of tumor growth in newborn mice by irradiation discovered in the present investigation is connected with the disturbance of T-suppressor function. However, it must be remembered that irradiation can also affect tumor growth by its action on nonimmunologic factors of the recipient. According to available data [6, 9], the acceleration of growth of weakly immunogenic tumors is unconnected with inhibition of immunocompetence of the recipients by sublethal irradiation. The authors cited attach great importance to the action of irradiation as a stress factor. Inhibition of growth of certain syngeneic and allogeneic tumors caused by preirradiation may be connected with inhibition of the connective tissue response to transplantation of the tumor which facilitates its survival and growth [1].

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IMMUNOFLUORESCENCE STUDY OF CHANGES IN THYMUS MYOID CELLS IN PATIENTS WITH MYASTHENIA

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Antibodies against antigens of thymus myoid cells common with antigens of muscle tissue are known to be present in high titer in the blood of patients with myasthenia gravis [2, 3, 9]. On the basis of these findings it has been suggested that such antibodies are evidently among the possible pathogenetic factors in this disease, for by injuring the myoid cells of the thymus under certain conditions they can lead to the development of an autoimmune thymitis, which is characteristic of several autoimmune diseases, including myasthenia [10]. Besides the formation of autoantibodies against antigens of myoid cells, other important evidence of damage to these thymus cells is demonstration of their immunomorphological changes in the thymus of patients with myasthenia.

This paper describes a comparative study, by the immunofluorescence method, of myoid cells in normal subjects and myasthenia patients with the aim of discovering possible changes in this heterologous tissue of the thymus in this disease.

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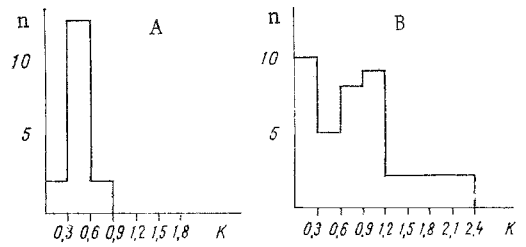

Fig. 1. Distribution of number of myoid cells in thymus of normal subjects (A) and patients with myasthenia gravis (B). Abscissa, number of myoid cells per field of vision (K); ordinate, number of cases falling within the given range of values of the parameter K (n).

EXPERIMENTAL METHOD

Sera from patients with myasthenia, reacting with antigens of myocardial and skeletal muscle fibers in a dilution of 1:1000, were used as the source of antibodies against antigens of thymus myoid cells. The specificity of reaction of the serum with antigens of myoid cells was verified by preliminary absorption of the serum with tissue homogenate of myocardium and other organs.

The immunomorphology of myoid cells was studied and their number counted in the thymus of myasthenia patients thymectomized at the age of 10-40 years (39 cases) and in the thymus of healthy persons dying from acute trauma at the age of 6-22 years (22 cases). Pieces of thymus tissue were frozen in petroleum ether, cooled in a mixture of acetone and dry ice to —70°C. Four or five nonserial sections, i.e., taken at different levels, were mounted on the same slide, fixed for 10 min in cold acetone, treated with serum from a myasthenia patient (dilution 1:200) for 2 h at room temperature, and rinsed for 20 min in a stream of 0.85% NaCl solution (pH 7.2). Sections of the thymus were then incubated for 45 min with antibodies against human IgG labeled with fluorescein isothiocyanate. Antibodies were isolated from donkey antiserum with the aid of an immunosorbent prepared from human IgG, treated with glutaraldehyde [6, 8]. The number of myoid cells in 500 fields of vision was counted in sections obtained from several pieces of the same thymus. The number of myoid cells in the thymus was assessed by the formula $K = K/a$, where K is the number of myoid cells found in the fields of vision. The mean value of this index (K_M and K_N respectively), the error of the mean and the standard deviation were determined for patients with myasthenia and normal subjects. Variability of the indices K_M and K_N was estimated by means of the coefficient of variation:

$$V = \frac{\sigma}{\overline{K}} \times 100\%$$

To assess the state of the lymphoid tissue (hyper- or hypoplasia) frozen sections of the thymus were fixed for 10 min in 96° ethanol and stained with hematoxylin—eosin.

EXPERIMENTAL RESULTS

According to the results of counting (Fig. 1) the mean number of myoid cells in the thymus of normal subjects is $K_N = 0.404 \pm 0.038$ per field of vision and is subject to small individual fluctuations (V = 35%). Diffusely luminescent round and oval structures measuring 15-20 mm could be seen in the subcortical and medullary zones of the lobules of the thymus (Fig. 2). As was noted previously [5], separation of granules from the surface of the myoid cells and attachment of degenerating structures to Hassall's corpuscles are observed comparatively rarely in the healthy human thymus.

In myasthenia the mean number of myoid cells per field of vision was about twice the normal value: $K_M = 0.760 \pm 0.11$; this index was characterized by considerable individual variations (V = 89%). All the cases studied could therefore be subdivided on the basis of the value of this index into three groups (Fig. 1). The results of analysis show that the heterogeneity of the test material was independent of the patients' age and extended not only to the number and morphological features of the myoid cells, but also to several other features of the thymus in patients with myasthenia and, above all, to the state of its lymphoid tissue.

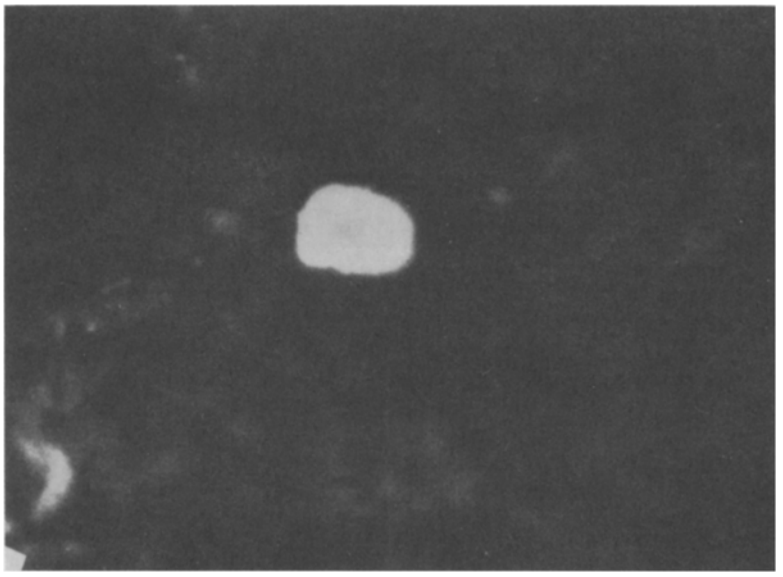

Fig. 2. Myoid cell in normal human thymus. Here and in Fig. 3: immunofluorescence method, 40× (water immersion), homal ×3.

It will be clear from Fig. 1 that the patients of group 1 (11 cases) were characterized by a marked decrease in the number of myoid cells in the thymus ($K_M \leqslant 0.03$). Single myoid cells detectable in sections through the gland were sharply reduced in size — not more than 10 μ. Histological examination showed that the lymphoid tissue of the thymus in the patients of this group contained rather more lymphoid cells than normal in the medullary zone of the lobules of the gland.

In the patients of group 2 (13 cases) the number of myoid cells per field of vision was $0.04 \leqslant K_M \leqslant 0.09$. The myoid cells were characterized by intense bright green luminescence of the cytoplasm, an increase in zone to 25-30 μ, a swollen appearance and, consequently, loss of their distinct outlines (Fig. 3). The considerably higher than normal intensity of luminescence of the cytoplasm, and also the fact that the sera reacted with the cytoplasm of myoid cells from the thymus of myasthenia patients in higher dilution than with myoid cells of the normal glands, are evidence of an increased antigen concentration in the cytoplasm of the thymus myoid cells in myasthenia. Granules containing myoid antigens separate from the surface of many myoid cells, and this evidently reflects increased secretory activity of these cells. Numerous granules of myoid cells also appeared in the internal medium of the gland at a considerable distance from the myoid cells. Together with cells showing signs of hypertrophy and increased secretory activity, the thymus of the patients of this group contained numerous myoid cells in the course of degeneration — so-called sarcoliths, and quantitative predominance of these structures was most characteristic of group 3. A study of preparations stained with hematoxylin—eosin showed a tendency toward hypoplasia of the lymphoid tissue of the thymus as a result of a decrease in the number of small lymphocytes in the medullary zone of the lobules of the gland.

In the thymus of the patients of group 3 (15 cases) the number of myoid cells was 3-6 times greater than normal ($0.9 \leqslant K_M \leqslant 2.4$). The myoid cells were represented mainly by sarcoliths, which were in the course of degeneration. Together with signs of hypertrophy such as an increase in size and swelling because of the sharply increased content of antigens in their cytoplasm, other characteristic features of the sarcoliths were a decrease in their secretory activity and the appearance of a distinctly yellowish color of luminescence of the cytoplasm, so that they came to resemble hyalinized masses contained in the cavities of the Hassall's corpuscles. In the internal medium of the thymus the sarcoliths were arranged in groups of three to eight or more elements of different sizes. Finally degenerating myoid cells disintegrated or attached themselves to Hassall's corpuscles (Fig. 3). The presence of transitional forms, with features of hypertrophy accompanied by more or less well-marked signs of degeneration, is evidence that despite their differences, the myoid cells in the thymus of myasthenia patients constitute a single population, the separate elements of which are in different physiological states. The lymphoid tissue of the thymus in this group of patients was characterized by marked hypoplasia as the result of a sharp decrease in the number of small

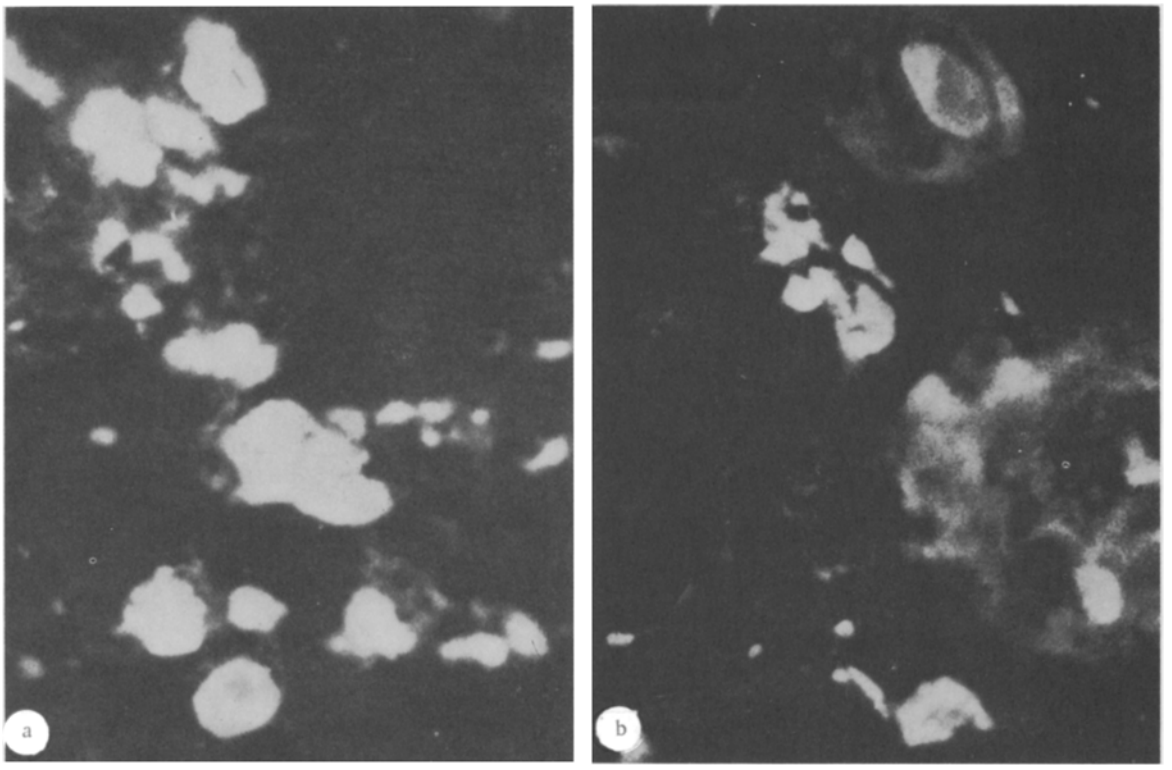

Fig. 3. Myoid cells in thymus of patients with myasthenia: a) group of myoid cells (sarcoliths) in the course of disintegration; b) group of myoid cells in process of attachment to a Hassall's corpuscle.

lymphocytes in the organ. The results of these investigations are thus evidence that in myasthenia the myoid cells of the thymus undergo profound changes, analysis of which enables the two main forms of their injury to be distinguished: practically complete depopulation and the more frequently observed hypertrophy and increase in number of the myoid cells. Meanwhile some degree of hyperplasia and hypoplasia (in most cases) of the lymphocytes is observed in the thymus of patients with myasthenia. As a result of the fact that hyperplasia of lymphoid tissue is accompanied by a deficiency of myoid cells, whereas hypoplasia, on the other hand, is accompanied by hypertrophy and an excess of myoid cells, the opposite relationship is observed in most cases between the numbers of myoid and lymphoid cells in the thymus of patients with myasthenia. This opposite character of the quantitative changes in myoid and lymphoid cells in the thymus can evidently be attributed to the fact that the mechanism controlling the numerical ratio between these cells in the gland is disturbed in myasthenia.

According to one view, a possible function of the heterologous structures of the thymus, including its myoid cells, is to provide the lymphocytes of the gland with information on the structure of antigens present in them in the course of formation of natural immunologic tolerance to the body's own antigens [1, 4]. In connection with this hypothesis it would be interesting to study the relationship between injury to the myoid cells and the development of the autoimmune process in myasthenia gravis.

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