Summary. Small pieces of heart tissue from young chick embryos coalesce to form a spherical body when brought into actual contact with each other in a mixture of chicken serum and Ringer's solution. The pieces must touch each other. A preliminary treatment of these fragments with "trypsin" increases the rate and percentage of coalescence. The method is serviceable for titration of cyto-antibodies in a medium, as these inhibit the coalescence in low concentrations.

The method is described, and its use is demonstrated by examples.

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Effect of a Single Dose of 3,3′-Methylenebis (4-Hydroxycoumarin) upon Blood Coagulation in Humans.*†

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The pharmacological and chemical activity of 3,3′-methylenebis (4-hydroxycoumarin) has been reported from agricultural experiment stations.[1,2] The most recent publication[3] by the Wisconsin workers describes the effect of a single dose of this dicoumarin upon the prothrombin time of the plasma of various animals. In this communication we describe the effect of single doses of this drug on blood coagulation in humans. Prandoni and Wright[6] have reported on the effect of daily doses in man. However, the few re-

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1 Roderick, L. M., and Schalk, A. F., *North Dakota Agr. Exp. Sta. Bull.*, 1931, 250.

2 Campbell, H. A., Smith, W. K., Roberts, W. L., and Link, K. P., *J. Biol. Chem.*, 1941, **138**, 1.

3 Overman, R. S., Stahmann, M. A., Sullivan, W. R., Hueber, C. F., Campbell, H. A., and Link, K. P., *J. Biol. Chem.*, 1942, **142**, 941.

4 Bingham, J. B., Meyer, O. O., and Pohle, F. J., *Am. J. Med. Sc.*, 1941, **202**, 563.

5 Butt, H. R., Allen, E. V., and Bollman, J. L., *Proc. Staff Meet. Mayo Clinic*, 1941, **16**, 388.

6 Prandoni, A. G., and Wright, I. S., presented before the N. Y. section of the Soc. for Exp. Biol and Med., Nov. 20, 1941.

ported instances[4, 5] of the use of 3,3'-methylenebis (4-hydroxycoumarin) in man have not yet provided enough information to warrant its general use.

Materials and Methods. Weighed doses of 3,3'-methylenebis (4-hydroxycoumarin)‡ were administered orally with water. Thirty-nine patients ranging from 12 to 70 years of age with most between 40 to 60 years, were selected for study. There were 21 with arteriosclerotic heart disease and a varying amount of cardiac failure, 9 with pneumonia, 5 convalescing from fracture of an extremity and others. The pneumonia patients were studied early in the course of their disease when the temperature was elevated and they were acutely ill. The weights of the patients varied from 100 to 180 lb, the mean being 140 lb.

Forty-five single doses of this dicoumarin were given. Prothrombin and coagulation times were performed on alternate days or more frequently. There were 5 doses of 100 mg or less, 5 of 200 mg, 6 of 300 mg, 10 of 400 mg, 11 of 500 mg, 4 of 600 mg, and 4 of 700 mg. The per kilo dose averaged 8 mg, varying from 1 mg per kilo to 12 mg per kilo. The patients were studied from 4 to 24 days after the drug was given; most were followed for 10 days.

Before the drug was administered 7 cc of venous blood was drawn with minimal stasis in a syringe and needle previously rinsed in 0.85% saline; 4.5 cc of blood was immediately mixed with 0.5 cc of 0.1 M sodium oxalate; the coagulation time was determined at room temperature with the remainder by a minor modification of the two-tube method of Lee and White.[7] All tubes used in this study were cleaned in green soap and cleaning solution, adequately rinsed and oven-dried. The oxalated blood was centrifuged several hours after collection and the plasma stored at 6°C. The patients were seen daily and examined for possible toxic effects of the drug.

The prothrombin determinations were performed by a modified Quick procedure[8] using acetone-treated rabbit brain as a source of thromboplastin and recalcifying the plasma and thromboplastin mixture with 0.0125 M calcium chloride. The brains were obtained from discarded Friedman pregnancy-test rabbits.

The reaction was carried out at 37°C with plasma diluted with normal saline to from 62.5% to 25% concentration. Duplicates gave close agreement. Normal plasma was used as a control whenever test plasma was titrated. The final result for

‡ Supplied by Dr. J. F. Biehn of Abbott Laboratories, Inc., North Chicago, Ill.

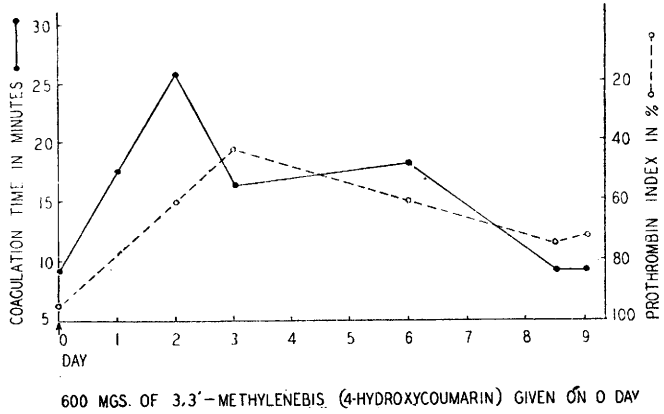
7 Lee, R. I., and White, P. D., *Am. J. Med. Sc.*, 1913, **145**, 495.

8 Quick, A. J., *J. Am. Med. Assn.*, 1938, **110**, 1658.

any dilution of plasma was expressed as: prothrombin index in % = 100 ($\frac{\text{control plasma in sec.}}{\text{test plasma in sec.}}$). Quick[8] and Pohle and Stewart[9] and others reported normal human prothrombin time of undiluted plasma of between 10 and 13 seconds. Our average undiluted plasma prothrombin time varied between 14 and 20 seconds.

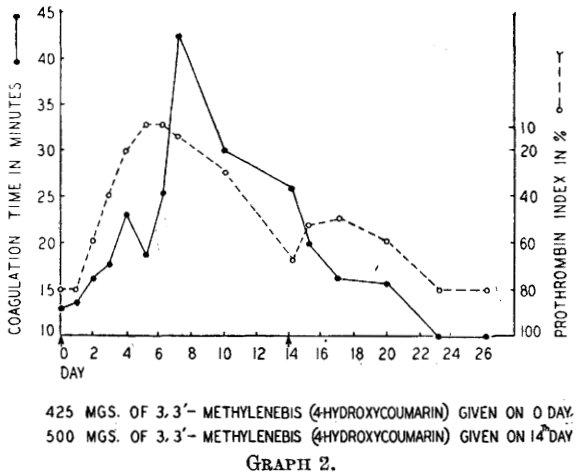
Results. Graphs 1 and 2 represent the effect of this dicoumarin on the coagulation time and prothrombin index of 2 different patients. The first detectable change in the coagulation mechanism occurred in from 24 to 72 hours after oral administration of 3,3′-methylenebis (4-hydroxycoumarin) and was manifest by a prolongation of the coagulation time or a fall in the prothrombin index. Though in most cases these changes occurred together, sometimes they were dissociated. The maximum effect from the drug was usually observed 1 to 3 days after the commencement of action, but this varied. Elevation of the coagulation time was quite variably maintained in different individuals and was unrelated to the dose. From a single dose, the duration of effect varied from 3 to 15 days averaging 6 days. The return of the clotting time to normal occurred either suddenly or gradually over a period of several days. It was unusual for a plateau to be reached in which the coagulation time remained evenly elevated and without considerable swings. As normal values were resumed, the coagulation time and prothrombin index were usually parallel but there were instances in which one became normal while the other still remained abnormal.

From these data it appears that the oral threshold dose of 3,3′-



GRAPH 1.
Effect of a single dose of 3,3′-methylenebis (4-hydroxycoumarin) upon coagulation time and prothrombin index of blood.

9 Pohle, F. J., and Stewart, J. K., *Am. J. Med. Sc.*, 1939, **198**, 622.



GRAPH 2.

Effect of single doses of 3,3′-methylenebis (4-hydroxycoumarin) upon coagulation time and prothrombin index of blood.

methylenebis (4-hydroxycoumarin) for adults is approximately 400 mg. Only one patient who received this amount failed to show the characteristic response. It is probable that 400 to 600 mg of this dicoumarin orally is the dose most likely to produce desired prolongation of clotting time safely. All cases receiving more than 5 mg per kilo of body weight showed a lowering of the prothrombin index and an elevation of the coagulation time.

In this study the initial control coagulation times before administration of 3,3′-methylenebis (4-hydroxycoumarin) varied between 6 and 19 minutes, averaging 11 minutes. At the height of drug action, the coagulation time varied between 16 and 42 minutes; most cases were between 22 and 26 minutes. All the pretreatment prothrombin indices ranged between 80% and 100%; during drug action they varied from 100% to less than 10% of normal.

When given in a single dose, there were practically no toxic manifestations. Two patients had minor epistaxes at a time when their coagulative mechanism was slightly abnormal. Both had been subject to such episodes prior to admission. The bleeding stopped spontaneously within one-half hour. It is difficult to interpret the evidence of toxicity in a third patient who entered in an advanced degree of cardiac failure and received 700 mg. Autopsy was performed and revealed, besides evidence of marked congestion due to heart failure, an unusual edema and intense hyperemia of the bronchi. There was a large quantity of hemorrhagic fluid in the bronchi. Three other patients who died of carcinomatosis or tuberculosis during or shortly after study showed no lesion that could

be interpreted as due to the dicoumarin administration. This pathological material is still under investigation. We have administered the drug to 20 other patients not included in this series and have not observed bleeding or other manifestations of toxicity. However, bleeding has been observed after the first week of major surgical procedures, as when a packing is removed. On this account, dicoumarin should be administered with caution to such patients.

Discussion. These observations indicate that the action of a single dose of 3,3′-methylenebis (4-hydroxycoumarin) is prolonged, and suggest the possibility that a few properly spaced doses may be sufficient to lengthen the coagulation time in humans for 2 weeks or longer. The therapeutic possibilities of this procedure are being investigated. During the latent period, 1 to 3 days before the action of the drug is apparent, heparin by continuous intravenous drip may be used. The heparin is discontinued as soon as the dicoumarin effect is seen. Further experimentation with these drugs in humans is desirable before they can be recommended for general use in thromboembolic states.

Summary. A prolongation in the coagulation time and a lowering of the prothrombin index of the blood for approximately 6 days beginning between 24 and 72 hours after the drug has been ingested will usually be produced by oral administration of a single dose of 400 mg of 3,3′-methylenebis (4-hydroxycoumarin) to a patient weighing 130 lb or less, or 600 mg to a patient weighing over 160 lb.

13701

Toxic Effects of Some Basic Proteins.

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Proteins which have an isoelectric point on the basic side of neutrality (protamine, histone), or near neutrality (globin) have recently been used in combination with insulin as depository therapeutic preparations. Their toxicities, however, have not been extensively studied. Vartiainen and Marble[1] reviewed the literature on the toxicity of protamine and determined its acute toxicity by

[1] Vartiainen, I., and Marble, A., *J. Lab. Clin. Med.*, 1941, **26**, 1416.