fluence of insulin [3]. Together with the known data on changes in the function of the plasma membranes in certain cellular tissues of the internal milieu [8, 9], the results now obtained point to the presence of functional changes in the plasma membranes of the fat cells as a special manifestation of a widely distributed phenomenon in these forms of chronic hypertension [1, 2].

Consequently, the increased corticosteroid secretion, reflected in the experiments described above in a general form as hypertrophy of the adrenal cortex in the hypertensive rats, can evidently be regarded as a compensating factor stabilizing the disturbance of the membrane function of the cells in the adipose and other tissues of the internal milieu.

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DYNAMICS OF SOME INDICES OF BLOOD CLOTTING AFTER INTRAPERITONEAL INJECTION OF THROMBIN INTO NONINBRED ALBINO RATS

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The possibility of giving thrombin by intraperitoneal injection as a test of the function of the blood clotting system (the in vivo thrombin test) was demonstrated in experiments on noninbred albino rats.

KEY WORDS: thrombin test; intraperitoneal injection of thrombin.

The in vivo thrombin test [2, 3, 7] involves intravenous injection of thrombin into animals, but this does not always satisfy the experimenter's needs, for intravenous injection may result in severe trauma to the animal and such trauma may itself give rise to changes in the blood clotting system. Intraperitoneal injection of thrombin is interesting from this standpoint [13]. In the investigation described below the results of the study of changes in some indices of blood coagulation after intraperitoneal injection of thrombin were examined.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats of both sexes weighing 160-180 g. Thrombin from Kaunas Bacteriological Preparations Factory was injected intraperitoneally in a dose of 25 units in 0.5 ml of 0.14 M sodium chloride solution, after 2 ml blood had been taken from each animal for control determinations. Later, in the various groups (each of 12 animals), blood was taken for testing after 30 min and 2, 3.5, and 24 h, and stabilized with sodium citrate (1 : 4). The recalcification time in the samples was determined by the method of Bergerhof and Roka in Baluda's modification [9], Quick's test by Tugolukov's method [9], plasma thromboplastin activity as in [10], the total fibrinogen concentration by coagulation [11] and nephelometric methods, fibrinogen B as in [12], and fibrinolytic activity as in [10].

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TABLE 1. Changes in Indices of Blood Clotting After Intraperitoneal Injection of Thrombin (M ± m)

Coagulation Indices	Before injection of thrombin, control	Time after injection of thrombin			
		30 min	2 h	3.5 h	24 h
Fibrinogen concentration, mg %:					
coagulation method	256±6,8	145±15,0	177±10,0	228±12,0	341±9,6
flocculation method	260±7,8	215±9,0	270±17,0	300±21,0	435±11,0
Quick's test, sec	26±1,2	26±3,2	27±3,4	26±1,9	28±2,1
Plasma thromboplastin activity, sec	34±1,7	39±4,1	36±3,5	30±1,7	38±2,9
Recalcification time, sec	112±3,1	108±10,4	140±7,6	112±5,7	119±3,6
Fibrinolytic activity, %	28±4,2	16±9,0	10±6,0	11±7,0	48±5,7
Concentration of fibrinogen B, mg %	0	165±12,0	67±22,0	22±12	0

Legend. Blood of 48 animals tested before injection of thrombin and blood of 12 animals tested at each time after injection of thrombin.

EXPERIMENTAL RESULTS AND DISCUSSION

In the first 30 min after injection of thrombin the fibrinogen concentration determined by the coagulation method was sharply reduced and fibrinogen B was detected in all the animals (Table 1). The shortening of the recalcification time and depression of fibrinolysis were not statistically significant, but in individual animals they were sufficiently clearly defined. The recalcification time was shortened 2 h after injection of thrombin, whereas activation of thromboplastin did not differ from the control values by either the external or the internal methods. The fibrinogen concentration was restored according to the results of nephelometric determination ("flocculating fibrinogen"), but when determined by the coagulation method the increase in the fibrinogen concentration was less marked. Fibrinogen B was found in seven of the 12 animals. After 3.5 h the normal recalcification time was restored, the concentrations of coagulating and flocculating fibrinogen continued to rise, and fibrinogen B was found in three of the 12 animals. After 24 h the concentrations of coagulating and flocculating fibrinogen were higher than the initial levels, no fibrinogen B could be found, and fibrinolysis was activated statistically significantly.

After intraperitoneal injection of thrombin the most significant changes were those affecting fibrinogen – the main coagulation substrate. During the first 30 min the concentration of coagulating fibrinogen fell sharply and its modified fraction (fibrinogen B) appeared. A smaller fall in the fibrinogen concentration was found when determined by the flocculation method. This is probably because in the course of the protective reaction to thrombin additional quantities of endogenous heparin are liberated into the blood stream and fibrinogen-heparin complexes resistant to the action of thrombin are formed [3]. The fact was noted that during the first 30 min after injection of thrombin a decrease in the fibrinogen concentration was discovered by both methods of determination; fibrinogen B also appeared in the plasma. At this stage it may be that true hypofibrinogenemia, developing as a result of partial fibrination [4-6], was observed at this stage. Later unstabilized microclots became fragmented with the formation of large fragments of fibrin, which were precipitated during the nephelometric method of determination, but which do not clot under the influence of thrombin in the coagulation method. Accordingly the fibrinogen concentration determined by the flocculation method was considerably greater than that determined by the coagulation method. Later, fibrinogen of this kind may gradually become incorporated into the fibrin clot, causing reversal of the results of determination, as has frequently been mentioned in the literature [1, 8]. Ultimately a picture of apparent hypofibrinogenemia was observed, due not to the absence of fibrinogen in the blood stream, but to its refractoriness to thrombin. This mechanism of development of apparent hypofibrinogenemia can be deduced from determination of fibrinolytic activity by comparing the fibrin content in clots formed as a result of exposure to thrombin for 1 and 24 h: In the presence of slowly coagulating fibrinogen in the clots, instead of a decrease in the fibrin concentration (as a result of breakdown by plasmin) after 24 h of coagulation an increase was observed. In the experimental models used in the present investigation this paradoxical increase in fibrin was observed in three cases 30 min after injection of thrombin, and in six cases in samples of blood plasma taken 2 and 3.5 h, respectively, after injection of thrombin. No such cases were observed with blood plasma taken 24 h after injection of thrombin.

Special attention must be paid to the dynamics of changes in the fibrinogen B concentration. The discovery of this protein, a product of semiconversion of fibrinogen into fibrin, is evidence of an increased threat or of the actual occurrence of intravascular clotting [2, 6]. The writer has shown that actual thrombus formation is accompanied by the appearance of fibrinogen B in concentrations exceeding 100 mg %, whereas during the threat of thrombus formation the concentration of this protein does not reach 100 mg % [12]. Fibrinogen B was found 30 min after intravenous injection of thrombin in concentrations exceeding 100 mg % in all the animals,

but in only seven of 12 animals after 2 h, and in three animals after 3.5 h. Fibrinogen B in a concentration of 60 mg% was found in one animal after 24 h. The differential diagnostic value of the discovery of fibrinogen B is thus limited to the acute period of action of thrombin, and in the late stages after injection of thrombin the likelihood of its discovery is small.

To conclude, intravenous injection of thrombin changes the state of the blood clotting system in the same direction as its intravenous injection; consequently, if it is necessary to carry out a thrombin function test, the thrombin may be injected intraperitoneally.

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