Clinical and Histopathologic Correlations in Acute Diarrheal Disease[1]

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This report describes recent work carried out at the Pakistan-SEATO Cholera Research Laboratory, the Walter Reed Army Institute of Research, and the Department of Pathology at Harvard Medical School. Observations will be presented on fatal cases of cholera, the pathogenesis of cholera in the guinea pig as investigated by light and electron microscopy, the mucosal pattern of cholera and its similarities and dissimilarities to mucosal alterations in other forms of acute diarrheal disease, and fatal diarrheal disease as it occurred in a hospitalized population of children in Guatemala.

Of the five fatal cases studied recently at the Cholera Research Laboratory in Dacca, four were children ranging in age 1½ to 10 years, and the fifth case was a woman aged 40. These observations are presented with the kind permission of Dr. Abram S. Benenson, director of the Cholera Research Laboratory. In all five cases the mucosal alterations were those which typify cholera (*1*), in that there was abundant cellular infiltration of the lamina propria with mononuclear cells predominating (fig. 1). The intestinal epithelium was intact, although mononuclear cells were found between the epithelial cells. Beneath the epithelium there were occasional spaces which sometimes contained eosinophilic granular material. There was invariably some bulging of the terminal portion of the villus, but the ratio of villus height to crypt depth appeared normal in all cases. Congestion of the vessels in the lamina propria and the submucosa was the rule.

In one case, that of a boy aged 10 (CRL 102), ulcerative lesions were noted in both the small and large intestine. These had the appearance of ischemic damage with a superimposed exudative response. The presence of these lesions may be related to the prolonged period of uncorrected shock and acidosis which had existed before death. Such lesions could account for the appearance of acute inflammatory cells and of blood in fecal specimens of cholera patients. Only the cholera vibrio, and no other

Figure 1.—Patient A. H., a 3½-year-old boy with acute cholera (Inaba); duration of clinical course, 18 hours (CRL Case No. 1505). This villus exemplifies the alterations seen throughout the small intestine. The epithelium is intact, with the brush border representing microvilli presenting no defects. Mononuclear cells of a variety of types are found among the epithelial cells and their large numbers produce prominence of the lamina propria. Subjacent to the epithelial layer and within the lamina propria there are spaces filled in part by a granular material (see arrows). (Hematoxylin and Eosin.)

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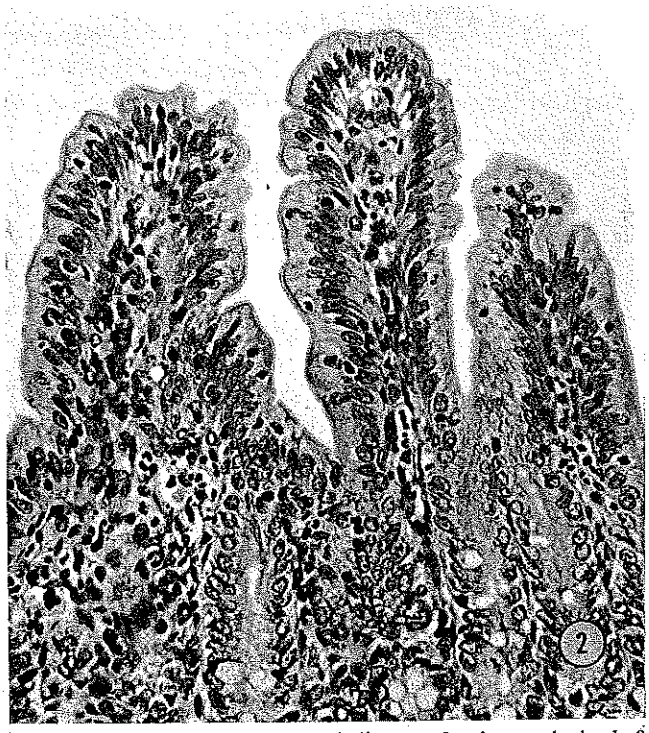

FIGURE 2.—Guinea pig, proximal ileum. Specimen obtained 8 hours following oral challenge with cholera vibrios. The epithelium with its brush border corresponding to microvilli is intact. There is hyperemia and a slight increase in cellularity of the tunica propria. Polymorphonuclear leucocytes are present. There is an increase in cellular debris and granular precipitate. There is a suggested space formation in the basal area of some epithelial cells and subjacent portion of the tunica (Gruenhagen-Mingazzini's spaces). (Hematoxylin and Eosin.)

enteric pathogen, was isolated from the intestine in this case.

Congestion of the pulmonary capillaries without pulmonary edema was common to all cases. There were occasional small foci of acute bronchitis with surrounding bronchopneumonia. Hyaline membranes lining the alveoli were noted in one portion of the lung in the case of the 10-year-old boy mentioned above. Focal myocardial damage was noted in all cases. This consisted of hypereosinophilia and swelling of myocardial fibers with loss of striations and the presence of pyknotic nuclei. Cellular infiltration surrounded such foci occasionally. In the liver there was a random distribution of degenerate hepatic cells with hypereosinophilic cytoplasm and pyknotic nuclei. Many of the portal areas showed infiltration with mononuclear cells. A similar degenerative alteration of parenchymal cells was noted also in the pancreas, the kidneys, and the adrenals. Focal dissolution of cells in the adrenal cortex resulted in a pseudotubular transformation. The lymphoid follicles in the intestinal wall showed active germinal centers, as did the follicles in the mesenteric lymph nodes. The spleen was congested, but showed little activity in the lymphoid follicles. In skeletal muscle there was occasional swelling, hypereosinophilia, and hyalinization of the muscle fibers.

The cellular alterations described above were encountered in various degrees in all cases. Since all patients had shown a period of hypotension, these changes may be a manifestation of an impaired circulation. However, the distribution of the abnormal cells in the organs mentioned suggests that cytotoxicity may also be a factor.

Because cytotoxicity was suspected, such cellular changes were sought in guinea pigs subjected to oral challenge with the cholera vibrio. In these experiments, which were carried out at the Walter Reed Army Institute of Research, the guinea pigs were starved for 96 hours and then 10 million vibrios were introduced by mouth, this being followed by the injection of tincture of opium to delay the passage of bowel contents. When examined 8 hours after challenge, a slight increase in cellularity of the jejunal villi and marked hyperemia of the tunica were evident (fig. 2). By this time, loss of fluid into the bowel lumen had commenced. At 18 hours after challenge there was a more intense cellular infiltrate of the lamina propria which, combined with an amorphous precipitate, resulted in bulging of the villi and in an appearance resembling that observed in acute cholera in man (fig. 3). At this time exsorption of fluid was larger, but the clinical state of the guinea pig appeared good. Examination at this time showed focal myocardial damage and degeneration of pancreatic acinar cells, as had been observed in the human cases. This would support the suspicion that cytotoxic damage may occur in cholera in-

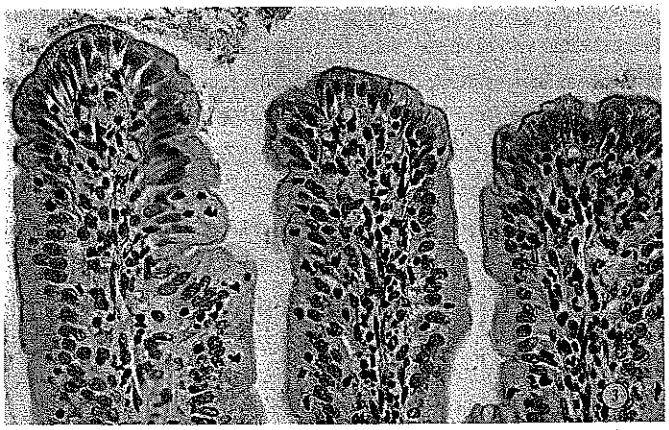

FIGURE 3.—Guinea pig. Specimen of proximal ileum obtained 18 hours after oral challenge with cholera vibrios. At this stage, there is an increase in the cellularity of the lamina propria. Many features of this preparation are shared with that of figure 1, which illustrates cholera at a comparable early stage in man. The epithelial covering of the villus with its brush border is intact. Between the epithelial cells are spaces which extend into the lamina propria. Maximum exsorption of fluid usually occurs at this stage following challenge. (Hematoxylin and Eosin.)

fection, and is in accord with the observations reported by J. K. Read (2) and J. P. Craig (3) at this symposium.

However, certain differences between the human and the experimental disease must be noted. Myocardial changes in guinea pigs were characterized by the presence of increased numbers of Anitschkow myocytes in the perivascular areas and occasionally in subendocardial zones. We noted a similar response in hearts of guinea pigs subjected to experimental shigellosis. Frank necrotic changes with lymphocytic infiltration, as noted in some of the human cases, were not seen. Pancreatic acinar cell changes were limited to fine vacuolization and swelling of the cytoplasm with rare nuclear pyknotic changes. Seifert (4) described similar changes in salivary glands following autonomic nervous stimulation. The role of the autonomic nervous system in cholera, particularly in its periphery, is currently under active investigation by the Walter Reed group (5).

Electron micrographs were prepared at the Walter Reed Army Institute of Research on specimens collected from guinea pigs subjected to oral challenge with cholera vibrios, as described above. At 8 hours after challenge major alterations were noted in the lamina propria of the small intestine and in the epithelial lining. Capillaries were distended and contained an electron-dense granular material. Macrophages and transmigrating neutrophils were the prominent cell types. The Golgi apparatus and endoplasmic reticulum membrane systems of the epithelial cells showed an initial swelling, while the remainder of the organelles appeared unchanged. At 18 hours there was less electron-dense granular material in the capillaries, with beginning signs of red blood cell packing. Spaces around the capillaries and other interstitial spaces had become widely patent. Neutrophils and macrophages continued to be present in large numbers, whereas lymphocytes were scarce or entirely absent. Epithelial cell damage consisted of sustained swelling of the Golgi apparatus and endoplasmic reticulum. Numerous microvesicles appeared in the cytoplasm at this time. By 24 hours an abrupt alteration had occurred in the capillaries, which now appeared densely packed with erythrocytes, suggesting an impaired capillary circulation in the form of sludging. Interstitial spaces, Golgi apparatus, and endoplasmic reticulum swelling continued at maximum levels. The cytoplasmic microvesicles were most numerous at this stage. Neutrophils and macrophages were prominent cell types of the lamina propria (figs. 4, 5, 6). By 48 hours the capillaries were empty; they were surrounded by large interstitial spaces which now communicated with spaces between the epithelial cells. At this time the swollen Golgi and endoplasmic reticulum membranes of the villus epithelium had returned to normal, but the numerous microvesicles persisted in the cytoplasm. Throughout the entire course of the infection no alterations as marked as those just described were noted in the microvilli, nuclei of the epithelial cells, or in the mitochondria. These studies, which place importance on the role of the capillaries, are in agreement with the findings of J. P. Craig (3), which implicate a vascular factor in the subcutaneous lesions produced by the cholera toxin in the guinea pig.

It should be noted that 8 hours after challenge, when fluid exsorption begins in the guinea pig intestine, the major abnormality seen by electron microscopy involves the capillaries and cytoplasmic membrane systems. Between 18 and 24 hours, when fluid exsorption is at its maximum, striking changes occur not only in the capillaries but also in the interstitial tissue and the cytoplasmic microvesicles, which become quite numerous. Between 24 and 48 hours many of the guinea pigs manifest an elevation in hematocrit, become moribund, and usually succumb by the end of the second postchallenge day. The concentration of major alterations in the capillaries, the interstitial tissue, and cytoplasmic vesicles, with only minor changes recognizable in the epithelial cells, suggests an abnormal hydrodynamic transport of fluid, possibly through a bypass mechanism.

The mucosal lesion observed in cholera infection in both man and the experimental animal differs from that seen in the other common acute diarrheal diseases, such as those caused by enteropathogenic *E. coli,* the Shigellas, and the Salmonellas. These are characterized by necrosis of epithelium, exudation of leukocytes and high protein-containing fluid, and ulceration (6). These bacteria are invasive and can be isolated from the lamina propria (7, 8). In enteropathogenic *E. coli* and *Salmonella* infections, bacteria can be isolated also from mesenteric lymph nodes and occasionally from the bloodstream. Thus, the infection produced by the cholera vibrio can be distinguished from that produced by those bacteria which cause an exudative enteritis, since the vibrio does not invade the mucosa and does not destroy it (9).

It is appropriate to conclude this report with reference to a form of diarrheal disease which we observed in Guatemala and which possesses some of the features of cholera vibrio infection (10, 11). In this childhood population, malnutrition and diarrheal disease were common, but specific enteropathogens were isolated in numbers too few to account for the diarrheal disease (12). Nor could it be established that malnutrition by itself was a cause of diarrheal disease. In our autopsy study of consecutive deaths on the pediatric service of the Roosevelt Hospital

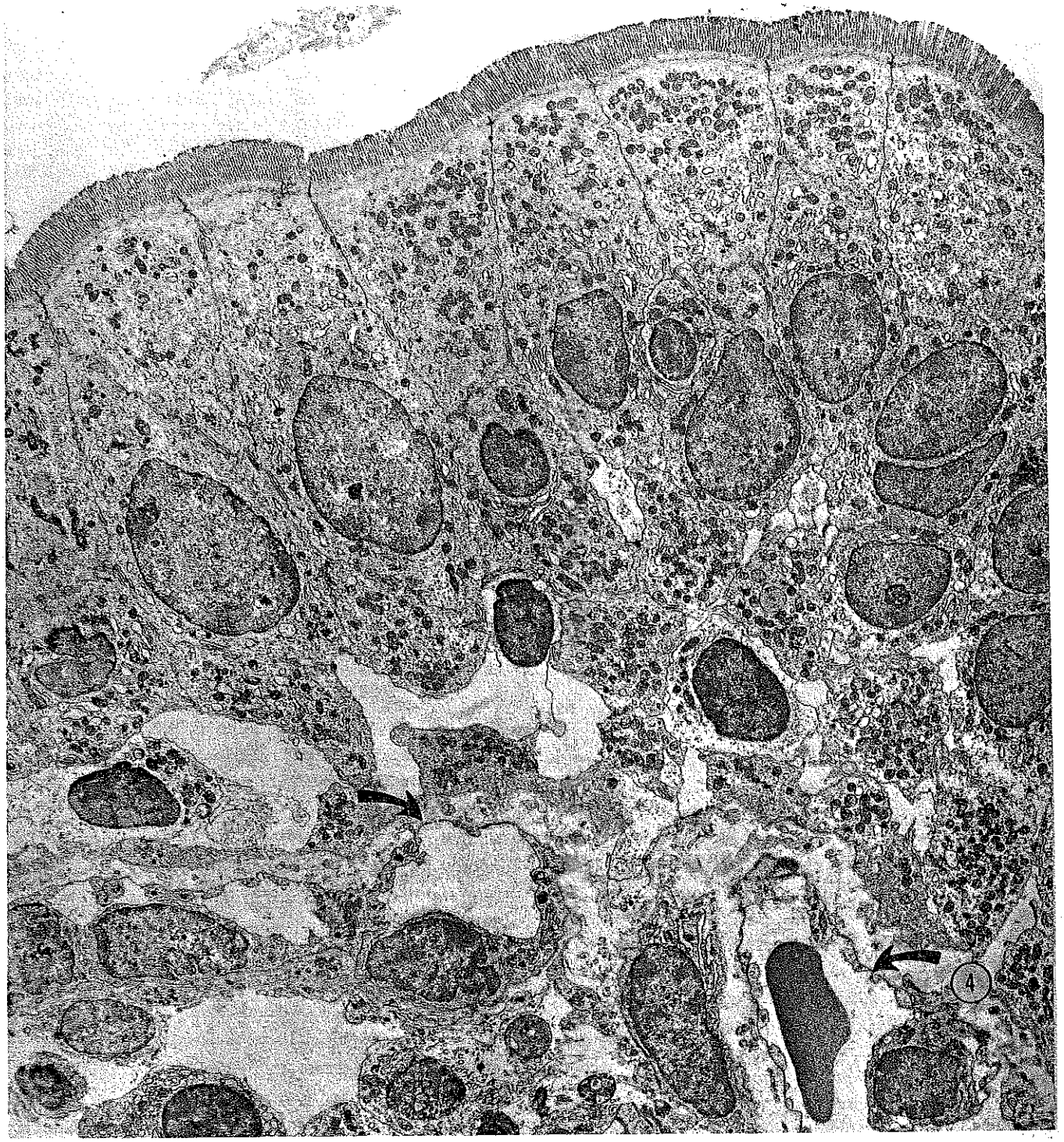

FIGURE 4.—Guinea pig (control). Ileum. Food but not fluid withheld for 96 hours. At the upper left, there are intact rows of microvilli. Capilalries are thin walled (see arrows) and contain no electron-dense material. (Magnification X6000).

in Guatemala City we noted that those patients who had malnutrition and diarrheal disease in the absence of specific enteric pathogens, harbored abnormally large populations of nonpathogens in the proximal small intestine, a portion of the intestinal tract which is usually sterile. Examination of the small intestine in these cases showed a histologic pattern resembling the nonexudative response which typifies cholera. We suspect, therefore, that in those parts of the world where malnutrition is common children may succumb to diarrheal disease which must be regarded at present as nonspecific, and which has some of the features of infection with the cholera vibrio.

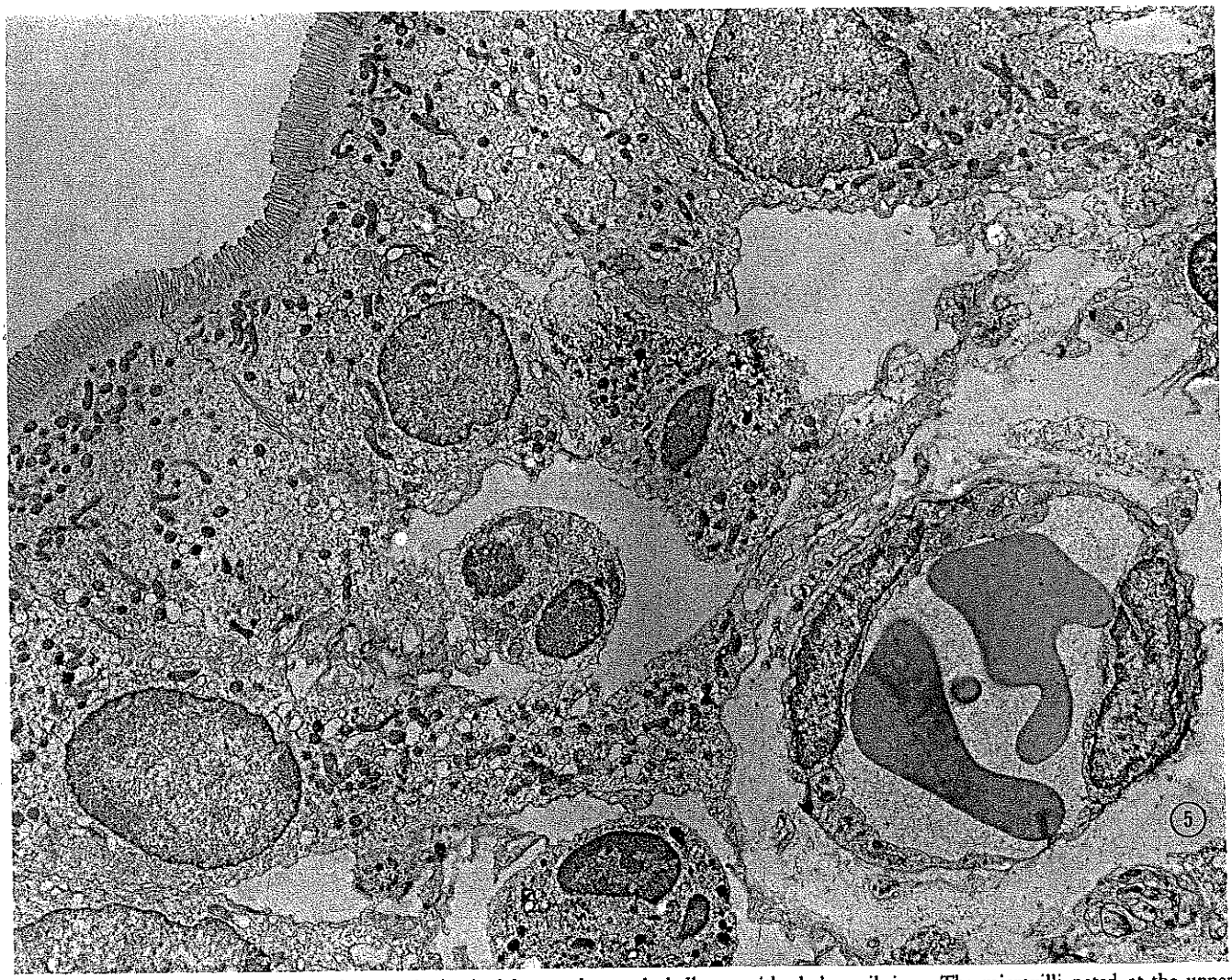

FIGURE 5.—Guinea pig. Specimen of ileum obtained 18 hours after oral challenge with cholera vibrios. The microvilli noted at the upper left are intact. The large capillary at the lower right shows the lumen uniformly filled with electron-dense granular material. Prominent spaces surround the capillary and the subepithelial portion of the villus. Within the cytoplasm of the epithelial cells there are numerous swollen vesicles. (Magnification X7000.)

SUMMARY

Fatal cholera in Dacca has been observed in patients who had no atrophy of the intestinal mucosa or other evidences of malnutrition or malabsorption. This contrasts with the intestinal mucosal pattern encountered in West Pakistan and Thailand.

In the fatal cases studied the mucosal pattern typical of cholera was present. There was also a disseminated cellular damage which may have resulted indirectly from the infection, e.g., from shock, hemoconcentration, effect on the autonomic nervous system, etc., or from direct cytotoxicity, or both. Similar cellular abnormalities were noted also in experimental cholera before circulatory derangement had become severe. The mucosal patterns in acute cholera of man and the guinea pig have many points of similarity.

The exsorption of fluid into the intestinal lumen of the cholera-challenged guinea pig is observed by electron microscopy to involve major changes in the mucosal capillaries, the interstitial tissue, and the cytoplasmic vesicles of the epithelial cells. These changes occur in the absence of any readily apparent modifications of the microvilli, nuclei, or mitochondria of the epithelial cell.

Among infants and children where malnutrition is common, fatal diarrheal disease may occur in the absence of specific enteric pathogens, but in the presence of abnormally high populations of bacterial nonpathogens in the proximal small intestine. The mucosal pattern observed in such cases resembles the nonexudative inflammatory response which is found in cholera.

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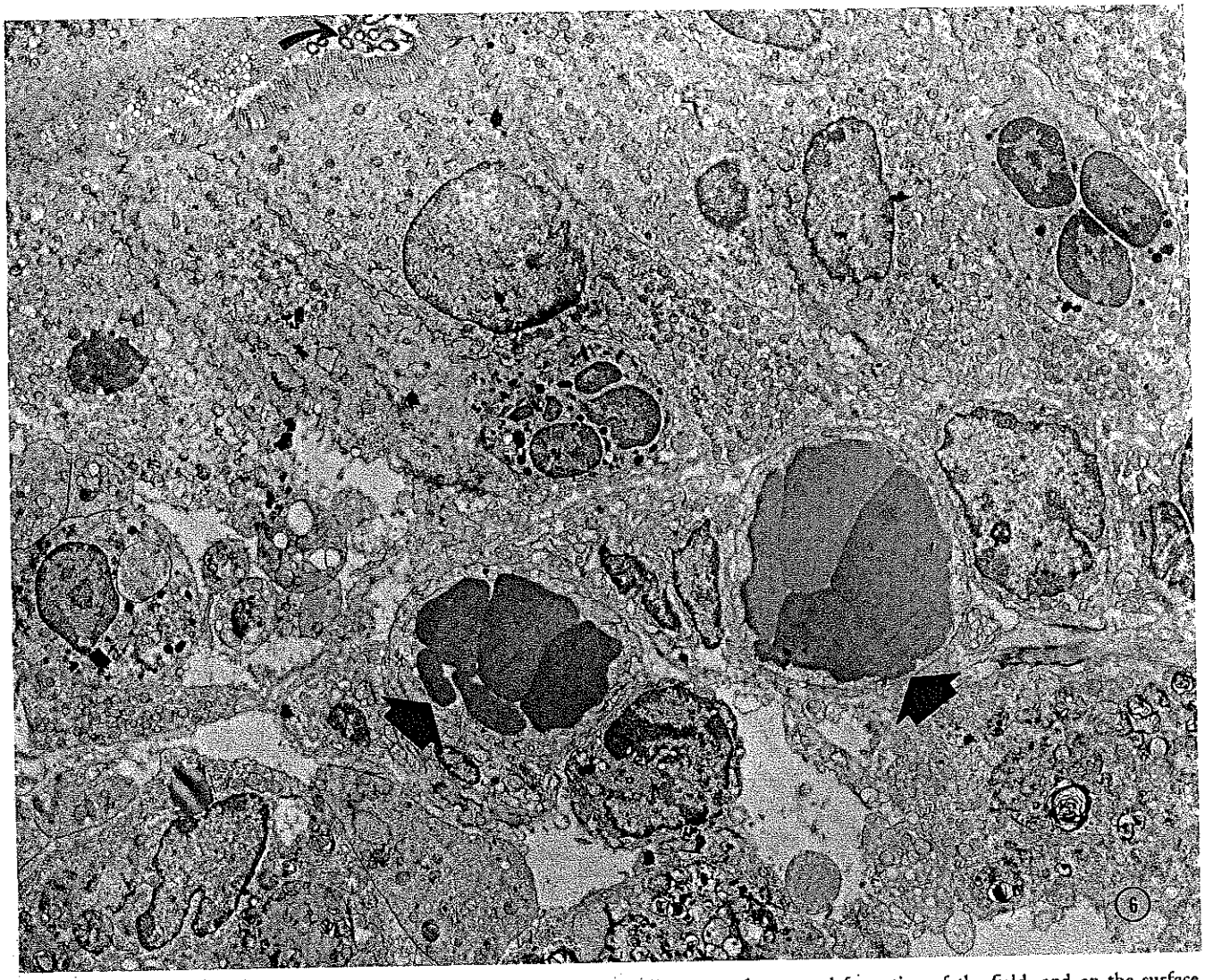

FIGURE 6.—Guinea pig. Ileum at 24 hours postchallenge. Intact microvilli occupy the upper left portion of the field, and on the surface of the microvilli there are several vibrios (see arrow). The prominent alteration is in the capillaries (see arrows). Note the packing of the erythrocytes and the disappearance of plasma from the capillary lumen. Spaces can be noted in the lamina propria and beneath the epithelial cells. Vesicles in the epithelial cells are as swollen and prominent as in the 18-hour stage (fig. 5). (Magnification X6000).

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Round Table Discussion: Management of Patients in Cholera Epidemics Where Ideal Facilities Are Not Available

Drs. C. C. J. Carpenter, R. N. Chaudhuri, J. Lindenbaum, A. Mondal, R. A. Phillips, A. K. M. Abdul Wahed, C. K. Wallace, K. Kobari, and R. Gordon, Chairman

Dr. Gordon. As a clinician I have a good deal of confidence in my own ability to treat cholera with success. On occasion, however, I have been in the unenviable position of finding my knowledge of no avail, because cholera was raging in a community where, for economic or merely logistic reasons, the facilities required for treatment were not available. It seemed appropriate, therefore, to hold a discussion of plans for simplified treatment that might be applicable in circumstances where ideal facilities cannot be realized.

With apologies to you all, I decided that no one person was qualified to tell us what the solution to this problem should be. The reason for this round table discussion is to bring together those ideas which we may have formulated. There may be places where we agree; there may be places where we disagree. We should be able to formulate here some plan of clinical studies to test these agreements and disagreements. The aim is to reconcile the ideal of no mortality with the practical problems posed by the social and economic realities of life in a cholera-endemic zone. The unpredictability of outbreaks, the inaccessibility of those outbreaks, and the acuteness of the disease pose the real problem. The facilities must be already on the site or must be moved into the affected area with great speed. Rarely can the suffering patient be moved out of his home to some large center for treatment. We then face logistic problems which require solution. We must minimize requirements for supplies, for bed space, and for highly trained personnel. Every step forward in this direction may save lives by the thousands.

I should like each participant to make a brief summary of his opinions on the subject, based on his personal experience in dealing with these problems. To start with, I shall call on Captain Phillips.

Captain Phillips. It seems to me that we must have some intravenous fluid available, and we would all agree that the use of antibiotics, if we have them, will shorten the disease and cut down the requirements for intravenous fluids. I think we would all agree that it is very simple to maintain hydration of the patient, because you know what goes out, and what must be put back in again. Of course, you have to allow for insensible water loss. I think the only place where we would differ with our colleagues from Dacca is in the method of assessing the degree of dehydration when the patient enters. We feel that specific gravity determinations provide the best method for accurate estimation of fluid requirements, and that clinical evaluation alone is not always adequate for this purpose. According to measurements of venous pressure and plasma volume a patient can be rather seriously dehydrated, but his systolic pressure, diastolic pressure, and pulse rate do not always reflect this.

Dr. Wallace. I would venture to say that, although there are some differences of opinion among all of us here about treating cholera in the field, all of us have had considerable success. We agree that the mainstay is fluid replacement. But how should this be given? Here we need an *objective* criterion to guide us. I agree with Captain Phillips that this should be the determination of plasma specific gravity. We have said in the past that

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