# THE REFLEX MECHANISM OF HEPARIN ACTION

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The unified physiological system of blood clotting comprises: 1) organs whose activity is associated with the synthesis, production, and utilization of the clotting and anticlotting factors of the blood (the liver, spleen, lungs, bone marrow, and vascular wall); 2) the dynamic equilibrium between the clotting and anticlotting factors in the circulating blood, maintaining the functional state of these organs; and 3) the neuro-humoral mechanism of regulation. It consists of a dynamic system capable of regulation, together with mechanisms of automatic regulation. The latter have not yet been studied. We have only a few facts concerning the changes in the rate of blood clotting during stimulation of the interoceptors of certain zones with different stimuli [1-5].

In order to elucidate the mechanisms of automatic regulation of the clotting system of the blood, it is necessary, in our opinion, to study the action of a physiological stimulus which is being formed continuously in the body and which is an essential factor in the process of blood clotting. It is only in these conditions that, in the course of evolution, specialized chemoreceptors have developed, capable of detecting a concentration of a particular substance or of others related to it. Such a substance is heparin. The first aim was therefore to determine whether heparin was capable of exerting a reflex influence on the process of blood clotting.

## EXPERIMENTAL METHOD

Experiments were carried out on sexually mature rabbits weighing from 2 to 3 kg. Blood for the investigations was taken from the hepatic vein. Under urethane anesthesia, the carotid sinus region from the general circulation was verified after the experiment by injection of methylene blue.

Heparin (Richter) or pure heparin, dissolved in physiological saline, was injected in a dose of 0.02-0.06 ml into raised edge of the common trunk of the carotid artery; it flowed freely into the region of the carotid sinus. The experiment began 1-1 $\frac{1}{2}$ h after the animals were anesthetized.

TABLE 1. Changes in Blood Clotting Indices after Injection of Heparin into Carotid Sinus

| Index | Initial data | After injection of heparin into carotid sinus | Significance of difference between means |
|---|---|---|---|
| | M± m | $M_1 \pm m_1$ | P |
| Clotting time of blood (beginning) . | 84±3.2 | 145.1±11 | 0.001 |
| Prothrombin time . . | 20.7± 0.5 | 26.7± 1.9 | 0.02 |
| Free heparin . . . . . | 9.5± 0.6 | 14.3± 1.6 | 0.05 |
| Heparin concentration (protamine sulfate titer) . . . . . | 58± 0.9 | 75± 3.7 | 0.001 |
| Fibrinogen . . . . . | 4.8± 0.1 | 1.8± 0.3 | 0.001 |

The clotting time of the blood was determined by means of a Sitkovskii-Egorov apparatus; the prothrombin time by Quick's one -stage method using thromboplastin prepared by the Leningrad Blood Transfusion Institute; free heparin by Sirmai's method; the heparin concentration by the protamine sulfate titer; the fibrinogen by a gravimetric method. The determinations were made before and 1-5 min after injection of heparin. Altogether 23 experiments were carried out on 21 rabbits.

## EXPERIMENTAL RESULTS

Injection of heparin into the isolated carotid sinus was followed by a clear slowing of blood clotting. One minute after the entry of heparin into the carotid sinus a considerable prolongation of the clotting time of the venous blood from the liver was observed, together with an increase in the prothrombin time and in the heparin

TABLE 2. Changes in Clotting Time of the Blood and in Fibrinogen Concentration after Injection of Heparin into the Carotid Sinus

| Expt. No. | Initial data | | | After injection of heparin into carotid sinus | | |
|---|---|---|---|---|---|---|
| | clotting time in sec | | fibrinogen (in mg) | clotting time in sec | | fibrinogen (in mg) |
| | beginning | end | | beginning | end | |
| 7 | 80 | 140 | 6 | 90 | 195 | 3.5 |
| 13 | 90 | 140 | 4.5 | 150 | 270 | 2.5 |
| 19 | 90 | 150 | 5 | 125 | 180 | 3 |
| 6 | 85 | 175 | 4 | 120 | 600 no clot | 0.5 |
| 9 | 110 | 330 | 5 | 160 | 600 no clot | 1 |
| 12 | 70 | 140 | 4.5 | 175 | 600 no clot | 0.5 |
| 16 | 80 | 140 | 5 | 120 | 600 no clot | 1 |
| 18 | 90 | 160 | 4 | 165 | 600 no clot | 1 |

concentration and a decrease in the fibrinogen concentration. These changes were statistically significant (Table 1).

Hypoprothrombinemia was observed in 9 of 12 experiments, and the prothrombin time was unchanged in only 3 experiments. The heparin concentration was increased in 7 of 12 experiments when estimated by the protamine sulfate titer, and in 7 of 8 experiments when determined as free heparin. The fibrinogen concentration fell distinctly in all experiments without exception, within 5 min of the injection. The mean reduction in the fibrinogen concentration was by 50-60%. In some experiments it fell by 80-89% of its initial value (Table 2). In these cases clotting was not complete even after 10 min.

Analysis of the results showed that when the fibrinogen concentration fell below 1 mg/ml plasma, the blood did not clot: a normal clot was not formed after 10-15 min. Evidently the reflex slowing of the coagulation of the blood in response to stimulation of the carotid sinus with heparin led, firstly, to hypofibrinogenemia and also to hypoprothrombinemia and to elevation of the heparin level.

An increase in the heparin-antithrombin concentration during stimulation of the pressor receptors (increased pressure in the carotid sinus) was observed by Perlick [8] and a delay in blood clotting in these conditions – by V. S. Zudin [3].

To rule out the suggestion that the slowing of the blood clotting observed after injection of heparin into the carotid sinus was associated with an increase in pressure in the sinus and not with the action of heparin, control experiments were performed. Injection of the same volume of physiological saline instead of heparin in the same conditions failed to cause changes in the clotting time of the blood in all of 4 experiments.

The reflex nature of the delay in blood clotting during stimulation of the carotid sinus with heparin was confirmed by the following experiments. In the experiments of series 1 the nerve to the carotid sinus was divided before the heparin was injected. The subsequent injection of heparin caused no changes in the clotting time of the blood. For example, in experiment No. 4 (March 3, 1962), the initial clotting time of the blood was 95 sec (beginning) and 155 sec (end). Injection of 0.06 ml heparin into the isolated carotid sinus led to a slowing of the clotting time: clotting began at 170 sec and was not complete even after 15 min. After 50 min the clotting time returned to its initial values. After division of the nerve to the carotid sinus and injection of heparin in the same dose as in the first case, the clotting time of the blood was unchanged (beginning 95 sec, end 155 sec).

In the second series of experiments, after a delay in the clotting of the blood had been established as a result of the heparin injected into the carotid sinus, the latter was washed out with Novocain, and a further injection of heparin was given. In these cases the clotting time of the blood was unchanged. As an illustration the results of experiment No. 7 (on April 6, 1962) are given below. The initial clotting time of the blood was 90 sec (beginning) and 145 sec (end). After injection of heparin into the isolated carotid sinus the time was considerably lengthened – clotting began after 130 sec but was not complete even after 300 sec. The clotting time of the blood was restored to normal after 1 h 45 min (beginning 80 sec, end 135 sec).

After washing out the carotid sinus with Novocain (0.5%) and again injecting heparin the clotting time of the blood was unchanged. After 45 min the carotid sinus was washed out with physiological saline and heparin again injected – this time the clotting time of the blood was clearly prolonged (beginning 105 sec, end 210 sec).

Hence, division of the nerve to the carotid sinus or blocking the nerve endings of the carotid sinus with Novocain abolished the effect of heparin, injected into the carotid sinus, in slowing the clotting of the blood.

It may be assumed that the carotid sinus contains chemoreceptors sensitive to heparin. These are stimulated by the concentration of heparin circulating in the blood. Impulses pass to the central nervous system, notably to

the reticular formation where, as A. A. Markosyan and G. A. Yakunin showed [6, 7], stimulation of certain areas is accompanied by slowing of blood clotting and by an increase in the heparin concentration. The efferent pathway terminates in the organs of the clotting system of the blood, and primarily in the liver, where many of the clotting and anticlotting factors are synthesized.

The reflex mechanism whereby heparin exerts its effect, which was investigated in this study, demonstrates that heparin may act both directly and indirectly. In the latter case reflex processes take place, which lead to a sharp fall, or to the total loss of the ability of the blood to coagulate. It will be the object of future investigations to examine the relationship between these processes.

## SUMMARY

An investigation was made of the mechanisms of the automatic regulation of the blood coagulation system. Heparin injected into the isolated carotid sinus, delayed blood coagulation and caused hypofibrinemia, hypoprothrombinemia and a rise in the heparin level. Division of the nerve to the L. C. S. or preliminary washing of the carotid sinus with novocain abolished the effect. Administration of physiological salines, instead of heparin confirmed the fact that the effect came as the result of heparin action and not of the pressure changes in the carotid sinus. The reflex mechanism of the heparin effect discovered by the authors, demonstrates the presence of a direct and indirect action. In the second case reflex processes lead to a marked reduction or a loss of the ability of the blood to coagulate.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

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