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Influence of Malnutrition and Alterations in Dietary Protein on Murine Rotaviral Disease[1] (41665)

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Abstract. The possible correlation between malnutrition and degree of severity of rotavirus-associated infantile diarrhea which appears to occur in human populations was studied using a mouse model. To determine the effects of general malnutrition or altered levels of dietary protein, female mice were fed throughout pregnancy and infection periods with diets diluted with 0, 300, or 600 g glucose/kg, designated as normal nutrient to calorie ratio (N/C) diet, 70% N/C diet, or 40% N/C diet or with diets containing 75, 150, or 300 g casein/kg, as low-, normal-, or high-protein diets. Murine rotavirus was given by gavage to the 2-day-old offspring of these dams, and the extent of infection determined. Marked increases in severity of diarrheal disease were seen in the infants from dams receiving the 40 and 70% N/C diets and the low-protein diet. Severity of infection was seen as increased deaths, reduced weight gain, and increased passage of diarrheic feces. Intestinal viral levels and intestinal diarrhea scores did not vary appreciably. Serum interferon remained below detectable limits throughout the studies, but serum antibody was determined in dams 30 days post-virus exposure. The latter titers were lower in the infected mice from dams fed the 40 and 70% N/C diets, but were essentially the same in all the protein diet groups. Cross-fostering was done using the 40 and 100% N/C diets, wherein mice from dams fed either diet were placed on mothers fed the opposite diet. Increased severity of infection was again seen when the virus was given 2 days after the exchange, although the greatest infection occurred in animals from dams fed 40% N/C diet which were then fostered by other similarly fed dams. The increased host sensitivity to the rotaviral infection appeared to be a result of both pre- and postnatal dietary effects.

Potentially fatal infantile diarrhea occurs at a high rate, especially among children aged 5 years or less in developing nations (1–3). World Health Organization studies indicate the rotavirus is associated with up to 50% of the hospitalized cases of these diarrheal diseases (2, 3). It is now recognized that malnutrition continues to be a problem in many of the developing nations, and a close association between diarrhea and malnutrition is apparent (3). Both conditions thrive in similar socioeconomic and cultural surroundings, and by acting together, contribute to high rates of childhood morbidity and mortality. Malnourished children are known to have a higher incidence of severe diarrhea resulting in high rates of diarrhea-related mortality (4–6). Close correlations have been established between nutritional status and immunity (7–9), suggesting at least one mechanism by which such enhancement of infection occurs.

We have undertaken studies to further relate malnutrition and rotaviral gastroenteritis, utilizing the mouse model for the disease as induced by a murine strain of rotavirus (MRV). In reported earlier studies (10) with this model, we have seen the disease to be readily characterized by diarrhea, altered intestinal appearance, villi destruction, weight loss or failure to gain weight, and sporadic deaths. Maximum virus production as determined by enzyme-linked immunosorbent assay (ELISA) occurred approximately 4 days after infection of the 2 to 3-day-old mouse. In the present report, the influence of malnutrition, as established by nutrient dilution, and alterations in dietary protein content on murine sensitivity to rotaviral infection is determined. In at least one case, a high level of dietary protein has been reported to increase mortality in a viral infection (11). Therefore, we included in this study an investigation of the effect of high

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protein on mice infected with rotavirus. Several parameters for evaluating the mouse infection are used; of especial interest is the enhancement of diarrhea-associated death in the animals by nutrient dilution of diet with glucose.

Materials and Methods. *Virus.* The murine rotavirus (MRV) was obtained from Dr. Michael Collins of Microbiological Associates, Inc. (Bethesda, Md.). It was a frozen stock used previously by Adams and Kraft (12), referred to in earlier work as epizootic diarrhea virus of infant mice (EDIM). We have shown the virus to be a rotavirus by comparison of the agent's RNA band patterns to similar patterns of bovine and human rotaviruses (13) and by positive reaction using enzyme-linked immunosorbent assay (ELISA) with bovine and simian rotaviruses used as antigens.[2] An MRV pool was prepared by homogenizing to 10% (weight/volume) the pooled infected intestines taken 4 days after gavage infection of 3-day-old specific pathogen-free Swiss Webster mice. Hank's balanced salt solution was used as diluent. The pooled homogenates were ampuled and stored at −90°C. Homogenate was centrifuged at 1900 rpm for 15 min and the supernate filtered through 4.0-, 1.0-, and 0.22-μm filters. This filtrate, inoculated by gavage, was capable of inducing the typical diarrheal disease to be described; filtered and nonfiltered intestinal homogenates prepared from uninfected mice from the same source did not induce this disease.

Mice. Designated "specific pathogen-free" Swiss Webster Mice [Crl: CFW(SW)BR] obtained from Charles River Labs (Wilmington, Mass.) were used. The animals had no detectable serum anti-rotavirus antibodies when assayed by either neutralization or ELISA tests using bovine rotavirus as antigen. Special precautions were taken to prevent accidental MRV infection of these mice. These precautions included housing them in a sterile disposable cage system using filtering bonnets (Lab Products, Inc., Federalsburg, Md.) on each cage or holding them in similar sterile cages kept in a 30-ft^3 laminar flow cage containment system (Lab Products) with sterile litter, water, and food provided. Personnel involved in MRV work did not enter the building wherein these uninfected mice were housed. Mice infected with MRV were kept in a containment room in a building separate from the university's animal care facility.

Diets. In the studies run to examine the effects of general malnutrition, one group of mice was fed a normal nutrient to calorie (100% N/C) diet. Malnourishment was achieved by feeding two other groups of mice a 100% N/C diet with the N/C ratio altered by addition of glucose to 70% N/C or 40% N/C. The 100% N/C diet contained (g/kg): casein 150, corn oil 60, trace mineral mixture 11.6, vitamin mixture 22, $CaCO_3$ 16.8, $NaH_2PO_4 \cdot H_2O$ 29.8, $FeSO_4 \cdot 7H_2O$ 0.173, wood α-cellulose 50, and glucose to make 1 kg. The trace mineral mixture contained (g/kg): KCl 296.7, $MgCO_3$ 121, $MnSO_4$ 12.7, $CoCl_2 \cdot 6H_2O$ 0.7, $CuSO_4 \cdot H_2O$ 1.6, $ZnSO_4 \cdot 7H_2O$ 38, KI 0.8, and glucose to make 1 kg. The vitamin mixture contained (g/kg): α-tocopherol 5.0, L-ascorbic acid 45.0, choline chloride 75.0, D-calcium panthothenate 3.0, *i*-inositol 5.0, menadione 2.25, niacin 4.5, *p*-aminobenzoic acid 5.0, pyridoxine-HCl 1.0, riboflavin 1.0, thiamine–HCl 1.0, biotin 0.02, folic acid 0.09, vitamin B_{12} 0.00135, vitamin A acetate 900,000 IU, vitamin D_2 100,000 IU, and glucose to make 1 kg. The nutrient dilution diets were administered to the animals in powder form, using metal-capped food cups placed in each cage. Both food and water were provided *ad libitum.*

The protein experiments utilized the same 100% N/C diet containing, as protein, 150 g casein/kg (designated as "normal" protein) and diets containing protein altered to 75 g/kg (designated as "low" protein or 50% of control) or 300 g/kg (designated as "high" protein or 200% of control). This diet was pelleted.

Nutrition study methods. Nutrient/calorie alteration effects: Groups of mice used as dams were fed in parallel the 100, 70, or 40% N/C diets beginning 24 hr prior to mating. The diet was then continued through birth of the infants and during their infection while lactation was occurring. Sufficient dams were used to provide statistically acceptable num-

[2] Neas E, Sidwell R, Barnett B, Spendlove R. Abstr American Soc Microbiology, p266, (1982).

bers of infants (60–70 infants, representing at least six litters) in each diet group. Shortly after birth, the litter sizes were adjusted to be approximately equal (8–10 pups/litter). Each infant was weighed and exposed to virus by gavage when 2 days old. Twenty infants marked at the time of infection were weighed daily and observed for signs of diarrhea; deaths were recorded. Of the unmarked remainder, at least 6 were randomly selected and killed on Days 2, 4, 6, 8, 10, or 15 post-virus exposure. The serum from each group of infants was pooled and frozen for later testing for interferon and anti-rotavirus antibodies. The intestines were removed, examined, and scored according to the degree of diarrhea visible, then stored at −90°C for eventual virus assay. Intestines were scored on a scale of 0 to 4, according to the following: 0: normal intestine, no diarrheal fluid; 1: diarrheal fluid in up to one-third of the length of the intestine, combined with solid feces; 2: same as 1, without solid feces; 3: diarrheal fluid in approximately one-half of the intestine without solid feces; small pockets of gas; 4: extensive diarrheal fluid throughout the intestinal area, combined with distention due to gas. Parallel to this experiment, similar numbers of infants from mothers fed each diet were exposed to sterile virus diluent and processed in an identical manner as the virus-infected mice. All values obtained in the nutritionally deprived groups were compared with those of the 100% N/C diet group. Increases in mortality were analyzed statistically using chi square analysis with Yate's correction. The *t* test was used for analysis of weight gain and mean survival time data. The Wilcoxan ranked sum test was used in evaluating differences in intestinal scores.

To distinguish between effects of malnourishment during the gestation period and that occurring after birth, a cross-fostering was achieved within 24 hr of birth. The dams readily accepted the new infants with less than 1% wastage. Four cross-fostering groups were studied: (i) animals conceived by dams receiving 100% N/C diet cross-fostered to dams also on the 100% N/C diet (designated 100/100); (ii) animals from dams receiving 40% N/C diet fostered to 100% N/C dams (40/100); (iii) mice from 100% N/C dams fostered to 40% N/C dams (100/40); (iv) mice from 40% N/C dams cross-fostered to other 40% N/C dams (40/40). The mice were infected or sham-infected and handled as in the initial malnourishment-infection study; at least three litters were used for each group.

Dietary protein alteration effects. Groups of mice used as dams were fed the low-, normal-, and high-protein diets, in parallel. The methodology in this experiment was the same as that described above for the malnutrition experiment. Pooled sera from both infants, killed 20 days after infection, and mothers, killed 30 days after infection, from each diet group were assayed for total protein, by the Coomassie brilliant blue G-250 assay (14) using a commercial kit (Bio-Rad Laboratories, Richmond, Calif.).

Titration of viral antigen. Viral antigen in the intestinal homogenates was assayed by ELISA using 96-well microtiter plates (polyvinyl V-well, Dynatech, Alexandria, Va.) coated with $(NH_4)_2SO_4$–purified guinea pig anti-simian (SA-11) rotavirus immunoglobulins. Serial half-log dilutions of homogenates in brain heart infusion (BHI) were incubated in the wells for 2 hr at 37°C. The wells were then washed and rabbit anti-human rotavirus-peroxidase conjugate (DAKO, Accurate Chemicals, Westbury, N.Y.) diluted in phosphate-buffered saline containing 20% fetal bovine serum and 2% normal guinea pig serum was added. After a 2-hr incubation at 37°C, the wells were washed and *O*-phenylenediamine · 2HCl, 2.56 mg/ml, diluted in citrate–phosphate buffer containing 0.02% H_2O_2 (Rotazyme, Abbott Laboratories, North Chicago, Ill.), was added, incubated 15 min at room temperature, and the reaction was stopped by adding 1 *N* HCl. The contents of each well were transferred to Gilford cuvettes, and the color change was measured spectrophotometrically at 490 nm in a Gilford PR50 automated EIA analyzer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Titers were expressed as the reciprocal of the highest dilution giving a $P/N \geqslant 2.1$, where $P = OD_{490}$ of sample and $N = 0.050$, a value greater than the average of four wells containing rotavirus negative intestinal homogenate.

Interferon assay. Serum samples were tested for presence and titer of interferon using the microplate procedure previously described

(15) in which mouse L-929 cells were exposed to varying dilutions of the serum and presence of interferon determined by challenge of the exposed cells to vesicular stomatitis virus. Interferon induced in L-929 cells by poly I·C was tested in parallel as a positive control.

Titration of serum antibody. Serum anti-rotavirus antibody was assayed using blocking of bovine rotavirus detection by ELISA. Equal volumes of Freon-extracted cell culture-propagated bovine rotavirus and varying twofold dilutions of the mouse serum to be tested for anti-rotavirus antibody were incubated together at 37°C. After 1 hr incubation, titration of bovine rotavirus in these mixtures was carried out as described above, for titration of viral antigen. Included were four wells of virus mixed with the diluent for the serum (BHI) to serve as virus controls, and four wells of BHI alone to serve as negative controls. Percentage reduction of each serum sample dilution was calculated using the equation: percentage reduction = [(mean virus control P/N − mean serum sample P/N)/mean virus control P/N − 1)] × 100. In this case, P = mean OD_{490} of indicated sample or control wells and N = mean OD_{490} of negative control wells. A 50% reduction was used as end point for indication of positive blocking antibody.

Results. *Effect of malnutrition.* Malnutrition, as induced by nutrient dilution, had a marked effect on the MRV infection, as summarized in Table I and Fig. 1. This effect appeared to be dependent upon the degree of nutrient dilution, with deaths occurring in 80% of the infected infants born from dams fed the 40% N/C diet and in 48% of the infected infants from dams fed the 70% N/C diet. Only 4% of the infants (3 of 70) died among those from mothers fed the 100% N/C diet. Among uninfected infants from 40% N/C-fed mothers, a 9% mortality occurred, suggesting this degree of malnutrition was approaching a minimum level for survival. Mean survival times were also markedly reduced in the malnourished animals. Weight gain was significantly retarded in the 40% N/C malnourished group. An important observation was that the initial (Day

TABLE I. EFFECT OF PRE- AND POSTNATAL NUTRIENT DILUTION ON ROTAVIRUS INFECTION IN INFANT MICE AND SERUM ANTIBODY TITER OF DAMS

	Percentage nutrients in diet[a] relative to control					
	40		70		100 (Control)	
	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
No. of mice	60	59	70	63	70	70
Avg initial weight (g)	1.8*	1.7*	2.0	2.2	2.4	2.5
Mortality (%)	80**	9	48**	0	4	0
Surv time[b] (days)	1.6	5.0	2.4	>15.0	7.0	>15.0
Intestinal scores[c]	3.5	0.0	3.5	0.0	3.7	0.0
Intestinal viral antigen titer[d]						
Day 2	$10^{1.8}$	$<10^{0.5}$	$10^{2.2}$	$<10^{0.5}$	$10^{2.4}$	$<10^{0.5}$
Day 4	10^{3}	$<10^{0.5}$	$10^{2.6}$	$<10^{0.5}$	$10^{2.9}$	$<10^{0.5}$
Serum antibody titer[e]	22	<4	16	<4	55	<4

[a] Diets of dams from which infants were taken. Diets were prepared by diluting the control diet by 0, 30, or 60% with glucose.

[b] Determined on animals dying on or before Day 15.

[c] Determined on mice killed on Days 2, 4, 6, 8, 10, and 15 post-virus exposure, except in the infected 40% N/C group, where the majority of the mice had died by Day 2. Scores defined as: 0, normal intestine; 1, diarrheal fluid in ~33% of intestine, with solid feces; 2, diarrheal fluid in ~50% of intestine; 3, appearance of gas, diarrheal fluid in ~50% of intestine; 4, distention due to gas, diarrheal fluid in ~75% of intestine.

[d] Expressed as reciprocal of highest dilution of intestinal homogenate with $P/N \geq 2.1$ by ELISA reaction.

[e] Reciprocal of highest dilution blocking ~50% bovine rotaviral ELISA reaction. Serum from dams killed 30 days post-virus exposure.

* $P < 0.01$, ** $P < 0.001$.

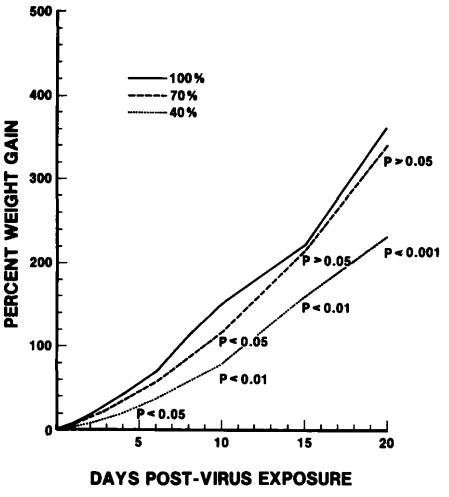

FIG. 1. Weight gain of rotavirus-infected infant mice from dams receiving 40, 70, or 100% nutrient/calorie (N/C) diets.

0) infant weights were significantly lower in the 40 and 70% N/C groups, which was indicative of a weakened condition at the time of infection. Since the majority of the infected infants in the 40 and 70% N/C groups died very early in the infection, this resulted in the later sacrifice of a somewhat select population for determination of intestinal scores and evaluation of intestinal viral antigen. It was, therefore, not surprising that the mean intestinal scores determined on Days 2 through 15, although indicative of severe diarrhea, did not appreciably vary between diet groups. Similarly, viral antigen titer was also not significantly different in each of the diet groups. Serum interferon levels remained below detectable levels in each group. The dams in each group were killed 30 days post-virus exposure and their serum removed and assayed for rotavirus antibody. The mean titers in the two nutritionally deprived groups were lower than those of the controls (Table I), suggesting the malnourishment may have lessened the animals' antibody-producing ability.

This study was essentially repeated and expanded upon when infants born by dams fed 40 or 100% N/C diets were cross-fostered to dams fed the same or the opposite diets (Table II, Fig. 2). The malnourished group again had a definitely enhanced response to the infection, with a 100% mortality occurring in the infected infants from 40% N/C-fed dams cross-fostered to other 40% N/C-fed dams (designated as the 40/40 group). The animals in this 40/40 group died very quickly after being infected. Mice from malnourished dams fostered by dams receiving full (100% N/C) nourishment, designated as the 40/100 group, appeared more resistant to the infection, with only 57% dying. Mice from fully nourished mothers fostered to the malnourished (40% N/C) mothers, designated as the 100/40 group, were more susceptible to the infection effects than the mice

TABLE II. EFFECT OF CROSS-FOSTERING TO DIFFERENTIATE BETWEEN PRE- AND POSTNATAL DIETARY EFFECTS ON ROTAVIRUS INFECTION IN INFANT MICE

	Percentage nutrients in diet relative to control							
	40[a]/40[b]		40/100		100/40		100/100 (Control)	
	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
No. of mice	16	20	17	20	48	45	32	40
Avg initial weight (g)	1.7**	1.7**	1.8**	2.0*	2.0*	2.0*	2.6	2.5
Mortality (%)	100**	0	57**	0	51**	17	0	0
Survival time, days[c]	1.4**	>15.0	3.5**	>15.0	4.7**	5.8	>15.0	>15.0
Intestinal scores[d]	2.8	0	2.0	0	3.2	0	3.0	0

[a] Diet of dams from which infants were taken.
[b] Diet of fostering dams.
[c] Determined on animals dying on or before Day 15.
[d] Determined on mice killed on Days 2, 4, and 8 post virus exposure, except in the infected 40/40 N/C group, where the majority of the mice had died by Day 2. Scores as described in footnote *c*, Table I.
* $P < 0.05$, ** $P < 0.001$ (compared to 100/100 group).

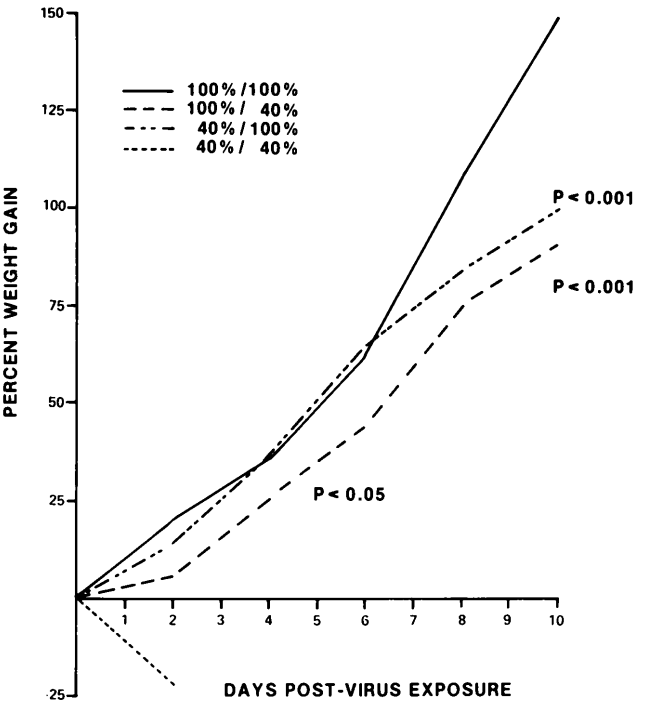

FIG. 2. Weight gain of rotavirus-infected infant mice cross-fostered from 100% N/C-fed dams to other 100% N/C dams (100/100), from 100% N/C dams to 40% N/C dams (100/40), from 40% N/C dams to 100% N/C dams (40/100), and from 40% N/C dams to other 40% N/C dams (40/40).

in the 100/100 group, but had fewer deaths than those in the 40/40 group. The weights of the offspring at the time of infection, 2 days after cross-fostering, did not appear to differ appreciably in the 40/40, 100/40, or 40/100 groups, although all were considerably lower than those weights in the 100/100 groups. Definite differences in weight gain were seen in the groups receiving the lower N/C diets (Fig. 2). Intestinal MRV antigen levels and intestinal diarrheal scores were not appreciably different in the four groups, although again the mice in the fully malnourished (40/40) group died so quickly that effective sampling could not be achieved. Serum antibody in the dams was not determined in this cross-foster study.

Effects of alteration in dietary protein. As seen in Table III, decrease in pre- and postnatal dietary protein had a significant enhancing effect on the infant mouse sensitivity to the infection, with a marked increased mortality occurring. Of importance was the observation that six litters of infants from dams receiving the low-protein diet were born dead or dying. Their birth was approximately 2 to 6 days premature, and the infants appeared very small. They were usually eaten by the dams shortly after birth. In this study, in addition to scoring of intestines taken from randomly sacrificed mice, the number of visibly diarrheal mice were also noted on Days 2–6 after virus exposure. The infants from mothers fed the low-protein diet had an increased ($P < 0.05$) incidence of diarrhea. The mean intestinal scores taken throughout the study, and the intestinal viral antigen titers did not vary substantially in the three diet groups, although, as occurred in the nutrient dilution experiments, the high number of animals dying limited the number and randomization of the animals killed later in the study for assay of viral antigen and intestinal scoring.

The weight gains of the three groups of infected mice are summarized in Fig. 3. Those mice from the low-protein-fed mothers weighed less initially; for the first 5 days of

TABLE III. EFFECT OF PRE- AND POSTNATAL ALTERATIONS IN DIETARY PROTEIN ON ROTAVIRUS INFECTION IN INFANT MICE AND SERUM ANTIBODY TITER OF DAMS

	Percent protein in diet[a] relative to control					
	50		100 (Control)		200	
	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
No. of mice	36	12	73	67	83	50
Avg initial weight (g)	1.3	1.5	1.8	1.7	2.0	1.9
Mortality (%)	76**	8	10	8	3	4
Surv time[b] (days)	2.8[c]	11.0[c]	5.6	8.4	2.2	7.0
Intestinal scores[d]	2.3	0.0	2.3	0.0	2.5	0.0
Visibly diarrheic[e] (%)	88**	0	50	0	39	0
Intestinal antigen titer[f]						
Day 2	$10^{3.1}$	$<10^{0.5}$	$10^{3.0}$	$<10^{0.5}$	$10^{3.0}$	$<10^{0.5}$
Day 4	$10^{3.0}$	$<10^{0.5}$	$10^{3.9}$	$<10^{0.5}$	$\geq 10^{4.0}$	$<10^{0.5}$
Serum antibody titer[g]	32	<4	32	<4	64	<4

[a] Diets of dams from which infants were taken. Protein expressed as casein.
[b] Determined on animals dying on or before Day 15.
[c] Six litters of mice from dams receiving 50% protein diet were dead or dying at birth. Their birth appeared to be 2–6 days premature.
[d] Determined on mice killed on Days 2, 4, 6, 8, 10, and 15, except in the infected 50% protein group where the majority of the animals had died prior to Day 4, and in the uninfected 50% protein group, which had relative few numbers from which to take samples. Scores as described in footnote *c*, Table I.
[e] Determined on Days 2–6.
[f] Reciprocal of highest dilution of intestinal homogenate having $P/N \geq 2.1$ by ELISA reaction.
[g] Reciprocal of highest dilution blocking ~50% bovine rotaviral ELISA reaction. Serum from dams killed 30 days post-virus exposure.
** $P < 0.001$.

infection these infants gained weight in an almost parallel manner to those infants in the normal- and high-protein groups. The weight gain in the protein-deprived group began to significantly lessen by 10 days post-virus exposure, and this decreased weight gain then continued throughout the remainder of the study. The infants from the dams fed the high-protein diet had a slightly lessened weight gain which became apparent by 10 days post-virus exposure.

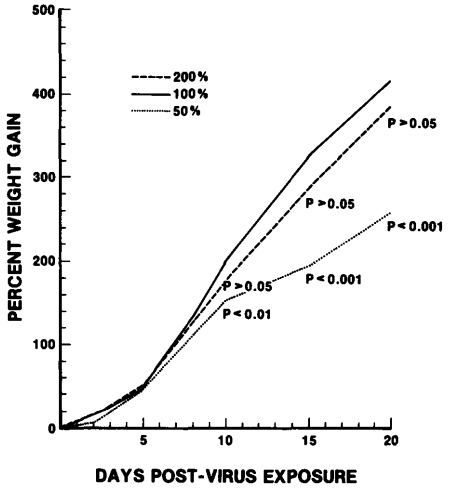

FIG. 3. Weight gain of rotavirus-infected infant mice from dams receiving low (50%), normal (100%), or high (200%) protein (casein)-containing diets.

At Day 30, serum anti-rotavirus antibody from the infected dams was at a relatively low level and did not vary appreciably in this experiment (Table III), suggesting that the protein alterations examined did not have a significant influence on this aspect of the animals' humoral immunity. Serum antibody from the infected infants killed on Day 20 post-virus exposure was below detectable levels, presumably because an adequate time to develop serum anti-rotavirus antibody levels had not yet elapsed.

The total serum protein values in the infected and sham-infected mice kept on each protein diet are seen in Table IV. These values in the sham-infected animals corresponded in

TABLE IV. TOTAL SERUM PROTEIN LEVELS[a] IN INFECTED AND UNINFECTED MICE FED PROTEIN-ALTERED DIETS

	Percentage protein in diet[b] relative to control					
	50		100 (Control)		200	
Animals	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
Infants[c]	10.5	7.8	8.6	10.1	10.0	14.3
Dams[d]	10.8	9.0	10.2	14.8	14.6	15.8

[a] g/dl. Pooled serum from at least five mice.
[b] Diets of dams from which infants were taken.
[c] Killed 20 days postvirus or sham virus exposure.
[d] Killed 30 days postvirus or sham virus exposure.

direct proportion to the amount of protein in the diet, indicating the dietary alterations were sufficient to cause differences in protein level in the serum. Those from the infected dams and infants varied considerably, with the infants from dams receiving the low-protein diet having higher levels of measurable total serum protein than those from the normal and high-protein groups. We attribute this to dehydration resulting from the severe diarrhea in the infants.

Discussion. The marked enhancing effect of malnutrition on the rotaviral disease in these experimentally infected infant mice strengthens the association reported in the clinic between increased incidence and severity of diarrhea in young children and the nutritional status of the infants (4–6).

Gastrointestinal infection is normally held in check by the alimentary microflora and the cellular integrity of the gut. In malnutrition, slowed cellular turnover may cause mucosal thinning, altering the normal relationship between the mucosa and flora, and thereby compromising this biological barrier (16). Once infection begins to damage the epithelial lining, it must be repaired by normal mechanisms. In the nutritionally deprived host, these mechanisms may be faulty, and repair is retarded. The loss of fluids and electrolytes, as well as malabsorption, may overcome the individual, especially if immunocompetence is decreased.

The lack of difference in viral antigen and overall intestinal diarrheal scoring, coupled with the lower initial weights in the mice receiving nutrient diluted diets suggests that a general weakening and stress of the animals occurred due to the malnourishment which caused them to succumb more readily to the disease. The occurrence of some deaths in the uninfected malnourished control infants seen in Table I strengthens this premise. Malnourishment is also known to have serious effects on immunological host defense mechanisms, including decrease in production of secretory immunoglobulins (17, 18), thymus involution, depletion of lymphocytes and germinal centers of the lymph node and spleen and reduction in size of lymphoid aggregation (19). A decrease in secretory antibodies would render the host more susceptible to antigen penetration across the mucosal barrier (20). Our finding that the mean serum antibody titers in the two nutritionally deprived groups were lower than those of controls are similar to those reported for bacterial antigen treated rats by Nalder *et al.* (21). Conversely, others have found no change in antibody response due to malnutrition (22). Studies into the influence of malnourishment on the various immunological factors in this model are currently underway in our laboratory.

By cross-fostering, it was shown that the effects of malnutrition on increasing sensitivity to rotaviral disease are not occurring only in the prenatal period; indeed, normal animals cross-fostered for only 2 days to severely malnourished mothers were markedly more sensitive to the infection than those cross-fostered to control, fully nourished, dams. However, the enhanced sensitivity to the infection was not as great as with those animals malnourished both pre- and postnatally.

Serum interferon apparently did not play a role in the response of these animals to ro-

taviral infection. In our earlier susceptibility studies (10), appreciable serum interferon increases after infection were not seen, and in the present study no increases were seen in interferon titers in mice from the various diet-altered groups. It is possible that interferon may be present in intestinal secretions, although a healthy intestinal mucosa may limit leakage of the material (23); further, fecal material has been shown to contain a substance or substances capable of inactivating interferon (24). We did not have adequate intestinal material to assay for interferon in the gut in the present study.

A pair-fed group was not included in the protein studies, which makes it difficult to attribute all the effects seen to changes in protein intake, as reduced protein intake may result in loss of appetite and consequently total energy intake. However, the increased mortality observed, when evaluated in comparison with the uninfected controls fed parallel diets would indicate a more severe infection in the protein-deprived animals.

Protein deprivation has been reported to enhance other viral infections as well as that induced in this study by murine rotavirus. Mice receiving a low-protein diet and injected with "infectious hepatitis agents" isolated from humans had twice the fatality rate of mice fed a diet with higher levels of protein (25). Coxsackie B3 virus infection in mice receiving low-protein diets after weaning was also increased as seen by more severe lesions and prolonged viral persistence (26). Chicks fed diets deficient or with excessive amounts of protein developed Newcastle disease of significantly increased severity (11). Prenatal protein deprivation of dogs also enhanced their susceptibility to distemper virus (7). Many reports also indicate similar disease-enhancing effects in animals infected with bacterial, mycotic, and parasitic organisms (7). A variety of mechanisms may account for such disease enhancement in protein-deprived animals. These include reduction in intraepithelial lymphocytes and submucosal plasma cells (27), decreased antibody production, impaired phagocytic killing and clearance, a reduced number of T lymphocytes, defects in cytotoxic immune function, low levels of complement, and possibly impaired interferon production (7). Of particular significance is the observation of Chandra and Newberne (7) that the number of mucosal epithelial cells in the gut are severely depleted in protein-undernourished human subjects. In the present study, we examined hematoxylin and eosin-stained sections of various portions of the intestines of sham-infected infant mice from dams on each protein diet, but no readily discernible defects were seen.

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