EXPERIMENTAL SHIGELLA INFECTIONS: CHARACTERISTICS OF A FATAL INFECTION PRODUCED IN GUINEA PIGS[1]

SAMUEL B. FORMAL, GUSTAVE J. DAMMIN, E. H. LABREC, AND H. SCHNEIDER

Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C., and the Departments of Pathology, Harvard Medical School and the Peter Bent Brigham Hospital, Boston, Massachusetts

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As long as man, the chimpanzee and the monkey are the only species in which enteric shigella infections can be studied, progress in problems concerning the pathogenesis of and immunity to bacillary dysentery will be seriously hampered. In recent years, successful attempts have been made to modify small laboratory animals in such ways as to make them susceptible to orally administered enteric pathogens. Thus Freter (1955) and Lowenthal (1953, *personal communication*), working independently, employed virtually identical methods to produce fatal infections in guinea pigs with orally administered cultures of *Vibrio comma*. The procedure involved starving the animal for four days and administering calcium carbonate prior to, and opium following, oral challenge. To modify the normal intestinal flora further, Freter treated his animals with streptomycin and used a streptomycin-resistant culture for challenge. Lowenthal found, however, that with the bacterial cultures and animals which he employed, treatment with streptomycin did not render the animals more susceptible. More recently Freter (1956) has described a long-term, nonfatal infection with either *V. comma* or *Shigella flexneri* 2a in guinea pigs which had been both (a) starved for 36 of the 48 hr prior to challenge, and (b) treated with antibiotics to alter the normal intestinal flora. No examination for intestinal lesions is mentioned in this report.

Using Lowenthal's method, we have produced a fatal infection in guinea pigs with an orally administered suspension of *S. flexneri* 2a. This communication describes some of the characteristics of this infection.

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MATERIALS AND METHODS

Cultures. S. flexneri 2a strain 2457 was isolated by Lt. Col. Oscar Felsenfeld in Tokyo, Japan, and was sent to us in the fall of 1954. It produces mucinase (Formal and Lowenthal, 1956) *in vitro* and is both serologically and biochemically a typical culture of *S. flexneri*. The culture has been maintained in the lyophilized state. In order to reduce variability from test to test, a new ampule was opened for each experiment.

Escherichia coli strains WS, HS, and EL were isolated from healthy human beings.

Guinea pigs. Hartley strain guinea pigs weighing 300 to 400 g were used in this work.

Infection of animals. Animals were deprived of food for 4 days but allowed water. They were then given, by stomach tube, 125 mg calcium carbonate suspended in 5 ml distilled water. After 3 hr, a known number of challenge organisms suspended in 10 ml brain-heart infusion broth was administered through a stomach tube. Tincture of opium, 1 ml, was inoculated intraperitoneally 1 hr after the culture was fed. The animals were then allowed food.

RESULTS

The results of an experiment to estimate the LD_{50} for Hartley strain guinea pigs are given in table 1. Since it was necessary to use small groups in such tests, this value is subject to wide error. Thus, in a series of experiments in which Hartley strain animals were orally challenged with doses ranging from 8×10^5 to 1.4×10^7 cells, variation in the percentage mortality was observed (table 2). Until the factors which determine the virulence of the infecting organism and the susceptibility of the host are better understood and controlled, variability will be inherent in the technique.

Animals which succumbed following oral chal-

TABLE 1

*Titration to determine the LD_{50} of Shigella flexneri 2a strain 2457, for Hartley strain guinea pigs**

Challenge Dose,† No. of Cells	Deaths/Total	LD_{50}‡
1.4×10^8	5/5	
2.7×10^7	3/5	
5.4×10^6	4/5	2.5×10^6
1.1×10^6	2/4	
3.0×10^5	0/4	
1.4×10^8 heat-killed organisms	0/4	

* Animals were starved for 4 days and received calcium carbonate prior to and tincture of opium following challenge.

† Challenge administered by the oral route.

‡ LD_{50} calculated by the Reed-Muench method.

TABLE 2

*Percentage mortality observed on different occasions in Hartley strain guinea pigs following the oral administration of Shigella flexneri 2a strain 2457**

Expt No.	Challenge Dose, No. of Cells	Deaths/ Total	Mortality
			%
1	1.4×10^7	10/24	38
2	1.2×10^7	2/5	40
3	9.6×10^6	2/5	40
4	1.0×10^7	4/5	80
5	6.1×10^6	2/5	40
6	8.1×10^6	3/10	30
7	1.1×10^7	2/5	40
8	6.2×10^6	0/5	0
9	6.5×10^6	3/5	60
10	8.0×10^5	11/14	79

* Animals were starved for 4 days and received calcium carbonate prior to and opium following challenge.

lenges in excess of 2×10^7 cells did so in 24 to 48 hr after the organisms were fed. No evidence of diarrhea was noted in these guinea pigs. However, when fewer than 10^7 cells of *S. flexneri* were administered some of the animals which died did so 72 to 96 hr after challenge. Diarrhea was observed in certain of these guinea pigs. Thus in experiment number 10 (table 2), 5 of the 11 animals which succumbed did so 72 to 96 hr following the oral administration of 8×10^5 cells of *S. flexneri* 2a, and 4 of these developed diarrhea before death.

The specific pathogenicity of *S. flexneri* 2a strain 2457, was demonstrated by several experiments carried out to compare pathogenicity of this strain with that of a limited number of strains of *Escherichia coli*. The latter caused neither intestinal lesions nor fatal enteric infections under the experimental conditions employed (table 3). The two deaths which occurred following the administration of *E. coli* strains WS and HS were, as far as could be ascertained, not related to the introduction of *E. coli* and no intestinal lesions were noted. The lesions observed in those animals receiving the *S. flexneri* 2a, are described below.

An experiment was next conducted to determine the distribution of *S. flexneri* in the intestinal tract at various intervals following a LD_{20} challenge. Groups of animals were sacrificed 1, 6, 24, 48 and 96 hr after the feeding. Portions of the proximal and distal small intestine, the caecum, and the proximal and distal large intestine were removed, weighed, ground in a mortar containing sterile sand and saline, and the number of shigella present determined by plating on SS agar. The findings (table 4) indicate that in one hour the organisms had reached the upper colon and by 6 hr could be found throughout the intestinal tract. By 24 hr, organisms could no longer be found in the proximal small intestine of some animals and by 48 hr were absent from this region in all animals tested. At this time the organism had also been cleared from the distal ileum of some animals. Organisms were not found in the small intestines of animals 72 hr post-challenge, except in one guinea pig which was moribund. In this animal, shigella were present throughout the

TABLE 3

*Susceptibility of Hartley strain guinea pigs to oral administration of cultures of Escherichia coli and Shigella flexneri**

Species and Strain	Avg Challenge Dose	Deaths/Total
E. coli WS	10^8	1/18
E. coli HS	10^8	1/14
E. coli EL	10^8	0/14
S. flexneri 2a	10^7	14/22

* Data pooled from 2 experiments.

TABLE 4

*Distribution of Shigella flexneri 2a in the intestinal tract of guinea pigs following the oral administration of a LD_{20} challenge**

Hr after Feeding	Upper Ileum		Lower Ileum		Caecum		Upper Colon		Lower Colon	
	No. positive/ total	Avg count per g material	No. positive/ total	Avg count per g materia	No. positive/ total	Avg count per g material	No. positive/ total	Avg count per g material	No. positive/ total	Avg count per g material
1	4/4	1.8×10^5	2/4	1.3×10^5	3/4	4.8×10^4	3/4	6.3×10^3	0/4	$<10^2$
6	4/5	2.3×10^6	5/5	3.9×10^6	5/5	7.5×10^3	4/5	1.7×10^5	5/5	2.7×10^4
24	2/5	3.8×10^4	4/5	8.0×10^6	5/5	7.7×10^6	4/5	1.8×10^7	5/5	1.5×10^6
48	0/5	$<10^2$	2/5	1.2×10^5	5/5	4×10^4	5/5	4.8×10^6	4/5	7.4×10^6
72	1†/5	4.8×10^5	1†/5	5.7×10^4	2/5	1.5×10^5	2/5	1.7×10^7	3/5	1.7×10^7
92	0/3	$<10^2$	0/3	$<10^2$	1/3	3×10^3	1/3	1×10^4	0/3	$<10^2$

* Animals were fed 10^6 cells; one of five control animals fed this dose died.

† Animal was moribund at time bacterial counts were made.

TABLE 5

*Distribution of Shigella flexneri 2a in the intestinal tract of guinea pigs following the oral administration of a LD_{40} challenge**

Hr after Feeding	Upper Ileum		Lower Ileum		Caecum		Upper Colon		Lower Colon	
	No. positive/ total	Avg count per g material	No. positive/ total	Avg count per g material	No. positive/ total	Avg count per g material	No. positive/ total	Avg count per g material	No. positive/ total	Avg count per g material
6	4/4	2.9×10^5	4/4	4.3×10^6	4/4	4.2×10^5	3/4	2.6×10^4	0/4	—
24	4/4	1.3×10^6	4/4	6.5×10^7	4/4	3.8×10^6	4/4	5.6×10^7	2/4	2.3×10^5

* Animals were fed 3×10^7 cells; 4 out of 5 control animals fed this dose died.

length of the intestinal tract. By 96 hr, the challenge strain was virtually cleared from the alimentary canal of all surviving animals.

Another experiment similar to the one above was performed in which a LD_{80} challenge was administered. Bacterial counts were made only at 6 and 24 hr after the bacteria were fed since most of the animals had succumbed within 48 hr. The data summarized in table 5 differ from the previous experiment in two respects. At 6 hr dysentery bacilli had not reached the distal large intestine in any of the animals, while in the previous test all animals were positive; and at 24 hr all animals fed the LD_{80} challenge still harbored dysentery bacilli in the proximal small intestine, while organisms were found in this region in only two of five guinea pigs receiving a LD_{20} dose. In animals succumbing to the infection, *S. flexneri* was found only in the large and small intestine and was not isolated from the kidney, heart, liver, spleen, gall bladder, lungs, or blood.

Pathological studies were made on material from (a) normal animals, (b) animals that were only starved for 4 days, (c) animals that were starved, given calcium carbonate, heat-killed bacilli and opium, and (d) on animals succumbing to infection after receiving similar treatment and oral challenge with living *S. flexneri*. The only consistent change produced by the four-day starvation period was a fatty metamorphosis of the liver. When calcium carbonate, heat-killed bacteria and opium were administered to starved guinea pigs, few further changes were noted. In some animals, however, small isolated areas of focal necrosis were seen in the liver. The submucosa of the intestinal tract was congested and hyperemic, while there were indications of lymphoid hyperplasia in the lower ileum, caecum and colon. The surface epithelium and lamina propria were normal and without evidence of leukocytic infiltration.

In animals succumbing after receiving live *S. flexneri* 2a, lesions of the intestinal mucosa were seen in the caecum and the colon. These lesions consisted of isolated areas of ulceration of the mucosa, hemorrhage and infiltration with inflam-

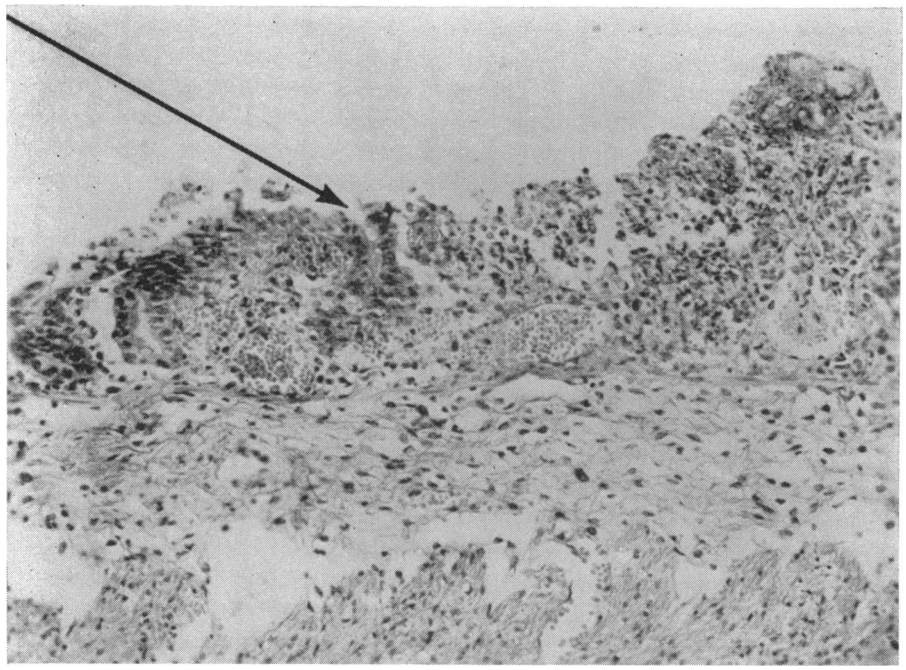

Figure 1. Animal received 10^7 living *Shigella flexneri* and succumbed 48 hr after infection. Ulceration and hemorrhage of the mucosa of the colon. At the right there is no glandular epithelium identifiable. Partially involved glandular epithelium is seen at the left (see arrows) (hematoxylin and eosin; original magnification × 160).

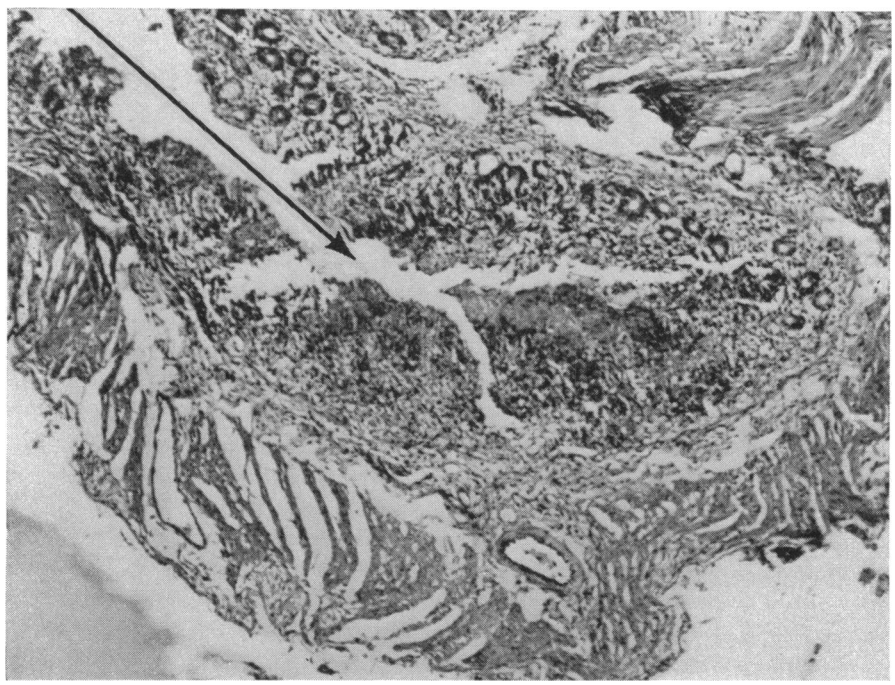

Figure 2. Animal received 6×10^7 living *Shigella flexneri* and succumbed 48 hr after infection. Ulceration of the colon. There is involvement of the entire mucosa below the lumen of the bowel and of the superficial portion of the mucosa above the lumen (hematoxylin and eosin; original magnification × 52). Lumen indicated by arrow.

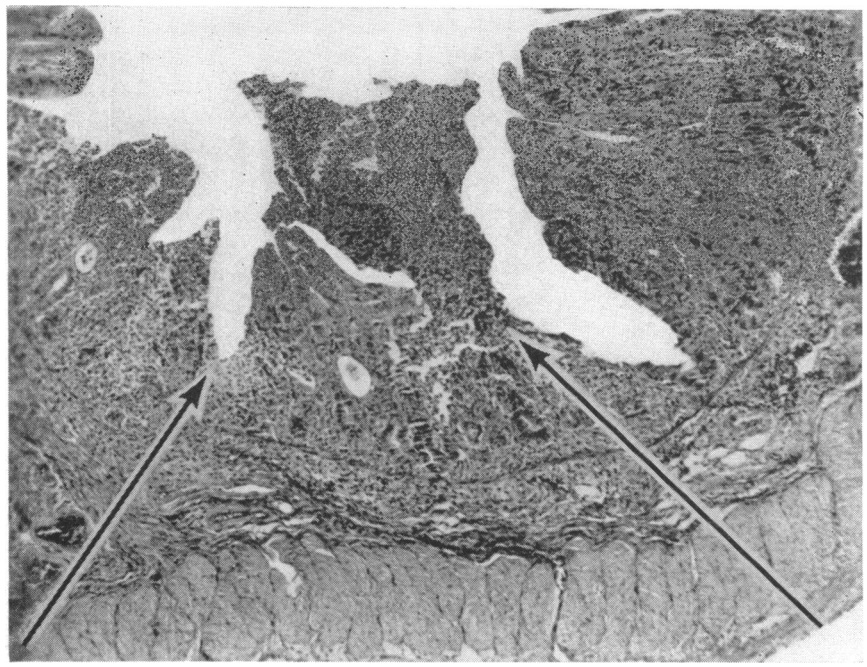

Figure 3. Animal received 7×10^5 living *Shigella flexneri* and succumbed 96 hr after infection. Multiple sites (see arrows) of ulceration with cellular exudate on the mucosal surface in the midportion of the field. The cellular inflammatory reaction extends through the muscularis mucosae into the submucosa (hemotoxylin and eosin; original magnification × 52).

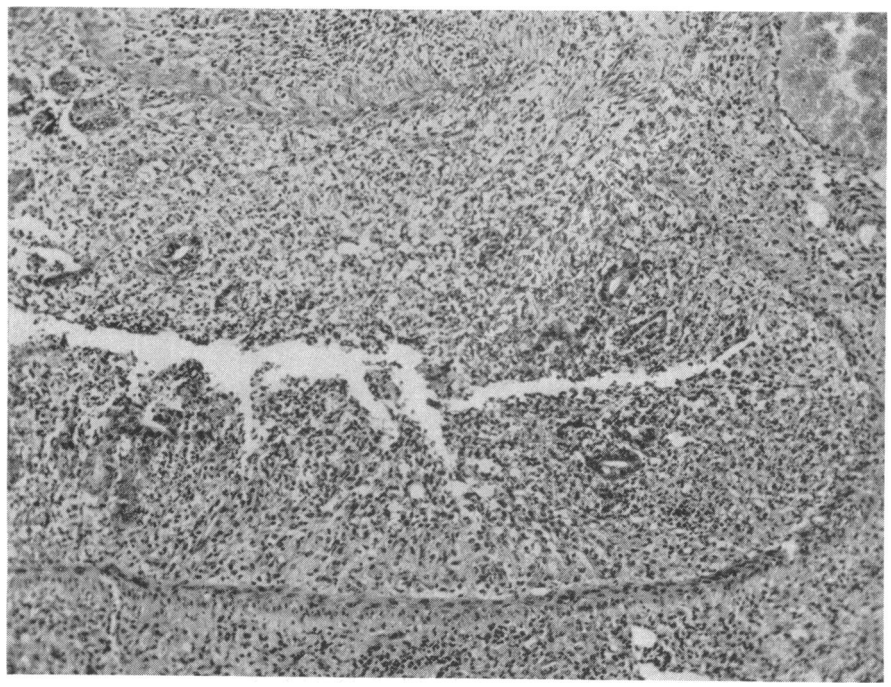

Figure 4. Animal received 5×10^7 *Shigella flexneri* and succumbed 72 hr after infection. There are necrosis and ulceration of the superficial portion of the mucosa of the colon. Only deeper portions of the glands are recognizable. The muscularis mucosae is intact but the cellular inflammatory reaction extends into the submucosa (hematoxylin and eosin; original magnification × 72).

matory cells. The inflammatory reaction was usually seen throughout the lamina propia and at times extended through the muscularis mucosae into the submucosa and the submucosal lymphoid tissue. Figures 1 to 4 illustrate these lesions. They resemble the lesions observed in Shigella infections in man, but are less diffuse and there is less fibrin in the exudate.

The liver cells of infected animals exhibited fatty metamorphosis, whereas the mesenteric lymph nodes displayed focal hyperplasia and dilated sinusoids. The spleen showed a prominant red pulp, lymphoid hyperplasia and congestion. No lesions were noted consistently in the kidney, pancreas, adrenals, heart, or lungs.

DISCUSSION

Including the present study, there are now reports that both *S. flexneri* and *V. comma* can cause either a long-term nonfatal or a short-term fatal enteric infection in guinea pigs (Freter, 1955; 1956). Whether the outcome of the infection is nonfatal or fatal depends, in large part, upon the pretreatment which the animals receive. Starvation for 36 of the 48 hr previous to challenge plus treatment with streptomycin, erythromycin, and nystatin results in a long-term nonfatal infection. On the other hand, depriving the animals of food for the 4 days previous to challenge without recourse to antibiotic treatment renders them susceptible to a short-term fatal infection (Lowenthal, 1953, *personal communication*).

The pretreatment the animals received is presumed to cause an alteration of the normal enteric flora which is considered capable of inhibiting the multiplication of potential pathogens introduced into the intestinal tract. Starvation and antibiotics are known to accomplish this. However, following starvation of 4 days the animal becomes susceptible to a fatal infection, while by the other regimen, apparently only multiplication of the invading organisms in the intestinal tract occurs. Thus one must assume either that the 4-day period of starvation is much more effective in altering the normal intestinal flora than is the procedure employing antibiotics, or that, in addition to affecting the enteric flora, starvation alters other host defense mechanisms. The factors which render animals susceptible to the fatal infection are now under study.

In addition to the fact that the method of preparation of the guinea pigs used in this study resulted in rendering them susceptible to a fatal infection with *S. flexneri*, our results differ from Freter's (1956) recent findings in two further respects. Most of our animals surviving a challenge cleared the invading organisms from the alimentary canal within 96 hr, while in Freter's study, dysentery bacilli persisted for at least two weeks. It is possible that the use of antibiotics results in a longer lasting alteration of the fecal flora than does starving alone and thus permits a longer period of multiplication by the dysentery bacilli. A second difference appears to be the presence of ulcerative lesions in the colon in our experiments, whereas no intestinal lesions were reported by Freter.

The infection with *S. flexneri* described, resembles that in man in that the tissue response is limited to the colon. The lesions produced in the large intestine in the guinea pig, while not as diffuse as those observed in man, demonstrate an essentially similar reaction in the experimental animal to orally administered *S. flexneri*. In addition, the pathogenicity of the dysentery bacilli is indicated by the inability of large numbers of viable *E. coli* to cause a fatal infection. It thus is possible that this method of enteric infection makes available a laboratory model for future studies concerning immunity to and pathogenesis of bacillary dysentery.

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SUMMARY

A fatal enteric infection with ulcerative lesions in the colon has been established in guinea pigs with a strain of *Shigella flexneri* 2a. To accomplish this, it was first necessary to deprive the animals of food for four days and to administer calcium carbonate before and opium following the challenge suspension. Animals receiving this treatment succumb following oral challenges of *S. flexneri* 2a (LD_{50} approximately 10^6 to 10^7 bacteria) but survive doses in excess of 10^8 cells of *Escherichia coli*. Some characteristics of the infection are described and an account of the intestinal lesions in animals succumbing to the infection is given.

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