GASTROINTESTINAL BARRIER PERMEABILITY FOR POLYETHYLENE-GLYCOL 4000 MACROMOLECULES: MECHANISM AND REPRODUCIBILITY

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Increased absorption of protein macromolecules in food and of bacterial toxins from the gastrointestinal tract (GIT) is regarded as one cause of food allergy, inflammatory diseases of the intestine, and other pathological states [3, 5, 7, 11]. The fact that GIT is the target for several dietary factors [4, 6, 8, 9] creates a basis for the possible dietetic correction of states accompanied by disturbed permeability for macromolecules. Many model compounds, including nonmetabolized sugars, chelation complexes of transitional metals, dextrans, and polyethylene-glycol, have been suggested for the evaluation of permeability of the GIT barrier [5]. The passage of these compounds from the lumen of the small intestine into the blood stream is determined on the basis of their excretion with the urine. The correctness of using most of the above-mentioned test compounds, which are essentially not macromolecules, has led to the expression of serious misgivings [10, 12-14]. Polyethylene-glycol with a molecular mass of 4000 (PEG-4000), widely used in gastroenterology as nonabsorbed indicator, has been suggested as a means of verifying permeability of GIT. As the writers showed previously [2] a number of aspects of absorption of perorally administered PEG-4000 into the blood stream are very similar to those of protein macromolecules.

The aim of this investigation was to verify the validity of the use of PEG-4000 as a model probe of the permeability of the GIT barrier from the standpoint of similarity of the mechanism of absorption of PEG-4000 and that of protein macromolecules.

EXPERIMENTAL METHOD

Experiments were carried out on 23 male Wistar rats. The animals received the standard animal house diet and water to drink ad lib.

In the 1st series of experiments reproducibility of determination of barrier permeability of the GIT for PEG-4000 was assessed when the substance was administered on several occasions to the same animals. For adaptation purposes five rats weighing initially 300 g were kept for 18 h in metabolic cages. Three days later, 500 mg of P13:G-4000 ("Serva," Germany, analytical grade) in 2 ml physiological saline (PS) was administered to the animals via a gastric tube, together with a small quantity of carmine ("Loba Chemie," Austria). The carmine was added to test the possibility of contamination of the urine collected for analysis with feces containing the two unabsorbed indicators. The animals were replaced in the metabolic cages and urine collected for 18 h. This test was repeated another twice with an interval of 2 days between repetitions.

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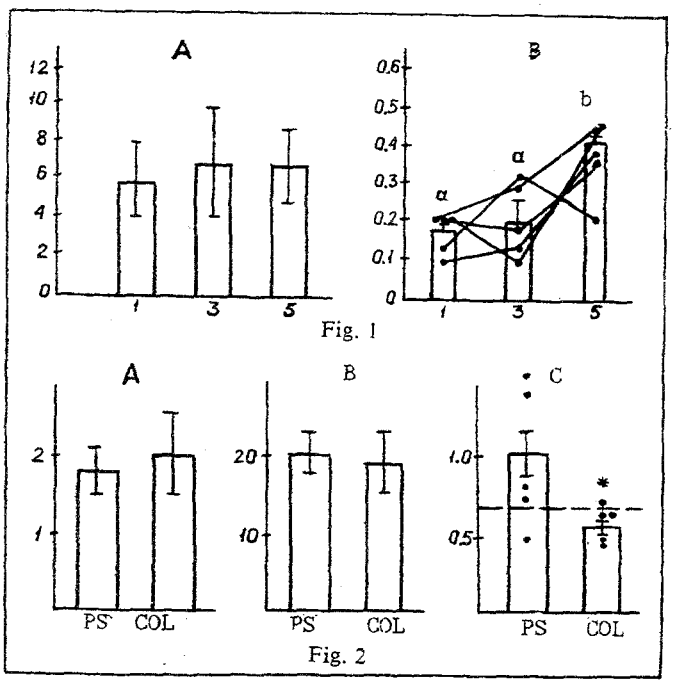

Fig. 1

Fig. 2

Fig. 1. Diuresis (A) and urinary excretion of PEG-4000, individual and mean values (B) during triple testing of permeability of GIT for PEG-4000, with intervals of 2 days. Abscissa, days of experiment; ordinate: a) volume of urine (in ml/18 h), b) PEG-4000 in urine (in % of administered dose). Samples indicated by lower case letters (a, b) differ significantly ($p < 0.01$).

Fig. 2. Diuresis (A), and urinary excretion of creatinine (B) and PEG-4000 (C) – individual and mean values in rats receiving of colchicine (COL) or physiological saline (PS) by intraperitoneal injection 3 h before injection of PEG-4000 by the intragastric route. Ordinate: a) volume of urine (in ml/15 h), b) creatinine in urine (in μmoles/15 h), c) PEG-4000 in urine (in % of administered dose). Asterisk indicates significant difference between groups ($p < 0.05$, by the median test). Common median of two samples (c) indicated by broken line.

The role of active intestinal transport in the absorption of PEG-4000, effected through the apical contractile system of the intestinal epithelium and intracellular displacement with the aid of the system of microtubules of the enterocytes, was studied in rats exposed to the systemic action of colchicine, an inhibitor of polymerization of the microtubules [1]. In a preliminary experiment on eight animals the injected dose of colchicine was titrated in a series of double dilutions. The sublethal dose of colchicine was 250 μg/100 g body weight, during observation for 24 h. Later a dose of colchicine of 125 μg/100 g, which does not cause significant pathological symptoms in the animals during observation for 24 h, was chosen.

The investigation was carried out on two groups, each consisting of five rats weighing 150-180 g. Rats of the experimental group were given an intraperitoneal injection of 125 μg/100 g body weight of colchicine, whereas animals of the control group received an injection of the solvent (PS) only. After 3 h the animals were given 200 mg of PEG-4000 in 1 ml PS by the intragastric route. The urine was collected for 15 h.

To determine excretion of PEG-4000 the urine was extracted quantitatively with an equal volume of distilled chloroform, with vigorous shaking 3 times, for 5 min each time. The chloroform extract was evaporated in vacuo, redissolved in 200 μl of a methanol–water mixture (1:1), and chromatographed on a column with TSK Gel Toyopearl HW50-F gel (Japan), equilibrated with the same solvent, and equipped with a differential refractometric detector. The content of PEG-4000 was determined from the area of the chromatographic peaks, with the aid of a standard curve.

Excretion of creatinine with the urine was determined with the "Creat-100" kit (Czechoslovakia). The significance of differences between the groups was determined by Student's t test and the median test (two-way test).

EXPERIMENTAL RESULTS

As the results in Fig. 1 show the excretory function of the kidneys, assessed on the basis of total diuresis, showed no significant change during triple testing of permeability of GIT for PEG-4000. Excretion of PEG-4000 did not differ significantly as a result of the 1st and 2nd injections of the indicator, separated by an interval of 2 days. However, after the 3rd injection of PEG-4000 its excretion with the urine increased significantly. The reasons for this are not quite clear although the possibility of a cumulative effect of the toxic action of PEG-4000 or of microcontaminants contained in it, such as ethylene oxide monomer, on the mucous membrane of the intestine cannot be ruled out. Thus PEG-4000 gives a reproducible result for GIT permeability in two repeated tests, but further testing of permeability can be done, evidently, only after a longer time interval, enabling the body to be cleared of trace amounts of the macromolecular indicator.

While the rats were kept for 18 h in the metabolic cages only very slight staining of the feces with carmine took place (observed in two of the five animals in one test), and staining of the urine was not observed at all. This state of affairs rules out any possibility of contamination of the specimens of the urine with feces containing large quantities of unabsorbed PEG-4000, which could distort the results of testing.

It follows from the data in Fig. 2 that injection of colchicine into the rats in a dose of 125 μg/kg body weight did not affect the excretory function of the kidneys, estimated on the basis of the volume of diuresis and creatinine excretion. As a result of this the differences in excretion of PEG-4000 between the experimental and control groups cannot be ascribed to a toxic action of the colchicine on the kidneys, but are evidently due entirely to differences in absorption of the indicator in the intestine. The dose of colchicine which we used was much smaller than the dose of 4800 μg/kg, injected locally (into the intestine), used previously [1], to inhibit absorption of glucose, effected through the system of microtubules, in an experiment of comparatively short duration (not exceeding 0.5 h). It is unlikely that the dose of the effector which we used would completely inhibit the process of assembly of the microtubules of the small intestinal enterocytes. Nevertheless, as follows from the data in Fig. 2, in rats treated with colchicine excretion of the indicator with the urine was significantly lower than in the control, and this in all probability is responsible for the marked inhibition of its absorption in the small intestine.

As we know from data in the literature [11], passage of protein macromolecules through the barrier of the small intestinal epithelium under normal conditions is effected through endocytosis, a process mediated by the system of microfilaments and microtubules of the enterocytes [1]. Meanwhile, for some low-molecular-weight probes of permeability of the intestinal epithelium a different mechanism of penetration has been postulated, namely passive transcellular diffusion [5, 13]. The data thus obtained do not necessarily agree with estimates of permeability for protein macromolecules [10, 12-14]. As the data in the present publication indicate, absorption of PEG-4000 in GIT is effectively inhibited by colchicine and is largely effected by a mechanism of endocytosis involving participation of the microtubular system. In this respect permeability of the intestinal epithelium for PEG-4000 can be regarded as an adequate model of permeability for protein macromolecules, in qualitative agreement with the results of our previous investigations [2]. Final proof of the endocytosis pathway of PEG-4000 transport through the enterocyte could be obtained by cytochemical methods, although at the present stage of the research, substantial technical difficulties would have to be overcome.

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