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TRANSLATIONS FROM MEDITSINSKAYA RADIOLGIYA

(Medical Radiology)

- USSR -

No. 11, 1962
FOREWORD

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ORGANIZATION OF WORK AND NORMS FOR THE WORK-LOAD

OF PERSONNEL ENGAGED IN THE DIAGNOSIS AND TREATMENT

OF DISEASES OF THE THYROID GLAND BY RADIOACTIVE IODINE

The Department of Roentgenology and Radiology (Chairman -- Docent A. P. Burkalov) of the Kishinev Medical Institute and Roentgeno-Radiological Center of the Moldavian SSR Clinical Hospital


Radioactive iodine (I\textsuperscript{131}) has occupied an important place in the diagnosis and treatment of diseases of the thyroid gland. This was facilitated by good diagnostics, positive therapy and simplicity of application (A. A. Atabek, M. M. Draznkin). In addition, the possibility of utilizing \( I\text{\textsuperscript{131}} \) is tied to the presence of isotope laboratories, organized and equipped in accordance with effective sanitation regulations.

Due to the prevalence of thyrotoxicosis in Moldavia, inter-regional anti-goiter dispensaries with radiological sections for applying radioactive iodine are now being organized in various regions of the republic. In this connection, the importance of organizing work in the isotope laboratory is growing in order to ensure maximum productivity with a minimum of work-connected injuries (S. M. Gorodinskii, V. T. Gusev).

The purpose of the present work is to thoroughly analyze the organization of labor and norms for the work load of personnel at the Isotope Laboratory of the Moldavian SSR Clinical Hospital.

The Isotope Laboratory of the Moldavian SSR Clinical Hospital is not typical, although it satisfies all the requirements of an institution of this type. Isotope laboratories to be organized in the republic in the near future will also be located in suitable buildings which differ one from another. Therefore, we are in no way making generalizations out of our conclusions. However, in view of the rather large volume of work completed in four years (over 12,000 diagnoses and 1,100 treatments), a familiarization with our organizational experience may be of some interest, especially in view of the light treatment of this problem in the literature.
The Isotope Laboratory of the Republic's Clinical Hospital consists of a storehouse, a washroom, a packing room, a treatment room, a waiting room for patients and a radiometric room. The annual expenditure of I131 is 3,600 mc (200 mc monthly). The isotope laboratory conducts diagnoses and treatment of patients with thyrotoxicosis. The following time intervals are used to measure thyroid absorption: 2, 3 and 24 hours after administration. The patients take internally an indicator quantity of I131 during the first two hours of the laboratory's work period and a subsequent radiometry after four hours. This coincides with the established six-hour work day. Treatments are conducted on the days when radioactive iodine are given (two to three times a month).

In calculating the isotope laboratory's capacity in diagnostics, one must consider the radiometric continuity of each patient for ensuring reliable results. As is known, a decrease in the count results in a reduction of the accuracy in measuring activity, characterized by a mean quadratic error (V. I. Spitsyn and co-authors). The determination of the measuring time is essential to obtaining the required degree of accuracy, and is made by using the formula:

$$t_{A+\phi} = \frac{J_{A+\phi} + \sqrt{J_{A+\phi} J_{\phi}}}{\sigma_{A} \sigma_{\phi}}$$

where $J_{A+\phi}$ is the activity of measured subject with background; $J_{\phi}$ is a background; $J_A$ is the activity of measured subject without background; $\sigma$ is the relative quadratic error of the activity measurement.

Below is a table of measuring times at a degree of accuracy of 0.05 (i.e., 5%) for various meanings for $J_{A+\phi}$ at $J_{\phi} = 40$ impulses/min (Table 1).

<table>
<thead>
<tr>
<th>$J_{A+\phi}$</th>
<th>$J_{\phi}$</th>
<th>$t_{A+\phi}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>imp/min</td>
<td>imp/min</td>
<td>min</td>
</tr>
<tr>
<td>75</td>
<td>40</td>
<td>0.05</td>
</tr>
<tr>
<td>100</td>
<td>40</td>
<td>0.05</td>
</tr>
<tr>
<td>150</td>
<td>40</td>
<td>0.05</td>
</tr>
<tr>
<td>200</td>
<td>40</td>
<td>0.05</td>
</tr>
<tr>
<td>250</td>
<td>40</td>
<td>0.05</td>
</tr>
<tr>
<td>300</td>
<td>40</td>
<td>0.05</td>
</tr>
<tr>
<td>350</td>
<td>40</td>
<td>0.05</td>
</tr>
<tr>
<td>400</td>
<td>40</td>
<td>0.05</td>
</tr>
<tr>
<td>500</td>
<td>40</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Upon using 3 mc of I131 under our conditions and measuring the thyroid gland on the B-2 or DP-100 counters with gas-charged counters, the count of impulses with background two hours after administration
was on the average between 150 and 250 (including measurements of from 400-500 up to 75 imp/min). As can be seen from Table 1, the optimal count continuity is from three to eight minutes, and on the average five or six minutes. Along with calculating the time needed for preparing the apparatus, calculating the background and standard, and deducting the absorption percent, one must consider that the work capacity of one radiometric instrument is 15 to 16 persons per working day. Any attempt to increase the laboratory's work capacity by reducing the measuring time of each patient inevitably leads to an increase in the relative error and a reduction in the reliability of the results obtained.

The widespread use of scintillation counters with a high recording efficiency of gamma-quanta makes it possible to reduce the indicator dose and decrease the count time without a loss of reliability. The most correct way of ensuring uniform reliability in radiometry is to calculate the time necessary for recording a given number of impulses.

In determining the norms of the personnel's work load, is it essential to consider the degree of irradiation incurred while carrying out diagnostic procedures? We estimated the radiation level at locations where the personnel were working at various stages of their work. For this purpose we used RK 0,1 microroentgenometer.

During the work in the radiometric room the only source of irradiation is the indicator portion of I\textsubscript{131} (in this case 3 mc) taken internally by the patient or found in the so-called standard. Dosimetric checks conducted by us show that the radiation level in direct proximity to the person internally receiving 3 mc of I\textsubscript{131} is lower than the instrument's sensitivity threshold. A measurement of the standard is likewise unrelated to any important external irradiation. In this way, the work in the radiometric room is practically safe.

The radioactive background is higher in other locations of the laboratory: 0.4 mr/sec in the storehouse (the irradiation dose reaches the daily maximum allowance in 12 hours) and 0.1 mr/sec in the remaining locations (the daily maximum dose is not reached). A chronometry showed that a laboratory worker is in a general complex for not more than 1 1/2 to 2 minutes, including one minute at the open safe, in the packing room for one hour and 53 minutes including five minutes of direct contact with I\textsubscript{131} (at 15 to 20 second per dose), five minutes in the treatment room of which 1 1/2 minutes is spent holding the container with I\textsubscript{131} (5 to 7 seconds per patient). In working with an indicator solution (total activity of the I\textsubscript{131} solution is 50 mc) the transfer of the iodine from the storeroom to the radio-handling table with the aid of remote instrumentation, and the pouring of the iodine with the aid of a remote forceps and pipette 30 cm long for transfer to the patient does not give a significant general or local irradiation (maximum strength of the dose is 0.1 mr/sec). Thus, the personnel's load in diagnosing is determined only by the time necessary for counting to ensure the essential reliability.
On the days when isotopes are administered and therapy is given, the radiation levels are markedly increased (Table 2).

Table 2

<table>
<thead>
<tr>
<th>Work Stage</th>
<th>General Irradiation</th>
<th>On the Hands</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum Daily Dose,</td>
<td>Maximum Daily Dose,</td>
</tr>
<tr>
<td></td>
<td>17,000 mr</td>
<td>63,000 mr</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dosage Strength, Time, Dose,</th>
<th>Irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose, mr/sec, seconds</td>
<td></td>
</tr>
</tbody>
</table>

| Extraction and transfer of one ampule with 25 mc from the container to the radio-handling table | 4 | 10 | 40 |
| Opening one ampule | 0.2 | 60 | 12 |
| Pouring out the first therapeutic dose, in 4-5 mc | 0.2 | 20 | 4 |
| Pouring out the last therapeutic dose in 4-5 mc | <0.1 | 20 | <2 |
| Transferring one therapeutic dose of 4-5 mc to the patient | 0.4 | 7 | 3 |

As can be seen from Table 2, the irradiation dose from pouring one ampule with 25 mc and administering therapeutic doses of 4 to 5 mc to 5 to 6 patients is generally equal to approximately 88 mr, i.e., 1/190 of the maximum allowed dose. Under these conditions, the irradiation dose of the hands is noticeably greater, approximately 7,260 mr, but is still only 1/11 of the maximum allowed dose. One must once again be reminded that the radiation levels presented by us were...
measures under conditions where protective and remote instrumentation was utilized. It is sufficient to say that without a remote pincette or in direct contact with an ampule containing 25 mc of $^{113}$I, the radiation level is 3,500 mr/sec (instead of 6) and the maximum allowable dose is reached in 23 seconds instead of three hours and 50 minutes. Therefore, great importance is acquired by individual dosimetric control, which reflects the factual dose of personnel irradiation, taking into account all possible errors and carelessness.

The radiation dosage strengths obtained by us are significantly lower than the calculated data presented by D. A. Ulitovskiy. This is explained by the fact that our investigations were conducted under specific concrete conditions. We consider that the dosimetric measurement more reliably reflects the radiation levels than tables which were calculated without relevance to a concrete situation.

Thus, the work load in pouring and administering therapy in each isotope laboratory must be estimated individually depending on the available protective equipment and remote instrumentation whose application reduces the radiation level to practically safe magnitudes.

The following order of work was adopted by us on the basis of calculating the radiation level in administering therapy and the essential duration of radiometry in diagnosis:

- 8 hours to 8 hours 30 minutes — preparation of the apparatus; measuring background and standard
- 8 hours 30 minutes to 10 hours — radiometry of patients 24 hours after administration ($^{113}$I taken night before)
- 10 hours to 10 hours 30 minutes — measuring the background and new standard
- 10 hours 30 minutes to 14 hours — radiometry of patients 2 to 4 hours after administration; cleaning premises, dosimetric control

Two laboratory workers operate each working counter: one for pouring, and the other for radiometry. Upon completion of the pouring, he helps the first technician calculate the percent of thyroid absorption. One technician is essential for each supplementary counter.

It is expedient to receive $^{113}$I once in ten days. Thus, therapeutic doses are administered in the course of two to three days every ten-day period. After pouring therapeutic doses, the laboratory technician works in the radiometric room for the remaining seven to eight days of the ten-day period, while his place is taken by the second technician — an alternate intake of $^{113}$I.
As was emphasized above, the cited descriptions ensue from our specific conditions and cannot be mechanically transferred to other isotope laboratories. The development of unified organizational methods of work and personnel norms in radiological sections can be made possible by developing nets of typical isotope laboratories, applying uniform protective equipment and remote instrumentation, and unifying the methodology of the diagnostic and therapeutic utilization of $^{131}$I.

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FIRST AID IN RADIOACTIVE IODINE POISONING

Supervisor  --  Corresponding Member of the Academy of Medical Sciences USSR, Professor V. A. Sanotskiy


A number of works has recently appeared in the literature which deals with the protective action of several preparations (6-methylthiouracil, BAL, iodides) in radioactive iodine (I131) poisoning of organisms due to overdoses. Several authors (L. A. Kashchenko) point out that stable iodine is a reliable prophylactic from the protective viewpoint, but is not at all a means for releasing I131 from the thyroid gland. Others (Ya. M. Kabak, I. N. Tal'skaya) consider that stable iodine and 6-methylthiouracil can have a protective action not only in precautionary but therapeutic (one hour after I131 poisoning) applications.

The present paper presents the results of experiments in investigating means for reducing the accumulation level of I131 in an organism as a first aid measure. The widespread use of I131 in therapeutic and diagnostic practice, and the industrial preparation of this and other radioactive isotopes of iodine demands an investigation of prophylactic and therapeutic means for the purposes indicated above.

The application of various anion exchange resins, permutite and other preparations used for distilling potable water, warrants interest for the prevention of iodine absorption from the digestive tract. The literature takes particular note of the increased adsorptive properties (relative to I131) of preparations such as activated charcoal or kaolin when mixed with trade amounts of silver. Analysis of chemical properties would indicate a similar action with salts of copper and mercury. There is even more data on the effect of various substances on thyroid gland function. Many preparations have been proposed for blocking the synthesis of thyroid hormone, such as thiourea, bromides, iodides, perchlorates, methylthiouracil, mercazolyl, cyanates, thiocyanates, resorcin, sulfidine and other sulfanilamide preparations, many of which are being used to treat diseases of the thyroid gland (M. N. Fateyeva, Ya. M. Kabak and I. B. Simon). From the first aid standpoint it was
necessary to check these preparations in order to establish possible doses and length of treatment necessary to ensure a proper effect.

The experiments were performed on rats and dogs. $^{1131}$I was administered into the stomachs of the rats with a needle-head catheter (as an aqueous solution), and the dogs were fed a sausage containing $^{1131}$I.

The radioactivity of the organs and tissues, as well as of the excretions was determined on beta-counters in comparison to a standard preparation of $^{1131}$I. In addition, gamma-irradiation from the thyroid gland were measured in vivo by a DP-11-B radiometer and a scintillation counter with a lead collimator. For the purpose of a primary approbation, the effectiveness of seventeen different substances was tested on 140 rats by intra-gastric or subcutaneous injections immediately following the administration of 4 mc of $^{1131}$I per rat. The results are presented in Table 1.

The data cited in Table 1 show that a particularly high degree of action is possessed by mercazolyl, 6-methylthiouracil, potassium iodide, Sajodin [calcium monoiodobenenate], silver iodide, silver bromide and calomel (3-7% of the control, i.e., 10-20 times lower). A noticeable effect was shown by sodium perchlorate, mercamine (betamercaptoethylamine hydrochloride) at a dose of 100 mg/kg (1 ml of a 2% solution per rat weight of 200 g) -- 13.6% of the control, i.e., almost an even reduction, and cystamine (18.2% of the control).

Table 1. Effectiveness of Thyrostatic and Radioprotective Preparations

<table>
<thead>
<tr>
<th>Name of Preparation</th>
<th>Dose Per Rat (in ml)</th>
<th>Method of Administration</th>
<th>Radioactivity of the Thyroid Gland (percent of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-methylthiouracil</td>
<td>2 (1%) Subcutaneous</td>
<td>3.0</td>
<td>1.9- 3.8</td>
</tr>
<tr>
<td></td>
<td>2 (1%) Gastric</td>
<td>16.1</td>
<td>13.7-18.5</td>
</tr>
<tr>
<td>Mercazolyl</td>
<td>1 (0.1%) Subcutaneous</td>
<td>10.7</td>
<td>9.6-12.7</td>
</tr>
<tr>
<td>6-methylthiouracil</td>
<td>2 (2%) Subcutaneous</td>
<td>25.7</td>
<td>25.6-25.8</td>
</tr>
<tr>
<td>Thiourea</td>
<td>2 (2%) Intra-peritoneal</td>
<td>11.6</td>
<td>9.4-13.8</td>
</tr>
<tr>
<td>Resorcin</td>
<td>1 (2%) Subcutaneous</td>
<td>13.6</td>
<td>11.9-15.2</td>
</tr>
<tr>
<td></td>
<td>1 (1%) Subcutaneous</td>
<td>41.6</td>
<td>35.3-51.1</td>
</tr>
<tr>
<td>Mercamime</td>
<td>1 (2%) Gastric</td>
<td>18.2</td>
<td>15.0-23.7</td>
</tr>
<tr>
<td>Cystamine</td>
<td>1 (2%) Gastric</td>
<td>21.5</td>
<td>15.2-27.3</td>
</tr>
<tr>
<td>KI</td>
<td>2 (0.1%) Subcutaneous</td>
<td>7.5</td>
<td>3.1-11.1</td>
</tr>
<tr>
<td>Sajodin</td>
<td>2 (0.1%) Subcutaneous</td>
<td>10.2</td>
<td>6.5-13.9</td>
</tr>
<tr>
<td>Potassium thiocyanate</td>
<td>1 (3%) Subcutaneous</td>
<td>18.2</td>
<td>13.4-22.8</td>
</tr>
<tr>
<td>Ammonium thiocyanate</td>
<td>1 (3%) Subcutaneous</td>
<td>17.1</td>
<td>12.6-20.6</td>
</tr>
</tbody>
</table>
The use of mercamine, cystamine (or their combination with more effective thyrostatic substances) warrants attention, since these preparations simultaneously afford protection against irradiation.

The following two series of experiments on 48 rats clarified the importance of dosage and particularly the duration of treatment with substances which block the accumulation of I\textsuperscript{131} in the thyroid gland.

The first series showed that only comparatively high (1-0.1 mg per rat) doses of iodides give the necessary effect. Smaller doses (0.01 mg per rat), on the contrary, increase the level of I\textsuperscript{131} resorption by the thyroid gland, i.e., here there is a two-phased action as is often observed in pharmacology (Table 2).

Table 2. 1.835 \cdot 10^{-4} \text{ mc of I}^{131} \text{ Administered Per Rat (average data obtained on the basis of examining three rats -- examination after five days)}

<table>
<thead>
<tr>
<th>Experimental Variant</th>
<th>Dose of KI Per Rat (in mg)</th>
<th>Activity (in mc \cdot 10^{-4})</th>
<th>Percent of the Thyroid Gland (in mc • of control)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (infected rats without treatment)</td>
<td>--</td>
<td>855.5</td>
<td>100.0</td>
<td>--</td>
</tr>
<tr>
<td>KI immediately after infection</td>
<td>0.01</td>
<td>1,191.3</td>
<td>139.2</td>
<td>107-174</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>184.4</td>
<td>21.6</td>
<td>15.2-27.3</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>45.3</td>
<td>5.3</td>
<td>4.0-6.0</td>
</tr>
<tr>
<td>KI three days before infection</td>
<td>1.0</td>
<td>346.6</td>
<td>40.5</td>
<td>35.4-43.6</td>
</tr>
</tbody>
</table>
As can be seen from Table 2, potassium iodide is effective in prophylactic and therapeutic applications, by accelerating the removal of $^{131}I$ even two hours after exposure when the latter is already partially fixed by the thyroid gland.

In the second series of experiments, treatments were administered after infecting the rats with $^{131}I$ (dose $= 6,240 \times 10^{-4}$ mc) in one variant immediately after exposure, and in another four hours after exposure. The rats were killed after 45 hours for comparing effectiveness; a part of the control rats was killed after four hours and 27 hours in order to have some idea of the accumulation level of $^{131}I$ in the thyroid gland during this period.

As is clearly seen from Table 3, potassium iodide and 6-methylthiouracil make it possible to accelerate the removal of $^{131}I$ from the thyroid gland soon after its deposition, i.e., these substances have both a prophylactic and therapeutic significance. A special balanced experiment on exchange cells showed that the blocking of $^{131}I$ accumulation in the thyroid gland results in the latter's non-accumulation in other organs and tissues, but its rapid removal from the organism, primarily via the urine (Table 4).

In practicality, in spite of the complete cessation of $^{131}I$ fixation in the thyroid gland during the application of the therapeutic preparations, the $^{131}I$ fixation in the remaining organs and tissues not only did not increase in comparison with the control, but on the contrary, decreased. The entire amount of the unfixed $^{131}I$ was removed from the organism in the first two days; the removal of $^{131}I$ increased from 10.7 to 75-87%. Hence it is once more apparent that it is important to simultaneously apply protective preparations of the cystamine or mercamine type. As is known from the pharmacodynamics of these preparations, they are removed from the organism primarily through the kidneys. Therefore, one may expect a particularly high degree of protection from cystamine or mercamine, namely for this organ during the intensive removal of $^{131}I$ (as well as from other radioactive isotopes eliminated through the kidneys).
Table 3. 6.240 $\times 10^{-14}$ mc of $^{131}$I Administered Per Rat (average data obtained on the basis of examining three rats)

<table>
<thead>
<tr>
<th>Experimental Variant</th>
<th>Thyroid Gland Activity (in mc $\times 10^{-4}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 hours after infection</td>
</tr>
<tr>
<td>Control (only $^{131}$I)</td>
<td>3,085</td>
</tr>
<tr>
<td>Treatment with KI immediately after infection</td>
<td>--</td>
</tr>
<tr>
<td>Treatment with KI 4 hours after infection</td>
<td>3,085</td>
</tr>
<tr>
<td>Treatment with methylthiouracil immediately after infection</td>
<td>--</td>
</tr>
<tr>
<td>Treatment with methylthiouracil 4 hours after infection</td>
<td>3,085</td>
</tr>
</tbody>
</table>

Table 4. A Balance Investigation of $^{131}$I Dynamics in the Organism. Gastric Administration of 61 $\times 10^{-2}$ mc of $^{131}$I (average data obtained on the basis of examining three animals)

<table>
<thead>
<tr>
<th>Examination Variant</th>
<th>Activity (in mc $\times 10^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Total found in the organism and excretions</td>
<td>57.94</td>
</tr>
<tr>
<td>Including:</td>
<td></td>
</tr>
<tr>
<td>In the thyroid gland</td>
<td>30.30</td>
</tr>
<tr>
<td>In the remaining organs and tissues</td>
<td>21.4</td>
</tr>
<tr>
<td>Removed in 48 hours</td>
<td></td>
</tr>
<tr>
<td>From the urine</td>
<td>5.45</td>
</tr>
<tr>
<td>From the feces</td>
<td>0.79</td>
</tr>
<tr>
<td>Total</td>
<td>6.24</td>
</tr>
<tr>
<td>Percent</td>
<td>10.74</td>
</tr>
</tbody>
</table>
The following experiments on dogs confirmed the effectiveness of several of the experimental preparations (KI, sulfidine, ammonium thiocyanate) as first aid measures. Table 5 shows the results of experiments on 33 dogs using potassium iodide.

Table 5. Reduction of the Thyroid Gland Radioactivity in Dogs After KI Treatment (0.05 g per treatment). 46 mc/kg I\textsuperscript{131} Were Administered (average data obtained on the basis of examining three dogs).

<table>
<thead>
<tr>
<th>Experimental Variant</th>
<th>Food With Infecting I\textsuperscript{131}</th>
<th>Water With Infecting I\textsuperscript{131}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2,159.0 100</td>
<td>2,381.6 100</td>
</tr>
<tr>
<td>KI treatment:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time after exposure to I\textsuperscript{131} -- 15 minutes</td>
<td>122.8 5.7</td>
<td>232.3 9.75</td>
</tr>
<tr>
<td>-- one hour</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>-- three hours</td>
<td>177.7 8.24</td>
<td>974.6 40.9</td>
</tr>
<tr>
<td>-- six hours</td>
<td>950.6 44.1</td>
<td>--</td>
</tr>
</tbody>
</table>

As the obtained results show, soon after infection (1-3 hours) it is possible to acquire a sufficiently good effect. Naturally, later on, the effect worsens and here it becomes essential to adopt a specific course of treatment, since a single 0.05 g dose of KI per dog is insufficient to rapidly decrease the amount of I\textsuperscript{131} in the organism.

Table 6 shows the data of an experiment on six dogs where such a course of treatment is taken (a triple administration of potassium iodide, 15 minutes, three hours and one day after infection). 54 mc/kg of I\textsuperscript{131} were administered. The effect was determined by a scintillation counter by means of in vivo measurements of thyroid gland radioactivity.

By the 96th hour the difference between the treated and control animals (according to the thyroid gland activity) was more than a hundred fold.

Thus, the known thyrostatic substances (mercazolyl, 6-methylthiouracil, thiocyanates, perchlorates, thiourea, resorcin), organic (Sajodin) and inorganic compounds of iodine (KI, AgI, AgBr), certain sulfanilamides (sulfodimezine), and radio-protective substances -- mercamine, cystamine, demonstrate a high degree of efficacy as a first
aid measure in I\textsuperscript{131} poisoning, by sharply reducing the deposition of this isotope in the thyroid gland and accelerating its elimination from the organism.

### Table 6. The Results of Treating Dogs Receiving 54 mc/kg I\textsuperscript{131} with Potassium Iodide

<table>
<thead>
<tr>
<th>Experimental Variant</th>
<th>Weight of Dog (in kg)</th>
<th>Activity of Administered I\textsuperscript{131} (in mc)</th>
<th>Activity of Thyroid Glands, in % of Administered Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.8</td>
<td>1,248</td>
<td>23.0, 43.9, 34.8</td>
</tr>
<tr>
<td>I\textsuperscript{131} infection</td>
<td>9.8, 17.0</td>
<td>543, 930</td>
<td>11.7, 54.0, 40.0</td>
</tr>
<tr>
<td>Treatment with KI</td>
<td>10.1, 13.0</td>
<td>485, 710</td>
<td>7.1, 5.9, 0.2</td>
</tr>
<tr>
<td>(three times, each 0.05 g)</td>
<td>15.5, 17.0</td>
<td>847, 930</td>
<td>5.4, 6.6, 0.6</td>
</tr>
</tbody>
</table>

One may recommend the use of KI or Sajodin in ordinary therapeutic doses (0.1-0.2 g per treatment) both as a prophylaxis and a first aid measure in I\textsuperscript{131} poisoning. To a certain extent, the use of mercamime (betamercaptoethylamine hydrochloride) and cystamine, thio-cyanate compounds and perchlorates may also be recommended. The preparations must be administered as soon as possible following the appearance of infection in order to minimize the degree of thyroid gland irradiation. As far as mercazolyl and 6-methylthiouracil are concerned, in spite of their great effectiveness in reducing the deposition of I\textsuperscript{131} in the thyroid gland, their recommendation should be reserved until additional experimental clinical investigations can be conducted. As is known, these compounds, particularly 6-methylthiouracil, cause distinct side reactions, especially leukopenia, agranulocytosis which are extremely undesirable in radiation sickness during infections by radioactive isotopes. These preparations are especially contraindicated for single applications for the purpose of first aid, but are rather for subsequent therapy in the form of a prolonged course of treatment.

Calomel warrants attention for the prevention of I\textsuperscript{131} absorption from the digestive tract. When indicating this purgative in poisoning by radioactive isotopes of iodine, one must consider the incompatibility of calomel and iodide salts. One must also refrain from using acid or salted foods in these cases.


6289

CSO: 8346-D
CHANGES IN PERIPHERAL BLOOD DURING RADIATION THERAPY

From the scientific-polyclinical section (Ye. M. Kagan) and the hematology laboratory (Ye. V. Karibskaya) of the State Scientific-Research Roentgenoradiological Institute of the Ministry of Health RSFSR.

Following is a translation of an article by Ye. V. Karibskaya and T. E. Matetskaya in the Russian-language periodical Meditsinskaya radiolonia (Medical Radiology), Vol. VII, No. 11, 1962, pages 39-45.

In the present work we assumed the task of studying changes in the peripheral blood of patients undergoing radiation therapy for various diseases. The blood of persons not undergoing radiation was examined for comparative purposes. We proposed a study of observable blood changes in daily dispensary work. We were primarily interested in the quantitative correlation between different cellular groups and the qualitative changes in the nuclei and protoplasma in patients during radiation therapy. To do this, we examined the amount of hemoglobin, the number of erythrocytes, the erythrocyte sedimentation reaction, the total number of leucocytes and a qualitative evaluation of a hemogram. The majority of the patients were observed dynamically, according to the course of the radiation therapy, and subsequently during return visits for examination.

One hundred and forty eight persons were under observation and underwent radiation therapy for the following maladies: malignant neoplasms in the lungs -- 31 persons, malignant neoplasms of the esophagus and stomach -- 21, malignant neoplasms of the mammary glands -- 5, of the genitalia -- 7, of the thyroid gland -- 4, of the rectum -- 12, dermal melanoma -- 6, lymphogranulomatosis of the lungs and mediastinum -- 13, leukemia, primarily lympholeucosis -- 4, erythremia -- 6, ostiosarcoma -- 13, malignant neoplasms of the cheeks, neck and upper jaws -- 16, non-tumorous maladies -- 10 persons.

As a rule, the patients undergoing radiation were given vitamins and hemo-stimulators during the course of treatment:

An analysis of our observations shows that specific changes appear in the peripheral blood as a result of radiation. The responsive reaction to penetrating radiation is of a singular character and independent of the form and character of radiation. First of all, one
must take note of the fact that ionizing irradiation effects a change in the leucocyte count, mainly a reduction, a reduction in the number of lymphocytes, a change in the granular neutrophils, a simultaneous increase in the color indicator, which is subsequently followed by a change in the state of the red blood. The erythrocyte sedimentation reaction in an overwhelming number of our patients remained high. We could be convinced that the time and degree of the changes in the peripheral blood depend on the method of radiation, the size of the radiation field and the individual sensitivity of the patient. The gravity of the tumorous process is also of great importance. In the terminal stage of the disease, the hemogram changes even without radiation therapy. Thus, we consider that the method of rotational remote gamma therapy is safer for hemopoietic organs than, for example, grid irradiation which is reflected in the pattern of the peripheral blood. In the rotational method, the content of hemoglobin is hardly reduced. The average number of erythrocytes was no less than 4,500,000 per one cu mm, and the leucocytes no less than 3,500 per one cu mm of whole blood, which made it possible to complete the course of irradiation. A non-stable reduction in the lymphocyte count is noted in this method of radiation therapy, both in percent and absolute relationships (360 per one cu mm of blood).

Morphological changes show a course toxic granulosity of the neutrophils and a measurable fragmentosis of neutrophil nuclei. The observed non-stable leucopenia apparently took place because of the reduced flow of leucocytes from the hemopoietic centers to the peripheral blood as a result of the rhythmic irradiation of bone marrow, ribs, etc., in irradiating lung tumors. The following observations serve as an illustration of peripheral blood changes in patients with malignant tumors treated by rotational remote gamma therapy.

Patient R, 60-year-old male, with cancer of the right lung, was given rotational remote gamma therapy with a focus dose of 7,617 r. A moderate reduction in the total number of leucocytes in the peripheral blood (examined approximately every 10 days) was observed after the first radiation treatments, primarily because of a fall in the lymphocyte content which, according to the patient's general improvement and reduction of tumor (determined by X-ray), leveled off, and even became somewhat higher than the original figure by the end of the treatment. At the same time, no changes in the red blood were observed. The erythrocyte sedimentation reaction constantly remained high. A course toxic granulosity of the neutrophils and fragmentation of neutrophil nuclei were morphologically observed in the blood cells (Diagram 1).

Patient K, male. Diagnosis: cancer of the supralobar bronchus. Subjected to rotational remote gamma therapy. Focus dose 7,615 r. The peripheral blood showed moderate leucopenia, a small drop in the lymphocyte count which leveled off by the end of the treatment equal to the initial counts, along with an improvement in the general condition of the patient. The red blood cells showed an
insignificant reduction in the hemoglobin content without a reduction of the erythrocytes. The erythrocyte sedimentation reaction was high. Qualitative changes in the cells showed up as toxic granulosity of neutrophil protoplasm and a moderate fragmentation of neutrophil nuclei (Diagram 2). Period of observation was 2 years.

Diagram 1. Hemogram of patient R.
- a) hemoglobin; b) time when analysis taken; c) erythrocytes; d) leucocytes; e) neutrophils; f) lymphocytes; g) erythrocyte count, millions; h) hemoglobin, %; i) total number of leucocytes, thousands; j) neutrophils; k) lymphocytes; l) beginning of observation.

Diagram 2. Hemogram of patient K.
- a) hemoglobin; b) time when analysis taken; c) erythrocytes; d) leucocytes; e) neutrophils; f) lymphocytes; g) erythrocyte count, millions; h) hemoglobin, %; i) total number of leucocytes, thousands; j) neutrophils; k) lymphocytes; l) beginning of observation.
Patient L, 55-year-old male. Diagnosis: cancer of the left lung. Subjected to rotational gamma therapy. Focus dose 6,600 r. The peripheral blood showed moderate leucopenia (total leucocyte count was not lower than 4,100 per 1 cu mm of blood), lymphopenia with relative neutrophilosis, a small transient reduction in the hemoglobin and erythrocyte content. The erythrocyte sedimentation reaction was markedly reduced. Morphological determination of a toxic neutrophil granulosity, uniform giant neutrophils (Diagram 3). Period of observation was eight months.

Diagram 3. Hamogram of patient L.
- a) hemoglobin; b) time when analysis taken; c) erythrocytes; d) leucocytes; e) neutrophils; f) lymphocytes; g) erythrocyte count, millions; h) hemoglobin, Z; i) total number of leucocytes, thousands; j) neutrophils; k) lymphocytes; l) beginning of observation.

The same kind of peripheral blood changes were observed in rotational remote gamma therapy of malignant tumors of the esophagus and stomach, where there was a reduction of the hemoglobin and erythrocyte content, moderate leucopenia and lymphopenia which disappears by the end of the treatment. At the same time there is a noticeable improvement in the patient's general condition and ability to swallow. The following example will be cited.

Patient S, a 50-year-old male. Diagnosis: esophageal tumor (at level D6). Rotational remote gamma therapy administered. The dose in the focus region was 6,712 r. Period of observation was 1 year and 3 months. The peripheral blood showed toxic granulosity of neutrophil protoplasm and fragments of neutrophil nuclei. The platelet count was in the normal range (Diagram 4).

The effect of grid radiation on the peripheral blood is certainly of interest, and is amply illustrated by the following observation.

Patient D, a 50-year-old woman. Diagnosis: malignant tumor of the middle third of the esophagus. Radiation given from four fields (two forward and two behind), each through a grid at 8,000 r. Period of observation was four months. Coincident with irradiation, the hemogram showed a gradual fall in the hemoglobin content (10.6 g % from 16),
a reduction in the erythrocyte count (3,000,000 per cu mm of whole blood from 5,000,000), a reduction in the total number of leucocytes (4,000 from 8,100) with an acute lymphopenia (320 cells in absolute numbers). Morphological demonstration of hypochromia, anisocytosis of the leucocytes, and toxic granulosity of neutrophil protoplasm.

Diagram 4. Hemogram of patient S.
a) hemoglobin; b) time when analysis taken; c) erythrocytes; d) leucocytes; e) neutrophils; f) lymphocytes; g) erythrocyte count, millions; h) hemoglobin, %; i) total number of leucocytes, thousands; j) neutrophils; k) lymphocytes; l) beginning of observation.

In this case the peripheral blood changes sharply differ from those observed in rotational remote gamma therapy. These changes are predictably less favorable (Diagram 5).

Diagram 5. Hemogram of patient D.
a) hemoglobin; b) time when analysis taken; c) erythrocytes; d) leucocytes; e) neutrophils; f) lymphocytes; g) erythrocyte count, millions; h) hemoglobin, %; i) total number of leucocytes, thousands; j) neutrophils; k) lymphocytes; l) beginning of observation.
In the radiation therapy of patients with malignant neoplasms of various organs, variable tumor localization as in all other cases of radiation therapy, one can notice a toxic granulosity of neutrophil protoplasm and a fragmentosis of neutrophil nuclei, whereas in cancer of the uterine cervix, rectum, and dermal melanoma there is a reduction in the content of hemoglobin, erythrocytes, leucopenia, lymphopenia, eosinophilia and monocyteosis. These indicators sometimes vacillate in a seemingly disorderly manner (and in the majority of cases they do not return to the initial magnitudes at the end of the observation period).

The following example is cited.

Patient A, a 51-year-old woman. Diagnosis: malignant tumor of the rectum, progressing to the rear wall of the vagina and perineum, complicated by paraproctitis. The period of observation was four months. The patient was given radiation therapy with a RUM-7 apparatus from two fields, each from which she received 5,715 r. The prognostic blood picture was not satisfactory, since there was a sharp fall in the hemoglobin content (to 6.6%), a reduction in the erythrocyte number (to 2,800,000), an increase in the total number of leucocytes where there was a two-phased leucocytosis (a drop phase and a new increase phase), a left shift in the hemogram, lymphopenia, hypochromia, anisocytosis and a course toxic granulosity of neutrophil protoplasm (Diagram 6).

![Diagram 6. Hemogram of patient A.](image)

In patients not undergoing irradiation and suffering from rheumatic carditis, gall stones, etc., the peripheral blood picture showed a reduction in the hemoglobin level and the erythrocyte number, leucocytosis with a moderate shift to the left. Morphological changes in the cells showed a toxic granulosity of neutrophil protoplasm, in individual cases there was fragmentosis of their nuclei, anisocytosis.
Patient P, a 32-year-old male. Diagnosis: thymoma of the anterior mediastinum. Operated on; no radiation therapy received. The peripheral blood picture coincided with that described above and became normal by the time the patient recovered (Diagram 7).

Diagram 7. Microphotograms of blood cells. The most frequently encountered qualitative changes in peripheral blood cells during irradiation of patients.

a) toxic granulosity of neutrophils, hypersegmentosis, angular shape of cell; b) giant neutrophil with hypersegmentosis (on the left), next to a neutrophil of a normal size (on the right); c) vacuolization of monocyte protoplasm; initial stage of nuclear rupture; d) degenerate lymphocyte with karyorxis.

Inasmuch as our purpose was to detect in as much detail as possible the morphological changes in peripheral blood cells subjected and not subjected to penetrating irradiation, we, in addition to describing
constantly observed qualitative symptoms in the cells, clarified a number of morphological details which are not usually noticed when examining blood in everyday dispensary practice (Diagram 7). Thus, for example, the round shape of a neutrophil changes to an angular form with five or six sharp corners. At the same time a shallow dust-like granulosity appears in the protoplasm, the shape of the nucleus changes and is often located in the wide section of the protoplasm and sometimes extends along the entire length of the cell creating distortion in relation to the nucleus and protoplasm. There also appear giant neutrophils and polycrystallinosis. The number of neutrophils with toxic granulosity rapidly increases and the granulosity becomes dense and coarse. Instead of an angular protusion of protoplasm the contours of the protoplasm begin to erupt and cytolysis takes place. These changes in the shapes of cells are also frequently noticed in eosinophils, lymphocytes and monocytes. Eosinophilia, observed in the beginning of treatments, gradually disappears, the number of eosinophils increase and vacuolization of the protoplasm takes place as well as lysis of the eosinophil nuclei. Non-granular forms of leucocytes also change their forms: wide-protoplasmic lymphocytes and lymphocytes with plasmotized protoplasm with vacuolization and nuclear fragmentation. Histiocytes are very often observed (three per 100 leucocytes). Among the monocytes Rider (degenerate) forms appear, and monocytes with nuclear and protoplasmic vacuolization are observed.

The qualitative changes in blood cells of patients not receiving radiation therapy and suffering from sepsis, rheumatic carditis and cholecystitis primarily show a toxic granulosity of neutrophil protoplasm which suggests the variable character and etiology of this granulosity. This situation must be the subject of subsequent and specialized research.

Conclusions

1. The peripheral blood picture definitely reflects the patient’s general condition and the organism’s reaction to irradiation.
2. The character of peripheral blood changes in irradiated patients depends on the dose, size of the radiation field, method of irradiation and the organism’s individual sensitivity.
3. Changes in the peripheral blood picture which are connected with the gravity of illness are observed near the terminal phase of the process.
4. The peripheral blood picture, in combination with other clinical data, can be of prognostic value.
5. Rotational irradiation is the safest method of radiating peripheral blood.
6. The toxic granulosity of neutrophil protoplasm observed in irradiation as well as in other conditions of persons not undergoing
radiative stimulation, must be a subject for further and specialized study.

6289
CSO: 8346-D
EARLY CHANGES IN THE SKIN CHRONAXY AND VISUAL ANALYSORS IN PERSONS EXPOSED TO A SINGLE LOCAL X-RAY TREATMENT

From the Pathological Physiology Laboratory (Chief -- Candidate of Medical Sciences V. S. Genes) of the Kharkov Institute of Medical Radiology.


The functional changes in the receptor apparatus during the ionizing radiation of an organism have not been sufficiently studied to date. Many authors attach great importance to the pathological impulse, developed in radiation sickness, from various receptors to the centers of the nervous system (Ye. I. Bakin, M. I. Livanov, A. B. Tsypin). The study of changes which occur both in the analysors as a whole and in their various links is essential to ascertaining the reasons and meaning of shifts occurring in the central nervous system under the effect of ionizing radiation. This problem has received particular attention during recent years.


A change in the vestibular analysor of irradiated animals was found by V. V. Petelina, Quastler, 0. I. Chulkova. Phase changes of the functional properties of an internal analysor were observed by V. A. Chernichenko, Yu. M. Zaretskaya, Ye. A. Komarov, N. S. Delitsina and
others. Ionizing radiation of an organism was observed to have also changed the functional state of the visual analyser's afferent component (Lipetz, A. B. Tsypin, G. G. Demirchoglyan, G. T. Adunts, T. M. Avakyan, Notokawa and coworkers).

Therefore, functional changes in analysors may take place either in direct ionizing radiation or in irradiation of remote segments of the body. The change can be brought about by either systematic irradiation in small doses or by short-termed irradiation in large doses. As a rule, the indicated changes are manifested by a reduction of analyser sensitivity to supplementary stimulation. However, in a number of cases (e.g., irradiation of visual and cutaneous analysors) the analyser reacts to the radiation by an increase of specific activity. A number of important problems related to functional changes in analysors during ionizing radiation has not been sufficiently studied. What is primarily unclear is the mechanism by which the changes occur in the receptors. The fact that they occur during irradiation of remote sections of the organism indicate the possibility of their intermediary origin. However, the correlation of direct and intermediary changes, the significance of changes occurring in irradiated internal organs have in fact not been studied to date. Therefore we assumed the task of investigating the correlation between changes in receptors subjected and not subjected to irradiation and related and unrelated functionally to the irradiated organs.

The investigation was carried out during a clinical physiological study of patients with cancer of the uterine cervix who underwent X-ray therapy. The cutaneous receptors and visual analyser were examined in 24 patients prior to irradiation and 30 minutes after irradiating the region of the parametry (300 r on both fields consecutively). The state of the receptors was examined by determining the sensitivity threshold to a constant electric current and by measuring the chronaxy with the aid of an ISE-O1 impulse electronic stimulator. An inert electrode of 100 cm in area was attached to the dorsum for determining the sensitivity threshold and chronaxy on the anterior surface of the trunk and visual chronaxy, or to the chest for determining the sensitivity threshold and chronaxy on the back. All investigations were conducted always under uniform conditions at the same hours of daylight illumination on the premises. We used an electrode 1 sq mm in area for determining the sensitivity threshold and cutaneous sensory chronaxy. During this examination the patients were placed in a horizontal position. The functional condition of the receptors were examined at strictly defined points, as practiced in Chinese medicine (Chzhen'-tszu therapy), reflexly related to the internal organs (lungs, liver, stomach, intestine and urogenital organs).

1. Point shu-fu, reflexly related to the lungs, located at the lower edge of the clavicle two proportional tsuns away from the medial line of the body (one proportional tsun for points located on the chest and back is equal to 1/7 of the distance between the xiphoid process
and the umbilicus, and for points located on the back, 1/5 of the distance between the umbilicus and the upper margin of the pubic symphysis.

2. Point lin-syui, reflexly related to the lungs, located in the third intercostal, two proportional tsuns away from the medial line.

3. Point shi-dou, reflexively related on the right side to the liver, located in the fifth intercostal, six proportional tsuns away from the medial line.

4. Point da-bao, reflexively related to the liver on the right side, located at the level of the middle subaxillary line in the sixth intercostal.

5. Point u-shu, reflexively related to the urogenital organs, located above the anterior iliac spine on a transverse line three proportional tsuns below the umbilicus.

6. Point tszyui-ts Yue, reflexively related to the stomach, located one proportional tsun lower than the xiphoid process.

7. Point tsyan-li, reflexively related to the stomach, located four proportional tsuns lower than the xiphoid process and two tsuns away from the medial line.

8. Point shui-fen, reflexively related to the intestine, located one proportional tsun above the umbilicus.

9. Point vai-lin, reflexively related to the intestine, located one proportional tsun below the umbilicus and two proportional tsuns away from the medial line.

10. Point shui-dao, reflexively related to the urogenital organs, located three proportional tsuns below the umbilicus, two tsuns away from the medial line.

11. Point guan-yuan, reflexively related to the urogenital organs, located three tsuns below the umbilicus.

12. Point fu-fen, reflexively related to the lungs, located away from the interval between the spinal processes of the II and III thoracic vertebra three proportional tsuns away from the medial line.

13. Point ge-khuan, reflexively related to the lungs, located three proportional tsuns away from the interval between the spinal processes of the IV and V thoracic vertebra.

The cutaneous sensory chronaxy was measured immediately after measuring the sensitivity threshold. The sensitivity threshold and chronaxy was determined from large to small magnitudes, since the subjects under study could determine the disappearance of the current sensation better and more accurately than the appearance of the current. We used non-differential skin sensation as the indicator of the current's stimulatory action. A special optical electrode, attached to the external third of the superciliary arc, was used to determine the sensitivity threshold of the optic analyser and optic chronaxy. The experimental subjects were seated with their backs to the light source (window). The investigation was conducted with eyes open.
Peripheral colorless phosphene, always appearing in the temporal region of the field of vision, was used as an indicator of the current's excitatory action.

The obtained data was processed statistically by the Fisher method for related selections (according to difference); the combined materials are presented in Tables 1 and 2. Table 1 shows the data on measuring the sensitivity threshold.

Table 1

<table>
<thead>
<tr>
<th>Point</th>
<th>Average Magnitude Before Irradiation</th>
<th>Average Magnitude After Irradiation</th>
<th>Difference</th>
<th>Percent Change</th>
<th>Probability of R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shu-fu</td>
<td>7.47</td>
<td>7.10</td>
<td>0.37</td>
<td>-5.00</td>
<td>0.433</td>
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<td>10.31</td>
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<td>17.60</td>
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<td>8.58</td>
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<td>Da-bao</td>
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<td>Vay-lin</td>
<td>11.87</td>
<td>11.38</td>
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<td>Shuy-dao</td>
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<td>Gao-khuan</td>
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<td>4.70</td>
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<td>Optic</td>
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<td>-0.04</td>
<td>0.89</td>
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Rheo-base fur the Medial Line

<table>
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<th>Point</th>
<th>Average Magnitude Before Irradiation</th>
<th>Average Magnitude After Irradiation</th>
<th>Difference</th>
<th>Percent Change</th>
<th>Probability of R</th>
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<td>11.04</td>
<td>-0.80</td>
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<td>0.284</td>
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Left Rheo-base

<table>
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<th>Average Magnitude Before Irradiation</th>
<th>Average Magnitude After Irradiation</th>
<th>Difference</th>
<th>Percent Change</th>
<th>Probability of R</th>
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<td>Fu-fen</td>
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<td>10.62</td>
<td>0.06</td>
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<td>Gao-khuan</td>
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<td>10.45</td>
<td>0.21</td>
<td>1.90</td>
<td>0.001</td>
</tr>
<tr>
<td>Optic</td>
<td>4.68</td>
<td>4.56</td>
<td>0.12</td>
<td>2.60</td>
<td>0.492</td>
</tr>
</tbody>
</table>
As can be seen from Table 1, reliable changes in the sensitivity threshold are related only to four points (da-bao from both sides, u-shu from the left side and gao-khuan from the left side). The remaining changes are not certain. Three of the points which showed reliable changes were not subjected to direct irradiation and were not reflexively related to the irradiated organs (da-bao from both sides and gao-khuan from the left side). The sensitivity threshold increased in the point da-bao from the right side, and decreased in the points da-bao and u-shu, both from the left side. Only one point (u-shu) proved to be reflexively related to irradiated organs (urogenital system).

Table 2, constructed analogously to Table 1, shows the data on chronaxy changes. All the average magnitudes, obtained after irradiation, were smaller than the original ones, and all shifts were reliable.

<table>
<thead>
<tr>
<th>Point</th>
<th>Average Magnitude Before Irradiation</th>
<th>Average Magnitude After Irradiation</th>
<th>Difference</th>
<th>Percent Change</th>
<th>Probability of R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Chronaxy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shu-fu</td>
<td>0.205</td>
<td>0.127</td>
<td>0.078</td>
<td>38.1</td>
<td>0.021</td>
</tr>
<tr>
<td>Lin-syui</td>
<td>0.170</td>
<td>0.117</td>
<td>0.053</td>
<td>31.1</td>
<td>0.000</td>
</tr>
<tr>
<td>Shi-dou</td>
<td>0.170</td>
<td>0.090</td>
<td>0.080</td>
<td>47.1</td>
<td>0.000</td>
</tr>
<tr>
<td>Da-bao</td>
<td>0.180</td>
<td>0.110</td>
<td>0.070</td>
<td>38.9</td>
<td>0.000</td>
</tr>
<tr>
<td>U-shu</td>
<td>0.214</td>
<td>0.110</td>
<td>0.104</td>
<td>48.6</td>
<td>0.003</td>
</tr>
<tr>
<td>Tszyan-li</td>
<td>0.216</td>
<td>0.121</td>
<td>0.095</td>
<td>44.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Vay-lin</td>
<td>0.200</td>
<td>0.122</td>
<td>0.078</td>
<td>40.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Shuy-dao</td>
<td>0.149</td>
<td>0.068</td>
<td>0.081</td>
<td>54.4</td>
<td>0.000</td>
</tr>
<tr>
<td>Fu-fen</td>
<td>0.208</td>
<td>0.145</td>
<td>0.063</td>
<td>30.3</td>
<td>0.000</td>
</tr>
<tr>
<td>Gao-khuan</td>
<td>0.186</td>
<td>0.130</td>
<td>0.150</td>
<td>30.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Optic</td>
<td>1.100</td>
<td>0.950</td>
<td>0.150</td>
<td>13.7</td>
<td>0.003</td>
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<tr>
<td>Chronaxy for the Medial Line</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tszyui-tsyue</td>
<td>0.230</td>
<td>0.140</td>
<td>0.090</td>
<td>38.1</td>
<td>0.000</td>
</tr>
<tr>
<td>Shuy-fen</td>
<td>0.220</td>
<td>0.120</td>
<td>0.100</td>
<td>45.5</td>
<td>0.000</td>
</tr>
<tr>
<td>Guan-yuan</td>
<td>0.228</td>
<td>0.104</td>
<td>0.124</td>
<td>54.4</td>
<td>0.040</td>
</tr>
<tr>
<td>Left Chronaxy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shu-fu</td>
<td>0.219</td>
<td>0.139</td>
<td>0.080</td>
<td>36.5</td>
<td>0.000</td>
</tr>
<tr>
<td>Lin-syui</td>
<td>0.200</td>
<td>0.141</td>
<td>0.059</td>
<td>29.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Shi-dou</td>
<td>0.175</td>
<td>0.103</td>
<td>0.072</td>
<td>41.2</td>
<td>0.001</td>
</tr>
</tbody>
</table>
The greatest changes (from 54 to 59%) were recorded in the points shuy-dao and guan-yuan from both sides, located in places of direct irradiation and related to the urogenital organs which were irradiated. Then, in order of decreasing chronaxy changes, come the points sjuy-fen, vay-lin, reflexively related to the irradiated intestine, the points tszyui-tyue and tszyan-li, reflexively related to the intestine not directly irradiated. The smallest changes were recorded in points lin-syu, fu-fen and gao-khuan from both sides. These points are reflexively related to the lungs. Their chronaxy changes were from 29.5 to 30.27. The chronaxy changes in all points were approximately symmetrical. The optical chronaxy showed the smallest changes, decreasing by 13.7% from the right, and by 20% from the left.

Five laboratory coworkers, whom we used as a control, were subjected to a dual measurement of sensitivity threshold and chronaxy at a one-hour interval. These indicators showed no noticeable changes.

The obtained results show that there is a change in the functional state of receptors in organisms undergoing ionizing radiation. As was seen from the brief review of the literature, ionizing radiation of receptors results in either an increase or decrease of the sensitivity threshold depending on the dose and period of irradiation following the change. Our investigations did not conclusively show any changes in the sensitivity threshold with the exception of four points which were not directly irradiated and reflexively related to non-irradiated liver, lungs and irradiated urogenital organs. Chronaxy, which is an index of lability and reflects both the properties of a nerve and the state of the central nervous system, is a more sensitive indicator of ionizing radiation's effect on the functional condition of the nervous system. As a rule, we observed a decrease in the chronaxy which indicates an increase in receptor lability during ionizing radiation.

Our investigations showed that the changes in the functional condition of receptors were observed both in the regions of irradiation.
and in remote points reflexively unrelated to irradiated organs.

This indicates that the initiating mechanism is affected by a change in the centers of the nervous system, reached by impulses from irradiated sections of the body or acted upon by substances formed in the irradiated regions and circulating in the blood. However, the irradiated sections reflected greater changes, which indicates the direct action of radiation on the receptors or on the tissues surrounding them, as well as the possible reflexive influence of irradiated organs.

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The luminescent method for studying the early reactions of organs and tissues to external radiation is quite promising. Data in the literature indicate definite morphological and cytochemical changes in the blood and bone marrow of irradiated animals, as shown by fluorescent dyes (M. N. Meysel', B. A. Sondak, T. M. Kondrat'yeva, O. S. Klimenko, M. Ya. Khodas and others).

However, the results obtained by various authors are not identical, and sometimes contradictory. O. S. Sergel' and A. A. Klimenko obtained a green coloring of nuclear cell elements under normal conditions as well as red, which several authors consider to be a consequence of cell injury (M. Ya. Khodas). What is still unclear is the correlation between the fluorescent color change upon coloring with acridine dyes and the extent of cell injury. Unstudied is the effect of a number of physical, chemical and biological factors, many of which influence the luminescence of the elements under study.

We studied the effect of general external X-ray irradiation on the luminescent pattern of the blood and bone marrow of white rats with respect to laboratory conditions and the activity of certain factors (pH medium, time of dyeing, mechanical compression of the preparation). The rats were irradiated at doses of 200, 500 and 700 r (180 kV, 15 mA, filter -- 0.5 mm Cu, distance -- 30 cm, dose concentration -- 69 g/min). A working solution of orange acridine fluorochrome in concentrations of 1:500 to 1:10,000 were prepared immediately before examinations in buffered isotonic mixtures. In order to obtain more stable results, the blood was fluorochromed in a blender with dyes at a ratio of 1:10. The time for dyeing was from two minutes to five hours.
The study was conducted with the aid of a MBR-1 microscope in the blue-violet region of the spectrum according to the method of crossed filters. The principal features of our methodology included the use of a dark-field condenser, an auxiliary filter which eliminates the basic ZhS-17 type fluorescence and a specially mounted illuminator with a K-22 cine-lamp (30 V, 400 W) which allowed the use of strong lens for magnifications of 600 to 800 times. This made it possible, under other similar circumstances, to use lens of less magnification but with a greater depth of acuity. Ordinary dark field and luminescent microscopy of bone marrow and blood in non-irradiated rats was used for basic control tests.

The effect of the reaction medium on the luminescent pattern was studied by preparing a working mixture of blood or bone marrow with fluorochrome in buffered solutions at pH 4, 5, 6, 7, 8, and dye concentrations of 1:10,000. In both the irradiated and control animals, at a pH of 4 the nuclei of the cellular elements were dyed moderately, but the cytoplasm had a faint green color. There was no red cytoplasmic granulosity of the leucocytes and bone marrow elements, nor the fibrous network of the reticulocytes. There was a significant "discoloring" of the preparation under the effect of blue-violet rays, which apparently depended on the small amount of dye related to the given pH.

As soon as the blood on the glass slide contacted the fluorochrome (primitive humid chamber), a brick-red luminescence of the entire mass of erythrocytes was obtained. A similar luminescence did not occur during a correspondingly equal blood dyeing time in the blender, but did show up only in significant concentrations of the dye (about 1:500) in the working mixture. Therefore, we started to dye the blood in the blender and examine it immediately after placing it under a cover slide. When the blood being dyed was left in the blender for more than 1 1/2 to 2 hours, radiation-type cell injury took place, as demonstrated by the protusion (potocytosis) of the nuclear content into the cytoplasm, and its homogenization without a color change in most cases. Large red granules and optically clear vacuoles appeared in the cytoplasm of the cells. In some of the cells with an ordinary green-colored nucleus, strong Brownian movement of granules took place in the cytoplasm, which, according to certain data (Ye. I. Freyfel'd), indicates cellular death. We observed green-colored nuclei in cells whose cytoplasmic contents had proliferated into the surrounding medium.

The acquired data make it necessary to cautiously evaluate the pattern of radiation injury to blood cells and bone marrow dyed with acridine orange only on the basis of color change. The data must be augmented by a consideration of the structural changes of the nucleus and protoplasm.

One must also remember the possibility of injuring the cells by compressing them under the cover slide for the purpose of increasing
the contrast and clarity of the elements under study. These types of cell changes are accompanied by a coarsening of the nuclear chromatic structure, and in more acute cases, "potocytotic" phenomena and the formation of variously sized non-luminescent zones of cytoplasm around the nucleus.

The above-described changes in blood cells and bone marrow also occurred in X-ray irradiation. Depending on the dose and time of initial irradiation, the cellular elements show initial destructive nuclear changes followed by fragmentation without color changes, destruction of the nuclear membrane and the passing of the nuclear contents into the cytoplasm. Large red granules were formed in the cytoplasm of peripheral leucocytes followed by the conversion of some cells into homogenous astructural formations of a red color. This conversion was accelerated by blue-violet ray illumination of the cells. The bone marrow demonstrated similar changes at an earlier period. Thus, in rats irradiated at a dose of 500 r and killed six hours after irradiation, there were red, astructural, diffusely colored cells along with the green colored injured cells. In some of them one could see clear red inclusions, similar to the residue from degenerated nuclei.

An important fact is the clear disappearance of the luminescent reticulocytes from the blood of the irradiated animals, which is related to the dose size. These elements were not demonstrated in rats irradiated at a dose of 700 r for three days. The disappearance of the reticulocytes was not observed in the blood of rats irradiated at a dose of 200 r (an analogous disappearance of reticulocytes was observed in blood remaining in the blender for more than 1 1/2 to 2 hours).

We did not observe luminescence of erythrocytes as a consequence of even small radiation doses -- 500 to 100 r (O. S. Sergel' and A. A. Klimenko). It is quite possible that it does occur at significantly greater doses, as described by N. N. Meysel' and V. A. Son-dak. However, in investigating the blood of persons with various color indicators and amounts of erythrocytes, luminescence of these elements took place only in high concentrations of dye (1:500 to 1:1,000) and did not depend on the indicators of erythrocytes.

Conclusions

1. pH 7 or 8 are optimal for fluorochroming of blood and bone marrow elements with acridine orange.

2. The luminescent color change of blood and bone marrow in irradiated animals upon dyeing with acridine orange cannot be a reliable criterion for evaluating the gravity of radiation sickness.

3. A proper evaluation of the degree of injury to cellular elements must include a consideration of the cellular structural changes.
4. In view of the high degree of sensitivity of the luminescent method to changes in the laboratory conditions, it is essential to carefully standardize them in order to obtain reliable and uniform results.

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The great interest expressed at the present time in transplantations of hemopoietic tissues in radiation sickness is primarily due to the protective action which they afford in this serious pathological condition. It is known that hemopoietic organs are very sensitive to ionizing radiation, and therefore many authors attribute a leading place to their affectation in the complex and varied pathogenesis of acute radiation sickness. It is therefore understandable that among the many various means and methods proposed for the treatment of radiation sickness, transplantation of hemopoietic tissues is considered to be most pathogenetically directed.

The utilization of hemopoietic tissues, particularly those of the bone marrow, as a protective against acute radiation sickness has been amply elucidated in the literature, so we shall merely cite the principal works which directly deal with transplanting spleen.

The first report on the protective role of spleen in radiation sickness was made in 1949 by Jacobson, Simmons, Block and was later confirmed by innumerable experiments with shielded spleen during X-ray irradiation of animals (N. I. Shapiro and co-authors, 1955; V. Ya. Lavrik and G. I. Lovchuk; L. I. Geller; Jacobson, 1952; Langendorff, Koch, Sauer; Jacobson, Simmons, 1960). These works showed that lead-shielded spleen, separated externally in surgery or located in the abdominal cavity, but shielded by various means (extra- or intra-abdominally), markedly increases animal survival during X-ray irradiation (up to 70 to 76% in comparison to 100% death in the control).

On the other hand, acquired data indicate that extirpation of the spleen leads to a significant increase in the mortality of irradiated animals (Rivento; Yu. M. Zaretskaya, 1960, and others). All of these observations initiated the concept of "the protective factor of the
spleen," the logical culmination of which was the attempt to transplant spleen for the purpose of treating acute radiation sickness.

Jacobson and coworkers (1951) showed that the implantation of spleen into the abdominal cavity of mice irradiated with a dose of 1,025 r markedly increases their survival. Even better results (up to 70% survival) were obtained by authors who injected spleen suspension intravenously.

Further work in this area, constituting different variations of Jacobson's experiments, was undertaken to clarify the protective action of spleen on an irradiated organism during implantation and plastic transplants, during administration of homo- and heterogenous spleen suspensions, and non-cellular extracts of spleen tissue. A number of authors succeeded in obtaining a definite protective effect during irradiation from spleen transplants into the abdominal cavity (Barnes, Loutit, 1953; Vogel and coworkers; V. P. Teodorovich), under the renal capsule (May, Thillard), and subcutaneously (A. G. Karavanov and coworkers). Thom observed a therapeutic effect when administering spleen homogenates both intravenously and intra-abdominally to white rats irradiated at a dose of 800 r. Several investigators obtained a good therapeutic effect by administering suspensions of spleen cells intra-abdominally to the irradiated animals (Cole, Ellis, Thom), and others, by intravenous administration (Soska, Brasil; Sullivan, Stecher, Sternberg). These experiments on various animals showed that spleen homogenates quickly restore the functions of hemopoietic organs and increase the survival of irradiated animals by 22, 75 and 100% (by various authors).

Of interest are the attempts to use non-cellular spleen extracts for the purpose of preventing acute radiation sickness (Ellinger, 1956, 1960). Goldfeder, Clarke observed a protective effect of these extracts when irradiating mice and guinea pigs at doses of 425 and 700 r, whereas Miya, Thorpe and Marcus did not get such good results. According to their observations, the mortality of mice treated with spleen extract was the same as that of the control animals. It is essential to emphasize that there was much contradiction among the research data of many authors on the protective effect of spleen transplants and preparations in the form of iso-, homo- and heterogenous homogenates and non-cellular extracts. Along with the favorable therapeutic effect obtained by experimenters in the above-indicated work, one may cite a number of instances where the authors could note no marked therapeutic effect from this method of treating radiation sickness. Other authors obtained a completely opposite result in the form of aggravation of the radiation reaction and an increase in the mortality of animals to whom splenic tissue was administered (Mandart, Lambert, Maisin; Rudali, Bonet-Maury; Santos, Cole, Yu. M. Zaretstakaya, 1958, 1961).

While we cannot thoroughly analyze the reasons for so many conflicting results, we would point out that in a number of works undertaken to clarify this point it was shown that the degree of the
spleen's protective activity during irradiation depends on the type of the experimental animal, the age and visual characteristics of the donor as well as the route of administering the spleen tissue (Friedell, Salerno; Jacobson, Marks, Gaston, 1956; Makinodan, Gengozian, Shekarchi; Daniell, Crosby). All of this indicates that the problem of transplanting splenic tissue in acute radiation sickness is still not solved, and requires further intensive study and research.

In the beginning of our own work we treated acute radiation sickness in rabbits by using homoplastic transplantations of native (fresh) spleen from a healthy donor, and after having developed a method for preserving it, conducted a series of transplants with preserved spleen.

The experiments were conducted on rabbits of the same species of five to six months of age, weighing from 2.6 to 3 kilograms. The animals were irradiated dorsally with a RUM-3 X-ray therapy machine under the following technical conditions: voltage -- 180 kV, current strength -- 10 mA, filter -- 0.55 mm Cu and 1.0 mm Al, layer of semi-dilution -- 0.8 mm Cu, focal distance -- 40 cm, irradiation dose -- 1,100 r. An evaluation of the gravity of the developing radiation sickness was made by estimating the following indicators: general condition and behavior of the animal, systematic examination of the blood and hemopoietic bone marrow, determination of body weight and skin temperature. In addition, we considered the survival of the animals and the pathological autopsy findings in the deceased animals. In order to clarify the protective mechanism of the spleen transplants against radiation sickness, we sectioned the animals at different times after irradiation for histological studies.

The control series of animals, irradiated at the same doses, consisted of fifty rabbits of which only seven survived. Five animals died during the first 24 hours, or more accurately, within the first few hours following irradiation from X-ray shock. The remaining 38 died from the second to 33rd day, with the majority (20) dying between the 11th and 18th day after irradiation. A severe form of radiation sickness developed in the control rabbits irradiated at a dose of 1,100 r. A worsening of the animals' general condition was observed at the onset of the third to fourth days: refusal to eat, severe thirst, catarrhal phenomena of the conjunctiva, nasal mucous, diarrhea in half of the animals, and an intensive loss of hair in the majority of animals. During the course of a week, the rabbits lost from 300 to 1,100 grams in weight, and up to an additional 250 to 300 grams by the 18th to 20th day after irradiation. The animals' temperature was sub-febrile for the first three days after irradiation, but from the fourth to fifth days, the temperature range was constantly between 39 to 41°. Regular changes were noted in the peripheral blood: during the first 24 hours after irradiation there was a neutrophilic leucocytosis of up to 21,600 cells per 1 cu mm of blood and a significant decrease in the lymphocyte count, a rapid decline in the leucocyte count in one to two
days which attained the lowest level by the third to fifth days (540 to 960), by this time the number of lymphocytes was so small that a percentage calculation could not be made, and a shift to the right was observed in the formula. From the fifth to seventh day following irradiation, the number of leucocytes began to gradually increase. As a rule, by the 12th to 15th day there was a second decrease in the number of leucocytes, but not as large as the first. Leukopenia did not exceed 1,500-2,000 cells per cu mm. A final normalization of the leucocytes took place by the 60th day after irradiation (see diagram).

Changes in the leucocyte count of peripheral blood of control and test rabbits (irradiation dose, 1,100 r).

- - - control

- - - test

The erythrocyte and hemoglobin content, beginning with the second to third day after irradiation, slowly decreased up to the 40th day after irradiation, but did not lower than 50-55% of the original level. The erythrocyte sedimentation reaction in the majority of the rabbits during the clinical development of the disease ranged from 25 to 40 mm per hour, and 60 to 70 mm in some of them. The suppression of bone marrow hemopoiesis in the control animals continued for an average of 10 to 13 days. The nuclear cell count of the bone marrow was the lowest during the seventh to thirteenth days after irradiation, from 3,000 to 12,000 cells per cubic millimeter (according to our data the norm was 10,500 cells per cubic millimeter). The severe course of acute radiation sickness in the control rabbits was responsible for their high mortality.

The second series of experiments, conducted on fifty rabbits, was for the purpose of explaining the protective action of homoplastic spleen transfer in acute radiation sickness. The method of irradiating the animals and the course of experiments were the same as in the control series of experiments, and the same tests were used to evaluate the severity of developing radiation sickness.

Twenty-four hours after irradiation, using local anesthesia, we transplanted a half spleen, 0.75 to 1 gram in weight, of a healthy rabbit-donor. The method of operation was the following: In the region of the animal's spine, a 1 to 1.5 cm incision was made of the
skin and subcutaneous tissue up to the aponeurosis. With the aid of a blunt instrument, a "pocket" was formed in the subcutaneous adipose tissue where half of a spleen, freshly removed from the donor, was transplanted. The wound was tightly sutured with 2-3 nodular silk stitches.

The survival of the irradiated animals after the fresh spleen transplant in comparison to the control data is shown in Table 1 (irradiation dose, 1,100 r).

<table>
<thead>
<tr>
<th>Character of Experiment</th>
<th>Number of Experiments</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
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<th>18th</th>
<th>19th</th>
<th>20th</th>
<th>31st</th>
<th>33rd</th>
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</thead>
<tbody>
<tr>
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<td>5</td>
<td>2</td>
<td>1</td>
<td>--</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Rabbits with transplanted spleen</td>
<td>50</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1</td>
<td>1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>2</td>
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</tr>
</tbody>
</table>

Of the 50 animals with the transplanted spleen, 38 survived and the remaining 12 perished between the fourth and twentieth days after irradiation. In analyzing the rabbit mortality of this series, we turned our attention to one interesting circumstance. As a rule, both rabbits receiving the transplanted spleen from the same donor died (one donated spleen was transplanted simultaneously to two irradiated rabbits). This gives us reason to assume that spleen does not have the same full protective properties in all rabbits.

The clinical pattern of acute radiation sickness in rabbits after spleen transplantation was significantly less severe than in the control animals. The maximum weight reduction in the first ten days after irradiation did not exceed 1,000 g, and ranged from 350 to 450 g in the majority of rabbits. Catarrh of the ocular conjunctiva and upper respiratory tract was noted in 12 and diarrhea in 9 animals. The temperature in 16 of the rabbits was sub-febrile, and the temperature curve in the remaining animals ranged between 38 and 39°. As a
rule, the wound suture at the site of the spleen transplantation was of primary taughtness. Only in two rabbits was there suppuration of the wound. They both subsequently died on the 10th and 14th days after irradiation.

Blood changes 24 hours after irradiation, immediately prior to transplantation were the same as in the control animals. In the following days the leucocyte count progressive decreased, and reached a minimum count on the fifth day after irradiation. In contrast to the control data, the minimal leucocyte count by this time ranged from 1,200 to 1,600 per cubic millimeter of blood. Only in a few rabbits did the leukopenia reach 1,000 cells per cubic millimeter. Lymphopenia was noted in all of the rabbits beginning with first day after irradiation, although the lymphocyte count in the majority was not lower than 10% in one week. Characteristically, the lymphopenia in the rabbits with the transplanted spleen was a stable symptom for a prolonged period of time, and held at a 10-15% level for a month. In 15 animals the lymphocyte count reached a normal level during the fifth week after irradiation. In the remaining cases, in spite of the good condition of the rabbits and virtually complete recovery, the lymphocyte count remained in the range of 50% of the original count for a period of 1 1/2 to 2 months. Normalization of the total leucocyte count took place in the rabbits after spleen transplantation by the 45th to 50th day after irradiation. The erythrocyte sedimentation reaction in 26 of the animals ranged from 30 to 50 mm per hour, and from 10 to 30 mm per hour in 12 animals.

The suppression of bone marrow hemopoiesis in the test rabbits lasted for the same time as in the control animals (10 to 13 days), although the maximum decrease in the bone marrow nuclear cell count did not exceed 13,500 to 19,000 per cubic millimeter during the 6th to 12th days after irradiation. Of additional interest was the prolonged (up to a month) decrease in the lymphocyte count of the bone marrow which indicated the persistent decrease in the number of these cells in the peripheral blood.

Therefore, the experiments with homotransplantation of fresh spleen to irradiated rabbits showed that this method of treatment is an effective means of protecting rabbits from radiation sickness and significantly increases their survival.

Nevertheless, we proposed that the practical value of the indicated method for treating radiation sickness would be much greater if a method for preserving hemopoietic tissue were developed, which would make it possible to retain their protective-biological properties against irradiation for as long a time as possible. Therefore, we assumed the task of developing an effective method for preserving and storing spleen.

Recent advances in the preservation of homotransplants were made by developing a method of preserving tissues by freezing at low temperatures. This method makes it possible to create conditions
which lower the antigenicity of the transplants and at the same time eliminate the biological incompatibility of the tissues as well as insure prolonged storage and retention of viability. The freezing method was given added impetus when it was established that glycerine solutions have a remarkable property of protecting live cells from injury during freezing up to -80°, after which they are able to resume normal activity (Polge, Smith; Smith, Sloviter; Barnes, Loutit, 1955; Billingham; Thomas, Lochte, Lu, Ferreebee; L. M. Spizharskaya and T. K. Hamysheva; Yu. P. Nekiasov; A. G. Fedotenkov, N. A. Mefedova, I. P. Dishkant).

We preserved spleen by freezing at -75° for 24 hours in various preserving media: in preservative TsOLIPK-7 (Tsentral'nyy ordena Lenina institut gematologii i perelivaniya krovi -- Central Institute of Hematology and Blood Transfusion of the Order of Lenin), both pure and with 30% glycerin added (by volume), in glycerin solutions of various concentrations (100, 30 and 15%) and in autolytic serum. The preserved material was transferred to a refrigerator after 24 hours where it was kept at 15° from 10 to 45 days up to the time of transfer. Prior to transfer, for the purpose of removing the preservative and defrosting, the spleen was placed in a sterile physiological solution for 10 to 15 minutes with a small amount of penicillin at a temperature of 37°, after which it was transplanted to the irradiated recipient (See Note/). The best results from the standpoint of preserving the morphology of the spleen tissue was obtained by preserving the material in TsOLIPK-7 with 30% glycerin and in a 30% glycerin solution, and somewhat worse in a 15% glycerin and blood serum solution.

(\textit{Note/}: A more detailed method of preserving hemopoietic tissue is described by us in the journal "Problemy hematologii i perelivaniya krovi" (Problems of Hematology and Blood Transfusion), 1961, No. 9.)

A study of the morphological changes that take place in the spleen as affected by the preservative in 30% glycerin and preservative TsOLIPK-7 with 30% glycerin showed that freezing at -75° in one day does not cause any changes in the histological pattern of the splenic tissue which in no way differs from fresh tissue. No morphological changes in the spleen were observed up to the thirtieth day. Beginning with the 31st or 32nd day, sections with nuclear cell deformations began to appear in the preparations alongside normal spleen tissue. The former took up dyes somewhat more weakly. The indicated changes were the only ones up to the 45th day of preservation. In spleen preserved in 15% solutions of glycerin and autolytic serum, nuclear cell deformations and their weaker dying capacity were observed on the 23rd or 25th day of preservation, although there were no signs of tissue necrosis or autolysis up to the 45th day.

Thus, our method of preserving spleen guarantees good preservation of structure up to 45 days. However, we were also interested in how well the protective-biological properties of splenic tissue were
retained during preservation and in acute radiation sickness after its transfer to irradiated recipients. To do this, one-half of a donor's spleen, preserved by the indicated method, was transplanted to 60 rabbits 24 hours after a total X-ray irradiation at a dose of 1,100 r. The technique of irradiating the animals and transplantations were analogous to those which were adopted in the preceding series of experiments. Thirty rabbits received spleen preserved in preservative TsOLIFK-7 with an addition of 30% glycerin preserved from 20 to 45 days. Thirty rabbits received spleen preserved in 30% solution of glycerin at the same period of preservation. The survival of the animals irradiated at a dose of 1,100 r following the transfer of the preserved spleen is indicated in Table 2.

Table 2

<table>
<thead>
<tr>
<th>Character of Experiment</th>
<th>Number of Experiments</th>
<th>Period of Preservation, days</th>
<th>Day of Death</th>
<th>Survived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfer of spleen, preserved in preservative</td>
<td>15</td>
<td>20</td>
<td>7th 10th 12th</td>
<td>1</td>
</tr>
<tr>
<td>TsOLIFK-7 + 30% glycerin</td>
<td>15</td>
<td>45</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Transfer of spleen, preserved in 30% glycerin</td>
<td>15</td>
<td>20</td>
<td>7th 10th 12th</td>
<td>2</td>
</tr>
</tbody>
</table>

Thus, of the 60 rabbits irradiated at a dose of 1,100 r, 43 survived in comparison to the survival of the control animals (7 out of 50). This indicates that the protective-biological properties of the spleen against irradiation is retained during its preservation process. A lengthening of the preservation period from 20 to 45 days has some
effect on the survival of the irradiated recipients, since 24 out of 30 animals survived in the transfer of spleen preserved for 20 days, and 19 out of 30 rabbits survived in the transplantation of spleen preserved for 45 days. Judging by the survival, the difference in the preserving medium (preservative TsOLIPK-7 + 30% glycerin or 30% glycerin) does not have an important effect on the retention of the "protective factor" of the spleen during its preservation.

The clinical pattern of acute radiation sickness in rabbits irradiated at a dose of 1,000 r and having received preserved spleen, is practically the same as that which was observed in rabbits after a transfer of fresh spleen, although certain symptoms would indicate a more severe course of the disease. The temperature in 23 animals was sub-febrile, in 25 it remained steadily between 38 and 39°, and in 12, varying up to 41°. The minimal reduction in the leucocyte count of the peripheral blood ranged from 1,000 to 1,400 cells per cubic millimeter. The leucocytes in the rabbits of this series became normal 45 to 55 days after the inception of irradiation.

In comparing the dynamics of the morphological changes occurring during various stages of the transplantation with the indicators of the functional condition of the irradiated recipients and their survival, a definite judgment can be made with respect to the mechanism of the protective action of the spleen transfer in acute radiation sickness. To do this, we made excisions of transplanted spleen in the surviving rabbits together with the surrounding tissue for histological studies on the 6th, 8th, 10th, 12th and 14th day after irradiating the animal. The excision was made under local anesthesia aseptically. The histological preparations of the spleen transplants were dyed by two methods: hematoxilin-eosin and azure II-eosin.

A serial study of these preparations showed that the spleen transplant, transferred to the subcutaneous tissue of the irradiated recipient, retains its structure for 4 to 5 days after irradiation. From the 7th to 8th day after irradiation certain necrobiotic processes begin to rapidly grow in the transplant. Eight days after irradiation the spleen transplant already looks like a astructural mass. Rarely does one find preserved walls of small arterioles around which are insignificant amounts of weakly dyed cells. On the 10th and 11th day after irradiation, the spleen transplant undergoes complete necrosis. The same changes take place in the transplants of preserved spleen, although the necro-biotic processes begin later than in the transplants of fresh spleen (on the 9th and 10th day), and the conversion into the astructural necrotic mass is observed on the 15th to 17th day after irradiation.

The results of the macro- and microscopic investigation of transferred spleen transplants showed that the mechanism of its protective action cannot be explained by the formation of extra-medullary foci of hemopoiesis in the irradiated recipient due to the adaption of the transplants. Humoral stimulation of the irradiated organism
and its hemopoietic system is a more probable path. This is indicated by the significantly lighter course of acute radiation sickness in these animals and the lesser suppression of hemopoiesis, and by the data from the histological study of the transplants which demonstrate that there is no adaption in the subcutaneous tissue of the recipient.

Conclusions

1. Transplantation of fresh spleen has a definite therapeutic effect in acute radiation sickness in rabbits irradiated at a dose of 1,100 r. This effect is manifested by an increase in animal survival and a less severe clinical course of radiation sickness.

2. Preservation of spleen by daily freezing at -75°C in glycerin media and preservative TsOLIPK-7 insures good retention of spleen tissue structure up to 45 days.

3. Preserved spleen retains its protective-biological properties against radiation sickness and transferred 20- and 45-day preserved spleen significantly increases the survival of irradiated animals and facilitates a less severe course of radiation sickness.

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EXPERIMENTAL EFFECT OF SEVERAL PHARMACOPEIAL PREPARATIONS
ON RADIATION SICKNESS

Following is a translation of an article by A. V. Shubina, Moscow, in the Russian-language periodical Meditsinskaya radiologiya (Medical Radiology), Vol. VII, No. 11, 1962, pages 83-85.

Two plant substances were tested: Aralia mandshurica and plowed restharrow (Ononias), as well as a chemical preparation, leucogen, in acute radiation sickness. The experiments were conducted on 300 white rats weighing from 159 to 170 g, irradiated by an X-ray unit RUM-3 at a dose of 700 r under the following conditions: voltage -- 180 kV, current strength -- 15 mA, distance -- 40 cm, filters -- 0.5 mm Cu and 1.0 mm Al; dosage strength -- 34 r/min. The aralia and restharrow were used in 0.8 ml decoctions and the leucogen in tablets at 0.003 g per rat. Indicators of the preparations' efficacy included the condition of the animals, their weight, general blood analysis with a strict consideration of changes in blood composition, reticulocyte count, thrombocyte count and bone marrow. The preparations tested on the non-irradiated rats showed that each of them has its own effect on the blood of healthy animals.

Two series of experiments were conducted on the irradiated rats. In the first series aralia and restharrow were administered immediately after completing the irradiation and subsequently every day for a period of 30 days. No positive action resulted from this routine, which was most clearly demonstrated by the beginning of the 15th day. The general condition of the rats was even worse than in rats which did not receive the preparations. Survival was 5% lower than the control with aralia and twice as low with restharrow. The weight of the rats having received the preparations was on the average 4 to 5% higher than in the control animals. The preparations showed no negative effect on the circulatory system.

Upon using restharrow on the 20th day after irradiation, the leucocyte content was 11.3% higher than the control, hemoglobin 8.3% higher and monocytes 27% higher, although the number of erythrocytes, thrombocytes and reticulocytes was lower than the control (by 7.34% and 26% respectively). Upon using aralia, the blood indicators were
somewhat worse than in the control (the erythrocyte and thrombocyte count decreased by 17% and lymphocytes by 21.6%), but the number of reticulocytes and leucocytes exceeded the level of the control group (by 37% and 5.7%). Qualitative changes in the blood composition in the rats from the experimental group were less. Aplasia was not observed in their bone marrow (which did take place in the control), restoration took place earlier and the megakaryocyte content was larger.

Six months after irradiation, complete restoration of hemopoiesis did not take place in the control irradiated rats and all the blood indicators were a little lower than in the non-irradiated rats which were tested simultaneously with the irradiated. The erythrocytes were restored better in the rats which received the preparations than in the irradiated control animals, although the restoration was slower than with the leucocytes. The reticulocytes vigorously regenerated (their content was several times greater than normal). The leucocyte count and the majority of leucocytic indicators were in the normal range by this time (only in the rats having received aralia was the leucocyte count lower than in the remaining groups). The lack of eosinophils in the irradiated control rats was indicative of radiation sickness. The thrombocyte count in the rats receiving retharrow was lower than in the other groups.

At an earlier date, we acquired data (Ye. A. Abaturova, G. N. Yelpat'yevskaya, N. K. Svirdov, A. B. Shubina) which showed that during the first days after irradiation it was better to administer preparations which do not increase the oxidative processes (e.g., leucogen). Aralia and retharrow, as was shown by Ye. A. Abaturova's experiments, increase the oxidative processes. Apparently, this explains the absence of a positive effect from these preparations in the first series of experiments.

In the second series of experiments leucogen was given to the rats immediately after irradiation, and aralia and retharrow were given six days later. The following groups of animals comprised the experiments: First -- control (irradiation); second and third -- administration of aralia and retharrow (on the sixth day); fourth -- administration of leucogen (on the first day); fifth and sixth -- immediate administration of leucogen, and aralia and retharrow on the sixth day.

The last two combinations of preparations proved to be the most favorable. During the course of the entire experiment the condition of the rats from these groups was best, the weight fell less and was regained faster than in the other animals. The survival of these rats was twice that of the control animals, and 1 1/2 times when using leucogen, aralia or retharrow alone. The blood indicators were significantly higher than the control even during the peak of the radiation sickness (14th day) upon administering leucogen with aralia, and especially with retharrow. The leucocyte count in these
animals was 73% higher than the control, hemoglobin 23% higher, lymphocytes almost two times greater and monocytes 1 1/2 times higher, which is indicative of lesser severity of the blood system infection as a result of the therapeutic action of the preparations.

Upon administering one preparation, the leucocytic indicators were also higher than the control (with the exception of the rats to whom aralia was administered), although the erythrocyte pattern in these animals changed more sharply. During the period of restoration (by the 20th day), many young cells appeared in the blood of rats given the preparations which is indicative of the vigorous regenerative processes. The group of animals which received restharrow with leucogen showed the smallest amount of qualitative changes in the blood.

Six months after irradiation, the irradiated control rats which received only restharrow showed alopecia and their weight lagged behind that of the non-irradiated animals. The general condition and blood indicators of the rats which received restharrow with leucogen, was closest to that of the healthy animals. Only the thrombocyte was lower by 10%. The number of reticulocytes in all groups was sharply increased (by 2 to 5 times) in comparison to the non-irradiated rats. The hemoglobin and erythrocyte counts were near normal, the leucocyte count somewhat exceeded the normal (with the exception of the rats given leucogen and leucogen + restharrow. Their leucocyte level was near normal). Qualitative changes were observed during this time, too, in all groups (cells with vacuolization of the nucleus and protoplasm, presence of atypical cells, etc.).

Thus, the preparations under study variously effect the organism in radiation sickness, dependent upon their period of use and combination of administration.
From the 29th to the 31st of May 1962 there convened a scientific conference of institutions of the Ministry of Health USSR, the Academy of Medical Sciences USSR, and other agencies on examining the restorative processes in radiation injuries.

In opening the conference, active member of the Academy of Medical Sciences USSR, Professor A. B. Lebedinskiy, noted the importance of studying in detail the restorative processes in an irradiated organism, since prophylactic and therapeutic means and recovery in radiation sickness is related to the restoration of functions disrupted by irradiation.

A large part of the 34 reports heard at the conference were devoted to explaining the mechanisms of recovery in radiation injuries at various levels, from molecules and cells to the various tissues and systems of the organism. Unfortunately, in a part of these reports interesting experimental data on changes occurring after irradiation were presented without a corresponding analysis of the restorative process.

The report of N. V. Luchnik, N. A. Poryadkova, L. S. Tserapkin, N. V. Timofeyev, "The Mechanism of Recovery From Radiation Injuries on the Cellular Level," dealt with proving the presence of a recovery from cytogenetic injuries and an examination of several mechanisms of this process.

The report of V. M. Mastryukova and A. D. Strzhizhovskiy contained data concerning neutron irradiation. A determination of the mitotic activity of corneal epithelium in white mice subjected to mixed gamma-neutron irradiation, showed that the restoration rate at a dose of 50 rad is 0.52 per 24 hours, and 0.26 per 24 hours at a dose of 200 rad.

G. P. Gruzdev, M. I. Fedotova and Ye. N. Shcherbova, in determining the mitotic index and amount of chromosome aberrations in the bone marrow cells in rats after gamma-irradiation at doses of 150 to 5,000 r, concluded that the restorative process in the bone marrow is
limited by the disruption of the cell division function where qualitative mitotic disruptions manifested as chromosome aberrations are the basic factors.

S. N. Aleksandrov in his report, "The Pathogenesis of Remote Consequences of Irradiation," indicated the partially or completely reversible and irreversible changes occurring at specific doses and conditions of irradiation. He examined the partially or completely irreversible changes which were primarily later consequences of ionizing irradiation.

I. K. Petrovich reported interesting data on experiments on rats beginning with the third to fifth day after irradiation, where regenerative processes were observed in the blood system of all animals both in survived animals after irradiation at doses from 50 to 400 r as well as in those which subsequently died (at doses of 1,000 r or higher).

T. M. Kondrat'yeva and V. G. Safronova, while not analyzing the restorative processes, consider that complete normalization of hemopoiesis does not occur after X-ray irradiation of rats at a dose of 500 r.

V. N. Strel'tsova, in the report, "Several Features of Restorative Reactions in Organs Containing Radioactive Isotopes," cited materials indicating the qualitative character and distortion of the restorative process in the liver stimulated by isotopes of Ce\textsuperscript{144}, Ru\textsuperscript{147}, Pu\textsuperscript{239} and La\textsuperscript{140}.

L. A. Afrikanova, in the report, "Disruption of the Restorative Processes in Healing the Focus of Acute Radiation Necrosis of the Skin," presented the morphological picture of various types of reparative processes after radiation necrosis of the skin, and showed the importance of the general state of the organism in total radiation and indicated that the tissue's proliferative capacity is not disrupted during the reparative phase after radiation injury, but the differentiation stage of forming tissue structures is affected.

G. A. Lebedeva's report, "Regenerative Processes in the Gastrointestinal Tract During Stimulation by Certain Radioactive Substances," showed general directions of restorative processes in radiation by various isotopes (Ce\textsuperscript{144}, Fo\textsuperscript{210}, Sr\textsuperscript{90}); inhibition of regeneration, delays in tissue structure differentiation and differences in the action of each of these isotopes.

N. N. Krushakova and A. Ye. Ivanov showed that the morphological restoration of cells and fibrous structures of lung tissue in irradiated rabbits takes place earlier than the complete restoration of various types of intracellular exchange when there is still a histochemical reduction of RNA and DNA in the cells, activity reduction of the cytochromo-oxidase and succinidehydrogenase, an increase in the activity of alkaline phosphatase, an increase in the mucopolysaccharides in the walls of the blood vessels, and increase in the polymerizability of hyaluronic acid.

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N. G. Akoyev and M. A. Lagun reported data on the reduction of resistance of irradiated mice to the toxin Bac. perfringens as a manifestation of irreversible radiation injuries.

I. M. Shur'yan, V. V. Andryushchenko, and G. M. Rekun noted the characteristic reaction of the hemopoietic system to various sources of ionizing radiation, and the slower restoration of the hemopoietic system in P32 radiation as opposed to X-ray irradiation.

The presence of polyurea and an increased chloride elimination in dogs as a result of increased glomerular filtration as compensatory mechanisms in relation to disruption of renal hemodynamics was demonstrated in the report by Z. I. Poluboyarinova, entitled "Compensatory Changes in Renal Function and Water-Salt Exchange in Dogs in Chronic Radiation Sickness Caused by Sr90."

D. I. Zakustinskiy, L. N. Burykina, Ye. N. Klimova, in the report "Characteristics of the Compensatory-Restorative Processes in Chronic Injury of Dogs by Sr90," indicated the possible appearance of compensatory and restorative processes in both chronic Sr90 exposure of animals (from 2 \times 10^{-4} to 2 \times 10^{-1} mc/kg per day for 3 to 4 years) and subsequent observation for two to three years, in the central nervous system, the blood system, endocrine, cardiovascular and other systems of the organism, which are unstable and combined with a prolonged retention of changes in the indicators under study.

Also heard were the report of V. S. Barsukov, O. V. Malinovskiy and M. I. Mityushov on the importance of cytoplasm in recovery from radiative genetic cell injuries; M. G. Chumak on the restoration of mitoses in variably radiosensitive tissues; T. S. Remzezova and V. P. Tret'yakova on the effect of the energy exchange on the recovery of irradiated yeast cells; A. L. Shabadash on the cytochemical characteristics of nervous system cells in radiation injuries; A. G. Khanin on the recovery of synaptic structures of the brain after irradiation.

V. P. Mikhaylov and N. F. Mayorova reported on the normal reparative regeneration of the mesothelium of the abdominal cavity in acute radiation sickness in rabbits, and K. M. Sveticova on the absence of any disruption of reparative regeneration of skin and intestinal epithelium in local irradiation at a dose of 1,000 r in rats.

Data presented in the report of V. N. Zvorykin were on the restoration of stomach's secretory functions after irradiation, N. A. Lapshin's report on the restoration of reflex reactions, A. G. Kuzkov on the restoration of functions of the hemato-encephalic barrier, N. Ye. Kuznetsova on the participation of the acetylcholine-cholinesterase system in compensatory reactions. V. A. Rezontov and others reported data on studies of restoration of disrupted indicators by secondary irradiation.

Interesting data on the stimulation of the restorative processes in the irradiated organism are presented in another group of reports. V. I. Korogodin demonstrated a need of energy exchange by the restorative process in irradiated yeast cells. By changing the
temperature and composition of the nutritive medium, it is possible to effect the restorative processes and alter cell survival from 0.65% to 46%.

N. N. Klemparskaya and G. A. Shal'nova, in the report "Restoration of Immunogenesis in Irradiated Animals," showed that the ability to produce antibodies and phagocytic activity of leucocytes as well as the normal reaction of the organism on the microbial antigens in survived animals in semi-lethal doses of irradiation is restored approximately one month after irradiation. Interesting data were also cited on the ways of compensating for the disruption of the immunological reactivity of an irradiated organism and acceleration of the restorative processes by combined inoculations prior to irradiation with revaccinations after irradiation, and by means of combining ordinary vaccination preparations with killed-cell vaccines of BCG and others.

The report of R. V. Petrova and Yu. M. Zaretshkaya examined the possibility and ways of using transplantations of hemopoietic tissues for compensation in radiation injuries and showed the role of this method in intensifying the restorative process in an irradiated organism.

G. S. Strelin and N. K. Shmidt showed that vascular administration of bone marrow cells taken from the femoral bone of the rat's extremity which was shielded during irradiation, significantly stimulates the restoration of the blood system and increases the survival of the irradiated animals.

In the report of L. V. Pozhelayev and his coworkers, data were reported on the fact that certain preparations prepared from skeletal muscle, liver, spleen and other tissues of a rabbit which were taken in a fresh, boiled, frozen and lyophilized state, and their basic acting components -- RNA and acid albumin, stimulate the regeneration of amputated extremities in irradiated axolotls.

N. F. Popova showed that skeletal muscle transformed to a plastic state by mechanical trauma or denervation becomes more radio-resistant along with neighboring muscles. After locally irradiating such muscles at a dose of 2,000 r, post-traumatic regeneration proceeds almost without disruption.

The report of A. S. Mozzhukhin was devoted to a description of experiments which showed the various degree of protective action of cystamine at various doses and strengths of irradiation. The authors consider that cellular protection is the basis of stimulating restorative processes with this preparation.

N. V. Traskunova reported on the effect of substances blocking the transmission of impulses in the various links of the autonomic nervous system, and the blood system in rabbits with acute radiation sickness.

In closing the conference, active member of the Academy of Medical Sciences USSR, Professor N. A. Krayevskiy, together with all in attendance, warmly congratulated A. V. Lebedinskiy on his 60th birthday.
and 40th year of scientific-pedagogical activity. N. A. Krayevskiy noted the value of domestic research on restorative processes in an irradiated organism.

The conference adopted a final resolution which particularly took note of the advances in the study of restorative mechanisms in radiation injuries and the necessity of further research in this field.

- END -

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