Virulence of *Escherichia coli* Strains for Chick Embryos¹

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ABSTRACT

POWELL, CALVIN J., JR. (Walter Reed Army Institute of Research, Washington, D.C.), AND RICHARD A. FINKELSTEIN. Virulence of *Escherichia coli* strains for chick embryos. *J. Bacteriol.* 91:1410-1417. 1966.—Fifty-three strains of *Escherichia coli*, freshly isolated from patients at Children's Hospital, Washington, D.C., were tested for virulence for 13-day chick embryos by allantoic inoculation of serial dilutions of viable cell suspensions. No clear-cut relationship could be demonstrated between inoculum size and response (death of the embryos) which would permit comparisons of virulence based on LD₅₀ determinations. However, the strains were classified into three groups according to the proportion of embryos which succumbed, regardless of inoculum size. There was no association between enteropathogenicity and embryo virulence, although there was high correlation between hemagglutinative activity for chicken erythrocytes and virulence of strains for embryos. Differences in virulence were not related to ability to multiply in the allantoic cavity nor did bacteremia appear to be essential for death of the embryos. Serial passage in eggs failed to alter virulence. Both the route of inoculation and the age of the embryos had a marked effect on the outcome of challenge with strains of varying virulence. The embryos were most susceptible to challenge by the intravenous route. The susceptibility of younger embryos decreased with increasing embryonic maturity, although some reversal of this trend was noted with 19-day embryos. The toxicity of culture filtrates and filtrates of allantoic fluid from infected embryos seemed to be related to strain virulence, suggesting that elaboration and availability of toxic factors may have an important bearing on the lethality of *E. coli* strains for chick embryos.

Some strains of *Escherichia coli* are known to cause disease in man (1, 15). Strains associated with infant diarrhea have been shown (3) to belong to a small number of serological types. However, other laboratory methods have not been successful in demonstrating differences between enteropathogenic and nonenteropathogenic serotypes (11, 13, 14, 17).

In screening some *E. coli* strains for virulence for the embryonated egg, Finkelstein and Ransom (8) found some strains to be highly virulent,

some virtually completely avirulent, and some with the ability to kill a percentage of the eggs when inoculated by the allantoic route. However, this model likewise did not appear to differentiate the enteropathogenic serotypes, but suggested that virulence for the chick embryo was, as noted with other experimental animals, a characteristic of the strain used rather than of the serotype in which it was classified. Turgeon et al. (17), however, found that when inoculated on the chorio-allantoic membrane (CAM), small inocula would kill chick embryos regardless of the strain of *E. coli* used.

There has been little other mention, in the literature, concerning the virulence of *E. coli* for the chick embryo. The present study was initiated to extend the observations of *E. coli* virulence in the chick embryo and to evaluate: (i) the relationship, if any, between embryo virulence and enteropathogenic serotypes, (ii) the effect of route

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of inoculation and age of embryos on the "virulence" of the strains, and (iii) the effect of variation among strains on their virulence for the chick embryo, and (iv) to obtain some observations which might relate to the pathogenic mechanisms of *E. coli* strains for chick embryos.

MATERIALS AND METHODS

Bacterial cultures. *E. coli* strains were isolated from normal and diarrheal stool specimens obtained from children at Children's Hospital, Washington, D.C. The cultures were maintained in the lyophilized state, and for everyday use, on meat extract-agar (MEA) or Trypticase Soy Agar (TSA) slants and plates. Methods outlined by Ewing and Edwards (4) were used for the isolation and identification of *E. coli* strains. Strains which were autoagglutinable in saline were regarded as rough.

Suspensions for inoculation into embryonated eggs were prepared by harvesting the overnight growth from petri dishes, heavily streaked with the cultures to be tested, with 10.0 ml of normal physiological saline. Serial dilutions in saline, 10-fold or 100-fold, or both, were made of the challenge suspensions for inoculation into the embryonated eggs. Viable counts were estimated by the drop-plate technique (12).

Filtrates and killed *E. coli* cells. Filtrates were prepared from 18-hr shaken broth cultures (Brain Heart Infusion), centrifuged, and passed through Millipore GS filters. Heat-killed cells were prepared by heating suspensions of harvested cells at 60 C for 30 min or boiling for 15 min. Sterility of filtrates and heat-killed cells was confirmed by inoculation of appropriate media.

Chick embryos. *Salmonella pullorum*-free, white Leghorn chick embryos from a single antibiotic-free flock were used. The eggs were incubated at 37 C in an electrically controlled incubator. The humidity of the incubator was maintained by open pans of water. The eggs were arranged with the air-sac end tilted slightly upward. The embryos were inoculated on the 13th day of incubation unless otherwise specified.

Inoculation procedures. Allantoic inoculations were performed by introducing 0.1 ml of appropriate dilutions of the challenge suspensions through a small hole, punched in an area free from large blood vessels, by means of a 25-gauge 0.25-inch (0.6 cm) hypodermic needle and a 1.0-ml tuberculin syringe. Unless otherwise indicated, 10 eggs were inoculated with each dilution. Death of embryos was determined by candling. To insure purity of inoculated strains, allantoic fluid of representative eggs was streaked on appropriate media. Intravenous inoculations were made by identifying a vein in the chorioallantois by candling. The shell immediately over the vein was removed after cutting three sides of the shell area around the vein. The shell was cut with a Moto-tool drill, furnished with a double cutting blade (model 1, Drexel Mfg. Co., Racine, Wis.). An inoculum of 0.1 ml was injected with a 27-gauge needle and 1.0-ml tuberculin syringe, in the direction of blood flow. Eggs that were observed to bleed internally immediately after inoculation were discarded. Moderate external bleeding

did not appear to have any adverse effects. No deaths resulted among control eggs inoculated with saline, in repeated tests.

For CAM inoculations, the CAM was dropped by punching one small hole in the shell over the chorioallantois and another at the air-sac end, and applying gentle suction from the air-sac end of the egg. The inoculum (0.1 ml) was then injected onto the dropped membrane through the hole punched in the shell.

Blood samples. Blood, free from allantoic fluid, was obtained from embryos after removing the shell over the air-sac end. A suitable blood vessel was selected from the exposed membrane and carefully nicked with the point of a scalpel blade while candling. The flowing blood was collected with a capillary pipette.

Hemagglutination test. The test was performed as described previously (6). A drop of physiological saline was applied with a 3-mm (diameter) bacteriological loop. Sufficient bacterial growth from agar cultures was emulsified in the drop to make a heavy milky suspension. A loopful of washed 3% chicken red blood cells in normal saline was then added, and the slide was tilted back and forth to mix the cells and bacterial suspension. If the test was positive, clumping of the erythrocytes was usually detectable almost immediately by the naked eye with transmitted illumination.

RESULTS

Characterization of the strain. A total of 52 strains was used in the investigation. Of the 52 strains, 11 belonged to serotypes which are regarded as enteropathogenic, and all of these were associated with diarrheal symptomatology. Twenty-four strains could not be typed with the antisera available. Of these, 21 were isolated from cases of frank infant diarrhea. Eight strains were regarded as rough.

Each of the strains was tested for its ability to kill chick embryos after allantoic inoculation of serial 10- or 100-fold dilutions of viable cell suspensions. From the killing patterns which emerged, it was not possible to derive clear-cut LD₅₀ values for each of the strains which could be used as a basis for comparison of virulence, since there was no clear relationship between dose and response. These findings were in contrast to the results of Lankford (10) and Gardner et al. (9), who in titrating *Vibrio cholerae* in the chick embryo were able to calculate LD₅₀ values for each of the strains tested because of a clear-cut dose-response relationship. Rather, it appeared that the strains were capable of killing a proportion of the inoculated eggs, regardless of the size of the dose inoculated.

From the results, the strains were classified as "virulent" (V) if 70 to 100% of the inoculated eggs succumbed within 3 days, as "partially virulent" (PV) if 20 to 70% succumbed, and as "avirulent" (AV) if less than one-third of the inoculated eggs succumbed during that interval.

It should be noted that upon repeated tests virulent strains always killed a definite high percentage of the inoculated eggs, and those regarded as avirulent never killed more than one-third of the inoculated eggs; however, strains classified as partially virulent would present a killing pattern fluctuating between 20 and 70%, depending upon when the tests were performed. Typical results with these strains are presented in Table 1. Thus, strain 6 is representative of the V group, strain 6P of the PV group, and strain 8 was regarded as typical of the AV group.

Of the 52 strains tested, 54% were regarded as AV, 40% were PV, and 6% were considered V (Table 2). Of the strains which belonged to enteropathogenic serotypes, one was virulent, three were in the PV class, and seven were essentially avirulent for chick embryos. Of the non-enteropathogenic serotypes, one was V, six were PV, and two were AV. Similar variations in virulence were observed among rough strains and those strains which could not be typed with the antisera available. Thus, there was no clear relationship between embryo virulence and enteropathogenicity.

Relationship between growth in ovo and virulence. It was also considered that the differences in ability to kill chick embryos could be a reflection of the ability of strains to multiply in the allantoic cavity. Accordingly, growth curve studies were performed with strains representative of the three virulence categories. The growth

curves (Fig. 1) indicate that the organisms multiply at essentially the same rate and reach similar maximal populations regardless of their ability to kill chick embryos.

Attempts were made to alter the partial virulence of strains 6P and 13 by serial passage in embryonated eggs. Single-colony isolates were made from the allantoic fluid of eggs which succumbed early and from eggs which survived longer than 72 hr. In addition, substrains were

TABLE 2. Comparison of serological classification with chick embryo virulence of 52 *Escherichia coli* strains

Serological classification	Virulence for chick embryos*			Total
	Virulent	Partially virulent	Avirulent	
Enteropathogenic†.....	1	3	7	11
Nonenteropathogenic‡	1	6	7	9
Not typed.....	0	9	15	24
Rough.....	1	3	4	8
Total.....	3	21	28	52

* Thirteen-day chick embryos inoculated allantoically.

† Enteropathogenic strains were distributed among the O antigen serotypes O25:B19, O26:B6, O55:B5, O111:B4, O119:B14, O125:B15, and O128:B12.

‡ Nonenteropathogenic strains were distributed among the O antigen serotypes O73, O75, O102, and O114.

TABLE 1. Virulence titration of representative strains of *Escherichia coli* in chick embryos

Strain	Dose (organisms)	No. dead/no. inoculated			
		24 hr*	48 hr	72 hr	96 hr
No. 6 (virulent)	10 ⁹	10/10	—	—	—
	10 ⁷	8/10	10/10	—	—
	10 ⁶	7/10	10/10	—	—
	10 ³	9/10	10/10	—	—
	10 ¹	8/10	10/10	—	—
No. 6p (partially virulent)	10 ⁹	1/10	4/10	5/10	7/10
	10 ⁷	0/10	2/10	2/10	3/10
	10 ⁶	0/10	0/10	2/10	2/10
	10 ³	2/10	6/10	6/10	7/10
	10 ¹	0/10	3/10	4/10	4/10
No. 8 (avirulent)	10 ⁹	0/10	0/10	0/10	0/10
	10 ⁷	0/10	0/10	0/10	2/10
	10 ⁶	0/10	0/10	0/10	1/10
	10 ³	0/10	0/10	0/10	0/10
	10 ¹	0/10	0/10	0/10	2/10

* Hours after allantoic inoculation of 13-day embryos.

FIG. 1. Comparison of growth rate of virulent, partially virulent, and avirulent strains of *Escherichia coli* after allantoic inoculation in 13-day-old embryos (mean of the log determined on two eggs).

established from positive early blood cultures of inoculated eggs. Three consecutive passages, in the same manner for each of the substrains of the two PV strains, failed to demonstrate any consistent alteration of the virulence characteristic (Fig. 2). There was, however, a suggestion of some reduction of virulence of strain 6P by selection of isolates from surviving embryos.

On the other hand, there was a highly suggestive correlation between virulence of the strains for the chick embryo and their ability to cause hemagglutination of chicken erythrocytes in slide agglutination tests (Table 3). Unfortunately, only three strains in the highly virulent category were available for this comparison.

Effect of route of inoculation on virulence. Thirteen-day-old chick embryos were inoculated, in parallel, allantoically, intravenously, and on the CAM with a dilution of a V, PV, and AV strain, to determine the effect of the various

routes on virulence (Table 4). By the intravenous route, all strains were uniformly lethal in 7 hr. The incidence of death after CAM inoculation was also extremely high for all strains, although deaths were not as rapid or uniform as when the intravenous route was employed. By the allantoic route, as was noted previously, there was a marked difference in the killing pattern of the three strains. Both the intravenous and CAM routes of inoculation tended to mask the differences in virulence which were demonstrated by the allantoic route. These results re-emphasize the importance of the route of inoculation used on studies of bacterial virulence for chick embryos.

After CAM inoculation with 10^8 viable cells of an embryo-virulent strain, the organisms could easily be recovered from allantoic fluid samples collected at 18-, 24-, and 48-hr intervals. This demonstrates that strains inoculated on the

FIG. 2. Effect of serial egg passage on virulence. Isolates from *Escherichia coli* strains 6P and 13 were obtained from embryos which succumbed early, those which survived longer than 72 hr, and from positive early blood cultures of inoculated 13-day-old embryos.

CAM possess the ability to invade the membrane and multiply allantoically.

Effect of age of chick embryos on susceptibility to V, PV, and AV strains. Chick embryos 8, 13,

16, and 19 days of age were inoculated with approximately 10^9 , 10^7 , and 10^5 cells of V, PV, and AV strains by the allantoic route. The results (Fig. 3) indicated that the age of the embryo had a marked effect on the susceptibility to challenge with the three representative strains. The younger embryos were extremely susceptible, whereas the embryos appeared to be most resistant at 16 days. This resistance was noticeably reduced in the later stages of embryo development. The relative position, with regard to virulence of the three strains, was, however, maintained in embryos of all ages tested, but the greatest differences in virulence were manifested in 13-day embryos.

Bacteremia after allantoic inoculation. Attempts were made to determine whether bacteremia was

TABLE 3. *Comparison between virulence of Escherichia coli strains for 13-day chick embryos and hemagglutinin activity**

Virulence category	Hemagglutinin activity (no. positive/no. tested)	Per cent positive
Virulent.....	3/3	100.0
Partially virulent.....	6/19	31.6
Avirulent.....	2/26	7.6

* Slide Test with 3% chicken red blood cells.

TABLE 4. *Effect of route of inoculation on virulence for 13-day chick embryos*

Route of inoculation	Strain*	Dose (organisms)	Mortality (no. dead/no. inoculated)				
			7†	17 hr	23 hr	52 hr	75 hr
Allantoic	6	10^9	3/10	5/10	7/10	7/10	10/10
		10^7	1/10	4/10	6/10	9/10	9/10
		10^5	0/10	5/10	5/10	8/10	9/10
	6P	10^9	0/10	0/10	0/10	3/10	5/10
		10^7	0/10	0/10	1/10	3/10	4/10
		10^5	0/10	0/10	0/10	4/10	4/10
	8a	10^9	0/10	0/10	3/9	3/9	3/9
		10^7	0/10	0/10	0/10	2/10	2/10
		10^5	0/10	0/10	1/10	2/10	2/10
CAM	6	10^9	0/10	10/10	—	—	—
		10^7	0/10	6/10	8/10	10/10	—
		10^5	0/10	3/10	5/10	10/10	—
	6P	10^9	1/10	10/10	—	—	—
		10^7	0/10	9/10	9/10	10/10	—
		10^5	0/10	2/10	6/10	10/10	—
	8a	10^9	0/7	6/7	6/7	6/7	—
		10^7	0/10	9/10	9/10	9/10	9/10
		10^5	0/10	2/10	5/10	6/10	6/10
Intravenous	6	10^9	6/6	—	—	—	—
		10^7	7/7	—	—	—	—
		10^5	5/6	6/6	—	—	—
	6P	10^9	6/6	—	—	—	—
		10^7	6/6	—	—	—	—
		10^5	5/5	6/6	—	—	—
	8a	10^9	6/6	—	—	—	—
		10^7	6/6	—	—	—	—
		10^5	6/6	—	—	—	—

* Strain 6, virulent; strain 6P, partially virulent; strain 8, avirulent.

† Hours after inoculation.

FIG. 3. Effect of age of chick embryos on susceptibility to virulent, partially virulent, and avirulent strains. Embryos were inoculated allantoically with approximately 10^7 cells of the indicated *Escherichia coli* strains. V = virulent; PV = partially virulent; AV = avirulent.

a prerequisite of death with *E. coli* infection (Table 5). Thirteen-day embryos were inoculated, allantoically, with representative strains and were killed and bled at intervals. Groups of embryos inoculated in parallel served as virulence controls.

In the first experiment, with the use of a massive inoculum, only 2 of 13 eggs inoculated with the virulent strain 6 gave evidence of bacteremia by 6 hr after inoculation, although 8 of 10 embryos in the control group had already succumbed. No bacteremia was demonstrated after inoculation of the PV and AV strains. In a second experiment, with the use of a moderate inoculum of strain 6, only 3 of 10 eggs exhibited bacteremia at 8 hr, although 8 of 10 control eggs were dead 4 hr later. These results do not support the hypothesis that bacteremia is a prerequisite for death in the chick embryo. If this were so, one would expect a higher proportion of embryos to present positive blood cultures several hours before death.

In additional experiments, embryos were inoculated via the allantoic cavity with approximately 10^9 viable cells from both virulent and avirulent strains. The embryo was removed, dipped in alcohol, flamed, and ground in a tissue grinder with normal saline to establish a 10% suspension. Plate counts made of the embryonic suspension from eggs killed at 5, 7, and 9 hr yielded counts of only approximately 10^2 cells per milliliter. The small number of organisms obtained from the embryo carcasses likewise indicates that bacteremia is probably not prerequisite for the greater percentage of embryo deaths. The low counts obtained could probably be attributed to contamination with extra-em-

TABLE 5. Bacteremia following allantoic inoculation of 13-day embryos

Expt	Strain	Hr observed	No. positive/ no. bled	Virulence control group
1*	6	3	1/10	0/10†
		6	1/3	8/10
		24		10/10
	6P	24	0/5	3/10
		48		5/10
	8	24	0/5	3/10
		48		3/10
2‡	6	4	1/10	0/10
		6	0/10	0/10
		8	3/10	0/10
		9		2/10
		12		8/10
		24		9/10
		48		10/10

* Inoculum 0.1 ml undiluted.

† Number dead/number inoculated.

‡ Inoculum 0.1 ml of a 10^{-4} dilution.

bryonic fluids while removing the embryos. In the control group, 75% of the eggs were dead at 9 hr.

Toxicity of heat-killed cells and filtrates for chick embryos. Since there was no apparent relationship between *E. coli* bacteremia and death, attention was turned to an examination of the toxicity of the whole cells and of their products for chick embryos. Eleven-day embryos have been reported to be more suitable for examination of toxicity of some products (5). Thus, some of the experiments were repeated in 11-day embryos.

A comparison of the toxicity on intravenous inoculation of heat-killed cell suspensions of representative V, PV, and AV strains (Table 6) failed to reveal differences which could account for the marked variation in virulence of the strains for chick embryos.

Chick embryos were also inoculated by the intravenous route with 0.1 ml of dilutions of 18-hr broth culture (Millipore) filtrates. In 13-day embryos, the technique revealed little or no difference between the V and PV strains tested. However, a difference greater than twofold was observed in the toxicity of filtrates from the AV culture as compared with the V culture. In the 11-day embryos, the same filtrates were much more active, and the differences in toxicity were in the same direction as the differences in virulence of the strains. The filtrate of the virulent culture was approximately 3 times as toxic as that from the partially virulent strain and 20

TABLE 6. Toxicity of heat-killed cells and filtrates for chick embryos*

Prepn	Age of embryo	Strain		
		Virulent	Partially virulent	Avirulent
	<i>days</i>			
Washed heat-killed cells	13	0.036†	0.04	0.045
Washed heat-killed cells	11	0.0029	0.0035	0.0018
Broth filtrate, 18-hr.	13	0.067	0.046	>0.1
Broth filtrate, 18-hr.	11	0.00038	0.001	0.0075
Allantoic fluid filtrate (8 hr)	13	0.01	0.078	>0.1
Allantoic fluid filtrate (24 hr)	13	—	0.01	0.025

* Inoculated intravenously with 0.1 ml of dilutions of cell suspensions or filtrates.

† The LD₅₀ values in milliliters.

times more active than the filtrates from the avirulent strains.

Comparisons were also made of the toxicity of filtrates of allantoic fluids of eggs infected intravenously with V, PV, and AV strains (Table 6). Fluids were collected from representative infected embryos at 8 hr and, in the case of PV- and AV-infected embryos, again at 24 hr, centrifuged, and passed through Millipore filters. Since the eggs infected with the V strain had already succumbed at 24 hr, filtrates from this group were not included. As was noted with broth culture filtrates, the toxicity of the allantoic fluid filtrates was directly related to the virulence of the infecting strains. The LD₅₀ values of the 8-hr allantoic fluid filtrates of strains infected with V, PV, and AV strains were, respectively, 0.01, 0.078, and >0.1 ml. Later filtrates of PV and AV strains exhibited a similar relationship.

However, when culture filtrates and filtrates of allantoic fluids of infected eggs were inoculated allantoically, large doses failed to kill 13-day-old chick embryos.

DISCUSSION

This report has shown that differences in virulence of *E. coli* strains may be demonstrated in the embryonated egg by use of the allantoic route of inoculation. Although the proportion of embryos succumbing did not bear a direct relationship to the size of the dose inoculated, the ability to kill certain percentages of the inoculated eggs showed a reproducible pattern for individual strains. Thus, grouping of strains into categories of virulent, partially virulent, and avirulent according to the percentage of embryos killed seems feasible.

Differences in virulence of strains were best demonstrated in 13-day-old embryos, although differences could also be shown in 8-, 16-, and 19-day-old embryos. The finding of increased susceptibility of 19-day-old embryos was an un-

expected one since, as other investigators have reported, resistance to infection tends to become greater as the chick embryo nears full development. Smith and Thomas (16) reported that the 10-day-old embryo is maximally susceptible to intravenous inoculation of endotoxin of several species, and that resistance increased with embryonic development. Finkelstein and Ramm (7), using allantoic inoculation of *V. cholerae*, reported a similar relationship of age to resistance. They associated the embryo resistance to *V. cholerae*, in the later development of the egg, to a shift in kidney function from the mesonephros (intermediate kidney) to the metanephros. Embryonic resistance to *V. cholerae* develops about the 15th day, which parallels the time of cessation of mesonephric function.

Comparison of growth rates of representative strains of the different virulence groups has shown that the differences in virulence are not the consequences of differences in bacterial growth. Cultures from embryos that survived longer than 72 hr revealed equally high counts in eggs infected with virulent, partially virulent, and avirulent strains. Thus, the possibility that fluids of 13-day-old embryos have some inhibitory activity for *E. coli* strains of lower virulence could be excluded.

Attempts to associate death with bacteremia in allantoically inoculated 13-day-old embryos were unsuccessful. However, when strains of *E. coli* were inoculated intravenously, at small inoculum was 100% lethal within a 7-hr period, regardless of the strain used. Infections initiated via the CAM were also uniformly lethal, although not as rapidly as when the inoculum was administered intravenously. The ability of small inocula of *E. coli* strains to kill both 13- and 16-day-old embryos when inoculated by these routes suggests that other pathogenic mechanisms may be involved in the mortality of embryos with systemic infection.

The observation that the toxicities of heat-killed cells of V, PV, and AV strains were not markedly different suggested that the reason for the difference in virulence of the strains must lie elsewhere, and it was found that the toxicities of culture filtrates and filtrates of allantoic fluids from infected eggs were directly related to the virulence of the strain for the chick embryos. This suggests that the elaboration and availability of toxic factors may have an important bearing on the lethality of *E. coli* strains for chick embryos. It remains to be explained why these toxic factors administered by allantoic inoculation failed to kill the embryos.

The highly suggestive correlation between virulence for chick embryos and hemagglutination activity in vitro merits additional consideration and investigation. It has not yet been determined whether hemagglutinins are formed by *E. coli* in the egg. The observation that only a portion of the embryos succumb to the allantoic infection initiated by the partially virulent strains suggests that chick embryos are not entirely homogenous with regard to their susceptibility. Whether genetic or other factors determine the susceptibility of individual embryos to infection with PV strains remains for further study.

This study emphasizes the potential of the chick embryo as a model for studying infection and virulence mechanisms.

LITERATURE CITED

- BURROWS, W. 1955. Textbook of microbiology, 16th ed., p. 372. W. B. Saunders Co., Philadelphia.
- DUGUID, J. P., I. W. SMITH, G. DEMPSTER, AND P. N. EDMUNDS. 1955. Non-flagellar filamentous appendages (fimbriae) and haemagglutinating activity in *Bacterium coli*. J. Pathol. Bacteriol. **70**:335-348.
- EWING, W. H. 1962. Sources of *Escherichia coli* cultures that belong to O antigen groups associated with infantile diarrheal disease. J. Infect. Diseases **110**:114-120.
- EWING, W. H., AND P. R. EDWARDS. 1957. Isolation and preliminary identification of *Escherichia coli* serotypes associated with diarrheal disease. Communicable Disease Center Lab. Manual Public Health Lab. **12**:75-81.
- FINKELSTEIN, R. A. 1964. Observations on mode of action of endotoxin in chick embryos. Proc. Soc. Exptl. Biol. Med. **115**:702.
- FINKELSTEIN, R. A., AND S. MUKERJEE. 1963. Hemagglutination: a rapid method for differentiating *Vibrio cholerae* and El Tor vibrios. Proc. Soc. Exptl. Biol. Med. **112**:355-359.
- FINKELSTEIN, R. A., AND G. M. RAMM. 1962. Effect of age on susceptibility to experimental cholera in embryonated eggs. J. Infect. Diseases **111**:239-249.
- FINKELSTEIN, R. A., AND J. P. RANSOM. 1960. Non-specific resistance to experimental cholera in embryonated eggs. J. Exptl. Med. **112**:315-328.
- GARDNER, E. W., S. T. LYLES, C. E. LANKFORD, AND S. J. HAGENS. 1963. *Vibrio cholerae* infection in the embryonated egg. J. Infect. Diseases **112**:264-272.
- LANKFORD, C. E. 1960. Factors of virulence of *Vibrio cholerae*. Ann. N.Y. Acad. Sci. **88**:1203-1212.
- LINDBERG, R. B., AND V. M. YOUNG. 1956. Observations on enteropathogenic *Escherichia coli*. Ann. N.Y. Acad. Sci. **66**:100-107.
- MILES, A. A., S. S. MISRA, AND J. O. IRWIN. 1938. Estimation of bactericidal power of blood. J. Hyg. **38**:732.
- MOLL, T., AND C. K. INGALSBE. 1955. The pathogenicity of certain strains of *Escherichia coli* for young mice and calves. Am. J. Vet. Res. **16**:337-341.
- NAMIOKA, S., M. URUSHIDO, AND R. SAKAZAKI. 1958. *Escherichia coli* isolated from transmissible gastroenteritis in pigs. Japan. J. Med. Sci. Biol. **11**:141-151.
- PARR, L. W. 1939. Coliform bacteria. Bacteriol. Rev. **3**:1-48.
- SMITH, R., AND L. THOMAS. 1956. The lethal effect of endotoxin on the chick embryo. J. Exptl. Med. **104**:217-231.
- TURGEON, F., A. F. BORDUAS, AND A. FRAPPIER. 1960. Pathogenicite pour l'embryon de poulet et la souris, de quelques souches d'*Escherichia coli* entero-pathogenes. Rev. Can. Biol. **19**:417-424.