Failure of Actinomycin D to Inhibit Appearance of Clotting Activity by Vitamin K in vitro

In vitamin K-deficient and coumarin anticoagulant-treated animals, vitamin K increases the plasma levels of vitamin K-dependent clotting factors (factors II, VII, IX and X). OLSON[1,2] has reported that this increase can be prevented by actinomycin D, an inhibitor of DNA-dependent (mRNA) synthesis. On the basis of this observation it was concluded that induction of mRNA formation is the mechanism by which vitamin K acts on plasma clotting factor synthesis. Such a mechanism would be consistent with current concepts of protein biosynthesis[3].

POOL and ROBINSON[4] have shown that incubation of liver slices from normal, but not from coumarin anticoagulant-treated rats, result in the appearance of plasma clotting factors in the medium. They failed, however, to induce the appearance in the medium of plasma clotting factors from slices of coumarin treated animals by the addition of vitamin K to the incubation medium[5]. This failure can be traced to insufficient solubilization of the fat-soluble vitamin, since in the present communication it is shown that when vitamin K is solubilized with Tween 80, addition of vitamin K induces the appearance of plasma clotting factors in vitro. This finding makes it possible to study the effect of actinomycin D on the action of vitamin K in vitro and thus avoid some of the complications that may have arisen in studies with intact animals.

Rats were given, by stomach tube, a single dose of 125 μg/100 g of body weight of warfarin (3-[α-acetonyl-benzyl]-4-hydroxycoumarin). After 18 h, when plasma levels of factor VII were less than 3% of normal, the animals were killed, the livers removed and slices approximately 0.5 mm thick prepared by the method of DEUTSCH[6]. The slices were washed 3 times with cold bicarbonate-buffered balanced salt solution[7], and 1.0 g of slices in 10 ml of buffered medium was incubated in a Dubnoff shaker at 37 °C in an atmosphere of 95% oxygen/5% carbon dioxide. At the times indicated (see Figures), 0.5 ml of medium was removed and mixed with 0.1 ml of 3.8% sodium citrate solution. Factor VII activity was determined on 0.1 ml of the citrated samples by the method of KOLLER et al.[8].

Because of its greater solubility in vitro, a vitamin K analogue with a polyisoprenoid side chain of 10 carbon atoms, 2-methyl-3-geranyl-1,4-naphthoquinone, was used. In coumarin anticoagulant-treated animals this compound has approximately 25% of the activity of vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone)[9]. The vitamin K analogue was brought into suspension with Tween 80, as described previously[10]. Neither Tween 80 nor actinomycin had any effect when added to the incubation system and none of the substances used (vitamin K, Tween 80, actinomycin D) had any effect when added directly to the factor VII assay. To prevent surface activation of factor VII, all glassware, including the Erlenmeyer flasks used for the incubation, were treated with silicone.

Incubation of slices from anticoagulant-pretreated animals did not result in the appearance of factor VII in the medium unless vitamin K (1.0 mg per flask) was added to the incubation system. Actinomycin, at a dose of 250 and 500 μg per flask, failed to inhibit the response to the vitamin (Figure 1, a) When the slices were pre-incubated with actinomycin (500 μg per flask) for $^1/_2$ or 1 h before addition of vitamin K, the response was still the same as that obtained with slices preincubated for the same period, but in the absence of actinomycin (Figure 1, b and c)

In the experiments reported by OLSON, actinomycin was administered 4 h before vitamin K. It is thus possible that inhibition of mRNA synthesis during the 4 h actinomycin pretreatment may have reduced the number of ribosomes programmed for plasma clotting factor

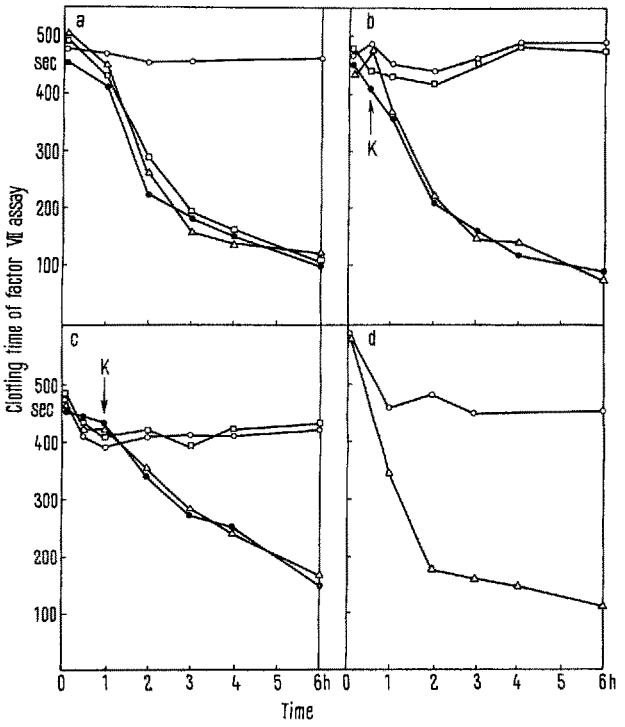

Fig. 1. Effect of actinomycin on vitamin K induced appearance of factor VII by liver slices from anticoagulant-treated rats. (a) Actinomycin and vitamin K added simultaneously. ○—○ no additions, ●—● vitamin K (1.0 mg), □—□ vitamin K (1.0 mg) and actinomycin (250 μg), △—△ vitamin K (1.0 mg) and actinomycin (500 μg). (b) Slices pre-incubated for $^1/_2$ h before addition of vitamin K. ○—○ no addition, □—□ actinomycin (500 μg), ●—● actinomycin (500 μg) and vitamin K (1.0 mg), △—△ vitamin K (1.0 mg). (c) Slices pre-incubated for 1 h before addition of vitamin K. ○—○ no additions, □—□ actinomycin (500 μg), ●—● actinomycin (500 μg) and vitamin K (1.0 mg), △—△ vitamin K (1.0 mg). (d) actinomycin (140 μg/100 g) administered 4 h before animals were killed. ○—○ no additions, △—△ vitamin K (1.0 mg). Since data from different experiments cannot be pooled numerically, the results shown are of typical single experiments. Confirmatory results were obtained for at least 2 additional experimental protocols. Doses refer to amount of substances added per flask.

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synthesis, so that at the time vitamin K was administered, a full response could not be obtained To test for this possibility, actinomycin (140 μg/100 g) was given i v. 4 h before the animals were killed. As shown in Figure 1, d, liver slices from such animals responded normally to vitamin K. OLSON also measured the change in plasma clotting factors 6 h after the i.p. injection of vitamin K. It has been found previously that when vitamin K is solubilized with Tween 80 and given i.v. to anticoagulant-treated animals, an increase of the plasma concentration of clotting factors can be detected already after 30 min and can be monitored in the same animal by the taking of blood samples at frequent intervals[11]. When actinomycin (350 μg/100 g) was given i.v. by injection into a tail vein 2 h before vitamin K_1 (50 μg/100 g) the rate of increase of the plasma levels of factor VII during the next 2 h was reduced only slightly (Figure 2). However,

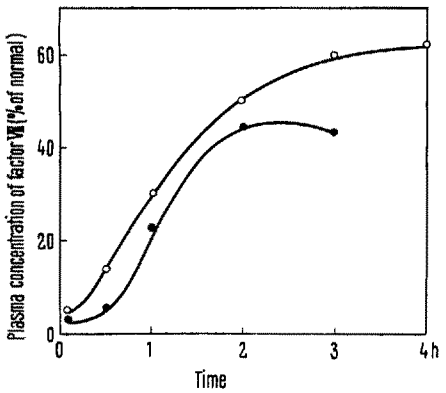

Fig. 2. Effect of actinomycin on the increase of the plasma concentration of factor VII by vitamin K_1 in anticoagulant-treated rats. O—O vitamin K_1 (50 μg/100 g), ●—● actinomycin (350 μg/100 g) given 2 h before vitamin K_1 (50 μg/100 g). Confirmatory results were obtained in 2 additional experiments.

the animals that had received actinomycin were definitely in a poor state and it became progressively more and more difficult to get blood samples by tail vein puncture. While the animals that had received actinomycin died within 24 h, in the control animals factor VII concentrations had returned to normal after 24 h.

The failure to demonstrate an effect by actinomycin in vitro and the likelihood that actinomycin can produce non-specific toxic effects under the conditions where inhibition has been reported in intact animals rules out the possibility that vitamin K acts at the level of mRNA synthesis. The present findings, therefore, do not support the hypothesis that vitamin K, and by analogy other fat-soluble vitamins, operate to control the synthesis of specific proteins by regulating mRNA formation[12,13].

Zusammenfassung. Lebergewebeschnitte mit Cumarin antikoagulentienvorbehandelter Ratten zeigen den Faktor VII im Medium nur beim Zusatz von Vitamin K. Die Vitamin-K-Wirkung wird bei gleichzeitigem Zusatz von Aktinomycin D nicht beeinflusst. Diese Beobachtung stimmt nicht überein mit der Hypothese, dass Vitamin K durch Beförderung der Auslösung der für die Synthese des Faktors VII spezifischen t-RNA eine Wirkung ausübt.

J. LOWENTHAL and ELIZABETH L. SIMMONS

Department of Biochemistry, Queen's University, Kingston (Ontario, Canada), 25th November 1966.

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The Effect of Basic Vital Dyes on the Acid Phosphatase Activity of the Granulated Juxtaglomerular Cells

The vital staining of the granulated juxtaglomerular cells (JGC) with neutral red was observed for the first time by SUGIYAMA[1], later HARADA[2], WORTHINGTON[3] and CORBASCIO[4] studied the details of the staining mechanism. ROSENBAUER[5] and SZOKOLY, GOMBA and SOLTÉSZ[6] found some other basic dyes staining the JGC vitally in addition to the neutral red. According to RUYTER'S[7] and our (GOMBA, SOLTÉSZ and SZOKOLY[8]) histochemical investigations the JGC of the mouse kidney contains acid phosphatase localized most probably to the secretory granules. Because, in the case of the neutral red, the vital staining properties of some intracytoplasmic granules has been found by HAFIEK and KOVÁCS[9] to be related with their acid phosphatase activity, therefore we examined the effect of some basic vital dyes on the acid phosphatase activity of the mouse JGC.

80 white mice of our own strain, of both sexes, weighing about 25 g, were used. The vital stains were the following: neutral red, neutral violet, acridin orange, Nile blue sulphate, brilliant cresyl blue. The dyes were dissolved in 1–2% concentrations in 0.85% NaCl. 1–2 ml of these solutions was injected into the tail vein or into the thoracic aorta (through the cranially ligated left common carotid artery) and the animals were killed 2–3 min after the injection. Each stain was tested also giving 1 ml 1% solution i.p. In those cases we killed the mice after 15, 30, 60 min or 6 and 8 h. In some cases the neutral red, Nile blue

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