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The importance of thymus preparations as a means of correcting disturbances of the T-cell component of immunity has already been confirmed by research workers in several countries, who have begun clinical trials of peptide thymus factors [1, 2, 4, 6, 9-11]. However, the preparations which have been used are molecularly heterogeneous. Accordingly, the detailed study of individual components of the therapeutic agents which may be used is of definite interest in connection with the elucidation of the mechanisms of cellular and humoral immunity.

This investigation was devoted to the study of a new antigenic component found in commercial immunostimulators prepared for bovine thymus tissue.

## EXPERIMENTAL METHOD

Antisera were obtained in rabbits, immunized with protein fractions obtained from the thymus (f4, f5) by the method described previously [7]. To potentiate the immunogenic properties of f4 and f5, these fractions were bound with bovine serum albumin, to serve as immunogenic carrier [3].

The immunochemical determination was carried out by the immunodiffusion method with a standard test system [5], whose sensitivity in these investigations was 1.5  $\mu\text{g/ml}$ . Electrophoretic mobility was studied by crossed immunoelectrophoresis in type 2 1% agarose gel (Sigma, USA), made up in 0.01 M Veronal-Medinal buffer (pH 8.6) [8].

The relative molecular weight of the protein was determined by gel-filtration through Sephadex G-100, and the isoelectric point was determined by chromatic focusing.

Extracts of organs and tissues were prepared in Tris-glycine buffer (pH 8.3) with the addition of 0.1% of the detergents Triton X-100 and Tween-80, and they were used in the work in a concentration of 50 mg of lyophilized product in 1 ml of physiological saline.

Commercial preparation with an immunostimulating action, obtained from bovine thymus, were used in the investigation: taktivin (Moscow, USSR); thymalin (Leningrad, USSR), thymosin fraction 5 (Texas, USA), TP<sub>1</sub>-Serono (Rome, Italy).

## EXPERIMENTAL RESULTS

Immunochemical analysis of antisera obtained by immunizing rabbits with protein mixture f4, after preliminary absorption with blood plasma and bovine kidney extract showed the presence of antibodies to thymus tissue protein with the electrophoretic mobility of  $\alpha_1$ -globulins. The identified antigen differed from thymus antigen-1 (Ag-1T) described by the writers previously [3], and it was accordingly called thymus antigen 2 (Ag-2T). Antisera obtained by immunization of rabbits with f5 formed a loose precipitate with extract of cow's thymus and with the fraction itself, and accordingly, work with them was extremely difficult.

Table 1 gives the results of immunodiffusion determination of Ag-2T in extracts of bovine organs and tissues. As Table 1 shows, the highest content of Ag-2T was found in extract of thymus, spleen, and lung of a fetal cow. In the tissue of the adult animal Ag-2T was found in extract of lung, large intestine, and liver. Consequently, Ag-2T can be classed with the group of interorgan proteins of narrow specificity, probably reflecting functional kinship of different organs. Redistribution of Ag-2T synthesis in the organs of

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TABLE 1. Results of Immunochemical Determination of Ag-2T in Extracts of Bovine Organs and Tissues

Test object	Concentration of Ag-2T, $\mu\text{g/ml}$	
	fetus	adult animal
Thymus	128	—
Spleen	16	0
Liver	16	Traces
Lung	0	32
Large intestine	0	8
Other organs and tissues	0	0

Legend. Results given in micrograms Ag-2T per milliliter tissue extract, containing 50 mg of lyophilized product of extract. Organs and tissues: brain, heart, kidney, stomach, large intestine, thyroid gland, adrenal, pancreas, blood vessels, skin, urinary bladder, amniotic fluid.

TABLE 3. Physicochemical Properties of Calf Thymus Ag-2T

Property	Ag-2T
Relative molecular weight	$12.0 \pm 2.1$ kD
Relative electrophoretic mobility	$0.70 \pm 0.03$
Thermostability	Withstands heating to $80^\circ\text{C}$ for 30 min
Precipitation with ammonium sulfate	Precipitated at 50-75% saturation
Precipitation with acetone	Precipitated at 60% saturation
Solubility in water	Soluble
Isoelectric point	pI 4.6

TABLE 2. Results of Immunochemical Determination of Ag-2T in Thymus Extracts of Man and Animals

Biological species	Number of tests	Concentration of Ag-2T, $\mu\text{g/ml}$
Man (fetus)	10	0
Cow (calf)	10	128
Pig (fetus)	6	120
Sheep (fetus)	8	120
Walrus (newborn)	1	100
Deer (newborn)	2	100
Chicken	10	40
Other animals	0	0

Legend. Results given in micrograms Ag-2T in 1 ml of tissue extract containing 50 mg of lyophilized product. Animals: dog, mouse, rat, rabbit.

TABLE 4. Results of Immunochemical Determination of Ag-2T Preparations Obtained from Calf Thymus

Preparation	Ag-2T concentration	
	$\mu\text{g/ml}$	%
Taktivin (Moscow, USSR)	48	1.6
Thymalin (Leningrad, USSR)	64	2.1
Thymosin, 5th fraction (Texas, USA)	96	3.2
TP <sub>1</sub> -Serono (Rome, Italy)	48	1.6

Legend. Protein concentration in preparations was 3 mg/ml.

the adult animal may be connected with maturation processes of the immune system during embryogenesis and its formation in the adult animal.

Immunodiffusion analysis of Ag-2T in extracts of the human and animal thymus revealed that this protein is interspecific. It was found in the cow, sheep, pig, walrus, deer, and chicken (Table 2). In man and in the species of animals studied, Ag-2T could not be found in tissue of the thymus.

Ag-2T is a thermostable microglobulin with relative molecular weight of  $12.0 \pm 2.1$  kilodaltons and with relative electrophoretic mobility of  $\alpha_1$ -globulins (Table 3).

The study of the physicochemical properties indicated that Ag-2T may be a component of therapeutic preparations obtained from bovine thymus tissue. Accordingly, it was decided to test for this protein in various immunocorrective preparations marketed both by Soviet industry and abroad (Table 4).

It will be clear from Table 4 that Ag-2T is a component of all the therapeutic preparations, and that its specific content in them varies from 1.6 to 3.2%.

The Ag-2T which we identified is thus a structural component of immunoregulatory organs and is also a component of all therapeutic preparations marketed for the purpose of immunocorrection of the T-cell factor of immunity. Although the biological role of this protein has not yet been studied, it is quite clear already that it can be used as an immunochemical marker for the production of biologically active preparations from the thymus not only of cattle, but also of certain other species of animals, so that the isolation and standardization of the resulting products may be facilitated.

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