corresponded to the time of remodeling of the bony callus, i.e., to the period of activation of mineral absorption. The data are summarized in Table 1.

Thus in intact (adjacent to the site of fracture) bone tissue changes in mineral exchange similar to those taking place in the zone of trauma are observed. This is evidently connected with the particular features of the action of breakdown products, metabolites, signals, and so on, proceeding from the zone of injury into adjacent tissues and inducing the changes observed in them. The results are evidence that this phenomenon is local rather than generalized in character. This zone can best be distinguished and defined as the "traumatic field." The traumatic field is the region nearest to the injured area, in which no morphological changes are present but, at the same time, specific biochemical reactions for the repair process are found in the region of injury at the moment of investigation.

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MORPHOLOGICAL ANALYSIS OF THE ZINC-ACCUMULATING CAPACITY OF THE DIGESTIVE ORGANS

Yu. G. Markov, N. I. Berzin', and A. R. Val'dman

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KEY WORDS: zinc excess; accumulation; target organs

The current annual production of zinc amounts to millions of tons. Industrial pollution of agricultural products by this element is one of the main factors leading to its toxic action on man. If zinc enters the body in excess, the content of this metal in the pancreas may be increased by 35 times (for comparison, by 11 times in the liver) [6]. Meanwhile the acinar cells (AC) do not possess a developed lysosomal apparatus [4], in which metals entering the cells usually accumulate. It is also difficult to explain the unique zinc-accumulating capacity of the pancreas purely by stimulation of synthesis of metallothioneines in it [12]. Accordingly, in order to study the adaptive changes in organs of the digestive system creating conditions for deposition of a large quantity of zinc in them, a comparative analysis was undertaken of the time course of accumulation and compartmentalization of an excess of zinc in the pancreas, liver, and ileal mucosa (IM).

Department of Histology, Latvian Medical Academy, Riga Laboratory of Animal Biochemistry and Physiology. Institute of Biology, Latvian Academy of Sciences, Salaspils.

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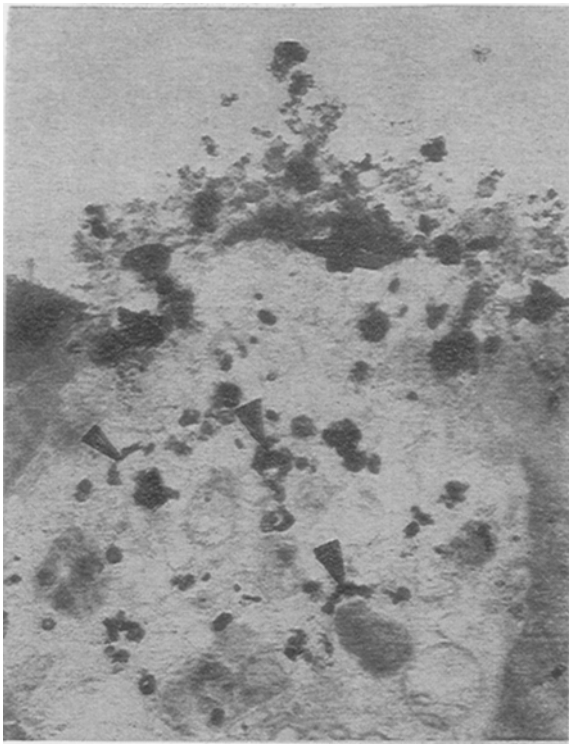

Fig. 1. BBE of control animal, desquamated into intestinal lumen. GRS in vesicles and tubules of endoplasmic reticulum in apical zone of cell (arrows). Magnification 17,000.

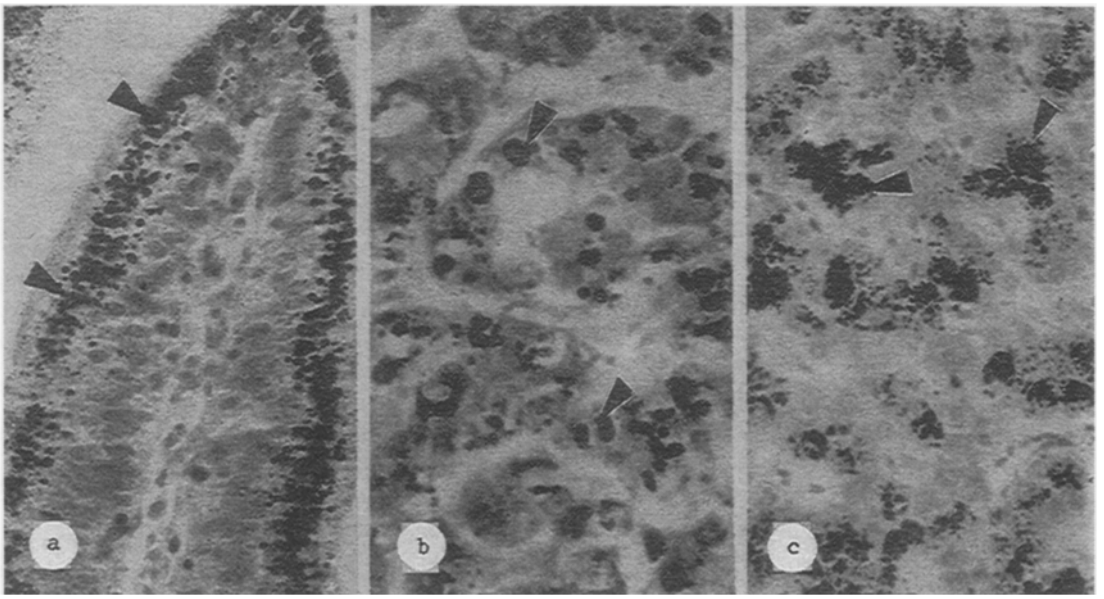

Fig. 2. Distribution of GRS in IM, pancreas, and liver of a group 6 bird: a) abundant deposition of GRS in supranuclear zone of BBE (arrows). Magnification 200; b) zinc-positive apoptotic bodies in acini of pancreas (arrows) Magnification 400; c) GRS filling perinuclear zone and biliary pole of hepatocytes (arrows). Magnification 200. Here and in Figs. 1 and 3, histochemical reaction for zinc.

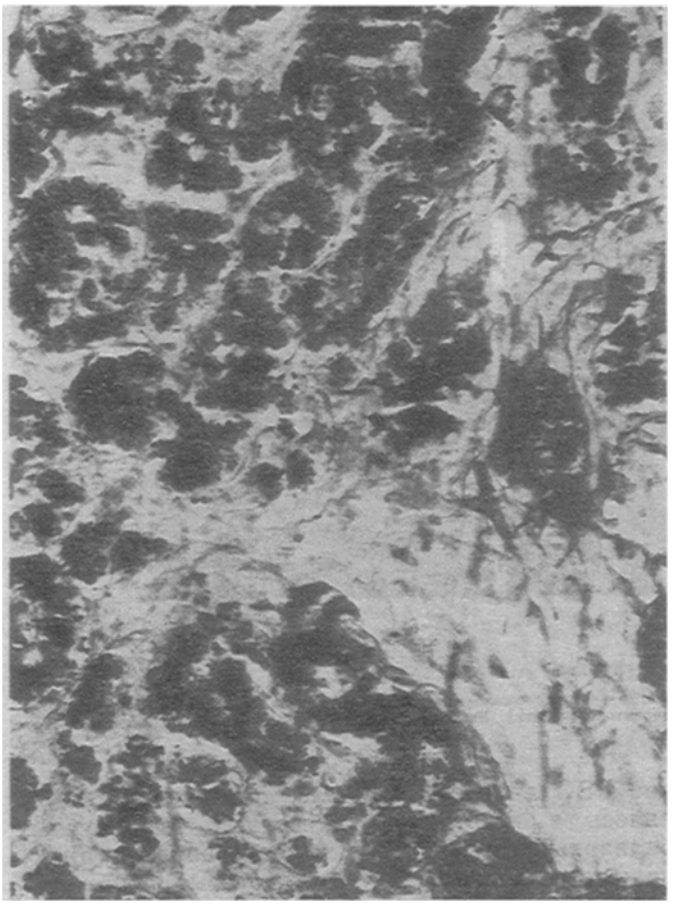

Fig. 3. Pancreas of a bird of group 6. Spreading of argyrophilic fibers between acini. Magnification 200.

EXPERIMENTAL METHOD

Experiments were carried out on White Leghorn chickens of the Hisex White Cross strain. From the 1st day of life the animals were given a basic diet containing 23 mg Zn/kg food. The 6-day chicks were divided into six groups (25 chicks in each group). The 1st group received the basic diet, and the diet of groups 2-6 was supplemented by the addition of 50, 500, 1000, 2000 and 4000 mg Zn/kg body weight respectively, in the form of the chloride. The experiment lasted 6 weeks. The zinc concentration in the liver, pancreas, and supernatant fraction of homogenate of IM was measured on a Perkin-Elmer atomic absorption spectrophotometer and calculated in mg/g dry substance (liver and pancreas) and in mg/liter (supernatant fraction of IM homogenate). The homogenate was obtained as follows: after decapitation of the chicks the ileum was quickly removed and washed with cold physiological saline (154 mM NaCl) IM was then curetted with a spatula and a homogenate (200 g/liter) prepared in 14 mM Tris buffer, pH 7.4, in the course of 1 min at 600*g*, and then centrifuged at 100,000*g* for 1 h. The results were subjected to statistical analysis by Student's and Fisher's tests. To study the distribution of zinc in IM, liver, and pancreas pieces of the organs were fixed in 70° ethanol, saturated with hydrogen sulfide, and embedded in paraffin wax, for subsequent treatment by the sulfide–silver method. The subcellular localization of zinc was determined by preparing the material in accordance with the modified sulfide–silver method described previously [2]. Sections were studied in the IEM-100B electron microscope.

EXPERIMENTAL RESULTS

Demonstration of the subcellular localization of the "histochemically active" zinc fraction in the intestinal epithelium showed that the end products of the reaction (granules of reduced silver – GRS) were found mainly in sites of the

TABLE 1. Zinc Content In Organs of Chickens Depending on Concentration of Zinc in the Diet (M ± m)

Group of birds	Amount of zinc added to food, mg/kg	Pancrease, mg/g dry substance	Liver, mg/g dry substance	Supernatant fraction of IM homogenate, mg/liter
1	—	0,19±0,02	0,102±0,020	4,5±0,3
2	50	0,16±0,01	0,120±0,010	8,4±0,2***
3	500	0,26±0,01**	0,152±0,009*	9,2±0,3***
4	1000	1,03±0,14***	0,342±0,069**	13,6±0,5***
5	2000	2,40±0,26***	0,690±0,094***	40,0±1,0***
6	4000	7,93±0,37***	1,702±0,126***	63,0±1,4***

Legend. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ Compared with group 1.

brushborder enterocytes (BBE) where the enzymes needing metal. ATP complexes for their activity are located. During hydrolysis of ATP inorganic phosphate (P_i) was released into the medium, where it probably combined with the metal to form the sparingly soluble zinc hydrophosphate, which is not eluted from the cell during fixation. The important role of P_i in "trapping" ink cations is indicated in particular by data obtained during study of this process with the aid of vesicles isolated from smooth myocytes [14]. In the control animals zinc was detected most effectively in damaged BBE, due to the increased accessibility of zinc-containing structures for molecules of the fixative. Large deposits of GRS were found under these circumstances in vesicles and tubules of the endoplasmic reticulum of the apical zone of BBE (Fig. 1), where glycerol kinase and acyl-CoA-synthetase, whose activity is dependent on the above-mentioned complexes, are involved in triglyceride resynthesis. This hypothesis is supported by the character of distribution of GRS on the inner side of the membrane of the microvilli of the brush border of the BBE, which resembled the distribution of products of the histochemical reaction for zinc-activated HCO_3-ATPase in this region of the cell [7, 13]. Moreover, deposits of GRS were found also by the present writers in other regions of BBE, where activity of ATPases also is observed (basolateral membranes, nuclear pores, membranes of the Golgi complex – GC –, and mitochondria) [10]. The increase in size of these GRS precipitates (to a particularly marked degree in the GC zone) occurred in the animals of groups 5 and 6 (Fig. 2a).

Demonstration of zinc in the liver of control chickens showed that GRS in the Kupfrer cells and endotheliocytes of the sinusoidal capillaries formed agglomerates close to the nucleus in the region of the dictyosomes of GC. In the hepatocytes, GRS surrounded the biliary capillaries in the form of punctate deposits. In the pancreas of the control chickens, the secretory granules of AC and insulocytes were distinguished by an intense reaction for zinc with an increase in the zinc content of the diet, the distribution of GRS in the liver showed the greatest changes in hepatocytes of birds of group 6, whose biliary pole was filled with zinc-positive vesicles, evidently reflecting hypertrophy and hyperplasia of the structures of GC and the lysosomes in response to the arrival of an excess of zinc (Fig. 2c). In the pancreas a similar reaction of GC was observed in birds of group 5, in whose acini agglomerates of GRS were found in the typical site of GC, namely the supranuclear zone.

An increase in the zinc concentration in the cytoplasm may also have a toxic action on the cell. This hypothesis is confirmed by the appearance of focal necroses of the hepatic parenchyma in individual birds of group 6 and of numerous AC in a state of apoptosis in the pancreas of birds of groups 5 and 6. Incidentally, it is possible to stain selectively AC damaged in this way, whose cytoplasm and nuclei are stained deep black with silver, by shortening the periods of incubation (Fig. 2b). Massive death of AC in the birth of these groups led to interstitial fibrosis of the pancreas. Excessive development of connective-tissue fibers under these circumstances was accompanied by their argyrophilia (Fig. 3). Similar changes also have been observed in the pancreas during development of zinc poisoning in ducklings [8]. Ultrastructures of BBE in the middle third of the villus during exposure to large doses of zinc (2000 mg or higher) also underwent changes of which the most characteristic were dilatation and vacuolation of cisterns of the rough endoplasmic reticulum and edema of the perinuclear space.

It follows from Table 1 that the zinc content in the pancreas of birds of group 6, in which destructive changes had developed, was increased by 41.7 times, whereas in the liver and IM it was increased by only 16.7 and 14 times respectively compared with the control. Such great differences in the zinc-accumulating capacity of these organs are probably due to the

more rapid passive uptake of zinc by the pancreas due to its higher sensitivity to the damaging action of this metal. It was found previously that more zinc accumulates in dying cells than in intact cells because of disturbance of the barrier function of the plasmalemma [2]. As the present investigation showed, apoptotic bodies also are inundated with zinc. The appearance of numerous apoptotic bodies in the parenchyma of the pancreas can be attributed to the bivalent action of excess of zinc on the cells. We know that initially compensatory-adaptive reactions develop in the cells, in the form of increased synthesis of metallothioneines and liposomes, accompanied by hypertropy of GC [5]. On the other hand, on exhaustion of the buffer capacity of these structures in AC, the aftereffects of the toxic action of zinc are exhibited particularly clearly, and lead to enzymic autolysis of the cell. The trigger mechanism of the latter is probably the inhibitory action of zinc cations on the proton ATPase of the secretory granules, accompanied by an increase in their pH and by release of pancreatic enzymes into the cytosol [11]. In this way a chain of autoenzymic reaction of injury to intracellular structures, including microtubules, may be triggered, and this itself may stimulate death of the cell by apoptosis [9].

In conclusion, attention must be drawn also to other important mechanisms of accumulation of an excess of metals in the digestive organs as a result of their excessive intake A review of data in the literature and the results of the present investigation indicates that these include: 1) complex formation with proteins, ATP, phospholipids, and nucleic acids; 2) sequestration by organoids (lysosomes, GC, mitochondria); 3) binding with extracellular structures (connective-tissue fibers and mucus) [1, 2, 3].

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