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THE RESPONSE OF LIVER AND SPLEEN TO ESCHERICHIA COLI BACTEREMIA IN TURKEYS

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The response of liver and spleen
to *Escherichia coli* bacteremia in turkeys

by

Lawrence Harry Arp

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major: Veterinary Pathology

Approved:

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*In Charge of Major Work*

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# TABLE OF CONTENTS

**GENERAL INTRODUCTION**

**LITERATURE REVIEW**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli infections of poultry</td>
<td>3</td>
</tr>
<tr>
<td>Role of liver and spleen in bacteremia of animals</td>
<td>4</td>
</tr>
<tr>
<td>Anatomy of the avian liver and spleen</td>
<td>6</td>
</tr>
<tr>
<td>Bacteremia and septicemia caused by <em>E. coli</em></td>
<td>8</td>
</tr>
<tr>
<td>Virulence factors of invasive <em>E. coli</em></td>
<td>10</td>
</tr>
</tbody>
</table>

**INTERACTION OF BLOOD-BORNE ESCHERICHIA COLI**

**WITH PHAGOCYTES OF SPLEEN AND LIVER IN TURKEYS**

**ABSTRACT**

**INTRODUCTION**

**MATERIALS AND METHODS**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli strains</td>
<td>17</td>
</tr>
<tr>
<td>Turkeys</td>
<td>17</td>
</tr>
<tr>
<td>Experimental design</td>
<td>17</td>
</tr>
<tr>
<td>Necropsy procedure</td>
<td>18</td>
</tr>
<tr>
<td>Quantitation of bacteria</td>
<td>18</td>
</tr>
<tr>
<td>Histopathologic examination</td>
<td>18</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td>19</td>
</tr>
</tbody>
</table>

**RESULTS**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial titers</td>
<td>20</td>
</tr>
</tbody>
</table>
ABSTRACT

INTRODUCTION

MATERIALS AND METHODS

Bacteria

Turkeys

Passive immunization

Necropsy procedure and specimen collection

Estimation of percentage of E. coli inoculum in selected tissues

Histology

Electron microscopy

RESULTS

Experimental design

Clearance of E. coli from circulating blood

Localization of E. coli in tissue

Histologic examination

Electron microscopic examination

DISCUSSION
GENERAL INTRODUCTION

Infections of young poultry caused by Escherichia coli are commonly seen as airsacculitis, pericarditis, and septicemia (14, 72). Bacteremia is recognized as an important feature of natural E. coli infections and of experimental infections initiated by the inoculation of E. coli parenterally or into the respiratory tract (4, 27, 57). In a recent study, virulent E. coli was detectable in blood of turkeys within 12 h of aerosol exposure but avirulent E. coli did not produce detectable bacteremia (4). It is unclear whether avirulent E. coli lacks the capacity to invade the bloodstream or whether avirulent E. coli is so rapidly cleared from blood that it is undetectable by blood culture.

Clearance of E. coli from the blood of mammals is accomplished most efficiently by the mononuclear phagocyte system (23) of the liver and spleen (7, 62, 78). In mammals, the efficiency with which bacteria are cleared from blood is dependent upon both bacterial and host factors. Encapsulated bacteria and bacteria having other antiphagocytic surface components tend to resist removal from the bloodstream (68, 93). However, animals with adequate levels of serum opsonins clear virulent E. coli from blood as efficiently as they clear avirulent strains (7, 78). Impaired opsonization is commonly observed in human patients with severe gram-negative bacteremia (20, 90).

Little is known of the response of avian species to E. coli bacteremia. The objectives of the present research were i) to compare the interaction of blood-borne virulent and avirulent E. coli with
phagocytes in spleen and liver of turkeys, ii) to determine the effect of specific antibody on the clearance of virulent E. coli from blood and localization in tissues, and iii) to characterize the lesions in spleen and liver of turkeys associated with E. coli bacteremia.

This dissertation is presented in alternate format including 3 manuscripts which are submitted or accepted for publication in scientific journals. The first manuscript has been accepted for publication in the American Journal of Veterinary Research. The second manuscript has been submitted to Infection and Immunity, and the third manuscript has been submitted to Avian Pathology. The format used for this dissertation is that of the journal, Infection and Immunity. A literature review precedes the first manuscript and a general summary and discussion follow the final manuscript. Literature cited throughout the dissertation is listed once, at the end of the dissertation.

The Ph.D. candidate, Lawrence H. Arp, was the principal or sole investigator for each of these studies. The coauthor of the first study, Norman F. Cheville, assisted with interpretation of histologic and electron microscopic results and in the preparation of the manuscript.
Escherichia coli infections of poultry. Escherichia coli has been recognized as a cause of disease in poultry (43) and other avian species (41) since the late 1800s. Numerous manifestations of E. coli infection in poultry have been described, including disease of chicks (16, 33), enteritis (56), salpingitis (21, 32), airsacculitis (27, 89), colisepticemia (27, 86), osteomyelitis and synovitis (25, 52), and coli-granuloma (34, 85). Diseases of poultry caused by E. coli have been reviewed by Sojka (72) and Gross (31). Manifestations of E. coli infection and their sequelae are sometimes grouped as acute colisepticemia, subacute fibrinopurulent serositis, and chronic granulomatous pneumonitis/hepatitis/enteritis (14). The natural disease is most commonly subacute and characterized by pericarditis, perihepatitis, airsacculitis, synovitis, panophthalmitis, and salpingitis (14, 73). The subacute disease is a common sequel in birds surviving acute colisepticemia.

Study and control of E. coli infections of poultry have been difficult due to the complex pathogenesis of the disease. Epizootics of E. coli infection are commonly associated with viral or mycoplasmal respiratory disease, vaccination, or environmental stress (28, 64, 65). Escherichia coli from poultry vary in virulence, susceptibility to antibiotics, and expression of surface antigens (73). Coincidently with the trend toward intensive confinement housing of poultry, there has been an increased incidence of poultry diseases caused by E. coli in the last two decades (57, 72).
The typical manifestations of the natural disease, septicemia and airsacculitis-pericarditis, have been reproduced experimentally by inoculation of poultry with virulent *E. coli* (28, 57, 73). Disease has been produced in poultry by virulent *E. coli* inoculated into air sacs (21, 30), intratracheally (21), intravenously (73), intraperitoneally (84), and by aerosol (4, 29). Oral or intranasal inoculation of *E. coli* does not produce significant disease in poultry (57).

Bacteremia is recognized as an important feature of natural *E. coli* infections and of experimental infections produced by inoculation of *E. coli* parenterally or into the respiratory tract (4, 27, 57). A large percentage of chicks are bacteremic 6 h after the inoculation of *E. coli* into air sacs (57). In a recent study, bacteremia was produced in young turkeys 12 h after aerosol exposure to virulent *E. coli* but not after exposure to avirulent *E. coli* (4). The mechanism whereby *E. coli* enter the bloodstream from respiratory passages remains a mystery (14), however, *E. coli* bacteremia is a common denominator for most, if not all, manifestations of *E. coli* infections in poultry.

Role of liver and spleen in bacteremia of animals. Transient bacteremia is thought to occur commonly in most animals. Bacteria may enter the bloodstream from periodontal tissues during chewing and dental procedures or from areas of local infection on skin and mucosal surfaces (8, 9). In spite of the absence of specific opsonins, most bacteria are readily cleared from circulating blood by the mononuclear phagocyte system (23) (reticuloendothelial system) of the spleen, liver, lung, bone marrow and lymphoid tissues (61). Bacteria which resist clearance
from blood are usually endowed with a capsule or other antiphagocytic surface component (68, 93). Generally, such bacteria behave as extracellular parasites and cause acute septicemic disease in animals (67, 93).

The rate of clearance from blood and the organ distribution of bacteria are determined by the bacterial species and strain, the presence of specific or nonspecific opsonins, and the species and age of the animal host (13, 61). The major portion of blood-borne bacteria are cleared by the mononuclear phagocytes of the liver (Kupffer cells) and spleen (7, 78). A minor portion of blood-borne bacteria are trapped in lung, kidney and bone marrow (6). Blood leukocytes are sometimes important in bacteremia, especially when clearance of bacteria by spleen and liver is slow (61). Profound leukopenia is commonly associated with bacteremia because of sequestration of leukocytes in capillary beds, particularly in the lung (62). Intravascular phagocytosis of bacteria by blood leukocytes is enhanced in the late stages of bacteremia by the presence of fibrin and platelet thrombi in the microcirculation (61).

Quantitative studies of organ distribution of blood-borne bacteria in mammals indicate that most bacteria are trapped in the liver when clearance is rapid (7, 61, 74). Bacterial trapping in the spleen is equally avid but quantitatively less important because of the small size of the spleen relative to the liver (7). Therefore, easily phagocytized and optimally opsonized bacteria are rapidly cleared from the blood in the liver; bacteria which resist clearance from blood are poorly phagocytized in the liver (7). In contrast, most bacteria are avidly
phagocytized in the spleen whether the bacteria are opsonized or not (7). The greater capacity of splenic macrophages, compared with hepatic macrophages, to phagocytize nonopsonized bacteria may be due to the sluggish flow of blood through the splenic parenchyma compared to the hepatic sinusoids (7). A slower blood flow may enhance the attachment of bacteria to fixed macrophages (7).

Anatomy of the avian liver and spleen. The avian liver consists of anastomotic sheets of hepatocytes which enclose a labyrinth of irregular sinusoids. In contrast to the mammalian liver, sheets of hepatocytes are a single cell thick and lobular structure is not discrete. Portal canals, composed of vessels and ducts within a scant fibrous stroma, irregularly penetrate the hepatic parenchyma. Blood enters the liver by way of the hepatic arteries and portal veins. Small terminal branches of the portal vein interdigitate with those of the hepatic venous system and both systems communicate through the network of sinusoids. Arterial blood enters the hepatic sinusoids both directly and by first passing through the peribiliary capillary plexus to the portal venous system (35, 59).

Walls of the sinusoids are composed of a discontinuous layer of fenestrated endothelial cells and scattered mononuclear phagocytes (Kupffer cells). Kupffer cells bulge into the lumen of the sinusoids and avidly phagocytize colloids and particulate material in circulating blood. Ultrastructurally, avian Kupffer cells have an irregular cytoplasmic border, an irregular shaped nucleus, numerous lysosomes, few mitochondria, and scant rough endoplasmic reticulum (35, 59).
The avian spleen consists of approximately equal amounts of red and white pulp superimposed on a delicate framework consisting of reticular cells and fibers. True trabeculae are absent, but the reticular framework is particularly dense around arteries of the white pulp. Red pulp is a loose, spongy tissue composed of cellular cords containing lymphocytes, macrophages and circulating blood cells. White pulp is composed of lymphoid tissue associated with branches of the splenic arteries. At the periphery of the white pulp, small penicillar arteries branch 3 to 5 times to form sheathed capillaries (ellipsoids, Schweigger-Seidel sheaths). Periarteriolar lymphoid tissue is primarily thymic dependent, but bursal dependent germinal centers are often found adjacent to central and penicillar arterioles. Periellipsoidal lymphoid tissue is bursal dependent (22, 36). Blood entering the spleen first traverses the white pulp via trabecular arteries, central arteries, penicillar arterioles, and sheathed capillaries to terminal arterial capillaries which enter the red pulp sinuses. Blood percolates through the red pulp to venous sinuses which empty into collecting veins and finally trabecular veins (22, 35).

An important function of splenic reticular sheaths (ellipsoids) is the trapping of particles 100 Å to 1 μm in diameter from circulating blood. Particles of carbon or latex and staphylococci are found within reticular sheaths minutes after intravenous injection, whereas Candida albicans (diameter 3 to 5 μm) are localized exclusively in the red pulp. In cross-section, reticular sheaths consist of 2 or 3 layers of macrophages with numerous pseudopodia intermingled with a network of reticular
fibers. At the center is the reticular sheath capillary with tall endothelial cells surrounded by a thick, discontinuous basal lamina and reticular fibers. Cytoplasmic protrusions of the endothelial cells commonly extend through the gaps in the basal lamina. Macrophages, laden with antigen, migrate from the reticular sheath back along pencycular arterioles to their bifurcation where germinal centers form in periarteriolar lymphoid tissue (92).

**Bacteremia and septicemia caused by* E. coli*.** Septicemia caused by* E. coli* is a serious, potentially fatal disease of poultry and caged birds, calves, pigs, foals (70, 72), and humans (42, 45). For many animal species, bacteremic* E. coli* is an important threat for the very young or the aged, and for animals with defective specific and nonspecific host defense mechanisms. Neonatal animals are at risk to coli-septicemia when transfer of maternal antibody to offspring is defective (70). Coli-septicemia of poultry is commonly associated with concurrent viral or mycoplasmal respiratory disease, environmental stress, vaccination and intensive confinement housing (72). In humans admitted to hospitals, the incidence of gram-negative bacteremia (*E. coli* is the most important agent) and associated fatality nearly doubled from 1965 to 1974 (42). The increase of gram-negative bacteremias of hospital patients is a reflection of increased patient age, extensive use of sophisticated diagnostic and therapeutic procedures and the use of antineoplastic agents (42). Impaired opsonization is commonly associated with gram-negative bacteremia in human patients (20, 90). The frequency of shock
or death, as a sequel to gram-negative rod bacteremia in humans, parallels an early reduction of the serum complement component, C3 (46).

*Escherichia coli* bacteremia has been studied experimentally in rabbits (7, 11, 62), mice (7, 74), guinea pigs (7), rats (63, 88) and nonhuman primates (24). When *E. coli* is injected into the ear vein of rabbits, approximately two-thirds of the circulating bacteria are removed in transit through the liver and spleen (62). Clearance of *E. coli* from blood is rapid for the first 20 min and moderate from 40 to 90 min at which time numbers of *E. coli* increase steadily. Progressive leukopenia is associated with early bacteremia due to sequestration of leukocytes in liver and lung. Pulmonary trapping of *E. coli* appears to follow pulmonary sequestration of leukocytes, particularly when bacteremia is of 3 to 5 h duration. There is little evidence for survival of *E. coli* within phagocytes (62).

Overwhelming bacteremia with pyrexia and vascular collapse (septicemia) occurs in rabbits fed *E. coli* in drinking water followed by treatment with nitrogen mustard and rectal instrumentation (11). Bacteremia does not occur in the absence of granulocytopenia or pelvic instrumentation with a rectal probe (11).

Clearance of $^{32}$P-labeled *E. coli* from the blood of mice is an exponential function of time until less than 10% of the injected bacteria remain; then the rate of clearance decreases progressively (7). The majority of $^{32}$P-labeled *E. coli*, when injected intravenously into mice, is phagocytized by mononuclear phagocytes of the liver and spleen. Small numbers of *E. coli* are found in lungs and traces in the kidneys (7).
Passive immunization of mice with specific antibody against *E. coli* lipopolysaccharide increases the rate of phagocytosis of blood-borne *E. coli*. A direct relationship exists between the antibody titer and the rate of phagocytosis until an antibody excess is reached (7). It appears that clearance of *E. coli* from blood of mice is inefficient in the absence of antibodies (7), and the rate of clearance of *E. coli* from germ-free mice is less than in conventional mice (7). Decomplementation of mice and nonhuman primates results in a markedly reduced rate of *E. coli* clearance from the blood (24, 74). Phagocytosis of *E. coli* by Kupffer cells in isolated, perfused rat liver is enhanced by specific antibody, complement, and another heat-labile factor which is absorbed by zymosan (88).

**Virulence factors of invasive *E. coli***. The capacity of bacteria to produce disease is often related to specialized structures and physico-chemical properties of the bacterial surface. Although properties of the bacterial surface are associated with invasiveness, resistance to phagocytosis, and colonization, the virulence of bacteria is rarely determined by a single factor (54). Most strains of *E. coli* which cause bacteremia in humans have a more negative surface charge than *E. coli* isolated from urine or feces (75). The negative surface charge of bacteremic *E. coli* is attributed to the capsular polysaccharide K antigens (75). However, in one study the amount of K antigen produced by bacteremic *E. coli* was not significantly greater than that produced by fecal and urine isolates (47). The poor correlation between bacteremic *E. coli* and K antigen production is explained in part by the fact that
virulence usually depends more on the overall properties of the bacterial surface than on the quantity of K and O antigens (76). Not all E. coli produce capsular antigens, but virulent strains usually do (54). Production of K antigens by E. coli of human origin is associated with resistance to phagocytosis, resistance to killing by complement and inefficient opsonization, particularly by the alternative complement pathway (10, 20, 38, 77).

Strains of E. coli having smooth hydrophilic O antigens tend to resist phagocytosis whether or not K antigens are present (75), and resistance to phagocytosis depends on the presence of complete polysaccharide side chains in the cell wall antigens (48, 67). An E. coli mutant lacking colitose in its cell wall was 100 times more susceptible to phagocytosis by mouse neutrophils than the parent strain (48). Mutations affecting the synthesis of the O-specific side chains may result in rough (R) mutants which are both more easily phagocytized and more sensitive to serum components than E. coli with complete O antigens (54). The superficial polysaccharide side chains of the lipopolysaccharide molecules may serve to keep antibodies and complement at a distance from the susceptible outer membrane and cytoplasmic membrane (15). Therefore, smooth E. coli strains resist the bactericidal effects of serum, whereas rough strains are susceptible to serum (54, 82).

A significant association exists in E. coli between the presence of a colicine V (Col V) plasmid and the ability to cause septicemia, particularly in cattle and chickens (71). Of 166 invasive strains of E. coli isolated from chickens, 126 strains (76%) produced Col V (70).
Pathogenicity trials of Col V+ and Col V- forms, of an O78:K80 E. coli strain, revealed that the Col V+ form is about 30 times more lethal than the Col V- form for chickens (70). Creation of Col V- forms of E. coli by treatment with sodium lauryl sulfate is accompanied by a decrease in pathogenicity (70). The increased virulence of Col V+ E. coli does not appear to be due to a lethal effect of colicine V or any other substance synthesized by the Col V plasmid, but it is conceivable that Col V+ E. coli are structurally different than Col V- E. coli (70). It was speculated that colicine V might form a proteinaceous coat around organisms that produce it and thus make them more resistant to phagocytosis and the effects of complement (70). In human volunteers, Col V+ E. coli have a greater ability to survive in, and to colonize, the alimentary tract (70).

The Vir plasmid, associated with some strains of E. coli and salmonella, is responsible for synthesis of a heat-labile, acid-sensitive, nondialyzable toxin which is lethal for rabbits, mice and chickens (69). Intravenous inoculation of chickens with membrane-sterilized supernatant from Vir+ E. coli cultures causes death within 6 to 24 h (69). At necropsy, chickens have large amounts of serous fluid in the abdominal cavity and pericardial sac (69). Of 166 invasive strains of E. coli isolated from chickens, none of the strains contained the Vir plasmid (70). Thus, the Vir plasmid, unlike the Col V plasmid, is not commonly found among invasive E. coli strains from poultry (70). A chick-lethal toxin, distinct from endotoxin and enterotoxins of E. coli, has been recovered from E. coli strains of bovine and avian origin. The toxin is heat-labile, antigenic, high in protein and inactivated by pronase,
trypsin, amylase and pancreatic lipase (83). The chick-lethal toxin resembles the toxin produced by *E. coli* containing the Vir plasmid (44). Both toxins are heat-labile, of high molecular weight and associated with the culture supernatant (70, 83).

Numerous types of pili (fimbriae) have been observed on *E. coli* and other bacteria (55). The natural function of pili is uncertain, but at least some types act as adhesins between the bacterial cell and host epithelium (55). Infections of the mammalian intestine and urinary bladder are often initiated by the specific attachment of *E. coli* to the mucosal surface mediated by pili (50, 80). In a recent study, most strains of *E. coli* which were virulent for turkeys were piliated when cultured *in vitro* (5). It was speculated that pili may be important mediators in the colonization of the respiratory tract by *E. coli* (5). In addition, production of pili has been attributed to possession of the Vir plasmid by some *E. coli* strains (44). On virulent *Neisseria gonorrhoeae*, pili enable the bacteria to resist phagocytosis (58). In contrast, piliated *E. coli* isolated from human blood and urine are more easily phagocytized than nonpiliated strains (66). Whether surface pili are important virulence factors of invasive *E. coli* has yet to be established.
INTERACTION OF BLOOD-BORNE *ESCHERICHIA COLI*
WITH PHAGOCYTES OF SPLEEN AND LIVER IN TURKEYS

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The response of splenic and hepatic macrophages to blood-borne virulent and avirulent *Escherichia coli* was studied in 3-week-old turkeys. Bacterial titers in blood, spleen, and liver were determined for 20 min after intravenous injection of *E. coli*. Spleen and liver were examined by light and electron microscopy. Blood titers of avirulent *E. coli* were reduced to 1/3,000 of their original level in 20 min, whereas titers of virulent *E. coli* were only slightly reduced. The *E. coli* localized in macrophages of hepatic sinusoids and splenic reticular sheaths (ellipsoids). In liver, phagocytosis was more efficient for avirulent *E. coli* than for virulent *E. coli*. In splenic macrophages, phagosomal membranes were separated from ingested avirulent *E. coli* by a prominent space, whereas phagosomal membranes surrounding virulent *E. coli* were wavy and closely apposed to the bacterial surface. The appearance of phagosomes may reflect the capacity of splenic macrophages to kill intracellular *E. coli*. Cultural and histopathologic results indicate that virulent *E. coli* resist trapping and killing by macrophages of spleen and liver.
INTRODUCTION

Diseases of turkeys caused by *Escherichia coli* vary from acute fatal septicemia of young birds to subacute disease in older birds with fibrinopurulent serositis or diffuse granulomatous inflammation (14). Strains of *E. coli* from turkeys vary greatly in their virulence for poults inoculated intravenously (i.v.) (14). The initial interaction of virulent bacteria with tissues of a nonimmune host is often dependent on specialized components of the bacterial surface (68, 93).

Virulent *E. coli* strains rapidly cause bacteremia in turkeys exposed by aerosol (4) and in chickens inoculated by the air sac route (57). Preliminary experiments with several *E. coli* strains from turkeys indicated that avirulent *E. coli* is rapidly cleared from blood of turkeys, whereas virulent *E. coli* resists clearance. In mammals, clearance of bacteria from circulating blood depends largely on the capacity of macrophages in spleen and liver to eliminate the bacteria before significant multiplication occurs (7).

The purpose of the present study was to compare the interaction of blood-borne virulent and avirulent *E. coli* with phagocytes in spleen and liver of turkeys. Clearance rates of *E. coli* from the bloodstream, sites of phagocytosis in spleen and liver, and the interactions between phagocytes and intracellular *E. coli* were investigated.
**MATERIALS AND METHODS**

**Escherichia coli strains.** The virulent *E. coli* serotype used was 078:K80:H9 (V078), and the avirulent *E. coli* serotype was 02:K-:H6 (AV02) (4). Both strains were isolated from liver of turkeys that died of colisepticemia. The V078 strain is piliated in broth culture and causes mortality in young turkeys when injected i.v. (5). The AV02 strain is weakly piliated or nonpiliated in broth culture and does not cause mortality in young turkeys (5). Each strain was grown 18 h in trypticase-soy broth, washed once in phosphate-buffered saline solution (PBSS, pH 7.2), and suspended to a concentration of 3 x 10⁹ colony-forming units (CFU)/ml for inoculation. The *E. coli* suspensions were quantitated and maintained at 5°C during use. The V078 inoculum was piliated and the AV02 inoculum was nonpiliated, as determined by D-mannose-sensitive hemagglutination (5).

**Turkeys.** One-day-old Broad-Breasted White turkeys were obtained commercially and raised to 3 weeks of age in isolation rooms as previously described (4).

**Experimental design.** At 2 weeks of age, 29 turkeys were randomly assigned to 2 groups of 12 birds each and a control group of 5 birds. At 3 weeks of age, the 12 birds of each group were inoculated i.v. with 1 ml of *E. coli* inoculum and control birds were injected with PBSS. Three birds each from the 2 principal groups were killed at postinoculation min (PIM) 2, 5, 10, and 20. Control birds were killed at PIM 2 (no. = 2) and 20 (no. = 3).
Necropsy procedure. At the given postinoculation times, a 1 ml blood sample was taken from the wing vein and then birds were immobilized (succinyl choline given i.v.) and killed (decapitation). Within 30 s, birds were dipped in a disinfectant solution and opened for collection of spleen and liver specimens. Sera of control birds were tested by tube agglutination for antibody against V078 and AV02.

Quantitation of bacteria. Blood samples were diluted 10-fold in PBSS 7 times. Appropriate dilutions (0.5 ml) were inoculated onto blood agar plates (BAP). The plates were incubated overnight at 37° C and CFU were counted.

A 1- to 2-g specimen of liver and half of the spleen of each bird were collected and placed in vials at 5° C. After the specimens were weighed, they were mixed with 9 ml of cold PBSS in TenBroeck grinders for trituration. Tissue specimens were triturated for 30 s, then serially diluted and processed as described for blood samples. Tissues were maintained at 5° C during processing and all were inoculated onto BAP within 2 h of collection. Bacterial isolates were confirmed as the inoculated strain by slide agglutination with rabbit antiserum against V078 and AV02.

Histopathologic examination. Specimens of spleen and liver were fixed in 10% neutral buffered formalin for 48 h. Blocks were trimmed, dehydrated in alcohols, embedded in paraffin, and sectioned at 5 μm. Sections were stained with hematoxylin and eosin (H & E), H & E and periodic acid-Schiff (PAS), Giemsa, and modified Dieterle (87) stains. Bacteria stained by the modified Dieterle technique were counted in
cross sections of splenic reticular sheaths [synonyms: ellipsoids, Schweigger-Seidel sheaths] (22) and in liver sinusoids with the aid of a grid reticle at 1,000 X magnification. Bacteria in 10 sheaths in spleen and 10 grid fields (0.12 mm x 0.12 mm) in liver were counted.

**Electron microscopy.** Cubes (0.5 mm) of spleen and liver were fixed in 2.5% buffered glutaraldehyde for 4 h, washed in sodium cacodylate buffer, postfixed in 1% buffered osmium tetroxide, washed in buffer, and dehydrated through graded ethanols and propylene oxide to embedment in epoxy resin (Epon 812). Ultrathin sections were cut with a diamond knife, stained with 2% uranyl acetate and Reynold’s lead citrate, and examined with an electron microscope. Sections from 1 or 2 blocks of each tissue were examined.
RESULTS

Bacterial titers. The clearance rates of virulent and avirulent *E. coli* from circulating blood were markedly different. Blood titers of AV02 were reduced to 1/3,000 of their original level in 20 min, whereas titers of V078 were reduced to 1/3 of their original level (Fig. 1). A reduction in V078 blood titers (P < 0.01) was not detected until PIM 20.

In livers, bacterial titers of AV02 were greater (P < 0.05) than titers of V078 at all sample times (Fig. 1). Titers of AV02 in livers did not increase significantly (P = 0.52) during the experiment. Titers of V078 in livers steadily increased (P < 0.05) during the experiment. The ratio of liver titers to blood titers (L:B) for AV02 increased from 9.3 at PIM 2 to 7,400 at PIM 20. However, the L:B for V078 increased from only 0.3 at PIM 2 to 3.6 at PIM 20.

In spleens, titers of both AV02 and V078 were generally equivalent and stable (P > 0.05) through PIM 10 (Fig. 1). The titer of V078 was slightly less (P = 0.046) at PIM 20 than at PIM 2. However, the ratios of spleen titers to blood titers (S:B) were different for virulent and avirulent *E. coli*. The S:B for AV02 increased from 33 at PIM 2 to 9,800 at PIM 20, whereas S:B for V078 at comparable times decreased from 11 to 4.5.

Histopathologic examination. Examination of tissue sections stained by the modified Dieterle technique revealed differences in the rate of localization of AV02 and V078 in livers (Fig. 2). At PIM 2, there were 6.5 X more (P < 0.01) AV02 than V078 bacteria observed in liver sinusoids (Fig. 3, A and B). Localization of AV02 was greater than V078 in livers
Fig. 1. Colony-forming units (CFU) of *Escherichia coli* per g in blood, spleen, and liver after i.v. inoculation.
at PIM 5 (P < 0.01) and PIM 10 (P = 0.038); numbers of AV02 and V078 were not significantly different at PIM 20 (P = 0.688). More V078 bacteria were observed in livers at PIM 20 than at PIM 2 (P < 0.05) whereas numbers of AV02 were similar (P > 0.10) at both times.

In spleens, both E. coli strains localized predominantly in the 2nd cell layer of the reticular sheaths (Fig. 3, C and D). At PIM 2, there were 3 X more V078 than AV02 bacteria (P = 0.018) observed in reticular sheaths (Fig. 2). Only occasional bacteria were found in red pulp areas. Numbers of V078 and AV02 were similar at PIM 5, 10, and 20 (P > 0.05).

At most sample times, spleens exposed to either E. coli strain were characterized by hyperemia with increased numbers of granulocytes in red pulp and small aggregates of heterophils in red pulp and adjacent to reticular sheaths. In contrast to other spleens at PIM 2, spleens exposed to V078 had fewer granulocytes in red pulp. At PIM 20, spleens exposed to AV02 were moderately hyperemic, whereas those exposed to V078 had fewer erythrocytes in red pulp and aggregates of heterophils within and adjacent to reticular sheaths. In spleens exposed to either E. coli strain, there were aggregates of small round cells with dense nuclei in red pulp, venous sinuses, and lumens of sheathed capillaries.

The response of livers exposed to either E. coli strain was characterized by slight hyperemia and more granulocytes in sinusoids at PIM 2 through 20. At PIM 20, aggregates of 3 to 5 heterophils were common in sinusoids.
Fig. 2. Number of *Escherichia coli* observed histopathologically in liver sinusoids and splenic reticular sheaths after i.v. inoculation.
Fig. 3. Liver and spleen collected 2 min after inoculation with *Escherichia coli*; modified Dieterle stain. (A) Large numbers of AV02 trapped in liver sinusoids (arrows) compared with (B) sparse numbers of V078. (C) AV02 trapped in splenic reticular sheaths compared with (D) greater numbers of V078 (arrow).
**Electron microscopic examination.** Splenic reticular sheath capillaries were characterized by tall endothelium incompletely surrounded by a basal lamina and a thick network of reticular fibers. Reticular sheaths were composed of 2 or 3 layers of macrophages with numerous pseudopodia intermingled with reticular fibers.

At PIM 2, bacteria were found within phagosomes of reticular sheath macrophages in all spleens exposed to *E. coli*. Occasionally, bacteria were in the process of being ingested or were located between macrophages (Fig. 4). Changes in spleens collected at PIM 5 and 10 were comparable with those at PIM 2, except that heterophils and eosinophils were commonly observed near reticular sheaths. Many phagosomes were surrounded by electron-dense homogeneous granular material (Fig. 4). Some phagosomes were in the process of fusion with lysosomes (Fig. 5). In general, phagosomes containing AV02 were larger and more regular than those containing V078 (Fig 6 and 7). Phagosomal membranes around V078 appeared wavy and closely apposed to the bacterial surface. Few bacteria were found within macrophages or heterophils of the red pulp.

At PIM 20, fewer bacteria were seen in spleens exposed to AV02 than to V078. Small round cells with dense, clumped chromatin were common in reticular sheaths and red pulp of all spleens exposed to *E. coli*. These cells occasionally contained concentric membranous structures similar to large myelin figures observed in thrombocytes of chickens (81) (Fig. 8A). Endothelium of sheathed capillaries was occasionally necrotic in spleens exposed to either *E. coli* strain.
Fig. 4. VO78 *Escherichia coli* trapped in splenic reticular sheath at PIM 2. Bacteria are within and between reticular sheath macrophages. (Insert) Bacterium surrounded by a macrophage pseudopod. Many phagosomes are surrounded by electron-dense granular material (arrow).
Fig. 5. AV02 *Escherichia coli* within macrophage phagosome in the process of fusion with lysosomes (arrows) at PIM 2. Spleen.
Fig. 6. Splenic reticular sheath macrophages containing (A) AVO2 and (B) V078 *Escherichia coli* at PIM 10. A space is between AVO2 *E. coli* and the phagosome membrane (arrow), whereas phagosome membranes around V078 *E. coli* are ruffled and closely apposed to the bacteria.
Fig. 7. Splenic reticular sheath capillary surrounded by macrophage containing V078 *Escherichia coli* within an indistinct phagosome. V078 *E. coli* surrounded by an irregular phagosome membrane at PIM 20.
Fig. 8. (A) Macrophage (left) and thrombocyte in splenic red pulp at PIM 20. Complex myelin figure type cytoplasmic inclusion of thrombocyte (81) (arrow). (B) Thrombocyte and W078 Escherichia coli contained in a liver sinusoid-lining macrophage.
In livers, the inflammatory responses to AV02 and V078 were similar, but slower to develop against the latter. At PIM 2, AV02 bacteria were found within large phagosomes of fixed macrophages lining liver sinusoids. Small cells with dense nuclei (thrombocytes), similar to those in spleens, were found free in sinusoids or within macrophages (Fig. 8B). Heterophils, eosinophils, and occasional mast cells were present in sinusoids. The response of livers to AV02 at PIM 5, 10, and 20 was similar to that at PIM 2 except that more thrombocytes and fewer bacteria were seen at PIM 20. In contrast, V078 bacteria were not found in livers by electron microscopy until PIM 5, and thrombocytes were not found until PIM 10 (Fig. 8B). Unlike phagosomes of splenic macrophages, those of liver macrophages appeared similar for either E. coli strain.

Antibody to AV02 and V078 was not detected (titer < 1:5) in sera from control birds.
DISCUSSION

The cultural and histopathologic results of this study indicate that V078 bacteria resist clearance from circulating blood, whereas AV02 bacteria are cleared at an exponential rate. Livers were more effective in trapping AV02 than V078. Due to the large size of the liver, effective trapping of AV02 in liver macrophages had a major effect on early clearance of these bacteria from blood.

Why there were 3 X more V078 than AV02 bacteria observed in reticular sheaths at PIM 2 is unclear, but at least 2 mechanisms are possible. First, bacteria of the V078 inoculum may not have been homogeneous with respect to expression of surface components affecting phagocytosis, thus a small population of bacteria did not resist phagocytosis. Second, initial rapid phagocytosis of V078 may have adversely affected these macrophages to inhibit further phagocytosis.

Different rates of killing of V078 and AV02 are indicated by the ratios of spleen and liver titers to blood titers (S:B, L:B). Organ titers reflect numbers of bacteria in circulating blood relative to fixed organ tissues plus trapped viable bacteria. However, organ titers probably underestimate the real number of viable bacteria since phagocytes laden with bacteria may not be sufficiently disrupted during trituration to register each bacterium as a CFU. Changes in organ titer are determined by the blood titer, plus the rate of trapping, minus the rate of killing. The present study was designed to exclude effects of bacterial multiplication since these strains have generation times of nearly 20 min after a 1 h lag phase in vitro (5). The fact that S:B and L:B were
much higher for AV02 than for V078 indicates greater bacterial trapping of AV02 in spleen and liver. Relatively stable spleen and liver titers concomitant with falling blood titers (AV02) indicate that rates of trapping and killing in spleen and liver are roughly equivalent and are at least partially responsible for the decrease of circulating bacteria. In early bacteremia, it is unlikely that significant numbers of bacteria are cleared from the blood by organs other than spleen and liver (7, 61). The low S:B and L:B of V078 indicate that V078 was slowly trapped in spleen and liver, slowly killed after phagocytosis, and therefore, slowly cleared from the blood stream.

The inflammatory process in spleens differed slightly depending on the E. coli strain used. Initial depletion of granulocytes from splenic red pulp at PIM 2 is probably a reflection of profound leukopenia that has been observed immediately after the i.v. injection of bacteria (61). The lack of hyperemia in spleens 20 min after exposure to V078 indicates that splenic blood flow was decreased compared with normal spleens and those exposed to AV02. Decreased blood flow through spleens could inhibit clearance, since a constant fraction of circulating bacteria are removed with each organ passage (61). The greater tendency for aggregates of heterophils to infiltrate reticular sheaths of spleens exposed to V078 indicates that V078 bacteria were not effectively neutralized by sheath macrophages.

The ultrastructure of splenic reticular sheaths in the present study was essentially like that described for chickens by White and Gordon (92). However, we did not distinguish 2 distinct reticular
sheath cell types. Numbers of AV02 and V078 observed in thin sections correlated with the histopathologic and cultural results. The electron-dense granular material observed around phagosomes was interpreted to be polymers of the contractile protein, actin (79). It was our impression that phagosomes containing V078 were often small, irregular, and indistinct compared with those around AV02 bacteria. Whether this finding reflects a defect of intracellular killing of V078 E. coli is unclear, however, the organ titers of V078 indicate that spleens are defective in both the trapping and killing of V078.

The capacity of V078 to resist clearance from the blood stream is probably due to specialized surface components that may be lacking on AV02. V078 was piliated and encapsulated, whereas AV02 lacked these surface components. The role of pili in bacterial-phagocyte interaction is variable. Studies with gonococci have indicated that pili are antiphagocytic (53, 58), whereas piliated E. coli are more susceptible to phagocytosis than nonpiliated strains (66). Type 1 pili are relatively hydrophobic (12) and would be expected to increase interfacial tension relative to phagocytes, thus facilitating phagocytosis. However, if piliated E. coli were to adhere to circulating erythrocytes as in hemagglutination in vitro (5), the exposure of bacteria to macrophages of spleen and liver may be altered. Trapping by splenic reticular sheath macrophages appears limited to particles less than 2 μm to 3 μm in diameter (92). Therefore, bacteria adherent to erythrocytes may be unable to pass through sheathed capillaries and contact macrophages of the reticular sheaths.
The role of a surface capsule (K antigen) on V078, like the role of pili, is not clearly understood. Strains of *E. coli* with sufficient K antigen to resist killing by complement are also poorly phagocytized when injected into mice (38). However, *E. coli* strains isolated from human patients with bacteremia do not have excessive amounts of K antigen (47). In a recent study, most *E. coli* organisms isolated from livers of turkeys produced K antigens, but production of K antigens did not correlate with virulence for turkeys (14). Two *E. coli* serotypes (01:K-:H7 and 02:K-:H5) were virulent for turkeys although they lacked K antigens. Stendahl et al. (76) found that strains of *E. coli* having smooth hydrophilic O antigens tend to resist phagocytosis whether or not K antigens are present. Further study is required to determine the nature of surface components of virulent *E. coli* that enable these bacteria to resist destruction by turkey phagocytes.
EFFECT OF PASSIVE IMMUNIZATION ON PHAGOCYTOSIS OF BLOOD-BORNE
ESCHERICHIA COLI IN SPLEEN AND LIVER OF TURKEYS

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No product endorsements are implied herein.
Clearance of *Escherichia coli* from blood and localization in tissues were studied in passively immune and nonimmune turkeys after intravenous inoculation. In passively immune turkeys, *E. coli* bacteria were rapidly cleared from blood and localized predominantly in liver and spleen. Bacteria were within or between macrophages of reticular sheaths and red pulp in spleen, and sinusoids of liver. Ultrastructurally, extracellular bacteria were covered by an extraneous coat. Moderate interstitial hemorrhage and aggregates of thrombocytes were found in lungs of passively immune birds. In nonimmune turkeys, persistence of large numbers of *E. coli* in blood and high mortality were associated with inefficient localization of *E. coli* in liver. These studies indicate that rapid clearance of virulent *E. coli* from blood requires antibody-dependent phagocytosis by hepatic macrophages.
INTRODUCTION

Bacteremia is a feature of clinical and subclinical infections of turkeys caused by *Escherichia coli*. Virulent strains of *E. coli* readily cause bacteremia in normal poultry exposed by aerosol (4) or air-sac inoculation (57). Colisepticemia may develop in birds exposed to large numbers of virulent *E. coli* at a time when their specific or nonspecific defenses are compromised.

In young turkeys, avirulent *E. coli* are rapidly cleared from circulating blood, whereas virulent *E. coli* resist clearance (3). Hepatic macrophages are the principal agents for the clearance of avirulent *E. coli* from blood, but these cells are less effective against virulent *E. coli* (3). A recent study showed that passive immunization with hyperimmune serum protected young turkeys from lethal challenge with a virulent *E. coli* strain (1). In contrast, other investigators have suggested there is no relationship between O and K serum agglutinins and protection against colisepticemia (17, 18). Although some experimental vaccines protected against challenge with virulent *E. coli*, sera from only 4 of 28 vaccinated birds had O or K agglutinin titers greater than 1:5 (17, 18).

The purpose of the present study was to determine the effect of antibody on: (i) the clearance of *E. coli* from circulating blood, (ii) the localization of blood-borne *E. coli* in spleen, liver, lung, and bone marrow, and (iii) the interaction of *E. coli* and phagocytes in spleen, liver, and lung.
MATERIALS AND METHODS

Bacteria. The *E. coli* serotype 078:K80:H9 was used (1, 3, 4). The strain was originally isolated from the liver of a turkey that died with colisepticemia. For each experiment, bacteria were grown for 24 h in trypticase-soy broth; washed once in phosphate-buffered saline (PBS), pH 7.2; and resuspended to the desired concentration for inoculation.

Turkeys. Three-week-old Broad-Breasted White turkeys were used in each experiment. Turkeys were obtained commercially and raised to 3 weeks of age in isolation rooms as previously described (4).

Passive immunization. Hyperimmune serum was produced in 8-week-old turkeys against the *E. coli* strain used in this study. Turkeys were inoculated intravenously (i.v.) 3 times at 5 day intervals with *E. coli* inoculum prepared as described above. The inoculum contained $10^6$, $10^7$, and $10^9$ colony-forming units (CFU) of *E. coli* for the first, second, and third inoculations, respectively. Serum was harvested 5 days after the third inoculation, diluted with PBS to an O-agglutinin titer of 1:256, and stored at $-70^\circ$ C until used. Treatment of a sample of the serum with 2-mercaptoethanol reduced the titer to 1:8. In each experiment, turkeys were injected i.v. with 1 ml of diluted hyperimmune serum 48 h before inoculation with *E. coli*.

Necropsy procedure and specimen collection. At the appropriate times after *E. coli* inoculation, a 1 ml blood sample was collected from the wing vein; birds were immobilized by i.v. injection of succinyl choline and killed by decapitation. Within 30 s, birds were dipped in a disinfectant solution and opened aseptically for collection of spleen,
liver, lung, and proximal tibia. Blood samples were diluted 10-fold in ice-cold PBS containing 1% Grobax® (Roche Diagnostics) as an anticoagu­lant. Blood was further diluted 10-fold in PBS 6 times. One-half ml of each dilution was inoculated onto blood agar plates. Plates were incu­bated overnight at 37° C, and CFU were counted. A 1- to 2-g specimen of liver, half of the spleen, and the right lung of each bird were collected aseptically, weighed, and processed to determine the number of CFU/g of tissue as previously described (3). Bacterial isolates were confirmed as the inoculated strain by slide agglutination tests.

**Estimation of percentage of E. coli inoculum in selected tissues.**

Total numbers of E. coli in spleen, liver, lung, and blood were calculated by multiplying the number of E. coli CFU/g of tissue by the estimated weight of the organ. The weight of spleen and liver were estimated from percentage body-weight means determined in 5, 3-week-old turkeys; expressed as the mean percentage of body-weight, the spleen was 0.0821% and the liver was 2.046%. The blood weight was estimated, based on values for White Leghorn chickens of similar weight (49), to be 8% of the body-weight. Since a whole lung was removed from each bird, actual lung-weight means were multiplied by 2 and used to estimate the total number of E. coli CFU in lungs. The percentage of E. coli inoculum in selected tissues was estimated by the following formula:

\[
% \text{ E. coli inoculum in tissue} = \frac{\text{No. of E. coli CFU in tissue}}{\text{No. of E. coli CFU in inoculum}} \times 100
\]
**Histology.** Specimens of spleen, liver, lung, and proximal tibia were fixed in 10% neutral buffered formalin for 48 h, and bone specimens were decalcified in Decalcifying Solution® (Scientific Products) for 4 h. Blocks were trimmed, dehydrated in ethanols, embedded in paraffin, and sectioned at 5 µm. Sections were stained with hematoxylin and eosin (H & E), Giemsa, and modified Dieterle (87) stain. Additional specimens of spleen, liver, and lung were fixed in Bouin's solution for 8 h, washed in 50% ethanol, dehydrated, embedded, sectioned, and stained with H & E.

**Electron microscopy.** Cubes (0.5 mm) of spleen, liver, and lung were fixed in 2.5% buffered glutaraldehyde for 3 h, washed in 0.1 M sodium cacodylate buffer with 0.05 M sucrose, postfixed in 1% buffered osmium tetroxide, washed in buffer, and dehydrated through graded ethanols and propylene oxide to embedment in epoxy resin (Epon 812). Ultrathin sections were cut with a diamond knife, stained with 2% uranyl acetate and Reynolds's lead citrate, and examined with an electron microscope. Sections from 2 or 3 blocks of each tissue were examined.
RESULTS

Experimental design. The study consisted of 3 experiments. Experiment 1 was designed to determine the clearance rate of E. coli from blood of passively immune (Ab+) and nonimmune (Ab-) turkeys. Experiments 2 and 3 were designed to determine sites of E. coli localization and E. coli-phagocyte interactions in tissues of Ab+ and Ab- turkeys. In experiment 1, 5 Ab+ and 5 Ab- turkeys were inoculated i.v. with $8 \times 10^7$ CFU of E. coli. For determining the number of E. coli CFU/ml of blood, a 0.5-ml blood sample was collected from the wing vein of each turkey at 1, 10, 30, 60, and 120 min after the injection of E. coli. In experiment 2, 3 Ab+ and 3 Ab- turkeys were inoculated i.v. with $6 \times 10^9$ CFU of E. coli. A 0.5 ml blood sample was collected, and the turkeys were killed 1 min after inoculation with E. coli. Numbers of viable bacteria in blood, spleen, liver, and lung were determined; specimens of spleen, liver, lung, and bone marrow were studied with light and electron microscopy. Experiment 3 was like experiment 2 except that the inoculum contained $10^9$ CFU of E. coli, and turkeys were killed 20 min after inoculation.

Blood was collected from 10 uninoculated control turkeys (5 Ab+ and 5 Ab-) for determination of serum agglutinin titers against the live E. coli inoculum. The Ab+ birds had agglutinin titers of 1:4 to 1:8, whereas Ab- birds had titers of less than 1:4. The 5 Ab+ birds were used as negative controls for bacterial culture and microscopic examination.
Clearance of *E. coli* from circulating blood. In experiment 1, *E. coli* was cleared from the bloodstream more efficiently in Ab+ birds than in Ab- birds (Fig. 1). The clearance of *E. coli* from blood of Ab+ birds was rapid during the first 10 min postinoculation (p.i.) and then moderate from 10-120 min p.i. In contrast, the clearance of *E. coli* from Ab- birds was moderate during the first 30 min p.i. followed by a period of increasing *E. coli* numbers. All Ab- birds died between 6 and 7 h p.i., whereas all Ab+ birds were clinically normal when killed and necropsied 24 h p.i.

Localization of *E. coli* in tissue. In experiments 2 and 3, clearance of *E. coli* from blood was more rapid in Ab+ birds than in Ab- birds. The greater concentration of *E. coli* in the tissue than in the blood was interpreted to be a result of bacterial trapping by the tissue. At 1 min p.i. (experiment 2), *E. coli* was trapped principally in spleens of Ab- birds but in spleens, livers, and lungs of Ab+ birds (Fig. 2). Compared with Ab- birds, Ab+ birds had significantly fewer *E. coli* CFU in blood (*P* = 0.0006) and spleen (*P* = 0.004) but more *E. coli* CFU in liver (*P* = 0.002) and similar numbers in lung (*P* = 0.07). At 20 min p.i. (experiment 3), *E. coli* was trapped in the spleen and probably the liver of Ab- birds, whereas it was avidly trapped in spleen, liver, and lung of Ab+ birds (Fig. 3). Compared with Ab- birds, Ab+ birds had significantly fewer *E. coli* CFU in blood (*P* < 0.0001) but similar numbers in spleen (*P* = 0.09), liver (*P* = 0.77), and lung (*P* = 0.77). Recovery of inoculum from birds of experiment 2 and experiment 3 is shown in Fig. 2,
Fig. 1. Clearance of *Escherichia coli* from blood of passively immune (Ab+) and nonimmune (Ab−) turkeys inoculated i.v. with $8 \times 10^7$ colony-forming units (CFU). Bar is standard deviation of CFU in 5 birds.
TIMES AFTER INOCULATION

CFU OF E. coli/ml OF BLOOD

10^7
10^6
10^5
10^4
10^3
10^2
10^1

0 10 30 60 120

MINUTES AFTER INOCULATION

NON IMMUNE

PASSIVELY IMMUNE
Fig. 2 Localization of *Escherichia coli* in tissues of 3 passively immune (Ab+) and 3 nonimmune (Ab-) turkeys 1 min after i.v. inoculation with $6 \times 10^9$ colony-forming units (CFU).

$^a$Estimated % of *E. coli* inoculum in whole tissue.

Fig. 3 Localization of *Escherichia coli* in tissues of 3 passively immune (Ab+) and 3 nonimmune (Ab-) turkeys 20 min after i.v. inoculation of $10^9$ colony-forming units (CFU).

$^a$Estimated % of *E. coli* inoculum in whole tissue.
NONIMMUNE TURKEYS • PASSIVELY IMMUNE TURKEYS

BLOOD  SPLEEN  LIVER  LUNG

<table>
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<tr>
<th>Nonimmune Turkeys</th>
<th>Passively Immune Turkeys</th>
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<td>58.0% / 1.8%</td>
<td>45.0% / 0.7%</td>
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<tr>
<td>1.1% / 16.6%</td>
<td>0.8% / 3.4%</td>
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CFU of E. coli / g of Tissue

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<tr>
<th>Nonimmune Turkeys</th>
<th>Passively Immune Turkeys</th>
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<tr>
<td>51.9% / 0.02%</td>
<td>5.2% / 11.0%</td>
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<tr>
<td>15.1% / 23.8%</td>
<td>1.0% / 0.8%</td>
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and Fig. 3, respectively. In each experiment, the recovery of viable \textit{E. coli} from tissues of Ab+ birds was about 1/3 of that from Ab- birds.

\textbf{Histologic examination.} At 1 min p.i. (experiment 2), spleens of Ab+ birds contained clumps of 5-20 \textit{E. coli} in reticular sheaths (ellipsoids) and red pulp (Fig. 4A). Spleens from Ab- birds contained large numbers of \textit{E. coli} evenly distributed in reticular sheaths but few \textit{E. coli} associated with red pulp (Fig. 4B). In livers from Ab+ birds, \textit{E. coli} were numerous, and clumps of 5-10 bacteria were associated with macrophages lining the sinusoids (Fig. 4C). Only scant numbers of \textit{E. coli} were observed in livers of Ab- birds. Lungs from Ab+ birds contained clumps of 5-20 \textit{E. coli} in small blood vessels associated with air passages (air capillaries) at the periphery of parabronchi (tertiary bronchi). Occasionally, \textit{E. coli} were contained within aggregates of thrombocytes. Bacteria were rarely observed in lungs of Ab- birds and then only individually, within small blood vessels. Small numbers of \textit{E. coli} were in vascular sinusoids of bone marrow from proximal tibias, however, differences between Ab+ and Ab- birds were not apparent. At 20 min p.i. (experiment 3), numbers of \textit{E. coli} were not adequate for microscopic interpretation of localization in tissues.

Lungs of Ab+ birds at 1 min p.i. (experiment 2) were characterized by diffuse interstitial hemorrhage, aggregation of thrombocytes in small blood vessels, hyperemia, and mild serous exudation into small airways (Fig. 4D). Lung lesions in Ab+ birds at 20 min p.i. (experiment 3) were similar but less severe. In all Ab- birds, lungs were normal except for hyperemia and occasional aggregates of thrombocytes in small blood vessels. Lesions in spleens were most pronounced at 20 min p.i.
Fig. 4. Histopathology and localization of *Escherichia coli* in turkeys killed 1 min after inoculation (experiment 2). A. Passively immune (Ab+) birds: clumps of *E. coli* (arrows) trapped in splenic reticular sheaths and red pulp. Modified Dieterle stain. B. Nonimmune (Ab-) birds: *Escherichia coli* trapped diffusely throughout splenic reticular sheaths. Modified Dieterle stain. C. Ab+ birds: numerous *E. coli* (arrow) associated with sinusoid-lining macrophages in livers. Modified Dieterle stain. D. Ab+ birds: aggregates of thrombocytes (a) in capillaries and interstitial hemorrhage (b) in lung. Lumen (c) of parabronchus. H & E stain.
(experiment 3). In spleens of Ab+ and Ab- birds, there was ischemia of red pulp, with increased numbers of heterophils and thrombocytes. Clumps of heterophils localized in red pulp of all birds, but in Ab- birds there were also accumulations of heterophils in and around reticular sheaths. Sinusoids of livers from Ab+ birds contained increased numbers of heterophils and thrombocytes. Heterophils or thrombocytes were often in clumps of 10-30 cells. Livers of Ab- birds at 1 min p.i. (experiment 2) were normal, but those at 20 min p.i. (experiment 3) contained increased numbers of heterophils and thrombocytes. Histologically, sections of bone marrow from Ab+ and Ab- birds did not differ appreciably from those of controls.

**Electron microscopic examination.** In tissues from Ab+ birds at 1 min p.i. (experiment 2), extracellular *E. coli* were enveloped in an extraneous coat of fibrillar/granular electron-dense material (Fig. 5A). In the case of *E. coli* phagocytized in Ab+ birds, the phagosomal membrane was closely apposed to the extraneous coat. No such coat was observed on *E. coli* from Ab- birds (Fig. 5B). In Ab+ birds phagosomes with *E. coli* often contained a homogeneous electron-dense substance between the phagosomal membrane and the bacterial surface. A substance of similar appearance was sometimes observed in lysosomes fused with or adjacent to phagosomes containing *E. coli* (Fig. 5C).

In spleen, most *E. coli* were found within phagosomes of reticular sheath macrophages. Some bacteria were incompletely surrounded by pseudopods of macrophages. Although extraneous coats were often continuous between extracellular *E. coli* from Ab+ birds, macrophage
Fig. 5. Interaction of *Escherichia coli* with macrophages in spleen and liver of turkeys 1 min after inoculation (experiment 2). A. Spleen, passively immune (Ab+) turkey. Extracellular *E. coli* covered with a fibrillar/granular, extraneous coat (a). Pseudopod (b) of reticular sheath macrophage and ingested bacterium (c). B. Ingestion of *E. coli* by a splenic macrophage, nonimmune (Ab−) turkey. C. Liver, Ab+ turkey. *Escherichia coli* contained in phagosomes of sinusoid-lining macrophage.
pseudopods were commonly observed between individual bacteria (Fig. 6A). Phagosomes containing \textit{E. coli} from both Ab+ and Ab- birds were often surrounded by granular electron-dense material. At high magnification, numerous filamentous structures were observed within pseudopods of macrophages and around phagosomes (Fig. 5A).

In liver of Ab+ birds, single (rarely multiple) \textit{E. coli} were contained in phagosomes of sinusoid-lining macrophages. Macrophages commonly contained 2 to 4 bacteria. Phagosomes were morphologically similar to those in spleen, except that fusion with lysosomes was more prevalent in liver macrophages. Too few \textit{E. coli} were found in livers of Ab- birds to reliably interpret the bacterial-phagocyte interactions ultrastructurally. Aggregates of 2-5 thrombocytes were frequently observed in hepatic sinusoids of Ab+ birds (Fig. 6B). Bacteria were not directly associated with the thrombocytes, but both bacteria and thrombocytes were sometimes found within a single hepatic macrophage.

In lung of Ab+ birds, parenchymal capillaries were frequently distended with erythrocytes and aggregated thrombocytes (Fig. 7). Clumps of \textit{E. coli} in capillaries were extracellular and coated with a coarse, granular material, or they were within mononuclear phagocytes. The architecture of small air passages and blood capillaries was destroyed and interstitial tissues were expanded. Thrombocytes with long filopodia were often found at the periphery of interstitial hemorrhages. No ultrastructural lesions were found in lungs from Ab- birds.
Fig. 6. Passively immune (Ab+) turkeys, 1 min after inoculation with *Escherichia coli* (experiment 2). A. Spleen. Macrophage pseudopods (arrow) between *E. coli* having contiguous extraneous coats. B. Liver. Aggregates of thrombocytes in hepatic sinusoid. Thrombocytes have dense, clumped chromatin and myelin figure inclusions (arrows). Mononuclear phagocyte (a).
Fig. 7. Aggregates of thrombocytes in lung capillary of passively immune (Ab+) bird (experiment 2). Disrupted capillary wall (arrows).
DISCUSSION

The results of the present study in turkeys concur with those of previous studies of bacteremia in mammals; that is, rapid clearance of virulent *E. coli* from blood requires antibody-dependent phagocytosis by hepatic macrophages. The role of antibody in the clearance of bacteria from circulating blood has been studied in small laboratory mammals (7, 37, 63, 74, 91). In mice and rabbits, *E. coli* is cleared from blood principally by mononuclear phagocytic cells of the spleen and liver (7, 61). The avidity with which bacteria are trapped is determined by the nature of the bacterial surface (68) and the presence or absence of serum opsonins (7). The efficiency with which *E. coli* is trapped in livers of mice and rats depends on the level of specific antibody (7, 39). Easily phagocytized bacteria and opsonized bacteria are efficiently cleared from blood by hepatic macrophages (7).

In a previous study, virulent *E. coli* resisted trapping by hepatic macrophages, whereas avirulent *E. coli* was avidly trapped in the liver (3). Although splenic phagocytes trap *E. coli* effectively in the presence or absence of antibody, the small size of the avian spleen, compared with the liver, limits its relative effect on severe *E. coli* bacteremia. Trapping of bacteria in the lung of mammals is minimal compared with that in the spleen and liver. In the present study, antibody enhanced localization of *E. coli* in the lung, but this enhancement may have been partially due to vascular effects of thrombocyte aggregation.
The presence of clumps of *E. coli* in tissues of Ab+ birds, but not in tissues of Ab- birds, suggests that antibody mediated the intravascular agglutination of bacteria. Such aggregation would be expected to increase the efficiency of phagocytosis. Finding clumps of *E. coli* in splenic reticular sheaths of Ab+ birds is inconsistent with the concept that in chickens particles greater than 2-3 μm in diameter are unlikely to pass between endothelial cells of reticular sheath capillaries (92). It may be that *E. coli* clumps are loosely arranged and therefore allow passage of bacteria between cells of the reticular sheath capillaries in single-file fashion.

Aggregation of platelets is sometimes associated with intravascular injection of large numbers of bacteria (37). Aggregates of thrombocytes are found in the splenic red pulp and hepatic sinusoids of turkeys injected i.v. with *E. coli* (3). In this study, the presence of antibody was associated with extensive aggregation of thrombocytes in lung capillaries of birds inoculated with $6 \times 10^9$ CFU of *E. coli*. When other Ab+ birds were inoculated similarly with *E. coli* (results not included), they died with severe respiratory distress in less than 10 min. The rapid development of thrombosis in lung capillaries was probably mediated by thrombocyte aggregation. The severity of lung lesions in Ab+ birds appeared to be directly related to the size of inoculum, since lesions were mild in experiment 3. It seems unlikely that a similar pulmonary vascular disorder would occur in the natural disease, since the onset of bacteremia is more gradual.
Although there was histologic and ultrastructural evidence of intravascular agglutination of bacteria in Ab+ birds, most E. coli were phagocytized individually. This is not unreasonable if one considers the "zipper" mechanism of phagocytosis proposed by Griffin et al. (26). These authors suggested that movement of the phagocyte membrane around a particle is dependent on sequential receptor-ligand interactions which enhance close apposition of the two surfaces. Granular electron-dense material observed around phagosomes and within pseudopods of macrophages is probably polymerized actin (79).

The extraneous coat observed on extracellular E. coli from Ab+ birds is similar to the "beard" described by Horn et al. (37) on staphylococci exposed to serum. Haemophilus influenzae, when complexed with type-specific antibodies and viewed with the electron microscope, has a similar appearing coat (60). Such coats were never observed on E. coli from Ab- birds. Therefore, the extraneous coats were probably composed of specific antibody against E. coli, although other plasma proteins may have contributed.
PATHOLOGY OF SPLEEN AND LIVER IN TURKEYS
INOCULATED WITH ESCHERICHIA COLI

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No product endorsements are implied herein.
Lesions of spleen and liver were studied in 3-week-old turkeys infected with Escherichia coli either intravenously or intratracheally. The character and severity of lesions depended on the magnitude and duration of E. coli bacteremia. Bacteremia resulting from intratracheal inoculation persisted 3 to 5 days at low titer and produced only mild lesions in spleen and liver. Spleens of birds inoculated intravenously had necrosis and fibrinopurulent exudates in reticular sheaths (ellipsoids). Red pulp and venous sinuses were hyperemic and contained aggregates of thrombocytes. Periellipsoidal and periarterial lymphoid tissues were hyperplastic beginning by the third day of infection.

Livers of birds killed in extremis were markedly hyperemic, and centrolobular hepatocytes were vacuolated or necrotic. Small hepatic veins were occluded by aggregates of thrombocytes and fibrin. Bacilli were numerous in hepatic sinusoids and phagocytes. Phagosomes of hepatic macrophages frequently contained up to 10 normal appearing bacteria, whereas bacteria within phagosomes of heterophils showed ultrastructural degenerative changes. These studies indicate that the liver may be an important site of bacterial colonization in turkeys with progressive E. coli bacteremia.
INTRODUCTION

Escherichia coli infections of young poultry are commonly seen as airsacculitis, pericarditis, and septicemia (72). Virulent E. coli readily produce bacteremia in poultry exposed by aerosol (4) or by airsac inoculation (57). Local infection of the avian respiratory tract by E. coli may progress to septicemia and death, or survival with systemic lesions (14).

Clearance of E. coli from the blood of mammals is primarily accomplished by fixed macrophages in the liver and spleen (7, 62, 780. In turkeys, the efficiency with which E. coli is cleared from blood in spleen and liver is influenced by the E. coli strain and the immune status of the bird (2, 3). Virulent E. coli resists phagocytosis by hepatic macrophages, whereas blood-borne avirulent E. coli is rapidly sequestered in liver (3). In the presence of specific antibody, virulent E. coli bacteria localize in liver, spleen, and lung (2). Avirulent and opsonized E. coli are effectively cleared from blood of mice by hepatic macrophages, but virulent E. coli is not (7).

The pathologic changes in spleen and liver of chickens with coli-septicemia has been briefly described by Truscott et al. (84). Recent studies of E. coli bacteremia in turkeys have focused on the initial response of spleen and liver to the intravenous inoculation of large numbers of E. coli (3). The purpose of this study was to determine the lesions in spleen and liver of turkeys with E. coli bacteremia.
MATERIALS AND METHODS

*Escherichia coli* strains. Two virulent strains (ECl and EC27) and one avirulent strain (EC3) were used. The serotype of strains ECl, EC27, and EC3 was 078:K80:H9, 078:K3:H9, and 02:K-:H6, respectively. ECl and EC3 were used in previous studies (3, 4). All strains were originally isolated from liver of turkeys that died of colisepticemia. Strains were grown in trypticase-soy broth 24 h at 37° C, washed once in phosphate-buffered saline (PBS, pH 7.2), and suspended in PBS to the concentration desired for inoculation (Table 1). The actual number of colony-forming units (CFU)/ml of each inoculum was determined by plate count.

Turkeys. In each experiment, one-day-old Broad-Breasted White turkeys were obtained commercially and raised to 3 weeks of age in isolation rooms (4).

Experimental design. Experiments were designed to determine the sequential changes in spleen and liver and the duration and magnitude of bacteremia in turkeys inoculated with *E. coli*. Turkeys were given 1 ml of *E. coli* inoculum intravenously (i.v.) in the wing vein or intratracheally (i.t.) through a polypropylene catheter passed 5 cm down the trachea. In each experiment, 2 birds were necropsied at intervals (Table 1) up to 7 days postinoculation (p.i.). From 4 to 6 turkeys in each experiment were injected with only PBS and served as controls.

Necropsy and collection of specimens. Immediately before necropsy, a 0.5 ml sample of blood was collected from the wing vein and placed in 4.5 ml of ice-cold PBS containing 1% Grobax® (Roche Diagnostics) as an
TABLE 1. Design of experiments used to determine the acute response of spleen and liver in 3-week-old turkeys inoculated with *Escherichia coli*

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>No. of birds</th>
<th>Strain of <em>E. coli</em></th>
<th>Inoculation procedure</th>
<th>Time of necropsy&lt;sup&gt;a&lt;/sup&gt; postinoculation (p.i.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Minutes</td>
<td>Hours</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>EC1</td>
<td>$10^6.7$ CFU, i.v.</td>
<td>10, 20, 40</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>EC3</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>EC1</td>
<td>$10^7.0$ CFU, i.v.</td>
<td>2, 4, 6, 12</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>EC27</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>EC1</td>
<td>$10^7.0$ CFU, i.t.</td>
<td>2, 8</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>EC27</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Three birds were necropsied *in extremis* at 8 to 10 h p.i. in experiment 3 and 1 bird was similarly necropsied in experiment 4.

<sup>b</sup>i.v. = intravenously; i.t. = intratracheally.
anticoagulant. Serial, 10-fold dilutions of blood in PBS were inoculated onto blood agar plates for determination of colony-forming units (CFU)/ml of blood (3). The remaining fraction of the 1/10 dilution of blood was mixed with an equal volume of brain-heart infusion broth and incubated at 37° C.

At necropsy, turkeys were immobilized by an i.v. injection of succinyl choline and killed by decapitation. Birds were dipped in a disinfectant solution and opened, gross lesions were recorded, and spleen and liver specimens were collected.

The development of bacteremia was studied in 10 turkeys inoculated i.t. with E. coli; sequential blood samples were collected up to 7 days p.i. (Table 2). Numbers of E. coli in blood were determined as described above.

Just prior to each experiment, sera from 10 to 20 percent of the turkeys were tested for antibody against the E. coli inoculum by tube agglutination.

Light microscopy. Specimens of spleen and liver were fixed in 10% neutral buffered formalin, dehydrated in alcohols, embedded in paraffin, and sectioned at 5-6 µm. Sections were stained with hematoxylin and eosin (H & E), Giemsa, and modified Dieterle (87) stains. Additional specimens of spleen and liver were fixed in Bouin's solution 12 h, washed in water and 70% ethanol, dehydrated, embedded, sectioned, and stained with H & E.
TABLE 2. Design of experiment used to determine the onset and duration of bacteremia in 3-week-old turkeys inoculated with *Escherichia coli*

<table>
<thead>
<tr>
<th>No. of birds</th>
<th>Strain of E. coli</th>
<th>Inoculation procedure(^a)</th>
<th>Time of blood collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>EC27</td>
<td>(10^7) CFU, i.t.</td>
<td>0, 2, 6, 12, 1, 3, 5, 7</td>
</tr>
<tr>
<td>5</td>
<td>ECl</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

\(^a\) i.t. = intratracheally.
Electron microscopy. Specimens of spleen and liver were collected from birds killed at 6 h p.i. and in extremis in experiment 3. Cubes (0.5mm) of tissue were fixed in 2.5% buffered glutaraldehyde for 2 h, washed in 0.1 M phosphate buffer pH 7.4, postfixed in 2% osmium tetroxide, washed in buffer, and dehydrated through graded ethanols and propylene oxide to embedding in epoxy resin (Epon 812). Ultrathin sections were cut with a diamond knife, stained with 2% uranyl acetate and Reynold's lead citrate, and examined with an electron microscope.
RESULTS

Response to i.v. inoculation of *E. coli* (experiments 1-4). Spleens were generally dark and slightly enlarged although some spleens exposed to ECl or EC27 were pale and mottled at less than 1 day p.i. Microscopically, spleens were characterized by hyperemia, heterophil infiltration, fibrinopurulent exudation and necrosis in reticular sheaths, necrosis of isolated cells, thrombocyte aggregation, and lymphoid hyperplasia. The onset, duration, and severity of lesions varied with the virulence of the *E. coli* strain (Fig. 1). Red pulp spaces were dilated and filled with erythrocytes. Heterophil infiltration was diffuse in red pulp but intense in reticular sheaths (ellipsoids) (Fig. 2). There was localization of heterophils in and around reticular sheaths accompanied by necrosis and fluid exudation, particularly in birds exposed to ECl or EC27. In birds inoculated with EC3, there was only slight cellular and fluid exudation into reticular sheaths. Aggregates of thrombocytes were common in the red pulp and venous sinuses of birds at 2 to 6 h p.i. Beginning at 3 days p.i., lymphocytes surrounding reticular sheaths (periellipsoidal) had abundant cytoplasm and large basophilic nuclei with a prominent nucleolus; mitotic figures were common (Fig. 3). Numbers of lymphoid follicles were increased in some birds as early as 5 days p.i.

Spleens of birds killed *in extremis* were diffusely necrotic and hyperemic. Small numbers of bacilli were scattered in necrotic tissue and rarely formed colonies. Ultrastructurally, cells associated with reticular sheaths of spleens were swollen and intercellular spaces were
Fig. 1. Acute pathology of spleens and livers of turkeys in experiments 1 and 2.

Comparison of response to virulent (ECl ------) and avirulent (EC3 ---) Escherichia coli bacteremia.
**Spleen**

- Hyperemia
- Heterophil infiltration (RP)\(^a\)
- Heterophil infiltration (RS)
- Thrombocyte aggregation
- Lymphoid hyperplasia

**Liver**

- Hyperemia
- Heterophil infiltration
- Heterophil aggregation

**Bacteremia**

- EC1
- EC3

<table>
<thead>
<tr>
<th>MINUTES</th>
<th>HOURS</th>
<th>DAYS</th>
</tr>
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<tbody>
<tr>
<td>10</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^a\)RP = red pulp; RS = reticular sheath; EC1 -------; EC3 ------.

\(^b\)At 3 and 5 days, 1 of 2 birds were bacteremic.
Fig. 2. Localization of heterophils (arrows) in splenic reticular sheaths 2 h after i.v. inoculation with *Escherichia coli* (EC27).  
H & E stain.

Fig. 3. Hyperplasia of periellipsoidal lymphoid tissue (arrows) and formation of lymphoid nodules (LN) in periarterial lymphoid tissues 5 days after i.v. inoculation with *Escherichia coli* (EC3).  H & E stain.
distended by serous exudates and fibrin at 6 h p.i. Birds killed in extremis had necrotic cells, erythrocytes, and thrombocytes scattered throughout the splenic parenchyma. Sinusoidal walls were disrupted. Reticular sheath macrophages had swollen mitochondria, fragmented endoplasmic reticulum, disrupted nuclear membranes, and clumped chromatin (Fig. 4). In lymphoid tissues, intercellular spaces contained erythrocytes and fibrin. Bacilli were occasionally seen in phagosomes of macrophages.

Livers of most turkeys exposed to E. coli were normal grossly. However, livers of all turkeys killed in extremis were swollen, firm, friable, and dark. One bird, killed at 1 day p.i., had a large pale area (5 mm x 20 mm) of coagulative necrosis in one hepatic lobe. Early microscopic lesions included hyperemia and diffuse heterophil infiltration of sinusoids (Fig. 1). Heterophil infiltration was greatest in livers of birds 40 min after inoculation with EC3. Aggregates of heterophils were common within sinusoids (Fig. 5) and adjacent to hepatic lymphoid tissue. Hyperplasia of sinusoidal macrophages and biliary epithelium was present in some birds at 3 and 5 days p.i.

Livers of birds killed in extremis were characterized by severe hyperemia, vacuolation and necrosis of centrolobular hepatocytes (Fig. 6), venous thrombosis (Fig. 7), and numerous bacterial colonies in sinusoids. Venous thrombi, composed of aggregates of thrombocytes, were common at the junctions of central veins with large hepatic veins (Fig. 8). Ultrastructurally, livers of birds killed in extremis had sinusoids dilated by erythrocytes, heterophils, thrombocytes, and fibrin; perisinusoidal
Fig. 4. Splenic reticular sheath of turkey killed in extremis. Degeneration of macrophages (M), infiltration of heterophils (HP), intercellular debris and fibrin (F), and blebbing of capillary endothelium (C). Heterophil and fibrin (inset) from reticular sheath 6 h after i.v. inoculation with Escherichia coli (ECl).
Fig. 5. Aggregates of heterophils (arrow) in hepatic sinusoids 4 h after i.v. inoculation with *Escherichia coli* (EC3). H & E stain.

Fig. 6. Vacuolation and necrosis of centrolobular hepatocytes in a turkey killed *in extremis* 10 h after i.v. inoculation with *Escherichia coli* (EC27). Islands of hepatocytes around portal canals (PC) appear normal. H & E stain.
Fig. 7. Thrombosis of a central vein (arrow), sinusoidal hyperemia, and centrolobular hepatic necrosis in a turkey killed in extremis 9 h after i.v. inoculation with Escherichia coli (ECl). Portal canal (PC). H & E stain.

Fig. 8. Thrombi composed of thrombocyte aggregates (arrow) at the junction of central veins with a large hepatic vein (HV). Same bird as that of Fig. 7. H & E stain.
hemorrhage; and hepatocellular degeneration (Fig. 9). Bacteria were numerous in hepatic sinusoids and macrophages (Fig. 10). Phagosomes of sinusoidal macrophages with lucid cytoplasm contained up to 10 normal appearing bacteria (Fig. 9). Occasionally, bacteria with altered outer membranes were observed within partially degranulated heterophils (Fig. 11). Heterophils, in various stages of degeneration, were also in the perisinusoidal space (Disse's space) and between hepatocytes. The cytoplasm of hepatocytes varied in electron density and contained swollen mitochondria and vaculated endoplasmic reticulum (Fig. 12).

In addition to lesions of spleen and liver, turkeys inoculated i.v. with EC1 or EC27 had serous airsacculitis at 8 h p.i. and some had fibrinopurulent pericarditis beginning at 1 day p.i. Birds killed in extremis had abundant serous fluid in the body cavities, generalized hyperemia of the viscera, edematous lungs, and petechiae on the proventriculus.

Response to i.t. inoculation of E. coli. Spleens were swollen and hyperemic beginning 1 day p.i. There were mild heterophil infiltration and fluid exudation into reticular sheaths at 1 day p.i., and lymphoid hyperplasia beginning at 3 days p.i. Livers were slightly enlarged at 1 day p.i. and hepatocellular glycogen was reduced. The liver of one bird killed at 7 days p.i. was swollen and bile stained. Airsacculitis, pneumonitis, and pericarditis were present in nearly all birds necropsied after 8 h p.i. (Table 3). The character of the exudates progressed from serous at 8 h p.i. to fibrinopurulent at 5 days p.i.
Fig. 9. Liver: Perisinusoidal space is expanded and contains erythrocytes (E) and a heterophil (HP). Sinusoidal macrophage (SM) has many normal appearing bacteria in a phagosome (inset). Acute cell swelling, hepatocytes (H). From a turkey killed in extremis 8 h after i.v. inoculation with *Escherichia coli* (EC1).
Fig. 10. Bacterial colonies (arrows) in hepatic sinusoids of a turkey killed in extremis 9 h after i.v. inoculation with *Escherichia coli* (ECl). Modified Dieterle stain.
Fig. 11. Bacteria in phagosomes of a partially degranulated heterophil from liver. Fusion (arrows) of granules with phagosome. Numerous blebs of the bacterial outer membrane (inset). Same bird as that of Fig. 9.

Fig. 12. Liver: Acute cell swelling, hepatocytes. Endoplasmic reticulum is severely dilated and cytoplasmic matrix is condensed. Sinusoid containing fibrin (F). From a turkey killed in extremis 8 h after i.v. inoculation with Escherichia coli (ECl).
TABLE 3. Gross lesions and bacteremia in turkeys inoculated i.t. with virulent *Escherichia coli*
strains EC1 and EC27<sup>a</sup> (experiments 5 and 6)

<table>
<thead>
<tr>
<th></th>
<th>Hours</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Airsacculitis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>Pericarditis</td>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>1+</td>
<td>0</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup>Right column = EC27, left column = EC1.

<sup>b</sup>Severity of lesions; 0 = normal, 1+ = detectable, 2+ = moderate, 3+ = severe.
The onset and duration of bacteremia in birds inoculated i.t. is expressed in Table 4. Bacteremia associated with EC27 had a more rapid onset, higher titer, and longer duration than that associated with ECl. Two turkeys inoculated with EC27 died between 1 to 2 days p.i. Except for the 2 birds which died, maximal bacterial titers were at 12 h p.i. when the mean CFU of *E. coli* per ml of blood was 1,200 for EC27 and 400 for ECl. Blood bacterial titers were generally less than 100 CFU/ml at other sample times, although the 2 birds which died had 14,000 and 5,000 CFU of *E. coli* per ml of blood at 1 day p.i. The duration of bacteremia correlated with the period of most severe visceral lesions seen in experiments 5 and 6 (Table 3).

Control turkeys of each experiment had neither lesions or bacteremia. Sera tested for agglutinating antibody against live *E. coli* inocula had titers less than 1:4.
TABLE 4. The development of bacteremia in turkeys inoculated i.t. with virulent *Escherichia coli* strains EC1 and EC27

<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>Hours</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>EC1</td>
<td>0/5(^a)</td>
<td>1/5</td>
</tr>
<tr>
<td>EC27</td>
<td>0/5</td>
<td>3/5</td>
</tr>
</tbody>
</table>

\(^a\)Number of turkeys with bacteremia per number inoculated. The sensitivity of bacterial detection was about 2 CFU/ml blood.

\(^b\)Two turkeys died between 1 and 2 days post inoculation (p.i.). Both turkeys had greater than 5,000 CFU of *E. coli*/ml of blood at 1 day p.i.
DISCUSSION

The spleen of most animals has a significant role in eliminating infectious agents from circulating blood. Blood-borne *E. coli* in turkeys is phagocytized by reticular sheath macrophages and less so by phagocytes in the red pulp (3). The splenic lesions associated with *E. coli* bacteremia in the present study are a reflection of the acute inflammatory response focused on the reticular sheaths. Concentration of fibrinopurulent exudates within and adjacent to reticular sheaths may be a response to potent chemotactic substances generated during the interaction of *E. coli* with plasma components (e.g. complement) and phagocytes. Heterophils appear to emigrate to reticular sheaths subsequent to more diffuse accumulation in the red pulp and adjacent lymphoid tissue. Such a response would be expected to a chemotactic gradient originating in reticular sheaths.

The more rapid onset of inflammation in spleen and liver of birds injected with avirulent *E. coli* (EC3) compared with virulent *E. coli* (EC1) was also seen in a previous study (3). This difference may be due to a greater capacity of avirulent *E. coli* to cause generation of chemotactic substances. Gonococci isolated from patients with local infection readily activate complement, whereas gonococci isolated from patients with disseminated gonococcal infection activate complement slowly and are poor stimulators of chemotaxis (19). Inefficient activation of the alternative complement pathway by *E. coli* isolated from patients with bacteremia has been associated with the presence of K capsular polysaccharide on the bacterial surface (10). Therefore,
the delayed inflammatory response to virulent *E. coli* in the present study may have been due to inhibition of complement activation caused by capsular antigens. The greater inflammatory response in livers against EC3 compared with EC1 may have been due to both greater localization of EC3 in liver (3) and greater generation of chemotactic substances.

The increased size and staining intensity of lymphoid tissue adjacent to reticular sheaths was probably due to blast transformation of lymphocytes. A similar response of splenic lymphoid tissue has been reported in turkeys infected with *Mycoplasma meleagris* and *E. coli* (51). Increased numbers of periarterial lymphoid follicles seen at 5 to 7 days p.i. may represent proliferating clones of B lymphocytes. In chicks, low levels of serum antibody are detectable within a week after inoculation with *E. coli* (40).

The pathogenesis of venous thrombosis in small hepatic veins of birds killed *in extremis* is speculative. In previous studies, rapid formation of thrombocytic aggregates and thrombosis of small vessels in lung, spleen, and liver occurred after i.v. injection of large numbers of *E. coli*, particularly in the presence of specific antibody (2, 3). The presence of thrombocytic thrombi, especially in small hepatic veins, may be due to severe endothelial damage in collecting branches of hepatic veins. Truscott et al. (84) attributed many vascular and parenchymal lesions in spleen and liver of chickens to the action of a chick-lethal toxin produced by some strains of *E. coli*. Also, small hepatic veins, like centrilobular hepatocytes, would be more vulnerable to hypoxic injury associated with severe septic shock. Scattered foci of hepatic
necrosis were likely the result of vascular occlusion since these areas were free of both bacteria and granulocytes. Thrombosis of hepatic veins in birds in extremis may have impaired sinusoidal circulation to the extent that severe sinusoidal congestion, bacterial colonization, and hepatocellular necrosis were inevitable sequelae. Extensive bacterial colonization of hepatic sinusoids, in contrast to splenic sinusoids, indicates that the liver may be an important site of bacterial seeding into the bloodstream in progressive E. coli bacteremia. The abundance of fibrin within hepatic sinusoids and spleens of birds in extremis indicates that systemic activation of the coagulation system may have also impaired the microcirculation.

Lesions of spleen and liver were much less severe in turkeys inoculated i.t. compared with those inoculated i.v. Thus, severity of lesions in spleen and liver appears to be a function of the duration and magnitude of bacteremia and the rate of E. coli localization within tissues. Low numbers of E. coli in the blood are apparently effectively managed in most birds by the mononuclear phagocytes of spleen and liver without the development of an intense granulocytic response. The death of 2 birds (Table 4), 1 to 2 days after i.t. inoculation with E. coli suggests that the stress of repeated blood sampling and handling may have impaired their capacity to control early E. coli bacteremia.

Although colisepticemia may occur secondary to the stress of primary disease or environmental factors (72), whether such stress causes dysfunction of the mononuclear phagocyte system is unknown. Since the liver is of major importance for removal of E. coli from blood (2, 3, 7),
impairment of hepatic circulation may predispose to progressive bac-
teremia. Hypothetically, hepatic circulation may be reduced in shock or
dehydration resulting from primary disease.
GENERAL SUMMARY AND DISCUSSION

Bacteria that cause acute systemic disease generally do so because of their capacity to resist ingestion by host phagocytes (67). Although similar numbers of virulent and avirulent E. coli localized in spleen, greater numbers of avirulent E. coli were present in liver. Studies of bacteremia in mammals (7) and the present study have shown that rapid clearance of bacteria from blood is dependent upon avid phagocytosis by hepatic macrophages. The greater importance of liver compared with spleen in clearance of blood-borne bacteria is probably due to the greater size and blood flow of liver (7). In turkeys, retention of bacteria by splenic macrophages is probably more important for presenting bacterial antigens to immunocompetent lymphocytes than for clearing large numbers of bacteria from blood.

Avirulent bacteria and opsonized bacteria are rapidly cleared from the blood of mammals by hepatic macrophages (7). In the present study, the clearance from blood and localization of opsonized virulent E. coli was similar to that of avirulent E. coli. Although serum agglutinin titers of passively immune turkeys were 1:8 or less, these turkeys were protected against large numbers of virulent E. coli. In contrast, other investigators have suggested that there is no correlation between O and K serum agglutinins and protection against colisepticemia (17, 18). They found that some experimental vaccines protected birds from challenge with virulent E. coli, but sera from only a small percentage of vaccinated birds had agglutinin titers greater than 1:5 (17, 18). It appears
likely from the present studies that humoral antibody may be pro-
tective even when the quantity is too little for **in vitro** detection.

Localization of *E. coli* in reticular sheath macrophages is con-
sistent with the findings of White and Gordon (92). They found that in
chickens blood-borne staphlococci and soluble protein antigens localize
in reticular sheaths whereas larger particles (*Candida albicans*) localize
exclusively in red pulp (92). In the present studies, virulent *E. coli*
localized predominantly in reticular sheaths but avirulent *E. coli* and
opsonized *E. coli* localized both in reticular sheaths and in red pulp
sinusoids. Phagocytosis of large numbers of virulent *E. coli* by macro-
phages of the reticular sheaths may be due to the very limited extracel-
lar space of these macrophages compared with those of sinusoids in
splenic red pulp and liver. The close association among these macrophages
would enhance bacteria-phagocyte contacts and reduce the repulsive effects
of antiphagocytic components on the surface of virulent *E. coli*.

Aggregation of thrombocytes was a common finding in turkeys killed
in **extremis** and in those inoculated i.v. with large numbers of *E. coli*,
particularly in the presence of antibody. Platelet aggregation may occur
in mammals inoculated i.v. with large numbers of bacteria (37). Throm-
mosis of pulmonary capillaries in passively immune birds inoculated with
*E. coli* is probably irrelevant to natural *E. coli* bacteremia because
numbers of *E. coli* are generally not great except in terminal stages of
disease. Thrombosis of small hepatic veins was seen in all birds killed
in **extremis**; however, a cause-effect relationship is difficult to es-
tablish between severe bacteremia and venous thrombosis. Thrombosis
of small hepatic veins may further impede sinusoidal circulation, enhance bacterial colonization, and ensure a fatal conclusion to the disease.

The lesions in spleen and liver reflected the magnitude of bacteremia and sites of bacterial localization in these tissues. That is, avirulent *E. coli* bacteria are trapped largely in liver and cause a transient hyperemia with multifocal accumulation of heterophils in sinusoids. Virulent *E. coli* localize in splenic reticular sheaths and incite an intense granulocytic response. Intratracheal inoculation is associated with a persistent bacteremia of low magnitude and only mild splenic and hepatic lesions in most birds.

Although these studies have contributed to our understanding of the role of bacteremia in the pathogenesis of colisepticemia in poultry, important questions remain. Why, in some birds, does *E. coli* bacteremia become progressive and fatal? Are virulent *E. coli* bacteria opsonized by "natural" antibody or components of complement? Where do predisposing factors operate in the pathogenesis of colisepticemia to make birds more susceptible to the disease? Is the capacity of the mononuclear phagocyte system of the spleen and liver to control bacteremia impaired by primary disease and dehydration?


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