Histopathology of the Upper Small Intestines in Typhoid Fever

Biopsy Study of Experimental Disease in Man

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THE RECOGNITION of the role of the small bowel as a target organ in typhoid fever goes back to the beginning of the 19th century. Special attention has been paid, ever since, to the morphologic alteration of the lymphatic nodules in the intestinal mucosa, in particular to the changes in Peyer's patches. In contrast, the histologic changes of the mucosa proper are still a largely unexplored aspect of the disease. One of the factors responsible for this state of affairs is the exclusive host specificity of man for *Salmonella typhosa.* Of all the animals tested so far in the laboratory, only the anthropoid apes respond to an oral typhoid infection with tissue changes akin to those seen in typhoid fever in man. This has resulted in almost complete reliance on human necropsy findings for the classic and standard descriptions of histologic changes of the gut in typhoid fever. Unfortunately, intestinal tissues are most prone to postmortem autolysis, a process defying a detailed analysis of mucosal alterations. More importantly, death in typhoid has to be considered a complication, usually occurring late in the disease, and findings obtained at autopsy do not necessarily reflect the lesions present in the early phases of the disease.

At present, an indication of the dynamic morphologic events can be gained by examining intestinal mucosal biopsies as infections proceed in the human subject. However, since intestinal biopsy technics are applied mainly in countries with a low incidence of typhoid, such cases have only rarely been studied, and these infrequent observations on randomly obtained specimens could not answer questions of the pathogenesis and histopathology of the early intestinal lesions in typhoid fever. It is the purpose of the present study to furnish additional information in this area.

This investigation was a by-product of a long-term study conducted in hu-

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This study was made possible through the wholehearted collaboration of the staff of the Maryland State House of Correction. The voluntary participation of the 6 inmates in this investigation is especially recognized.

mans; the primary purpose was to evaluate the role of vaccines in typhoid fever. The subjects of this study were 6 healthy male volunteers, between the ages of 29 and 48 years, inmates of the Maryland State House of Correction; all freely volunteered to participate in the study after its purpose and possible hazards had been explained.

The men received an oral challenge of 1.3×10^9 organisms of an 18-hr. culture of *Sal. typhosa*. The strain had originally been isolated from a human carrier. The challenge was given in milk after a 3-hr. fast. No medication was given until clinical illness developed. Chloramphenicol was then administered in a dose of 3 gm. immediately, followed by 1 gm. every 8 hr. The possibility that the intestinal changes which occurred may have been caused by this drug was ruled out by the study of 3 additional healthy subjects who received chloramphenicol alone in comparable doses for a longer period. Their intestinal biopsies were normal.

Biopsy specimens were obtained by the method of Crosby and Kugler,[1] using their instrument. The procedure was well tolerated and there were no ill effects. Tissue was obtained in each man prior to challenge, shortly after challenge or as soon after defervescence as the patient's condition permitted, and during convalescence.

A brief case report and morphologic findings in the biopsies of each subject follow:

CASE REPORTS

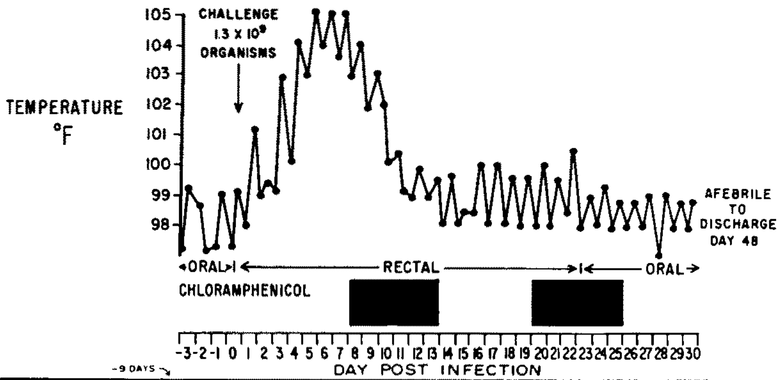
Subject 1 (R.S.)

This subject was a 42-year-old healthy Caucasian male. On the fifth day after the oral challenge, he complained of severe headache, chills, and abdominal pain. His temperature rose progressively to levels between 101° and 104° F. and remained in this range for 5 days. Stool and blood cultures were positive for *Sal. typhosa*. Treatment with chloramphenicol resulted in a gradual defervescence and eventual recovery. The subject's initial clinical course is summarized in Fig. 1.

Findings in the base-line biopsy, obtained 9 days prior to challenge, were entirely normal. The tunica propria contained a normal complement of plasma cells, monocytes, lymphocytes, mast cells, and occasional cellular debris.

The next biopsy was obtained 3 days following the ingestion of 1.3×10^9 of viable *Sal. typhosa*. At that time the individual had no subjective symptoms, the blood culture was sterile, and the temperature normal. Histologic examination of this specimen (Fig. 2 and 3) revealed an enteritis which was characterized by focal granulomatous lesions, in addition to a moderate degree of hyperemia and alterations of the intestinal epithelial lining. The cellular infiltrate was of a mixed type. It was more compact in the nonvillous and more basal portion of the mucosa but extended into the villi. The predominant cell type was the mononuclear histiocyte. There were also plasma cells, lymphocytes, and a few polymorphonuclear leukocytes. Eosinophilic leukocytes as well as tissue mast cells were moderately increased. The interstices, which were relatively clear in the base-line biopsy, were now filled with a delicate amorphous debris. There was a significant increase in the transmigration of predominantly mononuclear cells but also of occasional eosinophils through the epithelial lining. Crypt glands were slightly elongated, and their closely packed cells contained hyperchromatic nuclei. The crypts were also slightly dilated and largely empty. Extrusion of epithelial cells was not

only more prominent at the villus tip but was also noted toward the base of villi. Epithelial cells lining the villi showed a greater degree of variation in size and shape than was present in the initial biopsy, and in areas, the usually high columnar epithelium had become more flattened, almost cuboidal. The biopsy specimen included fragments of submucosa adhering



INTESTINAL BIOPSIES		■ ■ ■
WEIGHT POUNDS		145 142 145 141 139 141 144 144 144
CULTURES	BLOOD	0 0 + + + + + + 0 0 0
	STOOL	+ + + 0 + 0 + + + + 0 0 0 + 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	WBC	9250 9400 10900 11850 9650 5700 4550 3500 5750 4100 9500 9050 8000 6050 5100
ANTIBODY TITERS	O	40 40 40 1280 1280 1280 1280 1280 1280 2560
	H	80 40 80 640 2560 2560 5120 5120 5120 5120

U.S. Army photograph

Fig. 1. Clinical course, Subject R.S., after challenge with *Sal. typhosa.*

to the muscularis mucosa and showing minimal histologic changes. The inflammatory process was largely confined to the mucosa proper. It stopped short at the muscularis mucosa.

The third and last biopsy in this case was obtained after defervescence, 18 days after challenge, and prior to the start of a second course of chloramphenicol therapy. At this time the small intestinal mucosa showed some residual enteritis manifested by moderate hypercellularity of the tunica propria. The infiltrate was composed predominantly of monocytes, plasma cells, and lymphocytes. In comparison with the previous biopsy, both the numbers and the variety of cell types were greatly reduced, especially the numbers of large monocytes and large plasma cells. In comparison with the base-line biopsy, there was still an increased rate of transmigration of lymphocytes through the epithelial lining and some fine dust-like precipitate and cellular debris in the interstices of the tunica. While the number of mast cells was average, eosinophilic and neutrophilic leukocytes had again become exceptionally rare. The intestinal epithelial lining was regular.

Subject 2 (W.M.)

This subject was a 37-year-old Caucasian male who became ill 3 days after oral challenge with *Sal. typhosa.* He showed the following symptoms: headache, chills, abdominal pain, rose spots, and severe prostration. His temperature rose to 105° F. and remained elevated near this level for 4 days. Blood cultures and stool cultures were positive for *Sal. typhosa.* The clinical course is depicted in Fig. 4.

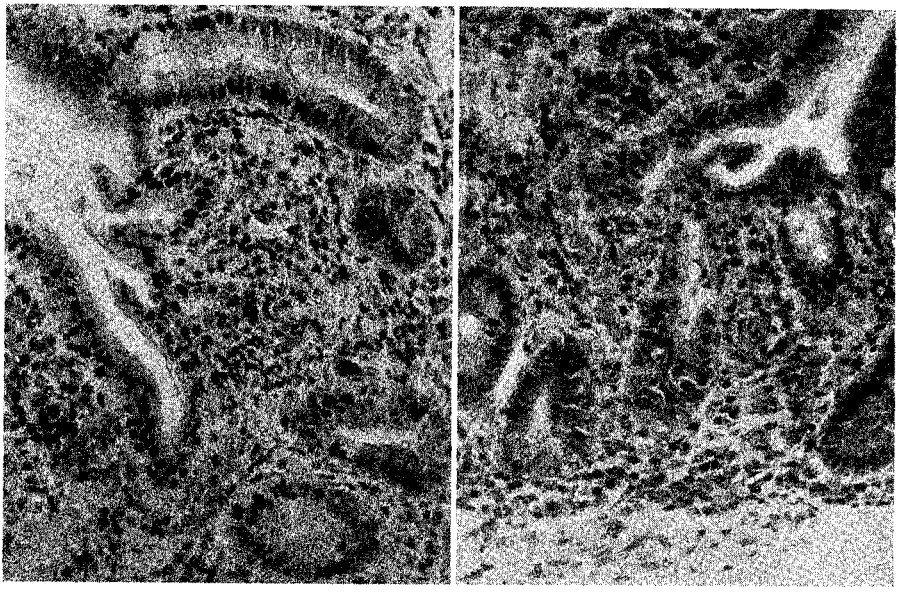
The biopsy obtained prior to the initiation of infection showed essentially regular findings.

The next biopsy was obtained 11 days following challenge and 4 days following the insti-

tution of chloramphenicol therapy. The temperature had fallen to 100° F.; the white blood cell count was 3500. Blood culture had been negative for 3 days, but the stool culture was still positive.

At this time the inflammatory reaction was essentially similar to that noted in the post-challenge specimen of the previous patient. There was a granulomatous enteritis with focal accumulations of swollen monocytic cells and minute areas of necrosis. Degenerative changes were noted in the crypt glands as well as in the epithelial lining of the villi. The epithelial cells showed great variability and irregularity. A distinct thinning of the epithelium had occurred in some areas near the crypt-villus junction, and there was evidence of precipitous extrusion of epithelial cells and the formation of occasional microulcers. The transmigration of predominantly lymphocytes was distinctly increased. The inflammatory infiltrate stopped short at the muscularis mucosa, and the underlying submucosa was relatively free of inflammatory cells.

The biopsy during convalescence was obtained 26 days after oral challenge with typhoid bacilli. The findings were essentially similar to those noted in the prechallenge biopsy. There was a slight over-all increase in cellularity of the tunica propria. Clusters of large plasma cells were conspicuous. Swollen monocytes were greatly reduced in number when compared with those in the postchallenge biopsy. The granulomatous inflammatory reaction had disappeared. Eosinophils and mast cells were rarely seen.

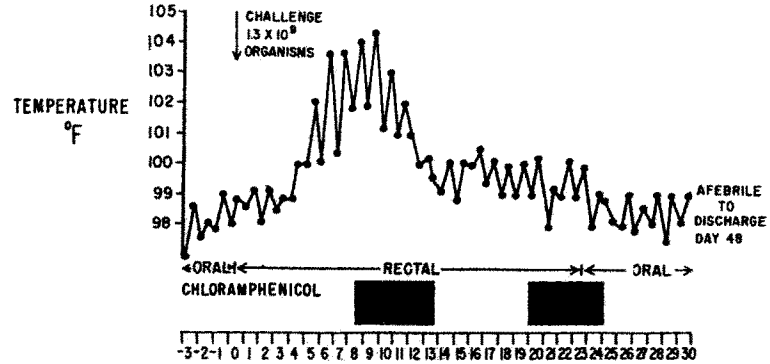


U.S. Army photographs

Fig. 2 (*left*). Three-day postchallenge biopsy of basal portion of jejunum, including non-villous portion of mucosa and bases of 2 villi (Volunteer R.S.). Granulomatous lesion occupies center of field. Over-all increased inflammatory cells in tunica propria transmigrate through epithelial lining; streamer-like projections of epithelial lining cells enter lumen, with evidence of abnormal and precipitous epithelial-cell extrusion. (H & E. × 180) **Fig. 3** (*right*). Another field from same biopsy shows prominent and abnormal cell extrusion from near base of villus. Crypt glands are infiltrated by inflammatory cells which, in places, have disrupted continuity of epithelial linings.

Subject 3 (L.T.)

This subject was a 34-year-old Negro male who was in good health until 9 days after challenge, when he developed severe headache, prostration, abdominal pain, and chills, coinciding with the appearance of typhoid bacteremia. Simultaneously, his temperature rose to 105° F. and remained elevated for 3 days. During this time blood and stool cultures were positive



		-9, -6 DAYS									
INTESTINAL BIOPSIES		■				■				■	
WEIGHT POUNDS		145	145	145	138	140	141	140	139		139
CULTURES	BLOOD		0 0 0 0	+ + + +	+ 0 0		0 0				
	STOOL		+ + + +	0 + 0 +	+ 0 0 0	0 0 +	+ 0 0 0	+ + + +	+ 0 0 0	0 0	
	WBC	6400	8350	6700	8300	9800	4300	7600	7850	4900	9500	9750	5800	11200	7400
ANTIBODY TITERS	O	80	80	160	160		320	320	320	320					640
	H	80	160	80	160		640	640	640	640					640

U.S. Army photograph

Fig. 4. Clinical course, Subject W.M., after challenge with *Sal. typhosa.*

for *Sal. typhosa.* Chloramphenicol therapy initiated at the height of fever resulted in a gradual decrease of his temperature and a return to health.

The control biopsy was obtained 2 days prior to oral challenge, when all base-line studies were in the range of normal. The histologic findings of the small bowel biopsy were within normal limits.

The postchallenge biopsy was obtained 15 days after oral challenge and 4 days following the height of fever and the start of antibiotic therapy, at a time when the subject's temperature was subsiding under the influence of chloramphenicol. A moderately severe enteritis was present, with focal accumulations of swollen monocytic cells, the hallmark of a granulomatous inflammatory reaction.[3] Likewise, the inflammatory cellular infiltrate of the tunica and the alterations of crypt glands and epithelial lining of villi were similar to the findings in the postchallenge biopsies in the 2 cases described above.

The third biopsy, obtained during convalescence on the thirty-fourth day following oral challenge, revealed some residual hypercellularity of the tunica propria. The cellular infiltrate consisted predominantly of monocytes, plasma cells, lymphocytes, mast cells, and very rarely, eosinophilic leukocytes. Changes in the epithelial cells which were noted in the postchallenge specimen were no longer present.

Subject 4 (R.G.)

This subject, a 48-year-old Caucasian male, did not develop the classic clinical picture of typhoid fever after challenge. His infection was characterized by low-grade temperature ele-

vation beginning 3 days after challenge, never exceeding 101° F., and persisting for 20 days. He was mildly symptomatic during this time, with complaints of headache, myalgia, and malaise. Stool cultures were positive for *Sal. typhosa* on 2 occasions during the first week after challenge. Blood cultures obtained daily for 17 days after challenge remained sterile. Blood counts ranged from 10,000 to 14,000 WBC, in contrast to the leukopenia which had accompanied the disease in the 3 patients discussed above. The subject received no antibiotic treatment and made an uneventful recovery. Intestinal biopsies were obtained 2 days prior to challenge, and 8, 15, and 21 days after challenge.

The control biopsy showed normal findings.

The second biopsy showed evidence of a moderately severe, diffuse, chronic nonsuppurative enteritis, without evidence of focal lesions or granuloma formation. The enteritis was manifested by a mixed infiltrate of lymphocytes, plasma cells, and large monocytic cells. In the nonvillous portion of the mucosa, histiocytes were prominent. Tissue mast cells were numerous. A small number of eosinophils were noted. Toward the villus tips, monocytes were fewer in number, and the predominant cell type was a large plasma cell characterized by a relatively large amount of cytoplasm. While the epithelium lining the villi was regular, crypt glands were elongated and, in places, showed a greater degree of pleomorphism and hyperchromasia than normally observed. Goblet cells were regular. No microulcers were noted. A small portion of submucosa included in the postchallenge biopsy showed only minimal histologic changes. As noted in the previous cases, the inflammatory reaction did not extend beyond the muscularis mucosa.

Fifteen days following oral challenge, the cellularity of the tunica propria was considerably diminished; in particular, the number of swollen histiocytes was greatly reduced. Plasma cells were numerous. Tissue mast cells and occasionally eosinophils were seen. The crypt gland epithelium at this time was more uniform and less cuboidal. Nuclei were more compact, oval-shaped, less rounded, and less vesicular than in the preceding biopsy.

At 21 days, the histologic findings were essentially within the range of normal. The biopsy could not be distinguished from the prechallenge biopsy.

Subject 5 (G.C.)

This patient, a 29-year-old Caucasian male, developed severe prostration, headache, chills, and abdominal pain 5 days after challenge, the symptoms coinciding with the appearance of the first positive blood culture. His temperature rose initially to 103° F. and then to 105° F. and remained elevated for 9 days. During this time he received first an analogue of chloramphenicol, but there was no measurable response, and the stool culture remained intermittently positive for *Sal. typhosa*. Then chloramphenicol administration was started (11 days after challenge), resulting in defervescence in 3 days and a gradual return to health.

The control biopsy, obtained 2 days prior to challenge, revealed essentially normal findings. The next biopsy was performed 8 days following oral challenge. At this time the tunica propria of the jejunal mucosa showed a moderately severe inflammatory cell infiltrate, characterized by many swollen histiocytes and plasma cells. Occasionally, the accumulation of monocytic cells in sheets revealed a focal granulomatous response. Segmented leukocytes were conspicuously absent. Tissue mast cells were plentiful, and their distribution was similar to that seen in the control biopsy of Subject 4. Eosinophils were rare. Crypt glands and villus epithelium showed only insignificant changes.

The second biopsy, obtained during convalescence 27 days following oral challenge, showed, in general, findings essentially similar to those described for the prechallenge biopsy. However, in addition to a slight increase in amorphous debris in the interstices of the tunica propria, there were focal accumulations predominantly of plasma cells, lymphocytes, and histiocytes.

Subject 6 (L.H.)

This subject was a 31-year-old Negro male who did not develop typical symptoms of typhoid fever after challenge. On the eighth day, his blood culture became positive for the first time, when there was also a mild elevation of temperature which lasted for about 1 week. His only subjective complaint was a mild headache on a few occasions. His stool and blood cultures were positive for *Sal. typhosa* on several occasions but reverted to negative during the last 3 weeks of hospitalization without antimicrobial therapy. The WBC dropped from a maximum of 14,800 on Day 1 after challenge to 7800 on the day the postchallenge biopsy was obtained; thereafter the count rose slightly and fluctuated between 8000 and 11,000 WBC.

The control biopsy was obtained 2 days prior to oral challenge. Except for a slight increase in cellularity of the tunica, the mucosa was within the range of normal.

The next biopsy was obtained at the height of a temperature rise to 102.4° F., 15 days following oral challenge. The subject was only mildly ill at the time. Blood and stool cultures had been intermittently positive for several days.

The salient feature of this biopsy, which was taken from the duodenum, consisted in an alteration of crypt-gland epithelium, which showed a greater degree of cellular pleomorphism, hyperchromasia, and alteration of shape and size of nuclei than normally seen. These changes were not associated with alterations in the epithelium lining the villi. There was a slight increase in cellularity of the tunica propria but no evidence of granuloma formation. The number of eosinophils remained unchanged. As noted previously, the inflammatory response did not extend beyond the muscularis mucosa. Brunner's glands and fragments of attached submucosa were not involved.

The third biopsy on this patient was obtained 30 days following oral challenge, when stools and blood culture were no longer positive. It showed essentially normal findings and was comparable to the control biopsy.

DISCUSSION

The postchallenge biopsies demonstrated a definite enteritis in all patients, indicating involvement of the upper small intestine even in the absence of any lymph follicles in the specimen. The enteritis affected the epithelial lining of the villi, the crypt glands, and the tunica propria, a fact which heretofore had not been documented, although there have been numerous reports on changes of solitary follicles and Peyer's patches. The enteritis was presumably the result of the invasion of the intestinal mucosa by virulent typhoid organisms. The exact mechanism of this invasion has so far not been investigated in typhoid. However, it is likely to be similar to the penetration of the mucosal barrier in guinea pigs by virulent *Shigella*[3] and *Salmonella typhimurium* organisms,[4] in which light- and electron-microscopic examinations demonstrated reactive and degenerative tissue responses of both epithelial lining and tunica propria. A similar mechanism of bacillary penetration would account for the histologic changes noted in typhoid. How many of the bacilli originally contained in the challenge dose actually penetrated the intestinal barrier and multiplied in the tissues cannot be stated. However, the nature of the lesion and analogous findings in the enteric infections cited suggest a random and diffuse penetration. The magnitude of the challenge dose possibly influenced the character of the histologic response. However, severe enteritis has been ob-

served in random biopsies of naturally acquired typhoid fever, which probably is caused by the ingestion of fewer organisms than employed in this study. We believe that the particular responsiveness of the human host to the typhoid organisms, rather than mere numbers of ingested bacilli, is a major factor determining the type of histologic lesion. It is an accepted tenet of general pathology that the production of a granulomatous reaction is not solely the result of a mass impact of the respective agent upon the tissues. The large challenge dose assured that a sufficient number of typhoid organisms penetrated the mucosal barrier and multiplied in the tissue to cause an overt disease after a relatively short incubation period. To what extent the local reaction observed in the intestinal mucosa reflects the barrier function of the gut, which may affect or modify the systemic disease, cannot be answered.

The enteritis was of a specific granulomatous nature in 4 patients and nonspecific in 2. Such variability could be anticipated, since the specific relationship which exists between an injurious agent—in the present instance, the typhoid bacillus—and the type of cellular response in the inflammatory reaction to it is modified by the immunologic responsiveness of the host. Enteritis was noted not only in the specimens obtained during the fully developed disease but also during the incubation period in Subject R.S., the only subject in whom a biopsy was performed at that time.

It was of particular interest that a granulomatous response was seen in this subject at a time when his blood culture was negative and humoral antibody response lacking. The inflammatory reaction is an indication of the antigenicity of typhoid organisms and their metabolic and breakdown products for the human host. *Sal. typhosa* does not elicit a granulomatous response in the gut of nonprimates if infection is attempted by the natural route. Even the germfree guinea pig, which is susceptible to an oral challenge with this organism, did not respond with a predominantly monocytic reaction but with a nonspecific, chronic, nonsuppurative enteritis.[4] The reaction was similar to that noted in germ-free guinea pigs monocontaminated with *Escherichia coli*.[5] Chimpanzees orally infected with a dose of a different strain of *Sal. typhosa* 100 times higher than that used in the present study proved susceptible.[6] However, specific granulomatous responses, when present in the intestinal mucosa, were confined to the solitary and aggregate lymphoid follicles.[7]

The enteritis was reversible, leaving no sequelae in the area studied, and in every patient the biopsy obtained during convalescence was comparable to the initial, control biopsy. Healing occurred without antimicrobial therapy in the 2 mild cases. A full course of chloramphenicol undoubtedly assisted in the histologic restitution in the 4 severely ill patients. The biopsy findings in the 2 patients who received drug treatment for only 4 days prior to the postchallenge biopsy were not materially different from those seen in the 2 patients whose illness was of comparable severity but in whom the biopsies were performed prior to institution of therapy.

TABLE 1. HISTOLOGIC RESPONSE, CLINICAL SYMPTOMS, AND LEUKOPENIA IN 6 VOLUNTEER SUBJECTS CHALLENGED WITH TYPHOID BACILLUS

	Subject					
	R.S.	*W.M.*	*L.T.*	*R.G.*	*G.C.*	*L.H.*
Granulomatous response	Pres.	Pres.	Pres.	Abs.	Pres.	Abs.
Severity of symptoms*	+++	+++	+++	+	+++	±
Leukopenia	Pres.	Pres.	Pres.	Abs.	Pres.	Abs.

*Graded on an arbitrary scale: ±, equivocal; + slight; +++, severe.

The histologic alterations of the intestinal mucosa were not associated with clinical symptoms directly attributable to the intestinal tract, such as diarrhea or constipation. A similar dichotomy occurs in other intestinal conditions—for instance, in celiac disease—even in the presence of sever atrophy of the intestinal mucosa. However, a positive correlation could be established between the severity of systemic clinical symptoms, leukopenia, and the severity of histologic lesions (Table 1). In particular, a granulomatous inflammatory reaction of the tunica propria was present only in the 4 subjects who developed typical typhoid fever with leukopenia. The 2 subjects who had a mild disease failed to develop either leukopenia or a granulomatous reaction, despite an intermittent bacteremia in 1 of them (L.H.).

SUMMARY

Volunteers infected orally with viable bacilli of a human carrier strain of *Salmonella typhi* were subjected to sequential small-bowel biopsies. The prechallenge, control biopsies were essentially normal in all 6 subjects. Biopsy specimens obtained shortly following the bacterial challenge or during the acute phase of the disease revealed an enteritis of the upper small intestines. Degenerative and proliferative changes were noted in the epithelial lining of villi, crypt glands, and tunica propria. These alterations are attributed to the direct effect of random bacterial invasion by *Sal. typhosa* from the gut lumen and the local tissue reaction to it, analogous to the sequence of events in *Shigella* and *Salmonella typhimurium* infections in experimental animals. The inflammatory cellular response of the tunica was of a specific granulomatous nature in 4 subjects, and nonspecific, chronic, and nonsuppurative in 2, a reflection of the variability in human host responsiveness. The enteritis was not associated with gastrointestinal symptoms. However, a correlation existed between the severity of systemic symptoms, leukopenia, and the severity of histologic lesions. The 4 volunteers who developed typical typhoid fever with leukopenia had a granulomatous inflammatory response, while 2 subjects who had a mild course did not.

The biopsy obtained during convalescence in all 6 volunteers showed evi-

dence of restitution and was essentially similar to the control biopsy. Typhoid enteritis leaves no scar in the region biopsied.

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