quired in a late step of the bactericidal reaction because the susceptible mucopeptide is found in the innermost layer of the cell envelope(10). The results of our studies on sequential steps in bactericidal serum reactions (Table III) now provide experimental evidence that the lysozyme-dependent reaction is indeed a late reaction, the uncovering of the substrate presumably requiring prior action of specific antibody and cation-dependent components of complement.

Summary. The inhibition of bactericidal and hemolytic human serum reactions by addition of certain fractions from a pool of normal human serum has been analyzed. The fractions with major inhibitory effects for bactericidal reactions, namely γ-globulins, proved to be qualitatively different from those which inhibited immune hemolysis, namely β-globulins and albumin. The inhibitory effects of these serum fractions were not on the cells but on different components of the complement system; addition of γ-globulin to whole serum affected C'2, whereas addition of β-globulin and albumin fractions affected principally C'4. Such differences have permitted identification of quantitative differences in requirements for components of complement by the hemolytic and bactericidal reactions. In addition, a qualitative difference in the requirement for lysozyme has been recognized.

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Immunological Reactivity of Thymic Autografts in the Rat.* (29589)

OSIAS STUTMAN AND SALVADOR B. ZINGALE† (Introduced by Carlos Martinez)
Instituto de Investigaciones Médicas, Universidad de Buenos Aires, Hospital Tornu, Buenos Aires, Arg.

After antigenic stimulation the lymphatic tissue undergoes several histological changes among which lymphocyte and plasma cell proliferation are the most characteristic. Notwithstanding its lymphatic structure the thymus does not develop the cellular changes that appear in other lymphatic organs after parenteral antigenic stimulation(1).

It has been clearly demonstrated that thymus grafts have the capacity to restore the immunological functions of thymectomized mice(2). This finding would indicate that the immunological functions of the thymus in an heterotopical position are similar to the functions observed when the organ is in its normal location. However, no studies have been reported of the immunological reactivity of the thymic cells in that abnormal localization of the gland. It seemed of interest to determine whether thymic autografts implanted in the subcutaneous tissue would not react to antigenic stimulation with any of the cellular changes associated with the immune response as it usually occurs when the organ is in its thoracic position. In this

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paper is reported an investigation showing that when the rat thymus is located in the subcutaneous tissue, parenteral antigenic stimulation leads to the appearance of the cellular changes that characterize immunological activity of other lymphatic organs.

Material and methods. Albino rats from the Instituto de Biologia Experimental were used throughout the experiments. Immediately after delivery each litter was divided into a control group and the experimental group. When the animals of the study group were in the fifth day of life, the thymus was removed and immediately implanted in the subcutaneous tissue of the left hemithorax. The control group was sham operated, opening the thorax without removing the thymus. The surgical procedure was performed according to the technique described by Gross (3) with minor modifications using hypothermia as anaesthetic. Animals were weaned at the 25th day of life, then fed with standard commercial pellets and tap water. Sixty days after operation all animals in both groups were subcutaneously inoculated in the interscapular region with 1 ml of aluminum activated diphtheria toxoid‡ containing 30 Lf per ml. A similar dose was administered weekly for 2 more weeks. All animals were sacrificed by exsanguination under light ether anaesthesia 2 weeks after receiving the last injection of toxoid. A control group of animals thymectomized at the same age as the study group and one of normal animals of comparable age were also immunized. As further controls, 2 groups of non-immunized autografted and sham operated animals of the same age were used. The number of available animals for the final analysis of the study was as follows: 24 autografted-immunized, 23 sham operated-immunized, 11 thymectomized-immunized, 14 normal-immunized, 22 autografted non-immunized and 22 sham operated non-immunized animals. Five rats which showed signs of infection in the graft and 7 with a residual thoracic thymus were not included in the study. Six animals in which the thymic graft could not be found at time of necropsy were also excluded.

Diphtheria antitoxin titers were determined by a tanned cell hemagglutination technique(4) using a microtiter kit(5).

A complete necropsy was performed on all animals. Before fixation cellular imprints of the thymus, spleen and lymph nodes were performed on several slides. These imprints were divided into 3 sets and used for: a) immunofluorescent studies, b) staining with methyl-green-pyronin and c) staining by the May Grünwald-Giemsa method. After formalin fixation the tissue sections were stained with hematoxylin and eosin.

Differential cell countings of the thymus and lymph node imprints stained with May Grünwald-Giemsa were done on all preparations. For this purpose the imprints were divided into a central and a peripheral zone and one thousand cells counted in each region. Morphologic classification was done dividing all the observable nucleated cells into 2 groups, one composed of "typical" plasma cells and a second group of cells not classifiable as "typical" plasma cells. The values obtained are the percental relation of "typical" plasma cells against the total number of counted cells.

A cell was defined as a "typical" plasma cell when it fulfilled the following criteria: a size of about 9 to 11 μ with an excentric nucleus measuring 5 to 7 μ; nucleus with reticular chromatin disposed in an alternate pattern of dense and clear zones; and a deeply stained basophilic cytoplasm usually with a clear perinuclear zone. Plasma cells with the above mentioned characteristics but acidophilic granules in the cytoplasm as well as reticular cytoplasm or vacuolated cytoplasm were also included.

For the differential cell countings performed on the slides stained with methyl-green-pyronin a different criterion was followed. All the nucleated cells with a clearly pyronin stained cytoplasm were included in the countings. Of these cells 35 to 45% were "typical" plasma cells.

For the immunofluorescent studies, purified horse diphtheria antitoxin containing 2400

‡ Diphtheria toxoid and antitoxin preparations were obtained from the Instituto Nacional de Microbiologia, Bueno Aires, Arg.

TABLE I. Diphtheria Antitoxin Titers in Autografted and Control Groups.

	No. of animals	Titer*	S.D.
Normal	14	10.1	3.1
Sham operated	23	9.7	2.0
Autografted	24	9.6	1.7
Thymectomized	11	2.4	1.7

* Arithmetic mean (log to base 2).
S.D. = Standard deviation.

UI per ml was labelled with fluorescein isothiocyanate as described by Marshall *et al* (6). The excess of dye was removed by passage through a column of Sephadex G25. The cell imprints were fixed with acetone for 10 minutes, then incubated with pure diphtheria toxoid containing 1500 Lf per ml during 30 minutes at room temperature. After 2 washings with buffered saline, pH 7.2, they were stained with fluorescent antitoxin previously absorbed with rat liver powder. Blocking controls with unlabelled antitoxin and direct staining with fluorescent antitoxin without previous treatment with toxoid were done in all cases. Different toxoid dilutions were also used. In some cases fluorescent tetanus antitoxin instead of diphtheria was used as further control of specificity. Fluorescence microscopy was performed with a Leitz 150 mercury lamp and Zeiss exciter and barrier filters.

Results. The diphtheria antitoxin titers are shown in Table I.

The mean weight of the autografted thymus was markedly diminished as compared with the weight of the thoracic thymus observed in the sham operated controls and in normal animals of the same age and sex. There were no differences between the weight of the autografted thymus in the immunized and non-immunized animals. The mean wet weight of the grafted non-immunized thymus was 67.1 mg $\pm$ 32.4 and of the immunized gland 70.5 mg $\pm$ 27.9. For the sham operated animals as well as the normal controls the mean wet weight of the thymus was about 350 mg.

The most noticeable histological finding in the autografted thymus of the non-immunized animals was a decrease of the cortical layer that in some cases was reduced to a thin rim of closely packed small lymphocytes. The normal looking medulla consisted mainly of reticulum cells and medium lymphocytes. Rare plasma cells were seen both in medulla and cortex. Newly formed vessels entering into the thymic parenchyma were also observed.

The thymus grafts of the immunized animals revealed the presence of large numbers of plasma cells mainly in the medulla (Fig. 1, 2) sometimes forming perivascular nest-like structures. Clusters of macrophages containing a refringent acidophilic substance were also seen in medulla and cortex. In some animals germinal center-like structures were found in the cortex. These formations consisted of a small group of large and medium sized lymphocytes with some macrophages containing nuclear debris, surrounded by a thin layer of small lymphocytes.

The thoracic thymus in both immunized and non-immunized groups showed no such changes. The cortical layer was well developed, rare plasma cells were found in the medulla, macrophages were not observed and very few Hassall corpuscles were found in both groups.

The other lymphatic organs of the autografted animals showed a normal histologic structure although in some cases there was a mild degree of small lymphocyte depletion.

The results of the differential cell countings are shown in Table II. It can be seen that the percentage of plasma cells and pyronin stained cells in the autografted immunized thymus is higher than that of the immunized thoracic thymus and comparable to the values obtained in the immunized lymph nodes.

The cytoplasm of many plasma cells as well as some other nucleated cells of the autografted immunized thymus contained diphtheria antitoxin as demonstrated by the immunofluorescence studies (Fig. 3). These studies were performed using several dilutions of diphtheria toxoid with comparable results. Similar studies performed on the thoracic thymus did not show any fluorescent cells.

Discussion. From the evidence obtained it can be inferred that under the conditions of our experiment the thymus responded to parenteral antigenic stimulation. When the thymus was located in the subcutaneous tissue, antigenic stimulation led to the appearance of a striking number of plasma cells which were involved in specific antibody production as revealed by the immunofluorescent studies. Since this pattern of cellular response as well as the location of antibodies within the cells is that usually seen in any lymphatic organ under these circumstances(7), it can be stated that the thymus was responding to the antigenic challenge. In some preparations the grafted thymus resembled a lymph node showing the presence of germinal center-like structures.

That these changes were actually induced by the antigenic stimulus is supported by the findings obtained in the control groups. None of the normally located thymus glands showed any cellular response. In addition no cellular changes were observed when the gland was autografted but the animals were not immunized.

Also our experiments demonstrate that the autografted thymus retains its capacity to control the maturation and normal function of the lymphatic tissue. This is indicated by the good antibody response along with the normal histologic structure of the lymphatic organs of the autografted animals. Further support for this observation was obtained from the data of the thymectomized controls which showed very poor antibody response and a marked lymphocyte depletion.

Why the thymus located in the subcutaneous tissue of the rat develops these cellular changes after antigenic stimulation can not be answered from our data. However, several possibilities may be considered. If, as has been postulated, the lack of response of the thymus to antigenic stimulation is due to the existence of a vascular barrier(1) it may be suggested that the new vessels giving the blood supply to the autograft allow an adequate contact between the antigen and the thymic parenchyma. Although in some mouse strains this vascular barrier seems to have a structural basis(8) it has been shown that bovine albumin enters easily into the rat thymus by way of perivascular

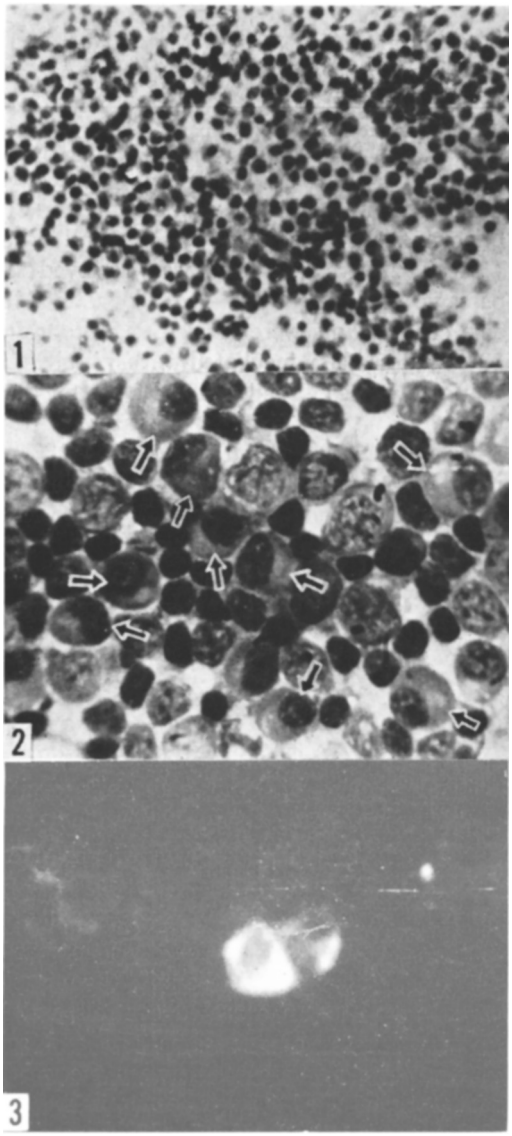

FIG. 1. Autografted immunized thymus. Medulla showing marked proliferation of mature plasma cells. H. & E. Optical system × 430.

FIG. 2. Cellular imprint of autografted immunized thymus. Small lymphocytes, abundant plasma cells (arrows) and some immature cells of the lymphatic series are seen. MG. G. Optical system × 980.

FIG. 3. Plasma cell from autografted immunized thymus with specific fluorescence of the cytoplasm. On its right upper side another mononuclear cell with specific fluorescence can be seen. Optical system × 500.

TABLE II. Differential Cell Countings of the Lymphatic Organs.

Non-immunized	No. of animals	% Plasma cells*	S.D.	% Pyronin cells*	S.D.
Thoracic thymus	22	1.1	.3	16.3	6.1
Autografted thymus	22	1.0	.3	17.0	2.0
Axillary node†	21	6.3	2.2	19.4	3.3
" " ‡	20	5.9	1.4	19.8	2.2
Immunized					
Thoracic thymus	23	1.2	.2	19.5	5.3
Autografted thymus	24	24.0	11.6	40.3	14.1
Axillary node†	21	28.9	7.6	41.6	14.5
" " ‡	20	28.3	9.7	46.8	13.0

* Mean. S.D. = Standard deviation. † These data correspond to sham operated animals.
‡ These data correspond to autografted animals.

channels after the intramediastinal injection (9).

An alternative and most attractive explanation is offered by the observations of Law and Miller(10). These authors using thymic autografts in DBA strain mice found that the graft suffers an initial massive death of the lymphoid cells with survival of the reticular and medullary epithelial elements. Later the graft is repopulated by lymphoid cells which normalize its structure. In our experimental model, it may be suggested that the original thymic lymphocytes of the autograft die initially, being later replaced by lymphocytes of unknown origin. These cells which repopulate the graft may represent the precursors of the plasma cells appearing after antigenic stimulation. This hypothesis implicates the acceptance of certain differences between "thymic lymphocytes" and "other lymphocytes" or as Gowans suggests(11) between immunologically "committed" small lymphocytes and "uncommitted" ones. Further investigation is in progress.

Summary. The immunological reactivity of the thymus of young adult rats in which the gland was removed from its normal location at the fifth day of life and implanted in the subcutaneous tissue was studied. As compared with control groups, these animals showed a normal histological structure of their lymphatic organs and also produced normal levels of circulating antibodies after immunization with diphtheria toxoid. The autografted thymus, by contrast with the normally located gland, revealed a cellular response comparable to the one observed in the lymphatic organs of normal animals after parenteral immunization. The most remarkable feature of the cellular response of the autografted thymus was a marked plasma cell proliferation producing specific antibody, as was demonstrated by differential cell counting and immunofluorescence studies.

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