response [3], and to activation of proliferation of connective tissue fibroblasts [11]. Finally, an aqueous solution of urotropine, introduced into the wound, on decomposing in the acid medium, forms formaldehyde and ammonia [6]; formaldehyde, which has a direct antiseptic action on the microflora, inhibits its growth, whereas the ammonia, making the wound medium alkaline, creates unfavorable conditions for the life of microorganisms, but favorable conditions for regenerative processes.

The combined, consecutive use of nialamide (internally), a 10% aqueous solution of urotropine for wound irrigation, and dressings with a 2% alcoholic solution of ionol, which is a pathogenetically based procedure, thus considerable quickens the course of healing of purulent wounds and shortens the time required for their complete healing.

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EFFECT OF VITAMIN A INTAKE ON ABSORPTION AND LOCALIZATION OF ZINC IN THE CHICK ILEUM

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KEY WORDS: vitamin A; zinc; ileum; absorption

The leading role in the maintenance of zinc homeostasis in animals is played by regulation of the absorption of this cation in the small intestine. Recent research has established a definite influence of vitamin A on the regulation of zinc metabolism [2, 5, 9, 12]. The small intestine is one of the target organs at which the action of the vitamin is directed. It has been shown that vitamin A [9], and also its active metabolite retinoic acid [4], stimulates zinc absorption in the chick small intestine. A vitamin A-dependent specific zinc-binding protein (ZnBP) [2, 9] has been isolated from the mucous membrane of the ileum, the site of maximal zinc absorption [8, 9, 11]. It is suggested that this protein participates in zinc transport through the intestinal epithelium into the blood.

The mechanism of absorption of zinc by the intestinal epithelium has received little study. There are no data on the role of particular intracellular organoids in this process.

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TABLE 1. Zinc Accumulation by Preparations of Chick Ileum (in nmoles/min·g) Depending on Vitamin A Intake and Presence of Mucous Deposits (incubation for 7 min in solution containing 0.3 mM $ZnCl_2$, 0.1 μCi/ml labeled ^{65}Zn)

Group	Accumulating preparations of chick ileal mucosa		p
	without removal of mucous deposits	after removal of mucous deposits	
Control	52,94±2,40	40,61±2,77	<0,01
1	57,45±2,52	46,94±3,70	<0,05
2	56,87±0,87	50,07±1,66	<0,002

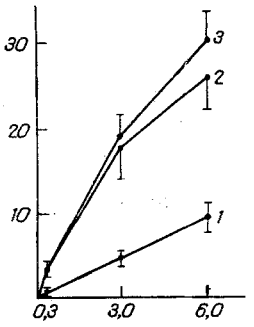

Fig. 1. Kinetics of zinc absorption in chick ileum depending on vitamin A intake. Abscissa, zinc concentration introduced into lumen of ileum (in mM); ordinate, rate of zinc transport (in nmoles/min/g intestine). Chicks of group 1, 2) of control group, 3) of group 2.

The main aim of this study was to use biochemical, physiological, and histochemical methods to determine the effect of vitamin A on absorption and localization of zinc in the epithelium of the chick ileum.

EXPERIMENTAL METHOD

Experiments were carried out on cockerels of the High Sex White crossbreed, divided into three groups: group 1 received a diet deficient in vitamin A; group 2 received 20,000 IU of retinyl acetate in oil per os 72 h before sacrifices (+A chicks); control chicks received a diet containing vitamin A (8000 IU/kg food). Chicks aged 30-35 days were used in the experiments. By that time the chicks of group 1 were showing signs of avitaminosis A. Before the experiments began the chicks were deprived of food for 15-16 h. Zinc accumulation was studied in the ileum in vitro by the method of Ugolev et al. [7]. Material was incubated in a buffer solution of the following composition: Tris 4 mM, NaCl 145 mM, KCl 4 mM, fructose 20 mM, pH 7.4, $ZnCl_2$ 0.06 mM, labeled ^{65}Zn 0.1 μCi/ml [1]. Accumulation of zinc in the intestine was studied in relation to the vitamin A intake of the chicks and the presence of mucous deposits. To remove some of the mucous deposits, the accumulating preparation, fixed with ligatures to a glass rod, was placed in a test tube containing 5 ml of cold buffer solution and shaken in it for 20 sec on a shaker at the rate of 3 cycles/sec. The effect of vitamin A was studied from the comparative aspect on chicks of groups 1 and 2. Zinc absorption was studied in the ileum in situ by the method described previously [3]. Absorption of zinc in the ileum was studied depending on the concentration of zinc introduced into the intestinal lumen (0.3, 3.0, and 6.0 mM) and the vitamin A intake of the animals. The results were subjected to statistical analysis by the Student–Fisher test. To study the ultrastructure of the enterocytes material was treated by the usual method for electron microscopy and studied in the Tesla BS 500 microscope. Histochemical tests were carried out on pieces of tissue taken 20 min after injection of the zinc solution into the intestine, fixed in 70° ethanol, saturated with H_2S, and embedded in paraffin wax. For the ultracytochemical study of zinc distribution in the enterocytes the tissue was fixed by the method described above, dehydrated in alcohols, and embedded

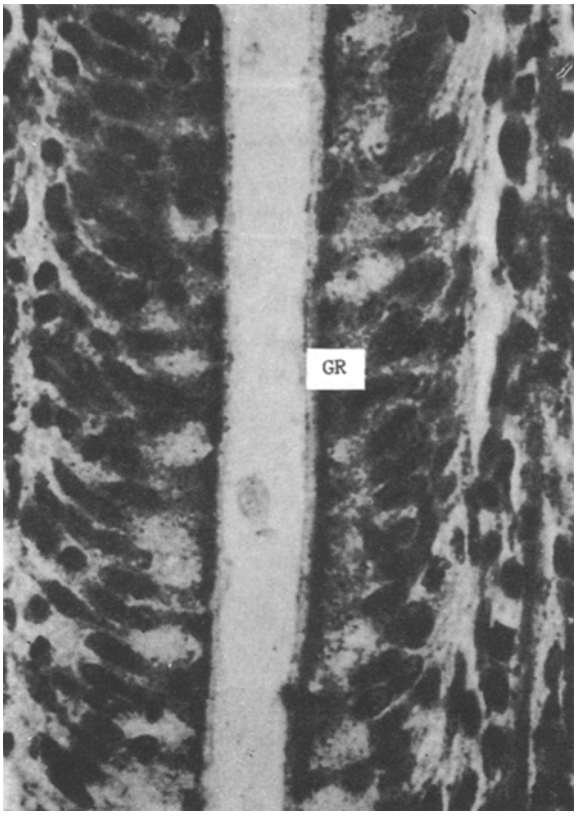

Fig. 2. Ileal mucosa of chicks of group 2. Here and in Fig. 3, histochemical test for zinc. 400×. Concentration of granules of reduced silver at apical pole of cell near brush border. GR) Granules.

in Araldite. Zinc was demonstrated in dewaxed and ultrathin sections by the silver sulfide reaction [6].

EXPERIMENTAL RESULTS

The results of biochemical investigations of zinc accumulation by the intestinal preparations showed that if a low concentration of zinc (0.06 mM) was present in the incubation solution, its accumulation by the intestinal preparations from the chicks of group 2 was significantly greater ($p < 0.001$) than from the chicks of group 1 (5.60 ± 0.28 and 3.36 ± 0.34 nmole/min/g respectively).

Experiments to study zinc accumulation by preparations of the ileum after partial removal of the mucous deposits (Table 1) revealed a significant decrease in zinc accumulation in the ileal wall.

Data on the kinetics of zinc absorption depending on the vitamin A intake of the chicks are summarized in Fig. 1. With all concentrations of zinc studied in animals receiving vitamin A the rate of absorption of the cation was significantly higher. The graph also shows that with an increase in the zinc concentration introduced into the intestinal lumen from 0.3 to 6.0 mM the rate of zinc absorption increased. However, the curves in Fig. 1 reflect different mechanisms of zinc absorption in the chick ileum. In chicks of group 1 the rate of zinc absorption was proportional to the concentration gradient of the cation introduced into the lumen of the ileum, whereas in the chicks of group 2 and the control group, dependence on the zinc ion concentration was nonlinear. This indicates that the process of zinc transport cannot be described as simple physical diffusion, but it takes place with the participation of an intermediate carrier. The role of this carrier may be played by vitamin A-dependent specific ZnBP.

The histochemical tests showed that in chicks of all three groups the apical pole of the enterocytes of the villi was filled with two or three layers of densely packed round granules

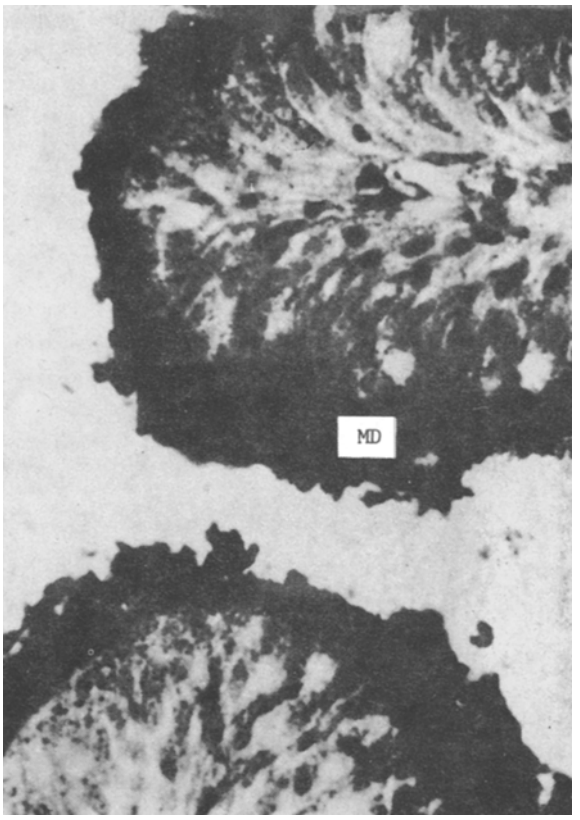

Fig. 3. Ileal mucosa of chicks of group 2 20 min after injection of 0.3 mM $ZnCl_2$ into intestinal lumen. 400×. Zinc-positive material in layer of mucous deposits (MD) of ileum and granules in apical zone of enterocytes.

of reduced silver, separated by a narrow band of cytoplasm from the brush border (Fig. 2). Ultrastructurally this zone consisted mainly of concentrations of vacuoles and tubules of the smooth endoplasmic reticulum (SER). On injection of a 0.3 mM solution of zinc chloride into the intestine most of the zinc-positive material was found in the form of bands, stained black, in the supramembranous layer of the enterocytes (Fig. 3). Intensification of zinc absorption was accompanied by an increase in the intensity of the reaction for this trace element in the granules of the apical zone, which often merged into a continuous black mass. The silver sulfide test on ultrathin sections showed that after injection of zinc chloride into the intestine precipitates of silver appeared on the membranes of the microvilli on the cytoplasmic side, and they were also found in the region of the tubules of SER.

Comparison of the results of the biochemical and histochemical tests of zinc accumulation in the ileum leads to the conclusion that the significant increase in zinc accumulation in the chicks of group 2 compared with those of group 1 from solutions with low zinc concentrations is due to the more highly developed layer of mucous deposits. The latter include in their composition acid and neutral glycoconjugates, forming the hydrophilic lining of the intestine and reversibly binding ions of metals, and ultimately transporting them to the membranes of the microvilli [14]. Incidentally, after partial removal of the mucous deposits, zinc accumulation was reduced by 12% in the chicks of group 1, by 19% in those of group 2, and by 24% in the control group of chicks. This is evidence both that mucous deposits can store large amounts of the metal entering the intestine and that synthesis of mucus by the goblet cells is depressed in avitaminosis A.

On the basis of these data on absorption and localization of zinc in the chick intestinal epithelium, one mechanism of transcellular transport of this cation, controlled by vitamin A (besides simple diffusion and transport of zinc bound with low-molecular-weight ligands) can be represented as follows. In the initial stage of absorption large amounts of zinc are retained in the supramembranous layer of the enterocytes, and this limits the amount of cations reaching the membranes of the microvilli and, within certain limits, prevents overloading

of the transmembrane zinc transport systems. Zinc cations then diffuse into the enterocytes where they form complexes with the specific vitamin A-dependent transport protein of the cytosol. Among the intracellular compartments, a leading role in the maintenance of zinc homeostasis under physiological conditions was probably played by the SER, which is located mainly close to the absorbing surface of the enterocyte, along the path of flow of cations entering the cell. To explain the vector flow of exogenous zinc cations in the direction of the basolateral membranes, it must be assumed that the latter contain a special ATPase, pumping zinc out of the cells and activated by the above-mentioned protein (or by vitamin A directly), by analogy with mechanism by which vitamin D controls the transepithelial transport of Ca^{++} [10].

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USE OF ACRIDINE ORANGE TO ASSESS LYMPHOCYTE MIGRATION IN VIVO

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KEY WORDS: migration; lymphocytes; transplantation; acridine orange; fluorescence

In the study of the mechanisms of repair processes, attention is increasingly being paid to lymphoid tissue. It has been shown that lymphocytes accumulate in intensively regenerating tissues as a result of their migration from lymphoid organs [1, 2, 5]. However, many mechanisms of this process remain unexplained, probably due to the absence of adequate methods of investigation.

Methods of studying lymphocyte migration based on the use of transplantation of donor's cells, labeled in vivo with radioactive isotopes, followed by autoradiography of squash preparations of organs, are known [8]. They have several disadvantages: the radioactive label is incorporated only by actively proliferating cells, and accordingly under physiological conditions not more than 20% of labeled lymphocytes can be obtained, or in the case of stimulation by phytohemagglutinin, not more than 40%. These cells possess the lowest level of functional activity, since they are less highly differentiated, and as a result the true picture of the distribution of the donor's lymphocytes is distorted. Also a radiological laboratory is required for work with isotopes such as ^{51}Cr, by means of which a substantially greater percentage of cells can be labeled [7].

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