CHAPTER ONE

INTRODUCTION
It is nearly three decades since anticoagulant drugs were introduced into medicine. Anticoagulants are substances which prolong the coagulation time of blood. It is axiomatic that circulating blood containing the proper balance of normal factors will not coagulate to any observable extent within the vascular system. The delicate balance is upset in response to local injury or when the blood is shed, and coagulation occurs. The proper coagulation response of shed blood is quite as important for the well being of the individuals as is the maintenance of fluidity within the vascular system. A complete elucidation of their mode of action is still lacking. However,
consideration of some of the reactions involved in the clotting of blood will aid in understanding the mechanism by which the anticoagulant effects are exerted. Blood coagulation is the result of a complex series of reactions. By the use of appropriate techniques, however, the process can be stopped at certain definite points, and it is common practice to discuss blood coagulation as though it takes place in three distinct stages.¹

1. Platelets + "Foreign Surface" $\rightarrow$ Thromboplastin

2. Thromboplastin + Prothrombin $\rightarrow$ Thrombin

3. Thrombin + Fibrinogen $\rightarrow$ Fibrin (Clot)

The first stage can be considered to be the formation of thromboplastin.² This is a controversial and poorly understood aspect of blood coagulation. The clotting factors that have been proposed to interact during thromboplastin formation are calcium ions, platelet material and some factors. The nature of this interaction and of those factors that actually participate have not yet been fully elucidated.

The second stage in blood coagulation is the conversion of prothrombin to thrombin. This reaction is believed to be entirely dependent upon the thromboplastin activity developed during the first stage.³ Calcium ions
are also required for prothrombin activation.

The third stage is the enzymatic conversion of fibrinogen to fibrin, catalysed by thrombin. Thrombin acts in a proteolytic manner, splitting off a small peptide from the fibrinogen molecule and the residual portion of the molecule is called "fibrin monomer". Many of these monomer units then polymerize to form the insoluble fibrin clot.

The anticoagulant drugs in use at the present time act by inhibiting the action or formation of one or more clotting factors. Since the inactivity of even a single clotting factor is considered a disease, one can say that the anticoagulant drugs exert their effects by creating a clotting defect resembling that of certain clinical diseases. Individualised treatment and frequent observations are imperative for patients on anticoagulant therapy.

The classic scheme has not been changed by modern theory, but it is being constantly expanded to include the numerous newly discovered factors involved in coagulation as in dissolution of the fibrin clot. These factors may be promoters or inhibitors of coagulation; indeed the system is so complex that in spite of considerable research it is understood only in limited aspects.
The discovery of the nature and function of the vitamin K provided a stimulus for further research into the mechanism of blood clotting as affected by these compounds and their antagonists. Vitamin K acts by promoting the formation of prothrombin, probably in the liver. No direct effect upon the blood in vitro has been demonstrated. Administration of vitamin K is of benefit in hemorrhagic disease of the new born and is widely used for this purpose in human and in veterinary medicine.

The anticoagulants which are known to-day and which are of clinical use are either of natural occurrence or are synthetic preparations. Heparin represents the oldest anticoagulant isolated from natural source.

(A) Heparin:

Heparin (I) is a natural anticoagulant in the circulatory blood and its effects can be readily demonstrated in vitro. In 1916, McLean⁸ at John Hopkin University, while extracting the thromboplastic substances from various tissues made the surprising discovery that some of these extracts not only contained thromboplastic activity but also a powerful anticoagulant activity. Howell and Holt⁹ (1918) described the characteristic of this anticoagulant in more detail and named it Heparin to indicate its abundant occurrence in liver. In Canada Best and his colleagues
showed that heparin was present in a number of tissues throughout the body, the lungs being the richest source (about 200 to 400 mg. present in a pair of human lungs). Through improved methods of extraction and purification Charles and Scott (1936) could prepare heparin in sufficiently pure form for human administration. The first reported clinical trials in the prophylaxis of thrombosis were by Crafoord (1937) and about that time heparin was identified as a mycolitin polysulphuric acid by Jorpes and Bergstrom.

Heparin is a mucopolysaccharide composed of sulphated D-glucosamine and D-glucoronic acid, the sulphonic acid group being present either on oxygen or nitrogen. The two hexose moieties are present in equimolecular amounts and appear to alternate along the polysaccharide chain. The molecular weight of heparin is not yet available although Lauront (1961) found that the smallest fraction possessing anticoagulant activity has a molecular

\[ \text{Heparin molecule} \]

\[ (1) \]
weight of 8000. Heparin differs from other mucopolysaccharides in that there are sulphate groups bound to amino groups to form sulphonlic linkages a type of linkage otherwise unknown in nature. Durant et al. 14 (1962) found that only one out of eight hexosamine units has a free amino groups and that the same proportion of glucoronic acid units has no O-sulphate groups. Such a structure makes heparin the strongest organic acid occurring within the body. Anti- coagulant activity is related to sulphuric acid content, and hydrolysis of the ester linkage results in a loss of activity. The strongly acidic groups of heparin react with certain basic compounds like protamine, toluidine blue, benzidine and quinine, thereby destroying the anticoagulant activity. Anticoagulant activity of heparin may be due to similar reactions with proteins that participate in the clotting process.

Heparin is found in mast cells and accounts for their characteristic metachromatic staining. Mast cells are widely distributed in connective tissue, and heparin can be extracted from many body organs, particularly from those with abundant mast cells.

The physiological function of heparin is the subject of great speculation. There is a suggestive evidence that it acts as a natural anticoagulant to aid in
maintaining the blood fluidity, as was postulated by Howell. However, this view has not found complete acceptance. The major pharmacological actions of heparin are almost entirely confined to the blood.

Heparin inhibits the clotting of blood both \textit{in vitro} and \textit{vivo}. Bleeding time is usually unaffected although clotting time is prolonged by therapeutic doses. The thrombin time and one-stage prothrombin time are prolonged. The action of heparin to an antithrombin effect was first ascribed by Howell and Holt\textsuperscript{9} (191\textsuperscript{g}). Heparin does not block prothrombin synthesis in the liver as coumarin anticoagulants but it inhibits certain factors involved in the conversion of prothrombin to thrombin. Heparin is ineffective when administered orally and is usually given intravenously in the form of its sodium salt. Its effect is very rapid, but of relatively short duration. An advantage of this fact is that hemorrhages are seldom encountered during clinical use.

Clinically heparin is used in the prevention of postoperative thrombosis\textsuperscript{15} and is preferred by some clinicians in spite of its evanescent effect, for prolonged therapy of thrombotic tendencies. It is of particular value in maintaining the fluidity of the blood during heart surgery.
Sulfonation of various natural polysaccharides has been carried out, the products being studied for heparin-like action. Some of these have approached heparin in their anticoagulant activity. But because of their higher toxicity and the availability of purified heparin from natural sources, none of these synthetic substitutes has received wide acceptance. Some such products are Partiol, Treburon, Dexulate and Kysate.

(B) Indandiones.

The other noncoumarin compounds which prolong the coagulation time are the indandiones. Their effects are less readily overcome by vitamin K. Phenindione was the first to be introduced and this was followed by diphenindione. Pivalin is the sodium salt of Pival, the 2-pivaloyl derivative and is used primarily as a rodenticide.

The hypoprothrombinemic effect of certain indandione derivatives was first recognised over 20 years ago. Since then phenindione (2-phenylindan-1,3-dione) (II) has become one of the most widely used anticoagulants. Both its onset and duration of action are shorter than those of bishydroxycoumarin. A therapeutically effective prothrombin time is attained in 24-48 hours. After discontinuation of maintenance therapy, the prothrombin time returns to normal in one to four days. Phenindione is
also known as hedulin, dindavan and danilone.

Following the introduction of phenindione, the compound 2-\((\text{diphenylacetyl})\)-indan-1,3-dione, diphenindione (III) was introduced into medicine. Prothrombin times in the therapeutic range are attained in 24 to 48 hours. However, its duration of action is one of the longest among the oral anticoagulants. Effects have been observed for as long as 15 to 20 days. The drug is far less toxic than phenindione.

Anisindione (IV) is the 2-\(\text{p}-\text{anisyl}\)-indan-1,3-dione, also known as miradon. Its action is similar to phenindione. No toxic or side effects have yet been reported. However, the drug has not had such extensive use as phenindione. A therapeutic prothrombin time response
is achieved in 24 to 72 hours, and the prothrombin time returns to normal about 36 to 72 hours after the peak effect.

Chlorophenindione (V) has so far shown no toxic or side effects. Its speed of action is relatively slow, a therapeutic response is attained in about 48 hours and persists for 4 to 6 days. It is also known as indaliton.

(C) Bishydroxycoumarins.

The history of common anticoagulants dates back to the early 1920s, when Schofield described the "sweet clover disease" that had been plaguing cattle in North Dakota and Alberta. This disease which was characterised by a severe bleeding tendency was shown by Schofield to be
caused by feeding the cattle with improperly cured sweet clover hay. The hay contained a toxic substance that interfered with the normal blood-clotting mechanism. Schofield was also able to reproduce this syndrome in rabbits fed with some spoiled fodder. Shortly thereafter, Roderick (1929) reported that the clotting defect was due to a prothrombin deficiency and the toxic agent in the hay was probably a decomposition product of coumarin. Large scale experiments on the disease were initiated at the Wisconsin Agricultural Experimental Station under the direction of Link and Campbell (1934). These investigators succeeded in isolating, identifying and synthesising the active principle of the spoiled sweet clover hay. It was shown to be 3,3'-methylene-bis-(4-hydroxycoumarin) (VI). It was first called dicoumarin and later dicoumarol. Its official U.S.P. designation is bishydroxycoumarin. The research was extended to include the synthesis of this and many related compounds and the study in animals of their anticoagulant properties. Finally bishydroxycoumarin (VI) was introduced into human therapy.
Simultaneously Quick\textsuperscript{27} developed a quantitative method for prothrombin determination, the one-stage prothrombin time, which has become quite useful for the control of anticoagulant therapy. The first clinical trials withbishydroxycoumarin were reported by Bingham et al.\textsuperscript{28} (1941) and Butt et al.\textsuperscript{29} (1941). During the past two decades hundreds of chemically related compounds have been studied, but only few are in use to-day. These drugs are often referred to as oral anticoagulants, because the oral route administration is used almost exclusively.

The establishment of the essential chemical characteristics of these type of compounds needed to produce anticoagulant activity has been rather difficult. It is known however, that the minimal structure requirements for activity are intact 4-hydroxycoumarin residue (VII) with the 3-position substituted by a carbon residue or a hydrogen atom.

Following the therapeutic acceptance of bishydroxycoumarin, other anticoagulants containing the hydroxy coumarin structure have been added. The considerable
delay in achieving an effective prolongation of prothrombin
time with bishydroxycoumarin led to efforts to obtain a
drug that would act more quickly. The first compound that
was developed for this purpose was bis-3-(4-hydroxy-
coumarinyl)-ethylacetate, ethylbiscoumarate also known as
tromexan (VIII) which has these properties.\textsuperscript{30}

\begin{center}
\textbf{(VIII)}
\end{center}

Other compounds of similar biological activity
containing only one hydroxycoumarin ring include warfarin
(IX), whose sodium salt was first used as a rodenticide\textsuperscript{31}

\begin{center}
\textbf{(IX)}
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and was later introduced into human therapy. The compound
is the sodium salt of 3-(\(\alpha\)-acetonylbenzyl)-4-hydroxy-
coumarin, and was originally employed as a rodent poison.
Sodium warfarin is effective by oral, intramuscular, intravenous
and rectal administration. Its aqueous solubility
permits parenteral use when the oral route cannot be
employed. Warfarin is somewhat more potent than bishydroxycoumarin. Vitamin K readily returns the prothrombin time to normal, although due to cumulative effects of warfarin, the prothrombin time may be increased for the succeeding three to four days.

Other related compounds are Coumechlor (X) (the p-chloro analogue of warfarin), Sintrom (XI) (its p-nitrophenyl analogue), Marcoumar (XII) and Cyclocoumarol (XIII).

Coumechlor is 3-(α-acetonyl-p-chlorobenzyl)-4-hydroxycoumarin and sintrom is 3-(α-acetonyl-p-nitrobenzyl)-4-hydroxycoumarin, also known as acenocoumarin. The onset of action in the case of latter is approximately same as that of bishydroxycoumarins, maximum prolongation of prothrombin time occurring in 36 to 48 hours. The only reported toxic effect has been ulceration of the mouth.

Marcoumar (XII) is 3-(1'-phenylpropyl)-4-hydroxycoumarin. It is one of the longest acting oral anticoagulants. Peak prolongation of prothrombin time is usually
seen in 48 to 72 hours and normal values do not return for one to two weeks. With shorter acting anticoagulants, a single dose of vitamin K is usually adequate to return the prothrombin time to normal.

Cyclocoumarol (XIII) also known as methopyranorin, coumopyron and Link compound 63 is one of the many coumarin congeners which was synthesised by Link during his studies on drugs related to bishydroxycoumarin. It is 3,4-\{(2'-methyl-3'-methoxy-4'-phenyl)-dihydropyranocoumarin. It has been administered to patients who have otherwise experienced gastrointestinal disturbances, over bishtydroxycoumarin and its long action may necessitate administration of repeated doses of vitamin K in cases of over dosage.
The biahydroxycoumarins and their congeners show no observable effect when mixed with blood in vitro. As in the case of vitamin K, tests for activity must be carried out by administration to animals, with subsequent determinations of "prothrombin time". They apparently act by interfering with the formation of prothrombin in the liver.

In view of their site of action and reversal of their effect by vitamin K, the hydroxycoumarins are also classified as vitamin K antagonists. Whether this antagonism can be explained on the basis of similarity in structure or whether the active material may be a metabolite produced from the compound administered is still a question. Members of a series of quinones related to phthiocol have been reported to exhibit antivitamin K activity.33,34

The danger inherent in the uncontrolled clinical use of the hydroxycoumarins is emphasised by the employment of some members as hemorrhagic rodenticides. But with proper safeguards they may be lifesaving in many cases. Their principal use is in the prevention of post-operative thrombotic complications and in the prophylactic treatment of patients who have previously suffered a thrombosis. Thousands of patients have been so treated.
within recent years, and the lives of many have probably been prolonged.

Very little work has been done on the synthesis of sulphur analogues of the well-known anticoagulants like, Dicoumarol, Warfarin, Tromexan, Coumechlor etc. Among the few analogues prepared, only one compound viz., 3,3'-methylenebis-(4-hydroxy-1-thiacoumarin) has been so far studied for its anticoagulant activity by Mentzer and Meunier, who have shown it to be active. Hence, a systematic study on the synthesis of various 4-hydroxy-1-thiacoumarins and related compounds has now been undertaken and their anticoagulant activity studied.
REFERENCES

20. Blaustein et al., Circulation, 1, 1195 (1950).
34. Chmielewska et al., Chem. Abstr., 47, 6387 (1953).