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PRINCIPLES OF THE MODE OF ACTION OF COUMARIN CONGENERS

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BIOSYNTHESIS of the four coagulation factors of the so-called prothrombin complex, i.e. factors II, VII, IX, and X, in the hepatic cell depends on the presence of vitamin K. Vitamin K-antagonists, and among these the coumarin congeners (comprising 4-hydroxy-coumarin and indan-1:3-dione derivatives), induce a reduction in the rate of synthesis of the four coagulation factors as judged from a decrease in their respective activities in the blood. The similarity to vitamin K of the chemical structure of the coumarin congeners (Fig. 1) suggests that they are likely to compete with vitamin K. Coagulation factor synthesis is supposed to be blocked at sites at which an interchange of the physiological vitamin K with the toxic

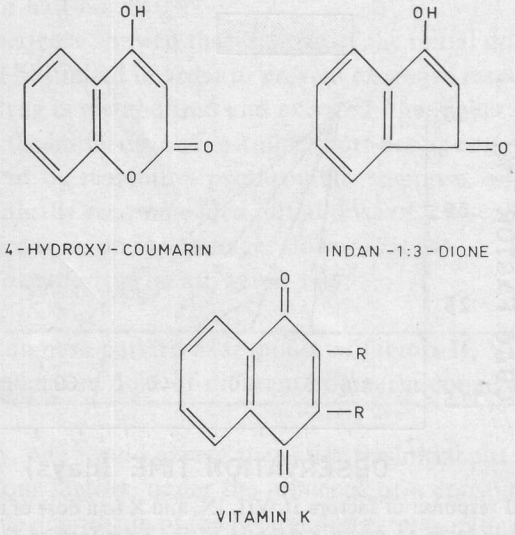


FIG. 1. Chemical structure of coumarin congeners and vitamin K.

coumarin congener molecule has occurred. So much for our present common knowledge.

Although coumarin congeners have now been used, experimentally as well as therapeutically, for more than 25 years, their biochemical and pharmacological mode of action is still a matter of opinion and thus open to discussion.

As an introduction to the present discussion, I would like to put before you some results of our work that might help to answer some pertinent questions.

One of the questions we attempted to answer was:

> Is there a common pattern of response of factors II, VII, IX, and X to an initial loading dose of different coumarin congeners?

In an *experimental study* in man, Van der Esch in our laboratory irrefutably demonstrated that different coumarin congeners, administered in doses sufficiently large to arrest synthesis of factors II, VII, IX, and X, induce identical reactions:[4] as shown in Fig. 2, 4–8 hours after oral administration the activities of coagulation factors II, VII, IX, and X start to decrease at rates defined by half-times of about 60, 6, 20, and 40 hours respectively.[4, 11] These half-times found in patients under normal metabolic conditions probably represent the normal metabolic rates of decay because we were able to provide evidence that the production rate and rate

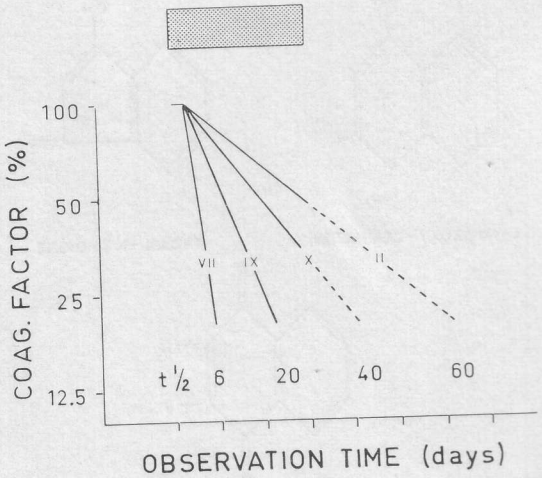


FIG. 2. Normal response of factors II, VII, IX, and X to a dose of coumarin congener sufficiently large to arrest synthesis of the four factors. On the ordinate coagulation factor activities are represented in a logarithmic scale.

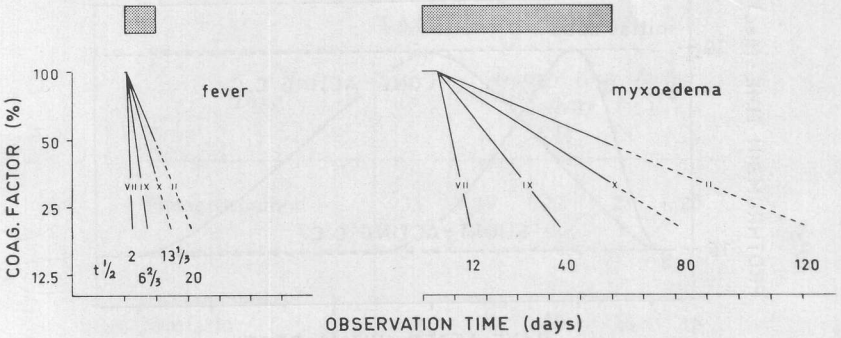


FIG. 3. Response of the four factors under pathological conditions: shortened lag phase and increased disappearance rates in fever, and prolonged lag phase and decreased disappearance rates in myxoedema.

of metabolic decay of the four factors are independent of the blood level.[12] It was also found that the rates of decay depend largely upon the metabolic condition of the person under study. Figure 3 shows that with increased catabolism, in hyperthyrotic and febrile states, accompanied by a considerable reduction of the initial lag phase (down to about 2 hours), a disappearance rate of up to three times the normal may be found; on the other hand, with decreased catabolism, as in hypothyroidism and possibly also during late pregnancy and the puerperium, the initial lag phase becomes longer and the disappearance rate of the coagulation factors appears to be much slower, down to half normal.[10]

Practical experience showed that the size of the initial dose of coumarin congeners must be limited in order to prevent excessive response. However, the faster the drug is metabolized and excreted, the higher the initial dose may be. Hence, the initial dose of so-called short-acting coumarin congeners is often followed by maximum prothrombin response, and the response to the therapeutically recommended initial dose of a so-called long-acting coumarin congener is, on the average, slower (Fig. 4).

The second question to be answered was:

> Is there a common pattern of response of factors II, VII, IX, and X to the maintenance dose of different coumarin congeners?

Theoretically, one would expect that after the initial phase of unequable lowering, the four factors, under the influence of a constant maintenance dose, i.e. a stable depression of the production rate, would reach a constant and mutually similar degree of lowered activity (Fig. 5). Actually, as

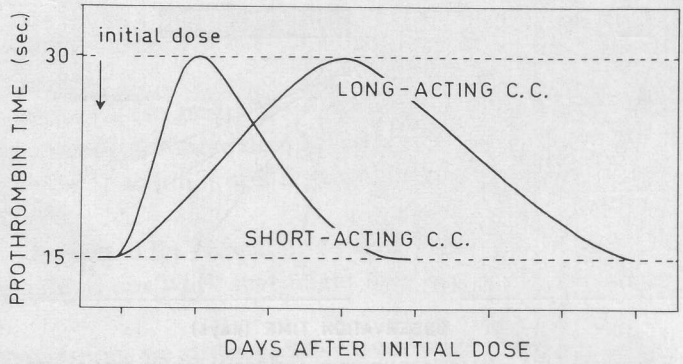


FIG. 4. Schematic representation of the prothrombin response to the initial dose of a short-acting and to that of a long-acting coumarin congener.

shown in Table 1, in patients treated for more than three months under supervision of our thrombosis service, we did find such a depression;[8, 9] the relatively high factor IX activity found in patients treated with Acenocoumarol, Warfarin and Dicoumarol, is probably due to the influence of age and the relatively low factor X activity due to a circulating anticoagulant to be discussed in answer to the next question.

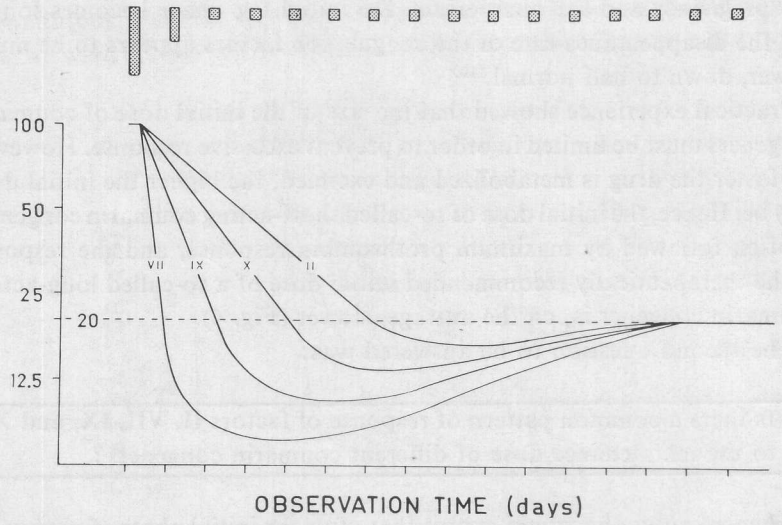


FIG. 5. Schematic representation of the unequable lowering of the activity of the four factors belonging to the prothrombin complex in response to the initial loading dose, and of the attainment of an equal depression of all four factors (down to 20%) during a constant maintenance dose.

TABLE 1

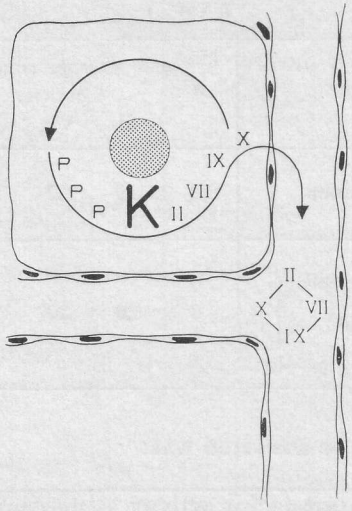
Drug	*n*	*Average coag. factor activity (%)* II	VII	IX	X
Phenprocoumon	33	19	22	21	20
Acenocoumarol Warfarin Dicoumarol	8	20	20	25	16

The third question to be answered was:

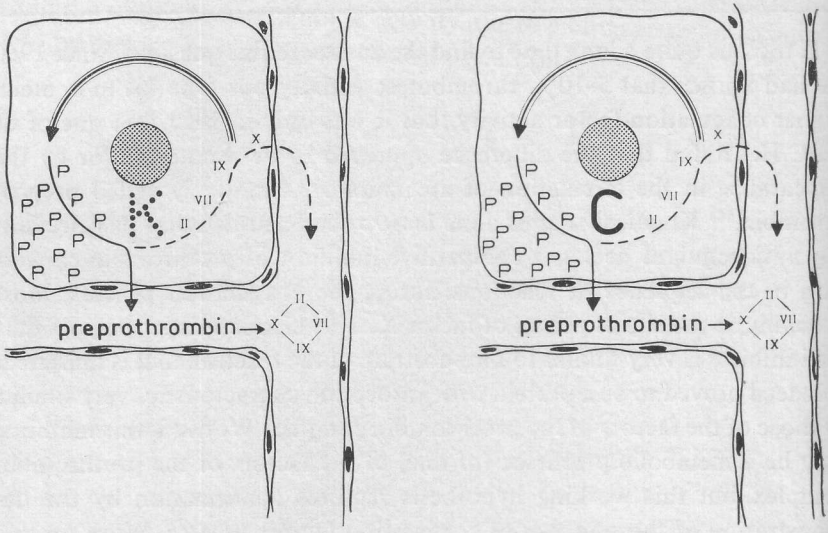
> Why is the coagulation-factor activity as measured by thrombotest considerably lower than that obtained from separate coagulation factor assays?

It took us quite a long time to find the answer to this question. Since 1960 we had known that 5–10% thrombotest activity corresponds to a much higher coagulation-factor activity, but it was only in 1963 that one of us (H.C.H.) found that the difference appeared to be accounted for by the appearance in the circulation of an inhibitor, tentatively called preprothrombin.[6] Kinetical studies done *in vitro* demonstrated that this circulating anticoagulant acts as a competitive inhibitor of prothrombin conversion in the sequence of reactions during the coagulation process, most probably at the site of action of factor X. This characteristic suggests that the inhibitor is very similar to the substrate of the reaction that is inhibited. It indeed proved to be a protein with adsorption characteristics very similar to those of the factors of the prothrombin complex. We think this inhibitor may be a metabolic precursor (of one) of the factors of the prothrombin complex, but this working hypothesis requires confirmation by the demonstration of the synthesis of coagulation factors *in vitro*. Work on this point is in progress in our laboratory.

An explanatory hypothesis for the appearance of preprothrombin in the circulation, shown schematically in Fig. 6, may be formulated as follows: in vitamin K-deficiency the metabolic precursor normally converted into (one of) the factors of the prothrombin complex in a vitamin K-dependent step is produced in excess owing to an intracellular feed-back stimulation of protein synthesis, the stimulus being the lowered intracellular levels



(a)



(b)

FIG. 6. Schematic representation of biosynthesis of factors II, VII, IX, and X in the hepatic cell under influence of vitamin K (Fig. 6a). In case of vitamin K-deficiency (Fig. 6b) the intracellular production of the four factors is lowered (as indicated by the smaller size of the figures), which feeds back protein synthesis (indicated by an agglomeration of P's). Preprothrombin is shed in the blood, where it acts as an inhibitor of the prothrombin complex (indicated by the arrow between preprothrombin and factor X of the prothrombin complex).

of factors II, VII, IX, and X. Excess production of this metabolic precursor results in intracellular accumulation and hence delivery into the circulation. The fact that the amount of inhibitor present in blood is found to be independent of the intensity of treatment indicates maximum feed-back stimulation of protein synthesis in vitamin K-deficiency.

The finding of a circulating anticoagulant offers one of the keys to the solution of the problem of anticoagulant check standards, for it is now clear that the result of a coagulation check depends on the sensitivity of the test system not only for prothrombin, factor VII, factor IX, and factor X, but also for the circulating anticoagulant under discussion. Thrombotest, which is widely used nowadays as a method of checking anticoagulant treatment, appears to be particularly sensitive to both the depression of coagulation factors and the presence of the anticoagulant. As a result of the action of the inhibitor the coagulation-factor activity in patients treated with coumarin congeners appears, if measured with thrombotest, to be only 2/5 of that expected from the amount of coagulation factors in the plasma to be tested. The reason why the inhibitor expresses itself especially in thrombotest is the fact that the final dilution of plasma under investigation is but 1/10, whereas it is 1/40 or even more in test systems used for the assessment of the activity of coagulation factors separately.

The fourth and last question to be answered was:

What is the therapeutically optimal range of hypocoagulability?

We have already known for a number of years that powerful prevention of venous thrombosis undoubtedly requires institution of strong and well-balanced hypocoagulability.[7, 14, 17, 18] The "therapeutic range" for patients who display no contra-indications for anticoagulant treatment is, in terms of thrombotest, 5–10%. If we look for data concerning the therapeutically optimal range for arterial thrombosis, there is very little evidence that in this field, too, the most favourable results can be obtained if a similar range of hypocoagulability is aimed at.[1, 5, 15] In a recent double-blind study[13] we were able to demonstrate that anticoagulant treatment, continued longer than one year after the occurrence of cardiac infarction, powerfully prevents reinfarction and other cardiovascular complications for an unlimited period of time. Phenprocoumon*, which is known to be superior to other coumarin congeners with respect to stability of hypocoagulability,[2, 3, 16] was used and tight supervision of patients by the

* We wish to thank the firm Hoffmann-La Roche for kindly furnishing both the phenprocoumon (Marcoumar) and the placebo tablets.

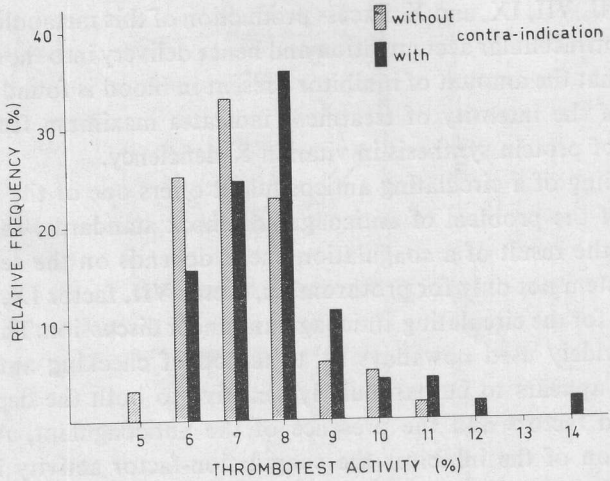


FIG. 7. Illustration of intensity and stability of treatment, based on representative samples of all 116 patients on phenprocoumon still in the trial after 12 months of treatment. The individual average thrombotest values are compiled in classes of one percent. Solid columns represent values found in the 59 patients without contraindications, and hatched columns those found in the 57 patients with contra-indications.

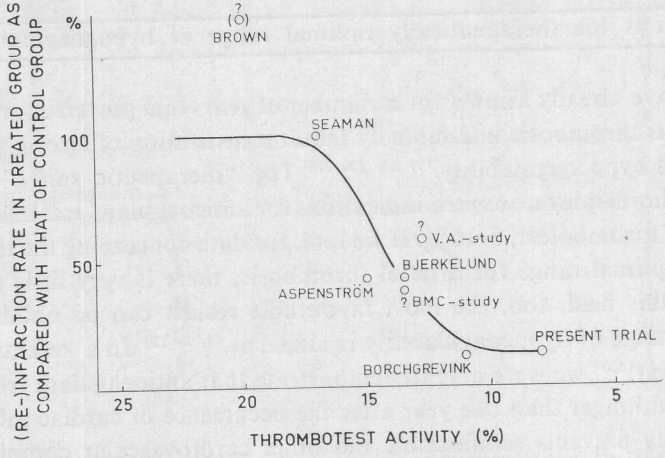


FIG. 8. Tentative curve of correlation between results obtained and intensity of treatment, achieved by different authors in controlled clinical trials. On the ordinate, the number of (re-)infarctions in patients while on coumarin treatment is given as percentage of the number found in the control group. On the abscissa, intensity of treatment is expressed in percentage thrombotest activity (grand means).

staff of the thrombosis service could be maintained. Under these circumstances, attainment and maintenance of a strong and stable hypocoagulability was found to be feasible without any risk of severe bleeding complications. The arithmetic mean of all thrombotest values was approximately 8%. The individual average values varied from 5% to 14%, as shown in Fig. 7, in which the solid black columns represented the values found in patients without contra-indications, and hatched columns those found in patients with relative contra-indications. A comparison of the clinical results of our study with those of other controlled trials made clear that the more intensively patients are treated, the more they benefit (Fig. 8). This may sound like a commonplace to pharmacologists; it is not, however, part of the common knowledge of investigators dealing with long-term anticoagulant treatment.

To conclude: coumarin congeners induce a state of hypocoagulability that is the result of a depression of the activity of the four coagulation factors of the prothrombin complex and the appearance in the circulation of a competitive inhibitor of prothrombin conversion. Depression of the factors takes place according to well-defined rules, whereas the circulating anticoagulant remains present in a constant amount. Therapeutic results depend wholly upon the degree and constancy of hypocoagulability achieved. Institution of a therapeutic range of 5% to 10% in terms of the commercially available standard thrombotest results in powerful prevention of venous as well as arterial thrombosis.

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DISCUSSION

B. G. FIRKIN, *(Sydney)*:

Could Dr. Loeliger give us some idea of the complication rate, number and age of patients in his trial?

E. A. LOELIGER:

The rate of bleeding complications was about 1 in 10 patient-treatment years. Table 1 illustrates severity of bleeding complications, whereas Table 2 shows their localization. These complications occurred in 128 patients treated with phenprocoumon, 112 of which were still in trial at the end of the observation period of 16 months. The control group consisted of 122 patients treated with placebo. Allocation to the trial took place randomly. The slight difference in number is due to occurrences between the time of selection of patients and the start of the trial. Only 45–75-year-old male patients, able to attend the laboratory of the thrombosis service, were included in the study.

G. ROSENFELD *(São Paulo)*:

Have you seen any time refractory cases with Phenylindandione? We have seen that in experiments in dogs, the drug used by the patients was inactive. Can you explain what can occur with the drug?

E. A. LOELIGER:

Phenylindandione is not used in our country, so I have no answer to your question. Perhaps Dr. Jaques or Prof. Born have an explanation.

DR. JAQUES:

It is known that the preparation of tablets is all important for reaction to oral treatment. If the tablet does not disintegrate in the intestinal tract, then resorption will not occur. In such instances non-reactivity may be misinterpreted as inactivity of or resistance to the drug.

TABLE 1

SEVERITY OF BLEEDING COMPLICATIONS

	Phenprocoumon	*Placebo*
No change in dosage	11	6
Temporary lowering of dosage	3	—
Administration of vitamin K_1	3*	1**
Blood transfusion	—	—

* Two macrohaematurias, one subarachnoidal bleeding (with complete recovery).
** Lethal intracerebral bleeding.

TABLE 2

LOCALIZATION OF BLEEDING

Localization	*Phenprocoumon*	*Placebo*
Cutaneous	5	0
Nasopharyngeal	7	5
Intestinal	1	1
Urogenital	2	0
Intracranial	1	1
Subconjunctival	1	0

J. D. P. GRAHAM, *(Cardiff):*

Would Dr. Loeliger care to comment on the mechanism of development of resistance to warfarin in a strain of wild rat in England which is now a serious problem in pest control.

E. A. LOELIGER:

Development of resistance to warfarin is as far as I can see a pharmacogenetical problem. But what I wonder about is the fact that the resistance in rats developed only about 25 years after the introduction of this rat-killer. In man a certain insensitivity to coumarin congeners is known to be a hereditary trait.*

In my personal experience with almost 20,000 patients treated with coumarin congeners, I have never observed a case of resistance to the drug. Incidentally we have patients who need up to 8 times the mean normal daily dose. We did not perform genetical studies.

* R. A. O'Reilly et al., Hereditary transmission of exceptional resistance to coumarin anticoagulant drugs. The first reported kindred. *New Eng. J. Med.* **271,** 809, (1964).