CHANGES IN GAS EXCHANGE IN ANIMALS DURING REGENERATION UNDER THE EFFECT OF PHENAMINE AND BARBAMYL

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The role of the central nervous system in performing restorative processes in skin has been shown in a number of investigations [2, 9-11]. The effect of the nervous system also propagates to processes of metabolism, which are the physiological basis of regeneration [1, 3, 4]. We can assume that the injection of phenamine (amphetamine) and barbamyl (amobarbital sodium) [5, 8], which enhances cortical excitation or inhibition in the central nervous system of animals, should lead to a change of metabolism and rate of healing.

The purpose of the present investigation was to study the changes of total gas exchange in animals having different functional states of the central nervous system during development of the regeneration process.

EXPERIMENTAL METHOD

The experiments were carried out on 187 white rats (average weight 152 g). The animals were divided into 3 groups. The animals of the I, control, series of experiments did not receive preparations; the rats of the II series of experiments were injected with phenamine (0.1 mg); in the III series of experiments the animals were given barbamyl (10 mg). The preparations were used for 10 days. Total gas exchange was determined in a Haldane apparatus. The quantity of absorbed oxygen and evolved carbon dioxide was expressed in milliliters per 100 g body weight per hour. In each determination we established the value of the respiratory quotient (RQ). At least 2 determinations were made for each animal to elicit the normal value of gas exchange. In all, 367 determinations were made.

Before using the preparations we determined the level of total gas exchange in the norm. After 3 day administration of the drugs we again established the magnitude of total gas exchange and removed a flap of skin measuring 225 mm^2. During the entire period of healing the basic indexes of exchange were determined repeatedly every 2, 4, 6, 8, 10, 12, and 16 days. During these periods we measured the area of the wounds and excised the skin from the wound edges. The material was subjected to the usual histological examination. The sections were stained with hematoxylin-eosin; at the same time glycogen was elicited by Shabadsh's method.

EXPERIMENTAL RESULTS

Before injecting the preparations the level of total gas exchange was normal in all animals (Table 1). The infliction of the wounds and the reparative phenomena in the damaged area caused an increase in the level of gas exchange. The quantity of absorbed oxygen only a day after the start of healing increased 26.8% as compared with the initial value. After 4 days the level of total gas exchange reached a maximal value (the quantity of absorbed oxygen increased 68.6% as compared with the normal). Then followed a drop in this level and its gradual approach to normal. The RQ value changed little during regeneration and was 0.70-0.75.

The considerable increase of the basic indexes of exchange which was observed after 1-2 and 4-8 days was associated with the beginning of the development of granulation tissue and growth of young epithelium. The return to the normal level of exchange ensued after the end of healing of the wound, when scars were at the injured site (Table 2).

TABLE 1. Alteration of the Gas Exchange Indexes of White Rats During Regeneration Under the Effect of Phenamine and Barbamyl

Series of Expts.	Before injection of preparations			3 Days after injection of preparations			One day after operation			After 4 days			After 8 days*			After 16 days		
	Absorption of O_2	Evolution of CO_2	RQ	Absorption of O_2	Evolution of CO_2	RQ	Absorption of O_2	Evolution of CO_2	RQ	Absorption of O_2	Evolution of CO_2	RQ	Absorption of O_2	Evolution of CO_2	RQ	Absorption of O_2	Evolution of CO_2	RQ
	In ml/100 gram h			In ml/100 gram h			In ml/100 gram h			In ml/100 gram h			In ml/100 gram h			In ml/100 gram h		
I	179	126	0.70	174	126	0.72	227	156	0.69	301	234	0.77	265	188	0.70	192	144	0.75
II	185	132	0.71	241	169	0.70	336	247	0.74	342	248	0.72	282	207	0.73	250	204	0.80
III	198	157	0.79	196	137	0.70	243	212	0.87	195	133	0.68	231	149	0.64	174	161	0.92

* The effect of the preparations was stopped.

The index of total gas exchange proved to be highest for animals that received phenamine. After only 3 days the injection of the preparation caused an increase in the level of gas exchange. The infliction of wounds led to a further enhancement of oxidative processes. A day after the trauma the quantity of absorbed oxygen increased 81.6%, i.e., more appreciably than in the control. After 4 days the indexes of exchange again rose, reaching a maximal value. After a brief decline a new increase in the intensity of the exchange was observed after 8 days. The intensity remained high even after completion of the reparative process. The RQ value and the effect of phenamine varied in wider limits, being 0.7-0.8. The high level of exchange that preceded the infliction of the wounds and accompanied proliferation of the young tissues, ensured accelerated healing of the injuries (see Table 2).

Barbamyl, enhancing the process of cortical inhibition, led to an appreciable drop of total gas exchange. Its injection for 3 days caused a drop in the intensity of metabolism, the degree of which severely varied in different animals. Wounding was accompanied by a noticeable increase in the quantity of absorbed oxygen (22.7% above the initial value). However, only 2 days after the infliction of the wounds the high level of exchange was replaced by low indexes of the quantity of absorbed oxygen and evolved carbon dioxide. It was precisely at this period that we noted large wound areas in the animals. Later there was a gradual increase in the level of gas exchange. After 8 days it was maximal for animals that received barbamyl, but it was appreciably lower than in the control and in the rats that were given phenamine. In spite of the enhancement in the intensity of oxidative reactions, healing of the wounds even at this period of the observation was greatly inhibited. Beginning with the 8th day the level of gas exchange continued to drop again and after 16 days was appreciably lower than the initial value. The low level of exchange during the first period of healing created adverse conditions for epithelization of the injuries. The area of the wounds in the 2nd half of the healing period contracted very slowly and after 16 days was 32 mm^2, whereas in the animals of the control group and in those that received phenamine, the reparative process by this time was completed by the formation of a scar. The low level of exchange in animals that received barbamyl was accompanied by low RQ values (to 0.6), which is evidently the result of a severe disturbance and perversion of the course of oxidative processes in the animals and gives us grounds to assume the possibility of the transformation of fats and proteins to carbohydrates [7].

A difference between the data obtained in the control and the experimental series, noted in the measurement of the area of the wounds, was also revealed in the microscopic study of the biopsy material of young tissues. For illustration we will cite the results of the microscopic study of the injured area 8 days post operation, i.e., at the period when distinct differences were noted in the level of basal metabolism.

The animals of the control series of experiments had developed granulation tissue of a typical structure. A large part of the granulations consisted of capillaries arranged vertically with respect to the wound surface. On treating the histological sections with periodates and Schiff's reagent it was established that the glycogen granules in the granulation tissue were situated primarily in neutrophils; the macrophages and fibroblasts contained traces of glycogen. The epithelium growing over the

TABLE 2. Area of Wounds (in mm^2) in Subsequent Stages of Healing

Series of expts.	Time after operation (in days)								
	0	2	4	6	8	10	12	14	16
I	225	195±4,11	183±3,76	120±3,36	95±2,01	81±3,23	76±1,73	30±1,96	Рубец
II	225	108±3.28	92+2,56	50±2,13	48±1,35	42±1,97	30±1,46	Рубец	—
III	225	197±5,21	184±2,99	121±2,55	121±2,55	90±2,39	83±2,23	58±0,87	32±2,52

wound surface and consisting of 6-8 layers of cells, grew under the scab. Glycogen in the form of granules was found in the regenerating epithelium, in the elements of the middle zone of the Malphigian layer of the epidermis (see figure, a). Glycogen was absent in the surface layers of the epidermis and in the epithelium of normal skin.

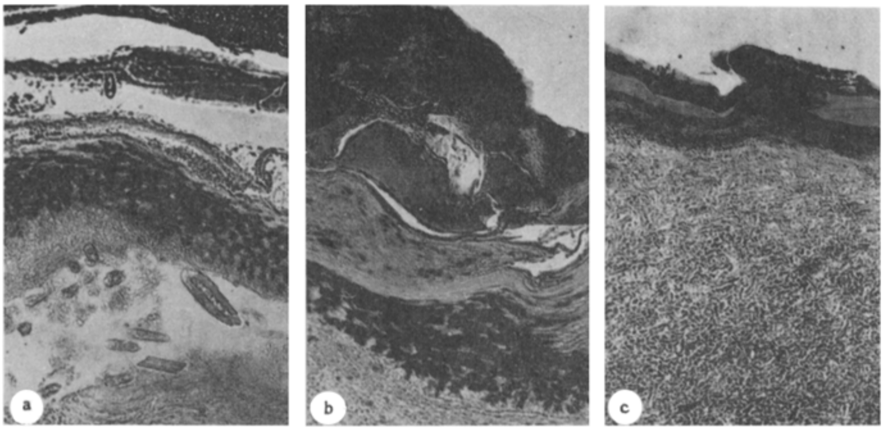
Under the effect of phenamine the layer of granulation tissue was characterized by an appreciable thickness as compared with its thickness in the animals of the 2 other series of experiments. The granulation tissue consisted primarily of a lyer of horizontal large fibroblasts and collagen fibers. On treating the histological sections with Schiff's reagent it was established that the glycogen in the granulation tissue was arranged in individual neutrophils which infiltrated the surface portions of the tissue, in macrophages, and in certain young fibroblasts. Under these conditions we noted an intense proliferation of young epithelium. The epithelial wedge was longer than in animals of the other experimental series. On treating the sections with Schiff's reagent many glycogen granules were detected in the epithelial regenerate. The basal layer of the regenerate and the cells of the undamaged epidermis did not contain glycogen (see figure, b). The absence of glycogen in normal skin and in skin taken at later periods indicated that even under the effect of phenamine the accumulation of glycogen is limited by the area of the epithelial regenerate under conditions of a high metabolic level. It is possible that resynthesis of glycogen in these areas occurs less energetically and only covers its expenditures. On the other hand, with intense proliferation of the epidermis there is an enhanced breakdown and consumption of glycogen under conditions of a maximal high level of metabolism. However, its synthesis proves to be excessive and it is detected in the young epidermis at the early stages of healing. Similar changes of carbohydrate metabolism were noted in brain tissue [6].

In the rats that received barbamyl the granulation tissue was very weakly developed and was situated only along the wound edges; in the central part the wound was filled with fatty cellular tissue and infiltrated with various wandering cells. The layer of horizontal fibroblasts, which was well represented in animals that received phenamine, was not developed in the rats that received barbamyl. The neutrophils frequently encountered in the superficial layers of the granulation tissue contained little glycogen. Sporadic small granules of glycogen were situated in microphages and young fibroblasts. Regeneration of the epithelium was weakly expressed. The thin epithelial wedge was short. Under the effect of barbamyl the content of glycogen dropped in the cells of the regenerating epithelium. Glycogen was situated only in 2-3 cell layers of the thin epithelial wedge. In certain cases we noted the presence of glycogen in the surface layers of the epidermis and its absence in the cells of the middle zone (see figure, c). At later periods the quantity of glycogen somewhat increased, but nevertheless it was less than in the control. Evidently with the injection of barbamyl under conditions of a low level of exchange there occurs not only an inhibition of glycogen synthesis but also a drop in its consumption. Even in the period of evident proliferation of the epithelium with a negligible enhancement of total metabolism there was no accumulation of glycogen in the cells of the middle zone of the epidermis, which was so characteristic for the control animals. The noted shifts in carbohydrate metabolism are an expression of the inhibition of glycolytic processes in tissues and a decrease of the oxidation-reduction processes in the organism in consequence of serious impairment of total gas exchange, which was observed in animals when barbamyl was injected [6].

Thus, an enhancement of the main nervous processes by injecting phenamine or barbamyl and a disturbance of their relationship changed the direction of the course of metabolic reactions and caused a change in the rate of healing of the wounds corresponding to the level of metabolism.

SUMMARY

Changes in the total gas exchange during healing of skin wounds were studied in albino rats in conditions of varying functional activity of the higher portions of their CNS. Administration of phenamine, which enhanced the cortical excitation process brought about a more intense exchange thus contributing to a more rapid healing of the



Newly formed structures of a healing wound: glycogen in cells of regenerating epithelium. a) Control; b) effect of phenamine; c) effect of barbamyl. Photomicrography. Treatment with periodata and Schiff's reagent. Magnification 120 ×.

wound. In these conditions there was a rise in the activity of cellular elements of mesenchymal nature, and also a more rapid alteration of individual phases of inflammation and regeneration. Barbamyl, while enhancing the inhibition in the cerebral cortex, led to reduction and inversion of the total gas exchange, depressing regeneration process in the skin.

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