SECRETORY AND IMMUNOCOMPETENCE FUNCTIONS OF THE PAROTID AND SUBMANDIBULAR SALIVARY GLANDS AFTER THYMECTOMY

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In recent years interest has grown in scientific developments revealing connections between the functions of tissues, organs, and systems. This applies in full measure to the system of immunity, whose autonomy is relative and whose connections with other tissues and organs are many and varied. An important aspect of this problem is the study of relations between the organs of digestion and of immunity, the phylogenetically determined morphological and functional close similarity of which was established originally by Mechnikov. The central position of the thymus in the hierarchy of the immunity system, as well as the fact that it produces hormonally active substances, are well known [11]. On the other hand, it has been shown that the salivary glands not only are involved in digestion, but also produce a whole range of biologically active substances, related to the phenomena of immune protection and immunoregulation of nonspecific resistance, including lysozyme, lactoferrin, transferrin, thymocyte-transforming factor, secretory immunoglobulins, and beta-lysine [8, 15].

The aim of this investigation was to study the effect of thymectomy on the secretory and immunocompetence functions of the parotid and submandibular salivary glands in noninbred albino rats.

EXPERIMENTAL METHOD

Experiments were carried out on 227 noninbred male albino rats weighing 150-170 g, on 115 of which thymectomy was performed by Racelis' method [16]; a mock operation with thoracotomy was performed on 57 animals, and the rest served as the intact control. The animals were decapitated all at the same time of day, 3 and 7 days and 1, 2 and 3 months after the operation. Pieces, of salivary glands were fixed in a 12% solution of neutral formalin and in Carnoy's fluid, and embedded in paraffin wax. Dewaxed sections 5-7 μ thick were stained with Ehrlich's hematoxylin and eosin; RNA was determined by Brachet's method and DNA by Feulgen's method. Total proteins in the structural components of the tissues were studied by Danielli's method in Shubich's modification [13]. Glycogen and neutral glycosaminoglycans (GAG_n) were studied by the PAS reaction (salivary amylase control, with acetylation). Acid phosphatase (AP) activity, by the azo-coupling method, and lactate dehydrogenase (LDH) activity were determined [5] in frozen sections. The relative activity of the enzymes was estimated on the LYUMAM I-3 microscope-photometer in transmitted light with a wavelength of 456 nm [1]. At each time of the experiment optical density was determined in at least 400 cells (250 cells in the experiment and 150 in each control) – in serocytes, mucocytes, and epitheliocytes of the granular, striated, and intralobular ducts. The numerical results were subjected to statistical an,analysis on a computer by Student's t test. Differences were considered significant at the $p < 0.05$ level. Immunomorphological analysis of the tissue was carried out in squash preparations, cells being differentiated in accordance with Pokrovskaya's nomenclature [6]. Meanwhile the total number of karyocytes of the salivary glands

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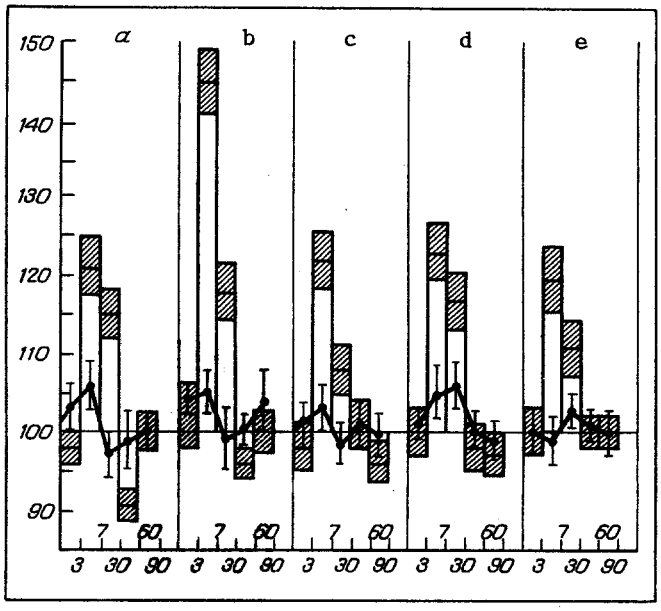

Fig. 1. LDH activity in submandibular salivary gland after thymectomy. Columns indicate thymectomy, shaded part of columns gives confidence interval, curve represents mock thymectomy; activity in control intact animals taken as 100%. a) Serocytes, b) mucocytes, c) granular ducts, d) striated ducts, e) intralobular efferent ducts. Abscissa, time of observation (in days); ordinate, LDH activity (in %).

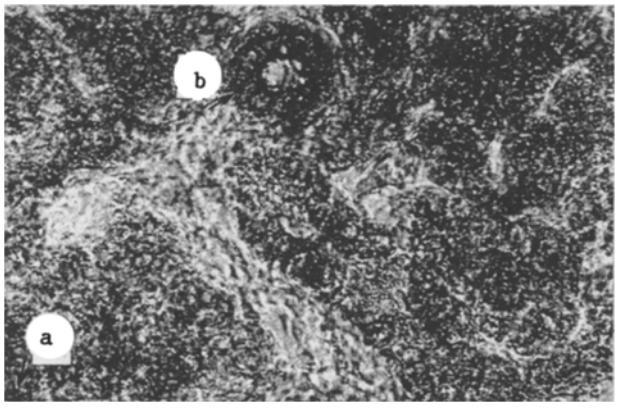

Fig. 2. AP activity in parotid salivary gland in control. Here and in Fig. 3: a) terminal divisions, b) efferent ducts. Burstone's azo-coupling reaction. 500×.

and their lysozyme content were determined by Dorofeychuk's method [3]. After preliminary homogenization of the parotid and submandibular salivary glands in physiological saline. In parallel tests the serum lysozyme concentration was determined by the same method.

EXPERIMENTAL RESULTS

In the early times of observation (3 and 7 days) an adaptive reaction developed in the salivary glands, accompanied by stimulation of their protein synthesizing activity. This was confirmed by the increased basophilia of the cytoplasm of the serocytes in the terminal divisions and of the serous demilunes, the agglomeration of nuclei of the epitheliocytes of the

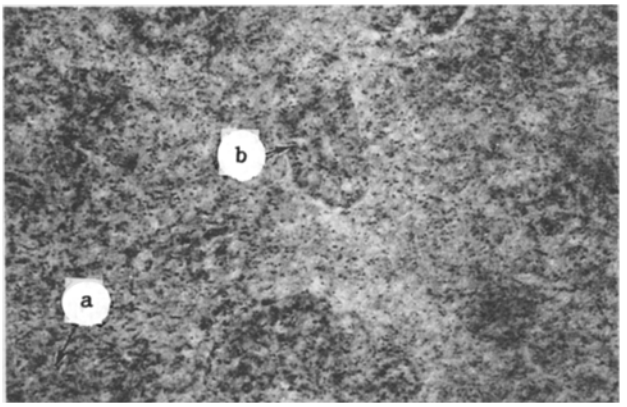

Fig. 3. Decrease in AP activity in parotid salivary gland 3 months after thymectomy.

TABLE 1. Time Course of Lysozyme Activity (conventional units) after Thymectomy on Mature Rats (M ± m)

Material studied	Control	Nature of operation	Time of observation				
			3 days	7 days	1 month	2 months	3 months
Tissue of submandibular salivary gland	74,8±3,7	Mock	71,4±4,1	67,2±4,1	74,0±3,9	74,6±13,8	74,8±3,8
		Thymectomy	44,5±3,3	31,4±2,9	73,9±4,0	115,6±7,6	127,4±7,9
		p	<0,01	<0,01	>0,05	<0,01	<0,01
		p_1	<0,01	<0,01	>0,05	<0,01	<0,01
Tissue of parotid salivary gland	39,9±2,8	Mock	38,4±3,1	42,4±3,7	39,5±3,4	38,2±2,9	39,0±3,0
		Thymectomy	29,6±2,7	67,0±5,9	74,0±5,7	86,7±6,5	57,5±4,1
		p	<0,05	<0,01	<0,01	<0,01	<0,01
		p_1	<0,05	<0,01	<0,01	<0,01	<0,01
Peripheral blood	37,4±1,8	Mock	38,6±2,4	39,5±2,5	38,5±2,3	36,9±2,3	37,3±2,1
		Thymectomy	35,9±2,7	35,3±2,6	38,5±3,0	46,6±3,7	58,3±3,6
		p	>0,05	>0,05	>0,05	<0,05	<0,01
		p_1	>0,05	>0,05	>0,05	<0,05	<0,01

Legend. p indicates significance of differences from control; p_1) differences from values obtained in animals undergoing mock operation.

granular and striated ducts, their displacement into the lumen, and the increase in the intensity of staining of the test portions of the salivary glands for RNA and total proteins. In cells of the terminal divisions and the efferent ducts of the salivary glands increased AP activity was found, followed after 7 days by increased LDH activity (Fig. 1). These enzymes not only synthesize and form the protein secretion in the serocytes, but they also play an active role in its release. The simultaneous increase in the content of GAG_n in the serocytes can be regarded as an adaptive reaction of these cells to new conditions of activity [12]. The second stage of the observations (1, 2, and 3 months after thymectomy) can be characterized as the period of diminution of protein-synthesizing activity of the serocytes. The nuclei of most serocytes were deformed and showed pycnotic changes. In some serocyte nuclei peripheral condensation of DNA was observed. The intensity of staining of the nucleoli and cytoplasm of the serocytes for RNA and total proteins was sharply reduced. Diminution of protein-synthesizing activity of the serocytes was combined with very active functioning of the epitheliocytes of the efferent ducts. Nuclei of epitheliocytes of the granular and striated ducts were displaced into the lumen. The intensity of staining of the nucleoli and cytoplasm of the epitheliocytes of the efferent ducts for RNA and total protein was within control limits. After 1 month the concentration of GAG_n in the serocytes and also concentrations of glycogen and GAG_n in the epitheliocytes of the efferent ducts were considerably increased, and they still remained high, although not quite as high, on subsequent days of observation. AP activity in the salivary glands, which was increased after 3 and 7 days and 1 month, decreased (Figs. 2 and 3) as LDH activity returned to normal (Fig. 1). The morphological changes which we observed were most marked in the parenchyma of the submandibular salivary gland. In the stroma of the organs, disturbances of the lymph and blood circulation were observed, and 2 and 3 months after thymectomy infiltration of lymphocytes and plasma cells was

observed, evidence of strengthening of the cellular component of the immunity system. Thus a late effect of thymectomy is a decrease in the content of the protein component and an increase in the mucous component of the secretion of the parotid and submandibular salivary glands.

At the same time, significant changes also were observed in the immunomorphologic parameters of the salivary glands. Activation of all cell populations involved in the immune response took place 1 month after thymectomy in the parotid and submandibular salivary glands. This was shown by a progressive increase in the relative and absolute numbers of medium-sized lymphocytes, lymphoblasts, neutrophils, reticular cells, and all forms of plasma cells, continuing until the end of the investigations. Changes were observed in both pairs of salivary glands, in agreement with the presence of histologically detected infiltration of lymphocytes and plasma cells. Reduction in the number of karyocytes (to 30-50%) in the salivary glands on the 3rd and 7th days after thymectomy was followed at 2 months by d significant ($p < 0.05$) increase in the parotid salivary gland by 23.4% and in the submandibular gland by 46.6%. So far as the serum and salivary lysozyme activity is concerned, the reaction observed in this case was identical in direction (Table 1): lowering of the serum lysozyme activity, whereas in the tissues of the parotid and submandibular salivary glands this was replaced by an increase in the later periods of observation (1, 2, and 3 months). Activation of the immunomorphological parameters of the salivary glands is evidently connected with the fact that they belong to the thymicocircumpharyngeal complex [4], whose components, judging by all the evidence, become involved as a compensatory-regulatory mechanism when the function of the thymus is depressed. This hypothesis is confirmed by the analogous changes taking place after removal of the thymus in the structure of the cell populations of another, immunocompetent organ, namely the spleen [14]. As regards inhibition of secretion of the protein component of the salivary glands, it is probably connected both with the endocrine imbalance [2] and with a redistribution of energy-yielding and plastic material for processes leading to the formation of immunity.

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