PULSATION OF LYMPHOID ORGANS

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Besides its extremely high capacity for repair, lymphoid tissue is characterized by ability to recirculate, i.e., for its lymphocytes to pass from the lymphoid organs into the circulation and back again. The mechanism of recirculation has been studied for a long time and a number of general reviews have been published [1, 5, 6], although much still remained to be explained. Besides the complexity of the problem, this is also largely due to the fact that the methods used to study the normal circulation of lymphocytes inevitably involve certain preliminary procedures on the experimental animals (injection of isotope-labeled cells or of a radioactive isotope itself, an operation, irradiation immunization, and so on), and these must inevitably give rise to artefacts [1]. Moreover, methods of studying a dynamic process such as recirculation are as a rule static.

This paper describes data showing that in the absence of preliminary procedures and by dynamic investigation in animals it is possible to detect periodic time-dependent fluctuations in the weight of the lymphoid organs, the number of cells contained in them, and the serum levels of factors modifying lymphocyte migration.

EXPERIMENTAL METHOD

Intact male CC57BR mice weighing 20-23 g (from the Nursery of the All-Union Oncologic Scientific Center) or female C57BL/6 mice weighing 19-21 g (from the Stolbovaya Nursery) were kept four in a cage. Every hour one cage was brought from the animal house. Blood samples were taken from the animals — 5 μl from the caudal vein, and in the case of C57BL/6 mice, 0.5 ml from the retro-orbital sinus. The mice were killed immediately after blood sampling (the time of taking blood and sacrificing the four animals did not exceed 10 min), and the lymphoid organs (thymus, spleen, inguinal lymph nodes) were removed and carefully freed from adipose tissue, and weighed on torsion scales. The organs were kept on ice in medium No. 199 with the addition of 2 mM HEPES buffer. Cells were then isolated from the lymphoid organs by means of glass homogenizers. Nucleated spleen, thymus, and lymph node cells (living and dead), and also peripheral blood cells suspended in 3% acetic acid, were counted in a Goryaev's chamber. Sera of individual animals (three mice at each point) were tested in the lymphocyte migration inhibition test from glass capillaries *in vitro* in the writers' micromodification [3]. Pools of lymphocytes or spleen cells obtained from five or six C57BL/6 mice were used as migrating cells. The sera were kept at 4°C and tested 1 or 2 days after the experiment in a dilution of 1:10 in medium No. 199 with the addition of 10% embryonic calf serum and antibiotics. Migration was estimated from the weight of the projection of the migration zone on paper, in milligrams.

EXPERIMENTAL RESULTS

Although within the same group of mice differences in the weight of the lymphoid organs in the number of cells were not significant and, as a rule, did not exceed 10%, considerable variations of these parameters were discovered between groups of animals tested every hour at different times of the 24-h period (Figs. 1 and 2). Changes in the weight of the organ and in the number of cells in it correlated directly with each other. In an experiment on 40 male CC57BR mice statistically significant ($P < 0.05$) variations were found in the weight of the lymphoid organs and the number of cells in them; for the thymus (Fig.

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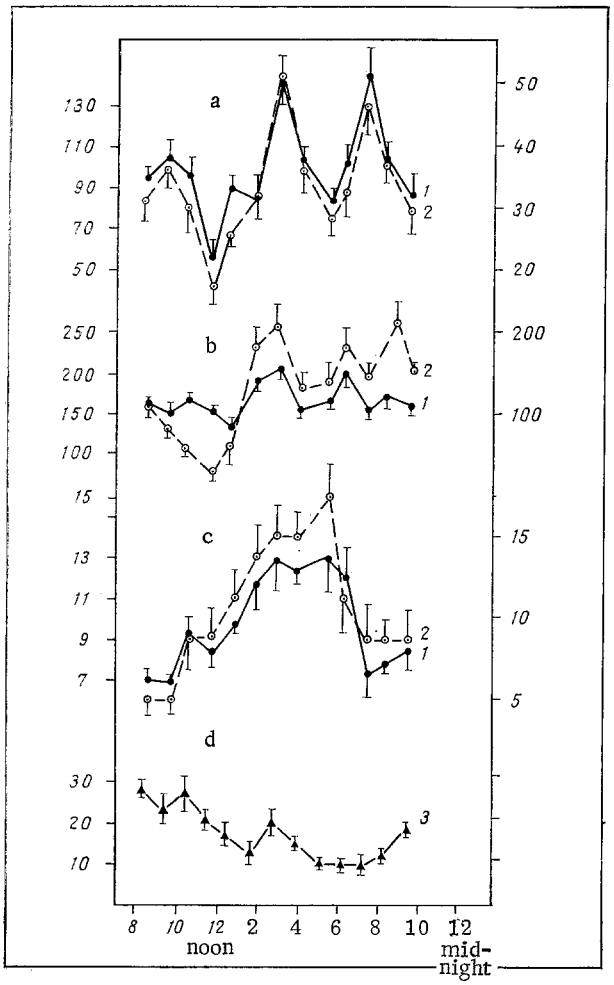

Fig. 1

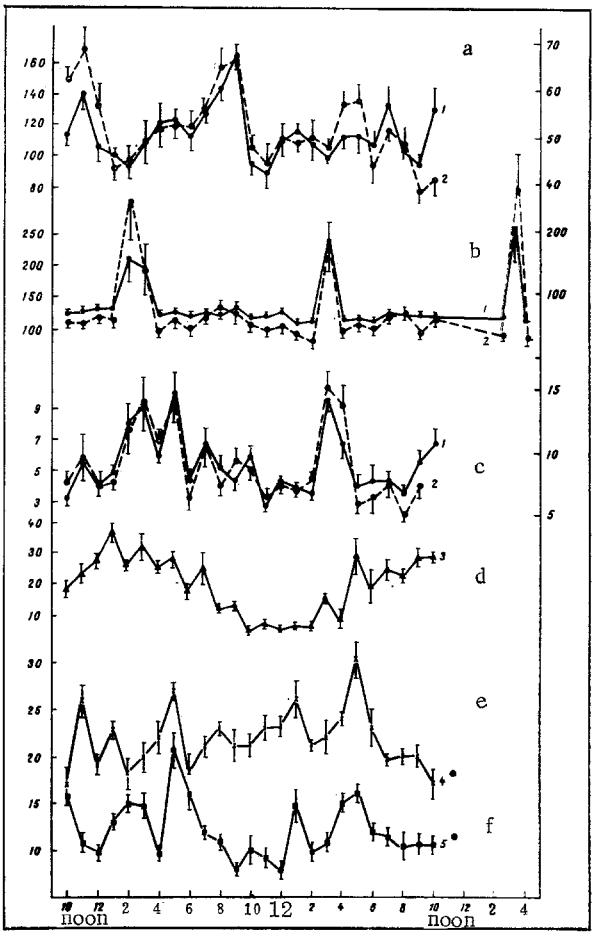

Fig. 2

Fig. 1. Variations in weight of organ (1), in number of nucleated cells contained in it (2), and peripheral blood leukocyte count (3), depending on time of day in CC57BR mice. a) Thymus, b) spleen, c) lymph nodes, d) peripheral blood leukocytes. Abscissa, time of day; ordinate: in a, b, c, on left — number of nucleated cells in organ, $\times 10^6$; d) number of leukocytes in blood (in 10^6/ml), M ± m; on right — weight of organ (in mg), M ± m.

Fig. 2. Variations in weight of organ (1), in number of nucleated cells contained in it (2), in peripheral blood leukocyte count (3), and in activity of factors modifying migration of spleen (4) and thymus (5) cells, depending on time of day in C57BL/6 mice. a) Thymus, b) spleen, c) lymph nodes, d) peripheral blood leukocytes, e) migration of spleen and thymus cells, f) weight (in mg) of projection of cell migration zone on paper in medium with and without sera of mice (4 and 5), M ± m. Remainder of legend as to Fig. 1.

1a) and spleen (Fig. 1b), moreover, these variations were distinctly periodic in character with a period of about 4 h. The number of leukocytes in the blood fell gradually in the course of the day and reached a minimum at 5-6 p.m. (Fig. 1d). On this change in the leukocyte count, which evidently reflects a circadian rhythm [2], were superposed fluctuations of a smaller order. In the experiments on 100 female C57BL/6 mice the most abrupt changes were found in the spleen (Fig. 2b). Three sharp peaks, reflecting a roughly threefold increase in the weight of the spleen and the number of nucleated cells in it, were found. Parallel with the increase in number of nucleated cells, the number of erythrocytes also increased. Maxima occurred with a period of 12 h at 2-3 p.m. and 3 a.m. In the intervals between maxima, variations in weight of the spleen were very small, both within the groups and between them. Only if the total number of cells in the spleen was counted were smaller variations discovered. Simultaneously with an increase in weight of the spleen, the weight of the lymph nodes and the number of cells in them also increased (Fig. 2c). This increase, just

as for the spleen, was considerable (by 2-2.5 times), but its duration was longer than for the spleen. The weight of the thymus and number of cells in it varied in this experiment with a period of about 10 h, and the maxima for the thymus did not coincide with the maxima for the spleen and lymph nodes (Fig. 2a). Just as in the first experiment, the blood leukocyte count varied in accordance with a circadian rhythm and fluctuations of a smaller order were superposed on the circadian variation (Fig. 2d).

Considerable differences also were found in the ability of sera from individual groups of mice to affect migration of thymus and spleen cells from glass capillary tubes *in vitro*. These differences were characterized by the periodic appearance of sharp peaks of intensification of cell migration in the presence of mouse sera compared with migration in medium without sera (Fig. 2e). The maxima of weight of the organs and number of cells in them corresponded as a rule to minimal mobility of lymphoid cells, whereas maxima of stimulation of migration appeared immediately after a decrease in weight of the organs.

Hourly estimation of simple parameters of the lymphoid system such as the number of cells in the organs and blood, and also the effect of sera on lymphocyte migration *in vitro* in individual groups of intact animals, revealed a phenomenon of pulsation of the lymphoid organs, the essence of which was that these organs periodically (and the spleen and lymph nodes synchronously with one another) accumulate cells to such an extent that they contain twice as many or more than in periods between accumulation. Since the increase in the number of cells in the spleen (about 20×10^7) at the maximum of pulsation exceeds the total number of cells in the mouse's blood, since it coincides in time with an increase in weight of the lymph nodes, and since it is not reflected in the number of cells in the blood, the most likely source of this large number of lymphoid cells for pulsations may be nonlymphoid organs, in which recirculating lymphocytes are diffusely distributed, although the possibility of an outflow of cells from the bone marrow cannot be ruled out. This hypothesis is supported by the existence of a "trapping" phenomenon, in which lymphocytes between 1 and 6 h after injection of an antigen are nonspecifically retained in regional lymph nodes, so that for a certain time the lymph is completely free from them [1]. Accumulation of lymphocytes in the lymph nodes may perhaps be due to activity of a soluble factor or factors, delaying the passage of the cells through this organ. Local injection of fractions containing macrophage migration inhibition factor (MIF) is known to lead to accumulation of cells in the lymph nodes, their adhesions to the walls of the peritoneal cavity and blood vessels [7, 9, 11]. MIF appears in the serum after 4 h, and in the lymph flowing from the lymph node within 24 h after injection of the antigen [8, 11]. There is evidence that factors regulating macrophage migration *in vitro* are produced by nonimmune spleen cells with a biological rhythm of 3-4 h [4]. Data in the literature, together with those now described showing periodic variations in the serum level of an active factor (or factors) modifying lymphocyte migration, and on variations in the number of cells in the lymphoid organs suggest that under normal conditions factors are periodically secreted in the lymphoid organs which have the effect of selectively holding up recirculating lymphocytes in them, and later facilitating their liberation from these organs. Fluctuation in the number of cells in the lymphoid organs could, on the one hand, facilitate the fastest possible accumulation of information in them and intensive exchange of information between the cells in the course of cooperation, and on the other hand, it could serve as a unique measuring device for the proper organization of division and differentiation of lymphoid cells.

The experiments thus showed that the weight of lymphoid organs may vary rhythmically (pulsate). This fact must be taken into consideration when experiments are planned in which the weight of the lymphoid organs or the number of cells in them have to be taken into consideration. The results may be of practical importance in chemotherapy, for the dosage and mode of administration of preparations could be significantly affected by biorhythms characterizing the state of the lymphoid system.

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REACTION OF SOME HYPOTHALAMIC CENTERS IN RATS TO A SINGLE INJECTION OF THYROTROPIN-RELEASING HORMONE

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The effect of exogenous thyrotropin-releasing hormone (TRH) on the level of thyroid stimulating hormone (TSH), prolactin, and thyroid gland hormones in the blood of animals and man has been investigated frequently [13, 14]. Information has been obtained on the effect of iontophoretic applications of TRH directly to neurons of various parts of the brain, including hypothalamic neurons [11, 13, 14].

It has not proved possible to show by these investigations which process in the neurosecretory cell was affected by administration of TRH: whether synthesis of the hormone, its transport along the fibers, or its liberation into the blood stream. The localization of the hypothalamic center or centers regulating thyroid gland function likewise has not yet been established. The investigation described below was devoted to the study of these problems.

EXPERIMENTAL METHOD

Male Wistar rats weighing 220-230 g were given an intramuscular injection of 8 μg synthetic TRH, dissolved in 2 ml physiological saline, 30 min before decapitation. Considering the high speed of TRH inactivation in the blood [12], its dose was chosen so that the TRH level remained high throughout the experiment, so that morphological and functional changes in the cells could be manifested. Control animals received an injection of 2 ml physiological saline.

The animals' brain was fixed in Bouin's fluid and sections 6 μ thick were stained with paraldehyde-fuchsin by the Gomori—Gabe method and counterstained with Heidenhain's azan.

The area of cross section of the nuclei and nucleoli in cells of the supraoptic, paraventricular, suprachiasmatic, ventromedial, and arcuate nuclei (SON, PVN, SCN, VMN, and AN respectively) of the hypothalamus was measured by a photographic method. Microfilming was carried out under a 60 × immersion objective, or 90 × for SCN. In each nucleus at least 35 cells were measured. The relative percentage of cells with no nucleolus or with one nucleolus and of the so-called polynucleolar cells, i.e., those containing nucleolus-like bodies, and fragments of cell nuclei also were counted in the sections among 400 cells in each animal. All measurements were made within the regions of the hypothalamic centers indicated in Fig. 1.

The significance of differences between control and experimental values was assessed by Student's t-test and the Mann—Whitney U test.

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