BBA 75957

INTESTINAL ABSORPTION AND GASTRO-INTESTINAL DIGESTION OF PROTEIN IN THE YOUNG RAT DURING THE NORMAL AND CORTISONE-INDUCED POST-CLOSURE PERIOD

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(Received November 30th, 1971)
(Revised manuscript received February 23rd, 1972)

SUMMARY

Experiments were performed on 12-day-old cortisone acetate-treated rats, their litter mate controls, and 24-day-old normal rats. While orally administered ^{125}I-labelled rat IgG passed freely into the circulation of 12-day-old rats, barely detectable amounts of this protein was transferred to the sera of 12-day-old cortisone-treated rats and normal 24-day-old rats. There is a large difference between the normal 12- and 24-day-old rat in respect of their peptic and tryptic activities, but the cortisone-treated 12-day-old rat is not significantly different from the normal animal of the same age. A comparison of the gel filtration results for the intestinal washes taken from normal and cortisone-treated 12-day-old rats implies an increased efficiency of the overall process of intra-luminal digestion in the latter animals, intragastric digestion of the administered rat IgG was complete in the 24-day-old rats. The recoveries of radioactivity from the stomachs and intestinal walls of rats fed 3 h previously with ^{125}I-labelled rat IgG differed between representatives of each group.

INTRODUCTION

The gut of the suckling rat is capable of transmitting orally administered antibodies into the circulation for the first 18 days after birth; thereafter this capacity declines rapidly and is completely lost by 21 days of age[1]. Concomitant with this cut off in antibody absorption, there was a marked increase in the alkaline phosphatase activity of the duodenum. However, any correlation between these phenomena remains obscure, as by far the greater absorption of orally administered proteins in the suckling rat occurs in the distal, rather than the proximal, regions of the small intestine[2,3]. An increase in the duodenal alkaline phosphatase, as well as closure can be induced prematurely in 9–12-day-old rats by the injection of large doses of cortisone acetate[4]. These effects became apparent 24 h after injection and maximal after 2 days.

The factors which bring about closure in young rats are complex and far from fully understood. Closure in normal rats could possibly be attributable to a reduction

in the level of pinocytosis shown by the absorptive cells of the intestinal mucosa[2], or to an increased efficiency of the lumenal digestive system[5], but none of these suggestions have been confirmed. The present work was therefore undertaken to investigate further the intestinal absorption and intra-lumenal digestion of protein in the normal and cortisone-induced post-closure period, in order to assess the validity of either or both of the above suggestions.

MATERIALS AND METHODS

Animals

Albino rats of a Wistar strain, bred in the laboratory, were used at 12 or 24 days of age. The younger rats were divided into control and experimental groups 48 h before the experiments, members of the latter group being injected with cortisone acetate. Injections were made intraperitoneally with 0.1 ml of a suspension of 5 mg cortisone acetate in ethyl oleate.

Each rat was fed with 0.5 mg of ^{125}I-labelled rat IgG in 0.1 ml physiological saline, and killed 3 h later. Blood was collected from the heart and the values of the isotope activity of the serum samples were expressed as concentration quotients (C.Q.); these being the ratio of cpm/ml serum protein to cpm/ml preparation fed. The isolated small intestine, from the pylorus to the ileo–caecal junction, was flushed with saline to remove any free radioactivity from the lumen, and the washings together with the stomach and small intestine were retained for analyses.

Preparation of labelled protein

Rat IgG prepared from pooled rat serum by DEAE-cellulose ion-exchange chromatography[6], was radioactively labelled with ^{125}I at a level of 0.5 atom of iodine per molecule of protein, by the electrolytic method of Rosa *et al.*[7]. Protein-bound radioactivity in the serum of fed rats was determined by precipitating the proteins with 10 vol. of 1 % tungstic acid, centrifuging, and estimating the radioactivity of the precipitate.

Molecular filtration

Gel filtration was performed on a 2.5 cm × 50 cm column of Sephadex G-100, using phosphate buffered saline (0.02 M sodium phosphate buffer (pH 7.0) in 0.9 % NaCl). The column was calibrated by running on it separately rat IgG, bovine albumin and cytochrome *c*, the effluent being collected in constant volume fractions of 5.0 ml.

Proteolytic activity

The stomach contents were diluted to 5.0 ml, and the intestinal lumen washes to twice this volume with physiological saline. These solutions were then assayed for their peptic and tryptic activities, respectively. In addition, the distal halves of the small intestines were extracted with saline (1 ml per 200 mg wet wt tissue), and the extracts examined for their catheptic activities. All the enzyme activities were determined by the method of Anson[8]. An assay mixture of 0.1 ml of enzyme extract and 0.5 ml of a 2.0 % haemoglobin solution in the appropriate buffer was incubated at 37 °C for a known time interval. The undigested substrate was precipitated with

2.0 ml trichloroacetic acid, and the amounts of amino acid recovered in the filtrate estimated spectrophotometrically at 580 nm using phenol reagent. A blank estimation was carried out at the same time by adding the trichloroacetic acid to the substrate solution before the enzyme extract. A known solution of tyrosine was used as a standard, and enzyme activities in the assay mixtures were expressed in terms of μmoles of tyrosine liberated per min. From these were calculated the total proteolytic activities of the stomach contents and intestinal lumenal washings, and the catheptic activities per 200 mg wet wt of intestinal wall. The latter unit of activity provides a valid comparison for both ages of rats used, since the wet wt/protein ratio in the intestinal wall does not change during post-natal developement[9].

RESULTS

Intestinal transmission of rat IgG

The mean C.Q. values obtained after feeding 0.5 mg of ^{125}I-labelled rat IgG to 12-day-old cortisone-treated rats and their litter mate controls, and to 24-day-old rats are given in Table I. It is observed that the rat IgG passed fairly freely into the circulation in the 12-day-old control rats, but not in the 24-day-old rats, or in the 12-days-old cortisone-treated rats where the C.Q.'s for transmission were 10-fold lower and near the limit of sensitivity of the technique.

Proteolytic activities of the gastro-intestinal tract

The total peptic and tryptic activities occurring in the stomach and intestinal lumen of several representatives from each group of young rats are summarised in Table II. Also included are the catheptic activities recovered in 200 mg wet wt of tissue from the intestinal walls of these animals. It is evident that the previous ad-

TABLE I

THE C.Q. VALUES FOR YOUNG RATS FED 3 h PREVIOUSLY WITH 0.5 mg OF ^{125}I-LABELLED RAT IgG

Rats fed	*Number fed*	*C.Q. ± S.E.*
12-day-old control rats	22	0.0122 ± 0.00078
12-day-old cortisone-treated rats	23	0.0011 ± 0.00012
24-day-old normal rats	25	0.0010 ± 0.00009

TABLE II

THE MEAN PROTEOLYTIC ACTIVITIES IN THE ALIMENTARY TRACT OF YOUNG RATS

Activities are given in terms of μmoles of tyrosine liberated per min from haemoglobin substrate under assay conditions. Figures in parentheses represent the number of determinations.

Animal	*Total peptic activity*	*Total tryptic activity*	*Catheptic activity per 200 mg wet wt of intestine*
12-day-old normal rats	0.19 ± 0.012 (20)	0.37 ± 0.033 (22)	0.13 ± 0.007 (8)
12-day-old cortisone-treated rats	0.20 ± 0.011 (21)	0.40 ± 0.035 (22)	0.11 ± 0.005 (12)
24-day-old normal rats	1.08 ± 0.10 (24)	3.96 ± 0.18 (27)	0.060 ± 0.003 (10)

ministration of cortisone acetate had no influence on the activities of these three enzymes in 12-day-old rats. There were marked increases in the peptic and tryptic activities in 24-day-old rats but a significant fall in the catheptic activity.

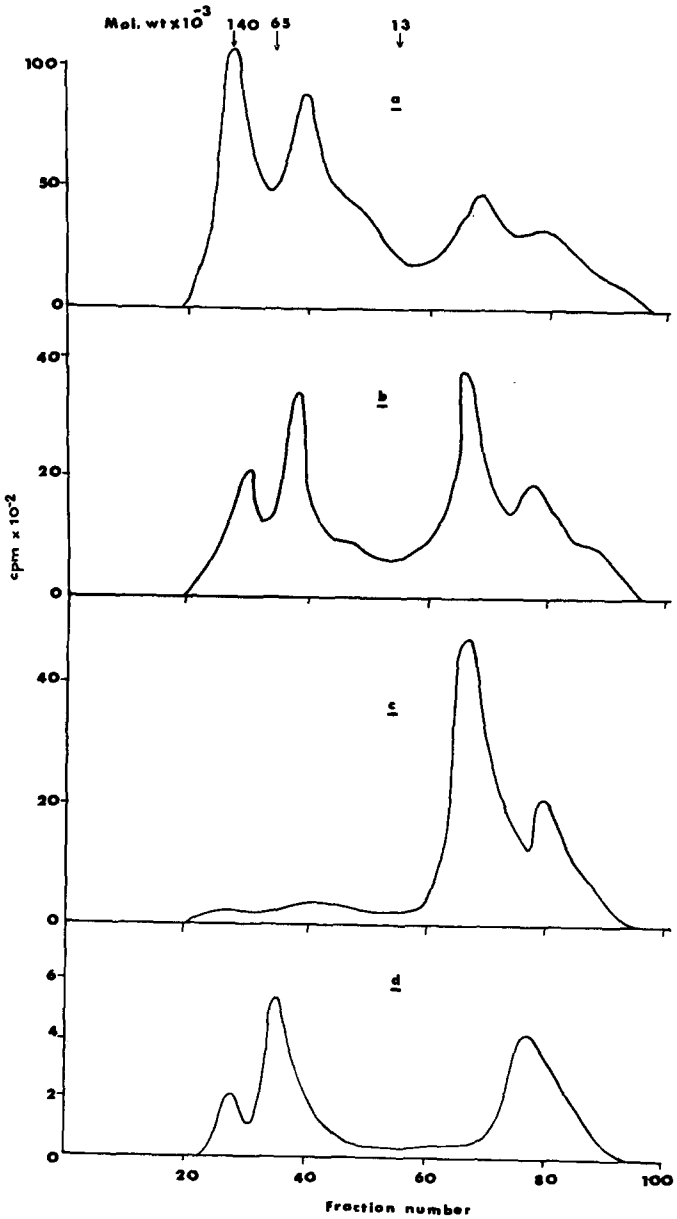

Fig. 1. The radioactivities of Sephadex G-100 eluates of intestinal washes (a, b, c) and intestinal wall extract (d) of young rats fed 90 min previously with ^{125}I-labelled rat IgG. a. 12-day-old normal rats. b and d. 12-day-old cortisone-treated rats. c. 24-day-old normal rats.

The H^+ concentration of the gastro-intestinal tract

The efficiency of all enzyme reactions depends on the H^+ concentration of the reacting medium. The pH prevailing in the stomach and at a point midway along the lumen of the small intestine was therefore investigated in 6 rats of each group by means of an Activon probe electrode. The average readings obtained are shown in Table III. The stomach contents appeared to be more acidic in the 24-day-old rats than in either the 12-day-old cortisone-treated or control animals, but there was no significant difference between the intestinal pH values in any of these animals.

Molecular filtration of the intestinal washes

The intestinal washes from representatives of the three groups of rats, each fed with ^{125}I-labelled rat IgG 90 min previously, were pooled and subjected to gel filtration (Fig. 1). Examination of the elution curves shows that more of the administered protein survives intra-lumenal digestion in normal 12-day-old rats, than in 12-day-old cortisone-treated animals. In the former, 27 % (measured planimetrically) of the total radioactivity in the lumenal wash was attributable to intact rat IgG, but only 17 % in the latter. The remaining activities in each case were due to degradation products of large molecular weight (approx. 50000) and to small peptides and amino acids.

Most (91 %) of the radioactivity remaining in the intestinal lumen of fed 24-day-old rats corresponded to degradation products with molecular weights less than that of cytochrome *c* (13000).

Distribution of radioactivity in the gastro-intestinal tract

The radioactivities remaining in the stomach, the intestinal lumen, and the intestinal walls of young rats 3 h after feeding with ^{125}I-labelled IgG were determined

TABLE III

THE AVERAGE pH VALUES OBSERVED IN THE STOMACH AND INTESTINAL LUMEN OF YOUNG RATS

Animals	*No. tested*	*pH*	
		Stomach	*Mid-intestine*
12-day-old normal rats	6	4.4	6.2
12-day-old cortisone-treated rats	6	4.2	6.0
24-day-old normal rats	6	2.7	6.1

TABLE IV

THE MEAN RADIOACTIVITIES AND THEIR STANDARD ERRORS RECOVERED IN THE STOMACH, INTESTINAL LUMEN, AND INTESTINAL WALL OF YOUNG RATS FED 3 h PREVIOUSLY WITH 0.5 mg OF ^{125}I-LABELLED RAT IgG

Animals	*No. tested*	*Percentage of administered radioactivity recovered in*		
		Stomach	*Intestinal lumen*	*Intestinal wall*
12-day-old normal rats	17	12.33 ± 1.09	1.54 ± 0.15	7.03 ± 0.71
12-day-old cortisone-treated rats	18	16.42 ± 1.17	1.45 ± 0.09	3.85 ± 0.37
24-day-old normal rats	20	19.40 ± 1.24	2.41 ± 0.21	1.45 ± 0.12

and expressed as percentages of the total radioactivity administered. The results are summarised in Table IV.

It is evident that the standard radioactive dose had been evacuated from the stomach in the normal 12-way-old rats at a faster rate than in the 24-day-old animals. The rate of evaculation in the cortisone-treated animals was intermediate. Important differences were also found in the amount of radioactivity remaining in the intestinal wall. Whereas 7 % of the administered dose could be recovered from the intestine of the normal 12-day-old rats only 3.8 % could be recovered in the cortisone-treated rats and 1.4 % in the 24-day-old animals.

DISCUSSION

Halliday's[1,4] finding that the administration of cortisone acetate to the 12-day-old rat caused a premature closure to immunoglobulin absorption has been confirmed. When 0.5 mg of ^{125}I-labelled rat IgG in 0.1 ml physiological saline was fed to young rats, the C.Q. in the blood after 3 h was 0.012 for normal 12-day-old animals, but was approximately an order lower for 24-day-old rats and 12-day-old rats treated with cortisone acetate 48 h previously. Assuming that the mean plasma volume of 12-day-old rats is 1 ml (ref. 10) then 12 % of the administered dose was accounted for as circulating protein in the normal 12-day-old rat, while only a little over 1 % could be recovered from cortisone-treated rats of the same age.

Whilst injection of cortisone acetate thus caused premature closure in young rats, this effect is clearly not mediated by a change in the pH of the gastric juice (Table III), nor by an increased efficiency of peptic or tryptic digestion (Table II). Although these enzyme activities are considerably higher in 24-day-old post-closure rats than in 12-day-old animals, they are similar in normal (pre-closure) and cortisone-treated (post-closure) 12-day-old rats. According to Taylor[11], pepsin has an additional pH optimum between 4.0 and 4.5, so that even at the relatively high pH found in the stomach of 12-day-old rats some proteolysis could be expected. The rate of evacuation of the radioactively labelled rat IgG molecules from the stomachs of normal 12-day-old rats was more rapid than from the stomachs of either the cortisone-treated or the 24-day-old rats. It follows therefore, that the administered dose was exposed to a shorter period of peptic digestion in the normal 12-day-old rats than when fed to either of the post-closure groups of animals.

A comparison of the gel filtration results for the intestinal washes taken from normal and cortisone-treated 12-day-old rats fed 90 min previously with ^{125}I-labelled rat IgG, reveals important differences between them (Figs 1a and 1b). There is a reduction in the proportion of intact IgG recoverable from the intestinal lumen in the cortisone-treated rats, and a corresponding increase in the proportions of the degradation products. This is clear evidence for an increased efficiency of intra-lumenal digestion in these animals. Gel filtration analysis of the small intestinal wall extracts from cortisone-treated fed rats, showed that the mucosal cells could still adsorb intact rat IgG (Fig. 1d). In 24-day-old fed rats the radioactivity in the intestinal wash represents only small peptides and amino acids (Fig. 1c). Thus, by this age gastric digestion is most efficient.

The recovery of radioactivity from the intestinal walls of young rats fed 3 h previously with ^{125}I-labelled rat IgG falls prematurely after cortisone treatment or

normally with increasing maturity (Table IV). This is probably a reflection of the increases which occur under these circumstances in the proportions of the radioactivities in the intestinal lumen attributable to small peptides and amino acids (Fig. 1) which may be absorbed into and transferred across the absorptive cells into the circulation more rapidly than intact IgG. Pinocytosis of intact IgG into the absorptive cells can occur when intact protein is available in the intestinal lumen, even in cortisone treated rats where subsequent transmission into the circulation is barely detectable. Whether or not pinocytosis of intact IgG could occur in 24-day-old rats, where intra-lumenal digestion of ingested protein seems to be complete, was not established, although the results of Bamford[12], and Morris and Begley[13], would indicate that it could, provided intact IgG reached the absorptive cells. 3 h after feeding only barely detectable levels of radioactivity were found in the large intestines of all three groups of rats, which suggested that no radioactivity had been lost in the faeces, while the low levels of radioactivities in the small intestinal lumen of these animals implied that absorption of the radioactive molecules was complete. However, even in the most favourable case (normal 12-day-old rats) only a small proportion of the administered immunoglobulin could be recovered intact in the circulation. Clearly protein digestion is an active process in the young rat, both during the pre- and post-closure period.

Brambell[14] has put forward an hypothesis to account for the selective digestion and transmission of immunoglobulins across the neo-natal intestines. Protein molecules absorbed pinocytotically into the absorptive cells and destined to be transported to the circulation become attached to specific receptor sites situated on the walls of the pinocytotic vacuoles. This attachment preserves the molecules from degradation when the vacuoles fuse with the lysosomes, while any molecules remaining free in the vacuolar sap are hydrolysed. Some of the rat IgG administered orally to cortisone-treated 12-day-old rats was absorbed intact by the intestine (Fig. 1d) but barely detectable amounts were transmitted intact into the circulation (Table I). It is possible that the cortisone treatment impaired the transfer mechanism, so that all protein molecules taken up by the mucosal absorptive cells were degraded by the lysosomal enzymes; the presumably rapid diffusion of the digestion products from the cells could account for the lower intestinal wall radioactivity in the cortisone-treated fed rats as compared to the 12-day-old rats (Table IV).

The presence of cathepsins in the extracts of the intestinal walls of suckling rats is important when it is considered that a property of these enzymes is the digestion of extra cellular protein which has been taken into the cell by pinocytosis[15]. The concentration of these enzymes decreases between the ages of 12 and 24 days but this deficiency is alleviated by the increasing efficiency of the gastro-intestinal digestion.

ACKNOWLEDGEMENTS

It is a great pleasure to acknowledge the interest of Dr W.A. Hemmings in this work. I am greatly indebted to Dr I. Morris for much helpful advice and discussion. Acknowledgements are also due to Mr E. Williams and Mrs J. Jenkinson for skilled technical assistance.

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