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G A Losonsky, ... , J A Winkelstein, R H Yolken

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Research Article

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Oral Administration of Human Serum Immunoglobulin in Immunodeficient Patients with Viral Gastroenteritis

A Pharmacokinetic and Functional Analysis

Genevieve A. Losonsky, John P. Johnson, Jerry A. Winkelstein, and Robert H. Yolken

Department of Pediatrics, Divisions of Infectious Diseases and Immunology, The Johns Hopkins University, Baltimore, Maryland 21205

Abstract

We examined the pharmacokinetics and immunological activity of human serum immunoglobulins (HSG) possessing anti-rotavirus activity which were orally administered to three children with primary immunodeficiency syndromes and prolonged gastrointestinal excretion of rotavirus. Detailed analysis of the excretion of immunoglobulins labeled with biotin or I^{125} revealed that ~50% of the recovered radioactivity was excreted in the stools over a 3-d period. Approximately half of the excreted radioactivity recovered in the stool was in a macromolecular form with immunological activity. The remainder of the recovered radioactivity was excreted in the urine as low molecular weight fragments or free iodide. In addition, immunological and chromatographic analyses revealed that the oral administration of HSG resulted in the generation of rotavirus-specific immune complexes in the gastrointestinal tract with a subsequent decrease in the presence of uncomplexed rotavirus antigen. These studies indicate that orally administered HSG can survive passage in the gastrointestinal tract in an immunologically active form, and that the oral administration of immunoglobulins with specific reactivities has potential for the prevention or treatment of gastrointestinal infections.

Introduction

Fastidious viral agents such as rotaviruses and enteric adenoviruses have been recognized as important causes of infantile gastroenteritis (1-4). While these viruses generally infect infants and young children, recent studies indicate that older children and adults with defects in host response to viral infections can have prolonged infections with these agents. Severe gastroenteritis associated with rotavirus has been encountered in immunocompromised children with severe combined immunodeficiency disease, X-linked agammaglobulinemia, and IgA deficiency (5, 6). Similarly, serious gastrointestinal infections due to rotavirus and adenovirus have been documented in adults undergoing bone marrow transplantation (7).

Effective antiviral chemotherapy is not yet available for

Address reprint requests to Dr. Yolken, Department of Medicine, Division of Infectious Diseases, The Johns Hopkins Hospital, 600 N. Wolfe St., CMSC 1109, Baltimore, MD 21205. The present address of Drs. Losonsky and Johnson is Department of Pediatrics, University of Maryland Hospital, Baltimore, MD 21202.

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treatment of infections due to these agents. In certain animal models of infectious diarrhea, oral administration of immunoglobulins has proved effective in preventing or modifying the disease (8, 9). In man, there is suggestive evidence that human milk may have a protective function against gastrointestinal pathogens (6, 10, 11). However, problems of availability, storage, and the possible transmission of infectious agents, such as cytomegalovirus, are factors that make widespread treatment or prophylaxis trials with human milk products difficult to accomplish. There have been several studies in man suggesting that passive immunity against infectious diarrheal agents may be achieved by the oral administration of immunoglobulin preparations derived from human or animal sera (12-14). To date, there have been no detailed studies of orally administered immunoglobulins in humans older than 13 wk of age or in patients with chronic infectious diarrhea. The purpose of this study was to evaluate the survival and immunologic activity of orally administered human serum globulin (HSG)¹ in the gastrointestinal tract of immunodeficient individuals with severe, prolonged rotavirus diarrhea.

Methods

Subjects. Two children, aged 4 yr (child A) and 16 mo (child B) with severe combined immunodeficiency disease, and one adolescent aged 18 yr (child C) with common variable immunodeficiency disease, participated in the trials. At the time of the study, all had been followed for extended periods of time for chronic diarrhea and found to be intermittent chronic excretors of serotype 1 rotavirus for a period of 2 mo-2 yr. Participation of these patients was approved by the Joint Committee of Clinical Investigation of the Johns Hopkins Hospital and informed consent obtained before entry into the study. All three children had abnormal gastrointestinal function as evidenced by fat malabsorption and decreased weight gain in spite of adequate caloric intake. Protein malabsorption was not evaluated in these children. The stools on all patients were negative for other intestinal viruses, pathogenic bacteria, and parasites as determined by standard microbiological methods.

Gammaglobulin preparations. Commercial HSG for intravenous use (Cutter Laboratories Inc., Berkeley, CA) containing 50 mg IgG/ml was used in all trials. In some trials, ~3% of the administered HSG was labeled with biotin by reacting the immunoglobulin with the *N*-hydroxy-succinimide ester as previously described (15). In additional trials, a small portion (0.1-0.5%) of the immunoglobulin was labeled with I^{125} by the method of Hunter et al. (16). Total radioactivity given was 10 μ Ci. The specific antibody titers of this gammaglobulin preparation to rotavirus antigen was 1:640 as measured by an enzyme-linked immunosorbent assay utilizing the WA-11 strain of the rotavirus (serotype 1) antigen (17). Potassium iodide was administered to the patients before treatment of radiolabeled immunoglobulin.

Specimen collection and processing. In each trial, all stools and urine were continuously collected for 1 wk before the trial and 1 wk thereafter. Stool was collected in stool cups in the two older patients. The 16-mo-old child had stool collected in diapers, a method of collection that does

1. Abbreviations used in this paper: HSG, human serum immunoglobulin.

Table I. Assays for Rotavirus Antigen, Biotinillated IgG, and Rotavirus IgG Complexes in Stool

Detection	Solid coating	Specimen	Intermediate antibody	Enzyme-linked antibody	Substrate
Rotavirus antigen	Chicken anti-rotavirus antibody	Lyophilized stool	Guinea pig anti-rotavirus antibody	Alkaline phosphatase rabbit anti-guinea pig IgG antibody	<i>p</i> -Nitrophenylophosphate
Rotavirus IgG complexes	Chicken anti-rotavirus antibody	Unabsorbed stool and stool absorbed with protein A rabbit anti-human IgG	Guinea pig anti-rotavirus antibody	Alkaline phosphatase rabbit anti-guinea pig IgG antibody	<i>p</i> -Nitrophenylophosphate
Biotinillated IgG*	Avidin	Lyophilized stool	None	Peroxidase rabbit anti-human IgG antibody	<i>o</i> -Phenylenediamine

* In immunologically recognizable form with solid phase avidin and anti-human IgG antibody.

not prevent urine contamination. Stool volume and wet weight were determined and the stools were subsequently lyophilized and reconstituted to a final concentration of 100 mg/ml using 0.01 M phosphate buffer, pH 7.4. The reconstituted stool was mixed for 30 min at 4°C and then centrifuged at 5,000 *g* for 15 min at 4°C. The resultant supernatant was dialyzed against phosphate buffer with 0.02% sodium azide and then stored at -70°C before use. Test serum was obtained from each patient once before each trial and then twice daily for 2 d posttreatment. Sera and urine were stored at -20°C before testing.

Pharmacokinetic analysis. Radioactive counts per minute were determined on serial pre- and post-dosage stool, serum, and urine samples using a gamma counter (model 1195, G. D. Searle and Co., Skokie, IL). Radioactivity was determined in both unprocessed and processed stool samples. In order to detect the proportion of activity present in a high molecular weight form, radioactive counts were determined on processed stool, urine, and serum samples precipitated with equal volumes of cold saturated ammonium sulfate. In addition, all body fluids collected were dialyzed through low molecular weight exclusion membranes (14,000-mol-wt exclusion, Scientific Products, McGraw Park, IL) and radioactivity determined on pre- and post-dialysis materials.

Molecular weight sizing of selected stool specimens was also performed by chromatography using a Biogel A5m column (Bio-Rad Laboratories, Richmond, CA), 25 × 2 cm, eluted at a flow rate of 30 ml/h with phosphate-buffered saline (PBS), pH 7.4. All specimens were dialyzed through low molecular weight exclusion membranes using 0.01 M phosphate buffer before loading of the column. Molecular weight standards used were Blue Dextran (Pharmacia Fine Chemicals, Uppsala, Sweden), β-lactamase (Sigma Chemical Co., St. Louis, MO) and unmodified biotinillated HSG. For these studies, the presence of eluted biotinillated

HSG was manifested by an inhibition of binding between solid phase avidin and the peroxidase-labeled biotin (18). For this assay, polyvinyl chloride microtiter plates (Dynatech Laboratories, Inc., Dynatech Corp., Alexandria, VA) were coated overnight at 4°C with 0.1 μg/ml of avidin (Sigma Chemical Co.) diluted in PBS. Control wells were coated only with PBS. 50 μl of twofold dilutions of eluted fractions was then incubated on the avidin-coated plates for 1 h at 37°C with gentle agitation. Peroxidase-labeled biotin (Vector Laboratories Inc., Burlingame, CA), diluted in PBS-Tween, was added for 1 h at 37°C followed by hydrogen-peroxide *o*-phenylenediamine substrate (Sigma Chemical Co.). The amount of biotinillated immunoglobulin in each fraction was determined by comparison of test results to the results of this inhibition assay using known amounts of standard biotinillated immunoglobulins.

Stool antigen and antibody detection. Enzyme immunoassays were used to detect rotavirus antigen, biotinillated IgG, and rotavirus-specific immune complexes in the processed stool samples (Table I). The assay for total rotavirus antigen has been previously described and is outlined in Table I (6). In addition, this assay was modified to allow for determination of stool rotavirus antigen bound to immunoglobulin in the form of immune complexes. This assay involved preincubating 150 μl of stool sample with rabbit anti-human IgG that had been complexed with insoluble protein A-bearing staphylococci (Sigma Chemical Co.). After preincubation for 1 h at 37°C, the protein A antibody mixture was pelleted by centrifugation and the resulting supernatant assayed for rotavirus antigen. The difference in rotavirus measured before and after absorption represented the amount of rotavirus that was excreted complexed to immunoglobulin. Total biotinillated IgG was also detected using avidin, at a concentration of 2 μg/ml in PBS, on the solid phase as the capture antigen. The biotinillated IgG bound to the solid phase

Table II. HSG Treatment Trials

Patient	Age	Wt kg	HSG dose	No. of doses given	Evaluation*
A	4 yr	9	150 mg/kg	10	R
A	4 yr	9	150 mg/kg double-label IgG	1	P, R, G, I
B	16 mo	13.5	150 mg/kg biotinillated IgG	1	P, R, G, I
C	18 yr	30	150 mg/kg double-label IgG	1	P, R, G, I

* Evaluation of HSG activity included pharmacokinetic analysis (P), rotavirus antigen excretion (R), IgG stool excretion (G), and specific immune complex formation (I).

Table III. Pharmacokinetics of HSG

Child	Total dose ^{125}I -HSG recovered	Distribution of administered radioactivity			Proportion of $(\text{NH}_4)_2\text{SO}_4$ precipitable activity*		
		Blood	Urine	Stool	Blood	Urine	Stool
		%	%	%	%	%	%
A	40	2	49	49	0	0.01	40
C	90	4	48	49	0	0	51

* Indicates percentage of recovered radioactivity precipitable by $(\text{NH}_4)_2\text{SO}_4$.

avidin was recognized by reaction with peroxidase-labeled rabbit anti-human IgG (Cappel Laboratories Inc., Cochranville, PA). Controls used in these assays included PBS-Tween, 10-fold dilutions of biotinillated immunoglobulin, nonbiotinillated immunoglobulin, rotavirus antigen, and preformed rotavirus-biotinillated immune complexes and rotavirus antigen. The limit of detectability in our assay for total immunoglobulin was 5 ng/ml as determined by the testing of known amounts of biotinillated immunoglobulin. In the immune complex assay, SA-11 antigen coupled with antibody could be detected at an amount of virus corresponding to 10^2 TCID₅₀ of infectious virus. Standard deviations in both of the antibody and immune complex assays using the above controls were 0.051 and 0.047 ODU, respectively.

Results

Treatment trials. The dosage schedule and laboratory analyses used for each trial can be seen in Table II. In the first trial, child A was given two courses of 150 mg/kg per dose of unlabeled HSG every 5 h for five doses. Each course was separated by 2 d. In subsequent trials, child A, B, and C were either given a single dose of 150 mg/kg of biotin and ^{125}I -labeled HSG (children A and C) or biotinillated HSG (child B).

Pharmacokinetics. The recovery of the orally administered radioactive labeled HSG was determined by radioactive counts of collected body fluids after a single dose of radiolabeled HSG (Table III). Total recovery of label was 40% in child A and 90% in child C. However, in both patients, the proportion of radioactivity in stool (48–49%), urine (48–49%), and blood (2–4%) was similar. The kinetics of recovery of radioactivity in urine and stool is depicted in Fig. 1, A and B. Although rapid recovery of a portion of radiolabel was found in the urine in both children after ingestion of HSG, only 1–2% of that recovered in the urine had a molecular weight > 14,000 as determined by dialysis, and only 0.01% of that recovered was present in a high molecular weight form as determined by ammonium sulfate precipitation. Approximately 2–4% of the administered radioactivity recovered was found in the blood, none of which was in a molecular weight form > 14,000. Peak recovery of radiolabel occurred later in the sequentially collected stool samples as compared with the urine. However, unlike the urine and serum findings, ~50% of the radiolabel recovered in the stools was in a high molecular weight form as determined by ammonium sulfate precipitation. Radioactivity persisted in the stools for 4–6 d after the single dose.

The recovery of biotinillated HSG in the stool was also evaluated in children A and B using an enzyme immunoassay. Approximately 30–40% of the administered immunoglobulin was recovered in an immunologically recognizable form as evidenced

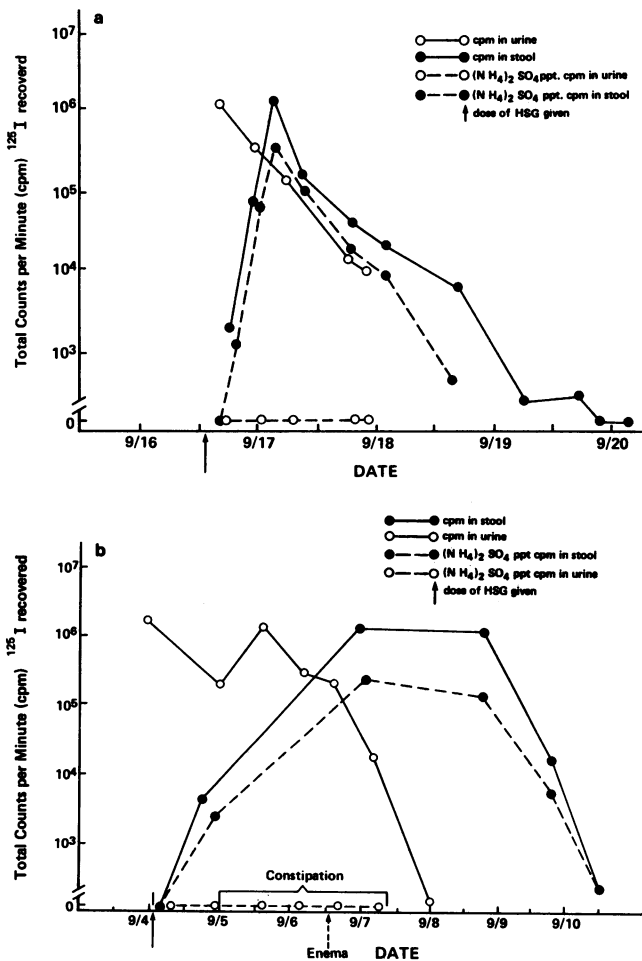


Figure 1. Pharmacokinetics of orally administered radiolabeled HSG in child A (a) and child C (b). Total and ammonium sulfate precipitable radioactive counts for urine and stool after HSG administration are depicted.

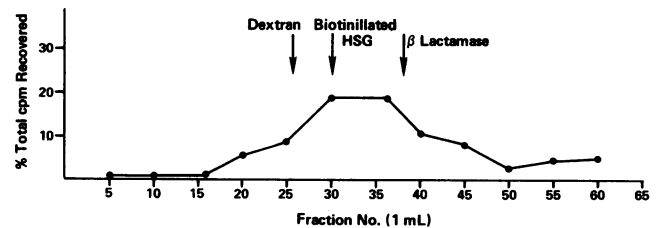


Figure 2. The total radioactivity of stool sample a, obtained 15 h after HSG administration in child A, as recovered after elution on a Biogel A5m column. Molecular weight standards used were Blue Dextran (2,000,000 mol wt), biotinillated HSG (200,000 mol wt), and β -lactamase (50,000 mol wt). Percentages of total radioactivity of stool samples applied to the column are depicted.

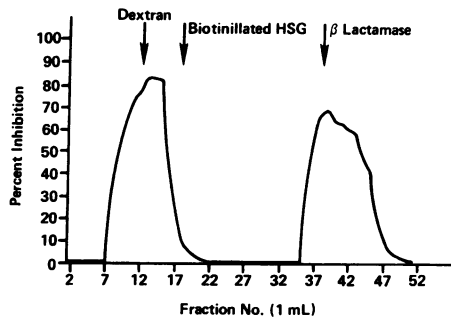


Figure 3. The form of biotinillated HSG recovered in stool sample b, obtained 23 h after HSG administration, in child A, as recovered in eluted fractions on a Biogel A5m column. Recovered fractions expressed as activity of biotinillated material in eluted fractions that inhibited binding of biotin-peroxidase to solid phase avidin. Molecular weight standards used were Blue Dextran (~2,000,000 mol wt), biotinillated HSG (~200,000 mol wt), and β -lactamase (~50,000 mol wt).

by immunoreactivity with antibody to human IgG (data on child A shown in Fig. 4, other subject data not shown).

To further analyze the components of these high molecular weight fractions, several of these stool samples obtained from children fed both radiolabeled and biotinillated immunoglobulin were chromatographed using gel exclusion chromatography and assayed for biotin or radioactivity (Figs. 2 and 3). In the sample a (9/17, 4 a.m.) obtained 15 h after dosing with labeled HSG, ~18% of the recovered radioactivity was in the fraction corresponding to a molecular weight greater than monomeric HSG (200,000-mol wt) (Fig. 2). Sample b, obtained 23 h after dosing with labeled HSG, had ~50% of the biotinillated material in such a high molecular weight form (Fig. 3). These chromatographic findings are consistent with those obtained by the non-chromatographed material assayed for uncomplexed and complexed HSG by immunoassays (Fig. 4, samples a and b).

Characteristics of stool rotavirus antigen excretion/immune complex formation. The patterns of uncomplexed rotavirus antigen excretion, presence of biotinillated IgG, and rotavirus-specific immune complexes in the single dose trials for child A and

B are depicted in Figs. 4 and 5, respectively. In both cases high levels of uncomplexed rotavirus antigen in the stool were detected before administration of HSG. After HSG administration, no rotavirus antigen was detected in the stools for 2–3 d. Concomitant with the disappearance of detectable antigen, rotavirus-specific IgG immune complexes appeared. Thereafter, uncomplexed rotavirus antigen reappeared in the stools as levels of detectable rotavirus-specific immune complexes declined and subsequently disappeared. Eventually only free antigen was detected in the stools of child A. However, in child B, rotavirus antigen could not be detected in stool samples for the last 4 d of surveillance. When multiple doses of HSG were administered over a short time period, duration of rotavirus antigen disappearance in the stools was similar to that seen in the single dosing trials (Fig. 6).

Clinical effects. No untoward side effects were experienced by any of the patients enrolled in the study. Because the trials were set up to establish the survival of serum immunoglobulins in the gastrointestinal tract, clinical efficacy could not be determined.

Discussion

Persistent diarrhea due to viral agents in patients with immunodeficiency diseases increases morbidity and is a challenge to physicians in terms of establishing treatment regimens useful in managing the diarrhea. Parenterally administered immunoglobulin does not appear to be effective in the prevention or treatment of mucosal viral infections in immunodeficient patients, although it may be helpful in preventing systemic dissemination (19). Local mucosal treatment with human breast milk may be beneficial, but breast milk can be difficult to obtain or standardize (6). On the other hand, serum immunoglobulin preparations are widely available, although their fate following oral administration has not been extensively investigated. We thus used I¹²⁵- and biotin-labeled immunoglobulins and a set of sensitive immunoassay systems to demonstrate that a major portion of HSG administered orally to patients with primary immunodeficiency diseases can survive in the gastrointestinal

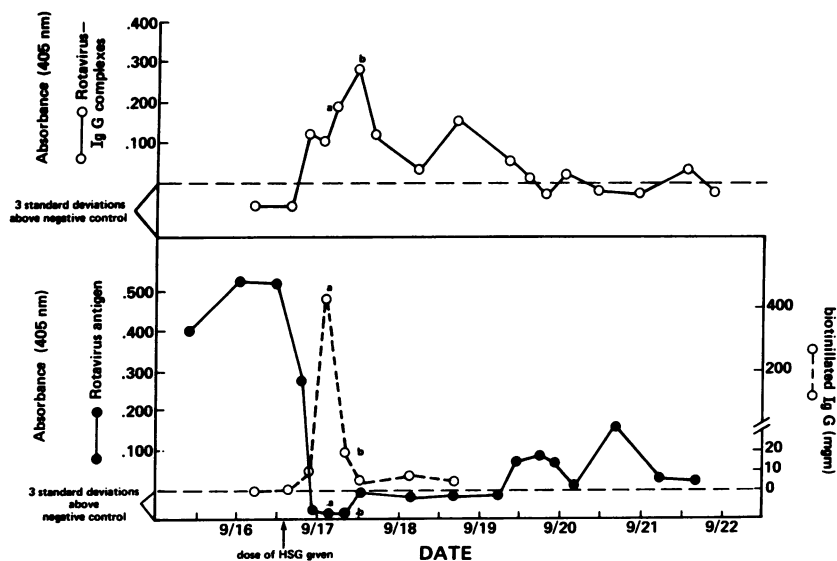


Figure 4. Patterns of recovery of measurable biotinillated IgG (--- o ---), uncomplexed rotavirus antigen (—●—), and rotavirus specific IgG complexes (—○—) after a single dose of biotinillated and radiolabeled HSG in child A. The levels of uncomplexed rotavirus antigen were determined by measuring the proportion of rotavirus antigen that could be precipitated by reaction with rabbit anti-human IgG and insoluble staphylococcal protein A as described above. Stool samples designated a, obtained 9/17, 4 a.m., and designated b, obtained 9/17, 11 a.m., were chromatographed and are those represented in Figs. 2 and 3, respectively.

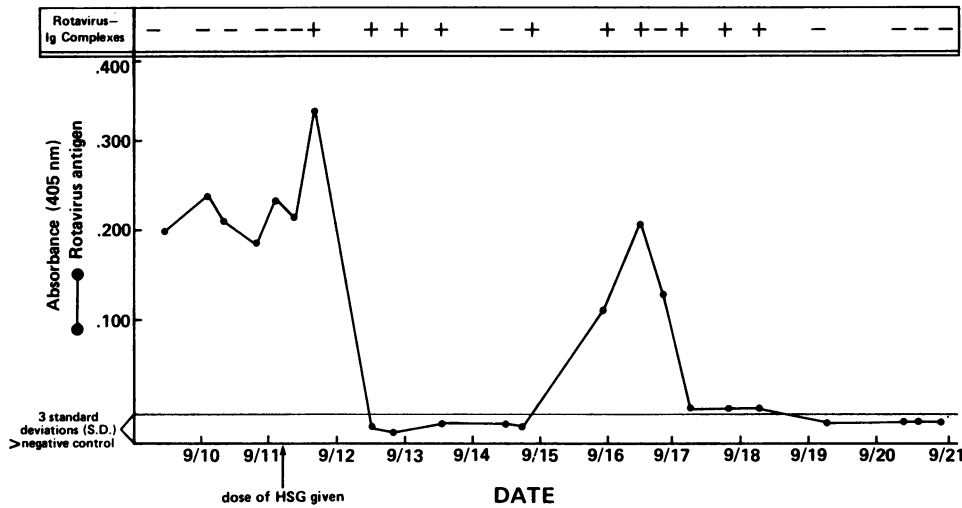


Figure 5. Pattern of recovery of stool rotavirus antigen and rotavirus specific immune complexes after a single dose of double label HSG in child B.

tract and maintain antigen binding capacity. These findings are in agreement with other investigators who found that orally administered HSG in immunocompetent infants maintains the ability to opsonize type III B streptococci after feeding to low birth weight babies (12). In our study, pharmacokinetic analysis demonstrated that radiolabeled HSG was not absorbed intact into the systemic circulation to any appreciable extent. This finding suggests that the activity of orally administered HSG may only be local, at the level of the mucosal surface. The gastrointestinal function of these three children is not entirely normal, as evidenced by all having fat malabsorption. This problem has been described in such children (6). The extent to which

these gastrointestinal abnormalities contribute to the survival of orally administered immunoglobulin in the gastrointestinal tract is not known and similar studies in normal controls are needed.

Several technical problems in our kinetic analysis are explainable. The difficulty in recovery of total radiolabel given in child A probably reflected loss of urine and stools in diapers. This problem, however, did not alter the proportional distribution of radioactivity present in high molecular weight form. Another problem was that constipation, produced by concomitant medical therapy with cholestyramine in child C, resulted in a delay of excretion of labeled HSG and a somewhat prolonged excretion of label in the urine. In this case, the survival of mac-

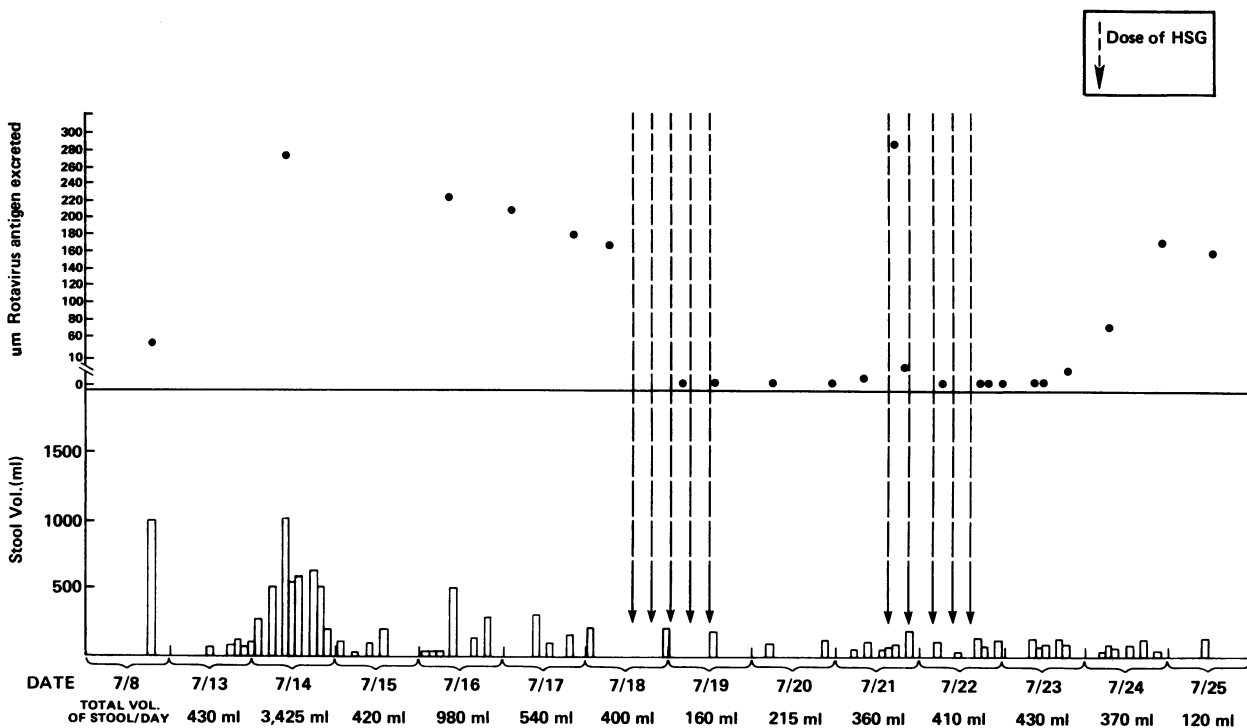


Figure 6. Stool rotavirus antigen excretion and stool output before and after HSG dosing trials in child A. Child A received two courses of HSG (five doses of HSG/course), each course separated by 2 d.

romolecular IgG was preserved even though transit time through the gut was lengthened, suggesting that HSG can survive for extended periods in the intestinal tract. The possible role of fat malabsorption, stomach acidity, and ion exchange resin administration on the survivability of orally administered immunoglobulin could not be determined in our study but should be the subject of additional investigations.

In addition to survival of HSG in the gastrointestinal tract after ingestion, we were able to document the retention of immunologic activity as evidenced by the formation of immune complexes between labeled orally administered immunoglobulin and endogenous rotavirus. During the first 24 h of oral immunoglobulin administration all rotavirus antigen present in the stool was in the form of specific immune complexes reflecting the presence of antigen in antibody excess. This phenomenon of antigen masking by specific immunoglobulin has been noted to occur in similar situations. For example, some patients with hepatitis B infection with the absence of detectable serum surface antigen have been found to have circulating specific immune complexes (20). In our study, these complexes continued to be excreted for several days after ingestion of a single dose of HSG. An inability to detect free rotavirus antigen after HSG administration was observed in all of the subjects, although a return of rotavirus antigen in the stool was noted in two of the three treated children several days after the administration of the oral immunoglobulin (often with immune complexes present in antigen excess) even when multiple doses of immunoglobulin were used. Whether this reflects a lack of total viral neutralization with HSG, a sequestered load of rotavirus in the intestinal tract unable to interact with the HSG, or reinfection with exogenous virus could not be determined by these studies but should be the subject of further evaluation. Our study demonstrates that orally administered HSG is capable of binding viral antigens in the human gastrointestinal tract and thus has the potential of being a prophylactic or therapeutic agent in the management of viral gastroenteritis in humans. The availability of such an agent might markedly affect the morbidity and mortality of viral gastroenteritis in immunodeficient children and other individuals who are unable to mount an adequate autonomous immune response to gastrointestinal pathogens.

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