Correlation between the presence of transport Ca-ATPase activity and ability of microsomal membrane vesicles to accumulate Ca^{++} in the presence of ATP has been demonstrated for sarcolemma-enriched membrane fractions of myometrium [1], small intestine [7], and blood vessels [8]. It is possible that ATP hydrolysis and Ca^{++} transport are coupled or performed by the same system. Such a system, in PM of smooth-muscle cells of rabbit small intestine could be Mg-dependent Ca-activated ATPase, activated by micromolar calcium concentrations.

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EFFECT OF EXCESS VITAMIN A INTAKE ON STATE OF THE EPITHELIUM OF THE RAT SMALL INTESTINE

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KEY WORDS: small intestine; vitamin A.

There is much evidence in the literature of the important role of vitamin A in the regulation of differentiation and also, evidently, proliferation of epithelial cells [3, 8, 11]. The effects of vitamin A are linked mainly with its influence on the epithelium of the skin, the upper respiratory passages, and the genitourinary tract [3, 9]. The mucous membrane of the small intestine is considered to be a tissue with low sensitivity to this vitamin. In recent years, however, evidence has been obtained that may compel a revision of this outlook. It has been shown that vitamin A deficiency in experimental animals leads to depression of synthesis in the intestinal mucosa of glycoproteins, which play an important role in intercellular interaction and cellular differentiation processes [9, 10], activation of the enzymes of their catabolism [5], a decrease in the number of goblet cells [9, 10, 12], and lengthening of the cell cycle of the epithelium of the crypts [15]. Only isolated communications have been published on structural changes in the intestinal mucosa following administration of large doses of vitamin A [6, 7]. Meanwhile biochemical studies undertaken previously by one of us (I. Ya. K. [4]) revealed significant changes in glycoprotein metabolism in the intestinal mucosa of rats with hypervitaminosis A.

With the above facts in mind it was decided to study the effect of large doses of vitamin A on the structure and cellular proliferation processes in the mucous membrane of the rat small intestine.

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TABLE 1. Effect of Excess of Vitamin A on Some Parameters of State of Epithelium of Rat Small Intestine

Parameter studied	Proximal part of intestine				Distal part of intestine			
	control	hypervitaminosis A	%	P	control	hypervitaminosis A	%	P
Depth of crypts, μ	238,0±10,9	199,1±9,2	83,6	<0,05	224,0±4,0	199,2±5,7	88,8	<0,01
Number of cells lining crypts	86,3±5,6	66,9±4,9	77,5	<0,05	79,1±4,8	64,9±2,9	82,0	<0,01
Number of mitoses per crypt	4,15±0,21	2,3±0,31	55,4	<0,01	4,25±0,1	1,9±0,26	44,7	<0,001
Number of mitoses per 500 epithelial cells of crypts	25,0±1,73	16,1±2,54	64,4	<0,02	26,0±2,89	14,7±2,43	56,5	<0,02

Legend. Mean values ($\bar{X} \pm S_{\bar{X}}$) of 10-11 experiments shown.

EXPERIMENTAL METHOD

Experiments were carried out on growing male Wistar rats weighing initially 50-60 g and kept on a balanced animal house diet. Hypervitaminosis A was induced in the animals, as described previously [4], by daily administration of an oily solution of retinyl palmitate in a dose of 50,000 IU per rat via gastric tube. Control animals were given 0.2 ml of sunflower oil. On the 8th day of the experiment, when marked symptoms of hypervitaminosis A appeared in the rats (total dose of vitamin 400,000 IU) the animals were decapitated and quickly autopsied. Segments of small intestine from its proximal and distal portions were fixed in Carnoy's fluid and 10% formalin. Paraffin sections 3 μ thick and frozen sections 5 μ thick were cut. The paraffin sections were stained with hematoxylin and eosin. RNA was revealed by Brachet's method. Besides ordinary survey methods, morphometric methods also were used: The size of the crypts and villi was measured in the sections, the number of mitoses and cells in the layer of epithelium lining the crypts and villi was counted. The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Examination of the survey sections of the intestinal mucosa of rats receiving excess of vitamin A revealed no gross pathological disturbances indicative of inflammation, atrophy, or necrosis. However, on the lateral surfaces of the villi of some animals foci of disturbance of the complex structure of the epithelial layer were observed, indicating the presence of degenerative changes. In these areas the regular arrangement of the nuclei was disturbed and changes were observed in the height of the epithelial layer of the villi and the intensity of its staining (compared with the remaining part).

The results of the morphometric study of the intestinal mucosa showed (Table 1) that administration of an excess of vitamin A to the animals caused marked changes in the crypts: They were less deep than in the control animals, and they were lined by fewer cells. These changes were observed in both proximal and distal parts of the intestine. Meanwhile, in crypts of both parts of the intestine in rats receiving an excess of vitamin A there was a considerable decrease (compared with the control) in the number of mitotically dividing cells, by 44.5 and 55.3% calculated per crypt and by 35.6 and 43.5% calculated per 500 cells of the epithelial layer in the proximal and distal parts of the intestine respectively. These results show that vitamin A, in large doses, depresses proliferative activity of the epithelial cells of the "cambial" zone of the mucous membrane of the small intestine considerably. It can be tentatively suggested that this is why crypts in the mucosa are less deep and are lined by fewer cells in rats receiving an excess of vitamin A. It was also noted that in the experimental rats dividing cells in the metaphase stage (including late prophase and early anaphase) were found more often in the experimental rats than in the control, and this evidently points to lengthening of this stage of the cycle. Under the conditions used, without special methods of investigation the chromosomes appeared to be fused. Presumably it was because of this that there was difficulty in proceeding into the next phase of the mitotic cycle, which is associated with separation of the chomosomes into two halves. The changes observed resembled the picture of "colchicine mitosis," which is considered to be due to structural disorganization of chromosomes and a disturbance of polymerization–depolymerization of tubulin [1].

The RNA level in the epithelium of the villi and crypts is known to reflect to some degree the intensity of cellular proliferation. Investigations conducted on these grounds showed that the RNA content in the cell cytoplasm of the control animals decreased in the direction from the base to the apex of the villi, in accordance with data in the literature [4]. The presence of this RNA concentration gradient is in agreement with the known fact that enterocytes mature gradually as they migrate along the villus. Excess of vitamin A caused an increase in the intensity of staining of the epithelial cells along the whole length of the villi and a more uniform distribution of staining along the villus. Disappearance of the RNA distribution gradient along the villi was most marked in the distal portion of the small intestine. These findings evidently indicate a disturbance of differentiation and maturation of enterocytes in rats receiving an excess of vitamin A.

The experiments thus revealed substantial changes in the state of the epithelium of the small intestine in rats receiving large doses of vitamin A. The most noteworthy results are those showing the inhibitory action of large doses of vitamin A on mitotic cell division in the intestinal epithelium. Data in the literature relate mainly to the effect of vitamin A on mitosis in the epithelium of the skin. These investigations showed that after administration of relatively small doses of vitamin A to animals the mitotic index rises, whereas large doses of the vitamin, on the contrary, cause it to fall [8, 3, 14]. Similar results were obtained in a study of the mitotic index in the mouse intestine, which rose in response to subcutaneous injection of vitamin A in doses of 100 and 1000 IU/kg body weight, but fell when the dose was increased to 3000 IU/kg [2]. The results of the present experiments consequently agree with data in the literature, and indicate for the first time that large doses of vitamin A inhibit mitosis in the epithelium of the mucous membrane of the small intestine when given perorally to rats.

An inhibitory action of retinol and its synthetic derivatives (retinoids) *in vitro* on cell growth and replication in cell and tissue culture has recently also been demonstrated, in agreement with data on the anticarcinogenic properties of these compounds [3, 8]. Since the intestinal epithelial cells, like bone marrow cells, are among the most rapidly renewed and proliferating cells in the body, and in this respect show a certain similarity to embryonic and tumor cells, the results must be considered in conjunction with those of investigation of the action of retinoids *in vitro*, which indicate the existence of a similar phenomenon, namely the antiproliferative action of retinol on rapidly renewed cells not only *in vitro*, but also *in vivo*. This conclusion assumes special significance if the possible role of vitamin A deficiency in the genesis of intestinal tumors in experimental animals is considered [13].

Bearing in mind the fundamental concept of reciprocity between cell proliferation and differentiation, it might be expected that inhibition of proliferation of enterocytes induced by excess of vitamin A would lead at the same time to acceleration of their differentiation in the course of migration from the crypts to the apices of the villi. However, the experimental results obtained provide no grounds for such an unequivocal conclusion. In fact, administration of large doses of vitamin A to rats was accompanied by disappearance of the RNA distribution gradient along the length of the villi and disturbance of the orderly arrangement of the nuclei. In addition, in a parallel study, a tendency was discovered for the relative number of goblet cells to decrease in both parts of the intestine. All these facts indicate that excess of vitamin A does not accelerate, but disturbs the normal course of differentiation of epithelial cells in the rat small intestine.

This investigation thus indicates undisputed changes in the state of the epithelium of the small intestine in rats receiving large doses of vitamin A. These changes are characterized by inhibition of proliferation of the epithelial cells of the crypts and disturbance of the program of their further differentiation. These results, in conjunction with those of the writers' previous biochemical investigations, confirm the view that the mucous membrane of the small intestine is one of the target organs for vitamin A.

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RESPONSE OF THE INFANTILE RAT OVARY TO INTRASPLENIC TRANSPLANTATION INTO ADULT CASTRATED DIABETIC ANIMALS

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In rats with experimental diabetes, compensatory hypertrophy of the ovary is weak or may be absent altogether [2]. It has been suggested that the response of the pituitary to a fall in the blood estrogen level is depressed in diabetes [2]. It was decided to study how the pituitary of diabetic rats responds to a sharper decline in the estrogen level, caused by transplantation of the ovaries into the spleen. Such transplantation is known to lead to inactivation of most of the estrogen produced by the ovary in the liver, and in turn this leads to increased secretion of gonadotrophins and to hypertrophy of the transplanted ovaries [1, 4]. The investigation described below was undertaken to study this problem.

EXPERIMENTAL METHOD

Adult female albino rats weighing on average 246 ± 4 g were castrated under pentobarbital anesthesia, after which one ovary, taken from an infantile animal, was implanted into their spleen. The weight of this ovary was 4-10 mg. All the animals 7-9 days after plantation, when survival of the graft could be expected, were divided into two groups. One group served as the control; animals of the other group received a subcutaneous injection of freshly prepared alloxan in a dose of 16 mg/kg. Vaginal smears were taken from all the rats, and in those receiving alloxan the diuresis and sugar concentration in the urine were studied. Rats with marked diabetes and control animals were decapitated 33 days after the injection of alloxan. The blood sugar of these animals was determined by the picrate method. The ovary was removed from the spleen and weighed. The uterus, pituitary, adrenals, and vagina also were weighed. The uterus and transplanted ovary were fixed in Bouin's fluid for histological treatment. The height of the uterine epithelium, epithelium of the uterine glands, and diameter of the follicles were measured. Only those animals with no adhesions between the spleen and peritoneum were taken into consideration. In addition, the total gonadotrophin level in the pituitary was determined in infantile cocks of the White Russian breed. Each cock received subcutaneous injections of saline extracts of pituitary glands twice a day for 5 days in a total dose of 8 mg. The degree of response was judged from the change in weight of the testes and comb.

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