CONCERNING THE PATHOGENESIS OF TYPHOID FEVER*

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The earliest phases of an infection, involving a portal of entry, a mechanism of invasion, and activities transpiring during the period of incubation, while of the utmost importance to an understanding of the disease as a whole, are the most difficult incidents to discover and are among the least well understood phenomena of most infectious processes.

Invasion of the host and, presumably, multiplication of the parasite, take place before there are manifest evidences of disease. The lesions which follow may at times obscure the pathways of the more cryptic progress of the earlier stages; and in experimental animals it may be difficult or impossible to reproduce conditions comparable to those existent in the original host naturally infected. Even though satisfactory conditions can be found there are technical difficulties that attend a study of these earliest stages of infection, hindering an exposition of them.

Typhoid fever offers a familiar illustration. Despite extensive knowledge of etiology, epidemiology, pathological anatomy and immunology of typhoid fever there is a broad hiatus between the entrance of the specific bacilli into the mouth and the development of manifest symptoms of infection, which is almost entirely unknown terrain. It is not known just where or how the bacilli invade the host and multiply during the incubation period. There are two main hypotheses.[1]

The hematogenic hypothesis supposes that the bacilli penetrate in an unknown way the mucosa of the throat, tonsils or gastrointestinal tract under natural conditions of infection, and on gaining entrance into the blood multiply in this medium and thereby are disseminated throughout the body to localize in the lymphoid tissue of the intestine and other places to induce the specific lesions of the disease as secondary phenomena. The enterogenous hypothesis

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assumes a penetration of the intestinal epithelial lining at some point or points unknown, possibly first that of the Peyer's patches in the lower ileum, followed by a local growth of the bacilli with the production of lesions and a secondary invasion of the blood stream passively through lymphatics and blood vessels. According to this assumption it would seem likely that the original foci of infection would be few and focal, followed by more extensive invasion of the intestinal mucosa after the bacilli begin to be excreted through the bile.

There seems to be no evidence that *E. typhi* actually multiplies within the lumen of the gastro-intestinal tract itself; and its demonstrable presence there is now assumed to be entirely a result of elimination through the biliary tracts.[2]

It has been surprisingly difficult to demonstrate by histological methods any exact relation between the lesions of typhoid fever, whether in the intestine or elsewhere, and the presence of the specific microorganisms. Small groups of bacilli, presumably *E. typhi*, have often been demonstrated in these lesions but they have not appeared to be definitely related to them; and not infrequently it is a question whether or not they represent postmortem growths. It seems to be accepted generally that the earlier the stage of the disease and the sooner after death the tissues are fixed, the more difficult it is to demonstrate bacilli. Mallory states in this connection: "Occasionally the characteristic colonies were found in the mesenteric lymph nodes and in the spleen, but not in any case that came to post-mortem examination very soon after death. In the earliest case (10 days after onset), in which postmortem examination was made one hour after death, no bacteria were found microscopically except along the edges of the beginning ulcerations of the intestine, and in that situation it is of course impossible to differentiate the typhoid from the colon bacillus. Certain it is that when typical colonies of the typhoid bacillus are found in the different organs they bear no intimate relation to the lesions present."[3] Although the typhoid bacillus may not be seen in the lesions their presence is usually demonstrable, by cultural methods, in the intestine, lymph nodes, spleen and bone marrow. It thus appears that the exact portal of entry and the primary site and mechanism of infection in typhoid fever are quite unknown.

Recent studies of bacterial infection of the chorio-allantoic mem-

brane of chick embryos in this laboratory have shown that *E. typhi* grows abundantly on the surface of the membrane at the site of inoculation in association with injured and necrotic membranal tissue and cellular exudate.[4] Furthermore, it was observed that this microorganism is capable of gaining admission into the cytoplasm of living entodermal epithelial cells of the membrane; and under these conditions it apparently will grow intracellularly, forming small intracytoplasmic colonies. The bacteria multiplying in association with necrotic tissue on the surface are quite large, resembling the large forms cultivable on dead media. Those within the entodermal epithelium are on the contrary relatively small and seem to be embedded in a matrix possibly of capsular material. The intracytoplasmic colonies in entodermal epithelium are the only morphological evidence of invasion and multiplication of the bacilli within the tissues of this host, although it was possible on several occasions to obtain positive blood and bile cultures.

This observation of the penetration of the lining entodermal epithelial cells by *E. typhi* and its seeming growth within this living medium suggested the possibility that a similar mechanism might be involved in the invasion of the human intestine by the bacillus, and was the occasion of a restudy of material from several cases of typhoid fever at our disposal.

Particularly suitable for such a study were the tissues from a recent case where death occurred on the 11th day of the disease. A postmortem examination was performed 1½ hours after death. The tissues were fixed immediately in Zenker's fluid, and paraffin sections were stained by immersing them for about 4 hours in Wright's stain (60 drops suspended in 100 cc. of distilled water). The stained sections were differentiated in absolute ethyl alcohol, cleared in xylol and mounted in cedar oil.

Report of Case

Clinical History: A 19 year old white male (V–34–147) was admitted to the Vanderbilt Hospital on Sept. 8, 1934. He had never had typhoid fever nor had he ever received antityphoid vaccine. He began complaining of general malaise 10 days before admission. Two days later after the onset of illness he had a mild chill and felt feverish. He was in bed for 6 days prior to admission and for 3 days vomited everything taken by mouth. At times he was delirious.

On physical examination the spleen was palpable. The temperature was 104.2° F., the pulse 110, and the blood pressure 130/70. Coupled pulse was

noted. The urine contained 2+ albumin with granular casts. White blood cells numbered 4350. The stools were tarry. Guiac test +. Blood culture showed 60 colonies *E. typhi* per cc. The stools and urine were negative for *E. typhi*. The Widal was 1:160.

In spite of all therapeutic efforts his temperature rose to 106° F. He became more restless, requiring restraint, developed signs of peripheral circulatory collapse with severe toxemia, and died on Sept. 9, 1934.

The Intestinal Lesion and Intracellular Parasitism

Autopsy was performed 1½ hours postmortem. Few of the solitary follicles and Peyer's patches in the intestine showed any gross evidence of ulceration, although all or most of them were considerably swollen.

Microscopically the intestinal lesions are typically those of early typhoid. Frequently a thin epithelial sheet completely covers a patch of Peyer without ulceration. The follicles themselves are swollen by edema and infiltration by numbers of the large mononuclear phagocytes so typical of the cellular response to this infection. There are also areas of necrosis, deposits of fibrin and cellular thrombi in dilated lymphatics and blood vessels. But throughout the agminated follicles there are persistent foci of lymphocytes, plasma cells and reticulo-endothelium which seem to represent original lymphoid nodules. These groups are still fairly abundant although many lymphocytes have been and are being removed by phagocytosis.

First a careful study was made of glandular and surface epithelium, both that covering the lymphoid nodules and that of intervening areas, to see if there were evidence of intracellular bacillary forms such as were found in the entoderm of the embryonic membranes of the chick. No such forms have as yet been found in sections of stomach, duodenum, jejunum, ileum and colon. Furthermore, in accordance with observations of others, no bacteria were found in or on this membrane in the unulcerated regions studied. Only a few large bacilli were found lying on the surface of shallow ulcerations. They had not penetrated below the surface and were not considered to be typhoid bacilli.

On examination of the cellular foci, composed chiefly of lymphocytes and plasma cells, it soon became apparent, however, that young plasma cells occasionally contain within their rather purplish cytoplasm circumscribed areas filled with a lighter staining material

embedded in which are numerous, small, rod-shaped and spherical structures that are obviously bacteria.

Owing to the relatively deeply staining cytoplasm of the plasma cells and the small size of the intracellular bacteria the cellular inclusions are quite inconspicuous, but when found they are very definite. Search was then made through many sections of the intestinal lesions, and although the number of demonstrably infected plasma cells is small in any one section, typical examples were found in each. Similar plasma cells with intracytoplasmic bacillary and coccoid inclusions were likewise found in mesenteric lymph nodes draining the intestinal lesions and showing the usual typhoidal lesions. In no other lesions, including those of the spleen, liver and bone marrow, were intracellular bacillary inclusions demonstrated, although a careful examination was made of several sections from each of these tissues.

The intracytoplasmic bacillary inclusions were found only in plasma cells, and in very rare instances in cells with more abundant and more pink staining cytoplasm which might be interpreted as being altered plasma cells.

The interpretation that the cells containing the inclusions are plasma cells is based on the fact that they possess an eccentric round or oval nucleus with chromatic material generally arranged about the periphery, and a relatively abundant basophilic cytoplasm sometimes exhibiting a crescentric, paranuclear, slightly acidophilic area. These cells are commonly and most numerously situated within the persisting lymphoid aggregates of the intestinal follicles, and sometimes exhibit multiple or lobulated nuclei. All of these are commonly known characteristics of plasma cells; but the cells under consideration are rarely classical mature plasma cells. Ordinarily they seem larger with more abundant cytoplasm and are apparently immature. Mitotic figures in such cells were occasionally observed. Cells having the characteristics as described are commonly accepted by pathologists as plasma cells in their descriptions of similar lesions of typhoid fever.[3] They are never seen to contain phagocytosed material other than the bacillary aggregates which I have described. It is important that the identity of these cells be recognized because they are the only cell in the typhoidal lesion that contains the inclusions and this, therefore, appears to be a cellular specific phenomenon.

After the discovery of the small bacillary aggregates within plasma cells search was made for bacillary groups such as have been described by others in lesions of typhoid fever — namely extracellular aggregates that appear to have no obvious relation to the lesion. Such groups, though quite rare and exceedingly small, consisting of about a dozen or less bacilli, were found after careful search but only in association with and practically always within a necrotic typhoidal macrophage. On rare occasions a normal appearing macrophage contained a few bacilli in relationship with the partially digested remains of a phagocytosed lymphocyte. The bacillary forms within the dead macrophage differ considerably from those within the plasma cells. They are composed of large, deeply staining rods which are vegetative, as judged from their fresh appearance and the linking of one rod to another. Bacillary forms of this kind were found only within or near the inflamed follicles of the Peyer's patches.

The question naturally arises whether or not any or all of the bacillary groups, including the large and small forms, are typhoid bacilli. Shortly before death in the case under consideration a blood culture yielded only *E. typhi*, 60 colonies per cubic centimeter. Clinically there was no evidence of a complicating infection. The case was such an early one and the tissues were fixed so shortly after death that there is no evidence microscopically of invasion of the intestinal lesions by any secondary bacteria. The bacillary inclusions within plasma cells are Gram-negative and resemble individually and collectively the small intracellular bacillary forms encountered in the entodermal epithelium of the chick embryonic membranes inoculated with *E. typhi* and from which pure cultures of this bacillus were recovered. Furthermore, the plasma cell is not known to be an active phagocyte, nor does it seem to migrate extensively. Those that contained bacterial inclusions in the intestinal lesions were most commonly situated near the central portion of follicular remnants, often remote from the intestinal surface, and they were also present in mesenteric lymph nodes that have been found to yield pure cultures of *E. typhi*.

Such considerations lead me to the conclusion that these represent an especially small bacillary form of *E. typhi* modified by their intracellular environment. That they are reproducing within the cytoplasm of the host-cell is judged by the fact that the focal aggre-

gates consist of numerous individuals. It often appears that they are embedded in a capsular material, possibly produced by themselves. There are no degenerating forms and they are not surrounded by a digestive vacuole, nor are they irregularly distributed in the cell, but occur as focal circumscribed inclusions. Furthermore, there are no detectable extracellular bacteria from which the incorporated forms might have been derived by ingestion.

It is of interest that in no instance has there appeared a large bacillary form or group derivable from a plasma cell. There is no evidence of an ante or postmortem transformation of the small forms into the large within the plasma cell. On the other hand only the large bacillary forms are found within the typhoid macrophage. Whether these represent postmortem growth or not cannot definitely be determined, but one has reason to judge that they do represent an ante mortem growth.

In the first place they are practically always associated with and incorporated by macrophages that exhibit evidences of necrosis. They are quite rare, more so than the plasma cell forms. There is no extracellular growth and in view of the abundant microorganisms in the blood stream it would seem, had postmortem growth occurred, that extracellular foci would be in evidence. Failure to find bacterial foci in the spleen and bone marrow is also opposed to an interpretation of postmortem growth.

Although the foregoing description is based largely on observations made on the tissues of the case reported, plasma cells containing characteristic intracytoplasmic groups of Gram-negative bacilli, judged to be *E. typhi*, have been found in Peyer's patches of 4 additional cases of typhoid fever. The late stages of the disease with complete ulceration, and tissues from cases examined several hours after death, are not suitable for such a study. No such inclusions have been found in tissues from other conditions, including lesions of bacillary dysentery, satisfactorily prepared for their demonstration.

Discussion

Observations of the phenomena of typhoid fever up to the present time have neither indicated the portal of entry of the typhoid bacillus into the tissues of the human host nor the sites of multiplication of this microorganism after invasion has taken place. Evidence

indicates that *E. typhi* does not multiply within the lumen of the gastro-intestinal tract, or within the blood stream.[5,2]

Although the observations I have recorded do not indicate the portal of entry, they may be interpreted to point out a site and mechanism of growth of the causative agent after invasion has taken place — namely within the cytoplasm of living young plasma cells, and within the cytoplasm of living or dead macrophages in the lymphoid tissue of the intestine and mesenteric lymph nodes.

The Gram-negative, small bacillary microorganisms found in groups within apparently normal and uninjured young plasma cells are interpreted to be intracellular forms of *E. typhi.* Similar structures have not been found in other cells, and it is tentatively concluded that the human host offers at least one living intracellular medium to which this bacterium may gain access, be thereby protected from unfavorable extracellular influences, and multiply. A few cells, presumably plasma cells, containing relatively large aggregations of bacilli appear to be necrotic, and one may assume that the enclosed bacilli may be liberated by rupture or disintegration of plasma cells thereby to gain access to the lymph and blood stream, or to become phagocytosed by other cells of the same kind in which the processes of multiplication may be continued in series.

On the other hand, the liberated bacilli could be phagocytosed by the macrophages, for Gram-negative bacilli, judged to be typhoid bacilli, have likewise been found in these cells. But the macrophage does not seem to be naturally a suitable medium for growth of the bacillus. In those instances where evidences of growth in macrophages are apparent the bacilli are associated with the digesting remains of a phagocytosed lymphocyte or are incorporated within the cytoplasmic remains of a dead macrophage.

Infection of embryonic chick membranes indicated that *E. typhi* multiplied under two conditions: (1) in association with necrotic tissue, and (2) within the cytoplasm of living entodermal cells. Within the intestinal lesions of typhoid fever similar conditions seem to exist. But here the living receptive and nourishing cell is the young plasma cell, and the necrotic material is the intracellular remains of a phagocytosed cell or the autolysing remains of the phagocyte itself.

In the infected embryonic tissue the bacilli multiplying within living entodermal cytoplasm are quite small and are embedded in an

incorporating material, while those growing in necrotic tissue are large and free. A similar distinction is found in the human lesions, namely the bacilli within plasma cells are small and lie within an incorporating material, while those within macrophages are large bacilli and free.

There are only a few bacilli, however, within a single macrophage, no more than might be considered to be subsisting on the dead material with which they are associated. It is rare to find a macrophage that contains bacilli, and one may reasonably judge that the microorganism is protected and nourished by the disintegrating cells for only a brief period. It seems probable that, following a short series of divisions, the bacilli become, through dissolution of the incorporating macrophage, exposed to the extracellular environment, whence they may reenter either young accessible plasma cells to repeat a generative cycle, or a macrophage (where they may be destroyed), or may regenerate, if the cell dies or is digesting a phagocytosed lymphocyte. On the other hand, if humoral bacteriolysins have developed the exposed bacilli may be rapidly dissolved, liberating thereby their endotoxins.

The "positively chemotactic" and susceptible young plasma cell within lymphoid tissue generally could come in contact with *E. typhi* and become infected, either from direct invasion of the intestinal wall or by indirect invasion through the blood stream. Consequently without knowledge of the earliest stages of infection it is not possible to be certain of the portal of entry. It would seem reasonable to conclude, however, that injury of lymphoid tissue resulting in proliferation of young plasma cells would predispose those tissues to the most extensive infection; and experience shows such injury occurs most prominently in the lymphoid tissue of the lower ileum and the mesenteric lymph nodes draining this area.

No bacillary groups were found in the early cases in any tissue examined other than the lymphoid tissue of the ileum and mesentery. It seems quite probable that extracellular bacilli would form small colonies in the dead tissues if they were permitted to incubate long enough, and observations of others indicate that they do so.

If it is assumed that the young plasma cells are the susceptible hosts for growth of *E. typhi* during the incubation period of typhoid fever, and from them the bacilli become disseminated, primary or metastatic infections might occur in various parts of the

body, notably in the tonsils, pharyngeal lymphoid nodules, intestine, lymph nodes, spleen and bone marrow, where plasma cells are normally present.

In the early case under examination no bacilli were found in the spleen, liver and bone marrow. The focal necroses present in these structures rather indicate the effects of endotoxins from phagocytosed and lysed bacilli from the circulating blood. Plasma cells are quite rare in the liver and *E. typhi* is almost never recognized in this organ although focal necroses are numerous. These cells, however, are more abundant in the spleen and bone marrow and, although no intracellular bacilli were found, it seems possible that cycles of generation could occur in these tissues within plasma cells.

Persistence of typhoid fever after the disappearance of the specific bacilli from the blood stream might be explained by a continuance of growth within plasma cells locally, although antibodies prevent their further dissemination.

The observation that the human plasma cell can be a susceptible cellular host for *E. typhi* may have a bearing on immunity to typhoid fever. It is generally conceded that recovery from typhoid fever is associated with a prolonged immunity to subsequent infection by *E. typhi*, while vaccination at best usually leads to less substantial and briefer immunity.

If infection is associated with intracellular multiplication of *E. typhi* it seems possible that antigens differing perhaps from those derivable from artificially cultivated bacilli may be liberated during the course of infection and that the susceptible plasma cell itself may become immune.

It is to be hoped that the observations I have recorded and the interpretation of them proposed may be subjected to more critical examination by others from investigation of the disease in man or in chimpanzees, in order that a better understanding of the pathogenesis of typhoid fever may be acquired and that the significance of relations between the cells of susceptible animals and parasitic agents may be demonstrated or excluded in this and other infectious processes.[6]

Summary and Conclusions

1. In early cases of typhoid fever small Gram-negative intracellular bacilli, judged to be *E. typhi*, have been found at autopsy

in the cytoplasm of young plasma cells, otherwise apparently unaltered, located in the lymphoid follicles of iliac and mesenteric lesions.

2. Larger Gram-negative bacilli have been found in macrophages of the intestinal lesions in association with the remains of phagocytosed lymphocytes, or the necrotic remnants of macrophages themselves.

3. It is concluded that *E. typhi* is capable of growing in both these situations and under conditions indicated.

4. The interpretation is proposed that the young plasma cell is an essential cellular host for *E. typhi* 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 the disease.

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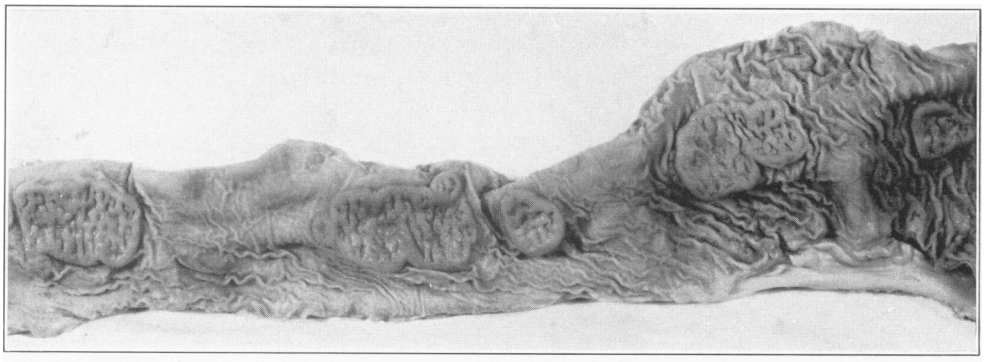
DESCRIPTION OF PLATES

PLATE 24

FIG. 1. Peyer's patches in ileum.

FIG. 2. Peyer's patch and solitary follicles just above ileocecal valve. Note slight ulceration.

FIG. 3. Peyer's patch showing partial covering with epithelial sheet. × 16.



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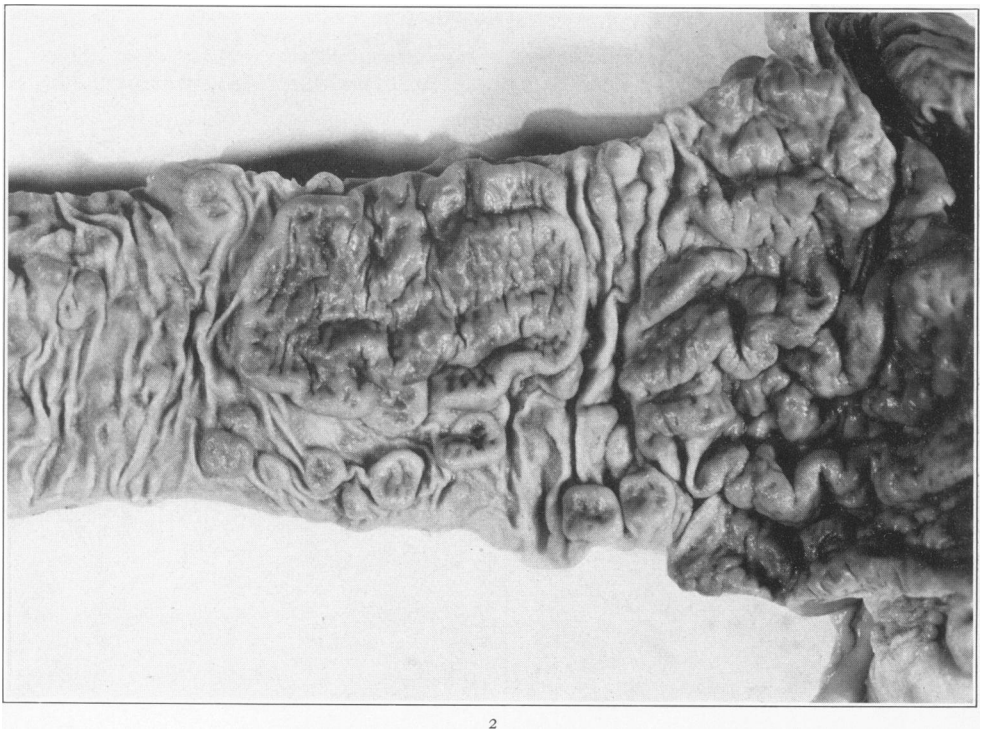

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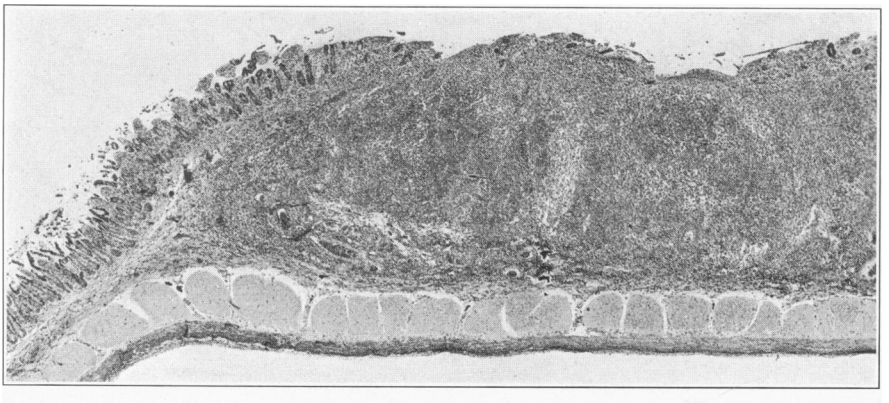

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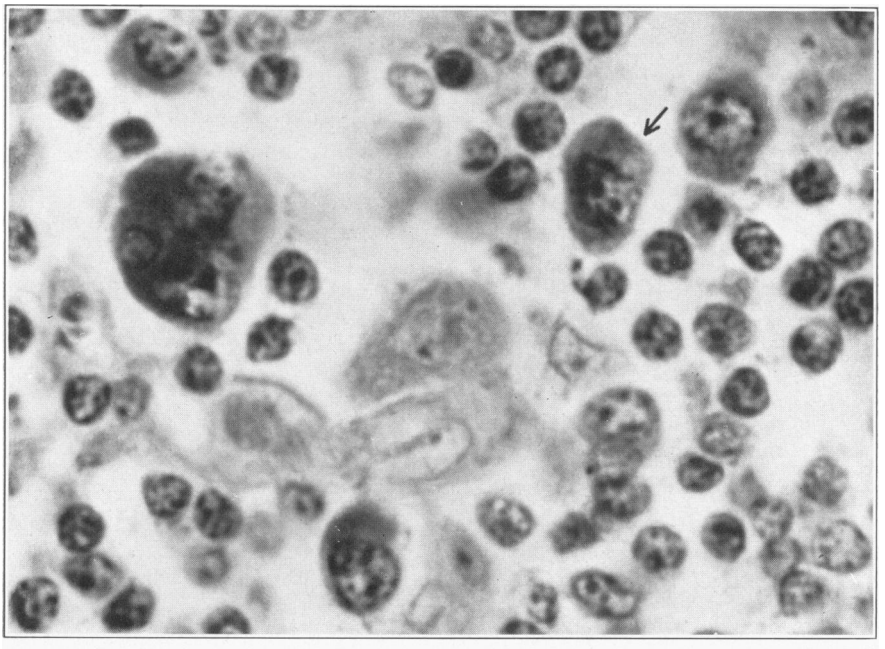
PLATE 25

FIG. 4. Photomicrograph showing young plasma cells near center of a persisting follicle in a Peyer's patch. Arrow points to ill-defined group of intracellular bacilli. × 1400.

FIG. 5. Arrow points to young plasma cell from a Peyer's patch containing a group of small bacilli. × 2500.

FIG. 6. Plasma cell with pyknotic nucleus. Intracellular group of small bacilli. × 2500.

FIG. 7. Necrotic macrophage containing group of large bacilli. × 2500.



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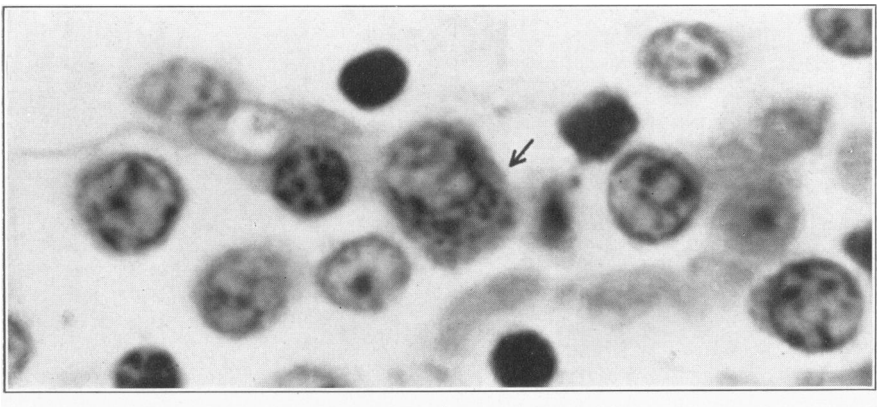

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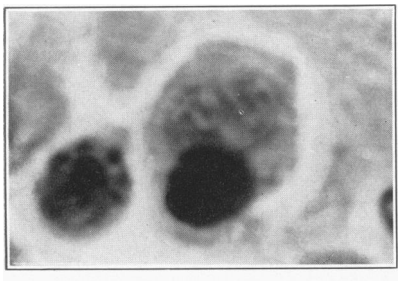

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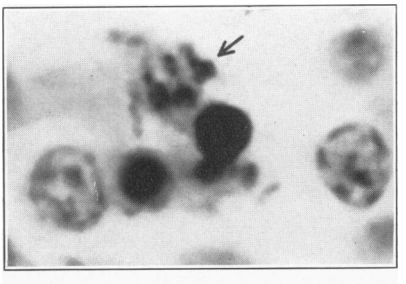

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PLATE 26

Cells from case reported. Figure 13 from a mesenteric node. Others from Peyer's patches.

FIG. 8. Plasma cell containing small bacilli. Large and small lymphocyte and macrophage.

FIG. 9. Group of young plasma cells. One near center contains a group of small bacilli.

FIG. 10. Plasma cell containing bacilli. Note encapsulating material.

FIG. 11. Necrotic plasma cell with encapsulated group of small bacilli.

FIG. 12. Plasma cell containing large group of bacilli.

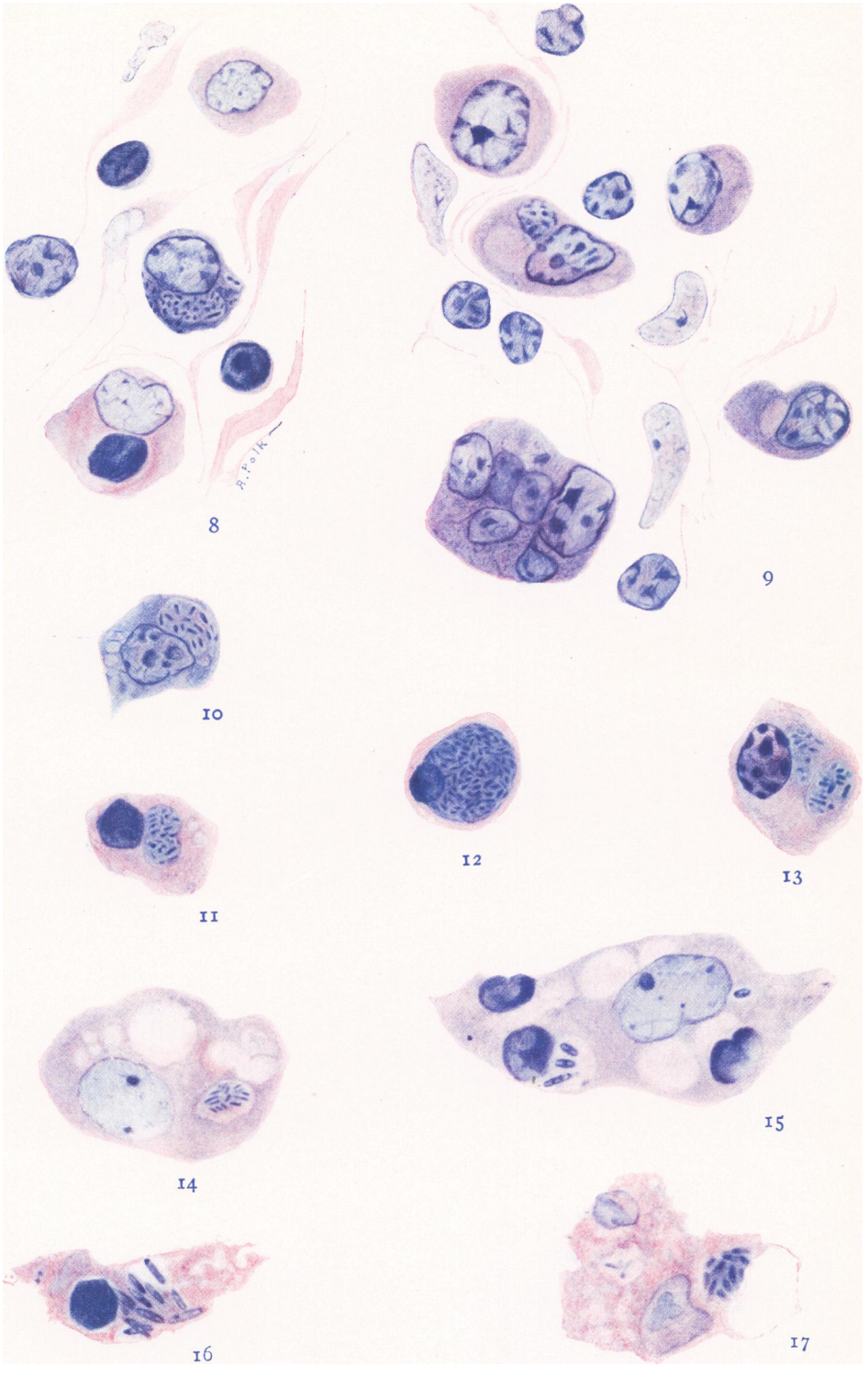
FIG. 13. Plasma cell containing two groups of small bacilli.

FIG. 14. Large cell, possibly transformed plasma cell containing group of small bacilli.

FIG. 15. Macrophage containing dead lymphocytes associated with one of which is a small group of large bacilli.

FIG. 16. Dead macrophage containing large bacilli.

FIG. 17. Dead macrophage containing large bacilli.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