Clinical Evaluation of Dicumarinyl Derivatives with a Metabolic Study of the Radioactively Labeled Anticoagulants in Animals

By Eric P. Hausner, M.D., Charles L. Shafer, M.D., Marion Corson, Ogden Johnson, Theodore Trujillo and Wright Langham

The common use of dicumarinyl derivatives for the treatment of thromboembolic diseases justifies further clinical evaluation of dicumarol and 4,4'-dihydroxydicumarinyl ethyl acetate [Pelentan, Tromexan, 3,3'-carboxymethylenebis (4-hydroxycoumarin) ethyl ester.] The need for a more thorough understanding of the pharmacodynamic action of dicumarinyl anticoagulants has prompted a study of their gross metabolism in animals, using C\textsuperscript{14}-labeled materials.

SINCE the introduction of anticoagulants for short and long term management of thromboembolic disease the search for a better anticoagulant has become more intensified. Heparin, dicumarol and the coumarin derivatives are the anticoagulants commonly used. Lately phenylindandione\textsuperscript{1} and Paritol with its heparin-like action\textsuperscript{2} have come into experimental clinical usage. Dicumarol, with its proved value as an anticoagulant, has the disadvantage of a long period of latency of action as well as a prolonged hypoprothrombinemic effect. The coumarin derivative, 4,4'-dihydroxydicumarinyl ethyl acetate [hereafter referred to as DEA; also known as 3,3'-carboxymethylenebis (4-hydroxycoumarin) ethyl ester; commercially available as Tromexan or Pelentan], widely described and favored in the European literature, is reported to have a shorter period of latency, a less sustained hypoprothrombinemic effect and is less toxic.\textsuperscript{3-7}

The initial step in our study was the comparative clinical analysis of dicumarol and the coumarin derivative DEA in healthy subjects and in patients with thromboembolic disease. Further studies were conducted on animals with the two anticoagulants, using radioactively labeled materials.

Dicumarol and DEA were labeled with C\textsuperscript{14} in the positions indicated by the asterisks in formulas I and II respectively.\textsuperscript{†} This method enabled us to study the absorption, metabolism and urinary excretion of the anticoagulants in relation to their hypoprothrombinemic effect.

Comparative Clinical Analysis of Dicumarol and DEA

Ten healthy subjects with normal liver function were given a single large dose of dicumarol. The dicumarol was administered in the early

\[\begin{align*}
\text{I. DICUMAROL} & \quad \text{OH} \\
& \quad \text{CH} \quad \text{CH} \\
& \quad \text{O} \quad \text{O} \\
\text{II. DEA} & \quad \text{OH} \quad \text{OH} \\
& \quad \text{COOC}_2 \text{H}_5 \\
& \quad \text{CH} \quad \text{CH} \\
& \quad \text{O} \quad \text{O}
\end{align*}\]

\[†\text{At the time this work was in progress in our laboratory Lee and co-workers\textsuperscript{8} independently labeled dicumarol with C\textsuperscript{14} and conducted studies of some of the aspects of the metabolism of this anticoagulant.}\]
morning hours, usually following a light breakfast. The individuals were kept on a normal diet throughout the experiment. No other medication was permitted. Prothrombin levels were determined by Quick's method for undiluted plasma prior to the administration of the drug, at 6 hour intervals for a period of 48 hours, and at 12 hour intervals from the forty-eighth through the ninety-sixth hour.

Curve A in figure 1 shows a steady decline in the prothrombin activity following the administration of a single dose of 400 mg. of dicumarol. In 34 hours average prothrombin activity was lowered to 30 per cent of normal, essentially the same time intervals following administration.

Curve B in figure 1 shows that the prothrombin level following the single dose of DEA declined at approximately the same rate as observed with dicumarol. However, 30 per cent of normal prothrombin activity was ob-

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**Table 1.**—Prolonged Management of a 45 Year Old Patient (313587) with Acute Myocardial Infarction Treated with DEA.

<table>
<thead>
<tr>
<th>Time Schedule of Prothrombin Determination and Treatment</th>
<th>Prothrombin Time (sec.)</th>
<th>Prothrombin Activity % of Normal</th>
<th>Dosage DEA (Gm.)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>Hour</td>
<td>Prothrombin</td>
<td>Prothrombin Dosage (mg.)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>15</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
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<td>20</td>
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</tr>
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<td>27</td>
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</tr>
<tr>
<td>5</td>
<td>30</td>
<td>32</td>
<td>13</td>
</tr>
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<td>6</td>
<td>31</td>
<td>14</td>
<td>0.3</td>
</tr>
<tr>
<td>7</td>
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<td>14</td>
<td>0.3</td>
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<td>0.3</td>
</tr>
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<td>0.3</td>
</tr>
<tr>
<td>10</td>
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</tr>
<tr>
<td>12</td>
<td>35</td>
<td>10.5</td>
<td>0.3</td>
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<tr>
<td>13</td>
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<td>10.5</td>
<td>0.3</td>
</tr>
<tr>
<td>14</td>
<td>37</td>
<td>13</td>
<td>0.3</td>
</tr>
<tr>
<td>15</td>
<td>31</td>
<td>10.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Medication was given after corresponding prothrombin values were reported.
† After the ninety-sixth hour prothrombin determinations were made twice daily unless otherwise indicated.

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Fig. 1—Comparative clinical analysis of dicumarol and DEA in healthy human subjects and in patients with thromboembolic disease. A. The hypoprothrombinemic effect of a single dose of dicumarol (0.4 Gm.) in 10 healthy human subjects. B. The hypoprothrombinemic effect of a single dose of DEA (1.5 Gm.) in 20 healthy human subjects. C. The hypoprothrombinemic effect of initial (1.5 Gm.) and (C') maintenance (0.3-0.6 Gm.) doses of DEA in 20 patients with thromboembolic disease.

Eight of the 10 subjects experienced mild and transient gastrointestinal symptoms. Twenty healthy subjects with normal liver function were given a single dose of 1.5 Gm. of DEA. Experimental conditions were kept identical to those in the dicumarol group and prothrombin determinations were made at the arbitrarily accepted therapeutic level. The maximal hypoprothrombinemic effect was observed at 36 hours. The prothrombin level remained below 30 per cent of normal activity for 24 hours. The return toward normal prothrombin values was gradual and at 96 hours the levels averaged 45 per cent of normal prothrombin activity.
served at 28 hours. The maximal hypoprothrombinemic effect occurred at 30 hours. The prothrombin level remained below 30 per cent of normal activity for only 4 hours as compared to 24 hours for dicumarol. The return toward normal prothrombin levels was far more rapid than in the dicumarol group, and normal levels were approached at 60 hours following administration of a single dose of this drug.

Twelve patients with thrombophlebitis (complicated in three instances by pulmonary embolism), 6 patients suffering with acute myocardial infarction, 1 patient with thrombangiitis obliterans, and 1 with multiple sclerosis comprised the group of 20 patients treated with DEA. All patients received an initial dose of 1.5 Gm. of DEA as in the controls. Additional DEA was given after the twenty-first hour when needed for the maintenance of therapeutic hypoprothrombinemia.

Curve C of figure 1 shows an even more rapid decline in prothrombin activity following the initial dose of 1.5 Gm. of DEA than was observed in the control group. Prothrombin activity was found to be reduced to 30 per cent of normal at 19 hours, and in the majority of patients the maximal hypoprothrombinemic effect was reached in 27 hours. In the average patient 0.3 to 0.6 Gm. of DEA daily in divided dosages maintained the prothrombin activity well within the therapeutic range (curve C'). For the sake of brevity, curve C-C' shows values only throughout the first four days of treatment.

Prolonged therapeutic management with DEA in a patient with acute myocardial infarction is illustrated in table 1. This is a representative case in our series of 20 patients with thromboembolic disease treated with DEA.

**ANIMAL EXPERIMENTS WITH C\(^{14}\)-LABELED DICUMAROL AND DEA**

**Methods.** Each of 15 male albino rats (Sprague-Dawley) weighing approximately 200 Gm. was given an oral dose of 25 mg. of radioactive dicumarol (specific activity, 77,000 counts per second per mg.). The anticoagulant was administered via stomach tube followed by 2 cc. of 5 per cent ethanol in water. To ensure the greatest possible absorption the rats were fasted for six hours prior to the administration of the drug. Three of the animals were placed in all-glass metabolism cages of the type reported by Roth and co-workers\(^9\) for the simultaneous collection of respiratory carbon dioxide, urine and feces. No significant radioactivity was found in the exhaled carbon dioxide up to 24 hours after oral administration of dicumarol so the remaining animals were placed in open metabolism cages for the collection of urine and feces samples only. The animals had free access to water throughout the experiment, and to food after an interval of 12 hours.

The rats, in groups of three, were sacrificed at 6, 12, 24, 36 and 48 hours. The prothrombin levels were estimated on blood obtained by heart puncture just prior to sacrifice, employing the Link-Shapiro modification of Quick's method.\(^11\) The estimations were made on 25 per cent plasma using .025 molar calcium chloride solution. Analyses for radioactivity were made in all groups on the following tissues: heart, lungs, liver, kidneys, entire gastrointestinal tract and contents, skin (including hair), feces, urine, blood and the remains, which included the carcass and all organs not mentioned above. A more detailed study was made of the 24 hour group. In addition to the above mentioned tissues, bone, bone marrow, muscle, spleen, thymus, testes, and brain were analyzed for radioactivity.

In order to obtain homogeneous samples, the gastrointestinal tract and contents, the feces and the remains were homogenized with normal sodium hydroxide in a Waring Blender and made up to a given volume with water. Uniform samples of all other tissues were obtained by freezing each tissue with liquid nitrogen and crushing it to a powder while still frozen. After thawing, the powdered tissue was mixed thoroughly and aliquots taken for analysis. All samples were oxidized to carbon dioxide, using a modification of the Van Slyke chromic acid oxidation procedure.\(^12\) The carbon dioxide was collected quantitatively in normal sodium hydroxide, precipitated as barium carbonate, plated and counted with a thin mica window Geiger tube using essentially the same techniques as described by Dauben and associates.\(^13\)

Blood samples were plated directly\(^14\) and counted. Direct activity measurements were also made on plasma and on washed cells. Urine samples were collected at 6 hour, and feces at 12 hour intervals. The individual samples were plated directly for relative activity measurements; pooled samples were then oxidized and analyzed to give the total radioactivity excreted in urine and feces during the period of the experiment.

Studies using radioactive DEA were made in essentially the same manner as described for radioactive dicumarol.

The C\(^{14}\)-labeled DEA (specific activity, 18,000 counts per second per mg.) was given in a dose of 100 mg. to each of 12 male albino rats weighing approximately 200 Gm. each. Since the 100 mg. dose
was too large to be given in one single administration with the stomach tube; it was administered in two 50 mg. portions 30 minutes apart. Samples of urine, feces, and expired carbon dioxide were collected and the animals sacrificed in groups of three at 6, 12, 24 and 36 hours.

One-dimensional filter paper partition chromatography was used to separate the metabolic products of dicumarol and DEA in urine, plasma, and in extracts of feces and the gastrointestinal tract plus its contents. Samples representing 100 counts per second were placed in a transverse line on strips of Whatman no. 1 filter paper 2 by 42 cm. The biological fluid was applied to the strip in very small amounts by means of a micropipette, allowing the fluid to dry between additions. The chromatogram was then developed in an upright, closed column in a solvent system of butanol-methanol (3:1) saturated with water. Fourteen different solvent systems were tried and the system of butanol-methanol-water was found to be most satisfactory. After development, the chromatograms were removed from the columns and allowed to dry. Autoradiographs were made from each chromatogram by exposing the filter paper strips to Blue Brand x-ray film for two weeks.

**Results.** Gross metabolic studies in 15 rats following oral administration of 25 mg. of C\(^14\)-labeled dicumarol and in 12 rats fed 100 mg. of C\(^14\)-labeled DEA gave an average recovery of approximately 99 per cent of the administered activity. The total recoveries ranged from 96.5 to 101.4 per cent (see tables 2 and 3).

An average of 71.9 per cent of the activity administered as dicumarol and 71.6 per cent of that administered as DEA was recovered from the feces plus the gastrointestinal tract and its contents. Autoradiographs of the filter paper chromatograms of fecal and intestinal extracts indicated that the activity in the feces and gastrointestinal tract was predominantly in the form of the original material.

If the net absorbed dose is taken as the amount of activity found in the tissues (excluding the gastrointestinal tract), urine and expired carbon dioxide, then the data in tables 2 and 3 show an average net absorption from the intestine of 27.1 per cent of the administered activity in the case of dicumarol and 27.5 per cent in the case of DEA. In both cases maximal net absorption occurred during the first six hours after administration. These data indicate that DEA, when given orally to rats, is not absorbed in significantly greater percentage nor at a significantly faster rate than is dicumarol.

Although filter paper partition chromatograms indicated that the activity in the intestinal tract and feces was probably predominantly in the form of the administered compounds, absorption from the intestine must be expressed as net rather than total absorption because of the possibility of excretion of absorbed material back into the gut via the hepatobiliary system. Studies by Lee and co-workers of the distribution of C\(^14\) activity following intravenous administration of labeled dicumarol to mice and rabbits showed appreciable amounts of activity in the bile and intestinal contents. Their report prompted us to conduct a preliminary study of the excretion of activity via the kidney and intestinal tract following intravenous injection of labeled dicumarol and DEA into rats.

Twenty-four hours after injection of 5 mg. of C\(^14\)-dicumarol into the tail vein of each of 3 200 Gm. rats, an average of 32.6 per cent of the activity was found in the feces plus the intestinal tract and its contents and 15.8 per cent was found in the total urinary excretion. This gave an over-all excretion of 48.4 per cent of the administered activity. An identical experiment using 5 mg. of C\(^14\)-DEA showed 49.7 per cent of the injected activity in the feces plus the intestinal tract and its contents and only 4.2 per cent in the urine giving a total excretion of 53.9 per cent.

The data in table 4 show the relationship between C\(^14\) activity in some of the tissues and organs, and in urine and expired air at various times after the oral administration of labeled dicumarol and DEA. Because of the fluctuations among individual animals in the net amounts of radioactivity absorbed from the gut during a given time interval, the results were computed as per cent of net absorbed activity per organ or tissue rather than as per cent of the administered dose. Table 5 shows the same data given in table 4 but expressed as per cent of the net absorbed activity per Gm. of tissue or organ.

These data show that the maximal tissue concentration of radioactivity occurred at or before six hours. The liver, blood and kidneys showed the highest concentration per Gm. of
tissue (table 5). It should, of course, be pointed out that some of the activity found in the various tissues was due to the activity of the blood contained therein since organs were not perfused. However, as much of the blood as possible was withdrawn by heart puncture in order to lower the blood content of the tissues.

Of the total radioactivity present in the blood of rats given oral doses of dicumarol, 97 to 99 per cent was found in the plasma fraction and the remaining 1 to 3 per cent was found in the cells. The ratio of the activity in the plasma to that in the cells remained unchanged throughout the 48-hour study. With DEA, on the other hand, 82 to 91 per cent of the total radioactivity in the blood was found in the plasma fraction at 6 and 12 hours and 9 to 18 per cent in the cells. The ratio of plasma to cell activity changed gradually with time until at 24 to 36 hours the per cent of activity in the plasma was the same as for dicumarol (97 to 99 per cent).

The data in table 5 show that up to 24 hours no significant activity was found in the exhaled carbon dioxide of rats given C14-dicumarol; therefore, collection of respiratory air was discontinued with the 36 and 48 hour groups. However, in rats given C14-DEA, approximately 0.1 per cent of the total administered radioactivity was found as expired carbon dioxide during the first 6 hours, and a total of 0.6 per cent during the first 24. Samples of respiratory air were collected at 30 minutes, 1, 2 and 3 hours, and thereafter every 3 hours for the first 24 hours. The activity in expired air increased to a maximum in the 6 to 9 hour sample. Computed on the basis of per cent of net absorbed dose, the following aggregate values were obtained: 0.53 per cent at six hours; 1.44 per cent at 12, and 2.58 per cent at 24 hours (table 4). The extrapolated estimate for the 36 hour carbon dioxide value was approximately 3.6 per cent of the absorbed dose.

Comparison (table 4) of the C14 activity in the tissues of the dicumarol-treated group with that of the DEA-treated group at various times reveals a rather significant point. The changes in tissue activity with time are similar but for a lag of 6 to 12 hours in the case of the dicumarol-treated animals. As an example, the average value for the blood of the dicumarol group at 24 hours was 7.77 per cent as compared with 7.53 per cent for the blood of the DEA group at 12 hours. The longer tissue retention of C14 in the dicumarol series was also reflected by a slower rate of urinary excretion.

The above observation is shown more clearly in figure 2. In this graph the urinary excretion rates of C14 activity following oral administration of the labeled compounds were compared by plotting the per cent values of the net absorbed activity excreted during successive six hour time intervals. The corresponding change in the activity retained by the body is shown in the upper curves of figure 2. Retention curves were established merely by subtracting the activity excreted in the urine (ignoring the small amount excreted as carbon dioxide in the case of DEA) from 100 per cent of the net absorbed activity. The curves demonstrate an earlier drop in retained activity following DEA than following dicumarol. The earlier drop in tissue retention is obviously paralleled by a greater rate of urinary excretion during the first 12 hours. Table 4 shows that 61.4 per cent of the net absorbed activity was excreted in the urine during the first 12 hours following oral administration of C14-DEA as compared with 20 per cent for C14-dicumarol. Both groups of animals showed a maximum urinary output of activity during the 6 to 12 hour period (fig. 2).

Autoradiographs of the filter paper chromatograms are shown in figure 3. The autoradiographs for urine and plasma from C14-dicumarol-treated animals indicate seven possible radioactive compounds in the urine and possibly four in the plasma. A control autoradiograph showing C14-dicumarol indicates that one of the radioactive compounds in the urine and one in the plasma may be unchanged dicumarol or a compound sufficiently similar in structure to have the same partition characteristics in the solvent systems used for the separation. The relative densities of the bands give a rough indication of the relative amounts of the substances present. The relative densities of the bands of the plasma autoradiograph suggest that unchanged dicumarol may account for a major part of the activity in the plasma. The autoradiographs for urine and plasma from
DEA-treated animals show only three separate bands in the urine and only two in the plasma. Again the control autoradiograph of C\textsuperscript{14}-DEA indicates that some unchanged DEA may be present in both urine and plasma. The various bands observed in the chromatograms of urine and plasma for both drugs as illustrated in figure 3 were in evidence at the six hour interval and remained essentially unchanged for the duration of the experiment.

It must be pointed out that the filter paper partition technic as applied in these studies is an indicator method only and is not meant to be taken as absolute. The difference in the number of bands shown by the chromatograms of the urine and plasma may be due to limitations in sensitivity of the method. The concentration of some of the metabolites in the plasma may be too low to be demonstrated chromatographically. Each band shown by the autoradiograph may result from a single radioactive substance or from two or more substances with very similar diffusion characteristics. Attempts to separate a mixture of C\textsuperscript{14}-dicumarol and C\textsuperscript{14}-DEA using fourteen different solvent systems were unsuccessful. Nevertheless, the material presented in figure 3 serves as an interesting preliminary observation regarding the detailed metabolism of DEA and dicumarol by the rat.

Prothrombin times were determined at 6, 12, 18, 21, 24, 27, 30, 36 and 48 hours following oral administration of 25 mg. of radioactively inert dicumarol and at comparable times after the administration of 50 mg. of inert DEA. A 50 mg. dose of inert DEA was used instead of 100 mg. (the dose comparable in hypoprothrombinemic effect to 25 mg. of dicumarol) because of the wide variation in response of rats to high
doses. With these dosages there were moderate variations in the hypoprothrombinemic response of rats to both dicumarol and DEA and it was necessary to use at least 5 animals per point. Response to DEA, particularly at higher effective dosage levels, was even more variable than response to dicumarol.

![Graph showing concentration of radioactivity in tissues as related to prothrombin activity following oral administration of C\textsubscript{14}-dicumarol.]

**Fig. 4**—Concentration of radioactivity in tissues as related to prothrombin activity following oral administration of C\textsubscript{14}-dicumarol.

![Graph showing concentration of radioactivity in tissues as related to prothrombin activity following oral administration of C\textsubscript{14}-DEA.]

**Fig. 5**—Concentration of radioactivity in tissues as related to prothrombin activity following oral administration of C\textsubscript{14}-DEA.

In figures 4 and 5 the hypoprothrombinemic response of rats to dicumarol and DEA is compared with the concentration of C\textsubscript{14} activity in liver, blood, kidney and balance (remainder of the animal) at various times after oral administration of the labeled compounds. Prothrombin time in seconds, as a function of time after oral administration, was compared with the per cent of net absorbed activity per gram of wet tissue. The results of the dicumarol study are given in figure 4. This graph shows that the peak of the hypoprothrombinemic effect occurred at approximately 24 hours after administration. During this time interval the concentration of radioactivity in the various tissues and in the balance steadily decreased to approximately one-third of the concentration at six hours. The results following oral administration of DEA are shown in figure 5. Maximal hypoprothrombinemic effect occurred at about 19 hours after administration. Again the concentration of radioactivity in the various tissues and in the balance had decreased to approximately one-third of the six hour concentrations.

**Discussion**

**Clinical Observations.** Our experience confirms the reports in the literature\textsuperscript{10-16} that DEA has a quicker and more transient hypoprothrombinemic effect in human subjects than has dicumarol. A single dose of 1.5 Gm. of DEA produced a therapeutic hypoprothrombinemia in our control series approximately six hours sooner than did a single dose of 400 mg. of dicumarol. It is interesting to note that therapeutic hypoprothrombinemia occurred at 19 hours in our series of DEA-treated patients with thromboembolic disease, as compared to 28 hours in our DEA controls. The therapeutic level in the controls was maintained for only 4 hours with DEA as compared to 24 hours with dicumarol. The return to normal prothrombin activity was far more rapid after the administration of DEA than after dicumarol. No gastrointestinal side effects were observed in our DEA group. Urinary and fecal excreta were analyzed for the presence of blood in the DEA group when prothrombin levels dropped below 10 per cent of normal activity. It was interesting to note that even with prothrombin times of 70 seconds evidence of bleeding was absent.

The management of patients receiving DEA frequently requires prothrombin determinations oftener than once a day for effective regulation of prothrombin levels. On the other hand, the management of patients with dicumarol is more easily controlled with less frequent prothrombin determinations. Although neither dicumarol nor DEA is an ideal
anticoagulant, DEA is somewhat superior to dicumarol in its speed of action and its less sustained hypoprothrombinemic effect.

Animal Experiments. The results of animal experiments using C\textsuperscript{14}-labeled dicumarol and DEA indicated that DEA had a slightly more rapid and more transient action than did dicumarol (figs. 4 and 5). The quicker action of DEA following oral administration does not seem to be attributable to a quicker or greater net absorption of the C\textsuperscript{14} activity from the intestinal tract (tables 2 and 3). In the case of DEA there does, however, seem to be a higher initial liver concentration and an earlier drop in the percentage of retained activity in the tissues of the animal, paralleled by a higher urinary excretion rate immediately following administration (fig. 2). In general, the slower rate of metabolic turnover of C\textsuperscript{14} activity following administration of labeled dicumarol may be responsible for its prolonged hypoprothrombinemic effect.

Maximal absorption and maximal concentration of activity in the liver, blood, kidney and balance occurred within six hours after the oral administration of both labeled dicumarol and labeled DEA (tables 4 and 5). In neither case was there any positive correlation between the time of maximal absorption or maximal concentration in the liver, blood, kidney and balance and the time of maximal hypoprothrombinemic effect of the drug (figs. 4 and 5).

The above observations indicate that the mode of action of dicumarol and DEA is essentially the same. The lack of positive correlation

### Table 2.—Gross Distribution of C\textsuperscript{14} Activity in Terms of Per Cent of Administered Dose at Various Times after Oral Administration of C\textsuperscript{14}-Dicumarol to Rats.

<table>
<thead>
<tr>
<th>Distribution of Administered Dose</th>
<th>Time After Oral Administration*</th>
<th>Average Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 Hr.</td>
<td>12 Hr.</td>
</tr>
<tr>
<td>Net Absorption, % Administered Dose</td>
<td>32.94</td>
<td>33.55</td>
</tr>
<tr>
<td>G.I. Tract &amp; Contents, % Administered Dose</td>
<td>66.44</td>
<td>61.65</td>
</tr>
<tr>
<td>Feces (Excreted), % Administered Dose</td>
<td>0.91</td>
<td>5.69</td>
</tr>
<tr>
<td>Total Recovery, % Administered Dose</td>
<td>100.29</td>
<td>100.89</td>
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</table>

* Each value represents the average of three animals.

† The net absorbed dose was taken as the amount of activity found in the tissues plus that excreted in the urine, allowing no excretion of absorbed material via the gastrointestinal tract.

### Table 3.—Gross Distribution of C\textsuperscript{14} Activity in Terms of Per Cent of Administered Dose at Various Times after Oral Administration of C\textsuperscript{14}-DEA to Rats.

<table>
<thead>
<tr>
<th>Distribution of Administered Dose</th>
<th>Time After Oral Administration*</th>
<th>Average Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 Hr.</td>
<td>12 Hr.</td>
</tr>
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<td>Net Absorption, % Administered Dose</td>
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<td>G.I. Tract &amp; Contents, % Administered Dose</td>
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<td>Feces (Excreted), % Administered Dose</td>
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<td>0.04</td>
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<tr>
<td>Total Recovery, % Administered Dose</td>
<td>100.98</td>
<td>97.39</td>
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</table>

* Each value represents the average of three animals.

† The net absorbed dose was taken as the amount of activity found in the tissues plus that excreted in the urine and as CO\textsubscript{2}, allowing no excretion of absorbed activity via the gastrointestinal tract.
(formula II). Only about 3.6 per cent of the net absorbed activity was exhaled as carbon dioxide in 36 hours which was equivalent to 7 per cent carboxyl group was the source of the C\(^{14}\)O\(_2\) rather than the carbon atom in the methylene bridge.

**Table 4.—Distribution of Activity in Terms of Per Cent of Net Absorbed* Dose Per Organ at Various Times after Oral Administration of C\(^{14}\)-Dicumarol and DEA to Rats.**

<table>
<thead>
<tr>
<th>Tissue or Excreta</th>
<th>Time in Hours after Oral Administration*</th>
<th>Dicumarol</th>
<th>DEA</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>6</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Blood</td>
<td>18.38</td>
<td>15.04</td>
<td>7.77</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1.97</td>
<td>1.98</td>
<td>1.11</td>
</tr>
<tr>
<td>Heart</td>
<td>0.46</td>
<td>0.47</td>
<td>0.15</td>
</tr>
<tr>
<td>Lungs</td>
<td>1.36</td>
<td>1.10</td>
<td>0.37</td>
</tr>
<tr>
<td>Skin and Hair</td>
<td>24.02</td>
<td>17.89</td>
<td>8.52</td>
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<tr>
<td>Remains</td>
<td>37.43</td>
<td>31.76</td>
<td>13.18</td>
</tr>
<tr>
<td>Urine</td>
<td>2.00</td>
<td>20.05</td>
<td>62.33</td>
</tr>
<tr>
<td>CO(_2)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* Net absorbed dose was taken as the amount of radioactivity found in the tissues plus that excreted in the urine and as CO\(_2\), allowing no excretion of absorbed material via the gastrointestinal tract.

† Each value represents the average on three animals.

‡ Of this fraction, the testes accounted for 0.26%; the spleen, 0.66%; the thymus, 0.67%; and the brain, 0.93% of the total absorbed dose.

§ 36 hour value for CO\(_2\) not measured but estimated for the purpose of calculating the other results at 36 hours.

**Table 5.—Distribution of Activity in Terms of Per Cent of Net Absorbed* Dose Per Gram of Wet Tissue at Various Times after Oral Administration of C\(^{14}\)-Dicumarol and DEA to Rats.**

<table>
<thead>
<tr>
<th>Tissue or Excreta</th>
<th>Time in Hours after Oral Administration*</th>
<th>Dicumarol</th>
<th>DEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Liver</td>
<td>1.60</td>
<td>1.42</td>
<td>0.56</td>
</tr>
<tr>
<td>Blood†</td>
<td>1.32</td>
<td>1.14</td>
<td>0.53</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1.08</td>
<td>1.16</td>
<td>0.59</td>
</tr>
<tr>
<td>Heart</td>
<td>0.60</td>
<td>0.57</td>
<td>0.19</td>
</tr>
<tr>
<td>Lungs</td>
<td>1.94</td>
<td>0.79</td>
<td>0.29</td>
</tr>
<tr>
<td>Skin and Hair</td>
<td>0.63</td>
<td>0.54</td>
<td>0.23</td>
</tr>
<tr>
<td>Remains</td>
<td>0.31</td>
<td>0.28</td>
<td>0.11§</td>
</tr>
<tr>
<td>CO(_2)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* Net absorbed dose was taken as the amount of radioactivity found in the tissues plus that excreted in the urine, allowing no excretion of absorbed material via the gastrointestinal tract.

† Each value represents the average on three animals.

‡ Values for other tissues incorporated into the remains but analyzed separately are: bone marrow, 0.21%; testes, 0.11%; spleen, 0.11%; muscle, 0.11%, thymus, 0.10%; bone, 0.09%; and brain, 0.02% of net absorbed dose per gram.

§ The values for blood are given as per cent of absorbed dose per cc.

of the C\(^{14}\) activity present in the carbethoxy group alone. As decarboxylation of organic acids is a rather common occurrence in biologic systems it seems reasonable to assume that the Filter paper partition chromatographic analyses of the urines and plasmas of animals treated orally with C\(^{14}\)-labeled dicumarol and C\(^{14}\)-labeled DEA indicated (but did not con-
CLUSIVELY prove) that unchanged dicumarol and DEA were present in appreciable amounts in both plasma and urine (fig. 3). The chromatographic analyses also indicated that there may be as many as seven metabolites of dicumarol and three of DEA present in the urinary excretion.

A comparison of the studies given in this report with those reported recently by Lee and co-workers of the metabolism of C14-dicumarol following intravenous administration to mice and rabbits indicates an appreciable difference in the mode of metabolism of dicumarinyl derivatives following intravenous and oral administration.

SUMMARY

1. Clinical observations in normal subjects and in patients suffering from thromboembolic diseases showed that 4,4'-dihydroxydicumarinyl ethyl acetate (Pelentan, Tromexan) has a quicker, more transient hypoprothrombinemic effect and less apparent side effects than does dicumarol. The quicker, more transient action of the former substance may provide an advantage over dicumarol for the control of thromboembolic diseases.

2. Animal experiments with C14-labeled dicumarol and 4,4'-dihydroxydicumarinyl ethyl acetate indicated that the quicker, more transient action of the latter substance is associated with a more rapid drop in body retention, paralleled by a higher urinary excretion rate in the first few hours following oral administration. In general, the slower rate of metabolic turnover of C14 activity following administration of labeled dicumarol may be responsible for its prolonged hypoprothrombinemic effect.

3. The experiments indicated that the mode of action of the two substances is essentially the same. Maximal absorption and the maximal concentration of the radioactivity occurred within six hours after oral administration. The time of maximal absorption and maximal tissue radioactivity was not positively correlated with the time of maximal hypoprothrombinemic effect. In the case of DEA, however, there seemed to be a higher initial liver concentration and an earlier drop in the percentage of retained activity in the tissues of the animal, paralleled by a higher urinary excretion rate immediately following administration.

4. Filter paper partition chromatographic analysis suggested the presence of unchanged dicumarol and unchanged DEA both in the urine and the plasma of animals treated orally with the radioactive materials. The analyses suggested the possible production of seven metabolites in the case of dicumarol and at least three in the case of 4,4'-dihydroxydicumarinyl ethyl acetate.

5. Preliminary studies following intravenous injection of the labeled materials suggested an appreciable difference in the mode of metabolism of dicumarinyl derivatives following oral and intravenous administration.

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