VI. Role of the Small Intestine in an Experimental Infection in Guinea Pigs

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ABSTRACT

FORMAL, Samuel B. (Walter Reed Army Institute, Washington, D.C.), G. D. ABRAMS, H. SCHNEIDER, AND H. SPRINZ. Experimental Shigella infections. VI. Role of the small intestine in an experimental infection in guinea pigs. J. Bacteriol. 85:119-125. 1963.—Shigella infection under the conditions of our experiment significantly involves the small intestine, producing lesions similar to those seen in human acute small intestinal dysentery. Previous work established that, for a rapidly fatal infection to occur, animals had to be pretreated by starvation or by administration of carbon tetrachloride, and, after oral challenge with S. flexneri, had to receive opium. Data are presented indicating that, in the guinea pig, the motility of the small intestine is a major defense mechanism in clearing bacteria from the gut. It is concluded that the two modes of pretreatment potentiate the ability of opium to decrease this motility, and hence increase susceptibility to enteric infection.

Although guinea pigs are normally resistant to enteric infection with dysentery bacilli, we have demonstrated that these animals, pretreated either by starvation or by a subcutaneous injection of carbon tetrachloride, suffer a fatal infection following oral administration of Shigella flexneri (Formal et al., 1958, 1959). To achieve this, it is also necessary to decrease intestinal motility with opium after challenge. Changes in the host responsible for this alteration in susceptibility are not clearly defined. One factor which has been implicated is an increase in sensitivity to endotoxin (Formal, Noyes, and Schneider, 1960). We also noted (Formal et al., 1959) that ability of the organisms to multiply in the small intestine might be another factor. The purpose of this work was to define the role of the small bowel in this experimental model, and to delineate further the essential changes in the host brought about by pretreatment.

MATERIALS AND METHODS

Cultures. S. flexneri 2a strain 2457 was employed, as in our previous work (Formal et al., 1958, 1959).

Infection of animals. Guinea pigs of the Hartley strain were used in all experiments. Procedures for pretreatment and oral challenge were similar to those previously described (Formal et al., 1958, 1959). When S. flexneri was administered directly into either the duodenum or the cecum, the peritoneal cavity was entered employing aseptic technique, and the desired area of the intestine exposed. The challenge organisms, suspended in 0.25 ml of Brain Heart Infusion (BHI) broth, were introduced into the lumen of the bowel by means of a syringe fitted with a 27-gauge needle, and the incision was closed with silk suture material. Deaths occurring in the 96-hr period after surgery were recorded.

Bacterial counts on the small intestine. Bacterial counts were determined for the entire small intestine, with the exception of a 4-cm segment of terminal ileum that was used for histological study. Animals were killed by a blow on the head. The small bowel was removed and ground in a mortar with sterile sand and saline. The volume was noted, and tenfold serial dilutions made. Appropriate dilutions were spread on the surface of nutrient agar and other suitable solid media, including MacConkey or Eosin Methylene Blue Agar for enumerating S. flexneri and Escherichia coli; LBS agar for lactobacilli; SF agar for enterococci; and 10% sheep blood agar for anaerobes. The nutrient, MacConkey, and blood agar plates were incubated both anaerobically and aerobically. The SF and LBS plates were incubated anaerobically and in candle jars.
Counts were determined and colonies stained with the Gram stain.

**Histopathological evaluation.** Segments of terminal ileum approximately 4 cm long were fixed in 10% neutral formalin and embedded in paraffin. In those experiments not involving ileal ligation, portions of cecum and colon were also secured and similarly processed. The degree of inflammation present was assessed in hematoxylin- and eosin-stained sections.

**RESULTS**

In the first series of experiments, various doses of *S. flexneri* were inoculated directly into either the duodenum or the cecum of normal, starved, or carbon tetrachloride-treated guinea pigs. The results (Table 1) show that the modified animals receiving challenge doses of $10^5$ to $10^8$ organisms in the duodenum died, whereas similarly treated animals receiving comparable numbers of organisms in the cecum survived. Thus, these data indicate that the small intestine is an important area of infection in this experimental model, serving as more than simply an avenue of entry of organisms into the cecum and colon.

The extent of multiplication of the challenge organism in the small intestine of pretreated, as compared with normal, animals was next determined. Groups of normal, starved, or carbon tetrachloride-injected animals were challenged *per os* with $10^5$ organisms, and killed 20 hr after challenge. The total number of viable *S. flexneri* in the small intestine was determined. In Table 2, it may be seen that small intestines of pretreated animals harbored significantly higher numbers of *S. flexneri* than did those of normal animals.

<table>
<thead>
<tr>
<th>Table 1. Deaths in normal, starved, or carbon tetrachloride-treated guinea pigs challenged with <em>Shigella flexneri</em> 2a in either the duodenum or the cecum.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Challenge dose</strong></td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>$10^8$</td>
</tr>
<tr>
<td>$10^7$</td>
</tr>
<tr>
<td>$10^6$</td>
</tr>
<tr>
<td>Broth</td>
</tr>
</tbody>
</table>

* Deaths/total.

The factors possibly responsible for this difference in growth of dysentery bacilli brought about by pretreatment of the host were then investigated. First, the influence of either starvation or carbon tetrachloride on the bacterial flora of the small intestine of unchallenged animals was studied in order to reveal any differences in the bacterial environment into which the dysentery bacilli would be introduced after pretreatment. In agreement with the work of Crecelius and Retter (1943), the flora of the small bowel was found to be simple, consisting for the most part of gram-positive rods and large gram-positive cocci which grew best under anaerobic conditions. The quantitative data are summarized in Table 3; we could detect no significant differences in flora of the three groups of animals. It may also be seen that the range of the counts varied greatly within each group.

Next, the possibility was considered that pretreatment with carbon tetrachloride or starvation might impair the ability of the small intestine to secrete some substance, present in normal animals, that would be inhibitory to the growth of *S. flexneri*. This was evaluated by comparing the growth of the organism in the small intestine of normal and pretreated animals subjected to ligation of the terminal ileum which, in effect, produced a closed tube for bacterial growth in the presence of any possible inhibitory substance. Ligation was accomplished by aseptic technique, with no significant interference with enteric blood supply. Immediately thereafter, the challenge dose of *S. flexneri* was given intraduodenally.
TABLE 3. Geometric mean counts of the predominant flora in the small intestine of normal, starved, and carbon tetrachloride-treated guinea pigs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gram-positive rods</th>
<th>Gram-positive cocci</th>
<th>Gram-negative rods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (9) ......</td>
<td>$5.5 \times 10^8 (2.3 \times 10^9-1.4 \times 10^9)$</td>
<td>$1.7 \times 10^9 (0-2.6 \times 10^9)$</td>
<td>$1 \times 10^8 (0-8.4 \times 10^8)$</td>
</tr>
<tr>
<td>Starved (8) ...</td>
<td>$1 \times 10^8 (3 \times 10^9-9.5 \times 10^9)$</td>
<td>$1.6 \times 10^9 (0-1.7 \times 10^9)$</td>
<td>$1.1 \times 10^8 (0-3.3 \times 10^9)$</td>
</tr>
<tr>
<td>CCl₄ (8) ... ...</td>
<td>$5.5 \times 10^8 (2.2 \times 10^9-7.4 \times 10^9)$</td>
<td>$9.1 \times 10^9 (0-5 \times 10^9)$</td>
<td>$0.8 \times 10^9 (0-5.5 \times 10^9)$</td>
</tr>
</tbody>
</table>

* Number of animals studied to compute geometric means given in parentheses.

* Range.

FIG. 1. Multiplication of Shigella flexneri 2a in the small intestine of normal, starved, and carbon tetrachloride-treated guinea pigs. The terminal ileum was ligated and the challenge inoculated into the duodenum. Each point represents the geometric mean of the counts obtained on two animals.

At various intervals after completion of the operation, animals were killed, and the total numbers of viable dysentery bacilli in the small intestine determined. The growth curves presented in Fig. 1 were constructed from these data. Each point represents the geometric mean count from two animals, and it can be seen that the organisms multiplied as well in normal as in pretreated guinea pigs. Histopathological evaluation likewise revealed no significant differences in the inflammatory reaction which developed in the three groups of animals. Thus, the small intestine of even the normal animal was found incapable of destroying or significantly inhibiting the growth of dysentery bacilli.

TABLE 4. Effect of opium on mortality and on number of Shigella flexneri 2a in the small intestine of carbon tetrachloride-treated or starved guinea pigs challenged per os with $1.2 \times 10^8$ organisms

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Carbon tetrachloride</th>
<th>Starvation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mortality*</td>
<td>Count†</td>
</tr>
<tr>
<td>Opium</td>
<td>9/12</td>
<td>$6.87 \times 10^8$ (8)</td>
</tr>
<tr>
<td>Saline</td>
<td>0/10</td>
<td>$6.38 \times 10^8$ (8)</td>
</tr>
</tbody>
</table>

* Deaths/total.
† Geometric mean of the number of viable $S$. flexneri 2a in the small intestine 20 hr after challenge.
§ Tincture of opium (1 ml) intraperitoneally 1 hr after challenge.
∥ Number of animals studied to compute bacterial count given in parentheses.

The role of intestinal motility in this experimental infection was next examined. As noted previously, administration of opium, which decreases the motility of the bowel, is necessary to achieve a fatal infection. In this series of experiments, bacterial counts and histopathological changes, as well as mortality rates, were compared in opiated and nonopiated animals, all of which had been pretreated by starvation or with carbon tetrachloride. If opium is not injected after oral challenge, the animals survive, and the number of viable dysentery bacilli in the small intestine 20 hr after challenge is significantly lower than when opium is employed (Table 4). Furthermore, microscopic examination of a portion of ileum from each of the animals in this series revealed inflammatory changes paralleling the bacterial counts. Thus, in the group of animals not treated with opium, the ileal mucosa
had a normal appearance, or, at most, showed patchy vascular congestion and slight focal leukocytic infiltration (Fig. 2). In contrast to this, in the group treated with opium after challenge, striking alterations of the mucosa were evident (Fig. 3). Blood vessels within the lamina propria were dilated and congested, and the edematous stroma was infiltrated by leukocytes. Within the epithelium, goblet cells normally containing abundant mucin discharged their content. The epithelium was flattened, and various degenerative changes were present, with formation of occasional pinpoint ulcers in areas of most severe damage. These changes were further reflected in the distortion of villi. Mucopurulent exudate and occasional traces of blood were present in the intestinal lumen. Even in the presence of severe inflammation in the ileum, corresponding changes in the cecum and colon were of irregular occurrence. Frequently, there is little, if any, inflammation in these latter areas, and generally, if present, it is milder than the process in the small intestine.

The fact that even pretreated animals must be given opium in order for a significant infection to ensue, combined with the above observation that even normal, nonpretreated animals will develop significant bacterial counts and inflammatory changes in the presence of mechanical obstruction of the intestine, points to the paramount importance of intestinal motility in this infection. Thus, in the attempt to explain the potentiating effects of starvation or of carbon tetrachloride in this model, the effect of these modes of pretreatment upon the response of the animal to opium was next examined. BHI broth (10 ml) containing 0.03 g of Pontamine sky blue dye was fed to normal, starved, and carbon tetrachloride-treated guinea pigs; 30 to 60 min after the dye was fed, 1 ml of tincture of opium was injected intraperitoneally. Animals were sacrificed at various intervals, and their small intestines examined for the presence of dye. The results of this study are summarized in Table 5; pretreated animals retained the dye approximately 10 hr longer than did normal animals.

FIG. 2. Ileum of carbon tetrachloride-treated guinea pig challenged with Shigella flexneri, but not injected with opium. The villi are slender and the epithelium is regular with scattered, lightly stained goblet cells. The lamina propria is essentially normal. Hematoxylin and eosin. Original magnification, X 144.
FIG. 3. Ileum of carbon tetrachloride-treated guinea pig challenged with Shigella flexneri and injected with opium. Villi are swollen and distorted. The congested, edematous lamina propria is infiltrated by leukocytes. The epithelium is altered, and goblet cells have discharged their mucus. Clumps of exudate are present in the lumen. Hematoxylin and eosin. Original magnification, × 144.

TABLE 5. Retention of Pontamine sky blue dye in the small intestine of normal, starved, or carbon tetrachloride-treated guinea pigs injected with tincture of opium

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Time (hr) after feeding dye&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Normal</td>
<td>2/2</td>
</tr>
<tr>
<td>Starved</td>
<td>2/2</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>4/4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Pontamine sky blue (0.03 g) suspended in 10 ml of Brain Heart Infusion broth.

<sup>b</sup> Number with detectable dye in small intestine/total examined.

<sup>c</sup> At 16 hr the presence of dye in the small intestines of normal animals was doubtful.

DISCUSSION

The present study demonstrates clearly that the small intestine is intimately involved in this experimental infection with S. flexneri. Most animals challenged per os as outlined above develop an acute, rapidly fatal infection, and, when examined within 24 hr of challenge, show severe inflammatory changes in the small intestine, with lesser or no alterations in the cecum and colon. The results of intraduodenal, as compared with intracecal, challenge with dysentery bacilli likewise emphasize the important role of the small intestine in this model. In some respects, injection of a given number of organisms into the duodenum with its sparse flora is not comparable to similar injection into the cecum with its many grams of living bacteria. However, the lethality of the former mode of challenge, as contrasted to the results with the latter, indicates that mere delivery of organisms to the cecum is not sufficient, and that the small intestine serves as far more than simply a conduit for the dysentery bacilli.

The finding that dysentery bacilli are 1,000-fold more numerous in the small intestine of pretreated, as compared with normal, animals 20 hr after challenge points to a marked change
in host defenses brought about by starvation or carbon tetrachloride. That these forms of pretreatment do not act by depressing the secretion or activity of some substance ordinarily destructive of bacteria is suggested by the fact that, in the ligated small intestine, dysentery bacilli grow as well in normal as in pretreated animals. In a study in rats, Dixon (1960) similarly concluded that the small intestine does not clear itself of organisms by bactericidal activity to any significant extent.

The study of Shigella infections in germ-free animals (Formal et al., 1961) and in antibiotic-treated animals (Freter, 1956) suggests that the normal bacterial flora of the intestinal tract acts as a defense against infection. We were not able in the present study, however, to assign a definite role to changes brought about in the pre-existing bacterial flora of the small intestine by carbon tetrachloride or starvation, since we could detect no consistent differences between pretreated and normal animals. However, our data, in agreement with those of Crecelius and Rettger (1943), emphasize the variability of the flora of this region. Thus, it is not possible to state that, in the few animals that fail to develop the rapidly fatal infection, the small intestinal flora did not have a protective effect.

The results of the present study serve mainly to emphasize that the motility of the small intestine constitutes a major defense against the establishment of significant infection with S. flexneri. Unless opium is administered to them shortly after challenge, even animals pretreated by starvation or carbon tetrachloride survive challenge with little or no histological alteration in the small intestine, and virtual elimination of dysentery bacilli from the small intestine within 1 day. Conversely, when mechanical obstruction of outflow of the small intestine is substituted, as in the ligation experiments, for the pharmacological impairment effected by opium, significant infection ensues even in nonpretreated animals. These data are in agreement with those of Dixon (1960), who concluded that the small intestine clears itself of organisms entering it largely by mechanical, i.e., peristaltic, means.

Finally, the fact that pretreated animals given opium retained a nonabsorbable dye marker in the small intestine approximately 10 hr longer than did normal animals demonstrates that starvation and carbon tetrachloride enhance the ability of opium to depress motility of the small bowel. It is felt that this longer period of opium-induced depression of motility of the bowel in pretreated, as contrasted to normal, animals allows multiplication of dysentery bacilli in the small intestine, and accounts, in large part, for the susceptibility of the starved or carbon tetrachloride-treated guinea pig to acute shigellosis.

This experimental model emphasizes the dominant involvement of the small intestine. The role of the small intestine in human bacillary dysentery is commonly overlooked, since the most familiar lesions are those noted during necropsy study of late or chronic stages of the disease, in which colonic involvement supervenes.

A more acute, small intestinal form of human bacillary dysentery, with changes paralleling those detailed in the present study, is, however, well documented (Letterer, 1944). The Ekiri syndrome described in Japanese children may represent a similar acute infection of the small intestine with dysentery bacilli (Ogasawara, 1955). Of interest is the fact that a predominantly colonic form of the disease has also been noted in guinea pigs which succumb with diarrhea much later than the more usual 24- to 48-hr period (Formal et al., 1958). Thus, the distribution of lesions observed in humans and guinea pigs appears to be a function of the stage of the disease studied. However, the precise factors that determine the sequence of events in a particular case, or the differences in vulnerability of one part of the bowel or another to the effects of infection, are as yet largely unknown. This is particularly true of our knowledge of the more usual, i.e., nonfatal, human cases in which the pattern of evolution of the disease might differ from that in those hyperacute cases terminating fatally. An intestinal biopsy study similar to that carried out in cholera patients by Gangarosa et al. (1960) would be of value in clarifying these points.

LITERATURE CITED


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