EFFECT OF VITAMIN A DEFICIENCY ON PERMEABILITY OF THE SMALL INTESTINAL MUCOSA FOR MACROMOLECULES IN ADULT RATS

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Vitamin A plays an important role in the structure and function of biomembranes [8, 13]. Under the influence of retinoyl in vitro, and also in hypo- and hypervitaminosis A considerable changes are found in the stability of cell membranes, coupled with marked disturbances of their permeability [2, 8, 11]. The importance of these shifts for the organism as a whole, however, has been inadequately studied. Since the barrier function of the intestinal epithelium plays an exclusive role in maintenance of the constancy of the internal milieu of the organism, it is interesting to study the effect of vitamin A on the state of this function, especially having regard to data indicating that the small intestine is the target organ for this vitamin [1, 9].

One of the parameters of the barrier function of the intestinal epithelium is its permeability for macromolecules [14]. In the normal adult animal unsplit protein is assimilated to only a very limited degree [4], but in certain pathological states its assimilation may be greatly intensified [12]. In this investigation the effect of experimental vitamin A deficiency on absorption of macromolecules of hen's ovalbumin (OA) in the intestine was studied. An electron-microscopic study of permeability of small intestinal enterocytes for particles of colloidal lanthanum hydroxide $La(OH)_3$ was carried out at the same time.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats. Vitamin A deficiency was induced in the animals by keeping lactating rats and also growing young rats on a semisynthetic diet not containing vitamin A [3]. Rats receiving in addition 0.2 ml of an oily solution of retinoyl palmitate in a dose of 1000 IU per rat, once a week by the intragastric route, served as the control. The animals were deprived of food 16 h before the experiment. OA in the form of a 30% solution in 0.01 M phosphate buffer, pH 7.5, with 0.15 M NaCl, was given by the intragastric route to animals of both groups in a dose of 150 mg protein/kg body weight. Blood was taken from the inferior vena cava of the animals under hexobarbital anesthesia 3 h later. The concentration of unsplit OA in the animals' blood was determined by competitive radioimmunoassay [5]. For this purpose samples of serum were incubated with indicator doses of ^{125}I-OA and with monospecific antiserum to this protein, after which the immune complexes were precipitated by the addition of polyethylene-glycol-6000 to a 10% concentration. Radioactivity of the precipitates was measured on an NRG-603 γ-spectrometer (Czechoslovakia). The OA concentrations were obtained from a standard curve in log-logit coordinates. The electron-microscopic study of permeability of the enterocytes for $La(OH)_3$ was carried out as described previously [6]. Tests were carried out in situ on animals under superficial ether anesthesia. Material for investigation was obtained 15 min after injection of the tracer. The tissues were fixed in a 4% solution of paraformaldehyde in cacodylate buffer, pH 7.4, and postfixed in a 1% solution of OsO_4, dehydrated in acetone, and embedded in a mixture of epoxide resins (Epon–Araldite). Ultrathin sections were examined in the H-300 electron microscope (Japan) without preliminary staining. Vitamin A was determined in the liver homogenates as described previously [3]. The experimental results were subjected to statistical analysis by Student's t test and also by the Mann–Whitney nonparametric rank test [7].

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TABLE 1. Effect of Vitamin A Deficiency on Blood Level of Immunoreactive OA in Rats

Group of animals	Blood concentration of OA, ng/ml	Absorption of OA into blood, per cent of dose administered	Significance of difference	
			student's t test	Mann Whitney test
Vitamin A insufficiency (n = 16)	66,4±20,9	$(1,33\pm0,42)\cdot10^{-4}$	—	—
Control (n = 20)	19,6±3,1	$(0,39\pm0,06)\cdot10^{-4}$	$<0,05$	$<0,025$

Legend. Mean values ± standard deviation are shown.

EXPERIMENTAL RESULTS

Vitamin A deprivation led to the appearance of typical symptoms of vitamin A deficiency in the animals after 40-50 days: disturbance of the hair cover, loss of apetite, retardation followed by arrest of growth, and reduced resistance to infectious diseases. The animals were used in the experiments at the "weight plateau" stage, indicating severe vitamin A deficiency [3]. This was confirmed by a sharp fall in the vitamin A concentration in the liver (38.4 ± 4.0 μg/g in the control and 2.1 ± 0.1 μg/g in the experimental animals, $P < 0.05$).

It will be clear from Table 1 that the blood levels of high-molecular-weight protein structures of OA, still able to react with specific antibodies, were significantly higher in animals with vitamin A deficiency. On average the fraction of absorbed unsplit OA was more than three times greater in the experimental rats than in the controls. Since values of absorption of food antigens into the bloodstream of experimental animals do not necessarily obey the normal law of distribution [5], significance of the difference between the groups was additionally verified by the Mann–Whitney nonparametric rank test.

The electron-microscopic investigation showed (Fig. 1) that the character of distribution of $La(OH)_3$ particles in the control animals corresponded to the picture obtained previously in a number of investigations of the small intestinal mucosa of rats kept on a standard animal house diet: the tracer was adsorbed uniformly on the glycocalyx of the microvilli [6]. Meanwhile no tracer could be found in the cytoplasm of the enterocytes or in the zone of tight junctions. A different pattern of distribution of $La(OH)_3$ was found in the small intestinal mucosa of rats with vitamin A deficiency. The weak development of the glycocalyx will be noted: where the glycocalyx was completely adsorbed, adsorption of the tracer likewise could not be detected (Fig. 2). Adsorption of the tracer also was very limited in other areas on the surface of the enterocyte. In cases when the tracer could nevertheless be observed, its distribution was uneven both between different enterocytes and between different parts of the surface of the same enterocyte. Under these circumstances it was found mainly in the depth of the space between the microvilli and was absent on their apex. Just as in the control animals, the tracer did not pass through the apical membrane of the enterocytes in the experimental rats and it was absent in their cytoplasm.

These experiments thus showed that in severe vitamin A deficiency the barrier function of the gastrointestinal trace relative to unsplit protein macromolecules is sharply depressed. Meanwhile, the electron-microscopic investigation showed that morphological changes in the state of the apical membrane of the enterocytes were manifested only as loss of the glycocalyx. This fact is in good agreement with the view that there is a marked disturbance of the synthesis of glycoproteins, which are a major component of the glycocalyx, in vitamin A deficiency [10]. Loss of the glycocalyx could result in disappearance of its function as a molecular sieve and weakening of the activity of the enzymes of contact digestion which, in turn, must evidently lead to an increase in the access of unsplit protein into the bloodstream. It can be postulated that the entry of foreign protein antigens in more than usual amounts into the bloodstream in vitamin A deficiency is one of the factors leading to sensitization of the body with food antigens and the development of manifestations of food allergy.

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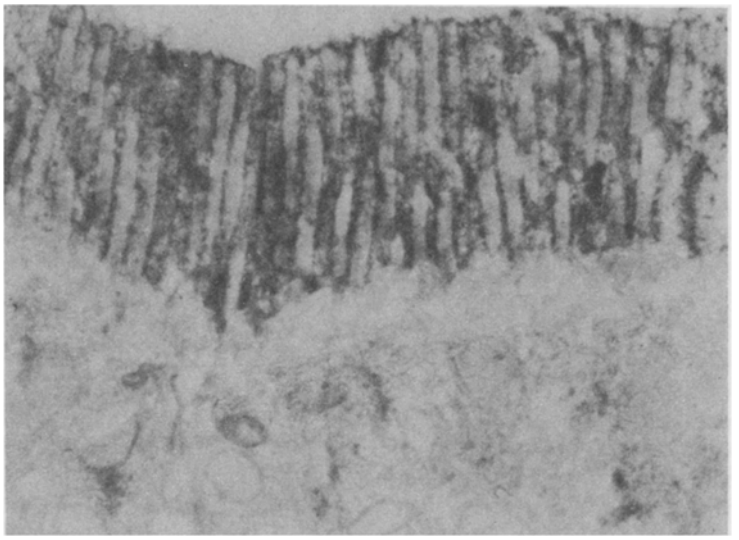

Fig. 1. Adsorption of colloidal lanthanum hydroxide particles on glycocalyx of small intestinal enterocytes of intact animal. Magnification 30,000×.

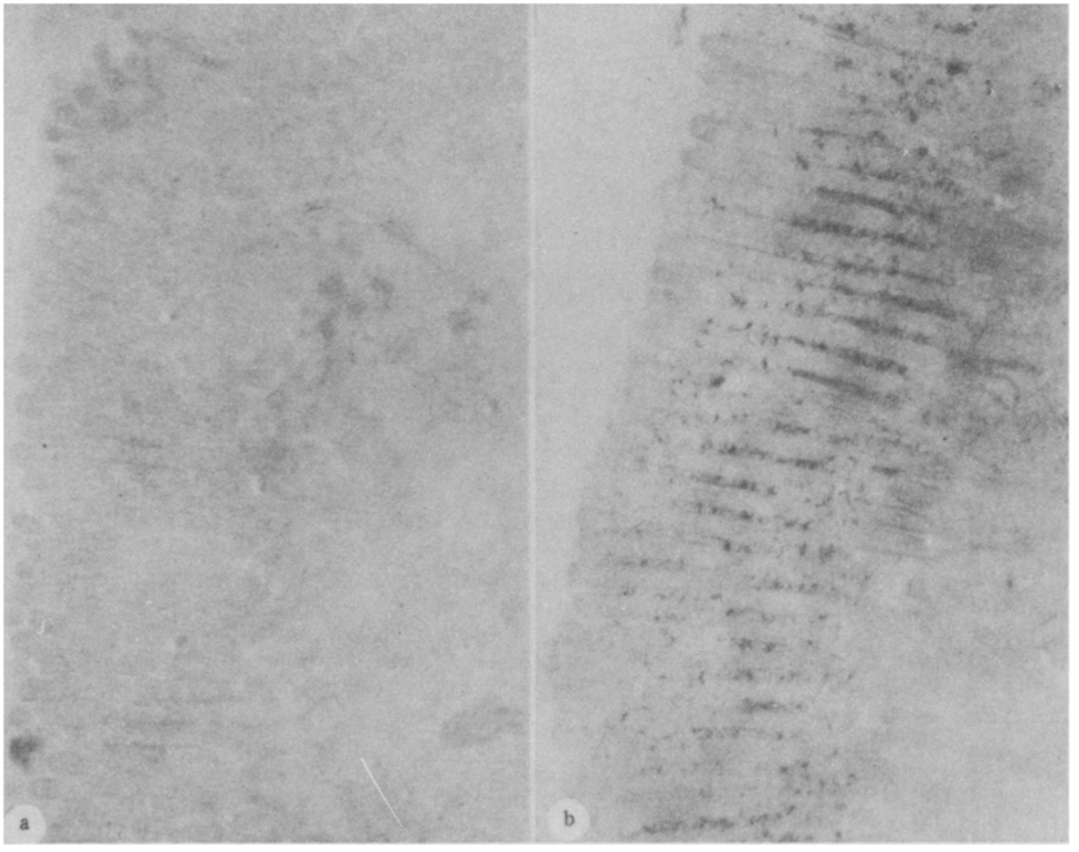

Fig. 2. Microvilli of small intestinal enterocytes of rats with vitamin A deficiency. a) absence of glycocalyx and of adsorption of tracer on apical membrane; b) limited adsorption of tracer present in depth of space between microvilli. Magnification 30,000×.

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EXPERIMENTAL DIABETES IN MICE INFECTED WITH COXSACKIE VIRUSES

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It has been shown that virus infections, together with heredity and autoimmune disturbances, can contribute to the development of insulin-dependent diabetes. The presence of virus-neutralizing antibodies in high titers in the early stages of the disease [1, 2], the discovery of specific antigens in the islets of Langerhans [3], isolation of viruses causing a diabetes-like syndrome in experimental animals from the pancreas of dying patients [5, 6] — all these and other factors confirm the parts played by virus infections in the development of the disease.

Among viruses that are most frequently stated to be a possible cause of human diabetes, Coxsackie viruses of the B group may be mentioned. These viruses can cause a disease resembling diabetes in genetically predisposed strains of mice. In some resistant strains of mice the disease develops after preliminary injection of subdiabetogenic doses of streptozocin into the animals [4]. No information could be found in the literature on the role of infection by Coxsackie A virus in the pathology of the pancreas.

The aim of this investigation was to compare the effect of Coxsackie B4 and A13 viruses on the pancreas of strains of mice sensitive and resistant to diabetes, using subdiabetogenic doses of aloxan in the second case.

EXPERIMENTAL METHOD

DBA/2 and (CBA × C57Bl/6)F_1 mice aged 3-4 months were used in the experiments. Altogether 247 animals were used. Male DBA/2 (sensitive) mice were infected with Coxsackie B4 or A13

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