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Direct evidence for pH-dependent Fc receptors on proximal enterocytes of suckling rat gut

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Summary. By means of an erythrocyte-antibody rosette technique, Fc receptors, functional at pH 6.0 but not at 7.2, were shown to be present on enterocytes isolated from duodenum and jejunum (but absent from ileum) of 12–20-day-old suckling rats.

In the rat, passive immunity is transferred from mother to young largely after birth and for a time period that extends up to 18–21 days post-partum[1]. When the young rat suckles during this time, immunoglobulin (IgG) present in the colostrum and milk has somehow to be selectively transported across the cellular barriers constituting the gut wall. The sites of transmission within the gut and the mechanism whereby selection is effected are still matters of controversy. A growing body of evidence based on ultrastructural[2,3], autoradiographic[4] and quantitative[5-7] assessment of IgG transport points to the proximal region (jejunum) of the small intestine and involvement of receptors specific for the Fc region of IgG that have dependency upon an acid pH for IgG-receptor binding[8,9]. However, a recent report[10] still places emphasis on the distal region (ileum) as a site of transport and suggests that specific receptors are not involved in the selective entry of immunoglobulin into enterocytes. In a previous study[11] it was shown that Fc receptors could be detected on endodermal cells of rabbit yolk sac by means of an erythrocyte-antibody (EA) rosette technique, and that such receptors were associated with selective immunoglobulin transport. In the present preliminary investigation we have applied the EA rosette technique to enterocytes removed from different regions of suckling rat gut to see whether or not they too have detectable Fc receptors.

Initial studies were carried out on 12-day-old suckling Wistar strain rats. These were isolated from the mother for 2 h and then killed by cervical dislocation. The intestine was dissected out, freed of mesentery, and the gut lumen perfused with cold isotonic phosphate buffered saline, pH 7.2, in order to remove debris. It was then divided into segments comprising duodenum, jejunum, lower jejunum and upper ileum, and ileum, and each segment everted over the sealed drawn-out end of a Pasteur pipette. The everted sacs so formed were tied with ligatures at both ends and incubated in serology tubes containing 0.25% trypsin (Difco) and 10% foetal calf serum (FCS) in Eagles minimal essential medium (MEM) for 45 min at 37 °C. The medium was then gently agitated for a few min to dislodge enterocytes and centrifuged at 250 × g for 3 min to pellet the cells. These were then washed 3 times in MEM/10 FCS, where necessary passed through a hypodermic needle to break up clumps, and finally re-suspended at $4–5 \times 10^5$ cells/ml. Although this isolation procedure resulted in some cell fragmentation, many cells were left intact with a readily visible brush border; ileal cells could be distinguished from jejunal cells by the presence of a large supranuclear vesicle

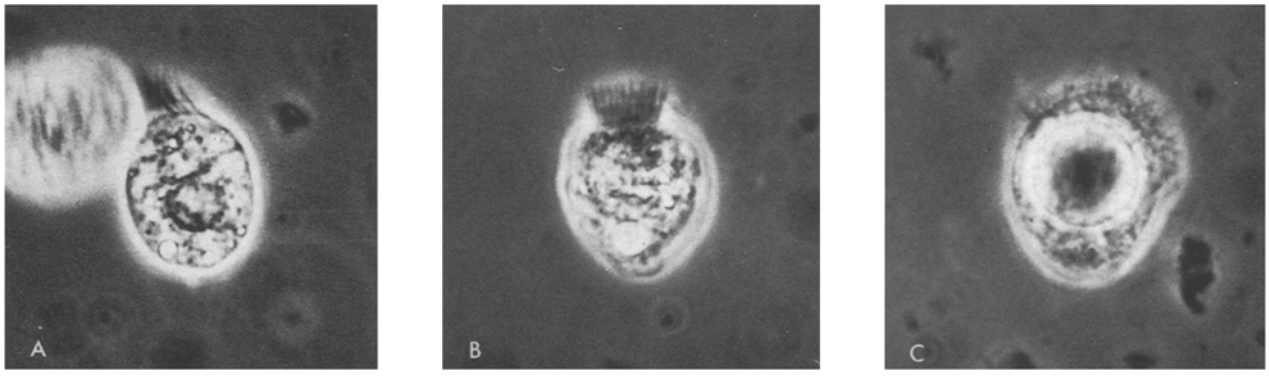

Fig. 1. Appearance of enterocytes obtained from *A* duodenum, *B* jejunum and *C* ileum, as seen under phase microscopy.

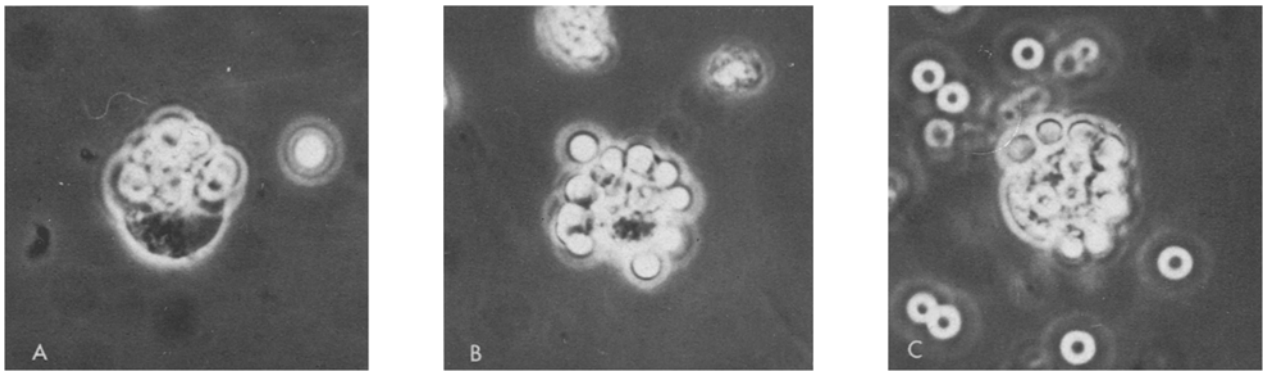

Fig. 2. Appearance of EA rosettes formed with *A* duodenal and *B* and *C* jejunal enterocytes at pH 6.0.

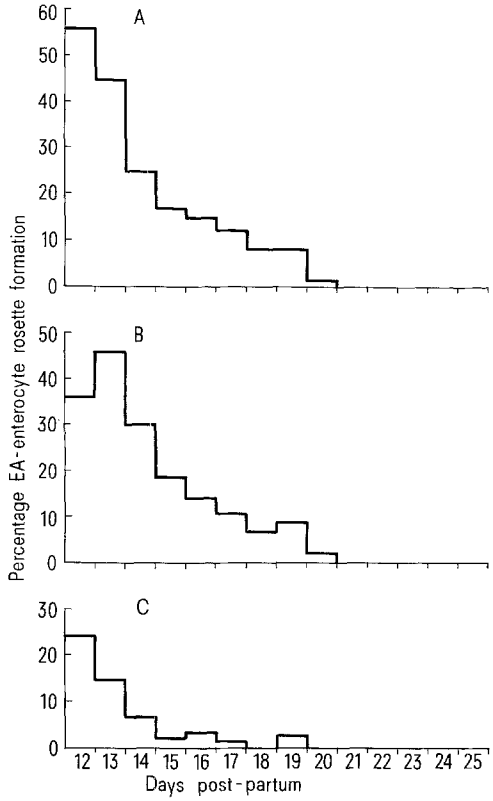

Fig. 3. EA rosette formation at pH 6.0 with enterocytes removed from *A* duodenum, *B* jejunum, *C* lower jejunum/upper ileum, at 12–25 days post-partum.

(figure 1). Indicator sheep red blood cells (SRBC) were coated with rabbit IgG or the F(ab')2 fraction of IgG. Appropriate dilutions of specific antibody fractions were made so as to give maximum sensitisation without agglutination. Rosetting procedures and counting were as previously described[11] except that the pH was adjusted to 6.0 in 1 of a pair of cell samples by adding 1 drop of Sorenson's phosphate buffer. The other sample was at the pH of the medium, namely 7.2.

At pH 7.2 no rosettes were formed between enterocytes and sensitized or control SRBC whatever the derivation of enterocytes from the intestine. However, at pH 6.0 (chosen because this gave maximal rosette counts when tests were made at 4.8–7.2) only enterocytes of the duodenal, jejunal and lower jejunal/upper ileal regions formed rosettes with IgG sensitized SRBC; no rosettes were formed with F(ab')2

Mean % EA rosette formation with enterocytes from different regions of 12-day-old suckling rat intestine (pH 6.0)

Sensitizing antibody	Duodenum	Jejunum	Lower jejunum/ upper ileum	Ileum
IgG	34 (67–8)*	37 (50–16)	25 (80–3)	0.2 (1–0)
F(ab')2	0	0	0	0
Control SRBC	0	0	0	0

* Brackets show range for 5 animals.

sensitized, or control SRBC (table). These results indicate the presence of Fc receptors on the surface of at least a proportion of enterocytes from these regions. Rosetting enterocytes (figure 2) had an appearence not unlike that of rabbit yolk sac endodermal cells[11], in that indicator SRBC became attached at the lateral and possibly basal cell surfaces and not necessarily on the apical brush border. It was our impression that the enterocytes with a large supranuclear vesicle (ileal cells) did not form rosettes in the mixed cell population of the lower jejunum/upper ileum.

These studies were extended to suckling rats ranging from 12 to 25 days post-partum. Again no EA rosettes formed at pH 7.2 with enterocytes from any region of the gut and Fc receptors were absent from ileal cells at all ages as detected by EA rosette formation at pH 6.0. Results for proximal intestinal regions revealed a gradual decrease in the proportion of cells forming rosettes as the animals aged, even though only single animals were studied and considerable variation might have been expected in the light of results shown in the table; at 21 days post-partum and later, Fc dependent EA rosette formation did not take place for cells from any region of the proximal small intestine (figure 3).

Our results provide direct evidence for what has been intimated by the work of Jones and Waldman[8] and Rodewald[9], namely that enterocytes of proximal small intestine of suckling rats have Fc receptors adapted to suit the acid conditions of this region of the gut in order to effect IgG binding and subsequent transport. If immunoglobulin is transported across the ileum, as has also been claimed[10], then it must be by some mechanism not involving Fc receptors. The use of rabbit IgG as the sensitizing antibody in our studies is justified on the grounds that this heterologous protein is well known to be transported across the gut of suckling rats[12] although it might be expected that SRBC sensitized with rat IgG would provide for better Fc receptor binding. Receptors for chicken IgG on chicken yolk sac have also been recently shown to be adapted to meet the acid (pH 6.0) conditions of the yolk to which the endodermal cells are exposed[13] and the suggestion has been made[14] that dissociation of IgG from the receptor is facilitated by

the more alkaline pH that may be experienced at the lateral cell surface when IgG is diacytosed. Such a simple explanation cannot hold for all IgG transporting epithelia since endodermal cells from rabbit yolk sac, exposed to the more neutral pH conditions of the uterine fluid, show little if any pH dependency for EA rosette formation between 6.0 and 8.0[15] and similar findings have been reported for binding of IgG to the Fc receptor on human syncytiotrophoblast exposed to maternal blood[16]. However, the Fc dependent binding of sensitized SRBC to the lateral and basal surfaces of proximal gut enterocytes indicates that receptor sites are regenerated here. We would favour the interpretation that these arise from coated vesicles that have previously selectively endocytosed IgG at the apical cell surface and then moved through the cell and subsequently fused with the lateral and basal plasmalemma so discharging their contents. Evidence for such a process has been obtained both in suckling rat gut[2,9] and rabbit yolk sac endoderm[17] at the ultrastructural level. The disappearance of Fc receptors (as indicated by decrease in EA-enterocyte rosette formation) as the young rat ages, correlates precisely with what is known about decrease and cessation of antibody transport to the blood of the suckling rat, and is in accord with studies of binding of ^{125}I-labelled mouse monomeric IgG by jejunal enterocytes of suckling rat gut as revealed by autoradiography[4]. In the latter studies[4] evidence has also been obtained that Fc receptors on jejunal enterocytes are trypsin-sensitive and restricted to those cells on the more apical regions of villi. This would explain why not all the enterocytes removed from proximal gut segments formed rosettes and why wide variations occurred in the number that did form rosettes when different rats at 12 days postpartum were compared.

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Shape change reaction of platelets in protein-free medium: ultramorphology

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Summary. Transmission and scanning electron microscopy indicate that rabbit platelets incubated in protein-poor medium retain their reactivity to shape change-inducing agents such as 5-hydroxytryptamine, adenosine-5′-diphosphate and chlorpromazine. Such platelets may be used as models for drug-membrane interactions, e.g. in neuronal cells.

During the shape-change, reaction blood platelets undergo a transformation from their normally discoid form into a spheroid shape, whereby the light absorption of the platelet suspensions is increased[1]. The shape-change reaction, i.e. the increase in light absorption, has generally been measured using plasma-containing media, which may however introduce sources of error (see below). Therefore, a method has been developed which allows the suspension of the isolated platelets in protein-free buffer without greatly impairing their reactivity to shape change-inducing agents such as 5-hydroxytryptamine (5HT), adenosine-5′-diphosphate (ADP) and chlorpromazine (CPZ)[2,3]. This paper describes the effects of these compounds on the ultramorphology of isolated platelets in protein-poor medium.

Materials and methods. Platelets of rabbits, isolated by centrifugation of platelet-rich plasma (PRP) on a discontinuous dextran T-10 gradient, were resuspended in about 4 vol. of citrate buffer containing glucose and saline, pH 7.4, 290 mosmol, as previously described[2]. After incubation in glass tubes for 30 min at 37°C, the platelets were stirred for 2 min at 37°C with a teflon-covered stirrer at 900 rpm. 5HT-creatinine sulfate (final concentration 5×10^{-5} M), ADP (disodium salt, 5×10^{-5} M), CPZ (hydrochloride 10^{-4} M) or solvent alone (H_2O) were then added[2,3]. When the light absorption had reached its maximum, i.e. after 60 sec (5HT), 30 sec (ADP) and 5 min (CPZ) an equal volume of 0.2% glutaraldehyde (in 0.1 M cacodylate buffer pH 7.3) was added and the platelets were prefixed for 30 min at room temperature. Thereafter, each specimen was divided into 2 parts and processed for transmission[2] and scanning[4] electron microscopy (TEM and SEM).

Results and discussion. With both TEM and SEM, the control platelets showed a discoid shape and the outline of the plasma membrane was smooth, except for occasional

Transmission (left) and scanning (right) electron micrographs of rabbit blood platelets isolated on a dextran gradient, resuspended in protein-free buffer and exposed to various compounds. Note the discoid shape of control platelets, the shape change and pseudopods induced by ADP and 5HT and the shape change with virtually no pseudopods after CPZ. Arrows identify highly electron-dense organelles.