JCI The Journal of Clinical Investigation

Protein Binding by Specific Receptors on Human Placenta, Murine Placenta, and Suckling Murine Intestine in Relation to Protein Transport across These Tissues

Jonathan D. Gitlin, David Gitlin

J Clin Invest. 1974;54(5):1155-1166. https://doi.org/10.1172/JCI107859.

Research Article

Human, rat, and mouse placentas and rat and mouse intestines were homogenized in buffered saline, and fraction consisting primarily of cell membranes was separated from each of the homogenates by differential centrifugation. Human, bovine, and guinea pig IgG, and human IgE, Bence-Jones protein, serum albumin, insulin, and growth hormone were labeled with ^{131}I or ^{125}I, and the binding of these proteins by the cell membrane fractions was investigated. Rat and mouse sucklings were given labeled proteins intragastrically, and the amount of each protein absorbed after a given interval of time was determined. It was found that the degree and specificity of protein binding by the cell membrane fractions from human and murine placentas strikingly paralleled the relative rate and specificity of protein transport from mother to fetus in the respective species at or near term. Similarly, the degree and specificity of protein binding by the cell membrane fractions from suckling rat and mouse intestines tended to parallel the rate and specificity of protein absorption from the gastrointestinal tract in these animals. However, some discordance between protein binding and protein transport was also observed. The data suggest that: (*a*) the binding of a protein by specific receptors on cell membranes may be a necessary first step in the transcellular transport of the protein; (*b*) specific protein binding by cell receptors does […]

Protein Binding by Specific Receptors on Human Placenta, Murine Placenta, and Suckling Murine Intestine in Relation to Protein Transport across These Tissues

JONATHAN D. GITLIN and DAVID GITLIN

From the Department of Pediatrics, University of Pittsburgh School of Medicine and the Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania 15213

ABSTRACT Human, rat, and mouse placentas and rat and mouse intestines were homogenized in buffered saline, and a fraction consisting primarily of cell membranes was separated from each of the homogenates by differential centrifugation. Human, bovine, and guinea pig IgG, and human IgE, Bence-Jones protein, serum albumin, insulin, and growth hormone were labeled with ^{131}I or ^{125}I, and the binding of these proteins by the cell membrane fractions was investigated. Rat and mouse sucklings were given labeled proteins intragastrically, and the amount of each protein absorbed after a given interval of time was determined. It was found that the degree and specificity of protein binding by the cell membrane fractions from human and murine placentas strikingly paralleled the relative rate and specificity of protein transport from mother to fetus in the respective species at or near term. Similarly, the degree and specificity of protein binding by the cell membrane fractions from suckling rat and mouse intestines tended to parallel the rate and specificity of protein absorption from the gastrointestinal tract in these animals. However, some discordance between protein binding and protein transport was also observed. The data suggest that: (*a*) the binding of a protein by specific receptors on cell membranes may be a necessary first step in the transcellular transport of the protein; (*b*) specific protein binding by cell receptors does not ensure the transport of that protein across the tissue barrier; and (*c*) specific transport mechanisms other than or in addition to specific cell membrane receptors are involved in the active transport of proteins across the human or murine placenta or the suckling murine intestine.

The work was presented in part before the American Pediatric Society, San Francisco, Calif., May 18, 1973.

Received for publication 28 February 1974 and in revised form 18 June 1974.

INTRODUCTION

The survival of most, if not all, mammalian species is dependent in part upon the successful transfer of maternal IgG from the mother to her offspring. This transference may occur either prenatally via the placenta as in primates (1–3), both prenatally via the placenta and postnatally during the neonatal period via the intestinal tract (4, 5) as in the rat and mouse, or primarily postnatally as in the pig and cow (6, 7). Although other maternal plasma proteins such as serum albumin and transferrin also traverse the primate and murine placentas and the proximal intestine of the murine suckling, these proteins do so much less readily than does IgG. Brambell (8) has suggested that the selective passage of IgG across these tissue barriers may be attributable to a preferential binding of IgG to hypothetical receptors present on the inner surface of the membrane which limits the pinocytotic vesicle: proteins in the external environment are brought into the cell by pinocytosis, and some are then presumed to be bound selectively by these receptors. The hypothesis requires that the receptors protect bound protein from intracellular digestion, whereas unbound protein is catabolized. Thus, proteins which are membrane bound would survive transit through the cell, and the amount of specific protein transferred across the tissue would be proportional to the degree of binding. Brambell (8) considered that the pinocytotic membrane might be either an extension or a derivative of the cell membrane and suggested that such receptors might also be present on the membrane of the microvilli. This concept of protein transport has recently received strong support from the observation by Jones and Waldmann (9) that cell membranes prepared from the intestines of suckling rats do indeed bind IgG preferentially over other plasma proteins.

In the study reported here, the selectivity of protein binding in vitro by cell membrane fractions prepared from human, rat, and mouse placentas and from suckling rat and mouse proximal (10, 11) small intestine was compared to the transport of the same or related proteins across these tissues in vivo.

METHODS

Tissues and cell membrane fractions. Fractions rich in cell membranes were prepared from homogenates of the following tissues: human placentas and fetal membranes obtained at term by hysterotokotomy, human placentas obtained at 7–8 wk of gestation by vaginal dilatation and forceps extraction, erythrocytes from venous blood of normal pregnant women at term, rat and mouse placentas and fetal membranes obtained at or near term, the liver and the proximal third of the small intestines from suckling rats 6–9 days of age and from suckling mice 5–8 days of age and 19 days of age, and the proximal third of the small intestines from adult mice and rats. The rats and mice were albino strains, the rats being descendants of a Wistar strain and the mice of a Swiss-Webster strain.

The cell membrane fractions were obtained by differential centrifugation using a modification of the method of Jones and Waldmann (9). The tissues were homogenized in a cold 5 mM EDTA at pH 6.5 with a Waring blender, using 10–20 ml of buffer per g wet weight of tissue. The homogenate was centrifuged at 500 *g* for 10 min at 2°C, and the supernate was carefully collected, avoiding both the uppermost layer and the sediment. The supernate was again centrifuged at 500 *g* for 10 min, collected, and then centrifuged at 60,000 *g* for 20–30 min at 2°C in polyallomer tubes using the fixed angle, type 40 rotor of a Spinco Model L preparative ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The sediment or pellet obtained in each tube represented from 0.5 to 0.7 g of wet tissue; the pellets were washed twice with cold 0.1 M NaCl in 5 mM EDTA buffer of pH 6.5 at 60,000 *g* for 20 min. The washed pellets appeared to consist almost entirely of cell membranes when examined with the electron microscope. However, since small amounts of debris from other cell structures were also observed, this fraction will be referred to as the "60,000-*g* fraction."

Only the villous portion of the placenta was used for the human placental homogenates. The whole placenta stripped of fetal membranes was used for the rat and mouse placental homogenates. A given homogenate of human placenta or fetal membranes contained the tissue from a single conceptus, whereas a given homogenate of murine placenta or fetal membranes represented the pooled tissue from 20 to 36 conceptuses. A given homogenate of murine intestines or liver contained the respective pooled tissue from 22 to 79 sucklings or from 3 to 6 adults. A given human erythrocyte homogenate represented the cells from a single individual.

Radiolabeled proteins. Crystallized human albumin (HA) [1] was obtained from pooled adult plasma by the low temperature ethanol-water fractionation method of Cohn, Hughes, and Weare (12). With columns of DEAE-cellulose (13), Bence-Jones protein (BJλ) consisting of light chains (λ-chains) was isolated from the urine of a myeloma patient; the amount of human IgG and/or γ-chain present in the preparation expressed as IgG was 0.1% as determined by radial immunodiffusion (14) using a rabbit antiserum specific for human γ-chain and using purified human IgG as the reference standard. With columns of DEAE-cellulose (13), human, bovine, and guinea pig IgG (H-IgG, B-IgG, and GP-IgG, respectively) were isolated from the appropriate normal serum. Human IgE (H-IgE) was separated from the plasma of a patient with IgE myeloma (15) using Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) (16) followed by zone electrophoresis of the partially purified IgE preparation on Geon (B. F. Goodrich, Chemical Co., Avon Lake, Ohio) in 0.1 M borate buffer, pH 8.6 (17). Crystallized human insulin (HI) was kindly provided by Dr. J. Schlichtkrull of the Novo Terapeutisk Laboratorium, Copenhagen, Denmark. The preparation of human growth hormone (HGH) used was obtained from the National Pituitary Agency, Baltimore, Md., through Dr. Frederic M. Kenny.

Aliquots of each of these proteins were labeled with ^{131}I, and additional aliquots were labeled with ^{125}I. The radioiodination method used was a modification of the nitrous acid method of Pressman and Eisen (18) as has been described elsewhere (19). Each of the radiolabeled proteins contained less than 0.5 atoms of iodine per protein molecule. Over 98% of the total radioactivity in each of these protein preparations was precipitable in 10% TCA, and from 92 to 97% of the radioactivity was precipitable immunochemically using specific antisera.

Assay of protein binding to the 60,000-×g fraction. Aliquots of each of the radiolabeled proteins containing from 3 to 8 μg of labeled protein with 0.05–0.2 μCi of radioactivity were added either to: (*a*) duplicate or triplicate 10-ml aliquots of supernate obtained after the second centrifugation of the tissue homogenate at 500 *g*, or (*b*) duplicate or triplicate tubes containing the washed 60,000-*g* fraction resuspended in 5 mM EDTA at pH 6.5, or (*c*) both. It should be noted that the aliquots of supernate were placed in the polyallomer tubes used (Beckman Instruments, Inc., Spinco Div.) before the addition of labeled protein. When the washed 60,000-*g* fraction was used, the fraction was resuspended in the same polyallomer tube in which it had been isolated from a 10-ml aliquot of the 500-*g* supernate, and then the labeled protein was added. In no instance was radiolabeled protein added to a polyallomer tube which did not or had not contained the 500-*g* supernate of a tissue homogenate. All subsequent operations were carried out in the same polyallomer tube. Although the amount of a given labeled protein added varied from 3 to 8 μg, differences in the amount of protein bound attributable to this degree of variation were not detected. The mixtures were incubated for 1–3 h at 37°C; incubation beyond 1 h had little apparent effect on the amount of labeled protein which was bound to the 60,000-*g* fraction. After centrifugation at 60,000 *g* for 20–30 min in the type 40 rotor, the supernates were collected, and the pellets were washed twice with 10 ml of 0.1 M NaCl in 5 mM EDTA at pH 6.5 (9). The washed pellets and aliquots of the initial supernates were assayed for radioactivity, and the percentage of the total radioactivity in the mixture that was bound to the washed 60,000-*g* fraction was determined. When a given radiolabeled protein was mixed with 500-*g* tissue supernate, the percentage bound to the resulting 60,000-*g* fraction was the same as that obtained when the protein was mixed instead directly with the washed 60,000-*g*

[1] *Abbreviations used in this paper:* B-IgG, bovine IgG; BJλ, Bence-Jones protein λ-chain; F/M, fetal to maternal serum concentration ratio; GP-IgG, guinea pig IgG; HA, human albumin; HGH, human growth hormone; HI, human insulin; H-IgE, human IgE; H-IgG, human IgG.

fraction. Binding of the labeled proteins to the pollyallomer tubes containing the pellets was determined by washing the pellets from the tubes with the EDTA buffer and then assaying the empty tubes for radioactivity; in no instance was this apparent binding greater than 5% of the radioactivity present in the pellet. Assay of radioactivity was performed using a 2- or 3-inch NaI crystal, each in conjunction with scalers capable of discriminating between ^{131}I and ^{125}I.

Inhibition of labeled protein binding with specific unlabeled proteins. The effect of protein concentration on binding was investigated by mixing a given radiolabeled protein with various amounts of the same or different purified unlabeled proteins. These mixtures were then added to aliquots of resuspended washed 60,000-*g* fractions, incubated at 37°C, and centrifuged at 60,000 *g* for 20–30 min in the type 40 rotor. The pellets were then washed twice with 10 ml of 0.1 M NaCl in 5 mM EDTA at pH 6.5. Both the initial supernate and the washed pellets were assayed for radioactivity.

Gastrointestinal absorption of labeled proteins by suckling rats and mice. Pregnant rats and mice near term were placed on drinking water containing 50 mg of NaI and 5 g of sucrose per 100 ml, and the animals were kept on this solution after delivery of their young. When the suckling rats and mice were 6–11 days old and 8–10 days old, respectively, they were weighed, and were then given an ^{131}I-labeled protein and an ^{125}I-labeled protein together intragastrically via a soft polyethylene catheter, 0.024 inches in outside diameter and having a 0.01-inch bore, passed into the stomach *per os*. Since the scalers and detectors used for radioactivity assay permitted easy differentiation between ^{131}I and ^{125}I, the absorption of two different proteins in the same animal could be estimated. Different amounts of either unlabeled H-IgG or HA were added to the radiolabeled protein mixture before intragastric instillation, so that the amount of H-IgG given varied from 2.4 μg to 28.8 mg in the rats and from 1.2 μg to 14.4 mg in the mice; the amount of HA given varied from 40 μg to 45 mg in the rats and from 20 μg to 22.5 mg in the mice. When the amount of H-IgG in the mixture was varied, the amount of HA given was kept constant at 40 μg per rat and 20 μg per mouse. When the amount of HA was varied, the amount of H-IgG given was kept constant at 2.4 μg per rat and 1.2 μg per mouse. All labeled protein mixtures were given in a volume of 0.3 ml per rat and 0.1 ml per mouse; a given mixture was given to six suckling rats and six to eight suckling mice. Half of each group was killed with ether anesthesia at 5 or 7 h after administration of the labeled proteins, and the other half was killed at 24 or 25 h. The entire gastrointestinal tract of each animal was removed immediately after the animal's death, and the carcasses were homogenized in 20% TCA using a Waring blender at high speed. After decanting, the blender was washed twice with 20% TCA, and the total volume of the homogenate including the washings was adjusted to 200 ml with 20% TCA. In the case of the suckling rats, each carcass was homogenized separately; in the case of the mice, the carcasses of the three or four mice comprising each group were homogenized together. The precipitates from duplicate 10-ml aliquots of each homogenate were collected by centrifugation, washed twice with 10% TCA, and then assayed for both ^{131}I and ^{125}I. Since some of the protein absorbed by the suckling was present in the blood and interstitial fluids removed with the gastrointestinal tract, the amount of labeled protein found in the carcass was considered as the minimum amount of protein absorbed.

Additional suckling rats and mice were given an injection of either unlabeled H-IgG or unlabeled HA subcutaneously 7.5 h before the intragastric instillation of the labeled protein mixture. The amount of H-IgG injected ranged from 2.4 to 9.5 mg/g of body wt in the rats and from 3.1 to 9.3 mg/g of body wt in the mice. The volume of distribution (19) of H-IgG in the murine suckling is approximately 20% of the body weight; the maximum serum levels of H-IgG achieved after distribution of the H-IgG in the body were calculated (19) to be from approximately 1,200 to 4,700 mg/100 ml in the rat and from 1,500 to 4,600 mg/100 ml in the mouse. The amount of HA injected was from 7.4 to 14.9 mg/g of body wt in the rat and from 4.8 to 6.0 mg/g of body wt in the mouse; the serum levels of HA reached after distribution were calculated to be about 3,700–6,400 mg/100 ml in the rat and from 2,400 to 3,000 mg/100 ml in the mouse. Subcutaneously injected H-IgG and HA reach a steady-state distribution between plasma and interstitial fluids (19) within 2–4 h in the rat and mouse. The intragastric dose of labeled protein contained less than 2.4 μg of labeled H-IgG and less than 40 μg of labeled HA. The animals were killed 23 h after the intragastric administration of labeled protein, and the carcasses, less the gastrointestinal tract, were assayed for TCA-precipitable radioactivity.

In some experiments, the concentrations of labeled protein in the sera of the sucklings after absorption, instead of in the carcasses, were determined. In these instances, the serum obtained from each of four rats in a given group was pooled as was the serum from each of six mice in a group, and aliquots of the pooled sera were assayed for TCA-precipitable radioactivity.

To determine if the labeled H-IgG absorbed via the gastrointestinal tract may have been drastically modified during the absorptive process, approximately 20 μCi of ^{131}I-labeled H-IgG were given to 8-day-old suckling mice either intragastrically or subcutaneously. Sera obtained 24 h later were injected intravenously into adult mice which were then counted daily in a whole body counter devised from a 2-inch NaI crystal probe coupled to a Picker scaler (Picker Briggs Corp., Cleveland, Ohio) with a pulse height discriminator. Other adult mice were given the labeled protein directly without prior passage through suckling mice. In this way, the half-life of the serum radioactivity found in the suckling mice which had been given labeled H-IgG intragastrically was compared with the half-life of the labeled H-IgG present in the serum of suckling mice which had been given the protein subcutaneously.

RESULTS

Protein binding to human placenta and fetal membranes. The binding of each of the radiolabeled proteins studied to the 60,000-*g* fraction from term human placenta is given in Table I in order of decreasing degree. As will be noted, BJλ was bound the most; approximately 10% of the radiolabeled BJλ added to the tissue homogenate fractions was fixed by the 60,000-*g* fraction. On the other hand, less than 0.5% of radiolabeled HA was found to be bound. The binding of HI, which was the second most bound protein, averaged 8.3 times that of HGH. Of the immunoglobulins investigated, H-IgG binding was slightly greater than that of GP-IgG and more than 3 times that of either B-IgG or H-IgE.

TABLE I

Binding of Radiolabeled Protein to the 60,000-×-g Fractions Obtained from Human Term Placentas, Term Fetal Membranes, Immature Placentas, and Erythrocytes

Radio-labeled protein	Term placentas	7–8-wk placentas	Term fetal membranes	Erythrocytes
	%	%	%	%
BJλ	10.6±0.75* (10)	—‡	—	—
HI	9.25±0.96 (4)	8.82±1.0 (3)	10.0±1.6 (4)	3.8±0.05 (3)
H-IgG	6.25±0.89 (19)	5.32±0.65 (3)	6.10±0.85 (4)	1.37±0.18 (3)
GP-IgG	5.40±0.42 (4)	—	—	2.41±0.32 (3)
B-IgG	2.03±0.13 (3)	2.15±0.23 (3)	4.31±0.52 (3)	0.81±0.09 (3)
H-IgE	1.70±0.13 (4)	—	1.10±0.10 (4)	0.58±0.08 (3)
HGH	0.77±0.10 (4)	0.89±0.05 (3)	0.52±0.06 (4)	0.32±0.05 (3)
HA	0.41±0.04 (12)	0.39±0.06 (3)	0.23±0.03 (4)	0.33±0.04 (3)

* Expressed as a percentage, ±SEM, of the total specific radiolabeled protein which was added to the tissue homogenate fractions; the parentheses indicate the number of individual placentas or other tissues studied; estimations were in duplicate or triplicate per individual tissue homogenate.
‡ Not done.

For those proteins studied, binding by the 60,000-*g* fraction from placentas of 7–8-wk gestation was virtually the same as the binding obtained with term placentas (Table I). In the case of the 60,000-*g* fraction from fetal membranes, when the proteins studied were listed in order of their degree of binding, the order was the same as that obtained with the placenta, but the absolute binding of B-IgG, at least, appeared to be somewhat different from that for the placenta (Table I). Protein binding to the 60,000-*g* fraction from human erythrocytes was different, both in relative terms and in absolute terms, from protein binding obtained with either the placenta or the fetal membranes (Table I).

When unlabeled H-IgG was added to radiolabeled H-IgG and the mixture was then added to suspensions of washed 60,000-*g* fractions from term placentas, the percentage of labeled H-IgG bound to the fraction was markedly affected (Fig. 1). At a total H-IgG concentration in the suspension of only 0.2 mg/ml, H-IgG binding was 2.1%, or about one-third of that obtained without the addition of unlabeled H-IgG. At a concentration of 1.0 mg of H-IgG/ml of suspension, H-IgG binding was 1.5%, and at an H-IgG concentration of 20 mg/ml, the binding was about 1.1% (Fig. 1). When unlabeled H-IgG was added to radioiodinated BJλ, a similar decrease, albeit relatively less marked, in the percentage of BJλ bound to the placental 60,000-*g* fraction was observed (Fig. 1). Unlabeled H-IgG inhibited the binding of B-IgG to the same relative degree as it did the binding of labeled H-IgG. The concentration of H-IgG appeared to have little or no effect on the binding of radiolabeled HA (Fig. 1). Conversely, unlabeled HA in concentrations up to and including 20 mg/ml, had no obvious effect on the binding of either H-IgG or BJλ. Although increasing amounts of unlabeled HA consistently decreased the amount of labeled albumin bound, from a mean of 0.40% without added albumin to 0.24% at a concentration of 20 mg HA/ml (Fig. 1), the difference between the means was not significant. Unfortunately, the amount of BJλ available was insufficient to permit an assessment of the effect of unlabeled BJλ on the binding of the labeled proteins.

The data in Fig. 1 also indicate that as the concentration of specific protein in the suspension increased, the *amount* of that protein which was bound by the 60,000-*g* fraction of term placenta actually increased, despite the fact that the *percentage* bound decreased. For example, it can be calculated from Fig. 1 that the amount of H-IgG bound at 1.0 mg/ml averaged 0.15 mg, or 1.5% of 1.0 mg/ml in a total volume of 10 ml; at a concentration of 10 mg/ml, the amount of H-IgG

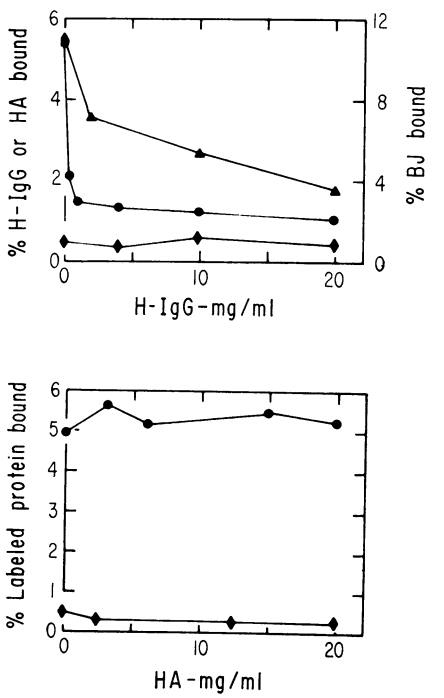

FIGURE 1 Percentage of labeled protein bound by the 60,000-*g* fraction from human placenta at different concentrations of either H-IgG (top) or HA (bottom). Symbols: ● = labeled H-IgG; ◆ = labeled HA; ▲ = labeled BJλ.

bound was calculated to average 1.24 mg, and at 20 mg/ml, the amount bound was 2.2 mg (Fig. 3).

Protein binding to placenta and fetal membranes of rats and mice. The binding of labeled BJλ, H-IgG, B-IgG, and HA to the 60,000-*g* fraction from term placentas of both rats, and mice was similar to that found for the human term placenta (Table II), although the binding of labeled HI seemed to be somewhat greater (Table II). The 60,000-*g* fraction from murine fetal membranes bound H-IgG and B-IgG to a degree similar to that seen for the murine placenta (Table II).

Protein binding to rat and mouse intestinal fractions. In sharp contrast to the binding of H-IgG to human, rat, or mouse 60,000-*g* placental fractions, the 60,000-*g* fraction from suckling rat, and mouse intestines bound approximately 50% of the labeled H-IgG (Table III). Although bound to a lesser degree than H-IgG, the binding of B-IgG to suckling murine intestines (Table III) was also at least fivefold greater than its binding to any of the placental 60,000-*g* fractions studied. On the other hand, the binding of HI and BJλ to the suckling intestinal fraction was approximately the same as or less than the binding of these proteins to the murine placental 60,000-*g* fractions. The degree of H-IgG binding to the 60,000-*g* intestinal fraction from adult rats and mice (Table III) was considerably lower than that for the suckling intestinal fraction, whereas the reverse was true for HI and BJλ. The binding of labeled proteins to the 60,000-*g* fraction from suckling murine liver (Table III) more closely resembled that for the placenta than the binding of these proteins by suckling intestines.

When mixtures of labeled and unlabeled H-IgG were incubated with suspensions of washed 60,000-*g* fraction

TABLE II

Binding of Radiolabeled Protein to the 60,000-×-g Fraction from Term Placentas and Fetal Membranes of Rats and Mice

Radiolabeled protein	Rat		Mouse	
	Term placenta	Fetal membranes	Term placenta	Fetal membranes
	%	%	%	%
HI	23.1±0.5 (4)*	—‡	—	—
BJλ	8.1±1.7 (3)	—	14.6 (1)	—
H-IgG	7.1±1.0 (5)	7.7 (2)	4.2±0.6 (3)	5.1±0.6 (3)
B-IgG	3.3±0.3 (3)	2.9 (2)	2.4±0.3 (3)	1.3±0.1 (3)
HA	0.53±0.12 (4)	—	0.65±0.08 (3)	—

* Parentheses indicate number of separate homogenates studied, each homogenate containing the placentas or fetal membranes from 20 to 36 conceptuses.
‡ Not done.

TABLE III

Binding of Radiolabeled Protein to the 60,000-×-g Fraction Obtained from Intestines and from Livers of Suckling Rats and Mice and from Intestines of the Adult Animals

Radiolabeled protein	Rat*			Mouse*		
	Suckling intestine	Adult intestine	Suckling liver	Suckling intestine	Adult intestine	Suckling liver
	%	%	%	%	%	%
H-IgG	54.1±10.7 (6)‡	20.6±1.0 (4)	10.5±1.4 (3)	49.9±6.1 (8)	14.6±1.5 (9)	10.7±3.0 (4)
B-IgG	17.6±3.5 (3)	15.6±1.2 (3)	4.1 (1)	16.8±1.8 (3)	9.83 (1)	— —
HI	15.2±0.8 (3)	22.8±4.5 (5)	19.9±0.8 (3)	—	—	—
H-IgE	13.6±3.2 (3)	11.9 (2)	7.0±0.8 (3)	—	—	—
BJλ	10.5±1.1 (5)	19.7±1.8 (3)	7.2±1.1	3.82±0.62 (3)	10.9±1.3 (3)	—
HA	2.3±0.5 (3)	—	—	0.48±0.04 (3)	0.44±0.02 (4)	—

* Suckling rats were 6-9 days old; suckling mice were 5-8 days old.
‡ Parentheses indicate the number of separate homogenates studied; each homogenate contained the pooled intestines or livers from 22 to 79 sucklings or the pooled intestines from 3 to 6 adults.

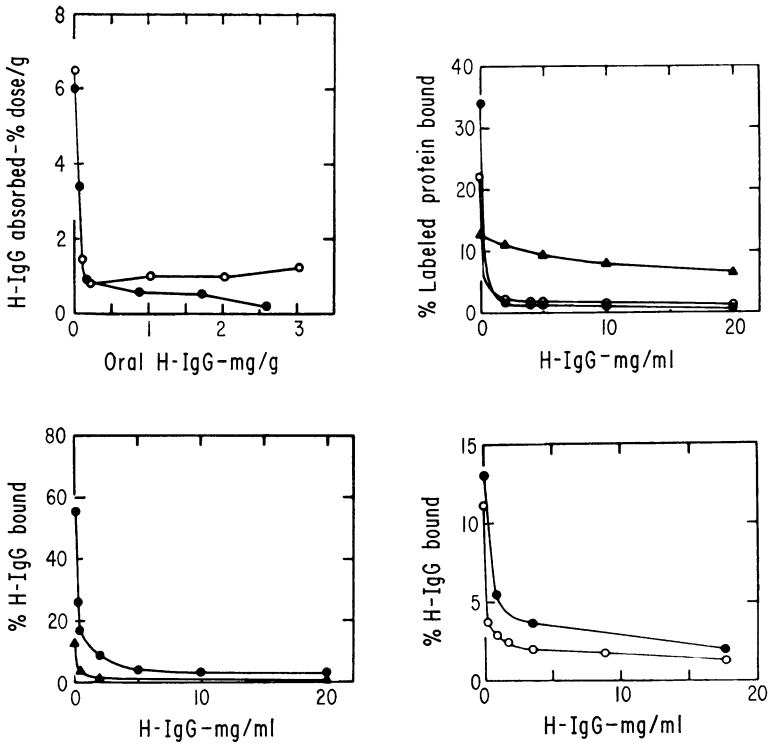

FIGURE 2 Top left: Percentage of the H-IgG dose given intragastrically that was absorbed per gram of rat (●) or mouse (○). Top right: Percentage of labeled H-IgG (●), B-IgG (○), and BJλ (▲) bound by the 60,000-*g* fraction from suckling rat intestine at different concentrations of H-IgG. Bottom left: Percentage of labeled H-IgG bound by the 60,000-*g* fraction from suckling mouse intestine (●) and by the 60,000-*g* fraction from suckling mouse liver (▲) at different concentrations of H-IgG. Bottom right: Percentage of labeled H-IgG bound by the 60,000-*g* fraction from intestines of 19-day-old (○) and adult (●) mice at different concentrations of H-IgG.

from intestines of 5–8-day-old mice, the percentage of labeled H-IgG bound by the 60,000-*g* fraction was found to be sensitive to the total H-IgG concentration (Fig. 2): an increase in total H-IgG concentration resulted in a decrease in the percentage of labeled H-IgG bound. Proportionately similar decreases in the percentage of labeled H-IgG bound were observed when the intestinal fractions from both adult mice and from 19-day-old mice were studied at various H-IgG concentrations (Fig. 2). Even the 60,000-*g* fraction from suckling mouse liver appeared to be H-IgG concentration dependent in terms of the percentage of labeled H-IgG bound (Fig. 2). The concentration of HA in the incubation mixtures, at least up to 20 mg of HA/ml of suspended 60,000-*g* fraction, had no apparent effect on the H-IgG binding by any of the mouse tissues. The binding of labeled H-IgG and labeled B-IgG by the 60,000-*g* fraction from intestines of 6–9-day-old rats was likewise sensitive to the total H-IgG concentration (Fig. 2). The binding of BJλ by intestinal 60,000-*g* fractions from suckling rats was dependent upon the H-IgG concentration, but much less so than was H-IgG or B-IgG binding (Fig. 2). The concentration of HA had no obvious effect on the binding of H-IgG, B-IgG, or BJλ by rat suckling intestines.

As with the human placental 60,000-*g* fractions, the *amount* of H-IgG bound by rat and mouse intestinal 60,000-*g* fractions appeared to increase when the concentration of H-IgG was increased, despite the fact that the *percentage* of the total H-IgG bound decreased (Fig. 3).

Absorption of labeled proteins in suckling rats and mice after intragastric instillation. The amounts of H-IgG found in the carcass of the 6–11-day-old suckling rat at 7 and at 25 h after different amounts of H-IgG were given intragastrically are shown in Fig. 3. It may be noted that as the amount of H-IgG administered was increased, the amount of H-IgG absorbed by 25 h increased and then remained relatively constant at H-IgG doses of 0.86 mg or more per g of body wt. In accord with this finding, when H-IgG serum concentrations were assayed instead of the amount of H-IgG in the

carcass, the serum levels of H-IgG increased and then tended to plateau at H-IgG doses greater than 1.06 mg/g of body wt. In the 8–10-day-old suckling mouse, however, the amount of H-IgG found in the carcass increased as the intragastric dose was increased; there was no evident plateau for the amount of H-IgG absorbed at doses up to and including 3.03 mg/g of body wt (Fig. 3). Similarly, the serum concentration of H-IgG in the

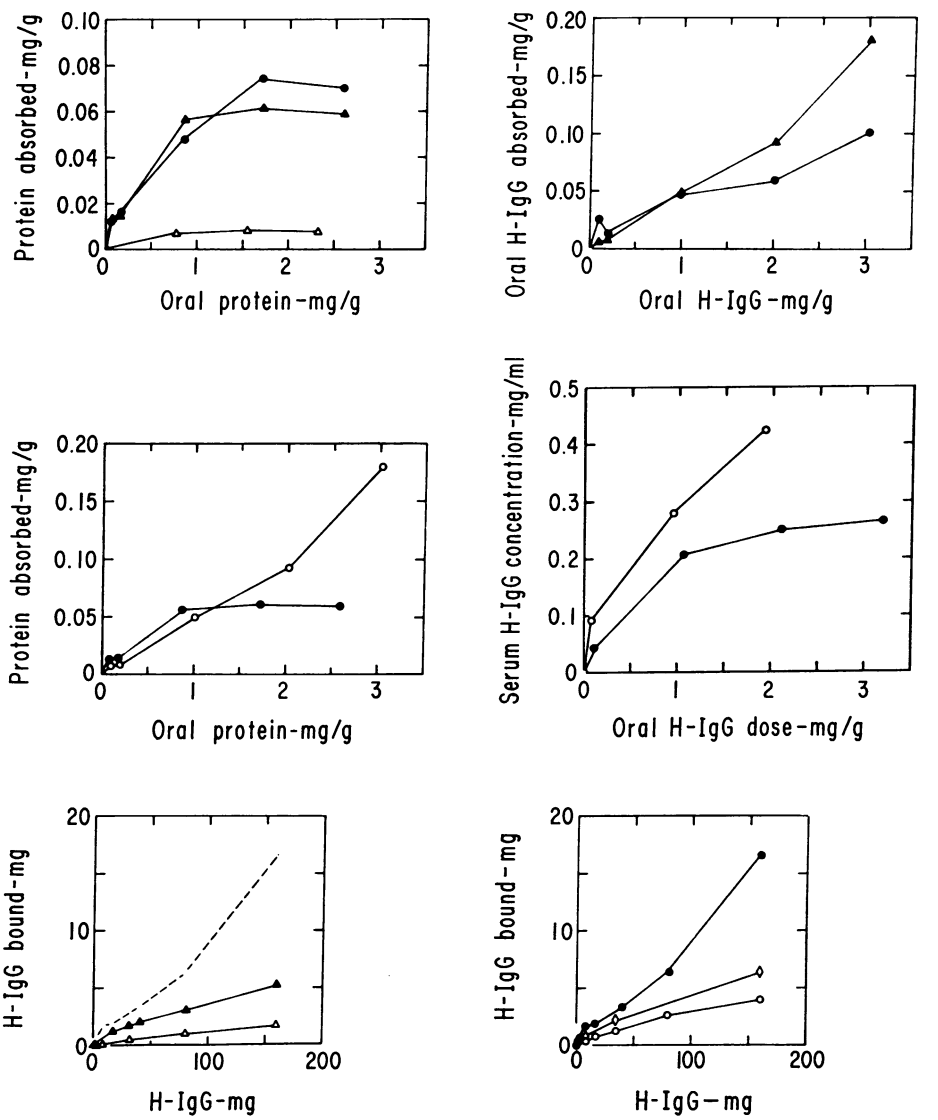

FIGURE 3 Top left: Amount of H-IgG found in the rat per gram of body wt 7 h (●) and 25 h (▲) after the intragastric administration of different amounts of H-IgG, and the amount of albumin found in the rat per gram of body wt (△) 25 h after intragastric instillation. Top right: Amount of H-IgG found in the mouse per gram of body wt at 5 h (●) and 24 h (▲) after intragastric administration. Middle left: Comparison of the amount of H-IgG found in the rat (●) and mouse (○) 25 and 24 h, respectively. Middle right: Serum concentrations of H-IgG found in the rat (●) and mouse (○) 25 h after intragastric administration. Bottom left: Amount of H-IgG bound to the 60,000-*g* fraction from suckling rat intestine (▲) and human placenta (△) when different amounts of H-IgG were present in the system; total volume of each aliquot of 60,000-*g* fraction with added H-IgG was 10 ml. For comparison, dashed line is the amount of H-IgG bound by suckling mouse intestine under the same conditions taken from figure to the right. Bottom right: Amount of H-IgG bound to the 60,000-*g* fraction from intestines of 5–8-day-old suckling mice (●), 19-day-old mice (○), and adult mice (◇).

TABLE IV

Relative Percentage of Labeled Protein Found in Serum of Suckling Rats and Mice after Intragastric Administration Compared to Relative Percentage Bound to the 60,000-×-g Fraction from Intestines of Same Age Sucklings

Radiolabeled protein	% of dose found per ml serum*	$\left(\frac{\%\text{ protein absorbed}}{\%\text{ H-IgG absorbed}}\right)$ ×100%	$\left(\frac{\%\text{ protein bound}}{\%\text{ H-IgG bound}}\right)$ ×100%
Suckling rats 6-9 days old			
H-IgG	31.7	100	100
B-IgG	18.4	58.0	32.5
HA	0.42	1.3	4.3
BJλ	0.01‡	0.32	19.4
Suckling mice 5-8 days old			
H-IgG	93.0	100	100
B-IgG	62.3	67.0	33.9
HA	1.52	1.64	0.96
BJλ	0.21‡	0.23	7.65

* Average for pooled sera of four rats or for pooled sera of six mice.

‡ Serum concentration obtained 5 h after administration of ^{125}I-labeled BJλ and ^{131}I-labeled H-IgG; serum concentration of labeled H-IgG at same time was 30.8% of dose in the rats and 67.3% of dose in the mice. All other serum concentrations are at 21 h after intragastric dose.

suckling mouse increased as the dose increased, the highest dose given in this case being 1.92 mg/g body wt (Fig. 3).

The percentage of the H-IgG dose that was absorbed was H-IgG dose dependent in both the rat and the mouse. As the dose of H-IgG in the rat was increased from the lowest dose studied, 0.22 μg/g of body wt, to 0.17 mg/g of body wt, the percentage of the H-IgG dose that was absorbed decreased dramatically (Fig. 2). Similarly, as the intragastric H-IgG dose was increased in the mouse from 0.25 μg/g, the smallest dose given, to 0.20 mg/g, the percentage of the H-IgG dose that was absorbed fell precipitously (Fig. 2). When unlabeled HA was substituted for unlabeled H-IgG in the intragastric dose, the percentage of the tracer dose of labeled H-IgG that was absorbed either remained unchanged or increased slightly over an HA dose range from 40 μg to 3.5 mg/g of body wt.

The amount of HA absorbed after intragastric administration was much less than the amount of H-IgG absorbed at equivalent doses of specific protein (Fig. 3). Loading the suckling intragastrically with amounts of unlabeled H-IgG up to 3.0 mg/g of body wt given together with labeled HA had no apparent effect on the absorption of labeled HA.

In the rat suckling, the subcutaneous administration of unlabeled H-IgG in amounts up to 9.5 mg/g of body wt given 7.5 h before the intragastric instillation of labeled H-IgG had little or no effect on the amount of H-IgG absorbed. Similarly, the administration of unlabeled H-IgG subcutaneously in the mouse suckling in amounts up to 9.2 mg/g of body wt had little apparent effect on the absorption of H-IgG given intragastrically.

The serum concentrations of labeled H-IgG, B-IgG, and HA which were found 21 h after intragastric administration in suckling rats and mice are given in Table IV, expressed as a percentage of the intragastric dose per milliliter of serum. The serum concentration of BJλ was estimated 5 h after intragastric administration instead of 21 h, because of the relatively short half-life of BJλ in the serum of these animals, i.e., approximately 1½ h. As a reference for the BJλ serum levels, the serum concentration attained by H-IgG at 5 h after intragastric instillation was also determined (Table IV). It will be noted that relatively little BJλ was absorbed via the gastrointestinal tract in either rats or mice; even assuming instantaneous absorption, the short half-life of BJλ cannot alone explain the relatively low serum concentrations found, since the maximum serum levels then attained would still be only about 8 times those observed.

An increase in the amount of H-IgG placed in the stomach of the suckling animal resulted in a decrease in the percentage of the H-IgG dose which was found in the serum: administration of only 1.5 mg of H-IgG/g of body wt resulted in a serum H-IgG level of only 1.65% of the intragastric dose per ml of serum in the rat and only 4.12% of the dose per ml of serum in the

mouse. Similarly, 1.5 mg of either H-IgG or B-IgG depressed the absorption of B-IgG, the amount of B-IgG found per milliliter of serum being less than 1% of the intragastric dose.

The radioactivity which was present in the serum of suckling mice after the intragastric instillation of labeled H-IgG had a half-life of 5.5 days in adult mice after intravenous injection. The radioactivity found in the serum of suckling mice given labeled H-IgG subcutaneously also had a half-life of 5.5 days in adult mice after intravenous injection. In both cases, the radiolabeled protein disappeared from the adult mice with a single exponential, and the disappearance curves were indistinguishable from each other. The half-life of labeled H-IgG injected intravenously into adult mice directly without being passed through suckling mice had a half-life of 5.5 days. Thus, labeled H-IgG did not appear to be drastically altered during its passage across the mouse suckling intestine.

DISCUSSION

When labeled H-IgG and labeled HA are given to the normal pregnant woman at term, the concentrations of these proteins in the serum of the fetus 1 day later are 0.07 and 0.006 times, respectively, their concentrations in maternal serum (3). Thus, both H-IgG and HA traverse the human term placenta in the direction of mother to fetus but at different net rates: the fetal to maternal serum concentration ratio, F/M, for labeled HA at 1 day after injection into the mother is only 8.6% of the F/M ratio for labeled H-IgG (Table V). It can be seen from the data in Table I that the binding of HA to the 60,000-*g* fraction from human term placenta is 6.6% of the binding of H-IgG (Table V), corresponding closely with the relative F/M ratios for these two proteins. A similar qualitative correspondence between maternofetal transfer and placental binding is apparent for BJ: immunoglobulin light chains isolated from normal human urine are, of course, BJ, and the F/M ratio for these chains in the pregnant women at term (3) 1 day after injection is 0.25, or approximately 3.6 times that for H-IgG (Table V); the binding of BJλ to the 60,000-*g* fraction from human term placenta (Table I) is 1.7 times that for R-IgG (Table V). Thus, the relative net transplacental transfer rates of H-IgG, BJλ, and HA in the pregnant women at term approximate the relative binding of these proteins to the cell membrane fraction from the human term placenta. Similarly, the relative net maternofetal transfer rates for H-IgG, B-IgG, and HA in the rat (20) and for H-IgG and BJλ in the mouse (20) correlate qualitatively with the relative binding of these proteins to the respective 60,000-*g* placental fractions (Table V). In addition, protein specificity in maternofetal transport is similar

TABLE V

Relative Net Maternofetal Transfer of Some Labeled Proteins in the Pregnant Human, Rat, and Mouse at Term and the Relative Binding of These Proteins In Vitro by the 60,000-×-g Fraction from the Respective Term Placentas

Radiolabeled protein	F/M*	$\left(\frac{\text{F/M for protein}}{\text{F/M for H-IgG}}\right)$ ×100%	$\left(\frac{\text{Percent protein bound}}{\text{Percent H-IgG bound}}\right)$ ×100%
Human			
H-IgG	0.07‡	100	100
BJ	0.25‡	360	170
HA	0.006‡	8.6	6.6
HGH	0§	0	12.3
Rat			
H-IgG	0.36‖	100	100
B-IgG	0.10‖	27.8	46
HA	0.028‖	7.8	7.5
Mouse			
H-IgG	1.50‖	100	100
BJ	5.40¶	360	348
B-IgG	0.15‖	9.3	57
HA	0.038‖	2.5	16

* F/M The concentration of labeled protein in fetal serum relative to that in maternal serum one day after intravenous injection in the mother (21).
‡,§,‖ Data taken from references 3, 23, and 21, respectivelv.
¶ Unpublished data.

to protein binding by the placenta: the maternofetal transport of labeled H-IgG, B-IgG, and BJλ, but not that of HA, is inhibitable with unlabeled H-IgG (21) just as the binding of labeled H-IgG, B-IgG, and BJλ, but not that of HA, to the 60,000-*g* fraction from term placenta is inhibitable with unlabeled H-IgG. This striking concurrence between maternofetal transport and binding would seem to support Brambell's hypothesis (8) that protein transport across tissue barriers is mediated by specific receptors on the cell membrane which bind the protein, and that the rate of transport is dependent upon the degree of binding.

However, it should be noted that HGH, although bound to human term placenta only one-eighth as well as H-IgG, is bound somewhat better than is HA; yet, HGH does not cross the human placenta (Table V), whereas HA does (22). Similarly, B-IgG is bound to the mouse placental 60,000-*g* fraction at least half as well as H-IgG, but passes from mother to fetus in the mouse (Table V) only 1/20th as rapidly as H-IgG (20). The occurrence of binding without corresponding maternofetal transport suggests either that placental receptors differ in function as well as in specificity, or that transport mechanisms besides receptor binding are involved, or both.

From the binding data obtained with the 60,000-*g* fraction from human placentas of 7–8-wk gestation, it is apparent that mechanisms other than or in addition to

receptor binding are necessary for the transplacental transport of proteins. Before 16 wk of gestation, H-IgG in the pregnant woman passes from mother to fetus primarily by diffusion (23). At approximately 22 wk of gestation there is a sudden and marked increase in the maternofetal transfer of H-IgG (24, 25), apparently due to the maturation or activation of a specific placental transport mechanism for H-IgG (26). Since the binding of H-IgG by the placenta of 7–8-wk gestation is the same as the binding of H-IgG by the term placenta, the difference in the maternofetal transport of H-IgG at the two different gestational periods cannot be explained by binding alone. Similarly, HI does not traverse the early human placenta (27), but may pass the term placenta from mother to fetus (28, 29). Yet, the binding of HI by the 60,000-*g* fraction from the placenta of 7–8-wk gestation is the same as from the term placenta. Obviously, binding cannot explain the gestational differences in the maternofetal transport of either H-IgG, or HI.

The binding data obtained with fetal membranes likewise indicate that protein transport is not accomplished through cell membrane receptors alone. Proteins such as H-IgG and HA traverse fetal membranes in vivo at rates which are *inversely* proportional to the square roots of their molecular weights (30). Not only is the binding of HA, which has a molecular weight of 65,000 daltons, much less than the binding of H-IgG, which has a molecular weight of approximately 165,000 daltons, but the binding of all the proteins studied to fetal membranes seems unrelated to protein molecular weight.

It is almost unnecessary to state, of course, that the binding of a protein by the placenta or fetal membranes in vivo may be quite different from the binding of that protein in vitro by the 60,000-*g* fraction. It is interesting to note, therefore, that the concentrations of HA, HGH, and HI in the villous portion of the human term placenta in vivo divided by the maternal serum concentration of the specific protein averages, respectively, 0.15, 0.27, and 2.90 (22); the relation HA : HGH : HI for these values is approximately 1 : 2 : 20. The relative binding of HA, HGH, and HI by the term placental 60,000-*g* fraction in vitro (Table I) is approximately 1 : 2 : 25. For these proteins and the placenta at least, relative binding in vitro does appear to approximate the relative "volume of distribution" (19) in vivo. In the rat intestine as well, the binding of labeled protein in vivo seems to be the same or similar to binding in vitro: Jones and Waldmann (9) have shown that the intestinal uptake of labeled H-IgG, sheep IgG, H-IgE, and HA in the suckling rat averaged 48.6%, 20.6%, 8.1%, and 3.3% of the administered dose, respectively, 4 h after the proteins were placed in the intestinal lumen. Binding of labeled H-IgG, B-IgG, H-IgE, and HA in vitro to the 60,000-*g* fraction from suckling rat intestine averaged 54.1%, 17.6%, 13.6% and 2.3%, respectively.

The binding of labeled proteins by the 60,000-*g* fraction from suckling murine intestine is greater than binding by the analogous fraction from placenta. In fact, the binding of H-IgG by the suckling rat and mouse intestinal fraction is 8–12 times, respectively, that by the corresponding placental fraction. Similarly, the transfer of IgG from mother to the offspring in the rat and mouse seems to be more effective via the intestine than via the placenta (31): the concentration of IgG in the murine neonate is only a small fraction of that in the mother, but it rapidly reaches the maternal concentration after a few days of suckling without a concomitant increase in neonatal endogenous IgG synthesis. Superficially, at least, the in vitro binding of immunoglobulins to suckling murine intestine as compared to the binding of immunoglobulins by murine placenta is in accord with Brambell's suggestion that specific receptors are important for protein transport.

Intestinal absorption of intact proteins in the rat and mouse seems to be related to protein binding in vitro in still other ways. The total amount of H-IgG bound by the 60,000-*g* fraction from suckling mouse intestine and the total amount of H-IgG absorbed in the mouse both increase as the amount of H-IgG in the system increases, at least up to levels of 20 mg H-IgG/ml in vitro and 22.5 mg H-IgG/animal in vivo. On the other hand, as the amount of H-IgG added to the 60,000-*g* fraction from suckling *rat* intestine increases, the total amount of H-IgG bound falls rapidly below that for the suckling mouse intestine at equivalent amounts of H-IgG in the system. This may be related to the observation that the total amount of H-IgG absorbed in the rat is readily saturated at doses of H-IgG which do not saturate the intestinal H-IgG transport system in the mouse. In the rat and in the mouse, both absorption and binding of labeled H-IgG seem to be specific, since the percentage of labeled H-IgG that is bound is easily inhibitable with unlabeled H-IgG but not with unlabeled HA. The observation that the relative serum concentrations of B-IgG and HA after administration to both rat and mouse approximate the relative binding of these proteins in vitro (Table IV) is still additional support for the receptor theory of protein transport. Interestingly, the amount of specific protein in the serum of the suckling is without effect on the amount of that protein absorbed, a fact which also can be interpreted as favoring the receptor theory.

In contrast to the increased maternofetal transport of H-IgG by the human or murine placenta as term approaches, murine absorption of intact H-IgG via the intestine is curtailed when the animal is 19–21 days of age, and little such absorption occurs in the adult ani-

mal. Yet, binding by the 60,000-*g* intestinal fraction from the 19-day-old or adult mouse is more than one-third of the binding observed with the intestinal fraction from young suckling animals. It is possible, that the decreased absorption of intact protein by the older animals may be due simply to increased intraluminal or intraepithelial protein digestion as has been suggested by others for the rabbit (32). Another possibility suggested by the data, however, is that an H-IgG transport system other than the cell receptor exists which is repressed or inhibited at 19–21 days of age.

There are some discrepancies between the absorption and binding data which indicate that specific receptors alone are not responsible for the transport of intact proteins across the suckling murine intestine. For example, a significant amount of labeled BJλ is bound by the 60,000-*g* fraction from suckling murine intestine, but little BJλ appears to be absorbed in the murine suckling. In addition, as was noted above, the transport systems for H-IgG absorption in the rat become saturated but the binding of H-IgG by the suckling rat intestinal fraction in vitro remains unsaturated at equivalent amounts of H-IgG in the two systems: if the binding of H-IgG to the rat intestinal 60,000-*g* fraction can be saturated, the amount of H-IgG to do so must be much greater than the amount which can saturate H-IgG transport mechanisms in vivo. Thus, there appears to be a saturable transport system in vivo which is not represented by the membrane receptor in vitro.

Although it may be argued that denaturation of the labeled protein might account for those instances where binding of the protein in vitro is much greater than the apparent transport of the protein in vivo, whether by placenta or intestine, this would seem unlikely. The same degree of binding by a given tissue was observed with different lots of labeled protein; the labeled proteins had normal half-lives of degradation in mice as assayed by total body counts after intravenous injection; the same labeled proteins, such as labeled BJλ and labeled H-IgG, were bound either to other tissues or to the same tissues in different species in vitro to a degree compatible with or even less than in vivo transport by that tissue.

The binding of labeled H-IgG to the 60,000-*g* fraction from suckling liver is inhibitable with unlabeled H-IgG but not with unlabeled HA, indicating that the H-IgG receptors in this system are specific for this protein.

ACKNOWLEDGMENTS

This study was supported by grants HD-00652 and HD-01031 from the National Institutes of Health.

REFERENCES

1. Bangham, D. R., K. R. Hobbs, and R. J. Terry. 1958. Selective placental transfer of serum-proteins in the rhesus. *Lancet.* **2**: 351–354.
2. Dancis, J., J. Lind, M. Oratz, J. Smolens, and P. Vara. 1961. Placental transfer of proteins in human gestation. *Am. J. Obstet. Gynecol.* **82**: 167–171.
3. Gitlin, D., J. Kumate, J. Urrusti, and C. Morales. 1964. The selectivity of the human placenta in the transfer of plasma proteins from mother to fetus. *J. Clin. Invest.* **43**: 1938–1951.
4. Quinlivan, L. G. 1964. Transplacental passage of gamma globulin-I^{131} in the rat. *Am. J. Physiol.* **207**: 782–786.
5. Halliday, R. 1955. Prenatal and postnatal transmission of passive immunity to young rats. *Proc. R. Soc. Lond. B. Biol. Sci.* **144**: 427–430.
6. Foster, J. F., R. W. Friedell, D. Catron, and M. R. Dieckmann. 1951. Electrophoretic studies on swine. III. Composition of baby pig plasma and sow's whey during lactation. *Arch. Biochem. Biophys.* **31**: 104–112.
7. Deutsch, H. F., and V. R. Smith. 1957. Intestinal permeability to proteins in the newborn herbivore. *Am. J. Physiol.* **191**: 271–276.
8. Brambell, F. W. R. 1966. The transmission of immunity from mother to young and the catabolism of immunoglobulins. *Lancet.* **2**: 1087–1093.
9. Jones, A. E., and T. A. Waldmann. 1972. The mechanism of intestinal uptake and transcellular transport of IgG in the neonatal rat. *J. Clin. Invest.* **51**: 2916–2927.
10. Rodewald, R. 1970. Selective antibody transport in the proximal small intestine of the neonatal rat. *J. Cell Biol.* **45**: 635–640.
11. Mackenzie, D. D. S. 1972. Selective uptake of immunoglobulins by the proximal intestine of suckling rats. *Am. J. Physiol.* **223**: 1286–1295.
12. Cohn, E. J., W. L. Hughes, Jr., and J. H. Weare. 1947. Plasma proteins. LIX. Preparation and properties of serum and plasma proteins. XIII. Crystallization of serum albumins from ethanol-water mixtures. *J. Am. Chem. Soc.* **69**: 1753–1971.
13. Fahey, J. L., and A. P. Horbett. 1959. Human gamma globulin fractionation on anion exchange cellulose columns. *J. Biol. Chem.* **234**: 2645–2651.
14. Mancini, G., A. O. Carbonara, and J. F. Heremans. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry.* **2**: 235–254.
15. Ogawa, M., S. Kochwa, C. Smith, K. Ishizaka, and O. R. McIntyre. 1969. Clinical aspects of IgE myeloma. *N. Engl. J. Med.* **281**: 1217–1220.
16. Ishizaka, T., K. Ishizaka, R. P. Orange, and K. F. Austen. 1970. The capacity of human immunoglobulin E to mediate the release of histamine and slow reacting substances of anaphylaxis (SRS-A) from monkey lung. *J. Immunol.* **104**: 335–343.
17. Miller, D. L., T. Hirvonen, and D. Gitlin. 1973. Synthesis of IgE by the human conceptus. *J. Allergy Clin. Immunol.* **52**: 182–188.
18. Pressman, D., and H. N. Eisen. 1950. Zone of localization of antibodies. V. Attempt to saturate antibody-binding sites in mouse kidney. *J. Immunol.* **64**: 273–279.
19. Gitlin, D., and C. A. Janeway. 1960. Some isotopic studies on the distribution and metabolism of plasma proteins. *Adv. Biol. Med. Phys.* **7**: 249–293.
20. Koch, C., M. Boesman, and D. Gitlin. 1967. Maternofoetal transfer of γG immunoglobulins. *Nature (Lond.).* **216**: 1116–1117.
21. Gitlin, D., and L. G. Morphis. 1969. Systems of maternofoetal transport of γG immunoglobulin in the mouse. *Nature (Lond.).* **223**: 195–196.

22. Gitlin, D., J. Kumate, and C. Morales. 1965. Metabolism and maternofetal transfer of human growth hormone in the pregnant woman at term. *J. Clin. Endocrinol. Metab.* **25**: 1599–1608.
23. Gitlin, D. Protein transport across the placenta and protein turnover between amniotic fluid, maternal and fetal circulations. *In* The Placenta, Biological and Clinical Aspects. K. S. Moghissi, editor. Charles C Thomas, Publisher, Springfield, Ill. In press.
24. Gitlin, D., and A. Biasucci. 1969. Development of γG, γA, γM, β_{1C}/β_{1A}, C'1-esterase inhibitor, ceruloplasmin, transferrin, hemopexin, haptoglobin, fibrinogen, plasminogen, α_1-antitrypsin, orosomucoid, β-lipoprotein, α_2-macroglobulin, and prealbumin in the human conceptus. *J. Clin. Invest.* **48**: 1433–1446.
25. Ewerbeck, H., and H. E. Levens. 1950. Die Bildung der Serumeiweisskörper des kindlichen Organismus bis zur Geburt und ihre Beziehund zum mütterlichen Serumeweissspektrum während der Schwangerschaft. *Monatsschr. Kinderheilk.* **98**: 436–440.
26. Gitlin, D. 1971. Development and metabolism of the immune globulins. *In* Immunologic Incompetence. B. M. Kagan and E. R. Stiehm, editors. Year Book Medical Publishers, Inc., Chicago. 3–16.
27. Adam, P. A. J., K. Teramo, N. Räihä, D. Gitlin, and R. Schwart. 1969. Human fetal insulin metabolism early in gestation. Response to acute elevation of the fetal glucose concentration and placental transfer of human insulin-I-131. *Diabetes.* **18**: 409–416.
28. Gitlin, D., J. Kumate, and C. Morales. 1965. On the transport of insulin across the human placenta. *Pediatrics.* **35**: 65–69.
29. Mintz, D. H., R. A. Chez, and E. O. Horger, III. 1969. Fetal insulin and growth hormone metabolism in the subhuman primate. *J. Clin. Invest.* **48**: 176–186.
30. Nencioni, T., B. Brambati, and P. G. Crosignani. 1970. Concentration d'albumine, de transferrine, IgA, IgG, IgM et bêta-lipoprotéines dan le sérum maternel, le liquide amniotique et le sérum funiculaire dans la grossesse normal près du terme. *Gynecol. Obstet.* **69**: 219–226.
31. Brambell, F. W. R. 1970. The Transmission of Passive Immunity from Mother to Young. North Holland Publishing Co., Amsterdam. 80, 102.
32. Kraehenbuhl, J. P., and M. A. Campiche. 1969. Early stages of intestinal absorption of specific antibodies in the newborn. An ultrastructural, cytochemical and immunological study in the pig, rat, and rabbit. *J. Cell Biol.* **42**: 345–365.