THE ROUTE OF ENTERIC INFECTION IN NORMAL MICE*

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The inability of *Salmonella typhi* to induce a progressive fatal infection in most laboratory animals, analogous to that seen in man, dictated that most experimental studies of typhoid immunity had to be performed in the mouse typhoid model. Ørskov and Moltke (1) carried out a classical study into the early distribution of *Salmonella* in the mouse gut after the ingestion of bread soaked in an *S. typhimurium* broth culture. However, these workers were unable to precisely delineate the primary site of bacterial invasion in these animals. Until relatively recently, surprisingly little more has been published which contributes to our understanding of the primary site of enteric infection in the mouse. Schlewinski et al. (2) demonstrated a very early systemic infection in orally challenged mice using *S. typhimurium*, suggesting that penetration by *Salmonella* occurs in the upper half of the gastrointestinal tract. This finding would be compatible with data obtained by Sprinz et al. (3) in humans. By way of contrast, however, Ozawa et al. (4) recently concluded that the primary site of *Salmonella* infection involved the cecum and the large intestine.

Earlier studies from this laboratory indicated that although the majority of orally introduced *S. enteritidis* had a rapid transit time through the normal mouse intestine, a small proportion of the inoculum established itself within the walls of the small intestine and in the cecum several days before a systemic infection could be demonstrated (5, 6). However, the primary site of the infection, and particularly, the route taken by the organisms to reach the liver and spleen, was unclear. Studies in opium-treated guinea pigs or in streptomycin-treated prestarved mice indicated a heavy early involvement of the lamina propria of the small intestine (7, 8). However, it was possible that the highly artificial experimental conditions used by these investigators may have contributed to this localization (5), so it was decided to follow the early distribution of an intragastric inoculum of virulent *S. enteritidis* in sequential sections of the normal undisturbed mouse intestinal tract. The fate of the inoculum was followed quantitatively throughout the gastrointestinal tract and the primary site of muscosal penetration was determined.

Materials and Methods

Animals.—6- to 8-wk old specific pathogen-free CD-1 (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) and B6D2 (C57Bl/6 × DBA/2 F_1) mice of either sex were

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maintained 10 to a cage, under isocaps, and on sterilized bedding (San-i-cel, Paxton, Ill.) with free access to sterilized diet (Charles River Rat/Mouse Formula, Country Foods, Syracuse, N. Y.) and water.

Bacteria.—*S. enteritidis* strains 5694 and a substrain, *S. enteritidis* 5694 SM^R (resistant to 20 μg of streptomycin/ml) were used. The oral mean lethal dose for *S. enteritidis* 5694 is 2×10^6 for CD-1 mice and 5×10^3 for B6D2 mice (6). The organisms were grown in trypticase soy broth (Baltimore Biological Laboratories, Cockeysville, Md.) at 37°C for 24 h, the logarithmic phase suspension was standardized turbidimetrically, diluted suitably in sterile saline, and inoculated immediately into the mice. The viability of the suspension was checked after inoculation by plating suitable saline dilutions onto trypticase soy agar (TSA) plates and incubating at 37°C overnight.

Methods of Infection.—Oral infection was accomplished by direct intragastric inoculation of the bacteria by means of a feeding tube (9). The rectal route of challenge was given 20 min after a warm saline enema. The challenge inoculum, suspended in 0.2 ml of saline, was deposited directly into the rectum through a stainless steel feeding tube, which had been moistened and gently inserted through the anus to a point 1.5 cm into the rectum. Direct injection of the challenge inoculum into the lumen of the intestinal tract was accomplished by exposing the duodenum, cecum, or colon of an ether-anesthetized mouse through a small incision in the abdominal wall. The challenge inoculum, in 0.1 ml of saline, was injected into the lumen of the intestinal segment via a 26 gauge needle, care being taken not to contaminate the serosa. The laparotomy incision was then closed with Autoclips (Clay Adams, Div. of Becton, Dickinson & Co., New York), and the animals allowed to revive from the anesthesia. Such mice were always checked for peritoneal infection at the time of sacrifice.

Bacterial Enumeration Technique.—Groups of five randomly selected mice were sacrificed at intervals and the liver, spleen, selected intestinal segments, mesenteric lymph nodes, and 0.1 ml of heart blood removed aseptically. The organs were homogenized separately in saline and plated on TSA or MacConkey agar (Difco Laboratories, Detroit, Mich.) as previously described (5). If the streptomycin-resistant strain of *S. enteritidis* was used, 20 μg of streptomycin was added/ml of media. In experiments where it was necessary to determine the number of viable *Salmonella* in selected Peyer's patches or individual lymph nodes draining a selected portion of the gastrointestinal tract, the entire tissue homogenate was plated to detect the small numbers of organisms usually present in these organs. The individual lymph nodes were dissected out aseptically, placed in 0.2 ml of sterile saline in a sterile glass test tube (10 × 76 mm), and crushed with a smooth metal rod. In the case of the Peyer's patches, the three most proximal Peyer's patches of the duodenum, the three most distal Peyer's patches of the ileum, and the apical Peyer's patch of the cecum were carefully excised from the intestinal wall in such a way that they remained free of intestinal contents, and were then homogenized in the same manner as the lymph nodes. The Peyer's patches from the duodenum were pooled and plated together, as were those from the ileum. In all experiments, questionable colonies appearing on the plate counts were checked as group D *Salmonella* by slide agglutination. The relative error for the bacterial counts was similar to that previously reported (5).

Dye Injections.—A preparation of Chicago blue 6B dye (K & K Laboratories, Inc., Plainview, N. Y.) made isotonic with mouse blood (17.0% wt/vol in distilled water [10]) was diluted 1:10 in saline and injected subserosally at various points along the gastrointestinal tract using a 30 gauge needle. Movement of the dye determined the lymphatic drainage of each injected area. Dye injections were made in ether-anesthetized mice or occasionally in freshly killed animals; in both cases, the results obtained were the same. The lymph nodes draining the head, the peritoneal cavity, and the appendages were also identified by injecting the appropriate site in unanesthetized mice. The animals were killed 5–10 min later and autopsied to determine the precise sites of dye localization within the tissues.

RESULTS

Early Distribution of Salmonella in the Gastrointestinal Tract.—The distribution of *Salmonella* within the gastrointestinal tract after an oral infection was determined by means of viable counts carried out at intervals on separate segments of gut over a 6-h period. The data in Table I is representative of several experiments showing that the challenge inoculum moved rapidly through the gut to infect the lower intestinal tract of the normal untreated animal within 1 h of infection. Less than 1% of the inoculum could still be detected 1 h after intragastric inoculation of 10^7 viable bacilli. The rate of decline subsequently slowed and by 6 h only 0.25% of the challenge inoculum could still be recovered from the entire gut. The greatest proportion of these surviving bacteria were in the cecum and large intestine (Table I). The number of viable *Salmonella*

TABLE I

Distribution of S. enteritidis SM^R in the Intestines of Normal CD-1 Mice after Intragastric Challenge

Time post-infection	Percentage of viable salmonella*									
	Stomach		Small intestine		Peyer's patches	Cecum		Large intestine		Total‡ recovery
	Wall	Contents	Wall	Contents		Wall	Contents	Wall	Contents	
h										%
1	0.1	1.0	40.0	30.0	3.4	3.1	17.3	1.0	4.1	100
3	0.0	4.4	0.0	0.0	0.6	4.0	30.0	3.0	50.0	92
6	0.1	1.0	2.0	0.1	0.1	3.0	10.0	0.3	6.4	23

* No viable *Salmonella* were detected in the blood, mesenteric lymph nodes, spleen, or liver at 1, 3, 6, 24, or 48 h. Five out of five mice died by day 10.

‡ Total per cent recovery in terms of the 1 h counts, which in turn represents 1% of the 10^7 viable organisms introduced into the stomach at time 0.

present in the stomach and small intestine 3 and 6 h postinfection was very small and may have been the result of coprophagy which was observed to occur with some of the animals.

The blood and the mesenteric lymph node remained infection free throughout this period despite the fact that 1.0 ml of heart blood and the entire saline homogenate of the mesenteric node was pour-plated for each time point. The spleen and liver contained less than 50 bacilli (the limit of accurate quantitation for these organs) up to 24 h, but by 48 h an increasing number of *Salmonella* appeared in both organs, after the pattern of growth described earlier for orally infected mice (5). All of the mice were dead 10 days postinfection.

Primary Site of Invasion in the Intestines of Normal Mice.—The bacterial infection in the intragastrically infected mouse very quickly reached the lower intestinal tract where it remained in larger numbers and for a longer time period than was the case for the small intestine or the stomach. It therefore

seemed reasonable that these viable bacilli which persisted in the lower intestinal tract were most likely responsible for the systemic infection of the host through penetration of the intestinal mucosa at this site. The relative paucity of lymphoid tissue, the smaller villi, and the relatively thinner mucosal layer in the cecum and large intestine as compared to the small intestine, possibly indicated sites more easily penetrated by virulent organisms (11). Also, the large populations of enterobacteria within this portion of the gut suggested an environment less hostile to the survival of the *Salmonella*. With this in mind, attempts were made to follow the fate of *Salmonella* introduced into the intestines by different experimental methods in the hope of pinpointing the primary site of bacterial penetration within the gastrointestinal tract.

Direct Intralumenal Inoculation.—In three separate experiments, B6D2 and CD-1 mice were injected with 10^8–10^9 viable *S. enteritidis* introduced directly into the lumen of the duodenum, cecum, or colon and exposed through a laparotomy incision. Determinations of the number of viable *Salmonella* in the spleens of animals from each of the infected groups were made 4, 8, and 12 days postinfection. In every experiment, a higher percentage of mice developed more severe infections of the spleen when the organisms were injected directly into the cecum or the large intestine than when the mice were infected via the duodenum. While these results fail to pinpoint the precise site of systemic entry by the infecting organisms, they do indicate that infection can be initiated by organisms deposited within the large intestine.

Rectal Infection.—Further support for the suggestion that *Salmonella* may invade the host by penetrating the mucosa of the lower bowel came from the successful infection of normal mice receiving *S. enteritidis* via a feeding tube inserted into the rectum. The bacterial inoculum was given 20 min after a saline enema and the animals were placed in a metal restrainer for 24 h with free access to food and water, but without the possibility of coprophagy. The addition of Chicago blue to the challenge inoculum indicated that the *Salmonella* remained within the large intestine and the cecum with no involvement of the small intestine. Saline washouts taken from the peritoneal cavities of the mice at the time of autopsy did not contain coliforms or *S. enteritidis*, thus removing the possibility that the rectal method of inoculation had contaminated the peritoneal cavity by the accidental perforation of the colon with the inoculating tube. The results of a representative experiment are shown in Table II. These data indicate that a systemic *Salmonella* infection did develop without the apparent involvement of the upper intestinal tract. The few *Salmonella* detected within the peritoneal cavity on day 11 of infection cannot be attributed to experimental perforation of the gut wall, since 100–200 *Salmonella* were also found at this time in the peritoneal cavities of mice infected intragastrically with 10^6 viable *S. enteritidis*.

Direct intralumenal or rectal inoculation of mice with *S. enteritidis* provided evidence that the organism was able to invade the tissues across the lower

TABLE II

Rectal Infection of B6D2 Mice with 10^7 S. enteritidis SM^R

Time postinfection	Animal no.	Log no. bacteria/organ			
		Spleen	Liver	Mesenteric lymph node	Peritoneal washout (1 ml)
2 h	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
4 days	5	5.4	5.8	5.1	0
	6	3.7	4.3	5.0	0
	7	3.2	3.8	5.0	0
	8	2.0	2.7	4.3	0
11 days	9	4.9	4.7	ND*	1.7
	10	4.2	4.3	ND	1.5
	11	4.1	4.1	ND	0.8

* ND, not done.

intestinal tract mucosa. However, the question remained whether this was the primary site of invasion in naturally infected animals. More direct information regarding the exact site of invasion was obtained by culturing lymph nodes which were shown by dye injections to be draining particular regions of the intestinal tract.

Determination of Drainage Areas in the Normal Mouse Intestine.—Chicago blue was injected subserosally at various points along the gastrointestinal tract to determine the lymphatic drainage of each area. The lymphatic capillaries quickly take up the dye, the lymphatics draining the injected area then become clearly defined, and finally, the regional lymph node(s) can be seen. In some instances, particularly those involving the right lumbar node (Fig. 1) the efferent lymphatics and cisterna chyli also became faintly stained.

A single dye injection was given per mouse and the nodes draining the area injected were recorded a few minutes later. The primary nodes draining the area injected appeared dark blue in color with the secondary nodes taking on a lighter blue stain. Use of this technique thus allows the definition of both the primary and secondary lymph nodes draining each section of the gastrointestinal tract and the same method can be used for other sites throughout the body. The specific areas of drainage for each section of the gastrointestinal tract are shown in Figs. 2–5. These are schematic representations of the various lymph drainage areas, the margins of which tend to overlap each other to some degree throughout the intestinal tract. Fig. 2 indicates that the pyloric lymph node drains only the stomach while the distal segments of the mesenteric node drain the distal ileum, the cecum, and ascending colon. The descending colon

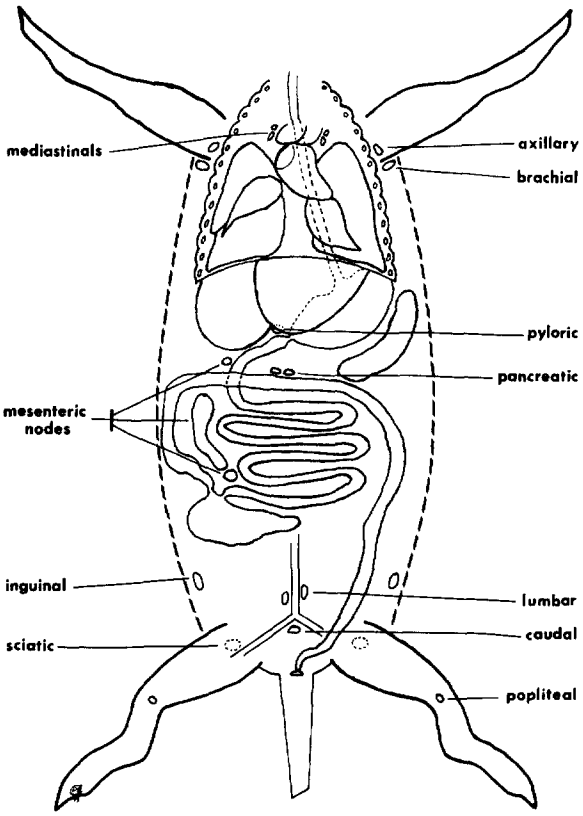

FIG. 1. Schematic representation of the major lymph nodes of the mouse, excluding the head, showing their relative locations and indicating their name as used in the text.

and rectum are drained primarily through the caudal lymph node. In Fig. 3, the duodenum is shown as draining to a small mesenteric node, which is embedded in pancreatic tissue. The majority of the ileum drains to the proximal end of the main body of the mesenteric node (Fig. 4). Two small nodes (which are buried in the pancreas, and are here referred to as pancreatic nodes) were the primary nodes draining the transverse colon (Fig. 5). Since a number of alternative inoculation routes (intraperitoneal as well as subcutaneous and intradermal) have also been used in studies of salmonellosis in the mouse, the lymphatic drainage pathways involved in these infection routes were also determined. As has generally been indicated by earlier studies (12), the peritoneal cavity drains into the mediastinal nodes which also are involved in drainage of the thoracic cavity and lungs. The mesenteric nodes were never involved in drainage from the peritoneal cavity. Four mediastinal nodes are depicted in Fig. 1, two on each side of the trachea. In some animals, particularly

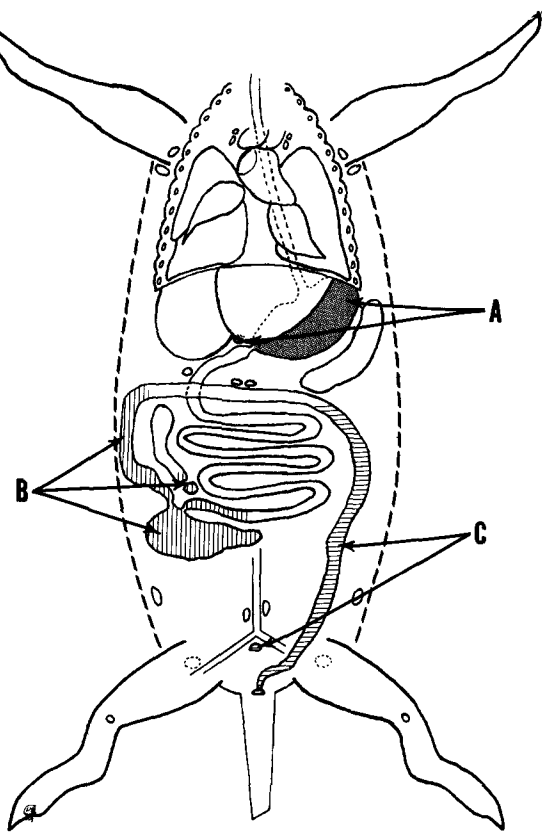

FIG. 2. The pyloric node draining the stomach (A), the mesenteric nodes draining the cecum and ascending colon (B), and the caudal node which drains the descending colon (C) are indicated by identical shadings.

those in which the nodes have been stimulated, three nodes may be found on the right side; one large node, inferior to the thymus and adjacent to the trachea and two small nodes lying next to the thymus.

Dye injected into the front footpads drained primarily into the brachial nodes and only secondarily reached the axillary nodes (Fig. 4). The head drains to the superficial cervical nodes and the tail is drained by both the right and left sciatic nodes with secondary involvement of the left lumbar node (Fig. 5). Injection into the hind footpads primarily involves the popliteal node and then the sciatic node, and little of the dye reached the inguinal nodes which were however intensely stained when dye was injected into flank skin.

Infection of the Peyer's Patches and Draining Lymph Nodes in Intragastrically Infected Mice.—Having determined the lymphatic drainage for the succesive sections of the intestinal tract, it was now possible to study further the site of

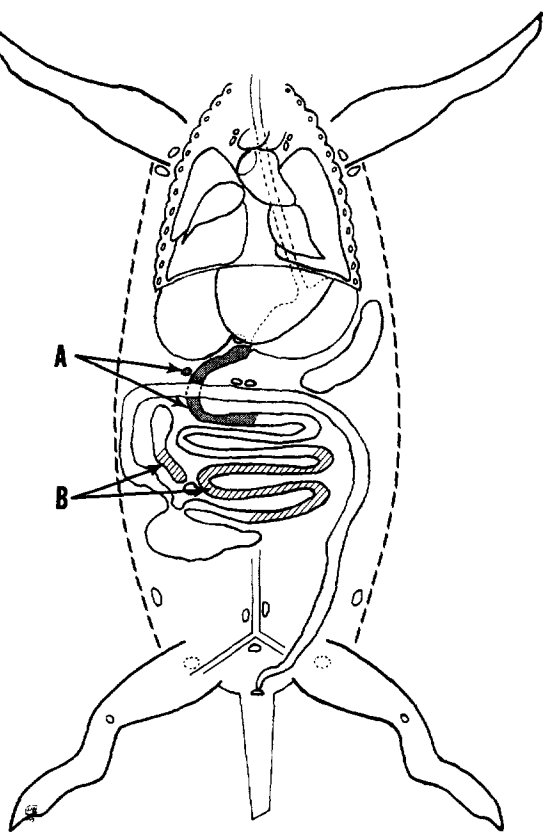

FIG. 3. The upper mesenteric nodes draining the duodenum (A) and the middle mesenteric node draining the ileum (B) are indicated.

infection in the orally challenged host. Normal mice were infected intragastrically with approximately 10^6 viable *S. enteritidis* and the various Peyer's patches and individual lymph nodes were excised carefully at increasing time periods. The number of viable *Salmonella* in the Peyer's patches from the different parts of the small intestine, as well as in the draining lymph nodes, are presented in Table III. These data demonstrate clearly that the primary site of infection is the distal ileum and possibly, the cecum. As early as 6 h postinfection, the Peyer's patches of the distal ileum and the cecum were infected in three out of four mice. None of the other Peyer's patches were involved at this time. The number of viable *Salmonella* in the infected Peyer's patches increased with time and by 48 h the lymph nodes draining these sites had also become infected. Presumably this infection then spread along the lymphatics, until the organisms reached the liver and spleen. None of the Peyer's patches associated with the duodenum nor the lymph nodes draining the various other areas of the intestinal

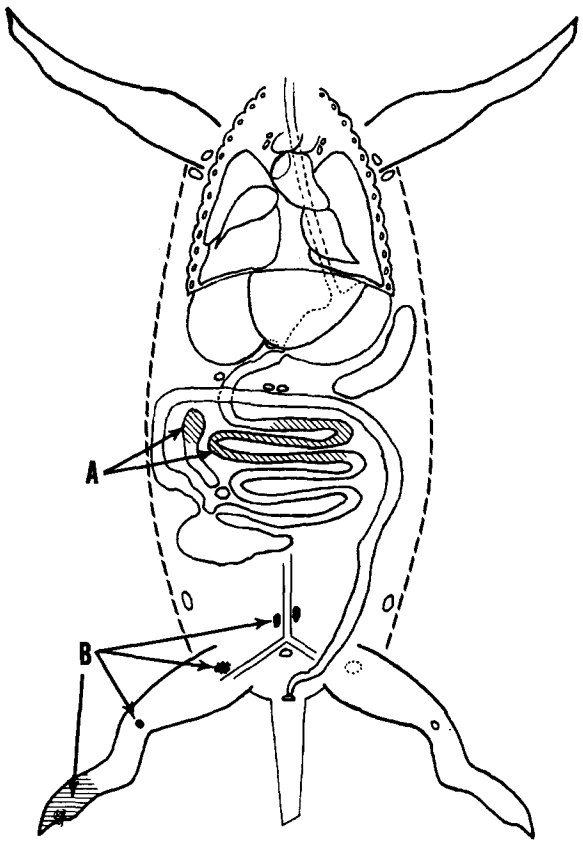

FIG. 4. The middle mesenteric node draining the jejunum (A) and the popliteal, sciatic, and lumbar nodes draining the rear feet (B) are indicated.

tract became detectably infected in any of the mice. Cultures of homogenates prepared from intestinal wall, taken from areas immediately adjacent to the ileal Peyer's patches, contained very few viable *Salmonella* (Table III) and serve to demonstrate the strict limitation of the infection to the Peyer's patch itself.

DISCUSSION

Intragastric inoculation of normal mice with a lethal dose of *S. enteritidis* was followed by a remarkably efficient rate of elimination of the organisms from the gut. It seems ironic that a normal host defense capable of eliminating more than 99% of the organisms from the gut within hours was still unable to prevent a severe systemic infection with the eventual death of most of the animals. Most of the *Salmonella* in the challenge inoculum have no pathogenic significance, since only a few organisms pass across the mucosa of the ileum during the initial

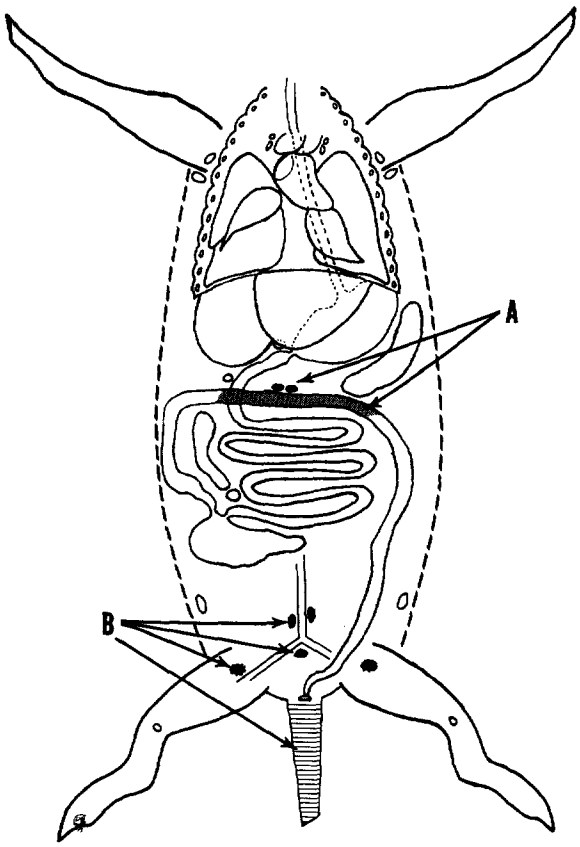

FIG. 5. The pancreatic nodes draining the transverse colon (A) and the caudal, sciatic, and lumbar nodes draining the tail (B) are indicated.

hours of the infection. Nevertheless, these few organisms have the potential to develop into a lethal systemic infection. The reason for the vulnerability of this relatively circumscribed area of gut to this infection is still unclear.

Schlewinski et al. (2) as well as Gerichter (13) reported a substantial bacteremia within minutes of administering massive oral challenge doses of *Salmonella* to mice. Attempts to verify these findings in the present experimental model were uniformly unsuccessful, even when 1 ml of heart blood was cultured at increasing times up to 1 h after intragastric infection. None of the cultures yielded viable bacteria and this, combined with an inability to infect mice orally with the highly mouse virulent *Pasteurella multocida* (9), argues strongly against the systemic infection resulting from "persorption" of the organisms immediately after oral challenge (2, 14).

The use of intralumenal and rectal inoculation resulted in the development of salmonellosis without the apparent involvement of the duodenum or jejunum.

TABLE III

Infection of Gastrointestinal Lymphoid Tissue after Oral Challenge with 2.5 × 10^6 S. enteritidis

Time post-infection	No. of viable *Salmonella* per organ										
	Peyer's patches			Lymph nodes draining							
	Duodenal	Ileal	Cecal	Duodenum	Jejunum	Ileum	Cecum	Transverse colon	Rectum	Spleen	Liver
h											
6	0	5±4*	2±2	0	0	0	0	0	0	0	0
12	0	23±18	8±7	0	0	0	0	0	0	0	0
24	0	1,500±1,130	9±9	0	0	0	0	0	0	0	0
36	0	1,400±1,200	0	0	0	0	0	0	0	0	0
48	0	5,700±2,800	0	0	0	4±2	46±20	0	0	0	0
54	0	27,000±9,500	8±4	0	0	42±34	48±43	0	0	0	25±25
72	0	32,000±17,000‡	230±225	0	0	840±150	620±190	0	0	150±45	800±140

* Mean ± standard error (groups of four mice).

‡ Intestinal wall immediately adjacent to Peyer's patches, 24 ± 15 organisms.

This provides experimental proof that the organism can gain entry to the body when introduced only into the cecum and the large intestine. While inoculation studies involving laparotomies may be criticized on the grounds of trauma and possible peritonitis, the rectal inoculation method suffered from no such shortcomings. Though it bears no resemblance to the natural route of infection, rectal challenge has also been reported to be successful by Wagner (15), indicating that the lower bowel is susceptible to bacterial infection. However, whether orally ingested organisms normally enter the body via the large intestine must be open to doubt, since it was not possible to culture viable *Salmonella* from the lymph nodes draining the transverse and descending colon or rectum (Table III).

The ileum as the primary invasion site would also be more consistent with the thesis that secretory antibodies play a major role in the intestinal defense system. Clearly, the potential value of such antibodies in preventing mucosal invasion would be greatly diminished if the primary site of invasion involved the stomach or duodenum, since it would permit the pathogen to essentially bypass this defense mechanism. By the same token, vaccination procedures which stimulate IgA antibody production should be largely ineffectual in enhancing host resistance to enteric infection.

Though the lymphatic drainage of the intestine has been extensively studied from a physiological point of view, we know surprisingly little about the infectious pathway taken by microorganisms entering different portions of the gut wall. Dunn (16) described the location and general characteristics of the lymph nodes throughout the mouse but did not delineate their individual drainage areas. Sanders and Florey (17) also used vital dyes as a means for defining

various lymph nodes in the rat but, in general, drainage areas in the mouse have not been described in detail, although such data on the rat have been available for some time (18, 19). Adaptation of the dye injection method described by Hudack and McMaster (20) enabled us to delineate the drainage pathways for sequential segments of the gastrointestinal tract and to trace the dye passage back as far as the central lymphatic trunk.

Some of the nodes, particularly those draining the proximal duodenum and the transverse colon, were so small and obscure that they had to be routinely visualized by injecting the respective gut section with dye before the node could be dissected out for bacterial counts. The intestinal segments were often injected by way of a Peyer's patch or lymphoid nodule since these sites facilitated intramucosal inoculation. Almost immediately after the Peyer's patch had been injected with dye, the lymphatic capillaries in the surrounding intestinal tissue became visible and then the lacteals began to accumulate the dye. Since lymphatic capillaries do not possess valves, the dye flow would not necessarily indicate the normal direction of lymph drainage. From examination of many such preparations, it appeared that lymph from a particular area of intestinal mucosa probably drains through the regional Peyer's patch or lymphoid nodule into the lacteals and on into the mesenteric lymph nodes.

Peyer's patches probably serve as the primary collecting point for antigenic material coming across the gut mucosa. In germ-free mice and rats the Peyer's patches remain virtually invisible until the animal is exposed to orally introduced antigens when the Peyer's patches of the lower small intestine and cecum rapidly develop to the point where they become grossly visible (21, 22). The Peyer's patches of the distal ileum in the conventionally raised mouse is always considerably larger than those found in other sections of the gut, and since there is a striking increase in the size of these Peyer's patches when germ-free animals are conventionalized (23), it may be inferred that the antigenic stimulus (and presumably the bacterial invasion of the intestinal mucosa) is greatest in this area. The present study indicated that the ileal Peyer's patches serve as a collection point since bacterial counts for these organs were invariably higher than in the surrounding ileal gut wall (Table III). In absolute terms, the cecal patch usually contained fewer *Salmonella* than the three nearest patches in the terminal ileum. However, the bacterial counts in the lymph nodes draining the ileum and the cecum were roughly equivalent. This is accounted for by the fact that lymph from the ileal Peyer's patch nearest to the ileocecal valve drains into the same lymph node that is draining the cecum. While bacteria may be passing from the cecal mucosa directly to the lymph node without involving the cecal Peyer's patch, counts carried out on the cecal wall itself argue against this. The cecal Peyer's patch actually becomes infected at a time when the numbers of *Salmonella* within the cecal mucosa were decreasing and before detectable counts were obtained for the draining lymph node. Thus, the conclusion seems to be that small numbers of *Salmonella* enter the ileal mucosa within hours of chal-

lenge and rapidly pass to the local Peyer's patches via the lymphatic capillaries. It is still not clear whether these organisms multiply within the lamina propria to continuously seed the Peyer's patches or whether most of the growth occurs within the patch itself. However, there is no question that growth and inflammatory changes occur within the cecal wall in *Salmonella*-infected gnotobiotic mice (24) and this data is consistent with fluorescence studies using tagged *S. typhimurium* which localized within the mucosal walls of the cecum and the colon (4).

It is concluded from the present study that the distal ileum is the primary site of invasion in normal mice infected intragastrically with virulent *S. enteritidis*. There is, at present, no explanation for such a circumscribed area of infection, nor do we know the mechanism by which the immune host is able to prevent or limit the entry of intestinal pathogen into this tissue. Since it is now known that the bacteria must run the gauntlet of host defenses in the small intestine before reaching the primary site of invasion, vaccines which stimulate these defenses might afford protection by reducing the number of pathogens reaching the distal ileum. Protection might also be afforded the host through enhanced bactericidal properties of Peyer's patch cells since the Peyer's patch seems to be intimately involved in the establishment of the primary infection focus.

SUMMARY

This study followed the early pathogenesis of orally induced murine typhoid fever. Intragastrically administered *Salmonella enteritidis* moves quickly through the normal undisturbed gut so that only a small residuum remains in the cecum and large intestine after the first few hours. Dye injection of the gut wall was used to show that lymph from discrete portions of the gastrointestinal tract drains to separate lymph nodes, probably via the regional Peyer's patches. Plating techniques capable of detecting a single colony-forming unit of *S. enteritidis* within the different Peyer's patches and draining lymph nodes indicate that, although the cecum and large intestine are exposed to large numbers of *Salmonella* for longer time periods than the small intestine, the primary site of bacterial penetration involves the distal ileum. This area of the small intestine as well as the cecum are both drained by the distal mesenteric lymph nodes, and were the only nodes which contained detectable numbers of viable *Salmonella* over the first 24 h of infection. Neither the pyloric nor the proximal mesenteric lymph nodes (which drain the stomach and duodenum) nor the pancreatic and caudal lymph nodes (which drain the transverse and descending colon) contained viable *Salmonella*.

Salmonella were observed to infect the ileal mucosa and its Peyer's patches. With time, this infection progresses to the draining lymph node and ultimately reaches the liver and spleen. Some of the implications of these findings relative to the development of acquired resistance to enteric disease are discussed.

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