

THE INFLUENCE OF PRELIMINARY RESERPINIZATION ON THE CHANGES OF BLOOD CELLS' NUMBER AFTER APPLICATION OF ULTRASOUND IN RATS

I. Investigation on the erythrocyte number changes

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It is a well-known fact that the blood system appears to be one of the most dynamic in the organism. Under the effect of a variety of factors, not merely the production of forming elements, but their number in the circulating blood and destruction as well (7) are altered. This is doubtlessly reflected in the blood functions, its respiratory function inclusive. The changes in the number of erythrocytes after application of ultrasound (US) are of particular theoretical and practical interest. We are perfectly well aware that US is being used very extensively in the diagnostical and therapeutical medical practice over the past several years. Still wider is its utilization in industry. All this implies further studies of the changes taking place under the effect of this biophysical factor, more particularly, the changes in the blood system. The literature data concerning the influence exerted by US on the number of erythrocytes are discrepant (1, 3, 4, 14). The mechanism of its action on the blood system, as well as its untoward side effects and late sequelae are likewise insufficiently investigated (3, 18).

With the present study we aimed to trace in experiment the influence of different biological US doses on the number of erythrocytes in albino rats after being subjected to preliminary treatment with reserpine. It is well-known that reserpine liberates catecholamines (KA) from the high-capacity intra-axonal depots of the sympathetic neurons and interferes with KA binding to granules, which depletes their reserves and leads to temporary disturbance of adrenergic transmission (6, 9, 13, 15, 16). On the other hand, proceeding from the definitely established sympathicolytic action of US (3, 20, 22), we presume that preliminary treatment with reserpine would allow us to analyze certain aspects of the mechanism of US action on the blood system, all the more that we failed to come across a similar approach to the problem in the literature surveyed.

Material and Method

The experiments were carried out on 44 healthy male albino rats, distributed in 6 groups: group I consisting of 8 experimental rats, treated with US at dose 0.1 watts/cm²; group II — 8 rats (experimental) treated

with US at 0.2 wats/cm² and group III — 8 animals, not treated with US — control of groups I and II; group IV — 9 rats (experimental), treated with US at dose 0.3 wats/cm²; group V — 4 rats, not treated with US — control of group IV and group VI consisting of 7 untreated with US rats. The US treatment in all instances lasted for 2 minutes, applied in the cervico-thoracic region and under the experimental setting described earlier (4, 5). All animals, following beforehand determination of the erythrocyte number, were subjected to single intramuscular sterile injection of «serpasil-Ciba», at dose 3 mg/kg body weight. The US treatment of the experimental groups was performed 24 hours thereafter. Besides the overall counting of erythrocytes, they were determined subsequently in all the groups at 24 hours after the reserpine injection, 1 hour after the US treatment, at 1, 3, 5, 8 and 10 days, and after that, every 5th day for a period of 25 days. The control animals were investigated in the same terms. The mode of blood obtaining and counting is described in a previous report (5). In the animals of group VI, in addition to the erythrocytes, also the reticulocytes were investigated after Heilmeyer (10).

Results and Discussion

In all the groups of animals, changes were established in the number of erythrocytes 24 hours after the single treatment with reserpine. These changes consisted in a tendency towards reduction of their number, in some of the groups rather considerable. Generally, this tendency was less pro-

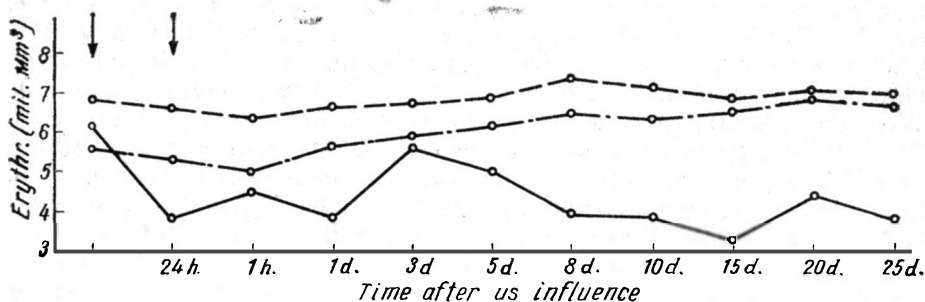


Fig. 1. The changes in the number of erythrocytes — mean values

First arrow — reserpine injection; second — ultrasound application.

The continuous line shows the changes in the animals treated with — 0.2 wats/cm²;
The interrupted line with dots and dashes — changes in treatment with 0.1 wats/cm²;
Interrupted line with dashes — changes in the number of erythrocytes in the control, untreated with US, animals.

nounced than the changes in the white blood cells (4, 5). It is evident (Fig. 1) that the most significant changes occurred in group II — injected with reserpine and US treated with 0.2 wats/cm². Twenty four hours after application of reserpine, they fall from 6.131 ± 0.608 millions/mm³ to 3.880 ± 0.688 mil/mm³, with statistical reliability $P < 0.001$. After the application of US, the red blood cells at 1 hour rise to 4.499 ± 0.580 mil/mm³, these show a second reduction at 1 day, and up to the end of the experiment din

close levels below the starting one. The changes in the erythrocytes of group I — injected with reserpine and US treated with 0.1 wats/cm² are the following: reduction after the reserpinization — from 5.592 ± 0.429 mil/mm³ to 5.306 ± 0.578 mil/mm³, reduction at 1 hour after the US treatment —

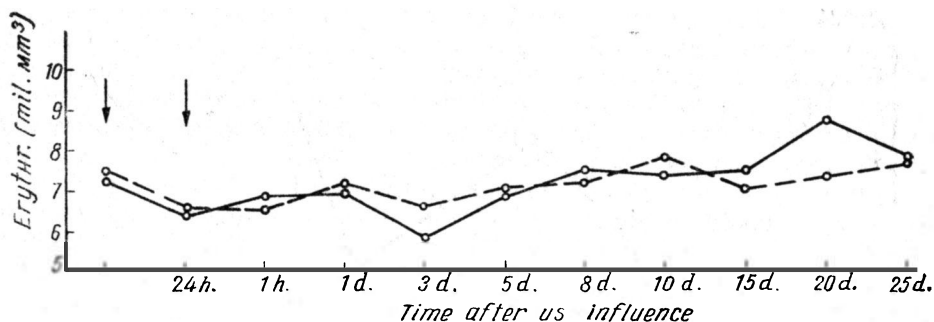


Fig. 2. Changes in the number of erythrocytes (mil/mm³) after US treatment with dose 0.3 wats/cm²

Continuous curved line — the US treated animals, and with interrupted curved line — the controls. The other indications are the same

5.016 ± 0.578 mil/mm³, reaction at 1 hour after the US treatment — 5.016 ± 0.578 mil/mm³, reaching the initial level at 1 day after US application and fluctuations above the starting level reaching the maximum at 20 days when their number amounts to 6.915 ± 0.827 mil/mm³. The changes in the control, untreated with US, group III, show: reduction of erythrocytes 24 hours after reserpine injection from 6.995 ± 0.640 mil/mm³ to 6.580 ± 0.520 mil/mm³, and 1 hour after the US treatment — 6.175 ± 0.320 mil/mm³. After the first day, their number was maintained at a relatively constant level within values near the initial one. The red blood cells in group V — experimental animals — treated with the highest US dose — 0.3 wats/cm² — exhibit the following changes: following reserpine injection, they decrease from 7.220 ± 1.723 to 6.320 ± 1.513 mil/mm³ at 24 hours. After the US treatment they increase — 6.920 ± 0.800 mil/mm³, and 1 day thereafter with maximum at the third day, their number again falls — 5.750 ± 2.026 mil/mm³, with statistical reliability at $P < 0.10$. Their number restores normal values at 8 days, rises at 20 days — 8.670 ± 1.800 mil/mm³, and reaches the starting value at 25 days. The changes recorded in the control group (V) up to 24 hours are analogical to those in group IV, with the subsequent fluctuations being insignificant, unreliable statistically and thus, considered as physiological (Fig. 2).

To clarify the influence of stored KA liberating effect of reserpine upon the production of young erythrocytes, in the last (VI) group, parallel to erythrocytes, the reticulocytes were also investigated. It was established that initially, their number (in relative and absolute values) shows insignificant reduction — from 26.2% to 20% at 1 day after reserpine injection. Within a day, the number of reticulocytes rises substantially — at 5 days they are 52.2%, i. e. showing a two-fold increase, and at 15 days — 63.3%, statistically reliable at $P < 0.05$, with their number maintaining

a level considerably exceeding the starting one until the end of the experiment. The latter fluctuations of reticulocytes are fully correlated with the erythrocyte number fluctuations (Fig. 3).

The explanation of the changes recorded is difficult owing to the complex action of reserpine (13, 15, 17) and intricate nature of the US effect

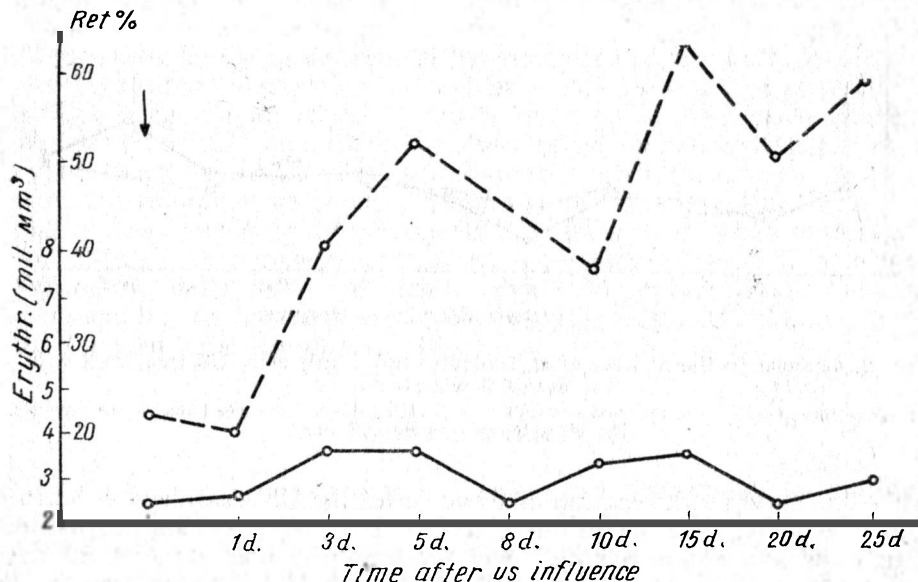


Fig. 3. Changes in the number of reticulocytes after single injection of reserpine
The arrow: reserpine injection. Interrupted line — reticulocytes (per 1000 erythrocytes).
Continuous line — number of erythrocytes in the same animals

(1, 2, 3, 8, 20). Literature reports concerning analogical experiments were not found, which deprives us of the possibility to study comparatively the changes described here. We are prone to explain the reduction of erythrocytes and reticulocytes, 24 hours after the application of reserpine, as occurring under the influence of two factors: a) firstly, as a result of the depleting action of reserpine on the intra-axonal sympathetic KA depots and damaging of the intra-axonal mechanism depositing KA — phenomena comprehensively described in the pertinent literature (6, 11, 13, 15); b) secondly, as a result of the increase of the parasympathetic tone. It has been proved that the central effects of reserpine are produced by the liberated serotonin, which is a CNS depressor, stimulating the central parasympathetic formations (6, 9, 11, 16, 17, 21). As a result of the former, in all likelihood, a transient damage occurs of the adrenergic conductivity along the blood-regulating neurons. Furthermore, the increased parasympathetic tone, most probably, contributes to a great extent for the reduction of erythrocytes, and these are the two factors which, in our opinion, determine the redistribution of erythrocytes. Much more difficult for interpretation are the changes, taking place following US treatment. The impression is one that the majority of experimental animals, with all three US doses utilized, display a tendency towards increasing the number of erythrocytes

1 hour thereafter, particularly clearcut at dose 0.2 and 0.3 wats/cm². This finding might be explained by the stimulating effect of ultrasound (3, 8), which is promptly exhausted, with ensuing inhibitory phase, and with the erythrocytes showing lower values than the control animals. This gives us sufficient reason to presume, that US against the background of disturbed adrenergic transfer in the blood-regulating neurons, leads to changes substantiating additional interference or facilitation of the conductivity, and in turn, to changes in the number of circulating erythrocytes. The increase of reticulocytes at 1 day, in group VI, is most probably due to the increased parasympathetic tone caused by the central and peripheral effects of reserpine. We feel that this might be the explanation of the increased proliferative activity of the red order cells in the bone marrow, followed by increase of reticulocytes in the peripheral blood. It is known that the proliferative processes in the bone marrow are stimulated by the parasympathetic system (19). Insofar the effect of US on bone marrow is concerned, it has been proved that the reticulocytes increase (1). Moreover, the influence of the endocrine and other humoral factors should by no means be ruled out, more particularly, the stimulators and depressors of erythropoiesis (12), produced as a result of erythrocytes' and reticulocytes' destruction by ultrasound.

Inferences

1. The single time injection of reserpine in albino rats, at dose 3 mg/kg body weight, leads to: a) pronounced reduction of erythrocytes within 24 hours; b) initial reduction, and considerable increase, from the 3rd day thereafter, of reticulocytes.

2. The effect of higher doses US — 0.2, 0.3 wats/cm² against the background of reserpine action, increases the number of erythrocytes 1 hour after its application, with subsequently their number being maintained at a lower level than in the control animals.

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ВЛИЯНИЕ ПРЕДВАРИТЕЛЬНОГО РЕЗЕРПИНИЗИРОВАНИЯ НА ИЗМЕНЕНИЯ ЧИСЛА КРОВЯНЫХ КЛЕТОК ПОСЛЕ ПРИМЕНЕНИЯ НА КРЫСАХ УЛЬТРАЗВУКА

I. Изучение изменений числа эритроцитов

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РЕЗЮМЕ

Авторы занимались исследованием изменений числа эритроцитов после применения биологических доз ультразвука на фоне предварительного однократного резерпинизирования 3 мг/кг веса. Устанавливается отчетливое снижение числа эритроцитов через 24 часа после введения резерпина. Исследование ретикулоцитов у одной из групп показывает первоначальное уменьшение их числа, а через три дня — значительное увеличение. Применение ультразвука через 24 часа после введения резерпина вызывает снижение числа эритроцитов уже через час при самой слабой дозе и увеличение — при более высоких дозах. В следующие дни число эритроцитов, у озвученных более высокими дозами (0,2, 0,3 ватта/см²) животных остается несколько ниже по сравнению с таковыми у контрольных.