# INTRACELLULAR BACILLI IN INTESTINAL AND MESENTERIC LESIONS OF TYPHOID FEVER *

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In 1937 Goodpasture [1] reported the occurrence of small Gram-negative intracellular bacilli, judged to be *Eberthella typhi*, within the cytoplasm of young plasma cells located in the lymphoid follicles of the ileum and in mesenteric lesions of early cases of typhoid fever. Larger Gram-negative bacilli were found concurrently in these lesions in necrotic macrophages. These observations were made on 5 cases of human typhoid fever which had come to autopsy early in the disease. It was concluded that *E. typhi* is capable of growing in both of these situations, and the inference was made that the young plasma cell is an essential cellular host in the typical human disease and serves as a nourishing and protecting medium, not only during the period of incubation but throughout the active course of typhoid fever. He was led to study the lesions of typhoid fever after the observation of small, intracytoplasmic colonies of bacilli in the entodermal epithelial cells of the chorio-allantois of chick embryos infected with *E. typhi*.[2]

It is the purpose of this paper to report a similar study of 6 additional cases of typhoid fever.†

Zenker-fixed paraffin sections stained in Wright's stain (60 drops to 100 cc. of distilled water) for about 4 hours, differentiated in absolute ethyl alcohol, cleared in xylol and mounted in cedar oil were used for study. With this method the intracytoplasmic bacillary forms are somewhat inconspicuous owing to the blue-staining properties of both the cytoplasm of the plasma cells and the bacteria, in conjunction with the relatively small size of the bacterial forms. The following staining method was therefore

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† Of the cases studied, 2 came from the Department of Pathology of Vanderbilt University. Material from 1 case was supplied by Dr. W. A. DeMonbreun from the Pathological Laboratory of the Nashville General Hospital. Sections from 3 cases were kindly sent by Dr. W. D. Forbus from the Pathology Department of Duke University Hospital.

devised whereby better contrast could be obtained between the bacteria and the cytoplasm of the cells.

1. Stain in Weigert's iron hematoxylin solution for 1 minute or less.
2. Wash in 50 per cent alcohol acidulated to 0.1 per cent hydrochloric acid.
3. Stain in Goodpasture's carbol-aniline-fuchsin solution for 1 minute.
4. Decolorize in a 5 per cent aqueous solution of acetic acid until the red color disappears or the sections remain a light pink.
5. Repeat steps 3 and 4 three times.
6. Wash in water.
7. Stain in a 0.01 per cent light green solution, acidulated to 0.2 per cent acetic acid, for about 1 minute or until the section has a light green hue. Overstaining is to be avoided.
8. Wash in water, differentiate in 95 per cent alcohol, dehydrate in absolute alcohol, clear in xylol and mount in balsam.

The nuclei are stained black, the nucleoli red, the cytoplasm a pale green, bacteria a brilliant red, red blood cells red and other structures green.

## Abstract of Cases

Case 1, V–38–111: A 56 year old negro was admitted to the Vanderbilt Hospital in a stuporous condition with a story of diarrhea and fever of 4 days duration. The blood culture was positive for *E. typhi*. The course of illness was one of profound toxemia and was steadily downhill. Death occurred on the 3rd hospital day.

Autopsy showed hyperplastic Peyer's patches and solitary follicles in the distal 36 inches of the ileum. The lesions were in an early ulcerative phase. There was a mesenteric lymphadenitis and focal necrosis of the liver, spleen and kidneys. The blood culture and culture of the intestinal lesions were positive for *E. typhi*.

Intracytoplasmic bacillary forms were demonstrated in the intestinal lesions without difficulty. Cells of this type were rarely found in sections of the mesenteric nodes.

CASE 2, G–38–127: A 22 year old negro was admitted to the Nashville General Hospital 13 days before death with a history of fever of 4 days duration. The Widal test showed agglutination at 1:640 and the stool culture yielded *E. typhi.*

Autopsy revealed classical, hyperplastic lymphoid lesions in the distal 12 inches of the ileum and the proximal 24 inches of the colon. The appendix was inflamed and the mesenteric nodes were swollen. Microscopic studies revealed classical, pre-ulcerative, early necrotic, typhoid intestinal lesions, mesenteric lymphadenitis, and focal areas of necrosis in the spleen, liver, pancreas and adrenal. A blood culture at autopsy yielded a pure culture of *E. typhi.*

Intracytoplasmic bacillary forms were relatively numerous in the plasma cells in the involved Peyer's patches. They were demonstrated in the colon and in the mesenteric nodes. None was found in the appendix.

CASE 3, Duke No. 173: A 20 year old negro was admitted to the Duke University Hospital 5 days before death with a history of headache for 15 days and fever for 8 days. The blood culture was positive for *E. typhi.*

Autopsy revealed early ulceration of Peyer's patches and hyperplastic follicles in the proximal colon. There was a mesenteric lymphadenitis, an inflamed appendix and focal areas of necrosis in the spleen and liver.

Intracytoplasmic bacillary forms were observed in plasma cells in sections of the involved areas of the ileum and colon. The mesenteric nodes were not studied and no bacterial forms were found in the appendix.

CASE 4, Duke No. 257: A 15 months old white boy was admitted to the Duke University Hospital 9 days before death with a history of fever of 2 weeks duration. The blood culture was positive for *E. typhi* on admission and at autopsy.

Ulcerative lesions were present, but not marked, in the Peyer's patches and in the colon. Microscopically the lesions were in the ulcerative stage. The cellular response was atypical in that there were many more large mononuclear cells and fewer plasma cells than usual. There was a marked mesenteric lymphadenitis.

Intracytoplasmic bacillary forms were demonstrated infrequently in occasional plasma cells in a section of a Peyer's patch. None was found in other sections.

CASE 5, Duke No. 298: An 8 year old negro boy ill for 2 weeks before admission to the Duke University Hospital died on his 2nd hospital day.

At autopsy ulcerated lesions were present in the cecum, ascending colon and Peyer's patches. Microscopically the hyperplastic and ulcerative lesions in the intestines showed a somewhat atypical cellular response. In some areas there were large collections of polymorphonuclear leukocytes. There was a marked mesenteric lymphadenitis.

Plasma cells in a section of a Peyer's patch showed infrequent intracytoplasmic bacillary forms. None was found in the colon. Typical intracytoplasmic colonies were observed in large plasma cells in a section of a mesenteric node.

A 6th case, in which no intracytoplasmic bacillary forms were found, may be compared with the above group.

CASE 6, V–37–144: A 15 year old girl was admitted to the Vanderbilt Hospital in a semistuporous condition with a history of fever of 2 weeks duration. On her 4th hospital day a perforation was suspected and an exploratory operation was performed, during which two perforated ulcers in the lower ileum were closed. The course of illness was downhill until she died on the 7th hospital day.

Autopsy showed a diffuse peritonitis, deeply ulcerated Peyer's patches in the ileum and ulcerated lesions in the cecum and ascending colon. *E. typhi* was isolated from the bile but was not recovered from cultures of the blood stream or from the ulcers. Microscopically the lesions were necrotic and deeply ulcerated.

## DISCUSSION

This study confirms the previous observations of Goodpasture that the intracytoplasmic bacillary forms within plasma cells of intestinal and mesenteric lymphoid lesions of typhoid fever are most numerous in early cases. They are found in areas where necrosis has not yet taken place or is limited in extent and where cellular hyperplasia and infiltration are marked. They are most frequently found in hyperplastic areas over which the mucosa is still intact.

The observations here recorded supplement the previous report in that intracytoplasmic forms were demonstrated in colonic lesions as well as in lesions in the ileum and in involved mesenteric nodes. They were also demonstrated in childhood lesions of

typhoid where the cellular reaction consists more of a large mononuclear infiltration with an associated polymorphonuclear invasion.

In one instance also the small bacillary forms were found within the cytoplasm of a lymphoblast or plasma cell in the process of mitosis. This observation indicates that the bacilli may cause little or no injury to the host cell, and that the latter, stimulated to mitosis, might give rise to two infected cells without reinfection. The frequent observation of two or more infected plasma cells in close proximity to each other suggests the possibility also of reproduction of infected cells.

The intracytoplasmic colonies of bacillary forms, as seen in plasma cells, are surrounded by a narrow clear zone. In some cells there is only a single colony composed of a few bacteria while in others there are as many as six colonies. Some colonies contain a large number of bacteria, perhaps 100 or more. In occasional cells containing large colonies the vacuoles in which they lie appear to have ruptured and the bacilli appear to be exuding into the adjacent tissue. The bacillary forms are Gram-negative and all indications are that they are small forms of *E. typhi*.

The apparent regularity in the occurrence of intracellular bacterial forms within young plasma cells in the intestinal and mesenteric lesions in early cases of typhoid fever indicates that they are an essential component of these classical early lesions and that they are of importance in the pathogenesis of this disease.

## Summary and Conclusion

1. Gram-negative, intracytoplasmic bacillary forms, judged to be *E. typhi*, have been found in the cytoplasm of young plasma cells located in the lymphoid follicles of the ileum, colon and mesenteric lymph nodes in 5 cases of early typhoid fever.

2. A method for staining intracellular bacteria is described.

3. It is concluded that the presence of these bacillary forms within the plasma cell is an essential part of the early, classical, intestinal and mesenteric lesions of typhoid fever.

## REFERENCES

1. Goodpasture, E. W. Concerning the pathogenesis of typhoid fever. *Am. J. Path.*, 1937, 13, 175–185.

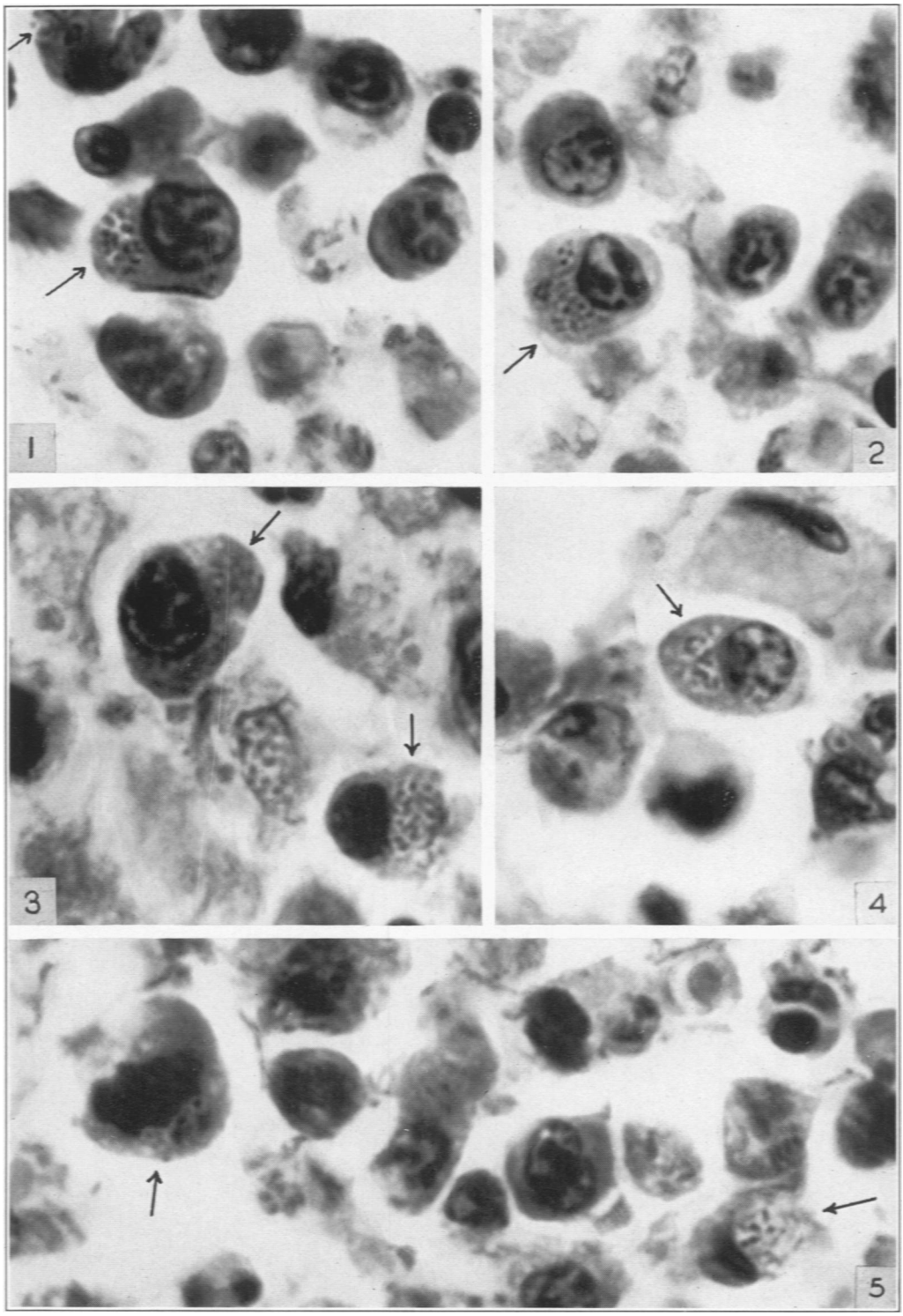
2. Goodpasture, E. W., and Anderson, K. The problem of infection as presented by bacterial invasion of the chorio-allantoic membrane of chick embryos. *Am. J. Path.*, 1937, 13, 149–174.

## DESCRIPTION OF PLATE

### PLATE 94

FIGS. 1–4. Microphotographs showing young plasma cells of a persisting follicle in a Peyer's patch. The arrows point to groups of intracellular bacilli. Note the encapsulating material about the bacilli. Basic fuchsin-light green stain. × 2500.

FIG. 5. The cell on the left containing intracellular bacilli is undergoing mitosis. × 2500.

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