DISTRIBUTION OF INDICES OF HEMOCOAGULATORY ACTIVITY IN KIDNEY ULTRASTRUCTURES

P. V. Sergeev, R. D. Seifulla, and A. I. Maiskii

UDC 612.115:612.46

Experiments on rats and rabbits showed that the microsomal fraction and, to a lesser degree, the mitochondrial and nuclear fractions of the kidneys possess clotting activity.

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It has recently been shown that besides their participation in regulation of water and salt metabolism, the kidneys also perform other functions. Blood clotting factors V, VII, fibrinogen, and prothrombin have been found in the urine of healthy persons. Disease of the kidneys is accompanied by a decrease in the concentration of procoagulants in the urine, thrombin formation in the blood is disturbed [4], the coagulation potential is considerably lowered [1], and both the fibrinolytic and the proteolytic activity in the blood are increased [10].

The object of the present investigation was to determine whether subcellular structures of the kidneys possess clotting or anticlotting activity and to study the distribution of these indices between the nuclear, mitochondrial, and microsomal fractions isolated from kidney homogenates.

EXPERIMENTAL METHOD

Experiments were carried out on 68 male rats weighing 150-200 g and on 12 rabbits weighing 2000-3000 g. The animals were decapitated and the kidneys carefully washed out through the inferior vena cava with cold physiological saline under pressure.

A sample of the organ weighing 1 g, dried on filter paper, was homogenized in 10 ml medinal buffer, pH 7.36. The homogenate was frozen 3 times to −20° and thawed (to destroy the subcellular structures), and then centrifuged on a refrigeration centrifuge. The supernatant was pooled and used to determine the total clotting activity of the organ.

The nuclear, mitochondrial, and microsomal fractions of the kidneys were isolated by homogenizing 1 g of the organ in 10 ml 0.25 M sucrose solution [7]. Consecutively, at different speeds, the nuclear, mitochondrial, and microsomal fractions were then sedimented and each of them was subsequently washed with sucrose. Cold medinal buffer was added to the separate fractions in a volume of 1 ml, after which the fractions were frozen to −20° and thawed 3 times. Intact subcellular fractions were used in a separate series of experiments.

The investigations were carried out on citrated donors' plasma from intact rabbits, from which the platelets had been removed. The plasma was standardized by determining the thrombin time and recalcification time.

We thus investigated the coagulating activity of the kidney homogenates, of the nuclear, mitochondrial, and microsomal fractions, and also of the supernatant (after sedimentation of the microsomes).

To determine the total effect of the subcellular structures on the various phases of blood coagulation, the thromboelastograms of citrated rabbit's plasma were recorded [0.36 ml citrated plasma + 0.036 ml of each of the fractions (in a dilution of 1:500) + 0.1 ml of 0.025 M $CaCl_2$ solution].

The clotting activity of the kidney homogenate and subcellular structures was measured in a silicone-coated test tube at 37°.

Department of Pharmacology and Toxicology, Faculty of Medical Biology, N. I. Pirogov 2nd Moscow Medical Institute (Presented by Active Member of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 66, No. 11, pp. 14-16, November, 1968. Original article submitted June 5, 1967.

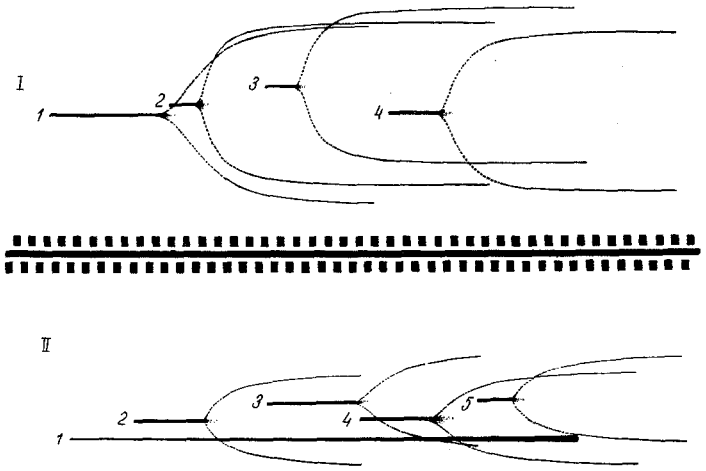

Fig. 1. Effect of subcellular kidney fractions on thromboelastogram indices. I: 1) control (physiological saline); 2) microsomal fraction; 3) mitochondrial fraction; 4) nuclear fraction. II: 1) heparin; 2) homogenate; 3) nuclear fraction; 4) mitochondrial fraction; 5) microsomal fraction.

Measurements of the kinetics of enzyme reactions [2] show that change in the rate of fibrin formation as a result of the polyenzymic proteolytic reaction in this closed system depends on the value of the coagulation potential of the tested fractions, for in all the experiments they were taken in equal quantities, and the constants of the donors' plasma, reagents, and $CaCl_2$ were the same. The activity of each index was expressed as a percentage determined from calibration dilution curves. Changes in the degree of coagulation potential brought about by subcellular kidney fractions were analyzed by statistical methods.

EXPERIMENTAL RESULTS

The experiments on 20 rats showed that after addition of homogenate, or of nuclear, mitochondrial, or microsomal fractions of the kidney or supernatant to the plasma a sharp decrease in the reaction time r was observed in all cases (by 3.4 times for the microsomal, 2.8 times for the mitochondrial, and 2.1 times for the nuclear fractions). This index reflects the rate of formation of active thromboplastin and the appearance of traces of thrombin in the plasma. The interval K, reflecting the beginning of clot formation, was shortened. The coagulation potential of the plasma was increased more distinctly if it had been considerably lowered before addition of the fractions (Fig. 1). Comparison of the clotting activity of the kidney fraction described showed that the most marked changes were produced by the microsomal suspension and supernatant, and less marked changes by the nuclear fraction and homogenate. It should be noted that in relation to the weights of these subcellular structures isolated from 1 g of the organ they were arranged in the opposite order.

The results of this series of experiments show that highly active substances concerned in the regulation of blood coagulation are present in the kidney, or more precisely in its microsomes, mitochondria, and nuclei. The results of thromboelastography show that these substances catalyze the phase of formation of active thromboplastin, thrombin, and fibrin. The elasticity of the clot in its initial state was lower because when this plasma clotted in the jar of the thromboelastograph blood cells were absent. It may thus be postulated that the blood cells and the subcellular structures of the kidney contain factors acting in a similar way on certain properties of fibrin reduced experimentally below their maximal amplitude, as a result of which the fibrin becomes more elastic.

The study of thromboplastic activity in experiments on 38 rats and 12 rabbits showed that addition of each of the tested kidney fractions to standard donors' plasma considerably shortened the plasma clotting time compared with control measurements for which physiological saline was used. The distribution of clotting activity among the ultrastructures in this series was similar to that in the experiments using thromboelastography. Increased clotting activity of the subcellular fractions was revealed when they were diluted in a ratio of 1:400-1:600.

Until recently it was considered that biosynthesis of the clotting factors of the blood system takes place in the liver mitochondria only [5, 6]. However, the view is also held that they are produced in a preformed state in the extrahepatic reticuloendothelial system [3, 8, 9].

The material described above shows that the kidney possesses a very high coagulation potential and is capable of accelerating various phases of blood coagulation. The clotting activity of individual kidney ultrastructures differs. The microsomal fraction plays a greater part than the other subcellular structures in catalysis of the phases of blood coagulation.

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