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Susceptibility of Mice to Rotavirus Infection: Effects of Age and Administration of Corticosteroids

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We examined susceptibility to the murine rotavirus, epizootic diarrhea of infant mice virus (EDIM), in normal suckling and weaned mice and in suckling mice treated with glucocorticoids. Normal mice 1 to 40 days old were inoculated by gastric intubation with high doses of EDIM and subsequently evaluated for rotavirus infection by solid-phase radioimmunoassay, by electron microscopy of intestinal tissue sections or by both. Radioimmunoassay and electron microscopy showed a concordance of 89.5% in the detection of rotavirus infection. After a period of low susceptibility to EDIM infection during the first 3 days after birth (23%), susceptibility was high for the next 11 days (95%), but decreased abruptly as mice approached weaning (41% on days 15 through 17). Mice 34 days or older did not develop EDIM infection after inoculation, but rotavirus antigen was detected in 12% of uninoculated mothers nursing inoculated litters. Administration of cortisone acetate to 8-day-old mice induced partial intestinal maturation prematurely. At 3 to 6 days after cortisone acetate treatment, susceptibility to EDIM infection decreased to 60% compared with 94% in age-matched controls. Our data suggest (i) that susceptibility of mice to EDIM infection is age dependent, decreasing in concert with intestinal maturation, and (ii) that glucocorticoids, which induce premature partial intestinal maturation, modulate susceptibility of mice to EDIM.

Rotaviruses cause enteritis with substantial morbidity and mortality in many vertebrate species, including humans (10, 25, 43). In all species studied, infection is more frequent and more serious in the young than in adults. However, why rotaviruses infect predominantly the young is not well understood.

Infection of mice with epizootic diarrhea of infant mice virus (EDIM) is a potentially useful animal model for studying the disparate morbidity and mortality in the young and adults after exposure to rotavirus. EDIM shares with other rotaviruses one or more common antigens on the inner capsid (18, 41), although one or more distinctive species-specific antigens are present on the outer capsid (41). Morphological changes have been described in the intestine in EDIM infection (1, 2, 32). These are very similar to the lesions induced in other species by other rotaviruses. EDIM infects primarily the intestinal villus epithelial cells rather than the crypt cells (1). Infected absorptive cells have vacuolated cytoplasm (32). In addition, there are many viral particles 70 to 120 nm in diameter with an outer membrane and particles 56 to 65 nm in diameter without a membrane in the endoplasmic reticulum and in viral factories (1, 2).

Experimental inoculation of mice with EDIM has helped to elucidate the course of illness. Approximately 2 days after being fed EDIM-containing stool filtrates, mice 1 to 11 days old developed diarrhea which resolved by day 17 (20). Although mice 12 days or older did not develop diarrhea (20), viral antigen has been detected in the intestine of 1-month-old mice 5 to 7 days after inoculation (40). Also, inoculated adult mice and uninoculated dams nursing infected pups have excreted virus in normal appearing stools (21, 22).

However, fundamental questions regarding EDIM infection remain unanswered. The actual frequency of infection which follows inoculation of mice of different ages with EDIM has not been defined. Moreover, it is not known what factors prevent obvious diarrheal disease and perhaps reduce the frequency of infection after EDIM challenge as suckling mice approach the weaning age. During this maturational period (15 to 17 days after birth), there are striking changes in intestinal structure and function. Clo-

sure occurs, with a resultant decrease in the capacity for macromolecular transport (4, 15). The brush border enzymes maltase (12, 29), sucrase (12, 29), and alkaline phosphatase (12, 27, 30) increase, whereas lactase decreases (12, 19, 27, 29). If these maturational processes play a role in mouse susceptibility to rotavirus, modulation of maturation might alter infectivity of EDIM. Glucocorticoid treatment of 8-day-old mice induces partial intestinal maturation with increased activity of the intestinal brush border enzymes maltase (29), sucrase (29), and alkaline phosphatase (28). Glucocorticoid treatment of 8- to 12-day-old rats also induces premature closure (4, 8, 16, 31). The reported effects of glucocorticoid treatment on lactase activity has been more variable, with both increases and decreases reported in mice (24).

In this report we describe studies designed to determine the susceptibility of mice of different ages to experimental inoculation with EDIM and the effect of glucocorticoid administration on susceptibility of mice to EDIM infection. We also compare the efficacy of a recently described radioimmunoassay (RIA) for rotavirus (6) and electron microscopy of intestinal tissue in detecting EDIM infection in mice.

MATERIALS AND METHODS

Animals. Pregnant CD-1 Swiss mice, dams with litters 5 to 21 days of age, and retired female breeders were obtained from Charles River Breeding Laboratories (Wilmington, Mass.). Pregnant mice gave birth naturally. Pups remained with their mothers and were allowed to suckle both before and during the experimental period. To avoid cross-contamination before EDIM inoculation, animals were housed in a separate building from inoculated animals. Mice were kept in cages covered with filter caps. Mice that were raised in our laboratory for the purpose of serological studies were housed in the same room as inoculated mice.

Rotavirus (EDIM). EDIM in a suspension of sonicated and coarsely filtered mouse intestine was obtained through the courtesy of Bernard Fields. The 50% infective dose (ID_{50}) was determined by administering 0.1 ml of serial 10-fold dilutions of suspension intragastrically via PE 10 tubing to 5- to 7-day-old mice. The dilution causing liquid stool in the distal colon in half the litter as determined by direct examination 5 days after inoculation was considered to be the ID_{50}. New crude viral stocks were established by inoculating nine litters of mice with a dose of $10^{5.7}$ ID_{50} in 0.1 ml of suspension. After 28 to 32 h, the entire small intestine and colon were removed and sonicated in gel saline (NaCl, 0.8 g%; CaCl, 0.003 g%; MgCl, 0.017 g%; boric acid, 0.12 g%; sodium borate, 0.005 g%; gelatin, 0.2 g%) diluted to 40% with 0.05 M tris(hydroxymethyl)aminomethane (pH 7.4) (Tris buffer). An ID_{50} of the new EDIM stock was determined as above. Then the intestinal suspension was diluted (1:50) with gel saline to contain the same infective dose as the original suspension. The suspensions were stored at −70°C. Only two EDIM stock pools were used for all experiments, and neither contained detectable viral particles other than rotavirus (determined by electron microscopy of negatively stained crude suspension).

Time course of development of EDIM antigen. The time after inoculation at which the maximum number of infected animals could be detected was determined in mice inoculated at 1, 5, 10, 15, and 20 days of age and also in mice at 11 and 13 days of age that had received 1.25 mg of cortisone acetate (The Upjohn Co., Kalamazoo, Mich.) intraperitoneally at 8 days of age. Mice were inoculated with EDIM as outlined above and killed at 12, 24, 30, 36, 48, 72, or 96 h. In the groups of mice without cortisone treatment, 3 to 6 animals were killed at each time. In the groups of cortisone-treated animals, 4 to 10 animals were killed at each time. The entire intestine from pylorus to anus was removed and stored at −70°C for subsequent study by RIA.

Susceptibility of mice to EDIM. To determine susceptibility of mice of different ages to EDIM, the following groups of mice were inoculated intragastrically with 0.1 ml of a suspension containing a dose of $10^{5.7}$ ID_{50} of EDIM: 6 litters (n = 44), days 1 through 3; 9 litters (n = 81), days 4 through 14; 7 litters (n = 66), days 15 through 17; 16 litters (n = 155), days 18 through 20; 3 litters (n = 38), days 34 through 35; and 7 adults. Animals were killed 28 to 32 h after inoculation, and the entire intestine from pylorus to anus was removed. The nature of intestinal contents (diarrhea versus normal) was recorded for each mouse. Small samples of duodenum and ileum were removed for fixation and embedment for electron microscopy from five animals in each litter. The remaining intestine was frozen at −70°C for subsequent RIA. Intestines from 67 uninoculated mothers were removed 28 to 32 h after inoculation of their pups for RIA.

Influence of glucocorticoids. For determination of the effect of glucocorticoids on susceptibility of mice to EDIM, 34 litters of mice received 1.25 mg of cortisone acetate (Upjohn) intraperitoneally at 8 days of age. Subsequently, 6 litters (n = 52) 8 to 10 days old and 28 litters (n = 241) 11 to 14 days old were inoculated with a dose of $10^{5.7}$ ID_{50} of EDIM and killed 28 to 32 h after inoculation. The intestines were removed and prepared for electron microscopy and RIA.

To assess the effect of this dose of glucocorticoids on intestinal maturation, one-half of the mice of 17 litters (n = 66) were injected with 1.25 mg of cortisone acetate intraperitoneally at 8 days of age. The other half of each litter (n = 64) served as controls. Mice were killed on days 9 to 16, and the intestine from the pylorus to the ileo-cecal valve was removed and sonicated in 1 ml of Tris buffer. A 100-μl sample was removed, diluted 1:10 in Tris buffer, and kept frozen at −20°C for protein analysis which was performed by the method of Lowry et al. (23). The remainder of the sonicate was frozen at −70°C for enzyme analysis.

Electron microscopy. Samples for electron microscopy were fixed in a mixture of 2.5% glutaraldehyde and 1.8% paraformaldehyde in 0.1 M phosphate buffer for at least 2 h and then washed in three changes of phosphate buffer for a minimum of 3 h, postfixed in

2% osmium tetroxide for 1.5 h, dehydrated, and embedded in Epon Araldite. Blocks from 57 randomly selected EDIM-inoculated mice of which 12 had and 45 had not received cortisone acetate were examined by electron microscopy. Thin sections were cut on a Sorvall Porter-Blum MT-2B ultramicrotome with diamond knives, contrasted with uranyl acetate and lead citrate, and examined with a Philips 300 electron microscope. Samples were scored as positive when rotavirus particles could be identified within intestinal epithelial cells.

RIA for detection of EDIM virus. Intestines were frozen and thawed three times, sonicated in 1 to 5 ml of Tris buffer, and diluted with Tris buffer to a final volume of 10 ml. The presence of rotavirus antigen was determined by a modification of a microtiter solid-phase RIA used to detect human rotavirus (6). Hyperimmune sera were prepared by inoculation of goats and guinea pigs with high-titered, sucrose gradient-purified simian rotavirus (SA11) grown in tissue culture. Wells of microtiter plates (220-25; Cooke Engineering Co., Alexandria, Va.) were coated with 75 μl of hyperimmune goat serum diluted 1:10,000 in phosphate-buffered saline (PBS) for 4 h at 4°C, washed twice with PBS, incubated with 1% bovine serum albumin in PBS overnight at 4°C, and washed twice again with PBS. Intestinal homogenate to be tested (50 μl) was placed in each of two precoated microtiter wells. The microtiter plates were incubated overnight at room temperature and washed five times with PBS. Purified antirotaviral guinea pig immunoglobulin labeled with ^{125}I served as the detection antibody. Detection antibody (50 μl) diluted in PBS to contain 2×10^5 cpm was added to each well and incubated for 4 h at 37°C. The wells were washed five times with PBS, the plates were cut apart, and the amount of radioactivity bound to each well was determined in a gamma counter (PRIAS; Packard Instrument Co., Downers Grove, Ill.). Intestinal homogenates prepared from six uninfected mice were used as negative controls. Radioimmunoassay of the intestine from inoculated mice was considered positive if the ratio of counts of inoculated to control uninoculated intestine was equal to or greater than 2:1. Purified SA11 virus and EDIM stock virus were positive in this assay. The sensitivity of the RIA was 3×10^4 ID_{50}.

RIA for serum IgG antibody to rotavirus. Serum samples were collected from mice bled by cardiac puncture. The presence of IgG antibody to rotavirus was determined by a modification of a microtiter solid-phase RIA used to detect IgA antibody to human rotavirus (7). Control antigen was prepared from mock-infected MA104 cells by following the method for the purification of SA11 virus. Nonspecific reactivity of goat anti-SA11 serum was absorbed with control antigen as follows. One volume of undiluted goat serum was incubated with 2 volumes of control antigen for 1 h at 37°C and then overnight at 4°C. The mixture was then centrifuged at 100,000 × *g* for 1 h. The supernatant was stored at −70°C until use. Wells of microtiter plates were coated for 4 h at 37°C with 100 μl of the absorbed anti-SA11 serum diluted 1:1,000 in PBS, washed three times with PBS, incubated overnight with 1% bovine serum albumin, and washed three times again with PBS. Each well was then incubated overnight at 4°C with either 25 μl of purified SA11 virus (10^7 ID_{50}/0.1 ml) or with 25 μl of control antigen, and then washed five times with 0.05% Tween 20-PBS. Test serum (25 μl) diluted 1:200 in PBS containing 1% bovine serum albumin and 0.05% Tween 20 was added to each of two virus-containing wells and two control wells and incubated for 1 h at 37°C. The wells were then washed five times with 0.05% Tween 20-PBS. The immunoglobulin G (IgG) fraction of rabbit antimouse IgG (Miles Laboratories, Inc., Elkhart, Ind.) was labeled with ^{125}I (6); 50 μl of the labeled antibody diluted with PBS to contain 2×10^5 cpm was added to each well for 4 h at 37°C. The wells were washed five times with 0.05% Tween 20-PBS, the plates were cut apart, and the amount of radioactivity bound to each well was determined in a gamma counter. A serum sample was considered antibody positive if the counts bound to the virus-containing wells were at least two times greater than the counts bound to the control wells. Previously described EDIM antibody-positive and -negative murine sera were used as controls in each test (40).

Intestinal enzyme assays. Sucrase, maltase, and lactase activities were determined by the methods of Messer and Dahlqvist (26) as modified by Grand et al. (14). A 0.04-ml sample of appropriately diluted intestinal homogenate was added to 0.2 ml of sugar substrate solution (sucrose for sucrase, β-D-maltose for maltase, and α-lactose for lactase) and incubated for at least 20 min at 37°C. The reaction was stopped by boiling for 4 min. To measure the liberated glucose, 0.2 ml of glucose oxidase-dianisidine-peroxidase reagent was added, and the mixture was incubated at 37°C for 10 min. The reaction was stopped by the addition of 0.4 ml of 50% (vol/vol) H_2SO_4, and the optical density was read spectrophotometrically at a wavelength of 530 nm. Results were expressed as units of enzyme activity, with each unit representing 1 μmol of substrate hydrolyzed per mg of protein per h.

Statistics. The frequency of infection in different groups was compared by the chi-square method. The influence of glucocorticoids on enzyme specific activities was analyzed using the two-tailed Student *t*-test.

RESULTS

Time course of development of EDIM antigen. As shown in Table 1, the highest antigen detection rates that encompassed all age groups of mice appeared at 30 h after inoculation and did not increase thereafter. Not shown in the table are data from mice that received cortisone acetate at 8 days of age and were inoculated with EDIM at 11 or 13 days of age, of which 63% ($n = 8$) were positive at 24 h, 72% ($n = 18$) were positive at 72 h, and 63% ($n = 8$) were positive at 96 h (not statistically significant). Therefore, for all experiments described below the time selected for sacrificing all mice to maximize antigen detection was 28 to 32 h after EDIM inoculation.

Efficacy of electron microscopy and radioimmunoassay in detecting EDIM infec-

tion. Electron microscopy revealed viral particles in infected epithelial cells of both duodenum and ileum 28 to 32 h after inoculation with EDIM. The cells showed typical features of EDIM infection including viral particles associated with elements of endoplasmic reticulum and viral factories in the cell cytoplasm (Fig. 1). Both large and small viral particles ranging from 130 to 65 nm in diameter were regularly observed (Fig. 2). Because damage to duodenal epithelial cells was extensive, viral particles were more abundant and more easily visualized in ileal mucosa. Therefore, ileal samples were generally used to detect EDIM infection by electron microscopy.

TABLE 1. *Time course of development of EDIM antigen*

Age at inoculation (days)	% Positive for rotavirus antigen by RIA at h post-inoculation[a]:						
	12	24	30	36	48	72	96
1	0	50	67	50	75	75	100
5	0	0	75	25	67	33	67
10	0	33	100	100	100	100	100
15	0	33	100	75	75	25	60
20	0	0	0	0	0	0	0

[a] Data are for three to six mice per group.

When the efficacy of RIA and electron microscopy in detecting EDIM infection in the small intestine from 57 mice inoculated with EDIM was tested, there was an overall concordance in 51 of 57 samples (89.5%). Concordance was found in 28 rotavirus-positive and 23 rotavirus-negative samples. Four of 32 samples (12.5%) were positive by RIA but negative by electron microscopy, whereas 2 of 25 samples (8%) were positive by electron microscopy but negative by RIA. The two samples that were positive by electron microscopy but negative by radioimmunoassay were both obtained from 1-day-old mice. Since the agreement between the two methods was high and RIA was much less time consuming than electron microscopy, RIA

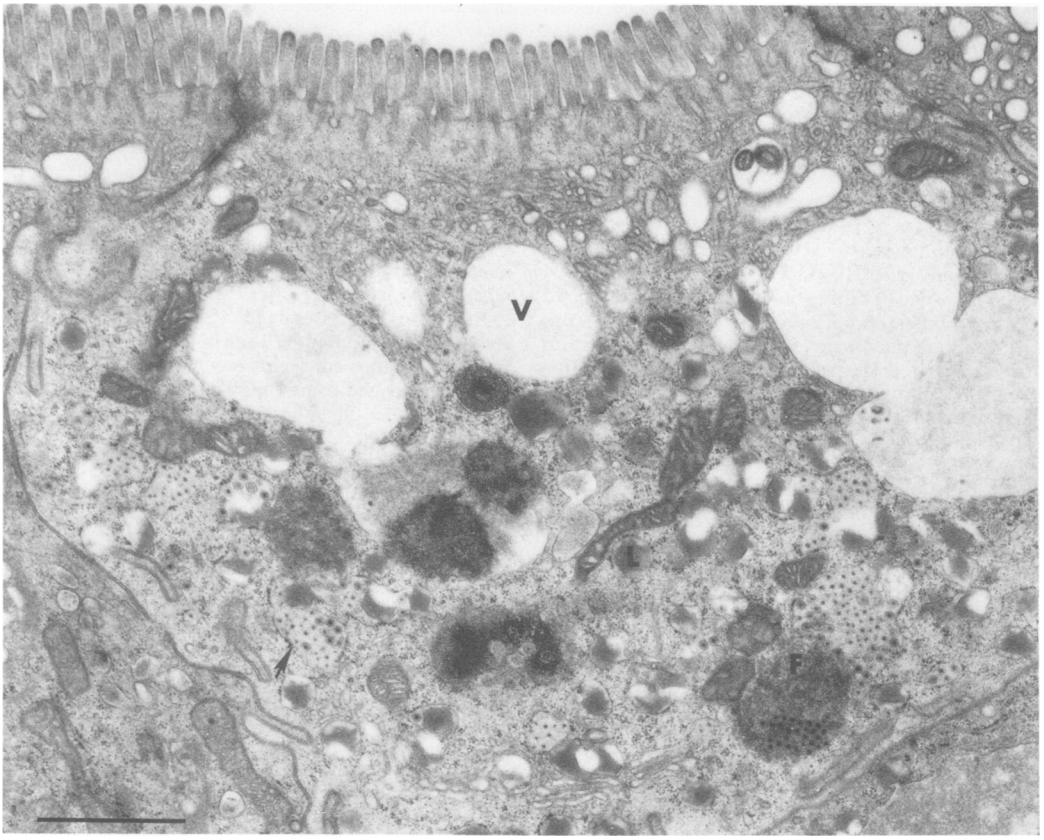

FIG. 1. *Electron micrograph of a typical mouse ileal absorptive cell infected with EDIM. Features shown are: viral factories (F), endoplasmic reticulum containing virus particles (arrow), vacuolization of the cytoplasm (V), and vacuoles containing "lipoid-like" material (L). Bar, 1 μm.*

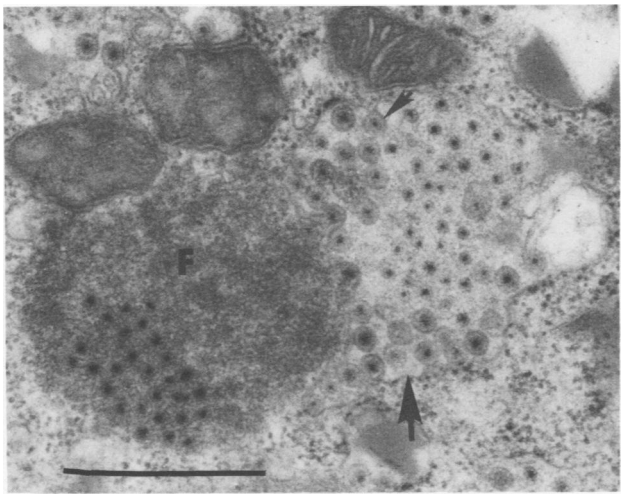

FIG. 2. *Higher magnification of a portion of Fig. 1 showing a viral factory (F) and a profile of endoplasmic reticulum (large arrow) containing viral particles 65 to 130 nm in diameter. Some, but not all, particles appear to have an outer membrane (small arrow). Bar, 0.5 μm.*

was used to determine the presence or absence of infection in the majority of EDIM-inoculated mice.

Effect of age on susceptibility of mice to EDIM infection. The results of the experiments designed to determine susceptibility of mice of various ages to infection after EDIM inoculation are summarized in Fig. 3. The frequency of infection was as follows: days 1 through 3, 23% (n = 44); days 4 through 14, 95% (n = 81); days 15 through 17, 41% (n = 66); days 18 through 20, 16% (n = 155), and day 34 to adult, 0% (n = 45). Of mothers that were not inoculated with virus but allowed to nurse their inoculated litters, 12% (n = 67) were positive for EDIM by RIA. The frequency of infection in inoculated mice between days 4 and 14 was significantly greater than that between days 1 and 3 ($P < 0.001$), the frequency between days 15 and 17 was significantly less than that between days 4 and 14 ($P < 0.001$), the frequency between days 18 and 20 was significantly less than that between days 15 and 17 ($P < 0.001$), and the frequency in animals older than 30 days (including adults) was significantly less than that between days 18 and 20 ($P < 0.05$). The 12% frequency of EDIM positivity in uninoculated mothers was significantly less than that in inoculated mice between days 4 and 14 and between days 15 and 17. Of mice negative for EDIM by RIA, only 4% had liquid stool and 5% had semiformed stool in the intestine when killed. Of mice 14 days or younger positive for EDIM, 71% had liquid or semiformed intestinal contents and 29% had normal intestinal contents. Only one 15-day-old mouse and no mouse older than 15 days had liquid intestinal contents after inoculation with EDIM.

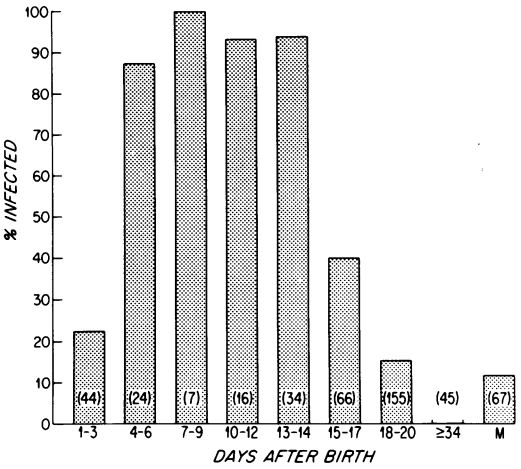

FIG. 3. *Effect of age on susceptibility of mice to EDIM infection. Mice were killed 28 to 32 h after intragastric inoculation with EDIM. A mouse was considered infected if radioimmunoassay of an intestinal homogenate or electron microscopy of an intestinal tissue section was positive. The number of mice in each group in this and subsequent figures is indicated within parentheses. M denotes uninoculated mothers nursing inoculated pups.*

Serum IgG antibody to rotavirus in mice. The results of tests for serum IgG antibody to EDIM are summarized in Table 2. Eleven dams were tested for the presence of IgG antibody in serum collected at the time their last infant was killed. Antibody was present in 55% of the mothers. The presence or absence of antibody in mothers did not influence susceptibility of their

TABLE 2. *Serum IgG antibody to rotavrus in CD-1 mice*

Mouse age	Days in laboratory	No. positive (%)
Retired breeder	1-2	15 of 20 (75)
Mother	13-30	6 of 11 (55)
22 to 23 days	22-23	2 of 7 (29)
21 days	1	0 of 20 (0)

infants to EDIM infection. The frequency of EDIM infection in 11- and 13-day-old mice injected with steroids on day 8 was the same in four litters from antibody-positive mothers and four litters from antibody-negative mothers. Moreover, in three litters inoculated on day 20 no animal developed EDIM infection, although one of three mothers did not have antibody to rotavirus. The percentage of dams positive for antibody was not statistically different from that of retired female breeders bled within 2 days of arriving in our facility; 75% (15 of 20) of the retired female breeders were antibody positive. None of 20 21-day-old mice had antibody on their arrival from Charles River Breeding facility. However, of mice born and housed in our EDIM-contaminated area, by 23 days of age 29% (two of seven) were positive for EDIM antibody.

Effect of cortisone acetate on susceptibility of mice to EDIM infection. The influence of cortisone acetate administration to 8-day-old mice on their susceptibility to infection upon subsequent inoculation with EDIM is summarized in Fig. 4. When mice pretreated with cortisone acetate at 8 days of age were inoculated with EDIM within 48 h after steroid administration, the frequency of infection was the same as in mice of identical age that were not pretreated with steroids (98 versus 100%). On the other hand, when such pretreated mice were inoculated with EDIM 72 to 144 h after steroid administration, the frequency of infection was significantly less than in mice of identical age not receiving cortisone (60 versus 94%, $P < 0.001$).

Effect of cortisone acetate on intestinal maturation. The effect of cortisone acetate administration to 8-day-old mice on induction of maltase activity is shown in Fig. 5. In 9- and 10-day-old mice (24 to 48 h after steroid treatment), maltase specific activity was 3.49 ± 0.43 U compared with only 1.27 ± 0.07 U in littermates not receiving steroids ($P < 0.001$). Similarly, in 11- to 14-day-old mice (72 to 144 h after steroid treatment), maltase specific activity was 4.04 ± 0.51 U compared with 1.42 ± 0.18 U in littermates not receiving steroids. In 15- to 16-day-old mice (7 to 8 days after steroid injection), there was no longer a significant difference between treated animals and control littermates (2.57 ± 0.32 U compared with 2.71 ± 0.38 U). The mean maltase specific activity in adult mice was 12.06 ± 0.61 U.

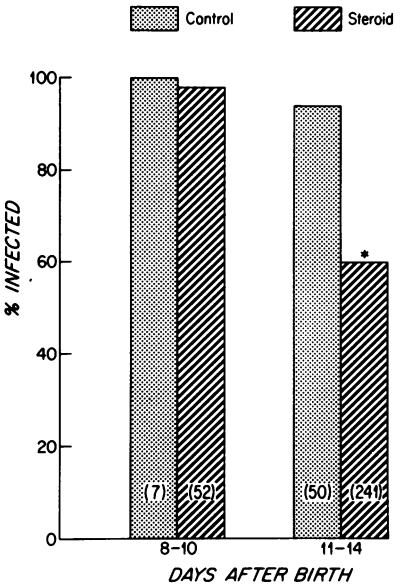

FIG. 4. *Effect of cortisone acetate on susceptibility of mice to EDIM infection compared with age-matched controls. All treated mice were injected with cortisone acetate on day 8. All mice were inoculated with virus on the days indicated on the abscissa and killed 28 to 32 h later.*

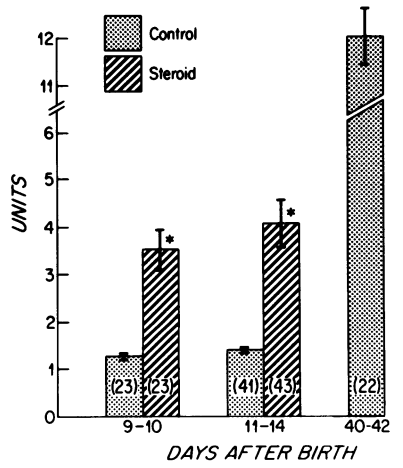

FIG. 5. *Maltase specific activity in sonicated small intestine of mice injected intraperitoneally with cortisone acetate on day 8, littermate controls, and normal young adults. Mice were killed on the days indicated on the abscissa. Maltase activity is expressed as units ± standard error of the mean. One unit equals micromoles of maltose hydrolyzed per milligram of protein per hour. *, $P < 0.001$.*

The effect of cortisone acetate injection in 8-day-old mice on intestinal sucrase activity varied from animal to animal. Whereas none of the control littermates had detectable sucrase activity until they were 14 days of age, 22% of steroid-injected animals had detectable sucrase activity at 9 and 10 days of age (24 to 48 h after steroids), and 56% had detectable sucrase activity at 11 to 14 days of age (72 to 144 h after steroids). The mean sucrase activity was 0.02 ± 0.01 U at 9 to 10 days and 0.20 ± 0.06 U at 11 to 14 days. By 15 to 16 days, there was no difference between the mean sucrase specific activity in control and cortisone acetate-injected animals (0.12 ± 0.05 U compared with 0.12 ± 0.04 U).

Intestinal lactase activity was unaffected by cortisone acetate injection both in animals 9 to 10 days and 11 to 14 days of age (Fig. 6). In both steroid-treated mice and untreated littermates 15 to 16 days of age (data not shown) mean lactase activity had decreased to levels which were not significantly different from those of young adult mice 40 to 44 days of age (0.72 ± 0.2 U compared with 0.62 ± 0.05 U).

DISCUSSION

The presence of rotavirus infection in experimentally inoculated mice was evaluated by two methods, RIA of homogenized intestine with antiserum to a closely related rotavirus (SA11)

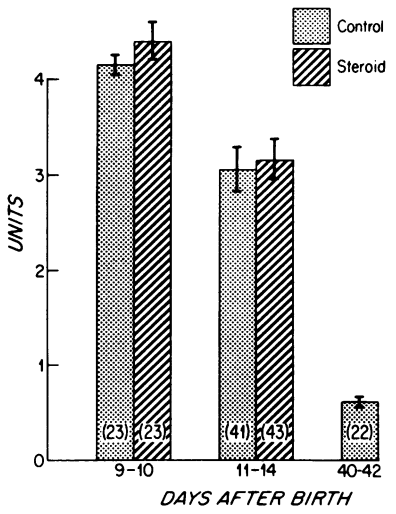

FIG. 6. *Lactase specific activity in sonicated small intestine of mice injected intraperitoneally with cortisone acetate on day 8, littermate controls, and normal young adults. Mice were killed on the days indicated on the abscissa. Lactase activity is expressed as units ± standard error of the mean. One unit equals micromoles of lactose hydrolyzed per milligram of protein per hour.*

and electron microscopy of thin sections of fixed intestinal mucosa. The two methods showed an overall concordance rate of 89.5%. Since only two of the samples which were positive by electron microscopy were negative by RIA (both from 1-day-old animals), the use of RIA instead of electron microscopy to detect EDIM infection appears justified. Although the sensitivity of the RIA is 3×10^4 ID_{50}, the cumbersome nature of infectivity studies as well as the problem of cross-contamination with EDIM precluded effective use of a biological assay in our relatively long-term studies involving multiple groups of animals.

Using RIA and electron microscopy, we have shown that the degree of susceptibility of mice to EDIM is an age-related phenomenon. Our experiments show three periods of differing susceptibility of mice to inoculation with EDIM virus. There is a low frequency of infection (23%) in 1- to 3-day-old mice, which rises to a very high frequency (95%) between days 4 to 14 and then falls abruptly at 15 to 17 days (41%) as the time of weaning is approached. The frequency of infection in adult mice is very low; no rotavirus infection was detected in any of the inoculated adults, but the detection of rotavirus antigen in the intestine of 12% of uninoculated mothers exposed to infected pups demonstrates that adult mice may, under some circumstances, retain the capacity to be infected with rotavirus. Alternatively, the presence of rotavirus antigen in the dam's intestine may reflect ingestion of EDIM in the excreta of their pups rather than true infection. Our results most likely represent a change in susceptibility to virus with age. Asymptomatic infection in some older animals could have been present and have remained undetected by our assays; however, no animal older than 15 days developed diarrhea after inoculation. The finding of liquid or semiformed stool contents in 71% of mice 14 days or younger that were positive for EDIM infection by RIA or electron microscopy (or both) is consistent with the reported average onset of diarrhea 2 days after inoculation (20). It is likely that a substantially larger number of mice would have developed diarrhea had they been killed at a later time.

Our findings raise two major questions: (i) what are the factors responsible for the low susceptibility of mice to EDIM in the 1- to 3-day period immediately after birth, and (ii) what are the factors responsible for the marked decrease in susceptibility as mice approach the weaning period?

One possibility for the low susceptibility to EDIM infection in 1- to 3-day-old mice could be

the presence of antibody to rotavirus in the colostrum and milk of the nursing mothers. Although there are no reported measurements of EDIM-specific colostral or milk antibodies in mice, specific rotavirus antibodies have been demonstrated in colostrum or milk at parturition in cows (42), ewes (39), and humans (7, 44); however, colostral and milk antirotavirus antibodies decrease rapidly after birth. Two calves fed colostrum with a neutralizing antibody titer of 1:320 to bovine rotavirus were protected from disease (42). Gnotobiotic lambs fed 450 ml, but not those fed only 100 ml, of colostrum on the first or second day of life and challenged 2 days later with rotavirus were protected from disease (37), as were lambs fed 40 ml of colostrum per kg for 4 consecutive days after birth and challenged with virus on the second day (36). In addition, two infant lambs fed high-titer antirotavirus hyperimmune serum on days 2 to 4 did not excrete rotavirus or develop diarrhea when challenged on day 2 with rotavirus (37). Thus, in species other than mice, oral ingestion of substantial amounts of antibody directed against rotavirus seems protective. In our studies, the presence or absence of serum IgG antibody to rotavirus in the mother did not appear to affect susceptibility to EDIM infection of 11- or 13-day-old mice injected with corticosteroids on day 8 or of 20-day-old mice.

A second possibility for the lower susceptibility of 1- to 3-day-old mice to EDIM infection may be low trypsin activity in the intestinal lumen. Trypsin has been shown to increase infectivity of rotaviruses in cells grown in tissue culture (3, 9, 13, 38). In rats, trypsin activity in the intestine is very low at birth, rises slightly on day 4, and then remains essentially unchanged until day 14, when activity increases further toward adult levels (33). If the intraluminal trypsin activity in the intestine of mice, a closely related species, should follow the same pattern during development, an increase between days 3 and 4 after birth may contribute to the very high susceptibility to EDIM infection observed between 4 to 14 days after birth.

Several factors may influence the striking decrease in susceptibility to EDIM infection which becomes apparent in mice age 15 days and older. In this study we have confirmed that administration of cortisone acetate to 8-day-old mice is followed by premature partial maturation of the intestinal absorptive epithelium (29). Moreover, we have shown that such premature maturation is associated with a significant decrease in the frequency of EDIM infection in 11- to 14-day-old mice 3 to 6 days after they received glucocorticoid injection. This decrease in susceptibility to EDIM infection was similar to that which occurs naturally in 15- to 17-day-old animals. The increase in maltase specific activity 24 h after steroid injection occurred 48 h before change in the susceptibility of mice to EDIM infection, implying that maturation of this enzyme is not of importance in the decrease. However, other events occurring during intestinal maturation in rodents, such as a decreased capacity for macromolecular transport (4, 8, 16, 31), may be important in modulating susceptibility to EDIM. Alternative explanations for the effect of cortisone on decreasing the susceptibility of mice to EDIM infection and the change in susceptibility at 15 days are possible and include maturation of the mouse's immune system or maturation of the pancreas.

A receptor for rat immunoglobulin has been demonstrated in the duodenal epithelium of suckling rats (15, 34). This receptor, which normally disappears at the time of weaning, can be made to disappear precociously by administration of glucocorticoids before weaning (8, 16, 31, 34). It has been suggested that an intestinal receptor for rotavirus may be important in rotavirus-induced enteritis, that the intestinal brush border enzyme, lactase, may serve as this receptor, and that lactase may also serve to alter the rotavirus, increasing its infectivity (17). This hypothesis is based on the observations that lactase specific activity tends to decrease in animals in concert with decreased susceptibility to rotavirus infection and that lactase partially uncoats bovine rotavirus (17). In addition, preincubation of bovine rotavirus with lactase was associated with a modest increase in infection of cultured cells in vitro (3). Our data do not preclude the presence in suckling mice of a rotavirus receptor which normally decreases or disappears at the time of weaning or can be induced to decrease prematurely after glucocorticoid administration. However, our data do indicate that lactase is an unlikely candidate for the receptor molecule and that the level of lactase activity is not a crucial determinant of mouse susceptibility to EDIM infection. Although susceptibility to EDIM infection was decreased significantly in our glucocorticoid-treated mice, intestinal lactase specific activity was not altered.

Rotavirus may not require a specific receptor, but may enter absorptive cells along with macromolecules by pinocytosis (4, 8). Such pinocytosis decreases abruptly at the time of weaning in mice and rats, and a decrease in pinocytosis can be induced prematurely by glucocorticoid administration (4, 8).

In rats, chymotrypsin and trypsin activities in the intestine increase strikingly shortly before

weaning (5, 33). Gastric pepsin activity also increases abruptly in the rat just before weaning (11). Although it is not known whether these changes take place in mice, rats and mice are closely related species, and there are studies which suggest that proteolytic enzymes may play a major role in rotavirus infectivity. Barnett et al. (3) noted that infectivity of bovine rotavirus was decreased 3-fold by prior incubation of rotavirus in medium containing 2.7 U of chymotrypsin per ml, although there was a 1.5-fold increase in infectivity after incubation in medium containing only 0.27 U of chymotrypsin per ml. Using reovirus, a closely related double-stranded RNA virus, Rubin and Fields (35) have shown that preincubation of type 3 reovirus with chymotrypsin reduces its capacity to infect cultured cells. The sensitivity of the reoviruses to chymotrypsin in vitro correlated with the amount of virus recoverable by plaque assay after mouse inoculation in vivo (35). The effect of gastric pepsin on rotavirus infectivity has not been examined in vivo or in vitro. It is possible that chymotrypsin or pepsin (or both) could decrease EDIM infectivity in the mouse in vivo at 15 days of age. It is less likely that increased trypsin activity is responsible for the decreased susceptibility to EDIM at 15 to 17 days since trypsin enhances rotavirus infection of cells grown in culture (3, 9, 13, 38).

In conclusion, it is clear that further studies are needed to elucidate the mechanisms which influence rotavirus infectivity in mammalian intestine. The precise characterization of susceptibility of mice of different ages to EDIM infection and the demonstration that glucocorticoids, which induce premature maturation of the intestine, modulate this susceptibility should aid in our understanding of the host-virus interactions in this in vivo model of rotavirus enteritis.

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