

Nature of prothrombin biosynthesis

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**NATURE OF PROTHROMBIN
BIOSYNTHESIS :
PREPROTHROMBINÆMIA IN
VITAMIN K-DEFICIENCY**

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NATURE OF PROTHROMBIN BIOSYNTHESIS: PRE-
PROTHROMBINAEMIA IN VITAMIN K-DEFICIENCY.
*H. C. Hemker, J. J. Veltkamp, A. Hensen and
E. A. Loeliger.* From University Hospital, Ley-
den, The Netherlands. *Nature*, London 200:589-
590, 1963.

BLOOD

Using a modified thrombotest (Owren) the authors found a difference in the behavior of plasmas with lowered clotting factors II, VII, IX and X derived from patients with hepatic damage as opposed to patients with vitamin K deficiency. They suggest that the data could be explained by the presence of an inhibitor in vitamin K deficiency which is active in both the intrinsic and extrinsic coagulation systems. This inhibitor might be a precursor of prothrombin normally present only in hepatic cells.—*I. C.*

Nature of Prothrombin Biosynthesis : Preprothrombinæmia in Vitamin K-deficiency

THE activities of the clotting factors II, VII, IX, and X are mutually lowered to a similar degree in chronic hepatic damage as well as during prolonged real or relative (coumarin-induced) vitamin K-deficiency^{1,2}. In the experiments described here, Owren's thrombotest (slightly modified, Fig. 1) is used for the assessment of the extent of depression. Each plasma sample is tested in a series of dilutions (D) and the coagulation time (t , mean of 4 readings) measured in a coagulometer^{3,4}. All plasmas and plasma dilutions are kept in carefully siliconized glass-ware or plastic material. When t is plotted against D , a straight line is obtained (Fig. 1).

The slope of the line is proportional to the substrate (complex of Factors II, VII, IX and X) concentration in the plasma tested. Extrapolation to infinite substrate concentration (intercept of the line obtained with the ordinate) will give the minimal coagulation time (t_{\min}). Plasmas with different substrate concentrations all display the same value for t_{\min} . This holds for plasmas from patients suffering from hepatic damage of different degree (Fig. 1, above). In vitamin K-deficiency, however, t_{\min} is generally found to be markedly elevated, suggesting the presence of an inhibitor (Fig. 1, below). A Lineweaver-Burk plot⁵ of values obtained from mixtures of a constant amount of plasma from a vitamin K-deficient patient with various amounts of normal plasma, as compared with values obtained with normal plasma alone, shows that a competitive inhibitor is present (Fig. 2).

The inhibitor appears to be active in both the intrinsic and extrinsic coagulation system. Addition of factors II, VII, and X, or of Russell's viper venom to the reaction mixture does not abolish the inhibition. The inhibitor is heat labile, undialysable and adsorbable on to barium sulphate, aluminium hydroxide, calcium phosphate and bentonite. There is a simple graphical way to estimate the amount of inhibitor (Fig. 1, below): When a line (1) is drawn through t_{\min} of normal plasma parallel to the abscissa, the line obtained from the values of a plasma containing the inhibitor (i) will intercept l at a certain distance (r) left of the ordinate. The length of r is proportional to the amount of inhibitor present, independent of the amount of substrate. One unit of inhibitor we define as the amount of inhibitor resulting in an r -value equal to the distance from the origin to $D=1$. As can be seen from Fig. 1b, the amount of inhibitor in elderly coumarin-treated patients displaying a clotting factor activity below 50 per cent seems to be independent of the intensity of treatment⁶.

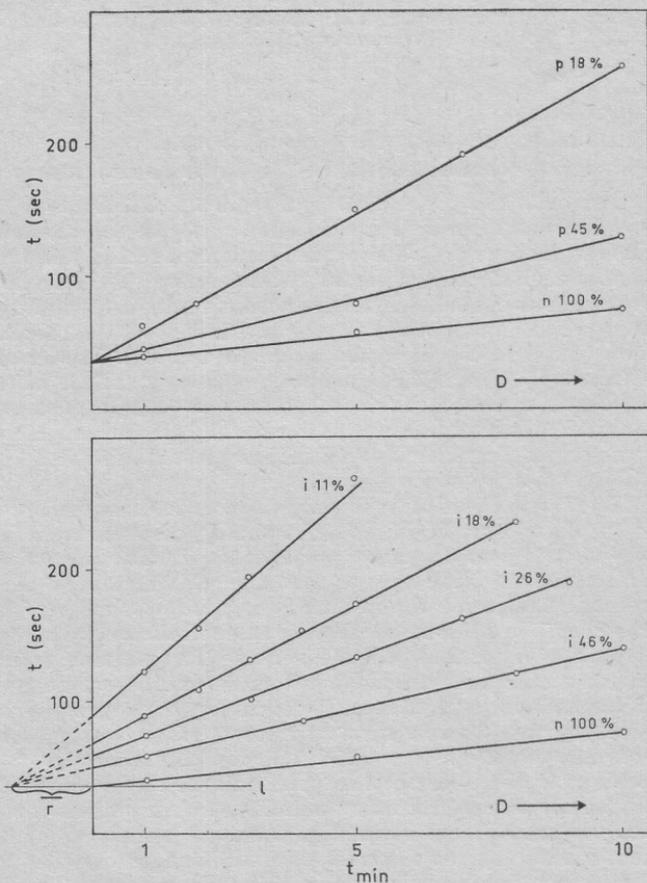


Fig. 1. Relation between plasma dilution and coagulation time. Above, in normal plasma (n) and chronic liver disease (p); below, in normal plasma and in plasma of dicoumarol-treated patients (i). Reaction medium: 0.25 ml. thrombotest reagent; 0.05 ml. plasma (at $D = 1$), or 0.05 ml. of a mixture of plasma with x times its volume of Michaelis buffer (at $D = x + 1$). The percentages indicate the relative amount of substrate present in the original plasma. For explanation of the letters see text

These findings fit in the following hypothesis: A protein precursor of prothrombin (tentatively called preprothrombin) is synthesized in liver cells. Under normal conditions this protein is converted to prothrombin in a vitamin K-dependent step. The blood-level of prothrombin is the feed-back signal for preprothrombin synthesis. When the vitamin K-dependent conversion is inhibited, the feed-back signal causes an excess production of preprothrombin which is shed into the bloodstream and acts as a competitive inhibitor of prothrombin conversion. Consistent with this view, a maximum feed-back signal

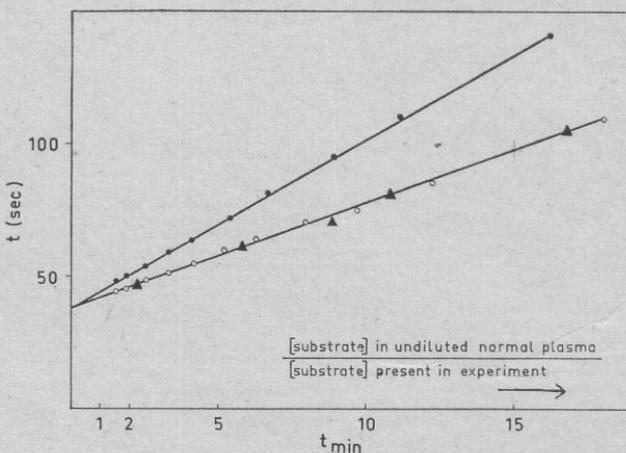


Fig. 2. Relation between substrate concentration and coagulation time. \circ , Normal plasma; \bullet , normal plasma with a constant amount of dicoumarol plasma in all dilutions; \blacktriangle , normal plasma with a constant amount of BaSO_4 -absorbed dicoumarol plasma. Reaction medium as in the experiments demonstrated in Fig. 1

(prothrombin-level below 50 per cent) establishes maximum synthesis of inhibitor, the amount of which is dependent on the protein-synthesizing capacity of the liver.

It is evident that demonstration of preprothrombin is a useful and rapid diagnostic procedure in vitamin K-deficiency. Preliminary clinical experience suggests that the amount of preprothrombin formed during coumarin treatment reflects liver function.

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¹Loeliger, E. A., van der Esch, B., Mattern, M. J., and den Brabander A. S. A., *Thrombos. Diathes. Hæmorrh.*, **9**, 74 (1963).

²Loeliger, E. A. (unpublished results).

³Schnitger, H., and Gross, R., *Klin. Wschr.*, **32**, 1011 (1954).

⁴Schnitger, H., M.D. thesis, Marburg (1956).

⁵Lineweaver, H., and Burk, D., *J. Amer. Chem. Soc.*, **56**, 658 (1934).

⁶Hemker, H. C., Veltkamp, J. J., Hensen, A., and Loeliger, E. A., *Trans. Conf. Intern. Comm. Blood Clotting Factors*, 1963 (F. K. Schattauer-Verlag, Stuttgart; in the press).