According to the results of a series of investigations, specific effects of preparations of exogenous RNAs, connected with selective activation of protein synthesis, are produced by the action of the fraction of template RNAs [8, 9]. Our own data are evidence in support of this conclusion. For instance, the increase in working capacity of the recipients after receiving an injection of the total RNA preparation from the liver of donors receiving hydrocortisone, was reproduced by the fraction of precursors of template RNAs, but not of ribosomal RNAs (Fig. 3). Possibly the precursors are converted in the recipient's body into ready-made template RNAs, which exert a specific action. Such a possibility is confirmed by reproduction of the effect of the total RNA preparation by the polysomal RNA fraction, which contains ready-made templates.

We used the exogenous RNA method to analyze effects not only of glucocorticoids, but also of other substrances [7]. This method appears to be very valuable, for it enables components of complex effects of biologically active substances, due to activation of protein synthesis in particular organs, to be studied separately.

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MORPHOLOGY AND FUNCTION OF THE THYMUS IN GUINEA PIGS RECEIVING THYMUS AND BONE MARROW POLYPEPTIDES

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In the modern view substances participating in regulation of the immunocompetent system are produced in the thymus and bone marrow [2, 3, 5]. In a previous investigation on mice the writers showed that the polypeptide preparation from the thymus (thymalin) stimulated thymocyte function [4].

The aim of the present investigation was to compare the effects of polypeptide preparations of thymus and bone marrow on morphology and function of the thymus gland in guinea pigs.

EXPERIMENTAL METHOD

Polypeptide preparations were isolated by acetic acid extraction followed by ion-exchange chromatography: thymalin from calf thymus, hemalin from bone marrow [1, 2].

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TABLE 1. Results of Morphometry of Guinea Pig Thymus (X ± m)

Experimental conditions	Time of investigation, days	Ratio between thickness of cortex and thickness of medulla of lobules	Mitotic coefficient, %	Dimensions of nuclei (in conven. units) of REC	
				of cortex	of medulla
Intact animals		0,91±0,12	4,5±1,25	0,1020±0,007	0,1377±0,005
Physiological saline	4-	0,87±0,025	3,9±0,25	0,1164±0,003**	0,1466±0,006**
Thymalin		0,70±0,07*	7,15±1,31*	0,1261±0,006*	0,1696±0,005*
Hemalin		0,90±0,07	4,37±0,68	0,1152±0,001	0,1555±0,004*
Physiological saline	11-	0,81±0,006	4,5±1,43	0,1208±0,001	0,1591±0,004
Thymalin		0,92±0,06*	5,3±0,01*	0,1229±0,002	0,1918±0,014*
Hemalin		0,89±0,011	4,3±0,8	0,1243±0,005	0,1677±0,003*
Physiological saline	21-	0 83±0,037	4,0±0,01	0,1159±0,006**	0,1407±0,01
Thymalin		1)7±0,43*	5,5±0,32*	0,1215±0,001	0,1650±0,006*
Hemalin		1,)3±0,078*	4,7±0,01	0,1213±0,026	0,1629±0,004*

Legend. Here and in Table 2: *P < 0.05 compared with control, **P < 0.05, compared with intact animals.

Experiments were carried out on 50 male guinea pigs weighing 200-250 g of which five remained intact, while the rest were divided into three equal groups. Thymalin was injected into the animals of group 1 and hemalin into the animals of group 2 in a dose of 0.5 μg/g, in 0.5 ml of physiological saline, intramuscularly daily for 3 days (five animals from each group were decapitated on the 4th day), and daily for 10 days (five animals from each group were decapitated on the 11th and 21st days). Animals of group 3 (control) received injections of 0.5 ml of physiological saline by the same scheme. The doses of these preparations were chosen such that when thymalin was injected in this dose in thymectomized guinea pigs the number of karyocytes and T lymphocytes in the animals' spleen was restored and no side effects were observed. For the comparative study of the effect of thymus and bone marrow preparations on the thymus gland, the conditions used were identical for both preparations and hemalin was injected in the same dose as thymalin.

The thymus was fixed in 10% neutral formalin solution and then embedded in paraffin wax: part of the gland was left for cutting frozen sections to demonstrate hydrolyases (by Burstone's method). Paraffin sections were stained with hematoxylin and eosin, axur II-eosin, and with methyl green and pyronine by Unna's method. For microscopic description of the material areas of cross section of the epithelial cells of the cortex and medulla of the lobules were measured by a gravimetric method, using the RA-7 drawing apparatus. The thickness of the cortex and medulla was measured with an acular micrometer and the ratio between them calculated. The numberical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

In animals receiving a 3-day course of injections of physiological saline, on the 4th day of the experiment the ratio of the thickness of the cortex and medulla and the mitotic coefficient were reduced compared with intact guinea pigs (P > 0.05; Table 1), and the total number of cells per conventional unit of area of the cortex showed a decrease (P < 0.05; Table 2). Meanwhile the size of the nuclei of the reticuloepithelial cells (REC) was significantly increased in both cortex and medulla (Table 1).

On the 4th day, animals receiving three injections of thymalin showed a decrease in thickness of the cortex of the thymus lobules compared with animals receiving physiological saline, adn the mitotic coefficient was increased by almost 1.5 times (P < 0.05; Table 1). The cell composition was almost unchanged as regards both quality and density of distribution. There was an even greater increase in size of the nuclei of REC in the cortex and medulla (P < 0.05; Table 1).

Compared with injection of physiological saline, three injections of hemalin caused an increase in thickness of the cortex of the lobules (P > 0.05) on the 4th day of the experiment, with a very small increase in the mitotic coefficient (Table 1) and an increase in the total number of cells per conventional unit of areas of the cortex, on account of lymphoblasts and, in particular, of medium lymphocytes (Table 2). Nuclei of REC in the medulla were larger than in the control (P < 0.05) but in the cortex they were almost unchanged (Table 1).

TABLE 2. Absolute Content of Cells per Conventional Unit of Area of Cortex of Thymus Lobules of Guinea Pigs (X ± m)

Experimental conditions	Time of investigation, days	Lymphoblasts	Lymphocytes		REC	Other cells	Total number of cells
			small	medium			
Intact animals		6,3±0,19	89,6±3,26	5,5±0,19	2,5±0,19	1,5±0,19	105,6±2,88
Physiological saline	4	8,0±0,38	86,0±0,77	2,7±0,19	1,3±0,19	1,3±0,19	99,6±1,15**
Thymalin		7,5±0,38	85,5±4,61	3,5±0,77	2,5±0,19	1,5±0,19	100,5±1,15
Hemalin		10,3±0,58	85,3±4,03	6,5±0,77	2,0±0,38	1,3±0,19	103,3±4,03
Physiological saline	11	7,3±1,37	96,0±2,5	3,3±0,38	1,7±0,19	1,7±0,19	110,6±0,58
Thymalin		5,5±0,96	107,0±1,54*	4,3±0,19	1,5±0,19	1,5±0,19	119,5±2,5*
Hemalin		8,7±0,38	98,3±2,69	8,3±0,38*	1,3±0,19	2,0±0,38	120,0±1,73*
Physiological saline	21	6,0±0,38	91,0±2,11	4,0±0,38	1,5±0,38	1,5±0,38	104,5±0,38
Thymalin		5,5±0,96	96,0±0,77	3,5±0,58	1,5±0,19	2,0±0,19	109,0±0,76
Hemalin		9,0±0,38	85,6±2,5	3,0±0,38	1,3±0,38	1,3±0,38	100,3±2,3

The content of acid phosphatase (AP) in cortical and medullary lymphocytes in animals receiving physiological saline was less than in intact guinea pigs ($P < 0.05$). After injection of thymalin and hemalin, the AP content in the cortical lymphocytes was significantly increased, whereas in the medullary lymphocyte only a tendency for it to increase compared with the control was observed.

Injection of thymalin and hemalin for 10 days caused thickening of the cortex of the lobules on the 11th day (in the case of injection of thymalin $P < 0.05$) and the mitotic coefficient was increased, only when thymalin was used ($P < 0.05$, see Table 1). Both preparations increased the total number of cells per conventional unit of area of the cortex: when thymalin was injected, on account of small lymphocytes, when hemalin was injected, on account of medium lymphocytes and lymphoblasts. Nuclei of REC in the medulla were larger than in the control in animals of both groups ($P < 0.05$), but it was much larger in animals receiving thymalin. Nuclei of cortical REC in the lobules of the thymus were unchanged compared with the control (Table 1). The AP content in the cortical and medullary lymphocytes on the 11th day of the experiment in animals receiving thymalin and hemalin remained the same as in the control.

On the 21st day of the experiment, after 10 injections of physiological saline many parameters in the animals approximated to the initial data (i.e., in intact animals) with respect to the mitotic coefficient, the total number of cells per conventional unit area of cortex, and cell composition. However, the thickness of the cortex remained a little less than that in intact guinea pigs, and the nuclei of the cortical REC were still larger than in intact animals ($P < 0.05$).

In animals receiving thymalin and hemalin, a significant increase in thickness of the cortex of the lobules, an increase in the mitotic coefficient (significant in the case of thymalin), and a tendency for this parameter to increase after injection of hemalin, took place on the 21st day after 10 injections of the preparations (Table 1). The total number of cells per conventional unit of area of the cortex was about the same as in the control, but when hemalin was given the number of small lymphocytes was reduced and the number of lymphoblasts increased (Table 2). Nuclei of REC remained larger than in the control. The AP content in cortical and medullary lymphocytes of animals of both groups was the same as in the control at the same time.

Comparison of the effect of the thymus and bone marrow preparations on morphology and function of the thymus in guinea pigs showed that thymalin and hemalin have a similar action, with only small differences for each of them. Both preparations led to population of the thymus with lymphoid cells during the period of observation, but whereas thymalin led to an increase in the total number of cells per conventional unit area of cortex mainly on account of small lymphocytes, the increase after hemalin was mainly due to medium lymphocytes and lymphoblasts, possible evidence of delay of differentiation.

Injection of thymalin caused a greater increase than injection of hemalin in the size of the REC nuclei, indirect evidence of the higher intensity of function of the epithelial cells of the thymus in animals receiving thymalin.

The impression was gained that thymalin and hemalin, as early as on the 4th day of the experiment, prevented the reaction to stress connected with the actual injection of the drugs and of physiological saline. Meanwhile the action of thymalin and hemalin continued to be reflected in the structure of the thymus even on the 21st day, after 10 injections: Thickening of the cortex of the lobules and an increase in size of the REC nuclei, in both cortex and medulla, were observed.

Thus, thymalin and hemalin act both on the lymphoid and on the reticuloepithelial component of the thymus. The action of the drugs is aimed at restoring the structure of the thymus through proliferation and differentiation of lymphoid cells; the intensity of the process is higher with thymalin than with hemalin.

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ROLE OF THE CYTOSKELETON IN RESTORATION OF NORMAL MORPHOLOGY OF TRANSFORMED CELLS IN CULTURE

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An important feature distinguishing tumor cells is their weakened adhesion to cellular and noncellular structures. The degree of spreading of tumor cells grown in culture is usually lower than that of their normal precursors [4]. It was shown previously [3] that artificial formation of multinuclear tumor cells leads to the appearance of features in them that are absent in mononuclear transformed cells, and by which they closely resemble in phenotype untransformed normal fibroblasts, i.e., to partial morphologic normalization.

It is generally accepted that the shape of cells is largely determined by cytoskeletal structures. In fact, agents which modify the structure of the cytoskeleton, such as cytochalasin B and colcemid, also affect the shape of cells in culture [1, 9],

The effect of changes in the cytoskeleton on the increase in the degree of spreading of transformed cells, caused by the multinuclear state, was investigated. The degree of spread (the area of the cell expressed per single nucleus) was chosen as criterion for evaluating the degree of the changes, because it can be estimated quantitatively and accurately. The sensitivity of the process of restoration of normal morphology of multinuclear tumor cells to colcemid, an alkaloid which destroys microtubules, was investigated. We also know that colcemid causes condensation of the system of intermediate filaments in the perinuclear zone of the cell [6, 7]. We showed that an increase in spreading of the cells in the multinuclear state is connected with reorganization of their microtubules and intermediate filaments.

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