

# CORRELATION BETWEEN CERTAIN MORPHOLOGICAL AND CYTOCHEMICAL REACTIONS OF THE SPLEEN AND PERIPHERAL BLOOD OF GUINEA PIGS TO INTRAVENOUS INJECTION OF LEUKOCYTE SERUM\*

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Experimental researches recently undertaken under the direction of G. K. Krushchov [1, 4, 5, 7-10] have shown that the injection of leukocyte serum into healthy mammals, without disturbing the "physiological background," causes an increase in the intensity of tissue regeneration in areas where this takes place naturally, increases the intensity of the metabolic processes in the tissues, and stimulates protective reactions.

The main peripheral blood indices and the relative proportions of the various categories of blood cells remain within physiologically normal limits. Only a transient increase in the monocyte count is observed for a certain period after the injection of leukocyte serum [6].

The study of certain cytochemical indices of the peripheral blood has revealed a considerable increase in the glycogen content of the neutrophils.

Examination of the bone marrow following administration of leukocyte serum reveals variations within normal limits [2].

The tissue and cellular reactions of the spleen to leukocyte serum, namely, an increase in the number and size of the Malpighian corpuscles, the appearance of large germinal centers in these corpuscles, increased mitotic activity of the lymphoblastic elements, and an increase in the number of polyblasts and macrophages, are distinguished by their obvious and prolonged nature. This is evidently attributable to the fact that the spleen participates in the formation of leukocyte factors in normal conditions [3].

The object of the present investigation was to study the glycogen content in the cells of the peripheral blood and spleen following injection of leukocyte serum into animals. Besides the neutrophils, cells of especial interest in this respect were the agranulocytic polyblasts and the macrophages of the spleen, cells related functionally to the formation of leukocyte factors [9].

## EXPERIMENTAL METHOD

Time (in days)	Before injection	After injection
1	3,5—6,5	3,5—5,5
4	3,0—6,0	4,0—9,5
7	1,0—4,0	5,5—9,5
14	2,5—3,0	2,0—2,5

Experiments were conducted on 40 male guinea pigs weighing from 300 to 450 g. Leukocyte serum, dissolved in distilled water and made up to its original volume, was injected intravenously in a single dose of 0.001 ml/g body weight into 16 guinea pigs (group 1). The controls consisted of 14 guinea pigs (group 2) receiving analogous injections of dried and reconstituted, but otherwise untreated, horse serum. The 10 guinea pigs of group 3 received no injections of serum.

\* Leukocyte serum or leukocyte preparation is the name given to horse serum initially exposed to the influence of leukocytes under certain specified conditions, and then centrifuged and dried.

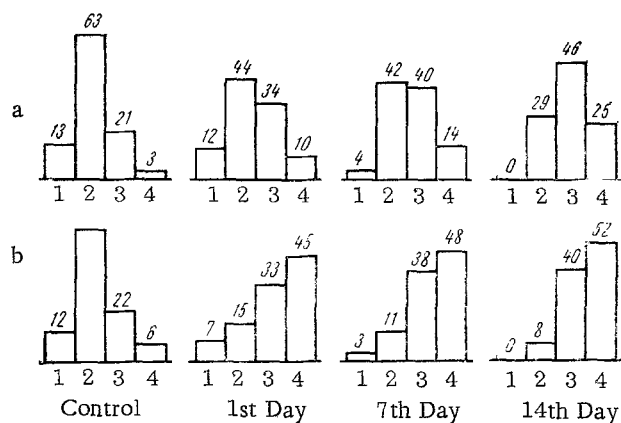


Fig. 1. Changes in relative percentages of groups of neutrophils (depending on their glycogen content) in the peripheral blood (a) and spleen (b) at various periods after intravenous injection of leukocyte serum into guinea pigs. Neutrophils of groups 1, 2, 3, and 4.

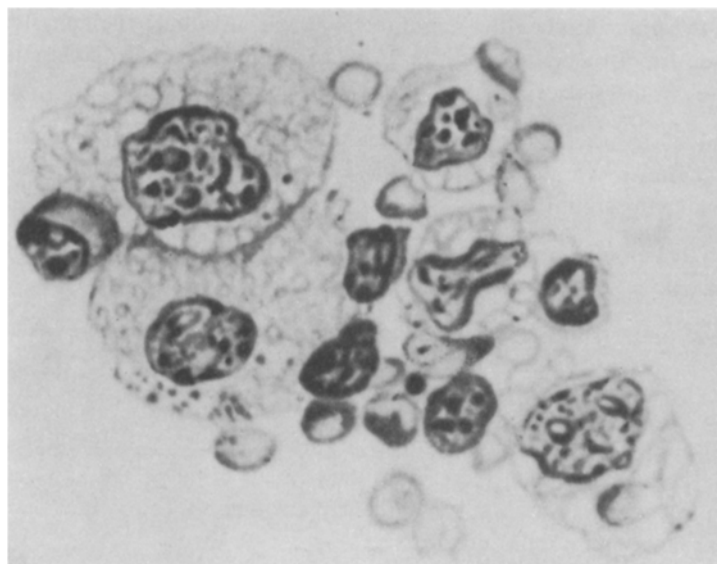


Fig. 2. Polyblastic reaction of spleen 24 h after injection of leukocyte serum. Drawing apparatus. Ocular 10X, objective 90X.

The animals were sacrificed 1, 4, 7, and 14 days after the injection. The total numbers of erythrocytes and leukocytes per  $\text{mm}^3$  of blood, the differential cell count, and the glycogen content in the neutrophils were determined for all the animals. Preparations of the spleen and blood were fixed with methyl alcohol and stained with azure-eosin. Some preparations were fixed by a modified Shabadash's method and glycogen was detected by means of the PAS reaction, using an ordinary control. Depending on the number of glycogen granules in their cytoplasm, the neutrophils of the blood and spleen were divided into 4 groups: group 1—neutrophils not containing glycogen; group 2—neutrophils containing solitary granules of glycogen; group 3—neutrophils containing an average number of glycogen granules; group 4—neutrophils containing the maximal amount of glycogen.

#### EXPERIMENTAL RESULTS

As a result of the determination of the total cell counts of the blood at different periods after injection of leukocyte and normal serum, no changes exceeding the limits of physiological variation were found. The differential count on the 4th and 7th days after the injection of leukocyte serum showed an increase in the number of monocytes.

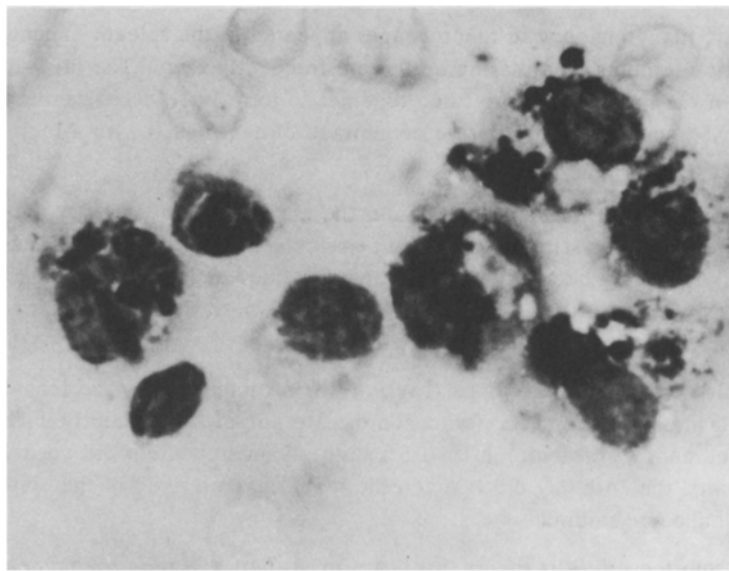


Fig. 3. PAS-positive reaction of polyblasts of the spleen 24 h after injection of leukocyte serum. Ocular 7X, objective 90X.

The number of monocytes in the peripheral blood of the guinea pigs before and after injection of leukocyte serum is given (in %) in the table.

Determination of the presence of glycogen in the leukocytes showed that the blood of the normal guinea pig contains: 8-17% of group 1 neutrophils, 55-65% of group 2 neutrophils, 18-30% of group 3, and 1-6% of group 4. No glycogen is found in the agranulocytes.

After the injection of leukocyte serum, the proportions of neutrophils of groups 3 and 4 gradually increase after the first 24 h. They reach a maximum on the 14th day (Fig. 1).

After the injection of normal serum, the increase in the number of neutrophils rich in glycogen took place later—on the 7th day after the injection. On the 14th day the numbers of neutrophils of groups 3 and 4 in the blood were increased, but were not as high as the corresponding values after injection of leukocyte serum.

In the spleen of normal guinea pigs the predominant form of nucleated cells are the lymphocytes, accounting for 55-78% of the total. The proportion of macrophages and polyblasts varies. The macrophages are mainly erythrophages. Neutrophils, mainly with segmented nuclei, amount to 8-14%; about 7-10% consists of destroyed cells—lymphocytes, neutrophils, reticulum cells. Other nucleated cells—monocytes, eosinophils, plasma cells, cells of the myeloid and erythroblastic series—amount to about 10%.

The neutrophils of all generations contained glycogen, the level of which increased with increasing maturity of the cell. Most of the neutrophils contained single glycogen granules. The relative percentages of the groups of neutrophils with different glycogen contents in the spleen in the control animals and at various times after injection of leukocyte serum are indicated in Fig. 1. Twenty-four hours after injection of the serum the lymphocytes were mainly large polyblastic forms, with an abundant, basophilic cytoplasm, the peripheral zones of which formed drop-like projections or elongated processes. Detached fragments of cytoplasm were scattered everywhere between the cells. Besides the lymphocytic polyblasts, monohistiocytes and reticulum cells were found, more numerous than in the controls. These were large cells with the characteristically round nucleus and an extensive, spread-out, basophilic cytoplasm (Fig. 2). Impressions of the spleen, stained for glycogen, differed appreciably in their overall intensively pink color from the control preparations of the corresponding times and from those from the group of animals receiving normal serum. The cytoplasm of the large polyblasts contained PAS-positive granules of different sizes (Fig. 3), not all of which responded to treatment with amylase, while the macrophages often contained remnants of neutrophils with glycogen granules. PAS-positive neutrophils could be distinguished. As regards their glycogen content, most of them could be classed in group 4, which differed in the greater number, the larger size, and the brighter staining of their glycogen granules than the corresponding group of neutrophils in the peripheral blood. On the 4th

and, especially, on the 7th day, many phagocytic macrophages appeared in the spleen. Young forms of neutrophils and eosinophils, erythroblastic elements, and plasma cells were frequently seen. The drop-like basophilic cytoplasmic fragments, resulting from clasmacytosis, were fused together to form large cytoplasmic complexes, staining a delicate pink color by the PAS reaction. The relative percentage of neutrophils with a high glycogen content was increased.

On the 14th day after injection of the leukocyte serum the majority of the cells of the spleen were lymphocytes. The macrophages were overlaid with fragments of leukocytes, erythrocytes, particles of cytoplasm of clasmacytic origin, and granules of pigment; their nuclei were often deformed and displaced. Macrophages of this type, having completed their phagocytic activity, no longer contained PAS-positive substances, as a rule. The overwhelming majority of neutrophils contained many glycogen granules.

The reaction of the spleen to injection of normal serum was distinguished by the long duration of the phenomena of cellular irritation: clasmacytosis, and the formation of bare nuclei, subsequently undergoing destruction, were prominent features. The number of neutrophils with a high glycogen content did not increase until the 7th day of the experiment, and not until the 14th day did it reach the level characteristic of the corresponding reaction in the group of animals receiving leukocyte serum.

It may be concluded from these results that a similarity exists between the cellular reactions of the spleen and of the peripheral blood to injection of leukocyte serum into animals. The polyblastic reaction of the spleen, gradually giving way to a macrophage reaction, is synchronized with the increase in the number of monocytes in the blood. The increase in the number of neutrophils with a high glycogen content in the spleen corresponds to the increase in the numbers of these categories of neutrophils in the blood; in these circumstances the reaction of the spleen is characterized by a sharp increase in the glycogen content of the neutrophils, while the reaction of the peripheral blood is gradual.

#### SUMMARY

It is shown that a single intravenous injection of leukocytic serum into guinea pigs causes an increase in the glycogen content of the neutrophils of the peripheral blood and spleen.

The polyblast reaction of the spleen, followed by a macrophage reaction, concurs with an increase in the number of monocytes in the peripheral blood.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. Some or all of this periodical literature may well be available in English translation. A complete list of the cover-to-cover English translations appears at the back of this issue.