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TRANSLATIONS FROM "PATLOGUESKAYA FIZIOLOGIYA I EKSPERIMENTAL 'NAYA TERAPIYA (PATHOLOGIC PHYSIOLOGY AND EXPERIMENTAL THERAPY)"

NO. 1, 1963

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- a -
THE ROLE OF HUMORAL FACTORS IN CHANGES OF THE CNS
FUNCTION IN EXPERIMENTAL RADIATION AFFECTIONS

[Following is a translation of an article by P. D. Gorizontov and Ye. N. Shcherbova, in the Russian-language journal Patologicheskaya Fiziologiya i Eksperimental'naya Terapiya (Pathologic Physiology and Experimental Therapy), No. 1, Moscow, 1963, pages 14-19.]

We have not found in the literature any data concerning the influence of humoral factors on the development of changes arising in the nervous system in radiation affections. It was shown in experiments that complicated disturbances of activity of the central nervous system are the result of reflex influences and the direct affect of ionizing radiation on nervous tissue (5, 6, etc.). We undertook to clarify the significance of the humoral environment in these processes.

The experiments were performed on parabiont rats. The parabiont pairs were created by means of the production of a musculocutaneous anastomosis. One week prior to this operation, electrodes were implanted in the rats by the modified method of Ukolova and Bordyushkov (8). The implanted electrodes were situated in bipolar fashion (at a distance of 2 mm) in the region of the visual zone of the cerebral cortex. Two to three weeks after operation, the rats were studied experimentally and their clinical condition observed, and biocurrents were recorded from the cerebral cortex of the animals before and after irradiation.

Irradiation was carried out in the RUM-3 apparatus; power of irradiation 28.5-34 r/min, current 15 mamp, voltage 180 kv. The total dose for each irradiated animal was 1100 r. The rats were irradiated in a special cage which permitted the screening of one of the partners. Screening was accomplished with the use of sheets consisting of 5 mm Pb and 3 mm Al. Measurements which were performed in a specially devised paraffin phantom enabled us to establish that the screened animal was exposed to the action of scatter radiation in a dose which total about 2 percent of the total dose, that is, not more than 25 r. In the same cage, that is, under identical conditions, two individual rats were irradiated. This enabled us to compare the changes which were observed in the parabiont rats with the changes in normal animals.
The experiments were performed only on pairs of parabionts which had learned after training to lie quietly without fixation of the head. The fulfillment of this requirement is associated with close selection of animals; as a result, we were able to perform this work only on three pairs of parabiont rats.

As controls, we irradiated three pairs of individual rats.

Individual rats irradiated with 1100 r died within 2-3 days. By the time of death they were showing marked diarrhea, and had lost considerable weight. Prior to death, all of the animals showed a reduced body temperature.

Individual rats irradiated in the screened cage, and which consequently received 25 r, were observed for a period of 1-2 weeks. The entire period of observation of these rats showed them to be in a good clinical state, they ate well, and they gained weight. The changes in the blood which are characteristic of radiation sickness did not develop.

Parabiont rats irradiated with 1100 r died within 3-5 days. The signs of disease in the irradiated rats appeared earlier than in the screened partner. By the end of the 2nd but usually on the 3rd day, the animal developed diarrhea. In the screened rats, there was no diarrhea. Prior to death, there was a reduction in body temperature in both of the irradiated animals. Beginning on the day of irradiation, the rats lost weight. Of the parabiont pairs, the irradiated animal died first, and then the screened partner.

Changes in the clinical indices and in the composition of the blood following irradiation were most pronounced in the individual rats irradiated with 1100 r, and slightly less pronounced in the parabiont rats receiving the same dose, and still less in the screened parabiont partner and were completely absent in the individual rats irradiated with 25 r. Our results are in accordance with the data published in the literature (2, 9, and others).

Different results were obtained in studying, in these animals, the functional changes of the central nervous system, which we assessed by means of the bioelectrical activity of the cerebral cortex.

Recordings of the biocurrents of the brain in all animals were performed in the laboratory of M. N. Il'yanov on several occasions prior to the experiment and then for 2-2 1/2 hours after irradiation, and again prior to death, and for a period of 7-8 days in individual rats irradiated with a dose of 25 r. Simultaneously, we recorded respirations by means of carbon transmitters. We did not limit ourselves to the recording merely of the spontaneous activity of the cerebral cortex, but also
studied the action of the dynamic stress of light flashes of increasing intensity (the method of Livanov). This method enabled us to determine the reactivity of the cortex with respect to the strength and character of the changes of the biocurrents in response to this stress. The reactions to visible minimal light enabled us to assess the threshold of stimulability. The use of weak and strong light stimuli is capable of demonstrating phase states.

In processing the data of the EEG, the function state of the cerebral cortex was characterized by the following indices: background activity (in microvolts), reactivity (in microvolts) and stimulability (threshold of stimulability in seconds).

In rats receiving 25 r, soon after irradiation the reactivity and stimulability of the cerebral cortex increased slightly. This was observed in rats No 2 and 6, whereas in rat No 4 there were virtually no changes in the bioelectric activity of the cortex. The background activity of the biocurrents did not change, except in rat No 2, in which it increased on the second day after irradiation.

It is known that small doses of ionizing radiation and even microdoses of the order of 0.05 r cause definite changes in the functional state of the cerebral cortex. The data of our experiments are in accord with those in the literature (1, 3, 4, 7, 9).

Total-body irradiation of individual rats with 1100 r caused considerable changes in the functional state of the cerebral cortex. These changes are of a phasic nature. In 2 or 3 rats following irradiation, we observed an intensification of the bioelectric activity of the cerebral cortex, with an increase in its reactivity and a decrease in the threshold of stimulability. Two to 2-1/2 hours after irradiation, the activity and reactivity of the cortex diminished. In the third rat, immediately after irradiation, there was depression of the biocurrents of the brain which, apparently, was due to individual peculiarities of this animal.

Hence, in individual rats irradiated with 1100 r, changes in the central nervous system were considerably greater than in individual animals receiving 25 r (Figure 1).

In parabiont rats, changes in the nervous system were of a phasic character and the degree of changes in the bioelectrical activity, reactivity, and stimulability of the cerebral cortex in irradiated and screened rats were approximately identical. In two of the three cases (4th and 6th pairs), the changes in the above mentioned indices in the irradiated and screened partners proceeded in parallel. Immediately after the post-irradiation brief period of depression, there was an intensification of the activity and reactivity of the cortex, which was then succeeded again by prolonged depression. Twenty-four hours after
Figure 1. Changes in the bioelectrical activity of the cerebral cortex in individual rats (1st pair).
1 - Background activity of the irradiated rat (1100 r);
2 - Reactivity of the irradiated rat (1100 r);
3 - Background activity of the screened rat (25 r);
4 - Reactivity of the screened rat (25 r).

Key:
1. Microvolts;
2. Irradiation;
3. Normal;
4. Minutes;
5. Days.
irradiation and on subsequent days, all animals showed a variable functional state of the cerebral cortex. In the irradiated partner of the 5th pair of parabionts (rat No 9), there was no stimulation of the cortex, but there was an immediate depression of biocurrents; in the screened partner (rat No 10), following a marked intensification of the bioelectrical activity, there was protective inhibition.

Characteristic of the irradiated screened and nonscreened parabionts was the greater degree of changes in the central nervous system than in the individual animals. In all parabiont rats, there were manifestations of weakness of the cells of the cerebral cortex, when the light stimulus caused a marked reduction in the bioelectrical activity, and the development of a profound depression or marked instability of the activity of the cerebral cortex which was more prolonged than in the individual rats (Figure 2). In the EEG of the fourth pair of animals (Figure 3), it was apparent that within two hours after irradiation, the light stimulus caused depression of the biocurrents of the brain in both rats. Within 24 hours in rats receiving 1100 r, there was a marked depression of the biocurrents, while in the screened animals there was instability of the cortical activity. In a number of cases, at various times after irradiation, it was possible to detect the appearance of a respiratory rhythm on the EEG of the parabiont rats (Figure 4).

In parabionts irradiated with with 1100 r, the changes were considerably more pronounced than in individual irradiated rats. Even greater was the difference between the individual rats receiving 25 r and the screened parabionts. It may be suggested that in the irradiated animal, certain substances of a hormonal nature were circulating in the blood stream which would influence the interoception and the brain of the partner. However, a modified hormonal environment alone does not cause such significant disturbances in the EEG of individual rats irradiated with 1100 r as in the irradiated parabiont rats. The concentration of hormonal products secreted in response to the stimulus must be greater in the individual irradiated rat than in the parabiont rat. Consequently, changes on the part of the central nervous system in individual rats must be more serious. However, this is not so. Hence, it is necessary to start with different assumptions.

The process of irradiation of one of the partners of the parabiont pair was continued in our experiments for a rather long time (32-38 minutes). During this time, the amount of circulating blood passing through the organism of the irradiated animal, as shown by calculations, must have been increased by approximately 1-1/2 times due to the blood of the screened partner. Consequently, the amount of toxic, radiochemical products formed in the circulating blood may be increased. On the other hand, the opposite may be true. The parabiont rats, with respect to their physiologic qualities, differ slightly from unoperated healthy...
Figure 2. Changes in the bioelectrical activity of the cerebral cortex following irradiation in parabiont rats (4th pair). Designations same as in Figure 1.

Key:
1. Microvolts;
2. Normal;
3. Minutes;
4. Days.
Figure 3. Changes in the EEG of the cortical end of the visual analyzer of parabiont rats in response to irradiation. 
A - Before irradiation; B - Two hours after irradiation; 
C - Twenty-four hours after irradiation; 1 - Marker for the light stimulus (arrow indicates the beginning of stimulation); 2 - EEG of the irradiated rat (1100 r); 
3 - EEG of the screened rat (25 r); 4 - Time marker (in seconds).

Figure 4. Changes in the EEG of the cortical end of the visual analyzer of parabiont rats 4 days after irradiation. 
1 - Pneumogram of the screened rat (25 r); 2 - Marker of the light stimulus; 3 - EEG of the screened rats; 
4 - EEG of the irradiated rat (1100 r); 5 - Pneumogram of the irradiated rat; 6 - Time marker (in seconds).
animals. This may be suggested on the basis of general ideas concerning a certain individual immunologic incompatibility, and also on the basis of factual material. In normal healthy rats, the number of leukocytes in the peripheral blood is 12,000-20,000. After the operation of parabiosis, they decrease to 8,000-5,000. It is possible that, due to changes in the physiologic state of the animals, the nervous system of the parabionts reacts to irradiation differently from the nervous system of the individual rats. But on the basis of this assumption, there is reason to believe that the humoral environment plays an important role in the reaction of the nervous system to irradiation. Of this, the following is evident: one of the parabiont rats received 1100 r, while the other received only 25 r; however, the reactions in them on the part of the central nervous system were practically identical.

The similar reactions of the nervous system in the parabionts in the presence of markedly different doses of X-rays can be explained only on the basis of a common humoral environment. On the basis of this, it is possible to conclude that the immediate effect of radiation on the tissues does not always play a decisive role in the changes of the central nervous system and that changes in the humoral environment, obviously, are also of great importance for interoception of the irradiated organism.

CONCLUSIONS

1) Irradiation with a dose of 1100 r causes marked changes in individual rats in the activity of the central nervous system, which take the form of phases of intensification and depression of the bioelectrical activity of the cerebral cortex, as well as changes in its reactivity and in the threshold of stimulability. Irradiation of parabiont rats with the same dose, contrary to what one would expect, causes considerably more pronounced changes of the same indices than in individual rats.

2) Irradiation of individual rats with 25 r causes slight changes in the activity of the cerebral cortex. However, irradiation with the same dose and under the same conditions of parabiont rats causes, in the latter, marked changes in the EEG, which are similar to a certain degree to the changes of the EEG of irradiated partners (dose 1100 r).

3) The results of these studies indicate that changes in the humoral environment of the organism are of great importance in the disturbances of central nervous system activity which develop following irradiation.

- 8 -
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IMMUNOTHERAPY AND DERMATOPLASTY IN COMBINED BURNS

Having taken an interest during the course of recent years in clarifying certain problems of the pathogenesis and treatment of burn and radiation disease, we made observations of the therapeutic effectiveness of blood and serum of patients convalescing from burns when used for patients with severe thermal burns (3-5).

These studies confirmed the findings of N. A. Fedorov and S. V. Skurkovich (1, 2) concerning the therapeutic effect of immunotherapy in extensive burns of third and fourth degree. We were convinced of the high effectiveness of immunotherapy also in cases of combined burns (thermal burns and radiation sickness), in which the resistance and the regenerative capacities of the organism were weakened to a considerable extent and burn shock was especially severe. The effectiveness of immunotherapy considerably increases when it is combined with necrectomy: the early removal of necrotic tissues increases the positive effect of the immune serum, increasing the survival of animals and to a certain extent (2-2.1/2 times) accelerating the processes of healing of the wounds.

The present study was undertaken in order to clarify further the problems of combined treatment using immunotherapy of burns of a combined nature. As is known, among the modern methods of treatment of deep thermal burns, an important place is occupied by free transplantation of skin. Hence, in the combined treatment of thermal burns in the presence of radiation injuries, we thought it necessary to study the effectiveness of immunotherapy in conjunction with necrectomy and subsequent skin transplantation, as well as to clarify the clinical and morphologic peculiarities of the adaptation of skin transplants under these conditions.

In 103 adult rabbits, we performed six series of experiments, of which two were controls, in which the burned animals were not subjected to skin transplantation. In one of the control series (Series A), necrectomy was not combined with immunotherapy, and in the other control series (Series B), the burned animals were given the blood of rabbits which had
recovered from burns. In all of the basic four series, early necrectomy was combined with transplantation of skin. In the first series, immunotherapy was not used; in the second series, the animals were given repeated infusions of the blood of convalescent animals; in the third series, the burned animals were transfused with the blood of healthy rabbits which had not been burned; and in the fourth series, transfusions of blood of convalescent rabbits were infused, and skin flaps were taken not earlier than 1-1 1/2 months after complete epithelialization of the wound surface of the donor.

Radiation sickness of moderate severity was induced by total body irradiation of animals, using a total dose of 501.84 r. Immediately after irradiation, burns of third and fourth degree were inflicted by a Bartel's lamp over an area equal to 10-12 percent of the entire body surface. Necrectomy was performed on the 5-6 day after combined injury. To the wound were applied fresh full-thickness skin homotransplants with perforations for the free release of wound exudate. The blood of convalescent rabbits was infused intravenously every day for 10 days.

The data are shown in Tables 1 and 2.

In the first series of experiments (transplantation of skin following necrectomy without immunotherapy), skin transplantation did not lead to any substantial changes in the course of the combined injury: the survival of animals was only slightly increased, and the length of life was not prolonged. The animals did not endure the operative procedure at all well; their general condition deteriorated markedly, especially during the first week after operation, they became weak, immobile, ate poorly, and showed elevations of body temperature of 1.5-2°. The majority of the rabbits died during the course of the first 2 weeks after operation. Operation accelerated the development of radiation sickness, and its course became grave. The changes in the peripheral blood developed more quickly and were more severe, and the number of white cells dropped by 90-95 percent; there was marked anemia, loss of weight of 30-35 percent of the original weight, and the hemorrhagic syndrome was striking. Operation itself was difficult due to the increased bleeding of the tissues, due to which the skin flap frequently was separated by a hematoma. Beneath the flap, secondary necrosis and hemorrhage developed. The skin transplant did not protect the wound against infection, and frequently there was suppuration with deep necrosis of tissues. The transplant itself, as a rule, shrivelled by the 4th-5th day, became desiccated, and assumed a brownish color, the edges of the flap began to be deformed, and a certain amount of seropurulent liquid accumulated beneath the flap. The inflammatory reaction at the sites of application of sutures was not very pronounced. The transplant by the 7th-10th day ordinarily showed complete necrosis, although it usually
# Table 1

Outcomes of Combined Treatment of Thermal Burns in the Presence of Radiation Injuries

<table>
<thead>
<tr>
<th>Series of experiments</th>
<th>Conditions of experiment</th>
<th>Number of animals</th>
<th>Average length of life of animals which died (in days)</th>
<th>Average length of time required for healing of wounds in surviving animals (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control A</td>
<td>Necrectomy without skin transplantation and without immunotherapy</td>
<td>19</td>
<td>3</td>
<td>92</td>
</tr>
<tr>
<td>Control B</td>
<td>Necrectomy without skin transplantation and with immunotherapy</td>
<td>19</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Primary</td>
<td>Necrectomy, transplantation of skin, without immunotherapy</td>
<td>18</td>
<td>7</td>
<td>46.2</td>
</tr>
<tr>
<td>Secondary</td>
<td>Necrectomy, transplantation of skin, with immunotherapy</td>
<td>18</td>
<td>3</td>
<td>41-16</td>
</tr>
<tr>
<td>Tertiary</td>
<td>Necrectomy, transplantation of skin, with skin transplantation for healthy animals</td>
<td>15</td>
<td>9</td>
<td>79</td>
</tr>
<tr>
<td>Quaternary</td>
<td>Necrectomy, transplantation of skin, with skin transplantation for healthy animals, immunotherapy</td>
<td>18</td>
<td>10</td>
<td>39.5</td>
</tr>
</tbody>
</table>

Key:

1. Series of experiments;
2. Conditions of experiment;
3. Number of animals;
4. In the series;
5. Died from shock;
6. Subjected to treatment;
7. Died from burns and radiation sickness;
8. Survived;
9. Average length of life of animals which died (in days);
10. Average length of time required for healing of wounds in surviving animals (in days);
11. Control A;
12. Necrectomy without skin transplantation and without immunotherapy;
13. Control B;
14. Necrectomy without transplantation of skin but with immunotherapy;
15. First;
16. Necrectomy, with transplantation of skin but without immunotherapy;
17. Second;
18. Necrectomy, with transplantation of skin and immunotherapy;
19. Third;
20. Necrectomy, with transplantation of skin and transfusions of blood of healthy animals;
21. Fourth;
22. Necrectomy, with transplantation of skin of animals recovering from burns and with immunotherapy.

Table 2

Outcomes of Skin Transplantation in the Combined Treatment of Combined Radiation Injuries (Thermal Burns and Radiation Sickness)

<table>
<thead>
<tr>
<th>Case number</th>
<th>Necrosis onset</th>
<th>Days immunoe</th>
<th>Days combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>Necrectomy, transplantation of skin; no immunotherapy</td>
<td>1-24</td>
<td>18-18-4</td>
</tr>
<tr>
<td>2nd</td>
<td>Necrectomy, transplantation of skin, immunotherapy</td>
<td>15-24-6</td>
<td>25-30-6</td>
</tr>
<tr>
<td>3rd</td>
<td>Necrectomy, transplantation of skin, perform biological skin of rabbits as a vehicle</td>
<td>7-13-6</td>
<td>21-23-6</td>
</tr>
<tr>
<td>4th</td>
<td>Necrectomy, transplantation of skin of animals recovering from burns and immunotherapy</td>
<td>25-37-6</td>
<td>30-34-6</td>
</tr>
</tbody>
</table>

Key:
1. Series of experiments;
2. Conditions of experiment;
3. Day of necrosis of skin transplant;
4. Day of complete sloughing of the skin flap;
5. First;
6. Necrectomy, with transplantation of skin but without immunotherapy;
7. Second;
8. Necrectomy, with transplantation of skin and immunotherapy;
9. Third;
10. Necrectomy, with transplantation of skin and transfusions of blood of healthy animals;
11. Fourth;
12. Necrectomy, with transplantation of skin of animals recovering from burns and immunotherapy.
remained for a certain length of time on the surface of the wound as a
dry piece of tissue; complete sloughing of it ordinarily occurred on the
15th-18th day. Cleansing and scarring of the wound were poor, and
stretched out over a long period of time (about 2-1/2 months); however,
as compared with the control series (without transplantation of skin),
healing of the wound occurred slightly earlier.

Histologic studies of the transplant and the surrounding tissues,
which was performed on the 9th-10th day after transplantation, showed
necrosis of the epidermis and dermis. The latter was homogenous (and
stained a rose color) and only in the deeper layers of it could fibrous
structures be distinguished. The layer of muscle adjacent to the
dermis was necrotic, the muscle fibers were homogenized, contained no
nuclei, and the cytoplasm sometimes showed a basophilic staining quality.
The more superficial muscle fibers were separated by exudate with multi-
ple degenerating nuclei. The walls of the vessels in the deeper layers
of the dermis were difficult to distinguish, and the blood in the lumans
was hemogenized. In some places, nerve fibers retained their structure
along the limits of the dermis and the muscle layers. At the periphery
areas of necrosis, there was a process of delimitation in the form of
the development of young granulation tissue, which was rich in vessels
and cellular elements, among which were many leukocytes. In many cases,
at the periphery of the areas of necrosis, there was a purulent degenera-
tion of the latter. In some places, colonies of bacteria could be seen
in the exudate. In the soft tissues adjacent to the transplant, there
was poor development of granulation tissue with a feeble inflammatory re-
action.

In the second series of experiments (skin transplantation against
a background of repeated transfusions of blood from animals convalescing
from burns), positive results were evident both with respect to the
clinical and morphologic peculiarities of the adaptation of the skin
transplant, as well as with respect to the outcomes of the burn-radiation
sickness. In this series of experiments, the results were more favorable
than in the control series B (immunotherapy without homotransplantation).
With this form of combined treatment, 75 percent of the experimental
animals and 66.6 percent of the control animals survived. There was an
increase (up to 27 days) in the length of life of those animals which
died. We did not observe any noticeable deterioration in the general
condition of the rabbits, although for 2-3 days after operation they were
slightly weak. The animals tolerated surgery well, and there were no
deaths in the postoperative period. In a majority of operations, homo-
transplantation lead to an improvement in the general condition, the
rabbits became more active, ceased losing weight, the body temperature
returned to normal, and leukopenia and anemia did not increase, and
normalization of the hematologic indices occurred more quickly. Immuno-
therapy was reflected in the condition of the skin transplant as well:
at first, it retained its viability, was warm to the touch, elastic, of a pale rose hue, hair grew on it, it was intimately adherent to the site of transplantation. Only later, by the 12th-15th day, when transfusions of the blood of convalescent rabbits were discontinued, the transplanted skin shrivelled, the edges pulled away, and complete necrosis of the flap occurred only by the 18th-21st day, that is, at a date which was three times later than in the case of skin transplantation without immunotherapy. Beneath the flap there was a slight serous exudate, and no suppuraton occurred. Complete sloughing of the flap ordinarily occurred on the 28th-30th day. Homotransplantation in combination with immunotherapy favored quicker healing of the wound, the growth of granulation tissue, and stimulation of growth of epithelium. Healing of the wound occurred twice as rapidly as in the case of skin transplantation without immunotherapy (the average time of healing in this series was 39.5 days, as contrasted with 79 days in the first series). Transplantation of skin also improved the effectiveness of immunotherapy; in the control series B, in which immunotherapy was not combined with skin transplantation, scarring of the wound occupied on the average 46.2 days; in the present series, this time was shortened to 39.5 days. This was clearly reflected also in the morphologic picture of the transplanted skin.

Histologic studies, which were performed on the 9th-10th day, showed only a partial coagulation necrosis of the tissues of the dermis, individual parts of which were structureless, and stained a bassophilic hue. In other parts, the nuclei in the cells were preserved, especially in layers of the skin which were situated further from the surface, but even here they were pyknotic. Nuclei were retained also in the adjacent muscles, the intermuscular connective tissues were homogenous and showed a basophilic staining property. In the deepest layers of the dermis, the vessels were markedly distended with blood. The structure of the walls of the blood vessels was unchanged. The process of delimitation of the necrosis was more marked than in the animals of the first series, with the development of granulation tissue; the latter was rich in histocytes.

In the tissues surrounding the transplant, there was an inflammatory reaction with marked growth of granulation tissue. Necrosis of the skin flap occurred considerably later, while the process of delimitation of it from the surrounding tissues, with the formation of young granulation tissue and a leukocytic infiltrate, were more pronounced, and in the underlying tissues, the formation of granulation tissue was more active.

Hence, although immunotherapy does not prevent necrosis of the skin flap, it considerably delays the time of its occurrence and favors the fulfillment by the transplant of its function of biological binding, which ensures the protection of the wound surface during the period of radiation sickness, and shortens the time of wound healing.
This favorable effect should be regarded only as a result of the combined skin transplantation and immunotherapy. We were further persuaded of this by the findings of the third series of experiments, in which, instead of the blood of convalescent rabbits, we transfused the blood of healthy rabbits. Despite skin transplantation, the survival of animals, the length of their life, the times of wound healing, and the fate of the transplant were the same as in the first series in which immunotherapy had not been used.

Our very best results were obtained in the fourth series of experiments, in which not only blood for transfusion but also the skin flaps for transplantation were taken from rabbits which had recovered from previous burns. Of 12 animals subjected to this type of treatment, only 2 died (of these 1 died as the result of postoperative hemorrhage). The general condition of the animals during the postoperative period remained completely satisfactory; they either did not lose weight or lost only a small amount, and the body temperature ordinarily exceeded normal by no more 0.5-1°. The transplanted skin retained its rose color and its soft elastic consistency for a long time, and necrosis did not occur until the 23rd-27th day. There was no inflammatory reaction at the sites of application of sutures; there was no purulent exudate, and a thin serous liquid was secreted from the surface of the flap only during the first 5-6 days after operation. Sloughing of the skin flaps began on the 21st-25th days and was completed by the 30th-34th days; and ordinarily, by this time, the wound had healed beneath the flap.

Hence, skin flaps taken from animals surviving burns, in conjunction with immunotherapy, are better than other homotransplants, and fulfilled the function of biological bandaging in cases of combined treatment of combined burns.

CONCLUSIONS

1) Skin transplantation in conjunction with immunotherapy improves the effectiveness of the latter in the combined treatment of thermal burns accompanied by radiation injuries, reducing the mortality, prolonging life of the affected animals, and shortening the healing times of the wounds.

2) Immunotherapy, while not preventing necrosis of the skin flap, significantly delays it, accelerates the formation of granulation tissue, and facilitates the fulfillment by the transplant of its biological function of bandaging and protecting the wound surface, during the height of radiation sickness.

3) The best results were obtained when immunotherapy was combined with transplantation of skin flaps taken from animals which had recovered from previous burns.
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EXPERIMENTAL THERAPY OF THERMAL BURNS OF THE UPPER RESPIRATORY TRACT AND LUNGS

In the literature there are only individual studies devoted to thermal burns of the upper respiratory passages and lungs. Primary attention has been given in these studies to the morphologic picture of the injury, whereas the problems of the clinical manifestations of the disease and its treatment are discussed only in very general terms (6-9).

Considering the great heat capacity of water vapor as compared to dry air, we induced burns of the respiratory passages and lungs with the use of steam under pressure.

Our experiments were carried out on 49 mongrel dogs of both sexes weighing from 8-21 kilograms. Thirty to forty minutes prior to the infliction of the burn, the dogs were given a 1 percent solution of morphine subcutaneously in a dose of 0.25 ml/kg. This dose of the narcotic under the conditions of our experiments did not prevent the development of burn shock, although it did slightly improve the course of the disease during the first day. The dogs were secured on their backs on a vivisection table. Into the nose, we introduced a tube with a cross-sectional area of 4.52 sq. mm. to a depth of 2 cm, and a tube with a cross-sectional area of 12.56 sq. mm. was placed in the area behind the root of the tongue. A hose conducting steam from an autoclave into a third tube was heated for several seconds with steam, which then emerged through a lateral valve. When the stop watch was started, the lateral valve was opened and steam under pressure of two atmospheres at a temperature of 120° C was introduced through the tubing into the cavity of the mouth into the respiratory passages and the lungs of the dogs.

In working out the conditions of the experiment, we performed observations on 14 dogs which had sustained burns by this method with different degrees of exposure to forced inhalation from 2-60 seconds. In our basic experiments, the length of exposure to the steam was 4 seconds.
This length of inhalation for dogs not receiving treatment proved fatal in all cases. In these experiments, burns of the upper respiratory passages and lungs were inflicted in 35 dogs: 26 dogs were subjected to treatment, and 9 did not receive treatment (controlled).

All of the animals dying following burns were autopsied and histologic studies were made of the organs and tissues.

Immediately after forced inhalation of steam, all the animals showed a brief cessation of respiration, with subsequent rapid breathing. After the dogs were untied, they jumped up and ran around the room for several seconds, then laid down on the floor, gradually becoming calmer, then seemed indifferent to their surroundings, and refused to eat and even to drink water. For the first 30 minutes after the burn, there was a thin rose colored fluid which dripped from the nose and mouth. Endoscopy showed areas of necrosis of the mucosa of the oral cavity, tongue, pharynx, and marked hyperemia of the mucosa of the trachea and moderate reddening of the mucosa of the bronchi; in some of the animals, during the first 2-3 days, one could see a foamy sputum and fibrinous clots in the trachea. As early as 1 day after the burn, abundant purulent exudate with an unpleasant odor appeared in the oral cavity. There were areas of necrosis at the root of the tongue, on the soft palate, and on the posterior wall of the pharynx. The free edge of the epiglottis and the surface of it toward the tongue, were covered with a fibrinopurulent film.

The temperature by the end of the day, and sometimes on the second day, was elevated by 2-3 degrees; it remained at these high figures for a while, and then abruptly dropped on the evening before or on the day of death of the animal.

The number of red cells in the blood during the 1st-3rd day increased by 1-2.5 million, then gradually declined, and by the 12th-13th day was below the original level by 1.5-2 million. The increase and decrease in the percentage content of hemoglobin corresponded to the curve of red cell changes in the blood. The ESR was accelerated as early as the first day, then slowed down along with the development of inflammatory changes in the mucosa of the upper respiratory passages and lungs. In individual cases, the ESR became prolonged from the third day to as long as 10 to 15 mm per hour, and by the fourth day was prolonged to 16-36 mm per hour. In the majority of animals, following the burn, the number of white cells in the blood rapidly increased to 11,000-33,000. The hematocrit during the first day, as a rule, increased by 10-20 percent, and by the 8th-9th day dropped to its original figures.
Very characteristic were the indices of hydrophilic of the tissues, which testified to the degree of dehydration of the organism. The rate of absorption of fluids following the burn rapidly increased and, by the 13th day, reached 10-15 minutes (the absorption rate prior to the burn was 44-50 minutes). Subsequently, the indices of hydrophilic slowly returned to normal; however, they did not reach the original levels even 39 days after the injury.

Upon capillaroscopy of the internal surface of the ear of the animals prior to the burn, there was a bright grayish yellow background with a network of fine, scarcely detectible capillaries and crisscrossing arterioles and venules; the light brown hair follicles could clearly be seen. After the burn, the background showed a marked darkening, became red, frequently with a cyanotic tinge; due to edema of the tissues, the capillaries, the lumens of which were markedly increased, were poorly distinguishable, the flow of blood in them was delayed (assumed a granular appearance), and the arterioles and venules were distended. Not infrequently we observed small hemorrhages and stasis. In the untreated animals, capillaroscopy prior to the end of observations showed no return to normal and the changes described above became progressively more severe.

The effect of hot steam was evident primarily on the mucosa of the upper respiratory passages; the degree and depth of the burn gradually decreased the further away the tissue was from the source of steam. Naturally, the degree and severity of the injury also depended on the duration of inhalation.

Upon exposure to steam for 12-15 seconds, there were deep necroses of the mucosa and submucosa of the respiratory passages as far as the bronchi of medium caliber, inclusive. There was also a partial necrosis of the cartilage of the epiglottis, the larynx and the trachea, as well as the soft tissues around the cartilages of the trachea. The mucosa of the small bronchi, even with prolonged exposure to steam, remained unchanged.

Exposure to steam for four seconds led to less profound and widespread necrosis. Necrosis was exhibited by the mucosa of the respiratory passages; changes in the cartilages, however, were negligible and were rarely seen. Foci of necrosis were observed as far as the bifurcation of the trachea, and these frequently disappeared even as high as the midportion of the trachea. The mucosa of the bronchi, even of the larger bronchi, rarely showed necrosis; the natural folds of the mucosa, for example in the larynx served as protection against the thermal effects (tissues at the bottom of the folds ordinarily remained undamaged). Sometimes there was a marked difference between the degree of necrosis above and below the vocal cords. In this case, apparently, an important
role is played by spasm of the vocal cords, which were capable of preventing, for a certain period of time, penetration of the hot steam into the areas of the respiratory passages below the vocal cords.

An inflammatory reaction in the respiratory tree did not develop immediately. On the first day after the burn, no such reaction could be observed, or else there was simply a small amount of edema of the mucosa with a light leukocytic infiltrate. Beginning on the second day and later, the picture of inflammation became more evident: edema, hyperemia, abundant leukocytic infiltration of the tissues, the frequent formation of a fibrinopurulent film on the surfaces devoid of epithelium. When the epithelium was preserved, the inflammatory infiltration was considerably less marked. In the small and medium bronchi, infiltration of the tissues was negligible or completely absent. Sometimes, accumulations of bacteria and fungi could be seen in the necrotic tissues.

In the lumen of the bronchi, there were frequently cells of desquamated epithelium, edema fluid, red cells, and white cells. On the second day after the burn, against a background of general congestion of the lung tissue, there were areas of edema, atelectasis, and acute emphysema, and following the second day, there were sometimes hemorrhages of varying size and shape. In some animals, on the day of the experiment, there were small foci of phenomena which were situated around the bronchi, with large accumulations of white cells in the exudate. Starting with the second day, these foci of phenomena were observed in the majority of animals (in 12 of 16 dogs), and later they increased in size, sometimes becoming confluent, while the exudate in the foci of inflammation remained catarrhal. After the third day, in many cases there were areas of necrosis of lung tissues with the presence of bacteria in them. In areas of inflammation and necrosis, after the third day, these processes also involved the walls of the small bronchi. In some cases, there was edema and leukocytic infiltration of the tissues surrounding the blood vessels. In animals which died, the parenchymatous organs (liver, kidneys, spleen) showed dystrophic changes and congestion.

Upon treatment of the burned animal, we gave principle attention to overcoming shock, evolving toxemia and septicemia, as well as phenomena.

For the treatment of the neurogenic shock of burn, a large number of methods are available at the present time; in order to prevent septicemia and the development of secondary foci of inflammation, antibiotics can be used. N. A. Fedorov and S. V. Skurkovich (4, 5) proposed immunotherapy for the prevention or control of rapidly developing burn auto-intoxication. The positive therapeutic effect of serum from convalescent patients recovering from burns has been proved by the authors under experimental conditions as well as under clinical conditions (2, 3).
our experiments we used: a) homoserum of convalescents in doses of 100 ml intramuscularly for 3 days after the burn (first series of experiments involving 9 dogs); b) the therapeutic serum of Belen'kiy (1), using the intravenous drip method in doses of 250 ml for 3 days after the burn (second series of experiments involving 8 dogs); c) homoserum of healthy dogs given in doses of 100 ml intramuscularly for 3 days after the burn (third series of experiments involving 9 dogs). All dogs received penicillin intramuscularly in doses of 100,000 units twice a day for 10-12 days, as well as spraying of the mucosa of the nose, mouth, and pharynx with a solution containing 50,000 units of penicillin twice a day for 10-12 days; d) the fourth series served as controls and involved 9 dogs which received burns but which were not treated.

We obtained our therapeutic antiburn serum from specially prepared donor dogs which had recovered from severe burns of the skin; the blood for preparation of the serum was taken 2-3 months after recovery of these animals.

The survival data of the experimental animals are shown in the Table. The most effective therapeutic measure proved to be the homoserum of convalescent animals in conjunction with the use of penicillin. Treatment of the burned animals with the serum of healthy animals and Belen'kiy serum in conjunction with penicillin was ineffective; all of these animals died, at early dates after burn.

The clinical manifestations of burn disease and the course of changes in the physiologic indices in the animals of the first series differed markedly from the evolution of burn disease in the untreated animals of the remaining series. Dogs treated with convalescent serum were more active, showed more initiative, and began to drink liquids and take food earlier than others. Within 5-7 days, these dogs showed a normalization of the body temperature, in distinction from the control animals and the animals treated with other methods. Also in contrast to these animals, the dogs of the first series showed less purulent and necrotic change in the areas of inflammation of the burned tissues.

As can be seen from the Figure, following the burn the content of red cells and hemoglobin increased markedly in all animals, but in dogs of the second, third, and fourth series, the hemoconcentration was greater than in the animals of the first series, in which the hemoconcentration by the fourth to fifth day was noticeably reduced. In dogs of the control, second, and third series, it continued to increase even further, and declined only by the seventh day.

An increase in the number of white cells and acceleration of the ESR in both groups following the burn continued for a period of 4-5 days.
Survival of Treated and Untreated Animals After Burning the Upper Respiratory Passages and Lungs

<table>
<thead>
<tr>
<th>Series of experiments</th>
<th>③ число животных после ожога</th>
<th>② число животных после ожога</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>выжившие в 1-й день</td>
<td>выжившие в 2-й день</td>
</tr>
<tr>
<td>Первая</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Вторая</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Третья</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Четвертая</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>

Key:
1. Series of experiments;
2. Number of animals in experiment;
3. Number of animals after the burn;
4. Surviving;
5. Dying;
6. On the first day;
7. On the second day;
8. On the third day;
9. On the fourth day;
10. On the fifth day;
11. On the tenth day;
12. On the eleventh day;
13. First;
14. Second;
15. Third;
16. Fourth.
The picture of the blood, the hematocrit, and hydrophilia of the tissues in burned dogs treated with convalescent serum (first series), Belen'kiy serum, and the serum of healthy dogs, or untreated (second, third, fourth series).

A - Changes in the number of red cells in the blood;
B - Percentage content of hemoglobin;
C - Changes in the number of white cells;
D - Changes in the ESR;
E - Changes in the hematocrit;
F - Changes in the hydrophilia of the tissues;
1 - Changes in the indices in dogs of the first series;
2 - In dogs of the second to fourth series. Indices prior to burn are taken as 100 percent.

Key:
1. Days.
Later, in animals of the first series, there began a slow reduction of these indices; however, in dogs of the second, third, and fourth series, the increase in the number of white cells and the ESR continued up to the time of death of the animals.

Of interest is the course of changes in the hematocrit. In dogs of the second, third, and fourth series, an increase in the hematocrit occurred quickly and reached a very high level, being as high as 118 percent by the 7th-10th day; whereas in the first series of experiments at this same time, the hematocrit had returned to the original values.

Similar changes were observed concerning the hydrophilicity of the tissues. Whereas in animals of the first series, the rate of absorption of fluid increased on the average of 30 percent with a gradual normalization, in animals of the second, third, and fourth series, by the 2nd-5th days, it had increased by 68-72 percent, and only by the 7th-10th days was there any decrease in it.

The Capillaroscopic picture following burns in control animals and in animals treated with Belen'kiy serum and serum obtained from healthy animals showed no normalization prior to the conclusion of observations. In the surviving animals of the first series, by the 10th-15th day the background gradually became lighter, more translucent, the edema gradually subsided, and the number and configuration of the capillaries returned to normal; by the 20th-30th day after the burn, the capillaroscopic picture, as a rule, could not be distinguished from normal.

The morphologic picture of the thermal injury in dying and sacrificed animals of the first series also showed differences from those in animals of the second, third, and fourth series. In animals treated with immune serum, and which either died or were sacrificed on the 13th-15th day after injury, the inflammatory reaction in the majority of areas of the mucosa had already disappeared, and at the site of necrosis there was granulation tissue with young epithelium, occasionally with persistence of the defects in the epithelium with inflammatory reaction in the underlying tissues. In dogs sacrificed on the 20th day after burn or later, the respiratory tree (excluding the nose) showed almost no traces of the burn; rarely there was a slight consolidation of the subepithelial tissues and small foci of small-cell infiltration in it. In animals which survived and which were sacrificed 13-27 days after the burn, we could no longer distinguish (with the exception of a single case) any traces of inflammation in the lung tissue.
THE ACID-ALKALINE BALANCE IN BLOOD LOSS COMBINED WITH A BURN

[FOLLOWING IS A TRANSLATION OF AN ARTICLE BY N. V. LUNINA, FROM THE CHAIR OF PATHOLOGIC PHYSIOLOGY (HEAD-PROFESSOR N. N. SMOY) OF THE LUGAN MEDICAL INSTITUTE, IN THE RUSSIAN-LANGUAGE JOURNAL PATOLOGICHESKAYA FIIOLOGIYA I EKSPERIMENTAL'NAYA TERAPIYA (PATHOLOGIC PHYSIOLOGY AND EXPERIMENTAL THERAPY), NO. 1, MOSCOW, 1963, PAGES 28-31.]

Affections of the organism in which two pathologic processes develop immediately are a phenomenon which is not infrequent in the practice of traumatologist and surgeons. Frequently, both processes occur in a definite interaction, which substantially alters the character of the course of each of them.

The few existing works in the literature indicate that different unfavorable effects on the organism (overheating, overcooling, and neuropain trauma), accompanied by loss of blood, may considerably aggravate its course (?). The authors relate this aggravation to the limitation under these conditions of the adaptive capacities of the organism. Upon loss of blood in amounts totaling 1-2 percent of the weight of the body, there is respiratory alkalosis and metabolic acidosis which lead to a reduction in the alkaline reserve of the plasma, not to speak of the reduction in the buffer properties of the blood due to the loss of hemoglobin (6). In posthemorrhagic anemias, respiratory alkalosis develops; however, due to the compensatory processes, the acidification of the urine remains normal. In experiments on rabbits it was shown that, with moderate blood loss, intermediary metabolism shows no substantial disturbances and there is no increase in the concentration of unoxidized substances in the urine; the oxidation coefficient of the urine may in this case even drop slightly due to compensatory activation of oxidative processes (1, 2, 6).

Thermal burns also disturb, to a certain extent, the normal course of oxidative processes. Burns of the skin occupying not less than 10 percent of the surface reduced the intensity of oxidative processes, which lead to a decrease in the alkaline reserve of the blood and to an increase in the acidity of the urine (5). Proportionate to the severity of the burn, there was an increase in the blood level of organic acids; burns of 10-20 percent of the body surface caused clotting of the blood;
the oxidative coefficient of the urine increased by the following day after the burn and was again normal by the second week (4). The cause of this is tissue hypoxia, caused by disruption of the oxidative enzyme systems in the tissues. Lactacidemia with metabolic acidosis has been observed in burns, along with an increase in the unoxidized components of the urine.

We investigated certain indices of disturbance in the acid-base equilibrium under conditions of combined blood loss and thermal burn. We were interested in determining, under these conditions, how thermal burns influence the resistance of the organism to the acute hypoxia caused by blood loss, as well as the compensatory processes elicited by it.

In order to study the disturbances in oxidative processes, we studied the alkaline reserve of the plasma, as well as the titrable and active acidity of the urine, since variations in the alkaline reserve alone do not always completely or even adequately reflect disturbances in acid base equilibrium.

Four series of experiments were carried out on 50 rabbits weighing 1.5-2.5 kilograms: the first with blood loss amounting to 0.4 percent of the body weight, without burn (controls); the second, the same with burn; the third, loss of blood amounting to 1.2 percent body weight, without burn (control), and fourth, same with burn.

The blood was withdrawn by means of removing it from the ear vein; in order to appraise the consequences of blood loss in the blood, we measured the content of hemoglobin and red cells. The alkaline reserve of the plasma was studied by the method of Van Slyke. The urine of the rabbits was collected from their cages into flasks, filtered, and the titratable acidity was determined by the use of phenolphthalein indicator and the active urine, that is the pH value, by the Michaelis colorimetric method.

Burns were imposed immediately after blood loss by applying to the skin of both sides of the chest two flasks containing water heated to 80° centigrade for five minutes. The total area of the burn was 77 sq. cm. In computing the percentage of burned surface, the total surface of the body was computed by the formula $C = kr \times 2/3$, where $C$ is the surface area (in square centimeters), $r$ is the weight (in grams), and $k$ is the coefficient (8.5-6.5 depending upon the weight of the rabbits, which range from 1.5-2.5 kg). The burned surface constituted, therefore, 8-10 percent; the intensity of the burn corresponded to the second degree. The alkaline reserve of the plasma and the acidity of the urine were determined before and 24 hours after the onset of the experiment, and the hemoglobin and red cell determinations were made before and 1.3 and 24 hours after the onset of the experiment.
The results of the studies are shown in the Table.

As can be seen from the table, changes in the number of red cells and in the amount of hemoglobin 1 and 3 hours after the onset of the experiment in cases of mild blood loss (0.4 percent of the animal's body weight) and burn, testify to a slight hemoconcentration of the blood which disappeared within 24 hours. Due to this, the posthemorrhagic anemia which was observed in the first series of experiments and which is characterized by a reduction in the number of red cells per cu. mm. of blood, was not detectible. Apparently, the complex interactions which are produced are capable both of reducing the output of fluid from the tissues (muscles, liver) into the blood (blood loss), and of increasing its passage into the tissues (burn). From the fact of the preservation of almost normal numbers of red cells in the blood following this slight blood loss in the presence of burn, it is necessary to postulate the presence in this group of rabbits of a general oligemia over the course of almost the entire day. This did not occur in cases of massive blood loss (1.2 percent body weight), combined with similar burns, in which case there was dilution of the blood. As to the acid base equilibrium, the small reductions noted in the first series in the alkaline reserve of the plasma and the reduction both of the titratable and of the active acidity of the urine (the pH of the urine was increased), serve as indications of the development of respiratory alkalosis, due to the accelerated respirations of the animal, which is a characteristic consequence of minor blood loss.

In the second series of experiments, in which, in the presence of similar blood loss, burns were administered to the rabbits, the alkaline reserve declined considerably more than in the first, while the acidity, both titratable and active, of the urine increased. These changes characterized the state of metabolic acidosis which obviously occurs as a result of the burn.

The results of the experiments of the third and fourth series were in complete agreement with these data. However, despite the fact that blood loss and the concomitant respiratory alkalosis in the third and fourth series were greater than in the first and second, the metabolic acidosis following the application of burn was more marked in the fourth series than in the second. If we admit that the influence on the acid base equilibrium of blood loss (alkalosis) and of burns (acidosis) to a certain extent cancel each other out, nevertheless one might sooner expect the opposite of this, for the conditions of production of the burn in the second and in the fourth series of experiments were identical. Hence we must admit that here, in actuality, we do not have two pathologic processes which, to a certain extent, act opposite to each other with respect to the acid base balance, but rather the creation of a single complex pathologic and protective and compensatory reaction in response to a combined injury. In the presence of great blood loss, compensation
Changes in the Amount of Hemoglobin, Red Cells, Alkaline Reserve of the Blood, and Fibrinogen in Conjunction with Blood Loss

<table>
<thead>
<tr>
<th>Procedure (in reference column)</th>
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<th>Test #2</th>
<th>Test #3</th>
<th>Test #4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (mg per liter)</td>
<td>10</td>
<td>55</td>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td>Red cells (x 10^4 per ccm)</td>
<td>+1.9</td>
<td>+2.2</td>
<td>+3.0</td>
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</tr>
<tr>
<td>Alkaline reserve of the blood</td>
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<td>4,800</td>
<td>4,800</td>
<td>4,800</td>
</tr>
<tr>
<td>Fibrinogen (mg per liter)</td>
<td>3,710</td>
<td>4,650</td>
<td>5,900</td>
<td>7,150</td>
</tr>
</tbody>
</table>

Key:
1. Sories of experiments;
2. Number of rabbits;
3. Hemoglobin in ml units;
4. Before the experiments;
5. After 1 hour;
6. After 2 hours;
7. After 3 hours;
8. After 24 hours;
9. Red cells (in thousands per cu. mm.);
10. Alkaline reserve of the blood;
11. Fibrinogen activity of the urine;
was less complete and less capable of counteracting the acidosis which developed in consequence of disturbances in oxidation-reduction processes which were caused by the burn trauma and by hemocoagulation of the blood.

CONCLUSIONS

1) In cases of acute loss of blood (0.4 and 1.2 percent of body weight) in rabbits, a state of respiratory alkalosis developed which was manifest as a reduction in the alkaline reserve of the plasma, as well as in the titratable and active acidity of the urine, being the more pronounced the greater the blood loss.

2) The infliction on the rabbits of second degree burns occupying 8-10 percent of the body surface caused in them the development of metabolic acidosis, which was manifest as a reduction in the alkaline reserve of the plasma and an increase in the titratable and active acidity of the urine.

3) The greater the blood loss which accompanied the burn, the more marked was the general acidosis which, apparently, may be related to the more extensive disturbances in protective and compensatory reactions of the organism arising in response to circulatory hypoxia caused by blood loss.

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