

# Hemorrhagic Agent 3,3'-Methylenebis (4-Hydroxycoumarin). V. Its Effect in Prevention of Experimental Thrombosis.\*

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The discovery of the hemorrhagic agent 3,3'-methylenebis (4-hydroxycoumarin) (Dicumarol) by Link and his associates<sup>1-5</sup> raised the question whether the reduction it caused in the prothrombin content of the blood would prevent intravascular thrombosis if the intima of the vessel wall had been damaged.

Bingham, Meyer, and Pohle<sup>6</sup> produced such damage by injecting the sclerosing agent Monolate (monoethanolamine oleate) Abbott into a segment of external jugular vein previously clamped off. They permitted circulation for one minute, reclamped, and checked the time necessary for clot formation. An increase in the necessary time was noted in Dicumarol-treated dogs, but no histological studies were done, and they recognized that other variables besides the Monolate were at work.

Dale and Jaques<sup>7</sup> administered 10 mg/kilo of Dicumarol intravenously to dogs. Sixty hours later they crushed the radial and saphenous veins with linen thread, and removed the veins after 2½ hours. They found 60% of the veins free of thrombi in contrast to 100% involvement in the controls.

Murray, Jaques, Perrett, and Best,<sup>8</sup> working with heparin, devised a method for experimental thrombus formation which has recently been used by Richards and Cortell<sup>9</sup> in studies with Dicumarol. The latter investigators used Monolate to produce thrombosis in the radial and saphenous veins in a series of 8 dogs, 4 of which had previously been given fatal doses of Dicumarol, 10 or 25 mg/kilo, daily for 3 to 5 days before injection of the veins with Monolate. Six to 10 days later the veins were removed and examined histologically; thrombi were absent in 10 of 12 veins in Dicumarol-treated dogs, in 3 of 14 veins in the controls.

Using a method similar to that outlined by Murray *et al.*<sup>8</sup> and Richards and Cortell<sup>9</sup> we made a study of the efficacy of Dicumarol in the prevention of venous thrombosis in dogs to which a single dose of the drug had been administered in very safe therapeutic dosage, 5 mg/kilo.

**Method.** A series of 30 dogs was studied. After the dogs were weighed, the prothrombin time was determined by the Pohle and Stewart<sup>10</sup> modification of Quick's technic on undiluted plasma, and the coagulation time by the 2-tube method of Lee and White at 37°-38°C. On the same day the dogs were matched by weight and divided into 2 groups. Half were kept as controls, and the other 15 were given Dicumarol, 5 mg/kilo, orally, in a gelatine capsule.

Two days later the determinations of prothrombin and coagulation time were repeated, and 0.25 cc of Monolate was injected into a

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<sup>9</sup> Richards, R. K., and Cortell, R., *Proc. Soc. Exp. Biol. and Med.*, 1942, **50**, 237.

<sup>10</sup> Pohle, F. J., and Stewart, J. K., *Am. J. Med. Sci.*, 1939, **198**, 622.

TABLE I.

| Control dogs |                                    |                                           |                                |      | Dicumarol-treated dogs |                                    |                                           |                                |      |
|--------------|------------------------------------|-------------------------------------------|--------------------------------|------|------------------------|------------------------------------|-------------------------------------------|--------------------------------|------|
| Dog          | Prothrombin time<br>(whole plasma) |                                           | Histological study<br>of veins |      | Dog                    | Prothrombin time<br>(whole plasma) |                                           | Histological study<br>of veins |      |
|              | Initial                            | At time<br>of inject.<br>with<br>monolate |                                |      |                        | Initial                            | At time<br>of inject.<br>with<br>monolate |                                |      |
|              |                                    |                                           | Right                          | Left |                        |                                    |                                           | Right                          | Left |
| 8            | 5.5                                | 6.5                                       | +                              | +    | 7                      | 6                                  | 20.5                                      | ?                              | —    |
| 10           | 6                                  | 7                                         | +                              | ?    | 9                      | 6                                  | 18.5                                      | —                              | —    |
| 12           | 6                                  | 6.5                                       | +                              | +    | 11                     | 6.5                                | 26                                        | —                              | —    |
| 14           | 6                                  | 6                                         | —                              | —    | 13                     | 5.5                                | 9.5                                       | ?                              | —    |
| 16           | 5.5                                | 5                                         | ?                              | —    | 15                     | 5.5                                | 12                                        | —                              | —    |
| 18           | 5.5                                | 6                                         | +                              | —    | 17                     | 6                                  | 14.5                                      | —                              | —    |
| 19           | 5                                  | 5                                         | —                              | +    | 20                     | 6                                  | 36                                        | —                              | —    |
| 21           | 6                                  | 6                                         | +                              | —    | 23                     | 6                                  | 46.5                                      | —                              | —    |
| 22           | 5.5                                | 6                                         | —                              | —    | 24                     | 6                                  | 18.5                                      | ?                              | —    |
| 27           | 5.5                                | 5.5                                       | —                              | +    | 25                     | 5                                  | 12                                        | —                              | —    |
| 29           | 5.5                                | 5.5                                       | —                              | —    | 26                     | 6                                  | 5.5                                       | +                              | —    |
| 30           | 6                                  | 6                                         | —                              | +    | 28                     | 5.5                                | 6                                         | ?                              | —    |
| 32           | 6.5                                | 6                                         | +                              | +    | 31                     | 6.5                                | 25.5                                      | —                              | —    |
| 33           | 6                                  | 5.5                                       | +                              | —    | 34                     | 6.5                                | 22                                        | —                              | —    |
| 36           | 6                                  | 6                                         | +                              | +    | 35                     | 6                                  | 18                                        | —                              | +    |

— No thrombus

? Doubtful

+ Thrombus present

3-inch segment of each radial vein. During the time of injection and for 3 minutes thereafter, the vein was compressed above and below the site of injection by the fingers, after which compression was released and the sclerosing agent permitted to enter the circulation.

Six to 9 days later, prothrombin and coagulation times were again determined. Then the previously injected segments of veins were removed under morphine and ether anesthesia. Hemostasis was secured by tying off branches to the vein and ligating both ends of the vein before removal. The skin was closed with interrupted sutures. The veins were placed in Helly's solution and histological study was made of all.

The results of the study are listed on Table I. The first group are the controls, the second the Dicumarol-treated animals.

**Discussion.** Of the 30 veins removed from 15 dogs which had received 5 mg/kilo of Dicumarol orally in a single dose, and which 2 days later had been subjected to the injection of a sclerosing agent, 0.25 cc Monolate (monoethanolamine oleate) Abbott into the radial vein, 2 veins (6.6%) showed definite thrombus formation. Four more (13.3%) were classed as inconclusive with respect to thrombus formation because it was difficult to decide

whether the reaction was entirely intimal or whether an organized mural thrombus was present. Thus a maximal total number of 6 veins (20%) showed involvement. In only one (Dog No. 35) of the 2 Dicumarol-treated dogs exhibiting a definite thrombus had the prothrombin time been increased. In one of the 4 exhibiting a questionable thrombus, the drug had not modified the prothrombin level.

In contrast, 15 (50%) of 30 veins in the controls showed thrombi, 2 (6.6%) were listed as inconclusive, the total involved being 17 (56.6%). Bilateral thrombosis occurred in 5 dogs, whereas no bilateral involvement occurred in the Dicumarol-treated group.

No excessive amount of bleeding was encountered at operation, and no post-operative hemorrhage was noted during two weeks of observation in any of the dogs.

**Conclusion.** A definite reduction in the incidence of thrombus formation was noted in dogs previously treated with Dicumarol in a dosage equivalent to a therapeutic and very safe quantity commonly administered to human beings.

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