

19. Dickinson KEJ, Nahorski SR, Willcocks AL 1981 Serotonin recognition sites are labelled in cerebral cortex by the β -adrenoreceptor antagonist 125 I-hydroxybenzylpindolol. *Br J Pharmacol* 72:165P
20. McMillian MK, Schanberg SM, Kuhn CM 1983 Ontogeny of rat hepatic adrenoreceptors. *J Pharmacol Exp Ther* 227:181-186
21. Noguchi A 1983 Normal ontogeny of α_1 adrenergic receptor in rat liver is thyroid hormone dependent. *Endocrinology* 113:672-676
22. Noguchi A, Jett PA, Gold AH cAMP independent stimulation of glycogen phosphorylase in newborn rat hepatocytes. *Am J Physiol* 248:E560-E566
23. Metz W, Forssman WG 1980 Innervation of the liver in guinea pig and rat. *Anat Embryol* 160:239-252
24. Reilly FD, McCuskey PA, McCuskey RS 1978 Intrahepatic distribution of nerves in the rat. *Anat Rec* 191:55-68
25. Forssman WG, Ito S 1977 Hepatocyte innervation in primates. *J Cell Biol* 74:299-313
26. Hartman H, Beckh K, Jungermann K 1982 Direct control of glycogen metabolism in the perfused rat liver by the sympathetic innervation. *Eur J Biochem* 123:521-526
27. Blazques E, Rubalcava B, Montesano R, Orci L, Unger RH 1976 Development of insulin and glucagon binding and the adenylate cyclase response in liver membranes of prenatal, postnatal, and adult rat: evidence of glucagon "resistance." *Endocrinology* 98:1014-1023

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Differential Toxicity of RCA_{II} (Ricin) on Rabbit Intestinal Epithelium in Relation to Postnatal Maturation¹

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ABSTRACT. The purpose of this work was to assess the toxic lectin ricin (RCA_{II}) as a probe for the study of intestinal permeability in the developing small bowel. Jejunal explants from suckling and adult rabbits were exposed to varying dosages of RCA_{II} for 30 min at 25° C and then cultured in toxin-free medium. The RCA_{II} dose required to inhibit protein synthesis during 6 h of culture increased from 0.1 μ g/ml in 4-day-old rabbits to 25 μ g/ml in weanling rabbits. RCA_{II} cytotoxicity was almost completely blocked by 0.1 M lactulose in all age groups. The kinetics of 125 I-RCA_{II} binding to purified microvillus membranes were determined by incubating a fixed concentration of membrane protein (30 μ g) with increasing concentrations of labeled lectin (2-18 μ g/ml). Binding attained saturation with adult but not with suckling animal membranes. The latter yielded a curvilinear relationship in Scatchard plots, suggesting either several classes of binding sites or negative cooperativity. RCA_{II} binding was confined to the delipidated fraction of the membranes and decreased by 42% from 6 days old to adult age. The extreme sensitivity of colostral epithelium to RCA_{II} is probably related to the high level of endocytosis exhibited by the immature membrane of suckling rabbits. The development of increasing resistance to the toxin, and associated decrease in binding, might be related to disappearance of saccharide sites in productive surface receptors occurring in the developmental course of intestinal glycosylation. (*Pediatr Res* 19: 868-872, 1985)

Abbreviations

RCA_{II}, ricin
PBS, phosphate-buffered saline

The early part of the suckling period in rodents is characterized by a high level of receptor-mediated responses in small intestinal epithelium. This has been clearly shown for the jejunal transport of breast milk immunoglobulin (1, 2) and is also inferred from the high content of trophic factors in breast milk (3, 4). The surface characteristics underlying this high receptor responsiveness in the intestine of neonatal animals are poorly understood. A marked developmental change in surface reactivities to lectins has been described in the small intestine of suckling rats (5-7). In this animal species, the intestinal microvillus membrane undergoes a progressive shift from sialylation to fucosylation of glycoproteins and glycolipids during postnatal development (7). In the present work, we have examined the response of rabbit intestinal epithelium to the toxic action of RCA_{II}. This toxin inhibits protein synthesis in intact cells (8, 9) through a mechanism that requires terminal nonreducing galactosyl (or N-acetyl-galactosaminy) residues (10) in a glycoprotein receptor capable of transmembrane signaling for toxin internalization (11). This toxin, therefore, "mimics" the mechanism of interaction of trophic hormones (12). The RCA_{II} membrane receptors may be part or be closely associated with cell surface components involved in cell-macromolecule and cell-cell recognition (13). The probing of intestinal epithelium with RCA_{II} may provide insight into the role of glycosylation on receptor-mediated responses in the developing small bowel.

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MATERIALS AND METHODS

Reagents and materials. Ricin (RCA₆₀) was obtained commercially (P-L Biochemicals, Inc, Milwaukee, WI). This protein was further purified by affinity chromatography on Sepharose 4B using 0.01 M D-GalNAc in the eluting buffer (10). The protein gave a single band with a molecular weight of ~65,000 on SDS-polyacrylamide gel electrophoresis. Lactose and lactulose (4-*O*- β -D-galactopyranosyl-D-fructofuranose) were purchased from Sigma (St. Louis, MO). [³H]-leucine (s.a. 59.2 Ci/mmol) and [¹⁴C]-leucine (s.a. 54.5 mCi/mmol) were obtained from New England Nuclear (Boston, MA); lactoperoxidase beads from BioRad (Richmond, CA); cellulose filters (0.45 μ pore size) from Millipore (Bedford, MA); organ culture dishes and stainless steel organ culture screens from Falcon (Cockeysville, MD). New Zealand White rabbits were purchased locally and maintained on Purina rabbit Chow (St. Louis, MO).

Preparation and culture of jejunal explants. Jejunal explants from New Zealand rabbits were obtained by two methods. In anesthetized adult and suckling rabbits older than 4 days, the explants from proximal jejunum were cut and prepared as described by Browning and Trier (14). From birth to 4 days of age, the animal was anesthetized with ether, the proximal jejunum was removed, washed with ice-saline, and sectioned into 2–3 mm long rings. The rings were opened with straight iris scissors and the whole tissue placed mucosal side up on a stainless steel grid. The thinness of the bowel in animals 1–4 days of age precluded the cutting of mucosal explants. The initial culture medium was serum-free RPMI 1640 with added glucose, insulin, and antibiotics.

Treatment of jejunal explants with RCA_{II}. After equilibration in culture medium for 15 min, the tissue explants were treated with varying dosages of RCA_{II} as follows: the grids with the mucosal explants attached were transferred to glass Petri dishes and immersed in serum-free culture medium (4.5 ml/dish) containing RCA_{II} or bovine serum albumin as controls. The dishes were placed in air-tight boxes, gassed with 95% O₂ and 5% CO₂, and shaken on a rotating platform (Fisher Rotator, Fisher Scientific Co. Dallas, TX) at 70 rpm for 30 min at 25° C. The grids were then removed, washed three times with 100 mM lactose in PBS and returned to culture dishes containing initial culture medium with added lactulose (5 mM) and 10% heat inactivated fetal calf serum. The culture times are indicated in the corresponding figures. Four hours prior to the end of the culture period, the medium was removed and replaced by Eagle's minimal essential medium without leucine to which 10% fetal calf serum, 1 mM lactulose, and 1 μ Ci/ml of [³H]- or [¹⁴C]-leucine had been added. After culture, the jejunal explants were removed from the grids, washed three times with 1 mM leucine in cold PBS and homogenized in warm 0.1 M KOH. Aliquots of the homogenates were taken for protein determination and for precipitation with cold 20% trichloroacetic acid. The precipitates were left at 4° C for 2 h and collected on Millipore filters, washed three times with 5% trichloroacetic acid and once with 95% ethanol, dried, and counted with 3 ml Aquasol in a Packard Tri-Carb scintillation counter.

Purification of the microvillus membranes. Microvillus membranes were prepared from the proximal half of the small intestine by the method of Schmitz *et al.* (15) and further purified as described by Ohsawa *et al.* (16). In 6-day-old rabbits, four litter mates were pooled for each membrane preparation. Enrichment of lactase (early suckling animals) and sucrase (late suckling and adult animals) (17) was 20 to 22 fold. Protein was measured by the method of Lowry *et al.* (18) using bovine serum albumin as standard.

¹²⁵I-RCA_{II} binding. In preliminary studies, we found that binding of ¹²⁵I-RCA_{II} to microvillus membranes reached equilibrium within 10 min at 25° C. In subsequent studies, microvillus membrane protein (30 μ g in 50 μ l) was added to 150 μ l of PBS

containing ¹²⁵I-RCA_{II} and incubated for 30 min at 25° C in plastic tubes (Falcon). The incubation was ended by adding 2 ml of PBS (4° C) and immediately filtering through 0.45 μ cellulose filters (these filters were found to retain 100% of the microvillus protein used in the binding assays); the filters were then washed twice with 4 ml of PBS at 4° C. The radioactivity retained by the filters was counted in a Tri-Carb spectrometer (Packard, Downers Grove, IL). Nonspecific binding was determined in the presence of 0.2 M lactose and was less than 1% of the total radioactivity bound in all ages. Binding to the filter was less than 1% of the total added radioactivity.

Preparation of ¹²⁵I-RCA_{II}. Iodination of about 0.5 mg of purified RCA_{II} was performed using lactoperoxidase from BioRad (Richmond, CA) as previously described (6). Each preparation of labeled RCA_{II} was used within 2 wk. The specific activity was about 5.5 \times 10⁶ cpm/mg protein (efficiency of ¹²⁵I counting: 49.4%).

RESULTS

Effect of RCA_{II} on rabbit jejunal explants. Figure 1 shows the time-dose relationships of the effect of RCA_{II} on jejunal explants from adult rabbits. The time required to reach 50% inhibition

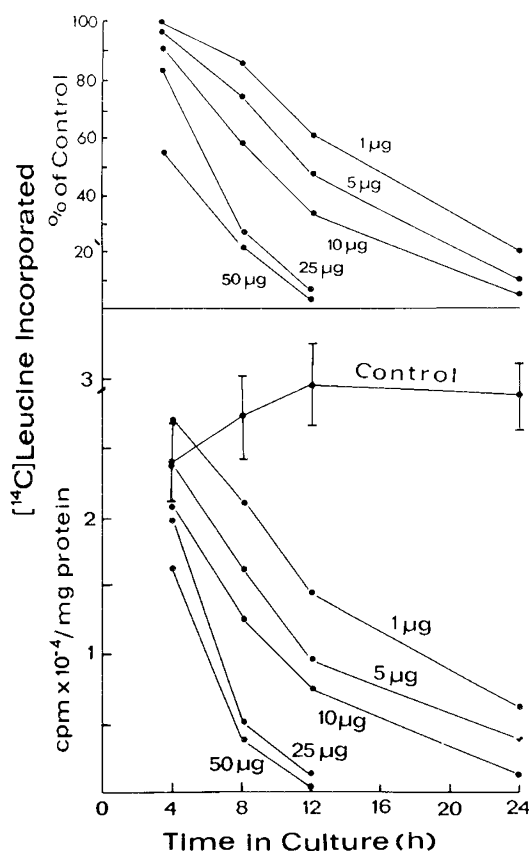


Fig. 1. Inhibition of protein synthesis in rabbit intestinal epithelium by RCA_{II}. Mucosal explants from proximal jejunum were pulsed (30 min) with RCA_{II} and then maintained in organ culture medium with 5 mM lactulose, as described in "Materials and methods." *Top*, the number in micrograms are the RCA_{II} concentrations used in each time-course experiment. Each *point* on the *curves* represents the average of eight RCA_{II}-treated explants (two explants per culture dish) from two animals. Paired control (albumin treated) explants were included for each set of RCA_{II}-treated explants. Each control value represents mean \pm SD for 40 explants from 10 animals. *Bottom*, the percentage of control values were calculated for each set of RCA_{II}-treated samples with respect to the corresponding set of albumin-treated samples.

of protein synthesis ranged from 4½–16 h for RCA_{II} doses of 50 and 1 µg/ml, respectively. Using this dose range, we then assessed the effect of RCA_{II} on jejunal explants from suckling rabbits during a 6-h culture period. As shown in Figure 2, the toxin dose required to cause 50% inhibition of protein synthesis was 0.5 µg/ml at birth, fell sharply to 0.1 µg/ml on days 4–7 after birth ($p < 0.001$), and then increased progressively to reach a value of 25 µg/ml on day 23. Extrapolation to 6 h in Figure 1 revealed the latter 50% inhibition dose to be similar in adult animals. Finally, we assessed the specificity of the effect of RCA_{II} by measuring the blocking action of lactulose—a sugar that is not hydrolyzed by intestinal mucosa. Figure 3 shows the toxin-hapten inhibitor relationships in mucosal explants from 6-day-old and adult rabbits. In both animal groups, the toxic effect of 0.5 µg/ml RCA_{II} could be almost completely abrogated with 100 mM lactulose.

In the next series of experiments, we compared the histological damage caused by RCA_{II} on mucosal explants of 6-day-old and weanling rabbits (Fig. 4). On light microscopy, control explants showed normal cellular morphology, slightly shortened and broadened villi, and mild edema of the lamina propria. These changes were similar to those previously reported by Kagnoff *et al.* (19). In 6-day-old pups, mucosal explants treated with RCA_{II} showed marked stunting of the villi, as well as shortening and pyknotic degeneration of the epithelial cells. By contrast, jejunal explants from weanling animals similarly treated showed only slight pyknotic changes and retained a near-normal cell shape.

Binding of ¹²⁵I-labeled RCA_{II} to the microvillus membranes. The binding of ¹²⁵I-labeled RCA_{II} to the microvillus membranes

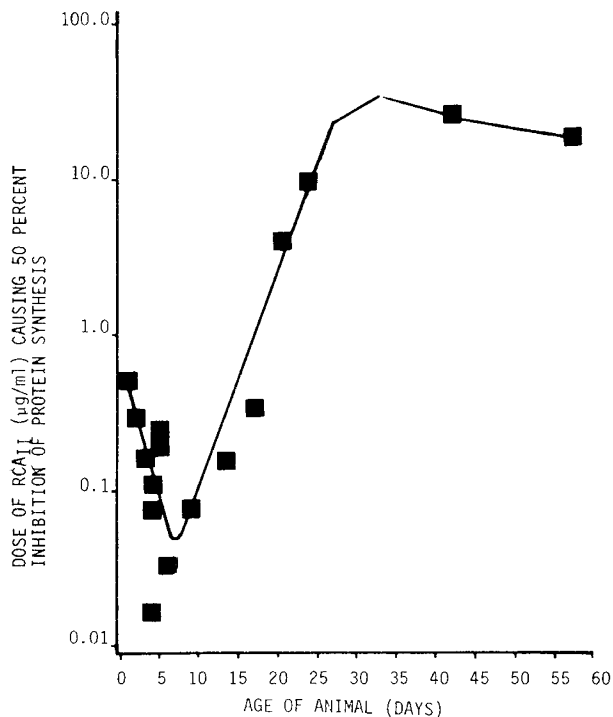


Fig. 2. Developmental change in sensitivity to the toxic effect of RCA_{II} in jejunal explants from suckling rabbits. Each point represents the dose of RCA_{II} required to give 50% inhibition of protein synthesis after a 30-min pulse of RCA_{II} followed by 6 h in culture. Triplicates of organ culture grids each containing three explants were exposed to varying doses of RCA_{II} or bovine serum albumin as described in "Materials and methods." The dose of RCA_{II} which gave 50% inhibition of protein synthesis was determined for each experiment from semi-log plots of RCA_{II} concentration against percentage inhibition of protein synthesis. Each point represents a single animal from different litters (except for day 1, where three animals gave results within the dose range covered by the symbol).

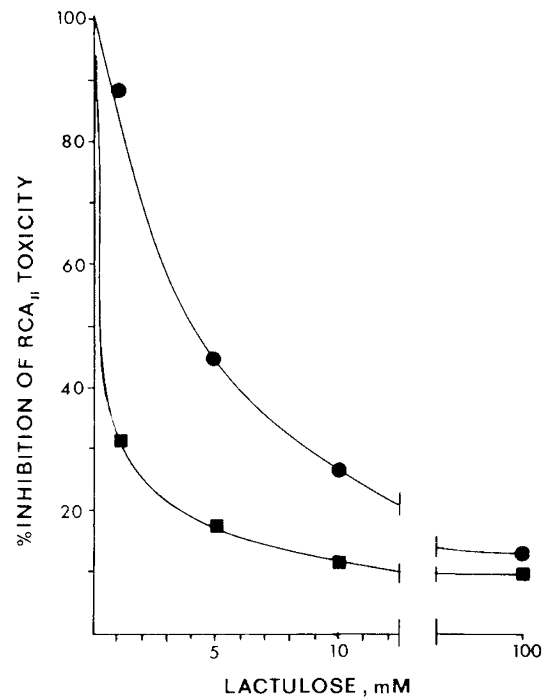


Fig. 3. Inhibition of RCA_{II} cytotoxicity by lactulose. Explants from 6-day-old (●) and adult (■) rabbits were pulsed with 0.5 µg/ml RCA_{II} (30 min, 25° C) in the presence of variable concentrations of lactulose (0–100 mM). The tissues were then removed, washed with 100 mM lactose in phosphate-saline buffer pH 7.38, and cultured for 24 h as described in "Materials and methods." Control samples in which bovine serum albumin substituted for RCA_{II} were also cultured for 24 h.

of suckling animals was higher than those of adult animals over a wide range of lectin concentrations (Fig. 5). When the results were represented according to the Scatchard equation (20) (Fig. 5, *inset*), the plot from adult rabbit membranes yielded a straight relationship at concentrations above 2 µg/ml of free lectin ($n = 23 \mu\text{g mg}^{-1}$). In contrast, the membranes from suckling animals exhibited a curvilinear relationship throughout the plot, suggesting either the existence of several classes of binding sites of decreasing affinities, or the presence of additional nonsugar interactions.

To assess the approximate distribution of RCA_{II} binding sites between glycoproteins and glycolipids, about one-half of the membrane preparations (4–5 mg protein) was delipidated (Table 1). Binding to intact and delipidated membranes was then compared within the same binding assay, using equal amounts of membrane protein and labeled lectin. RCA_{II} binding was almost identical in both membrane preparations, indicating that most of the receptor sites were contained in the glycoprotein fraction of the membrane. Under the assay conditions, from early suckling to adult ages, RCA_{II} binding to delipidated membranes decreased by 42%.

DISCUSSION

The experimental approach used in these studies—a short pulse of RCA_{II} followed by organ culture in toxin-free medium in the presence of the blocking sugar—allowed us to define accurately the cytotoxic effect of RCA_{II} on rabbit jejunal mucosa *in vitro*. The intestinal epithelium was highly sensitive to the effect of RCA_{II} during the colostral period, and became increasingly resistant with growth and maturation. The cytotoxic effect of RCA_{II} could be completely abrogated by lactulose in both colostral and weanling animals, indicating that a receptor-mediated process was required throughout development for toxin

penetration into the epithelial cells.

The results of our protein inhibition and histological studies indicated a marked age-related difference in the susceptibility of the intestinal villus cells to RCA_{II} cytotoxicity. The decrease in sensitivity of these cells to the effect of RCA_{II} was accompanied by both qualitative and quantitative changes in RCA_{II} binding to the microvillus membrane. The kinetic characteristics of RCA_{II} binding to membranes from suckling animals could be explained through the presence of several classes of binding sites of decreasing affinities; however, an alternative explanation could be negative cooperativity induced by nonsugar interactions. A similar phenomenon has been observed in the differential toxicity of RCA_I toward chick fibroblasts in relation to the stage of embryo development (21). The kinetic behavior of the RCA_{II} interaction with immature membranes might be related to the unique molecular organization of the intestinal apical membrane of suckling animals (22, 23). In this regard, it is interesting that the binding of ¹²⁵I-cholera toxin to the microvillus membrane of suckling and adult rabbits exhibited kinetic differences similar to those shown herein for RCA_{II} (24). There is increasing evidence (25) that lectins and bacterial toxins share common properties in their ability to mimic hormonal interactions with the membrane to gain access into cells. The increased mucosal uptake of RCA_{II} during the rabbit colostrum period might occur through interaction with membrane structures involved in the

high level of endocytosis characteristic of this period (1, 2, 26).

The marked decrease in the binding of RCA_{II} to delipidated membranes of weanling animals suggested either the disappearance of specific glycoproteins from the membrane or a change in the glycosidic structure of certain glycopeptides. Conceivably, the specific glycopeptide involved in transmembrane signaling and internalization of RCA_{II} (11) might be affected by such change in glycosylation. Similar to what we previously described in the rat (7), the microvillus membrane of suckling rabbits experiences a decrease in sialic acid and increase in fucose content during postnatal development (unpublished observations). This leads to a complete reversal of the sialic acid to fucose molar ratios in glycoproteins and glycolipids at weaning. It remains to be determined if this shift from sialylation to fucosylation plays a role in decreasing the accessibility of RCA_{II} to the receptor saccharide sites in the membrane.

In summary, the intestinal epithelium of suckling rabbits exhibits high sensitivity to the toxic effect of RCA_{II} during the colostrum period. At this time, the lectin-receptor interaction might be influenced by membrane structures primarily involved in enhanced endocytosis during the rabbit neonatal period. The epithelium loses considerable sensitivity to the toxin with age-related maturation. This might be related to disappearance of saccharide binding sites in a productive membrane receptor, as a consequence of developmental changes in glycosylation.

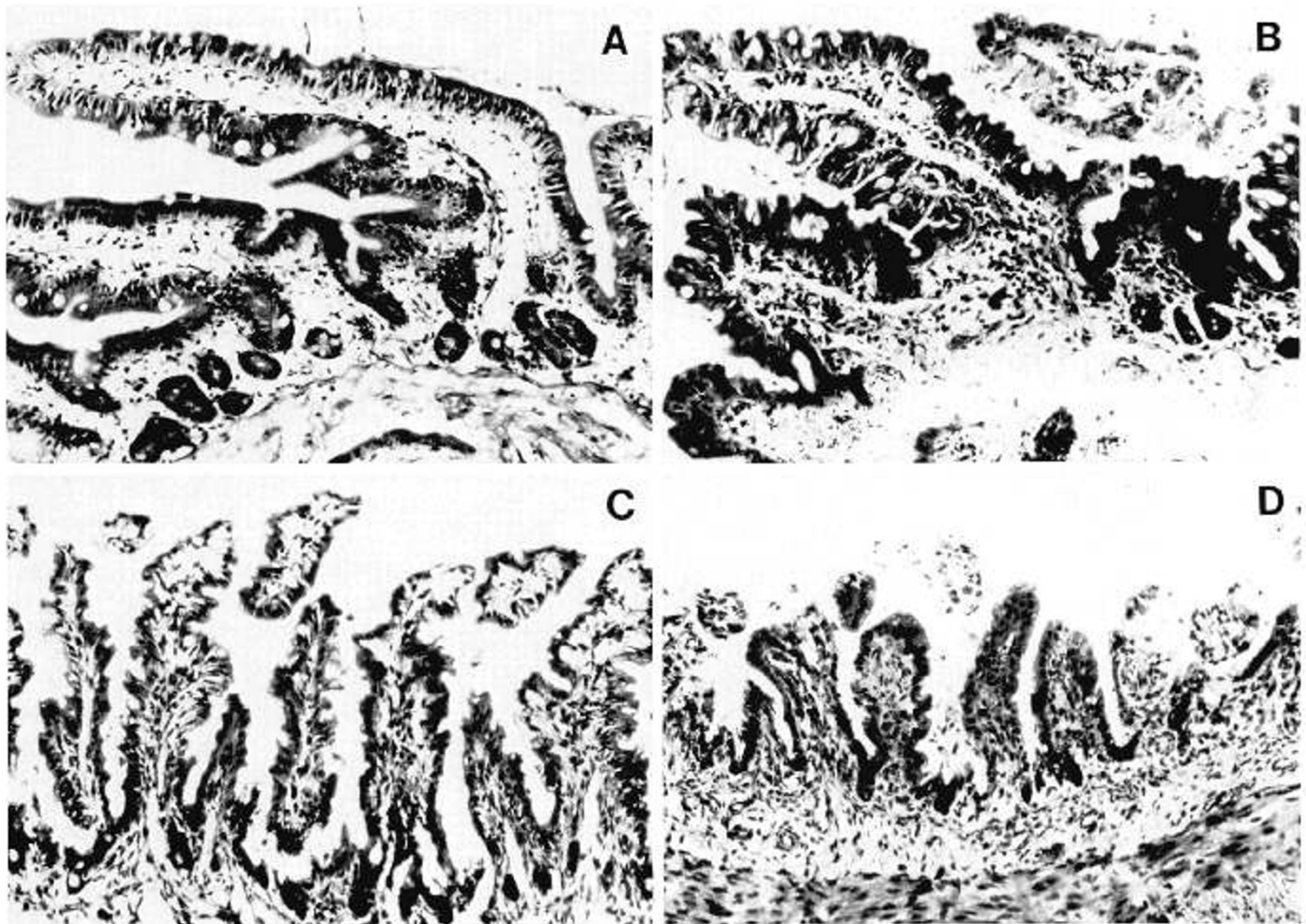


Fig. 4. Appearance of explants after 30-min exposure to albumin (controls) or RCA_{II} followed by 8-h culture. *A*, Adult rabbit intestine exposed to albumin. *B*, adult rabbit intestine exposed to RCA_{II}. *C*, 8-day-old rabbit intestine exposed to albumin. *D*, 8-day-old rabbit intestine exposed to RCA_{II}. Note that albumin-exposed explants tolerated *in vitro* conditions well. RCA_{II} exposure resulted in both focal necrosis and alterations in surviving cells, the latter manifested principally by intercellular edema in explants from adult bowel, but marked rounding of cells and nuclei in explants from suckling bowel. Hematoxylin and eosin. $\times 500$.

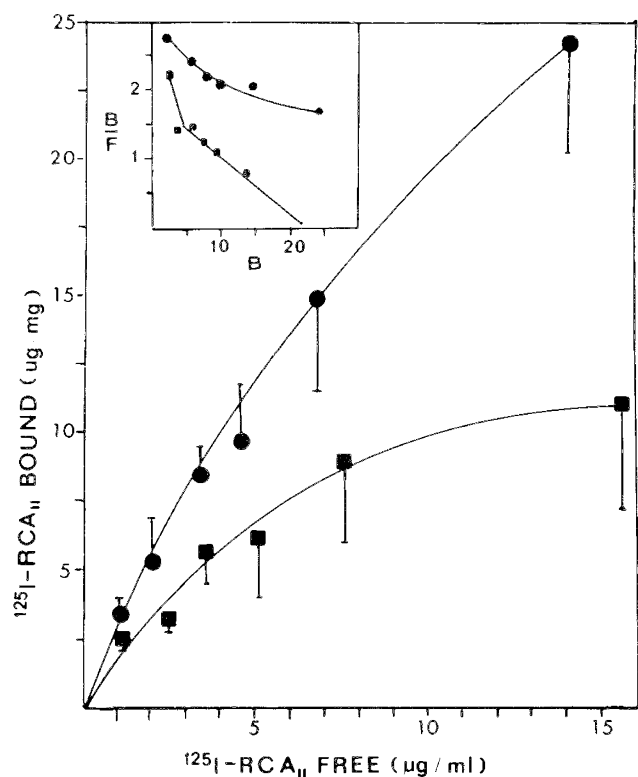


Fig. 5. Specific binding of ^{125}I -labeled RCA_{II} to intestinal microvillus membranes of 6-day-old (●) and adult (■) rabbits. The binding assays were performed as described in "Materials and methods." Each point represents the mean \pm SD of eight to 10 binding assays with microvillus membranes from three animals. Specific activity of ^{125}I - RCA_{II} : 5.5×10^3 cpm/ μg protein. *Inset*. Scatchard plots of data from main body of the figure. *B*, ^{125}I - RCA_{II} bound in micrograms per milligram of microvillus membrane protein; *F*, free concentration of ^{125}I - RCA_{II} in micrograms per milliliter.

REFERENCES

- Abrahamson DR, Powers A, Rodewald R 1979 Intestinal absorption of immune complexes by neonatal rats: a route of antigen transfer from mother to young. *Science* 206:567-569
- Rodewald R 1973 Intestinal transport of antibodies in the newborn rat. *J Cell Biol* 58:189-211
- Keenan TW, Patton S 1975 The milk fat globule membrane. *Biochim Biophys Acta* 415:273-309
- Carpenter G 1980 Epidermal growth factor is a major growth-promoting agent in human milk. *Science* 210:198-199
- Etzler ME, Branstrator ML 1979 In: *Ciba Foundation Symposium 70* (new series). Excerpta Medica, Amsterdam, pp 51-68
- Mahmood A, Torres-Pinedo R 1983 Postnatal changes in lectin binding to microvillus membranes from rat intestine. *Biochem Biophys Res Commun* 113:400-406
- Torres-Pinedo R, Mahmood A 1984 Postnatal changes in biosynthesis of microvillus membrane glycans of rat small intestine. I. Evidence of a developmental shift from terminal sialylation to fucosylation. *Biochem Biophys Res Commun* 125:546-553
- Benson S, Olsnes S, Pihl A 1975 On the mechanism of protein-synthesis inhibition by abrin and ricin. Inhibition of the GTP-hydrolysis site on the 60-S ribosomal subunit. *Eur J Biochem* 59:573-580
- Olsnes S, Pihl A 1974 Different biological properties of the two constituents peptide chains of ricin, a toxic protein inhibiting protein synthesis. *Biochemistry* 12:3121-3126
- Nicolson GL 1974 Characterization of two plant lectins from *Ricinus communis* and their quantitative interaction with a murine lymphoma. *Biochemistry* 13:196-204
- Nicolson GL, Poste G 1978 Mechanism of resistance to ricin toxin in selected

Table 1. Specific ^{125}I - RCA_{II} binding to native and delipidated microvillus membranes from suckling and weanling rabbit intestine

Rabbit age (day)	Membrane preparation*	^{125}I - RCA_{II} binding [†] ($\mu\text{g}/\text{mg}$ protein)
6	Native	16.4 ± 2.7
	Delipidated	16.1 ± 2.0
40	Native	$8.9 \pm 2.5\ddagger$
	Delipidated	$9.4 \pm 3.1\ddagger$

* In comparison to the whole mucosal homogenates, the purified microvillus membranes showed the following enrichment in the insoluble fraction of alkaline phosphatase: 6 day old, 23-fold (± 1); 40 day old, 21-fold (± 2). Membranes were delipidated by a slight modification of the method of Svennerholm and Fredman (27). In brief, to 3 vol of membrane suspension (3-5 mg protein) 8 vol of methanol and 4 vol of chloroform were added with mixing. After stirring for 30 min at room temperature, the mixture was centrifuged ($15,000 \times g$ 15 min) and the pellet resuspended in water-methanol-chloroform (3:8:4) and centrifuged as above. The pellet was washed with absolute ethanol, which was removed by lyophilization. The moist delipidated material was finally dried under reduced pressure and suspended in 0.05 M sodium maleate pH 6.5 to a protein concentration of 1.0-2.0 mg/ml. Protein recoveries were 50-70%.

[†] The binding assay mixture contained 1.8 μg of ^{125}I - RCA_{II} (sp. act. 5.5×10^6 cpm/mg protein) and 30 μg of microvillus membrane protein in a total of 200 μl . Values were mean \pm SD of 8-10 binding assays from four pairs of animals. Statistical significance of differences between ages.

[‡] $p < 0.05$.

- mouse lymphoma cell lines. *J Supramol Struct* 8:235-245
- Carpenter G, Cohen S 1977 Influence of lectins on the binding of ^{125}I -labeled EGF to human fibroblasts. *Biochem Biophys Res Commun* 79:545-552
- Hughes RC 1978 Lectin receptors and cell surface recognition. In: Marchesi VT, Ginsburg V, Robbins PW and Fox CF (eds) *Progress in Clinical and Biological Research*, Vol 23. Alan R. Liss, Inc, New York, pp 657-668
- Browning TH, Trier JS 1969 Organ culture of mucosal biopsies of human small intestine. *J Clin Invest* 48:1423-1432
- Schmitz JS, Presier H, Maestracci D, Ghosh BK, Cerda JJ, Crane RK 1973 Purification of the human intestinal brush border membrane. *Biochim Biophys Acta* 323:98-112
- Ohsawa K, Kano A, Hoshi T 1979 Purification of intestinal brush border membrane vesicles by the use of controlled-pore glass-beads column. *Life Sci* 24:669-678
- Dahlqvist A 1964 Method for assay of intestinal disaccharidases. *Anal Biochem* 7:18-25
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ 1951 Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265-275
- Kagnoff MF, Donaldson RM, Trier JS 1972 Organ culture of rabbit small intestine: prolonged *in vitro* steady state protein synthesis and secretion and secretory IgA secretion. *Gastroenterology* 63:541-551
- Scatchard G 1949 Attractions of protein for small molecules and ions. *Ann NY Acad Sci* 51:660-672
- Bernard B, Aubery M, Bourrillon R 1979 Changes in the sensitivity of chick fibroblasts to ricinus lectin (RCA_{II}) toxicity in relation to the stage of embryo development. *Biochem J* 182:33-38
- Bouhours D, Bouhours JF 1983 Developmental changes in hematoside of rat small intestine. Postnatal hydroxylation of fatty acids and sialic acid. *J Biol Chem* 258:299-304
- Moog F 1979 Differentiation and redifferentiation of the intestinal epithelium and its brush border membrane. In: *Development and Mammalian Absorptive Processes*, Ciba Foundation Symposium 70 (new series), Excerpta Medica, Amsterdam, pp 31-50
- Bresson JL, Pang KY, Walker WA 1984 Microvillus membrane differentiation: quantitative differences in cholera toxin binding to the intestinal surface of newborn and adult rabbits. *Pediatr Res* 18:984-987
- Hughes RC 1979 How do toxins penetrate cells? *Nature* 281:526-527.
- Udall JN, Pang K, Fritze L, Kleiman R, Walker WA 1981 Development of gastrointestinal mucosal barrier. I. The effect of age on intestinal permeability to macromolecules. *Pediatr Res* 15:241-244
- Svennerholm L, Fredman P 1980 A procedure for the quantitative isolation of brain ganglioside. *Biochim Biophys Acta* 617:97-109