pH-DEPENDENT BINDING OF IMMUNOGLOBULINS TO INTESTINAL CELLS OF THE NEONATAL RAT

RICHARD RODEWALD. From the Department of Biology, University of Virginia, Charlottesville, Virginia 22901.

Neonatal rats are able to absorb maternal immunoglobulins from milk in the small intestine and to transport these proteins intact to the circulation. An important feature of this transfer is that only immunoglobulins of the IgG class are transported whereas other milk proteins as well as immunoglobulins of other classes are apparently digested within the intestine (1, 8). Experiments with tracers for electron microscopy (9–11) have revealed that the Fc portion of the IgG molecule binds selectively to the liminal surface of the epithelial cells in the proximal one-third of the small

intestine, presumably the site of the specific immunoglobulin receptors whose existence was originally postulated by Brambell et al. (2). Immunoglobulins are then absorbed by pinocytosis and transported to the abluminal surface of the cells within a specialized system of small vesicles (10, 11). Recently Jones and Waldmann (6) observed that isolated fragments from the brush borders of intestinal cells retain the capacity to bind immunoglobulins selectively. More importantly, these investigators noted that this binding occurs at pH 6.5 but is mostly lost at pH 7.4 or higher. We report here studies with immunoglobulins conjugated to horseradish peroxidase as a histochemical tracer for light and electron microscopy which indicate a similar pH-dependent binding to intact cells from the proximal intestine. We suggest that a pH change probably plays an important role in the normal transport of immunoglobulins across the cells.

MATERIALS AND METHODS

A preliminary experiment was undertaken to estimate the normal pH within the stomach and intestinal lumen of rats 9–18-days old, ages at which IgG is normally transported to the circulation (4). A simple colorimetric assay with pH paper was adapted for the very small volumes of fluid present in the lumen at these ages. A mid-ventral incision was made in the abdomen of etherized rats to expose the gastrointestinal tract. Short lengths of PE90 polyethylene tubing (Clay-Adams, Inc., Div. of Becton, Dickinson & Co., Parsippany, N. J.) which contained small pieces of narrow range pH paper (Micro Essential Laboratory, Inc., Brooklyn, N. Y.) were inserted through incisions of various points along the intestine. just enough fluid, 1–2 μl, was withdrawn with the aid of 1-ml syringe into each tube to moisten the pH paper. Tubes were immediately compared to reference tubes standardized with 0.01 M phosphate buffers of known pH. Fluid samples were assayed in this manner from the stomach, duodenum, proximal jejunum, jejunal-ileal transition region, distal ileum, and caecum. By this method, the pH of samples could be estimated to within ± 0.2 pH units.

For tracer experiments, IgG immunoglobulins (Miles Laboratories, Inc., Elkhart, Ind.) were conjugated to horseradish peroxidase (Type VI, Sigma Chemical Co., St. Louis, Mo.) by the method of Kawaoi and Nakane (7). After dialysis against 0.1 M phosphate buffer with 0.05 M NaCl at pH 6.0 or 7.4, small samples of the conjugates at a concentration of 10 mg/ml were injected at these two pH values into 2–3-cm long segments of jejunum which had been removed from 10–12-day old rats and had been cooled to 0°C in physiological saline. After an incubation of 10 min at 0°C, the segments were rinsed briefly with 1–2 ml of buffer at the same pH as the injected conjugate. Small pieces of tissue were then excised and fixed with 2% glutaraldehyde in 0.1 M phosphate buffer, again at the same pH as the conjugate. Fixed tissue was rinsed briefly and incubated with 3,3'-diaminobenzidine tetrahydrochloride and H_2O_2 according to the method of Graham and Karnovsky (3) to reveal sites which contained the peroxidase conjugates. Tissue was then postfixed in osmium tetroxide, dehydrated with alcohol, and finally embedded in epoxy resin. Unstained sections, 1–2 μm thick, were observed with a Zeiss WL light microscope. Thin sections, 70–100 nm thick, were stained for 1 min in lead citrate and examined with a Philips 200 electron microscope.

RESULTS AND DISCUSSION

Results of pH measurements are summarized in Table I. A pH gradient was found to extend the length of the small intestine from a mean of pH 6.2 in the duodenum to near pH 7.0 in the distal ileum. The pH within any given region did not appear to change over the age period studied. The proximal one-third of the intestine, the region where the major portion of immunoglobulins are transported to the circulation (9), always exhibited a pH between 6.0 and 6.5.

When jejunal segments were exposed at pH 6.0 to rat IgG conjugated to peroxidase, a dark brown reaction product due to peroxidase was evident by light microscopy on the luminal surface of those absorptive cells which lined the upper one-half of the villi (Fig. 1 a). Goblet cells showed no binding. When jejunal segments were incubated with this same conjugate at pH 7.4, no binding was found on any cells (Fig. 1 b). Similarly, if the cells were allowed to bind the conjugate at pH 6.0 but were rinsed briefly with buffer at pH 7.4 just before fixation, no binding was subsequently observed. An identical pattern of pH-dependent binding was found when segments were exposed to peroxidase conjugated to rabbit IgG, an immunoglobulin which is transported across the intestine in the

TABLE I

Luminal pH of the Gastrointestinal Tract of 9–18-Day Old Rats

	No. of animals	Mean pH	Standard deviation
Stomach	20	5.1	(± 0.6)
Duodenum	16	6.2	(± 0.2)
Jejunum	19	6.3	(± 0.2)
Transition	15	6.5	(± 0.3)
Ileum	20	6.9	(± 0.3)
Caecum	15	7.4	(± 0.3)

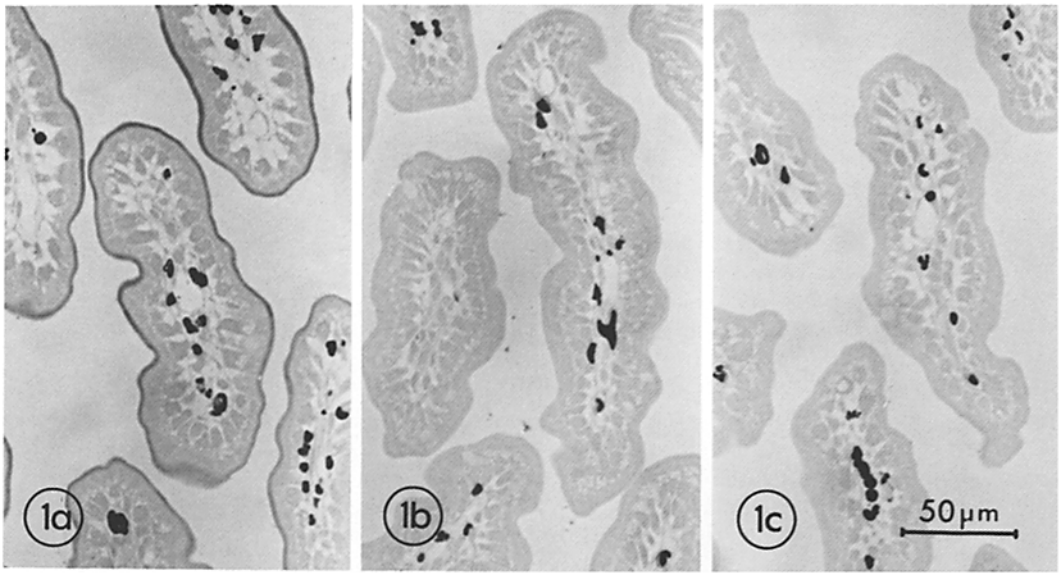

FIGURE 1 Light micrographs of neonatal jejunum exposed to peroxidase conjugates. Sections have cut the villi obliquely near their tips. Sections are unstained except for peroxidase reaction product. Erythrocytes, which contain endogenous peroxidase activity, appear as dense particles in the lamina propria of each villus. × 300. (*a*) Tissue exposed to the peroxidase conjugate of rat IgG at pH 6.0 for 10 min at 60°C. Reaction due to the conjugate appears as a dark band on the luminal surfaces of the villi. (*b*) Tissue exposed to the same conjugate at pH 7.4. (*c*) Control tissue exposed to a peroxidase conjugate of chicken IgG at pH 6.0.

intact rat (4, 11). As controls, jejunal segments were exposed at pH 6.0 to unconjugated peroxidase or peroxidase conjugated to (*a*) chicken IgG, (*b*) sheep IgG, or (*c*) bovine serum albumin, proteins which are not normally transported in the neonatal rat (4, 5, 10). None of these proteins bound to the luminal surface at this pH (Fig. 1*c*). As a further control, segments from the ileum, a region which does not participate in immunoglobulin transport in the intact animal (9, 10), were exposed to the rat IgG conjugate at pH 6.0 or 7.4. No binding could be demonstrated at either pH on any cells in this region.

Electron microscopy of jejunal segments revealed that at pH 6.0 the rat IgG conjugate bound to the entire luminal surface of individual absorptive cells. Electron-dense reaction product was distributed over the microvillar membranes and within surface pits at the bases of microvilli (Fig. 2). The conjugate was not found within vesicles of the cell interior nor within the extracellular spaces between cells, presumably because transport of the conjugate across the cell was inhibited at 0°C. No reaction product was seen on the surface of or within goblet cells.

These results demonstrate that selective binding to the luminal surface of absorptive cells is specific for the peroxidase conjugates of rat and rabbit IgG in the same manner that overall transport is selective for the unconjugated immunoglobulins from these animal species (4, 5). Therefore, at least qualitatively, the presence of peroxidase does not appear to influence the binding of immunoglobulins to the receptors on the membrane surface. This conclusion suggests that the sensitivity to pH with which the rat and rabbit conjugates bind to the luminal surface is a property of the normal interaction between the membrane receptor and immunoglobulins. Although the presence of the peroxidase could conceivably alter the pH-dependent binding of the immunoglobulins, we assume that the peroxidase conjugates have the same binding properties as do unconjugated immunoglobulins or iodinated immunoglobulins such as those employed by Jones and Waldmann (6). Since the Fc but not the Fab portion of rabbit IgG is able to bind to the cell surface (6, 11), it is likely that changes in pH preferentially affect either the Fc region of the immunoglobulin or the membrane receptor itself.

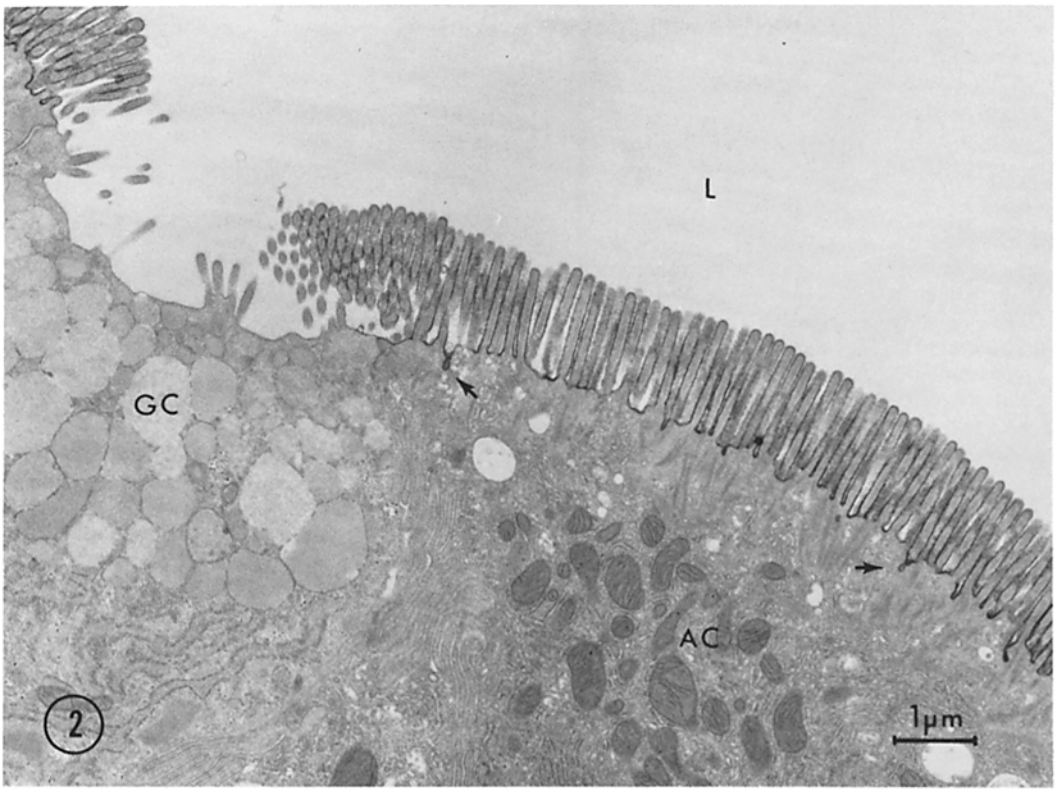

FIGURE 2 Electron micrograph of the luminal surface of jejunal cells exposed to rat IgG conjugated to peroxidase. Reaction product appears on the microvillar membrane and within small surface pits (arrowheads) of an absorptive cell (*AC*). The luminal surface of a goblet cell (*GC*) is devoid of reaction product. *L*, lumen. × 10,000.

It is not surprising that the conjugates of rat and rabbit IgG bind efficiently at pH 6.0 which is near the normal pH of the luminal contents in the region of the proximal intestine where transport occurs (9). However, it is quite intriguing that no binding can be demonstrated at pH 7.4, presumably very close to the pH of the plasma which bathes the cells at their abluminal surfaces. If, as is likely, IgG is absorbed and transmitted across the cell as IgG-receptor complexes on the surfaces of pinocytotic vesicles (6, 11), the immunoglobulins must necessarily be released from these receptors during discharge at the abluminal plasma membrane. Although release from receptors could occur at an earlier step during transport, a change in pH at the abluminal surface appears to be the simplest and most reliable mechanism to achieve this end. Such a mechanism has also been suggested by Waldmann and Jones (12). This sensitivity of binding to pH also insures that transport is efficient and essentially unidirectional with little or no back transfer of IgG from the circulation into the lumen of the intestine.

SUMMARY

Rat and rabbit IgG immunoglobulins conjugated to horseradish peroxidase as a histochemical marker bind at 0°C to the luminal surface of absorptive cells in isolated segments of jejunum from 10–12-day old rats. Binding is observed at pH 6.0, near the normal luminal pH of the duodenum and jejunum at this age, but not at pH 7.4. Furthermore, no binding occurs when cells are exposed at pH 6.0 to either free peroxidase or peroxidase conjugated to chicken or sheep IgG immunoglobulins or bovine serum albumin. The sensitivity of binding to pH suggests a means whereby immunoglobulins which are selectively absorbed by the cells can be released efficiently at the abluminal surface.

The author thanks Al Powers and Toby Zakin for their excellent technical assistance.

This work was supported by grant AI 11937 from the National Institutes of Health.

Received for publication 9 February 1976, and in revised form 28 June 1976.

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