

PASSIVE HEMAGGLUTINATION REACTION AFTER BOYDEN IN ADRENALECTOMIZED RATS WITH EXPERIMENTAL MYOCARDITIS

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The role played by autoimmune reactions of the organism in rheumatic conditions is the subject of numerous clinical and experimental studies (Kaluzhenko, 1963, 1964; Seifert, 1963, Cecchi, 1963; Ivanov, 1964; Joffe, 1964; Lampert, 1963, 1964). Whilst the presence of autoantibodies is beyond any dispute, the problem of their species' specificity is still an item of lively discussions. Kaplan (1963), Markovich and Lange (1964), Mazina and Rasohina (1966) point out that this is due to the common antigenic substances present in the cardiac tissue of man and animals, on one hand, and to the *Streptococcus* β haemolyticus, on the other. Berezhnaya and assoc. (1966) attribute a predominant role to the specific immune reactions. In allergic reactions of the organism, a great importance is attached to the adrenal gland (Valdman, 1956; Smirnov and Beletzkaya, 1955; Argynskiy, 1962; Kemileva, 1962 and others).

Proceeding from the data thus outlined, we made an attempt to induce a model (sample) of experimental myocarditis by introducing antigen of homologous heart tissue, obtained from animal, subjected to repeated previous treatment with *Streptococcus* β haemolyticus against the background of adrenalectomy in rats.

Experimental material and method

The experiments are carried out on a series comprising 54 rats, aged 3 $\frac{1}{2}$ to 4 months. We chose this particular age-group because the animals have completed maturity and, regardless of that, destructive changes characteristic for advanced ages have not yet occurred in the myocardium. The animals were distributed in three groups: experimental group comprising 32 rats, previously subjected to bilateral adrenalectomy by retroperitoneal access; second group of 12 animals not subjected to preliminary intervention and third group of 10 control animals, similarly treated with adrenalectomy. In the first two groups experimental myocarditis was provoked by means of triple antigen injection, obtained from the heart tissue of rat, previously subjected to triple contamination with *Streptococcus* β haemolyticus. The antigen represents an extract of blood washed cardiac tissue in physiological saline solution. This extract is subjected to fivefold freezing at -72° and the clear filtrate resulting is injected intravenously (0.5 cc per animal). The ECG recordings after each single inoculation of the antigen and the histological finding after killing the animals accounted for the changes established in the myocardium. Sacrification was performed by exsanguination and the serum obtained was used for the passive hemagglutination reaction after

Boyden. The antigens used in the reaction were: a) the same antigen responsible for the induction of the process, b) antigen, prepared in analogous way, from the normal cardiac tissue of rat and c) antigen from *Streptococcus* culture, prepared in the same manner.

Results and Discussion

The observations disclose that in addition to the changes in the myocardium, alterations are also present in the joints of the antigen injected animals. All the antigen treated animals included in the first two groups, exhibited modified gait pattern and altered external appearance of the joints, which, in compliance with data of previous studies by the authors (1962), might be accepted as the manifestation of an allergic articular reaction. These changes occur as early as after the first antigen treatment and are more pronounced in the group of adrenalectomized animals (in 30 of a total of 32 animals) as compared to the group not subjected to adrenalectomy (in 6 of a total of 12). The subsequent injections of the antigen produced a similar reaction in a great number of the animals, among those reacting after the first injection and among those which did not react alike. It is beyond any doubt that such a reaction was absent in the third — control group — which was not treated with antigen. Another index for judging the severeness of the process was the mortality rate of the animals in the different groups. Of the not adrenalectomized and subjected to triple injection with antigen animals, three died and, it should be emphasized, the death occurred after the third antigen injection. Out of the adrenalectomized and subsequently injected with antigen, 16 animals died (4 after the first injection, 4 after the second and 8 after the third). Such a high mortality rate in this group is certainly related to the adrenalectomy, carried out in combination with the injection of the noxious agent employed, for the animals of the control group remained alive till the end of the experiment.

The results of the electrocardiographic investigations reveal a reduced voltage of the ECG deflections — changes already referred to in previous publications by the authors (1963). These changes are chiefly manifested and most frequently met with in the group of animals with adrenalectomy + + antigen injection (the antigen used is personally prepared). Merely two animals (No. 3 and 7) of the group not subjected to adrenalectomy displayed a slight lowering of the R deflections in all three leads and, at that, only after the first injection of the antigen. After the second and third antigen injection the values of the deflections returned to normal. Twenty three animals of the experimental group showed a fall of the R deflection voltage in all the leads. In 18 of the total group, this reduction exceeded 2 mm, and in the remainder five it was more weakly manifested. The changes described were established after the threefold antigen injection. In 5 animals of this group no ECG changes were established at all, owing to the fact that they died after the first injection of antigen, i. e. prior to the ECG investigations. Of the group subjected to adrenalectomy alone, a fall of the ECG deflections was found merely in two animals. The histological changes in the myocardium were likewise more frequently met with and heavier in the animals of the experimental group as compared to the group without adrenalectomy.

They reveal the characteristics of focal myocarditis, already described by the authors in injecting living culture from *Streptococcus* β haemolyticus (Fig. 1).

The agglutination occurring at dilution exceeding 1:40 was accepted as specific in accounting for the passive hemagglutination reaction after Boyden,

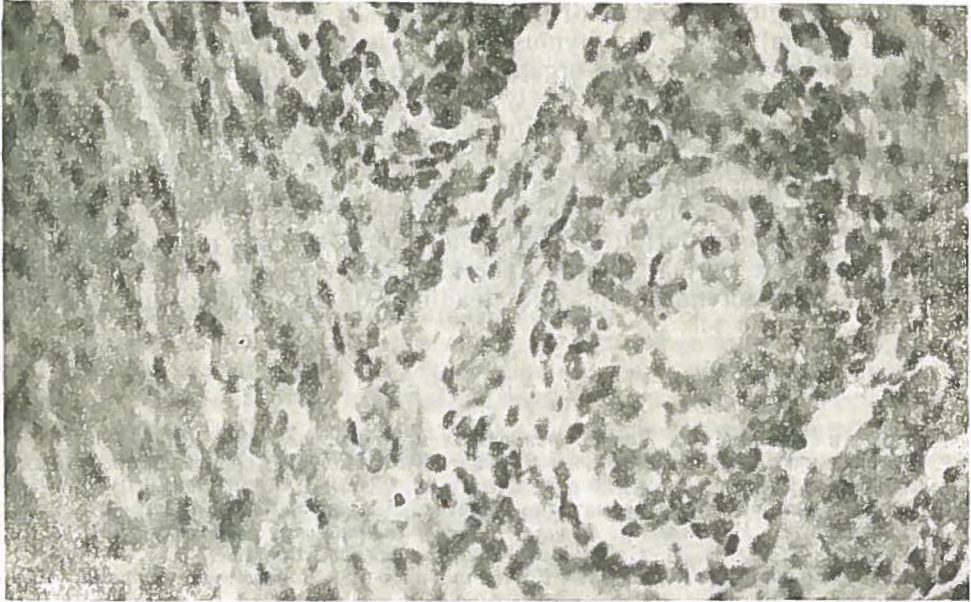


Fig. 1. Mucoïd turgescence within the myocardium and lymphocytic infiltrate around a blood vessel.

which is in agreement also with the opinion expressed by other authors. The test in the animals, injected with antigen but not subjected to beforehand adrenalectomy as well as in those merely adrenalectomized and not injected with antigen did not produce agglutination titers exceeding the accepted dilutions. A demonstrative agglutination reaction towards the antigen (used in the treatment of the animals) was produced merely in 10 animals of the experimental group in which the experimentally induced myocarditis was preceded by adrenalectomy. In some animals (No. 43) agglutination was produced at rather high titers (1:1280). In others (No. 32, 41) hemagglutination was obtained from antigens prepared from normal heart tissue, whereas in No. 32 — also with respect to antigen prepared from *Streptococcus* β haemolyticus culture. In two of the animals (No. 32, 48) agglutination was produced only from normal cardiac tissue antigen.

Our results demonstrate that the threefold administration of antigen prepared from the heart tissue of rat, previously intravenously injected with living *Streptococcus* haemolyticus culture, does not show a specific organotropic effect in terms of heart tissues but rather causes inflammatory reactions in other tissues (articular). The latter reaction is substantially

stronger in the animals subjected to preliminary adrenalectomy treatment. The stronger reaction referred to might be related, on one hand, with the absence of adrenal hormones (mainly cortical) which are known to exert an antiallergic effect. On the other hand, it might be determined by the antagonism, existing between the adrenal gland and the thymus (Comsa, 1959). Miller (1963, 1964) established the basic function of the thymus gland within the immunological processes of the organism. In all likelihood, in the absence of adrenal glands, the thymus' activity is intensified and the immunologic responsiveness of the organism accordingly increased. On similar terrain the allergic reaction is likely to occur as early as at the first contact with the antigen, provided a preliminary, nonspecific sensitization of the organism against the effect of various external, environmental factors was present. A similar conjecture is also supported by the positive reaction for passive hemagglutination mainly among the animals with adrenalectomy. The negative agglutination test with the control animals might be related, firstly, with the normal thymus functions and secondly, with the normal function of the adrenal glands and steadiness of the immunologic reactions in rats.

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**РЕАКЦИЯ ПАССИВНОЙ ГЕМОАГГЛЮТИНАЦИИ ПО БОЙДЕНУ,
ПРИ ЭКСПЕРИМЕНТАЛЬНОМ МИОКАРДИТЕ У ЭПИНЕФРЭКТОМИРОВАННЫХ
КРЫС**

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РЕЗЮМЕ

У крыс вызван экспериментальный миокардит путем внутривенного вливания антигена, приготовленного из сердечной ткани крысы с экспериментальным миокардитом, вызванным бета-гемолитическим стрептококком. Антиген готовится путем пятикратного замораживания гомогенизированной ткани при -72°C . Животные разделены на три группы — одной предварительно сделана эпинефрэктомия, а затем вызван процесс, второй — без предварительного вмешательства, а третья является контрольной. У всех животных прослеживаются изменения в электрокардиограмме, а после их убийства сыворотка крови исследуется в отношении пассивной гемоагглютинации по Бойдену.

Исследования показывают, что введенный антиген не имеет специфического действия на миокард, а дает признаки и обще алергизирующего фактора. Устанавливается наличие положительной реакции пассивной гемоагглютинации по Бойдену не только в отношении введенного антигена, но и в отношении антигена, приготовленного аналогичным способом из нормальной сердечной ткани крысы, а также и в отношении антигена из бета-гемолитического стрептококка.