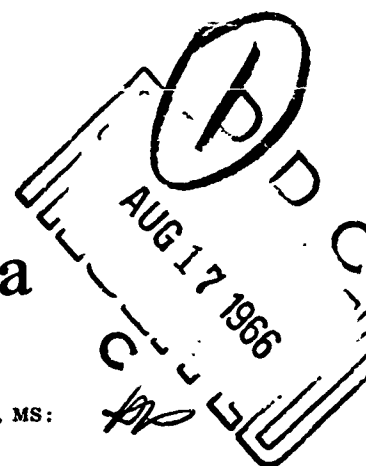




Experimental Cholera

Morphologic Evidence of Cytotoxicity

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Pretreated guinea pigs fed a relatively heavy dose of *Vibrio cholerae* in a young broth culture have shown acute enteritis with degenerative lesions of the endothelial cells of the lamina propria. In addition, focal cardiac, hepatic and pancreatic lesions have been found. The findings suggest that a systemic toxic effect occurs in this model.

IN 1855, Horatio Jameson¹ suggested that a pathologist seeking to understand the cause of cholera by interpreting postmortem tissue changes is like a person who finds the old clothes of one who has run away. Recent conceptual advances in pathology and instrumentation, however, have allowed more exact studies of the pathogenesis of this disease. The majority of the recent investigations of the pathophysiologic alterations have been applied largely to studies of the remarkable fluid and electrolyte losses which occur into the gastrointestinal tract both in man and in experimental animals.

For over 100 years it has been argued whether, in addition to or aside from, electrolyte and fluid imbalance or a "toxin" or "toxins" cause or influence the death of cholera victims at various clinical stages of their disease. Although the cholera vibrio does not invade the hosts' tissues, several observations have been made implicating toxic activity. De et al have proposed that absorbed toxins may explain the hemolysis that exists in many cases² and the hyper-

trophy of lymphoid tissue in the presence of lymphopenia.³ Cholera endotoxin is known to have a hypotensive effect when injected intravenously⁴; it is injurious to mammalian cells in tissue culture⁵; and may be the cause of elevation of C-reactive protein reported in human cholera.⁶ Similarly, toxicity may well cause "cholera sicca" in which there is little loss of fluid in the face of a profound state of collapse. There is no proof yet that endotoxin absorption occurs in the natural or experimental disease. Clinical cases of cholera have been observed, however, in which the severity of the disease state could

Fig 1.—Midileum section 18 hours after infection showing congestion and cellular infiltration of the lamina propria with uneven staining of epithelial cells (hematoxylin and eosin; $\times 300$, reduced about 15%).



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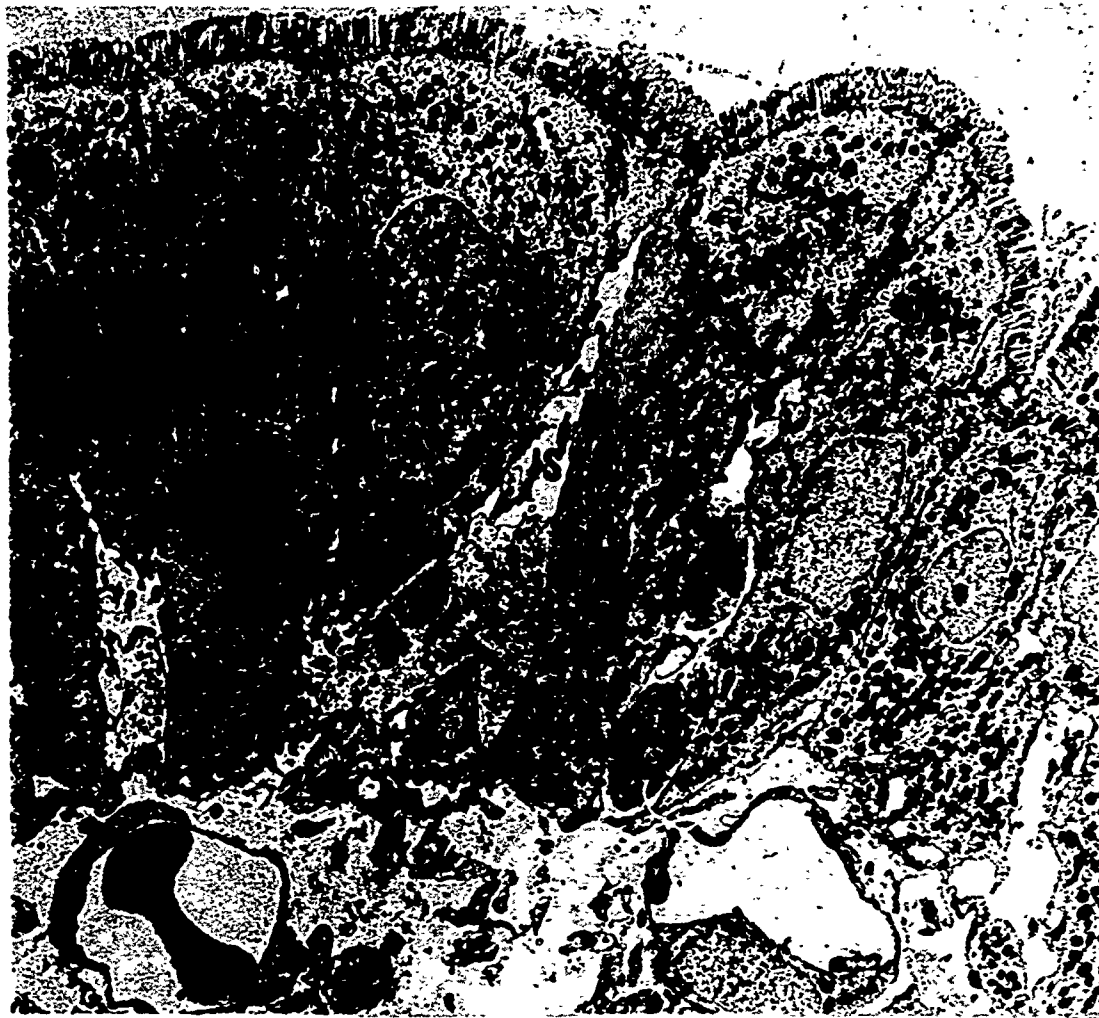


Fig 2.—*Top*, Electron micrograph of the ileum eight hours after cholera infection. Epithelial cells are relatively unaffected except for swelling of the endoplasmic reticulum and increased number of lysosomes (*L*) and dense bodies (*DB*). Several transmigrating neutrophils appear between epithelial cells. Separation of intercellular borders have created large inter-epithelial spaces (*IS*) from which fluid has been extracted. Distended interstices of the tunica propria are similarly empty, also due to fluid extraction (osmium fixation, Epon-Araldite embedding, $\times 4,200$). *Bottom*, Portions of two adjacent epithelial cells showing swollen appearance of the Golgi apparatus (*G*) and endoplasmic reticulum (*ER*) in detail (osmium fixation, Epon-Araldite embedding, $\times 24,000$).

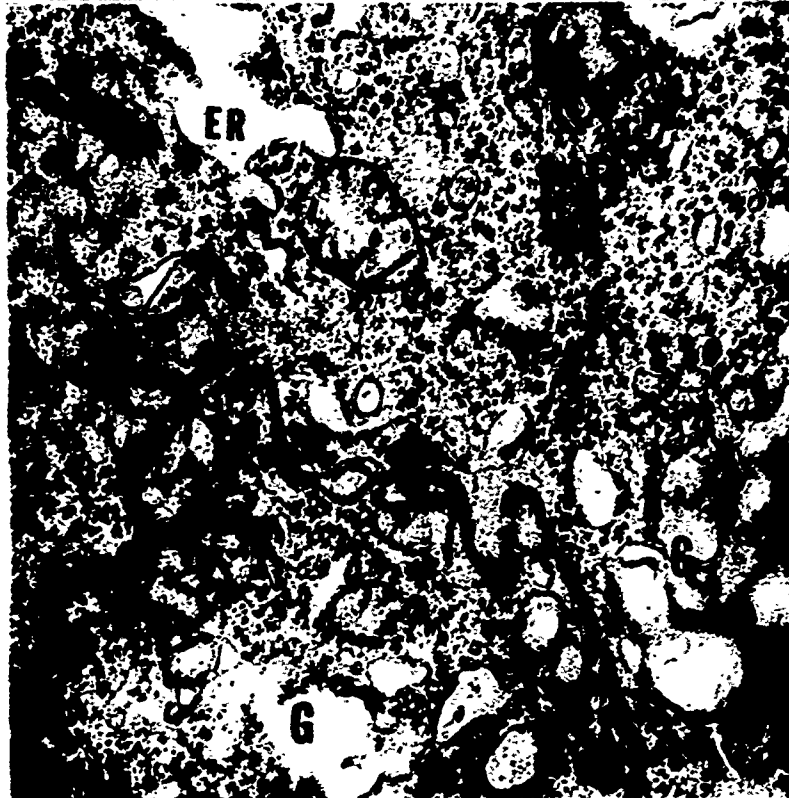




Fig 3.—Ileal capillary eight hours after cholera infection. Endothelial cytoplasm of one cell (E_1) has lost most of its organelles, is swollen and rarefied. The other less severely affected endothelial cell (E_2) contains only degenerating mitochondria (M) and numerous ribosomes. Swollen endothelial cells have almost completely obliterated the capillary lumen (L). At this magnification intercellular junction separation is not too apparent ($\times 9,000$). *Inset*, Higher magnification of endothelial cell cytoplasm (E_2) showing the degenerating mitochondria in greater detail ($\times 15,000$).

not be attributed to fluid loss alone. A toxic factor has therefore been postulated. We believe that a reexamination of this controversy is in order. This report is part of a morphologic study of the systemic effects of cholera infection utilizing the guinea pig model.

Materials and Methods

Adult Walter Reed strain guinea pigs of either sex previously maintained on a normal chow diet were deprived of food but not of water for 96 hours.

After this period of starvation approximately 10^8 vibrios of an 18-hour old culture of *Vibrio cholerae* (Inaba 2867) suspended in brain-heart infusion broth were fed to them by stomach tube. To retard peristaltic activity, 1.0 ml of tincture of opium was then given to the animals intraperitoneally. The animals were sacrificed at 8, 12, 18, 24, and 72 hours by a blow to the head. A complete autopsy was done in each instance. Tissues were fixed in formalin. Ileal specimens for electron microscopy were fixed in phosphate buffered OsO_4 or glutaraldehyde followed by OsO_4 , and were embedded (Epon-Araldite mixture). Virulence controls re-



Fig 4.—Myocardial arteriole, 24 hours after cholera infection, showing Anitschkow myocytes at the periphery (hematoxylin and eosin, $\times 198$).

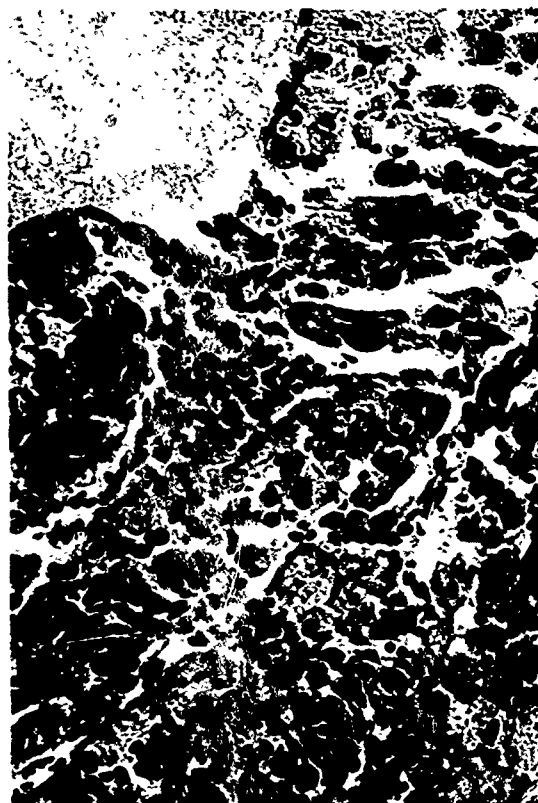


Fig 6.—Myocardial lesions adjacent to a papillary muscle with predominantly lymphocytic infiltration: 24 hours postinfection (hematoxylin and eosin, $\times 384$).



Fig 5.—Higher magnification of Fig 4 to show Anitschkow myocytes in greater detail (hematoxylin and eosin, $\times 700$).

vealed up to 85% mortality within 72 hours after feeding of the cholera culture. There were 72 controls and 68 experimental animals used in this study.

Results

Although many organs were sampled, we were able to find pathologic alterations only in the small intestine, the heart, pancreas, liver, and occasionally in the kidneys.

Intestine.—The earliest intestinal changes

by light microscopy are an increase in the cellularity of the lamina propria associated with congestion of the submucosal blood vessels (Fig 1). The cellular infiltrate, predominantly neutrophils and macrophages, increased steadily during the first 24 hours. The villi appeared to bulge somewhat. The epithelial lining remained intact but individual cells tended to stain unevenly and in some areas the lining had a "scalloped" appearance. Electron micrographs revealed changes in the endothelial cells of venules and capillaries within the lamina propria which we believe represent a toxic degenerative effect. The intestinal epithelial cells exhibit minimal changes of the Golgi apparatus and of the endoplasmic reticulum (Fig 2). The continuity of the striated border is preserved. Interepithelial spaces are found in areas where they do not normally appear. At such sites transmigrating inflammatory cells are seen. At this time the formerly empty interstices of the tunica are filled with plasma-like fluid which extends beyond the epithelial basement membrane into the

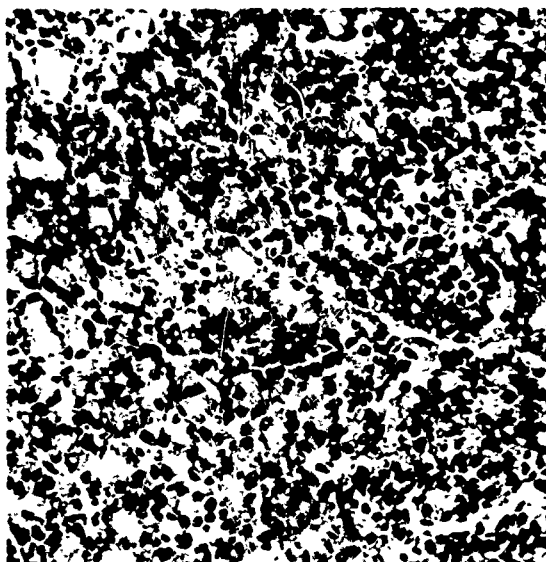


Fig 7.—Guinea pig pancreas 24 hours postcholera infection showing diffuse acinar cell vacuolization (hematoxylin and eosin, $\times 204$).

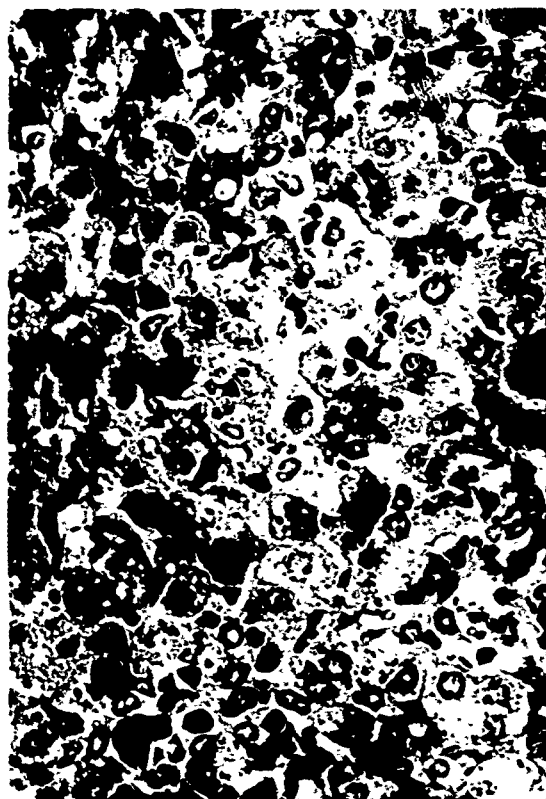


Fig 8.—Area of hepatic necrosis 24 hours postcholera infection (hematoxylin and eosin, $\times 318$).

widened epithelial spaces. In the endothelial cells the mitochondria and Golgi bodies are very often degenerated and there are many pinocytotic vesicles in the cytoplasm. A separation of the endothelial cell junctions has on occasion been noted. These changes exist prior to any significant changes in the epithelial cells. An extreme degree of en-

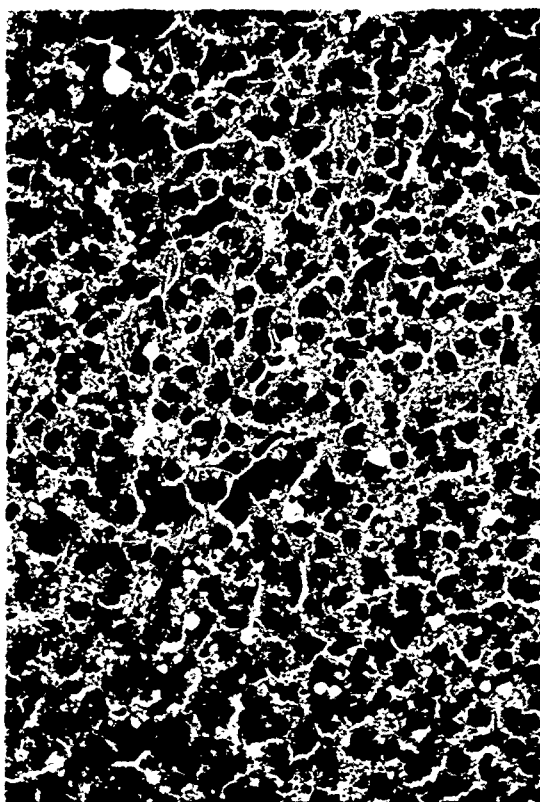


Fig 9.—Hepatic damage 24 hours postcholera infection showing necrotic changes manifested by hyper eosinophilia and pyknotic nuclei (hematoxylin and eosin, $\times 260$).

dothelial cell damage was noted at the eight-hour period (Fig 3). The injured cell exhibits swelling of the cytoplasm with severe loss of cytoplasmic components. During the 24-hour period postinfection, there were increasing amounts of clear fluid found in the small intestinal lumen. This is considered to be evidence of choleragenic activity.

Heart.—Frank necrosis was only rarely seen in the guinea pig. In 78% there existed a striking increase in Anitschkow myocytes. These cells appear to originate at the periphery of arterioles within the myocardium (Fig 4 and 5) and are scattered through the heart especially in subendocardial areas by 24 hours postinfection. No prevalent area for myocardial damage could be detected. Mononuclear cells and Anitschkow myocytes were the dominant cell types noted in damaged areas (Fig 6). Cardiac damage in the guinea pig was not fully comparable to that described by Dammin et al⁷ to occur in the human disease. They described mild diffuse myocarditis in five human autopsies characterized by foci of coagulation necrosis,

enlargement of myocardial nuclei and an increase in the number of interstitial cells present.

Pancreas.—Sections of the pancreas did not show alterations of the islets of Langerhans. The exocrine pancreas, however, exhibited diffuse fine vacuolization and occasional swelling of the cytoplasm in 45% of the animals (Fig 7). Nuclear pyknotic changes were also found although less frequently. There was no inflammatory infiltration associated with these changes.

Liver.—The liver sections evidenced variable degrees of fatty change which can be attributed to the lack of food during the preinfection period of the experiment. By 24 hours, there were focal areas of necrosis in 62% of the cholera animals (Fig 8). All lobular zones were affected although the majority of the lesions were midzonal. Such areas contained acute inflammatory cells. As in the human cholera cases,⁷ the cytoplasm of other hepatic cells at times had increased affinity for eosin (Fig 9). Such cells were probably in an early stage of death and were often finely and diffusely vacuolated with hyperchromatic nuclei. The biliary ducts were unremarkable.

Kidney.—Renal cortical ischemia has been described by De⁸ to occur in some cases of human cholera. Ischemia was not recognized in the guinea pig. Parenchymatous lesions were quite rare and were limited to a non-specific diffuse vacuolization and granularity of the tubular epithelium. Such an appearance cannot be charged to the presence of

toxemia but may reflect the acute loss of fluids and electrolytes into the intestinal lumen.

Comment

In the guinea pig as well as in the human disease, the vibrio is not invasive and so the factors causing fluid loss and cellular damage must be absorbed. The degeneration of the endothelial cell is only appreciated visually through electron microscopy and is possibly one of the underlying factors in the initiation of intestinal fluid loss. Similar changes were also seen in two recent cases of human cholera that were biopsied and studied in our laboratory. The heart lesions may have been a direct effect of a cholera toxin. The Anitschkow myocyte is among the earliest of cells to respond to toxic influences upon the myocardium.^{9,10} The increased numbers of these cells and the focal myocardial injury seen suggest the existence of a toxic myocarditis. Interpretation of autopsy findings in human cholera is hampered as by the time the victim dies, many factors such as shock and fluid and electrolyte imbalance may exist and have great influence upon the histopathologic features of the tissue. Fluid loss alone cannot be considered the sole causative factor for the various anatomic changes described in the guinea pig.

The focal tissue changes found in this experimental model suggest that a systemic toxic effect exists in acute cholera infection.

Dr. Samuel Formal assisted this study. SFC Thomas Burley, Mr. Smiley Austin, and Mr. Giles White provided technical assistance.

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