CHANGES IN HISTOCHEMICAL AND MORPHOMETRIC PARAMETERS OF THE MOUSE MAMMARY GLAND IN RESPONSE TO DIFFERENT DOSES OF VITAMIN A

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When treating diseases with vitamin preparations in doses some tens of times greater than the body's physiological requimements of them, investigators have concentrated their attention on the effect and mechanism of hypervitaminization [4, 5, 8, 9, 10, 14, 17]. After administration of a physiological dose of vitamin A to mice, biosynthesis and secretion in the salivary glands are intensified [3, 6]. Toxic doses of retinol acetate (RA) causes a sharp fall in all parameters of metabolism (nuclear RNA, cytoplasmic RNA, total cytoplasmic protein, body weight, weight of the salivary glands, a decrease in size of the nuclei and cytoplasm) in mouse salivary glands.

The object of this investigation was to study several parameters of metabolism in the body as a whole and in the nonlactating mammary gland in response to administration of different doses of RA, paying particular attention to the body weight of the animals, the weight of their mammary gland, RNA and total protein in the epithelial cells of the gland, and the area of the nuclei and cytoplasm of the epitheliocytes.

EXPERIMENTAL METHOD

Experiments were carried out on 60 female CBA/C57BL mice weighing 22-24 g and aged 3-4 months. The animals were divided into four groups, with 15 in each group. Group 1 consisted of intact mice which served as the control, the animals of group 2 received soy oil in a dose of 0.1 ml, and those of group 3 received RA in a total dose of 30,000 IU (3000 IU daily for 10 days). The animals of group 4 received RA in a total dose of 80,000 IU over a period of 10 days. A 3.44% solution of RA in factory-produced soy oil was used as the vitamin A preparation. RA was administered by gastric tube. At the end of its administration, the physiologically active phase (the phase of estrus) was identified in the animals by the vaginal smears method and the animals were killed during that phase. The right inguinal mammary glands were dissected for investigation. After sacrifice the animals and the mammary gland were weighed and the ratio between them (the organosomatic index) was calculated. The glands were coiled into a "helix" on filter paper, fixed in 10% buffered formalin and Carnoy's fluid, and embedded in paraffin wax. Serial sections 6 μ thick were stained with hematoxylin–eosin. Karyometry and cytometry of the epithelial cells of the glands were carried out with an ocular micrometer and the area of the nucleus and cytoplasm was determined [1].

To determine RNA the material was stained with gallocyanin and chrome alum by Einarson's method, with treatment with DNase (control sections were not treated with the enzyme), and to detect total protein they were stained with Amido black 10B. The sections thus obtained were subjected to photometry on a Reichert microphotometer by the single-wave multiple-points method, at a wavelength of 570 nm for RNA and 600 nm for total protein. To determine the quantity of the substance in a cell, the product of optical density of the substance in the cell and its area was calculated. The area of the nucleus and cytoplasm was expressed in conventional units. All the numerical results were subjected to statistical analysis with the determination of the arithmetic mean and its error. The significance of differences between groups was estimated by the Student–Fisher t test.

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TABLE 1. Changes in Body Weight and Weight of Nonlactating Mammary Gland in Response to Various Doses of Vitamin A

Group of animals	Body weight of mouse at sacrifice, g	Weight of mammary glands at sacrifice, mg	Organosomatic index
1 (Control)	21,6±0,3	128,8±4,1	0,59±0,01
2	21,3±0,4	127,8±3,5	0,58±0,01
P	>0,01	>0,05	>0,05
3	27,3±0,27	206,1±5,2	0,76±0,02
P	<0,01	<0,01	<0,01
	(126,5)	(159)	(131)
4	12,1±0,5	40,7±1,5	0,32±0,01
P	<0,05	<0,05	<0,05
	(56)	(31)	(55)

Legend. 1) Here and in Tables 2 and 3 P given relative to control. 2) Percentage of control shown in parentheses.

TABLE 2. Changes in Area of Epitheliocytes (S) of Nonlactating Mammary Gland in Response to Various Doses of Vitamin A

Mammary glands	Group 1 (control)		Group 2		Group 3		Group 4	
	S of nucleus	S of cytoplasm	S of nucleus	S of cytoplasm	S of nucleus	S of cytoplasm	S of nucleus	S of cytoplasm
Growth buds	6,2±0,5	17,7±1,1	6,0±0,3	17,2±1,5	10,1±1,0	25,8±1,3	4,9±0,9	11,0±0,8
P			>0,01	>0,01	<0,05	<0,01	<0,05	<0,05
Small ducts	9,8±1,1	23,0±1,4	9,9±1,2	23,2±1,0	13,4±1,5	30,6±1,4	8,7±1,0	17,2±1,5
P			>0,05	>0,05	<0,05	<0,01	0,05	<0,01
Medium-sized ducts	7,5±0,4	18,0±1,2	7,0±0,3	18,0±1,1	11,0±0,9	23,7±1,2	6,2±0,4	14,3±1,0
P			>0,05	>0,01	<0,01	<0,01	0,05	<0,05

EXPERIMENTAL RESULTS

Administration of vitamin A in a dose not giving rise to clinical symptoms of toxic hypervitaminosis A (30,000 IU) was accompanied by an increase in the body weight and weight of the mammary gland of the animals (Table 1). To make the results more demonstrative they were converted into percentages. Intact animals (group 1) served as the control. In animals receiving the vitamin in a dose of 30,000 IU the body weight was 26.5% greater than that of the control animals, the weight of the mammary gland was 59% greater, and the organosomatic index was 31% higher. In animals receiving soy oil (group 2) no significant differences were found compared with group 1. In animals receiving vitamin A in a dose of 80,000 IU toxic manifestations were observed: a decrease in body weight and weight of the mammary gland, inertia and photophobia, loss of hair and baldness, accompanied by hyperkeratosis. The body weight of the mice was reduced by 44% compared with animals of group 1, the weight of the mammary gland by 69%, and the organosomatic index by 45%.

Karyocytometric investigations showed (Table 2) that the area of cytoplasm and nuclei of the epithelial stroma of the mammary gland in the animals of group 2 was not significantly different from the control group. In group 3 a statistically significant increase was observed in the area of the nuclei and cytoplasm of the epithelial cells compared with group 1. In the animals of group 4 there was a statistically significant decrease in the area of both nuclei and cytoplasm, evidence of the unfavorable action of toxic doses of vitamin A.

Vitamin A in nontoxic doses (animals of group 4) had a stimulating effect on the RNA and total protein content in the epitheliocytes of the nonlactating mammary gland. This is shown clearly in Table 3 and Fig. 1. In the animals of this group, receiving vitamin A in a dose of 30,000 IU, the content of nuclear and cytoplasmic RNA and also of cytoplasmic total protein was increased. In the case of toxic doses (animals of group 4), on the other hand, the vitamin inhibited synthetic activity of the glandular cells, as shown by a decrease in the content of both nuclear and cytoplasmic RNA and total cytoplasmic protein. The RNA content

TABLE 3. Changes in Total Cytoplasmic Protein, Nuclear RNA (RNA_n), and Cytoplasmic RNA (RNA_c) in Nonlactating Mammary Gland in Response to Various Doses of Vitamin A

Groups of animals	Component determined	Nonlactating mammary gland					
		growth buds	P	small ducts	P	medium-sized ducts	P
1 (Control)	Total protein	11,5±1,0		13,34±0,9		10,3±1,0	
	RNA_n	2,91±0,3		4,21±0,1		2,7±0,2	
	RNA_c	7,43±0,5		9,43±1,2		5,22±0,8	
2	Total protein	11,08±0,8	>0,05	13,11±0,7	>0,05	10,0±0,9	>0,05
	RNA_n	2,73±0,1	>0,05	4,1±0,2	>0,05	2,4±0,1	>0,05
	RNA_c	7,0±0,4	>0,05	9,3±0,8	>0,05	5,0±0,7	>0,05
3	Total protein	23,7±1,6	<0,001	26,01±1,5	<0,05	18,96±1,3	<0,05
	RNA_n	6,4±0,6	<0,001	8,1±0,7	<0,01	5,94±0,8	<0,01
	RNA_c	13,6±1,1	<0,01	15,9±1,4	<0,001	11,4±1,1	<0,05
4	Total protein	5,94±0,6	<0,05	11,7±0,8	<0,05	9,58±0,9	0,05
	RNA_n	0,83±0,07	<0,01	2,09±0,3	<0,05	1,3±0,1	<0,05
	RNA_c	2,53±0,45	<0,01	4,47±0,3	<0,001	3,15±0,2	<0,05

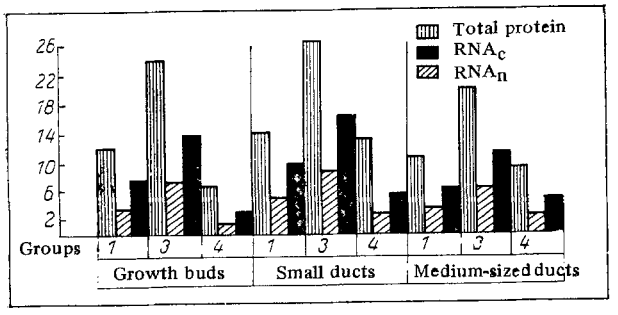

Fig. 1. Content of total protein, RNA_n, and RNA_c in epithelial cells of nonlactating mammary gland.

in the nucleus and cytoplasm and the total cytoplasmic protein in the animals of group 2, receiving soy oil, showed no significant difference from the control group.

Hypervitaminosis A is known to cause changes in the nucleuc acid and total protein content in cells of the liver, lungs, and other organs [3, 5, 12]. Some workers [12, 15] found a marked increase in the RNA content in hepatocytes after administration of vitamin A in a dose of 50,000 IU. Potentiation of nucleic acid synthesis also was found after treatment of a cell culture with vitamin A [11]. Retinoic acid, by local application in a physiological dose, stimulated RNA biosynthesis by 60% in cells of the guinea pig epidermis [13]. After administration of vitamin A in a dose of 50,000 IU biosynthesis was stimulated in mouse salivary glands [2, 3]. The data described above suggests that vitamin A is a regulator of nucleic acid metabolism in epithelial cells, although the mechanisms of this action are not yet sufficiently clear. There is only indirect evidence that the effect of vitamin A on nucleic acid metabolism may be indirectly connected with a change in the level of function of the endocrine glands (adrenals, thyroid, ovaries [7, 8, 15]), and also with changes in the structure and function of biological membranes [5]. Some workers [12, 15] consider that the mechanism of action of vitamin A resembles that of steroid hormones.

The results of the present investigation indicate that administration of vitamin A affects the mammary glands, and the degree and character of the influence depend on the dose of the vitamin given.

Administration of vitamin A in the form of RA to animals in a dose of 30,000 IU, which does not give rise to toxic hypervitaminosis, thus stimulated metabolic activity of the cells, as shown by an increase in area of their nuclei and cytoplasm, an increase in the total cytoplasmic protein and nuclear and cytoplasmic RNA content, an increase in weight of the mammary gland, and an increase in body weight. Vitamin A in a dose of 80,000 IU, at which signs of toxic hypervitaminosis begin to appear in the recipient, depresses the cell metabolism of the nonlactating mammary gland in mice, as shown by a decrease in body weight,

the weight of the mammary glands, the area of the nuclei and cytoplasm of the epitheliocytes, and a fall in the total cytoplasmic protein and nuclear and cytoplasmic RNA contents.

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