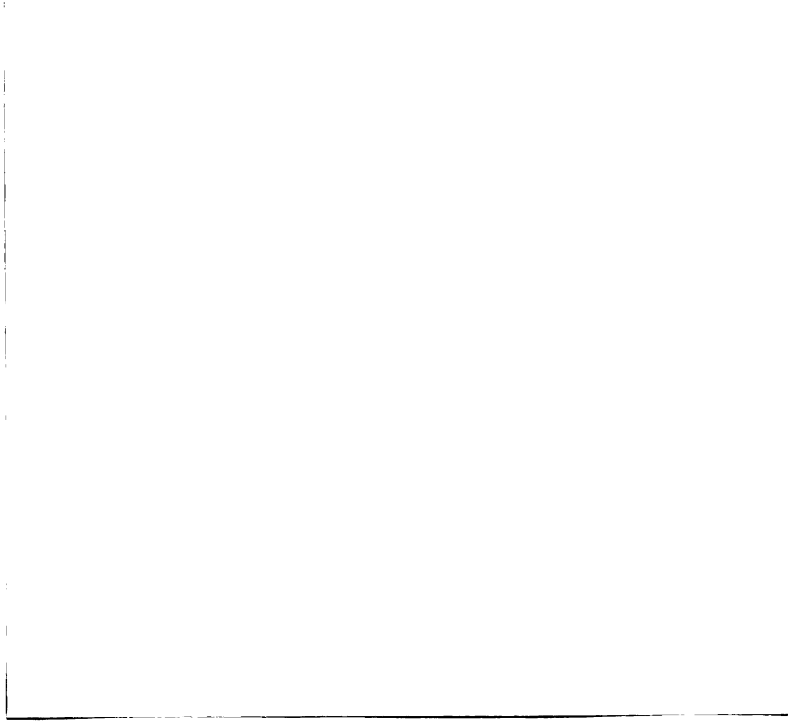


سجده



ABSTRACT

PATHOLOGIC EFFECTS OF

ESCHERICHIA COLI 083:K.:NM IN

GNOTOBIOTIC PIGS

By Alfred Lenneth Britt

In a series of 6 experiments, 58 gnotobiotic pigs from 6 litters were utilized in investigation of the pathologic effects of oral exposure to Escherichia coli serotype 083:K.:NM in gnotobiotic pigs. The pigs were germfree or monocontaminated with Staphylococcus sp. or Bacillus sp. at 69 to 148 hours of age, when they were fed E. coli inoculums in volumes varying from 1.25 ml. to 5.0 ml. of brain-heart infusion or thioglycolate liquid mediums. The inoculums were incubated at 37 C. for 24 or 36 hours just prior to their being fed to the pigs.

In the first 4 experiments there was 100% morbidity and 84% mortality among 25 pigs between 10 and 162 hours after exposure to E. coli at ages ranging from 69 to 148 hours. The most prominent lesions consisted of serofibrinous to fibrinopurulent polyserositis and/or polyarthritis in 100% of the 25 principals.

Invasiveness of the organism was indicated by the septicemic nature of the infection. Four principals were severely ill but not moribund when they were euthanatized between 99 and 236 hours after exposure to E. coli. Septicemia was confirmed by post-mortem microbiological recovery of E. coli from the exudates in serous and synovial cavities of the euthanatized principals as well as from the principals that had died.

Macroscopic and microscopic morphologic evidence of gastroenteritis was usually mild or absent, and results of clinicopathologic examinations did not reveal evidence of anemia, hemoconcentration, or serum electrolyte (sodium and potassium) derangement in the pigs.

Clinical signs were nonspecific, rather than pathognomonic, and included fever, hypothermia, diarrhea, partial anorexia, leukopenia, and leukocytosis. The occurrence of fever and leukocytosis indicated that colostrum-deprived gnotobiotic pigs are capable of responding defensively to bacterial stimuli in much the same manner as other animals.

In Experiments 5 and 6, anti-hog cholera serum was fed to one-half of each group of principals at 6 hours of age or earlier as a substitute for colostrum. Some biological protection was manifested by the absence of mortality and reduced incidence of gross lesions in the pigs fed anti-hog cholera serum as compared with the principals not fed anti-hog cholera serum. Infection following exposure to a contaminated fomite resulted in the only death among the principals in Experiment 6. Evaluation of the degree of protection against E. coli infection provided by feeding anti-hog cholera serum was complicated by an unexplained reduction of mortality in the group of principals not fed anti-hog cholera serum. Also, some pigs in both groups were able to eliminate E. coli from the serous cavities after the acute septicemic phase of infection.

Serums collected from the pigs at 5 to 12 days of age, before and after exposure to E. coli in Experiment 6, were negative for O agglutinins to whole cell E. coli 083 somatic antigen, alone or combined with latex particles, in tube agglutination tests in final dilutions of 1:10 and 1:20.

Results of electrophoresis indicated a range of 0 to 13% gamma globulin with similar ranges in pigs irrespective of the feeding of anti-hog cholera serum. Also, there was no difference between groups in (1) hemoglobin values and packed cell volumes, which increased to a peak at 12 days of age, (2) declining α_1 globulin levels with increasing age, and (3) serum potassium values, which increased with increasing age of the pigs.

PATHOLOGIC EFFECTS OF
ESCHERICHIA COLI 083:K.:NM IN
GNOTOBIOTIC PIGS

By

Alfred Lenneth Britt

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Pathology

1967

G 48436
3-7-68

To my wife Jean
and our children

Nancy Jean
Rebecca Ann
Mary Lynn
Daniel Lenneth
David Henry
Kevin Victor

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to his major professor, Dr. Glenn L. Waxler, for continued invaluable assistance, advice, and encouragement in the conduct of the research and the writing of this manuscript.

Appreciation is expressed to Dr. C. C. Morrill, Chairman of the Department of Pathology, and Dr. R. F. Langham for assistance and instruction during the period in which this research was conducted and the manuscript written. Drs. C. K. Whitehair and C. K. Smith have been especially helpful with counsel and assistance in the area of gnotobiotic research in addition to serving on the author's guidance committee.

Dr. S. D. Sleight and other members of the Department of Pathology have earned the author's humble appreciation of their professional and technical assistance.

Miss Janice Fuller has been most helpful in performing the essential service of typing the manuscript. The patience and encouragement of the author's wife and children have inspired the completion of this work.

TABLE OF CONTENTS

	Page
INTRODUCTION.	1
REVIEW OF LITERATURE.	3
General Characteristics of <u>Escherichia coli</u>	3
Pathogenicity of <u>E. coli</u>	4
Some Factors in Susceptibility to Enteric Disease.	8
Clinical Signs, Clinicopathologic Findings and Necropsy Findings.	11
Prevention of Disease.	15
Experimental Reproduction of Disease in Pigs	18
Artificial Rearing of Pigs	21
Germfree (Gnotobiotic) Research.	24
Chronology of Development of Germfree Research.	25
Gnotobiotic Pigs as Laboratory Animals.	27
MATERIALS AND METHODS	31
Experimental Animals	31
Equipment and Technique for Procuring and Rearing Gnotobiotic Pigs	31
Clinical Observations, Clinicopathologic Examinations and Necropsy Procedures.	39
Preparation of Infective Agent and Exposure of Pigs.	41
RESULTS	44
Experiment 1	44
Experiment 2	53
Experiment 3	58

	Page
Experiment 4	65
Experiment 5	82
Experiment 6	87
DISCUSSION.	96
Bacterial Status	96
Experiments 1, 2, 3, and 4	96
Experiments 5 and 6.	102
SUMMARY	107
BIBLIOGRAPHY.	110
VITA.	119

LIST OF TABLES

Table		Page
1	Distribution of pigs, conditions of oral exposure to <u>Escherichia coli</u> and clinicopathologic examinations employed in each experiment.	43
2	Distribution of pigs and clinical signs observed in Experiment 1	45
3	Body temperatures (°F.) of pigs in Experiment 1.	46
4	Hemograms of surviving pigs and body temperatures of pigs at the time blood samples were collected in Experiment 1	48
5	Distribution of pigs and clinical signs observed in Experiment 2	54
6	Distribution of pigs and clinical signs observed in Experiment 3	59
7	Distribution of pigs and clinical signs observed in Experiment 4	66
8	Distribution of pigs and clinical signs observed in Experiment 5	83
9	Distribution of pigs and clinical signs observed in Experiment 6	88

LIST OF FIGURES

Figure		Page
1	Side view of flexible plastic film isolator used to house gnotobiotic pigs.	32
2	Surgical isolator floor after modification by installation of rigid fiberglass ring	32
3	Fiberglass ring being secured to the sow's flank by means of cotton cords	34
4	Allis tissue forceps being held out of the surgical field after they were applied to the incised plastic and the sow's skin.	36
5	Pig before removal from uterus. Uterine wall being incised with scissors	36
6	Pig G4480, 58 hours after exposure. Fibrinous to fibrinopurulent exudates cover the surfaces of abdominal viscera	62
7	Pig G4482, 75 hours after exposure. Polyserositis with inflammatory exudates on peritoneal, pleural and pericardial surfaces.	62
8	Pig G4481, 72 hours after exposure. Gastric serositis with fibrin, subserosal edema and inflammatory cells extending to involve outer muscularis (M). Hematoxylin and eosin. x 190.	64
9	Pig G4480, 58 hours after exposure. Subserosal edema, hyperemia, and leukocytic infiltration of spiral colon. Hematoxylin and eosin. x 190.	64
10	Pig G5228. Germfree control pig which weighed 2100 Gm. when it was euthanatized at 15 days of age (Experiment 4).	69
11	Pig G5226. Emaciated carcass of pig euthanatized 162 hours after oral exposure to <u>Escherichia coli</u>	69
12	Pig G5226. Polyserositis in the pig carcass illustrated in Figure 11. Note exudates on peritoneal, pleural, and epicardial surfaces	70

Figure		Page
13	Pig G5224, 120 hours after exposure. Mesenteric lymph node. Note absence of lymphoid follicles. Hematoxylin and eosin. x 75	72
14	Higher magnification of mesenteric lymph node in Figure 13. Note small number of lymphocytes, edema (a), and hyperemia (b). Hematoxylin and eosin. x 190.	72
15	Pig G5224, 120 hours after exposure. Superficial inflammation at hilus of spleen with serositis. Note mass of neutrophils (N). Hematoxylin and eosin. x 75	73
16	Pig G5224, 120 hours after exposure. Pancreas with focal parenchymal necrosis, interlobular edema and extension of serositis. Hematoxylin and eosin. x 75. . . .	73
17	Pig G5224, 120 hours after exposure. Hepatic serositis in proximity to gallbladder. Note hepatic parenchyma (a), edematous Glisson's capsule (b), and subserosal leukocytic infiltration (c). Hematoxylin and eosin. x 75.	74
18	Pig G5224, 120 hours after exposure. Duodenal serositis. Note subserosal edema and leukocytic infiltration	74
19	Normal duodenal mucosa from section in Figure 18. Hematoxylin and eosin. x 190.	75
20	Cuboidal mucosal epithelium with subepithelial edema also from section in Figure 18. Hematoxylin and eosin. x 470.	75
21	Pig G5224, 120 hours after exposure. Fibrinopurulent serositis of the mid-jejunum. Hematoxylin and eosin. x 75.	77
22	Pig G5227. Mid-jejunal section. Note single layer of serosal cells and the hydropic appearance of the mucosal epithelium in a germfree control pig 15 days of age. Hematoxylin and eosin. x 75	77
23	Pig G5224, 120 hours after exposure. Subserosal edema and serositis of the ileum. Leukocytic infiltration of the lamina propria is only slightly greater than in the controls. Hematoxylin and eosin. x 75.	78
24	Pig G5224, 120 hours after exposure. Fibrinopurulent pericarditis and epicarditis with adhesions between the pericardium (p) and epicardium (e). Hematoxylin and eosin. x 75	79

Figure		Page
25	Pig G5226, 162 hours after exposure. Suppurative myocarditis. Note neutrophils infiltrating the myocardium. Hematoxylin and eosin. x 470.	79
26	Pig G5224, 120 hours after exposure. Interstitial pneumonia with interlobular edema and neutrophilic infiltration. Alveolar septa are thickened but alveoli and bronchioles are free of cellular exudates. Hematoxylin and eosin. x 190.	80
27	Pig G5222, 94 hours after exposure. Synovitis of the elbow joint. Note fibrinous and cellular exudates on the synovial intima accompanied by intimal hyperplasia and subintimal edema with leukocytic infiltration. Hematoxylin and eosin. x 190.	81
28	Pig G5227. Histologic features of the synovial membrane from the elbow of a germfree pig. Hematoxylin and eosin. x 190.	81

INTRODUCTION

Enteric infectious diseases of swine constitute a major problem in swine production. The role of Escherichia coli in enteric infections of swine has been the subject of considerable discussion and has been investigated by several groups and individuals. Among the causes for the lack of certainty regarding the significance of E. coli in enteric diseases has been the cosmopolitan distribution of E. coli as a member of the intestinal flora in apparently healthy pigs and other species of warm-blooded animals. Another cause of confusion has been the difficulty encountered in attempts to determine which bacterial species constitute the "normal intestinal flora" of pigs. Serological grouping of E. coli strains isolated from cases of enteric disease has shown that certain serological groups or serotypes are more frequently isolated than other serotypes.

The development of techniques and equipment for rearing pigs germ-free, or with specifically known associates as gnotobiotics, has made it possible to investigate the effect of suspected enteropathogenic organisms in pigs in the absence of other bacteria or in association with certain known bacterial species.

The main objective of this study was to determine the effects of E. coli 083 on gnotobiotic pigs by exposing the animals orally to cultures of the organism and observing the clinical signs, the results of clinico-pathologic laboratory tests, and the gross and microscopic

lesions. Another objective was to determine the effect of anti-hog cholera serum on the course of the disease produced by this organism.

REVIEW OF LITERATURE

General Characteristics of Escherichia coli

Definition of Escherichia coli. Escherichia coli is a species of straight, Gram-negative, nonsporulating, rod-shaped bacteria of the genus Escherichia, tribe Escherichieae and family Enterobacteriaceae according to Breed, Murray and Smith (1957) in the 7th edition of Bergey's Manual. Many species of the family Enterobacteriaceae live in the intestines of man and other animals, frequently causing intestinal disturbances. Escherichia coli is usually a short, plump rod, but its form is variable under varying conditions and sometimes rather long filaments may be seen (Hagan, 1947). Hagan also wrote that Bacterium coli (E. coli) is

"...strictly parasitic in its habits and, therefore, is not found abundantly anywhere in nature except in intestinal tracts, in feces, and in materials which have been subjected to fecal pollution."

Classification of E. coli. Recommended procedures for biochemical differentiation and classification of E. coli were presented by Breed et al. (1957) and Edwards and Ewing (1962). Edwards and Ewing (1962) said that the E. coli group is composed of bacteria that give similar biochemical reactions but which may be separated into many different serotypes by means of serological methods.

Serological typing is based upon the varied and complex antigenic structure which is similar to that of Salmonella and Shigella. Kauffmann (1947) developed an antigenic scheme for serological differentiation or

serotyping of E. coli based upon his own previous work and that of Knipschildt and Vahlne. Since 1947, many investigators have studied E. coli serotypes (Edwards and Ewing, 1962), especially those associated with cases of infantile diarrhea, and as a result of their efforts, the E. coli scheme has been extended to include 145 O antigen groups, 86 K antigens, and 49 H antigens. There are 3 classes of antigens that are important in E. coli serology. Edwards and Ewing (1962) listed them as: (1) the O antigens, which are somatic antigens not inactivated by heat at 100 C. or 121 C.; (2) the K antigens, which are somatic antigens that occur as sheaths, envelopes or capsules and which act as masking antigens that inhibit O agglutination. The K antigens are inactivated by heat at 100 C. or 121 C.; (3) the H antigens or flagellar antigens which are inactivated by heat at 100 C. Typing of an unknown E. coli serotype is dependent upon the determination of its O, K, and H antigens. The K antigens are divisible into at least 3 varieties, L, A, and B antigens, which are tabulated by Kauffmann (1947, 1966) and Edwards and Ewing (1962). All of the K antigens inhibit agglutination of living enterobacteria in O antisera.

Pathogenicity of E. coli

The problem of pathogenicity of E. coli. Because of the cosmopolitan distribution of E. coli in the intestines of healthy animals (Hagan, 1947), it has been difficult to evaluate the significance of E. coli isolations from diseased animals. Hagan (1956) cited the work of Jensen (1903) who elucidated many of the facts regarding overwhelming E. coli septicemia, which he termed "colibacillosis" in colostrum-deprived calves. Many others have confirmed Jensen's findings and have studied

the coliform organism, endeavoring to learn whether they differed in any way from the same species found in normal animals. The earlier workers were unable to find any distinguishing characteristics of these "pathogenic" strains. McBryde (1934) reported that E. coli was recovered quite consistently in cultures taken from the mesenteric lymph nodes, kidney, spleen, and heart blood from 31 selected cases on a large garbage-feeding ranch in southern California where there was high mortality in pigs 2 to 5 days of age. From the bacteriological and histological studies, it appeared that the disease was the result of invasion by Bacterium coli (E. coli) which had assumed pathogenic and invasive properties.

Kauffmann (1947) indicated that there are certain serological E. coli types which possess a particular pathogenicity. Ewing (1956) said investigations in the past have made it clear that the diarrheal diseases of infants may be caused by a variety of microorganisms. In a large number of otherwise unexplained epidemics of diarrheal disease of infants, however, certain E. coli serotypes are recovered. The designation enteropathogenic E. coli has been suggested for these particular serotypes.

Strains of the organism, which have been freshly isolated from a diseased animal (Merchant and Packer, 1958), will produce death in guinea pigs, mice, rats, and rabbits within 48 hours. The animals die with a fibrinopurulent peritonitis and, in some cases, a septicemia. In the larger animals, the organism has been isolated from numerous tissues affected with disease. In some cases, it appears to be the only organism present, but, in many, it occurs as a secondary invader. Colibacillosis or diarrhea of baby pigs was described by Dunne (1958). In an analysis

of enteric conditions encountered in routine diagnosis, Roberts and Vallely (1959) found that hemolytic E. coli was a common inhabitant of the intestines of pigs of all ages. High concentrations of these organisms, usually found throughout the intestines, were specifically correlated with gastroenteritis, which occurred chiefly in 2- to 4-month-old pigs. The hemolytic E. coli serotypes in such cases were found to belong principally to 5 serotypes, whereas strains isolated in small numbers from the intestines in other diseases had much greater serological variation. Clinical and epidemiologic data implicated a disease syndrome resembling gut edema in many ways, but with edema as an infrequent post-mortem feature. An analysis of enteric conditions encountered in routine diagnosis underlined the importance of hemolytic E. coli in 2- to 4-month-old feeder-pig mortality, but indicated that "baby piglet enteritis" was more typically associated with nonhemolytic E. coli. Sojka, Lloyd and Sweeney (1960) also found that in very young piglets the strains isolated from enteritis were often nonhemolytic and that the commonest serotypes were E68 and G7.

Escherichia coli was considered significant by Saunders et al. (1960) in 58 outbreaks of disease causing high mortality in baby pigs. The syndrome was consistently associated with certain serotypes of E. coli and evidence was presented to suggest that they were of primary etiologic importance. They reported that lesions of acute hemorrhagic enteritis were not present, and they were unable to demonstrate the presence of clostridial toxin. Blood tests did not indicate anemia in the piglets, and vitamin A deficiency seemed very unlikely as most sows had grazed during pregnancy. There was no sign of hemolytic disease, and very few of the dams had been vaccinated against hog cholera with crystal violet vaccine.

Stevens (1963a) estimated that less than one-fourth of the porcine enteritis outbreaks are associated with known specific causes (such as swine fever, salmonellosis, transmissible gastroenteritis, inorganic toxicoses, or deficiency of vitamin A or iron) and that many of the remaining cases are likely to be associated with pathogenic E. coli serotypes. Lecce and Reep (1962) identified E. coli 08 as a pathogenic serotype in pigs which were kept under excellent environmental conditions.

In studies on the pathogenicity of E. coli, Namioka and Murata (1962) observed the effect on the organism of a substance obtained from pseudoeosinophils of rabbits. Obvious differences of sensitivity of various E. coli cultures to phagocytin's bactericidal effect allowed them to be divided into 2 groups. The behavior of the Salmonella organisms was quite similar to that of the resistant E. coli groups. Their data indicated that the organisms isolated from pathological materials had a tendency to resist the bactericidal action of phagocytin, whereas the cultures derived from apparently healthy animals did not.

Moon, Sorensen, and Sautter (1966a) reported that 4 E. coli serotypes isolated from newborn pigs with diarrhea were enteropathogenic as determined by their ability to cause ligated loops of the small intestine in the newborn pig to become distended with fluid. Gossling and Rhoades (1966) identified 2 serotypes "as possible specific etiologic agents of enteritis in baby pigs".

Host specificity of E. coli. Saunders et al. (1960) stated that it is now evident that E. coli strains are host-specific. Ingram and Lovell (1960) listed 3 serotypes originally isolated from scouring calves and subsequently from cases of piglet enteritis. Taylor (1961) reported that serotypes isolated from calf scours have not been found to affect

man or other animals; similarly, serotypes related to pig diseases appear to be concerned solely with this animal. Human pathogenic types have been isolated from domestic pets. Taylor listed serotypes 026, 0114, and 0126 as having been isolated from babies with infantile diarrhea and from calves with calf scours, but only those serotypes isolated from infants had the ability to cause dilatation of ligated rabbit gut loops.

Some Factors in Susceptibility to Enteric Disease

Age of host. Pigs are highly susceptible to E. coli infections only during the first few days of life (Dunne, 1958). Dillard (1964) stated that colibacillosis is the greatest disease hazard during the pig's first week, with the incidence being determined mainly by age of the host. However, Saunders et al. (1960) reported that the disease sometimes started in 3-week-old pigs and progressed to the younger ones.

Colostrum deprivation. Lecce, Matrone and Morgan (1961) stated that colostrum fed to piglets is rich in antibodies (gamma globulin) to coliform organisms. Lecce and Reep (1962) found that specific pathogen-free (SPF) pigs, deprived of colostrum and reared in isolation, often developed diarrhea when 3 days old and sometimes half of the litter was lost within 2 weeks. Eighty-five per cent of the bacteremia and death correlated with E. coli isolations from blood or liver occurred in pigs fed diets lacking bovine and porcine gamma globulin. Agglutinins for E. coli 08 and other E. coli serotypes were demonstrated in bovine and porcine colostrum by tube agglutination tests. These agglutinins were practically absent from porcine and bovine gamma globulin.

Immunologic behavior of baby pigs. In studies on the electrophoretic mobility of serum protein of colostrum-deprived baby pigs, Bauriedel et al. (1954) showed that there was no measurable gamma globulin at birth and that none was present before 6 weeks of age.

Baby pigs under 8 weeks of age, deprived of colostrum, did not produce a measurable serologic response to the Brucella abortus bacteria and ovine RBC antigens used by Hoerlein (1957). Baby pigs that received normal (nonimmune) colostrum responded to antigens when inoculated at 3 weeks of age. This response increased markedly to 6 weeks of age. Passive transfer of antibodies through "immune" colostrum interfered with active antibody production between 3 and 6 weeks of age. Segre and Kaeberle (1962) reported that colostrum-deprived pigs could form tetanus, but not diphtheria antibodies at birth. Colostrum-fed pigs formed diphtheria antibodies after intraperitoneal inoculation of a toxoid at birth, but their antibody response to tetanus toxoid was depressed. The results were interpreted as evidence that a degree of transplacental transfer of maternal antibody took place and that the sow's blood contained anti-tetanus antibodies, but not antidiphtheria antibodies. Myers and Segre (1963) used a double-diffusion agar precipitation system against anti-swine gamma globulin serum of rabbit origin to demonstrate the presence of gamma globulin in the blood of newborn, colostrum-deprived pigs.

Miller et al. (1962) found that Salmonella pullorum antibodies acquired by newborn pigs fed serum from hyperimmunized swine were retained in measurable concentration for as long as 7 days, but not for 17 days. Colostrum-derived S. pullorum antibodies reached a peak in the pigs' serum at 24 hours of age and diminished logarithmically thereafter

with a half life of 4.7 days. Kaeberle and Segre (1964) administered porcine, ovine, and equine globulin concentrate by oral or intraperitoneal routes to colostrum-deprived pigs within 12 hours of birth. With the exception of the intraperitoneally administered bovine globulin, the homologous and heterologous globulins were eliminated at logarithmic rates. Elimination of heterologous globulin at a logarithmic rate and the absence of detectable antiglobulin were accepted as evidence that immunologic tolerance had been induced.

Concurrent disease. McBryde (1934) suggested that inbreeding and the presence of brucellosis in the sows had contributed to the birth of weak pigs highly susceptible to colibacillosis. However, one of the criteria for selection of the 58 outbreaks of disease investigated by Saunders et al. (1960) was the absence of any identifiable disease other than E. coli infection.

Environmental factors. Gordon and Luke (1960) found that gastroenteritis was not always associated with poor sanitation. Lamont, Luke and Gordon (1950) discussed the effects of chilling and the relationship of housing to the health of the pig in general. Dunne (1958) recommended ample dry bedding, elimination of drafts, provision of heat lamps in chilly weather, clean dry pens, and a good sow ration, in control of colibacillosis. Sojka (1965) assumed that predisposing factors must play an important role in the development of enteritis. These predisposing factors are largely unknown and may be of infectious or noninfectious nature, but they may be of prime importance in initiating disease.

Clinical Signs, Clinicopathologic Findings, and Necropsy Findings

Fever, depression of appetite, and dullness. Saunders et al. (1963a) reported that fever did not occur in their "pathogen-free" piglets experimentally infected with E. coli E68 Type I, but the body temperatures were often subnormal. Carpenter (1965) and Smith, Conant, and Overman (1964) reported that the response of experimental animals to endotoxin (lipopolysaccharide from Gram-negative bacteria) is complex. Among the reactions to a single injection listed by Carpenter were: fever, hypothermia in some species; leukopenia, followed by leukocytosis; thrombocytopenia; hyperglycemia followed by hypoglycemia; impeded phagocytosis by reticuloendothelial cells; disturbances of the reactivity of terminal blood vessels; peripheral vasoconstriction and vasomotor shock. Depression of appetite might be expected as a part of the listlessness and dullness described by Dunne (1958) in pigs with colibacillosis.

Diarrhea. Colibacillosis, white scours, diarrhea neonatorum, or diarrhea of baby pigs, is an acute, sometimes highly fatal disease of suckling pigs characterized by diarrhea with yellowish-white, watery feces and often accompanied by septicemia (Dunne, 1958). Within the first few days after birth, pigs become listless and develop diarrhea with watery, yellowish-white feces having a fetid odor. Infected animals lose weight rapidly and become very weak. The death rate of infected untreated pigs varies but may be as high as 100%. Saunders et al. (1960) accepted a high incidence of diarrhea and/or mortality in the herds as one of their criteria for investigation of E. coli infection in piglets. Smith and Jones (1963) reported a 10^2 to 10^4 increase over the usual number of

E. coli in the small intestines of newborn pigs with E. coli infection, and bacteremia was absent in the diarrheal disease.

Moon et al. (1966b) concluded that E. coli infection of the newborn pig was most frequently a diarrheal disease without bacteremia or morphologic evidence of enteritis. The E. coli associated with the diarrheal syndrome were of different serotypes than those associated with the generalized infections encountered in their study.

Hematologic and serologic alterations. Saunders et al. (1960) reported that blood tests did not indicate anemia in the piglets with E. coli infection, and there was no sign of hemolytic disease. Roy et al. (1959) investigated the effect of white scours on the sodium and potassium concentration in the serum of newborn calves. Sodium and potassium values from those calves that died from E. coli septicemia were similar to those of calves that scoured, but recovered. Serum sodium values of calves that died with localized intestinal E. coli infection were similar to the sodium values of calves that died from E. coli septicemia. However, serum potassium values of calves dying with localized intestinal E. coli infection increased until death. The authors discussed the possibility of cardiac arrest being caused by increased serum potassium.

Enteritis and gastroenteritis. Enteritis in pigs 2 to 5 days of age was reported by McBryde (1934) and in very young piglets by Sojka et al. (1960). Gordon and Luke (1960) described their investigation of an acute gastroenteritis affecting piglets as young as 6 hours to a few days of age in which the same serotype was involved in 5 of 7 farms. The sows remained normal, the pigs scoured badly, mortality was heavy, and sometimes whole litters died. On some farms the disease started

in 3- to 4-week-old pigs and progressed to the younger ones. E. coli was recovered from all levels of the gastrointestinal tract but rarely from the viscera. Baby piglet enteritis, associated with E. coli infection, was included in a report of investigations by Roberts and Vallely (1959). Kernkamp (1950) noted that gastrointestinal disorders in which coliform organisms are an etiologic factor are not manifested at necropsy by diphtheritic exudates and necrosis that reach deeper than the epithelium. In acute E. coli infection in suckling pigs, post-mortem examination revealed catarrhal inflammation of the stomach and intestine and reddening, with swelling, of the intestinal mucous membrane (Glässer, Hupka and Wetzell, 1950, cited by Sojka, 1965).

Bacteremia and septicemia. McBryde (1934), in examination of 31 selected cases, reported that in every case in which enteritis had developed in 2- to 5-day-old pigs, cultures showed invasion by B. coli (now E. coli). Dunne (1958) noted that E. coli can usually be isolated from the parenchymal organs of pigs which have died following severe diarrhea or which have been killed in a moribund condition. Jubb and Kennedy (1963) mentioned that the infection is septicemic and the organisms can be isolated from all tissues. The intestinal tract seems to be the constant site of infection, but other viscera may be invaded in the terminal stages of disease (Stevens, 1963b).

Polyserositis and arthritis. The term "polyserositis" was used by Roberts, Switzer, and Ramsey (1963a) to refer to peritonitis, pleuritis, pericarditis and arthritis which are frequently observed in young swine at necropsy. Lecce (1960) reported on porcine polyserositis with arthritis in SPF pigs which were transferred at 8 weeks of age from isolation

cages to a pig farm where they became infected with a pleuropneumonia-like organism and Hemophilus influenzae suis. Polyserositis was caused by Pasteurella multocida, Hemophilus suis, and/or Mycoplasma hyorhinis, according to Roberts et al. (1963a, 1963b).

Pneumonia, pleuritis and peritonitis may be present as complications in colibacillosis (Dunne, 1958) and abscessed joints sometimes occur as a secondary infection after the disease appears to have abated. Jubb and Kennedy (1963) stated that E. coli infection is septicemic and localization can occur in meninges, joints and lungs. Localizations may accompany the enteric phase, but their manifestations are usually delayed. Also, the bacteria may enter through the umbilicus to localize in these sites.

Edema disease and anaphylaxis. Edema disease usually occurs in weanlings or weaned pigs, but occasionally may be seen in any pigs (Schofield and Davis, 1955). According to Lamont et al. (1950), so-called "gut oedema" in the pig was first recognized in Northern Ireland in 1932. Shanks and Lamont (1938) described the condition as "an unusual condition affecting the digestive organs of the pig". The symptomatology and post-mortem findings of gut edema were described by Lamont et al. (1950). They indicated that edema of the stomach wall between the muscular coat and the mucosa was the most consistent post-mortem finding. The folds of the mesentery supporting the spiral coil of the colon was another common site of edema which was noted as having occurred in 1 pig only 1 week old and was restricted to the mesentery without involvement of the wall of the colon. Subcutaneous edema, extending along the belly, has been noted in many cases. Edema of the eyelids was also

reported as a frequent lesion and the ears, throat region, and elbow and hock joints were sometimes edematous.

Sojka, Erskine and Lloyd (1957) stated that there was evidence of specific serotypes of E. coli associated with edema disease, thus suggesting a toxin is produced by E. coli. The serotypes appear to be noninvasive and are not common in pigs except with edema disease.

Lemcke, Bellis and Hirsch (1957) gave antiserum to pigs affected with gut edema and encountered a peculiar hypersensitivity which required that the pigs be desensitized to prevent anaphylaxis. The sensitivity of the pigs was shown to be specific to the E. coli antibodies. Thomlinson and Buxton (1963) reported the production of active anaphylactic shock using egg albumin, and reversed passive anaphylaxis using E. coli (0138) in pigs. The signs and lesions closely resembled those of edema disease and hemorrhagic gastroenteritis. Catarrhal enteritis was also observed. There was a relationship between the character of the lesions which were produced and the severity and duration of the anaphylactic symptoms. Chopra, Blackwood, and Dale (1964) injected cell-free extracts of E. coli 0139:K92(B) and produced edema disease in pigs raised in disease-free and conventional environments. The severity of clinical signs was proportional to the amount of extract administered. Two injections, administered 48 hours apart, produced signs of the same magnitude on both occasions, which is in contrast to what would be expected if edema were caused by a hypersensitivity reaction.

Prevention of Disease

In a treatise on coliform infections in the young pig and a practical approach to the control of enteritis, Stevens (1963b) gave a general description of a coliform enteritis syndrome in baby pigs and suggested

that the disease arises when there is a lack of balance between passive immunity and certain strains of E. coli. His recommendations for control of enteritis in piglets a few days old encompassed 3 general areas: (1) reducing the numbers of E. coli; (2) increasing the immunity; and (3) improving the management. Thorough disinfection of farrowing houses will reduce the numbers of E. coli present. Hysterectomy techniques have offered the promise of total elimination of some pathogens. These could include "pathogenic" serotypes of E. coli, but the ability of the organisms to survive for long periods outside the host and in the soil makes continuous freedom unlikely. The use of antibiotics is the most frequent method attempted for reducing the number of E. coli, but it may cause other procedures to be ignored. Dosing by mouth is preferable to injection (Stevens, 1963b; Kohler and Bohl, 1964), because the action of the E. coli is believed to be largely in the intestines, and some drugs, given by injection, fail to produce high concentration in the intestines. Stevens considered intramuscular injection of one gram of oxytetracycline into the sow just prior to farrowing as wasteful and unreliable because a comparatively small proportion of the antibiotic finds its way into the milk. Also, it is not always easy to time the injection to be just prior to farrowing.

For increasing the immunity of the dam and the antibody content of the sow's colostrum, there should be advantage in keeping a closed herd (Stevens, 1963b) and allowing new gilts or sows to mix with the older sows for several weeks before farrowing. Gordon and Luke (1958) reported that an autogenous bacterin, administered to the dams during gestation, protected the pigs. But Jones, Sellers and Smith (1962) failed to reduce the incidence of scours in litters born subsequent to vaccination of the sows with living and dead autogenous vaccines.

The use of specific immune serum (Dunne, 1959) has proved effective in the treatment of diarrhea caused by E. coli infection in baby pigs. Lecce and Reep (1962) found that gamma globulin in the diet was apparently important in preventing disease only in the first 2 days when the neonatal pig was still absorbing large molecules from the gut. They suggested that gamma globulin might function by promoting the localized inhibition of the bacteria in the gut, by inhibiting the stray bacteria that passed the first line of defense and by influencing the rapid maturation of the piglets' serum protein profile. Switzer, Preston and Morrissey (1962) administered 10 ml. of commercial swine serum concentrate orally by stomach tube and another 5 ml. subcutaneously, plus 2 ml. of a polyvalent Cl. perfringens antitoxin to SPF pigs to substitute for colostrum in biological protection of the pigs.

Management factors contributing to the apparent increase of the baby piglet enteritis syndrome during the last decade were listed by Stevens (1963b) as the increase in intensive pig breeding, the use of large farrowing houses, and the births of a rapid succession of litters providing a regular supply of susceptible hosts. McBryde (1934) recommended improved sow rations and elimination of disease in the sows to prevent the birth of weak pigs. Dunne (1958) recommended treatment of the umbilicus with iodine at the time of birth in controlling joint infection. Stevens (1963b) recommended good creeps with warmth and protection for the pigs, control of agalactia and "farrowing fever" in the sows and good pre-farrowing rations to include vitamin A and iron. Stevens advocated a water supply for the baby pigs since a pig with diarrhea is adversely affected by dehydration. Parenteral injection of glucose and electrolyte solutions may save some sick pigs.

Experimental Reproduction of Disease in Pigs

In naturally reared pigs. Saunders et al. (1963b) were successful in experimentally reproducing a naturally occurring syndrome which was described by them in 1960 as being associated with certain serotypes of E. coli and characterized by diarrhea and death in piglets under a week of age. They found it necessary in view of their results to modify their methods with each succeeding experiment and to devise means of limiting cross infection of the controls. Large numbers of freshly isolated organisms of the 2 most common E. coli serotypes from typical outbreaks were given orally to newborn colostrum-deprived piglets which were the offspring of gilts. There was positive correlation between the time of appearance of the test serotype in rectal swabs and the onset of diarrhea which usually followed exposure by 12 to 24 hours. Body temperatures were never elevated, but often subnormal. All 21 newborn piglets exposed to the 2 different serotypes, designated G7 and E68 Type I, scoured and 11 of them died. In the fatal infections the pigs continued to eat until near death in spite of obvious illness, and death frequently occurred within 3 days of exposure. In those pigs which survived, disappearance of the test serotype from rectal swabs was usually followed by clinical recovery. The clinical course and gross lesions at necropsy in all 5 experiments closely resembled those of field cases. Post-mortem bacteriological recovery of the test organisms in profuse pure culture, not only from the intestines, but frequently from other viscera, revealed the septicemic nature of the infection with a propensity for infection of the brain.

Saunders et al. (1963b) undertook an additional experiment to assess the effect of giving a "non-pathogenic" strain designated P50 to 7 piglets. At 12 to 24 hours after the pigs were orally exposed, serotype P50 appeared in profuse pure culture in the rectal swabs of the 7 inoculated pigs, but there was no diarrhea. Post-mortem examination of one piglet which died suddenly at 34 hours after exposure without having scoured revealed fibrinous peritonitis, congestion of the lungs and gastroenteritis. The results of bacteriologic examination suggested a fatal septicemic infection. No other evidence of pathogenicity was displayed by serotype P50, and the gross lesions were not typical of cases of E. coli infection observed in the field. Serotype P50 had not spread to the control group of piglets and was rapidly displaced in the exposed group following accidental infection by E68 Type I. The controls also became infected with E68 Type I and all pigs died within 2 days of first excreting that organism and the onset of diarrhea. E68 Type I was recovered from most visceral and intestinal sites of 5 piglets. Two piglets in each of the experiments 5 and 6 (Saunders et al., 1963b) were allowed to take colostrum before dosing but did not exhibit increased resistance to infection. All piglets which survived to the end of each experiment at 7 to 15 days were killed and blood was collected for serological examination. The 26 serum samples were all negative on the rapid slide test for bacterial agglutinating antibodies to the respective serotype used.

In pathogen-free pigs. The term "pathogen-free" or specific pathogen-free (SPF) pigs is used to designate pigs which have been obtained by hysterectomy or cesarotomy and prevented from nursing the sow. These

colostrum-deprived pigs are then reared in an environment designed to prevent the infection of the pig with specifically known pathogenic viruses or bacteria such as the agents causing atrophic rhinitis and virus pig pneumonia.

In reporting their investigations of E. coli associated with colostrum-free neonatal pigs raised in isolation, Lecce and Reep (1962) indicated that E. coli strain 08 was a part of the "normal" fecal flora in their piglet population and was also the strain most frequently isolated in bacterial cultures of blood or liver from moribund or dead pigs. In a litter of healthy colostrum-deprived, gamma globulin-free pigs raised in isolation, E. coli serotype 08 was identified by Lecce and Reep in 17% of the fecal cultures taken from the 9 pigs at 4 and 8 days of age. When the same pigs were 2 weeks of age, they were fed an inoculum of 10^9 E. coli 08, but did not develop signs of disease. Agar immunoelectrophoresis of 2 of the piglets' sera taken at the time of feeding the E. coli indicated that gamma globulin was not present at 2 weeks but was present by 3 weeks of age. At serum dilutions of 1:10 and 1:40, blood serum samples taken at 2, 3 and 6 weeks of age were negative for agglutinins to the 08 isolate fed. Lecce and Reep suggested that resistance to infection was not dependent upon antibody (gamma globulin) except during the first 2 days of a pig's life when it is capable of absorbing large molecules from the gut.

Saunders et al. (1963a) experimented with pigs obtained by hysterectomy, deprived of colostrum and artificially reared in individual incubators in strict isolation. They were able to avoid outside contamination in their study of the effects of feeding a pathogenic serotype (E68 Type I) of E. coli at varied dosage rates to pigs of different ages.

Also, they studied the effect of giving a pathogenic serotype 6 days after feeding a supposedly nonpathogenic serotype. The experimental pathogen-free pigs of Saunders et al. were equally susceptible to disease whether the pigs were 2 or 12 days of age at the time of oral inoculation with E. coli E68 Type I in doses ranging from 10^3 to 10^8 bacteria. In their experiments disease production by invasion of a pathogenic serotype was not prevented by the previous introduction of a nonpathogenic E. coli. Two experiments in which colicinogenic E. coli (C18) were established in pigs weaned at 10 and 11 days of age showed that the predominant hemolytic strains (E4 and E57) were not suppressed by the colicinogenic strain (Tadd and Hurst, 1961).

Saunders et al. (1963a) found that the disease experimentally produced in their artificially reared animals did not differ in any essential respect from disease produced by the same E. coli serotypes in naturally reared animals. Saunders and his associates considered that their experiments clearly indicated the value of pathogen-free piglets for this type of research.

Artificial Rearing of Pigs

Dietary need for colostrum and hexose sugars. Early workers attempting to rear pigs artificially from birth (Bustad, 1948) on a synthetic milk diet containing all known vitamins found that colostrum, or plasma or serum as a substitute for colostrum, in the diet was essential for the pigs' survival. Without the colostrum or the colostrum substitute in their synthetic milk diet for the first day of life, the pigs developed severe diarrhea and died shortly after birth. A diet of casein, lard, sucrose, minerals and vitamins was used successfully by Wintrobe (1939)

for rearing pigs weaned at 2 to 23 days of age. Pigs allowed access to colostrum for 24 hours were unable (Becker et al., 1954) to utilize sucrose as a source of carbohydrates. When sucrose was replaced with glucose as the source of carbohydrate in the synthetic diet, satisfactory results were obtained. Pigs weaned at a minimum of 24 hours (Johnson, James, and Krider, 1948) were reared on a diet containing casein, cere-lose, lard, salt and vitamins.

Function of colostrum. Young and Underdahl (1949) used a hemagglutination-inhibition test to demonstrate the absence of antibodies for swine influenza in the serum of newborn pigs prior to ingesting colostrum and the appearance of those antibodies in the pigs' serum 30 minutes after obtaining colostrum by nursing. In addition to proving the colostral transfer of hemagglutinin inhibitors (antibodies) for swine influenza virus from dam to offspring, they also showed that the HI titer in pig serum continued to increase to 6 hours of age, but there was very little increase after that time, although the colostral content of antibody remained high. Tiselius and Kabat (1939) demonstrated by electrophoresis that bacterial antibody in pig serum was contained in the gamma globulin fraction. Hoerlein (1957), Rutqvist (1958), Lecce et al. (1961), Miller et al. (1962) and Segre and Kaeberle (1962) are among the investigators who have confirmed the function of colostrum in the transfer of antibody or gamma globulin to the newborn pig. Evidence has accumulated (Young and Underdahl, 1949; Speer et al., 1959; Lecce et al., 1961; Miller et al., 1962) to indicate that there is a cessation of gamma globulin absorption from the pig's intestines after the first 24 hours.

Diets and techniques for rearing colostrum-deprived pigs. Young and Underdahl (1951) described a diet and technique for starting pigs without colostrum. Young and Underdahl (1951) and Hoerlein (1957) recommended protective isolation of the colostrum-deprived pigs to prevent their exposure to infections which may be fatal to them although they may be enzootic or inapparent in pigs that have had colostrum. Morrill (1952) showed that fatal hypoglycemia in fasting newborn pigs was delayed by elevating the environmental temperature from 60 to 90 F. Young and Underdahl (1951) collected pigs in sterile bags at birth and fed them a diet based on cow's milk in an isolated room where the temperature was maintained at 80 to 90 F. Young, Underdahl and Hinz (1955) reported a technique for procurement of baby pigs by hysterectomy from full-term sows. Improvements in apparatus, technique and diet were described by Underdahl and Young (1957a, 1957b). Among other changes, they kept the isolation units at 90 to 100 F. during the first few days and then gradually lowered the temperature. The diet for starting the young pigs consisted of 1 quart of homogenized milk, 1 whole egg and 5 ml. of a mineral mixture containing ferrous sulfate, copper sulfate, manganese chloride and potassium iodide. At one week of age, the pigs were transferred to an isolation brooder large enough to hold 12 pigs. The starting diet was continued and gradually changed to a commercial pig starter. The pigs were transferred at 4 weeks of age to a clean area. These "disease-free" pigs were used to repopulate herds where atrophic rhinitis and virus pneumonia of pigs had been problems (Young et al., 1959).

Whitehair and Thompson (1956) took pigs by hysterotomy rather than hysterectomy and reared the cesarean-derived pigs in individual metabolism cages on a diet composed of casein, fat, lactose, minerals and vitamins. More recently, commercially prepared diets for rearing SPF pigs have become available (Waxler, Schmidt, and Whitehair, 1966; Betts, Lamont, and Littleworth, 1960).

Saunders et al. (1963a) obtained pathogen-free pigs by hysterectomy after the methods reported by Betts et al. (1960). Betts and his associates had procured pathogen-free, colostrum-deprived pigs by hysterectomy using the techniques which had been developed by Young and Underdahl (1953) and Young et al. (1955). The sows were stunned electrically and then anesthetized with carbon dioxide gas at 110 to 113 days of gestation. The entire uterus was removed aseptically and transferred through an antiseptic lock into a sterile operating hood. The piglets were removed from the uterus by 2 operators working through long rubber gloves. The operators tore open the uterus, removed the pigs, ligated the umbilical cords and dried the pigs. The pigs were reared in sterile incubators supplied with a source of heated, filtered air. The diet was a commercially available milk substitute dissolved in water and sterilized by heat. Betts et al. (1960) reported a survival rate of 82.3% of the pathogen-free colostrum-deprived pigs they obtained in 39 litters.

Germfree (Gnotobiotic) Research

Terminology. The problem of terminology for the expanding discipline of research with germfree life forms was discussed by Reyniers et al. (1949) and their recommendations were published in Lobund Reports No.

2, "The Need for a Unified Terminology in Germfree Life Studies". They suggested the term "gnotobiotics", which was derived from two Greek words (gnos, known and bios, life), as a designation for the field of investigation concerned with growing living organisms by themselves or in association with other known organisms. The term "gnotobiote" may then be used to refer to an animal whose bacterial flora is known, and gnotobiotics may be designated as germfree, monocontaminated, dicontaminated, or in some other classification based upon the number of species of bacteria with which the gnotobiote shares its environment.

Chronology of development of germfree research

Theoretical phase. According to Reyniers (1959), a definite date for the first pure culture concept is not established but must have preceded the recognition that microbes might be involved in the health and disease of man and animals. The first reported direct experimentation on germfree life was done by Duclaux, who reported in 1885 on his attempts to grow peas and beans as pure cultures in a sterile medium. Pasteur commented in the same journal in his introduction to Duclaux's work that he thought microbes were necessary to the life of animals and that animal life without microbes would probably be impossible. Reyniers speculated that Duclaux's work might have been forgotten except for the controversy stirred by Pasteur's remarks. Pasteur's negative philosophy was supported by the experiments of Schottelius (Gordon, 1959) at the turn of the century.

The school of supporters of the feasibility of germfree life was headed by Nencki who, in 1886, attacked Pasteur on theoretical grounds (Gordon, 1959). Nuttall and Thierfelder in 1895 and 1896 sought to

prove that Nencki was right by means of the first experiments on germ-free mammals. Metchnikoff (1903) supported the philosophy that the host may be unfavorably affected by substances produced by various normal intestinal flora, and Cohendy (1912) successfully reared germfree chickens of remarkable quality.

Basic research phase. Gordon (1959) stated that Schottelius fed inadequate diets to his germfree chickens as did Nuttall and Thierfelder to their guinea pigs, and little information was gained in terms of the effects on the body of the missing bacterial flora. However, each investigator contributed much to the methodology of rearing germfree animals. Kuster reared germfree goats in 1914 and proved that the weight gain of the germfree animals was not due to retention of water only, but represented true growth. Glimstedt (1936) found moderate inhibition of development in various organs and reduced amount of lymphoid tissue in approximately 30- and 60-day-old germfree guinea pigs. The difference from organs and tissues of normally reared guinea pigs was greatest in organs that are normally in close association with the intestinal flora.

Instrumentation of basic and applied research. With the exception of the Lobund group at Notre Dame (Reyniers, 1959), there were only 8 investigative attempts to use higher animals in germfree research between 1895 and 1950. Early research in the field of gnotobiotics was directed toward solving the academic question of whether life and growth were possible for animals in the germfree condition. To progress from the theoretical phase to one of basic and applied research in gnotobiotics required the development of dependable apparatus (Reyniers, 1959) and

techniques for procuring germfree animals and maintaining them germfree from birth to old age to death. The apparatus developed by Küster is the basis for most of that now used for rearing and studying of germfree higher animals (Trexler, 1959). Gustaffsson (1959) described the light-weight germfree rearing systems and instrumentation for rearing germfree vertebrates used at the University of Lund, Lund, Sweden, and Miyakawa (1959a) described the remote controlled germfree rearing unit developed by Miyakawa and associates in Japan. Reyniers (1959) reported on the design and operation of germfree systems developed and standardized in use over a period of 25 years at the Lobund Institute, University of Notre Dame, Notre Dame, Indiana. The adaptation of plastic flexible film apparatus for the rearing and use of germfree animals was described by Trexler and Reynolds in 1957. Waxler (1961) reviewed the development of techniques and equipment and the use of germfree animals in gnotobiotic investigations from 1885 through 1960. In most instances (Waxler et al., 1966), efforts to rear mammals free of demonstrable microorganisms have been limited to the smaller laboratory animals, although Smith (1961) reared germfree lambs and goats and Reyniers (1942) reported the rearing of a germfree monkey.

Gnotobiotic pigs as laboratory animals

Technique for rearing gnotobiotic pigs. The swine industry derived much benefit from the SPF pig programs which provided a means of interrupting the disease cycle for some specific diseases and supplying pigs for herd repopulation (Waxler et al., 1966). While the SPF and "disease-free" pigs were satisfactory for disease control through repopulation and for some research purposes, other phases of research required the rearing

of pigs under even more carefully controlled conditions to reduce the number of variables encountered in such things as studying the action of specific infectious agents. Landy et al. (1961) discussed the use of large germfree animals in medical research. Meyer, Bohl and Kohler (1964) published an account of procurement and maintenance of germfree swine for microbiological investigations. The technique, equipment and diet developed by Waxler (1961) made it possible for him to rear gnotobiotic pigs to ages of 17 to 52 days in the presence of not more than 2 isolates of bacteria. His results indicate that the technique can be used in producing gnotobiotic pigs for use in research. Two problems which persisted at the end of the experiments were: (1) determining the proper time to perform the hysterotomy to obtain strong, healthy pigs and (2) development of a diet adequate not only for supporting life and slow growth rates, but one which will produce weight gains comparable to those of pigs reared by the sow.

Characteristics of gnotobiotic pigs. The need for characterization of germfree animals exists and it can be justified in at least 3 ways according to Gordon (1959). First, there is a need to determine whether the available germfree animals are truly the long-anticipated standardized biological tool. Secondly, parameters should be established to supply some standards for comparison of morphological and physiological differences between conventional and germfree stock which occur as a result of absent bacterial stimuli. Thirdly, the effects on the host caused by the environmental presence of the normal bacterial flora should be clarified.

Wostmann (1959) reported decreased gamma globulin in the electrophoretic serum protein profile of rats, mice, chickens and rabbits, while gamma globulin was completely lacking in germfree guinea pigs. The data of Wostmann indicated the presence of circulating antibodies to protozoan and helminthic parasites in the alpha and beta fractions of serum protein in rats and mice. These data seemed to be paralleled in the case of bacterial antibodies and the gamma fraction also showed an increase with a lag time of at least 2 weeks following bacterial invasion.

Wagner (1959) reported that antibacterial agglutinins were absent from the serum of rats and young germfree chickens long after conventional rats and chickens had developed positive titers. Glimstedt (1936) and Miyakawa (1959b) found the lymphoid tissues to be less developed in germfree guinea pigs than in conventional guinea pigs. Thorbecke (1959) concluded that such differences are only quantitative and not qualitative. Thorbecke and Benacerraf (1959) found that blood clearance of intravenously injected E. coli was slower in germfree mice than in immunized germfree, conventional control and immunized control mice. Further, they concluded that qualitative differences regarding development of lymphoid tissue, serum gamma globulin levels and phagocytic indices for bacteria may become apparent in germfree animals only after it becomes possible to rear the animals not only free of demonstrable living bacteria, but also free of antigenic stimuli from dead bacteria and other antigenic substances.

After reviewing the literature concerning the histology of the lymphatic system and the liver of the pig, Waxler (1961) found only quantitative histologic differences of the lymph nodes in germfree pigs as compared with those of pigs raised under farm conditions. There were

no significant differences in the histology of the spleen of various groups of pigs reared artificially or naturally, but artificially reared pigs appeared to have more iron-containing pigment in the spleen. The hepatic interlobular septa, which are prominent fibrous bands in the farm-raised pigs, were often absent or reduced to a few reticular fibers in the germfree pigs.

Schmidt (1961) investigated the hematologic characteristics and the morphology of the intestinal tract in germfree pigs. Again, only quantitative differences were found between the germfree pigs and naturally raised pigs.

MATERIALS AND METHODS

Experimental Animals

The pigs utilized in this series of 6 experiments were all sired by the same purebred Yorkshire boar and were collected by cesarotomy on the 112th day of gestation, or later, from related purebred Yorkshire sows and gilts. Medroxyprogesterone acetate* was added to the feed at the rate of 500 mg. per day for the sows in Experiments 2 through 5 for periods of time ranging from 9 to 26 days immediately prior to the scheduled date for cesarotomy procedures.

Equipment and Technique for Procuring and Rearing Gnotobiotic Pigs

Equipment. The isolator systems used in this work consisted of 60 x 60 x 180 cm. flexible plastic film isolators, each equipped with 1 pair of shoulder-length gloves on each side; an air lock at 1 end to permit exchange of supplies, pigs, and accessory items; and a filtering system for the exchange of bacteria-free forced air under slight positive pressure (Figure 1). The preparation and sterilization of the equipment and techniques for rearing gnotobiotic pigs were described by Waxler et al. (1966).

The surgical isolator was modified by installing a rigid fiberglass ring 30 cm. in diameter by 10 cm. in length in the center of the plastic

* Repromix, The Upjohn Company, Kalamazoo, Mich.

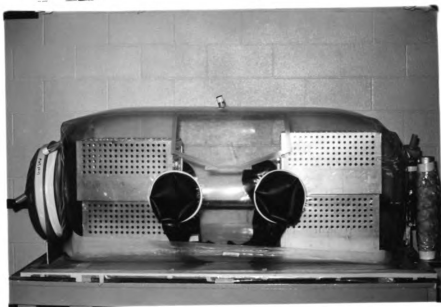


Figure 1. Side view of flexible plastic film isolator used to house gnotobiotic pigs.



Figure 2. Surgical isolator floor after modification by installation of rigid fiberglass ring.

film forming the floor of the isolator (Figure 2). Holes of 3 mm. diameter were drilled at 5 cm. intervals around the periphery of the fiberglass ring 5 mm. ventral to its uppermost edge. Sterilized rubber bands were looped through the holes to anchor Allis tissue forceps during the surgical procedures. Three complete turns of plastic film tape served to fasten the fiberglass ring in the opening provided for it in the center of the floor of the isolator, as described by Waxler et al. (1966). The lower end of the ring was covered with a cap of 20 mil vinyl plastic which formed the only area of contact between the sow's skin and the plastic isolator when it was sealed to her flank in preparation for surgery. The ring was further secured in place by means of 3 cotton cords passed beneath the sow and anchored to 3 eyelets provided in each side of the flange (Figure 3).

Surgical procedures. On approximately the 112th day of gestation the sows or gilts were scrubbed with water and detergent. For preoperative sedation promazine hydrochloride solution* was injected intravenously at the rate of 1 ml. per 100 pounds estimated body weight and 20 to 25 ml. of 2.5% procaine hydrochloride solution** were injected epidurally at the lumbosacral articulation. When the epidural anesthesia had become effective, the sow was placed on the operating table surface with her left side uppermost and elevated to working height for completion of preoperative preparation.

* Sparine, Wyeth Laboratories, Cleveland, Ohio.

** Epidural, Haver-Lockhart, Kansas City, Mo.



Figure 3. Fiberglass ring being secured to the sow's flank by means of cotton cords.

The hair was shaved from the surgical site and adjacent surrounding skin. The shaved area was scrubbed 3 times with a detergent solution* and rinsed with water, dried with sterile towels after the 3rd rinse, and subjected to 3 alternating floodings of 70% alcohol and phenylmercuric nitrate solution. In Experiments 5 and 6, chloroform was added as a final cleansing and defatting rinse before the surgical site was sprayed with a sterile adhesive compound.** The surgical procedure was continued according to the method of Waxler et al. (1966). When the bottom of the isolator and the sow's skin were incised with electric cautery, Allis tissue forceps were applied at approximately 2 cm. intervals to hold the incised edges of the plastic and the skin in close apposition (Figure 4). The handles of the Allis tissue forceps were secured in the rubber bands at the edge of the fiberglass ring to prevent their obstructing the field of surgery. Only one man of the team working from opposite sides of the surgical isolator actually performed the laparotomy and lifted the pigs individually but still within the uterus into the surgical isolator (Figure 5). The second member of the veterinary surgical team refrained from touching the gloves of the other operator and anything else that might have come into contact with the skin of the sow. Scissors were employed to incise the uterus and placental membrane over the elevated pig from its head to midway of its back. As the incised uterus and placental membranes retracted ventrally along the sides of the pig, the

* Liquid Germicidal Detergent, Parke, Davis and Company, Detroit, Mich.

** Vi-Hesive, Aeroplast Corp., Dayton, Ohio.

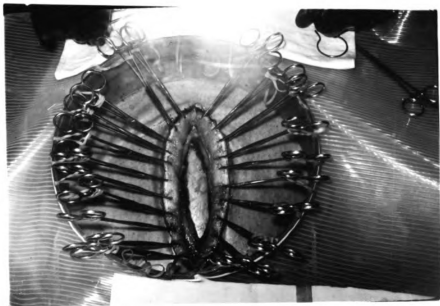


Figure 4. Allis tissue forceps being held out of the surgical field after they were applied to the incised plastic and the sow's skin.



Figure 5. Pig before removal from uterus. Uterine wall being incised with scissors.

assisting surgeon was able to grasp the pig without touching the uterus, lift the pig from the uterus, and apply 2 hemostatic forceps to the umbilical cord. He then severed the cord between the forceps and passed the pig into the attached receiving isolator where umbilical ligatures were applied and the hemostatic forceps removed by 2 additional assistants working at the receiving isolator. All other surgical procedures were according to the method described by Waxler et al. (1966). As soon as the surgical procedures and identification by ear notching of the pigs was completed, the receiving isolator was separated from the surgical isolator and used to transport the pigs to the rearing room.

Rearing technique and diet. In the rearing room, the pigs were distributed in approximately equal groups among 3 isolators where they were kept individually in stainless steel cages and reared according to established methods (Waxler et al., 1966). The pigs in one isolator served as controls and the pigs in the other isolators served as principals in these experiments. The pigs were weighed at 24 hours of age. The diet consisted of a liquid milk substitute* which was supplemented with 5 ml. of sterilized 50% dextrose solution at each feeding during the first 3 days. The pigs were fed 3 ounces SPF Lac 5 times daily until the 4th day, when the feedings were reduced to 4 times daily and the volume increased to 4 ounces per feeding.

In Experiments 5 and 6 a commercial anti-hog cholera serum was added to the first feeding for 6 pigs in each experiment. In Experiment 5 the first feeding at 5-1/2 hours after the birth by hysterotomy

* SPF Lac, Borden Co., New York, N.Y.

consisted of 50 ml. anti-hog cholera serum and 2 ounces of the liquid diet with 5 ml. of 50% dextrose solution for each of 6 pigs. On the third day, the liquid diet was increased to 5 ounces per feeding. In Experiment 6, the first feeding to 6 pigs at 4 hours of age was 25 ml. of anti-hog cholera serum plus 2 ounces of liquid diet and 5 ml. of 50% dextrose solution, while the other 6 pigs received 3 ounces liquid diet and 5 ml. 50% dextrose solution but no anti-hog cholera serum.

Bacteriologic procedures. Rectal swabs for bacteriologic cultural determinations were collected from the pigs at birth and 3 times weekly thereafter. Three tubes of thioglycollate medium* were inoculated for culture at 25, 37, and 55 C. Then the swabs were streaked directly onto 5% bovine blood agar* plates and eosin-methylene blue* (EMB) plates for incubation aerobically at 37 C. The culture mediums were examined twice daily for evidence of bacterial growth. When growth occurred in the liquid mediums they were subinoculated onto blood agar and EMB plates, then final cultural examination and determinations were made. In Experiments 5 and 6, the use of thioglycollate liquid medium was discontinued in favor of brain-heart infusion (BHI) broth,* and all cultures of rectal swabs were incubated only at 37 C. The blood agar and EMB plates were incubated for 72 hours and brain-heart infusion tubes for 1 week. Slides were prepared according to standard methods from thioglycollate liquid medium or BHI broth and from typical discrete colonies on the blood agar and EMB plates and were stained by Gram's method and examined at high dry and oil immersion magnifications by transmitted light microscopy.

* A Bacto dehydrated base for each of the mediums was obtained from Difco Laboratories, Detroit 1, Mich.

Clinical Observations, Clinicopathologic Examinations and Necropsy Procedures

Clinical observations. Body temperatures were recorded daily from rectal thermometer readings from all pigs. Observations of appetite, physical activity and general appearance of the pigs, and the character of the feces were recorded daily or at each feeding when changes were observed.

Clinicopathologic examinations. Blood samples were collected 3 times weekly in volumes of 3 to 5 ml. from the anterior vena cava according to the method of Carle and Dewhirst (1942). One milliliter of blood from each pig was put into a vial containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and the remaining volume of blood was put into a vial without anticoagulant. Hematologic examinations of specimens from the nonclotted blood were according to the methods of Benjamin (1961) for micro-hematocrit determination of packed cell volume of erythrocytes (PCV), cyanmethemoglobin estimation of hemoglobin (Hb.) and total and differential leukocyte counts. Examinations of serum samples were according to methods described by Frankel and Reitman (1963) in Gradwohl's Clinical Laboratory Methods and Diagnosis and included the biuret method for determination of total serum proteins, paper electrophoresis* for quantitative determination of serum albumin and globulins, and sodium and potassium determinations by flame spectrophotometry.**

* Beckman-Spinco paper electrophoresis, Duostat Power Supply, and Analytrol Automatic Densitometer, Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.

** Coleman Model 21 Flame Photometer, Coleman Instruments, Inc., Maywood, Ill.

The serums from pigs in Experiment 6 were examined in tube agglutination tests for O agglutinins to E. coli 083 somatic antigens. Porcine antiserum to E. coli 083 was obtained from the blood of 2 conventionally reared and weaned pigs immunized with O antigens prepared according to the method of Campbell et al. (1963). Polystyrene latex particles* were used experimentally as indirect indicators to improve the sensitivity and visibility of the tube agglutination test as described by Singer and Plotz (1956) and Muraschi (1958).

Necropsy procedures. Necropsy of each pig was done as soon as possible after death whether death occurred as the result of experimental infection or euthanasia. External appearance of the carcass was noted before a midventral skin incision was made from the mandibular symphysis to the posterior pelvic region. The skin and limbs on each side were dissected from the trunk and reflected laterally. In all experiments except Experiment 1, a flame-heated spatula was used to sear the exposed subcutaneous tissues covering the elbow and stifle joints preparatory to incising the joint capsules with a flamed scalpel. Sterile inoculating loops were inserted deeply into the opened joints to contact the synovial surfaces and then withdrawn to streak identified areas on prepared blood agar and EMB plates. The pleural and peritoneal cavities were similarly sampled for bacteriologic examination, then the cavities were fully opened and the pericardial cavity exposed for bacteriologic examination.

* Bacto-Latex 0.81 (Code 3102), Difco Laboratories, Detroit, Mich.

The viscera were observed for lesions and, in some cases, were photographed. The organs were then removed, and blocks of tissue no more than 0.5 cm. thick were collected from the mandibular, superficial inguinal, prefemoral and mesenteric lymph nodes; adrenal glands; pancreas; spleen; liver; midportion of the kidneys; urocyt; cardiac, fundic and pyloric regions of the stomach; 5 equidistant levels of the small intestine; cecum; spiral colon; terminal colon; heart; lungs. Rarely, sections were taken from the elbow and/or stifle joints. The tissues were fixed in 10% neutral buffered formalin, retrimmed and processed for histopathologic examination according to procedures outlined in the Manual of Histologic and Special Staining Technics of the Armed Forces Institute of Pathology, Washington, D.C. (1957). Paraffin-embedded tissues were sectioned at 7 microns thickness, mounted on glass slides, stained with hematoxylin and eosin, and coverslipped for histopathologic examination. In the 6th experiment, blocks of liver tissue were fixed in Carnoy's solution for subsequent staining with Best's carmine and examination for glycogen.

Preparation of Infective Agent and Exposure of Pigs

The original culture of E. coli 083 was isolated from a pig which developed pericarditis and died at the Veterinary Research Farm at Michigan State University. Serially transferred cultures were maintained on nutrient agar* slants stored at 25 C. and were serotyped in 1963 at The University of Minnesota** and again in 1965 at The Pennsylvania State

* Nutrient Agar 1.5% (B69), Difco Laboratories, Detroit, Mich.

** Bacterial cultures serotyped as 083 (Minnesota #344) by Dr. Harley Moon, The University of Minnesota, St. Paul, Minn.

University.* Inoculums for the pigs were prepared by inoculating screw-capped vials containing 5 ml. quantities of thioglycollate liquid medium or 5 ml. of brain-heart infusion broth with organisms picked from at least 3 typical colonies on 5% bovine blood agar which had been inoculated from the storage tubes and incubated for 24 hours aerobically at 37 C. The inoculated thioglycollate and BHI liquid mediums were incubated at 37 C. for 24 hours in Experiments 2, 5 and 6 and 36 hours in Experiments 1, 3 and 4. The inoculums were transferred after incubation into the isolators containing the principals.

Gnotobiotic pigs of different ages in separate experiments were fed varied volumes of the cultures of E. coli mixed in their liquid diet. The sex distribution and mean 24-hour body weights of the controls and principals, age of the pigs at exposure to E. coli, volume of inoculum fed, culture medium and length of time the inoculum was incubated, and the types of clinicopathologic examinations conducted in each experiment are given in Table 1.

* Bacterial cultures serotyped as O group 83 (083:K.:HNM) by courtesy of Dr. Paul J. Glantz, The Pennsylvania State University, University Park, Pa.

Table 1. Distribution of pigs, conditions of oral exposure to Escherichia coli, and clinicopathologic examinations employed in each experiment.

Exp. No.	Controls		Principals		Anti- serum fed	Bac- teria in Age of environ- ment at exposure	Inoculum			Clinicopathologic examinations						
	No.	Sex	Wt. (kg.)	No.			Sex	Wt. (kg.)	Vol.	Incubated (hr.)	Cul- ture medi- um	Hema- to- log- ic	Serum elec- tro- lytes	Serum elec- tro- phor- esis	Latex agglu- tina- tion test	Bacteri- ologic examina- tion at necropsy
1	1	M	1.0	4	M	1.0	No	<u>Staph.</u> <u>sp.</u>	72	5.0	36	Thio*	Yes	No	No	Yes
	2	F		2	F											
2	1	M	1.1	3	M	1.3	No	<u>Staph.</u> <u>sp.</u>	69	5.0	24	Thio	Yes	Yes	No	Yes
	1	F		3	F											
3	0	M	0.8	2	M	0.9	No	<u>Staph.</u> <u>sp.</u>	72	1.25 or 5.0	36	BHI**	Yes	No	Yes	Yes
	3	F		5	F											
4	0	M	0.9	1	M	0.9	No	None	148	5.0	36	BHI	Yes	Yes	No	Yes
	2	F		5	F											
5	1	M	1.4	4	M	1.3	Yes	<u>Bacillus</u> <u>sp.</u>	98	2.5 or 5.0	24	BHI	Yes	Yes	No	Yes
	2	F		4	F											
6	3	M	1.0	5	M	1.1	Yes	<u>Bacillus</u> <u>sp.</u>	120	5.0	24	BHI	Yes	Yes	Yes	Yes
	1	F		2	F											

* Thio - Thioglycollate liquid medium

** BHI - Brain-heart infusion broth

RESULTS

Experiment 1

The details of the pigs' identification numbers, sex and body weight of the principals and controls with clinical signs of illness and mortality are tabulated (Table 2). Bacteriologic examination of the pigs at birth revealed no bacterial contamination, but monocontamination of pigs in all 3 isolators with Staphylococcus sp. was detected in culture of rectal swabs collected when the pigs were 72 hours of age and immediately prior to their exposure to E. coli. Escherichia coli was recovered from the principals in culture of rectal swabs collected at 20 hours post-exposure.

One of the controls, Pig 5, had been weakened by loss of blood when the umbilical ligature loosened. The pig remained weak and maintained a subnormal body temperature until it died at approximately 84 hours of age. Hypothermia was recorded for principal Pigs 1, 2, and 3 at 24 hours of age and body temperatures became more depressed after exposure to E. coli. Pig 3, in Isolator 1, and Pigs 7, 8, and 9, in Isolator 3, all developed fevers following exposure to E. coli. The body temperatures are given in Table 3.

Clinical signs of illness in the principals included listlessness, fever, rough hair, partial to complete anorexia, and moderate diarrhea.

Table 2. Distribution of pigs and clinical signs observed in Experiment 1.

Iso-lator No.	Pig No.	Necropsy No.	Sex	Body wt.** (kg.)	Fever	Leuko-penia	Leuko-cytosis	Anorexia	Diarrhea	Death	Gross lesions at necropsy	le-Postexposure survival time (hr.)
2	4*	G2632	F	1.00	-	-	-	-	+	-	-	(Euth.) 99
2	5*	G2584	M	1.04	-	-	-	+	-	+	+	12
2	6*	G2633	F	0.95	-	-	-	-	-	-	-	(Euth.) 99
1	1	G2583	M	1.04	-	+	-	+	-	+	+	45
1	2	G2582	F	0.82	-	+	-	+	-	+	+	46
1	3	G2608	M	1.04	+	+	+	+	+	+	+	60
3	7	G2630	F	1.09	+	+	+	+	+	+	+	94
3	8	G2631	M	1.04	+	+	+	-	+	-	+	(Euth.) 99
3	9	G2622	M	1.00	+	+	+	+	+	+	+	84

* Control pigs

** Pigs were weighed within 24 hours of birth

Euth. Pigs euthanatized at termination of experiment

- = Condition was absent

+ = Condition was present

Table 3. Body temperatures (°F.) of pigs in Experiment 1.

Iso- lator No.	Pig No.	Pre-exposure (hr.)	Postexposure			
		52	24	44	72	93
2	4*	100.0	99.4	101.0	101.6	101.4
2	5*	97.0	**	---	---	---
2	6*	101.6	102.0	101.2	100.8	102.0
1	1	98.0	97.8	96.6	Dead	---
1	2	96.0	95.2	94.0	Dead	---
1	3	98.0	99.0	105.0	Dead	---
3	7	100.6	102.6	106.4	106.0	98.8
3	8	100.4	100.2	102.5	104.0	106.0
3	9	99.0	100.2	100.6	105.2	Dead

* Control pigs

** Died at 84 hours of age after being weakened by blood loss

Hematology. The hemograms are shown in Table 4 together with the pigs' body temperatures at the time the blood samples were collected. The arithmetic means of blood values for all 9 pigs at 24 hours of age were:

Hemoglobin (Gm./100 ml.)	7.9
Packed cell volume (%)	27.7
Corrected total leukocytes ($\times 10^3/\text{cu. mm.}$)	5.49
Differential leukocyte count (%)	
Segmented neutrophils	46.5
Nonsegmented neutrophils	7.8
Lymphocytes	41.0
Monocytes	4.4
Basophils	0.2
Eosinophils	0.0
Nucleated erythrocytes (/100 leukocytes)	1.7

At 40 hours postexposure the 2 hypothermic principals had leukopenia, which was essentially absolute neutropenia, and the other 4 principals had leukocytosis, which represented both relative and absolute neutrophilia and lymphocytosis. The lymphocytosis persisted in the 2 surviving principals at 93 hours postexposure, but neutrophilia was changed to severe absolute neutropenia (Table 4).

Electrophoretic examination of serum samples collected terminally from pigs 4, 6, and 8, prior to euthanasia, resulted in poor separation of the beta and gamma globulin fractions, but the albumin and globulins were well separated and were calculated as being in the ratios of 0.56:1, 0.74:1, and 0.19:1, respectively.

Necropsy findings. There were dried brownish feces on the tail and perineal area of one of the controls, but the other control was entirely free of signs of diarrhea. There were no grossly visible inflammatory exudates in the serous cavities. The spleens were smaller and much brighter red than in the principals. Each of the stomachs contained

Table 4. Hemograms of surviving pigs and body temperatures of pigs at the time blood samples were collected in Experiment 1.

Time in hours relative to exposure	Pig No.	Body tempera- ture (°F.)	Hb. (Gm./ 100 ml.)	PCV (%)	Corrected total WBC	Corrected differential leukocyte count							
						Seg.	Neutrophils		L	M	B	E	NRBC**
							NS						
50 before exposure	4*	100.0	6.7	24.0	4417	42	5	48	5	0	0	3	
	5*	97.0	8.6	27.5	6699	66	6	24	4	0	0	3	
	6*	101.6	8.6	31.0	6633	47	10	33	10	0	0	1	
	1	98.0	8.0	27.5	5150	48	6	45	1	0	0	0	
	2	96.0	8.0	30.0	3932	40	3	53	4	0	0	3	
	3	98.0	7.7	24.5	4117	55	7	33	5	0	0	2	
	7	100.6	6.7	25.0	5098	48	12	36	4	0	0	2	
	8	100.4	6.7	24.5	8118	34	16	43	5	2	0	1	
	9	99.0	10.4	35.5	5250	39	5	54	2	0	0	0	
44 after exposure	4*	101.0	8.9	30.5	5817	49	13	36	0	1	1	4	
	5*	Dead	---	---	---	---	---	---	---	---	---	---	
	6*	101.2	10.4	33.5	6250	32	6	58	3	0	1	0	
	1	96.6	8.6	24.0	3271	3	1	84	11	0	1	7	
	2	94.0	10.1	29.5	2251	12	0	82	6	0	0	4	
	3	105.0	6.7	24.0	14504	24	22	45	9	0	0	1	
	7	106.4	8.0	25.5	15700	21	36	33	9	0	0	0	
	8	102.5	8.0	25.0	15882	42	22	35	1	0	0	2	
	9	100.6	10.1	32.0	12050	59	13	27	1	0	0	0	
93 after exposure	4*	101.4	8.6	31.0	7772	51	12	36	1	0	0	1	
	6*	102.0	11.0	35.0	7864	52	10	37	1	0	0	3	
	7	98.8	7.7	25.0	5666	1	0	97	1	0	1	5	
	8	106.0	7.1	21.0	5450	1	1	92	6	0	0	11	

* Control pigs

** Seg = segmented, NS = nonsegmented, L = lymphocytes, M = monocytes, B = basophils, E = eosinophils, NRBC = nucleated erythrocytes.

approximately 2 ounces of milk curd. The livers were slightly yellow, the gallbladders were not edematous, and all other organs were without visible lesions.

Pig 2 died 45 hours after exposure to E. coli. Grossly, the skeletal muscles were pale, and the prefemoral lymph nodes were edematous. There were several milliliters of serosanguineous fluid in the pleural cavity and serofibrinous fluid in the peritoneal cavity. The liver was faintly yellow, and the spleen was enlarged and dull red. The stomach was distended with gas but contained no food. There was hyperemia of the mesenteric lymph nodes and the small intestine, especially in the upper jejunum 2 to 5 feet posterior to the pylorus. The kidneys were pale, and the urinary bladder was faintly petechiated on the serous and mucous surfaces.

Pig 1 died 46 hours after exposure, and the lesions were similar to those in Pig 2. However, there was a small amount of milk curd in the stomach and a small amount of fluid in the small intestines, which were more edematous than hyperemic. There was marked subserosal edema of the spiral colon.

There were dried feces on the tail and rear legs of Pig 9, which died 84 hours postexposure. Edema or serous inflammation involved the muscles and subcutis of the posterior ventral abdominal wall and the associated lymph nodes. There was severe serofibrinous inflammation of the peritoneal, pleural, and pericardial membranes. Fibrin covered the epicardium, and the myocardium was pale. There were scattered focal areas of emphysema in the lungs with some congestion and with consolidation of the ventral parts of the apical lobes. Fibrinous adhesions occurred between the visceral and parietal layers of the pleura. The

spleen was enlarged and dark. The liver was enlarged, dark red and faintly mottled, but the margins of the lobes were thin. The gallbladder was edematous. There was excess mucus but no food in the stomach. The abdominal viscera were firmly adherent to each other due to a heavy coating of fibrinopurulent exudate. The mesenteric lymph nodes were edematous. The small intestine was hyperemic and the contents were more fluid than in the 3 principals previously examined. The colon and rectum contained yellow, mucinous, viscous, nongaseous fluid. There was apparent diffuse petechiation of the cortical surfaces of the pale kidneys. The urinary bladder was empty and slightly thickened.

Lesions observed in principals 3, 7, and 8 were similar to those described for Pig 9, with the exceptions that the gallbladders were not edematous and one stomach contained some milk curd. Experiment 1 was terminated at 99 hours postexposure with euthanasia of Pigs 8, 4, and 6.

Bacteriologic findings. Post-mortem bacteriologic examination of the controls revealed no growth in mediums inoculated from the peritoneal and pericardial cavities, but Staphylococcus sp. grew on the blood agar plate subinoculated from the primary culture of a rectal swab from Pig 6.

Escherichia coli was recovered from the principals from all sites sampled, which included the peritoneal cavity and rectal swab from Pig 7 and peritoneal and pericardial cavities and rectal swab from Pig 8.

Histopathologic findings. The tissues from Pigs 4 and 6 appeared to be normal, but the other control (Pig 5), which died at 3 days of age, had edema in all lymph nodes. Lymphoid tissue in the controls was not dense, and secondary nodules were absent. The lymphoid tissue was composed of plasma cells, lymphocytes and medium-sized to large reticuloendothelial

cells with some mitoses. There were a few foci of medium-sized dark lymphocytes, but small dark lymphocytes were infrequent. Myeloid elements, which were mostly polymorphonuclear neutrophils (PMN) with some eosinophils, were found in clusters or diffusely scattered between the lymphoid nodules and, sometimes, mixed with cells in the lymphoid nodules.

The mandibular lymph nodes from the principals were characterized by mild edema, faint follicular pattern, infrequency of small dark lymphocytes, and increased granulocytes, which were primarily neutrophils with a few prominent eosinophils. There was a fibrinoid substance in the mandibular lymph node sinuses of Fig 9.

The lesions in the mesenteric lymph nodes were edema, hyperemia and depletion of lymphocytes, with increased prominence of eosinophils and neutrophilic granulocytes, some of which were necrotic. The superficial inguinal and prefemoral lymph nodes were similar to the mesenteric lymph nodes.

The adrenal glands were not hyperemic, except in Fig 3, which had fibrinous to fibrinopurulent serositis of the adrenal capsule, and in Fig 8, which was similarly affected and had focal hemorrhages in the capsule. The pancreatic parenchyma was essentially normal, although there were lesions of serositis which were most severe at the hilus. There were no significant lesions in any of the thyroid gland sections.

Lymphoid tissue of the spleens resembled that of the lymph nodes. The Malpighian corpuscles were poorly developed and usually consisted of 2 or 3 concentric layers of lymphocytes. Serositis of varying severity was found in all the splenic sections but did not appear to penetrate into the parenchyma.

Hepatic lobular outlines were more distinct than in the controls and perilobular connective tissue was not demonstrated by the hematoxylin-eosin stain. There were scattered foci of hematopoiesis in the hepatic sinusoids. Serositis of Glisson's capsule was of varying severity, and the relative composition of fibrinous and leukocytic elements in the exudate also varied. The cytoplasm of the hepatocytes was usually lightly stained and the cellular outline was often indistinct. Lesions less frequently seen in the liver sections were: scattered focal necrosis, hemorrhage, and some fatty metamorphosis. In one liver section there was moderate edema with neutrophils surrounding the cholecyst.

The most common renal lesion was cloudy swelling of the epithelium of the convoluted tubules. Fibrinopurulent serositis was seen in 4 pigs and there was subserosal extension of the inflammation at the renal hilus in one section, but the pelvis was free of cellular exudates. There was fibrinopurulent serositis of the urinary bladder in 5 principals and one pig had a few discrete submucosal hemorrhages. There were no lesions of urocystitis in the bladder lumens.

There were lesions of serositis of varying severity and character in some sections from the stomach of each of the principals, but there were no lesions of severe mucosal hyperemia, hemorrhage, necrosis, or leukocytic infiltration in the gastric sections. There was an apparent increase of mucous secretion.

Duodenal serositis was seen in 5 principals. The inflammation penetrated subserosally in one pig and through the muscularis into the submucosa in another pig. Fibrinopurulent serositis was seen in most of the sections from 3 levels of the jejunum, and hyperemia of the mucosa and submucosa was seen in approximately 50% of the sections. There was

fibrinopurulent serositis of the ileum in sections from 3 pigs. Ileal enteritis was manifested in one pig by leukocytic infiltration of the lamina propria, neutrophilic granulocytes in the lacteals of some villi, and increased eosinophils in the lymphoid tissue. Lesions of fibrinopurulent serositis were features of the cecum in 4 pigs, of the spiral colon of 3 pigs, and of the terminal colon of 5 pigs. Subserosal edema of the spiral colon was severe in 2 pigs.

Pericarditis and epicarditis were fibrinous in one pig and fibrinopurulent in 2 pigs. Pleuritis varied from fibrinous to fibrinopurulent in 3 pigs, and in one pig there were interlobular fibrinopurulent exudates in the lung and interstitial pneumonia with septal thickening. One of 3 pigs with focal infiltration of neutrophils in the mesenchymal stroma of the umbilicus had developed a microabscess with early peripheral fibroplasia.

Experiment 2

Information concerning distribution of the controls and principals, their identifying numbers, sex, and 24-hour body weights is included with clinical signs in Table 5. Bacteriologic examination of rectal swabs collected from the pigs at 48 hours of age revealed Staphylococcus sp. in pure culture from 7 of the 8 pigs and no bacterial growth from the remaining pig. Rectal swabs collected 24 hours after exposure yielded E. coli from all the principals.

Hematology. No pattern was established in the hemograms of blood samples collected 1, 4, and 6 days after the pigs were exposed to E. coli. Severe neutropenia (neutrophils - 3 and 13%, lymphocytes - 97 and 87%, corrected total leukocyte counts - 24,260 and 22,850) was coexistent

Table 5. Distribution of pigs and clinical signs observed in Experiment 2.

Iso-lator No.	Pig No.	Necropsy No.	Sex	Body wt. (kg.)	Fever	Leuko-penia	Leuko-cytosis	Anorexia	Diar-rhea	Death	Gross lesions at necropsy	le-Postexposure survival time (hr.)
2	4*	G3695	M	1.13	-	-	-	-	-	-	-	(Euth.) 236
2	6*	G3696	F	1.04	-	-	-	-	-	-	-	(Euth.) 236
1	1	G3533	F	1.45	+	-	-	+	+	+	+	96
1	2	G3534	F	1.09	+	-	+	+	+	+	+	124
1	3	G3531	M	1.27	+	-	-	+	+	+	+	72
3	5	G3557	M	1.23	+	-	+	+	+	+	+	162
3	7	G3697	F	1.59	+	-	-	-	+	-	+	(Euth.) 236
3	8	G3532	M	1.09	-	-	-	+	+	+	+	72

* Control pigs

Euth. Pigs euthanatized at termination of experiment

- = Condition was absent

+ = Condition was present

with lymphocytosis in samples from Pigs 2 and 5, respectively, approximately 100 hours after exposure. The controls and principals had very similar hemoglobin and erythrocyte PCV values in each day's blood samples.

Fever and other clinical signs. The maximum body temperature recorded for either of the controls was 103.0 F. Five of the 6 principals had at least one temperature reading of 104.0 F. or above, with 106.8 F. being the maximum. The feces were noticeably more fluid in the principals than in the controls from the first postexposure day onward. Decreased appetite seemed to be associated with pain or stiffness and reluctance to move rather than with decreased alertness or fever, as some of the principals would not arise when fed and would protest when lifted but would then eat a part or all of the feeding.

Serum electrolytes in 28 samples were found to be essentially the same in both groups of pigs. Sodium values in flame photometry ranged from 131 to 152.5 milliequivalents/liter for the principals and in the controls the range was from 135 to 151 mEq./liter. Serum potassium increased with age of the pigs. The extremes were 4.35 and 8.45 mEq./liter, with the highest concentration being from one of the controls. Separation of the serum protein fractions in electrophoresis was not sufficient to permit interpretation of the results.

Necropsy findings. There were no gross lesions in the controls. Signs of diarrhea, cachexia, enlarged external lymph nodes, and fibrinous to fibrinopurulent polyserositis were constant findings in the principals. Inflammatory edema of the ventrolateral abdominal areas was extensive in 3 pigs and a fourth pig had subcutaneous edema of the shoulder, neck

and head regions. It also had swollen leg joints with excess fluid in the synovial sacs. Splenic enlargement was present in all principals. Hepatic enlargement or congestion and mesenteric lymph node edema and/or hyperemia were associated lesions in 4 pigs. There was mild catarrhal gastritis, and 3 pigs had milk or curd filling their stomachs. Hyperemia of the small intestine was seen in only 1 pig. The small intestinal contents were scanty yellow fluid and similar material of greater quantity filled the cecum and colon. The cecum of one pig was distended with gas, but the feces were much alike in all the principals. Petechiation of the renal cortical surfaces was apparent in the principals but not in the controls. One principal pig had polycystic kidneys and the urinary bladder contained about 10 ml. of viscous urine. In the male pigs there was extension of the peritonitis into the scrotal sac. Escherichia coli was recovered in culture of the 3 serous cavities and the humeroradial synovial sacs from all the principals, including one that was euthanatized with electricity when it was seriously ill but not yet moribund.

Histopathologic findings. Lymphoid tissue from the controls was more dense than in the pigs from Experiment 1, but secondary nodules were still not seen. Granulocytes were prominent in the lymph nodes and in the spleens. There were some foci of hematopoiesis in the spleen and liver sections. No lesions were seen in the gastric tissues. The duodenum had a small number of granulocytes in the lamina propria and mitoses in the mucosal epithelium. There was hyperemia of the mucosa in the upper, middle and lower levels of the jejunum. The epithelial cells of villi in the lower section of the jejunum appeared hydropic

but were not desquamating, and there were very few leukocytes in the lamina propria. Peyer's patches in the ileum were composed of nondense lymphoid tissue and there were small numbers of granulocytes in the ileal stroma. The cecum, spiral colon, and terminal colon presented no unusual features. Other organs were also without lesions, except there appeared to be some mild hyperemia of the adrenal cortex.

The principals had a tendency toward suppurative lymphadenitis in addition to the lesions of edema, hyperemia, and depletion of lymphocytes viewed in tissues in Experiment 1. Fibrinous to fibrinopurulent serositis was found in most of the sections of the viscera. Congestion of the hepatic sinusoids was found in sections from 5 pigs and 2 sections had edema around the gallbladders. Mild leukocytic infiltration was found in one pancreatic section.

Sections from the stomachs of the principals had lesions of mild to severe serositis but only one section had signs of inflammation of the muscularis. The mucus formed a thicker layer on the mucosa in the principals than in the controls. There was mucosal necrosis in one duodenal section and inflammation of the muscularis in another, but the remainder appeared to have only serositis. Two sections of the upper jejunum were hyperemic and a third section had edema and leukocytic infiltration of the muscularis. Pig 8 had lesions of enteritis consisting of hyperemia, edema, mixed leukocytic infiltration of the lamina propria, and necrosis of the mucosal epithelium, especially at the tips of the villi in the middle and lower jejunum and ileum. Leukocytic infiltration of the lamina propria in the middle and lower jejunum in the other pigs was mild or absent, but catarrhal changes of increased mucous secretion and mucosal hyperemia were found in some of the sections. Two sections of the ileum

had necrosis of the mucosal epithelium with leukocytic infiltration of the lamina propria and some necrosis of Peyer's patches. The outer muscularis of the ileum was affected by serocellular inflammation which appeared to be an extension of the serositis. Mitoses were frequent in the ileocecal lymphoid tissue in one section and necrosis was evident in another section. Submucosal and subserosal edema was mild, but fibrinopurulent serositis was severe in 2 sections. Severe fibrinopurulent serositis with subserosal edema and some leukocytic infiltration were common lesions of the spiral colon and the terminal colon. Heart and lung lesions resembled those in Experiment 1. Pig 7 had acute exudative synovitis of the elbow and stifle joints.

Experiment 3

Bacteriologic examination of rectal swabs from 11 pigs at birth revealed no bacterial growth after 72 hours' incubation but the original germfree status of the pigs was converted to monocontaminated when Staphylococcus sp. grew in cultures of rectal swabs taken from the pigs at 48 hours of age. The pigs in Isolator 1 (Table 6) were fed 5 ml. of the E. coli inoculum at 72 hours of age, and the pigs in Isolator 3 were fed only 1.25 ml. of the inoculum. Both groups of principals were positive for E. coli in culture of the rectal swabs collected the next day.

Although 11 pigs were transferred into the receiving isolator, only 10 pigs were distributed among the isolators in the rearing room. Pig 2 had escaped from the uterus into the peritoneal cavity of the sow during the surgical proceedings and it died about one hour afterward. Pigs 7 and 9 were weak and had poor appetites; one of them had lost a large volume of blood from loosening of the umbilical ligature.

Table 6. Distribution of pigs and clinical signs observed in Experiment 3.

Iso-lator No.	Pig No.	Necropsy No.	Sex	Body wt. (kg.)	Fever	Leuko-penia	Leuko-cytosis	Anorexia	Diar-rhea	Death	Gross lesions at necropsy	le- Postexposure survival time (hr.)
2	5*	G4484	F	0.91	-	-	-	-	-	-	-	(Euth.) 144
2	6*	G4485	F	0.77	-	-	-	-	-	-	-	(Euth.) 144
2	7*	G4476	F	0.77	-	-	-	**	-	+	-	None
1	1	G4479	F	0.95	+	-	-	+	-	+	+	58
1	3	G4481	F	0.91	+	-	-	+	+	+	+	72
1	4	G4478	M	0.82	+	-	-	+	+	+	+	34
3	8	G4482	F	0.91	-	-	-	+	+	+	+	75
3	9	G4477	F	0.95	-	-	-	**	+	***	+	10
3	10	G4483	F	0.91	-	-	-	-	+	-	+	(Euth.) 144
3	11	G4480	M	0.69	-	-	-	+	+	+	+	58

* Control pigs

** These pigs were weak and had poor appetites from birth until death.

Euth. Pigs euthanatized at 9 days of age

- = Condition was absent

+ = Condition was present

Fever and other clinical signs. The clinical signs are tabulated with the details of distribution of the pigs in Experiment 3 (Table 6). Body temperatures of 104.0, 105.6, and 106.3 F. were observed in Pigs 4, 1, and 3, respectively, approximately 12 hours before they died. Blood samples from all 10 pigs 24 hours before exposure of 7 pigs and from all 8 remaining pigs 21 hours after the principals were exposed had only minor changes in individual and group values. The mean hemogram values for the 10 pigs at 48 hours and 8 pigs at 93 hours of age were:

	Age	
	48 hours	93 hours
Hemoglobin (Gm./100 ml.)	8.18	6.79
Packed cell volume (%)	26.50	24.25
Total leukocytes ($\times 10^3$ /cu.mm.)	7.68	7.30
Differential leukocyte counts (%)		
Segmented neutrophils	65.10	45.75
Nonsegmented neutrophils	11.2	26.0
Lymphocytes	22.3	26.6
Monocytes	1.5	1.4
Basophils	0.0	0.0
Eosinophils	0.0	0.25
Nucleated erythrocytes (/100 leukocytes)	1.6	2.62

The hemogram values for the one surviving principal at the next blood sampling and the mean values for the 2 remaining controls 6 days after exposure were:

	Controls	Principal
Hemoglobin (Gm./100 ml.)	10.2	9.2
Packed cell volume (%)	33.0	28.0
Total corrected leukocytes ($\times 10^3$ /cu.mm.)	6.65	9.80
Differential leukocyte counts (%)		
Segmented neutrophils	46.5	36.0
Nonsegmented neutrophils	5.5	15.0
Lymphocytes	46.0	49.0
Monocytes	0.5	0.0
Basophils	0.5	0.0
Eosinophils	0.5	0.0
Nucleated erythrocytes (/100 leukocytes)	3.0	0.0

Serum electrolytes were not determined in this experiment. Lack of separation of the serum proteins in electrophoresis produced highly erratic results which are not presented here.

Necropsy findings. No gross lesions were found in the controls and all their body cavities were negative for bacterial growth on culture. Gross lesions in the principals were similar to those in pigs on Experiment 2. There were external signs of diarrhea in 5 pigs and 3 carcasses were dehydrated. Subcutaneous ventral abdominal edema was severe in 3 pigs, and the edema extended to the neck in one of them. Hyperemia and edema were seen in the external and internal lymph nodes. Polyserositis in 6 pigs varied from serofibrinous to fibrinopurulent. The extensive peritoneal exudates in Pig 11 (G4480) are illustrated in Figure 6. That pig also had 20 ml. pleural serous exudate. Peritoneal exudates, pleural adhesions, and pericardial exudates (Figure 7) are shown in the photograph of Pig 8 (G4482). Pig 9 was cyanotic at the time of exposure and died some 10 hours later with signs of diarrhea, intestinal hyperemia, and rounded diastolic heart. Cultures of the pericardial and pleural cavities were negative for bacterial growth. Three pigs had 30 to 60 cc. of milk curd in the stomach, but others were empty. There was mild hyperemia of the small intestine in 5 pigs and in approximately the first 125 cm. of the jejunum in Pig 10, which was euthanatized. The latter pig also had lesions of polyarthritis and mild fibrinous peritonitis. The liver and spleen were enlarged and dark in 5 pigs. The kidneys were usually pale and the urinary bladder was adherent to the ventral parietal peritoneum. Lesions of acute pneumonia were patchy or confined to the apical and cardiac lobes. Septicemic infection was indicated by the recovery of E. coli from the 3 major serous cavities



Figure 6. Pig G4480, 58 hours after exposure. Fibrinous to fibrinopurulent exudates cover the surfaces of abdominal viscera.

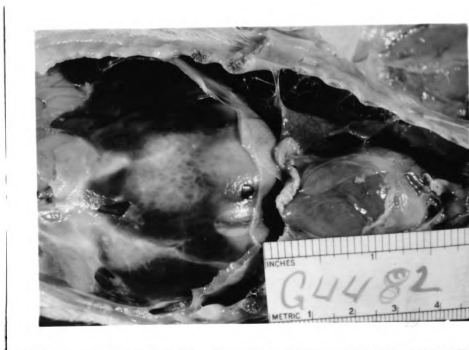


Figure 7. Pig G4482, 75 hours after exposure. Polyserositis with inflammatory exudates on peritoneal, pleural and pericardial surfaces.

and the left elbow synovial surface in 5 pigs, peritoneal cavity and elbow in one pig (Fig 9) and from the hock and elbow but not from the serous cavities in the 9-day-old Fig 10.

Histopathologic findings. In most of the lymph node sections there were lesions of edema, hyperemia, and/or depletion of lymphocytes, as in the previous experiments. Diffuse suppurative lymphadenitis occurred in the superficial inguinal and mesenteric lymph node sections of 2 pigs. Four pigs had serositis of the adrenal capsule, and one of the 4 had hyperemia of the zona glomerulosa accompanied by superficial necrosis. Two pancreatic sections had interlobular edema and some leukocytic infiltration deeper than the serosa. There was congestion in some splenic sections and some lymphocytic depletion. There was some cloudy swelling of the hepatic parenchyma and congestion of the sinusoids in most sections. In one liver there was edema of the gallbladder, and fatty vacuolation was seen in another section. Gastric serositis did not usually penetrate deeper than the serosa, but 2 sections were found to have edema and leukocytic infiltration of the outer muscularis (Figure 8). Mucosal hyperemia was found in one or more levels of the small intestine in 5 pigs and 1 had mild hemorrhagic enteritis in the middle and lower jejunum. Serositis was the most prominent lesion of the cecum. Subserosal edema of the spiral colon with hyperemia and leukocytic infiltration accompanied inflammation of the colonic muscularis in one pig (Figure 9). Fibrino-purulent pericarditis and epicarditis were severe in 3 pigs. Pig 9 had the most severe epicarditis and myocarditis of this group. Fibrino-purulent pleuritis, in some of the lung sections, was accompanied by septal thickening and atelectasis which resembled incompletely expanded fetal lung tissue.

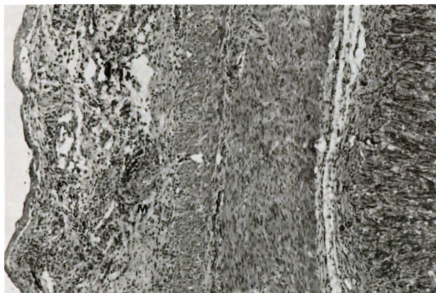


Figure 8. Pig G4481, 72 hours after exposure. Gastric serositis with fibrin, subserosal edema and inflammatory cells extending to involve outer muscularis (M). Hematoxylin and eosin. x 190.

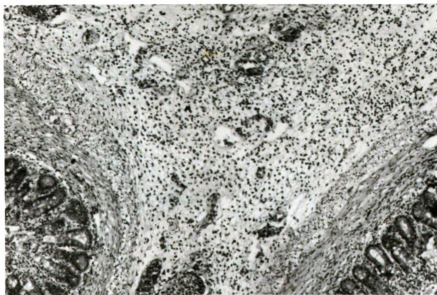


Figure 9. Pig G4480, 58 hours after exposure. Subserosal edema, hyperemia, and leukocytic infiltration of spiral colon. Hematoxylin and eosin. x 190.

Experiment 4

Bacterial status and clinical observations. The distribution of the pigs and the clinical signs observed are shown in Table 7. The pigs in Experiment 4 were greml-free at birth and remained free of any demonstrable bacteria throughout the experiment, except for the inoculum which was fed to the principals when they were 6 days (148 hours) of age. Viability of the inoculum was confirmed by the recovery of typical E. coli from rectal swabs from the principals at 40 hours after exposure.

At 18 hours after exposure the body temperatures ranged from 102.4 to 103.0 F. The pigs were all eating normally at 21 hours after exposure, but at 24 hours 4 of the 6 principals did not eat all their feeding at once, and Pigs 6 and 7 were shivering. By 40 hours after exposure Pigs 6, 7, and 3 had body temperatures of 107.0, 104.5, and 107.0 F., respectively. Twenty-four hours later, Pigs 1, 2, and 4 had temperatures of 105.6, 106.0, and 104.6 F., respectively. At that time Pig 6 was dead and none of the principals ate their complete feeding within 30 minutes. Pig 4 maintained body temperatures above 105.0 F. for another 4 days until it was euthanatized. Lameness or soreness and reluctance to move about was manifested by 3 pigs. Diarrhea was severe in only 3 of the 6 principals, although the feces were more fluid in all the principals than in the controls.

Blood samples collected at 40 hours after exposure were calculated to have the following mean values with total leukocyte counts corrected for nucleated erythrocytes in the controls and principals:

Table 7. Distribution of pigs and clinical signs observed in Experiment 4.

Iso-lator No.	Pig No.	Necropsy No.	Sex	Body wt. (kg.)	Fever	Leuko-penia	Leuko-cytosis	Anorexia	Diar-rhea	Death	Gross le-sions at necropsy	Postexposure survival time (hr.)
2	8*	G5227	F	0.95	-	-	-	-	-	-	-	(Euth.) 208
2	9*	G5228	F	0.82	-	-	-	-	-	-	-	(Euth.) 208
1	1	G5223	F	0.91	+	-	+	+	-	+	+	104
1	2	G5225	F	0.95	+	-	+	+	-	+	+	127
1	3	G5224	F	0.82	+	-	+	+	-	+	+	120
3	4	G5226	M	1.13	+	+	+	-	+	-	+	(Euth.) 162
3	6	G5221	F	0.82	+	-	+	+	+	+	+	54
3	7	G5222	F	0.91	+	-	+	+	+	+	+	94
3	5	G5220	F	0.91	---	---	---	---	---	---	---	(Euth.) **

* Control pigs.

** Pig 5 was euthanatized before exposure to E. coli. Contents from the colon did not produce bacterial growth on blood agar.

- = condition was absent

+ = condition was present

	Controls	Principals
Hemoglobin (Gm./100 ml.)	8.75	8.03
Packed cell volume (%)	26.50	26.17
Total corrected leukocytes ($\times 10^3$ /cu.mm.)	5.11	9.73
Differential leukocyte counts (%)		
Segmented neutrophils	44.0	41.67
Nonsegmented neutrophils	2.0	9.33
Lymphocytes	53.0	49.0
Monocytes	0.5	0.0
Basophils	0.0	0.17
Eosinophils	0.5	0.0
Nucleated erythrocytes (/100 leukocytes)	14.5	14.3

While the other principals had leukocytosis, Fig 4 had leukopenia with relative and absolute lymphopenia. At the next blood sampling Fig 4 was the sole surviving principal and the leukopenia had been converted to leukocytosis. The hemogram was:

Hemoglobin (Gm./100 ml.)	9.0
Packed cell volume (%)	28.0
Total leukocyte count (/cu.mm.)	17,100
Total corrected leukocyte count (/cu.mm.)	11,958
Absolute differential counts (/cu.mm.)	
Segmented neutrophils	2,870
Nonsegmented neutrophils	1,315
Lymphocytes	7,773

The results of determinations for serum electrolytes in 19 samples indicated similar ranges of values for the controls and the principals. The results of electrophoresis of 23 serum samples from 7 pigs at 5 days of age (1 day before exposure) and at 8 days of age (2 days after exposure) indicated that albumin remained nearly constant and the alpha globulin component increased slightly in percent of total protein in the 5 principals, while albumin increased sharply and the alpha globulin component decreased almost proportionately in the 2 controls. Change in the beta globulin component was variable in the principals and controls.

The gamma globulin component remained unchanged in the principals but decreased in both controls. The lack of total protein determinations for these samples prevented quantitative evaluation.

Necropsy findings. Pig 5 was found in opisthotonic convulsions and was removed from the isolator for euthanasia when it was 3 days of age. There was no ligature on the umbilical stump. Subcutaneous edema of the limbs and ventral abdomen was the only grossly visible lesion. Tissues were not saved for histopathologic examination, but contents from the colon were streaked on a blood agar plate and no bacterial growth occurred during 72 hours of aerobic incubation at 37 C.

The controls had no visible gross lesions and had approximately doubled their birth weights when they were euthanatized at 15 days of age (Figure 10). The cecum and colon in each of the controls were larger than in the principals. The carcasses of the principals were emaciated (Figure 11) in contrast to the controls. Diarrheal feces covered the rear quarters of Pigs 4 and 6. Elbows, hocks and points of jaw contact with the cages were denuded of hair in some pigs. Hyperemia and edema of the external lymph nodes varied from none to moderate. Polyserositis varied from serofibrinous in the pigs which died earlier to fibrinous or fibrinopurulent in the pigs which survived longer (Figure 12) but was the most prominent lesion in each of the pigs. Hepatic congestion and splenic enlargement were consistent lesions. Each of 5 principals had about 1 ounce of dry milk curd in the stomach; Pig 4 had about 2 ounces. Mesenteric lymph node hyperemia or edema was found in 4 pigs but was mild in 3 of them. Intestinal hyperemia was absent or mild. There was subserosal edema of the spiral colon in 2 pigs. The cecum

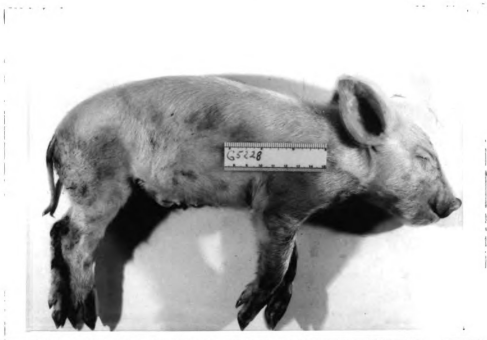


Figure 10. Pig G5228. Germfree control pig which weighed 2100 Gm. when it was euthanatized at 15 days of age (Experiment 4).



Figure 11. Pig G5226. Emaciated carcass of pig euthanatized 162 hours after oral exposure to Escherichia coli.



Figure 12. Pig G5226. Polyserositis in the pig carcass illustrated in Figure 11. Note exudates on peritoneal, pleural, and epicardial surfaces.

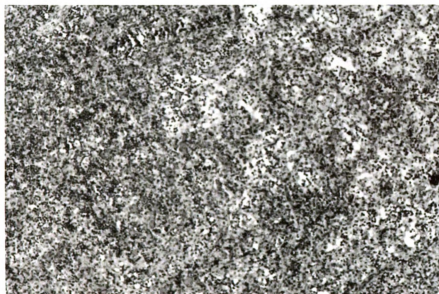


Figure 13. Pig G5224, 120 hours after exposure. Mesenteric lymph node. Note absence of lymphoid follicles. Hematoxylin and eosin. x 75.

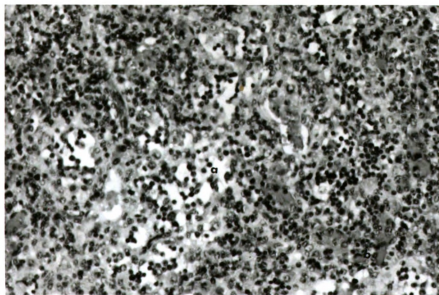


Figure 14. Higher magnification of mesenteric lymph node in Figure 13. Note small number of lymphocytes, edema (a), and hyperemia (b). Hematoxylin and eosin. x 190.

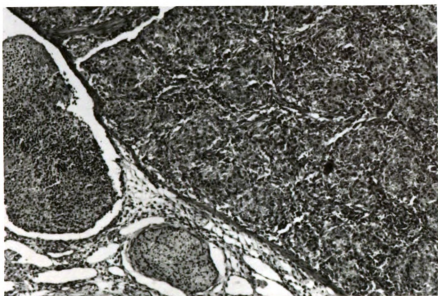


Figure 15. Pig G5224, 120 hours after exposure. Superficial inflammation at hilus of spleen with serositis. Note mass of neutrophils (N). Hematoxylin and eosin. x 75.

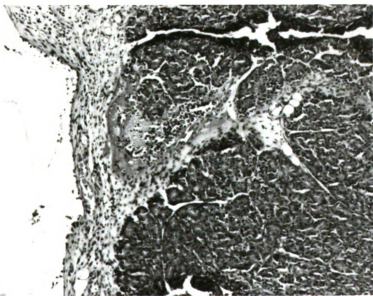


Figure 16. Pig G5224, 120 hours after exposure. Pancreas with focal parenchymal necrosis, interlobular edema and extension of serositis. Hematoxylin and eosin. x 75.

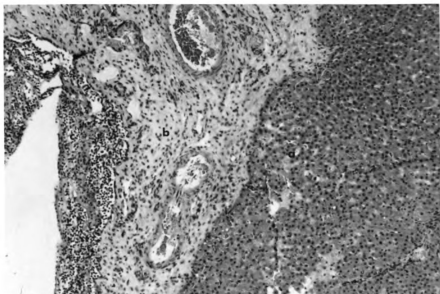


Figure 17. Pig G5224, 120 hours after exposure. Hepatic serositis in proximity to gallbladder. Note hepatic parenchyma (a), edematous Glisson's capsule (b), and subserosal leukocytic infiltration (c). Hematoxylin and eosin. x 75.

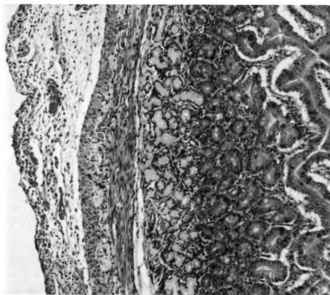


Figure 18. Pig G5224, 120 hours after exposure. Duodenal serositis. Note subserosal edema and leukocytic infiltration.

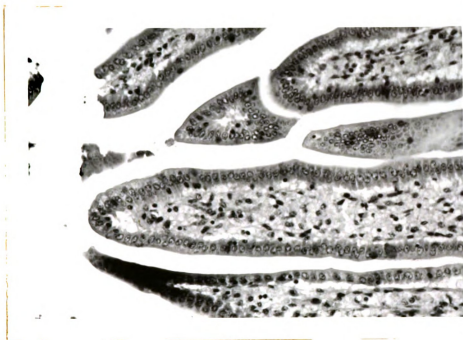


Figure 19. Normal duodenal mucosa from section in Figure 18. Hematoxylin and eosin. x 190.

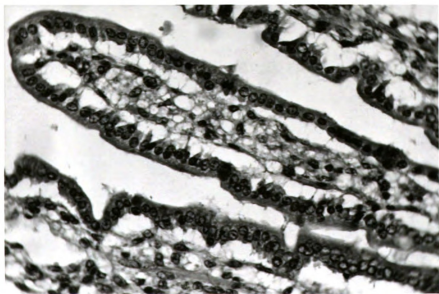


Figure 20. Cuboidal mucosal epithelium with subepithelial edema also from section in Figure 18. Hematoxylin and eosin. x 470.

edema (Figure 20). One pig had lesions of catarrhal enteritis in the upper jejunum with increased mucus, epithelial debris, and degenerating neutrophils in the lumen; another pig had severe lesions of fibrinopurulent serositis with some extension to the mucosa. Similar lesions were found in the middle and lower jejunal regions of the same 2 pigs (Figure 21). A normal serosa and the hydropic appearance of the lower jejunal mucosal epithelium in a control pig are illustrated in Figure 22. Fibrinopurulent serositis of the ileum varied from mild to severe in 3 pigs (Figure 23), which had only slightly greater leukocytic infiltration of the lamina propria than in the controls. The same 3 principals had lesions of similar nature and severity in sections of the cecum, spiral colon, and terminal colon. All but one principal had lesions of fibrinous to fibrinopurulent pericarditis (Figure 24), and Pig 4 had lesions of suppurative myocarditis (Figure 25). All the principals had lesions of pleuritis ranging from fibrinous to suppurative with mixed leukocytic infiltration, and 2 of the pigs had lesions of interstitial pneumonia with interlobular edema and neutrophilic infiltration (Figure 26) accompanied by alveolar septal thickening but without alveolar and bronchiolar cellular exudates. Two of 3 elbow joints had lesions of synovitis. The most severe synovitis (Figure 27) was represented by fibrinocellular exudates on the intimal surface near the limbus, synovial intimal hyperplasia, and subintimal edema with focal necrosis and leukocytic infiltration. Figure 28 illustrates the histologic features of the synovial membrane of the elbow from a similar site in a control pig.

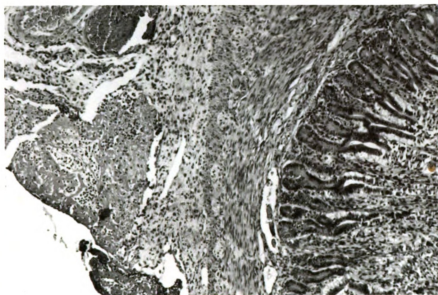


Figure 21. Pig G5224, 120 hours after exposure. Fibrino-purulent serositis of the mid-jejunum. Hematoxylin and eosin. x 75.

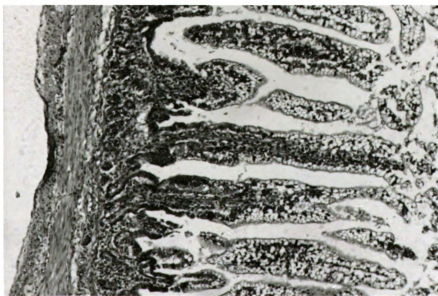


Figure 22. Pig G5227. Mid-jejunal section. Note single layer of serosal cells and the hydropic appearance of the mucosal epithelium in a germfree control pig 15 days of age. Hematoxylin and eosin. x 75.

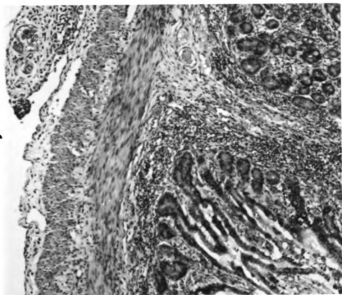


Figure 23. Pig G5224, 120 hours after exposure. Subserosal edema and serositis of the ileum. Leukocytic infiltration of the lamina propria is only slightly greater than in the controls. Hematoxylin and eosin. x 75.

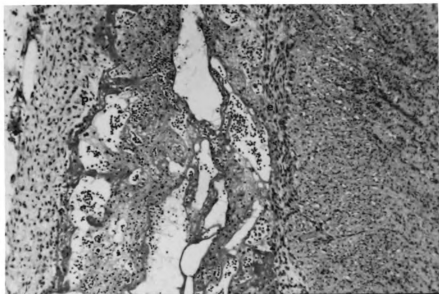


Figure 24. Pig G5224, 120 hours after exposure. Fibrinopurulent pericarditis and epicarditis with adhesions between the pericardium (p) and epicardium (e). Hematoxylin and eosin. x 75.

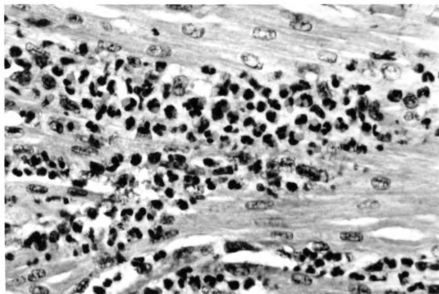


Figure 25. Pig G5226, 162 hours after exposure. Suppurative myocarditis. Note neutrophils infiltrating the myocardium. Hematoxylin and eosin. x 470.

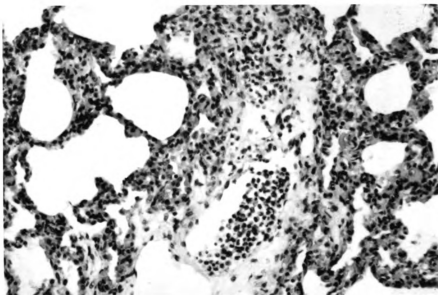


Figure 26. Pig G5224, 120 hours after exposure. Interstitial pneumonia with interlobular edema and neutrophilic infiltration. Alveolar septa are thickened but alveoli and bronchioles are free of cellular exudates. Hematoxylin and eosin. x 190.

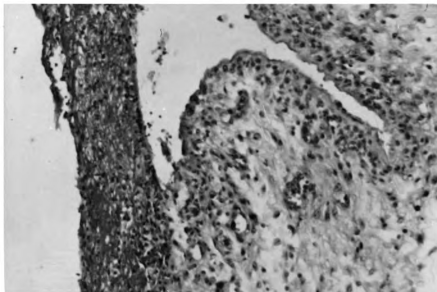


Figure 27. Pig G5222, 94 hours after exposure. Synovitis of the elbow joint. Note fibrinous and cellular exudates on the synovial intima accompanied by intimal hyperplasia and subintimal edema with leukocytic infiltration. Hematoxylin and eosin. x 190.

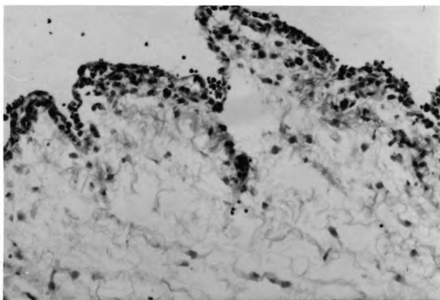


Figure 28. Pig G5227. Histologic features of the synovial membrane from the elbow of a germfree control pig. Hematoxylin and eosin. x 190.

Experiment 5

Anti-hog cholera serum was fed to each of 6 pigs in Experiment 5 to determine whether this would provide passive immunity against infection by E. coli 083 in colostrum-deprived gnotobiotic pigs and to investigate some of the ways the course of the disease might be altered by the anti-hog cholera serum.

Rectal swabs taken before the pigs' first feeding were negative on culture for bacterial growth. Monocontamination with a Gram-positive, spore-forming rod which grew in chains and was classified as Bacillus sp. was disclosed in culture of rectal swabs collected from the pigs at 4 days of age. Escherichia coli was recovered from all the principals at 44 hours after oral exposure. Also, the principals and controls still had the Gram-positive bacilli but they were reported as few or rare in cultures of the principals' rectal swabs from 44 hours postexposure onward, and none was recovered from Pig 6 at one week after exposure.

Clinical observations. The controls' body temperatures of 103 to 104.6 F. were generally higher than in previous experiments, but fever was observed in only one principal (106.8 F. at 5 days postexposure). Anorexia was of short duration in one pig and persistent but incomplete until near death in another. Diarrhea or erythema of the perineum was observed in 3 pigs. Details of the pigs' distribution and the clinical observations are shown in Table 8.

Hematologic and serologic findings. In 5 successive samplings between 96 and 311 hours of age, the controls and principals had increasing hemoglobin values -- 9.8 to 11.4 Gm./100 ml. -- and packed cell volumes -- 30.7 to 34.1%. The maximum mean of nonsegmented neutrophils observed

Table 8. Distribution of pigs and clinical signs observed in Experiment 5.

Iso- lator No.	Pig No.	Necropsy No.	Sex	Body wt. (kg.)		Fever	Leuko- penia	Leuko- cytosis	Anor- exia	Diar- rhea	Death	Gross le- Age when	
				Birth	Necropsy							sions at	tized (days)
1	5#	H1967*	F	1.50	2.19	-	-	-	-	-	-	-	11
1	7#	H1969*	F	1.41	2.56	-	-	-	-	-	-	-	11
1	11#	H1973	M	1.36	2.27	-	-	-	-	-	-	-	11
2**	6	H1968	F	1.18	1.25	-	-	+	+	+	+	+	8.5 (died)
2**	8	H1970*	M	1.23	2.79	-	-	+	-	-	-	-	10
2**	9	H1971*	M	1.41	2.42	-	-	+	-	-	-	-	10
2**	10	H1972	F	1.23	2.59	-	-	+	-	+	-	-	10
3**	1	H1963*	F	1.41	2.48	-	-	+	-	-	-	-	10
3**	2	H1964*	F	1.45	2.30	+	-	+	+	+	-	+	10
3**	3	H1965	M	1.45	2.32	-	-	+	-	-	-	-	10
3**	4	H1966	M	1.32	2.29	-	-	+	-	-	-	-	10

* Pigs 1, 2, 5, 7, 8 and 9 were each fed 50 ml. anti-hog cholera serum with 5 ml. 50% dextrose solution and 60 ml. SPF Lac as the first feeding at 6 hours of age. Pigs 3, 4, 6, 10 and 11 were fed 5 ml. 50% dextrose solution with 120 ml. SPF Lac but no antiserum.

** Each of the pigs in Isolator 3 was fed only 2.5 ml. of the inoculum. Each of the pigs in Isolator 2 was fed 5.0 ml. of the inoculum.

Control pigs.

in the differential leukocyte counts for the controls was 1.0 and for the principals was 2.2; therefore, they were included with the segmented neutrophils in the data above. Corrected mean total leukocyte counts and differential neutrophil and lymphocyte counts for the controls and principals in 5 successive samplings were:

Age of Pigs (hr.)	<u>Total WBC/cu.mm.</u>		<u>Differential (% of total)</u>			
			<u>Neutrophils</u>		<u>Lymphocytes</u>	
	Controls	Principals	Controls	Principals	Controls	Principals
96	6513	5772	43.6	39.5	55.7	58.7
140	6429	9541	28.7	26.2	71.0	73.1
192	7078	14469	25.0	18.6	74.3	81.0
264	6139	15784	26.0	24.9	72.3	74.5
311	4471	13075	19.6	18.4	79.3	81.1

Monocytes and basophils were generally absent, and eosinophils were less than 1.0% except in the fourth sampling of the controls when the mean of the eosinophils was 1.67% of the leukocytes counted in the differential.

Among the principals, the pigs fed anti-hog cholera serum had the lowest (7573) and the highest (28,300) corrected total leukocyte counts after the day of exposure. However, the pigs not fed anti-hog cholera serum (-) and those fed the antiserum (+) alternated in having the higher mean total corrected leukocyte counts on successive days of sampling: (-) 10,806, (+) 15,704, (-) 16,606, and (+) 13,256. Relative neutropenia and moderate absolute neutropenia were observed only in Pigs 6 and 10 at 146 hours after exposure.

The total serum protein tended to increase gradually from a mean of 2.76 Gm./100 ml. (range - 2.3 to 3.3) in the samples from 11 pigs 96 hours of age to a mean of 4.38 Gm./100 ml. (range - 4.2 to 5.2) in samples from 10 pigs 311 hours of age. Ten samples collected terminally, just prior to euthanasia, ranged from 3.6 to 4.4 Gm./100 ml. total serum protein.

Paper electrophoresis of 64 serum samples revealed that there was not a correlation between the distribution (percentage) of serum proteins migrating in the albumin and globulin fractions when anti-hog cholera serum was fed in the first feeding and compared to control serum samples. The albumin fraction decreased from 32% before exposure to 20% terminally in the pig that died, but the total serum protein increased from 2.6 to 4.6% to indicate an actual increase in the total amount of albumin in the terminal serum sample. The results of electrophoretic analysis of protein (expressed as percentage of total serum protein) in the first sampling of 11 pigs at 4 days of age (96 hours) and in the terminal samples from 10 pigs at 14 and 15 days of age ranged as follows:

Age of pigs	Albumin	Globulins			
		Alpha ₁	Alpha ₂	Beta	Gamma
4 days	23-35	4-11	23-40	13-36	6-10
14 and 15 days	45-63	2-4	22-37	10-18	2-5

The albumin:globulin ratio and total amount of serum albumin increased with the age of the pigs. Gamma globulin was decreased in proportion (ratio) to total proteins but was maintained nearly constant in total amount in the samples in the 10 surviving pigs.

In 54 samplings representing 11 pigs in each of the first 4 groups, and 10 pigs in the fifth sample group, the flame photometer analysis indicated a range from 139 to 155 mEq.Na/liter in the first group of serums, and 140 to 145 mEq.Na/liter in the final group. Potassium ranges in the same groups of serum samples were 4.6 to 7.0 mEq.K/liter in the first group and 7.4 to 10.5 mEq.K/liter in the final group. The mean sodium values in the 5 successive groups of samples were 147, 144, 150, 145, and 143 mEq.Na/liter. The means for serum potassium were 5.9, 6.1,

6.3, 8.1, and 8.8 mEq.K/liter. No differences were noted between the experimental groups.

Necropsy findings. Gross lesions were found in 2 pigs at necropsy. At the ventral margin of the left central lobe of the liver in Pig 2 there was a pale yellow, 1-cm.-diameter, circumscribed lesion that resembled inspissated fibrin but seemed to be an integral part of the liver. No other lesions were seen in Pig 2, which had been fed anti-hog cholera serum.

Pig 6 was similar to principals in previous experiments in having severe fibrinous polyserositis. In all 3 cavities, the exudates appeared dry and organizing. There was diffuse pulmonary congestion. There were mild catarrhal gastritis and enteritis.

In bacteriologic examination, pure cultures of E. coli were recovered from the pleural, peritoneal and pericardial cavities of Pig 6. Peritoneal swabs from Pigs 3, 7, 8 and 11 produced no growth in culture. Gram-positive rods with spores (Bacillus sp.) were isolated from the peritoneal swabs of Pigs 5, 9, and 10. Similar Bacillus sp. and E. coli were isolated from Pigs 1, 2, and 4.

Histopathologic findings. The lesion from the liver of Pig 2 was composed of a central zone of necrotic neutrophils with a peripheral zone of lymphocytes in a mass of fibrin. The tissues of Pig 6 were similar to those from principals in previous experiments with fibrinopurulent polyserositis. There were signs of edema, hyperemia, and depletion of lymphocytes in the mandibular and mesenteric lymph nodes. Mucous secretion was increased in the stomach and lower intestinal sections. There were adhesions between the visceral and parietal pleural surfaces

in the lung section which was taken to include the parietal pleura and intimate subjacent tissues in the ventral thorax. No lesions were seen in sections from the other principals and the controls, but the liver sections presented the usual pale, glycogen-rich appearance of the hepatocytes. The middle and lower jejunal sections frequently had hydropic appearing mucosal epithelium.

Experiment 6

Anti-hog cholera serum (25 ml./pig) was fed to each of 6 pigs in their first feeding at 4 hours of age. The details of distribution of the pigs and the clinical signs observed in Experiment 6 are shown in Table 9, together with comparative body weights at birth (24 hours) and at necropsy. Fifteen pigs were delivered by hysterotomy, but 3 of them were discarded because of small size and weakness. Pig 9 died at 3 days of age following probable thermometer perforation of the rectum which caused intraperitoneal retention of sterile feces.

No microorganisms were demonstrated in swabs from the liquid diet and rectal swabs from 11 of the 12 pigs at 16 hours of age. However, one pig's rectal swab yielded a Gram-positive Bacillus sp. and 8 of the pigs were monocontaminated with the Bacillus by their fifth day.

Prior to exposure to E. coli at 5 days (115 hours) of age, Pigs 11 and 12 were each fed 2.5 ml. of a 36-hour BHI culture of the monocontaminant Bacillus which was already present in their environment. Pig 11 was never fed the usual inoculum of E. coli but was given the empty feeding tray from the cage of Pig 13 some 20 hours and 2 feedings after the E. coli inoculum had been fed in it to Pig 13. In addition to the contaminant Bacillus sp., E. coli was recovered from the rectal swabs of all

Table 9. Distribution of pigs and clinical signs observed in Experiment 6.

Iso- lator No.	Pig No.	Necropsy No.	Sex	Body wt. (kg.)		Fever	Leuko-		Leuko- cytosis	Anor- xia	Diar- rhea	Death	Gross le-		Age when euthana- tized (days)
				Birth	Necropsy		penia	Leuko-					sions at necropsy	Age when euthana- tized (days)	
1	1	H2477#	F	0.91	2.21	-	-	-	-	-	-	-	-	-	23
1	2	H2478#	M	0.91	2.48	-	-	-	-	-	-	-	-	-	23
1	3*	H2479#	M	1.04	2.55	-	-	-	-	-	-	-	-	-	23
1	5*	H2480#	M	1.18	2.73	-	-	-	-	-	-	-	-	-	23
2	6*	H2481	M	1.13	2.42	-	-	+	+	+	-	-	-	-	22
2	7*	H2482	M	1.18	2.66	+	-	+	+	+	+	-	+	+	22
2	8	H2483	F	1.13	1.94	+	-	+	+	+	+	-	+	+	22
2	9	H2484	M	1.13	**	---	---	---	---	---	---	---	---	---	---
3	10*	H2485	M	1.27	2.46	-	-	+	+	+	-	-	-	-	22
3	11	H2487	M	1.36	1.75	+	-	+	+	+	-	+	+	+	5.5 (died)
3	12*	H2486	F	0.73	2.19	-	-	+	+	-	-	-	-	-	22
3	13	H2488	M	0.68	2.06	+	-	+	+	+	+	-	+	+	22

* 25 ml. of a commercial anti-hog cholera serum was fed to each of these pigs approximately 4 hours after completion of the hysterotomy.

** The rectum was accidentally perforated by the thermometer and the pig died at 3.5 days of age.

Control pigs.

the principals at 45 hours after the first exposure to E. coli. With the latter group of rectal swabs, clean swabs were collected from Isolators 2 and 3, and curdled liquid diet from Isolator 1 for microbiological examination and all were sterile on culture.

Clinical observations. All 3 pigs in Isolator 2 were exhibiting signs of illness at 40 to 48 hours after exposure. The body temperature of Pig 8 was 106.4 F., Pigs 7 and 8 had diarrhea, and Pigs 6, 7 and 8 had transient anorexia. In Isolator 3, Pigs 10 and 13 also ate slowly the second day after exposure to E. coli. Three more of the pigs developed fevers in excess of 106.0 F., Pig 11 at 4 days, Pig 13 at 5 days, and Pig 7 at 9 days, with recurrence in Pig 7 at 16 days after exposure. Fever and incomplete anorexia persisted in Pig 11, which died at approximately 5.5 days after being fed from a tray that had been contaminated with E. coli. All the other pigs survived the exposure to E. coli and were euthanatized to terminate the experiment when the principals were 22 days and the controls were 23 days of age.

Hematologic findings. Leukopenia was not observed in the 44 postexposure samples from the principals, except that relative neutropenia at 4 days after exposure followed relative neutrophilia, which had occurred at approximately 2 days after exposure and in which there was also an absolute increase in numbers of nonsegmented neutrophils. In that first postexposure group of samples the differential neutrophils, including the nonsegmented neutrophils, ranged between 49 and 76 for the principals and 39 to 46 for the controls. The nonsegmented neutrophil range in the controls was 0 to 3, while the principals had 3 to 21. Two principals had total corrected WBC counts at least 50% above the mean of the controls;

they were: 10,693 for Pig 10 at 42 hours after exposure, and 19,400 for Pig 11, which had been exposed to E. coli only 24 hours previously. In 5 samplings, a total of 30 blood samples was collected from the 6 surviving principals between 6 and 16 days after exposure. The lowest individual corrected total WBC count was 8020 and the highest was 29,612. Both extremes occurred in samples from pigs that had been fed anti-hog cholera serum. In 20 comparative samples from the 4 controls, the corrected total WBC range was 1974 to 6820, and both extremes were from pigs that had not been fed anti-hog cholera serum.

Hemoglobin levels in all 11 pigs at 5 days of age and just prior to exposure ranged between 7.7 and 10.5, with a mean of 8.8 Gm./100 ml. The PCV range was 24 to 32% for the group of 11 at the same time. The 4 pigs that remained the controls had an average mean corpuscular hemoglobin concentration (MCHC) of 29.75%, and the 7 principals averaged 33.53% MCHC. Hemoglobin and packed cell volumes reached their peak at 12 days of age, when the controls had means of 10.6 Gm. Hb./100 ml. and 33.75% PCV. Comparative mean values for the 6 surviving principals were: Hb. - 9.8 Gm./100 ml. and PCV - 32.5%. Conversely, at 12 days of age, the values for MCHC were reduced (controls - 31.4%, principals - 30.15%) to the lowest in the 8 groups of samples taken from the pigs between 5 and 21 days of age, excepting the 29.75% MCHC in the first sampling from the controls. At 21 days of age the MCHC averages and the hemoglobin and PCV ranges were:

	Controls	Principals
MCHC average (%)	33.55	32.64
Hemoglobin range (Gm./100 ml.)	7.2-8.6	5.5-9.0
Packed cell volume (%)	22.0-24.0	18.0-30.0

Only 2 hemoglobin estimations in the 83 samples were below 7.0 Gm./100 ml.

and only one was above 11.6 Gm./100 ml. The principals had slightly lower hemoglobin levels than the controls in all except the samples taken on Day 21. Also, the principals had the lower MCHC values in the last 5 samplings. Trends were similar and ranges overlapped in measurements of hemoglobin, packed cell volume, and MCHC in the principals and controls, whether they had or had not been fed anti-hog cholera serum.

Serologic findings. In 9 successive samplings, the total serum protein was found to range between 2.8 and 4.0 Gm./100 ml. in the controls and 2.6 to 5.1 Gm./100 ml. in the principals. The total serum protein quantities were similar in range in the controls and principals irrespective of the previous feeding of anti-hog cholera serum.

In paper strip electrophoresis the mean gamma globulin in 21 samples from principals not fed anti-hog cholera serum was 5.0%, and in 36 samples from the principals fed anti-hog cholera serum the mean was 4.92% of the total serum protein. In 18 samples from 2 controls that had been fed anti-hog cholera serum there was 4.33% gamma globulin as compared with a mean of 3.66% in the same number of samples from the 2 controls not fed anti-hog cholera serum. The gamma globulin range in 36 samples from the controls was between 1 and 9%, and in 57 samples from the principals the range was 0 to 13%.

The principals had higher beta globulin mean values than the controls in all days except at 2 days after exposure to E. coli. Also, with the exception of that one day, whenever the beta globulin value was increased or decreased in the principals, it was paralleled in the controls. Ninety-three individual samples ranged between 10 and 25% beta globulin, while the means of the groups ranged from 11.5 to 18.5% in the controls and 11.7 to 21.5% of the total serum protein in the principals.

Alpha-1-globulin decreased in all pigs as their age increased, and declined from a range of 6 to 14% in 11 pigs at 115 hours of age to a range of 2 to 5% with a mean of 3.2% for 10 pigs at 22 and 23 days of age.

Alpha-2-globulin and albumin were approximately equal in the pre-exposure and first postexposure groups of blood samples. However, by the twelfth day of age (7 days after exposure) the albumin:alpha-2 ratio had been increased to 2.38:1 in the controls and was only 1.12:1 in the principals. Also, at the seventh day after exposure, the 2 principals not fed antiserum had 28% albumin and the antiserum-fed principals had an average of 49% albumin with albumin:alpha-2 ratios of 0.58:1 and 1.53:1, respectively. By 16 days after exposure, the ratio of albumin to alpha-2-globulin had declined to 1.66:1 in the controls and to 0.96:1 in the principals. The 2 principals not fed anti-hog cholera serum now had the higher albumin:alpha-2 ratio of the 2 groups of principals, 1.02:1 for those not fed antiserum and 0.93:1 for the antiserum-fed principals.

Albumin reached a peak at a mean of 57.25% of total serum protein in the controls and 42.16% in the 6 principals at 12 days of age. After 12 days of age the albumin fraction fluctuated. There were means of 58% in the controls and 37% in the principals at 23 days and 22 days of age, respectively, when they were anesthetized and exsanguinated.

Serum potassium increased in all the pigs as their age increased. The means at 2 days after exposure were: 4 controls - 6.4 mEq.K/liter, 7 principals - 6.14 mEq.K/liter. At 16 days after exposure the means were: 4 controls - 9.25 mEq.K/liter, 6 principals - 9.2 mEq.K/liter. The extreme range in individual measurements of 93 samples was 4.2 to 9.8 mEq.K/liter. Serum sodium concentrations were similar among all groups of pigs, ranging between 136 and 154 mEq.Na/liter in 72 samples

from pigs between 7 and 21 days of age. In 10 additional samples at 22 and 23 days of age, 2 of the principals had lower concentrations of 133 and 128 mEq.Na/liter.

Tube agglutination tests employing systems of 0.1 ml. gnotobiotic pig serum diluted 1 to 10 in isotonic sodium chloride aqueous diluent before adding 1.0 ml. of diluted whole-cell E. coli 083 somatic antigen, which made the final serum dilutions 1 to 20, failed to demonstrate 0 agglutinins for E. coli 083:K.:HNM in 43 serums collected from the gnotobiotic pigs at 5 to 12 days of age and tested in duplicate. Eleven serums from the 5-day-old (115 hour) pigs were also tested at final dilutions of 1:10 and 1:20 with combined E. coli-latex particle antigen, and with the plain E. coli antigen in glycine-buffered saline solution diluent. All the gnotobiotic pig serums were negative for agglutination. Positive control serums from immunized conventionally reared weaned pigs agglutinated fully at the above dilutions.

Necropsy findings. There were no lesions in the controls. Four of 7 principals had lesions of polyserositis which varied from serofibrinous to fibrinopurulent and, in one pig, organizing fibrinous serositis with firm intestinal adhesions. In the latter case a peritoneal swab produced no bacterial growth on culture, whereas E. coli was recovered in culture of peritoneal swabs from the other 3 pigs which had gross lesions, and only from those pigs with lesions of serositis. One of the pigs with lesions had been fed anti-hog cholera serum but had diffuse serofibrinous peritonitis with effusion, pleuritis with approximately 10 ml. of gray serous exudate, and severe fibrinous pericarditis.

Histopathologic findings. Lymphoid follicular development was more distinct in the 3-week-old controls than it had been in younger pigs in previous experiments, and secondary nodules were becoming distinguishable. However, no differences were noticed between those controls fed anti-hog cholera serum and those not fed the antiserum. There was less distinct follicular development in the lymph nodes and spleens of the principals which had gross lesions than in those without gross lesions. Granulocytes were prominent in the lymph nodes of the controls and principals. Eosinophils were less numerous than neutrophils but were obvious because of the brightly staining granules in their cytoplasm.

Pale, vesicular, parenchymal cytoplasm in hematoxylin-eosin stained liver sections was characteristic of all except 2 pigs. Moderately large amounts of glycogen were visible in those livers with pale parenchyma when they were fixed in Carnoy's solution and stained with Best's carmine.

Moderate numbers of mixed leukocytes in the dilated sinusoids were accompanied by eosinophils and lymphoid cells in the hepatic portal areas of Pig 7. It also had extensive fibrinopurulent exudates on the serosal surfaces of all levels of the intestines and stomach, spleen, liver, testicle and epididymis, lung and heart. Although Pig 10 had no grossly visible lesions, there was an area of organizing purulent debris on the serosa of the jejunum, and there were increased numbers of leukocytes in the lamina propria of the jejunum. In the middle and terminal jejunum the villi were broader and shorter than in the controls. Some glandular crypts in the terminal jejunum and ileum in Pig 10 and in the ileum of Pig 7 contained cellular debris and neutrophils. In Pig 11 there was prominent post-mortem decomposition in addition to lesions of fibrinopurulent polyserositis. There was severe fibrinopurulent serositis

in one of 2 liver sections from Pig 13. In addition, there was a thick layer of mucus covering the mucosa of the esophageal and cardiac regions of the stomach, and fibrinopurulent serositis was present in the fundic region. The small intestine was essentially normal, but the epithelium of the lower jejunum was greatly vacuolated and resembled hydropic degeneration as it had in the controls. There was very mild serositis of the spiral colon, and moderate subacute serositis of the terminal colon. There was fibrinopurulent serositis in sections of the testicle, epididymis, and lung.

DISCUSSION

Bacterial Status

In 6 experiments, each of the first 3 experimental litters had a Staphylococcus sp. in their isolator environment. The fourth litter was entirely free of any demonstrable contaminant bacteria. Modifications of equipment and technique, as described in the section on materials and methods and by Waxler et al. (1966), controlled the problem of staphylococcic contamination. Experiments 5 and 6 were contaminated by a spore-forming Bacillus sp. which had seemed to survive in the cages in single passage through the steam sterilizer.

Experiments 1, 2, 3, and 4

Confirmation of the hypothesis that E. coli 083:K.:NM would be pathogenic for gnotobiotic pigs was achieved in the first 4 experiments, when clinical signs of illness occurred in 100% of the 25 principals exposed to E. coli but in none of the 10 controls. Twenty-one of the 25 principals (84%) died between 10 and 162 hours after exposure to E. coli, and all 25 (100%) had gross lesions at necropsy. Twenty-four pigs had lesions of polyserositis, and the remaining pig had fibrinous peritonitis accompanying polyarthritis when it was euthanatized. Dunne (1959) doubted the significance of E. coli isolations from pigs which had died or were killed while in a moribund condition. The present work, however, agrees with the report of Jubb and Kennedy (1963) that the infection can be septicemic. Escherichia coli was isolated from the serous

cavities and/or synovial cavities of pigs which were euthanatized while they were still ambulant, as well as from all the exposed principals that died. The outstanding lesions of serofibrinous to fibrinopurulent polyserositis were the result of inflammatory reaction, which could occur only in the living host.

Overt polyarthrititis was first observed in Experiment 2, when lameness in one or more swollen leg joints was observed in 4 of the 6 principals. The post-mortem recovery of E. coli in cultures of the elbow synovial fluid confirmed the suspicion of infectious arthritis in 16 of the 19 principals in Experiments 2, 3, and 4. The problem of localization of E. coli in the joints as a complication in colibacillosis of pigs was recognized by Dunne (1958, 1964) and Jubb and Kennedy (1963).

Lesions of gastritis were never more severe than catarrhal. Moon et al. (1966b) reported that the lesions of enteritis in diarrheal disease of pigs were usually limited to hyperemia of the jejunum, and several pigs had no morphologic evidence of enteritis. Lesions of enteritis in the pigs in these experiments were usually minor and were overshadowed by the lesions of serositis. Subserosal edema of the spiral colon was a frequent lesion. Ventral abdominal edema in several of the pigs resembled lesions which occurred in gnotobiotic pigs experimentally infected with E. coli 0138:K81 by Christie (1967).

Varying the volume of the inoculum from 1.25 ml. to 5.0 ml. of E. coli and the age of the pigs at exposure from 69 to 148 hours of age did not establish a relationship between the volume of inoculum or the age of the pigs and susceptibility to infection. The pigs which were youngest at exposure had the greatest mean survival time. Developmental age of the pigs, however, may not have coincided with their chronologic age based

on the number of elapsed hours or days from the time they were delivered by hysterotomy. Feeding medroxyprogesterone to the sows in Experiments 2 through 5 allowed the hysterotomies to be performed at more advanced stages of gestation than might have been possible without the progestational hormone in the sow's diet. In order of decreasing mean postexposure survival time, Experiments 2, 4, 1, and 3 consisted of litters delivered at 115, 114, 112, and 112 days' gestation, respectively. This small number of experiments can hardly be conclusive, but it serves to illustrate that fetal maturity may be one of the variable factors in gnotobiotic research. Altered placental permeability related to the feeding of a progestational hormone could constitute another variable, particularly if larger molecules such as gamma globulin were transferred across the placental barrier. Pigs in Experiment 3, which were from a sow fed progesterone, were at least as susceptible as those pigs in Experiment 1, in which the sow had not been fed medroxyprogesterone. Saunders et al. (1963b) cited reports to indicate that pigs from gilts are more susceptible to infection than pigs from sows. In Experiments 1 and 3, the pigs, which were from sows, did not survive as long as pigs from a gilt in Experiment 4.

Clinical signs were not sufficiently characteristic to be pathognomonic for infections with E. coli but conformed to the nonspecific effects ascribed to the endotoxins of the Enterobacteriaceae by Smith et al. (1964) and Carpenter (1965). Fever was recorded on at least one day for each of 18 pigs. This contrasts with the report by Saunders et al. (1963a) that no fevers, but frequent subnormal body temperatures, were observed in their "pathogen-free" pigs infected with a different serotype of E. coli. Subnormal body temperatures recorded for several of the

gnotobiotes in these experiments usually were observed shortly before the pigs died. Diarrhea was only moderately severe in most cases but was observed in the majority of the principals. Anorexia appeared to be related more to soreness and pain induced by movement than to decreased alertness until near death. Some of the pigs which did not stand and approach their trays when fed would protest being moved but would eat after being lifted to their feet. In Experiment 1 leukopenia followed by leukocytosis conformed to the sequence of these reactions in laboratory animals inoculated with endotoxins of Gram-negative bacteria as reported by Carpenter (1965). Leukopenia was observed in 7 of the 25 pigs and was primarily neutropenic, with differential counts as low as 3% neutrophils coexisting with relative lymphocytosis. In Experiment 1 leukocytosis occurred only in those pigs which became febrile. The occurrence of leukocytosis in approximately 50% of the principals indicated that gnotobiotic pigs are capable of a neutrophilic response to oral challenge with E. coli. Circulating neutrophils were the cells most depleted in the leukopenic phase. Also, in the later experiments a frequently observed pattern was leukocytosis followed by leukopenia or neutropenia, which often preceded death when the pig was overwhelmed by infection.

The mean hemoglobin of 8.33 Gm./100 ml. for all 35 pigs at 1 to 2 days of age and before exposure in the first 4 experiments was approximately 10% less than the means reported by Miller et al. (1961b) for 2-day-old naturally reared pigs. However, at 7 to 9 days of age the mean hemoglobin of 12 surviving principals in Experiments 2, 3, and 4 was 8.4 Gm./100 ml., which exceeded the mean values for normal pigs of similar age as reported by Schalm (1965) and Gardiner, Sippel, and McCormick (1953); and the mean MCHC of 30.5% was greater than those in the report of Miller et al.

(1961b). The preceding data permit the assumption that these gnotobiotic pigs were not predisposed to infection because of anemia. Also, it is evident that hemoconcentration was not a pathologic effect of E. coli infection in the pigs, since the principals had slightly lower PCV and hemoglobin values than controls of similar ages.

In electrophoresis the principals in Experiment 4 did not parallel the increase in albumin per cent of serum protein which occurred in the controls. Frankel and Reitman (1963) and Coles (1966) included decreased hepatic function in disease, decreased protein intake, decreased protein digestion and absorption, and nephritis and nephrosis as causes of decreased serum albumin levels. The relative decrease in the alpha globulin component observed in the controls did not occur in the principals, and their beta and gamma globulin remained essentially unchanged. The lack of results of total protein determinations for the foregoing groups of serums prevented quantitative evaluation of the relative serum protein changes.

Serum electrolytes, sodium and potassium, in 28 samples in Experiment 2 were essentially the same in principals and controls. The range of sodium values from 131 to 152.5 mEq./liter in the principals and from 135 to 151 mEq./liter in the controls was similar to that presented by Benjamin (1961). The serum potassium was of slightly less concentration than that reported by Widdowson and McCance (1956) for newborn pigs. The serum potassium increased with age of the pigs, and the maximum of the extremes (4.5 to 8.45 mEq./liter) was from one of the controls. Therefore, it seems unlikely that elevated serum potassium was associated with cardiac arrest, as suggested by Roy et al. (1959) in calves which died with localized intestinal E. coli infections. Histopathologic examination of

tissues from the controls revealed great similarity between these gnotobiotic pigs and those described by Waxler (1961) and Schmidt (1961). The lymph nodes appeared immature and did not have secondary lymphoid nodules. Liver lobulation was indistinct. Vacuolization resembling hydropic degeneration of the epithelium in the mucosa of the jejunum and ileum occurred in controls as well as principals. Sprinz et al. (1961) stated that the intestinal mucosa of the germfree guinea pig at 42 days of age resembled the mucosa of the intestine in the fetal guinea pig. Kenworthy and Allen described the influence of diet and bacteria on small intestinal morphology with special reference to early weaning and Escherichia coli. They related the differences in morphology to alterations of metabolic pathways associated with the absence of bacterial enzymes from the substrate in the digestive tract. Sprinz et al. (1961) discussed the morphogenetic influence of bacteria on the intestinal mucosa and concluded that bacteria, diet, hormones, etc., probably act as a combination of factors influencing the intestinal mucosal morphology.

Lymphadenopathy in the principals was characterized by varying severity of edema, hyperemia, and depletion of lymphocytes. Depletion of lymphocytes also occurred in the spleen. In germfree guinea pigs (Sprinz et al., 1961), after oral challenge with E. coli, the lymph nodes were less dense than in the controls and appeared as though the lymphocytes had been washed out of the lymphoid nodules leaving a reticular network with prominence of reticulum cells.

Serositis was noted in a majority of the sections from parenchymatous organs but rarely penetrated to the subcapsular areas, as did the serositis in experimental mycoplasmosis reported by Roberts et al. (1963a). Foci of hematopoiesis were infrequent in the liver but more frequent in

the spleen. Cloudy swelling was evident in most of the kidney sections, but they did not have signs of proteinuric casts in tubules, focal interstitial leukocytic infiltration, or hemorrhages. The petechial hemorrhages noted in gross examination were possibly blood-filled superficial glomeruli. Cloudy swelling was noted in many sections of the kidneys and probably represented a mild response to toxemia or to terminal hypoxia.

The gastric sections were devoid of severe mucosal lesions, although most of the pigs had some lesions of serositis. Lesions of enteritis in the small intestine, cecum, spiral colon, and terminal colon were usually mild or absent and were not prominent in comparison with the lesions of serositis which infrequently extended into the muscularis.

In male pigs the peritonitis extended to involve the tunica vaginalis. Other lesions of septicemia included fibrinous to fibrinopurulent pericarditis and pleuritis with occasional focal suppurative myocarditis and interstitial pneumonia as well as synovitis.

Experiments 5 and 6

Septicemic E. coli infections in colostrum-deprived artificially reared pigs was recognized as a problem by Lecce and Reep (1962). Switzer et al. (1962) recommended the use of substitutes for colostrum to provide biological protection to SPF pigs, which were much more susceptible to ordinary infections than their conventional counterparts. Experiments 5 and 6 were designed to investigate (1) how much protection against E. coli infection could be obtained by feeding commercially available anti-hog cholera serum to the pigs as a substitute for colostrum, and (2) how the course of disease would be altered in those pigs fed anti-hog cholera serum. Speer et al. (1959) demonstrated a declining rate of colostral

antibody absorption from the digestive tract of the newborn pig after 6 hours of age and virtual cessation of absorption of the large molecules by 24 hours of age. Therefore, these pigs were fed anti-hog cholera serum at 6 hours or less of age to insure maximum antibody absorption.

Four of the principals in Experiment 5 were fed anti-hog cholera serum and 4 were not fed anti-hog cholera serum. All the principals developed leukocytosis, but a neutropenic phase was observed only in 2 pigs not fed anti-hog cholera serum. Among the 4 pigs fed anti-hog cholera serum no deaths occurred; 1 pig had clinical signs of illness and when euthanatized had a single gross lesion (liver abscess).

Escherichia coli was isolated in culture of peritoneal swabs from this and one other pig fed anti-hog cholera serum. In the 4 principals not fed anti-hog cholera serum, one pig developed clinical signs of illness and died 4.5 days after exposure. The pig had severe lesions of polyserositis, and E. coli was isolated in culture of swabs from 3 serous cavities; but E. coli was not septicemic in the other 3 principals not fed anti-hog cholera serum.

In Experiment 6 severity of the disease following oral exposure to E. coli 083 at 5 days of age was much greater in the 3 pigs not fed anti-hog cholera serum than in the 4 pigs fed anti-hog cholera serum. The one pig that died had not been fed anti-hog cholera serum nor a measured inoculum. It had been given the empty feeding tray as a contaminated fomite from the cage of another pig. Signs of illness developed sooner after exposure in the pig given the contaminated fomite than in other pigs fed measured and, presumably, larger inoculums, suggesting that quantity of inoculum was not a major factor in susceptibility in this experiment.

Only one of the anti-hog cholera serum-fed principals had gross lesions at necropsy, but the severity of those lesions was as great as that in the antiserum-devoid principals, of which 100% had gross lesions. Histopathologic findings were correlated with the gross observations in the pigs with gross lesions. Also, one of the antiserum-fed pigs which had had clinical signs of illness but no gross lesions at necropsy did have resolving subacute histopathologic lesions of peritonitis. In post-mortem microbiologic examination the antiserum-devoid pig which had resolving gross lesions of firm intestinal adhesions had apparently eliminated E. coli from the peritoneal cavity, since its peritoneal swab produced no growth in bacterial culture. In fact, E. coli was recovered in culture of peritoneal swabs only from those pigs with acute lesions of peritonitis.

Hematologic trends and ranges were similar in measurements of hemoglobin and PCV, which reached their peak in the pigs at 12 days of age and of MCHC, which was lowest when hemoglobin and PCV were highest in the principals and controls, irrespective of the feeding of anti-hog cholera serum. The increasing levels of hemoglobin and PCV to a peak at 12 days of age contrasted with the reports of Gardiner et al. (1953) and Miller et al. (1961b) for hemoglobin trends in conventionally reared pigs from 1 to 10 days of age and may have been related to the smaller increase of body weight and body fluids in the gnotobiotic pigs as compared with the conventionally reared pigs. One interpretation of the decreasing MCHC while the hemoglobin and PCV were increasing would be that there was a degree of macrocytic normochromic anemia. However, as mentioned previously, the mean hemoglobin values in these gnotobiotic pigs exceeded the mean values for normal pigs of similar age reported by Schalm (1965) and

Gardiner et al. (1953). So there was no anemia in comparison with available standards. Leukocytosis and transient neutropenia in the anti-serum-devoid principals were paralleled in those principals fed the antiserum.

Gamma globulin and total serum protein values were essentially the same for the principals and the controls and for those pigs fed or not fed anti-hog cholera serum. The anti-hog cholera serum was a commercially available nonconcentrated product and would have contained a relatively small amount of gamma globulin. Miller et al. (1961a) found 6.5% gamma globulin in newborn pig serum with a mean total serum protein of 2.22 Gm./100 ml. in 47 pigs. The results of experiments by Nordbring and Olsson (1957, cited by Miller et al., 1961a) were similar to those of Miller et al. (1961a) and to those reported here. Miller et al. (1961a) called attention to the subjective judgment required to differentiate the beta and gamma globulin components which are characteristically incompletely separated in electrophoresis of serum from conventionally reared pigs 6 to 48 hours of age. Indistinct separation of the beta and gamma globulin fractions was also a problem in these experiments. Lecce and Reep (1962) demonstrated agglutinins for E. coli 08 and other E. coli serotypes in bovine and porcine colostrum by tube agglutination tests. They also found that agglutinins were practically absent from porcine and bovine gamma globulin. This may account for the failure to demonstrate agglutinins for E. coli 083 in ordinary tube agglutination tests and latex agglutination tests of the serums from all of the pigs in Experiment 6 between 5 and 12 days of age. Carpenter (1965) discussed factors other than agglutinins which may function in the phagocytic elimination of bacteria in infections.

Serum potassium levels increased as the pigs' age increased in all groups of principals and controls in Experiments 5 and 6, as they had in Experiment 2. The erythrocytes were not subjected to fragility tests, but it is possible that there was increased permeability of the erythrocytic membranes with resultant osmotic transfer of potassium to extracellular location in the serum in the absence of actual hemolysis.

SUMMARY

In a series of 6 experiments, 58 gnotobiotic pigs from 6 litters were utilized in investigation of the pathologic effects of oral exposure to Escherichia coli serotype 083:K.:NM in gnotobiotic pigs. The pigs were germfree or monocontaminated with Staphylococcus sp. or Bacillus sp. at 69 to 148 hours of age, when they were fed E. coli inoculums in volumes varying from 1.25 ml. to 5.0 ml. of brain-heart infusion or thioglycolate liquid mediums. The inoculums were incubated at 37 C. for 24 or 36 hours just prior to their being fed to the pigs.

In the first 4 experiments there was 100% morbidity and 84% mortality among 25 pigs between 10 and 162 hours after exposure to E. coli at ages ranging from 69 to 148 hours. The most prominent lesions consisted of serofibrinous to fibrinopurulent polyserositis and/or polyarthrititis in 100% of the 25 principals.

Invasiveness of the organism was indicated by the septicemic nature of the infection. Four principals were severely ill but not moribund when they were euthanatized between 99 and 236 hours after exposure to E. coli. Septicemia was confirmed by post-mortem microbiological recovery of E. coli from the exudates in serous and synovial cavities of the euthanatized principals as well as from the principals that had died.

Macroscopic and microscopic morphologic evidence of gastroenteritis was usually mild or absent, and results of clinicopathologic examinations did not reveal evidence of anemia, hemoconcentration, or serum electrolyte (sodium and potassium) derangement in the pigs.

Clinical signs were nonspecific, rather than pathognomonic, and included fever, hypothermia, diarrhea, partial anorexia, leukopenia, and leukocytosis. The occurrence of fever and leukocytosis indicated that colostrum-deprived gnotobiotic pigs are capable of responding defensively to bacterial stimuli in much the same manner as other animals.

In Experiments 5 and 6, anti-hog cholera serum was fed to one-half of each group of principals at 6 hours of age or earlier as a substitute for colostrum. Some biological protection was manifested by the absence of mortality and reduced incidence of gross lesions in the pigs fed anti-hog cholera serum as compared with the principals not fed anti-hog cholera serum. Infection following exposure to a contaminated fomite resulted in the only death among the principals in Experiment 6. Evaluation of the degree of protection against E. coli infection provided by feeding anti-hog cholera serum was complicated by an unexplained reduction of mortality in the group of principals not fed anti-hog cholera serum. Also, some pigs in both groups were able to eliminate E. coli from the serous cavities after the acute septicemic phase of infection.

Serums collected from the pigs at 5 to 12 days of age, before and after exposure to E. coli in Experiment 6, were negative for O agglutinins to whole cell E. coli 083 somatic antigen, alone or combined with latex particles, in tube agglutination tests in final dilutions of 1:10 and 1:20.

Results of electrophoresis indicated a range of 0 to 13% gamma globulin with similar ranges in pigs irrespective of the feeding of anti-hog cholera serum. Also, there was no difference between groups in (1) hemoglobin values and packed cell volumes, which increased to a peak

at 12 days of age, (2) declining α_1 globulin levels with increasing age, and (3) serum potassium values, which increased with increasing age of the pigs.

BIBLIOGRAPHY

- Armed Forces Institute of Pathology: Manual of Histologic and Special Staining Technics. Washington, D.C. 1957.
- Bauriedel, W. R., Hoerlein, A. B., Picken, J. C., Jr., and Underkofler, L. A.: Selection of diet for studies of vitamin B₁₂ depletion using unsuckled baby pigs. J. Agr. Food Chem., 2, ¹²(April, 1954): 468-471.
- Becker, D. E., Ullrey, D. E., Terrill, S. W., and Notzold, R. A.: Failure of the newborn pig to utilize dietary sucrose. Science, 120, (1954): 345-346.
- Benjamin, M. M.: Outline of Veterinary Clinical Pathology. The Iowa State Univ. Press, Ames, Iowa. 1961.
- Betts, A. O., Lamont, P. H., and Littlewort, M. C. G.: The production by hysterectomy of pathogen-free, colostrum-deprived pigs and the foundation of a minimal-disease herd. Vet. Rec., 72(24), (1960): 461-468.
- Breed, R. S., Murray, E. G. D., and Smith, N. R.: Bergey's Manual of Determinative Bacteriology, 7th ed. Williams and Wilkins Co., Baltimore, Md. 1957.
- Bustad, L. K., Ham, W. E., and Cunha, T. J.: Preliminary observations on using a synthetic milk for raising pigs from birth. Arch. Biochem., 17, (1948): 249-260.
- Calhoun, M. L., and Smith, E. M.: Hematology and hematopoietic organs, in Diseases of Swine, 2nd ed. H. W. Dunne (ed.). The Iowa State Univ. Press, Ames, Iowa. 1964.
- Campbell, D. H., Garvey, J. S., Cremer, N. E., and Sussdorf, D. H.: Methods in Immunology; A Laboratory Text for Instruction and Research. W. A. Benjamin, N. Y. 1963.
- Carle, B. N., and Dewhirst, W. H., Jr.: A Method for bleeding swine. J.A.V.M.A., 101, (1942): 495-496.
- Carpenter, P. L.: Immunology and Serology, 2nd ed. W. B. Saunders Co., Philadelphia and London. 1965.
- Chopra, S. L., Blackwood, A. C., and Dale, D. G.: Anaphylactic aspects of edema disease. Canad. J. Comp. Med. and Vet. Sci., 28, (Nov., 1964): 272-278.

- Christie, B. R.: Experimental Colibacillosis in Gnotobiotic Pigs. M.S. Thesis, Michigan State University, East Lansing. 1967.
- Coles, E. H.: Veterinary Clinical Pathology. W. B. Saunders Co., Philadelphia, Pa., and London. p. 178-179. 1967.
- Dillard, W. M.: Colibacillosis in baby pigs. Mod. Vet. Prac., 45(1), (Jan, 1964): 47-49.
- Dunne, H. W.: Streptococcosis and colibacillosis, in Diseases of Swine, H. W. Dunne (ed.). The Iowa State Coll. Press, Ames, Iowa. 1958.
- Dunne, H. W.: An expanding concept of colibacillosis with emphasis on the disease in swine. Canad. J. Comp. Med. and Vet. Sci., 23, (1959): 101-104.
- Dunne, H. W.: Streptococcosis, colibacillosis and bordetellosis, in Diseases of Swine, 2nd ed. H. W. Dunne (ed.). The Iowa State Univ. Press, Ames, Iowa. 1964.
- Edwards, P. R., and Ewing, W. H.: The Escherichia coli group, in Identification of Enterobacteriaceae, 2nd ed. Burgess Pub. Co., Minneapolis, Minn. 1962.
- Ewing, W. H.: Enteropathogenic Escherichia coli serotypes. Ann. N. Y. Acad. Sci., 66, (1956): 61-70.
- Frankel, S., and Reitman, S.: Gradwohl's Clinical Laboratory Methods and Diagnosis, 6th ed., Vol. 1. The C. V. Mosby Co., St. Louis, Mo. 1963.
- Frankel, S., and Reitman, S.: Gradwohl's Clinical Laboratory Methods and Diagnosis, 6th ed., Vol. 2. The C. V. Mosby Co., St. Louis, Mo. 1963.
- Gardiner, M. R., Sippel, W. L., and McCormick, W. C.: The blood picture in newborn pigs. Am. J. Vet. Res., 14, (Jan., 1953): 68-71.
- Glässer, K., Hupka, E., and Wetzell, R.: Die Krankheiten des Schweines, 5th ed. M. and H. Schaper, Hanover, Germany. 1950.
- Glimstedt, G.: Bakterienfreie Meerschweinchen. Acta path. et microbiol. Scand. Suppl. 30, (1936): 1-295.
- Glimstedt, G.: The germfree animal as a research tool. Ann. N. Y. Acad. Sci., 78(1), (1959): 281-284.
- Gordon, H. A.: Morphological and physiological characterization of germfree life. Ann. N. Y. Acad. Sci., 78(1), (1959): 208-220.
- Gordon, W. A. M., and Luke, D.: Gastroenteritis in young piglets associated with Escherichia coli. Vet. Rec., 70, (1960): 542-543.

- Gossling, J., and Rhoades, H. E.: Serologic types of Escherichia coli isolated from certain pigs with enteric disorders. Cornell Vet., 56, (1966): 344-353.
- Gustafsson, B. E.: Lightweight stainless steel systems for rearing germ-free animals. Ann. N. Y. Acad. Sci., 78(1), (1959): 17-28.
- Gustafsson, B. E., and Laurell, C.-B.: Gamma-globulin production in germfree rats after bacterial contamination. J. Exp. Med., 110, (1959): 675-684.
- Hagan, W. A.: Bacterium coli, in The Infectious Diseases of Domestic Animals. Comstock Pub. Co., Ithaca, N. Y. 1947.
- Hagan, W. A.: Diarrheal diseases in animals: an appraisal. Ann. N. Y. Acad. Sci., 66, (1956): 14-17.
- Hoerlein, A. B.: The influence of colostrum on antibody response in baby pigs. J. Immunol., 78(2), (1957): 112-117.
- Ingram, P. L., and Lovell, R.: (3) Infection by Escherichia coli and Salmonella. Vet. Rec., 72(51), (1960): 1183-1185.
- Johnson, B. C., James, M. F., and Krider, J. L.: Raising newborn pigs to weaning age on a synthetic diet with attempt to produce pteroylglutamic acid deficiency. J. Ani. Sci., 7, (1948): 486-493.
- Jones, J. E. T., Sellers, K. C., and Smith, H. W.: The evaluation of live and dead E. coli vaccines administered to the pregnant female in the prevention of scouring in piglets. Vet. Rec., 74, (1962): 202-204.
- Jubb, K. V. F., and Kennedy, P. C.: Pathology of Domestic Animals, Vol. 2. Academic Press, N. Y. 1963.
- Kaeberle, M. L., and Segre, D.: The persistence of passively administered antibodies of homologous and heterologous origin in the circulation of baby pigs. Am. J. Vet. Res., 25(107), (July, 1964): 1103-1110.
- Kauffmann, F.: The serology of the coli group. J. Immunol., 57, (1947): 71-100.
- Kauffmann, F.: The Bacteriology of Enterobacteriaceae. Scand. Univ. Bucks, Munksgaard, Copenhagen, Denmark. 1966.
- Kenworthy, R., and Allen, D.: Influence of diet and bacteria on small intestinal morphology with special reference to early weaning and Escherichia coli. Studies with germfree and gnotobiotic pigs. J. Comp. Path., 76, (1966): 291-296.
- Kernkamp, H. C. H.: A reply to a reader's question concerning the lesions caused by coliform infections. N. Am. Vet., 31, (1950): 336.

- Kohler, E. M., and Bohl, E. H.: Prophylaxis of diarrhea in newborn pigs. J.A.V.M.A., 144, (June, 1964): 1294-1297.
- Lamont, H. G., Luke, D., and Gordon, W. A. M.: Some pig diseases. Vet. Rec., 62, (1950): 737-743.
- Landy, J. J., Growdon, J. H., and Sandberg, R. L.: Use of large, germfree animals in medical research. J.A.M.A., 178, (1961): 1084-1087.
- Landy, J. J., and Ledbetter, R. K.: Delivery and maintenance of the germfree pig, in Swine in Biomedical Research. Frayn Printing Co., Seattle, Wash. p. 619-632. 1966.
- Lecce, J. G.: Porcine polyserositis with arthritis: isolation of a fastidious pleuropneumonia-like organism and Hemophilus influenzae suis. Ann. N. Y. Acad. Sci., 79, (1960): 670-676.
- Lecce, J. G., Matrone, G., and Morgan, D. O.: The effect of diet on the maturation of the neonatal piglet's serum protein profile and resistance to disease. Ann. N. Y. Acad. Sci., 94, (1961): 250-264.
- Lecce, J. G., and Reep, B. R.: Escherichia coli associated with colostrum-free neonatal pigs raised in isolation. J. Exp. Med., 115(3), (1962): 491-501.
- Lemcke, R. M., Bellis, D. B., and Hirsch, A.: Preliminary observations on the relation of Escherichia coli to gut oedema of baby pigs. Vet. Rec., 69, (1957): 601-603.
- Lemcke, R. M., and Hurst, A.: Antibody content of colostrum and piglet serum following vaccination of the sow. J. Comp. Path., 71, (1961): 268-278.
- McBryde, C. N.: Acute enteritis in young pigs due to infection with colon group. J.A.V.M.A., 84, (1934): 36-50.
- McCance, R. A., and Widdowson, E. M.: The effect of colostrum on the composition of newborn piglets serum. J. Physiol., 145, (1959): 547-550.
- Merchant, I. A., and Packer, R. A.: Veterinary Bacteriology and Virology, 5th ed. The Iowa State Coll. Press, Ames, Iowa. 1958.
- Meyer, R. C., Bohl, E. H., and Kohler, E. M.: Procurement and maintenance of germfree swine for microbiological investigation. Appl. Microbiol., 12(4), (1964): 295-300.
- Miller, E. R., Ullrey, D. E., Ackerman, I., Schmidt, D. A., Hoefer, J. A., and Luecke, R. W.: Swine hematology from birth to maturity. I. Serum proteins. J. Ani. Sci., 20, (Feb., 1961a): 31-35.
- Miller, E. R., Ullrey, D. E., Ackerman, I., Schmidt, D. A., Hoefer, J. A., and Luecke, R. W.: Swine hematology from birth to maturity. II. Erythrocyte population, size, and hemoglobin concentration. J. Ani. Sci., 20, (Nov., 1961b): 890-897.

- Miller, E. R., Harmon, B. G., Ullrey, D. E., Schmidt, D. A., Luecke, R. W., and Hoefer, J. A.: Antibody absorption, retention and production by the baby pig. *J. Ani. Sci.*, 21, (May, 1962): 309-314.
- Miyakawa, M.: The Miyakawa remote-control germfree rearing unit. *Ann. N. Y. Acad. Sci.*, 78(1), (1959a): 37-46.
- Miyakawa, M.: The lymphatic system of germfree guinea pigs. *Ann. N. Y. Acad. Sci.*, 78(1), (1959b): 221-236.
- Moon, H. W., Sorensen, D. K., and Sautter, J. H.: Escherichia coli infection of the ligated intestinal loop of the newborn pig. *Am. J. Vet. Res.*, 27(120), (1966a): 1317-1325.
- Moon, H. W., Sorensen, D. K., Sautter, J. H., and Higbee, J. M.: Association of Escherichia coli with diarrheal disease of the newborn pig. *Am. J. Vet. Res.*, 27(119), (1966b): 994-995.
- Morrill, C. C.: Studies on baby pig mortality. X. Influence of environmental temperature on fasting newborn pigs. *Am. J. Vet. Res.*, 13, (July, 1952): 322-324.
- Muraschi, T. F.: Latex leptospiral agglutination test. *Proc. Soc. Exp. Biol. and Med.*, 99, (1958): 235-238.
- Myers, W. L., and Segre, D.: The immunologic behavior of baby pigs. III. Transplacental transfer of antibody globulin in swine. *J. Immunol.*, 91(5), (1963): 697-701.
- Namioka, S., and Murata, M.: Studies on the pathogenicity of Escherichia coli. II. The effect of a substance obtained from pseudoeosinophils of rabbits on the organism. *Cornell Vet.*, 52(3), (1962): 289-296.
- Pirtle, E. C., and Deyoe, B. L.: Electrophoresis of serum from specific-pathogen-free swine. *Am. J. Vet. Res.*, 24, (July, 1963): 762-765.
- Reyniers, J. A.: Rearing of a caesarian-born M. rhesus monkey under sterile conditions. *J. Bact.*, 43, (1942): 778.
- Reyniers, J. A.: The pure-culture concept and gnotobiotics. *Ann. N. Y. Acad. Sci.*, 78(1), (1959): 3-16.
- Reyniers, J. A., Trexler, P. C., Ervin, R. F., Wagner, M., Luckey, T. D., and Gordon, H. A.: The need for a unified terminology in germfree life studies. *Lobund Reports No. 2*. Univ. Notre Dame Press, Notre Dame, Ind. p. 151-162. 1949.
- Roberts, E. D., Switzer, W. P., and Ramsey, F. K.: Pathology of the visceral organs of swine inoculated with Mycoplasma hyorhinis. *Am. J. Vet. Res.*, 24(98), (Jan., 1963a): 9-18.

- Roberts, E. D., Switzer, W. P., and Ramsey, F. K.: The pathology of Mycoplasma hyorhinis arthritis produced experimentally in swine. *Am. J. Vet. Res.*, 24(98), (Jan., 1963b): 19-31.
- Roberts, H. E., and Vallely, T. F.: An investigation into the relationships of hemolytic Escherichia coli to disease in pigs. *Vet. Rec.*, 71, (1959): 846-850.
- Roy, J. H. B., Shillam, K. W. G., Hawkins, G. M., Lang, J. M., and Ingram, P. L.: The effect of white scours on the sodium and potassium concentration in the serum of newborn calves. *Brit. J. Nutr.*, 13, (1959): 219-226.
- Rutqvist, L.: Electrophoretic patterns of blood serum from pig fetuses and young pigs. *Am. J. Vet. Res.*, 19, (1958): 25-31.
- Saunders, C. N., Stevens, A. J., Spence, J. B., and Betts, A. O.: Escherichia coli infection: reproduction of the disease in "pathogen-free" piglets. *Res. Vet. Sci.*, 4(2), (April, 1963a): 347-357.
- Saunders, C. N., Stevens, A. J., Spence, J. B., and Sojka, W. J.: Escherichia coli infection in piglets. *Res. Vet. Sci.*, 1, (1960): 28-35.
- Saunders, C. N., Stevens, A. J., Spence, J. B., and Sojka, W. J.: Escherichia coli infection: reproduction of the disease in naturally-reared piglets. *Res. Vet. Sci.*, 4(2), (April, 1963b): 333-346.
- Schalm, O. W.: *Veterinary Hematology*, 2nd ed. Lea and Febiger, Philadelphia, Pa. 1965.
- Schmidt, D. A.: Some Micromorphologic Features of the Intestinal Tract of the Young Pig. I. Mitotic Activity of the Epithelium. II. A Micromorphologic Comparison of Gnotobiotic and Farm-Raised Pigs. Ph.D. Thesis, Michigan State University, East Lansing, Mich. 1961.
- Schofield, F. W., and Davis, D.: Oedema disease in swine. II. Experiment conducted in a susceptible herd. *Canad. J. Comp. Med. and Vet. Sci.*, 19, (1955): 242-245.
- Segre, D., and Kaeberle, M. L.: The immunologic behavior of baby pigs. I. Production of antibodies in three-week-old pigs. *J. Immunol.*, 89(6), (Dec., 1962): 782-789.
- Segre, D., and Kaeberle, M. L.: Antibody production in baby pigs. *Am. J. Vet. Res.*, 105, (March, 1964): 413-419.
- Shanks, P. L.: An unusual condition affecting the digestive organs of the pig. *Vet. Rec.*, 50, (1938): 356-358.

- Singer, J. M., and Plotz, C. M.: The latex fixation test. I. Application to the serologic diagnosis of rheumatoid arthritis. *Am. J. Med.*, 21, (1956): 888-893.
- Smith, D. T., Conant, N. F., and Overman, J. R.: Microbiology (Zinsser). 13th ed. Appleton-Century-Crofts, N. Y. 1964.
- Smith, H. W., and Jones, J. E. T.: Observations on the alimentary tract and its bacterial flora in healthy and diseased pigs. *J. Path. Bact.*, 86, (1963): 387-412.
- Smith, C. K.: Rearing gnotobiotic (germfree) goats and lambs. Conf. on Application of Cesarean-derived Animals, Michigan State Univ., March, 1961.
- Sojka, W. J.: Escherichia coli infections in pigs, in Escherichia coli in Domestic Animals and Poultry. Commonwealth Agricultural Bureau, Farnham Royal, Bucks, Eng. 1965.
- Sojka, W. J., Erskine, R. G., and Lloyd, M. K.: Hemolytic Escherichia coli and "oedema disease" of pigs. *Vet. Rec.*, 69, (1957): 293-301.
- Sojka, W. J., Lloyd, M. K., and Sweeney, E. J.: Escherichia coli serotypes associated with certain pig diseases. *Res. Vet. Sci.*, 1, (1960): 17-27.
- Speer, V. C., Brown, H., Quinn, L., and Catron, D. V.: The cessation of antibody absorption in the young pig. *J. Immunol.*, 83, (1959): 632-634.
- Sprinz, H., Kundel, D. W., Dammin, G. J., Horowitz, R. E., Schneiper, H., and Formal, S. B.: The response of the germfree guinea pig to oral challenge with Escherichia coli and Shigella flexneri. *Am. J. Path.*, 39(6), (1961): 681-695.
- Stevens, A. J.: Enteritis in pigs -- a working hypothesis. *Brit. Vet. J.*, 119, (1963a): 520-526.
- Stevens, A. J.: Coliform infections in the young pig and a practical approach to the control of enteritis. *Vet. Rec.*, 75, (1963b): 1241-1245.
- Switzer, W. P., Preston, K. S., and Morrissey, T. J.: Factors in SPF program ... Substitutes needed for biological protection. *Iowa State Coll. Vet.*, 25(1), (1962): 5-10.
- Tadd, A. D., and Hurst, A.: The effect of feeding colicinogenic Escherichia coli on the intestinal E. coli of early weaned pigs. *J. Appl. Bact.*, 24(2), (1961): 222-228.
- Taylor, J.: Host specificity and enteropathogenicity of Escherichia coli. *J. Appl. Bact.*, 24(3), (1961): 316-325.

- Thomlinson, J. R., and Buxton, A.: Anaphylaxis in pigs and its relationship to the pathogenesis of oedema disease and gastroenteritis associated with Escherichia coli. Immunol., 6, (1963): 126-139.
- Thorbecke, G. J.: Some histological and functional aspects of lymphoid tissue in germfree animals. I. Morphological studies. Ann. N. Y. Acad. Sci., 78(1), (1959): 237-246.
- Thorbecke, G. J., and Benacerraf, B.: Some histological and functional aspects of lymphoid tissue in germfree animals. II. Studies on phagocytosis in vivo. Ann. N. Y. Acad. Sci., 78(1), (1959): 247-253.
- Trautmann, A., and Fiebiger, J.: Fundamentals of the Histology of Domestic Animals. Comstock Pub. Assoc., Ithaca, N. Y. 1952.
- Trexler, P. C.: The use of plastics in the design of isolator systems. Ann. N. Y. Acad. Sci., 78(1), (1959): 29-36.
- Tiselius, A., and Kabat, E. A.: An electrophoretic study of immune sera and purified antibody preparations. J. Exp. Med., 69, (1939): 119-131.
- Underdahl, N. R., and Young, G. A.: An improved hood for swine hysterectomies. J.A.V.M.A., 131, (1957a): 222-224.
- Underdahl, N. R., and Young, G. A.: An isolation brooder for raising disease-free pigs. J.A.V.M.A., 131, (1957b): 279-283.
- Wagner, M.: Serologic aspects of germfree life. Ann. N. Y. Acad. Sci., 78(1), (1959): 261-271.
- Waxler, G. L.: Studies on Gnotobiotic Pigs. Part One. A Technique for Rearing Gnotobiotic Pigs. Part Two. A Comparison of Body Weights, Organ Weights, and Some Histological Features of Gnotobiotic and Farm-Raised Pigs. Ph.D. Thesis, Michigan State University, East Lansing, Mich. 1961.
- Waxler, G. L., Schmidt, D. A., and Whitehair, C. K.: Technique for rearing gnotobiotic pigs. Am. J. Vet. Res., 27, (Jan., 1966): 300-307.
- Waxler, G. L., and Whitehair, C. K.: Germfree Swine in Biomedical Research (Symposium 1965). Swine in Biomedical Research. Frayn Printing Co., Seattle, Wash. p. 611-618. 1966.
- Whitehair, C. K., and Thompson, C. M.: Observations on raising "disease-free" swine. J.A.V.M.A., 128, (1956): 94-98.
- Wintrobe, M. M.: Nutritive requirements of young pigs. Am. J. Physiol., 126, (1939): 375-387.

- Wostmann, B. S.: Serum proteins in germfree vertebrates. *Ann. N. Y. Acad. Sci.*, 78(1), (1959): 254-260.
- Young, G. A., and Underdahl, N. R.: Swine influenza as a possible factor in suckling pig mortalities. II. Colostral transfer of hemagglutinin inhibitors for swine influenza virus from dam to offspring. *Cornell Vet.*, 34, (1949): 120-128.
- Young, G. A., and Underdahl, N. R.: A diet and technique for starting pigs without colostrum. *Arch. Biochem. Biophys.*, 32, (1951): 449-450.
- Young, G. A., and Underdahl, N. R.: Isolation units for growing baby pigs without colostrum. *Am. J. Vet. Res.*, 14, (1953): 571-574.
- Young, G. A., Underdahl, N. R., and Hinz, R. W.: Procurement of baby pigs by hysterectomy. *Am. J. Vet. Res.*, 16, (1955): 123-131.
- Young, G. A., Underdahl, N. R., Sumption, L. J., Peo, E. R., Jr., Olsen, L. S., Kelley, G. W., Hudman, D. B., Caldwell, J. D., and Adams, C. H.: Swine repopulation. I. Performance within a "disease-free" experiment station herd. *J.A.V.M.A.*, 134, (1959): 491-496.

VITA

The author was born August 24, 1924, in Pulaski County, Illinois. He attended public schools and graduated from Mound City Community High School in May, 1941. After attending Draughon's Business College in Paducah, Kentucky, he was employed as a shop clerk in Peoria, Illinois. He served in the U. S. Army Air Corps from May, 1943, until September, 1945.

During assignment to the College Training Detachment for aviation students at Michigan State College he met Jean Marie Black, whom he married at Okemos, Michigan, in December, 1944. Upon release from active military service as a pilot of multiengine aircraft he enrolled at Michigan State College in the preveterinary curriculum and received the D.V.M. degree from there in June, 1950. After a year of large animal practice at Montfort, Wisconsin, he joined the faculty of the College of Veterinary Medicine, University of Georgia, at Athens, Georgia, where he remained for one year and returned to Illinois, where he worked in bovine brucellosis and tuberculosis control for Union County and the State of Illinois. In April, 1953, he moved to Albion, Indiana, and conducted a private rural veterinary practice until August, 1960, when he matriculated at the University of Michigan, Ann Arbor, Michigan, and earned a Master of Public Health degree from the School of Public Health in June, 1961.

In September, 1962, he enrolled as a part-time graduate student in the Department of Pathology at Michigan State University, where he was

employed as an assistant instructor and later as an instructor. He is currently employed as pathologist for the Florida Department of Agriculture, Animal Disease Diagnostic Laboratory, at Kissimmee, Florida.

The author is a member of the A.V.M.A., Florida V.M.A., Phi Zeta, and Sigma Xi.