it has for the redifferentiation of the intestine of the suckling rat. In rat pups deprived of the hypophysis (*21*) or of the adrenal glands (*22*) in early neonatal life, T4 alone promotes cytogenetic differentiation of the absorptive cells and elevates the activity of the brush-border enzymes that normally rise during the transition from the suckling to the mature state.

In the chick embryo in ovo, the differentiation of goblet cells, as well as of the absorptive epithelium, is no doubt under the control of several hormones. In thyroprivic embryos, HC promotes intestinal development only if T4 is also administered (*23*). In decapitated embryos, intestinal development is not affected by T4 and cortisone in concert (*2*), but can be restored by factors secreted by the cephalic portion of the adenohypophysis (*5*). Our results, while indicating that thyroid hormone may play a dominant role in promoting the production of goblet cells, strongly suggest that an inhibitory factor also participates in the control of intestinal development, perhaps by regulating the time at which critical events occur.

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Stimulation by Immune Complexes of Mucus Release from Goblet Cells of the Rat Small Intestine

Abstract. *Immune complexes (bovine serum albumin with rat antibodies to bovine serum albumin) formed in twofold antibody excess were injected into the duodenum of normal rats. In comparison to controls injected with antigen only, there was a marked increase in the percentage of disrupted goblet cells (an index of mucus release) in segments from the intestine of rats exposed for 3 hours to immune complexes in vivo. Similarly, there was a significant increase in ^{35}S-labeled mucus recovered by filtration of intestinal wash, rinse, and mucosal homogenate fluids from rats exposed to immune complexes compared to those from rats exposed to bovine serum albumin or purified rat antiserum to bovine serum albumin alone. These findings suggest that certain immune complexes can stimulate mucus release from intact rat small intestine; enhanced mucus release may have a role in clearing the surface of complexes.*

In the course of testing the uptake of immune complexes by everted segments of rat small intestine in vitro, we noted that complexes prepared in twofold antibody excess were absorbed in significantly smaller quantities than was antigen alone (*1*). Complexes prepared in antibody excess appeared to stimulate the secretion of mucus, and complexes were found to be associated with the mucus fraction. These experiments suggested that release of mucus might have a role in reducing contact between immune complexes and the surface of the gut. The present study tested the ability of immune complexes to stimulate release of mucus under physiological conditions in intact rats.

Female Sprague-Dawley rats (Holtzman) were maintained on rat chow (Ralston Purina) containing no cow's milk proteins. Those weighing 150 to 175 g were used for the intestinal infusion experiments in vivo, and those weighing 250 to 350 g were used as a source of homologous antiserum. Animals were immunized by intraperitoneal injection of crystalline bovine serum albumin (BSA, Nutritional Biochemicals) emulsified in incomplete Freund's adjuvant (Difco) according to an immunization schedule previously described (*2*). The rats were injected five times at 10-day intervals and were exsanguinated by cardiac puncture 10 days after the last injection. The antigen-binding capacity (ABC) of individual serum was tested by a modification (*3*) of the ammonium sulfate method of Minden and Farr (*4*); serums with an ABC-33 (at 0.1 μg of ^{125}I-labeled BSA per milliliter) greater than 125 μg/ml were combined (*5*). Purified antibodies to BSA were prepared by an immunoadsorption technique (*6*). Based on the results of quantitative precipitin tests with the purified antibody (*7*), complexes were prepared at twofold antibody excess as previously described (*1*).

At the time of study, animals fasted for 24 hours were subjected to laparotomy under ether anesthesia and the small intestine was identified. Test materials, including BSA (10 μg), complexes of BSA (10 μg) with antibodies to BSA (1060 μg). and cholera toxin (50 μg) (Schwarz/Mann) in 2 ml of phosphate-buffered saline (PBS), and PBS alone, were injected by syringe through a "purse-string" duodenal ligature (*8*). The abdominal cavity was closed with surgical clips and the animals were allowed to resume normal activity. At 3 hours after infusion, the rats were again anesthetized, the small intestine was removed, and 5-mm (full thickness) rings of jejunum and ileum were prepared for morphological studies according to standard methods (*9*). Sixty 6-μm sections of jejunal and ileal rings were cut, and every tenth section was stained with hematoxylin and eosin and examined by light microscopy at ×40 magnification. The total number of goblet cells per villus, and the number of goblet cells showing mucus release

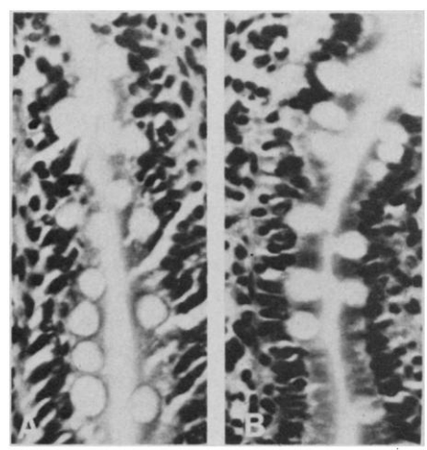

Fig. 1. Microscopic sections of representative intestinal villi from jejunum of rat 3 hours after exposure to BSA alone (A) or to complexes of BSA with rat antibodies to BSA (B). Note the increased number of disrupted goblet cells in (B) compared to (A) (×22).

(mucus is released from goblet cells by disruption of the cell surface) were determined in 20 villi on each of six sections of jejunum and ileum examined. The average percentage of disrupted goblet cells and the standard error of measurements were calculated. In order to prevent the introduction of a bias, all sections were examined as unknowns.

In other groups of infused rats, the amount of ^{35}S-labeled mucus released into the intestinal lumen was determined. Rats were injected in the tail vein with 20 μc of $Na_2{}^{35}SO_4$ (New England Nuclear) in 0.6 ml of saline (*10*); these injections were administered 1 hour after the intestinal infusions described above. Two hours later, the rats were exsanguinated under anesthesia and the entire small intestine was removed; its contents were recovered as "wash fluid" by washing with 10 ml of iced PBS. Thereafter, the gut was everted and rinsed by agitation in 25 ml of PBS (rinse fluid). The mucosal surface was then scraped and homogenized in 10 ml of PBS as described (*1*) (mucosal homogenate fluid). The mucus fractions from the wash, rinse, and mucosal homogenate fluids were recovered by filtration (Whatman filter paper No. 2); the filters were washed five times with 10-ml volumes of PBS. Radioactivity remaining on the filter paper was measured and expressed as counts per minute per milligram of mucosal protein. Mucosal protein was determined by the method of Lowry *et al.* (*11*). Controls for the filtration process were prepared by applying $Na_2{}^{35}SO_4$ (1×10^6 count/min), either alone or mixed with fresh intestinal mucus, to filter paper which was then rinsed as above. Under these conditions, less than 5 percent of the total radioactivity added remained on the filter paper.

The release of goblet cell mucus was examined morphologically in three groups of four rats given BSA, complexes of BSA with rat antibodies to BSA, cholera toxin, or saline alone. Figure 1B shows several goblet cells with disrupted cell surfaces in a section of small intestine exposed to complexes for 3 hours; nearly all goblet cells are intact in the section of small intestine exposed to BSA alone (Fig. 1A).

Figure 2 shows that the percentage of disrupted goblet cells was significantly greater in sections of intestine from rats exposed to complexes compared to those from rats exposed to BSA alone ($P < .001$). Cholera toxin was used as a positive control since this enterotoxin has been shown to cause the release of mucus from goblet cells (*12*). In further experiments, there was no difference in the percentage of disrupted cells in sections from intestine of rats exposed to BSA solution compared to those from rats exposed to saline alone.

The amount of ^{35}S-labeled mucus released into the intestinal lumen, adherent to the intestinal surface or recovered from mucosal homogenates, was determined in groups of five rats exposed to BSA, purified rat antibodies to BSA, complexes of BSA with antibody to BSA, cholera toxin, or saline. The results are shown in Fig. 3. Instillation of saline solution alone resulted in the release of ^{35}S-labeled mucus in amounts similar to those released by instillation of BSA alone or antibodies to BSA.

The present study indicates that immune complexes prepared in twofold antibody excess can stimulate the "release" of mucus from goblet cells of the intact rat small intestine. We have not determined whether the increase in mucus release results from increased secretion alone, from increased synthesis of mucus by goblet cells, or from a combination of these events. We also have not determined whether the ^{35}S-labeled mucus released after stimulation by antigen-antibody complexes is entirely the product of goblet cells. However, in view of the morphological demonstration of mucus release from goblet cells after exposure of the intestine to immune complexes (Fig. 1), it seems likely that at least a portion of the labeled mucus measured in wash, rinse, and mucosal homogenate fluids was contributed by goblet cells.

Several lines of evidence suggest that mucus present on the surface of the intestinal epithelium contributes to protection of that surface against penetration by antigens and microorganisms (*13*). This protection may depend on the role of the mucus coat as a physical barrier to the migration of large molecules from the intestinal lumen to the epithelial surface, as well as on the presence of specific moieties in the mucus. For example, Gibbons *et al.* (*14*) have reported that salivary glycoproteins inhibit the adherence of bacteria to epithelial cells and thus prevent colonization of the mucosa. Strombeck and Harrold (*15*) have shown that gastric mucins interfere with the binding of toxins to receptors on enterocytes, thus preventing toxin from stimulating the active secretion into the lumen of electrolytes and water. With respect

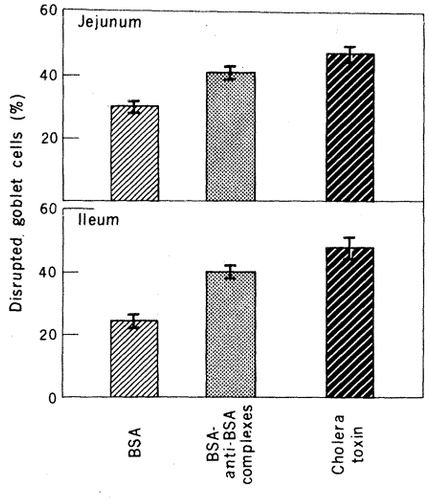

Fig. 2. The average percentage of disrupted goblet cells (± standard error) in 20 villi from each jejunal and ileal section of intestine from three groups of rats exposed to BSA, complexes of BSA with rat antibodies to BSA, or cholera toxin. A significant increase in the number of disrupted goblet cells was noted in rat jejunum and ileum exposed to immune complexes compared to those exposed to BSA alone ($P < .001$). The difference in percentage of disrupted goblet cells in intestine exposed to complexes or cholera toxin was not significant ($P > .1$).

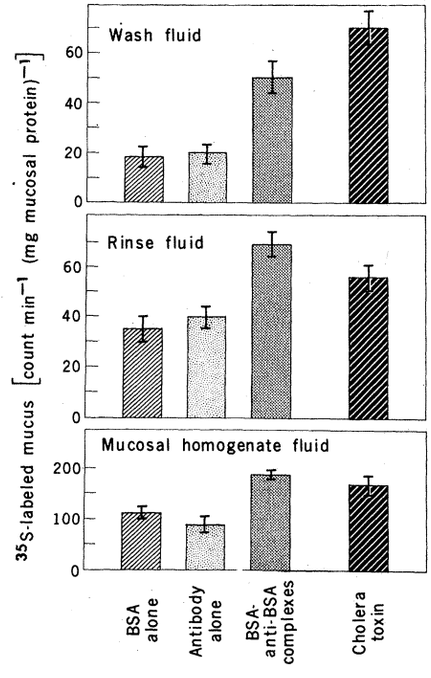

Fig. 3. The average radioactivity per milligram of mucosal protein (± standard error) in wash, rinse, and mucosal homogenate fluid from the small intestine of rats exposed to BSA, purified antibodies to BSA, complexes of BSA with rat antibodies to BSA, or cholera toxin is shown. A significant increase in ^{35}S-labeled mucus release occurred in intestines exposed to immune complexes compared to those exposed to BSA ($P < .001$) or to antibodies to BSA alone ($P < .001$). Intestine exposed to cholera toxin also showed a significant increase in the volume of ^{35}S-labeled mucus compared to intestine exposed to BSA ($P < .001$) or antibodies to BSA alone ($P < .001$).

to the uptake of soluble protein antigens, antibodies present in the mucus layer may combine with antigens to form complexes and thereby prevent the molecules from reaching the surface of the enterocyte from whence uptake by pinocytosis is initiated (*2*). With respect to the uptake of preformed antigen-antibody complexes or those forming in the lumen of the intestine, mucus may serve as a mechanical barrier to the diffusion of the complexes, thereby again limiting the access of complexes to the surface of the enterocyte (*1*). Our experiments suggest an additional relation between immune complexes and mucus, that is, the ability of the former to stimulate release of mucus by the intact small intestine of the rat. Release of mucus may in turn serve to clear the surface of the gut of adherent immune complexes.

The mechanisms by which immune complexes stimulate the release of goblet cell mucus is still to be determined. Certain antigen-antibody complexes might interact with receptors on the surface of goblet cells and thereby stimulate release of mucus, or interaction of complexes with epithelial cells might stimulate such cells to release unknown mediators which in turn activate goblet cells. Alternatively, it is known that the first component of complement and possibly others are produced by the intestine (*16*), and that rat IgG-1 antibodies are capable of activating the complement sequence (*17*). Therefore, it is possible that components of the complement system might be involved in mediating the release of mucus.

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Circulation of H^+ and K^+ Across the Plasma Membrane Is Not Obligatory for Bacterial Growth

Abstract. Streptococcus faecalis *grows normally in the presence of gramicidin and other ionophores under conditions such that there is no gradient of* pH *or of electrical potential across the plasma membrane and that currents of* H^+, K^+, *and* Na^+ *are short-circuited. Growth requires a rich medium, a slightly alkaline* pH, *and a high concentration of external* K^+. *The proton circulation maintains the cytoplasmic* pH *and pools of ions and other metabolites but is not obligatory for biosynthetic functions including DNA replication, cell division, or assembly of the structural framework of the cell.*

It is now generally accepted that a central feature of energy metabolism in bacteria is the circulation of protons across the plasma membrane (*1*). Several major metabolic pathways, including the respiratory chain and the membrane-bound adenosine triphosphatase (E.C. 3.6.1.3) complex, mediate electrogenic transport of protons outward; the current loop is completed by an array of molecular devices that allow protons to return to the cytoplasm while performing useful work. Examples of proton-linked processes are oxidative and photosynthetic phosphorylation, transhydrogenation of pyridine nucleotides, motility, and the transport of many metabolites (*1*, *2*). In order to determine whether ion currents are involved in other essential functions, particularly in the construction and replication of the fabric of bacterial cells, we have examined the growth of *Streptococcus faecalis* in the presence of ionophores. This fermentative organism normally maintains a circulation of protons across the membrane by means of a proton-translocating adenosine triphosphatase (*2*, *3*); gramicidin and other ionophores were used to short-circuit the proton current and to dissipate gradients of K^+ and Na^+. The results confirm that the proton circulation is required to maintain cytoplasmic pools of ions and metabolites as well as a neutral *p*H. However, it is not obligatory for the synthesis of macromolecules and organelles (cell wall, membranes, nucleoid and other structural elements), for DNA replication, or for cell division.

The basic observation is illustrated in Fig. 1A. Growth of *Streptococcus faecalis* (*faecium*) (American Type Culture Collection 9790) in the standard complex medium NaTY (*4*) was completely blocked by gramicidin D, an ionophore that renders the cytoplasmic membrane permeable to K^+, Na^+, and H^+ (*5*, *6*). By contrast, in the medium designated KTY2XH (*4*) exponential growth continued at a rate not much less than that of control cells. This particular medium is buffered at *p*H 7.7 and contains 0.28*N* K^+ and less than 0.01*N* Na^+. Similar results were obtained with complex media containing as much as 1*N* K^+ and buffered with bicine, tricine, or Hepes (*7*) between *p*H 7.5 and 8.2. Other ionophores, including gramicidin A, valinomycin plus nigericin (1 μg/ml each), and the proton conductors tetrachlorosalicylanilide and carbonylcyanide *m*-chlorophenylhydrazone ($10^{-5}M$) also block growth in NaTY but not in media enriched with K^+ (data not shown).

Although the properties of the ionophores are well documented (*5*, *6*), it is important to establish that cells growing in the presence of gramicidin on medium KTY2XH maintain neither an electrical potential across the cytoplasmic membrane nor concentration gradients of H^+, K^+, or Na^+. With methods presently available (*8*) these measurements cannot be made directly on growing cells. We therefore compared cells that had been grown for at least four generations in KTY2XH in the absence or presence of gramicidin under the conditions of Fig. 1A; the cells were then washed and resuspended in buffer (0.14*M* K_2HPO_4, 0.05*M* Hepes, *p*H 7.5). Cells grown with gramicidin retain the antibiotic through