flurothyl and ISO, revealed a marked difference which might be considered responsible for their respective pharmacologic responses. The surfactant phenomenon was not a constant finding with all fluorinated, convulsive ethers. We were unable to establish any relationship between this physical property and pharmacologic response; however, it was sufficiently unusual and interesting to warrant exploration.

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Received November 16, 1966. P.S.E.B.M., 1967, v124.

A Vascular Permeability Defect in Experimental Cholera. (31862)

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The nature of the pathophysiologic lesion in cholera has remained undefined following the demonstration by Gangarosa *et al*(1) that the intestinal epithelial lining remained intact in the human disease. The introduction of an experimental model (the suckling rabbit) faithfully reproducing almost all facets of cholera in man(2,3), and the discovery of a cell-free vibrio product, choleragen, which causes cholera in this animal model(4) as well as in the human(5) have allowed laboratory investigation of the two major hypotheses of pathogenesis: *1*) inhibition of sodium pump activity(6), and *2*) increased vascular permeability(7).

Choleragen has been shown to have no significant effect upon ion transport systems in the short circuited frog skin (Neptune, E. M., personal communication), or the everted rabbit ileal loop (Field, M., Schultz, S. G., personal communication). Intact sodium pump activity has recently been demonstrated in adjacent control and choleragen treated ileal loops of adult rabbits (Norris, H. T., Schultz, S. G., Curran, P. F., Finkelstein, R. A., submitted) showing that enterosorption of fluid in the choleraic loop proceeds without inhibition of pump activity. In contrast, choleragen is an exceedingly potent permeability factor, causing a "delayed-prolonged" response in rabbit skin when injected in sub-microgram amounts(8). The present communication adds to the evidence that experimental cholera involves an alteration of vascular permeability by the demonstration of such an effect in the intestinal villi of infant rabbits made choleraic with choleragen.

Methods.‡ The technique of "vascular labelling"(9) was employed. This involves intravenous injection of a visible colloidal marker, for example India ink, which is trapped by the leaking vessel, thereby identifying it. In the present study labelling with carbon black could not be demonstrated. Instead a commercial iron-dextran complex (Imferon, Lakeside Laboratories) was successfully utilized. This material contains iron coupled to dextran and appears to be of high molecular weight, exceeding 150,000 and approximately 3-4 mμ in size(10). Purified choleragen was prepared and fed *via* stomach tube to infant rabbits pretreated by gastric lavage as previously described(7). At intervals following administration of 50 μg of choleragen, a single dose of iron-dextran was injected intravenously in a volume of 0.1 cc through a

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‡The principles of laboratory animal care as promulgated by Nat. Soc. for Med. Res. were observed.

cut down on the femoral vein, and the animal was sacrificed. The interval between injection and sacrifice was not critical, and animals were sacrificed between 10 and 60 minutes following iron-dextran. The abdominal wall was opened and 2-3 cm lengths of intestine, including duodenum, first portion of jejunum, mid jejunum, and ileum were removed, opened lengthwise, and pinned out on paraffin under formalin. After 24 hours of fixation, villi were scraped off with a scalpel blade and fixed to glass slides with Mayers egg albumin. The tissue was stained for iron by the Gomori technique, and examined by transmitted light. Titration of iron-dextran in the untreated control animal established that a dose containing 2.5 mg of iron per 100 g of animal failed to produce detectable staining of iron in the villus vessels, even in animals sacrificed immediately following injection. A dose of iron-dextran equivalent to 1.25 mg iron per 100 g of animal was selected and used throughout the experiments to be described. Gomori stained sections from control and choleragen treated animals not given Imferon were negative.

Results. Sixty-two animals were examined (Table I). Iron was detected in villus vessels beginning 90 minutes after administration of choleragen (Fig. 1). Only 1 of 9 animals examined between 90 and 120 minutes was negative for iron. At this time, approximately 2 hours before initiation of overt choleraic diarrhea, fluid was just beginning to accumulate within the intestinal lumen. The iron was localized almost exclusively in the venular loop (compare Fig. 1 and 2) and was demonstrable during the entire course of the choleraic syndrome. All but 4 of 34 experimental animals sacrificed between 2 and 24 hours after choleragen were positive for iron. Of these 4 animals, 3 were sacrificed between 2 and 2½ hours, and did not reveal evidence of response to choleragen. The fourth animal did not manifest diarrhea, although a moderate intraintestinal accumulation of fluid was found at sacrifice 23 hours following choleragen. It is possible that the intestinal lesion in this mild illness healed prior to injection of iron-dextran resulting in a paradoxical failure to "label" in the presence of residual evidence of disease.

TABLE I. Summary of Results in Experimental and Control Animals.

Category		No. positive for iron/ Total No. of animals
Control		0/12
Choleragen treated*	Sacrificed before 2 hr	8/16
	Sacrificed after 2 or more hours	30/34

* 50 μg of purified choleragen(7) administered *per os* in phosphate buffer. All animals received intravenous iron-dextran, 0.025 ml per 100 g of animal.

With this single exception, the distinction between the control and choleraic animal was clear. However, even in choleraic animals involvement was incomplete and not all villi were positive for iron. Staining was spotty and gave a discrete mottled appearance (Fig. 3), tending to diminish in degree in sections obtained from progressively distal sites, particularly in animals sacrificed in the first few hours after choleragen administration. Because all portions of the small bowel were involved, indicating the action of choleragen to be general, the differences observed between proximal and distal sites may represent dilution, proximal binding, or inactivation of choleragen as it proceeds through the intestine. No staining of colonic vessels was observed, consistent with the concept that cholera is a small bowel disease and supporting the specificity of these findings.

Discussion. This study clearly demonstrates an alteration of vascular permeability in the intestinal villus in experimental cholera initiated by choleragen. It is noteworthy that a series of experiments conducted with colloidal carbon yielded entirely negative results. Colloidal iron-dextran was then tested because, as a smaller particle, it might demonstrate leaks too small for the passage of carbon. As carbon labelling has been successfully used to demonstrate choleragen induced vascular lesions in skin(8) the response of the villus vessel appears different in this regard. This situation is unique and the reasons are not known at present.

Electron microscopic studies of intestinal tissue in cholera(11,12) have shown separation of the endothelial intercellular

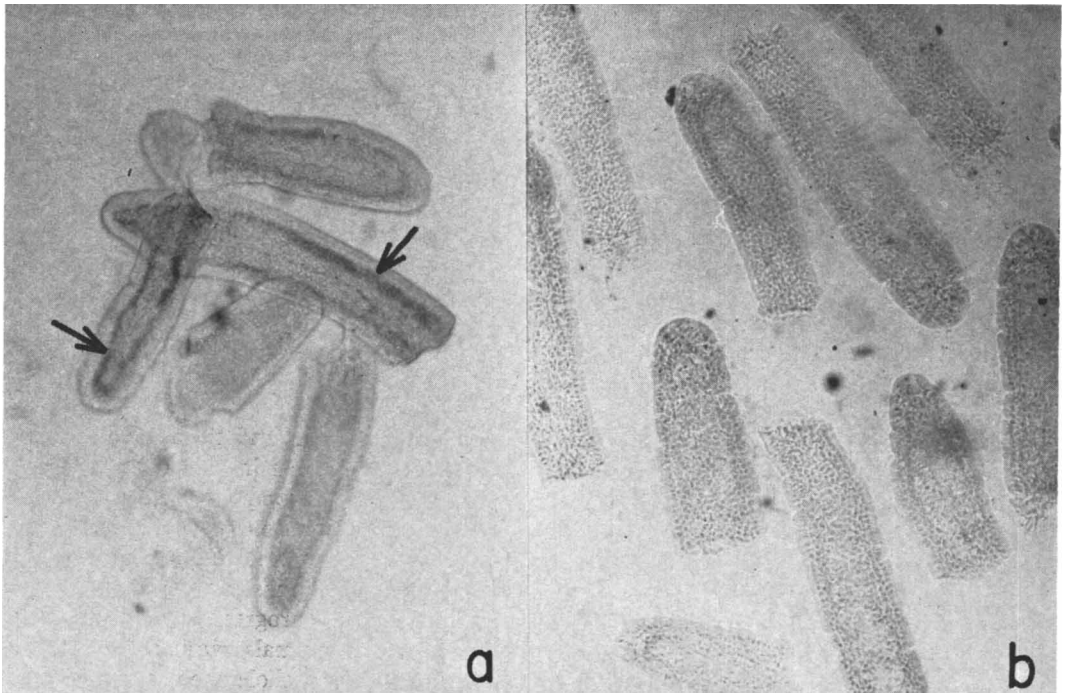

Fig. 1. Gomori stained villi from Imferon injected animal with cholera (1 a.) and Imferon injected control animal (1 b.). Arrows indicate vessels with stainable iron; no iron can be seen in the control. 76 X, No. 29 red filter.

junction, similar to the findings in histamine injury in other tissues(13). The discrete mottled nature of the deposition of a colloidal marker, such as seen with iron-dextran in the present study, is said to be characteristic of such open intercellular junctions(14). Electron microscopic study of tissue from Imferon injected rabbits should resolve this point in the animal model.

The time course of the response is one of delayed onset and prolonged duration, similar to thermal injury(15) or vascular leaks induced by other bacterial permeability factors (16). The vessel involved, however, is primarily the venule as seen in histamine mediated responses(9). The significance of this is not known.

The permeability change detailed in this communication is consistent with the clinical course of cholera. Choleraic diarrhea continues for 24 or more hours following elimination of viable vibrios by effective antimicrobial therapy(17,18), indicating that the pathophysiologic event once initiated cannot be quickly shut off, even after elimination of the responsible organism. In experimental disease induced with choleragen in a human volunteer, diarrhea continued for 72 hours following a single oral dose(5). A delayed-prolonged vascular leak can explain these findings.

Summary. A method for demonstrating vascular leakage, utilizing colloidal iron-dextran, was applied in the study of experimental cholera in infant rabbits. An increase of vascular permeability, delayed in onset and prolonged in duration, consistent with the clinical course of cholera was demonstrated in this model. The reaction resembled histamine-mediated responses in that it involved primarily the post capillary venule. However the time course differed from the usual immediate-type histamine reaction. It is suggested that this permeability alteration is a major factor in experimental choleraic diarrhea. It may be possible to test this hypothesis in human cholera with the technique described.

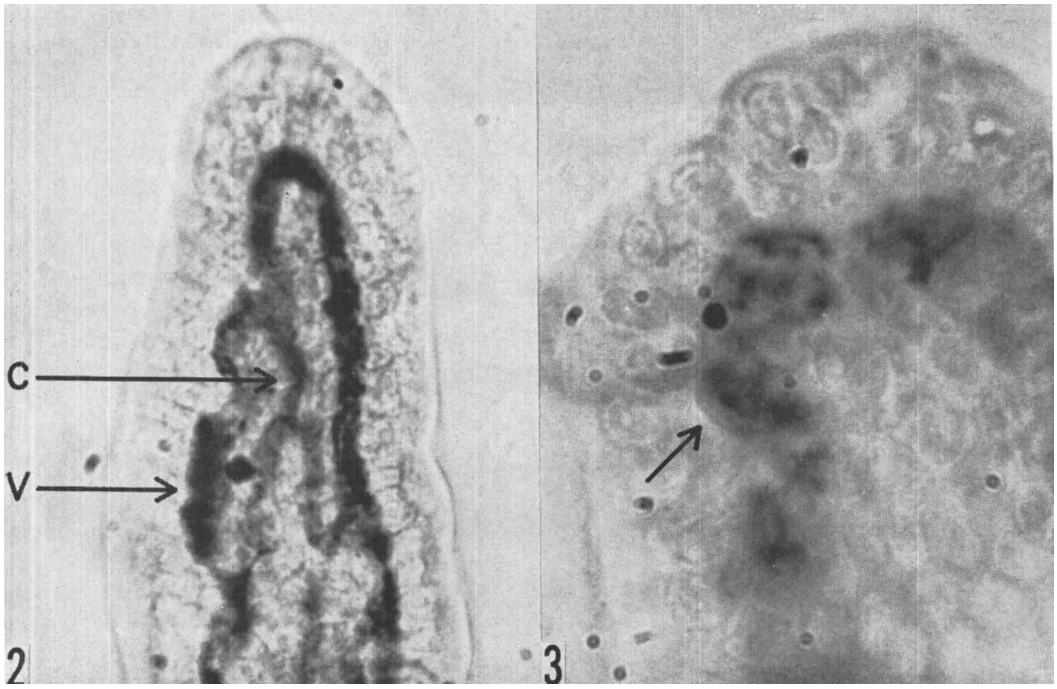

Fig. 2. Villus from a conrol animal overloaded with iron given intravenously to outline the vasculature. V is the venular loop, and C indicates capillaries. The arteriole which penetrates the core of the villus giving rise to the capillary bed is not seen. 304 X, No. 29 Red filter.

Fig. 3. Detail of villus from Imferon injected, choleragen treated animal showing the discrete, mottled nature of the stainable iron. 707 X, No. 29 Red filter.

We thank Dr. Guido Majno for his helpful suggestions. Mr. Waykin Nopanitaya, Miss Kannikar Punyashthiti and Miss Mallika NaSongkhla furnished technical assistance. The photographs were taken by Richard Buchanan, Capt., MC Dept. of Pathology, SEATO Med. Research Laboratory.

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Received November 14, 1966. P.S.E.B.M., 1967, v124.