DEPRESSION OF FIBRINASE ACTIVITY OF BLOOD PLASMA BY VITAMIN K AND NEODICOUMARIN

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Investigations conducted in the author's laboratory showed that in vitamin K deficiency not only is the biosynthesis of procoagulants depressed, but changes also occur in myosin A: its ATPase activity and the contractile power of the myosin filaments are reduced; these disturbances are abolished by administration of the water-soluble vitamin K analogue vikasol (2-methyl-1, 4-naphthoquinone bisulfite) to animals with avitaminosis K [3, 4]. The effect of vikasol on the other fibrillary protein, fibrin, was therefore investigated. Administration of vikasol to rabbits led to a sharp increase in the elasticity of the fibrin clots of the blood plasma. A similar effect was observed in experiments in vitro—on the addition of vikasol to oxalated human plasma [2, 5]. The hypothesis was put forward that this effect may be connected with the influence of vitamin K on the process of polymerization of fibrin and that it may take place through a change in the activity of fibrinase, an enzyme strengthening the bonds between the fibrin molecules, as a result of which the fibrin clot becomes insoluble in urea and monochloroacetic acid: by the action of fibrinase the urea-soluble fibrin s is converted into urea-insoluble fibrin i.

This paper describes experiments in which the effect of administration of the water-soluble vitamin K analogue, vikasol, and the antivitamin K, neodicoumarin, on the activity of the fibrinase of the blood was investigated in vivo.

EXPERIMENTAL METHOD

Experiments were carried out on 34 adult male rabbits weighing 2.5-3 kg. In 19 animals the fibrinase activity was determined before and 24 h after administration of vikasol, which was given orally to the animals once daily in a dose of 2 mg/kg body weight. After determination of the prothrombin time and fibrinase acticity, 15 animals received neodicoumarin twice daily by mouth in a dose of 2.5 mg/kg body weight. The initial values of the prothrombin time were 23-26 sec, and on the 3rd-4th day of feeding with neodicoumarin it increased to 40-45 sec, and during this period the fibrinase activity was estimated repeatedly.

To determine the fibrinase activity,the method of Sigg and Duckert [14] was used. This is based on the ability of monoiodoacetic acid to block the change from fibrin s into fibrin i under the influence of fibrinase. This method was used in all the experiments as originally described, and in some experiments in the author's modification also.

Blood was taken from the marginal vein of the ear, mixed with a 1.34% solution of sodium oxalate in the ratio of 9:1, and quickly centrifuged for 10 min at 2000 rpm. The plasma was aspirated and investigated at once. Into each of 7 test tubes 0.2 ml plasma was poured, and into the first six tubes 0.1 ml of a solution of monoiodoacetic acid of descending concentrations was added (in tubes Nos. 1-6 from 0.6 to 0.1% respectively). Into all seven tubes 0.4 ml of 0.277% calcium chloride solution was added, followed by 0.1 ml thrombin purified from plasminogen (activity 15 sec). After being kept at 20° for 20 min, the contents of the tubes were treated with 2 ml of 5 M urea solution and the degree of solution of the fibrin was determined. In accordance with the original description given by Sigg and Duckert, this was expressed by a scale showing the character of the contents of the tubes after the addition of the calcium chloride and thrombin to the plasma containing monoiodoacetic acid: 0—no clot formed, 1—clot completely dissolved on addition of urea, 2—small floccules, 3—large floccules, 4—the clot did not dissolve. In control tube No. 7 (not containing monoiodoacetic acid) a compact clot was present in all the experiments.

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TABLE 1. Plasma Fibrinase Activity before and 24 h after Administration of Vikasol to Rabbits in a Dose of 2 mg/kg Body Weight

Concentration of monoiodoacetic acid (in %)	Estimation of fibrinase by original Sigg-Duckert method (19 experiments)				Estimation of fibrinase by modified Sigg-Duckert method (8 experiments)			
	degree of solution of clot		t	P	extinction (in % of control)		t	P
	before administration of vikasol	after administration of vikasol			before administration of vikasol	after administration of vikasol		
0,6	2,2	2,2			44	46	0,64	0,5
0,5	2,2	2,1	1,90	<0,1	51	47	0,75	0,5
0,4	3,3	2,4	4,20	<0,001	50	47	4,67	<0,02
0,3	3,8	2,6	5,61	<0,001	70	51	3,75	<0,01
0,2	4,0	3,1	11,84	<0,001	80	64	3,22	<0,01
0,1	4,0	3,5	4,13	<0,001	84	71	2,63	<0,02

TABLE 2. Plasma Fibrinase Activity before and after Administration of Neodicoumarin to Rabbits

Concentration of monoiodoacetic acid (in %)	Estimation of fibrinase by original Sigg-Duckert method (15 experiments)				Estimation of fibrinase by modified Sigg-Duckert method (15 experiments)			
	degree of solutions of clots		t	P	extinction (in % of control)		t	P
	before administration of neodicoumarin	after administration of neodicoumarin			before administration of neodicoumarin	after administration of neodicoumarin		
0,6	2,0	2,0			46	36	3,36	<0,01
0,5	2,0	2,0			52	41	4,02	<0,02
0,4	2,6	2,0	2,80	<0,01	59	43	4,45	<0,01
0,3	2,7	2,2	2,32	<0,05	75	60	2,25	<0,05
0,2	3,6	3,0	2,60	<0,01	85	75	3,91	<0,001
0,1	3,8	3,7	1,16	<0,2	91	84	2,17	<0,01

Dissatisfied with the visual method of reading the results, the author modified the Sigg and Duckert method as follows. The investigation itself was carried out completely as described by them, but the degree of solution of the fibrin after addition of urea was recorded on the basis of the decrease in the protein content in the residue (the clot) instead of by describing the state of the clot. For this purpose the clot was separated by centrifugation, dissolved in 2 ml 0.1 N NaOh, and the protein was estimated by Lowry's method using a type FEK-M photoelectric colorimeter with a filter S-66 for the measurements. The extinction in control tube No. 7 was taken as 100.

EXPERIMENTAL RESULTS

Effect of Vikasol. The results of this group of experiments are given (as mean values) in Table 1.

In the left portion of the table the results given were obtained by the original Sigg–Duckert method, and in the right portion by the author's modification of this method. Since the investigations before and after administration of vikasol were carried out repeatedly on the same animals, the significance of the differences was determined by the difference method [6]. The values of the index of significance of the difference t, and the level of probability of the difference P, showed that both methods were capable of yielding consistent and statistically significant results: when the original Sigg–Duckert method was used the values of the conventional scale index were reduced, suggesting an increase in the solubility of the fibrin clots in urea; when the modified method was used a decrease in the extinction values, i.e., in the protein content in the residues (clots), was detected. The use of both variants of the method thus showed that after administration of vikasol to the animals relatively smaller amounts of monoiodoacetic acid were required to inhibit the fibrinase, i.e., the activity of this enzyme was depressed. The somewhat smaller

absolute values of the indices t and P in the right half of Table 1 are attributable to the fact that, when the modification was used, fewer animals (8) were investigated than when the original Sigg–Duckert method was used (19) [14]. Nevertheless, both variants of the method led to statistically significant differences between the experiment and the control.

Experiments with Neodicoumarin. The results of this group of experiments are shown in Table 2.

It is clear from Table 2 that the administration of neodicoumarin to rabbits also led to a reduction of the fibrinase activity. When the original Sigg–Duckert method was used this was revealed in the test in which three concentrations of monoiodiacetic acid (0.4, 0.3, and 0.2%) were added. The modified method detected differences with all six concentrations of added fibrinase inhibitor.

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