

# REACTION OF SERA OF RHEUMATIC FEVER PATIENTS WITH THYMUS EPITHELIAL CELLS

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Sera of patients with rheumatic fever, which contain antibodies against muscle fiber disks, when tested by the immunofluorescence method react with the cytoplasm of some epithelial (myoid) cells of normal animal thymus. Absorption of the sera by heart tissue homogenate abolishes the fluorescence of the elements. These results show that the cytoplasm of thymus epithelial cells and muscle fiber disks of the myocardium evidently contain identical antigens.

The sera of patients with myasthenia gravis, which contain antibodies against skeletal muscle fiber antigens, have been shown to react with the epithelial cells of the thymus of healthy animals and man [10, 11]. The workers cited consider that the antigens which they discovered in thymus cells are similar to the antigens of striped muscle, and for this reason they regard the diffuse and focal lymphocytic infiltration around the epithelial cells, in the form of follicles with germinal centers, observed in the thymus in myasthenia gravis, as a manifestation of autoimmune thymitis [10].

The picture of thymitis is characteristic not only of myasthenia, as is generally accepted, but also of other diseases in which autoimmune processes are concerned. Similar follicles and foci of infiltration around epithelial cells are found in the thymus of patients with rheumatic fever [12]. Antibodies reacting with various components of myocardial muscle fibers have been found by the immunofluorescence method in the blood serum of patients with rheumatic fever [3, 4, 13]. Antibodies reacting with myocardial muscle fiber disks have been found in the sera of some patients with rheumatic fever in tests on sections of animal tissues [3].

The object of the investigation described below was to study the reactions of the blood sera of patients with rheumatic fever with thymus cells and to compare these reactions with those observed in myasthenia gravis.

## EXPERIMENTAL METHOD

The sera of 20 patients with rheumatic fever and 16 patients with myasthenia gravis were studied and six donor sera were used as the control.

Sections of the thymus, myocardium, skeletal muscle, and liver of rabbits and of bovine myocardium were cut from frozen unfixed tissue in a cryostat at  $-20^{\circ}\text{C}$  and fixed for 10 min in absolute acetone or used in the native form.

Tests were carried out by the indirect immunofluorescence method using pure antibodies against human  $\gamma$ -globulin labeled with fluorescein isothiocyanate. The  $\gamma$ -globulin was obtained by Baumstark's method [5, 6], and the pure antibodies were isolated by the use of immunosorbent by the method of Avrameas and Ternynck [5] and conjugated with fluorescein isothiocyanate by Riggs' method [14] in the modification of

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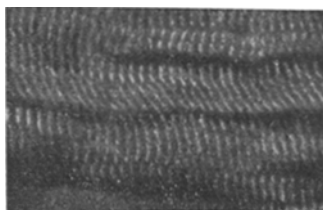


Fig. 1. Section through bovine myocardial tissue treated with serum of patient with rheumatic fever. Fluorescence of muscle fiber disks, objective 40 $\times$ , Homal 3 $\times$ .

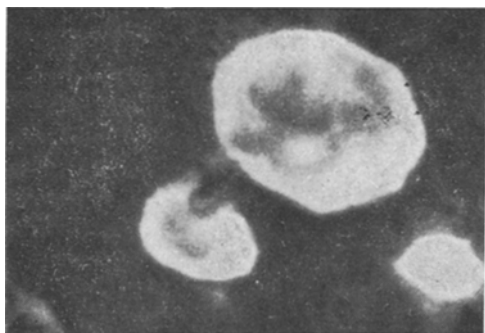


Fig. 2. Section through rabbit thymus tissue treated with serum of patient with rheumatic fever. Fluorescence of cytoplasm of epithelial cells, objective 90 $\times$ , Homal 3 $\times$ .

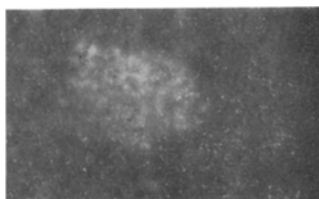


Fig. 3. Section through rabbit thymus tissue. Preliminary absorption of serum with heart tissue homogenate. Fluorescence of cytoplasm absent, objective 90 $\times$ , Homal 3 $\times$ .

Blagoveshchenskii and Kul'berg [1]. To remove nonspecific fluorescence the test sera, in a working dilution (1:4), were absorbed by mouse or pigeon liver powder. In some cases thymus sections were stained with hematoxylineosin and by the PAS reaction.

## EXPERIMENTAL RESULTS

The sera of patients with rheumatic fever reacted on tissue sections of the rabbit or bovine heart with components of the myocardial muscle fiber disks in dilutions of 1:8 to 1:32 (Fig. 1). If the sera were poured on liver tissue sections, used as the control, no reaction was observed. The donor sera either failed to react with the myocardial tissue sections at all or did so only in low dilutions (1:4). When serum containing antibodies against myocardial muscle fiber disks was poured onto thymus sections fluorescence of the cytoplasm of the large cells situated principally in the medullary layer of the gland was observed in all cases (Fig. 2). In their morphological features (large size, weakly acidophilic cytoplasm, content of PAS-positive material) these cells must be regarded as epithelial. Absorption of the sera with heart tissue homogenate partly or completely abolished the fluorescence of the epithelial cells (Fig. 3). Absorption of the serum by liver homogenate did not effect the character of the fluorescence. When sera of patients with rheumatic fever were tested on skeletal muscle tissue sections, fluorescence of the muscle fiber disks was observed, while sera of patients with myasthenia gravis reacted with myocardial muscle fiber disks. After absorption of the sera from patients with myasthenia gravis on heart tissue homogenate and absorption of sera from patients with rheumatic fever on skeletal muscle tissue homogenate, fluorescence of the muscle fibers was partly or completely prevented.

After treatment of rabbit thymus sections with sera from patients with myasthenia gravis fluorescence of cells morphologically and topographically analogous to those detected by the sera of patients with rheumatic fever was observed. After adsorption of sera from patients with myasthenia gravis by heart tissue homogenate, fluorescence of the cytoplasm of the epithelial cells was partly or completely prevented.

The sera of donors containing antibodies against the components of myocardial muscle fibers also reacted, although in lower dilutions (1:4), with the cytoplasmic components of the epithelial cells.

The blood serum of patients with rheumatic fever thus contains antibodies against antigens present in the epithelial cells of the healthy animal thymus. The results obtained by absorption of the sera indicate that these antigens are evidently analogous to myocardial muscle fiber antigens.

The results do not agree with those obtained by other investigators [11] who, after studying many sera from patients with various diseases, claim that antibodies against components of thymus epithelial cells are present only in the sera of patients with myasthenia gravis.

Further investigations are required to determine whether the antigens found are identical with those against which antibodies are present in myasthenia gravis. Some antigens of myocardial and skeletal

muscle fibers are known to be identical. However, these antigens include components which are specific for each tissue only [2, 7, 9]. Heterogeneity probably also exists among the antigenic components contained in the cytoplasm of thymus epithelial cells.

Further research is required to elucidate the connection between the development of diseases such as rheumatic fever and myasthenia gravis and the presence of myoid cells in the thymus. At present it can only be postulated that the presence of organ-specific components of certain organs in the thymus epithelium is related to the formation of immunological tolerance of the organism to its own tissue proteins.

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