

## THE INFLUENCE OF THYMUS ON EXPERIMENTAL MYOCARDITIS AND ARTHRITIS (MORPHOLOGICAL STUDY)

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The intensive experimental studies on the thymus, performed in recent years, have shown its outstanding role in the formation of immune response in the organism (Miller, 1961, 1963, 1965; Aisenberg and assoc., 1964; Pinnes and assoc., 1966; Metcalf and assoc., 1967). It has been established that neonatal thymectomy in mammals results in reduction of the number of lymphocytes in the peripheral blood and lymph circulation, lymphoid hypoplasia, lowered antibody production and inhibition of hypersensitivity reactions of a delayed (cellular) type (Atnason and assoc. — 1962, 1964; Waksman and assoc., 1962; Jankovic and assoc., 1962; Lujan and Candiolo, 1965). Lately, rheumatism also was ranked among the latter type of reactions (Bibikova and assoc., 1965; Borisova and assoc., 1966; Borisova, 1967).

The aim of the present work is to investigate the influence exerted by neonatal thymectomy on the morphological changes in myocarditis and arthritis in rats, assumed as an experimental model of rheumatism in humans.

### Material and Method

The experiments were performed on a series of 59 rats of both sexes, divided in two groups: control group, comprising 28 animals, and experimental — 31 animals. The rats of the latter group were subjected to thymectomy through aspiration within 48 hours of birth. The number of animals of the experimental group does not include those in which, following autopsy, residual thymus or septic processes were established at the site of operative intervention. Experimental myocarditis and arthritis were induced in all the animals after the third month of life, according to a method, elaborated at the Chair of Pathophysiology — Higher Medical Institute, Varna. Twenty-four-hour culture of Beta-streptococcus hemolyticus, group A, was administered three times via i. v. injection, at dose 0,2 ml of 1000 million suspension, at 15-day intervals. Material for histological investigation was taken after killing the animals, two weeks after the last introduction of culture. The paraarticular connective tissue and myocardium of all the animals was subjected to histological investigation. The material for histological investigation was fixed in 10% neutral formalin and treated according to the paraffin method. The histological preparations were stained with hematoxylin eosin, and with toluidine blue for demonstration of the collagen destruction, at different pH of the solutions.

## Results and Discussion

Inflammatory changes in the paraarticular connective tissue and mucoïd turgescence of the connective tissue was established in 21 control animals (75%) and in 13 of the group of thymectomized — 42 per cent (Fig. 1). The inflammatory reaction of the paraarticular tissue revealed a product-

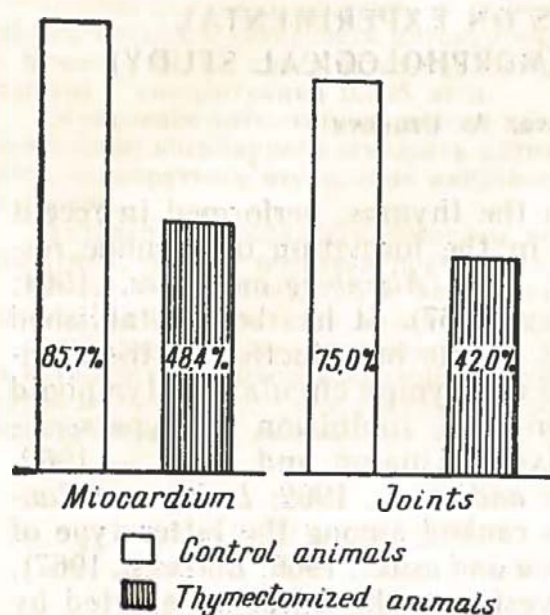


Fig. 1. Percentage of inflammatory changes established in the paraarticular connective tissue and myocardium.

ive-proliferative character and was manifested by infiltrates of lymphocytes, histiocytes and mastocytes, agglomerated around the blood vessels in a cuff-like fashion. The walls of the blood vessels in these particular zones appear thickened, homogeneous, with pale-pink coloration (Фиг. 2). The connective tissue fibres are swollen, with slight basophilic tinge and deleted structural outlines. The changes in the paraarticular connective tissue of the thymectomized animals do not differ in nature from those in the controls, except that they are comparatively less defined.

The changes in the myocardium display the same regularity, namely, affection of a greater number of animals of the control group. Well manifested inflammatory changes and disorganization of the connective tissue were established in 24 out of a total of 28 rats in the control group (85,7%). Among the thymectomized animals, inflammatory changes were discovered in 15 out of a total of 31 animals in the group (48,4%) (Fig. 1). The changes in the myocardium were manifested by focal perivascular ammassments of oval cells (lymphocytes, histiocytes, monocytes and mastocytes), displaying resemblance to rheumatic granulomas and absence of typical Aschoff's cells. In some animals, diffuse round-cellular infiltration was present within the interstitial tissue of the myocardium (Fig. 3). The connective tissue, surrounding the blood vessels of the myocardium was characterized by mucoïd turgescence.

The cellular infiltrates and the signs of mucoïd turgescence observed afford the possibility for making analogy between the pathological process described and the first stage of rheumatic reaction in humans. The latter concept is corroborated by the fact that nowadays, the Aschoff—Talalaev granuloma is not considered a mandatory sign in the diagnosis of rheumatism (Mitin, 1966).

The presence of a greater number of infiltrates in the control animals may be due to the preserved immunologic reactivity, related to the function of the thymus. The studies of Kosunen and coworkers (1963) on the mechanism of cellular hypersensitivity, by way of autoradiography, show that the cellular infiltration is caused by migrating lymphocytes, which subsequently become transformed into histiocytic elements and stimulate

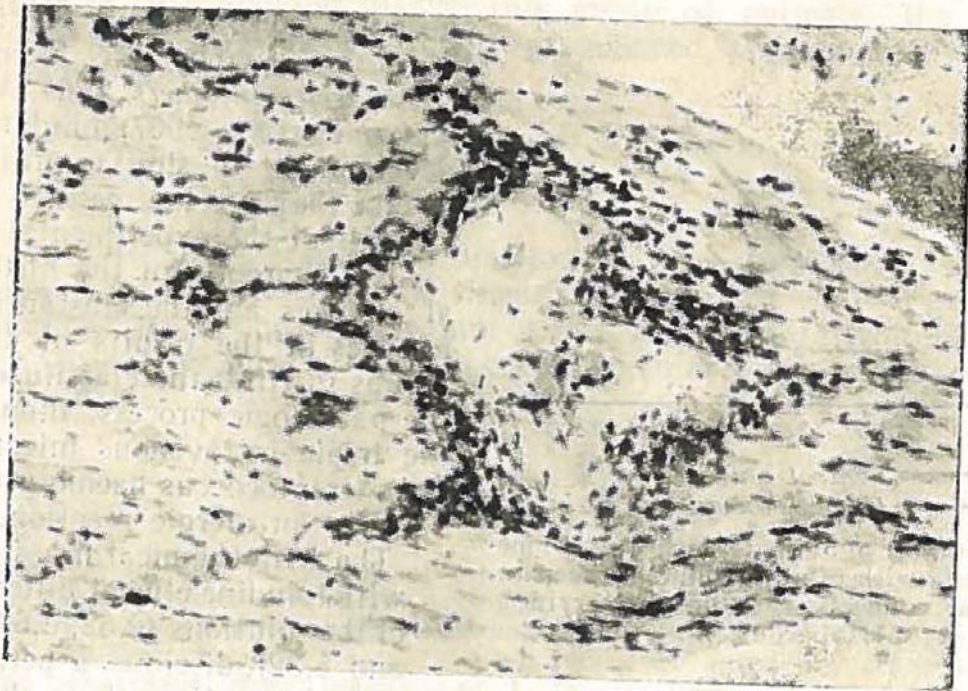


Fig. 2. Pararticular connective tissue — muroid turgescence and proliferation of ovoid cells surrounding a blood vessel. Stain H. E., magnif.  $10\times 20$ .

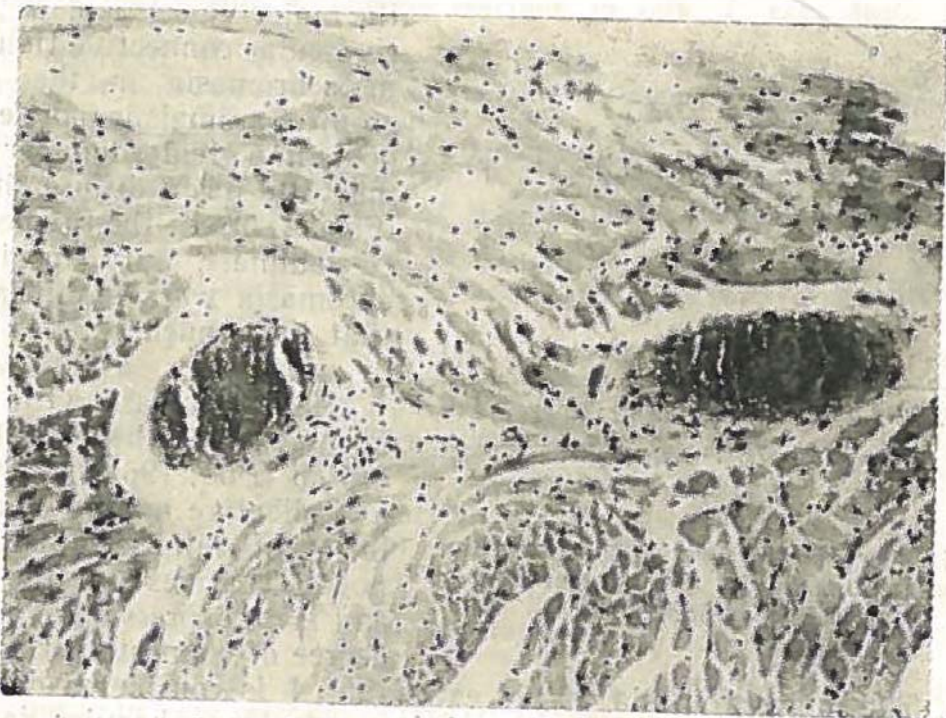


Fig. 3. Myocardium — focal perivascular ammassment of cells, resembling rheumatic granulomas.

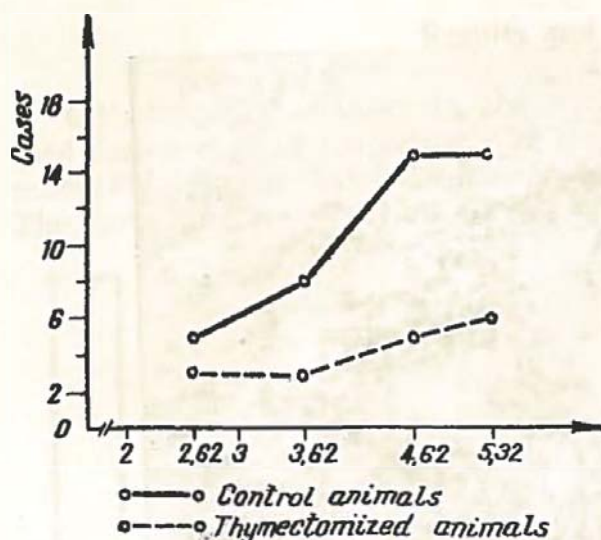


Fig. 4. Number of animals with proved metachromasia with toluidine blue in the para-articular connective tissue at varying pH of solutions.

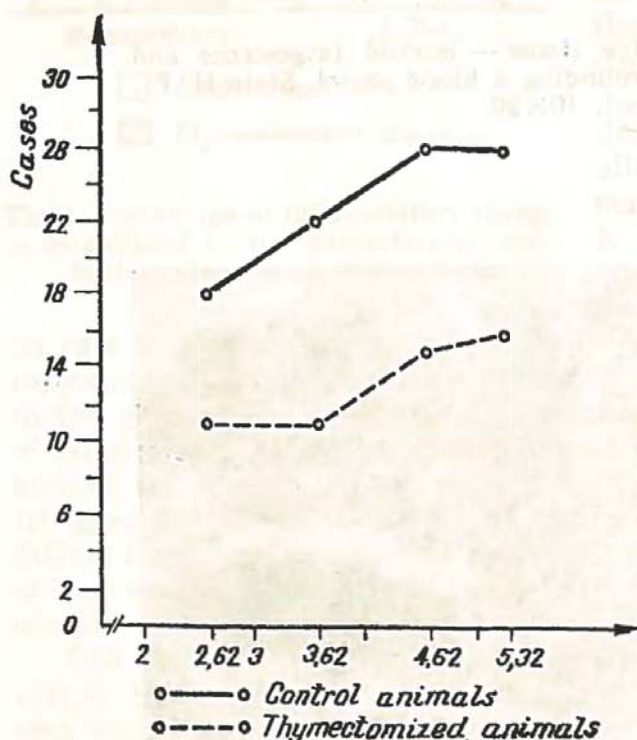


Fig. 5. Number of animals with toluidine blue proved metachromasia in the myocardium at various pH of solutions.

the local proliferation of cells. The neonatal thymectomy which provides for lymphocytic hypoplasia (Aisenberg and others, 1964, Metcalf and co-workers, 1967) obviates the development of similar infiltrates, or else they are less defined in the animals of the experimental group.

The character of the histological changes and the substantial differences in the results of the two groups justifies the classification of the pathologic process, induced by the triple intravenous injection of *Beta-streptococcus haemolyticus* in rats, as an allergic reaction.

The histochemical investigation with toluidine blue at different pH of the solutions (2,62, 3,62, 4,62, and 5,32), discloses a permanent metachromatic scale in the para-articular tissue and myocardium. It is more pronounced in the myocardium and is found mainly in the parietal endocardium, in the fibrous ring of the valvular openings, in the walls of the major vessels and in the perivascular connective tissue. The metachromasia in the control group of animals is more evident, occurs in a greater number of animals and has a diffuse character (Figs. 4, 5). In the thymectomized animals, only focal metachromasia was established and that in a much smaller number of animals.

Metachromasia with toluidine blue at pH beneath 4 is accepted (Pire, 1961, Mitin, 1966) as indicative mainly of accumulation of sulfatized acid mucopolysaccharides in the tissues and is an expression of a slighter degree of connective tissue destruction.

The metachromasia at pH exceeding 4 is considered due to the accumulation of hyaluronic acid and demonstrates a heavier damage of the connective tissue. The strongly pronounced, diffuse metachromasia with toluidine blue in the para-articular tissue and myocardium of the con-

trol animals, at varying acidity of solutions, is indicative of the presence equally of sulfatized acid mucopolysaccharides and also of hyaluronic acid. This is an indication that in this group of animals, the varying degrees of lesion are more strongly pronounced as compared to experimental animals.

### Inference

1. The threefold intravenous injection of Beta-streptococcus haemolyticus, group A, provokes signs of tissue allergic reaction in the paraarticular connective tissue and myocardium of rats. 2. Neonatal thymectomy inhibits the tissue allergic reaction in the paraarticular tissue and myocardium of the animals.

### REFERENCES

1. Библикова, Г. А., А. М. Борисова, С. С. Мутин, Я. А. Сигидин. Опыт дальнейшего изучения аллергии замедленного типа при ревматизме. *Современные проблемы ревматологии АМН СССР*, М., 1965, 56—60.
2. Борисова, А. М., Я. А. Сигидин. Об аллергии у сердечной ткани при ревматизме. *Вопр. ревматизма*, 1, 13—17, 1966.
3. Борисова, А. М. Некоторые итоги изучения аллергии замедленного типа при ревматизме. *Вопр. ревматизма*, 3, 61—66, 1967.
4. Митин, К. С. Гистохимия соединительной ткани сосудов при ревматизме, «Медицина», М., 1966.
5. Пирс. Гистохимия, 1962, 224—231.
6. Aisenberg, A. C., B. Wilkes. Immunologic status of thymectomized adult rats. *J. Immunology*, 1964, 93, 75—80.
7. Arnason, B. G., B. D. Jankovic, Waksman, Wennersten. Role of the thymus in immune reactions in rats. *J. exp. Med.*, 1962, 116, 177—178.
8. Arnason, B. G., B. D. Jankovic, B. H. Waksman. The role of the thymus in immune reactions in rats. In the thymus in Immunobiology. Ed. by R. A. Good and A. E. Gabrielsen, Hoeber—Harper, New York, 1964.
9. Jankovic, B. D., B. H. Waksman, B. G. Arnason. Role of the thymus in immune reactions in rats. I. The immunologic response to bovine serum albumin in rats thymectomized or splenectomized at various times after birth. *J. exp. Med.*, 1962, 116, 159—176.
10. Kosunen, T. U., B. H. Waksman, M. H. Flak, W. S. Tihen. Radioautographic study of cellular mechanisms in dilated hypersensitivity. *J. Immunology*, 1963, 6, 276.
11. Lujan, M. A., B. Candiolo. Influence of thymectomy on experimental poliartthritis. *Acta physiol. Latino Americana*, 1965, 15, 32—37.
12. Metcalf, D., M. Brumby. The role of the thymus in the ontogeny of the immune system. *J. cell. Physiol. sup.*, 1967, 1, 149—168.
13. Miller, J. F. A. P. Immunological function of the thymus. *Lancet*, II, 1961, 748—749.
14. Miller, J. F. A. P. Immunity and the thymus. *Lancet*, 1963, 1, 43—45.
15. Miller, J. F. A. P. The role of the thymus in immune processes. *Int. Arch. Allergy*, 1965, 28, 61—70.
16. Pinnas, J. Z., F. W. Fitch. Immunologic competence of thymectomized rats to several soluble and particulate antigens. *Int. Arch. Allergy*, 1966, 30, 217—230.
17. Waksman, B. H., B. G. Arnason, B. D. Jankovic. Role of the thymus in immune reactions in rats III. Changes in the lymphoid organs of thymectomized rats. *J. exp. Med.*, 1962, 116, 187—206.

## ВЛИЯНИЕ ТИМУСА НА ЭКСПЕРИМЕНТАЛЬНЫЕ МИОКАРДИТ И АРТРИТ

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### РЕЗЮМЕ

Исследованы гистологически и гистохимически околоуставная соединительная ткань и миокард у 31 неонатально тимэктомированных и 28 контрольных крыс, у которых в 3-месячном возрасте были вызваны экспериментальные миокардит и артрит. Полученные инфильтраты продуктивно-пролиферативной природы и мукоидное пропитывание напоминают Ашов—Талалаевский гранулем в начальной стадии ревматизма. Неонатальная тимэктомия подавляет развитие аллергической воспалительной реакции клеточного типа в околоуставной ткани и миокарде. Эта реакция связывается с лимфоидной гипоплазией и со сниженной иммунной реактивностью тимэктомированных животных.