THE EFFECT OF PAIN STIMULATION ON THE PHAGOCYTIC POWER OF THE LEUKOCYTES IN HORSES

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It is clear from data in the literature that the phagocytic function of the leukocytes is regulated by the nervous system and is increased by pain stimulation [4, 5, 7, 9, 11, etc.]. Although the leukocytes are feed blood cells and have no anatomical innervation, their phagocytic activity is regulated by mediators secreted into the blood stream by the nerve endings of the vegetative nervous system. Sympathin causes increased phagocytosis and "vagus-stoff" causes its depression.

METHOD

The changes in the phagocytic function of the leukocytes during the healing of wounds were studied in 18 horses under various conditions: without anesthesia, with ordinary lumbar paravertebral block, and with prolonged lumbar paravertebral block.

The phagocytic index was determined by the following method: Into two sterile agglutination tubes, each containing 0.05 ml of a 6% solution of sodium citrate, was poured 0.2 ml of whole blood obtained from the jugular vein. After agitation of the contents of the tubes, to one of them was added 0.25 ml of a standard suspension of streptococci, and to the other, the same volume of a standard suspension of Staphylococcus aureus. The suspensions consisted of washings of a 24-hr culture of these microorganisms, and contained 2×10^9 bacterial cells/ml. The blood was mixed with the suspension of the microorganisms and then allowed to stand in the incubator at 38° for 30 minutes. It was essential that a fresh bacterial suspension be prepared before the reaction by washing a 24-hr culture of streptococci or staphylococci with physiological saline. The bacterial suspension was prepared from permanent strains of streptococcus and staphylococcus, which we obtained from the Department of Microbiology of the Khar'kov Veterinary Institute. The ingested bacterial cells were counted in 200 leukocytes.

During the experiments the animals were kept on the same diet and in the same conditions. The phagocytic index was investigated before the infliction of incised or crushing wounds of the skin and muscles in the iliac region, and subsequently after 15-30 min, 1-2-3-4-6-8 hr and 1-2-3-4-5-6-7-10-12-15-20 days of the injury (only individual examples are given in the tables). We investigated the phagocytic index so frequently because, in our experiments, it was an indirect sign of the degree of stimulation of the nervous system as the result of pain impulses from the region of the wound.

RESULTS

In all the experiments the phagocytic index rose sharply immediately after infliction of the wounds, being 1.5-2 times larger after only 15 minutes. After ordinary anesthesia, the phagocytic index remained essentially unchanged at the end of 4-6 hours by comparison with the first and second hours, and it then began to increase gradually again (Table 1).

In the horses in which anesthesia was not used, the index continued to rise during the next 2-4-6-8 hours. Usually in these cases the phagocytic index rose until 24 hours. Occasionally the increase was less pronounced. After 24 hours a fall in the phagocytic index was observed, with a return to its initial value at the end of 4-5 days. In animals in which prolonged anesthesia with a 5% solution of procaine in horse fat (or in peach oil) was used, the phagocytic index rose only very slightly to 6-8 hours by comparison with the first and second hours; it then gradually fell and reached its initial level at 24 hours or, occasionally, at 48 hours (see Table 1).

The phagocytic index changed especially demonstratively when an additional stimulus was used—the instillation of concentrated sulfuric acid onto the wound surface (Table 2).

After infliction of the wounds, as in the previous experiments, the phagocytic index increased sharply—by 1.5-2 times in comparison with the initial values; then, after instillation of sulfuric acid on the wound surface without anesthesia, it continued to rise sharply for the first two days.

With ordinary anesthesia, the increase in the phagocytic index was delayed immediately after anesthesia and for the next 2-4 hours, and it then continued to rise until 24-48 hours. In both cases the phagocytic index be-

TABLE 1. Results of Determinations of the Phagocytic Index in Experiments on Horses

Name of horse and record no.	Conditions of wound healing	Phagocytic index[1]																
		before wounding	after															
			15-30 min	1 hr	2 hr	4 hr	6 hr	8 hr	1 day	2 days	3 days	4 days	5 days	6 days	7 days	10 days	15 days	20 days
The mare Astra, No. 1	Without anesthesia	15	25	29	36	38	38	40	55	25	20	16	14	15	15	14	13	14
The mare Galka, No. 4	With ordinary anesthesia. . .	7	12	12	14	14	15	19	24	11	10	10	7	8	7	8	9	7
The mare Kukla, No. 13	With prolonges anesthesia. .	11	25	28	28	28	27	28	14	10	10	12	10	10	10	12	11	10

[1]The number of ingested microorganisms was counted in 200 leukocytes

TABLE 2. Results of Determinations of the Phagocytic Index in Experiments on Horses Using An Additional Stimulus – Sulfuric Acid

Name of horse and record no.	Conditions of wound healing	Phagocytic index[1]																
		before wounding	after															
			15-30 min	1 hr	2 hr	4 hr	6hr	8 hr	1 day	2 days	3 days	4 days	5 days	6 days	7 days	10 days	15 days	20 days
The mare Lastochka, No. 24	Without anesthesia + sulfuric acid.	25	52	56	60	70	79	90	98	98	65	34	28	25	23	22	23	24
The stallion Linok, No. 21	Sulfuric acid + ordinary anesthesia.	23	42	44	60	60	68	90	94	99	67	35	29	26	27	29	25	25
The stallion Myach, No. 21	Sulfuric acid + prolonged anesthesia	25	47	50	55	57	66	70	40	22	22	23	23	24	23	24	24	23

[1]The number of ingested microorganisms was counted in 200 leukocytes

gan to fall at the end of the second day after infliction of the wounds, and returned to its initial values at the end of the fifth or sixth day. It remained perceptibly unchanged during the next 10-15 days.

With prolonged anesthesia, the phagocytic index rose only in the first 6-8 hours after infliction of the wounds and instillation of sulfuric acid, after which it began to fall and reached its initial values at the end of 24-48 hours. In the next 15-20 days it was almost unchanged.

What is the explanation of the changes in the phagocytic index after the infliction of wounds without anesthesia and with the subsequent use of ordinary and prolonged anesthesia?

Pain is one of the factors causing excitation of the sympathetic nervous system. It is well known that the sympathetic nervous system is distinguished by its very high excitability [8]. It is pertinent to quote A. N. Bekulev's statement that "a wound is an extensive receptor surface which is the source of pathological impulses to the cerebral cortex, causing its exhaustion" [1].

Among the many pathological impulses, those due to pain occupy a prominent place. From the data in the literature and our personal investigations, we consider that, immediately after the infliction of wounds, especially with the subsequent instillation of sulfuric acid onto the wound surface, the intensity of pain in the region of the wound increases. The pain stimulation, acting reflexively through the nervous system, causes a series of defensive reactions, including an increase in the phagocytic power of the leukocytes. In our experiments the increase in this power was observed for 48 hours. It may be suggested that, during this period, the strongest pathological impulses (with pain dominant among them) pass from the wound to the central nervous system, and that these caused the well-marked increase in the phagocytic index. As these pathological impulses, including those due to pain, weakened, the phagocytic power of the leukocytes diminished.

Bearing in mind the changes in the phagocytic index, it must be considered that the pathological impulses (including those due to pain) were strongest in the first phase of healing (5-7 days after infliction of the wounds), especially when sulfuric acid was subsequently instilled onto the wound surfaces. They later became weaker, so that at the end of 5-7 days the phagocytic index returned to its initial value; after 5-7 days, i.e., in the second and third phases of the healing process, it remained substantially unchanged.

The ordinary or prolonged lumbar paravertebral block which we used prevented the transmission of pain stimulation to the central nervous system. Under local anesthesia the peripheral receptors in the injured tissues are blocked, so that the reflex influence of the "products" of pain is decreased. Removal of the pain and other impulses, passing from the region of the wound to the central nervous system, delays the increase in the phagocytic activity of the leukocytes.

With ordinary anesthesia the phagocytic index did not change appreciably for two hours, and then continued to rise. It must be assumed that this was due to the fact that the peripheral receptors in the injured tissues were blocked for two hours, as a result of which the humoral "products" of pain were not formed, and their reflex influence was thereby prevented. With prolonged local anesthesia, the block of the peripheral receptors continued longer; during this time no humoral "products" of pain were evidently formed, in consequence of which the phagocytic index not only did not increase, but returned comparatively quickly to its initial level. In this connection it has to be pointed out that most authors [2, 3, 6, 10, etc.] consider the opsono-phagocytic reaction to be very sensitive, reflecting the condition of the body.

It will be apparent from the findings described that, in animals in which no anesthesia was used, the phagocytic index rose sharply immediately after infliction of the wounds and during the next two days, after which it gradually fell and reached its initial values at the end of 5-7 days, i.e., when the acute inflammatory processes had subsided and granulation tissue had begun to develop in the wound. In the second and third phases of healing of the wound the phagocytic index remained substantially unchanged.

Ordinary and prolonged anesthesia delayed the increase in the phagocytic index. We may remark that during this time the number of phagocytes in the wound itself increased perceptibly, from our observations made on impressions from the wound surface, in those animals in which the phagocytic index was studied.

The method of taking impressions enabled us to judge objectively the intensity of the measures taken by the body to resist infection, and the course of cleansing of the wounds from microorganisms and necrotic tissues, both with and without anesthesia. For the sake of brevity we will describe the comparative findings for the neutrophil group alone.

During the systematic analysis of the wound impressions in all the animals, we observed the following. In horses in which especially prolonged anesthesia was used during healing of the wounds, the cytogram during the first 4-6 days showed a large number of neutrophils in various stages of phagocytic activity. The number of dead leukocytes in a state of disintegration was relatively small. Free-lying microorganisms were almost nonexistent or were found as isolated specimens. The microorganisms were completely ingested by the neutrophils and showed a varying intensity of staining, indicating the active bacteriolysis (digestion) of the microorganisms, a sign of the high protective power of the animal. At the same period of wound healing without anesthesia, the impressions showed a less marked phagocytosis, many neutrophils without signs of phagocytosis and many dead leukocytes in various stages of disintegration. Around the neutrophils were seen large collections of microorganisms that had not undergone phagocytosis.

SUMMARY

As shown by investigations, the phagocytic index may to a certain extent objectively reflect the removal of pain and other pathological impulses coming from the wound area into the central nervous system. Proceeding from the literature data and his own observations, the author also considers that the phagocytic activity of leukocytes is an objective indication of the condition of the organism, the course of the wound process, and the efficacy of the therapeutic measures.

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