INCREASE IN PLASMA FIBRIN CLOT ELASTICITY IN VIVO AND IN VITRO DUE TO MENADIONE SODIUM BISULFITE

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It is usually considered that vitamin K plays an indirect role in blood clotting associated with the stimulation of the biosynthesis of certain procoagulants in the liver – prothrombin, proconvertin, and factors IX and X. If vitamin K is added to blood in vitro it has no effect on its clotting power.

Investigations in the authors' laboratory have shown that in vitamin K deficiency the properties of some functional proteins are modified [1, 4], and in particular, the contractile power of the myosin filaments and the ATPase activity of the myosin are reduced [3]. The contractile properties and ATPase activity of myosin in animals with avitaminosis K can be increased both by administration of vitamin K (the soluble preparation) and after its direct addition to myosin solutions [3].

It was therefore decided to investigate the effect of menadione sodium bisulfite on the properties of another fibrillary protein, namely fibrin. The results of experiments to investigate the changes in the elastic properties of fibrin clots in blood plasma are described in this paper.

EXPERIMENTAL METHOD

Experiments in vivo were carried out on male rabbits weighing 2.5-3.5 kg receiving a normal laboratory diet. Blood was taken from the marginal vein of the ear, mixed with 1.34% sodium oxalate solution in the ratio of 9 : 1, and centrifuged at 2000 rpm to separate the plasma. The elasticity of the fibrin clot (E) was measured by means of a thromboelastograph, the result being calculated from the formula:

$$E=\frac{100\times a_{\text{M}}}{100-a_{\text{M}}},$$

where a_m denotes the maximal amplitude of the thromboelastograph [8]. To obtain a clot, 0.5 ml of plasma was placed in the receiver of a thromboelastograph, 0.1 ml of a 2.7% solution of $CaCl_2$ was added, and the thromboelastogram was recorded. The animal was then given menadione sodium bisulfite by mouth in a dose of 2 mg/kg, and 3 and 24 h later the elasticity of the recalcified plasma clots was again measured. In 5 rabbits the observations were continued for 3 days, and these animals received menadione 24 h before each measurement of the elasticity of the clot.

Experiments in vitro were performed on citrated blood plasma from human donors. The plasma was divided into two portions. In the control tests, 0.5 ml of plasma was treated with 0.1 ml of physiological saline, and in the experimental tests with 0.1 ml of a 2.5% solution of menadione sodium bisulfite in physiological saline. In this way the same concentrations of the vitamin K substitute was obtained in the plasma as that which in a solution of myosin, in P. V. Lidina's experiments [3], caused an increase in the contractile power of the filaments. The experimental and control samples were incubated for 30 min at 20°, after which measurements were made of the elasticity of the clot obtained as a result of recalcification of the plasma by the addition of 0.1 ml of a 2.7% solution of $CaCl_2$ to it.

EXPERIMENTAL RESULTS AND DISCUSSION

Experiments in vivo. Changes in the character of the thromboelastograms were found after administration of the soluble vitamin K substitute to the animals (Fig. 1). The increase in amplitude demonstrated an increase in the elasticity of the fibrin clot. The experiments on rabbits Nos. 1-6 were carried out with the apparatus working in conditions so that the initial (before administration of vitamin K) indices of

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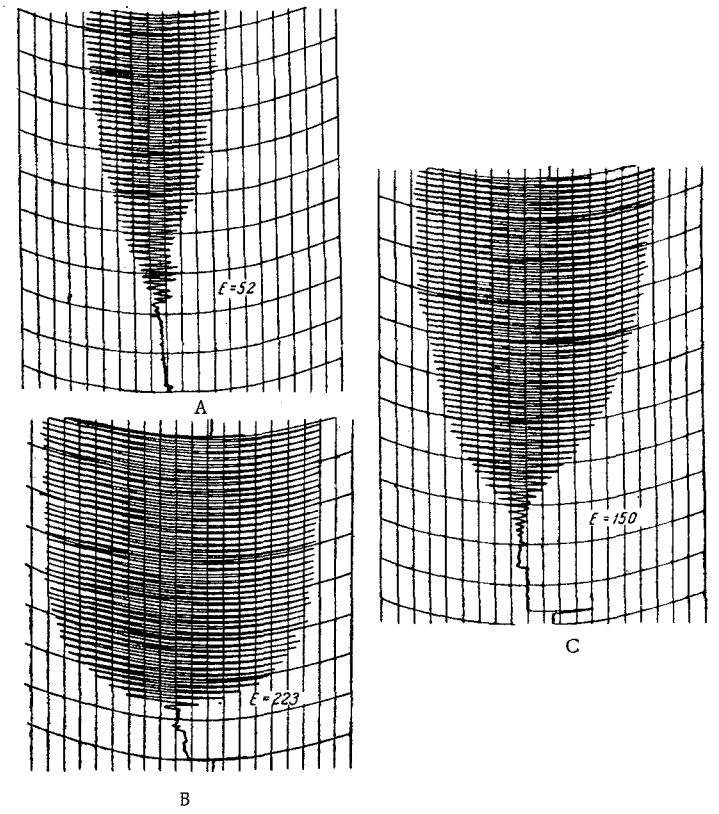

Fig. 1. Thromboelastogram of rabbit's plasma before administration (A) and 3 h (B) and 24 h (C) after administration of menadione sodium bisulfite.

elasticity in the animals varied from 50 to 82. Having discovered an increase in the value of E in the animals fed with vitamin K, it was decided that for the next experiments the apparatus should be adjusted to a different setting which would then be left undisturbed throughout the remainder of the experiment on a particular subgroup of animals. For the experiments on rabbits Nos. 7-11, the initial values of the elasticity were between 122 and 150, and for the experiments on rabbits Nos. 12-15 they were between 177 and 245.

With all three settings of the apparatus, the elasticity of the plasma fibrin clots from all 15 animals increased. The elasticity of the fibrin clots before administration of vitamin K averaged 123, increasing to 205 ($P = 0.001$) 3 h after administration of the preparation, to 221 ($P = 0.001$ 24 h, and to 256 ($P < 0.02$) 48 h after administration, while after 72 h its value was 206 ($P > 0.05$).

The increase in elasticity of the fibrin clots was not accompanied by any similar regular decrease in their time of formation. The latent period on the thromboelastogram (r) 3 h after administration of menadione sodium bisulfite to the rabbits was shorter than initially in 7 experiments, unchanged in 3 ex-

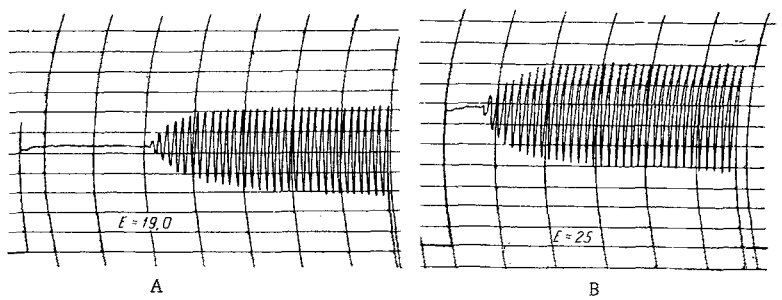

Fig. 2. Thromboelastogram of human blood plasma before (A) and 30 min after (B) addition of menadione sodium bisulfite to the plasma.

periments, and longer in 3 animals. The latent period 24 h after administration of menadione sodium bisulfite was shortened in 7 experiments, unchanged in 3 rabbits, and lengthened in 2. The mean value of r (M ± m) before administration of menadione sodium bisulfite was 5.6 ± 0.54 min, diminishing to 5.1-0.58 min 3 h after administration ($P > 0.2$) and to 4.7 ± 0.52 min 24 h after administration ($P > 0.05$).

The absence of any significant effect of menadione sodium bisulfite on the latent period of formation of the clot was reflected in the absence of changes in the prothrombin time, the mean value of which was 25 sec before administration and 24.6 sec 24 h after administration of the vitamin K substitute.

The dose of menadione sodium bisulfite used in these experiments, when administered to rabbits, thus caused a sharp increase in the elasticity of the plasma fibrin clot, but had no significant effect on the activity of the procoagulant responsible for the formation of thromboplastin and thrombin.

Experiments in vitro. A typical result of this series of experiments is illustrated by the thromboelastograms shown in Fig. 2.

Only in two experiments (Nos. 4 and 16) was the elasticity of the fibrin clots the same in the experimental and control tests. In the other 18 experiments, after addition of menadione sodium bisulfite to the plasma the elasticity of the fibrin clots was increased. The index of elasticity in the test with addition of menadione sodium bisulfite increased on the average from 25.5 to 32.3, i.e., by 26% ($P < 0.001$).

While the result of the experiments of series 1 provided weighty evidence that menadione sodium bisulfite affects not only the synthesis of procoagulants in the body but also the third final phase of the process of blood clotting, the results of the experiments of series II, in which this effect was observed in vitro, prove this hypothesis conclusively. Clearly the point of application of vitamin K (or at least of the bisulfite derivatives of 2-methyl-1,4-naphthoquinone) in the animal body is not only the synthesis of procoagulants in the liver, but also the actual process of conversion of fibrinogen into fibrin.

These results are in good agreement with those of experiments showing that administration of vitamin K_3 to patients increases the plasticity of thrombin [2]. The positive effect of vitamin K on the healing of wounds and ulcers observed by A. V. Palladin even in patients with normal prothrombin activity and clotting power of the blood [5] may be dependent on its effect on the properties of the fibrin in the wound.

In connection with the experiments described above it is interesting to note that in 1956 it was shown that if vitamin K_1, labelled with C^{14}, is administered to rats, it is bound in the blood with the protein fraction containing fibrinogen [9], and it has recently been reported [7] that aqueous extracts of fibrin contain a substance which, although not thrombin, promotes coagulation of the blood and during chromatographic investigation behaves like vitamin K_3; it is possible that this is a vitamin K derivative directly concerned in fibrin formation.

The increase in the elasticity of fibrin clots in blood plasma following administration of vitamin K may provisionally be associated with the effect of this substance on the polymerization of fibrin, an effect probably brought about through its influence on the activity of the fibrinase (fibrin-stabilizing factor) of the plasma.

There is another possible mechanism of this influence of vitamin K on the properties of the fibrin clot. A protein, thrombostenin, responsible for clot retraction, has recently been isolated from platelets. This protein is very similar to the actomyosin of muscles; possesses high ATPase activity. Just as from actomyosin, filaments can be obtained from it which contract in the presence of ATP and Mg^{2+} [6]. Bearing in mind the action of vitamin K on ATPase activity and the contractile properties of myosin discussed above, it may be assumed that the effect of this vitamin (menadione) on the properties of fibrin may be brought about through its action on the ATPase activity and contractility of thrombostenin.

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