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Research Article

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The Mechanism of Intestinal Uptake and Transcellular Transport of IgG in the Neonatal Rat

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ABSTRACT The transport of immunoglobulins across the intestinal mucosa of neonatal rats provides an excellent model for the study of transcellular protein transport. The mechanism of intestinal uptake and transcellular transport of plasma proteins has been studied in 12–14-day old rats using intraduodenally administered radioiodinated proteins. Appreciable quantities of rat IgG, mouse IgG, rabbit IgG, and all four subclasses of human IgG were taken up by the intestinal wall (19–54% of administered dose at 4 hr) and transported to the animal (10–35% of administered dose at 4 hr). In contrast there was little or no uptake of human IgM, IgA, and IgE and little or no transport of human IgM, IgA, IgD, IgE, albumin, transferrin, and ceruloplasmin. Both the uptake and transport of labeled IgG were significantly inhibited by unlabeled IgG. Further insight into the transport process was obtained from the observation that an appreciable proportion of the label of IgG in intestinal wall homogenates, but not in plasma or intestinal washings, migrated in a sucrose ultracentrifugation gradient much more rapidly than did the administered 7S molecules. This pattern was not observed with other proteins studied. This apparent binding of labeled IgG was also markedly inhibited by unlabeled IgG. In subcellular fractionation studies of intestinal homogenates the complexed labeled IgG was shown to be associated predominantly with cell membrane rather than cell sap fractions. In addition IgG could be shown to bind to purified enterocyte microvillous membranes in vitro.

It is concluded that in the neonatal rat: (*a*) the major processes involved in both intestinal uptake and transport of IgG are specific and saturable; (*b*) intestinal transport is associated with complexing of IgG molecules with membranes, most probably with enterocyte microvillous membranes; and (*c*) the part of the IgG structure involved in this process is probably similar to that involved in the concentration-catabolism effect but is not identical to that mediating other nonantigen combining functions of IgG. Our data are consistent with the existence of specific receptors for IgG on enterocyte microvillous membranes of the neonatal rat. Such receptors would be necessary for the specific uptake and transport of these molecules.

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INTRODUCTION

The mechanisms involved in the cellular uptake and transport of many small molecules have been extensively investigated. However, cellular uptake and transport of large molecules, in particular proteins, have been the subject of much less study. The transmission of immunoglobulins from mother to young appears to provide an excellent model for the study of such transcellular protein transport.

Transference of passive immunity from mother to young occurs predominantly during the first 21 days after birth in the rat (1, 2). Antibodies in maternal colostrum or milk are transported across the intestinal mucosa into intestinal lymphatics (3). It has been demonstrated that this process is selective, shows species specificity, can be competitively inhibited, and is associated with protein catabolism (4–10). Based on these experimental findings an hypothesis for the mechanism of intestinal protein transport in the neonatal rat has been put forward (2, 6, 11, 12). It was proposed that there is initial nonspecific uptake of protein molecules into pinocytotic vacuoles in the enterocyte. It was also

assumed that the proportion of the protein molecules taken up that are selectively transported would become bound to specific receptors, adapted for homologous protein, on the walls of these vacuoles and that such binding would protect protein molecules from catabolism. So far this hypothesis has not been substantiated by experimental demonstration of specific binding of antibody molecules to the walls of pinocytotic vacuoles in enterocytes.

The studies in the neonatal rat reported in this paper were designed to determine: (*a*) whether there is selective intestinal transport of one or more specific classes of protein molecules; (*b*) whether one or more subclasses of human IgG are selectively transported; (*c*) whether both intestinal uptake and transport of any selectively transported proteins are both specific and saturable processes; and (*d*) whether any complexing of protein molecules consistent with binding to specific receptors can be demonstrated in association with transport. All the studies were conducted in 12–14-day old rats, as at this age the animals are a convenient size and passive transference of immunity from the mother is still occurring (1).

METHODS

Materials. Human IgG for labeling was prepared from fresh human serum by DEAE-cellulose column chromatography. Human IgG not required for labeling, sheep IgG and human transferrin were obtained from Mann Research Laboratories, Orangeburg, N. Y. Rabbit IgG and rat IgG were obtained from Pentex Biochemicals, Kankakee, Ill. The sheep IgG was passed through a DEAE-cellulose column before being labeled. Mouse IgG was isolated from the serum of mice with an IgG myeloma by block electrophoresis. Human IgM, IgA, IgD, and the four subclasses of IgG were isolated from the sera of patients with appropriate types of myeloma or, in the case of IgM, macroglobulinemia, by block electrophoresis, DEAE-cellulose chromatography and Sephadex G200 gel filtration as previously described (13–16). Human IgE was isolated from the serum of a patient with IgE myeloma by DEAE-cellulose chromatography and Sephadex G200 gel filtration. Human albumin was obtained from Behringwerke, Marburg/Lahn, West Germany. Human ceruloplasmin was prepared from Cohn-Fraction IV of pooled human plasma with DEAE-cellulose column chromatography and precipitation by an ethanol-chloroform mixture (17). Hemocyanin was obtained from the keyhole limpet by the method of Campbell, Garvey, Cremer, and Sussdorf (18). Rabbit IgG Fc and IgG Fab fragments were prepared by the method of Porter (19).

Polyvinylpyrrolidone (PVP)[1]-^{125}I was obtained from Amersham/Searle, Des Plaines, Ill. in lots of 4 ml containing 32 mg PVP, and 1 mCi ^{125}I. The average molecular weight of the PVP was 30–40,000.

^{131}I and ^{125}I for radioiodinating proteins were obtained in the form of concentrated solutions of the sodium salt of iodine, carrier free, from, respectively; E. R. Squibb & Sons, New Brunswick, N. J., and New England Nuclear Corp., Boston, Mass.

Animals. 12–14-day old NIH Sprague-Dawley rats were used. Mothers were given water containing potassium iodide for at least 48 hr prior to experiments in which animals were sacrificed 4 hr after the administration of iodinated proteins.

Solutions containing labeled proteins. The iodine-monochloride method (20) was used to label proteins with either ^{131}I or ^{125}I. The degree of iodination of proteins approximated to a statistical average of one atom of iodine per molecule of protein. All labeled proteins and labeled PVP were dialyzed for at least 24 hr against 0.15 M saline (two changes) before use. The radioactivity in the preparations of labeled proteins, which was not precipitable by 10% trichloracetic acid (TCA), did not exceed 1.5%.

Solutions used in experiments contained either 10 mg albumin/ml or when specifically indicated different concentrations of human IgG. They also usually contained a mixture of an ^{131}I-labeled protein and an ^{125}I-labeled protein or PVP, the ratio of ^{131}I to ^{125}I radioactivity in each mixture approximating unity.

Experimental protocol. Animals were subjected to a laparotomy under ether anesthesia. Two black silk sutures were placed around the duodenum and one around the terminal ileum. The gut was divided between the duodenal ligatures and an accurately weighed quantity, approximately 0.3 ml, of a solution containing labeled proteins was injected into the duodenal lumen distal to its point of division. The abdominal wall was sutured. After the desired period of time animals were sacrificed in a chloroform jar. The small intestine, from the terminal ileal ligature to the distal duodenal ligature, was dissected free of mesentery and removed, taking care not to puncture the intestine between the two ligatures. The removed small intestine was then divided longitudinally and the lumen washed twice by agitating in two 5–10 ml lots of 0.15 M saline at 4°C. The intestine was then cut into small, approximately ½ inch long fragments, which were placed in 2–5 ml saline at 4°C and homogenized for about 5 min in a TenBroeck homogenizer. The volumes of the pooled intestinal washings and the intestinal homogenate were measured. The eviscerated carcass was added to 200 ml distilled water and homogenized for about 3 min in a Waring-Blender.

The ^{125}I and ^{131}I radioactivity contents of the following experimental samples were determined: (*a*) portions of the intestinal homogenate, intestinal washings and carcass homogenate, and the administered solution, and (*b*) the supernatants of the same portions as in *a* after precipitation of proteins by 10% trichloracetic acid. Values for protein bound radioactivity in the intestinal wall, intestinal washings, and carcass were obtained by subtracting values for non-protein-bound radioactivity from the total radioactivity in each portion.

In these studies transport is defined as the proportion of the administered dose of labeled protein transferred from the intestinal lumen to the circulation over a particular time interval. Protein-bound radioactivity in the carcass as a percentage of the administered dose was taken as the experimental estimate of transport. This is a minimum value as a small proportion of the transported protein would have been catabolized. To determine the maximum amount of transported protein that could have been catabolized (i.e. that amount catabolized if transport from the lumen to the carcass were instantaneous) we determined the 4 hr survival of intravenously administered protein as de-

[1] *Abbreviations used in this paper:* PVP, polyvinylpyrrolidone; TCA, trichloracetic acid.

TABLE I

Intestinal Transport of Radio

	45 min*		240 min						
								Human	
	Human IgG	PVP	Human IgG	Rat IgG	Mouse IgG	Rabbit IgG	Sheep IgG	G1	G2
Number of estimates	11	4	12	4	4	4	6	4	3
Mean	4.9	0.2	29.8	35.1	21.3	32.3	6.6	20.8	32.2
SEM	1.4	0.0	2.4	2.9	2.2	2.7	0.9	3.4	2.4

* Time between intraduodenal administration of radioactivity and the time the animals were sacrificed.

scribed previously by Wochner, Strober, and Waldmann (21). In brief this was done by determining the fraction of the intravenous dose of radiolabeled protein that remained protein bound (i.e. 10% TCA precipitable) in a portion of whole homogenized animal sacrificed at 4 hr. The fraction of the initial radioactivity remaining protein bound was considered to be the fraction of the injected protein not yet metabolized. Less than 15% of each of the various proteins used in this study, with the exception of IgE and IgG Fab piece, were catabolized in the 4 hr study period. 25% of the IgE and 54% of the IgG Fab piece were catabolized in the 4 hr study period.

The effect of the addition of unlabeled IgG on the transport of labeled IgG was studied in groups of 12-day-old rats. In these studies the percentage of intraduodenally administered IgG transported by 4 hr was determined after administration of radiolabeled IgG either without or with unlabeled IgG in varying concentrations. The absolute quantity of IgG transported in 4 hr was also determined from the product of the fraction transported and the quantity of IgG in mg introduced into the duodenum.

In these studies uptake of proteins is defined as the proportion of the administered dose of labeled protein taken up by the intestinal mucosa over a particular time interval. Protein-bound radioactivity in the carcass plus that in the intestinal wall as a percentage of the administered dose was taken as the experimental estimate of uptake. This is a minimum value as a proportion of that taken up by the intestinal wall and of that transported would have been catabolized by the time the animals were sacrificed.

Sucrose gradients. To determine whether the protein label becomes associated with complexes having a higher molecular weight than that in the starting material, portions of experimental samples were subjected to sucrose gradient ultracentrifugation analysis. 0.3 ml portions of the intestinal wall homogenate, intestinal washings, eviscerated carcass homogenate, and starting solution were applied to the top of linear 10–45% sucrose gradients, made up in cellulose nitrate centrifuge tubes. The gradients were then subjected to 90,000 *g* for 18 hr in a SW 50.1 rotor in a Spinco ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.). After centrifugation the bottom of each tube was punctured and successive portions of the sucrose solution, each of 10 drops, were collected together with 3 ml of saline in separate tubes for radioactivity counting.

Subcellular fractionation. Analysis of the subcellular distribution of labeled protein was carried out to determine if any high molecular weight protein complexes observed represented protein bound to a carrier protein, or to a membrane receptor. Small bowel obtained 90 min after intraduodenal administration of labeled protein was subjected to homogenization as described above. The homogenate was centrifuged at 300 *g* for 100 min. The crude "nuclear" pellet was washed twice with cold saline. The supernatant from the initial centrifugation of the homogenate and the initial wash of the "nuclear" pellet were pooled and centrifuged at 106,000 *g* in a preparative ultracentrifuge for 30 min. The supernatant was removed and retained as the cell sap fraction. The remaining pellet was washed twice with 3 ml of cold saline and was termed the "membrane and organelle" fraction. The protein-bound radioactivity content and the distribution of radioactivity on sucrose ultracentrifugation gradients of the cell sap, and "membrane and organelle" fraction were determined. Also the ability of the protein label in the cell sap and "membrane and organelle" fraction to pass through a 0.45 μ Millipore filter was determined.

In addition purified small intestinal brush borders and purified small intestinal microvillous membranes were prepared from the small intestine of a group of 25 12-day old rats. The small intestine was obtained from rats 45 min after intraduodenal administration of ^{125}I-labeled human IgG. The procedure used to purify the brush borders and intestinal microvillous membranes was identical to that used by Forstner, Sabesin, and Isselbacher (22) with the exception that all EDTA buffers were adjusted to pH 6.5 rather than the pH 7.4 used by these workers. The more acid pH was used since it was shown [2] that the binding of radiolabeled IgG to membranes was critically pH dependent. 85% of the activity bound to membranes at pH 6.5 was released on incubation at pH 7.4, and 100% of the activity was released on incubation at pH 8.0 using tris (tris [hydroxymethyl] methylamine HCl), phosphate, or barbital buffers.

Counting of radioactivity. All experimental samples were counted in the well of a two channel autogamma counter. The gains and windows of the two channels were set for counting ^{131}I and ^{125}I radioactivity. Virtually no ^{125}I counts appeared in the ^{131}I channel but about 7% of the ^{131}I counts appeared in the ^{125}I channel. The ^{125}I radioactivity content of a sample was determined by subtracting the counts due to ^{131}I from the total counts in the ^{125}I channel.

[2] Waldmann, T. A., E. A. Jones, R. Bruce, S. Balestra, and M. C. Jost. Unpublished observations.

iodinated Proteins and PVP

240 min									
Human		Human	Human	Human	Human	Human trans-	Human cerulo-	Human	
G3	G4	IgM	IgA	IgD	IgE	ferrin	plasmin	albumin	PVP
10	8	4	4	4	4	6	6	7	8
9.9	36.3	1.5	3.6	4.9	3.0	2.1	3.7	2.5	2.3
1.7	5.3	0.3	1.4	0.8	0.5	0.7	1.1	0.6	0.6

RESULTS

Transport. Estimates of transport of different proteins and PVP are shown in Table I. There was little transport of human IgG by 45 min. However, the mean values for human, rat, mouse, and rabbit IgG and all four subclasses of human IgG were all appreciably greater than 5% of the administered dose at 4 hr, whereas the corresponding values for human IgM, IgA, IgD, IgE, transferrin, ceruloplasmin, and albumin and PVP were less than 5% at this time. The transport of rabbit IgG Fc piece was considerable (12.6%) whereas that of IgG Fab piece was minimal (1.7%).[3] The transport of rat, mouse, rabbit, and human IgG was significantly greater than that of sheep IgG ($P < 0.01$), confirming the species specificity of this process in rats found by others (1, 6, 12). The transport of human IgG-^{131}I at 4 hr was significantly reduced by the presence of unlabeled human IgG ($P < 0.001$) (Table II, Fig. 1). The percentage of the intraduodenally administered IgG transported by 4 hr was reduced from 29.8% in animals receiving human labeled IgG (less than 0.01 mg IgG in administered dose) without excess unlabeled IgG to 1.4% in animals receiving the radiolabeled IgG in the presence of 8 mg of unlabeled human IgG. The absolute quantity of IgG transported in 4 hr increased with increasing IgG concentrations approaching a limit of 0.12 mg when 1 mg of IgG was administered along with the radiolabeled IgG. Further increases in the quantity of unlabeled IgG administered did not result in increases in the absolute quantity of IgG transported. These data suggest that a major process involved in IgG transport is saturable. However they do not exclude the possibility that IgG is also transported by a minor process shared with other proteins and macromolecules that is nonspecific and not inhibitable by unlabeled IgG molecules.

Uptake. Estimates of uptake of different proteins and PVP are shown in Table III. The mean uptake of human IgG at 45 min was 50.7% of the administered dose whereas the mean uptake of PVP was only 4.3% at this time. The mean uptakes of human, rat, mouse, rabbit, and sheep IgG and all four subclasses of human IgG were all appreciable at 4 hr (19–54% of the administered dose) and were all significantly greater than

[3] It should be noted that 54% of an intravenously administered dose of radioiodinated IgG Fab piece was catabolized in the 4 hr study period. Thus, the 1.7% of the administered dose of Fab piece in the carcass as precipitable counts at 4 hr in the transport study could correspond to the transport of up to 3.7% of the administered dose.

TABLE II

Effect of Excess Unlabeled IgG on Intestinal Uptake and Transport of Radioiodinated IgG and PVP*

	Uptake						Transport					
	45 min		240 min				45 min		240 min			
	Human IgG	Human IgG and excess unlabeled IgG	Human IgG	Human IgG and excess unlabeled IgG	PVP	PVP and excess unlabeled IgG	Human IgG	Human IgG and excess unlabeled IgG	Human IgG	Human IgG and excess unlabeled IgG	PVP	PVP and excess unlabeled IgG
Number of estimates	11	6	11	6	8	8	11	5	12	20	8	8
Mean	50.7	17.1	48.6	25.9	22.1	30.7	4.9	2.4	29.8	2.8	2.3	1.1
SEM	3.1	3.0	2.2	2.4	7.2	5.9	1.4	1.0	2.4	1.0	0.6	0.2
P	<0.001		<0.001		>0.15		>0.3		<0.001		>0.025	

* The radioiodinated IgG or PVP was administered intraduodenally in 0.3 ml of 16 mg/ml IgG for those animals receiving excess unlabeled IgG.

TABLE III
Intestinal Uptake of Radio

	45 min		240 min						
	Human IgG	PVP	Human IgG	Rat IgG	Mouse IgG	Rabbit IgG	Sheep IgG	Human G1	Human G2
Number of estimates	11	4	11	4	4	4	6	4	4
Mean	50.7	4.3	48.6	49.0	40.7	54.4	20.6	37.1	45.9
SEM	3.1	1.1	2.2	2.6	3.6	4.2	1.5	4.4	2.4

the mean uptakes of human IgM, IgA, IgD, IgE, transferrin, ceruloplasmin, and albumin at this time (<9% of the administered dose). The percentage of the administered dose that remained as protein-bound radioactivity in the intestinal lumen was 79.5% for IgM, 71.9% for IgA, and 67.2% for IgE, whereas it was only 18.4% for IgG. These results imply that the uptake process for IgG is specific. There was appreciable uptake of Fc piece (53.8%) whereas the uptake of Fab piece was small (8.5%). Uptakes of human, rabbit, rat, and mouse IgG were also significantly greater than that of sheep IgG at 4 hr ($P < 0.01$). Uptake of human IgG-^{131}I was significantly inhibited by the presence of unlabeled IgG at both 45 min (reduced from 50.7% in controls to 17.1% in presence of unlabeled IgG, $P < 0.001$) and 4 hr (Table II). In addition 46.1% of the administered dose of human IgG was retained as protein-bound radioactivity in the intestinal lumen in animals receiving the radiolabeled IgG together with unlabeled IgG (50 mg/ml) whereas the corresponding figure was only 18.4% in animals receiving radiolabeled IgG without excess unlabeled IgG. Thus the uptake of labeled IgG is at least partially saturable by unlabeled IgG.

IgG binding during transport. Portions of experimental samples obtained after administering labeled human IgG intraduodenally were subjected to preparative sucrose gradient ultracentrifugation. An appreciable proportion of the labeled IgG in intestinal wall homogenates migrated more rapidly in the sucrose gra-

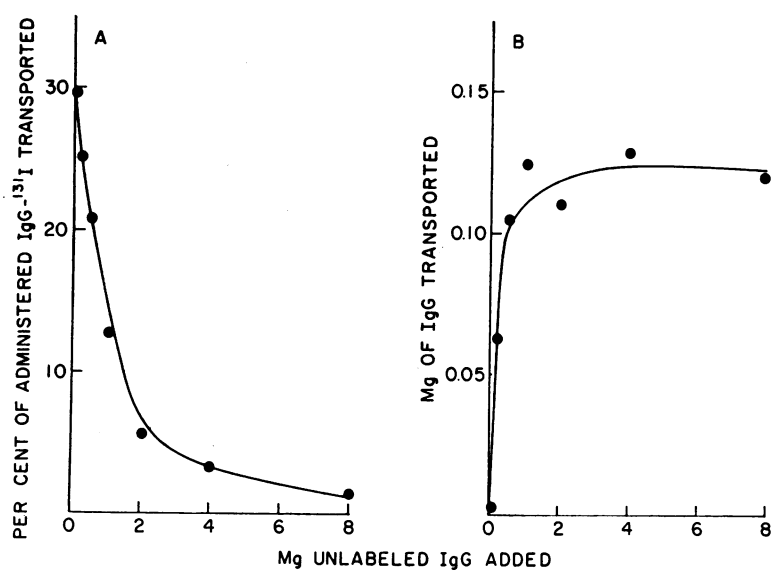

FIGURE 1 The transport of intraduodenally administered IgG in the 12 day old rat. Each point represents the mean of the results obtained in four rats. Panel *A*: The percentage of the intraduodenally administered labeled human IgG transported by 4 hr was reduced in the presence of increasing quantities of unlabeled human IgG. Panel *B*: The absolute quantity of IgG transported during the 4 hr study period increased to a limit of 0.12 mg when 1 mg of unlabeled IgG was administered. Further increases in the quantity of unlabeled IgG administered did not result in increases in the absolute quantity of IgG transported. These data suggest that a major process involved in the transport of IgG is saturable.

iodinated Proteins and PVP

240 min								
Human		Human	Human	Human	Human	Human trans-	Human cerulo-	Human
G3	G4	IgM	IgA	IgD	IgE	ferrin	plasmin	albumin
10	8	4	4	4	4	6	6	7
19.3	51.6	8.3	6.8	7.1	8.1	5.0	6.0	3.3
2.6	3.2	2.2	1.2	0.9	1.5	1.0	1.0	0.6

dient than did the administered 7S molecules (Fig. 2). This observation suggests that there was complexing of the labeled IgG with other proteins or with cell fragments in the homogenates. A similar pattern was also given by labeled sheep IgG, but the degree of complexing was relatively less for sheep IgG than human IgG. All four subclasses of human IgG showed evidence of complexing in intestinal homogenates. IgG1 and IgG2 tended to give relatively more striking evidence of complexing than IgG3 and IgG4 with the particular labeled preparations of the subclasses used. Complexing was also observed after administering labeled rabbit Fc piece (Fig. 3). Evidence of complexing was not observed in intestinal homogenates after the administration of PVP-^{125}I (Fig. 4), even though uptake of PVP-^{125}I by some of the intestinal homogenates subjected to sucrose gradient analysis was appreciable. Also complexing was not observed after administering labeled human IgA, IgM, IgE, albumin, transferrin, and ceruloplasmin and rabbit Fab piece. Thus, those proteins which showed evidence of complexing were also those which showed marked intestinal uptake and transport. Simultaneous sucrose gradients of intestinal washings, intestinal wall and plasma, 2 hr after the administration of labeled human IgG showed that complexing of labeled IgG occurred only in the intestinal wall (Fig. 5). Complexing of labeled IgG could be markedly inhibited by administering excess unlabeled IgG along with the labeled preparation (Fig. 6).

Evidence consistent with complexing was also observed when intestinal homogenates, crude intestinal membrane preparations, or purified microvillus membranes were incubated in vitro with labeled human IgG (Fig. 7). The complexing of radiolabeled IgG with membrane preparations was completely inhibited if unlabeled IgG (16 mg/ml) was included in the incubation mixture. In addition no evidence of complexing was observed when rat liver membranes were similarly incubated with labeled IgG in vitro (Fig. 7).

Analysis of the subcellular distribution of labeled IgG in intestinal wall homogenates was carried out to determine if the high molecular weight complexes observed represented IgG bound to a carrier protein or to a membrane receptor. From 65 to 92% of the IgG label in homogenates was associated with fractions containing membranes and organelles. Virtually all of the radioactivity in the "membrane and organelle" fractions migrated as high molecular weight complexes on sucrose ultracentrifugation gradients (Fig. 8). The small amount of IgG label found in cell sap fractions showed a distribution comparable to that of 7S molecules in the starting solution with no evidence of complexing (Fig. 8). In an effort to identify the nature of the cellular material complexing with IgG, small intestinal microvillous membranes were purified after the intraduodenal

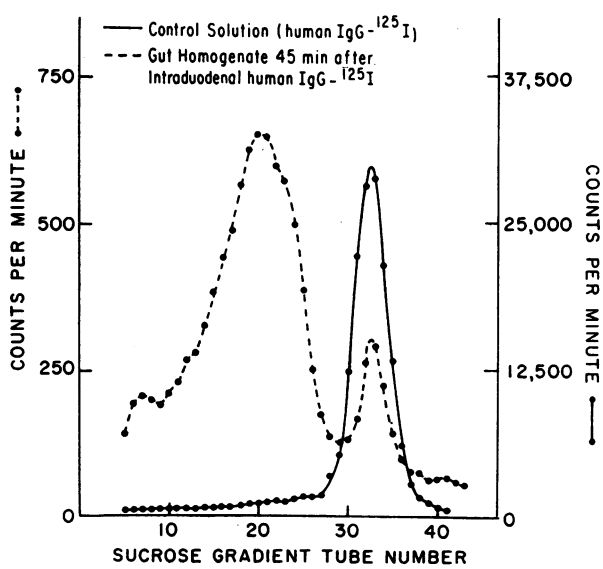

FIGURE 2 Distribution of the label of human IgG in a linear 10–45% sucrose ultracentrifugation gradient of an homogenate of the small intestine obtained 45 min after the intraduodenal administration of labeled human IgG. Sedimentation is from right to left. A large amount of the label in the homogenate is associated with higher molecular weight complexes than the 7S molecules in the starting solution.

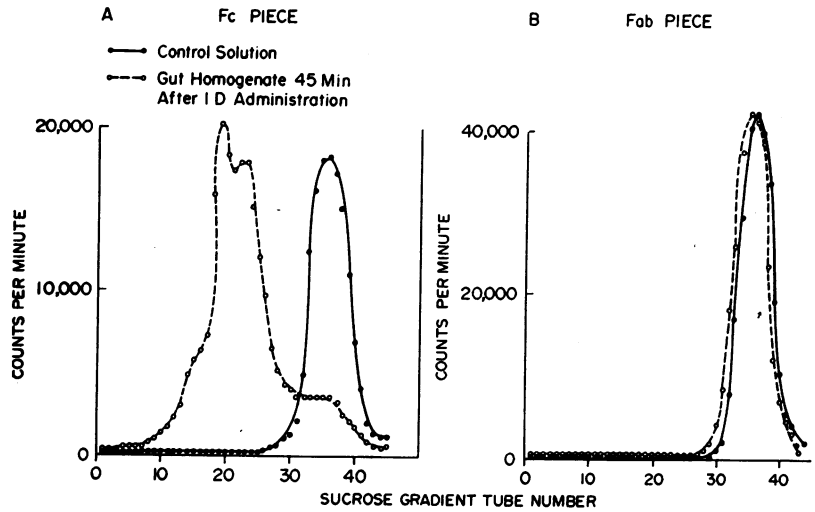

FIGURE 3 Sucrose ultracentrifugation gradients of homogenates of small intestine 45 min after the intraduodenal (ID) administration of labeled rabbit IgG Fc piece and Fab piece. (*A*) In contrast to the starting (control) solution there is a well-defined peak of radioactivity associated with higher molecular weight complexes in the intestinal homogenates after giving labeled Fc piece. (*B*) In contrast the distribution of the label of Fab piece in both the intestinal homogenate and the starting solution was similar.

administration of radioiodinated IgG to 12-day old rats. Significant quantities of the label of IgG were demonstrated to be bound to these purified enterocyte microvillous membranes. All of the label of IgG associated with microvillous membranes migrated as high molecular weight complexes. The IgG label associated with "membrane and organelle" fractions and with purified microvillous membranes did not pass through a 0.45 μ Millipore filter, whereas over 97% of the IgG label in cell sap passed through the filter. In contrast to labeled human IgG in intestinal homogenates, more than 90% of labeled hemocyanin and labeled human IgM was found in cell sap fractions, and over 96% of these proteins with molecular weights of 800,000 or greater

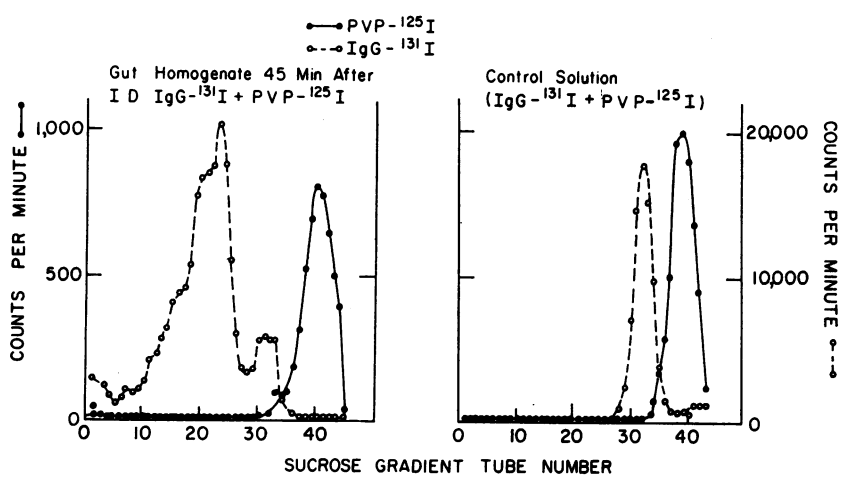

FIGURE 4 Sucrose ultracentrifugation gradient patterns in an homogenate of small intestine 45 min after administering a mixture of labeled human IgG and PVP intraduodenally (ID). The different positions of the single discrete peaks given by both labeled substances in the starting solution (right panel) reflect the different mean molecular weights of IgG and PVP. In the homogenate appreciable IgG label is associated with complexes having higher molecular weights than 7S molecules. The distribution of the PVP label in the homogenate is similar to that in the control solution.

passed through a 0.45 μ Millipore filter. Finally a number of studies were carried out to confirm that the radioactivity bound to purified membranes did represent labeled IgG. 85% of the radiolabel associated with purified membrane preparations, that were isolated after intraduodenal administration of radiolabeled IgG was precipitable with an antihuman IgG antiserum made in sheep. As indicated above it was shown that the binding of the label of IgG to membranes was critically dependent on the pH of the media. When high molecular weight complexes containing the label of IgG, obtained either in vivo from intestinal homogenates subjected to sucrose gradient analysis or in vitro from purified enterocyte microvillous membranes, were incubated with pH 8.0 tris buffer all of the radiolabel was released from the complexes. All of this released label showed a distribution comparable to the 7S starting material on sucrose ultracentrifugation gradients and 95% of it was precipitable with antihuman IgG antibodies. These studies support the conclusion that the high molecular weight labeled complexes do represent labeled IgG bound to a membrane receptor rather than to a soluble carrier protein.

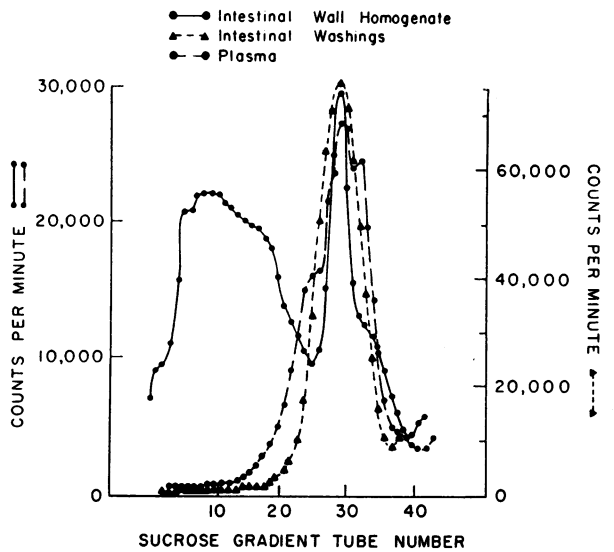

FIGURE 5 Simultaneous sucrose ultracentrifugation gradient patterns of the intestinal washings, intestinal wall homogenate, and plasma 2 hr after the intraduodenal (ID) injection of labeled human IgGl. Evidence of complexing of the labeled IgGl was only found in the intestinal wall homogenate.

DISCUSSION

The present studies confirm that the transmission of immunoglobulins across the intestinal mucosa of neonatal rats is indeed a valuable model for studying transcellular protein transport. They provide data on the mechanisms involved in the intestinal uptake and transport of IgG in the neonatal rat. In addition they indi-

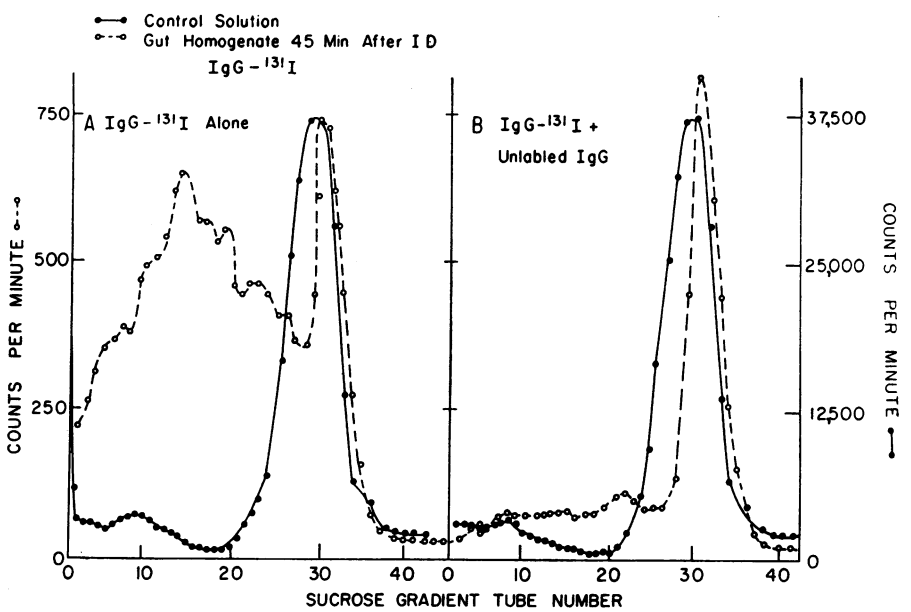

FIGURE 6 Sucrose ultracentrifugation gradient patterns of small intestinal homogenates 45 min after the intraduodenal (ID) administration of (*A*) labeled human IgG alone and (*B*) labeled human IgG with excess unlabeled IgG. There is evidence of appreciable complexing after giving labeled IgG alone. However when excess unlabeled IgG was present, the distribution of the label in the homogenate was similar to that in the starting solution.

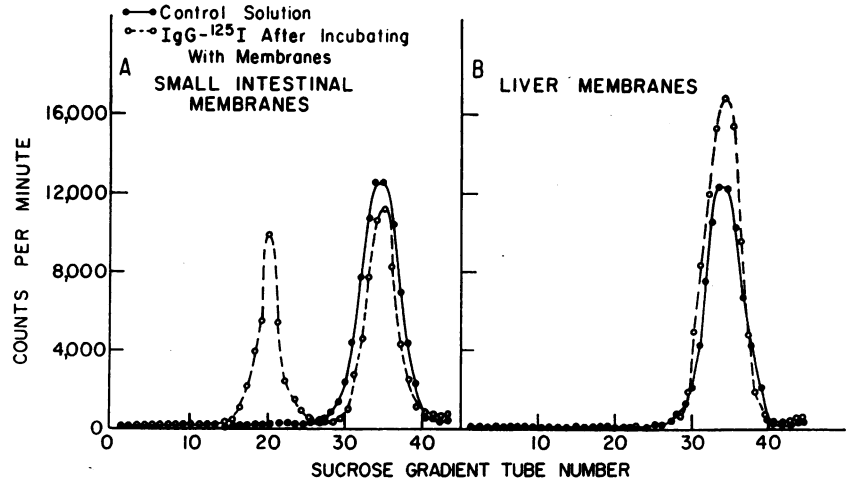

FIGURE 7 Sucrose ultracentrifugation patterns of labeled IgG after incubation in vitro for 3 hr with (*A*) small intestinal membranes and (*B*) liver membranes from a 14 day old rat. Evidence for the association of the IgG label with higher molecular weight complexes was present with the intestinal membranes but not with the liver membranes.

cate which of several classes of human proteins are involved in this transport process. Our results are consistent with and extend the data obtained in earlier studies (1, 2, 4–10, 23–25) by demonstrating the highly selective nature of intestinal transport of proteins in the neonatal rat. They indicate that appreciable quantities of rat IgG, mouse IgG, rabbit IgG, and all four subclasses of human IgG are selectively transported to the animal. In contrast there is little or no transport of any of the other proteins studied, including the other four immunoglobulin classes. It is of particular interest to note that IgA, which in some species occurs in colostrum and milk at concentrations that are much higher than those of the other immunoglobulins (26), was not selectively transported.

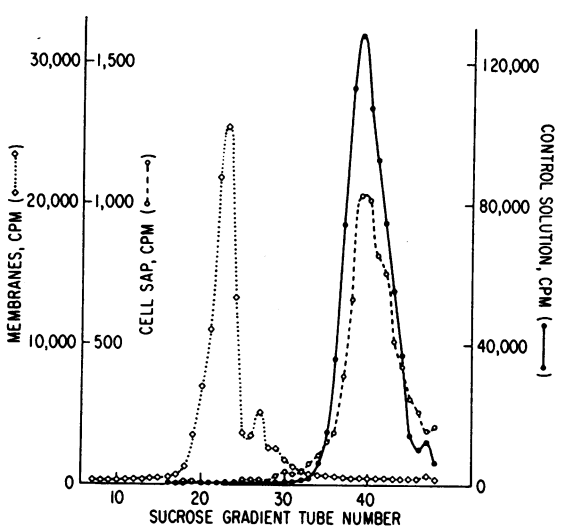

FIGURE 8 Sucrose ultracentrifugation gradient patterns of subcellular fractions of an intestinal homogenate 45 min after the intraduodenal administration of labeled human IgG. Evidence for the association of the IgG label with higher molecular weight complexes was present in the fraction containing microvillous membranes but not the fraction containing cell sap.

The studies utilizing isolated IgG fragments indicate that the selective transport of IgG is dependent on the Fc, but not the Fab region of the molecule, a conclusion consistent with the data of Brambell, Hemmings, Oakley, and Porter (27) and Kaplan, Catsoulis, and Franklin (28) in the rabbit and Morris (29) in the mouse. In contrast Gitlin, Kumate, Urrusti, and Morales (30) conclude that both the Fab and the Fc piece are transported across the human placenta at a similar rate.

The data of Gitlin and Koch (31) suggest that maternofetal transport of human IgG in the mouse may be mediated by two phenomena, one a first order process and the other consistent with a carrier or enzymatic process. It has been previously shown that the transference of antibodies to the neonatal rat could be inhibited by human serum or by a fraction of serum containing gammaglobulins obtained by electrophoresis (6, 12). Our data extend these observations by demonstrating that there is a marked inhibition of intestinal transport of labeled IgG by purified unlabeled IgG thus indicating that a major process involved in IgG transport is saturable.

The studies reported in this paper provide for the first time quantitative data on the process of uptake of IgG by the intestinal mucosa. It has been assumed that the initial intestinal uptake of proteins, including

immunoglobulins, was by a nonspecific process. This assumption was based on morphological, immunofluorescent, and isotopic studies on the uptake of various macromolecules and colloids by entodermal cells of the yolk sac or neonatal rodent intestine (3, 32–37). In none of these studies was a quantitative comparison of the uptake of different proteins or other substances made. In the light of the present study, in which the uptake of different proteins was quantitated, it is apparent that there is some nonspecific uptake of all the proteins studied and of PVP. However, the intestinal uptake of IgG appeared to involve a specific process in addition, since intestinal uptake of this particular labeled protein was not only significantly greater than that of other protein classes, but also significantly inhibited in the presence of unlabeled IgG. All subclasses of human IgG, rat IgG, mouse IgG, and to a lesser extent sheep IgG participated in this process. This was so not only when estimates of uptake as defined above were considered but also when figures for protein-bound radioactivity in the intestinal wall alone or protein-bound radioactivity of IgG, IgM, IgA, and IgE retained in the intestinal lumen were considered. In addition, the data obtained with isolated IgG fragments indicate that, like transport, the selective uptake of IgG is dependent on the Fc, but not the Fab region of the molecule.

The studies of the migration of the radioiodinated molecules on sucrose ultracentrifugation gradients indicate that IgG molecules form complexes in the intestinal wall, which have higher molecular weights than 7S molecules. This complexing of IgG molecules does not occur in the intestinal lumen, but only after these molecules are taken up by the intestinal mucosa. After transport the labeled IgG molecules in the plasma are no longer associated with complexes but again migrate as 7S molecules. The observed complexing could be due to combination of IgG molecules with specific membrane receptors, with a selective transport protein, or with some other compound unrelated to both uptake and transport. Against the last of these possibilities is the observation that the proteins which apparently form complexes are the same as those participating in specific uptake and transport. In addition, all three of the processes studied, uptake, transport, and complexing of labeled IgG molecules, are at least partially saturable by unlabeled IgG molecules, suggesting competition of labeled and unlabeled IgG molecules for a limited number of receptors. In favor of the binding of IgG to a receptor on a cell membrane and against binding to a soluble transport protein is the finding that the IgG label associated with high molecular weight complexes was found in a purified enterocyte microvillous membrane fraction and in a fraction of intestinal homogenates containing membranes and organelles but not in the corresponding cell sap fraction. In addition, the labeled IgG associated with purified microvillous membranes and the "membrane and organelle" fraction was retained by a 0.45 μ Millipore filter, whereas less than 4% of such large proteins as IgM and hemocyanin were retained by the filter. Finally binding of labeled IgG to purified microvillous membranes was demonstrated in in vitro incubation studies.

It follows that our data are consistent with a modification of Brambell's hypothesis (6, 11, 12). It is proposed that there are specific receptors for IgG on the microvillous membrane of the cell surface of the enterocyte of the neonatal rat to which IgG molecules become attached before the formation of pinocytotic vacuoles. The attachment of IgG molecules to cell receptors in this way could prevent the degradation of IgG molecules by the contents of both the intestine and pinocytotic vacuoles. After transcellular movement the pinocytotic vacuoles probably release the IgG molecules into the villous lacteals (3) or into the intercellular spaces.

The process of specific transport of IgG molecules across the intestinal mucosa of the newborn rat may be profitably related to other phenomena also mediated by the Fc region of the IgG molecule. Certain well recognized non-antigen combining activities of human IgG such as complement fixation, passive cutaneous sensitization, binding to monocytes, and the concentration-catabolism effect are also known to be mediated by the Fc region of the IgG molecule. However, it is apparent that not all of these various phenomena are mediated by all four subclasses of human IgG. It has been demonstrated that there are specific receptor sites for only IgG1 and IgG3 molecules on human monocytes (38). Passive cutaneous sensitization in the guinea pig is mediated by IgG1 and IgG3 and to a lesser extent IgG4 (39, 40). In addition, IgG1, IgG3, and to a lesser extent IgG2 fix complement (41). Since all four subclasses of IgG participate in selective transport in the neonatal rat, this process probably depends on a different submolecular structure of the Fc piece than those involved in these other processes.

One process that shares many characteristics with the intestinal transport of IgG in the neonatal rat is the concentration-catabolism relationship which affects the metabolism of the IgG molecules. The concentration-catabolism effect refers to the direct relationship between the fractional catabolic rate for IgG molecules and the serum concentration of IgG in both man and rodents such as the mouse and rat (42, 43). That is, as the concentration of IgG rises by endogenous production or by infusion, the fractional catabolic rate increases (or alternatively the survival $t_{\frac{1}{2}}$ decreases) until a limiting concentration is

reached. This phenomenon is unique to IgG among the immunoglobulins (43). Both the concentration-catabolism effect and intestinal transport in the neonatal rat show IgG specificity (43), involve all four subclasses of human IgG (16), are mediated through the Fc portion of the IgG molecule (42, 43), and have similar species specificities. In the mouse and rat, human IgG and rodent IgG are equally effective in both processes; sheep IgG is significantly less effective; whereas the other immunoglobulin classes are not effective at all (1, 5, 36, 42–46). However, appreciable kinetic differences between IgG catabolism and maternofetal transmission of IgG have been found in the mouse (47).

A mechanism similar to that proposed to explain the selective transport of IgG across the newborn rodent gut, that is a saturable protective system specific for IgG molecules, is also the most attractive hypothesis that has so far been suggested to account for the concentration-catabolism effect observed with IgG. In this model (12, 43, 48) a fraction of the plasma IgG is isolated from the circulating protein pool into a catabolic pool. It was proposed that some of the IgG molecules become specifically attached to a limited number of protective receptors, perhaps on the walls of pinocytotic vacuoles. These molecules would be ultimately returned to the circulation whereas all remaining IgG molecules would be degraded. At a low serum concentration most isolated IgG molecules would be protected and returned to the circulation, producing a long survival of the protein, whereas at a high serum concentration the converse would be true. Studies in adult germfree mice involving the intravenous injection of labelad IgG and techniques comparable to the ones used in the present study have provided evidence for the formation of high molecular weight IgG complexes consistent with membrane binding of IgG in the eviscerated carcass of this species.[4] Thus, it would appear that a major process involved in both IgG transport across the intestine of the neonatal rat and the IgG concentration-catabolism effect may well involve competition for a limited number of saturable membrane receptors which are specific for IgG and protect this molecule from catabolism.

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