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

II. Investigation on the changes of leukocytes' number

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Although the infavourable influence of ultrasound (US) on the central and vegetative nerve system is well known, the mechanism of its action has not been clarified hitherto (1, 3, 7, 14, 24). The wide application of US in medicine and industry implies more comprehensive studies of the changes, taking place in the organism and elucidation of the mechanism of its action. This would make possible not only to determine with greater precision the indications and contraindication for US therapy, but also would cast additional light on the problem of preliminary selection of those working under conditions of US utilization in the production. The changes within the blood system appear to be of utmost importance — in particular the white blood cells, implementing a protective function in the organism. The literature data concerning the changes in the number of leukocytes following US application are contradictory (3, 6, 9, 14, 19, 23). In the present study, we set out to trace in experiment the changes in the number of leukocytes after application of biological doses US, against the background of a single-time injection of reserpine. It is a well known fact that reserpine leads to a depletion of the high-capacity intra-axonal catecholamine (KA) stores in the sympathetic neurons, and simultaneously interferes with KA binding (8, 11, 18, 21). All this, in the long run, renders difficult, for a certain period of time, the adrenergic transmission. Proceeding from the fact that US may stimulate or inhibit (depending on the dose and time) the biological processes, we assumed that this would allow for performing analysis of certain aspects of the mechanism of action of US upon the blood system, more particularly, on the number of leukocytes in the circulating blood. In the literature reviewed we failed to come across a similar approach to the problem.

Material and Method

The experiments were conducted on 64 healthy, male unbred albino rats, weighing 180—270 g, divided into 9 groups:

Group I — 8 rats, treated with US 0.1 wats/cm^2 — experimental group.

Group II — 8 rats, treated with 0.2 wats/cm^2 — experimental group.

Group III — 8 rats, untreated with US — control group of groups I and II.

Group IV — 9 rats, treated with 0.3 wats/cm² — experimental group.

Group V — 4 rats, untreated — control group. The animals of the end experimental groups alike were injected i. m. under sterile conditions, a single time, with 3 mg/kg weight «Serpasil» — Cibá» each, whereas US was applied 24 later to the experimental animals only. To determine the importance of the degree of KA intra-axonal depletion of the sympathetic neurons and the effect of US on the number of leukocytes, we studied further 4 groups of animals:

Group VI — 7 rats, treated with US 0.3 wats/cm², 5 hours after reserpine injection at identical dose — experimental group.

Group VII — 6 rats — control, untreated with US group, subjected to reserpine injection only.

Group VIII — 7 rats, treated with US 0.3 wats/cm², 10 hours after reserpine injection at identical dose — experimental group.

Group IX — 7 rats, untreated with US, control group, injected with reserpine.

Ultrasound, in all instances, was applied on the cervico-thoracal region, at 2 minutes duration of the session and experimental setting as described in earlier publications (6). The leukocytes were investigated twice prior to reserpine injection, before US, 1 hour after US treatment, at 1, 3, 5, 8 and 10 days, and thereafter at 5-day intervals until the 25th day. The method of blood obtaining and the technique of counting are described in a previous work (6). In addition, differential counting of the white blood cells was performed in the animals of group VIII, with a view to clarify the influence of reserpine on the number of the various types white blood cells. The effect of US on the differential blood picture has been studied previously (4).

Results and Discussion

All animals of the experimental groups showed considerable changes in the number of white blood cells 24 hours after the injection of reserpine — pronounced leukopenia, statistically reliable at $P<0.01$. Differences were established between the changes of white blood cells immediately after the US treatment, depending on the US dose applied.

At 0.1 wats/cm² dose of US, the leukocytes were reduced from $8938\pm\pm3973$ mm³ to 6450 ± 1310 after the injection of reserpine, and 1 hour after US treatment they were insignificantly increased — 6942 ± 1391 mm³, and 1 day thereafter they reached values close to the starting one. In the following days, their number was reduced with a maximum shown on the 5th day — 7093 ± 1990 (statistically reliable at $P<0.01$) and trereafter gradually returning to norm.

The changes of the white blood cells in animals treated with 0.2 wats/cm² US dose are analogical: from 16250 ± 4067 before the beginning of the experiment they are reduced to 8913 ± 3472 mm³ 24 hours after the reserpine injection, and 1 hour after the US treatment they reveal a tendency towards increase — 10350 ± 2890. Here the lowest value of the cells is re-

corded on the 3rd day — 7913±1553 mm³, while the normalization occurs, after some fluctuations, at 25 days. The leukocytes in the control group III from 11725±3750 mm³ are reduced, 24 hours after reserpine injection, to 7068±3720 mm³, and 1 hour thereafter do not show significant chan-

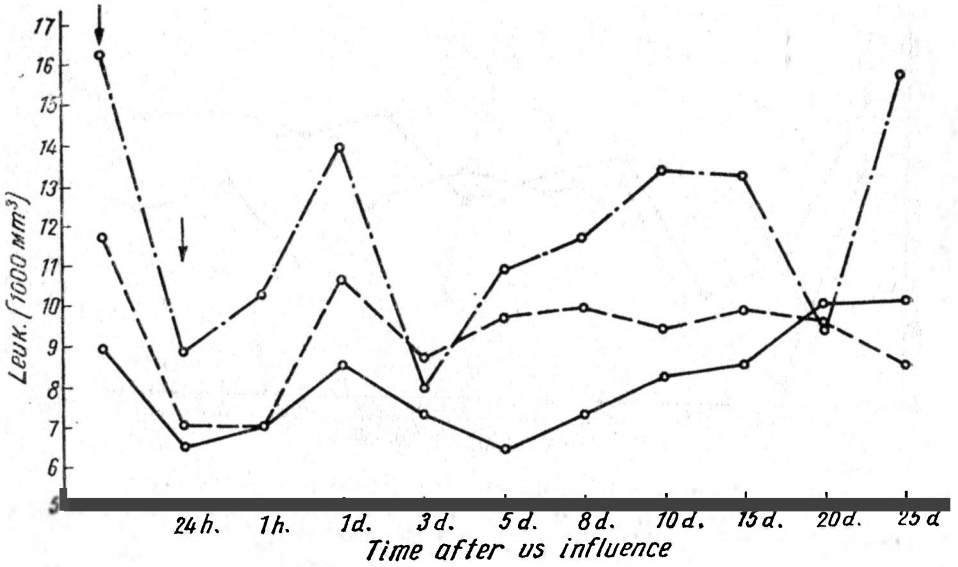

Fig. 1. Changes in the leukocytes of animals of groups I, II and III

The first arrow — reserpine injection. The second — US treatment of the experimental groups — I and II. Continuous curved line — the leukocytes of group I, treated with US at 0.1 watts/cm². The interrupted (with dots) curved line — the leukocytes of group II, treated with 0.2 watts US. The interrupted curved line — leukocytes of the control animals — group III untreated

ges. At the first day, their number approximates the starting value — 10637±3740 mm³, shows a slight reduction at 3 days — 8618±2920 mm³ (statistically unreliable and rises in the following days with fluctuations close to the normal values up to the end of the experiment. The results described are illustrated in Fig. 1.

Upon US treatment with the highest dose — 0.3 wats/cm² — shown in Fig. 2, the leukocytes in the experimental animals revealed the following changes: from 11758±2201 mm³, they were reduced to 7642±2301 mm³ 24 hrs after the injection of reserpine, and 1 hour after US treatment they did not rise as in groups I and II, but revealed a more substantial reduction — 6684±2220 mm³. Their number, after insignificant fluctuations up to the 5th day, was gradually increased, reaching the starting value after 25 days. In the control group of animals (V), reduction was not recorded of leukocytes during the investigation at 1 hour, but rather a tendency for increase — from 4593±1520 to 5443±2580 mm³. After the fifth day, the leukocytes in this group displayed lower values as compared to the experimental group, regaining their normal level at the end of the experiment.

It is evident from the above data that the number of white blood cells shows a considerable reduction 24 hrs after reserpine injection. We are prone to explain these changes in the number of leukocytes after the application of reserpine with the temporary impairment of the adrenergic trans-

mission, subsequent to the depletion of the high-capacity intra-axonal KA depots in the sympathetic neurons (8, 11, 18, 21), most probably, occurring in the blood-regulating neurons also. On the other hand, the increased parasympathetic tone is, in all likelihood, a contributing factor for the

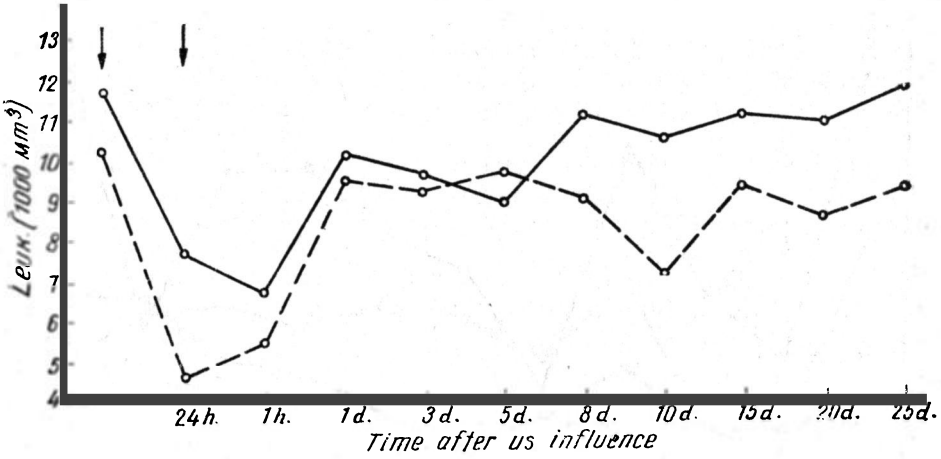

Fig. 2. Changes in the leukocytes of the animals of gr. IV and V
The continuous curved line — leukocytes of the experimental IV group — treated with 0.3 watts/cm² US. Interrupted curved line — leukocytes of the control group (V) animals, untreated. The arrow indications — the same

leukopenia. We know that reserpine liberates serotonin in the CNS, which being a CNS depressor, stimulates the central parasympathetic formations (11, 13, 22, 28). The processes referred to, in our opinion, substantiate the re-distribution of leukocytes, which in turn conditions the number of circulating cells during the first 24 hours after reserpine injection. After the 24th hour, the leukocytes in practically all untreated with US animals showed a tendency towards approximation, although with fluctuations, of the starting value.

Rather difficult for interpretation are the changes in the number of white blood cells, taking place after the application of US. The tendency for slight increase of leukocytes 1 hour after application low US doses (0.1 and 0.2 wats/cm²) may be explained by its stimulating effect at low and medium doses on the biological structures (1, 3, 10). At the highest US dose — 0.3 cm² — the number of leukocytes reveals a tendency for substantial decrease, evident in Figs. 2, 3 and 4. We believe that such a reduction might be explained by the sympathicolytic effect of US (1, 3, 27, 29). It is probable that the increased parasympathetic tone, acting synergetically on the sympathicolytic effect of US, contributes to a great extent to the occurrence of leukopenia. It is worth noticing that the reduction of leukocytes against the background of reserpine action, is much weaker at this particular US dose in comparison with the animals untreated with reserpine (4, 6). It is our impression that restoration of the white blood cells upon treatment with the highest US dose occurs comparatively early — at 8 days, whereas at lower doses it takes place rather later. It is possible

that during application of the highest US dose in our experiment (0.3 wats/cm²) additional KA amounts are being liberated by the intra-axonal stores of the blood-regulating sympathetic neurons. This is exactly the basis substantiating the occurrence of most of the reflexive effects of US, more par-

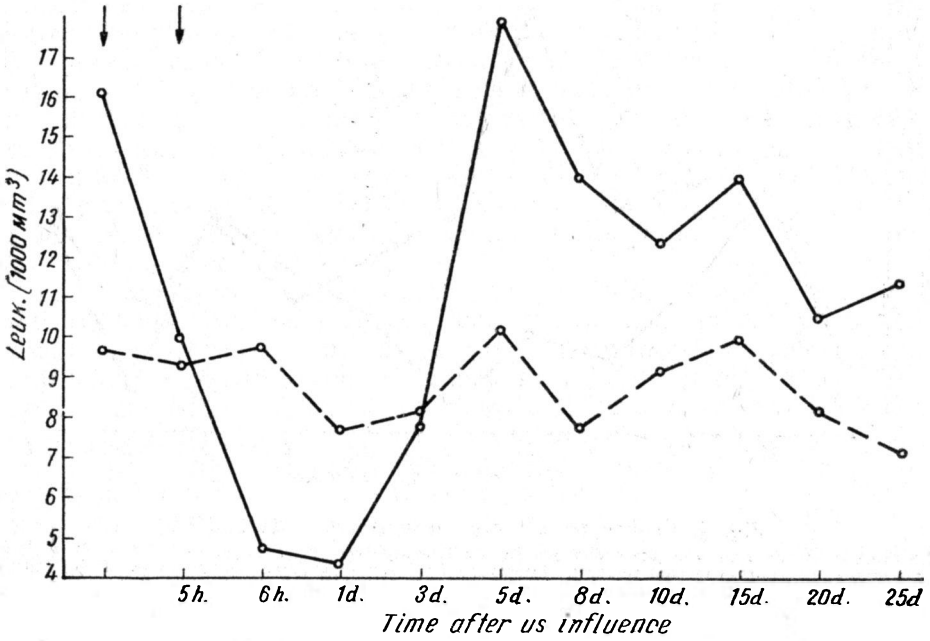

Fig. 3. The leukocytes in the animals of groups VI and VIII

The thick continuous curved line — changes in the number of leukocytes after US treatment with 0.3 watts/cm² at 5 hours after reserpine injection. The interrupted line — leukocytes of the control untreated with US VII group. The arrow indications — the same

ticularly, the re-distributional changes of the circulating leukocytes in the initial hours. The increase of KA in a number of tissue after US application is in support of the latter concept (1, 3, 20).

With the purpose to determine the influence of the degree of KA intra-axonal depletion of the sympathetic neurons on the number of leukocytes, they were investigated after US treatment at dose 0.3 wats/cm², 5 and 10 hours after reserpine injection (Figs. 3, 4). The following results were obtained: the single injection of reserpine at dose 3 mg/kg body weight (group VI) accounted for a statistically reliable ($p < 0.05$) reduction of leukocytes at 5 hours — from 16057 ± 2578 mm³ after 5 hours they fell to 9929 ± 2884 mm³, and 1 hour after US application their number was further reduced — 4743 ± 1236 mm³. On the following day the leukocytic number was still lower — 4300 ± 0.670 mm³, whilst at 5 days their values slightly exceeded the starting level. In the subsequent days and up to the end of the experiment, the leukocytic number revealed fluctuations beneath the starting value, being 11277 ± 6466 mm³ at 25 days. In the control (untreated with US) seventh group, 5 hours after injection of reserpine, the leukocytes showed a reduction, and 1 hour thereafter they were increased, while in the same time, their number in the experimental group disclosed a pronounced leukopenia.

The injection of reserpine and US application at identical dose, at 10 hours in group VIII (experimental) did not show a reduction of leukocytes, but rather intense leukocytosis (statistically reliable at $P > 0.05$) — from 6742± ±1535 mm³ they were increased on the 10th post-reserpine hour to 11157± ±3442 mm³, thereafter reaching the starting value at 1 day, showing a minimum at 5 days — 5657±1696 mm³ (statistically unreliable reduction), increasing in the following days with maximum on the 15th day — 10457± ±1828 mm³ and showing elevated values up to the end of the experiment — 10000±4299. In the control group (IX), the reserpine injection caused a lesser increase of the leukocytic number after 10 hours, and 1 hour thereafter their number was insignificantly reduced; at 1 day it showed values 10614±3837 mm³, with subsequent pronounced rise at 3 days — 15516± ±10720 mm³ (statistically unreliable), and up to the end of the experiment it revealed fluctuations, in some of the animals much exceeding the starting value.

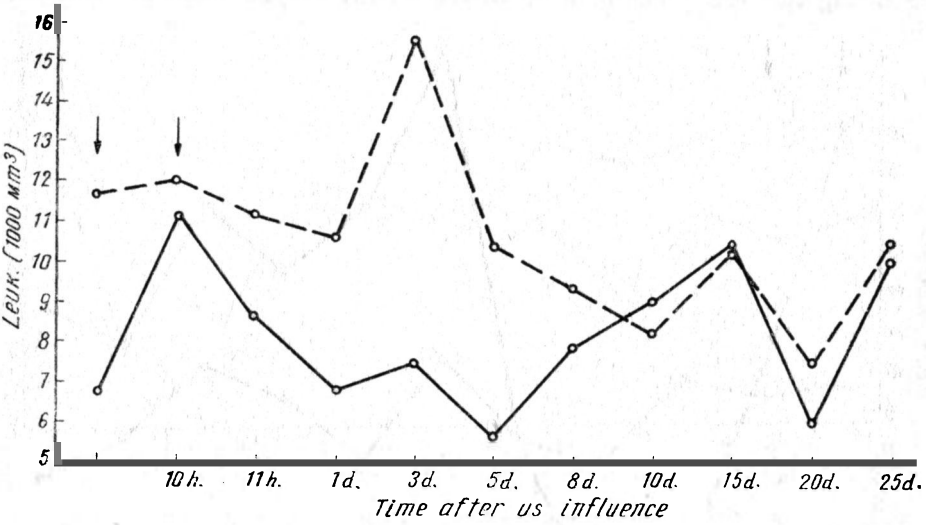

Fig. 4. Leukocytes of animals in groups VIII and IX

The thick continuous curved line— changes in the leukocytes of group VIII — US treated with 0.3 watts/cm² at 10 hours after the reserpine injection. The interrupted line — leukocytes of the control, untreated with US group. The arrow indications — the same

The comparative study of the results in the animals of the experimental groups, injected with reserpine and treated at various intervals with the highest US dose — 0.3 wats/cm² — at 5, 10 and 24 hours (Figs. 2, 3 and 4) shows that the degree of intra-axonal depletion of KA stores exerts a substantial influence on the US action as regards the number of white blood cells. The difference in the number of leukocytes involves **early** changes — in the initial hours and days — up to the 5th day, and **late** changes — after the 5th day and up to the end of the experiment. The US application 5 and 24 hours after reserpine injection leads to reduction of leukocytes 1 hour thereafter. The low leukocytic number is maintained in both

groups up to the 3—5th day. After that, their number is increased, fluctuating around the starting value, and, not infrequently, beneath the same. The US application 10 hours after reserpine injection similarly resulted in reduction of the leukocytic number at 1 hour, but their final value remained above the starting one, and was maintained up to the 5th day, when an insignificant decrease was revealed. After 5 days, the leukocytes were considerably increased, and in all the investigations, except for that at 20 days, they showed values above the starting level. The general impression was that the greatest reduction of leukocytes was recorded 1 hour after US when the latter is applied 5 hours after the commencement of KA intra-axonal depletion. Rather insignificant is this reduction at 1 hour, when US was applied 24 hours after the reserpine injection. In the latter case, a comparatively quicker increase of leukocytes occurs — as early as at 1 day their values are close to the starting, whereas in the former, the restoration is accomplished later — at 5 days.

The interpretation of the results submitted is difficult, the more so, that in the literature surveyed we did not come across similar reports, which renders impossible the comparative study. Nevertheless, we feel that there is sufficient reason to assume that they are substantiated by the influence of ultrasound effect exerted on the sympathetic neurons and their KA intra-axonal stores. The state of KA-depositing and liberating structures following reserpine injection is the most essential, according to US, and it is just the factor determining the variable effect exerted by US upon the number of white blood cells. The assumption of such an activity of US is justified by the facts proving that it increases the permeability of the membranes, produces microflows and microdisplacements within the cytoplasm and subcellular structures, activies certain enzymes, creates new active centers, in the membranes inclusive, whereas in higher doses it might account for morphological alterations and inhibition of a number of enzymes. Besides that, the US might bring about also to phasic changes — initially stimulating, and subsequently inhibiting a number of processes and vice versa (1, 2, 3, 10, 15, 16, 17, 26).

The analysis of the differential blood picture in the animals of group VIII, after the reserpine injection (Fig. 5), shows that (in absolute values): 10 hours after the reserpine injection absolute lymphopenia occurred (statistically reliable at $P<0.01$). The lymphocytes were normalized at 3 days and up to the end of the experiment maintained low values. The segmented nuclear neutrophilic granulocytes disclosed considerable increase at 10 hours (reliable at $P<0.001$), and after the 5th day revealed fluctuations beneath the starting value and normalization at 25 days. The stafflike granulocytes were increased at 10 hours (reliable at $P<0.01$). Thereafter, their number fluctuates but anyway, it is quite beneath the starting value up to the end of the experiment. The monocytes at 10 hours are insignificantly reduced, and at 1 day, they reach the starting value. At 3 days they show a secondary reduction (statistically reliable at $P<0.01$), whilst from the 5th to 20th day they are absent in the peripheral blood, reappearing only at 25 days, although in considerably reduced quantity. The changes in the eosinophils are of interest — 10 hours after reserpine injection they are reduced (statistically reliable at $P<0.02$), thereafter reaching the starting level and again showing a decrease at 5 days (statistical reliability

at $P<0.02$). In the following days they are closer to the starting value, and towards the end of the experiment disclose rather low values, with statistical reliability at $P<0.05$.

On the 3rd day after the reserpine injection, in the peripherial blood of some of the animals metamyelocytes and plasmatic cells were establi-

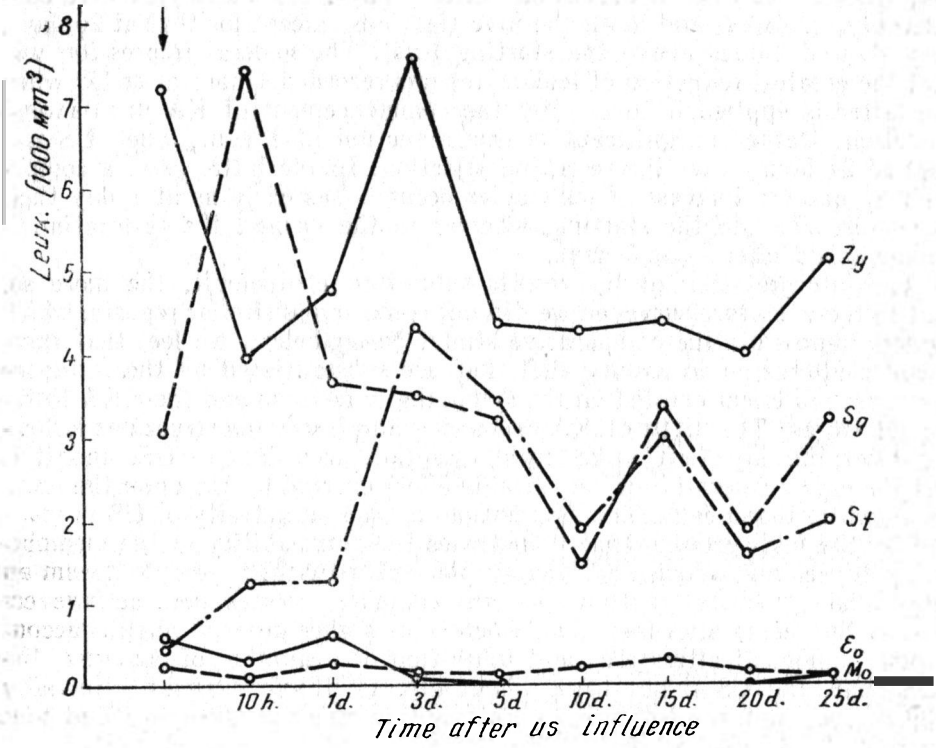

Fig. 5. Differential blood picture of the animals of group VIII — untreated with US, injected with reserpine

The leukocytes are submitted in absolute values. The arrow — reserpine injection

shed. The latter finding along with the increase of staff-like granulocytes might be considered as an expression of increased bone marrow activity, whereas the early changes in the number of lymphocytes and segmentonuclear granulocytes are assumed as an expression of re-distributional mechanism. Of course, further bone marrow studies are needed, which will be the subject of future publications.

The data submitted in the present work are in accordance with our earlier investigations, where we established an increase of reticulocytes after reserpine injection (5). It is by no means ruled out that in the above processes, parallel to the temporary desympathetization, the parasympatheticus also takes part. It is a well known fact that the parasympathetic nerve stimulates the proliferative processes within the bone marrow (25). It is possible that the enhanced parasympathetic tone under the circumstances existing accounts for further intensification of the cellular proliferation of certain derivatives of the myelogenous row in the bone marrow.

Inferences

1. The single time reserpine injection, at dose 3 mg/kg body weight, in albino rats leads to reduction of the leukocytes after 5 and 24 hours, and to an increase after 10 hours.

2. The application of the ultrasound, at doses 0.1 and 0.2 watts/cm², 24 hours after reserpinization accounts for slight increase of the number of leukocytes at 1 hour, whilst at dose 0.3 watts/cm², their number shows a substantial fall.

3. The US treatment at dose 0.3 watts/cm² at 5, 10 and 24 hours after reserpine injection produces a reduction of leukocytes 1 hour after the US treatment, most significant at 5 hours, whilst their values in the following days, with US treatment on the 10th hour after the reserpine injection, are maintained at a substantially higher level as compared to the US treatment at 5 and 24 hours, i. e. the degree of KA intra-axonal depletion of the sympathetic neurons has an essential bearing on the regulation of the number of circulating white blood cells under the specific conditions.

4. The injection of reserpine produces absolute lymphopenia, eosinopenia and monocytopenia within 10 hours whereas the segmented nuclear granulocytes and the staff-like cells are increased.

5. The changes in the number of leukocytes after US treatment are explained by the stimulating, and in some instances, inhibitory effect of ultrasound exerted upon the sympathetic neurons, assumed as developing on the basis of changes in the intra-axonal KA.

6. A simultaneous influence on the parasympathetic nerve is also considered, since it increases its tone after the reserpine injection, and most probably, also after treatment with higher ultrasound doses.

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ВЛИЯНИЕ ПРЕДВАРИТЕЛЬНОГО РЕЗЕРПИНИЗИРОВАНИЯ НА ИЗМЕНЕНИЕ ЧИСЛА КРОВЯНЫХ КЛЕТОК ПОСЛЕ ПРИМЕНЕНИЯ НА БЕЛЫХ КРЫСАХ УЛЬТРАЗВУКА

II. Изучение изменения числа лейкоцитов

Т. Ганчев, Д. Георгиева

РЕЗЮМЕ

Авторы изучают действие ультразвука на число лейкоцитов у крыс, получавших однократно резерпин. Ультразвук используется через 5, 10 и 24 часа после инъекции резерпина. Устанавливается значительное снижение числа белых кровяных клеток через 5 и 24 часа после введения резерпина, в то время как к 10-му часу отмечается их увеличение. Озвучение 0,1 и 0,2 ватта/см² через 24 часа после введения резерпина вызывает слабое увеличение лейкоцитов через 1 час, а при дозе 0,3 ватта/см² число их уменьшается. Озвучение дозой 0,3 ватта/см² на 5-м, 10-м и 24-м часу после введения резерпина вызывает снижение лейкоцитов уже через час, причем оно наиболее выражено на следующий день при озвучении на 5-ом часу. Лейкоциты при озвучении на 10-ом часу у животных остаются несколько выше чем у животных, озвученных на 5-ом и 24-ом часу. Введение резерпина в дозе 3 мг/кг веса к 10-ому часу приводит к снижению лимфоцитов, эозинофилов и моноцитов. Сегментоядерные гранулоциты и палочкоядерные имеют максимальные величины на 3-ий день, когда у некоторых животных появляются метамиэлоциты и плазматические клетки. Описанные изменения объясняются действием резерпина и ультразвука на симпатикусовые нейроны.