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DETERMINATION OF THE NUMBER OF T AND B LYMPHOCYTES IN GUINEA PIGS

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Determination of T and B lymphocytes and of subpopulations of these cells is an essential condition for the study of the immune status of an individual. Guinea pigs are widely used for models of pathological processes, or the study of the action of biologically active substances, and so on. Recently data have been published on the property of the T lymphocytes of these animals of fixing rabbits' erythrocytes [8]. However, there is no information of the optimal conditions for formation of E rosettes by T lymphocytes of guinea pigs. There are likewise no data on whether these animals possess a fraction of "active" T lymphocytes, able to form rosettes directly after the addition of a suspension of xenogenic erythrocytes to them.

To determine β-lymphocytes of guinea pigs, the method of detecting receptors for C_3 complement on their surface was used [7, 8]. The effect of the concentration of complement, the class of antibodies used to form the antibody–complement complex, and centrifugation of a mixture of erythrocytes sensitized by this complex and lymphocytes on the course of this reaction likewise was not studied.

The aim of the investigation was to study the basic parameters of the method of determination of T and B lymphocytes of guinea pigs and the "active" fraction of T lymphocytes, and also to study the distribution of T and B lymphocytes *in vivo*.

EXPERIMENTAL METHOD

To obtain a suspension of lymphocytes the lymph nodes and bone marrow of guinea pigs weighing 250–300 g were placed in Hanks' solution, teased with dissection needles, and the suspension was filtered through four layers of Kapron gauze. The cells were washed 3 times with Hanks' solution for 10 min at 200g, resuspended in medium 199, and counted in a Goryaev's chamber. The viability of the cells was determined in the test with trypan blue. Before washing off, the bone marrow cells were treated with 0.85% ammonium chloride solution. Blood was collected by cardiac puncture, mixed with heparin (25–30 Units/ml blood) and diluted with Hanks' solution in the ratio of 1:3. The lymphocytes were isolated by the method in [5] and a suspension containing 2.5×10^6 cells/ml was prepared.

Erythrocytes were obtained from a rabbit and a sheep as follows. Blood was collected in an equal volume of Alsever's solution. The rabbit's erythrocytes were kept at least 4 days before the experiment. They were then washed 3 times before use and suspensions of rabbit's (10^8 cells/ml) and sheep's erythrocytes (5×10^8 cells/ml) were prepared.

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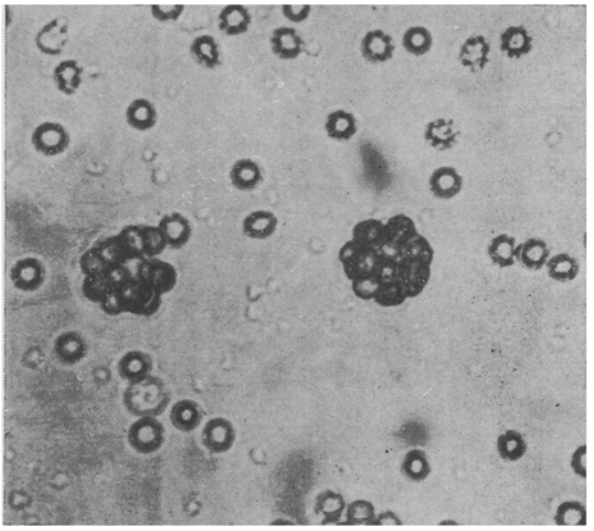

Fig. 1. E rosettes formed by guinea pig's T lymphocytes with rabbit's erythrocytes. Phase contrast, 6b objective, 20×, ocular 7×.

TABLE 1. Effect of Conditions of Incubation on Formation of E Rosettes by Lymphocytes from Guinea Pig Blood with Rabbit's Erythrocytes and Distribution of T Lymphocytes in Systems and Organs

System or organ	Duration of incubation at undermentioned temperature, °C		Number of animals in experiment	Mean number of T lymphocytes and limits of variations, %	P	Criterion
	37	4				
Blood	Not incubated		15	25,4 (1—46)		
	15 min	60 min	15	31,9 (12—55)	<0,01	T
	30 min	18 h	9	29,5 (20—49)	<0,01	T
Lymph nodes	Not incubated		7	27,0 (19—34)		
	15 min	60 min	7	41,0 (30—54)	<0,01	T
Bone marrow	Not incubated		6	1,0 (0—1)		
	15 min	60 min	6	1,0 (0—2)		

Legend. Level of significance P calculated according to [2]; significance of differences tested on data for paired experiments.

Antiserum against sheep's erythrocytes, containing chiefly antibodies of the IgM class, were obtained by a single intravenous immunization of rabbits with 5 ml of a 50% suspension of sheep's erythrocytes. Blood for obtaining serum was collected on the 5th day. The hemagglutination titer of this serum when tested in the panel of a Takachi microtitrator was 1:32. Hemolytic serum, supplied by the I. I. Mechnikov Moscow Research Institute of Vaccines and Sera, with a hemagglutination titer of 1:256, was used as the source of class IgG antibodies. The localization of the antibodies in both antisera was determined by rapid chromatography on DEAE-cellulose by a no-column method [3]. Before the experiments the sera were inactivated by heating for 30 min at 56°C.

To sensitize the sheep's erythrocytes 1 ml of the erythrocyte suspension was mixed with 1 ml of diluted antiserum and incubated for 30 min at 37°C. The erythrocytes were washed 3 times with Hanks' solution, resuspended in 1 ml of the same solution, and treated with an equal volume of fresh mouse serum, the source of complement. Incubation was repeated for 30 min at 37°C, the cells were washed 3 times, and a suspension containing 10^8 cells/ml was prepared in medium 199.

To determine T lymphocytes 0.2 ml of the lymphocyte suspension (2.5×10^6 cells/ml) was treated with an equal volume of rabbit erythrocyte suspension (10^8 cells/ml). After incubation and centrifugation this mixture was introduced into a Goryaev's chamber where at least 400 cells were counted and the number of lymphocytes with three or more erythrocytes fixed on their surface was determined. When determining B lymphocytes 0.4 ml of guinea pig lymphocyte suspension (2.5×10^6 cells/ml) was treated with an equal volume of sheep's erythrocyte suspension (10^8 cells/ml), sensitized with complement. After incubation and, in certain

TABLE 2. Effect of Conditions of Incubation and Centrifugation on EAC Rosette Formation by Guinea Pig Lymph Node Cells

Duration of incubation at 37°C, min	Centrifugation for 5 min at 100×g	Keeping at 4°C	Number of animals in experiment	Number of EAC rosettes (M ± tm), %
30	—	—	6	16,0±3,7
30	—	60 min	6	15,0±4,1
30	+	—	6	17,0±2,5
30	+	60 min	6	17,2±5,0

Legend. Here and in Table 3, statistical analysis as in [1].

TABLE 3. Distribution of B Lymphocytes in Systems and Organs of Guinea Pigs

System or organ	Number of animals in experiment	Number of B lymphocytes (M ± tm), %
Blood	7	10,3±5,0
Lymph nodes	7	17,7±2,3
Bone marrow	6	4,6±1,7

cases, after centrifugation also the mixture of cells was introduced into a Goryaev's chamber. The number of rosettes was counted in the same way as when determining T lymphocytes.

EXPERIMENTAL RESULTS

T lymphocytes formed rosettes with rabbit's erythrocytes. Significant differences ($P_T < 0.01$) in the relative numbers of these rosettes were observed under the conditions specified in Table 1 (lines 1 and 2, 1 and 3, and 4 and 5). Correlation was found between the number of rosettes formed by blood and lymph node lymphocytes under the same conditions. Bone marrow cells formed practically no such rosettes.

Determination of B lymphocytes in experiments with cells isolated from lymph nodes showed that the type of antibodies (IgM or IgG) used to sensitize the erythrocytes had no effect on the relative number of EAC rosettes. The mean data for 17 experiments were 17.5 ± 2.8 and 17.7 ± 2.8% respectively. The number of rosettes likewise was unchanged when mouse serum was used in different dilutions: 17.5 ± 5.0% of rosettes with a dilution of 1:4, 18.0 ± 5.4% with 1:8, and 16.0 ± 5.4% with 1:10.

In the experiments whose results are given in Tables 2 and 3, rabbit serum against sheep's erythrocytes, containing class IgM antibodies, and mouse serum in a dilution of 1:8 were used. The experiments showed (Table 2) that centrifugation of the mixture of cells with sensitized erythrocytes and additional incubation at 4°C likewise did not affect the number of EAC rosettes. The largest number of B lymphocytes was found in lymph nodes, the smallest in bone marrow (Table 3).

The experiments thus confirmed data [8] on the property of guinea pigs' lymphocytes of forming rosettes with intact rabbit's erythocytes. At the same time it was found that, just as in man, there exists in guinea pigs a subpopulation of lymphocytes which form "early" rosettes. However, whereas to detect "early" rosettes in man the lymphocytes must be incubated with sheep's erythrocytes for 15 min at 37°C [4], "early" guinea pig rosettes are formed without any preliminary incubation (Table 1). The significance of determination of "early" rosettes as a characteristic of the immune status of guinea pigs is a matter for further study. The presence of correlation between the number of T lymphocytes in the blood and lymph nodes is evidence that blood can be used as material for determination of the state of the T system.

Commercial hemolytic serum was used to determine B lymphocytes of guinea pigs [7, 8]. The present experiments showed that similar results are obtained by the use of class IgM antibodies, but if the latter are used it evidently reduces the possibility of detecting EA rosettes along with EAC rosettes. The relatively small number of B lymphocytes found in bone marrow is in agreement with data on the number of these cells in other species of rodents [6]. The possibility likewise cannot be ruled out that some of the B lymphocytes contained in bone marrow are in an earlier stage of antigen-dependent differentiation and do not possess receptors for C_3.

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