CHANGES IN SOME PARAMETERS OF THE HEMOSTASIS SYSTEM IN HEALTHY DOGS DURING HEMOPERFUSION

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Hemoperfusion — a method of extracorporeal detoxication of the patient by passing his blood through a column containing a sorbent [3, 6, 8], — is widely used in modern medical practice.

Contact between blood and a foreign surface induces activation of the blood clotting system and thus leads to thrombus formation. To prevent thrombus formation during the extracorporeal circulation procedure under clinical and experimental conditions it is customary to use heparin, a direct-acting anticoagulant with powerful universal anticlotting activity. The optimal level of heparinization is an essential condition for successful hemoperfusion: Too little anticoagulant may complicate the hemoperfusion procedure because of deposition of fibrin in the system of connecting tubes, and it is also the cause of disseminated intravascular clotting (DIC) in the patient, whereas an excess of heparin increases the risk of bleeding [2].

The aim of this investigation was to assess the state of the hemostasis system after hemoperfusion, associated with hypocoagulation procedures with the associated risk of hemorrhagic complications, and also to assess late results of this procedure.

EXPERIMENTAL METHOD

Experiments were carried out on mongrel dogs of both sexes weighing 15-20 kg. Hemoperfusion was carried out on 11 dogs, using the SKN-2M charcoal sorbent manufactured by Kiev Institute of General and Inorganic Chemistry. As the control, six dogs underwent extracorporeal hemoperfusion through a system of connecting tubes but without a column containing sorbent.

Under thiopental anesthesia (0.35-1.1 g thiopental sodium in physiological saline, intravenously) the femoral artery and vein of the animals were isolated unilaterally and cannulated. Heparin was injected intravenously in a dose of 10,000 units, and perfusion began 10 min later. After passing through the system of connecting tubes the arterial blood was returned into the vein. The blood flow (70-80 ml/min) was controlled by means of a roller pump. Before perfusion the system was washed out with sterile physiological saline containing heparin in a closed circuit. During hemoperfusion (at the beginning of the procedure) an additional 10,000 units of heparin was introduced into the column. The volume of the column was 250 ml and the perfusion time 1 h. After perfusion the vessels were tied, the wound was closed without drainage and no protamine sulfate was injected.

The state of the hemostasis system was estimated from the platelet count in the peripheral blood, determined by the phase contrast method, the fibrinogen concentration (Bidwell) the euglobulin clot lysis time (Kowarzik), plasma recalcification time (Howell), prothrombin time, fibrinase activity (Baluda), and ethanol and protamine sulfate tests. The tests were carried out before and 24 h after the operation, and also in five dogs subjected to hemoperfusion on the 3rd and 6th days.

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TABLE 1. Platelet Count (thousands/μl) in Peripheral Blood (M ± m)

Group	Times of investigation					
	initial value	10 min after injection of heparin	10 min after end of procedure	days		
				1st	3rd	6th
Hemoperfusion with sorption	286±8 (n=16)	183±17* (n=9)	115±10* (n=10)	117±17* (n=9)	210±29* (n=5)	479±42* (n=4)
Hemoperfusion without sorption			249±27 (n=4)	253±35 (n=3)	—	—

* Here and in Table 2 P < 0.05 compared with initial value.

TABLE 2. Effect of Hemoperfusion With (I) and Without (II) Sorption on Blood Clotting System Parameters (M ± m)

Parameter	Group	Time of investigation, days			
		initial value	1st	3rd	6th
Plasma recalcification time, sec	I	62,0±4,6	64,5±8,1	58,0±4,1	47,0±3,2
	II		58,3±5,1	—	—
Fibrinogen, mg %	I	434±22,5	657±25,5*	818,0±87*	655,0±72,0*
	II		703±30,0*	—	—
Euglobulin clot lysis time, min	I	31,3±2,7	48,3±3,1*	55,0±6,5*	50,0±9,4*
	II		58,3±5,1*	—	—
Prothrombin time, sec	I	13,5±0,3	14,6±0,4*	13,5±0,3	13,5±0,5
	II		—	—	—
Fibrinase activity, i.u.	I	57,7±3,9	55,8±5,5	53,8±2,4	55,3±1,9
	II		—	—	—
Ethanol test	I	Negative	Negative	Negative	—
	II		"	—	—
Protamine sulfate test	I	"	Positive in two cases, negative in two	Positive in three cases, negative in one	—
	II		—	—	—

Legend. Initial values in both groups, after analysis by Student's t test, were combined as belonging to the same general set.

EXPERIMENTAL RESULTS

A significant decrease was found in the number of circulating platelets 10 min after intravenous injection of 10,000 units of heparin (Table 1). This may be due to the aggregating action of heparin, in the doses used, on platelets and subsequent retention of the aggregated platelets in the capillaries of organs and tissues [5, 10, 12].

Toward the end of extracorporeal perfusion the circulating platelet count was back to normal, evidently because of disaggregation of the previously aggregated platelets. By contrast, immediately after hemoperfusion and sorption the platelet count in the peripheral blood was considerably reduced. In some cases the platelet count was reduced by 75%, in agreement with data in the literature [14], and as a rule in these cases packing on the sorbent was observed, as shown by elevation of the hydrodynamic pressure (resistance to the blood flow), and the presence of clots in the column containing the sorbent. This suggests mechanical retention of the aggregated platelets by charcoal particles in the upper layer of the sorbent and adhesion of the platelets to the particles during local fibrinogenesis. In the present experiments, it will be noted, no case of thrombocytopenia below the "critical" level of 30,000 cells/μl was observed [1].

Thrombocytopenia, caused by mechanical removal of platelets from the blood stream, is long-lasting in character and is followed by thrombocytosis, evidently due to stimulation of

the megakaryocytic series of medullary hematopoiesis [9]. In fact, 24 h after hemoperfusion the circulating platelet count was not back to normal, but after 6 days the peripheral blood platelet count was significantly higher than initially.

Immediately after hemoperfusion the tests used to determine factors of the blood clotting system were impracticable on technical grounds because of the heparinemia. Only in two dogs could the prothrombin time be measured: It was 64 and 65 sec, corresponding to a prothrombin index of about 20%. In some cases the prothrombin time could not be determined.

No clinical signs of a bleeding tendency could be observed in any of the dogs 24 h after extracorporeal circulation procedure. The plasma recalcification time, reflecting the overall clotting activity of the blood, did not differ significantly from the initial value at any time of the investigation (Table 2). This indicates the absence of any marked changes in the hemostasis system after hemoperfusion, with or without sorption, under the conditions described.

On the first day after hemoperfusion a significant lengthening of the prothrombin time was observed, evidently due to adsorption of some of the proteins of the prothrombin complex on the column or their utilization during local fibrinogenesis, occurring on contact between blood and artificial surfaces. Such a small decrease in the prothrombin level could not be the cause of hemorrhages and could not maintain a state of increased liability to bleeding.

The fibrinogen concentration was increased after hemoperfusion, with or without sorption, by about 1.5 times. The increase in the fibrinogen concentration was lasting in character, and was evidently one manifestation of an inflammatory reaction to the operation or was due to the effect of heparin on fibrinogen metabolism.

The euglobulin clot lysis time was lengthened, reflecting changes in the plasma fibrinogen concentration. It seems more likely to link lengthening of the euglobulin clot lysis time with an increase in fibrinogen concentration, altough the possibility of a decrease in the concentration of plasminogen or its activators cannot be ruled out.

Fibrinase activity did not differ significantly from the inital level at any time of the investigation. The ethanol test was constantly negative at the times it was carried out. In some dogs on the 1st and 3rd days after hemoperfusion a weakly positive protamine sulfate test was recorded (this test is positive in hyperfibrinogenemia [4]).

Lengthening of the euglobulin clot lysis time, the negative ethanol test, and also the increase in fibrinogen concentration during a progressive rise in the circulating platelet count suggest absence of the DIC syndrome.

When activated charcoal is used as the sorbent, often it undergoes "packing" [6, 7], due to adsorption initially of plasma proteins and later of blood cells, chiefly platelets and leukocytes, on the foreign surface, and consequent local fibrinogenesis. This phenomenon is observed even when heparin is used in doses considered to be optimal for hemoperfusion. In the present experiments the doses of heparin were known to be on the high side. The aim was to test the hypothesis that the more marked hypocoagulation achieved by injection of an additional quantity of heparin would prevent packing of the sorbent. The possibility of active absorption of heparin by the SKN-2M sorbent also was taken into account [6].

The experiments showed that the high doses of heparin did not prevent packing of the sorbent, nor did absorption of heparin by the sorbent take place in amounts which could be reflected in performance of the procedure. During hemoperfusion on activated charcoal for 1 h, heparin administration can evidently be limited to a dose of 500 units/kg [3].

Destruction of platelets during hemoperfusion leads to liberation of procoagulants into the plasma, increasing the risk of development of the DIC syndrome. For this reason it seems advisable not to neutralize the heparin with protamine sulfate after hemoperfusion, especially in the absence of any clinical data indicating an increased bleeding tendency. Gradual neutralization of the heparin *in vivo* creates conditions for disappearance of the excess of procoagulants; protamine sulfate, moreover, induces neutralization of heparin, considerably reduces the sensitivity of platelets to aggregating factors, and this impairs their hemostatic functions [11].

Stimulation of thrombocytopoiesis after hemoperfusion, revealed by this investigation, deserves special attention. Activation of thrombocytopoiesis can be regarded as a component of the many sided therapeutic action of hemoperfusion.

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MECHANISM OF DISTURBANCE OF ANDROGEN PRODUCTION IN STRESS

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There is abundant clinical and experimental evidence [10-13] of weakening of reproductive function and lowering of fertility during stress. A fall in the plasma testosterone concentration and in the excretion of its metabolites with the urine, especially of the androsterone fraction, has been demonstrated in surgical or traumatic stress [2, 4, 8, 9]. Sexual function is known to be depressed in "hunger stress," and this also is connected with a fall in the plasma testosterone level and stimulation of the luteinizing function of the pituitary [6]. The absence of progeny of animals in captivity (in a state of alarm and fear) also is due to despression of spermatogenesis associated with depression of androgen activity. Data in the literature thus reveal depression of reproductive function and androgenic status in stress, but the mechanism of the inhibition of androgenic activity under these conditions still remains largely unstudied. In particular, it has not yet been settled whether testosterone production is blocked in the testes, whether the disturbances of androgen production are connected with structural changes, and specifically, whether the disturbances of androgen production are connected with structural changes in Leydig's interstitial cells or whether they are physiological in character.

The aim of the present investigation was to study functional and structural changes in the testes in stress.

EXPERIMENTAL METHOD

Experiments were carried out on 28 young sexually mature male rabbits. Stress was induced in the animals by daily immobilization for 1 h and simultaneous electrical stimulation (frequency 100 Hz, duration 1 msec) from an ÉI-1 pulse generator for 2 weeks. The strength of the current was chosen arbitrarily so as to produce contraction of the hind limb muscles. Testicular androgen activity was judged from the concentration of testosterone and androstenedione measured by a spectrofluorometric method [7] in blood from the spermatic vein [14]. Plasma sterods were identified on the Ultrachemiscope apparatus (UFS-1 filter) and determined quantitatively on the BIAN fluorometer at wavelengths of 436 and 510 nm. Changes in weight of the gonads and prostate gland and also the state of the spermatogenic epithelium and inter-

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