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INTESTINAL ABSORPTION AND DEGRADATION OF RAT AND BOVINE γ-GLOBULINS IN THE SUCKLING RAT

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SUMMARY

When ^{125}I-labelled rat IgG and ^{131}I-labelled bovine IgG were fed together to 12-days-old rats, they appeared to pass intact into the circulation, the homologous globulin being transmitted preferentially. 3 h after feeding the immunoglobulin mixture, the maximum distribution of radioactivities occurred in the distal rather than the proximal portion of the small intestine, and there was a significant change in the ratio of the concentration quotient values (^{125}I:^{131}I) from 0.81 at the pyloric to 1.46 at the distal end of the small intestine. The results of the gel filtration of the luminal contents of the small intestine of fed young rats showed that an effective proteolysis of the administered immunoglobulins occurred whithin the alimentary tract. The optimum pH for proteolysis was 2.0 for stomach contents, 8.0 for intestinal washes, and 3.4 for intestinal wall extracts.

INTRODUCTION

The young rat for the first few weeks after birth acquires maternal antibodies from the colostrum (HALLIDAY[1,2]). The ability to absorb colostral antibodies into the circulation persists for the first three weeks after birth; during this period, serum antibodies produced in other species and fed to young rats are also transmitted intact across the epithelial cells of the small intestine (HALLIDAY[1]). Transmission is selective, in that rabbit agglutinins are transmitted less readily than rat agglutinins, whereas bovine or fowl agglutinins are not transmitted in detectable quantitities. Possibly the latter are absorbed from the gut, but are degraded before they reach the circulation. The work of BANGHAM AND TERRY[3], and BRAMBELL *et al.*[4], on the transmission across the gut in 14-days old rats of orally administered homologous and heterologous γ-globulins radioactively labelled with ^{131}I has thrown some light on this possibility. Whereas the absorption of the radioactively labelled proteins from the gut was nearly complete, over 80 % of the administered doses were broken down, presumably within the absorptive mucosal cells. Our knowledge of the cellular absorption of ingested proteins in the young rodent was advanced by CLARK[5] using the electron microscope. He located the absorbed proteins within the pinocytotic vesicles of the mucosal epithelial cells of the jejunum and ileum.

The present work was undertaken to study further the intestinal absorption

and degradation in the young rat of bovine and rat γ-globulins administered orally together with the object of determining whether these proteins are transmitted by common or disparate transport systems.

MATERIALS AND METHODS

Animals

Most of the previous data from this laboratory concerning the transfer of immunoglobulins across the gut of the young rat relates to the 10 to 14 days old animal (BRAMBELL[6]). At this age, the transfer efficiency is maximal (JORDAN AND MORGAN[7]). For comparative reasons, the experiments in this study were performed on 12-days-old albino rats of a Wistar strain. They were fed with radioactively labelled IgG of rat or bovine origin, using a polythene stomach tube. Each animal was killed (with chloroform) 3 h after feeding and bled from the heart. The radioactivity of the serum was expressed as a concentration quotient (C.Q.) (BATTY *et al.*[8]), which is the ratio of the activity in the serum to that in the dose administered. The small intestine from the pylorus to the ileocaecal junction was gently stripped from the mesentery and flushed with 5 ml of ice cold 0.9% saline. These washings, together with the stomach and the small intestine, were retained separately for counting.

Preparation of proteins

Rat and bovine IgG were separated from pooled adult sera by Na_2SO_4 fractionation (KECKWICK[9]) and ion-exchange chromatography on DEAE-cellulose (REISFELD AND HYSLOP[10]), using 0.02 M phosphate buffer of pH 8. The chromatographic eluates were concentrated by negative pressure dialysis against 0.9% saline and their purity confirmed by immunoelectrophoresis. These rat and bovine proteins were radioactively labelled with ^{125}I and ^{131}I, respectively, at a level of 0.5 atoms of iodine per molecule, by the electrolytic iodination procedure of ROSA *et al.*[11]. Electrolysis was continued for 20 min at 0.5 mA and the unbound iodide removed by dialysis against several changes of 0.9% saline at 2°.

Estimating radioactivity

Samples for counting were placed in 4-ml disposable tubes and their radioactivities determined with a Panax double channel scintillation well counter. Protein-bound and protein-free radioactivities were estimated in liquid samples after precipitating the proteins with 10 vol. of 1% tungstic acid, centrifuging, and counting the radioactivities of the sediment and supernatant, respectively. Chromatographic examination of the tungstic acid soluble fractions of the gut contents of fed rats, on Whatman No. 1 paper using an ascending solvent (butanol–acetic acid–water, 65:17:17, by vol.), established that the major proportion of the radioactivity was attributable to mono- and di-iodotyrosine. These degradation products indicated that the release of non-protein radioactivity was due to proteolysis and not simply to dissociation of radio-iodine from labelled protein.

Molecular filtration

Gel filtration was performed on a 2.5 cm × 50 cm column of Sephadex G-100 at room temperature with phosphate buffered saline (0.02 M sodium phosphate buffer

(pH 7.0), 0.9 % NaCl). The column was first calibrated for molecular weight determinations by running on it 10-mg samples of bovine albumin (65 000), pepsin (36 000) and cytochrome *c* (13 000).

Proteolytic assays

The pH optima for the proteolytic activities of various extracts were determined using a series of 0.2 M acetate (pH 2–6) or phosphate (pH 6–9) buffers. Volumes of 0.1 ml of a given extract were incubated for 30 min at 37° with 0.5 ml of a 1.5 % heat-denatured haemoglobin solution in the appropriate buffer. After incubation, undigested protein was precipitated with 2.5 ml of 0.3 M trichloroacetic acid and the concentration of the liberated amino acids in the filtrate estimated in terms of the absorbance (*A*) at 280 nm. A reagent blank was carried out at the same time by adding trichloroacetic acid to the substrate before the enzyme extract. The distribution of catheptic activities in 0.9 % saline extracts of intestinal segments (200 mg wet tissue/ml) were determined in a similar way at a pH of 3.6 but in the presence of 0.01 M cysteine (PRESS *et al.*[12]).

RESULTS

Intestinal transmission of IgG

Young rats were fed with 0.1 ml of a mixture containing 0.5 mg of both radioactively labelled rat and bovine IgG. They were killed and bled after 3 h, and their

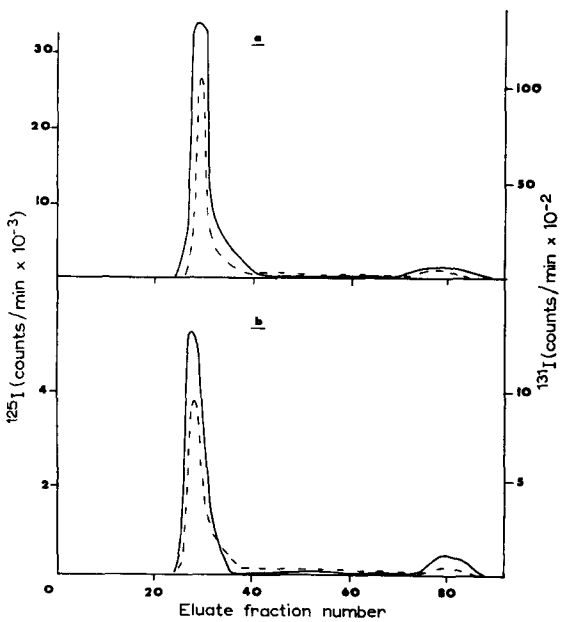

Fig. 1. The radioactivities of Sephadex G-100 eluate fractions of (a) a mixture of ^{125}I-labeled rat IgG (——) and ^{131}I-labelled bovine IgG (- - -), and of (b) the pooled sera of young rats fed with this mixture.

sera pooled and subjected to gel filtration. Another portion of the IgG mixture used for feeding was also subjected to gel filtration. The radioactivities of the column eluate fractions are given in Fig. 1. Evidently the bulk of the radioactivities appearing in the circulation of fed rats (Fig. 1b) is due to labelled components of molecular weights similar to those of the administered globulins (Fig. 1a). The remaining activities are attributable to small molecular weight degradation products such as labelled amino acids. No intermediate molecular weight degradation products were separated, which supports the contention that the radioactively labelled rat and bovine proteins appearing in the circulation of fed rats were transmitted across the gut intact.

The transmission of labelled rat and bovine IgG across the gut of young rats was then estimated quantitatively in each of 23 aminals fed with 0.1 ml of the mixture of these proteins. The mean protein-bound radioactivities of the sera of the fed rats, expressed as C.Q's, and their standard errors were 0.0122 ± 0.0004 for rat IgG and 0.0093 ± 0.0003 for bovine IgG. It is evident from the ratio of the C.Q's that the rat IgG was transmitted intact across the gut 1.32 times more readily than the bovine IgG. On the assumption that the mean plasma volume of the 12-days-old rats is 1 ml (BRAMBELL[6]), only 12% of the rat IgG and 9% of the bovine IgG administered could be recovered intact in the circulation.

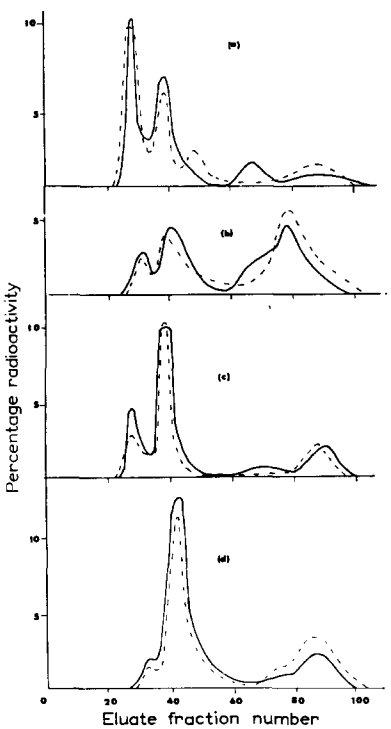

Fig. 2. The radioactivities of Sephadex G-100 eluate fractions of washings of the intestinal lumen (a,b) and of intestinal wall extracts (c,d) from young rats fed 90 min (a,c) or 180 min (b,d) previously with a mixture of ^{125}I-labelled rat IgG (——) and ^{131}I-labelled bovine IgG (- - -). For each filtration, the radioactivity of each fraction is expressed as a percentage of the total activity eluted from the Sephadex column.

The distribution of radioactivity in the gut lumen of young rats fed with radio-iodinated IgG

A determination of the protein bound and free radioactivities of the stomach contents of young rats, fed 3 h previously with radio-iodinated rat (^{125}I) and bovine (^{131}I) IgG, showed that the mean proportion of non-protein to protein bound radioactivities (6:4) had increased considerably over that in the dose administered (1:9). As evidenced by paper chromatography of the degradation products, this was due to proteolysis and not to the dissociation of radio-iodine from the labelled proteins. The pooled small intestinal washings from several young rats, fed 90 or 180 min previously, were subjected to gel filtration (Fig. 2a, b). The distribution of the radioactivities in the eluate fractions were similar for ^{125}I and ^{131}I. Most of the radioactive protein remaining in the intestinal lumen 90 min after feeding was

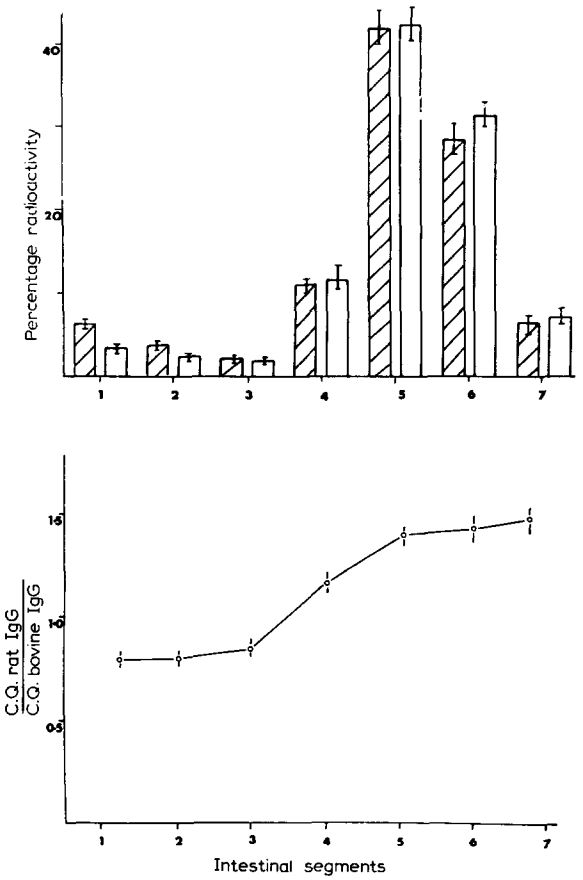

Fig. 3. The distribution of radioactivity in the walls of Segments 1 (pyloric end) to 7 (ileo-caecal end) of the small intestines of 15 young rats fed 3 h previously with an equimolecular mixture of ^{125}I-labelled rat IgG and ^{131}I-labelled bovine IgG. The histogram shows the distribution of ^{125}I (open columns) and ^{131}I (hatched columns) activities along the small intestine, whilst the curve shows the ratio of ^{125}I to ^{131}I activities (corrected to unit value of the radioactivities administered, *i.e.* C.Q. rat IgG:C.Q. bovine IgG) in the segments. Mean values and their standard errors are shown.

attributable to intact labelled IgG, although considerable activities were due to smaller (50 000) molecular weight components. 90 min later, no intact IgG remained in the lumen. The radioactivities were then due partly to 80 000 and 50 000 molecular weight components, but mainly to labelled peptides and amino acids. At this stage, measurements on six young rats showed that 30–40 % of the radioactivity administered could still be accounted for between the stomach and small intestine.

The distribution of radioactivities in the intestinal wall of fed rats

The flushed small intestines from the pylorus to the ileo-caecal junction from each of 15 young rats, fed 3 h previously with the standard mixture of radio-iodinated rat and bovine IgG, were laid out against a centimetre scale, and each cut into seven equal lengths. These segments numbered 1 to 7 from the pyloric to the ileo-caecal end were each approximately 5 cm long. The radioactivities of the segments were determined, and expressed as percentages of the total activities recovered in the intestinal wall, and also in the form of C.Q's. The mean percentages and the mean relative C.Q's are shown in Fig. 3. Most of the radioactivities ($^{125}I + {}^{131}I$) in the small intestinal wall are located in the distal part, mainly Segments 5 and 6. Although the percentage distributions of the radioactivities in the small intestine is similar for ^{125}I and ^{131}I, there is a significant change in the relative C.Q. value ($^{125}I:{}^{131}I$) from 0.80 in the proximal segments to 1.46 in the distal segments ($t = 9.85$, $n = 28$, $P < 0.001$). Pooled Segments 4, 5 and 6 of the small intestines of rats fed 90 or 180 min previously, were homogenised in 0.9 % saline. These two homogenates were frozen and thawed 3 times to effect complete cellular disintegration and then centrifuged. The supernatants were subjected to gel filtration (Fig. 2c, d). The distribution of ^{125}I and ^{131}I radioactivities in the eluate fractions were similar to each other. In rats fed 90 min previously some intact rat and bovine IgG still remained in the

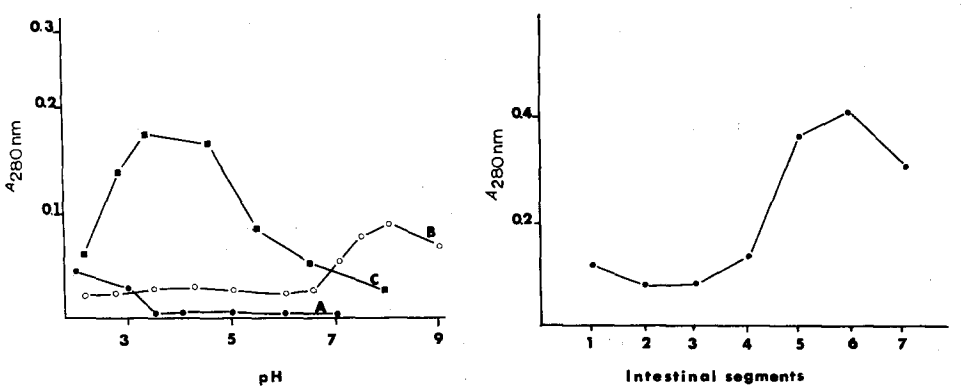

Fig. 4. The proteolytic activities at various pH values of (A) stomach contents, (B) intestinal luminal washings and (C) intestinal wall extracts of 12-days old-rats. The activities are expressed in terms of the optical densities of the amino acids liberated from denatured heamoglobin incubated with the gut preparation under assay conditions.

Fig. 5. The distribution of catheptic activity in the intestinal wall of the 12-days -old rat. The relative activities per 20 mg of wet weight of tissue are expressed in terms of the optical densities of the amino acids liberated when 0.5 ml of a 1.5% solution of denatured haemoglobin is incubated for 30 min at pH 3.6 and 37° with 0.1 ml of the intestinal wall extracts.

intestinal wall, but in rats fed 180 min previously only intermediate molecular weight products (80 000 and 50 000) could be detected in appreciable amounts.

Sites of proteolysis

Considerable proteolysis of ingested IgG is seen to occur in the alimentary tract of the young rodent. The pH optima for the proteolytic activities of stomach contents, small intestinal luminal washings and of small intestinal wall extracts from young rats were determined (Fig. 4), and found to be pH 2.0 for stomach contents, pH 8.0 for the intestinal washings and pH 3.4 for the small intestinal wall extracts. These values possibly correspond to the pH optima for pepsin, trypsin and cathepsin, respectively. Saline extracts of pooled Segments 1–7 of the small intestine from several young rats were also prepared separately and assayed for their catheptic activities at pH 3.6. The segments were homogenised in 0.9 % saline (1 ml per 200 mg of wet tissue) and frozen and thawed 3 times to complete cellular disruption and to release latent cathepsins. Portions of 0.1 ml of the supernatants (corresponding to 20 mg wet weight of tissue) from these homogenates were used for assay (see MATERIALS AND METHODS) and the results are shown in Fig. 5. The most marked catheptic activities were located in Segments 5, 6 and 7.

DISCUSSION

The work described above was facilitated by labelling bovine and rat γ-globulins with radioactive ^{131}I and ^{125}I, respectively, and feeding the labelled proteins together as a mixture. This had the advantages of reducing the number of experimental animals required for the study as well as providing controls for eliminating the differences between the responses of individual animals. These advantages were considered to outweigh any inaccuracies arising from the mutual interfering effects of the IgG's on one another's transfer across the gut, since at the concentration used in these experiments interference between these particular proteins has been shown to be minimal (HALLIDAY[13]) and (BRAMBELL *et al.*[14]). It was assumed during the course of these experiments that any radioactive iodine released by digestion was not re-incorporated into other proteins.

Oral administration of the radio-iodinated rat and bovine IgG mixture to suckling rats resulted in homologous and heterologous labelled protein reaching the circulation. The molecular sizes of the transferred proteins were similar to those of the administered globulins, which supports the contention that a certain proportion of both the rat and bovine molecules escaped digestion and were transferred across the gut intact. However, in agreement with the results of BANGHAM AND TERRY[3] and of BRAMBELL *et al.*[4], quantitative studies showed that only a small proportion of the administered protein reached the circulation intact, with the radioactively labelled rat IgG being transferred only slightly more readily than the labelled bovine IgG. This result contrasts markedly with that of HALLIDAY[1] on the transmission of orally administered serum antibodies in rats between 4 and 23 days of age, when appreciable amounts of rat agglutinins but no bovine agglutinins were transferred intact into the circulation. Possibly, in the latter case, the antibody investigated belonged to a non-IgG class of immunoglobulins and was not trans-

mitted, or was slightly altered during transfer, thereby losing its antibody activity.

Cytological evidence by CLARK[5] and work involving the undigestable colloid polyvinyl pyrrolidone by CLARKE AND HARDY[15], suggest that the distal part of the small intestine in the suckling rat is the main site of macromolecular transport. The present work agrees with these findings in that 3 h after feeding, the distributions of the radioactivities along the intestinal wall were limited almost exclusively to the distal portion.

BAMFORD[16], in his studies on the *in vitro* passage of ^{131}I-labelled rat and bovine IgG across the walls of everted sections of the ileum of the 18-days-old rat, has clearly shown that while both proteins were transmitted to the serosal fluid in appreciable quantities, the rat IgG was transmitted at more than twice the rate of the heterologous globulin. An attempt to demonstrate a similar selection *in vivo* in 12-days-old rats by measuring the ratios of ^{125}I to ^{131}I activities in segments of the intestinal wall yielded results which were considered to be significant, as the determination of these ratios is a very sensitive measurement. The transition from an excess of bovine protein at the pyloric end to an excess of rat protein at the distal end of the intestine remains to be explained. Two main types of theory spring to mind; the first is compatable with the assumption that the heterologous protein is taken up by pinocytosis in equal amounts with the homologous protein, but is degraded and cleared more quickly from the mucosal cells of the ileum. Equally possible in our state of knowledge, is the assumption that quantitatively different mechanisms operate in the different regions of the intestine. This is in keeping with the finding of the very greatly dissimilar absolute activities in the different sections of the tissue.

It has been generally assumed that the immaturity of the luminal digestive system in the suckling rat as demonstrated by HILL[17] ensured the non-digestion of the immunoglobulins as they traverse the stomach and small intestine. The presence of pepsin and trypsin have been confirmed in the gastro-intestinal tract of the 12-days-old rat, and whereas their enzymatic activities may be low, the results of the gel filtration of the gut washes suggest that these activities cannot be ignored. After 3 h in fed rats, a large proportion of the administered radioactivity could still be accounted for between the stomach and small intestine. However, gel filtration of the intestinal washes at this stage indicated that an effective proteolysis had occurred, suggesting further transmission of intact γ-globulin into the circulation was unlikely.

The maximum distribution of cathepsins in the absorptive region of the small intestine could be significant as these enzymes are considered to be typical of lysosomes which have the property of digesting material that has been taken into the cell by endocytosis (DE DUVE[18]). It has also been suggested by BRAMBELL[19] that the lysosomal system could have an important function in the selective transport of immunoglobulins across the intestine of the young rat. Whereas the ileal lysosomal system of the suckling rat may primarily be concerned with the digestion of nutrients at a time when the luminal digestive system is immature, the transmission of immunoglobulins by the same system may be a secondarily derived adaptation, as suggested by JEAL[20] and by WILLIAMS AND BECK[21].

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