PROTEOLYSIS AND ABSORPTION IN THE ALIMENTARY TRACT OF TWO FOOD ANTIGENS ADMINISTERED SIMULTANEOUSLY TO ADULT RATS

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KEY WORDS: serum albumin; ovalbumin; antigens; immunosorbents; proteolysis; absorption in the intestine.

Food and intestinal bacteria are the source of continuous immune stimulation of the organism [10]. The quantity of protein antigen entering the internal medium of the body evidently depends to some degree on the structure of the protein and its resistance to the action of digestive enzymes [5]. Comparison of the processes of proteolysis and absorption for different proteins is of great interest, especially when their activity as food allergens is compared [6]. In the investigation described below proteolysis and absorption of two food antigens administered simultaneously to the same experimental animal were compared. Selective determination of two different antigens in extremely low concentrations was carried out by the use of the method of analytical immunosorption of tritiated proteins [7]. The results were subjected to statistical analysis by comparing random samples with tied pairs [4].

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-180 and 250-280 g were used. The animals were deprived of food for 24 h before the experiment. The mixture administered to the animals through a gastric tube included 100 mg unlabeled human serum albumin (HSA) and 1 mg each of bovine serum albumin (BSA) and hen ovalbumin (OVA), labeled with ^3H. The total volume of the mixture was 1.5 ml and the solvent was 0.01 M Na-phosphate buffer with 0.15 M NaCl (PSB). The dose of radioactivity of the labeled proteins was 1-5 μCi for each. The proteins were labeled as described in [3]. After 20, 60, or 180 min laparotomy was performed on the animals under hexobarbital anesthesia. In the animals of groups 1 and 2 (20 and 60 min) the stomach, proximal and distal portions of the small intestine, and the large intestine were perfused with PSB in a volume of 5 ml. The perfusates were quickly cooled to 0°C, 10 μl of diisopropyl fluorophosphate in isopropyl alcohol was added, and the mixture was cleared by centrifugation in an OPN-3 centrifuge at 3000 rpm and kept at −30°C until required for analysis for not more than 7 days. Serum was taken from the posterior vena cava of the animals of groups 2 and 3 (60 and 180 min) and biopsy material was taken from the spleen and liver. The tissues were homogenized in a homogenizer with Teflon pestle in 9 volumes of PSB, the homogenates were centrifuged at 105,000g in a UTsP-65 centrifuge for 1 h, and the transparent aqueous layer of supernatant was withdrawn for analysis (saline cell extract). The perfusates of the various parts of the gastrointestinal tract, peripheral blood serum, and saline cell extracts of the organs were applied to columns with sepharose immunosorbents against BSA and against OVA. Sorption of heterologous protein on the immunosorbents did not exceed 0.3% of the quantity applied; a 300-fold excess of HSA by weight did not inhibit binding of BSA and OVA with the corresponding immunosorbents. After application of the preparations, ^3H-label not bound with antibodies was washed off with 20 ml PCB (about 100 free volumes of the column), and the immunospecific structures were then eluted from the immunosorbent by 0.1 M acetic acid. Radioactivity in the eluates was determined on an Intertechnique SL-30 liquid scintillation β-spectrometer (France) in Bray's scintillator.

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TABLE 1. Content of (immunoreactive) BSA and OVA, Retained by Specific Immunosorbent in Chyme of Rats 20 and 60 min after Simultaneous Gastric Tube Feeding with Proteins (in % of dose administered, calculated per whole organ)

Part of gastrointestinal tract	After 20 min			After 60 min		
	BSA	OVA	P	BSA	OVA	P
Stomach	2,5±0,7 (4,6±2,0)	5,9±1,9 (5,3±2,7)	<0,1 >0,1	0,25±0,03	0,9±0,2	<0,1
Proximal part of small intestine	2,8±1,2 (2,5±0,4)	4,4±0,2 (6,2±0,8)	>0,1 <0,001	0,10±0,03	0,62±0,08	<0,01
Distal part of small intestine	0,42±0,08 (1,1±0,1)	1,0±0,1 (3,6±0,4)	<0,001 <0,001	0,36±0,04	5,2±1,2	<0,02
Large intestine	0,52±0,08 (0,52±0,05)	0,67±0,08 (1,42±0,26)	>0,1 <0,01	0,10±0,02	0,54±0,16	<0,05
Total gastrointestinal tract	6,3±1,9 (8,7±2,8)	12,0±1,8 (16,4±3,4)	<0,001 <0,001	0,81±0,09	7,4±1,5	<0,02

Legend. Numbers without parentheses show data for animals weighing 150-180 g; numbers in parentheses are data for animals weighing 250-280 g. After 20 min four rats weighing 150-180 g and four rats weighing 250-280 g were used; after 60 min five rats weighing 150-180 g were used; standard deviations of values and significance of differences between data for OVA and BSA are shown.

TABLE 2. Content of (immunoreactive) BSA and OVA Retained by Specific Immunosorbent in Peripheral Blood Serum and Extracts of Liver and Spleen Cells of Rats 60 and 180 min after Feeding (in % of fed dose, calculated for weight of organ)

Tissue	After 60 min			After 180 min		
	BSA	OVA	P	BSA	OVA	P
Blood serum	0,022±0,003	0,11±0,01	<0,01	0,013±0,002	0,11±0,02	<0,01
Liver	0,042±0,002	0,31±0,05	<0,01	0,069±0,006	0,523±0,038	<0,001
Spleen	0,0063±0,0007	0,040±0,002	<0,001	0,0035±0,0001	0,036±0,008	<0,05

Legend. In both groups five rats weighing 150-180 g were used; the standard errors of the mean values and significance of differences between data for OVA and BSA are shown.

EXPERIMENTAL RESULTS

The results of determination of the antigenic structures of BSA and OVA in perfusates of the gastrointestinal tract of rats of two age groups weighing 150-180 and 250-280 g, 20 and 60 min after feeding, are given in Table 1. The degree of preservation of the antigenic determinants in all parts of the gastrointestinal tract 20 min after administration of the mixture was less for BSA than for OVA; for the distal part of the small intestine and for the gastrointestinal tract as a whole, the difference was highly significant. The difference in the content of antigenic structures of BSA and OVA in the large intestine after 20 min of observation was not significant, and this was evidently due to the fact that the very small fraction of the liquid food mixture entering this part of the intestine has passed rapidly through the small intestine without having undergone intensive luminal proteolysis. The content of immunoreactive OVA in the chyme 60 min after gastric tube feeding was substantially greater, as before, than the content of immunoreactive BSA.

The results of determination of the antigenic structures of BSA and OVA in the animals' peripheral blood serum, liver, and spleen are given in Table 2. The results indicate that antigenic determinants of OVA penetrate into the internal medium of the body in much larger quantities than those of BSA. The time course of accumulation of the two antigens in the internal organs is quite similar. Both OVA and BSA accumulate with time in the liver ($P < 0.01$), whereas no such accumulation is observed in the blood and spleen.

It can be concluded from these results that antigenic determinants of OVA are much more resistant in the body than antigenic determinants of BSA when these proteins are administered perorally. The immunoreactive protein determined in these experiments consisted evidently of intact molecules [8] or, at least, of large fragments with a molecular weight of not less than 12,000 daltons [9]. The high yield of such products in the course of metabolism of OVA may be connected with its lesser vulnerability to attack by proteinases. Vulnerability of OVA to attack by pepsin, determined in vitro [1], was 1.3 times less than that of BSA ($P < 0.01$). A definite

role in protection of the antigenic determinants of OVA may also be played by its greater resistance to acid denaturation in the stomach compared with BSA [2].

The greater quantity of OVA than of BSA entering the internal medium of the body may lead to differences in the immune response to these proteins. It was shown, in particular, in [12] that, depending on the dose of food protein assimilated, it may behave both as an immunogen and as a tolerogen.

During separate administration of different proteins to animals in experiments to study digestion and absorption, interpretation of the results may be made difficult by the considerable dispersion of the experimental data [8, 11]. It can be concluded from the present investigation that the simultaneous administration of two labeled food antigens to an animal, followed by selective immunosorbent antigen determination, enables the permeability of the protective barriers of the body for different antigenic structures to be effectively compared.

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THYMUS TARGET CELLS UNDER MACROPHAGE CONTROL DURING THE FORMATION OF GRAFT VERSUS HOST REACTION EFFECTORS

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Thymocytes (TC) are weak inducers of the graft versus host reaction (GVHR) [2]. Meanwhile the writers have shown that maturation of thymus cells to functionally active effectors can take place under the influence of short-term incubation with peritoneal exudate macrophages [1]. The thymus is known to contain different populations of lymphoid cells, differing not only in their location in the organ (cortex, medulla), but also in the antigenic properties of their surface membranes, immunocompetence, sensitivity to corticosteroids and irradiation, and so on.

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