Tromexan—3,3’-Carboxymethylenebis (4-Hydroxycoumarin) Ethyl Ester
Experimental and Clinical Properties

By Grafton E. Burke, M.D., and Irving S. Wright, M.D.

The use of heparin and dicumarol has provided important information regarding the effectiveness and the difficulties of anticoagulant therapy. Neither, however, is an ideal anticoagulant; therefore a search for new substances with advantages over them must be continued. Tromexan, which is administered orally, appears to have the advantages of more rapid utilization and more rapid cessation of action than dicumarol, although the mode of action is similar in that it produces a hypoprothrombinemia. It does require accurate prothrombin tests for satisfactory clinical use. It appears to be less prone to producing hemorrhagic complications. Animal and clinical experiences with Tromexan are herewith presented.

The value of anticoagulant therapy in the treatment of thromboembolic disease has been established by many workers. However, certain properties which prevent both heparin and dicumarol from being ideal anticoagulants have spurred the search for new anticoagulants which might have advantages over them. Clinical and experimental reports concerning the anticoagulant activity of the 3,3’-carboxymethylenebis (4-hydroxycoumarin) ethyl ester (Tromexan*) have appeared from Czechoslovakia, Switzerland, England, and France.

Working on the hypothesis that one reason dicumarol is poorly absorbed is the difficulty in splitting the coumarin molecules, Rosicky weakened the methylene linkage between the two coumarin groups by adding the carboxyl radical.

Overman and Link had originally investigated the acid in 1940 but, finding that it was much less active than dicumarol, did not esoterify the compound. The anhydride of dicumarol has been shown to be inert biologically.

According to von Kauila, Reinis and Kubik in 1948 first reported the synthesis of this new anticoagulant. In their animal experimental work they noted that the administration of the drug caused a rapid decrease in the prothrombin activity with a relatively quick return to initial values after cessation of administration. They reported that patients showed a similar response following the administration of a single dose of 900 mg. In their experience this dose caused a measurable prolongation of the prothrombin time within one or two hours, reaching a maximum in 12 to 24 hours.

Von Kauila and Pulver in subsequent experimental work reported that the minimal lethal dose of Tromexan was approximately 750 and 1000 mg. per Kg. of body weight for mice and rabbits respectively. (The minimal lethal dose for dicumarol was 150 to 250 mg. per Kg.). It was also noted that after feeding 100 mg. per Kg. of Tromexan daily to mice, death usually occurred within a range of 26 to 51 days. At autopsy pathologic changes, mainly fatty infiltration, were noted in the liver. Less frequent lesions of the same nature were found in the kidney. In rats daily doses of 50 mg. per Kg. could be tolerated well over a period of one month or more. On the basis of clinical experience they concluded that Tromexan was tolerated well but that it must be administered at

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This work was carried on under the auspices of the Committee on Anticoagulants of the American Heart Association and aided by grants from the Kress Foundation, the Lasker Foundation, the Hampil Foundation and the Hyde Foundation.

* Known as Pelentan in Czechoslovakia. Tromexan is sometimes referred to as DEA, from an alternate name, 4,4’-dihydroxydicumarinyl ethyl acetate. The Tromexan was supplied through the courtesy of Geigy, Inc.
approximately three times the dosage level of dicumarol in order to achieve a corresponding prolongation of the prothrombin time.

These workers\textsuperscript{17, 18} also studied the pharmacology of the drug and demonstrated its breakdown into two degradation products: (1) Tromexan acid A, with a melting point of 216 C. and (2) Tromexan acid B, with a melting point of 171 C. They isolated these relatively inactive and nontoxic metabolic products in significant quantity from the urine. They also described a method for the quantitative determination of Tromexan in serum and in urine by coupling the drug with diazotized paranitroaniline at a pH of 6 to form a yellow dye. Following separation from the serum protein, Tromexan was extracted with benzene and was measured colorimetrically. This method can be applied to other coumarins. Tromexan itself, as well as its degradation products, is measured by this method. Throughout the above work it was found that rabbits tolerated Tromexan better and that it was more rapidly absorbed than dicumarol. The maximum blood concentration of the drug was somewhat less for Tromexan than for dicumarol with the same mg. dosage. The elimination of Tromexan was tested by giving 900 mg. to 4 patients. Maximum concentration in the serum was obtained at three to six hours. No Tromexan could be determined in the serum 24 hours after administration whereas traces of dicumarol could be detected as long as seven days after administration.

Reinis and Kubik,\textsuperscript{19} de Nicola,\textsuperscript{19} Pulver and Von Kaulla\textsuperscript{18} and Della Santa\textsuperscript{20a} all described additional clinical experience with Tromexan in the therapeutic management of thromboembolic disease. The dose used varied from 900 to 1200 mg. for the first two days, followed by a maintenance dose of 300 to 900 mg. daily. They originally gave this in a single daily dose but later concluded that the best method of administration was in the form of a tablet of 300 mg. three times a day.

Burt, Wright and Kubik\textsuperscript{14} in England reported experiences with the use of Tromexan in 126 cases of thromboembolic disease. They too used the dose of 900 to 1200 mg. for the first two days followed by maintenance doses of 300 to 600 mg. In most instances a prothrombin percentage of 20 to 30 per cent was maintained. In some a level of 40 to 50 per cent was used. Daily prothrombin times were usually determined but experience suggested that once a maintenance dose had been established, determination on alternate days was sufficient. The period of treatment averaged 11 days but in 1 patient the prothrombin level was maintained at from 20 to 40 per cent for 10 months. In over 80 per cent of the cases the prothrombin activity decreased to at least 50 per cent within 36 hours and returned to normal within the same period after discontinuance of the drug. In 20 patients evidence of this response lasted for 60 hours or more. Slow initial response was not, however, invariably followed by prolonged effect. Postoperative patients as well as postpartum patients were also treated. No excess puerperal or postoperative hemorrhage was observed in cases treated for varying periods following operation or delivery. In addition they noted that infants suckled by mothers receiving Tromexan manifested no bleeding tendencies.

Because of these European reports,\textsuperscript{12-15, 17-20b} the experimental and clinical evaluation of Tromexan was undertaken at New York Hospital-Cornell Medical Center. A preliminary report of our early experience has appeared.\textsuperscript{21}

\textbf{Studies in Animals}

As shown by Overman, Stahmann, Link and their co-workers\textsuperscript{22a, b} with dicumarol there is an individual variation in rabbits and hence the animals used were standardized according to the technic described by them. By this means resistant animals were eliminated from the

\fig{0.5}{thrombin_curve.png}{Prothrombin curve following single dose of 400 mg. of Tromexan to rabbits.}
series. A total of 36 rabbits were fed a sodium hydroxide solution (pH 11) of Tromexan in doses of 100, 200, 300, 400, 500, 600 mg. by stomach tube in a single dose. Prothrombin times were done at six hour intervals around the clock according to the Link-Shapiro technic. Determinations were twice checked by different technicians, and the samples were treated as unknowns. The results are tabulated in figure 1. No changes were noted in the first six hours. Slight elevations were noted in rabbits at the end of 12 hours, while at 18 and 24 hours consistent and reproducible prolongation of the prothrombin time was evident. The normal

<table>
<thead>
<tr>
<th>Table 1.—Thromboembolic Diseases Present in Patients Treated with Tromexan.</th>
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<tbody>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Thrombophlebitis without embolism .............. 37</td>
</tr>
<tr>
<td>Legs (deep and superficial) ....................... 34</td>
</tr>
<tr>
<td>Arm (axillary vein) ................................ 3</td>
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<tr>
<td>Antepartum phlebitis ............................... 3</td>
</tr>
<tr>
<td>Thrombosis with embolism to lung .................. 8</td>
</tr>
<tr>
<td>Peripheral embolism (rheumatic heart disease with auricular fibrillation) .......... 3</td>
</tr>
<tr>
<td>Saddle embolus ................................... 1</td>
</tr>
<tr>
<td>Popliteal embolism ................................ 2</td>
</tr>
<tr>
<td>Cerebral embolism ................................ 2</td>
</tr>
<tr>
<td>Cerebral thrombosis ................................ 2</td>
</tr>
<tr>
<td>Retinal vein thrombosis .............................. 1</td>
</tr>
<tr>
<td>Myocardial infarction ................................ 48</td>
</tr>
<tr>
<td>Arteriosclerosis obliterans .......................... 6</td>
</tr>
<tr>
<td>Thromboangitis obliterans ........................... 2</td>
</tr>
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<td>Total ............................................. 112</td>
</tr>
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time in rabbits, 10.5 to 11.5 seconds, was doubled in a period of 18 to 24 hours, with average readings of the undiluted plasma of 22 at 24 hours and of 26 to 35 at 24 hours depending upon the dose. It was noticed that with increased dosage there appeared to be a total increased rise in the prothrombin time as well as a prolonged total excretion time. With increased doses the prothrombin rose more precipitously.

In addition, mice and rats were fed Tromexan. The half lethal dose for mice was found to be 750 mg. per Kg, and for rats and rabbits, 1500 to 1800 mg. per Kg. Rats fed 100 mg. doses daily by stomach tube for 14 to 20 days failed to show any evidence of toxic symptoms.

Studies in Man

Twenty-four normal individuals used as controls were first studied with arbitrarily selected single doses of Tromexan of 1200, 1500 and 1800 mg. Twenty-four hours after the administration of single doses of 1500 to 1800 mg. the prothrombin time was found to have uniformly reached a level of 20 to 30 seconds (undilute plasma) and 65 to 100 seconds (dilute plasma). The control readings were 15 seconds ± 1.0 second with undilute plasma and 38 seconds ± 2 seconds with dilute plasma. With doses of 1200 mg. correspondingly lower levels were achieved. With the undilute plasma method consistent prolongation of the prothrombin times was obtained in the minimum time of 15 to 18 hours. However, in some cases slight elevation of prothrombin time in the undilute plasma and elevation of from 6 to 12 seconds in the dilute plasma was found within 12 hours following the initial dose. In the normal subjects no evidence of toxicity was found. Following a single dose the prothrombin time returned to normal within 48 to 60 hours.

In order to evaluate Tromexan clinically, a number of patients with thromboembolic disease were treated. These included a variety of conditions (table 1), chief among which were peripheral thrombophlebitis of the veins of the extremities, with and without pulmonary infarction, and coronary occlusion with myocardial infarction. The following studies were conducted on each patient before, during and after the use of Tromexan: daily urine examinations and periodic phenolsulfonphthalein tests; bromosulfalein tests; cephalin flocculation; thymol turbidity; total protein and albumin-globulin ratio; the alkaline phosphatase determinations; counts of the red and white blood cells; estimation of hemoglobin; determination of erythrocyte sedimentation rate and examination of stools for occult blood. Three of 112 cases showed evidence of microscopic hematuria while taking the drug. In all 3, however, there was evidence of pre-existing renal disease. Further discussion of these cases will follow.

Four patients showed symptomatic or chemical evidence of toxicity. One patient with liver disease showed slight alteration with the cephalin flocculation test and there was also a
slight alteration of the albumin-globulin ratio. These were known to be altered prior to the onset of therapy and following the cessation of Tromexan they returned to their previous abnormal levels.

Following an initial dose of 1200, 1500 or 1800 mg. the prothrombin time rose as indicated in figures 2, 3, and 4. A maintenance dose of 600 to 900 mg. per day was given. For the most part this was given in a single dose, and it should be emphasized that a period greater than 24 hours should not elapse between the administration of each dose if a therapeutic level is to be maintained. In individuals who showed marked fluctuation in their daily prothrombin times, divided doses of 300 mg. three times a day or 600 mg. twice a day were given with satisfactory results. Rare individuals required doses as high as 1200 mg. twice a day. About 15 to 20 per cent of the patients studied required this split dosage schedule (see figs. 5 and 6). Cases which showed fluctuations in daily levels were placed on the split doses and were well maintained. In only 1 case was it noted that the prothrombin time could not be considered to have been held within therapeutic range and in this patient there was extension of the thromboembolic process. This individual with a pulmonary infarction had clinical evidence of extension or a new pulmonary embolus to the lungs after three days of therapy. The dosage was increased to produce a satisfactory hypoprothrombinemia and thereafter the patient had an uneventful clinical course. The patients on Tromexan showed a good clinical response which was fully comparable to that experience with dicumarol.

Two patients showed a rather precipitous
rise in their prothrombin time following the administration of Tromexan over a period of two or three days. Both these patients had overt signs of renal disease with elevated blood urea nitrogen, fixed concentrations of specific gravity of the urines and other evidence of a chronic glomerulonephritis. One patient showed a rise of the prothrombin time (undilute plasma) to 120 seconds while on a maintenance dose of 900 mg. per Kg. per day. Prothrombin time was then determined at six hour intervals and the prothrombin time rapidly returned toward normal. Twenty-four hours following the high peak of 120 seconds the prothrombin time was 26 seconds; 30 hours later, 22 seconds; and in 36 hours it was normal, namely 15 seconds with undilute plasma. There was no evidence of bleeding and no vitamin K was administered.

One patient was mistakenly given an extra dose of 1200 mg. of Tromexan and thus received 3000 mg. in 24 hours; his prothrombin time rose to 66 seconds. This was watched carefully and within 24 hours had returned to 23 seconds.

The above cases suggest that vitamin K will rarely be necessary in cases of accidental overdosage (unless evident hemorrhage is present) because of the rapid excretion rate of Tromexan and the fact that the prothrombin time returns to the normal range in a relatively short period of time. However, the effect of vitamin K was studied in 3 normal patients. Tromexan was pushed so that the prothrombin time rose to levels of 35 to 53 seconds at which points 50 mg. of water soluble vitamin K were given intravenously. The prothrombin time returned to within normal limits in a period of 12 hours which is faster than we have noted if no vitamin K is given. Control studies on the same subjects without using vitamin K showed a return to normal from an average peak of 50 seconds after 24 to 48 hours. Vitamin K does apparently effectively combat the anticoagulant activity of Tromexan.

**DISCUSSION**

These studies have confirmed the reports that Tromexan acts and is excreted more rapidly than dicumarol. From the structural formula of this coumarin derivative it could be hypothesized that the weakening of the bond between the two coumarin groups would tend to make it more soluble and the addition of the carboxyl at this point would from the pharmacologic basis tend to make the drug more readily absorbed. This was been borne out by our clinical and animal experimentation. The metabolic route of excretion and absorption previously studied by Della Santa and von Kaulla is now being investigated by Brody, Shapiro and Weiner. Further pharmacologic studies are being carried on by Gruber in Philadelphia. On the basis of work done heretofore by Gianella and von Kaulla and others, and communications from Brody, Shapiro and Weiner it appears that Tromexan probably has different routes of excretion and metabolic breakdown from dicumarol, and its method of detoxification is still obscure. The difference in potency between dicumarol and Tromexan may well be explained on this basis. Tromexan appears to be about one-fifth as potent as dicumarol on the basis of weight and hence four to five times the dose must be given. In addition, Tromexan is so rapidly absorbed that prothrombin time determinations done at 18 to 24 hours show a consistent prolongation. The toxicity of the drug appears less when compared in terms of therapeutic dosage with dicumarol. No patients showed any evidence of urticaria, rash, idiosyncrasy to the drug or intolerance except as cited. The tablets are bitter and should be swallowed intact. The dosage given in our series has ranged from 600 to 1800 mg. Occasionally an individual will be found who requires an even larger dose. We have found no cases who showed a true drug resistance by failing to respond to the careful administration of an increased dosage. The regulation of the patient on Tromexan is usually easier than that of the patient on dicumarol because each prothrombin test is a direct reflection of the dose given the previous day. If this time is too low the dose can be increased with a relatively prompt response. There is little cumulative effect of the drug, particularly if it is given in single doses at an interval of 24 hours, or less. In general, however, an interval greater than 24 hours should not elapse between

* Personal communications.
the administration of the drug if an effective level is to be consistently maintained. Certain individuals may require the drug at more frequent intervals. Our results differ from those of the European investigators in that we have been unable to show appreciable prolongation of the prothrombin time in six hours as they reported.

CONCLUSIONS

1. The coumarin derivative 3,3'-carboxymethylenebis (4-hydroxycoumarin) ethyl ester (Tromexan) produces a significant prolongation in the prothrombin time when administered in therapeutic doses.

2. It has approximately one-fifth the potency of dicumarol, when compared mg. per Kg., in terms of effect on the prothrombin time obtained.

3. Tromexan has a faster absorption and utilization rate than dicumarol as measured by the level of hypoprothrombinemia produced within 18 to 24 hours after the initial dose.

4. The duration of the anticoagulant effect is about one-half to one-fourth that of dicumarol.

5. Prothrombin times return to normal 48 to 60 hours after a single initial dose.

6. Satisfactory maintenance of a therapeutic level of hypoprothrombinemia can be achieved by single daily doses of 600 to 2400 mg. or a dose of 300 to 900 mg. administered in two or three divided doses.

7. The requirements are dependent on the individual response which must be determined by prothrombin tests.

8. In the presence of renal damage hematuria was noted in 3 cases.

9. Further alteration of abnormal liver function and the albumin-globulin ratio was noted in 1 case of liver disease.

10. Of 112 cases treated only the reactions cited under 8 and 9 were noted.

11. Further clinical trials, on a cooperative basis, are now in progress in order to evaluate its usefulness as a rapidly acting anticoagulant in the treatment of thromboembolic disease.

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Circulation. 1951;3:164-170
doi: 10.1161/01.CIR.3.2.164

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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