INFECTION AND IMMUNITY, Feb. 1970, p. 195-199

Vol. 1, No. 2
Printed in U.S.A.

Intestinal Strangulation in *Escherichia coli*-Monocontaminated Gnotobiotic Rats[1]

CHARLES E. YALE AND ALAPAKKAM R. VIVEK
Department of Surgery, University of Wisconsin Medical School, Madison, Wisconsin 53706

Received for publication 4 August 1969

The natural course of ischemic and hemorrhagic intestinal strangulation in previously germ-free rats individually monocontaminated with one of five separate strains of *Escherichia coli* was investigated. In addition, the effect of intraintestinal homologous heparinized blood upon the course of ischemic intestinal strangulation in *E. coli*-monocontaminated rats was studied. It was found that (i) *E. coli* could be an important lethal factor in ischemic and hemorrhagic intestinal strangulation, (ii) hemorrhagic was more deadly than ischemic strangulation, (iii) intraintestinal blood did not augment the action of the *E. coli* in these experiments, and (iv) some strains of *E. coli* were more toxic than others in the presence of intestinal strangulation.

The fundamental importance of bacteria in intestinal strangulation has been dramatically demonstrated in our laboratories by studies with conventional and germ-free rats (7, 8). *Escherichia coli* is one of the most common of the many species of intestinal bacteria in man and animals, and it is frequently believed to be a major lethal factor in intestinal strangulation, peritonitis, and septic shock. Blood, or one of its breakdown products, appears to augment the action of *E. coli* (1, 5, 9).

This paper summarizes the results of two groups of experiments. The first was an investigation of the natural course of ischemic and hemorrhagic intestinal strangulation in rats, each monocontaminated with only one of five separate strains of *E. coli*. The second was a study of the effect of intraintestinal blood upon the course of ischemic intestinal strangulation in *E. coli*-monocontaminated rats.

MATERIALS AND METHODS

One hundred, 256- to 530-gm germ-free Sprague-Dawley rats of mixed sexes were individually caged, 20 animals in each of five separate flexible-film housing isolators with individually attached operating isolators. On the day of the operations, a rubber-stoppered 50-cc vial of *E. coli* containing approximately 10^9 viable cells per cc was introduced into each of the five isolation systems, each system receiving a different *E. coli* strain. The first 12 of the 20 animals in each isolator were used in experiment 1 and the remaining eight animals for experiment 2.

[1] Part of this study was presented at the 69th Annual Meeting of the American Society for Microbiology at Miami Beach, Fla., 4-9 May 1969.

Experiment 1. The first 12 germ-free rats in each isolator were anesthetized with sodium pentobarbital (25 mg/kg of body weight) and, through a midline abdominal incision, the distal small intestine was identified. The animals were subdivided into three equal groups. A 2-cc amount of sterile normal saline solution was injected through a no. 30 needle into the lumen of the distal ileum in one rat of the first group, and 2 cc of the *E. coli* suspension was injected into the ileum of the other three. The abdominal wall was closed in two layers with continuous 5-0 chromic catgut.

In each of the second group of four rats, a 10-cm segment of distal ileum was isolated by ligating its ends with no. 2 silk. The bowel was divided above and below the ligatures, and an end-to-end anastomosis with interrupted inverting 6-0 silk was done to restore the continuity of the bowel. The venous return from the test segment was ligated, producing a hemorrhagic strangulation. A 1-cc amount of sterile normal saline solution was injected into the lumen of the test segment of one animal, and 1 cc of the *E. coli* suspension was injected into the segment in each of the other three rats. The abdominal wall was closed as before.

A test segment of distal ileum 25 cm long was created in each of the third group of four rats. After completion of the bypassing end-to-end anastomosis, the arterial and venous supply of the test segment was ligated, producing an ischemic strangulation. A 2-cc amount of sterile normal saline was placed into the lumen of the test segment in one animal, and 2 cc of the *E. coli* suspension was placed into the segment of each of the other three animals. The abdominal wall was closed as before.

In each isolator, the operations divided the 12 animals into one of six separate groups—i.e., hemorrhagic, ischemic, or no strangulation, with or without *E. coli* contamination.

Postoperatively, the rats were given feed and water

ad lib. Initially, at 1, 2, 4, and 7 days and weekly until death or sacrifice at 4 weeks, the activity, weight, temperature, white blood count (WBC), and hematocrit were determined. An autopsy was performed at death or sacrifice.

Experiment 2. The eight remaining germ-free rats in each of the five isolation systems were used in experiment 2. All animals were anesthetized and a midline abdominal incision made as in experiment 1. An 8-cc amount of blood was withdrawn from the inferior vena cava of one rat into a syringe containing 2 cc of normal saline and 100 units of heparin.

A test segment 20 cm long with ischemic (arterial and venous) strangulation was fashioned in each of seven rats following the techniques of experiment 1. After completion of the bypassing anastomosis, 0.5 cc of the *E. coli* suspension was injected into the lumen of the test segment, followed by either 1 cc of sterile normal saline or 1 cc of the homologous heparinized blood. The abdominal wall was closed and the rats were observed postoperatively as in experiment 1.

Bacteriology. The five strains of *E. coli* used in these experiments as typed at the National Communicable Disease Center (NCDC), Atlanta, Ga., were O-X12:H14; O-26:B6:nonmotile (NM); O-86a:B7:NM; O-111a, 111b:B4:NM; and O-128a, 128c:B12:H12. The O-X12:H14 was isolated from a normal, conventional rat in the University of Wisconsin Medical Center Animal Care Unit; the other four strains were obtained from the NCDC. The strain from each isolator system was retyped at the conclusion of the experiment at the NCDC.

The *E. coli* suspensions were obtained by spreading two loopfuls of a 24-hr nutrient broth (Difco) culture of a given strain onto each of a series of nutrient agar (Difco) plates. After incubation for 24 hr at 37 C, the growth from a minimum of six plates was scraped off the agar surface with a rubber policeman and was suspended in tryptose saline [1 gm of tryptose (Difco) plus 5 gm of sodium chloride in 1 liter of distilled water.] The suspension was centrifuged at 14,000 $\times$ *g* (model HN, International Equipment Co., Needham Heights, Mass.) for 10 min; the supernatant fluid was discarded and the cells were resuspended in tryptose saline. After two additional similar washings, the cells were suspended in approximately 50 cc of 8.5% sucrose solution to adjust the concentration of the suspension to approximately 10^9 viable cells per cc. The suspension was then placed in a 50-cc rubber-stoppered vial for introduction into the isolator. The concentration of the number of viable cells was determined initially and at the conclusion of all the operations in a given isolator by the serial plate count dilution method.

Samples of the ileal stool at operation, the feces and material in each isolator at weekly intervals, and the test segments or peritoneal cavities at death or sacrifice were cultured in the following media: (i) fluid thioglycollate medium (Difco) aerobically and anaerobically at 37 C, (ii) tryptose blood-agar (Difco) base plates both aerobically and anaerobically at 37 C, and (iii) Sabouraud dextrose-agar (Difco) slants aerobically at room temperature (22 C).

RESULTS

Experiment 1. Fifty-nine animals were suitable for analysis (Table 1). All 20 of the animals without a strangulated test segment lived to be killed after 4 weeks, showing that contamination alone was not lethal. Four of the six germ-free rats with hemorrhagic and all six with ischemic strangulation lived until sacrifice. In contrast, none of the 14 *E. coli*-monocontaminated rats with hemorrhagic strangulation and only 2 of the 13 animals with ischemic strangulation lived for 4 weeks. A rank-order test indicates that the *E. coli*-monocontaminated hemorrhagic and ischemic groups were significantly different ($P < 0.05$). All of the five strains of *E. coli* were shown to be lethal in conjunction with either hemorrhagic or ischemic intestinal strangulation.

Postoperatively, the weight, temperature, WBC, and hematocrit of the germ-free animals, with and without strangulation, were similar to that reported in our previous studies (7, 8). Monocontamination without strangulation led to (i) a 35-g weight loss at 96 hr followed by a gradual gain during 4 weeks to a value equal to or greater than their preoperative weights, (ii) no significant change in the temperature or WBC, and (iii) a five-point drop in hematocrit to 45 at 96 hr and a return to preoperative levels by 1 week. Monocontamination with hemorrhagic strangulation resulted in early deaths; therefore, long-term post-

TABLE 1. *Intestinal strangulation in rats monocontaminated with Escherichia coli*

Type of strangulation	Type of O antigen	No. of animals for analysis	Survival time of individual animals[a] (hours)
None	Germ free	6	(6)
	X12	2	(2)
	26	3	(3)
	86a	3	(3)
	111a, 111b	3	(3)
	128a, 128c	3	(3)
Hemorrhagic (venous)	Germ free	6	356, 74 (4)
	X12	2	25, 15
	26	3	30, 16, 10
	86a	3	12, 10, 10
	111a, 111b	3	13, 11, 11
	128a, 128c	3	15, 10, 8
Ischemic (arterial and venous)	Germ free	6	(6)
	X12	2	24, 18
	26	3	472, 58, 48
	86a	3	24, 24 (1)
	111a, 111b	2	20, 12
	128a, 128c	3	206, 99 (1)

[a] Numbers in parentheses indicate animals killed 4 weeks after operation.

operative data was unobtainable. Monocontamination with ischemic strangulation produced (i) a steady weight loss occasionally exceeding 100 g at death, (ii) a slight (0.5 C) increase in temperature followed by a fall to 33 to 35 C approximately 12 hr before death, (iii) a variable change in WBC with an occasional very low count (3,000 to 4,000) just before death, (iv) a small drop in hematocrit to about 45 at 24 hr followed by a gradual rise until death or recovery, and (v) occasional diarrhea lasting up to 1 week.

At autopsy, the germ-free and monocontaminated animals without strangulation had equally large cecums; the findings were otherwise unremarkable except for an occasional thin adhesion from the omentum to the peritoneal surface of the anterior abdominal wound. The test segments of the contaminated animals with hemorrhagic strangulation were dark red and hemorrhagic and were distended with intraintestinal gas, but usually were not perforated. There was 2 to 4 ml of clear, dark-red fluid within the peritoneal cavity. The test segments of the contaminated animals which died with ischemic strangulation were pale white, but again distended with intraintestinal gas and usually not perforated. There was 1 to 2 cc of cloudy, pale-yellow peritoneal fluid. If premortem diarrhea had been extensive, the cecum was collapsed. If the contaminated rats with ischemic strangulation lived to be killed after 4 weeks, there was invariably a mass 3 to 4 cm in diameter in either the right or left lower abdominal quadrant, adherent on all sides to the omentum and visceral and parietal peritoneum. This mass consisted of a shell 0.5 mm thick of viable, fibrous tissue filled with a moderately thick yellow to "blue cheese" type of material in which could be seen the ghost-like outline of the nearly completely dissolved ischemic test segment and its heavy silk ligatures. There was no free peritoneal fluid and, in general, there were very few adhesions. The cecums were still enlarged to the size of the normal, germ-free rat. All anastomoses were open and the wounds were well healed. After 4 weeks, the test segments of the germ-free animals with ischemic or hemorrhagic strangulation were difficult to find—especially the former. Usually, an irregular, firm cyst 1 cm in diameter containing thick, yellow, caseous material was all that remained.

Experiment 2. Thirty-three animals were suitable for analysis (Table 2). All had ischemic strangulation of an *E. coli*-monocontaminated distal ileal test segment 20 cm in length, whereas 16 had normal saline and 17 had homologous heparinized blood injected into the lumen of the test segment. Although eight animals with intraintestinal normal saline against only four with intraintestinal blood lived to be killed after 4 weeks, the other eight with normal saline lived an average of 32 hr against the other 13 with blood that lived an average of 40 hr after operation. Looking at the individual results, we conclude that intraintestinal, homologous, heparinized whole blood does not potentiate or enhance the action of *E. coli* in ischemic intestinal strangulation.

The survival times of all of the *E. coli*-monocontaminated rats with ischemic strangulation from experiments 1 and 2 and the concentrations of the *E. coli* suspensions are listed in Table 3. Although at least one animal lived until sacrifice

TABLE 2. *Ischemic intestinal strangulation in Escherichia coli-monocontaminated rats with intraintestinal blood*

Type of intraintestinal fluid	Type of O antigen	No. of animals for analysis	Survival time of individual animals (hours)[a]
Normal saline	X12	3	18, 15 (1)
	26	3	(3)
	86a	3	51, 42, 15
	111a, 111b	3	41, 17 (1)
	128a, 128c	4	54 (3)
Homologous, heparinized blood	X12	4	24, 19, 13, 11
	26	4	22 (3)
	86a	4	61, 47, 40, 30
	111a, 111b	2	61, 52
	128a, 128c	3	76, 58 (1)

[a] Numbers in parentheses indicate animals killed 4 weeks after operation.

TABLE 3. *Ischemic intestinal strangulation in Escherichia coli-monocontaminated rats*

Type of O antigen	Concn of *E. coli* suspension[a]	No. of animals for analysis	Survival time of individual animals[b] (hours)
X12	2.5×10^9	9	24, 24, 19, 18, 18, 15, 13, 11 (1)
26	3.8×10^9	10	472, 58, 48, 22 (6)
86a	20.5×10^9	10	61, 51, 47, 42, 40, 30, 24, 24, 15 (1)
111a, 111b	1.1×10^9	7	61, 52, 41, 20, 17, 12 (1)
128a, 128c	16.2×10^9	10	206, 99, 76, 58, 54 (5)

[a] Expressed as number of viable cells per cubic centimeter of suspension.

[b] Numbers in parentheses indicate animals killed 4 weeks after operation.

after contamination with each strain, six and five animals, respectively, contaminated with strains O 26:B6:NM and O 128a, 128c:B12:H12 lived to be killed after 4 weeks, and those that died lived longer, on the average, than did the animals contaminated with the remaining three strains. This longevity could not be related to animal sex or size or to the concentration of the *E. coli* suspensions. We conclude that some strains of *E. coli* are more toxic than others in the presence of ischemic intestinal strangulation.

Postoperatively, the weight, temperature, WBC, hematocrit, and postmortem findings of the animals in experiment 2 were similar to those described for the monocontaminated rats with ischemic intestinal strangulation of experiment 1. There was no recognizable difference in the postoperative and autopsy findings between the group with intraintestinal, normal saline and the group with intraintestinal, homologous, heparinized blood, nor was there any difference among the animals with the various strains of *E. coli*.

Bacteriology. At the time of operation, all the samples from the ileal test segments or the anus (in those animals without strangulation) were sterile. Postoperatively, the feces and material from each isolator, as well as those from the test segments or peritoneal cavities at death, were monocontaminated with *E. coli*. No other organism was found in any of the many cultures from each of the five separate isolation systems during the 5 weeks of these experiments.

The preoperative typing of the *E. coli* suspension introduced into each isolator and the postoperative typing of a sample from one of the dead animals from the corresponding isolator were performed by NCDC and found to be identical and without cross-contamination.

The method of preparing the *E. coli* suspension appeared to be satisfactory. The initial and final (48 to 72 hr later) concentrations were averaged and were found to vary from 1.1×10^9 to 20.5×10^9 viable cells per cc (Table 3). The final was from 25 to 80% of the initial concentration, but all samples of the *E. coli* suspensions contained at least 10^9 viable cells per cc. This variation in concentration did not appear to be significant.

DISCUSSION

In the germ-free rat, up to 15% (about 15 cm) of the small intestine can be hemorrhagically strangulated, and at least 75% can be ischemically strangulated without fatalities (7, 8). In contrast, in rats with a conventional bacterial flora, much smaller intestinal segments either hemorrhagically or ischemically strangulated are uniformly fatal, although the animals with ischemic strangulation live, on the average, several days after operation (8). The *E. coli*-monocontaminated rats of the present study responded to both ischemic and hemorrhagic strangulation similarly to animals with a conventional bacterial flora containing a large variety of organisms. It is clear that *E. coli* alone can be an important lethal factor in ischemic and hemorrhagic intestinal strangulation.

Hemorrhagic is more deadly than ischemic strangulation because of the added stress of a nonlethal blood loss and possibly, as suggested by others (1, 5, 9), a potentiating action of blood, hemoglobin, or a hemin pigment upon the intestinal bacteria. The nonlethal course of ischemic strangulation in germ-free rats (i.e., animals without a significant blood loss) is not altered by placing homologous, heparinized blood into the strangulated intestine or the free peritoneal cavity (7). In the present report, the introduction of homologous, heparinized blood into the ischemically strangulated intestine monocontaminated with *E. coli* did not appear to increase the severity of the insult. This suggests that any bacterial potentiating action of blood in hemorrhagic intestinal strangulation is of minor importance compared to the stress of blood loss.

E. coli is a normal inhabitant of the intestinal tract of man and animals at concentrations up to 10^{10} organisms per g of wet feces (2). Therefore, the concentrations of the *E. coli* suspensions were placed in this range. All of the strains produced large amounts of gas which greatly distended the necrotic test segments. This distention probably increases the incidence of frank rupture of the closed loops and may also increase the rate of passage of *E. coli* across the bowel wall. The hemolytic strains of *E. coli* are frequently of pathological origin (6), but none of the five strains of this experiment was hemolytic. Four of the five strains of *E. coli* (those obtained from NCDC) were isolated from humans with diarrhea and are known to belong to serotypes that frequently cause severe diarrheal disease (3). Although diarrhea was noted in a few instances, these enteropathogenic strains were no more lethal in the present study than was the strain isolated from a normal rat. None of the strains resulted in a permanent reduction of the cecum, as noted after contamination of germ-free rats with other organisms (4). The reason for the decreased toxicity of the O-26 and O-128 serotypes is unexplained.

This study forcefully demonstrates the usefulness of the germ-free animal as a method of studying the importance of bacteria, singly or in combinations, in intestinal strangulation.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-06956 from the National Institute of Allergy and Infectious Diseases.

We gratefully acknowledge the assistance of William H. Ewing of the National Communicable Disease Center, Atlanta, Ga., in typing the strains of *E. coli*.

LITERATURE CITED

1. Bornside, G. H., P. J. Bouis, Jr., and I. Cohn, Jr. 1968. Hemoglobin and *Escherichia coli*, a lethal intraperitoneal combination. J. Bacteriol. 95:1567–1571.
2. Donaldson, R. M., Jr. 1964. Normal bacterial populations of the intestine and their relation to intestinal function. New Eng. J. Med. 270:938–945.
3. Edwards, P. R., and W. H. Ewing. 1962. Identification of Enterobacteriaceae, p. 67–69. Burgess Publishing Co., Minneapolis, Minn.
4. Hudson, J. A., and T. D. Luckey. 1964. Bacteria-induced morphologic changes. Proc. Soc. Exp. Biol. Med. 116:628–631.
5. Simmons, R. L., J. W. Diggs, and H. K. Sleeman. 1968. Pathogenesis of peritonitis. III. Local adjuvant action of hemoglobin in experimental *E. coli* peritonitis. Surgery 63:810–815.
6. Wilson, G. S., and A. A. Miles. 1964. Topley and Wilson's principles of bacteriology and immunity, p. 824. Edward Arnold (Publishers) Ltd., London.
7. Yale, C. E. 1969. Ischemic intestinal strangulation in germfree rats. Arch. Surg. 99:397–400.
8. Yale, C. E., and W. A. Altemeier. 1965. Intestinal obstruction in germfree rats. Arch. Surg. 91:241–247.
9. Yull, A. B., J. S. Abrams, and J. H. Davis. 1962. The peritoneal fluid in strangulation obstruction. The role of the red blood cell and *E. coli* bacteria in producing toxicity. J. Surg. Res. 2:223–232.