the human buccal epithelial cells and bacteria are mixed, incubated, and excess bacteria removed by washing. The buccal cells are then placed on a slide, air dried and fixed in methanol for two min. The slide is then stained with specific rabbit antisera, washed and stained with goat anti-rabbit antiserum which has been conjugated with fluorescein isothiocyanate. Following a final washing, the slides are examined using a fluorescent microscope. This method has the inherent advantage of directly visualizing

attachment and needing only a small number of buccal cells. However, it has the disadvantage of requiring specific antisera and leaves open the possibility of changes in distribution of the various cells during both the washing and drying periods. This assay also has very finite upper and lower limits. The lower limits are defined by the small number of buccal cells examined (ca 25-50). Using this assay, binding cannot be accurately determined when on the order of 10% of buccal cells have bacteria attached. The upper limit of this assay is defined by the number of attached bacteria per buccal cell that can be counted. In practice this upper limit is on the order of 20 bacteria/buccal cell. This method is, therefore, most suitable for studying gross changes in binding capability with a small number of strains. We feel strongly encouraged that this method directly shows the attachment of E. coli pathogenic for humans to human buccal epithelial cells (Table 10). The binding of E. coli 334 to human buccal cells decreases with extended incubation times and is reduced by growth in the presence of glucose which also abolishes pili formation and the agglutination of guinea pig red blood cells in the presence of mannose at 4C, as has been discussed in previous progress reports.

We have modified this basic assay system to measure the binding of radioactively labelled E. coli to the buccal cells. The first method attempted used direct counting of the pelleted material following separation of the buccal cells with adherent bacteria and free bacteria by low speed centrifugation (Table 11). Because the relative fraction of bacteria found to the buccal cells is typically low and high levels of bacteria (ca 10^9 bacteria/ml) are used, the lack of complete separation of free and attached bacteria limits this assay to relatively high levels of binding. The major problem with this assay involves

formation of aggregates by bacteria along (see E. coli 193-4, Table 11). These aggregates are then packed together by centrifugation and tend to remain clumped throughout further washing procedures.

In order to solve these problems, we have recently begun to monitor binding using a Nucleopore filtration method (Table 12). The filters used (8 μ Nucleopore) have a large pore size combined with inherently low surface-adsorptive properties so that there is a lower background level of non-specific binding of bacteria to the filters. The filter assay should allow us to pursue kinetic studies and appears to be a potentially satisfactory system.

TABLE 1

INOCULATION OF INFANT RABBITS WITH
LOG-PHASE BHI GROWN CELLS. 6 HOUR ASSAY

STRAIN	METHOD OF INPLANT[1]	INPUT	OUTPUT[2]
334	I	2.2×10^6	5.25×10^7
	I	2.2×10^6	3.1×10^7
	S	9×10^5	1.13×10^7
	I	1.9×10^5	3×10^5 *
	S	3.1×10^5	4.3×10^5 *
334-3	I	4.2×10^5	1.8×10^7
	S	2.1×10^5	1.6×10^8
	S	2.1×10^5	2.4×10^8
	S	1.9×10^5	4×10^8 *
1111A	S	2.7×10^5	6.5×10^7
	S	1.5×10^5	8×10^6

[1] I = INTUBATION

S = SURGERY

[2] Entire small intestine was excised, homogenized, diluted and plated for total viable bacterial counts

*Only the proximal 15 cm of the small intestine were examined

TABLE 2.

INTUBATION OF INFANT RABBITS WITH OVERNIGHT EEC GROWN CELLS - 6 HOUR ASSAY

STRAIN	INPUT	OUTPUT [1]
334	6.8×10^9	2.3×10^8
	8×10^9	3.25×10^8
	8×10^9	2.75×10^8
	8×10^9	5.5×10^8
	3.6×10^8	6.1×10^8 *
	3.6×10^8	4.5×10^8 *
	3.6×10^8	5.5×10^8 *
	2.9×10^9	5.8×10^7
	2.36×10^9	1.0×10^8
	2.36×10^9	9.0×10^7
	4.6×10^9	5.8×10^8
TD427c$_2$	6.8×10^9	2.9×10^5
	2.7×10^9	2.0×10^7
	7.6×10^9	3.6×10^8
1111A	1.5×10^{10}	2.9×10^5
	3.6×10^9	4.85×10^8 *
	3.6×10^9	5.9×10^8 *
	2.6×10^9	2.4×10^8
393-4	N.C.	4.0×10^8
	2.2×10^9	2.2×10^7
334-27	N.C.	9.9×10^8
	N.C.	1.55×10^8
	1.78×10^9	1.52×10^6 *
	3.12×10^9	2.0×10^7 *

TABLE 2 (continued)

STRAIN	INPUT	OUTPUT
334-3	2.68×10^9	2.1×10^9 *
	2.68×10^9	1.2×10^8 *
	N.C.	4.0×10^8
10405	1.0×10^9	6.4×10^8
	1.0×10^9	4.6×10^7
JS F⁻ *E. coli* K-12	2.16×10^9	9.2×10^7
	3.5×10^9	3.4×10^6

1. Entire small intestine was excised, homogenized, diluted and plated onto MacConkey agar to determine total viable bacterial cells in the tissue.

* Only the proximal 15 cm. of tissue were examined.

TABLE 3.

GROWTH OF LOG PHASE EEC - GROWN CELLS IN LIGATED SMALL BOWEL OF INFANT RABBIT

STRAIN	INPUT	OUTPUT (6 hour assay)
334	1.4×10^5	2.7×10^7
	1.4×10^5	1.3×10^8
	8.7×10^4	4.7×10^7
	8.7×10^4	2.35×10^7
	2.4×10^5	1.63×10^7
1111A	1.05×10^5	2.1×10^7
	1.05×10^5	1.6×10^7
	2×10^5	1.07×10^8
	1.3×10^5	5.2×10^7
TD427c. LT-only	1.0×10^5	8×10^5
	1.0×10^5	7.5×10^5
334LL (plasmid-free)	6.7×10^5	5.3×10^7
	6.7×10^5	3.0×10^7
	2.2×10^5	8.2×10^5

TABLE 4. INTUBATION OF INFANT RABBITS WITH EEG GROWN CELLS (18 HOUR ASSAY)

STRAIN	INPUT[1]	OUTPUT[2]
334 (ST/LT)	8.0×10^6	1.1×10^5
	8.0×10^6	4.5×10^5
	2.0×10^6	$<10^3$
	3.4×10^6	3.4×10^7
	8.0×10^5	9.0×10^5
	8.0×10^5	1.1×10^5
	1.1×10^6	1.2×10^4
	1.0×10^6	0
	4.8×10^6	3.7×10^5
	4.8×10^6	5.6×10^5
334 LL (Plasmid-free derivative of 334)	2.4×10^6	2.0×10^3
	2.4×10^6	$<10^3$
	2.6×10^6	2.0×10^4
	2.6×10^6	2.5×10^3
	2.8×10^5	5.0×10^5
	2.8×10^5	1.5×10^2
	2.0×10^5	5.0×10^2
	2.0×10^5	1.2×10^4
193-4	1.5×10^6	1.2×10^4
	1.5×10^6	2.7×10^4
	1.5×10^6	2.2×10^5
	3.5×10^6	9.4×10^4
	3.5×10^6	6.0×10^2
	2.6×10^6	2.0×10^2
	2.6×10^6	4.0×10^5
	4.4×10^6	5.0×10^5
	4.4×10^6	7.9×10^4

TABLE 4 Continued (2)

STRAIN	INPUT[1]	OUTPUT[2]
H10405	1.1×10^6	5.6×10^6
	1.1×10^6	2.6×10^6
	3.2×10^6	2.5×10^5
	3.2×10^6	2.2×10^6
341	4.8×10^6	1.0×10^6
(0101:K99, ENT$^+$ (ST)	4.8×10^6	1.1×10^8
K12 (K88ab)	7.6×10^5	1.0×10^6
	7.6×10^5	2.3×10^3
	2.8×10^5	6.5×10^1
	9.2×10^5	$<10^3$
	9.2×10^5	6.0×10^2
	1.9×10^6	1.8×10^5
	1.9×10^6	4.0×10^5
	2.4×10^6	6.0×10^4
	2.4×10^6	2.8×10^4
	3.2×10^6	2.8×10^5
	3.2×10^6	2.8×10^6
K12	1.3×10^6	3.1×10^1
	1.3×10^6	8.5×10^1
	8.0×10^5	2.4×10^2
	8.0×10^5	5.2×10^2
	2.6×10^6	$<10^2$
	2.6×10^6	$<10^2$
RDEC - 1	2.4×10^6	4.0×10^5
	2.4×10^6	1.7×10^6
	2.4×10^6	2.3×10^6
	4.8×10^6	7.4×10^7
	1.9×10^6	8.0×10^7
	1.9×10^6	3.6×10^7
	1.9×10^6	6.2×10^7
	1.9×10^6	1.3×10^7
	1.9×10^6	1.7×10^7

TABLE 4 CONTINUED (3)

STRAIN	INPUT[1]	OUTPUT[2]
HS non-enteropathogenic control strain	3.3×10^6	5.1×10^6
	3.3×10^6	1.0×10^8
	3.3×10^6	1.2×10^7
	3.8×10^6	1.3×10^5
	3.8×10^6	1.8×10^5
	3.9×10^6	4.6×10^6
	3.9×10^6	1.9×10^5
	3.9×10^6	2.0×10^6
	3.9×10^6	2.2×10^5
	3.9×10^6	1.4×10^6
	3.9×10^6	3.8×10^5

[1] All animals received their challenge dose by stomach intubation under light ether anesthesia.

[2] Entire small intestine excised, homogenized, diluted, and plated in MacConkey agar for total viable bacterial counts.

TABLE 5.

STRAIN	TOXIN PROFILE	RABBIT LOOP (18 hr)[1]	NO. RABBITS	SUCKLING MOUSE[2]	NO. MICE	HA TEST[3]
1111A		-	16	-	6	-
TD427c$_2$	LT	+	16	-	3	-
408-4		-	1	NT		-
TD327c$_2$		-	4	NT		-
410G-1		-	2	-	4	-
334	LT/ST	+	4	+	2	+
10407	LT/ST	+	12	+	3	-
10407P	LT/ST	+	12	±	3	-
339t5	LT/ST	+	2	+	2	-
1105F	ST?	-	3	±	6	-
16719		-	3	-	3	-
17799		-	3	NT		-

TABLE 5 (continued)

STRAIN	TOXIN PROFILE	RABBIT LOOP (18 hr)[1]	NO. RABBITS	SUCKLING MOUSE[2]	NO. MICE	HA TEST[3]
408-3	LT/ST	+	3	+	2	-
17060		-	2	NT		-
16717		-	1	-	5	-
PM-11		-	1	-	3	-
SS-3		-	1	NT		-
SS-5		-	1	NT		-
474B-5		-	1	NT		-
434A-4		-	1	-	5	-
474B-1		-	1	NT		-
002002-1		-	3	NT		-
029001-3		-	3	NT		-
135004-5		-	3	NT		-
193-4	LT?/ST	NT		+	2	+

TABLE 5 (Continued)

STRAIN	TOXIN PROFILE	RABBIT LOOP (18 hr) [1]	NO. RABBITS	SUCKLING MOUSE [2]	NO. MICE	HA TEST [3]
117005-6		-	3	NT		--
117005-61		-	3	NT		-
029001-3		-	3	NT		-
040006-5		-	3	NT		-
006005-1		-	3	NT		-
134004-5		-	3	NT		-
166006-3		-	2	NT		-
034003-4		-	2	NT		-
122008-9		-	2	NT		-
034003-5		-	3	NT		-
047010-4		-	3	NT		-
151006-8		-	3	NT		-

TABLE 5 (continued)

STRAIN	TOXIN PROFILE	RABBIT LOOP (18 hr)[1]	NO. RABBITS	SUCKLING MOUSE[2]	NO. MICE	HA TEST[3]
151006-5		-	3	NT		-
152005-74	ST	+		-	8	-
091004-4		-		NT		-
041005-6		-		NT		-
TD213C$_2$	ST only	NT		+	6	-
TD514C$_4$	ST only?	NT		+	1	-

NT = Not tested

[1] + = ratio of ml fluid/cm loop ≥1

[2] + = ratio of weight of intestine/weight of carcass ≥.083
± = .075 - .082
- = <.074

[3] Hemagglutination of guinea pig red blood cells was carried out in phosphate buffered saline with 1% mannose at 4°C

TABLE 6.

STRAIN	SUCKLING MOUSE[1]	NO. MICE	HA TEST[2]	Y1 ASSAY[3]
12 RODRIGUEZ	-	8	-	-
CHICAGO INFANT	-	5	-	NT
FING	-	6	-	-
H10407 (BRAZIL)	+	3	-	NT
9985	-	6	-	NT
3990	-	7	-	-
1111A	-	3	-	-
8926	-	6	-	-
8828	-	4	-	NT
#29 230-3	+	5	-	NT
116002-8	-	3	-	NT
134003-2	-	3	-	NT
075005-3	-	1	-	NT
126004-6	±	2	-	NT
123005-8	±	2	-	NT
334	+	5	+	+
334-LL	-	3	-	±?
334-SL	-	4	-	±?
334-3	+	2	-	+
334-21	NT		-	+
334-27	+	1	-	+
193-4	+	5	+	-?
334 P^+15	±?	3	+	±
10405	-	3	-	-
B2C	NT		-	+
B7A	NT		-	-
JG262	NT		-	-

TABLE 6 CONTINUED

STRAIN	SUCKLING MOUSE[1]	NO. MICE	HA TEST[2]	Y1 ASSAY[3]
CONTROLS:				
VIBRIO CHOLERAE				
VC 569 B	-		-	+
E. COLI LT ONLY				
TD $427C_2$	-		-	+

[1] + = Ratio of weight of intestine/weight of carcass > .083

± = .075 - .082

- = <.074

[2] Hemagglutination of guinea pig red blood cells was carried out in phosphate buffered saline with 1% mannose at 4°C

[3] The Y1 adrenal cell assay was carried out according to the methods of D.A. Sack and R.B. Sack. Infect. Immunity 11:334-336,1975.

Table 7.

OD_{280} of specific pili preparation fractions

Eluted from guinea pig red blood cells with	fraction	temperature used (°C)	OD_{280}
PBS + 1% mannose	1	0	.492
	2	0	.598
	3	0	.222
PBS	1	37	.426
	2	37	.209
	3	45	.924
	4	55	2.177

TABLE 8.

OD_{280} OF PBS FRACTIONS DESCRIBED IN TABLE 6
AFTER SUCCESSIVE ULTRACENTRIFUGATION STEPS

	PBS PREPARATION			
ULTRACENTRIFUGE FRACTION	1	2	3	4
2 HR PRECIPITATE	.245	.053	.320	2.550
4 HR PRECIPITATE	.045	.032	.066	.101
6 HR PRECIPITATE	.089	.037	.264	.516
6 HR SUPERNATANT	.314	.585	.646	.946

TABLE 9. TITERS OF RABBIT ANTISERA, AGAINST VARIOUS E. COLI STRAINS

E. coli strain	RABBIT ANTISERAS 334	partly purified pili[1]	334-334LL[2]	193-4
334	2560	2560	2560	320
334+ glucose[3](A)[4]	1260			640
(B)	320	320	160	
193-4	320	80		160
193-4+ glucose[3]	40	10		80
334LL	-	-	-	
334-3	-	-	-	
334-27	-	-	-	
334 P^+15	2560	160	1280	720
B2c	-	-	-	-
B7A	-	-	-	-
TD 427C2	-	-	-	
TD 213C2	-	-	-	
H10407	-	-	-	
H10407P	80	80	40	
H10405	-	-	-	
H5	-	-	-	
RDEC-1	-	-	-	

[1] pili purified by specific adsorption to guinea pig red blood cells in the presence of mannose

[2] antiserum against 334 adsorbed twice with 334LL

[3] grown in the presence of glucose

[4] grown different days

TABLE 10

FLUORESCENT MICROSCOPY ASSAY OF BINDING OF E COLI TO HUMAN BUCCAL CELLS

ORGANISM	GROWTH TIME (HR)	GROWTH MEDIUM	AVE. NO. OF BACTERIA/BUCCAL SLIDE A	SLIDE B
334	24	Peptone agar	4.44	3.00
334	72	Peptone agar	.267	.235
334	24	Peptone/glucose agar	.562	2.714
1111A	24	Peptone agar	.333	.666

Bacterial cells were grown at 37°C on slants of peptone agar, supplemented with 0.5 ml of 20% glucose when indicated. Bacteria adherent to buccal cells following washing were visualized using indirect fluorescent antibody methods. The averages were determined after observation of 25 randomly chosen buccal cells.

TABLE 11

BINDING OF RADIOACTIVELY LABELLED E COLI TO HUMAN BUCCAL CELLS. WASHING BY DIFFERENTIAL CENTRIFUGATION.

EXPERIMENT	ORGANISM	TOTAL CPM USED	AFTER WASHING +MUCOSAL CELLS	AFTER WASHING -MUCOSAL CELLS	CPM BOUND	# BACTERIA BUCCAL CELL
I	334	547,176.5	11,005.0	5,067.3	5,937.7	187.5
	334LL	383,232	269.1	168.8	100.3	4.2
	HS	410,982	683.3	462.3	176.0	6.4
	RDEC-1	429,925	154.3	330.9	0	0
II	334	530,670	14,704.8	5,101.4	9,603.4	544.1
	334LL	469,292	68.0	78.7	0	0
	HS	532,900	352.6	207.1	145.5	7.7
	RDEC-1	424,037	708.5	533.0	175.5	9.5
III	193-4	752,405	191,744.0	56,573.1	135,370.9	1808.0
	H10407	200,860.5	716.5	379.3	337.2	17.1
	H10407P	532,254	69.7	48.8	20.9	0
	H10405	576,517	158.3	123.9	34.4	0

Human buccal cells were collected by scraping and washing five times. Bacteria were labeled by growth in the presence of ^{3}H-labeled amino acids. The mixture was incubated for 15 minutes at 37°C and buccal cells and attached bacteria were removed by low speed centrifugation. After five washes, the pelleted material was counted in a liquid scintillation counter using Aquasol-2.

TABLE 12

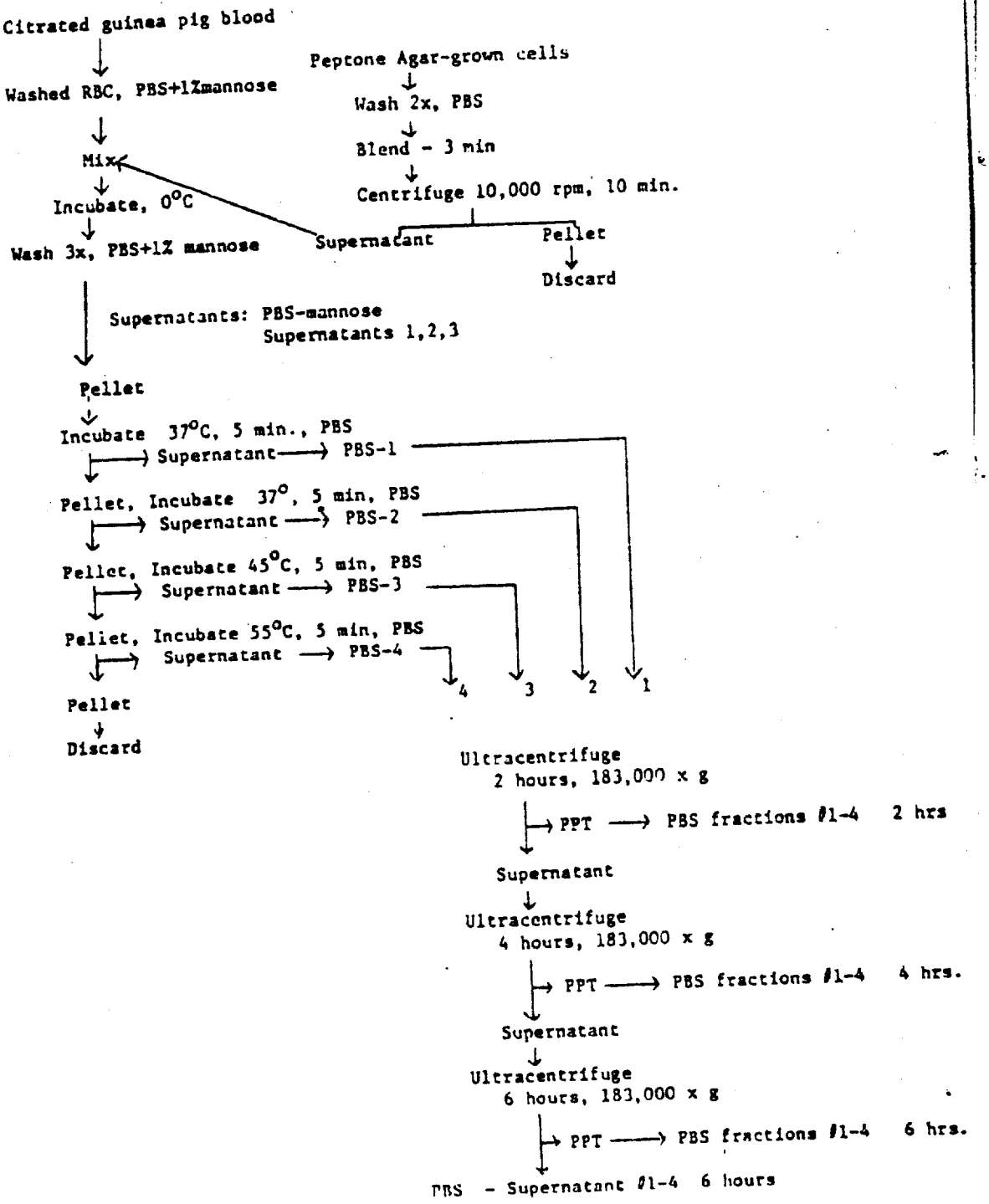
BINDING OF RADIOACTIVELY-LABELED E. COLI
TO HUMAN BUCCAL CELLS USING FILTRATION ASSAY

EXPERIMENT	ORGANISM	CPM BACTERIA BOUND	# BACTERIA / BUCCAL CELL
I	334	8205.5	331.7
	193.4	24496.3	813.5
	HS	1840.2	102.6
	H10405	997.2	52.4
II	334	3766.4	56.2
	334LL	0	0
	193-4	1769.8	35.6
	Mex.	1298.5	19.9
	HS	248.4	4.3
	B2C	179.1	3.5
	B7A	0	0
	P+15	0	0
	H10405	0	0
	H10407	256.6	5.5
	H10407P	145.8	2.7
	Dec 1	0	0

Bacteria were grown on peptone agar slants supplemented with 25 uCi each of H^3 alanine and H^3 leucine. Following incubation at 37°C for 15 minutes in phosphate buffered saline (pH 7.2) with human buccal cells, samples of 0.5 ml were filtered and washed twice with 5 ml of phosphate buffered saline. CPM bacteria bound was calculated from the difference between the CPM on the filter with and without mucosal cells.

Figure 1.

Purification of Specific Pili from E. coli 334



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