

# CHANGES IN VITAL STAINING OF THE MARROW CELLS OF THE OCCIPITAL BONES AFTER DESTRUCTION OF THE GASSERIAN GANGLION

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The object of this investigation was to determine ways of studying the trophic function of the nervous system in regulating the functions of bone marrow tissue.

The problem of the trophic function of the nervous system is attracting considerable attention at the present time. The changes arising after disturbances of the innervation of tissues and organs are expressed by the development of degenerative processes: disturbances of the structure and function of the tissues, loss of the power of complete regeneration of the tissues, and disturbances of metabolism. The most marked trophic disturbances arise in the tissues after deafferentation.

The morphological composition of the bone marrow of the long bones of the limbs after division of the sciatic nerve has now been studied reasonably fully [1-5].

A disturbance of the innervation of the bone marrow has a marked effect on its cell composition, leading to a decrease in the number of young cells of the myeloid series in the marrow, an increase in the percentage of mitoses and mature forms, and the appearance of plasma cells and other changes in the tissue structure of the marrow. These changes were found not to be restricted to the denervated area of the marrow, but to extend also into its intact region [1, 5, 6]. Changes in the function of medullary hemopoiesis after disturbance of the innervation of the marrow, leading to generalized changes in the peripheral blood, are the result of a disturbance of the trophic function of the nervous system.

In the present investigation a study was made of the ability of the bone marrow cells of the occipital bone of the rabbit to undergo vital staining with fluorescein before and at various periods after destruction of the gasserian ganglion.

Puncture or destruction of the gasserian ganglion leads to the development of a neuroparalytic keratitis, a typical degenerative process. In this case trophic ulcers also developed on the mucous membrane and skin of the lips on the side of the operation. Undoubtedly all tissues on the deafferented side, not excluding the bone tissue, undergo trophic changes after the procedure described.

## EXPERIMENTAL METHOD

Fluorescein was injected intravenously into the rabbit in a dose of 250 mg (5 ml of a 5% solution)/kg body weight. Thirty minutes after injection of the dye the marrow was extracted from both occipital bones, and films of the marrow were examined under the microscope in a transmitted ultra-violet spectrum from a type PRK-4 lamp. The bone marrow cells with different intensities of fluorescein were counted and the ratio between the number of brightly fluorescent cells to 100 cells with weak fluorescein was determined.\*

Injection of fluorescein and extraction of the marrow from the occipital bones on both sides were carried out once only on the various animals at intervals of 1 h, and 1, 2, and 3 weeks after destruction of the gasserian ganglion on the right side. Proof of the correct performance of the operation was total loss of sensation in the right cornea and the skin of the right half of the head.

\* To avoid the possibility of subjective error, all films taken from the side of the operation or the control were mixed and the identification marks on the films were read only after the brightly and weakly fluorescent cells had been counted.

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Director of Research, Active Member of the Academy of Medical Sciences of the USSR Professor A. V. Lebedinskii (Presented by Active Member of the Academy of Medical Sciences of the USSR A. V. Lebedinskii). Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 62, No. 11, pp. 112-114, November, 1966. Original article submitted December 21, 1964.

# Character of Vital Staining of Marrow Cells of the Occipital Bones of the Rabbit after Destruction of the Right Gasserian Ganglion

| Time of experiments | No. of animals | No. of bone marrow films | No. of bone marrow cells counted | Number of fluorescent marrow cells     |        |                                  |        |
|---------------------|----------------|--------------------------|----------------------------------|--|--------|----------------------------------|--------|
|                     |                |                          |                                  | On the right, on the side of operation |        | On the left, on the control side |        |
|                     |                |                          |                                  | brightly                               | weakly | brightly                         | weakly |
| Normal conditions   | 2              | 12                       | 1807                             | 25                                     | 100    | 26                               | 100    |
| After operation     |                |                          |                                  |  |        |                                  |        |
| 1 h                 | 2              | 12                       | 1611                             | 32                                     | 100    | 24                               | 100    |
| 1 week              | 3              | 17                       | 2279                             | 44                                     | 100    | 22                               | 100    |
| 2 weeks             | 2              | 12                       | 1734                             | 35                                     | 100    | 18                               | 100    |
| 3 weeks             | 3              | 20                       | 2797                             | 29                                     | 100    | 24                               | 100    |

Altogether 12 animals were used in the experiment, 73 films were made, and more than 10,000 cells were counted.

## EXPERIMENTAL RESULTS

In normal conditions the ratio between brightly and weakly fluorescent cells was found to be 25:100 on the right side and 26:100 on the left (see table). A slight decrease in the ratio between brightly fluorescent and weakly fluorescent cells was observed on the control side in the animals undergoing the operation. On the side of the operation in the same animals the ratio between the number of brightly fluorescent and weakly fluorescent cells increased. The maximal increase (to twice the figure) was observed in films taken one week after destruction of the gasserian ganglion. By the end of the experiment 3 weeks after the operation the number of brightly fluorescent bone marrow cells on the side of the operation fell and the ratio between brightly and weakly fluorescent cells came close to the normal value.

In these experiments the trophic degenerative disturbances of the superficial tissues on the side of the operation appeared after the maximal intensity of changes in the character of vital staining of the marrow cells had appeared. Trophic ulcers in the mucous membrane and skin of the right lip developed in individual rabbits two weeks after destruction of the gasserian ganglion. Neuroparalytic keratitis, developing in rabbits kept in ordinary conditions on the 2nd-3rd day after destruction of the gasserian ganglion, in the present experimental conditions, in which the insensitive cornea of the animals undergoing the operation was protected against mechanical injury by fixing the lids together, also appeared no sooner than 2 weeks after the operation.

The changes in the myelograms of the degenerated bone marrow of the long bones is achieved, according to She Yink [5, 6] on the 30th-50th day after removal of the final ganglia. É. I. Terent'eva and co-workers [3] found the most marked signs of degeneration of the bone marrow between one and three months after denervation of the hind limbs. A characteristic finding in this case was that the period of most severe depression of myelopoiesis coincided with the appearance of degenerative changes in the tissues of the denervated hind limb; loss of hair, the edema, ulcers and scabs on the skin, necrosis of muscles and bone tissue [1].

In the present investigation, the most marked changes in the morphological picture of the marrow cells\* also coincided with the appearance of trophic degenerative processes in the skin and cornea, two weeks after destruction of the gasserian ganglion. The changes in the myelograms were expressed by an increase in the proportion of granulocytes and acceleration of their maturation, and an increase in the percentage of adult forms and in the percentage of mitoses. No difference could be found between the changes in the myelograms from the right and left occipital bones. In other words, the character of the morphological disturbances of the marrow from the occipital bones after unilateral destruction of the gasserian ganglion was analogous to the changes in the myelograms of the long bone of the hind limbs observed after division of the sciatic nerve or removal of the spinal sensory ganglia of the lumbar segments.

\* The myelograms of the marrow films from both occipital bones of the rabbits at different times after destruction of the right gasserian ganglion were compared by Senior Scientific Assistant V. A. Gubin.

It may, thus be concluded that the development of the trophic disturbances in the deafferented marrow tissue takes place in a certain order. The initial disturbances take the form of an increase in permeability and in the sorption properties of the cytoplasm of the marrow cells, accompanied by more intensive staining of the marrow cells after intravenous injection of the dye. These changes reach their maximum one week after destruction of the gasserian ganglion. Rather later, two weeks after the operation, trophic ulcers appear in the mucous membrane and skin of the lips, and also in the deafferented cornea, although protected against mechanical injury. This moment coincides with the development of marked degeneration of the medullary cells of the myeloid series, giving rise to a picture of atrophy and degeneration of the bone marrow.

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