

## HUMORAL FACTORS OF BLOOD WITH APPLICATIONS OF MUD

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In the previous communication [1] it was shown that there are two phases in the mechanism of action of mud applications: neuro-reflex and humoral. In its turn the humoral influence is effected through the nervous apparatus.

In the present work we were faced with the task of studying the character of the biologically active substances appearing in the blood in mud techniques and their influence on the hemodynamic.

### EXPERIMENTAL METHODS

The investigations were conducted on dogs in acute and chronic experiments. Altogether 45 chronic and 35 acute experiments were conducted.

In order to detect the biologically active substances we took an isolated frog heart and the dorsal muscle of a leech as test objects. The heart of a frog was isolated according to the method of Straub. The specimens of the eserine-treated dorsal muscle of the leech, freed from the nerve formations, were used in the detection of acetylcholine by means of the commonly accepted method. Eserine was added to the Ringer solution used for washing the leech dorsal muscle preparation at a ratio of 1:200,000. In order to protect the acetylcholine from destruction, 0.1-0.05 mg/kg eserine was also introduced in the organism of the experimental animals. In order to detect the humoral factors in the blood the latter was taken from the femoral vein or from the marginal vein of the ear. Plasmatic serum was obtained from the freshly obtained blood by means of centrifugalization. In a number of experiments whole blood was added directly to the Ringer solution. Both the serum and the whole blood used to influence the test objects were taken at attenuations of 1:10, 1:20, 1:40, 1:50, 1:100.

The acute experiments were carried out on the dogs under morphine-ether narcosis. We used the method of the isolated (from a humoral point of view) carotid sinus. Arterial blood pressure was recorded in the femoral artery with a mercury manometer, respiration with a Marei capsule, connected to a pneumograph and the volume of the spleen also with a Marei capsule, connected through a rubber tube to an oncograph in which the spleen was placed.

Medicinal mud from the Karasov lake (Karagan region) was applied to the shaven skin of the abdomen, the back or hind paw for 30 minutes. The medicinal mud was heated to 40-42°C.

### EXPERIMENTAL RESULTS

The blood taken from the experimental animals before application of mud, on addition to the Ringer solution, used to wash the dorsal muscle of a leech, never produced contraction of the muscle, but as a rule, had a marked positive inotropic effect on the isolated heart of a frog.

The serum or blood, obtained from the dogs 5-10 minutes after the commencement of the procedure, also did not produce contraction of the dorsal muscle of the leech, while addition of one drop of the serum or blood

(at an attenuation of 1:10, 1:20) to the Ringer solution produced an intensified inotropic effect on the isolated heart of the frog. Thus, for example, while before application of mud the serum or the blood, at an attenuation of 1:10, 1:20, intensified contraction of the heart on average by 110-120% (Fig. 1a), addition of the same amount of blood or blood serum taken after application of mud increased the contraction of the isolated heart by 150-250% (Fig. 1b).

Blood taken 30-40 minutes after use of the mud application produced contraction of the leech muscle by 10 mm and more. The largest contraction of the leech muscle was observed with the influence of blood taken in acute and chronic experiments at the 30th minute of the procedure and for 30-60 minutes after removal of the mud application. This blood exerted a predominantly positive inotropic influence on the isolated heart. In rare cases we noted at first a negative inotropic effect, changing into a positive one. Determination of the concentration of substances in the blood, producing contraction of the dorsal muscle of the leech, by the method of biological assay, showed that it corresponded roughly to a concentration of acetylcholine from  $10^{-5}$  to  $10^{-9}$ . With atropinization of the test objects this effect did not manifest itself.

The indices of the hemodynamic shifts (increase in blood pressure, bradycardia, contraction of the spleen, increased excitability of the depressor apparatus), arising under the influence of mud applications, upon introduction of eserine in the experimental animals, was more marked than without eserine introduction.

On the basis of the findings obtained one may assume that the substance appearing in the blood under the influence of mud application and producing contraction of the dorsal muscle of the leech was acetylcholine.

Blood taken 2 and 3 hours after mud application contained a considerably smaller quantity of acetylcholine. Therefore, acetylcholine a few hours after use of the mud application was not always found in the blood, whereas the biological activity of the blood, producing a positive inotropic influence, was maintained for many hours.

Thus, under the influence of mud applications an increase in the blood content both of acetylcholine-like and sympathico-like substances was observed. The increase in their content corresponds in terms of time to a manifestation of the more considerable shifts in the hemodynamic indices.

It is necessary to note that uniform results were not obtained in all the experiments. Whereas almost always sympathico-tropic substances were found in the blood, acetylcholine-like substances were found in only about 60-70% of the cases. The negative results of the experiments do not always indicate absence of acetylcholine in the investigated blood. They can be explained in some experiments by the slight sensitivity of the dorsal muscle of the leech, and in others by its small content or destruction in the blood as a result of introduction of an insufficient amount of eserine in the organism of the experimental animal.

For the purpose of a more precise assessment of the manifestation of humoral substances in the blood and their role in the organism's reaction to mud application, a series of experiments was carried out with blood transfusion, the blood being taken from a dog-donor before and after mud was applied to it. In all the chronic experiments of this series conducted on 10 dogs, a prior determination of biological blood compatibility was made and also 0.05-0.1 mg eserine per kg animal weight was introduced in the organism of the experimental animals. The influence of blood, taken from the donor before mud application, on the blood circulation of the recipient was studied. 10 ml blood, taken from the femoral artery, was introduced in the jugular or femoral vein of the recipient. Introduction of such an amount of blood in the recipient was accompanied by a transitory change in its blood pressure and in the number of cardiac contractions. Arterial blood pressure, measured in the recipient in the exposed common carotid artery with a cutaneous flap rose on average by 10-15 mm Hg. Cardiac contractions in the majority of cases were accelerated. These changes in pulse and blood pressure disappeared in 20-30 minutes. Blood, taken from the donor 30-60 minutes after depositing mud on its skin and introduced intravenously in the recipient, produced more marked and durable changes in the hemodynamic. In all experiments except for one, arterial blood pressure in the recipient rose against a background of fairly clearly marked bradycardia and did not fall for 1-2 hours (Fig. 2).

Control experiments showed that the effects noted were not connected with thermal influences.

Thus, the results with blood transfusion before and after mud application suggest that the blood of the donor after subjection to mud application acquires a marked biological activity, which is capable, despite the small content of active substances, of producing pronounced changes in the blood circulation system.

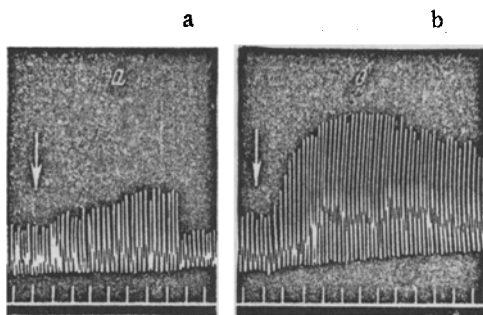


Fig. 1. Change in cardiac contractions in frog under the influence of the blood of the donor, taken before (a) and after (b) mud application. Indication of time (5 seconds), ↓ moment of influence of mud application.

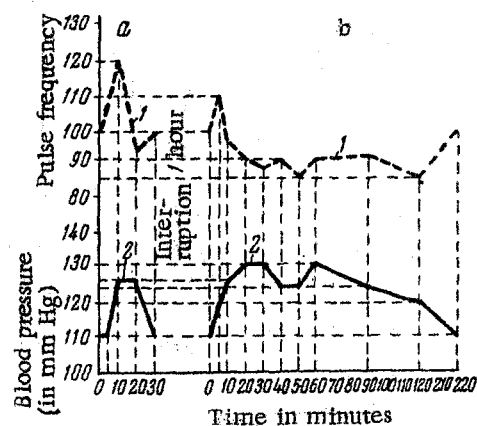


Fig. 2. Change in pulse and maximum blood pressure in dog-recipient after entry of 10 ml of donor's blood taken before (a) and 30 minutes after commencement (b) of application, 1) pulse; 2) blood pressure in common carotid artery.

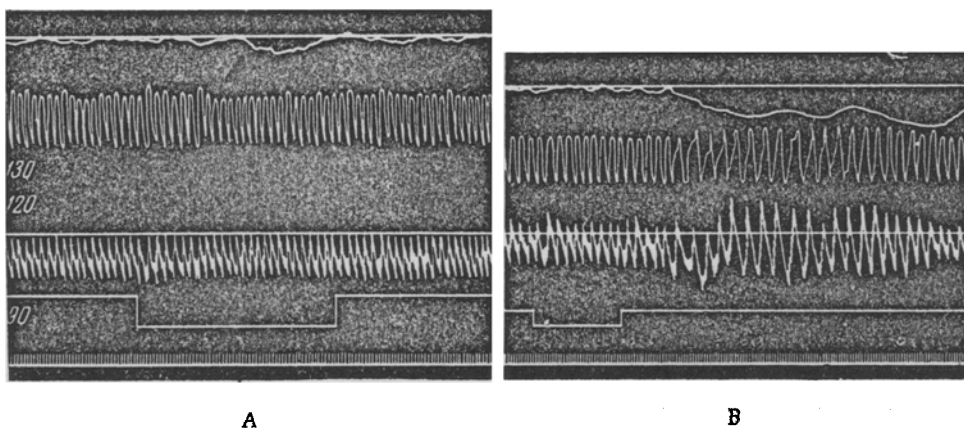


Fig. 3. Changes in volume of spleen, respiration and blood pressure upon perfusion of carotid sinus with blood (dilution 1:5), taken from donor before (A) and 1 hour after commencement (B) of mud application. Significance of tracings (top to bottom): change in volume of spleen, respiration, blood pressure, indication of stimulation, indication of time (1 second).

Those prolonged and perceptible shifts in the blood circulation system which occurred as a result of placing medicinal mud on the skin are therefore conditioned and maintained not only by the reflex effect of mud through the skin directly on the cardio-vascular system, but by those biologically active substances which in turn form and mobilize in response to these reflex changes. The biologically active substances appearing do not remain merely a product of reflex activity of the organism but themselves are incorporated in the complex chain of cardio-vascular reflexes.

Thus, by perfusing the isolated carotid sinus, with blood taken from the experimental animals, after application of medicinal mud to their skin, we succeeded in establishing reflex shifts in the cardio-vascular and respiratory systems. Upon perfusion of the sinus with blood, diluted 1:5, 1:10 with Ringer-Locke solution, in 1-2 minutes it was possible to observe deeper respiration, increased arterial blood pressure and a reduction in the volume of the spleen (Fig. 3A). Blood, taken from the experimental animals before mud application or 4-5 hours after it, on perfusion in the carotid sinus, either produced no reflex shifts at all in the cardio-vascular system or the latter were weakly expressed and were of an ephemeral character (Fig. 3B).

Thus, biologically active substances appearing in the blood after mud application are an intermediary link in the performance of the complicated neuro-reflex reactions of the organism.

#### LITERATURE CITED

- [1] Uzbekov, A. A. *Byull. Eksptl. Biol. i Med.* 36, 6, p. 14 (1953).