Studies on the Immunoglobulin-G Fc-Fragment Receptor from Neonatal Rat Small Intestine

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1. A method for preparing the small-intestinal brush-border membrane of neonatal rats is described in which enzymic methods are used to remove associated polysaccharide and cell nuclei. 2. ^{125}I -labelled IgG (immunoglobulin G) and ^{125}I -labelled IgG Fc fragment have high specific binding and low non-specific binding to brush borders prepared in this way. F(ab)_2 fragment however, does not bind, indicating the existence of a specific receptor for the Fc fragment of IgG. The receptor system is saturable, and the affinity (K_A) for the binding of rat IgG was determined by both equilibrium and kinetic methods. 3. The binding of heterologous IgG species (human and bovine) was compared and demonstrated a close similarity between human IgG and rat IgG in their receptor affinities. 4. Kinetic results are presented that are consistent with previously proposed models of ligand-induced receptor aggregation.

In order to explain the high selectivity of protein transport by the neonatal rat small intestine Brambell *et al.* (1958) proposed that the columnar epithelial cells bear receptors for homologous IgG. Subsequently Jones & Waldmann (1972) showed that unlabelled rat IgG competed with ^{125}I -labelled IgG in the transfer of this molecule through the neonatal intestine, and Rodewald (1973) established that the absorption of homologous IgG (fluorescein isothiocyanate-labelled) was restricted to the proximal epithelial cells (the jejunum) whereas the distal cells (the ileum) absorbed both homologous IgG and heterologous IgG with equal facility. Since only the brush-border membrane of the proximal cells was coated with IgG, Rodewald (1973) postulated the existence of a receptor for homologous IgG in these cells and non-specific protein absorption by the distal cells. Again, the results obtained by Morris & Morris (1974) seemed to confirm this idea, since these authors noted that transport of undegraded IgG is only observed in the jejunum whereas the transport products of the ileum consist of a mixture of large and small fragments of IgG.

The existence of a specific saturable receptor for IgG has been demonstrated in transporting tissues of other species [Guyer *et al.* (1976) for the neonatal mouse intestine; McNabb *et al.* (1976) and Rees & Wallace (1979a) for the human placenta], and the

origin of this specificity has been ascribed to the Fc region of the IgG molecule and its interaction with an Fc-fragment receptor (for a review see Rees & Wallace, 1979b).

In the present paper we report a method for the preparation of purified brush borders from neonatal rat intestine and the properties of the Fc-fragment receptor in this membrane.

Experimental

Materials

Common laboratory reagents were obtained from BDH Chemicals (Poole, Dorset, U.K.) and were AnalaR grade. Phenylmethanesulphonyl fluoride, pepstatin, *O*-methyltyrosine, sodium deoxycholate, hyaluronidase (EC 3.2.1.35) and deoxyribonuclease (EC 3.1.21.1) were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.). Bovine serum albumin and both rat IgG and human IgG (mixed subclass) were obtained from Miles Laboratories (Slough, Berks., U.K.). Na^{125}I was supplied by The Radiochemical Centre (Amersham, Bucks., U.K.) at 100 mCi/ml (3.7 GBq/ml). Sephadex was supplied by Pharmacia (London W.5, U.K.).

Wistar rats were obtained from various sources (Olac, Bicester, Oxon, U.K., the Department of Pathology, University of Oxford, and our own Department). Rat IgG fragments were a gift from Dr. Alan Williams (M.R.C. Unit of Cellular Immunology, Sir William Dunn School of Pathology, University of Oxford, Oxford, U.K.).

Abbreviation used: IgG, immunoglobulin G; for nomenclature of IgG fragments see World Health Organisation (1964).

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Membrane preparation

The method of Forstner *et al.* (1968) for the preparation of adult rat small-intestinal brush borders was considerably modified for the neonatal animal. Suckling Wistar rats (9–17 days old) were killed and the proximal half of the jejunum was removed into ice-cold 5 mM-EDTA, pH 7.4, containing phenylmethanesulphonyl fluoride (2 µg/ml) and pepstatin (1 µg/ml) as proteinase inhibitors. The mucosa was scraped off and the suspension thereof dispersed by using a Pasteur pipette. This suspension largely consisted of cells and debris trapped within amorphous material. The subsequent steps leading to the preparation of mucus-free and DNA-free brush borders are outlined in Scheme 1. The importance of the order of the steps in this scheme is illustrated in the Results section, where equilibrium binding data for membranes prepared in different ways are compared. Also shown in Scheme 1 is a typical membrane protein composition produced on SDS/polyacrylamide-gradient-gel electrophoresis by the method of Laemmli (1970).

IgG fragment preparation

Both Fc and F(ab)₂ fragments from human IgG were prepared by the methods described by Stanworth & Turner (1973).

Protein determination

The Lowry method as modified by Hartree (1972) was used, with 1% sodium deoxycholate as solubilizing agent for the membranes; bovine serum albumin was used to prepare standard curves in the presence of deoxycholate.

Protein iodination

The chloramine-T method was used to label IgG and the Fc fragment with ¹²⁵I according to the following protocol.

Protein (0.07 nmol) in 10 µl of phosphate-buffered saline, pH 7.4 (Dulbecco medium A; Dulbecco & Vogt, 1954), was added to 1 µl of Na¹²⁵I (100 mCi/ml), and 10 µl of chloramine-T (0.17 mg/ml) was then added rapidly and the mixture agitated for 60 s. *O*-Methyltyrosine (100 µl of 0.04% solution) was then added and the mixture fractionated on a column (20 cm × 0.7 cm) of Sephadex G-25 equilibrated with phosphate-buffered saline containing bovine serum albumin (2 mg/ml). The labelled protein was stored in 100 µl portions at -20°C. By use of the above procedure an iodine/protein molar ratio of 0.4:1 was routinely obtained. If this ratio is exceeded there is danger of a decrease in affinity of the IgG or Fc fragment for its receptor (K. H. Wallace & A. R. Rees, unpublished work). In addition, if the iodinated protein is stored for more than 1 week the percentage of shed ¹²⁵I increases significantly. Thus for

optimum results the protein should be used within this period.

Binding assays

Affinity constants (K_A) for the binding of IgG and Fc fragment were determined by the direct competition method. ¹²⁵I-labelled IgG (or Fc fragment) was added at a final concentration of approx. 0.5 nM to 190 µg of membrane protein, and unlabelled protein was added over the range 3 nM to 10 µM. The total volume of each sample was 1.5 ml in either Tris or phosphate buffer as indicated, containing bovine serum albumin (2 mg/ml) and at pH 6.6 unless otherwise stated. The assay was carried out by shaking the reaction mixture in capped vials for 1 h at 37°C, then layering on to ice-cold buffer containing bovine serum albumin solution (22 mg/ml) contained in sealed-off Pasteur pipettes. These were then centrifuged at 1000 *g*_{av} for 20 min at 4°C, and the supernatants were aspirated off, leaving a small pellet. The tips of the pipettes containing the pellets were broken off and their radioactivities counted in a Hewlett-Packard γ-radiation counter. Non-specific binding was defined as that labelled protein which could not be displaced with unlabelled protein at 0.5 mg/ml.

Data were analysed by the method of Scatchard (1949). The parameters of the Scatchard equation (K_A and n) were evaluated by using a computed least-squares fit according to the method of Klotz & Hunston (1971).

Determination of kinetic constants

(a) *Dissociation experiments.* If the dissociation of labelled IgG from its receptor:

(where L is IgG and R is receptor) is assumed to follow a simple first-order process, then the determination of k_{-1} can be performed by a dilution process. In addition, the presence or absence of site-site interactions can be assessed by performing the dissociation in the presence of unlabelled IgG (cf. insulin; DeMeys *et al.*, 1976). If the k_{-1} value is the same in each instance, then this would suggest either that Fc-fragment receptors do not interact in the membrane or that any interaction does not influence the affinity constant for the ligand-receptor interaction.

The dissociation experiments were performed by allowing membrane and labelled IgG to come to equilibrium as described above, centrifuging and diluting the pellet at least ten times in the presence and absence of unlabelled IgG (10 nM). At the stated time intervals portions were removed and quickly centrifuged in a Beckman Microfuge B. Exactly half the supernatant was then removed to a separate tube and the radioactivities of both halves were counted

in a γ -radiation counter. The amount of radioactivity bound to the membrane pellet was then determined by a simple difference procedure. The rate constants were obtained from first-order rate plots of the data.

(b) *Association experiments.* A number of duplicate samples were set up at 37°C as described for binding assays, containing membrane and ^{125}I -labelled IgG at 0.5 nM. At various times from 40 s to 60 min portions were removed to ice-cold Microfuge tubes and centrifuged rapidly in a Microfuge. The radioactivity associated with the pellet was measured as described above. The data were analysed by assuming a bimolecular association between IgG and Fc-fragment receptor. A first-order kinetic plot was used to obtain an apparent pseudo-first-order rate constant, from which a second-order rate constant was calculated.

Results

Brush-border membranes

The combination of small cell size and a considerable glycocalyx component makes the neonatal columnar epithelial cell very difficult to handle. This is even more apparent when the cell is lysed and the nuclei are disrupted. The steps outlined in Scheme 1 are essential for the preparation of a homogeneous sample of brush borders that exhibit a high degree of binding of IgG or Fc fragment and a low non-specific binding. The latter is subject to large fluctuations

when either DNA or glycocalyx is incompletely removed. In addition, the importance of the order of the steps cannot be overemphasized. A summary of our experiences with this system and the effects on non-specific binding is shown in Table 1.

In addition, we observed a considerable variation (10–35%) in the degree of specific binding of IgG between different litters (age range 9–17 days), although the scatter in the non-specific binding component was small (1.5–2.5%). Since the variation in the specific binding of IgG to membranes from the same litter, prepared simultaneously but split into five separate batches, was small ($\pm 3.5\%$), we conclude that the inter-litter differences are significant. Since the affinity of the IgG binding was the same in all experiments, whether from the same litter or not, we consider that this variation may reflect a loss of receptors with increasing age. Alternatively it is possible that the variation is due to differences in the maturity of the transport system, and hence the receptor status, for rats of the same age.

Equilibrium binding

Fig. 1 shows a curve for the binding of labelled rat IgG to purified brush-border membranes. The corresponding Scatchard curve is seen to be biphasic and was analysed as described in the Experimental section. The presence of two sites with K_A values between one and two orders of magnitude apart raises the question of receptor homogeneity. By way of comparison Rees & Wallace (1979a)

- Step 1. Intestinal mucosa, from proximal half of small intestine of three rats, scraped into 50 ml of 5 mM-EDTA, pH 7.4.
- Step 2. Scrapings repeatedly drawn into Pasteur pipette until a uniform opaque creamy-yellow suspension obtained; any muscle tissue fragments removed, as these will block syringe needle in step 4.
- Step 3. Hyaluronidase added, as a 10 mg/ml solution in 5 mM-EDTA, pH 7.4, to a final concentration of 0.5 mg/ml; mixture swirled repeatedly at room temperature for 30 min.
- Step 4. Suspension forced through a 23-gauge hypodermic needle, from a 50 ml plastic syringe, by hand as fast as possible.
- Step 5. Suspension centrifuged at 1000 g for 20 min at 4°C, and the supernatant discarded.
- Step 6. Pellet resuspended in a small volume (1–3 ml) of 90 mM-NaCl/0.8 mM-EDTA, pH 7.4, containing deoxyribonuclease 1 (0.2 mg/ml); left at room temperature for 10 min.
- Step 7. Step 5 repeated.
- Step 8. Pellet resuspended in binding-assay buffer of choice.

Scheme 1. Method for the preparation of brush-border membranes from the neonatal rat small intestine

Also shown is a typical sodium dodecyl sulphate/polyacrylamide-gel electrophoretogram of the glycoproteins from the brush border. This was obtained by electrophoresis of a sodium deoxycholate-soluble fraction on a 9–20%-acrylamide gradient gel. The gel was stained with Coomassie Blue, destained and then washed for 24 h in phosphate-buffered saline containing 0.4 M-NaCl. It was incubated for 18 h with ^{125}I -labelled concanavalin A, washed with several changes of phosphate-buffered saline containing 0.4 M-NaCl (total concn.), dried and radioautographed.

Table 1. Comparison of non-specific binding of ^{125}I -labelled IgG for membranes prepared in different ways
Non-specific binding was defined as the percentage of total radioactivity not displaceable by unlabelled IgG (0.5 mg/ml). The binding conditions were 35 mM-Tris/HCl buffer, pH 6.6, containing bovine serum albumin (2 mg/ml). The buffer contained proteinase inhibitors. Percentage binding is quoted $\pm$ s.d.

Membrane preparation and assay details	Non-specific binding (% of total binding)	No. of litters	No. of preparations
Normal preparation; ^{125}I -labelled IgG; <1 week old	1.85 ± 0.12	3	15 (duplicate determinations)
Syringe step omitted; ^{125}I -labelled IgG; <1 week old	9.79 ± 0.25	1*	6 (single determinations)
	10.42 ± 0.15	1*	1 (duplicate determinations)
	11.22 ± 1.48	1*	1 (duplicate determinations)
Syringing before hyaluronidase treatment; ^{125}I -labelled IgG; <1 week old	6.81 ± 0.18	1*	1 (duplicate determinations)
Normal preparation; ^{125}I -labelled IgG; >1 week old	6.46 ± 0.31	1*	1 (duplicate determinations)
Syringing before hyaluronidase treatment; ^{125}I -labelled IgG; >1 week old	11.70 ± 0.30	1*	4 (duplicate determinations)

* All different litters.

Fig. 1. Equilibrium binding of ^{125}I -labelled IgG to brush-border membranes prepared as indicated in Scheme 1. Each tube contained membrane protein (190 μg), ^{125}I -labelled IgG (10^5 c.p.m.; approx. 0.5 nM), unlabelled IgG (in the range 3 nM–10 μM) and binding buffer [35 mM-Tris/HCl buffer, pH 6.6, containing bovine serum albumin (2 mg/ml) and proteinase inhibitors] to a total volume of 1.5 ml. Tubes were shaken at 37°C for 1 h, then centrifuged for 15 min at 1000 g at 4°C, and the radioactivities of the pellets were counted. Error bars represent $\pm$ 1 s.d. The binding curve was drawn by hand. The Scatchard curve was computer-fitted by the method of Klotz & Hunston (1971).

have shown that two types of Fc-fragment receptor are present in human placental plasma membranes. Qualitative experiments with fluorescein isothiocyanate-labelled IgG by Johnson & Matre (1979) are consistent with there being one receptor for monomeric IgG and a second receptor for heat-aggregated IgG.

Although we do not dismiss the possibility that two different receptor populations are present in the brush-border membrane [uptake experiments by

Borthistle *et al.* (1977) suggest that monomeric IgG and heat-aggregated IgG bind to different receptors, the former predominating in the neonatal animal], kinetic data presented below suggests an alternative interpretation of the equilibrium binding results.

Dissociation experiments

In Fig. 2 dissociation data for the receptor system in the presence and absence of unlabelled IgG are plotted. Both curves are complex. In the

Fig. 2. Time course of dissociation of ^{125}I -labelled rat IgG from brush-border membranes

Membrane and labelled IgG were incubated as described for Fig. 1. Pellets were resuspended in 10 times the volume of incubation buffer with or without unlabelled IgG at 20 nM. Then 1 ml samples were withdrawn at the various times, pelleted in a Beckman Microfuge B and the radioactivities of the pellets counted. All data points are means of duplicates. O, Without unlabelled IgG in the resuspension medium; ●, with IgG in the resuspension medium; ▲, non-specifically bound radioactivity.

absence of unlabelled IgG 90% of the bound radioactivity dissociates with a rate constant $k_{-1} = 0.0065 \text{ min}^{-1}$, the remainder dissociating with a value at least an order of magnitude larger. In the presence of unlabelled IgG about 50% of the bound radioactivity has $k_{-1} = 0.008 \text{ min}^{-1}$, the remaining 50% having an approximate k_{-1} of 0.225 min^{-1} . The increase in the rate of dissociation suggests that unlabelled IgG on binding to the membrane is in some way decreasing the affinity of those labelled molecules already bound. This phenomenon has already been observed for many hormone systems, and has been variously explained by ligand association, ligand-induced receptor aggregation (negative cooperativity) or non-specific effects (for discussion see Cuatrecasas & Hollenberg, 1976). We propose that our results are consistent with a ligand-induced receptor aggregation, and consider the reasons for this proposal in the Discussion section. Association data are also presented in the Discussion section.

Ionic-strength and pH effects

Fig. 3(a) shows the effect of ionic strength on the binding of IgG. Maximum binding is observed at relatively low ionic strength, with an optimum at 60 mM, where the difference between specific and non-specific binding is greatest. The degree of binding is independent of the buffer ion used (e.g. Tris/HCl or phosphate buffers, or NaCl in combination with either of these), although we have not investigated this effect with any other buffer systems.

Fig. 3. Dependence of IgG binding on (a) ionic strength and (b) pH

Specific binding was determined as described in the Experimental section and Fig. 1. (a) ●, Specific binding with ^{125}I -labelled IgG present only; O, non-specific binding with ^{125}I -labelled IgG and unlabelled IgG (0.5 mg/ml) present. Inset: percentage non-specific binding expressed as a percentage of specific binding [NS (% of S)]. The pH for all the determinations was 6.75 and all data points are means of duplicates. Sodium phosphate buffers gave similar results, with an optimum at 33 mM (the equivalent ionic strength to 60 mM-Tris/HCl buffer). (b) ●, Specific binding as defined above; O, non-specific binding as defined above. Inset: percentage non-specific binding expressed as a percentage of specific binding [NS (% of S)]. The buffer was 33 mM-sodium phosphate containing bovine serum albumin (2 mg/ml).

This observation, which has been noted by Tsay & Schlamowitz (1978) for rabbit membranes, suggests that there are important ionic interactions between IgG and its receptor, although it is not clear whether the dependence is due to an effect on the receptor or the IgG molecule (for further discussion see Rees & Wallace, 1979a).

We cannot say whether the dependence of binding on ionic strength is physiologically relevant. Other workers (e.g. Rodewald, 1973) have assumed that the intraluminal fluid is iso-osmotic and have successfully demonstrated uptake *in vivo* of labelled IgG from phosphate-buffered saline. Our results suggest that, under these conditions, binding of IgG to receptor would be very inefficient and probably less discriminatory than at lower ionic strengths. This may suggest either that *in vivo* the bulk fluid is much less than iso-osmotic or that the local environment experienced by the Fc-fragment receptor is different to that of the bulk fluid, or alternatively that the behaviour of the receptor system in intact cells *in vivo* is different to that of the brush border *in vitro*, where it is missing much of the material it is in contact with in the whole cell.

The pH optimum of binding (Fig. 3b) was measured in sodium phosphate buffer containing bovine serum albumin (2mg/ml). The pH for optimum binding was observed at 6.6. This is consistent with the value quoted for the lumen of the neonatal rat (Rodewald, 1973), and agrees with the results obtained by Jones & Waldmann (1972), who showed that IgG binding to brush borders was sensitive to pH. It is significant that, as the pH is lowered, the ratio of non-specific to specific binding increases and could contribute to the loss of specificity of protein transport with increasing maturation. In addition, since as the animal ages the pH of its intestinal lumen gradually decreases and approaches a value of about 4 in the adult, the activation of acid proteinases and their degradation of ingested proteins will undoubtedly serve to increase the redundancy of the receptor selection system. That the behaviour that we have observed is probably a consequence of simple ionic interactions between IgG and its receptor is verified by the ease with which the pH and ionic-strength effects can be reversed with regain of full binding potential at the optima.

Binding of heterologous IgG and proteolytic fragments

IgG from various species was tested for its ability to displace labelled rat IgG. The results are shown in Fig. 4(a). The fact that human IgG is as efficient in competition as rat IgG has proved useful in that we have been able to use the more easily obtainable Fc fragment of human IgG to establish the specificity of the receptor interaction. Fig. 4(b) compares the relative ability of human Fc and F(ab)₂ fragments to displace ¹²⁵I-labelled human Fc fragment. The affinity of human Fc fragment for the receptor is almost exactly the same as that of rat IgG [ED₅₀ (Fc) = 20nM; ED₅₀ (rat IgG) = 50nM], whereas that of F(ab)₂ fragment is less than 1% of the value for Fc fragment. The fact that F(ab)₂ fragment com-

Fig. 4. Comparisons of the relative abilities of (a) IgG from various species to displace ¹²⁵I-labelled rat IgG and of (b) rat F(ab)₂ fragment, rat IgG and human Fc fragment to displace ¹²⁵I-labelled human Fc fragment. The assay conditions were as for Fig. 1. (a) ○, Rat IgG; ●, human IgG; ▲, bovine IgG. (b) ●, Rat F(ab)₂ fragment; ○, rat IgG; ▲, human Fc fragment.

petes at all could be explained by a trace contamination (<1%) of the preparation with IgG, as shown by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, and we therefore conclude that the receptor is specific for the Fc region of IgG.

Discussion

It is clear from the results presented that the binding of IgG to the rat brush-border membrane satisfies all the criteria required of a receptor molecule. The interaction is highly specific for the Fc fragment, is saturable and has a high binding affinity relative to those values quoted for other monomeric IgG receptors (Unkeless & Eisen, 1976; Rees & Wallace, 1979a). Preliminary experiments (K. H. Wallace & A. R. Rees, unpublished work) on a soluble form of the receptor suggest that the specificity of the molecule is retained in solution.

With respect to the variation in receptor number in membranes prepared from different litters but assayed under the same conditions, we conclude that a loss of specificity of IgG transport with increasing age of the neonatal animal is not simply the result of a changing ionic environment. The ionic-strength and pH results suggest that this may be a contri-

butory factor, but what seems more likely is that there is a gradual decrease in the number of receptors as the neonatal animal approaches closure.

The somewhat lower affinity of bovine IgG for the receptor reinforces the observation made by Hemmings & Williams (1976), who showed a much lower rate of transport in the neonatal rat for this species compared with homologous IgG. We have not investigated the binding of bovine IgG subclasses, and it is possible that the diminished affinity that we observe is a consequence of using a mixture of subclasses in which there may be a component with a very low affinity for the receptor.

Various explanations of an increased dissociation rate in the presence of unlabelled ligand have been discussed by Cuatrecasas & Hollenberg (1976). We can eliminate causes such as re-binding of labelled IgG and non-specific binding, which we have corrected for, and the 'retention effect' discussed by Silhavy *et al.* (1975), since the critical ratio of receptor concentration to dissociation constant is much less than 1. The lowering of affinity in the presence of unlabelled IgG could reflect an aggregation process involving IgG-receptor complexes similar to the negative co-operativity effect observed by other workers with hormones (Cuatrecasas & Hollenberg, 1976). It may seem curious that a transport system actually experiences a decrease in the affinity of its ligand before the transport even formally begins, but the fact that we are dealing with an incomplete system may explain this. *In vivo*, internalization may follow receptor binding far faster than the time taken for ligand to dissociate even when high concentrations are present. This internalization has been suggested to be mediated by a specialized system of coated vesicles (for a review see Rees & Wallace, 1979b), the formation of which may well not be possible in an isolated brush-border membrane.

To compare the behaviour of the receptor system under both equilibrium and non-equilibrium conditions we have estimated the affinity constants for IgG-receptor interaction from data from both methods. We have measured the rate of association of ^{125}I -labelled IgG with the receptor preparation and have obtained an estimate of the pseudo-first-order rate constant as $4 \times 10^{-2} \text{ min}^{-1}$. The calculated second-order rate constant of $8.4 \times 10^5 \text{ mol}^{-1}$ can be used to estimate the equilibrium constant K_A for the high-affinity site, since $K_A = k_{+1}/k_{-1}$. By using the two k_{-1} values of the dissociation curve with unlabelled IgG present (the slow k_{-1} is in fact the same both in the presence and in the absence of unlabelled IgG), K_A values are obtained ($1.2 \times 10^8 \text{ M}^{-1}$ and $5 \times 10^6 \text{ M}^{-1}$) that are in close agreement with those of the equilibrium method ($1.3 \times 10^8 \pm 0.43 \times 10^8 \text{ M}^{-1}$ and $5.51 \times 10^6 \pm 0.69 \times 10^6 \text{ M}^{-1}$) and suggest that the affinity of ^{125}I -labelled

IgG is not dissimilar to that of native IgG. We do not consider that the interaction of monomeric IgG with a receptor for antigen-antibody complex is an explanation of the binding heterogeneity, even though such a receptor has been proposed for the neonatal rat by Borthistle *et al.* (1977). Our own results (Rees & Wallace, 1979a) and those of others (Johnson & Matre, 1979) suggest that monomeric IgG is very poor at binding to sites for antigen-antibody complexes, although the reverse does not seem to be true.

In conclusion, we are confident that we are dealing with a specific receptor system for IgG in the neonatal rat intestine, although at present we are unable to shed any light on the events that occur subsequent to binding. The biphasic nature of the equilibrium Scatchard analysis and the ability of unlabelled IgG to enhance the dissociation rate of bound IgG may be taken to support a receptor aggregation model, this aggregation being an obligatory preliminary to internalization by a specialized vesicle (coated or otherwise) system.

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