Oral anticoagulants—the totem and the taboo*

A BRECKENRIDGE

For many years one of the main aims of pharmacology has been to promote rational drug treatment. Most new drugs that are introduced today originate in a chemist’s test tube and enter therapeutic via an all-embracing animal screening programme and studies in normal volunteers. A few drugs have a slightly less stereotyped origin, which brings me, of course, to the oral anticoagulants.

The discovery of these drugs by Karl Paul Link has been cited as a triumph of logic. His group identified, isolated, purified, and synthesisedbishydroxycoumarin, the first oral anticoagulant. After a brief study of animal toxicity, which would have appalled drug regulatory authorities today, bishydroxycoumarin "was grabbed from Link’s hands by the clinicians" and given to man in 1941.

The first long-term studies using bishydroxycoumarin in myocardial infarction were reported in 1946. The controversy about the efficacy of oral anticoagulants then began and the logic of their discovery rather vanishes. But over the years, in the quieter backwaters of pharmacology, oral anticoagulants have become a model system for the study of drug action, and I shall address myself to both these facets—the pharmacological and the therapeutic. Our work with the oral anticoagulant warfarin started in 1967 and I shall describe here some of the lessons in clinical pharmacology that I have learnt from our studies.

Is there a clinical problem to investigate?

Many research programmes in clinical pharmacology have their beginnings in apparently isolated clinical incidents involving drug treatment, and this was certainly the case with our oral anticoagulant work. In the summer of 1967 our attention was drawn to two patients taking warfarin who were readmitted to Hammersmith Hospital 10 days after discharge because of life-threatening haemorrhage. Their histories were remarkably similar. Both had been stabilised on warfarin when inpatients some 10 days earlier, and on discharge their anticoagulant control was immaculate. But during this first admission each had been given a nightly hypnotic, dichloralphenazone (Welldorm) in one case and amylobarbitone in the other. On discharge they stopped the hypnotic in their quieter home environment, but warfarin was continued in its previous dose.

Today every candidate for the MRCP will recognise this as a prescription for disaster, but in 1967 information on the frequency and time course of this type of drug–drug interaction in man was small. We therefore planned a series of studies to define which hypnotics and sedatives could be safely given to patients on warfarin without altering anticoagulant control and where interactions did occur to study their mechanism, magnitude, and time course. The patients we studied were already stabilised on warfarin and were about to end their course of treatment.

Since dichloralphenazone had been one of the drugs incriminated we gave this to a group of patients and showed that its

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FIG 1—Administration of dichloralphenazone (1300 mg nightly) for 33 days. Effect on Thrombotest result and plasma warfarin concentration.
administration was associated with a fall in plasma warfarin concentrations and a decrease in anticoagulant effect (fig 1). On stopping the dichloralphenazone it took some 20 days for plasma concentrations and effect to return to previous levels. Dichloralphenazone—as its name implies—contains chloral hydrate and phenazone (better known as antipyrine) in the ratio of 2:1. Phenazone is added to chloral to improve its stability and, more importantly from the patient’s point of view, to mask the bitter taste and gastric irritation of chloral hydrate.

When chloral hydrate alone was given to patients on warfarin, there was no change in the anticoagulant response, but there was a fall in plasma warfarin concentrations. This apparent paradox was explained by Soeligers and Koch-Weser.7 Chloral hydrate is metabolised to trichloroacetic acid,8 which accumulates in plasma. Since it is more strongly protein-bound than warfarin, the anticoagulant is displaced and there are two consequences. Firstly, the increased free (unbound) warfarin temporarily enhances the anticoagulant response, but, secondly, at the same time the free drug is metabolised more readily by the liver. Thus one sees only a temporary increase in effect but a sustained fall in plasma warfarin. Nevertheless, the clinical importance and the magnitude of this interaction between chloral hydrate and warfarin remain in question.9,10

When phenazone was given both plasma warfarin concentration and its therapeutic response fell, as we had seen with dichloralphenazone, and these changes followed the same time course. This effect of dichloralphenazone and phenazone was due to a stimulation of the rate of warfarin metabolism in the liver, by the process of liver microsomal enzyme induction, and we produced evidence for this in both man and rats.11 Contrary to previous reports we found no evidence in either man or rat to suggest that chloral hydrate stimulated rates of drug metabolism.12 None of the three benzodiazepines that we studied—chlordiazepoxide, diazepam, and nitrazepam—had any effect on either anticoagulant response or warfarin kinetics.13 These compounds, of course, are the most widely used tranquillisers and hypnotics today and may safely be given to patients taking warfarin.

Pharmacological principles in experimental design

If the stimulus to studies in clinical pharmacology often comes from clinical medicine then these studies must be designed along accepted pharmacological lines. Two basic principles in pharmacology were important in planning studies with oral anticoagulants. Firstly, different subjects may respond in different ways to the same dose of a drug—a topic discussed by Dollery in his Bradshaw Lecture of 1974.14 Secondly, if one increases the dose of a drug one must expect increasing effects.

When quinalbarbitone 100 mg nightly for 33 days was given to six patients the fall in plasma warfarin ranged from 6% to 65%, which represented variations in the extent of induction in the different subjects.13 This has been noted by others,15 but the reason for the variation is not clear. Environmentalists claim that our drug metabolising enzymes are constantly stimulated by the polluted air we breathe and the food additives we consume17 and thus it is tempting to postulate that those subjects who respond least to barbiturate administration are those who have already been exposed most to inducing agents, but there is little evidence to support this. Neither have we found a correlation between the rate of elimination of the inducing agent and the degree of induction produced.

When we gave an increased dose (200 mg for a similar period) to two subjects who showed a small fall (5% and 15-3%) in steady state plasma warfarin concentrations on 100 mg quinalbarbitone the percentage fall in plasma warfarin concentrations was greater (40-5% and 51%, respectively). When one of these two subjects took 300 mg quinalbarbitone for a further 30 days, there was no further fall in plasma warfarin (49%) (fig 2). In other words, enzyme induction may have dose-dependant charac-

teristics, like many other pharmacological responses, and one can construct a dose-response curve in individual subjects.18

There are differences in this dose-response relationship with different agents.15 Groups of rats were treated with increasing doses of four inducing agents—phenobarbitone, quinalbarbitone, amylobarbitone, and phenazone. Preparations of liver microsomal enzymes were made from these rats and the rate of metabolism of the substrate ethylmorphine examined. With each of the four drugs, the greater the dose the greater the rate of ethylmorphine metabolism (fig 3). Furthermore, phenobarbitone was a better inducing agent than amylobarbitone, quinalbarbitone, or phenazone. This related partly to the elimination half-life of the inducing agent and partly to the liver:plasma ratio. We did not find that relative lipid solubility was important in our experiments, although this has been challenged.19

The lesson from these studies is that to classify a drug as an inducing agent—in man or any other species—on the basis of a single dose level is unwise. What may be an inducing dose of a barbiturate in one patient may have no effect in another. Enzyme induction is not an all-or-nothing phenomenon.

Importance of chemical expertise

Clinical pharmacology is a hybrid discipline. If ideas originate in clinical medicine, and studies are carried out in accordance
with pharmacological principles, then unless one works closely with a chemist interpretation of one's data may be fallacious. This is important from two points of view. Warfarin is metabolised by enzymes of the liver and to a lesser extent by other tissues such as the kidney. Metabolites are inactive or have an activity considerably less than that of the parent drug but accumulate in plasma, and thus in studies to correlate plasma and tissue drug concentrations with effect specific analytical techniques are mandatory. Early studies of the kinetics of warfarin used assays for warfarin that failed to distinguish drug from metabolite, and in our initial studies we too used non-specific methods. But we were soon corrected by our chemist colleagues and we adopted the method of Lewis et al, in which drug is separated from metabolites before assay, and these metabolites can themselves be measured.

Specificity is one aspect of methodology, sensitivity is another. In Liverpool we are currently engaged on studies in which small doses of warfarin are given to subjects and we must thus measure warfarin in plasma in nanogram quantities, and thus even more sensitive chemical assays must be devised.

Chemical expertise is not necessary only from the analytical standpoint. Warfarin has an asymmetric centre, and this implies that there are two isomers, or enantiomers. In the rat S warfarin is about five times more potent than R warfarin. When we examined the rate of elimination of these enantiomers in the rat we found that S warfarin was eliminated less rapidly than R warfarin. The difference in the rate of elimination was only twofold, whereas the difference in potency was some fivefold, and in our studies we used non-specific methods. But we were soon corrected by our chemist colleagues and we adopted the method of Lewis et al, in which drug is separated from metabolites before assay, and these metabolites can themselves be measured.

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Which species to study?

A perennial problem in clinical pharmacology is to decide how much reliance should be placed on data derived from animal studies and, further, which animal species should be used. We made extensive use of measurements of rates of in-vitro drug metabolism in rat liver preparations in studies of enzyme induction with hypnotics, and the results were used to complement clinical observations, but even in this field there are significant differences between man and the rat. There are other circumstances when this type of extrapolation may be unwise, as is illustrated with studies of the enantiomers of warfarin.

Commercial warfarin is a racemic mixture. In many respects the enantiomers are different drugs. In man they differ in potency (S warfarin being about three to four times more potent than R warfarin), rate of elimination (S warfarin being eliminated more rapidly than R, unlike in the rat), and pathway of metabolism. The principal metabolic product of S warfarin is 7-hydroxycumarin, while that of R warfarin is a warfarin alcohol, formed by reduction of a ketone group on the warfarin side chain (fig 4). Since the configuration of warfarin clearly influences its pharmacological effect, it seemed relevant to explore further the relation between the configuration and metabolism of warfarin. We thus re-examined a well known drug interaction—that of phenylbutazone and warfarin. Phenylbutazone administration in man will augment the anticoagulant effect of warfarin. The explanation usually proposed for this potentiation is that phenylbutazone will compete with warfarin for binding sites on circulating albumin and displace it, leading to an enhanced effect. In in-vitro studies when phenylbutazone is added to plasma containing warfarin as much as 30% of warfarin is in the free form. But, if displacement from albumin were the sole mechanism of the drug interaction, logically there should be more rapid metabolism and elimination of warfarin in vivo. There was, however, no change in the clearance of racemic warfarin in the presence of phenylbutazone. Plasma concentrations of 7-hydroxywarfarin were, however, consistently lower in phenylbutazone studies than controls when racemic warfarin was given, and plasma concentrations of warfarin alcohols were higher. The explanation for these differences was found in studies where the isomers were given separately. The fractional clearance of S warfarin (the more potent) was slowed down, while that of R warfarin (the less potent) was increased by phenylbutazone (fig 5) Differences in rates of elimination have been shown between the isomers of methadone, propranolol, amphetamine, and hexobarbitone. It does not seem unreasonable to assume that drug interactions with these agents, like those with warfarin, might be stereochrmically dependent, and where the isomers show differences in pharmacological potency such changes may have clinical significance.

When one examines drug interactions with the isomers of warfarin in other species, such as the rat and the dog, the results are quantitatively and qualitatively different from those described above, and clearly definitive studies must be done in man.

More than just a study of pharmacokinetics

Dollery has paraphrased the suggestion that there is more to clinical pharmacology than just a study of pharmacokinetics by posing the question “Pharmacokinetics—servant or master?”
He implied that there has been an increasing tendency by many clinical pharmacologists to regard the study of pharmacokinetics—that is, the way the body handles drugs—as an end in itself, rather than to consider it as complementary to a study of what drugs do to the body. I am uncertain if he had our studies with oral anticoagulants in mind when he coined this phrase, but if so let me hasten to redress the balance and consider the nature of the response to oral anticoagulant treatment.

If one measures the plasma warfarin concentration in patients who have all had the same optimal anticoagulant response a fivefold to sixfold range is found. These differences in plasma concentration may be equated with differences in tissue (receptor) sensitivity, provided there are no appreciable differences in the liver:plasma warfarin ratio.

Over the last few years several groups11-14 have examined the nature of response to warfarin administration and the warfarin "receptor." Warfarin interferes with the hepatic synthesis of clotting factors II, VII, IX, and X. Their synthesis is closely linked to a shuttle involving vitamin K (which is active) and an inactive metabolite vitamin K, 2:3 epoxide (κ, oxide). The cycle is mediated by two enzymes—a reductase and an epoxidease—both of which are membrane-bound and found in the microsomal fraction of liver homogenate. According to this scheme, generation of clotting factors occurs during the conversion of K, to the epoxide while the reductase is concerned with the regeneration of vitamin K. This evidence has been deduced from studies in rats, but is supported by studies in man. Shearer et al15 have shown that the metabolic pattern of vitamin K, is altered in man by therapeutic doses of warfarin in that there is an accumulation in plasma of K, oxide (fig 6). The clearance of vitamin K, from the plasma is not affected.

![Graph](image)

**FIG 6.—**Plasma concentration of radioactivity, expressed as disintegrations per minute per ml of plasma, after intravenous injection of 1 mg (11, 21, 3-H) vitamin K, in one subject. O—Control studies. A—Studies after warfarin administration. (Reproduced by kind permission of the author and the editor of British Journal of Haematology.)

By administering various doses of warfarin to different subjects and measuring the accumulation of vitamin K, oxide in plasma, it is possible to study the relationship between doses of warfarin and the vitamin K,--K, oxide response.

A dose-response curve can be constructed which has a sigmoid shape, indicating that above a critical dose of warfarin (equivalent to about 70 mg/day) there is no corresponding increase in K, oxide accumulation in plasma but that over the therapeutic range the relationship is linear.16

While clearly the action of warfarin is mediated through vitamin K,, many important questions remain unanswered. Can one postulate that there is a receptor for warfarin? Is this receptor a series of enzymes or a single enzyme in the vitamin K,--K, oxide cycle? Is genetic resistance to warfarin, well documented in the rat and in man, a nearerer, provided change in this enzyme system? The answer to these and many other questions might be forthcoming more easily if vitamin K could be measured. Surprisingly, vitamin K cannot be measured and we have to rely on studies with labelled vitamin K,.

This aspect of oral anticoagulant treatment is of great importance and shows the potential we have for monitoring a biochemical aspect of the response to oral anticoagulants.

**Importance of clinical trial design**

So far I have addressed myself to problems of the pharmacology of oral anticoagulants, but now I must turn to a more contentious aspect—namely, their use in clinical medicine. It has been said that perhaps the greatest use that anticoagulants may yet have in clinical medicine is their ability to cause controversy.17 There are those who claim that oral anticoagulants are effective rat poisons and there their usefulness ends. At the other end of the spectrum are the workers who find that anticoagulation produces a fourfold decrease in mortality in patients with acute myocardial infarction.15 16

Few would now gainsay their place in the treatment of venous thrombosis and pulmonary embolism, established by the classical studies of Sevitt and Gallacher18 and Barritt and Jordan.9 8 The place of these drugs in treatment of patients with myocardial infarction and cerebrovascular disease, however, remains controversial. When one overviews critically the design of the clinical trials on which entrenched attitudes have been adopted about the efficacy or otherwise of oral anticoagulants in these conditions, many of these trials are deficient by modern standards. Although statistics were first used in the scientific evaluation of treatment as long ago as 1828,19 in a study to show the inappropriateness of blood letting as a therapeutic measure, the principles of conducting therapeutic trials were not laid down until the work of Bradford Hill in the 1930s.20 Moreover, studies designed and executed in the 1940s and 1950s are no longer acceptable in the 1970s.

Gifford and Feinstein21 and Gross et al22 have recently analysed all trials of anticoagulants in acute myocardial infarction reported in the English language between 1948 and 1972. Forty-three studies were reviewed. Seven criteria were chosen which would today be generally acceptable as necessary in the design of a therapeutic trial. Not one of these 43 studies fulfilled all criteria, and, interestingly, the lower the standard of the trial the more common was the finding that oral anticoagulant treatment was better than no treatment. The number of studies fulfilling each of the criteria is shown in the table.

<table>
<thead>
<tr>
<th>Number of trials fulfilling each of seven criteria considered necessary to the design of a trial</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic criteria for:</td>
<td></td>
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</tr>
<tr>
<td>Myocardial infarction</td>
<td>13</td>
<td>30</td>
</tr>
<tr>
<td>Pulmonary embolus</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Perspective clinical trial</td>
<td>24</td>
<td>56</td>
</tr>
<tr>
<td>Concurrent controls</td>
<td>34</td>
<td>79</td>
</tr>
<tr>
<td>Controls treated in same hospital as test group</td>
<td>5</td>
<td>12</td>
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<tr>
<td>Random allocation to test or control groups</td>
<td>11</td>
<td>26</td>
</tr>
<tr>
<td>Double blind methodology</td>
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</tr>
<tr>
<td>Allocation of bad-risk patients to test and control groups</td>
<td>19</td>
<td>44</td>
</tr>
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With hindsight, of course, it is an easy task to criticise these studies, which assessed many thousands of patients. If one does criticise the next question must be: is a new properly designed trial now justified?

In my opinion the answer is no. If the introduction of oral anticoagulants was logical, perhaps their administration to patients with arterial thrombosis is not entirely rational. The fundamental role of the platelet in the genesis of arterial thrombosis, and the failure of oral anticoagulants to lessen platelet function suggest that on theoretical grounds alone, these drugs might be ineffective.23 Nor is a possible diminution in thromboembolic events a valid reason for resuscitating large-scale trials of oral anticoagulant treatment in myocardial infarction. The
main causes of death in acute myocardial infarction are pump failure, cardiac arrhythmias, cardiac rupture, and thromboembolism. Only the last of these would be influenced by anticoagulants, and they account for some 20%, of the deaths. To prevent deaths due to thromboembolism would reduce the overall death rate only from 20% to 16%, and to detect this the size of the trial and its complexity would indeed have to be great. If there is controversy about the use of anticoagulants in acute myocardial infarction the same arguments rage in cerebrovascular disease. By using the same criteria as those proposed for myocardial infarction, 16 studies of the use of oral anticoagulants in studies of cerebrovascular disease have been analysed recently. None fulfilled all the criteria. But, as in trials in myocardial infarction, the better the design the more common was the conclusion that anticoagulant treatment was of no benefit.

Totem and taboo

The totem, Freud's guardian spirit,* I have equated with the pharmacology of oral anticoagulants. Because of the precision of measurements of both effect and concentration, these drugs have become a pharmacologist's touchstone. The taboo, Freud's ill-understood and dangerous being, is obviously their place in therapeutics. To improve treatment with these drugs, not only must their pharmacokinetics be understood, the role of vitamin K in determining their response must be appreciated, trials must be appropriately designed, and, of course, clinical judgment must be exercised.

I wish to express my appreciation to those colleagues with whom these studies were carried out and for the many helpful discussions we have had, particularly, Dr Michael Orme, Dr Donald Davies, Professor Colin Dollery, Dr Martin Shearer, Dr Richard Lewis, and Professor Malcolm Rowland. This work was supported by the Medical Research Council, the Wellcome Trust, and Roche Pharmaceuticals Limited.

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