LOCAL IMMUNOPROPHYLAXIS IN ENTERIC COLIBACILLOSIS

By

LANE DOYLE CORLEY

Bachelor of Science
East Central Oklahoma State University
Ada, Oklahoma
1968

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1971

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of the Oklahoma State University
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Thesis Approved:

Richard E. Corstvet
Thesis Adviser

L. Wynn Gay

Helen Jordan

Marion J. Frey

Richard M. Hyde

N. M. Durham
Dean of the Graduate College

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INTRODUCTION

Diarrheal diseases are a major problem of infants and children (8). Data on disease prevalence indicates that 15 million annual deaths in children are attributed to the combination of malnutrition and intestinal infection (36). In domestic animals, especially ungulates, neonatal diarrhea is also a major problem (45). Investigations reveal that diseases characterized by diarrhea may result from both infectious and noninfectious processes (29). Although the order of importance may vary among investigators, enteropathogenic Escherichia coli, Salmonella spp., and Shigella spp. are bacterial pathogens commonly recovered from stools of patients with diarrhea (29,20). Crambott, et al. (20) found recovery rate of E. coli to be consistently greater than Salmonella and Shigella species. It has long been known that E. coli is a major contributor to diarrheal disease in neonatal animals including man (71,11). Of the numerous E. coli serotypes known only a few appear to be pathogenic to man and animals, and in general the pathogenic serotypes appear species specific (72). Bacteriologic examination of dead pigs in the United Kingdom revealed that 36% of deaths in young pigs could be attributed to E. coli and that the same 6-8 serotypes were found to be associated with neonatal diarrhea (71,72).

Since comprehensive reviews of Escherichia coli infections have been published recently (70,71,11,49,63) this communication will be

limited to a brief overview of neonatal colibacillary diarrhea.

The classification of enteric colibacillosis as indicated by Nielsen et al. (49) will be used, namely 1) neonatal colibacillary diarrhea (diarrhea in pigs less than a week old), 2) weaning colibacillary diarrhea (diarrhea associated with weaning), and 3) edema disease usually associated with weaning. The present paper will deal only with aspects of neonatal colibacillary diarrhea, which is characterized by diarrhea during the first few days of life. Although E. coli has been incriminated in 10-36% of the deaths of neonates (72,7) morbidity and mortality varies greatly within individual litters and farms (11). Diarrhea is evident in affected litters within hours or at most within a few days after birth. Affected pigs may be found dead, may become progressively dehydrated until death, or recover (11,70).

Pathogenesis of E. coli infections is the subject of recent reviews (71,72,49,63). Available evidence suggests that prerequisites for enteric colibacillosis, while not limited to, must include the following: 1) the E. coli strain must colonize and proliferate in the upper small intestine, 2) the E. coli strain must be able to produce an extracellular enterotoxin, and 3) the host must be susceptible to E. coli enterotoxin (72,49). It has been observed that many E. coli recovered from pigs with colibacillosis contain K88 antigen (70,30). Furthermore, K88 positive E. coli were found to adhere to and colonize intestinal mucosa while K88 negative bacteria did not (38). Truszczynski and Ciosek (80) found that K antigens were neither toxigenic in ligated intestinal loops nor toxic for white mice. Enterotoxigenic capabilities were strain dependent and not related to K type.

Smith and Halls (67) found complete correlation between enterotoxi-

genic activity of cell-free filtrates and live E. coli in ligated intestinal loops.

Although efforts to purify E. coli enterotoxins have been unsuccessful, at least two E. coli enterotoxins have been identified on the basis of heat stability. Heat labile enterotoxin which loses activity after 15 minutes at 60°C has a molecular weight (MW) of 50,000, and appears to be antigenically related to enterotoxin produced by Vibrio cholerae (33,32,10). Heat stable enterotoxin resists heating for 30 minutes at 100°C and has a MW of 1,000 - 10,000 (67,10,15). Despite these apparent differences heat-labile and heat-stable enterotoxins have at least two properties in common: (1) Ability to produce heat-labile and heat-stable enterotoxin may be transmissible by plasmids (31,68), (2) When exposed to mucosa of the small intestine both types of toxin cause excessive secretion of fluid and electrolyte which is clinically manifested as diarrhea (10). The precise mechanism by which enterotoxins elicit diarrhea remains unclear. Current knowledge indicates that enterotoxins stimulate adenylate cyclase to catalyze the formation of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (28). Intracellular accumulation of cAMP causes a net secretion of fluid and electrolytes with consequent dehydration (23).

Many factors modify the proliferation of microorganisms in the gastrointestinal tract. Past attempts to control enteric pathogens by immunoprophylactic methods have met with limited success (65). Administration of homologous hyperimmune serum to piglets prevented diarrhea when piglets were given an experimental E. coli challenge (43,41). Recent investigations reveal that vaccination of pregnant sows with certain coliform bacterins or modified live cultures prevent piglet diar-

rhea and decrease the necessity for treatment (42,85,17,75). This protection was attributed to colostral and milk antibodies produced by the sow subsequent to vaccination. Despite the generally inconsistent results of immunotherapy involving exogenous antibodies little has been done to explore the potential of endogenous immunologic capabilities of neonatal and weaning animals to increase resistance against enteric diseases. This is somewhat surprising because 1) there is evidence that newborn calves and pigs (21) may not be able to absorb adequate amounts of protective antibody during the first 2 days of life (21), 2) pigs born late in the farrowing process are at an immunologic and physiologic disadvantage (14), 3) passive antibody is at minimal levels near weaning age (54), and 4) fetal and neonatal animals possess immunogenic potential (13,73,39). Previous attempts to control enteric disorders have been directed toward attaining high levels of circulating antibodies, which afford little protection against some bacterial induced diarrheal diseases (48,9). Tomasi and Bienenstock (77) proposed a local (surface) immune system in humans believed to be distinct from that which produces circulating antibodies. In addition they suggested that local immunity may be of primary importance in defense against infectious processes involving mucous membranes, viz., digestive, respiratory, genito-urinary tracts and mammary gland. The predominant immunoglobulin type in most secretions, termed secretory IgA (SIgA), is synthesized in underlying plasma cells and a glycoprotein "secretory component" originates from the epithelial cells (18). Recent literature is replete with physical and chemical characteristics as well as proposed function of SIgA (82, 87). An important feature of the local immune system, as its name implies, is its localized nature. Studies on the gastrointestinal (51)

and respiratory (52) tracts emphasize the advantages of direct application of antigens to the mucosal surface so that maximal antigenic stimulation may be realized. Recent investigations reveal another feature which appears unique to the local immune system. Available data indicates that the local immune system lacks immunologic memory and in absence of live antigenic stimulus requires repeated stimulation (55,51).

Researchers (81) have recently presented evidence for existence of SIgA and a local immune system in domestic and laboratory animals (81). Porter (55,56,2,57) has further characterized the SIgA system in the calf and pig. These studies suggest that the local immune system contribute to the well being of the host by both passive (colostral) and active immune mechanisms. However, immunology of secretory surfaces is a new area of investigation and many questions remain unanswered, viz., specificity, mode of action, method(s) of stimulation, and relationship to immune deficiency diseases (82).

Little is known of the qualitative or quantitative contributions of the gut to the body's defense mechanisms. Although it is generally known that the gut and its embryonic derived lymphoid tissues (thymus) confer immunity to the whole organism by cell-mediated and humoral systems (62) only recently have investigators recognized the immunologic potential of the gut. The lamina propria of the gut is one of the richest lymphoid tissues of the body. It constitutes the first line of defense against infection, primarily by being a mandatory point of passage for perorally absorbed antigens and toxins (62,34). Like the peripheral lymphoid tissue, the lymphoid elements of the gastrointestinal tract may experience an antigenic challenge and contribute to the body's defense with an immunologic response (50). Furthermore, the aggregated

and non-aggregated lymphoid tissue of the gut synthesize both serum and coproantibodies (62,12). Perhaps the intimate embryonic association of gut and peripheral lymphoid tissue led previous investigators to believe that, regardless of route of administration, an immunogen would elicit a comparable response in both humoral and local (gut level) immune systems. On the contrary, however, investigators have been unable to relate cellular and serological findings in gastrointestnal diseases (83).

The present study was undertaken to characterize further local (intestinal) immunity in the young pig, with particular reference to local antigens, intestinal antibody levels and resistance to enteric infection.

MATERIALS AND METHODS

Maintenance of bacterial cultures. Laboratory cultures of Escherichia coli 055:B5:H7 (055) were maintained on brain heart infusion agar[1] slants in the dark and at room temperature. Abbotstown strain of E. coli 0149:K91,88a,c:H10 (0149) was obtained,[2] passed through 5-7 day chick embryos, lyophilized in egg yold and stored at -20°C. Additional stock cultures were grown in brain heart infusion broth and suspended in skim milk prior to lyophilization.

Preparation of bacterins and modified cultures. Bacterins were prepared by adding formalin to provide a final concentration of 0.5% (V/V) to 8 hour E. coli 0149 cultures (1 x 10^8 E. coli/ml) which had

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[1] Difco Laboratories, Detroit, Michigan.

[2] Courtesy of Drs. D. A. Barnum and M. R. Wilson, University of Guelph, Ontario, Canada.

been grown in trypticase soy broth[3] (TSB) and then incubated at 37°C for an additional 15-18 hours. Cultures of E. coli 0149 were modified according to the method described by Wilson and Svendsen (86). Formalin (0.04% V/V) was added to an 8 hour TSB culture which was incubated for an additional 15 hours at 37°C. Cultures thus modified failed to grow for 12-15 hours when plated on blood agar. Bacterins (10 ml) and modified (2 ml) cultures were prepared directly from rehydrated lyophilized stock cultures and administered via stomach tube.

Challenge cultures. Tubes containing 10 ml TSB were inoculated with lyophilized stock cultures and incubated for 18-24 hours at 37°C. Counts revealed 1 x 10^9 E. coli per ml of culture as determined by pour plate method. Cultures were administered via stomach tube.

Serum collection. Pigs were bled from the anterior vena cava and the blood allowed to clot at room temperature. Serum and cells were separated by centrifugation. Serum was frozen and stored at -20°C.

Intestinal secretions. Intestinal secretions were collected by modification of the method described by Plaut and Kesnil (53). Piglets were anesthetized with sodium pentobarbital[4] and the small intestine exteriorized via a midline incision. Intestinal secretions were expressed into a sterile container surrounded by dry ice and stored at -4°C. Prior to assay the frozen intestinal secretions were placed in an ice bath, refrigerated at 4°C and allowed to melt slowly overnight, and then placed in a preheated flask and allowed to oscillate slowly at

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[3]Baltimore Biological Laboratories, Baltimore 18, Maryland.

[4]Nembutal, Abbott Laboratories, North Chicago, Illinois.

56°C for 30 minutes. When not being used the fluids were maintained at 4°C. Immunoglobulins in this intestinal fluid were concentrated by cold centrifugation at 3000 x g. Ten ml of the supernatant were treated with an equal volume of saturated ammonium sulfate while continuously stirred at 4°C. The precipitate was removed by centrifugation at 3000 x g, redissolved in 1 ml 0.85% NaCl, and dialyzed overnight with frequent changes against 0.85% NaCl at 4°C. The contents of the dialysis bags were adjusted to 2 ml with 0.85% NaCl.

Bacterial agglutination. Antigens for titration of K antibody activity were prepared by the method of Edwards and Ewing (22). Blood agar plates were streaked and allowed to incubate initially for 24 hours at 37°C and subsequently for an additional 24 hours at room temperature (26°C). A smooth, mucoid colony was selected from the plates and inoculated into a tube of brain heart infusion broth. The broth culture was incubated at 37°C for 5-6 hours and formalized (final concentration 0.5%). These antigens were added to 2 fold dilutions of serum or intestinal secretions (16). The dilutions were incubated at 37°C for two hours and antibody activity determined following overnight refrigeration. A positive test was indicated by disc formation in the bottom of the tube. Titers were recorded as a reciprocal of the highest dilution giving a positive agglutination reaction.

Immunoglobulin assay. Antisera to immunoglobulins of human origin are known to react with animal proteins (81). Quanti-plate Immunoglobulin Test Kits[5] were utilized to determine porcine immunoglobulin (IgM, IgA, IgG) levels by single radial diffusion. Monospecific antiserum to

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[5]Kallestad Laboratories, Inc., Chaska, Minnesota.

immunoglobulins of human origin is incorporated in agar medium. Capillary pipettes were used to fill wells with serum or intestinal secretions and then the plates were incubated for 18-24 hours at room temperature. The radius of precipitate formed was dependent on quality of specific immunoglobulins present. Sample precipitates were compared to precipitates formed by reference sera.

Immunoelectrophoresis. Immunoelectrophoresis (IEP) was performed by Klaus' modification (40) of Scheideggar's technique (66) using an Immunophor[6] electrophoresis unit. Special Noble Agar[7] (1.5%) was dissolved in 0.03 ionic strength, pH 8.6 veronal buffer and poured onto six 1 x 3 inch microscope slides arranged on a tray. Standard two-well, single trench patterns were cut in the agar. Electrophoresis was carried out at 250 V for 60 minutes. Antiserum to porcine whole serum was applied to the trenches and the slides were incubated in a humid chamber for 18-24 hours at room temperature.

Antiserum production. Antiserum to porcine serum was produced in healthy young rabbits purchased locally. Porcine serum was emulsified in an equal volume of either complete or incomplete Freund's adjuvant prior to subcutaneous injection of 5-10 mg protein per dose. Following an initial injection with complete Freund's and pig serum, immunization was repeated with incomplete Freund's and pig serum. Rabbits were bled from the ear vein 45-60 days after the initial injection.

Clinical observations. Following intragastric challenge with E.

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[6] LKB-Produkter, A-B Stockholm, Sweden.

[7] Difco Laboratories, Detroit, Michigan.

coli pigs were fed and observed every 6 hours for 144 hours. Observations included: feces, appetite, temperature, degree of activity, signs of dehydration (sunken eyes, elasticity of skin), morbidity, mortality and time of death when applicable.

Experimental animals. Gnotobiotic pigs were delivered from gravid sows of Hampshire or Yorkshire breeding by germfree hysterotomy at 112 days gestation. Anesthesia was induced with intravenous sodium thiopental[8] (4 mg/kg body wt) and maintained with 2-3% halothane in nitrous oxide-oxygen (2:1) via a to and fro system using a partial rebreathing technique (5). Preanesthetic medicants were not used. Following anesthesia, germfree pig procurement was carried out following the general recommendations of Landy, et al. (44), Myer, et al. (47) and Trexler (79). Pigs were fed a synthetic milk diet[9] according to manufacturer's suggestion. Conventionally farrowed pigs were allowed to suckle for 48 hours, at which time they were assigned to gnotobiotic isolators according to treatment. After transfer to isolators, conventional pigs also received the synthetic diet.

Sterility check of gnotobiotic systems. Isolator contents (flooring, excreta, feces) and piglets feet were cultured for bacteria each time materials were introduced and/or removed from the isolators. Gram stains were made from the original swabs and examined for bacteria. Culture media, which included bovine blood agar,[10] Endo Agar,[11] and

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[8] Pentothal, Abbott Laboratories, North Chicago, Illinois.

[9] SPF-lac, Borden Company, New York, New York.

[10] Difco Laboratories, Detroit, Michigan.

[11] Ibid.

Thioglycollate[12] medium, were inoculated in duplicate and incubated at room temperature and at 37°C. Cultures were observed for 1 week before negative cultures were discarded. Contaminated litters were not used for experiments.

Experimental procedure. Experiment I was planned to determine if intragastric exposure to live E. coli would induce appearance of specific antibodies in intestinal secretions and serum of young gnotobiotic pigs. Immunoglobulin levels were measured in serum and intestinal secretions. At 3 weeks of age twenty-five gnotobiotic pigs were dosed intragastrically with 10 ml of live E. coli 055:B5:H7 culture (1×10^9/ml). Pigs were enthanatized at 4, 8 or 12 days following E. coli inoculation and serum and intestinal secretions were collected. Four uninoculated pigs served as controls.

Experiment II was designed to determine if prior exposure to killed E. coli enhanced resistance to a homologous challenge. At birth and 3, 6 and 9 days post parturm fourteen gnotobiotic pigs received 10 ml of an intragastric dose of 1.0×10^9 formalin-killed E. coli. Eleven pigs which did not receive the bacterin were utilized as controls. At 14 days of age all 25 pigs were challenged with (1.0×10^9 in 10 ml) enteropathogenic E. coli 0149.

Experiment III was initiated to evaluate local antigenic stimulus of live formalin-modified E. coli on conventionally farrowed pigs subsequently challenged with a homologous enteropathogenic strain. Twenty-five pigs were transferred to gnotobiotic isolators at 48 hours of age at which time 13 received (1×10^9 in 2 ml) live formalin-modified E.

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[12] Ibid.

coli 0149. The remaining 12 pigs were maintained as controls. Homologous challenge cultures of 10×10^9 E. coli were administered when the pigs reached 14 days of age. Observations were recorded as indicated in Experiment II.

RESULTS

Antibody levels in intestinal secretions and sera (Experiment I). Agglutination tests on intestinal secretions revealed antibody activity as early as 4 days following exposure to live Escherichia coli 055 (Table I). Five of eight pigs had positive titers of 40 or greater. Two were positive at 10-20 and only 1 of eight was negative for specific antibody activity. At eight days post exposure 5 of 12 pigs had titers of 40-80 while titers of 7 of 12 piglets were 10-20. Titers of 2 pigs had reached 160 or greater 12 days following E. coli exposure. Fourteen of 23 pigs possessed positive titers of 40-80; seven of 23 had measurable activity in the range 10-20 while no pigs in this group were negative. Intestinal secretions from 4 of 5 control pigs tested at 12 days were negative whereas one had a titer of 10.

Tests on sera of eight pigs collected 4 days after E. coli infection revealed two pigs with positive titers of 40 and 160 respectively (Table II). Four of eight titers were 10-20 and 2 of 8 were negative. However, by 8 days after exposure to E. coli sera from all pigs were positive. Two, six and four pigs had titers of 10-20, 40-80 and 160 respectively. By 12 days post inoculation (PI) 21 of 25 pigs had serum titers of 160 or greater. Three of twenty-five demonstrated titers of 40-80, 0 of 25 had titers 10-20, while antibody activity was absent in 1 of the 25 pigs. Sera from all control pigs were negative.

TABLE I

ANTIBODY TITERS OF INTESTINAL SECRETIONS FOLLOWING GASTROINTESTINAL INOCULATION OF THREE WEEK OLD GNOTOBIOTIC PIGS WITH LIVE *E. COLI* 055

Days After *E. coli* Inoculation	Titer				Total Samples Tested
	0	10-20[a]	40-80	160 or >	
4 days	1/8[b]	2/8	5/8	0	8
8 days	0/12	7/12	5/12	0	12
12 days	0/23	7/23	14/23	2/23	23
Untreated Controls 12 days	4/5	1/5	0	0	5

[a]Reciprocal of highest dilution of intestinal secretions that were positive.

[b]No. samples positive/total.

TABLE II

ANTIBODY TITERS IN SERUM FOLLOWING GASTROINTESTINAL INOCULATION OF THREE WEEK OLD GNOTOBIOTIC PIGS WITH LIVE *E. COLI* 055

Days After *E. coli* Inoculation	Titer				Total Samples Tested
	0	10-20[a]	40-80	160 or >	
4 days	2/8[b]	4/8	1/8	1/8	8
8 days	0/12	2/12	6/12	4/12	12
12 days	1/25	0/25	3/25	21/25	25
Untreated Controls 12 days	5/5	0	0	0	5

[a] Reciprocal of highest dilution of serum that were positive.

[b] No. samples positive/total.

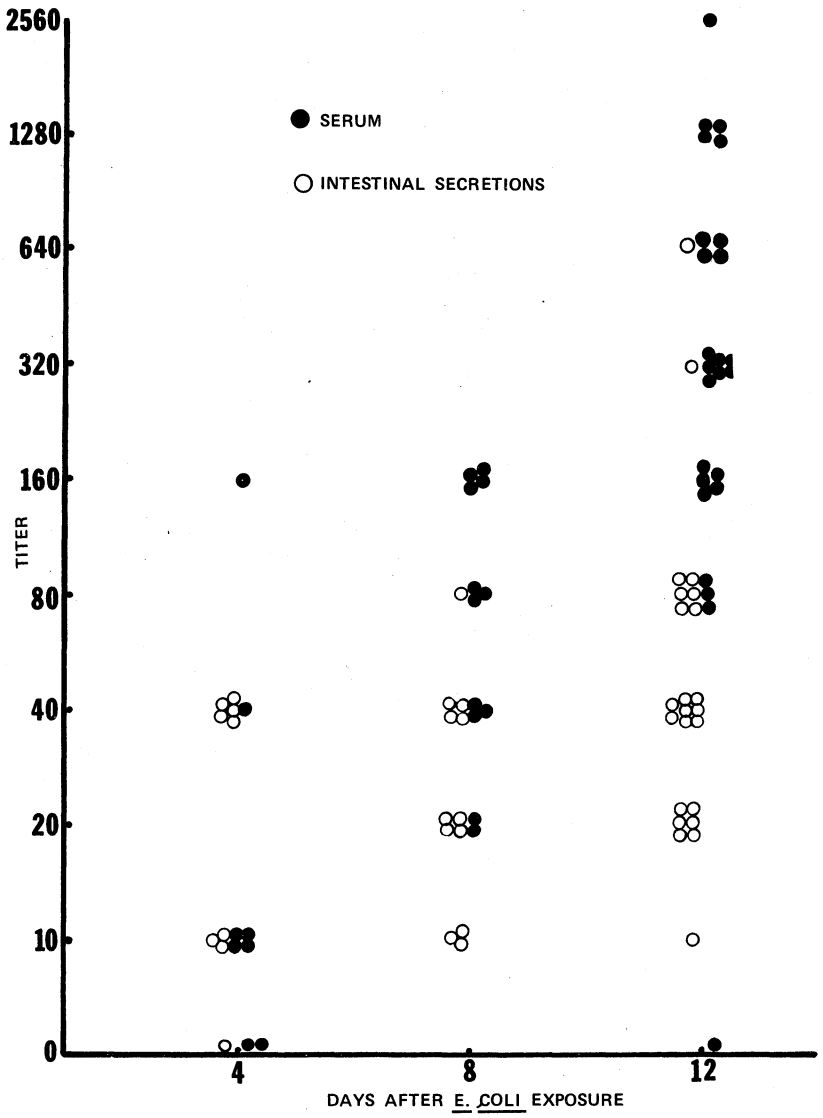

Figure 1. Relationship Between Agglutinins in Sera and Intestinal Secretions of Three Week Old Gnotobiotic Pigs Following Intragastric Administration of Live E. coli 055

Immunoglobulin levels (IgG, IgM, IgA) also were measured in intestinal secretions and sera of the pigs at 4, 8 and 12 days PI with E. coli 055:B5:H7. Sera from four of the 8 pigs killed 4 days PI revealed a mean IgG concentration of 411 mg/100 ml (Table III). IgM and IgA were not detected in sera 4 days PI. Intestinal secretions of the 8 pigs killed 4 days PI did not contain IgG, IgM or IgA. Mean IgG concentration in sera of the 11 samples taken from pigs killed 8 days PI was 509 mg/100 ml; IgM and IgA were not detected in sera of these pigs. Intestinal secretions from 6 pigs in the 8 day group were tested and found to contain 0, 9 and 0 mg/100 ml IgG, IgM and IgA respectively. Sera collected 12 days PI revealed a mean concentration of 852 mg IgG per 100 ml of serum and 1 mg of IgM per 100 ml of serum. IgA was not detected in the sera of these pigs. All 3 immunoglobulin classes IgG ($\bar{X}$ = 73 mg/100 ml), IgM ($\bar{X}$ = 18 mg/100 ml), and IgA ($\bar{X}$ = 23 mg/100 ml) were found in intestinal secretions of pigs sampled 12 days PI. With the exception of IgG ($\bar{X}$ = 410 mg/100 ml) in sera, immunoglobulins could not be detected in sera or intestinal secretions of control pigs. Immunoglobulin levels of individual pigs are listed in the Appendix. A typical radial immuno-diffusion cross reaction of pig intestinal secretions with antisera directed against immunoglobulins of human origin is shown in Figure 2. Immunoelectrophoresis of intestinal secretions collected from 23 of 25 pigs 12 days PI revealed precipitin arc(s) in the gamma region (Figure 3).

Clinical observations on pigs receiving bacterin or modified live culture prior to challenge with Escherichia coli 0149 (Experiments II and III). Twenty-nine percent (4 of 14) of the pigs that received killed E. coli 5, 8, 11 and 14 days prior to administration of a homolo-

TABLE III

MEAN IMMUNOLGOBULIN CONCENTRATION (mg/100 ml) IN SERA AND INTESTINAL SECRETIONS OF 3 WEEK OLD GNOTOBIOTIC PIGS THAT RECEIVED AN INTRAGASTRIC DOSE OF LIVE E. COLI 055

Days After E. coli Inoculation		IgG	IgM	IgA
4 days	serum	411[a] ± 112[b] (4)[c]	0 (4)	0 (40
	intestinal secretions	0 (6)	0 (6)	0 (6)
8 days	serum	509 ± 117 (11)	0 (11)	0 (11)
	intestinal secretions	0 (6)	9 ± 9 (6)	0 (6)
12 days	serum	852 ± 123 (22)	1 ± 1 (22)	0 (21)
	intestinal secretions	73 ± 24 (24)	18 ± 8 (24)	23 ± 8 (23)
Untreated Controls 12 days	serum	410 ± 110	0	0
	intestinal secretions	0 (2)	0 (2)	0 (2)

[a] Immunoglobulin levels of individual pigs are listed in the Appendix.

[b] Standard error.

[c] Number of animals.

Figure 2. Radial Immunodiffusion Assay of Sera and Intestinal Secretions From 3 Week Old Gnotobiotic Pigs 12 Days After Inoculation With Live E. coli 055. Antisera Incorporated in the Agar was Directed Against Immunoglobulins of Human Origin. Left Well in Each Plate Contains Human Reference Serum. Top Plate: IgG Antiserum Vs. Pig Intestinal Secretions; Bottom Plate: IgA Antiserum Vs. Pig Intestinal Secretions

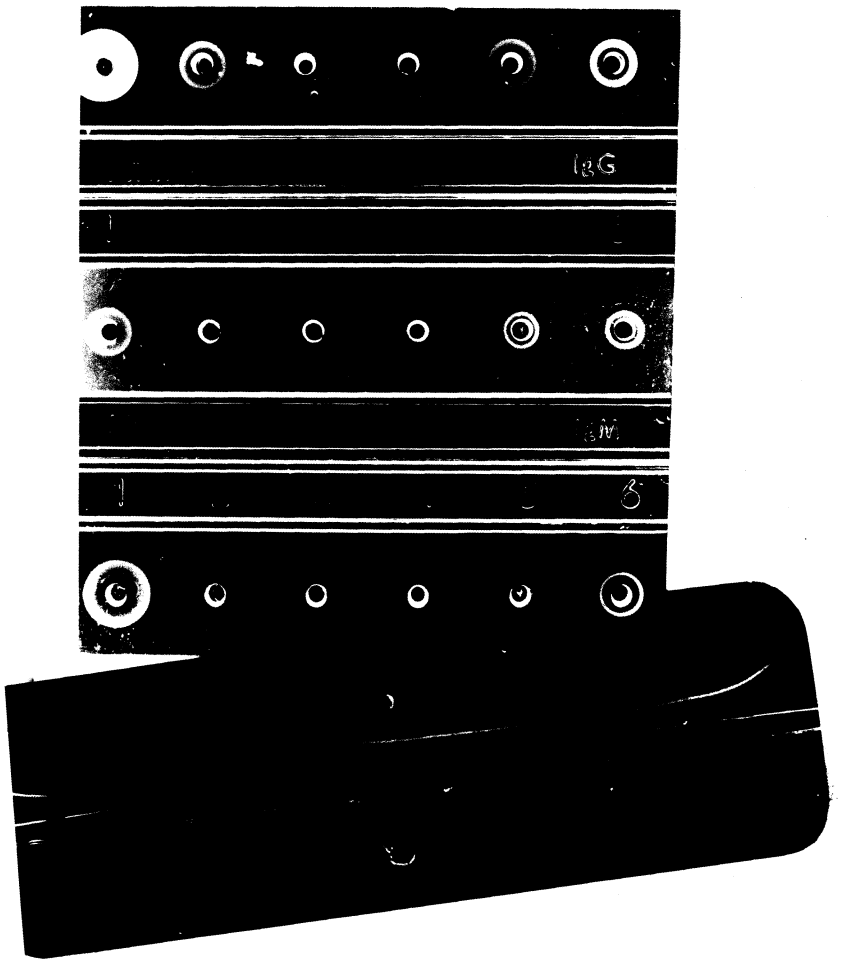

Figure 3. Immunoelectrophoresis of Serum From a Normal Adult Pig; Intestinal Secretions Collected From a 3 Week Old Gnotobiotic Pigs 12 Days After Inoculation With Live E. coli 055. Top Well, Normal Adult Pig Serum; Trough: Antisera Raised in Rabbit Against Normal Adult Pig Serum; Bottom Well; Pig Intestinal Secretions 12 Days PI

gous enteropathogenic E. coli survived the challenge (Table IV). Survival time of pigs that died ranged from 18-60 hours (mean of 39 hours) after receiving live E. coli 0149:K91,88ac:H10. Two pigs died less than 24 hours following challenge; 6 died within 48 hours and 2 died within 60 hours. Four pigs had a mild transient diarrhea 24-48 hours after challenge but were fully recovered 60-72 hours after challenge. Diarrhea was much more severe in the 11 nonvaccinated pigs that didn't receive killed E. coli prior to challenge, the resulting mortality rate (Table V) being 100%. Survival time of the nonvaccinated group ranged from 18-36 hours with a mean of 28 hours. Four pigs died within 24 hours after receiving live E. coli and the remaining seven died 36 hours after exposure to live E. coli.

Observations on 13 conventionally farrowed pigs receiving intragastric formalin-modified live E. coli 0149 12 days prior to challenge with the homologous strain is summarized in Table VI. Two vaccinated pigs and 1 control died prior to inoculation with the enteropathogenic culture. Two of the remaining 11 (18%) pigs which had previously received the modified E. coli culture were ill at 12-18 hours and died within 30 hours after receiving the E. coli challenge. Four of the remaining 9 were slightly lethargic at 36 hours after challenge but were clinically healthy by 48-60 hours after inoculation of E. coli.

Clinical observations on the 11 control pigs which did not receive the formalin-modified E. coli culture were similar to those that received the formalin-modified culture prior to challenge. Seventy-three percent of the control pigs survived the challenge with enteropathogenic E. coli. Two pigs found in lateral recumbency 12 hours after challenge were dead 12 hours later. One pig was recumbent at 84 hours after challenge with

TABLE IV

CLINICAL OBSERVATIONS ON TWO WEEK OLD GNOTOBIOTIC PIGS THAT RECEIVED AN E. COLI 0149 BACTERIN 5, 8, 11, AND 14 DAYS PRIOR TO CHALLENGE WITH LIVE E. COLI 0149

Pig #	Clinical Observations	Peak Temperature °F	Outcome
53-1	Found dead; no previous symptoms	102°	Dead at 18 hours
65-5	None	101°	Dead at 24 hours
65-1	Camatose at 24 hours[a]	98.4°	Dead at 30 hours
65-3	Anorexia at 30 hours PI	101.5°	Dead at 36 hours
63-7	Lateral recumbency at 30 hours	101°	Dead at 36 hours
63-1	Lateral recumbency at 30 hours	101°	Dead at 36 hours
59-3	Anorexia at 42 hours	104.6°	Dead at 48 hours
59-11	Diarrhea at 24-48 hours	104°	Dead at 48 hours
63-9	Found dead; no previous symptoms	101.4°	Dead at 54 hours
65-7	Comatose at 54 hours	100.8°	Dead at 60 hours
53-9	Apparently healthy throughout experiment	102°	Survived, healthy at 144 hours
59-5	Mild diarrhea at 12-24 hours	103.5°	Survived, healthy at 144 hours
59-13	Active, alert, distended abdomen at 24-48 hours	102.5°	Survived, healthy at 144 hours
63-3	Mild diarrhea, transient anorexia at 24 hours	101.5°	Survived, healthy at 144 hours

[a] Hours after challenge.

TABLE V

CLINICAL OBSERVATIONS ON TWO WEEK OLD GNOTOBIOTIC PIGS THAT HAD NOT RECEIVED A BACTERIN PRIOR TO INOCULATION WITH LIVE E. COLI 0149

Pig #	Clinical Observations	Peak Temperature °F	Outcome
53-2	Anorexia at 6 hours[a]	102.5°	Dead at 18 hours
53-4	Anorexia at 6 hours	103°	Dead at 24 hours
53-6	Anorexia at 6 hours	102.7°	Dead at 24 hours
65-4	Anorexia at 18 hours	100.5°	Dead at 24 hours
59-2	Lateral recumbency at 24 hours	103.2°	Dead at 30 hours
59-8	Lateral recumbency at 24 hours	102.6°	Dead at 30 hours
63-8	Anorexia at 24 hours	101.5°	Dead at 30 hours
65-6	Comatose at 24 hours	98.4°	Dead at 30 hours
59-6	Lateral recumbency at 30 hours	105.2°	Dead at 36 hours
59-10	Lateral recumbency at 30 hours	103°	Dead at 36 hours
65-2	Anorexia at 18 hours, lateral recumbency at 24 hours	101.2°	Dead at 36 hours

[a]Hours after challenge.

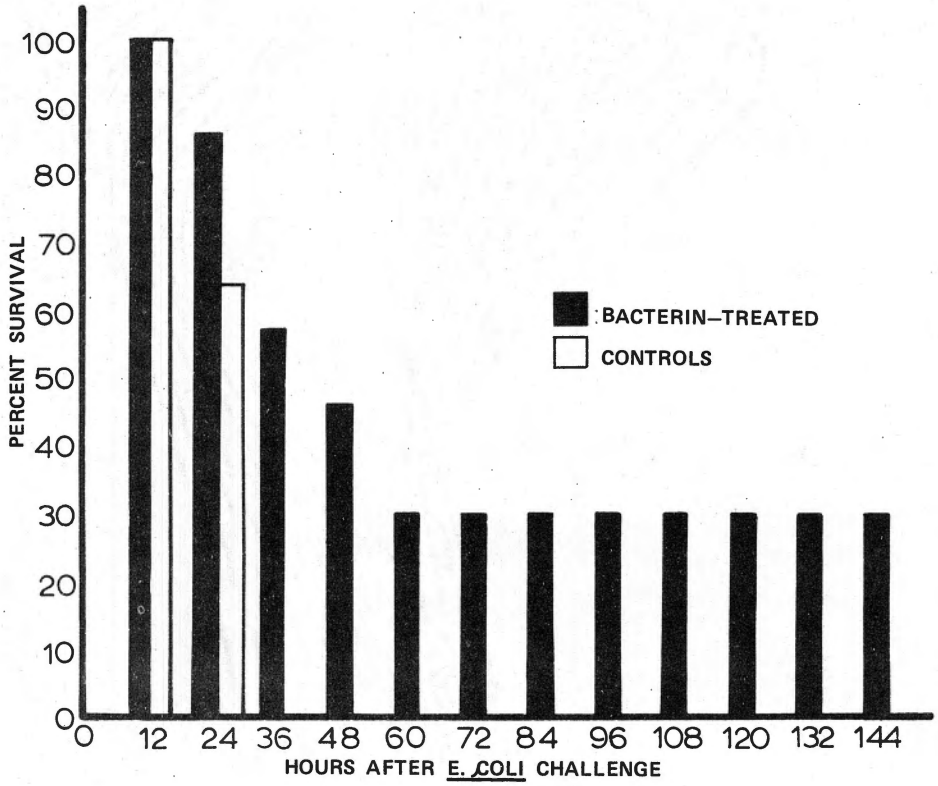

Figure 4. Comparison of Survival in Percent of Two Week Old Gnotobiotic Pigs That Either Had Been Vaccinated With an E. coli 0149 Bacterin 5, 8, 11, and 14 Days Prior to Challenge With Live E. coli 0149 or Had Not Received a Bacterin (Controls) Before Inoculation With Live E. coli 0149

TABLE VI

CLINICAL OBSERVATIONS ON CONVENTIONALLY FARROWED PIGS THAT RECEIVED FORMALIN-MODIFIED *E. COLI* 0149 BY GASTROINTESTINAL INOCULATION AT TWO DAYS OF AGE AND THEN CHALLENGED WITH LIVE *E. COLI* 0149 AT TWO WEEKS OF AGE

Pig #	Clinical Observations	Outcome
68-5	Listless, slight anorexia at 12 hours[a]	Dead at 18 hours
68-4	Listless, slight anorexia at 18 hours	Dead at 30 hours
68-6	No clinical signs of disease	Survived, healthy at 144 hours
69-1	No clinical signs of disease	Survived, healthy at 144 hours
69-2	No clinical signs of disease	Survived, healthy at 144 hours
69-4	No clinical signs of disease	Survived, healthy at 144 hours
70-1	Slightly inactive at challenge	Survived, healthy at 144 hours
70-2	No clinical signs of disease	Survived, healthy at 144 hours
70-3	Slightly inactive at 36 hours	Survived, healthy at 144 hours
70-4	Slightly inactive at challenge	Survived, healthy at 144 hours
70-5	Slightly inactive at challenge	Survived, healthy at 144 hours

[a]Hours after challenge.

TABLE VII

CLINICAL OBSERVATIONS ON CONVENTIONALLY FARROWED PIGS THAT HAD NOT RECEIVED FORMALIN-MODIFIED *E. COLI* 0149 BY GASTROINTESTINAL INOCULATION AT TWO DAYS OF AGE AND THEN INOCULATED WITH LIVE *E. COLI* 0149 AT TWO WEEKS OF AGE

Pig #	Clinical Observations	Outcome
69-8	Lateral recumbency at 12 hours[a]	Dead at 18 hours
68-2	Listless at 12 hours, lateral recumbency at 18 hours	Dead at 24 hours
69-9	Lateral recumbency at 84 hours	Dead at 90 hours
68-1	No clinical signs of disease	Survived, healthy at 144 hours
68-3	No clinical signs of disease	Survived, healthy at 144 hours
69-6	No clinical signs of disease	Survived, healthy at 144 hours
69-7	No clinical signs of disease	Survived, healthy at 144 hours
69-10	No clinical signs of disease	Survived, healthy at 144 hours
70-7	No clinical signs of disease	Survived, healthy at 144 hours
70-8	No clinical signs of disease	Survived, healthy at 144 hours
70-9	No clinical signs of disease	Survived, healthy at 144 hours

[a]Hours after challenge.

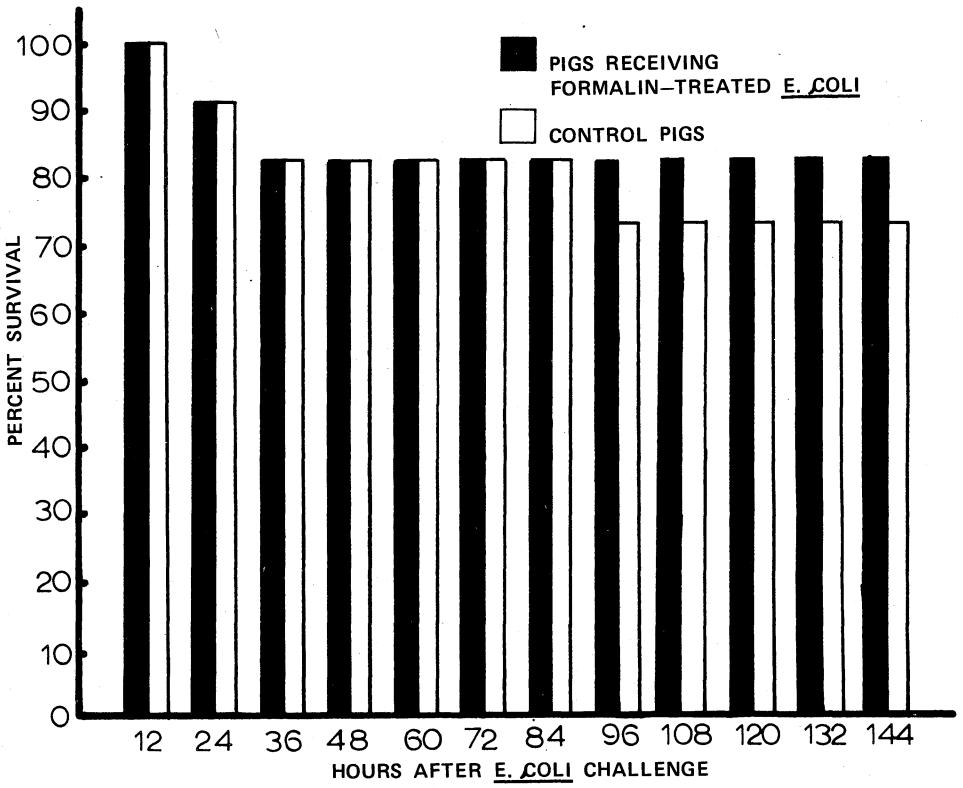

Figure 5. Comparison of Survival in Percent of Two Week Old Conventionally Farrowed Pigs That Either Had Received Formalin-Modified E. coli 0149 at 2 Days of Age and Then Challenged With Live E. coli 0149 or Had Not Received Formalin-Modified E. coli 0149 Before Inoculation With Live E. coli 0149

E. coli and was discovered dead 6 hours later. The remaining 8 pigs did not show clinical signs of disease during the 144 hour observation period.

DISCUSSION

The study reported herein indicates that intragastric administration of live Escherichia coli 055 to 3 week old gnotobiotic pigs is followed by the appearance of specific "K" agglutinins in serum and intestinal secretions (Tables I and II). At 4 days PI agglutination tests revealed that antibody activity was greater in intestinal secretions than in serum. By 8 days PI antibody activity in intestinal secretions remained at intermediate levels (10-80), in contrast tests on serum revealed most pigs possessed titers ranged from 40-160. By 12 days PI serum antibody activity was 160 in 21 of 25 pigs tested while intestinal secretions from only 2 of 23 attained titers of 160 or greater. Low levels of agglutinating antibodies were detected in intestinal secretions of 1 control pig. These findings revealed several points of interest. 1) Other previous investigators failed to demonstrate significant levels of serum "O" agglutinins by the bacterial agglutination method and the investigators concluded there was no correlation between E. coli serum agglutinins and bactericidal activity in swine (43). The "K" bacterial agglutination test was successfully used in the present study and has been reported by others to correlate with protection tests in mice (70). The passive hemagglutination assay was found unsatisfactory for measuring antibody activity in intestinal secretions of piglets (19). Porcine intestinal secretions contained hemolysins to human 0 and sheep red blood cells. Attempts to remove hemolytic activity by absorption and

extended dialysis were not successful. Workers have previously reported non-specific hemolysis associated with gut lymphoid tissue (61), whereas others have successfully used the hemagglutination assay to demonstrate antibodies to E. coli in intestinal secretions of conventional pigs (56). It was not clear, however, whether these antibodies were induced by a specific immunogen or if hemagglutinating activity was due to "natural antibodies". 2) The early appearance of agglutinins in intestinal secretions suggests the response to intragastric administration of E. coli was initiated in local (gut) lymphoid elements, although direct evidence to substantiate this was not presented. Investigators cannot agree on whether antibodies in intestinal secretions are produced at the gut level or whether they are derived by serum transudation (12,18). Findings of the study reported herein are in accord with other findings (6) that reveal presence of pyroninophilic blast cells in local lymphoid elements one day after oral administration of E. coli to gnotobiotic pigs; germinal centers were evident seven days after E. coli exposure (6). Immunofluorescent and histochemical studies of salivary and intestinal tissues revealed antibody laden cells (predominantly IgA) in lymphoid tissues beneath the mucosal surface of animals observed, including the pig (78,4). Cell transfer experiments suggest that local lymphoid elements play an active role in local antibody production (76). Mesenteric lymph node cells from intravenously or orally immunized rabbits were capable of antibody production when transferred to non-immunized recipients. However, cells from peripheral nodes of orally vaccinated rabbits did not transfer the response to recipients, but cells from peripheral lymph nodes of intravenously immunized animals were capable of transferring antibody producing ability to the recipi-

ents (76). Data suggesting that the major portion of intestinal antibodies may not be produced locally has been introduced recently (84). These workers found that serum IgG antibodies, and to a lesser extent IgA and IgM, were transported across the intestinal mucosa into the gut lumen. Additional information from studies involving rats and mice indicate intestinal antibodies originated in serum of conventional animals but not in germfree animals (27). These authors concluded that serum derived IgG and IgM were not detected in intestines of germfree animals because of high concentrations of intestinal enzymes. The fact that they found serum derived antibodies in intestines of conventional animals was attributed to the normal enteric flora protecting intestinal antibodies by reducing activity of intestinal enzymes (27). 3) Another point of interest is that observations on samples collected 8 and 12 days PI suggest the local antibody response peaks soon after antigenic stimulation and intestinal antibody levels are soon surpassed by circulating (serum) antibodies. This observation lends support to the agglutinin studies in Experiment I which suggest that following gastrointestinal antigenic stimulation the initial response is at the gut level. Although the kinetics of the local immune response have not been elucidated, it seems reasonable to assume that local lymphoid cells could have synthesized antibodies which subsequently appeared in serum. This hypothesis is supported by work of others who have reported that intestinal lymphoid tissues have the potential of forming both serum and coproantibodies (83). Additional evidence has been presented which indicates circulating antibodies appear prior to detection of antibody producing cells in peripheral lymphoid tissues of orally immunized animals (61).

IgM was the first immunoglobulin to appear in intestinal secretions

after intragastric administration of E. coli. Other workers found that the number of cells producing IgM exceeded IgA cells in the lamina propria from small intestines of young pigs (3). They hypothesized that, like humoral immunity local (intestinal) immunity may begin with an IgM response. Other studies of preweanling and adult pigs revealed that IgM remained at very low levels in intestinal fluids (79,56). Porter (55) proposed that a selective transport mechanism may inhibit transport of IgM into the gut lumen from cells underlying the mucosa. IgG and IgA were not detected in intestinal secretions until 12 days PI. This observation supports the concept that there may be a switch from IgM synthesis as the local antibody system matures (55).

Formation of multiple precipitin rings and non-immunoglobulin precipitins in intestinal secretions make interpretation of data difficult and somewhat subjective (46,60). The problem of multiple ring formation was not encountered in the present study. Precipitin rings were not observed when intestinal secretions from controls and pigs killed 4 days PI were reacted with antisera. Therefore, it seems reasonable to assume that nonimmunoglobulin precipitates were not a problem. Earlier studies indicate that non-immunoglobulin precipitins have been consistently associated with use of goat antisera (60). Another problem commonly encountered in study of secretory immunoglobulins is lack of a homogenous standard (77). Use of a 7S instead of an 11S standard for IgA assay of saliva resulted in values which are only 1/3 of the actual immunoglobulin concentration (77). Since a 7S standard was used for assay of IgA in the data presented herein, intestinal IgA levels could have been considerably higher.

Results from Experiment I indicate that the 3 week old pig is

capable of producing luminal (intestinal) and circulating antibodies after intragastric administration of live E. coli. Experiments II and III were initiated to determine if intragastric administration of E. coli 0149 (bacterin or formalin-modified culture) enhanced resistance of the preweanling (2 week old) pig to subsequent homologous challenge. Results suggest that pigs that received an intragastric dose of killed E. coli antigens prior to challenge with live homologous culture were more resistant to enteric disease than were their control counterparts. This resistance is reflected in the 29% survival rate and extended survival time of pigs that previously received the bacterin. Mortality was 100% and deaths were more acute in control pigs when they received the challenge culture. It is tempting to speculate that many of the pigs receiving the E. coli bacterin responded with production of intestinal and serum antibodies which helped protect the host. Advantages of local immunization could probably be demonstrated better by using fewer E. coli for challenge. In view of the fact that others used 0.2 ml of 7×10^8 ml. E. coli for bacterial challenge of gnotobiotic pigs (81), 10 ml of 1.0×10^9 ml E. coli may have been an overwhelming challenge for "immunized" pigs. It would seem reasonable to conduct another trial utilizing a lower challenge dose. Investigators have reported benefits of intestinal antibodies in combating weanling diarrhea in pigs (59). Also, recent studies indicate that addition of E. coli antigens to creep feeds enhances growth of pigs during the stressful period of weaning (58). These authors concluded that increase in growth of bacterin-fed pigs over control pigs could be attributed to intestinal antibodies helping maintain integrity of the intestinal mucosa during bacterial insult. The precise mechanism by which intes-

tinal antibodies contribute to the well-being of the host has not been elucidated.

Mechanism(s) of action of secretory antibodies (primarily IgA) have received considerable attention recently, but with exception of virus neutralization, results are inconclusive and contradictory (26). Johnson (37) suggested secretory antibodies may serve primarily as opsonins while others have indicated that 11S secretory IgA of human origin does not opsonize (87). It is generally agreed that IgA antibodies do not fix complement (26,74). Nevertheless, Hill and Porter (35) report that bactericidal activity of IgA in porcine colostrum occurred only in the presence of complement and lysozyme. It has been reported previously that human colostral IgA has been shown to lyse E. coli in the presence of lysozyme and complement (1). Studies of intestinal loops in rabbits suggest that intestinal antibodies protect against Vibrio cholerae by decreasing absorption of Vibrio cholerae onto the intestinal mucosa (25). These workers concluded that the antibacterial effect occurred on the mucosal surface in conjunction with a factor supplied by mucosal cells. Other investigators were able to demonstrate increased resistance to E. coli enterotoxin challenge after rabbits had been immunized with homologous enterotoxin (64). Increased resistance appeared to be associated with presence of antitoxin. Whether the antitoxin originated from peripheral or local (intestinal) lymphoid elements is not known.

Observations from Experiment III were difficult to interpret. Morbidity in control and vaccinated pigs remained low throughout the six day experiment. Perhaps ingestion of "normal colostrum" by the pigs during the first 48 hours of life accounts for the low morbidity. This conclusion seems unlikely because the E. coli 0149 culture used in the

present experiment originated from a strain which was highly virulent for colostrum-fed pigs (75). The low morbidity could also be attributed to loss of a plasmid which is responsible for enterotoxin production. Smith and Gyles (69) have shown the production of heat labile and heat stable E. coli enterotoxins may be transmitted by plasmids. Another possible explanation for low morbidity could be the manner in which the stock 0149 cultures were stored. Cultures used for Experiment II were lyophilized and maintained in egg yolk until ready for use. Due to a laboratory accident the cultures lyophilized in egg yolk were lost, and cultures from the same stock lyophilized in skim milk were used for Experiment III. Gnotobiotic pigs were not used in Experiment III because the formalin-modified E. coli 0149 was found to be lethal to colostrum deprived gnotobiotic pigs (19). Also naturally farrowed colostrum fed pigs are better representative of a conventional environment than are gnotobiotic pigs.

Results from the present investigation and others suggest that local application of antigens may be the most effective method for stimulating production of antibodies in milk and intestinal secretions (42,58). These investigations emphasize the fact that mucosal surfaces and underlying lymphoid tissue have great potential for rendering protection against infectious organisms (including bacteria, viruses and helminths) that gain access to the host via mucosal surfaces. It seems logical that vaccination against pathogens should be directed toward their point of entry. Despite recent advances in immunotherapy of diseases involving mucosal surfaces, further characterization of the secretory immune system is indicated. 1) The relationship of passive immunity and local immunogens remains to be clarified. 2) It has not

been clearly established whether or not an antigen must penetrate the mucosa to stimulate local lymphoid elements. 3) Immunotherapy of enteric colibacillosis presents a problem because several distinct serotypes have been incriminated in the etiology of neonatal diarrhea (70). Thus heterogeneity of intestinal antibodies needs to be determined. An encouraging note is the apparent similarity of enterotoxins of E. coli and Vibrio cholerae (32). 4) Efforts should be continued toward development of a live oral bacterial vaccine. Studies of oral immunization of man with Vibrio cholerae vaccine revealed that luminal antibodies persist for short periods of time in absence of antigenic stimuli (24). In addition intranasal administration of polio vaccine indicated the secretory immune system probably does not have immunologic memory as we know it in the humoral system (51,50). Similar findings were reported when E. coli were administered locally to the intestines of pigs (55); lack of an anamnestic response was indicated by a similar low intensity response of short duration following a second antigenic challenge. A live vaccine that would colonize the small intestines would provide a continuous antigenic stimulus and overcome some of the apparent shortcomings of the secretory immune system.

SUMMARY

Specific agglutinins were detected in sera and intestinal secretions after intragastric administration of live E. coli to 3 week old gnotobiotic pigs. Rising IgG levels correlated well with the appearance of specific agglutinins in piglet sera. IgM was detected in intestinal secretions 8 days after intragastric administration of live E. coli to piglets. Intestinal secretions of pigs tested 4 days later re-

vealed IgG and IgA along with IgM. When challenged with virulent E. coli at 10 days of age, pigs which had received intragastric doses of E. coli from birth were more resistant to enteric colibacillosis than were their untreated counterparts. Continuation of studies involving the secretory immune system seems justified.

LITERATURE CITED

(1) Adinolfi, M., A. A. Glynn, M. Lindsay and C. Miline. 1966. Serological properties of IgA to Escherichia coli present in human colostrum. Immunology 10:517-526.

(2) Allen, W. D. and P. Porter. 1973. Localization by immunofluorescence of secretory component and IgA in the intestinal mucosa of the young pig. Immunology 24:365-374.

(3) Allen, W. D., and P. Porter. 1973. The relative distribution of IgM and IgA cells in intestinal mucosa and lymphoid tissues of the young unweaned pig and their significance in ontogenesis of secretory immunity. Immunology 24:493-501.

(4) Allen, W. D., C. E. Smith, and P. Porter. 1973. Localization of intracellular immunoglobulin A in porcine intestinal mucosa using enzyme-labelled antibody. Immunology 25:334-340.

(5) Anderson, I. L. 1973. Anaesthesia in the pig. Aust. Vet. J. 49:474-477.

(6) Anderson, J. C. 1974. The response of the tonsil and associated lymph nodes of gnotobiotic piglets to the presence of bacterial antigen in the oral cavity. J. Anat. 117(1):191-198.

(7) Anon. 1959. A study of the incidence and causes of mortality of pigs. Vet. Rec. 71:777-786.

(8) Anon. 1966. Infant, fetal and maternal mortality. National Center for Health Statistics, Series 20, Number 3.

(9) Arbuckle, J. B. R. 1968. The occurrence of Escherichia coli somatic antibody in pig serum, colostrum and milk and an investigation of its possible significance in immunity. Br. Vet. J. 124(7):273-281.

(10) Banwell, J. G. and H. Shen. 1973. Effect of bacterial enterotoxins on the gastrointestinal tract. Gastro. 65(3):467-497.

(11) Barnum, D. A., P. J. Glantz, and H. W. Moon. 1967. Colibacillosis. Ciba Veterinary Monograph Series/Two.

(12) Bazin, H., P. Maldague, E. Schonne, P. A. Crabee, H. Bauldon and J. E. Heremans. 1971. The metabolism of different immunoglo ulin classes in irradiated mice. V. Contribution of the gut to serum IgA levels in normal and irradiated mice. Immunology

20:571-595.

(13) Binns, R. M. 1967. Bone marrow and lymphoid cell injection of the pig foetus resulting in transplantation tolerance or immunity, and immunoglobulin production. Nature (London) 214: 179-181.

(14) Bourne, F. J. 1969. Studies on colostral and milk whey proteins in the sow. 1. The transitions of mammary secretion from colostrum to milk with natural suckling. Anim. Prod. 11: 337-343.

(15) Bywater, R. J. 1972. Dialysis and ultrafiltration of a heat stable enterotoxin from Escherichia coli. J. Med. Microbiol. 5:337-343.

(16) Cambell, D. H., J. S. Carvey, N. E. Cremer, and D. H. Sussdorf. 1964. Methods in Immunology. Wa. A. Benjamin Co., New York.

(17) Cardella, M. A., R. G. Hugn, and R. G. Wilson. 1974. Immunity to neonatal colibacillosis: field studies. J.A.V.M.A. 164(3): 299-303.

(18) Cebra, J. J. 1969. Immunoglobulins and immunocytes. Bact. Rev. 2:159-171.

(19) Corley, L. D., T. E. Staley and E. W. Jones. 1973. Uptake and pathogenesis of Escherichia coli 055B5 in the young gnotobiotic pig. Res. Vet. Sci. 14:69-74.

(20) Cramblett, H. G., P. Azimi, and R. E. Haynes. 1971. The etiology of infectious diarrhea in infancy, with special reference to enteropathogenic E. coli. Ann. N. Y. Acad. Sci. 176:80-92.

(21) Dunne, H. W. 1972. Comments on immunity in control of neonatal colibacillosis in pigs. J.A.V.M.A. 160(4):591-592.

(22) Edwards, P. R. and E. H. Ewing. 1962. Identification of Enterobacteriaceae. Burgess Publishing Co., Minneapolis 15, Minnesota.

(23) Evans, D. J., Jr., L. G. Chen, G. T. Curlin, et al. 1972. Stimulation of adenyl cyclase by Escherichia coli enterotoxin. Nature (New Biol.) 236:137-138.

(24) Freter, R. 1962. Detection of coproantibody and its formation after parenteral and oral immunization of human volunteers. J. Inf. Dis. 111:37-48.

(25) Freter, R. 1970. Mechanisms of action of intestinal antibody in experimental cholera. II. Antibody mediated antibacterial reaction at the mucosal surface. Inf. and Immun. 2(5):556-562.

(26) Freter, R. 1971. Locally produced and serum derived antibodies in "local immunity". New Eng. J. Med. 285(24):1375-1376.

(27) Fubara, E. S., and R. Freter. 1972. Availability of locally synthesized and systemic antibodies in the intestine. Inf. and Immun. 6(6):965-981.

(28) Goldberg, N. D. 1974. Cyclic nucleotides and cell function. Hosp. Pract. 9(5):127-142.

(29) Gordon, J. E. 1971. Diarrheal disease of early childhood--world-wide scope of the problem. Ann. N. Y. Acad. Sci. 176:9-15.

(30) Gossling, J. and H. E. Rhoades. 1967. Identification of certain Escherichia coli strains from baby pigs in North Central United States. Amer. J. Vet. Res. 28:1615-1617.

(31) Gyles, C. L. 1971. Heat-labile and heat-stable forms of the enterotoxin from E. coli strains enteropathogenic for pigs. Ann. N. Y. Acad. Sci. 176:314-322.

(32) Gyles, C. L. 1974. Immunological study of the heat-liable enterotoxins of Escherichia coli and Vibrio cholerae. Infect. and Immun. 9(3):564-570.

(33) Gyles, C. L. and D. A. Barnum. 1969. A heat-labile enterotoxin from strains of Escherichia coli enteropathogenic for pigs. J. Infect. Dis. 120:419-426.

(34) Heremans, J. F. 1969. Peroral stimulation of IgA antibody formation in mice. Conf. on Secretory Immune System. U.S. Dept. of Health, Education and Welfare, PHS, NIH, Bethesda, Md., 309-324.

(35) Hill, I. R. and P. Porter. 1974. Studies of bactericidal activity to Escherichia coli of porcine serum and colostral immunoglobulins and the role of lysozyme with secretory IgA. Immunology 26:1239-1250.

(36) Howard, L. M. 1971. The relevance of parasitology to the growth of nations. J. Parasitol. 57(4):143-147.

(37) Johnson, J. S. 1970. Immunologic mechanisms in the gastrointestinal tract. J. Inf. Dis. 121:5115-5117.

(38) Jones, G. W. and J. M. Rutter. 1972. Role of K88 antigen in the pathogenesis of neonatal diarrhea caused by Escherichia coli in piglets. Infect. and Immun. 6(6):918-927.

(39) Kim, Y. B., S. G. Bradley and D. W. Watson. 1966. Ontogeny of the immune response. I. Development of immunoglobulins in germfree and conventional colostrum-deprived piglets. J. Immun. 97:52-63.

(40) Klaus, G. G. B. 1968. The immunoglobulin response in intact and splenectomized calves infected with Anaplasma marginale. Master's Thesis, Oklahoma State University.

(41) Kohler, E. M. 1967. Studies of Escherichia coli in gnotobiotic pigs. V. Evaluation of the effects of oral and parenteral administration of immune serum. Can. J. Comp. Med. and Vet. Sci. 31:283-289.

(42) Kohler, E. M. 1974. Protection of pigs against neonatal enteric colibacillosis with colostrum and milk from orally vaccinated sows. Amer. J. Vet. Res. 35(3):331-338.

(43) Kohler, E M. and E. H. Bohl. 1966. Studies of Escherichia coli in gnotobiotic pigs. II. The immune response. Can. J. Comp. Med. and Vet. Sci. 30:169-174.

(44) Landy, J. J., J. H. Growdon, and R. L. Sandberg. 1961. Use of large germfree animals in medical research. J.A.M.A. 178(11): 1084-1087.

(45) Lovell, R. 1955. Intestinal diseases of young calves with special reference to infection with Bacterium coli. Vet. Rev. and Annot. 1(1):1-32.

(46) McClelland, D. B. L., D. M. Parkin, R. R. Samson, and D. J. C. Shearman. 1972. Intestinal immunoglobulins in Shigellosis. Gastroenterology 62(4):693-694.

(47) Meyer, R. C., E. H. Bohl, R. D. Henthorne, V. L. Tharp, and D. W. Baldwin. 1963. The procurement and rearing of gnotobiotic swine. Lab. Anim. Care 13:655-664.

(48) Miniats, O. P. and D. G. Ingram. 1967. Antibody response of young to vaccination with Escherichia coli. Can. Vet. J. 8 (11):260-268.

(49) Nielsen, N. D., H. W. Moon and W. E. Roe. 1968. Enteric colibacillosis in swine. J.A.V.M.A. 153:1590-1606.

(50) Ogra, P. L. 1969. The secretory immunolgobulin system of the gastrointestinal tract. Conf. on Secretory Immune System, U.S. Dept. Hlth., Ed. and Welfare, PHS, NIH, Bethesda, Md., 259-279.

(51) Ogra, P. L. and D. I. Karzon. 1969. Distribution of poliovirus antibody in serum, nasopharynx and alimentary tract following segmental immunization of lower alimentary tract with poliovaccine. J. Immunol. 102:1423-1430.

(52) Perkins, J. C., D. N. Tucker, and H. L. Knopf. 1969. Comparison of protective effect of neutralizing antibody in serum and nasal secretions in experimental rhinovirus type 13 illness. Amer. J. Epidemiology 90:519-526.

(53) Plaut, A. G. and P. Keonil. 1969. Immunoglobulins in human small intestinal fluid. Gastroenterology 56(3):522-530.

(54) Porter, P. 1969. Porcine colostral IgA and IgM antibodies to Escherichia coli and their intestinal absorption by the neonatal piglet. Immunology 17:617-626.

(55) Porter, P. 1973. The functional role of secretory antibody systems in early development of the pig and the calf. Proc. Nutr. Soc. 42:217-222.

(56) Porter, P., D. E. Noakes, and W. D. Allen. 1970. Intestinal secretion of immunoglobulins and antibodies to Escherichia coli in the pig. Immunology 18:909-920.

(57) Porter, P., D. E. Noakes, and W. D. Allen. 1972. Intestinal secretion of immunolgobulins in the preruminant calf. Immunology 23:299-312.

(58) Porter, P., R. Kenworthy, D. W. Holme, and S. Horsfield. 1973. Escherichia coli antigens as dietary additives for oral immunisation of pigs: trials with pig creep feeds. Vet. Rec. 92:630-636.

(59) Porter, P., R. Kenworthy and W. D. Allen. 1974. Effect of oral immunization with E. coli antigens on post weaning enteric infection in the young pig. Vet. Rec. 95:99-104.

(60) Reed, W. P. and R. C. Williams. 1971. Intestinal immunoglobulins in Shigellosis. Gastroenterology 61(1):35-45.

(61) Rothberg, R. M., S. C. Kraft, S. M. Michalek. 1973. Systemic immunity after local antigenic stimulation of lymphoid tissue of the gastrointestinal tract. J. Immunol. 111:(6)1906-1913.

(62) Roy, C. C. and R. S. Dubois. 1971. The human gut and immune homeostatis. A brief survey of current concepts. Clinical Pediatrics 10(5):275-281.

(63) Rutter, J. M. 1975. Escherichia coli infections in piglets: pathogenesis, virulence and vaccination. Vet. Rec. 96:171-175.

(64) Sack, R. B. 1973. Immunization with Escherichia coli enterotoxin protects against homologous enterotoxin challenge. Inf. and Immun. 8(4):641-644.

(65) Saunders, C. N., A. J. Stevens, J. B. Spence and W. J. Sojka. 1960. Escherichia coli infection in piglets. Res. Vet. Sci. 1:28-35.

(66) Scheideggar, J. 1955. A micromethod of immunoelectrophoresis. Int. Arch. Allergy 7:102.

(67) Smith, H. W. and S. Halls. 1967. Studies on Escherichia coli enterotoxin. J. Path. Bact. 93:531-543.

(68) Smith, H. W. and S. Halls. 1968. The transmissible nature of the genetic factor in Escherichia coli that controls enterotoxin production. J. Gen. Microbiol. 52:319-334.

(69) Smith, H. W. and C. L. Gyles. 1970. The relationship between two apparently different enterotoxins produced by enteropathogenic strains of Escherichia coli of porcine origin. J. Med. Micro. 3:387-401.

(70) Sojka, W. J. 1965. Escherichia coli in domestic animals and poultry. Commonwealth Agricultural Bureau. Farnham Royal. Bucks, England.

(71) Sojka, W. J. 1971. Enteric diseases in new-born piglets, calves and lambs due to Escherichia coli infection. Vet. Bull. 41:509-522.

(72) Sojka, W. J. 1973. Enteropathogenic Escherichia coli in man and farm animals. Can. Inst. Food Sci. Technol. J. 6:52-63.

(73) Solomon, J. B. 1971. Foetal and Neonatal Immunology. American Elsevier Publishing Co., Inc., New York.

(74) South, M. A., M. D. Cooper, F. A. Wollheim, R. Hong and R. A. Good. 1966. The IgA system. I. Studies of the transport and immunochemistry of IgA in the saliva. J. Exp. Med. 123: 615-627.

(75) Svendsen, J. and M. R. Wilson. 1971. Immunity to Escherichia coli in pigs: Effect of feeding colostrum or serum from vaccinated sows to Escherichia coli-infected pigs. Amer. J. Vet. Res. 32(6):899-904.

(76) Thind, K. S. 1966. Antibody formation against Vibrio cholerae by mesenteric lymph node cell transfer. Immunology 11:59-65.

(77) Tomasi, T. B. and J. Bienenstock. 1968. Secretory Immunoglobulins. Advances in Immunology, Vol. IX, 1-97.

(78) Tomasi, T. B., E. M. Tan, A. Soloman and R. A. Pendergast. 1965. Characteristics of an immune system common to certain external secretions. J. Exp. Med. 121:101-125.

(79) Trexler, P. C. 1963. An isolator system for control of contamination. Lab. Anim. Care 13:572-581.

(80) Truszcrynski, M. and D. Diosek. 1972. The influence of the contents of different K antigens and of physical factors on toxicity of enterotoxins and water extracts of Escherichia coli. Res. Vet. Sci. 13:212-218.

(81) Vaerman, J. P., J. F. Heremans and G. Van Kerckhoven. 1969. Identification of IgA in several mammalian species. J. Immunol. 103:1421-1423.

(82) Waldman, R. H. and R. Ganguly. 1974. Immunity to infections of secretory surfaces. J. Inf. Dis. 130(4):419-440.

(83) Watson, D. W. 1969. Immune responses of the gut. Gastroentereology 56(5):944-965.

(84) Wernet, P., H. Brew, J. Knop, and D. Rawley. 1971. Antibacterial action of specific IgA and transport of IgM, IgA and IgG from serum into the small intestine. J. Inf. Dis. 124(2):223-226.

(85) Wilson, M. R. 1974. Immunity to Escherichia coli in pigs: efficacy of a live formalized vaccine under field conditions. Br. Vet. J. 130:599-605.

(86) Wilson, M. R. and J. Svendsen. 1971. Immunity to Escherichia coli in pigs: serologic response of sows given formalin-treated live Escherichia coli vaccine. Amer. J. Vet. Res. 32(6):891-898.

(87) Zipursky, A., E. J. Brown, and J. Bienenstock. 1973. Lack of opsonization potential of 11S human secretory IgA. Proc. Soc. Exp. Biol. Med. 142:181-184.

A P P E N D I X

TABLE VIII

ANTIBODY ACTIVITY AND IMMUNOGLOBULIN LEVELS IN SERUM AND INTESTINAL SECRETIONS OF CONTROL GNOTOBIOTIC PIGS 33 DAYS OF AGE NOT EXPOSED TO *E. COLI* ANTIGENS

Pig #	Agglutination Titer		Immunoglobulin Concentration (mg/100 ml)					
	Serum	Intestinal Secretions	Serum			Intestinal Secretions		
			IgG	IgA	IgM	IgG	IgA	IgM
48-271	0[a]	0	NT[b]	NT	NT	0	0	0
272	0	0	300	0	0	NT	NT	NT
273	0	10	520	0	0	0	0	0
280	0	0	NT	NT	NT	NT	NT	NT
281	0	0	NT	NT	NT	NT	NT	NT

[a] No meausrable agglutinins and/or immunoglobulins.

[b] Not tested.

TABLE IX

ANTIBODY ACTIVITY AND IMMUNOGLOBULIN LEVELS IN SERUM AND INTESTINAL SECRETIONS OF GNOTOBIOTIC PIGS 4 DAYS AFTER EXPOSURE TO LIVE *E. COLI* 055

Pig #	Agglutination Titer		Immunoglobulin Concentration (mg/100 ml)					
	Serum	Intestinal Secretions	Serum			Intestinal Secretions		
			IgG	IgA	IgM	IgG	IgA	IgM
43-224	NT[a]	40	NT	NT	NT	NT	NT	NT
225	0[b]	0	NT	NT	NT	0	0	0
226	0	NT	NT	NT	NT	NT	NT	NT
48-274	10	40	720	0	0	0	0	0
275	10	10	410	0	0	0	0	0
276	10	40	NT	NT	NT	0	0	0
277	40	40	315	0	0	NT	NT	NT
278	160	40	200	0	0	0	0	0
279	10	10	NT	NT	NT	0	0	0

[a] Not tested.

[b] No measurable agglutinins and/or immunoglobulins.

TABLE X

ANTIBODY ACTIVITY AND IMMUNOGLOBULIN LEVELS IN SERUM AND INTESTINAL SECRETIONS OF GNOTOBIOTIC PIGS 8 DAYS AFTER EXPOSURE TO LIVE *E. COLI* 055

Pig #	Agglutination Titer		Immunoglobulin Concentration (mg/100 ml)					
	Serum	Intestinal Secretions	Serum			Intestinal Secretions		
			IgG	IgA	IgM	IgG	IgA	IgM
44-231	640	40	260	0[b]	0	0	0	0
232	80	20	NT[a]	NT	NT	0	0	0
233	160	20	260	0	0	NT	NT	NT
234	160	80	260	0	0	0	0	0
235	160	40	260	0	0	NT	NT	NT
47-264	40	20	285	0	0	NT	NT	NT
265	20	40	475	0	0	NT	NT	NT
266	80	10	400	0	0	NT	NT	NT
267	80	10	1050	0	0	0	0	52
268	40	10	410	0	0	0	0	0
269	20	40	490	0	0	NT	NT	NT
270	40	20	1450	0	0	0	0	0

[a]Not tested.

[b]No measurable agglutinins and/or immunoglobulins.

TABLE XI

ANTIBODY ACTIVITY AND IMMUNOGLOBULIN LEVELS IN SERUM AND INTESTINAL SECRETIONS OF GNOTOBIOTIC PIGS 12 DAYS AFTER EXPOSURE TO LIVE E. COLI 055

Pig #	Agglutination Titer		Immunoglobulin Concentration (mg/100 ml)					
	Serum	Intestinal Secretions	Serum			Intestinal Secretions		
			IgG	IgA	IgM	IgG	IgA	IgM
43-227	160	320	1300	0[b]	0	0	0	0
228	80	80	2500	0	0	0	0	0
229	320	20	1700	0	0	0	0	0
44-236	320	80	635	0	0	180	49	26
237	320	40	NT	NT	NT	0	0	0
238	160	20	410	0	0	270	75	57
239	320	80	310	0	0	0	0	0
240	640	80	470	0	0	340	145	160
241	160	20	410	0	0	180	45	23
45-243	2560	40	410	0	0	310	77	80
244	80	10	550	0	0	0	0	0
245	0	NT[a]	1300	0	0	0	0	0
246	640	40	720	0	0	0	110	36
247	320	40	635	0	0	0	0	0
248	1280	NT	710	0	0	0	0	0
249	1280	40	900	0	0	0	0	0
46-255	1280	80	NT	NT	NT	NT	NT	NT
256	1280	40	NT	NT	NT	0	0	0
257	80	40	785	0	0	0	0	0
258	160	40	1200	0	0	0	0	0
259	640	20	570	0	0	0	0	0
260	160	20	390	0	0	0	0	0
261	320	640	650	0	0	0	0	0
262	320	80	280	0	0	0	0	0
263	640	20	1900	0	20	260	0	27

[a]Not tested; [b]No measurable agglutinins and/or immunoglobulins.

VITA

Lane Doyle Corley

Candidate for the Degree of

Doctor of Philosophy

Thesis: LOCAL IMMUNOPROPHYLAXIS IN ENTERIC COLIBACILLOSIS

Major Field: Veterinary Parasitology and Public Health

Biographical:

Personal Data: Born in Claremore, Oklahoma, February 19, 1943, the son of Mr. and Mrs. Raymond Corley.

Education: Graduated from Claremore High School, Claremore, Oklahoma, in May, 1961; attended East Central State College from 1961-1965; Who's Who in American Colleges and Universities, 1962-1963; attended Central State College in the Summer of 1965; Medical Technology Internship at St. Anthony Hospital, Oklahoma City, Oklahoma , January 1966-February 1967; received the Bachelor of Science degree from East Central Oklahoma State University in 1968; completed requirements for the Master of Science degree at Oklahoma State University in May, 1971.

Professional Experience: Medical Technologist, College of Veterinary Medicine, Oklahoma State University, February 1967-July 1967; Research Assistant, July 1967-September 1968; Research Associate, September, 1968 to present.