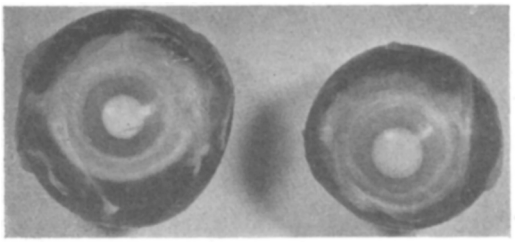

FIG. 1.
Contrast in size of eyeballs. The eyeball on the left is from a 7-week-old chick which received a diet containing 8% of glycine; its body weight was 395 g. The eyeball on the right is from a normal 7-week-old chick; its body weight was 574 g.

tion, a peculiar enlargement of the eyeballs developed which interfered with normal vision and functioning of the nictitating membrane. Eight percent of glycine as the peptide produced no such effect. The action of glycine appeared to be confined to the growing chicken. The feeding of 12% of glycine to hens over an extended period produced no apparent ocular abnormalities.

We are indebted to Merck & Co., Rahway, N. J., for crystalline vitamins; Wilson and Co., Inc., Chicago, Ill., for 2X gelatin; Allied Mills, Inc., Portsmouth, Va., for soybean oil; Abbott Laboratories, North Chicago, Ill., for haliver oil; U. S. Industrial Chemicals, Inc., Baltimore, Md., for *DL*-methionine; and to Lederle Laboratories, Pearl River, N. Y., for pteroylglutamic acid.

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Effect of Dicumarol on Ac-Globulin and Prothrombin Activity.*

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(Introduced by Walter H. Seegers.)

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The administration of dicumarol to prevent the occurrence or extension of intravascular thromboses has given encouraging results.[1-3] The rationale of this treatment is based upon the ability of dicumarol to lower the prothrombin concentration in the circulating plasma.[3] Dicumarol has been thought to act principally upon the prothrombin portion of the coagulation mechanism and thus to depress the clotting activity of whole blood. It is now apparent that another factor, Ac-globulin, warrants study in connection with dicumarol therapy. Ac-globulin[4] is a plasma protein normally found in the circulating plasma. It acts to assure normal physiological conversion of prothrombin to thrombin during the process of blood coagulation. A deficiency of this factor impairs thrombin formation and a bleeding tendency may develop.[5]

The existence of Ac-globulin was unknown until recently, and previous reports on prothrombin measurements by both the one- and two-stage tests have not considered the possible role of this factor. With accurate and specific methods now available, a study was undertaken of both prothrombin and Ac-globulin levels as they are influenced by dicumarol administration.

Methods. Blood was obtained by venipuncture. In order to reduce contamination by tissue fluids the initial 3 cc of blood drawn into the syringe were discarded. The sample to be analyzed was received into a second syringe containing the anticoagulant. In this

* Aided by grants from the United States Public Health Service and the Ortho S. A. Sprague Memorial Institute Fund.

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2 Allen, E. V., Hines, E. A., Kvale, W. F., and Barker, N. W., *Ann. Int. Med.*, 1947, **27**, 371.

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manner 9 volumes of blood were added to 1 volume of 3.2% sodium citrate. After centrifugation at 3,000 rpm for 30 minutes the plasma was carefully removed from the cellular elements and frozen at –20°C until analyzed. Quantitative prothrombin determinations were carried out by means of a modified 2-stage test in which Ac-globulin is supplied.[6] By adding an excess of accelerator in this modification, maximum conversion of prothrombin to thrombin is assured under the conditions specified in the 2-stage prothrombin analysis.[7]

Ac-globulin was measured by a method in which the concentrations of prothrombin, thromboplastin, and other known variables are controlled with the result that the rate of thrombin formation is dependent upon the amount of Ac-globulin present.[4] Because this test for Ac-globulin is a measure of a reaction rate (prothrombin to thrombin) and quite sensitive to minor changes in the reacting medium, all determinations were related to a control plasma of the same species. The normal control plasmas were carefully collected and frozen at –20°C until they were measured at the same time as the unknown samples.

Experimental. 1. *Canine species.* Seven normal healthy dogs were selected and the normal levels of plasma prothrombin and Ac-globulin determined. On successive days dicumarol was administered orally either as 2 doses of 14 mg per kg of body weight or as 4 doses of 4 mg per kg. Samples of blood for analysis were obtained regularly either from the saphenous or antecubital vein.

The results of these experiments indicate a sharp drop in plasma prothrombin which is maintained for a period of about a week. Following this a gradual restoration of prothrombin activity takes place. In addition, there was generally an initial decrease in Ac-globulin activity upon dicumarolization, though the magnitude of fall was much less than that of the prothrombin. This reduction

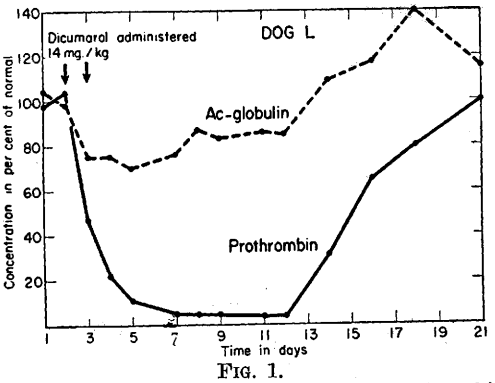

FIG. 1.

Changes in plasma Ac-globulin and prothrombin concentrations from dicumarol administration to a dog.

in Ac-globulin is only moderate, rarely falling under 65% of normal, and is in contrast to the severe fall in the prothrombin concentration. Even with the larger doses of dicumarol the Ac-globulin level did not fall below 50% of normal as compared to prothrombin titers reduced to as low as 1% of normal. In Fig. 1 are illustrated the results of a typical experiment employing a 9.0 kg female dog.

Considerable individual variation in the depth and duration of the response of Ac-globulin was observed. The drop generally began on the day following the first dose of the drug but on one occasion it did not occur until the 4th day. Recovery usually followed in 10-14 days. Interestingly, the increase in Ac-globulin concentration extended to values above the original levels, in one instance going as high as 150%, before returning to the normal range. This rise generally coincided with the period of marked prothrombin restoration. The dosage of 4 mg per kg of body weight approximates the original dose employed clinically in dicumarol therapy, though a total of 4 such doses is seldom utilized. In order to achieve maximal effects, 2 doses of 14 mg per kg were given to 5 of the dogs.

The prothrombin response to dicumarol in all dogs studied followed a pattern similar to that shown in Fig. 1, with the maximal reduction varying from 6% to less than 1% of normal. Both dosage schedules resulted in marked reductions of prothrombin. The smaller doses given over a greater period of time usually produced a slightly longer de-

[6] Seegers, W. H., and Ware, A. G., Josiah Macy, Jr., Conference on Blood Clotting and Allied Problems, N. Y., 1948.

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pression of Ac-globulin.

2. *Human Patients.* Preliminary investigations were carried out on 8 patients to whom dicumarol was administered after the occurrence of intravascular thromboses. Prothrombin values were reduced under this therapeutic regime and maintained at the desired low levels. Whenever possible, samples for analysis were obtained from the patients before dicumarol therapy was instituted. The prothrombin and Ac-globulin levels were followed as systematically as possible in each case, usually at 2- or 3-day intervals.

These studies indicate that in the human being as well as in the experimental animal the Ac-globulin level is lowered following the first doses of dicumarol. The concentration may be reduced to half of that normally found in human beings. Some individual variation was observed. As the prothrombin concentration was brought to the desired level of about 20% and the dicumarol doses reduced to maintain it so, the Ac-globulin titer returned to normal values. This restoration was found to be complete after about 3 weeks of therapy. Even when followed into the fourth week of dicumarol administration none of these patients showed a concentration of Ac-globulin above normal. The prothrombin and Ac-globulin levels of a patient, recorded in Table I, are representative of our general experience.

In addition to those patients followed from a time prior to or early in therapy, plasma samples were also analyzed from 12 out-patients who had received dicumarol for periods ranging from 1 to 14 months. Prothrombin levels had been reduced as rapidly as was safely possible and for the most part were maintained within a range of between 15 and 30% of normal. These patients who had received dicumarol for long periods of time showed no appreciable variation in Ac-globulin from normal levels.

TABLE I.
Plasma Ac-globulin and Prothrombin Levels in a Human Patient Receiving Continuous Dicumarol Therapy.

Days following initial administration of drug	Prothrombin % of normal	Ac-globulin % of normal
1	77	—
2	34	49
3	56	52
5	35	49
7	24	68
9	20	59
12	21	64
14	26	67
16	26	89

Discussion. These observations in general agree with those of Owen and Bollman[8] who report on the effect of dicumarol in dogs. However, those authors do suggest a disappearance of the accelerator in the early stages of dicumarolization whereas no such marked alteration was found by us. The difference between the marked fall in accelerator indicated by their work and the relatively small decreases in Ac-globulin reported in this present study, even though higher dicumarol doses were utilized, may possibly be attributed to the methods of analysis used. The test developed in this laboratory is believed to be more sensitive to Ac-globulin and less sensitive to other factors in the plasma than is the method utilized by Owen and Bollman. The period of increased convertibility noted in their work in the recovery stages agrees with our observations which indicate that Ac-globulin concentrations in the canine species may reach 150% of the normal during that time.

Study of the Ac-globulin level in dogs is beset with certain problems which unless controlled may lead to erroneous results. The blood of a normal dog when removed from the vein clots rapidly. The Lee-White time of dog blood is 3-5 minutes compared to 6-9 minutes for normal human blood. If incipient initiation of the clotting process occurs in the sample to be tested the small amount of thrombin formed will partially convert the plasma Ac-globulin to the active form, serum Ac-globulin.[9] Experience has shown that this effect is easier to avoid when citrate is used as anticoagulant. The presence of a small amount of serum Ac-globulin will indicate an apparent but false increase in the plasma Ac-

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[9] Ware, A. G., and Seegers, W. H., *Am. J. Physiol.*, 1948, **152**, 567.

globulin present.[9] On the other hand, if a considerable amount of thrombin is released prior to the effect of the anticoagulant it may actually destroy part of the Ac-globulin. To guard against these difficulties the procedure described in this paper for drawing blood was followed carefully. Sodium citrate is also preferable to oxalate as an anticoagulant because Ac-globulin is more stable in stored citrated dog plasma than in oxalated plasma.

It is certainly possible, or even probable, that the effect of dicumarol on the coagulation mechanism is not restricted to prothrombin and Ac-globulin alone. Conflicting reports[10,11] have been presented as to the susceptibility of fibrinogen to dicumarol and a variation in serum antithrombin titer has also been proposed.[12] From the observations reported here it is seen that dicumarol has, in addition to its paramount function of lowering the prothrombin concentration, a secondary effect upon the coagulation mechanism through its reduction of Ac-globulin early in the period of dicumarol therapy. The definite drop in Ac-globulin which occurs following initiation of therapy should contribute at that time to the prevention of clot formation.

Summary. In human patients receiving dicumarol the plasma Ac-globulin level may be depressed by 20-50% following initiation of therapy. Individual variation was noteworthy. A gradual return to normal concentrations of Ac-globulin occurs within 3 weeks as therapy is continued and the prothrombin is maintained at a low titer. No appreciable difference from normal was found in the Ac-globulin values of patients who had been on dicumarol therapy for 1 to 14 months.

Dogs receiving larger dicumarol doses than were administered to human patients showed a similar Ac-globulin response and a more marked reduction in prothrombin. A period of slightly lowered Ac-globulin activity in the dog is followed by a temporary rise to levels above normal.

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Indoleacetic Acid Studies in Man.*

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Corn products have usually been present in diets which have been associated with the etiology of pellagra in man,[1] blacktongue in dogs,[2] and nicotinic acid deficiency in rats.[3] It has been shown that the role of corn in the development of these deficiency syndromes is not due only to the low nicotinic acid content of the corn.[2,4,5] Other factors may be

* A preliminary report was presented at the meeting of the American Society of Biological Chemists, March, 1947. (*Fed. Proc.*, 1947, **6**, 288).

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