CHANGE IN THE TISSUE RESPIRATION IN REGENERATING TISSUES AGAINST A BACKGROUND OF ADMINISTRATION OF PHENAMINE AND BARBAMYL

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We established earlier that phenamine, which produces an increase in the excitability, both in the cerebral cortex and in the subcortical regions [2], leads to a substantial intensification of the basal metabolism and thereby promotes an accelerated healing of wounds. Barbamyl, which intensifies the cortical inhibition, produces a decrease in the level of metabolism and inhibition of the activity of the cellular elements of the connective tissue participating in healing [3, 4].

In this communication we present the results of a study of the tissue respiration in the regeneration of skin in animals against a background of the administration of phenamine and barbamyl.

PROCEDURE

In the experiments we used 136 albino rats, 150-200 g in weight. The animals were divided into three groups. The rats of the control group (first) did not receive the preparations. The animals of the second group received daily injections of 0.1 mg of phenamine, those of the third group daily injections of 10 mg of barbamyl. The administration of the preparations was begun three days before wounding and was continued for 10 days.

The tissue respiration of skin slices (including the wound and its edge) was measured manometrically according to the Warburg method; the skin slices were incubated for 2 h at 37° in a Krebs-Ringer phosphate buffer solution (pH 7.4) in an atmosphere of oxygen. The amount of oxygen absorbed in 2 h and calculated with respect to 1 mg of dry matter of the investigated tissue characterized the respiratory intensity and was denoted as the respiratory quotient, Q_{O_2}.

Before the beginning of the experiment, the respiratory intensity of normal, nonregenerating skin was determined, after which the animal was wounded over an area of 225 mm^2. At different stages of healing—1,3,5,7,9,13, and 17 days after the injury, the respiration of the skin was determined, the area of the wound was measured, and its edges were biopsied. The material was subjected to the usual histological treatment. The height of the leukocytic torus, granulation tissue, and young epithelium was measured with an ocular micrometer.

RESULTS

Preliminary experiments, conducted on a large number of animals of different sexes and weights, showed that the respiratory intensity of normal, undamaged skin varies negligibly and lies within the range 1.8-2.9 ml of oxygen per milligram of dry tissue weight.

Wounding and the subsequent recovery process in the skin was accompanied by a change in the level of the tissue respiration (Table 1). A certain increase in the absorption of oxygen was noted five days after the operation. A substantial increase in the level of the tissue respiration was observed 11-13 days after healing began. During this period, the endogenous respiration of the regenerating skin was a maximum. During the following days, at the end of the healing period, there was a gradual lowering of the activity of the tissue respiration.

TABLE 1. Amount of Absorbed O_2 (in mm^3 per mg of dry weight of skin) at Various Periods After Wounding

Group of animals	Period after injury (in days)								
	1	3	5	7	9	11	13	15	17
Control	2.1	2.2	4.2	1.9	2.1	6.3	11.4	6.1	4.0
Received phenamine	2.2	8.0	7.0	10.6	24.5	11.8	20.2	21.5	6.9
Received barbamyl	1.6	2.0	1.45	1.5	1.6	4.1	10.3	4.2	2.0

TABLE 2. Dimensions of the Wound Surface and of Certain Structures of the Wound

Group of animals	Area of wound (in mm^2) after				Height (in μ)			
	3 days	7 days	9 days	11 days	Leukocytic torus	Layer of granulation tissue	Epithelium at boundary of defect	Epithelial layer
Control	130.5	84.9	56.1	24.9	124.8	1264.8	88.2	58.4
Received phenamine	81.4	50.5	39.9	7.3	72.9	1521.5	125.7	94.8
Received barbamyl	176.6	97.3	84.3	70.2	249.5	627.0	31.2	22.5

Under the influence of phenamine, the respiratory intensity was increased at all stages of healing of the wounds. After three days, a strong increase in Q_{O_2} had already occurred. After five days, a negligible decrease was observed, and subsequently the respiratory intensity again increased sharply, reaching the greatest value after nine days. After a transitory decrease in the respiratory level, it increased sharply a third time, and decreased after 17 days, still remaining considerably higher than in the control.

The administration of barbamyl produced a lowering of the intensity of the tissue respiration. A low level of metabolism was observed during the entire first half of the healing period. Intensification of the respiratory activity of the skin was noted only 11-13 days after the operation. However, the amount of oxygen absorbed by the tissues was even smaller than in the animals of the other series. The increase in the respiratory intensity was transitory, and was replaced by a sharp inhibition after 15-17 days.

The change in the cortical activity of the animals, induced by the administration of phenamine or barbamyl, finds a sufficiently objective reflection in the level of the tissue metabolism and in the course of the recovery process in the skin. Data on a measurement of the area of the wound and information on the magnitude of the newly formed structures (11 days after the operation) are presented in Table 2.

The increase in the intensity of tissue respiration, observed in the animals of the control group three to five days after wounding, is related to the development of granulation tissue. Eleven days after the operation, the region of the injury was filled with differentiating granulation tissue, in which three layers were distinctly pronounced: leukocytic-necrotic, a layer of vertical vessels, and a layer of horizontally arranged fibroblasts. The bulk of the granulation tissue was made up of the layer containing the vertical vessels, with a large number of hematogenic cellular elements. The epithelial layer, consisting of from four to six layers of cells, grew under the scab along the young connective tissue. Eleven days from the beginning of healing, when various structures were differentiated and the granulation tissue was actively growing, a substantial reduction of the area of the wounds was observed, which corresponded to the highest level of tissue respiration of the regenerating skin.

During the process of the healing of wounds in animals that received phenamine, three waves of increase in the level of tissue respiration were noted. The first (negligible intensification of the activity of the tissue respiration) was observed three days after the operation and corresponded to the development of a luxuriant granulation layer. The second (a more pronounced increase in the tissue respiration) was noted after 7 to 9 days, when the region of the injury was filled with differentiated granulation tissue, consisting of three layers, differing from the control in their degree of expression. The granulation tissue situated below the small leukocytic torus consisted primarily of a layer of horizontally stratified fibroblasts. A substantial portion of the wound surface was covered with

young epithelium. The regenerate took the form of a wedge, consisting of 8 to 10 rows of cells. The basal surface of the young epithelium formed projections into the underlying connective tissue. The intensive growth of the epithelium and acceleration of the formation of granulation tissue into connective tissue led to a sharp reduction of the wound area.

Eleven days after the operation, the region of the injury was filled with connective tissue of typical structure and covered with newly formed epithelium.

In this series of experiments, the third increase in the respiratory intensity of the regenerating skin 13 to 15 days after the operation was paralleled by the formation of skin derivatives: sebaceous glands and hair follicles.

Our data correspond to the results of the investigations of Mann and Quastel [8], who showed that under the influence of phenamine, the oxygen uptake of the brain tissue is sharply increased.

In the rats that received barbamyl, the intensity of the tissue respiration remained especially low during the first half of the period of healing of the wound. At the same time, a strong inhibition of the recovery process was also observed. An increase in the tissue respiration intensity did not occur until 11-13 days after the operation, when granulation tissue developed with a great time lag in the region of the injury. In this group of experiments, thinning of the layer of granulation tissue was observed: the thickness of the leukocytic-necrotic layer increased, and the width of the layer of vertical vessels was substantially reduced; the layer of horizontal fibroblasts was underdeveloped. The later formation of the granulation tissue also had an effect on the nature of the epithelization of the wound surface. Great thinning of the epithelial regenerate and a decrease in the number of cellular layers was observed; the basal surface of the epithelium was even; no projections into the underlying tissue were formed.

The tissue respiration was increased for four to five days, and then was sharply reduced. In spite of a transitory increase in the level of respiration, the recovery process was inhibited, which is evidently related to profound changes in the trophics of the tissues, caused by a prolonged state of cortical inhibition under the influence of barbamyl.

A number of the newest investigations also indicate serious disturbances of the metabolic processes in the tissues of animals under conditions of drugged sleep [1, 5, 6, 7].

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