

the three patients and 0.39 seconds in normal infants (Table 1). Thus, there was no evidence that an abnormal QT_c interval contributed to death of these patients.

End-tidal P_{CO_2} , reflecting the extent of alveolar ventilation, was 40, 37, and 44 mm-Hg, respectively, compared with 35.1 ± 1.9 in normal infants (Table 2). The change in minute ventilation [BTPS (5)] was corrected for body weight to permit comparison. The response to CO₂ breathing in each affected infant was less than the mean minus 2 standard deviations for normal infants.

Therefore, these three infants manifest alveolar hypoventilation and impaired regulation of ventilation. This finding is similar to the physiological defect in two infants with congenital alveolar hypoventilation who exhibit no response to CO₂ breathing, maintain normal ventilation while awake, but require mechanical ventilation to support life during sleep (6). We have not defined the cause of abnormal ventilatory control in any of

these five patients. The data indicate that impaired regulation of alveolar ventilation rather than impaired cardiac conduction was the cause of death in the three infants of the present study. Alveolar hypoxia resulting from hypoventilation could explain the pathological findings described by Naeye in SIDS (1).

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Goblet Cells in Embryonic Intestine: Accelerated Differentiation in Culture

Abstract. *If duodenal tissue from 14-day chick embryos is cultured in chemically defined medium, more than twice as many goblet cells appear within 48 hours as in intact embryos during the same time. The increase in goblet cell number is further accelerated by 10^{-9} molar thyroxine but is prevented by 10^{-6} molar hydrocortisone. The results suggest that differentiation of intestinal epithelium is regulated in part by a circulating inhibitor.*

During the third week of development in ovo, the simple epithelium lining the intestine of the chick embryo develops the morphological and biochemical characteristics required for function (1-4). The greater part of the epithelium is made up of absorptive cells, but a part differentiates as mucus-producing goblet cells. The latter, barely detectable at 14 days, increase in number and in mucus content during the third week (2, 4). That these changes are hormone-dependent is indicated by the failure of goblet cells to exceed the 17-day level in embryos deprived of the hypophysis by removal of the forebrain at 33 hours of incubation (2, 4). An increase in goblet cell number in such embryos may be brought about by grafts of the cephalic but not the caudal part of the adenohypophysis (5). Since the cephalic region is the only site of adrenocorticotropin and thyrotropin output (6), this result may imply that these hormones influence goblet cell production by inducing secretion of glucocorticoid, thyroxine, or both.

The profound effects of early decapitation on the growth and morphogenesis of the intestine (2), however, suggest that glucocorticoids or thyroxine may act only as part of a complex system affecting growth and metabolism. This possibility is supported by the fact that whereas exogenous hydrocortisone accelerated the increase of goblet cell number in intact embryos (7), neither cortisone nor thyroxine, alone or in combination, tended to normalize epithelial differentiation in decapitated embryos (2). We therefore decided to examine goblet cell differentiation and its hormonal control apart from the embryonic environment, by culturing intestinal tissue in a chemically defined medium. Medium 199, which supports intestinal differentiation (8), was utilized.

White Leghorn eggs of the Babcock strain, obtained from Ken-Roy Hatchery, Berger, Missouri, were kept in a forced-draft incubator at 37.5°C. At 14 days, the duodenal loop was excised, placed in sterile Ringer solution, and

freed from pancreatic tissue and mesentery. The duodenum was then transferred to a petri dish containing culture medium and cut in eight to ten segments 1 to 2 mm in length. Each segment was split open, allowing it to curl into a tube with the luminal surface outside. The fragments obtained from a single duodenum were divided between a control and an experimental culture flask, giving approximately 5 mg of tissue per flask.

Each 25-ml flask contained 2.9 ml of medium 199 (Grand Island Biological Co.), 0.03 ml of penicillin-streptomycin solution (50 unit/ml; Gibco), and 0.1 ml of hormone solvent (control culture) or of hormone solution (experimental culture). Thyroxine solutions were prepared by dissolving 3,3',5,5'-tetraiodo-DL-thyronine (Sigma) in sterile 0.01M NaOH; hydrocortisone 21-sodium succinate (Sigma) was dissolved in sterile 0.9 percent NaCl. Hormone concentrations in the experimental culture flasks were 10^{-9} M thyroxine (T4) or 10^{-6} M hydrocortisone (HC). After addition of the tissue, the flasks were gassed with a mixture of 95 percent O₂ and 5 percent CO₂, stoppered tightly, and incubated at 38°C. The tissue maintained normal morphology for 48 to 72 hours, as ascertained from paraffin sections of the whole tissue and from electron micrographs of the epithelial cells.

To study the course of goblet cell differentiation in vivo, duodena were collected from embryos at 14 to 19 days of development. Freshly excised duodena were cut into segments and split open as described above. To compensate for possible regional differences in goblet cell numbers, two pieces of each duodenum, from proximal and distal ends, were used. Collected tissue was fixed for 2 to 3 hours in Carnoy fixative, embedded in paraffin, sectioned at 5 μ m at right angles to the longitudinal axis, and stained by the periodic acid-Schiff (PAS) procedure.

Up to 17 days, only previllous ridges or primordial villi (2) are present, with true villi beginning to grow thereafter. Goblet cells were counted on ridges (or villi) that were sectioned perfectly from tip to base. Counts were made on every third section of a specimen until 100 suitable cross sections of ridges were counted. Results were recorded as total number of goblet cells per 100 ridge (or villus) sections, and also as number confined to the basal halves of the ridges or villi.

Goblet cells increase in vivo from 10 per 100 ridge sections at 14 days to 236 at 18 days (Fig. 1) and 596 at 19 days. The exponential nature of the rise suggests

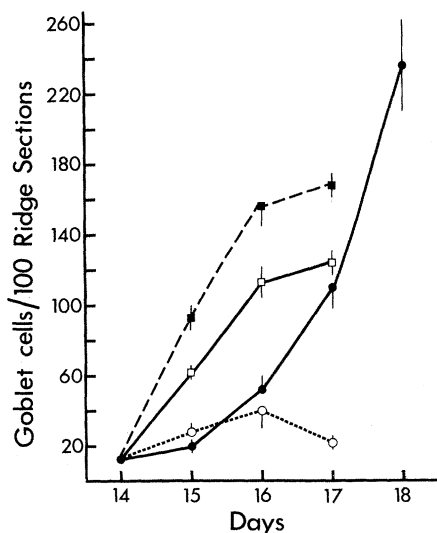


Fig. 1. Increase of numbers of goblet cells in intact embryos (●) and in duodenal fragments cultured in unsupplemented medium 199 (□) or in medium supplemented with $10^{-9}M$ thyroxine (■) or $10^{-6}M$ hydrocortisone (○). For each day of development, each point represents the average number of goblet cells per 100 ridge sections (see text) for 10 specimens from intact embryos, 24 specimens from unsupplemented medium, and 12 specimens each from medium containing thyroxine or hydrocortisone. Vertical lines indicate standard error of the mean.

that division of goblet cells may be principally responsible for the increase, a possibility supported by the observation that these cells frequently occur in pairs in the embryo. Moreover, many of the goblet cells on the embryonic ridges contain mucus only in the apical region, in this respect resembling the immature goblet cells found in the crypts in the adult intestine (9), in which goblet cell division is limited to the crypts.

Goblet cells are at first confined to the ridge bases, but after 15 days become more evenly distributed between the proximal and distal halves of the ridges or villi (Fig. 2). This finding is in agreement with that of Hart and Betz (5). Since cell extrusion does not begin until after hatching (2), and the epithelial cells clothing the embryonic villi are bound together by junctional complexes (3), it is unlikely that goblet cells are able to migrate up the villi. Thus the appearance of goblet cells on the distal halves of the villi must be due to differentiation of undifferentiated precursors, an event which would be concomitant with the loss of mitotic activity from the distal region (3). The production of new goblet cells both by division of immature cells and by differentiation of undifferentiated precursors is consistent with current views of goblet cell renewal in the mammalian intestine (10).

During the first 48 hours in culture in

medium 199, previllous ridges increase in height to about the same extent as in the intact duodenum (11, 12). The number of goblet cells increases significantly faster than in vivo (Fig. 1), however, and a larger proportion of the goblet cells appears on the distal halves of the ridges (Fig. 2), indicating that the increase in number is due in part to the accelerated differentiation of undifferentiated precursors. The intensity of PAS staining is also enhanced. The changes in cell number and distribution are accentuated by the addition of $10^{-9}M$ T4 to the medium, with the result that goblet cell numbers are significantly higher ($P < .01$) than in control cultures at each time interval. Accelerated goblet cell formation in culture is not an artifact of altered growth rates in the presence or absence of T4, since explant ridges differ little in length from those found in vivo at 15 and 16 days.

If $10^{-6}M$ HC is added to the medium, goblet cells do not accumulate faster than in the intact embryo (Fig. 1). During the first day in culture, however, there is a large increase in goblet cells on the distal halves of the ridges (Fig. 2). This change in distribution suggests that HC may act preferentially by stimulating differentiation of undifferentiated precursors, rather than by influencing the rate of cell division. This view is in harmony with the fact that only HC depresses mitotic counts significantly below in vivo levels after 24 hours in culture (11). The effect cannot be more than initiation of differentiation, however, for mucus accumulation, as revealed by PAS staining, is less than in control cultures. The acceleration of chemostructural differentiation of the absorptive cells that also occurs in culture (12) is not inhibited by HC.

The factors responsible for accelerated goblet cell formation in unsupplemented medium are obscure. The effect is not specific for goblet cells, since both morphological and biochemical differentiation of the absorptive cells also proceed at a rate higher than that in the intact embryo (11, 12). This phenomenon is an exceptional one, for cultures of embryonic chick skin and retina differentiate at subnormal rates in a variety of defined media with or without serum, but without added hormones (13, 14). In embryonic pancreas, amylase activity failed to increase over the course of 6 days in embryo extract plus horse serum, unless HC was added (15). None of these studies involved the use of medium 199, some ingredient of which might have had a stimulatory effect on goblet cell production in our intestinal explants.

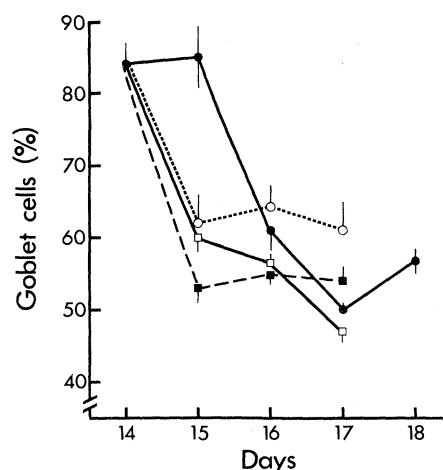


Fig. 2. Percentage of goblet cells located in basal halves of previllous ridges in duodenum of intact embryos, or in duodenal fragments cultured in medium 199 with or without hormones. Symbols are described in Fig. 1.

Medium 199 has, however, failed to support even normal differentiation in embryonic endocrine glands (16), and other media of almost equal complexity have not caused accelerated differentiation in a variety of tissues (14, 17). The only reported case of precocious differentiation is the rapid rise of uridine diphosphate glucuronosyltransferase activity in 5-day embryo liver explanted to Eagle medium (18), a less complex mixture than medium 199. When the 5-day livers were grafted to the chorioallantoic membranes of 12-day-old embryos, no acceleration occurred, suggesting that the effect in culture was due to removal from the influence of an inhibitor circulating in the intact system (18).

Our findings might similarly indicate the presence of an inhibitor of goblet cell production during the last week of incubation. The fact that HC suppresses the increase of goblet cell number in vitro might implicate this hormone as the inhibitor in vivo. This possibility must remain moot, however, because the concentration found effective in these studies ($10^{-6}M$) is 100 times higher than serum corticosterone levels reported for 14- to 17-day chick embryos (19).

Goblet cell formation is subject to stimulation as well as inhibition, as the results obtained with T4 show. The effect is probably a physiological one in this case, for the concentration of $10^{-9}M$ employed is well within the range of thyroid hormone concentrations in adult mammalian serum (20). In addition to its influence on goblet cells, T4 further accelerates the morphological and biochemical differentiation of absorptive cells, indicating that this hormone may have the same significance for the differentiation of the embryonic intestine as

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 adeno-hypophysis (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 ³⁵S-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 ho-

mologous 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 ¹²⁵I-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

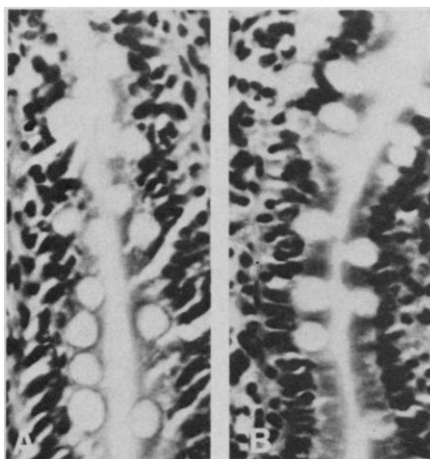


Fig. 1. Microscopic sections of representative intestinal villi from jejunum of rat 3 hours after exposure to BSA alone (A) or to complexes of BSA with rat antibodies to BSA (B). Note the increased number of disrupted goblet cells in (B) compared to (A) (×22).