Protein Turnover

Ciba Foundation Symposium 9 (new series)

1973

Elsevier · Excerpta Medica · North-Holland
Associated Scientific Publishers · Amsterdam · London · New York

Protein Turnover

The Ciba Foundation for the promotion of international cooperation in medical and chemical research is a scientific and educational charity established by CIBA Limited – now CIBA-GEIGY Limited – of Basle. The Foundation operates independently in London under English trust law.

Ciba Foundation Symposia are published in collaboration with Associated Scientific Publishers (Elsevier Scientific Publishing Company, Excerpta Medica, North-Holland Publishing Company) in Amsterdam.

Associated Scientific Publishers, P.O. Box 1270, Amsterdam

Protein Turnover

Ciba Foundation Symposium 9 (new series)

1973

Elsevier · Excerpta Medica · North-Holland
Associated Scientific Publishers · Amsterdam · London · New York

ISBN Excerpta Medica 90 219 4010 8
ISBN American Elsevier 0-444-10414-3

Library of Congress Catalog Card Number 72-96519

Published in 1973 by Associated Scientific Publishers, P.O. Box 1270, Amsterdam, and 52 Vanderbilt Avenue, New York, N. Y. 10017.
Suggested series entry for library catalogues: Ciba Foundation Symposia.

Ciba Foundation Symposium 9 (new series)

Printed in The Netherlands by Van Gorcum, Assen

Contents

Participants

Symposium on Protein Turnover held at the Ciba Foundation, London, 9th-11th May 1972

A. S. MCFARLANE (*Chairman*) MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ

C. A. ALPER The Blood Grouping Laboratory, 320 Longwood Avenue, Boston, Mass. 02115, USA

S. B. ANDERSEN Institute of Medical Physiology A, University of Copenhagen, Juliane Mariesvej 28, DK-2100 Copenhagen Ø, Denmark

L. DONATO Fisiologia Clinica, Laboratorio del Consiglio Nazionale delle Ricerche, presso l'Università degli Studi di Pisa, Via Savi 8, I-56100 Pisa, Italy

A. H. GORDON MRC National Institute for Medical Research, Mill Hill, London NW7

* R. HOFFENBERG MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ

J. H. HUMPHREY MRC National Institute for Medical Research, Mill Hill, London, NW7

K. N. JEEJEEBHOY Department of Medicine, Division of Gastroenterology, University of Toronto, Room No. 6352, Medical Sciences Building, Toronto 181, Ontario, Canada

K. BIRGER JENSEN Medical Department P 2132, Gastroenterological Unit, Rigshospitalet, Blegdamsvej, DK-2100 Copenhagen Ø, Denmark

* *Present address:* Department of Medicine, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH

* E. A. JONES Medical Unit, The Royal Free Hospital, North Western Branch, Lawn Road, London, NW3

G. MILHAUD Université de Paris, Faculté de Médecine Saint-Antoine, Service de Biophysique, 27 rue Chaligny, Paris 12, France

L. L. MILLER Department of Radiation Biology and Biophysics, The University of Rochester School of Medicine, Rochester, NY 14620, USA

H. N. MUNRO Department of Physiological Chemistry, Massachusetts Institute of Technology, Cambridge, Mass. 02139, USA

B. NOSSLIN Isotope Laboratory, General Hospital, S-214 01 Malmö, Sweden

M. ORATZ Radioisotope Service and Nuclear Medicine, Veterans Administration Hospital, First Avenue at East 24th Street, New York, NY 10010, USA

D. K. PETERS Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London, W12

B. POOLE The Rockefeller University, New York, NY 10021, USA

E. B. REEVE Division of Laboratory Medicine, University of Colorado Medical Center, 4200 East Ninth Avenue, Denver, Colorado 80220, USA

E. REGOECZI Department of Pathology, McMaster University, Hamilton, Ontario, Canada

M. A. ROTHSCHILD Nuclear Medicine and Radioisotope Service, Veterans Administration Hospital, First Avenue at East 24th Street, New York, NY 10010, USA

W. STROBER Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014, USA

Y. TAKEDA Department of Medicine, University of Colorado Medical Center, 4200 East Ninth Avenue, Denver, Colorado 80220, USA

A. S. TAVILL MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ

P. VESIN 31, rue de Moscou, Paris 8, France

T. A. WALDMANN Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014, USA

Editors: G. E. W. WOLSTENHOLME and MAEVE O'CONNOR

* *Present address:* Department of Medicine, University of Liverpool, P.O. Box 147, Liverpool L69 3BX

Chairman's introduction

A. S. MCFARLANE

MRC Clinical Research Centre, Northwick Park, Harrow, Middlesex

This group has been meeting to discuss aspects of protein metabolism biennially for the last ten years and a chairman's introduction to this particular symposium is hardly necessary. The main idea of the organizers of the first meeting in Paris was to bring together clinicians and basic scientists interested in the application of isotopes. At Paris and at subsequent meetings, the subject of leakage of plasma proteins into the gastrointestinal tract dominated the proceedings. At Stockholm and later in New York, it became evident that plasma protein metabolism was beginning to assume pride of place, but there were also increasing numbers of papers describing results obtained with the perfused liver. The present meeting is termed 'protein turnover' rather than 'plasma protein turnover' because we knew that people would inevitably speak about plasma proteins, but we wanted them to feel free to discuss also the intracellular biochemistry of protein biosynthesis.

Another innovation was to invite some immunologists, among whom John Humphrey was the only one able to accept. It has become obvious in recent years that the combination of the sensitivity of isotopic techniques with the specificity of immunological ones forms an unusually powerful tool in every aspect of biological research and we may yet find that immunologists will provide a new slant on protein turnover.

You will be able to judge from what follows whether these two modifications to the normal procedure were wise. However, the group cannot meet indefinitely without having some kind of descriptive title to indicate its general interests. It is not satisfactory to continue using a vaguely defined charter relating to the applications of isotopes in clinical medicine and veering with the wind of change towards whichever application happens to be popular at the time. Some of us feel that the time is approaching, and may even come at this meeting, when a general title must be found which need not necessarily

govern each individual symposium but which should have enough continuity to cover broadly the group's activities over the next 20 or 30 years. I would venture to suggest that possibly the subject of protein turnover might be considered.

I was quite a young man when turnover was discovered by Schonheimer, Rittenberg and others, and my feeling was, and still is, that this discovery was inevitable at the time. It seems to me that the essential contributions of these pioneers lay in persuading the chemists to synthesize the labelled amino acids, in knowing just enough about the crude counting or mass spectrometric techniques available at that time to be able to use them, and in having enough biochemical insight to realize that they were not dealing just with contamination of their proteins by the injected labelled amino acids. With those three essentials the qualitative discovery of turnover came naturally. Since then, it has taken more pedestrian workers practically a lifetime to try to introduce quantitative aspects into the subject of protein turnover. In particular, it has taken us nearly 30 years to demonstrate, even in one instance, and by independent isotopic techniques, the truth of a simple corollary of turnover, namely that in an adult animal in nutritional equilibrium, the absolute amount of a substance synthesized in a given time must equal the absolute amount catabolized.

I wonder how much longer it will take us to measure, in patients, the difference between these two rates. After all, disease, at least in any nutritional context, implies a difference between synthetic and catabolic rates, and we as a group ought to be able to measure this. So I hold that protein synthesis will be important in clinical medicine for a long time to come and I expect that even 50 years from now papers will still be appearing on the intermediate biochemistry of protein synthesis. If a title is necessary, this is one that might be considered. I don't think our founder members who originally had the idea of discussing the application of isotopes in clinical medicine need fear that such a title might represent too serious a diversion into the field of academic biochemistry. Whether we want biochemistry or not, we are going to get it in the next 20 or 30 years. It is interesting in this particular programme that only the last three papers deal mainly with patients—a surprising number of our clinical members having elected to present or participate in papers concerned with the isolated perfused liver, and largely dealing with protein synthesis. A title such as protein turnover would therefore still cover their activities and I put it to you as a suggestion. Towards the end of the meeting, Dr Rothschild will have something to say about the need for forming an international group with some continuity about it, and then of course the question of a title may become important.

Previous meetings

(1) [Paris, 1961]: Schwartz, M. & Vesin, P. (eds.) (1962) *Plasma Proteins and Gastrointestinal Tract in Health and Disease*, Munksgaard, Copenhagen
(2) [Bruges, 1963]: Peeters, H. (ed.) (1964) *Protides of the Biological Fluids* (12th colloq.) Elsevier, Amsterdam
(3) [Grindelwald, 1964]: Koblet, H. *et al.* (ed.) (1965) *Physiology and Pathophysiology of Plasma Protein Metabolism*, Huber, Berne
(4) [Stockholm, 1967]: Birke, G., Norberg, R. & Plantin, L.O. (eds.) (1969) *Physiology and Pathophysiology of Plasma Protein Metabolism*, Pergamon, Oxford
(5) [New York, 1969]: Rothschild, M. & Waldmann, T. (eds.) (1970) *Plasma Protein Metabolism: Regulation of Synthesis, Distribution and Degradation*, Academic Press, New York

This Page Intentionally Left Blank

The role of cell-surface receptors in the transport and catabolism of immunoglobulins

THOMAS A. WALDMANN and E. ANTHONY JONES*

Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Abstract The transport of serum proteins across the gastrointestinal tract of neonatal rats was used as a model for studying the mechanism of transcellular transport of proteins and, by inference, one of the factors controlling the rate of protein catabolism. The uptake of immunoglobulin G (IgG) molecules by intestinal cells and their transcellular transport involved specific saturable processes not shared by other classes of immunoglobulins. The first step in intestinal transport of IgG appears to include the formation of a complex of high molecular weight between these molecules and an IgG-specific receptor on enterocyte microvillous membranes. The Fc piece is the submolecular region of the IgG molecule that is required for interaction with the cell surface receptor and for specific uptake and transport of the whole molecule.

In related studies, IgG complexes of high molecular weight were also formed in carcass homogenates after radioiodinated IgG had been administered intravenously to germ-free mice. Because of the IgG-specific concentration–catabolism effect these mice had a low serum concentration of IgG and a long survival of IgG. A major process in both the specific IgG transport between the mother and neonatal rat and the IgG concentration–catabolism effect may be competition for a limited number of saturable receptors on cell membranes that are specific for the IgG molecule. Such receptors would protect the immunoglobulin from catabolism and would be necessary for the specific cellular uptake and transport of this molecule.

The different classes of immunoglobulin molecules have different patterns of distribution and different rates of synthesis and catabolism. For example, in normal individuals the fractional catabolic rate of the immunoglobulins ranges from 6.3% of the intravascular pool for IgG to 72% for IgE (Waldmann & Strober 1969). Physiological factors have been described that affect the survival of different classes of immunoglobulin molecules in different ways. The effect of

* Present address: Department of Medicine, University of Liverpool.

IgG concentration on the fractional catabolic rate of IgG molecules is an example of such a physiological factor affecting the metabolism of one immunoglobulin class (Fahey & Robinson 1963; Waldmann & Strober 1969). In addition, certain immunoglobulin molecules are transported across cells by predominantly saturable processes that are specific for one immunoglobulin class (Brambell 1970; Jones & Waldmann 1972). Finally abnormalities in the concentration of an immunoglobulin in the serum may result from a variety of pathophysiological mechanisms, including disorders of endogenous catabolic mechanisms affecting a single immunoglobulin class (Wochner *et al.* 1966).

Processes of transport or destruction, which affect all plasma proteins equally, clearly cannot explain these findings adequately. In addition, known differences in the size or charge of immunoglobulin molecules or differences in specificity of protein catabolic enzymes do not appear to be sufficient to explain the marked differences in the patterns of catabolism and transport between different classes of immunoglobulins. Accordingly, we have directed our studies towards defining other mechanisms. In particular we have studied the interaction of immunoglobulin molecules with cell membranes to determine whether cell-surface receptors are of significance in controlling the metabolism of these molecules.

The transport of serum proteins across the gastrointestinal tract of neonatal rats was used as a model for studying the transcellular transport of proteins and, by inference, the mechanisms controlling the rate of immunoglobulin catabolism. Transference of passive immunity from mother to young occurs predominantly during the first 21 days after birth in the rat (Halliday 1955). Antibodies in the maternal colostrum or milk are transported across the intestinal mucosa into intestinal lymphatics (Clark 1959). This process is selective, shows species specificity, can be competitively inhibited and is associated with protein catabolism (Halliday 1958; Bangham & Terry, 1957; Brambell *et al.* 1958, 1961). A hypothesis for the mechanism of intestinal protein transport in the neonatal rat (Brambell *et al.* 1958; Brambell 1966) has been based on these findings. It was proposed that there is initial non-specific uptake of protein molecules into pinocytic vacuoles of the enterocyte. It was also assumed that those 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 pinocytic vacuoles in enterocytes. The studies reported in this paper were designed to determine, in the neonatal rat: (*a*) whether there is selective intestinal transport of one or more specific classes of immunoglobulin molecules; (*b*) whether

intestinal uptake and transport of any selectively transported protein are both specific and saturable processes; and (*c*) whether any binding of protein molecules to specific cell surface membrane receptors can be demonstrated in association with transport; and, in the germ-free mouse: (*d*) whether the formation of IgG–membrane complexes can be related to the concentration–catabolism effect that regulates IgG survival.

TRANSCELLULAR TRANSPORT OF PROTEINS IN THE NEONATAL RAT

All the studies reported here on the transport of proteins were conducted in rats aged 12—14 days, as at this age the animals are a convenient size and passive transference of immunity from the mother is still occurring (Halliday 1955). Solutions of purified undenatured proteins, labelled with either ^{131}I or ^{125}I and containing unlabelled albumin, were injected into the duodenal end of a segment of gut consisting of almost all the small intestine isolated by ligatures at laparotomy. The animals were usually killed four hours after injection. The small intestine was removed, divided, washed with saline and homogenized. The eviscerated carcass was also homogenized. Samples of the intestinal homogenate, intestinal washing and carcass homogenate were counted for radioactivity. The supernatants of the same samples were also counted after removal of proteins by precipitation with 10% trichloroacetic acid and the protein-bound radioactivity in each sample was calculated. In these studies transport is defined as the proportion of the administered dose of labelled protein transferred from the intestinal lumen to the circulation over a particular time interval.

Appreciable quantities of rat IgG, mouse IgG, rabbit IgG and all four subclasses of human IgG had been transported to the animal's circulation (10—35% of administered dose) by four hours after injection. In contrast, there was little transport of human IgM, IgA, IgD, IgE, transferrin, ceruloplasmin, albumin and polyvinylpyrrolidone by this time (1.5—4.9% of administered dose). Our results are thus consistent with data obtained in earlier studies (Halliday 1955; Bangham & Terry 1957; Morris 1967; Brambell 1970) and extend them by demonstrating the highly selective IgG-specific nature of intestinal transport of protein in the neonatal rat. Transport of the Fc piece of rabbit IgG was considerable (12.6%), whereas that of the Fab piece was minimal (1.7%). The transport of rat, mouse, rabbit and human IgG was significantly greater than that of sheep IgG, which confirms the species-specificity of the process in rats reported by others (Halliday 1955; Brambell *et al.* 1958).

The transference of antibodies to the neonatal rat can be inhibited by human

serum or by a fraction containing gammaglobulins obtained by electrophoresis (Brambell *et al.* 1958; Brambell 1966). Our data extend these observations by demonstrating that intestinal transport of labelled IgG is markedly inhibited by purified unlabelled IgG. The percentage of intraduodenally administered IgG transported by four hours decreased from 29.8% in animals receiving labelled human IgG (less than 0.1 mg IgG in administered dose) without an excess of unlabelled IgG, to 1.4% in animals receiving labelled IgG and 8 mg of unlabelled human IgG. The absolute quantity of human IgG transported in four hours, determined from the product of the amount (in mg) of IgG introduced into duodenum and the fraction transported, increased with increasing IgG concentrations and approached a limit of 0.12 mg when 1 mg of IgG was administered along with the labelled IgG. Further increases in the quantity of unlabelled IgG administered did not result in increases in the absolute quantity of IgG transported. These data suggest that a major process in IgG transport is saturable.

INTESTINAL UPTAKE OF PROTEINS

It has generally been assumed that the initial intestinal uptake of macromolecules, including immunoglobulins, is by a non-specific 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 (Clark 1959; Hemmings 1958; Padykula *et al.* 1966). In none of these studies was a quantitative comparison made of the uptake of different proteins or other substances. In contrast, in the present study uptake of different serum proteins—uptake being defined as the proportion of the administered dose of labelled protein taken up by the intestinal mucosa over a particular time interval—was measured by estimating the protein-bound radioactivity in the carcass plus that in the intestinal wall as a percentage of the administered dose. The mean uptakes of human, rat, mouse, rabbit and sheep IgG and all four subclasses of human IgG were all appreciable by four hours (9—54% of the administered dose) and were all significantly greater than the mean uptakes of human IgM, IgA, IgD, IgE, transferrin, ceruloplasmin and albumin by this time (less than 9% of the administered dose). There was appreciable uptake of rabbit Fc piece (53.8%) whereas the uptake of rabbit Fab piece was small (8.5%). The uptake of human ^{125}I-labelled IgG was significantly reduced by the presence of unlabelled IgG. Thus uptake of labelled IgG involves a process that is inhibitable by unlabelled IgG and is mediated by the Fc region of IgG molecules.

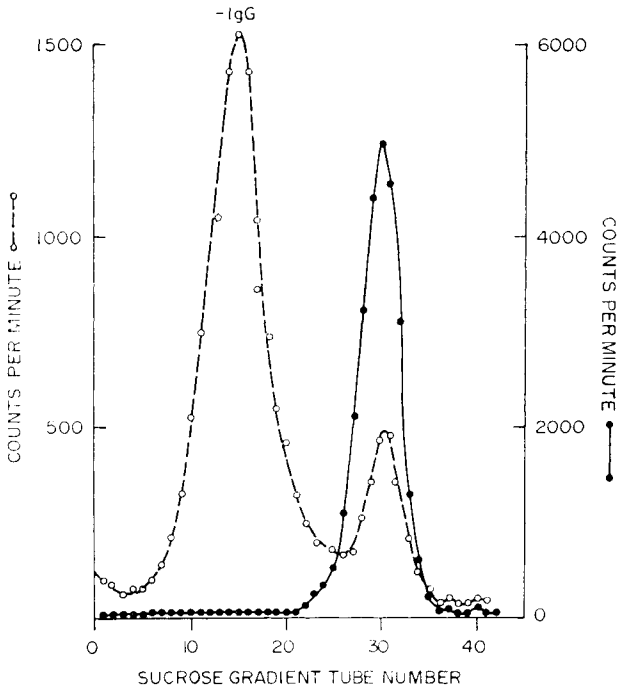

FIG. 1. Distribution of the label of human IgG in a linear 10–45% sucrose ultracentrifugation gradient of a homogenate of the small intestine obtained 45 minutes after the intraduodenal administration of ^{125}I-labelled human IgG (○– – –○). Sedimentation is from right to left. A large amount of the label in the homogenate is associated with complexes of higher molecular weight than the 7S molecules in the starting solution. ●—● Control solution (^{125}I-labelled IgG).

BINDING OF IgG MOLECULES TO RECEPTORS ON ENTEROCYTE MICROVILLI DURING TRANSPORT

Intestinal homogenates obtained after labelled human IgG had been intraduodenally administered were subjected to preparative sucrose-gradient ultracentrifugation. Samples were applied to the top of linear sucrose gradients (10–45%) and then subjected to 90000 *g* for 18 hours (Jones & Waldmann 1972). An appreciable proportion of the labelled IgG in intestinal wall homogenates migrated more rapidly in the sucrose gradient than did the administered 7S molecules (Fig. 1). This suggests that some of the IgG complexed with other proteins or with cell fragments in the homogenates. All four subclasses of human IgG showed evidence of complexing in intestinal homogenates. Complexing was also observed after administration of labelled rabbit Fc piece. No evidence of

complexing was seen in intestinal homogenates after the administration of radioiodinated polyvinylpyrrolidone (PVP), human IgA, IgM, IgE, albumin, transferrin, ceruloplasmin or rabbit Fab piece. When ^{125}I-labelled PVP and ^{131}I-labelled IgG were administered simultaneously, complexing was only observed with the IgG molecules. Thus, those proteins which showed marked intestinal uptake and transport were also those which showed evidence of complexing.

Several studies were designed to define the site within the gastrointestinal tract where complexing occurs, and to identify some of the factors affecting the formation of IgG complexes. In order to determine the site of formation, intestinal washings, homogenates of intestinal wall and plasma were subjected to simultaneous sucrose-gradient analysis two hours after the labelled human IgG had been administered intraduodenally. Complexing of labelled IgG occurred only in the intestinal wall. Thus IgG molecules do not complex in the intestinal lumen but only after these molecules are taken up by the intestinal mucosa. After transport, the labelled IgG molecules in the plasma are no longer associated with complexes but again migrate as 7S molecules. When radioiodinated IgG was placed in different segments of the gastrointestinal tract isolated by ligatures, complexing of IgG in the intestinal mucosa as well as appreciable transport from the lumen to the carcass could be demonstrated only when the labelled IgG was placed in the upper third of the small intestine. No evidence of IgG complexing or significant transport to the carcass was found when the labelled IgG was placed in the stomach, the lower third of the small intestine or the large intestine. No formation of IgG complex in the mucosa could be demonstrated when labelled IgG was placed in the small bowel of animals older than 21 days, that is after transport had ended. Studies of the effect of cortisone acetate on intestinal IgG transport and on IgG complex formation provided further support for the close association of these two processes. Halliday (1959) had reported that in young rats large doses of adrenal steroid hormones prematurely terminated transmission of antibodies from the gut lumen to the circulation. We administered cortisone acetate (2.5 mg) intraperitoneally to rats on the 11th, 12th and 13th day after birth. On the 14th day radioiodinated IgG was administered intraduodenally to these animals and to control rats. The transport of IgG was reduced from 44% of the dose in the control rats to 1.8% in the treated animals. Marked complexing of labelled IgG was demonstrable in the gut wall homogenates of the untreated controls whereas no complexing was seen in the animals treated with cortisone acetate. As already noted (p. 8), transport of labelled IgG was inhibited by an excess of unlabelled IgG. Complexing of labelled IgG could also be virtually completely inhibited by the administration of an excess of unlabelled IgG along

with the labelled preparation. Thus the specific site and physiological conditions associated with significant transport of IgG were also associated with the formation of IgG complexes.

The observed complexing of IgG molecules could theoretically be due to a combination of IgG molecules with specific membrane receptors, with a specific transport protein or with some other compound unrelated to either uptake or 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. No complexing occurs in sites of the gastrointestinal tract where there is no appreciable transport of IgG, and no complexing of IgG can be observed in the gastrointestinal mucosa after selective transport has terminated. In addition, all three processes studied (uptake, transport and complexing of labelled IgG molecules) can be inhibited by unlabelled IgG molecules, suggesting that labelled and unlabelled IgG molecules compete for a limited number of receptors.

The subcellular distribution of labelled IgG was studied to determine whether the observed complexes of high molecular weight represented IgG bound to a carrier protein or to a membrane receptor. Small bowel, excised 90 minutes after intraduodenal administration of labelled protein, was homogenized and then fractionated into cell sap, membrane and organelle, and nuclear fractions by differential centrifugation (Jones & Waldmann 1972). From 65 to 92% of the IgG label in homogenates was associated with fractions containing membranes and organelles. Virtually all the radioactivity in the membrane and organelle fraction migrated as complexes of high molecular weight on sucrose ultracentrifugation gradients. The small amount of IgG label found in the cell-sap fraction showed a distribution comparable to that of 7S molecules in the starting solution, with no evidence of complexing. The IgG label associated with the membrane and organelle fraction did not pass through a 0.45 μm Millipore filter whereas over 97% of the IgG in the cell sap and over 96% of such large labelled proteins as IgM and haemocyanin with molecular weights of 800000 or greater passed through this Millipore filter. These findings support the conclusion that the IgG complexes represent IgG label bound to a membrane receptor rather than bound to a soluble carrier protein.

In an effort to identify the nature of the cellular material complexing with IgG, small intestinal microvillous membranes were purified after radioiodinated IgG had been administered intraduodenally to 12-day-old rats. Enterocyte microvillous membranes, uncontaminated with lysosomes, mitochondria or cell sap, were purified from homogenates of small intestine by the method of Forstner *et al.* (1968). Significant quantities of the label of IgG were bound to these purified membranes. All the radioactivity associated with the microvillous membranes

migrated as complexes of high molecular weight on sucrose ultracentrifugation gradients. These results support the conclusion that the labelled IgG complexes of high molecular weight represent IgG label bound to a membrane receptor rather than to a soluble carrier protein. The presence of receptors for IgG on the surface of enterocytes was further supported by the observation that complexing occurred when labelled IgG was incubated *in vitro* with purified enterocyte microvillous membranes obtained from 12-day-old rats. The complexing of labelled IgG with membrane preparations was completely inhibited if unlabelled IgG (16 mg/ml) was included in the incubation mixtures. No evidence of complexing was observed when rat liver homogenates or membranes, or small intestinal membranes from adult rats, were similarly incubated with labelled IgG *in vitro*.

The interaction between IgG and the receptors on purified microvillous membranes was studied further to confirm that radioactivity bound to the membranes does indeed represent labelled IgG. Complexes of high molecular weight containing the label of IgG obtained either *in vivo* from intestinal homogenates subjected to sucrose gradient ultracentrifugation or *in vitro* from purified enterocyte microvillous membranes were used in these studies and gave essentially identical results. The label of IgG was not released from the complexes of high molecular weight after freezing and thawing, incubation in distilled water or treatment of these fractions with a non-ionic detergent (0.01 M Triton), thereby confirming that the label was not merely trapped in lysosomal vesicles. The binding of the label of IgG to purified membranes or to complexes of high molecular weight was shown to be critically dependent on the pH of the incubating media. All the activity remained bound to membranes when incubation was in buffers of pH 4.0 to 6.5. When the pH was raised to 7.4, 85% of the activity bound to the membranes or complexes was released and at pH 8.0 all the activity was released. All the label released by alkaline buffers showed a distribution comparable to the 7S starting material on sucrose ultracentrifugation gradients and 95% of this released label was precipitable with an antihuman IgG antibody. These studies indicate that the label associated with membrane complexes of high molecular weight is still associated with IgG molecules.

Our data are consistent with a modification of Brambell's hypothesis (Brambell *et al.* 1958; Brambell 1966). We propose that there are specific receptors for the Fc region of IgG molecules on the enterocyte microvillous membrane to which IgG molecules become attached before pinocytic vacuoles form (Fig. 2). The presence of such receptors on the cell surface would explain the specificity of the uptake as well as transport processes. The inhibition of uptake and transport of labelled IgG by unlabelled IgG would reflect competition for a

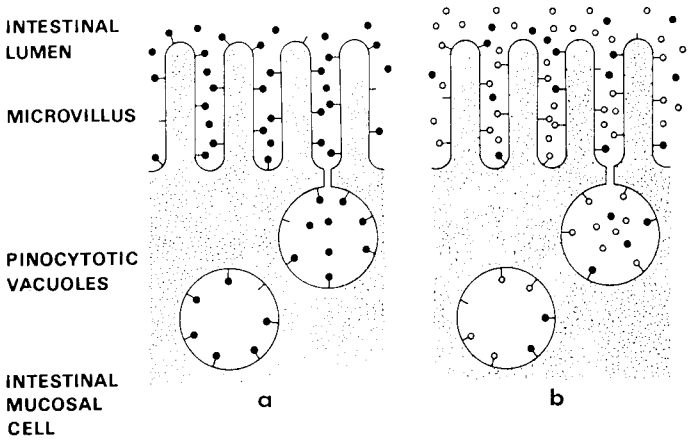

FIG. 2. Schematic representation of the uptake and transport of IgG by the enterocyte of the neonatal rat. It is proposed that there are specific receptors for the Fc region of IgG molecules on the enterocyte microvillous membranes to which IgG molecules become attached before pinocytic vacuoles form. The inhibition of uptake and transport of ^{131}I-labelled IgG (solid circles) by unlabelled IgG (open circles) reflects competition for a limited number of cell surface receptors. Left: ^{131}I-labelled IgG alone; right: ^{131}I-labelled + unlabelled IgG.

limited number of cell-surface receptors. The attachment of IgG molecules to the cell receptors could prevent the degradation of IgG molecules by the contents of both the intestine and pinocytic vacuoles. After transcellular movement pinocytic vacuoles probably release the IgG molecules into the villous lacteal or via the Golgi apparatus into the intercellular spaces. The major effects of minor changes in pH on the binding of IgG to membranes may be of physiological importance in the transport process. Binding of IgG to membranes would be facilitated by the slightly acidic medium of the neonatal small intestine and the release of IgG from receptors would be facilitated by the slightly alkaline extracellular fluids.

THE ROLE OF CELL-MEMBRANE RECEPTORS IN IgG CATABOLISM

The process of specific transport of IgG molecules across the intestinal mucosa of the newborn rat may be related profitably 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, not all these phenomena are mediated by all four subclasses of human IgG. Thus, it has been demonstrated that there are specific receptor sites for only IgG1 and IgG3 on human monocytes (Huber & Fudenberg 1968). Passive cutaneous sensitization in the guinea pig is mediated by IgG1 and IgG3 and to a lesser extent by IgG4 (Terry 1965; Ishizaka *et al.* 1967). In addition IgG1, IgG3, and to a lesser extent IgG2, fix complement (Müller-Eberhard 1968). Since all four subclasses of IgG participate in selective transport in the neonatal rat, this process probably involves a different submolecular structure of the Fc piece than those involved in these other processes.

One process that shares many characteristics with the transport process is the concentration–catabolism relationship, which affects the metabolism of IgG molecules. The concentration–catabolism effect refers to the observation that the fractional catabolic rate for IgG molecules varies directly with the concentration of IgG in the serum in both man and rodents such as the mouse and rat (Fahey & Robinson 1963; Waldmann & Strober 1969), i.e. as the concentration of IgG rises by endogenous production or by infusion, the fractional catabolic rate increases until a limiting concentration is reached. In man the fraction of the intravascular pool of IgG catabolized daily rises from 2% in patients with extreme hypogammaglobulinaemia to a limit of 16–18% in patients with high concentrations of IgG in the serum (Fig. 3). This phenomenon is unique to IgG among the immunoglobulins (Waldmann & Strober 1969). The effect of the serum concentration of IgG on IgG catabolism cannot be explained by shifts of proteins between compartments, nor is it the result of induction of catabolic enzymes (Waldmann & Strober 1969). Both the concentration–catabolism effect and the intestinal transport of IgG molecules in the neonatal rat show IgG specificity, involve all four subclasses of IgG (Morell *et al.* 1970), are mediated through the Fc portion of the IgG molecule (Fahey & Robinson 1963) and have similar species-specificity. In the mouse and rat, human IgG and rodent IgG are equally effective in both processes; sheep IgG is significantly less effective, while the other immunoglobulin classes and other serum proteins are not effective at all (Halliday 1958; Hemmings & Morris 1959; Fahey & Robinson, 1963; Sell 1964; Waldmann & Strober 1969).

A mechanism similar to that proposed to explain the selective transport of IgG across the newborn gut of the rat, that is a saturable protective system specific for IgG molecules, is the most attractive hypothesis so far suggested to account for the concentration–catabolism effect observed with IgG (Brambell *et al.* 1964; Waldmann & Strober 1969). In this model a fraction of the plasma pool is isolated from the circulating protein into a catabolic pool. It is proposed that some IgG molecules become attached to a limited number of specific

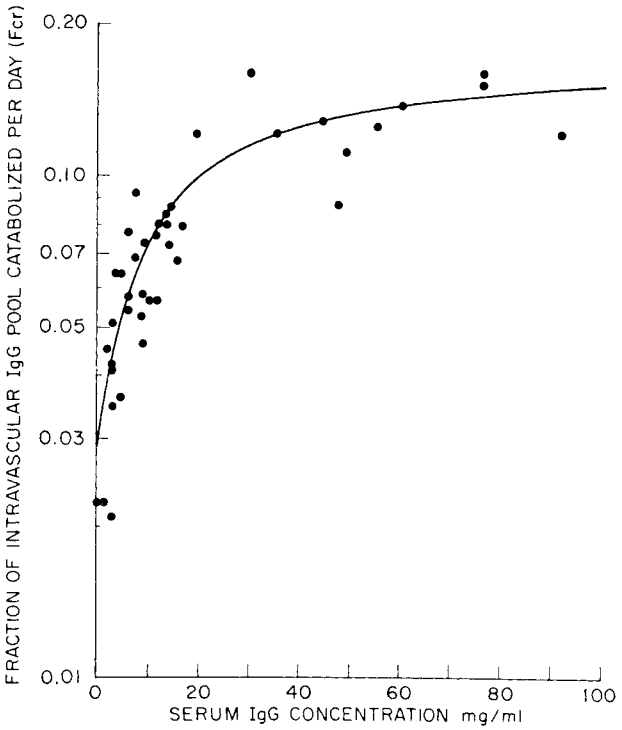

FIG. 3. The relationship between the fraction of the intravascular pool of IgG catabolized per day and the IgG concentration in serum obtained from patients with a wide range of serum concentrations of IgG. The fraction of the intravascular pool of IgG catabolized daily rises from 2% in patients with extreme hypogammaglobulinaemia to a limit of 16–18% in patients with serum IgG concentrations over 30 mg/ml.

protective receptors, perhaps on the walls of pinocytic vacuoles. These molecules are ultimately returned to the circulation whereas all remaining IgG molecules are 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. To provide evidence in support of such membrane binding of IgG we gave germ-free mice intravenous injections of labelled IgG and then used techniques, including sucrose-gradient ultracentrifugation, comparable to those used in the studies of IgG transport (pp. 9–10). IgG complexes of high molecular weight were observed in homogenates of the eviscerated carcasses of germ-free mice four hours after labelled IgG had been administered intravenously (Fig. 4). No such complexes of high molecular weight were

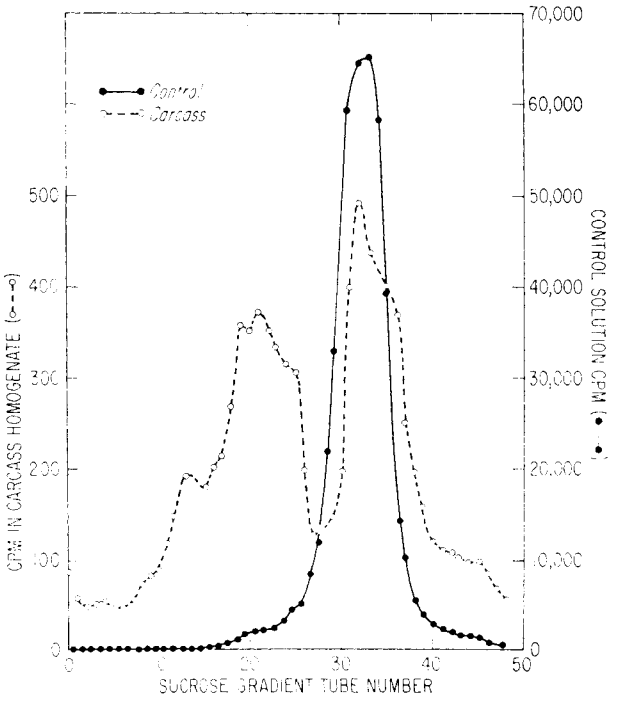

FIG. 4. Sucrose-gradient ultracentrifugation pattern of a homogenate of the eviscerated carcass of a germ-free mouse 4 hours after the intravenous administration of ^{125}I-labelled IgG. An appreciable quantity of the label in the homogenate is associated with complexes of higher molecular weight than the 7S molecules in the starting solution.

demonstrable in similarly treated mice that had received 50 mg of IgG intraperitoneally the day before labelled IgG was injected. Thus, it appears that a major process in both the transport of IgG between the maternal and neonatal rat and the concentration–catabolism effect for IgG may well involve competition for a limited number of saturable membrane receptors that are specific for IgG and protect this molecule from catabolism.

ACKNOWLEDGEMENT

E. A. Jones was in receipt of a United States Public Health Service International Post-doctoral Fellowship.

References

BANGHAM, D. R. & TERRY, R. J. (1957) The absorption of ^{131}I-labelled homologous and heterologous serum proteins fed orally to young rats. *Biochem. J.* **66**, 579-583

BRAMBELL, F. W. R. (1966) The transmission of immunity from mother to young and the catabolism of immunoglobulins. *Lancet* **2**, 1087-1093

BRAMBELL, F. W. R. (1970) *The Transmission of Passive Immunity from Mother to Young* (*Front. Biol.*, vol. 18), North-Holland, Amsterdam

BRAMBELL, F. W. R., HALLIDAY, R. & MORRIS, I. G. (1958) Interference by human and bovine serum and serum protein fractions with the absorption of antibodies by suckling rats and mice. *Proc. R. Soc. Lond. B Biol. Sci.* **149**, 1-11

BRAMBELL, F. W. R., HALLIDAY, R. & HEMMINGS, W. A. (1961) Changes in ^{131}I-labelled immune bovine γ-globulin during transmission to the circulation after oral administration to the young rat. *Proc. R. Soc. Lond. B Biol. Sci.* **153**, 477-489

BRAMBELL, F. W. R., HEMMINGS, W. A. & MORRIS, I. G. (1964) A theoretical model of γ-globulin catabolism. *Nature* (*Lond.*) **203**, 1352-1355

CLARK, S. L. (1959) The ingestion of proteins and colloidal materials by columnar absorptive cells of the small intestine in suckling rats and mice. *J. Biophys. Biochem. Cytol.* **5**, 41-50

FAHEY, J. L. & ROBINSON, A. G. (1963) Factors controlling serum γ-globulin concentration. *J. Exp. Med.* **118**, 845-868

FORSTNER, G. G., SABESIN, S. M. & ISSELBACKER, K. J. (1968) Rat intestinal microvillous membranes: purification and biochemical characterization. *Biochem. J.* **106**, 381-390

HALLIDAY, R. (1955) The absorption of antibodies from immune sera by the gut of the young rat. *Proc. R. Soc. Lond. B Biol. Sci.* **143**, 408-413

HALLIDAY, R. (1958) The absorption of antibody from immune sera and from mixtures of sera by the gut of the young rat. *Proc. R. Soc. Lond. B Biol. Sci.* **148**, 92-103

HALLIDAY, R. (1959) The effect of steroid hormones on the absorption of antibody by the young rat. *J. Endocrinol.* **18**, 56-66

HEMMINGS, W. A. (1958) Protein selection in the yolk-sac splanchnopleur of the rabbit: The total uptake estimated as loss from the uterus. *Proc. R. Soc. Lond. B Biol. Sci.* **148**, 76-83

HEMMINGS, W. A. & MORRIS, I. G. (1959) An attempt to affect the selective absorption of antibodies from the gut in young mice. *Proc. R. Soc. Lond. B Biol. Sci.* **150**, 403-409

HUBER, H. & FUDENBERG, H. H. (1968) Receptor sites of human monocytes for IgG. *Int. Arch. Allergy Appl. Immunol.* **34**, 18-31

ISHIZAKA, T., ISHIZAKA, K., SALMON, S. & FUDENBERG, H. H. (1967) Biologic activities of aggregated γ-globulin. *J. Immunol.* **99**, 82-91

JONES, E. A. & WALDMANN, T. A. (1972) The mechanism of intestinal uptake and transcellular transport of IgG in the neonatal rat. *J. Clin. Invest.* **51**, 2916-2927

MORRELL, A., TERRY, W. D. & WALDMANN, T. A. (1970) Metabolic properties of IgG subclasses in man. *J. Clin. Invest.* **49**, 673-680

MORRIS, I. G. (1967) The transmission of bovine anti-*Brucella abortus* agglutinins across the gut of suckling rats. *Immunology* **13**, 49-61

MÜLLER-EBERHARD, H. J. (1968) Chemistry and reaction mechanisms of complement. *Adv. Immunol.* **8**, 1-80

PADYKULA, H. A., DEREN, J. J. & WILSON, T. H. (1966) Development of structure and function in the mammalian yolk sac. I. Developmental morphology and vitamin B_{12} uptake of the rat yolk sac. *Dev. Biol.* **13**, 311-348

SELL, S. (1964) Evidence for species differences in the effect of serum γ-globulin concentration on γ-globulin catabolism. *J. Exp. Med.* **120**, 967-986

TERRY, W. D. (1965) Skin-sensitizing activity related to γ-polypeptide chain characteristics of human IgG. *J. Immunol.* **95**, 1041-1047

WALDMANN, T. A. & STROBER, W. (1969) Metabolism of immunoglobulins. *Prog. Allergy* **13**, 1-110

WOCHNER, R. D., STROBER, W. & WALDMANN, T. A. (1966) Accelerated breakdown of immunoglobulin G (IgG) in myotonic dystrophy: a hereditary error of immunoglobulin catabolism. *J. Clin. Invest.* **45**, 321-329

Discussion

Vesin: Do you think that transport of protein across the gastrointestinal tract, as in your model, plays a role in IgG metabolism *in vivo*?

Waldmann: Yes, I think that this is how antibodies are transported to the neonatal rat. I also think that the same mechanism of transcellular transmission is involved in the specific placental transport of IgG between the mother and the foetus in humans. In addition the studies of IgG metabolism in germ-free mice suggest that binding to receptors may play an important physiological role in controlling the catabolism of IgG molecules in normal man and rodents.

Vesin: Your studies were done with normal enterocytes. Did you try giving neomycin, which would produce a lesion of the small intestinal villi, to see whether any specific structure plays a part in this transport?

Waldmann: No, but we are using affinity chromatography, with IgG bound to columns, to try to isolate a specific enterocyte membrane receptor that interacts with IgG.

Munro: Your very beautiful demonstration suggests that there are two steps: binding and subsequent transfer to the pinocyte. Could a pulse-chase type of experiment be done to validate the sequence? The labelling at the membrane is deleted by a cold chase, whereas the pinocytes in the isolated cell fraction continue to accumulate label.

Waldmann: We have not done the pulse chase experiments you suggest, but we have found that on incubation at 37°C unlabelled IgG will gradually displace labelled IgG from the membrane.

Munro: Is there any evidence to show which part of the Fc fragment is involved? Is it runs of lipid-soluble amino acids similar to those which the membrane enzymes appear to have for their anchorage?

Waldmann: We do not know which part of the Fc region is involved in the membrane binding.

Oratz: I was particularly intrigued by the pH effect and the binding. Is the Fc piece particularly rich in imidazole groups, that is histidine residues, as one might think from the sensitivity of the binding between pH 7 and 8?

Waldmann: I do not know the amino acid sequence of the Fc region of IgG molecules, so I cannot define the chemical groups that are of importance in determining the critical pH effect.

Humphrey: Professor Brambell would have been very pleased to hear of what looks like the detailed solution to his general hypothesis about regulation of immunoglobulin catabolism (Brambell *et al.* 1964).

Waldmann: In this field we are standing on the shoulders of a giant. The excellent work and incisive thinking of Professor Brambell form the basis of these studies.

Humphrey: These receptors must be receptors for common features between IgG in different species, which already provides a clue. Dr Eva Orlans has been immunizing birds with different mammalian IgGs and she then easily finds cross-reactions, which suggest that there are common structural features. This sort of reagent might also give you a clue as to what the receptor on those IgG molecules is. I am very interested by the clear demonstration from the varying binding of Ig sub-classes that the receptor involved in this transport mechanism must be different from those on the macrophage, and from that on the mast cell which binds IgE, even from other species. This adds one more dimension to the varying qualities of the immunoglobulin molecule. Various reagents seem to be able to attach themselves to immunoglobulins—presumably via carbohydrate groups—such as the staphylococcal protein, phytohaemagglutinin, concanavalin and so on. You might have some chance of seeing whether one or other of these in small doses would compete with the receptors on the villi. You have many different available approaches with this very elegant technique.

Waldmann: One may also cleave the Fc region of the IgG molecule with cyanogen bromide, then radioiodinate the fragments and determine which ones bind to the enterocyte receptor. In addition one can put ferritin on the IgG molecules and follow the phases of IgG binding, uptake and transport by electron microscopy.

Milhaud: Does the transport process more or less stop at the 22nd day of life? Do all parts of the small intestine display the same pattern as you showed? You showed quite conclusively that displacement by the same protein occurs when it is unlabelled, but what do other proteins do in your system?

Waldmann: The process of IgG transport begins to decline at about the 18th day of life in the rat and is essentially absent by the 21st day of life. Only the upper third of the small intestine can clearly be shown to be involved in IgG uptake, transport or binding. Proteins such as albumin or transferrin *in vivo* or *in vitro* do not inhibit this phenomenon.

Milhaud: Why does it stop at 21 days?

Waldmann: Many other alterations in the gastrointestinal function of the rat occur when transport ceases. At this time the stomach begins to secrete hydrochloric acid and proteolytic enzymes. The alkaline phosphatase of the small intestine increases markedly and goblet cells begin to appear. The primary

reason for the cessation of specific uptake and binding may be the synthesis of new cells at the base of the villi that do not have receptors for IgG molecules. Some immunofluorescence studies show that during the first 18 days IgG is present in all enterocytes, but in the subsequent three days only the cells nearer to the tips of the villi are capable of incorporating IgG. I think that with steroids one is causing a premature differentiation of enterocytes, with production of a different line of enterocytes that do not have IgG receptors on their surface.

Rothschild: Does the age limitation indicate that other mechanisms regulate catabolism after 21 days?

Waldmann: There is no age limitation in the concentration–catabolism effect of germ-free animals. We have shown that a process requiring interaction of IgG molecules with cell surface receptors is involved in this concentration–catabolism effect that continues throughout life and in the transport process that is limited to the neonatal period.

Regoeczi: Secretory IgA, as compared with serum IgA, is markedly resistant to proteolysis and this difference has been attributed to the presence of the secretory component. Could the secretory component or some similar attachment be responsible for the protection of IgA against catabolism during transport across the gut? Is it only a membrane that is attached to the IgG, or could there be a protein component in addition? If the membrane is lysed completely, for example, would the molecular weight of the transported immunoglobulin molecule be normal?

Waldmann: I feel that the receptor for IgG is a protein. In contrast to the secretory component of secretory IgA the receptor protein for IgG appears to be an integral part of the enterocyte membrane. We reach this conclusion on the basis of studies indicating that the IgG bound to the receptor is very large. The complex appears in the pellet fraction after centrifugation at 2000 *g* for 30 minutes, it does not pass through a 0.45 μm Millipore filter and is precipitable with an anti-enterocyte antibody. In contrast IgA coupled to the secretory piece is a small unit of 390000 molecular weight that passes through the filter easily. When we disrupt the membrane–IgG complex with sodium dodecyl sulphate or high concentrations of the detergent Triton the released radioactive material has the same mobility on sucrose ultracentrifugation as does the IgG starting material. Thus it is either free IgG or IgG with a very small part of the membrane attached to it.

Vesin: Have you established a relationship between this mechanism and the binding of immunoglobulin to circulating lymphocytes?

Waldmann: Immunoglobulins or aggregates of immunoglobulins have been shown to bind to receptors on B-type, thymic-independent lymphocytes. I do not know the sub-class specificity of this lymphocyte binding and therefore