DRUG INTERACTIONS WITH WARFARIN: STUDIES WITH DICHLORALPHENAZONE, CHLORAL HYDRATE AND PHENAZONE (ANTIPYRINE)

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SUMMARY

1. Administration of dichloralphenazone, a complex of chloral hydrate and phenazone (antipyrine) caused a fall in steady-state plasma warfarin concentration and loss of anticoagulant control in five subjects.

2. This effect of dichloralphenazone is due to stimulation of the drug-oxidizing enzymes of the liver endoplasmic reticulum by antipyrine, the non-hypnotic part of the complex. Administration of antipyrine caused a fall in steady-state plasma warfarin concentration in five subjects, a shortening of the plasma warfarin half-life, with increased urinary excretion of the metabolites of $^{14}$C-labelled warfarin in two subjects and increased urinary excretion of 6β-hydroxycortisol which is formed in the liver endoplasmic reticulum.

3. Administration of chloral hydrate, the hypnotic part of dichloralphenazone, caused no change in anticoagulant control but a fall in steady-state plasma warfarin concentration in five subjects. This is due to the accumulation of trichloroacetic acid which displaces warfarin from plasma protein binding sites.

4. Individual differences in the extent of enzyme induction have been shown to be related to the subjects’ rates of drug oxidation.

5. In the rat administration of dichloralphenazone and antipyrine, but not chloral hydrate, caused shortening of pentobarbitone sleeping time and of the plasma $[^{14}$C]pentobarbitone half-life, shortening of the zoxazolamine paralysis time and increase in the maximal velocity of N-demethylation of ethylmorphine.

The intensity and duration of action of many lipid-soluble drugs including coumarin anticoagulants such as warfarin is controlled by the rate at which these drugs are oxidized by the enzymes of the liver endoplasmic reticulum. Agents such as hypnotics increase the rate of drug oxidation in animals (Conney, 1967) but corresponding information in man is scanty and many hypnotics are prescribed in situations where their ability to act as inducers of drug
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oxidation is potentially dangerous. Since patients taking anticoagulant drugs are frequently given hypnotics either in hospital or at home, it is important to find agents that can safely be given to patients without disturbing anticoagulant control.

We have examined the effects of the administration of the hypnotic drug dichloralphenazone (Welldorm, Smith and Nephew) on plasma warfarin concentrations and anticoagulant control in patients on long-term therapy. Dichloralphenazone, as its name suggests, is a complex of two parts chloral hydrate and one part phenazone (antipyrine). The reason for this formulation is that chloral hydrate has a bitter taste and tends to cause gastric irritation: complexing it with antipyrine minimizes these properties.

In addition to studying the effects of dichloralphenazone in patients on long-term warfarin therapy, we have also examined the effects of its two component substances, chloral hydrate and antipyrine, on plasma warfarin concentration and anticoagulant control and we have studied the mechanism of the interactions observed. These three drugs, dichloralphenazone, chloral hydrate and antipyrine, have also been tested in the rat for their ability to act as inducers of drug metabolism.

METHODS

Patients studied

Ten patients were studied, eight of whom were on long-term anticoagulant therapy with warfarin. In five of these patients more than one study was carried out, and patients G.L., K.W. and B.W. were given dichloralphenazone, chloral hydrate and antipyrine in sequence with a period of 3 months between each study. Six patients were taking no drugs other than warfarin, and four others were taking digoxin with or without the benzothiadiazine diuretic, hydrochlorothiazide. A preliminary study was therefore done to see if the administration of digoxin and hydrochlorothiazide affected warfarin metabolism.

All long-term studies were done with patients in their home environment. Venous blood samples were taken twice weekly at the same time of day and were collected in plastic syringes into sodium citrate. Plasma warfarin concentration was measured by the method of Corn & Berberich (1967). Dichloralphenazone, chloral hydrate and phenazone did not interfere with the assay of warfarin. Anticoagulant control was measured by Thrombotest, Owren, on the day of sample collection. Each study took approximately 100 days and during this time the dose of warfarin taken by the patient was not changed. The daily dose of warfarin was always taken by the patient in the morning. The first 20–30 days were a control period when anticoagulant control was maintained within the therapeutic range (Thrombotest 6–12%); for the next 30 days, dichloralphenazone, chloral hydrate or antipyrine was introduced into the patient's drug regimen and for the last 30 days of the study the same measurements were continued.

Dose of drugs

The standard hypnotic dose of dichloralphenazone, 2 tablets (1300 mg), was given. For studies with chloral hydrate a dose of 1000 mg was given since approximately two-thirds of dichloralphenazone is chloral hydrate. It was given at night in liquid form, flavoured with blackcurrant to disguise the taste. The dose of antipyrine was 600 mg/day; this is slightly more than the quantity of antipyrine in 1300 mg of dichloralphenazone, but was chosen because of the size of the antipyrine tablets available. In the initial study to determine the effects of
Digitalis and hydrochlorothiazide on the plasma warfarin concentration, 0.25 mg of digoxin twice daily and hydrochlorothiazide 50 mg/day were given.

**Plasma half-life of [14C]warfarin [1-(4'-hydroxy-3'-coumarinyl)-1-phenyl[1-14C]butan-3-one; 23 μCi/mg]**

This was measured after the oral administration of 0.5 mg/kg of warfarin (35 μCi) on two occasions in each of two subjects (A.M. and T.T.) before and during treatment with 600 mg of antipyrine daily for 30 days. Both subjects were given intramuscular vitamin K and there was no change observed in anticoagulant control. It has been shown that administration of vitamin K does not alter warfarin metabolism (O'Reilly, Aggeler, Hoag & Leong, 1962). Plasma samples were collected over 8 days and radioactivity was measured by liquid-scintillation spectrometry. Warfarin and its metabolites were extracted from acidified plasma with ethylene dichloride, and separated by t.l.c. as described by Lewis & Ilnicki (1969). Radioactivity was localized by using a Packard Chromatogram Scanner (Model 7210) fitted with a disc integrator. The percentage activity due to unchanged warfarin was thus measured in each sample. From this the plasma half-life of [14C]warfarin was estimated by a least-squares method with a computer program which gave in addition the 95% confidence interval.

Urine was collected for 8 days after warfarin administration. Warfarin and its metabolites were extracted from urine by a modification of the method of Lewis & Trager (1970). Samples (15 ml) of urine were acidified with 1 ml of 3 M-HCl and shaken with 15 ml of ethylene dichloride. The organic layer was separated by centrifugation and evaporated to dryness by vacuum distillation and the residue was taken up in a small volume of acetone. A 25 μl sample was spotted on to a silica gel thin-layer plate which was developed in ethylene dichloride–acetone (9:1, v/v). The distribution of radioactivity on the plate was compared with a chromatogram of [14C]warfarin added to urine and carried through the same procedure.

Faeces were collected for 5 days in both subjects before and during antipyrine administration. Daily faecal collections were homogenized and made up to 1000 ml. Samples (0.1 ml) were dried on filter paper and burnt in an atmosphere of oxygen in plastic bags as described by Gupta (1968).

**Urinary 6β-hydroxycortisol**

This was estimated in three 24 h collections made before and three collections made during the last 3 days of administration of 600 mg of antipyrine daily for 30 days to three subjects (A.M., T.T., B.W.) and 1300 mg of dichloralphenazone (one subject G.L.)

A 50 ml sample of each 24 h urine collection was extracted by the method of Frantz, Katz & Jailer (1961) and duplicate one-fifth samples of the extracts were applied as small spots to 1.5 cm-wide lanes of a paper chromatogram (Brooks, 1960). The paper chromatograms, containing also eight lanes of 6β-hydroxycortisol standards, were run in the system toluene–ethyl acetate–methanol–water (7:3:5:5, by vol.) for 16 h. The fluorescence of the spots was developed and quantitated as described by Brooks (1960). By this method the recovery of added 6β-hydroxycortisol is 97% and the coefficient of variation is 6%. The urinary 17-oxosteroids and 17-oxogenic steroids were estimated by the method of Gray, Baron, Brooks & James (1969).

6β-Hydroxycortisol is a polar metabolite of cortisol formed in the endoplasmic reticulum.
of the liver. The concentration of urinary 6ß-hydroxycortisol has been shown to be a reflection of the hydroxylating capacity of the liver endoplasmic reticulum, and to be increased on the administration of certain drugs (Conney, 1967).

**Plasma antipyrine half-life**

This was measured in five subjects given dichloralphenazone by using the assay of Brodie, Axelrod, Sokerman & Levy (1949). The half-life was calculated by a least-squares method from six points obtained over 24 h.

**Plasma concentrations of chloral hydrate, trichlorethanol and trichloroacetic acid**

These were measured in three subjects on long-term warfarin therapy given 1000 mg of chloral hydrate/day for 30 days. Blood samples were taken twice weekly into sodium citrate, and plasma warfarin concentrations were measured in the same samples. Chloral hydrate, trichlorethanol and trichloroacetic acid were measured by the method of Cabana & Gessner (1967). The effect of trichloroacetic acid on the protein binding of warfarin was measured as follows: fresh plasma was incubated with [14C]warfarin (5 ìg/ml) for 30 min at 37°C. To 10 ml samples of this was added trichloroacetic acid in concentrations from 0 to 300 ìg/ml and incubated for another 30 min. These samples were ultrafiltered in an MSE 4L centrifuge modified to maintain a temperature of 37°C. The samples were gassed with O₂ + CO₂ (95:5) to maintain the pH of the plasma. Approximately 0.8 ml of ultrafiltrate was collected and the percentage of warfarin bound to protein in the absence and presence of trichloroacetic acid was calculated by measuring the 14C radioactivity in the ultrafiltrate (non-protein bound) and in the sac after ultrafiltration (total) and expressing the former measurement as a percentage of the latter.

**Animal studies**

Dichloralphenazone (80 mg/day per kg), chloral hydrate (80 mg/day per kg) and antipyrine (40 mg/day per kg) were screened for their ability to act as inducers of drug metabolism in 100 g male rats (RPGMS strain).

The drug under study was given by intraperitoneal injection twice daily for 3 days and once on the fourth day. On day 5 the following measurements were made.

(a) *Sleeping time.* Pentobarbitone (30 mg/kg) was administered by intraperitoneal injection to test and control animals. Sleeping time was estimated as described by Breckenridge, Davies, Orme & Thorgeirsson (1969).

(b) *Paralysis time.* The duration of paralysis after zoxazolamine (100 mg/kg) was measured as described by Breckenridge et al. (1969). The purpose of this test was to detect an inducing agent such as 3,4-benzpyrene which increases the rate of zoxazolamine metabolism but not pentobarbitone metabolism (Arcos, Conney & Buu-Hoi, 1961).

(c) *Plasma half-life of pentobarbitone.* The plasma half-life of [14C]pentobarbitone (New England Nuclear Corp.) was measured in treated and control animals. Ten animals from each group were given an intraperitoneal injection of [14C]pentobarbitone (30 mg/kg and 3-125 ìCi). At selected time-intervals one rat from each of the treated and control groups was killed and unchanged pentobarbitone was extracted by the method of Cooper & Brodie (1957) and the radioactivity was counted in a Packard Liquid-Scintillation Spectrometer. The plasma half-life of [14C]pentobarbitone was estimated by computer analysis of these results by using a least-squares method.
Liver microsomal enzyme activity. The N-demethylation of ethylmorphine was used as a measure of microsomal enzyme activity. Rats were killed by cervical dislocation. The livers were removed and homogenized in a Teflon–glass homogenizer with 4 vol. of ice-cold 1·15% (w/v) KCl solution containing 0·02 M-tris–HCl buffer, pH 7·4. The homogenate was centrifuged at 9000 g for 15 min and the resulting supernatant fraction was then centrifuged at 156000 g for 30 min in an MSE Superspeed 50 ultracentrifuge. The supernatant was discarded and the microsomal pellet was suspended in tris–KCl solution. Microsomal protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). The microsomal suspension was diluted to contain 4·5 mg of protein/ml and was used immediately for the determination of enzyme activity.

The N-demethylation of ethylmorphine was measured as described by Davies, Gigon & Gillette (1969) except that protein was precipitated with 1 ml of M-HClO₄ before measurement of formaldehyde.

Apparent affinity constants (Kₘ) and maximal velocities (Vₘₐₓ) were calculated for each of eight rats in the test and control groups by using the computer program described by Davies et al. (1969).

Statistical variations among the experiments were evaluated by the Student t-test.

RESULTS

Human Studies

An initial study on a single patient showed that a combination of digoxin and hydrochlorothiazide had no effect on plasma warfarin concentrations or anticoagulant control.

Long-term studies

(a) Dichloralphenazone. Dichloralphenazone was found to cause a fall in plasma warfarin concentration in five patients and a corresponding loss of anticoagulant control as shown by a rise in thrombotest percentage. Withdrawal of the hypnotic was followed by a return to control values. Fig. 1 shows an example of this effect. Table 1 shows the mean plasma warfarin concentration during the last 14 days of the control period and the last 14 days of the dichloralphenazone period in the five patients. Plasma warfarin concentrations fell to 20·2–68·5% of control values.

(b) Chloral hydrate. Chloral hydrate caused a fall in plasma warfarin concentration in all five cases. An example is shown in Fig. 2. The change in steady-state plasma warfarin concentration was less than that produced by dichloralphenazone, varying from 66·0% to 89·2% of control values (Table 1). In none of these five cases was there a change in the thrombotest.

(c) Antipyrine. Antipyrine caused a significant fall in plasma warfarin concentration in all five patients (Table 1) and a corresponding fall in thrombotest. As with dichloralphenazone, when antipyrine administration was stopped, plasma warfarin concentrations and thrombotest returned to control values. Fig. 3 shows an example of this effect of antipyrine.

Evidence for induction of drug-oxidizing enzymes

(a) Plasma warfarin half-life. In two subjects the administration of antipyrine (600 mg/day) for 30 days caused the plasma warfarin half-life to change from 55·1 ± 5·2 h to 25·9 ± 7·5 h (subject T.T.) and from 39·1 ± 9·2 h to 24·3 ± 5·6 h (subject A.M.).
(b) **Urine \[^{14}C\] warfarin.** In the two subjects, T.T. and A.M., 80% of the dose of \[^{14}C\] warfarin was recovered in the urine collected over 8 days before and during the administration of antipyrine. The urine was subjected to chromatography (see the Methods section) before and during antipyrine administration and no unchanged warfarin was detected. Four radioactive peaks were found with \( R_F \) values 0·24, 0·33, 0·35, 0·40, whereas the \( R_F \) value of warfarin added to urine was 0·66.

Thus the \(^{14}C\) radioactivity in urine is a measure of the metabolites of warfarin. The rate of excretion of radioactivity and therefore of warfarin metabolites in urine was greater after administration of antipyrine, although the total recovery was not changed (Table 2).

(c) **Faecal radioactivity.** The percentage of the dose of radioactivity excreted over the 5-day period in both patients before and during antipyrine was 5·56% and 5·37% (T.T.) and 6·44% and 6·32% (A.M.) respectively. This indicates that diminution of plasma warfarin concentrations during antipyrine administration was not due to a change in absorption. However, we have not determined whether faecal radioactivity was in the form of the unchanged drug or its metabolites.

(d) **Urinary 6β-hydroxycortisol excretion.** In four subjects, administration of antipyrine (three subjects) and dichloralphenazone (one subject) caused an increase in the urinary excretion of 6β-hydroxycortisol (Table 3). There was no change in 17-oxosteroid or 17-oxogenic steroid excretion when these drugs were given.

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**FIG. 1.** Patient Mrs K.W. Effect of administration of dichloralphenazone (Welldorm) 1300 mg nightly on plasma warfarin concentration and thrombotest percentage.
## Drug interactions with warfarin

### TABLE 1. Plasma warfarin concentrations measured for 14 days before and during dichloralphenazone, chloral hydrate and antipyrine administration

<table>
<thead>
<tr>
<th>Patient</th>
<th>Plasma warfarin concentration (µg/ml)</th>
<th>Dichloralphenazone (µg/ml)</th>
<th>Percentage of control values</th>
<th>Significance of change in plasma warfarin*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O.H.</td>
<td>3.30 ± 0.09</td>
<td>2.10 ± 0.06</td>
<td>63.6%</td>
<td><em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td>B.W.</td>
<td>2.16 ± 0.11</td>
<td>1.48 ± 0.02</td>
<td>68.5%</td>
<td><em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td>V.H.</td>
<td>4.06 ± 0.25</td>
<td>0.82 ± 0.04</td>
<td>20.2%</td>
<td><em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td>K.W.</td>
<td>4.40 ± 0.11</td>
<td>1.30 ± 0.08</td>
<td>29.5%</td>
<td><em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td>G.L.</td>
<td>3.20 ± 0.17</td>
<td>1.37 ± 0.06</td>
<td>47.5%</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chloral hydrate (µg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.H.</td>
<td>2.27 ± 0.06</td>
<td>1.71 ± 0.04</td>
<td>75.3%</td>
<td><em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td>V.H.</td>
<td>2.96 ± 0.06</td>
<td>1.60 ± 0.11</td>
<td>54.2%</td>
<td><em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td>B.W.</td>
<td>2.50 ± 0.08</td>
<td>1.65 ± 0.10</td>
<td>66.0%</td>
<td><em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td>G.L.</td>
<td>2.35 ± 0.07</td>
<td>1.79 ± 0.17</td>
<td>76.2%</td>
<td><em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td>K.W.</td>
<td>3.14 ± 0.30</td>
<td>2.80 ± 0.17</td>
<td>89.2%</td>
<td><em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>2.65 ± 0.11</td>
<td>1.91 ± 0.12</td>
<td>72.1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antipyrine (µg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K.W.</td>
<td>3.88 ± 0.15</td>
<td>1.56 ± 0.02</td>
<td>40.2%</td>
<td><em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td>C.F.</td>
<td>2.32 ± 0.12</td>
<td>1.23 ± 0.15</td>
<td>53.0%</td>
<td><em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td>G.L.</td>
<td>2.55 ± 0.05</td>
<td>1.36 ± 0.09</td>
<td>53.3%</td>
<td><em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td>O.H.</td>
<td>3.43 ± 0.17</td>
<td>1.84 ± 0.12</td>
<td>53.6%</td>
<td><em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td>B.W.</td>
<td>2.46 ± 0.11</td>
<td>1.09 ± 0.07</td>
<td>44.3%</td>
<td><em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>2.93 ± 0.12</td>
<td>1.41 ± 0.09</td>
<td>48.8%</td>
<td></td>
</tr>
</tbody>
</table>

* Student's *t*-test.

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**Fig. 2.** Patient Mr. G.L. Effect of administration of chloral hydrate 1000 mg nightly on plasma warfarin concentration and thrombotest percentage.
Alteration of protein binding of warfarin by trichloroacetic acid

Administration of chloral hydrate for 30 days caused a small but significant fall in plasma warfarin concentration in man but we were unable to show that chloral hydrate induced microsomal enzymes in the rat (see below). Thus another explanation for the change was sought. Trichloroacetic acid, a major metabolite of chloral hydrate, is known to accumulate in the plasma after chloral hydrate administration (Marshall & Owens, 1954). Plasma concentrations of trichloroacetic acid, trichlorethanol and chloral hydrate were measured in three subjects given 1000 mg of chloral hydrate nightly for 30 days. No trichlorethanol or chloral hydrate was detected but trichloroacetic acid was found in all samples. In Fig. 4 plasma trichloroacetic acid and plasma warfarin concentrations are shown for one study. Trichloroacetic acid was detectable in the plasma for some 15 days after chloral hydrate administration was stopped.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T.T.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>8.3%</td>
<td>31.4%</td>
<td>46.8%</td>
<td>58.3%</td>
<td>65.5%</td>
</tr>
<tr>
<td>During</td>
<td>26.0%</td>
<td>44.9%</td>
<td>54.0%</td>
<td>69.0%</td>
<td>73.5%</td>
</tr>
<tr>
<td>A.M.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>22.8%</td>
<td>41.0%</td>
<td>53.2%</td>
<td>61.1%</td>
<td>66.0%</td>
</tr>
<tr>
<td>During</td>
<td>30.0%</td>
<td>53.3%</td>
<td>66.8%</td>
<td>69.5%</td>
<td>73.8%</td>
</tr>
</tbody>
</table>
Drug interactions with warfarin

Since trichloroacetic acid is bound to plasma albumin and to the same group of binding sites as warfarin (Sellars & Koch-Weser, 1970), it may displace warfarin from those sites. Table 4 shows that a greater percentage of warfarin is displaced from protein in an ultrafiltration system in vitro, as the concentration of trichloroacetic acid is increased.

### Table 3. 24 h urinary excretion of 6β-hydroxycortisol before and during drug administration

<table>
<thead>
<tr>
<th>Patient</th>
<th>Drug</th>
<th>6β-Hydroxy cortisol (µg/day)</th>
<th>6β-Hydroxy cortisol (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>G.L.</td>
<td>Dichlorphenazone</td>
<td>130</td>
<td>120</td>
</tr>
<tr>
<td>B.W.</td>
<td>Antipyrine</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>A.M.</td>
<td>Antipyrine</td>
<td>77</td>
<td>50</td>
</tr>
<tr>
<td>T.T.</td>
<td>Antipyrine</td>
<td>60</td>
<td>71</td>
</tr>
</tbody>
</table>

**Fig. 4.** Patient Mrs B.W. Accumulation of trichloracetic acid (TCA) after chloral hydrate administration (1000 mg/day).

**Plasma antipyrine half-lives**

The plasma antipyrine half-life in the five subjects given dichlorphenazone ranged from 7.2 ± 0.4 h to 25.7 ± 4.3 h. There was a significant correlation (Fig. 5) between the antipyrine half-life and the percentage change in the steady-state plasma warfarin concentration as shown in Table 1.

**Animal Studies**

**Pentobarbitone sleeping time**

The mean pentobarbitone sleeping time for ten rats treated with dichlorphenazone was 43.1 ± SD 9.4 min, for ten rats treated with chloral hydrate 121.8 ± SD 39.1 min, and for ten
rats treated with antipyrine $55.7 \pm SD 9.4$ min. The value for the control group of ten rats was $124.2 \pm SD 23.6$ min. The sleeping time is thus shorter ($P<0.001$) in the dichloralphenazone- and antipyrine-treated animals, but not significantly different in the chloral hydrate animals, as compared with controls.

**Zoxazolamine paralysis time**

The duration of paralysis after the injection of zoxazolamine in nine rats treated with dichloralphenazone was $278.0 \pm SD 59.7$ min, for ten rats treated with chloral hydrate $472.8 \pm SD 124.8$ min, and for nine rats treated with antipyrine $290.0 \pm SD 63.5$ min. The duration of paralysis, like the sleeping time, is thus significantly shorter ($P<0.001$) in the dichloralphenazone-treated and antipyrine-treated animals, but not in the chloral hydrate-treated group, when compared with ten control animals, whose mean was $462.8 \pm SD 124.8$ min.

**Table 4. Ultrafiltration of warfarin with trichloroacetic acid**

<table>
<thead>
<tr>
<th>Conc. of warfarin ($\mu g/ml$)</th>
<th>Conc. of trichloroacetic acid ($\mu g/ml$)</th>
<th>% warfarin free</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>—</td>
<td>1.48%</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>1.61%</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>2.11%</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>2.41%</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>2.91%</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>3.24%</td>
</tr>
<tr>
<td>5</td>
<td>300</td>
<td>4.38%</td>
</tr>
</tbody>
</table>

![Fig. 5. Correlation between fall in plasma warfarin concentration and plasma antipyrene half-life after administration of dichloralphenazone.](image)

Plasma half-life of $[^{14}C]$pentobarbitone

The plasma half-life for the control group of animals was $139 \pm SD 52.2$ min. For the dichloralphenazone-treated animals, the half-life was $87.3 \pm SD 31.1$ min, for chloral hydrate-treated animals $126.5 \pm SD 86.6$ min and for antipyrine-treated animals $62.3 \pm SD 41.3$ min.
The plasma half-life of $[^{14}C]p$entobarbitone in dichloralphenazone-treated and antipyrine-treated animals is considerably shorter than in control animals, whereas that of chloral hydrate-treated animals is approximately the same as controls.

Liver microsomal enzyme activity

Table 5 shows the values of apparent affinity constant ($K_m$) and maximal velocity constant ($V_{max}$) for ethylmorphine $N$-demethylation in experiments with animals pretreated with dichloralphenazone, chloral hydrate and antipyrine. In each experiment measurements of these kinetic constants were made in test and control animals. In the dichloralphenazone and antipyrine experiments, the $V_{max}$ was significantly higher ($P<0.01$) in the test group than the control group. The $V_{max}$ in the chloral hydrate-treated group of animals did not differ significantly from the control group ($P>0.3$). In none of the three experiments was the $K_m$ value significantly different in the test and control groups of animals ($P>0.1$).

**DISCUSSION**

These studies suggest that dichloralphenazone is not a suitable hypnotic for patients on warfarin therapy, since its administration causes a significant fall in steady-state plasma warfarin concentration and a corresponding loss of anticoagulant control. This fall was observed in all five patients studied.

Since dichloralphenazone is composed of two compounds, chloral hydrate and antipyrine, we have examined the effects of administering each of these two substances to human subjects and to rats. The administration of chloral hydrate to human subjects caused a slight but significant fall in plasma warfarin concentration but no change in thrombotest. These findings agree with those of McDonald, Robinson, Sylvester & Jaffe (1969), who found that administration of 1.74 g of chloral betaine/day for 19 days shortened the plasma half-life of warfarin in ten volunteers, but did not alter the hypoprothrombinaemic response as measured by prothrombin time. Sellars & Koch-Weser (1970) showed that chloral hydrate administered to three volunteers on warfarin therapy caused a fall in plasma warfarin and an increase in prothrombin time. There are several possible reasons for the lack of change in thrombotest in our studies and of prothrombin time in the experiments of McDonald et al. (1969). Chloral hydrate may have a hypoprothrombinaemic effect independent of its ability to lower plasma warfarin. However, we have been unable to show that administration of 1.0 g of chloral hydrate nightly for 14 days
caused a change in thrombotest in three patients not taking warfarin. Another possible reason is that chloral hydrate or one of its metabolites may displace warfarin from protein binding sites. Chloral hydrate is metabolized to trichlorehanol and trichloracetic acid (Marshall & Owens, 1954). Trichloroacetic acid which is 85-95% bound to serum albumin accumulates with prolonged administration of chloral hydrate; plasma trichloroacetic acid concentrations up to 300 µg/ml were found in our studies. Equilibrium-dialysis studies (Sellars & Koch-Weser, 1970) suggest that trichloroacetic acid and warfarin compete for the same binding site on serum albumin, and ultrafiltration studies presented here confirm that trichloroacetic acid can displace warfarin from protein. At a concentration of 300 µg of trichloroacetic acid/ml the free warfarin percentage was increased approximately threefold. Such an increase makes more warfarin available for metabolism and a fall in steady-state plasma warfarin concentration results; also more warfarin is available for diffusion to active sites to produce a greater pharmacological effect. Sellars & Koch-Weser (1970) have shown an increase in prothrombin time in patients on warfarin given chloral hydrate. In our studies we have shown that the expected decrease in prothrombin time commensurate with the change in plasma warfarin did not occur.

We have been unable to show any change in the rate of drug oxidation in the rat on administration of chloral hydrate. Antipyrine increases the enzyme activity of the endoplasmic reticulum in the rat, producing a two- to three-fold increase in $V_{\text{max}}$, but no significant change in $K_m$ for ethylmorphine $N$-demethylation. It also produces significant shortening of the pentobarbitone sleeping time, zoxazolamine paralysis time and plasma half-life of $[^{14}\text{C}]$pentobarbitone. The evidence for a similar effect on enzyme activity in man is a shortening of plasma warfarin half-life after pretreatment with antipyrine and an increased excretion of urinary metabolites of warfarin. The nature of these metabolites was not determined in this study, but Lewis & Trager (1970) have found four metabolites of warfarin in human urine, two steroisomeric alcohols of warfarin, 6-hydroxy- and 7-hydroxy-warfarin. Further indirect evidence of enzyme induction with antipyrine and dichloralphenazine is shown by the two- to three-fold increase in urinary excretion of 6β-hydroxycortisol after the administration of these two drugs.

Evidence that antipyrine does not interfere with warfarin absorption is given by data in two subjects in whom the percentage of the dose of $^{14}\text{C}$ radioactivity excreted in the faeces over a 5-day period did not change significantly before and during antipyrine administration. Hepatobarbitone has been shown to interfere with the absorption of bishydroxycoumarin when the two drugs are given together (Aggeler & O'Reilly, 1969). Bishydroxycoumarin is less readily absorbed than warfarin (O'Reilly, Aggeler & Leong, 1964).

Thus there are two mechanisms by which dichloralphenazone causes the steady-state plasma warfarin concentration to fall, enzyme induction and displacement of warfarin from plasma albumin. The change in anticoagulant control observed in subjects given dichloralphenazine is presumably a reflection of the stimulation of warfarin metabolism, since less will be available to interfere with the synthesis of vitamin K-dependent clotting factors. Displacement of warfarin by trichloroacetic acid might be expected to have the opposite effect, but the displaced warfarin will also be metabolized at a more rapid rate (Gillette, 1967). This may be the reason for the predominant influence of enzyme induction on anticoagulant control in the present studies. A similar potentiation of the anticoagulant effect of warfarin by phenylbutazone, accompanied by a shortening of the warfarin half-life has been shown by Aggeler, O'Reilly, Leong & Kowitz (1967).
Drug interactions with warfarin

It is a matter of clinical importance to attempt to define the rate of onset and offset of these changes in plasma warfarin concentrations. The decrease was brisk in patients taking dichloralphenazone and antipyrine but slower with chloral hydrate. After withdrawal of the drug, there is a delay of 18–24 days before the plasma warfarin concentration returns to its previous value. Thus at a time when the plasma warfarin concentration is rising after the withdrawal of a hypnotic, it is obviously undesirable for the physician to increase the dose of warfarin that a patient is taking.

We have shown that subjects with a long plasma antipyrine half-life show a more marked fall in plasma warfarin concentration when given dichloralphenazone than subjects with a short plasma antipyrine half-life. This suggests that subjects who metabolize drugs slowly will show the phenomenon of enzyme induction to a greater extent than subjects who metabolize drugs quickly. The concept of genetic control of enzyme induction has been fully discussed by Vesell & Page (1969). Variations in the patient’s environment may alter the rate of drug metabolism, but by studying subjects in their homes we have tried to eliminate such variable factors.

Our findings demonstrate the value of long-term studies in man to assess the clinical importance of drug interactions.

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REFERENCES

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