FINE STRUCTURAL LOCALIZATION OF PEROXIDASE ACTIVITY IN THE EPITHELIUM OF LARGE INTESTINE OF RAT

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The method of Graham and Karnovsky (1) for the fine structural localization of peroxidase has been used for visualization of endogenous peroxidases in a variety of cells (2–5) known to contain peroxidase by biochemical analysis.[1, 2] In leukocytes (2), eosinophils (3, 4),[1] and macrophages,[1] as well as in thyroid and salivary gland epithelial cells (5),[2] Kupffer cells of the liver (5),[3] and epithelial cells of the rat endometrium (6), the enzyme has been demonstrated in the cisternae of endoplasmic reticulum. We now report on the

fine structural localization of peroxidase activity in mucus-secreting cells lining the crypts of rat large intestine. Except for brief mention in a histochemical study on the gerbil (7), large intestinal cells have not been shown to contain peroxidase.

MATERIALS AND METHODS

Segments of colon, duodenum, ileum, and stomach from male and female rats (Charles River strain), weighing 150–200 g and kept on a normal diet, were fixed by intraluminal instillation of a fixative containing 4% paraformaldehyde and 2% distilled glutaraldehyde in 0.15 M cacodylate buffer pH 7.4. Thin slices of tissue were then excised and further immersed in the same fixative for a total of 30 min. After thorough washing in the same buffer, 40-μ-thick sections were cut on a Smith-Farquhar tissue chopper (Ivan Sorvall, Inc., Norwalk, Conn.) and incubated in a medium containing 10 mg of 3,3'-

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[1] R. S. Cotran and M. Litt. Ultrastructural localization of endogenous and exogenous peroxidase activity in guinea pig peritoneal macrophages. Submitted for publication.

[2] J. Strum and M. J. Karnovsky. Personal communication.

[3] H. D. Fahimi. Cytochemical localization of peroxidase activity in rat Kupffer cells. In preparation.

diamino-benzidine (DAB), 10 ml of 0.05 M Tris-HCl buffer, pH 7.6, and 0.02% H_2O_2. Reactions were carried out for a maximum of 1 hr at room temperature. For control experiments, companion sections were preincubated for 20 min in Tris-HCl buffer at pH 7.6 containing different concentrations of KCN, NaN_3, H_2O_2, and 3-amino-1,2,4 triazole, followed by incubation in the complete media containing the same inhibitory agents. Some sections were boiled for 5 min in the fixative, washed, and then incubated in the complete medium. Other sections were incubated in a medium containing Tris-HCl buffer and DAB without H_2O_2. Sections were then washed in Tris-HCl buffer, postfixed in 1.3% osmium tetroxide in 0.2 M s-collidine buffer pH 7.2 for 90 min, dehydrated in alcohol, and embedded in Epon. Representative sections were also mounted for light microscopy.
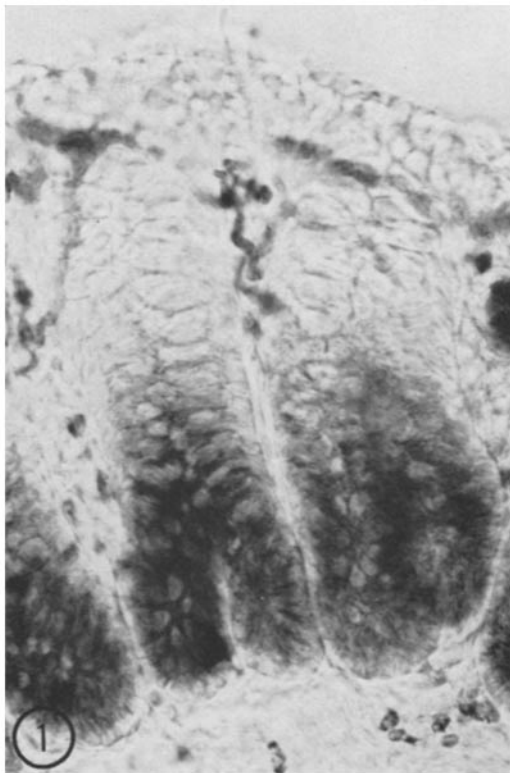
Scale line on all figures indicates 1 μ.

FIGURE 1 Light micrograph of a 40 μ section of rat large intestine, reacted for peroxidase activity. Note the positive reaction in cells in the lower portion of the crypts. The white and red blood cells in lamina propria also show some staining. X 400.

RESULTS

Examination with the light microscope revealed prominent brown staining of epithelial cells in the lower portion of the crypts of Lieberkühn of colon (Fig. 1). The reaction developed within 15 min and increased in intensity until the end of incubation at 1 hr.

By electron microscopy, the cells in the lower half of colonic crypts contained an electron-opaque reaction product in cisternae of the endoplasmic reticulum including those of the nuclear envelope (Fig. 2). Whereas the staining was observed mainly in the granular endoplasmic reticulum, a few apparently agranular segments also contained reaction product. Furthermore, some Golgi saccules (Fig. 3), numerous Golgi vesicles, measuring 0.05–0.1 μ in diameter (Figs. 3, 4), and some larger membrane-bounded granules measuring up to 0.8 μ in diameter (Fig. 5) stained prominently. Occasionally, some extensions of the endoplasmic reticulum, containing reaction product, appeared in close proximity to the saccules of the Golgi complex (Fig. 4). In many cells, even in sections not counterstained with lead or uranyl acetate, free ribosome-like particles in the vicinity of endoplasmic reticulum and mucus droplets stained positively (Fig. 3). This staining was somewhat patchy since in the same cells nonreactive particles could still be recognized in

TABLE I

Controls for the Specificity of Peroxidase Reaction in Large Intestine of Rat

Incubation mixture*		
H_2O_2	Special conditions	Black reaction product in ER‡
%		
0.02	0	++++
None	0	--
0.02	Boiling	--
0.02	NaN_3 10^{-5} M	±
0.02	NaN_3 10^{-6} M	++
0.02	KCN 10^{-1} M	++
0.02	Aminotriazole 10^{-1} M	±
0.02	Aminotriazole 2 X 10^{-2} M	+
2.00	Excess H_2O_2	—

* All incubation media contained 10 mg of DAB in 10 ml of 0.05 M Tris-HCl buffer, pH 7.6.
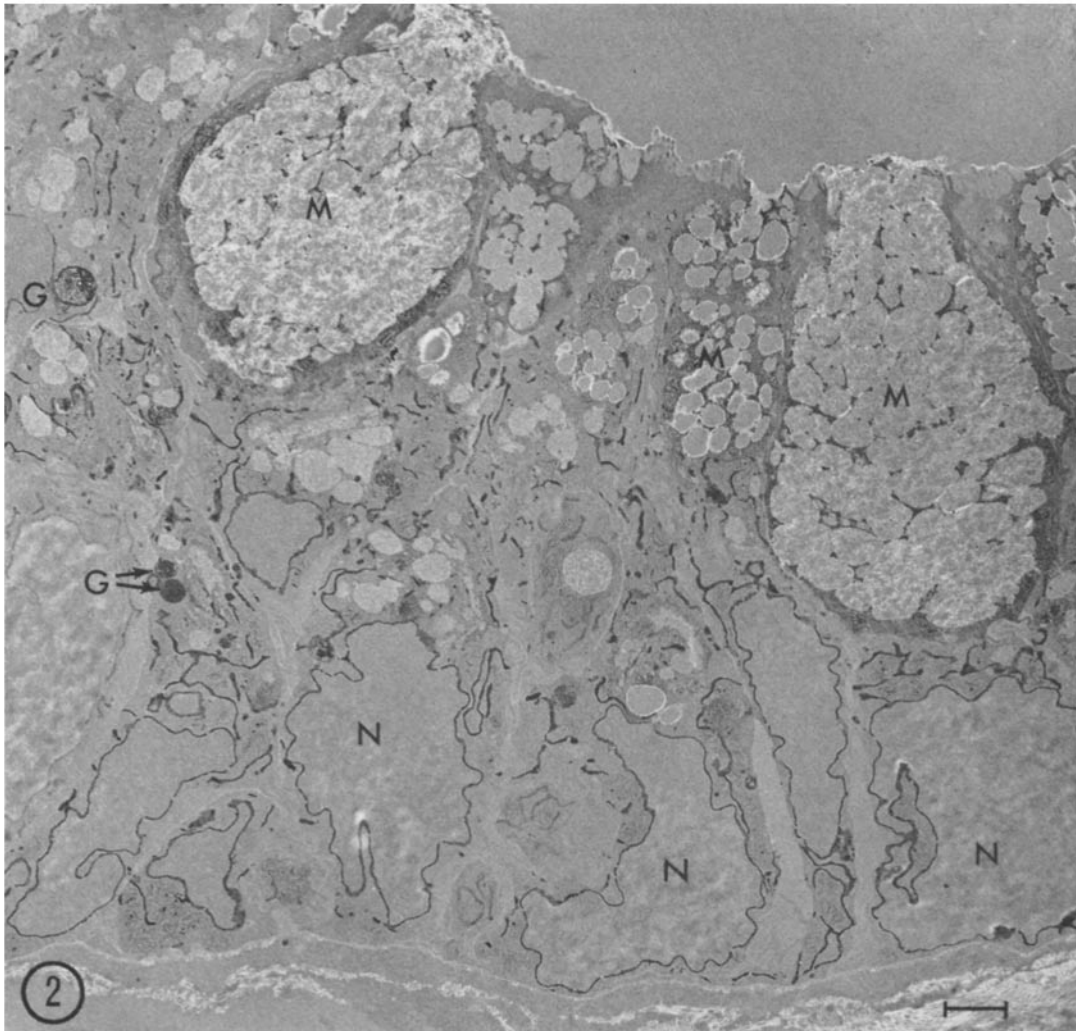‡ The intensity of staining in endoplasmic reticulum arbitrarily graded from 1+ to 4+.

FIGURE 2   Low-power electron micrograph showing general distribution of peroxidase in colonic epithelial cells. Enzyme is present in endoplasmic reticulum, including the nuclear envelope, large granules (G), and as a diffuse deposit around some clumps of mucus (M). Section not counterstained. N, nucleus. × 6,500.

many areas. Mitochondria often showed staining in the cristae (Fig. 3). Similar staining of mitochondria has been observed in other tissues (8, 9). In contrast to colon, the gastric, duodenal, and ileal glands did not stain for peroxidase activity.

The results of control experiments are recorded in Table I. The inhibition of staining by boiling, exclusion of H_2O_2, excess H_2O_2, and by azide strongly suggests that the reaction is enzymatic in nature and that the enzyme is most probably a peroxidase. The sensitivity of the staining to very

low concentrations of NaN_3 is in contrast to its resistance to high concentrations of KCN.

DISCUSSION

Palade and coworkers have established that secretory proteins such as pancreatic enzymes are synthesized on ribosomes, transferred to cisternae of the endoplasmic reticulum and packaged within the Golgi complex (10–12). The localization of peroxidase in the endoplasmic reticulum and in parts of the Golgi apparatus of rat colonic epi-
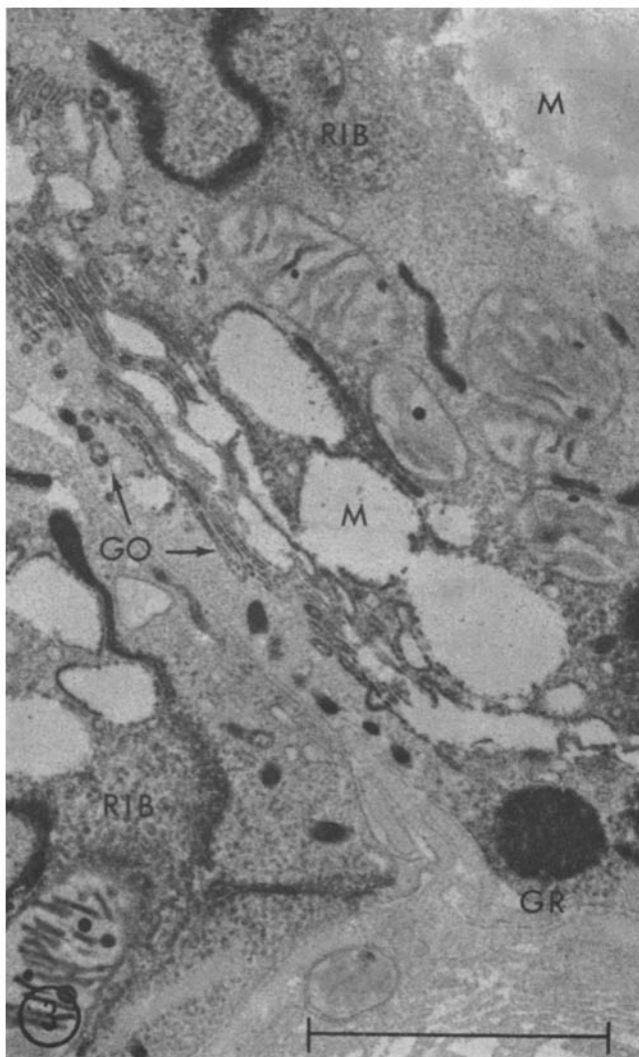
FIGURE 3 Electron micrograph of colonic epithelial cells, showing reaction product in saccules and vesicles of Golgi complex (GO), and larger granules (GR). There is also a diffuse staining of free ribosome-like particles (RIB). Moderate staining of some mitochondrial cristae is also noted. M, mucus. Unstained. × 41,000.

thelial cells thus strongly suggests that these cells synthesize peroxidase. The localization of enzyme in free ribosomes may, indeed, reflect actual synthesis of the peroxidase by these particles, but the possibility that such staining may represent artifactual adsorption of enzyme or its reaction product that had leached out from membrane-bounded organelles, cannot be ruled out. Recently, however, a similar observation of peroxidase activity in ribosome-like particles has also been made in the rat submaxillary gland.[2] It is commonly acknowledged that cells at the base of intestinal crypts are in a process of continuous

maturation and migration towards the surface (13). Our findings, therefore, indicate that synthesis of peroxidase occurs mainly in maturing epithelial cells at the base of colonic crypts.

At present, the function of colonic peroxidase must remain conjectural. It is possible that the enzyme may participate in certain metabolic functions in the endoplasmic reticulum, similar to those suggested recently for peroxidase in rat uterus (6); however, the localization of peroxidase in the Golgi region and in large "secretory" vacuoles, as well as its presence around mucus droplets, suggests that at least some of the syn-
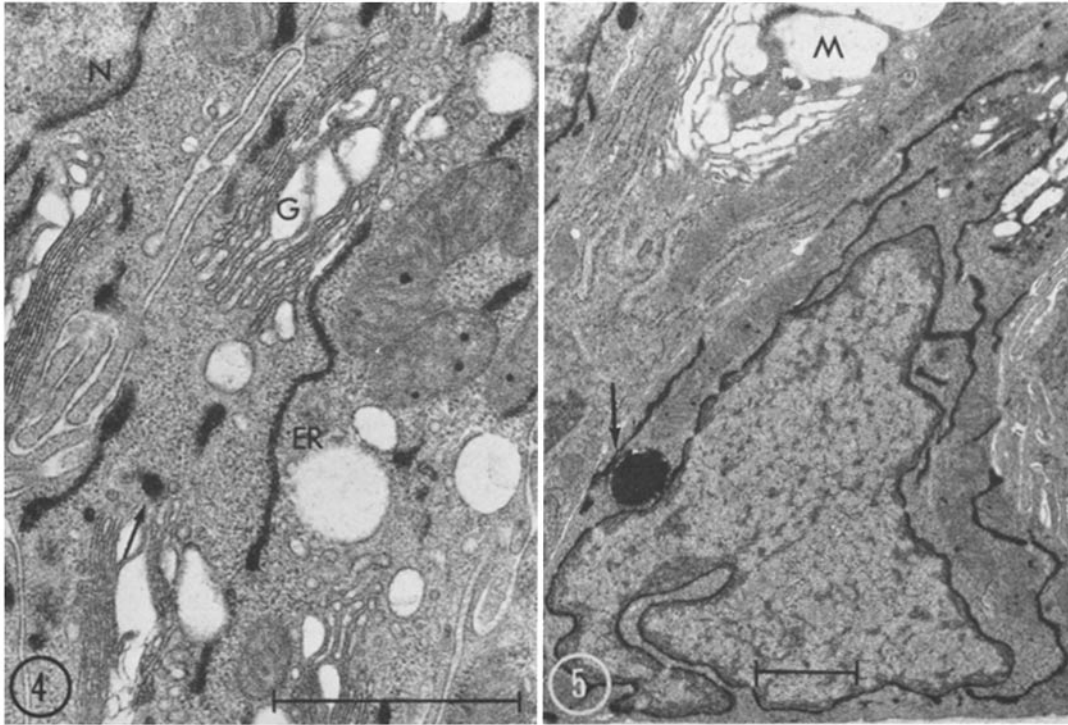
FIGURE 4 This micrograph illustrates the localization of peroxidase reaction in the perinuclear cisternae and in a segment of ER which is in close proximity to Golgi saccules and in several small vesicles in periphery of the Golgi complex (arrow). Golgi saccules, themselves, however, are void of reaction product. *N*, nucleus. *G*, Golgi complex. × 31,000.

FIGURE 5 Note the presence of reaction product in the cisternae of endoplasmic reticulum and in a large granule (arrow). *M*, mucus. × 14,000.

thesized peroxidase is destined for secretion. Evidence obtained from experiments on neutrophils (myeloperoxidase), lactoperoxidase, and salivary gland peroxidase has suggested that the bactericidal action of neutrophils, milk, and saliva may depend on the effect of peroxidase in the presence of H_2O_2 and certain ions such as iodide and thiocyanate (14). Colonic mucosa has been shown to concentrate thiocyanate (15). Thus, the possibility that peroxidase in large intestine may participate in a bactericidal mechanism serving to form a "mucosal barrier" to microorganisms which inhabit the intestinal lumen, deserves further investigation.

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