lems is beyond the scope of the present inquiry. The same is true of the explanation for the evident interference in thrombin formation by our weak HF solutions and the only conclusion, for the present, is that "antihemophilic globulin" lacks the accelerator effect.

We have shown elsewhere[12] that HF contains a protease precursor which, when activated by streptokinase, evinces proteolytic (*e.g.*, fibrinogenolytic, etc.) and "thromboplastic" (aiding thrombin formation) properties, similar to other plasma protease preparations. The negative clot-lysis tests included in Table II, A, show that, for reasons of dilution and lack of activation such protease plays no part in the present results.

AcG has been studied in several types of experiment with completely negative results as regards any indication that it may be related to the plasma protease[17] system, whether as active enzyme or precursor, or as activator or inhibitor of added precursor, streptokinase, or active protease. It is an important point, therefore, that the aid which the "accelerator globulin" affords to thrombin formation has no demonstrable relation to the plasma protease system (*cf.* [1]).

It is also of interest to note that AcG has no significant "antihemophilic" activity, which, of course, is to be expected from the normal prothrombin clotting times obtained in hemophilia, indicating that hemophilic blood has adequate amounts both of prothrombin and the accelerator factor.

*Conclusions.* 1. There is a new factor, or group of factors, (Quick's "labile factor," Owren's "factor V," Ware, Guest and Seegers' "accelerator globulin"), to be recognized in the first phase of the blood-clotting system. At present it may be considered an "accessory" activator distinct from calcium, thromboplastin, and thromboplastic enzyme. 2. This factor is found abundantly in fresh plasma and prothrombin (purified by the usual methods, *e. g.* [13]), but deteriorates on "aging," independently of the prothrombin. High dilution may be necessary to demonstrate this. The addition of fresh AcG will restore the original potency of the prothrombin, but aged AcG loses this property. 3. AcG is not "antihemophilic" and so-called "antihemophilic globulin" is not able to restore the activity of aged prothrombin. 4. "Owren's disease,"[18] a specific hemorrhagic disorder associated with a plasma deficiency of this new factor, is, in all probability, a true clinical entity, which suitable tests can differentiate from "idiopathic hypoprothrombinemia," hemophilia, and other bleeding diseases.

[17] Ferguson, J. H., *Science*, 1943, **97**, 319; 1947, **105**, 488.

[18] Owren, P. A., *Lancet*, 1947, **252**, 446.

## 16262

## Prothrombin Conversion Factor of Dicumarol Plasma.*

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Dicumarol *in vivo* is generally believed to cause a deficiency of plasma prothrombin. The therapeutic value of serum[1,2] in the treatment of cattle with "hemorrhagic sweet clover disease," a dicumarol-induced diathesis is therefore puzzling.

An investigation was conducted on dogs to which dicumarol was administered orally or

* Abridgment of a portion of the thesis submitted by Dr. Owen to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Ph.D. in Medicine.

[1] Schofield, F. W., *J. Am. Vet. M. A.*, 1924, **64**, 553.

[2] Roderick, L. M., *Am. J. Physiol.*, 1931, **96**, 413.

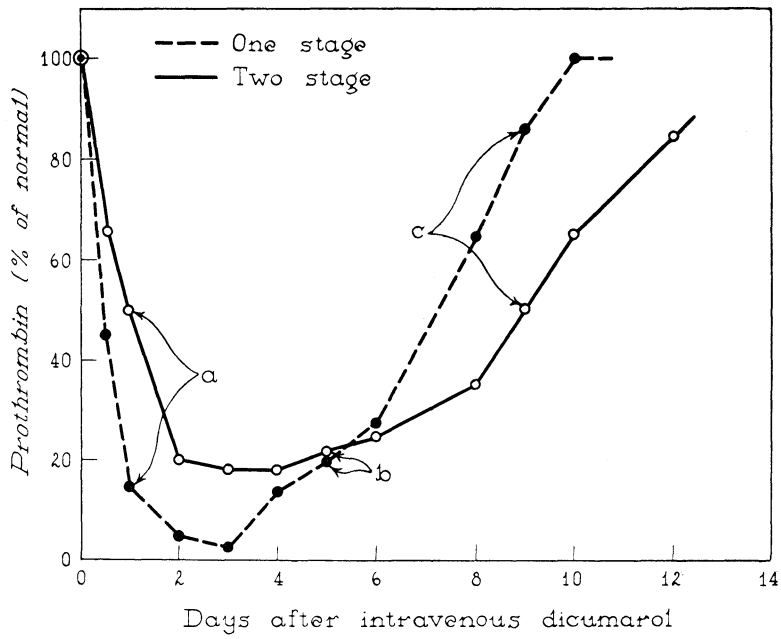


FIG. 1.

Changes of the prothrombin level as determined by the one-stage and the 2-stage methods after intravenous administration of dicumarol. For explanation of *a*, *b*, and *c* see text.

intravenously. Prothrombin determinations were performed by the one-stage test of Quick[3] and the 2-stage test of Warner, Brinkhous, and Smith.[4]

To increase the sensitivity of the Quick test, plasmas were studied at a number of dilutions, 0.3% fibrinogen being used as diluent; percentages were interpolated from the dilution curves of control samples.

In the 2-stage test, plasma is freed of fibrinogen by the addition of weak thrombin, which in turn is rapidly inactivated. The defibrinated plasma is diluted; calcium and thromboplastin are added and portions of the solution are mixed with fibrinogen, the clotting times being noted. Under standard conditions a coagulation time of 15 seconds denotes 1 unit of thrombin per milliliter, which is presumed to have arisen from 1 unit of prothrombin per milliliter. Since normal dog plasma, diluted about 300-fold, yields 1 unit of thrombin per milliliter, the undiluted plasma is said to contain about 300 units of prothrombin per milliliter. By determination of the concentration of thrombin at frequent intervals during its evolution not only the amount of thrombin formed but also the time for its formation are measurable.

As expected, the prothrombin level dropped after dicumarolization—the extent of the fall and its duration varying with the dose.[5] Fig. 1 shows the characteristic course in a female dog, weighing 8.5 kg, given 10 mg of dicumarol per kilo of body weight, by the intravenous route.

According to the Quick test the prothrombin fell more rapidly, to lower levels, but returned to normal sooner than by the 2-stage test. Thus, a sample of blood taken 24 hours after the drug had been administered (*a*) showed 50% prothrombin by the 2-stage test and 15% by the one-stage test. On the fifth day (*b*) the two methods were in close agreement. However, on the ninth day (*c*), with

[3] Quick, A. J., Stanley-Brown, Margaret, and Bancroft, F. W., *Am. J. M. Sc.*, 1935, **190**, 501.

[4] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

[5] Bollman, J. L., and Preston, F. W., *J. A. M. A.*, 1942, **120**, 1021.

the level determined by the 2-stage test at 50% again, the prothrombin as determined by Quick's method had returned to almost normal levels.

Repetition of this experiment invariably yielded similar results. If smaller doses of dicumarol were employed, a point was reached (1 mg per kilo of body weight) at which the prothrombin, as measured by the 2-stage test, fell very little (to not less than 80%) while by the Quick test it dropped to a 40 or 50% level.

Study of the rate of conversion of prothrombin to thrombin seemed to indicate the cause of the discrepancy between the two methods. "Conversion times" represent the time required for conversion of 80% of the prothrombin into thrombin after addition of calcium and thromboplastin to plasma diluted to a prothrombin concentration of 1 unit per milliliter. It was found that when the Quick test yielded lower results than the 2-stage test, conversion of the prothrombin was delayed; when the tests agreed, conversion rates were normal; or, when the one-stage results were higher, conversion rates were accelerated above normal. Thus, in Fig. 1, at the predicumarol level (100%) conversion of 1 unit of prothrombin was 80% complete in 30 seconds; at (*a*) in 80 seconds; at (*b*) in 30 seconds; at (*c*) in 20 seconds.

In an attempt to discover the cause of the variable conversion rates of prothrombin it was noted that mixture of as little as one part of normal plasma with nine parts of slowly converting "dicumarol plasma" completely corrected the hypoconvertibility (from 80 seconds to 30 seconds) and likewise closed the gap between the one-stage and 2-stage tests. For example, at point (*a*) in Fig. 1, when 9 parts of the "dicumarol plasma" were mixed with one part of control plasma, the 2-stage test now read 52% (theoretically 55%); a Quick test on this mixture yielded 55% instead of the expected 24%. Further, addition of normal serum, free of prothrombin, thrombin, and fibrinogen, was equally corrective. Thus, some factor would seem to be present in normal serum, as well as in plasma, which can accelerate delayed convertibility.

Ammonium sulfate fractionation of normal dog serum revealed traces of the "conversion factor" in 25% and 33% saturation fractions, with the highest concentration at about 40 or 50% saturation. Dilution of serum and acidification to pH 5.3, followed by exposure to magnesium hydroxide, revealed little adsorption unless a great excess of the insoluble hydroxide was used. The factor, like prothrombin, is associated with the pseudoglobulins, but differs from prothrombin by being poorly adsorbed by magnesium hydroxide. Its deterioration at extremes of pH (5.5, 10.0), with moderate heating (50°C), and on exposure to air is much greater than that of prothrombin.

An analysis of dog plasma kept at 4°C for several weeks confirmed the findings reported in human and ox plasma;[6] that is, a rapid fall of prothrombin as measured by Quick's method, a slower fall as measured by the 2-stage test. Study of the rate of conversion of prothrombin of dog plasma kept at 4°C was made at intervals for a period of more than 60 days. Progressive hypoconvertibility was found to be the cause of the discrepancy between the methods. Ware, Guest and Seegers[6] have attributed the disparity to deterioration of fibrinogen. However, their data show incomplete correction when the fibrinogen of the plasma was replenished. While the fibrinogen must be adequate for performance of the Quick test, we find that correction of delayed conversion and of the difference between the prothrombin methods is possible only on addition of the conversion factor—such as in plasma, serum, or their pseudoglobulins.

There is a close resemblance between this conversion factor and Owren's factor V,[7] present in Seitz-filtered plasma, or the activators of Fantl and Nance[8] and of Ware, Guest, and Seegers,[9] as well as Quick's "pro-

[6] Ware, A. G., Guest, M. M., and Seegers, W. H., *Am. J. Physiol.*, 1947, **150**, 58.

[7] Owren, P. A., *Acta med. Scandinav.*, 1947 (suppl.), **194**, 327 pp.

[8] Fantl, P., and Nance, M., *Nature*, London, 1946, **158**, 708.

[9] Ware, A. G., Guest, M. M., and Seegers, W. H., *J. Biol. Chem.*, 1947, **169**, 231.

thrombin A."[10] The presence of the factor in serum might explain Schofield's[1] and Roderick's[2] therapeutic findings—findings difficult to understand if deterioration of prothrombin alone were present in cases of dicumarol or toxic sweet clover poisoning.

*Summary.* Evidence is presented that suggests the disappearance not only of prothrombin in dogs treated with dicumarol, but also of a factor important in the conversion of prothrombin to thrombin. Like prothrombin, this factor is associated with the plasma pseudoglobulins, but unlike prothrombin it is plentiful in serum. Addition of plasma, serum or serum pseudoglobulin corrects the delayed prothrombin convertibility of early dicumarolization or of stored plasma. The discrepancy between the one-stage and 2-stage methods of estimating prothrombin seems to reside in this conversion factor.

[10] Quick, A. J., *Am. J. Physiol.*, 1943, **140**, 212.

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## Immunological Studies of Newcastle Virus.

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A California strain of Newcastle virus, No. 11,914, which was adapted to the Syrian hamster,[1] proved valuable as an immunizing agent in chickens against the virulent unmodified virus of this strain.[2] This successful immunization prompted continued experiments to study the relationship of the living modified virus as an immunizing agent, not only against the California strain but also against a Colorado strain, No. 8518, and a Connecticut strain, secured from the Bureau of Animal Industry, Washington, D.C.

Healthy 6-week-old New Hampshire Red chickens were used, and all birds were identified by wing bands. Serum from these chickens showed no neutralizing antibodies for Newcastle disease when tested by the chick embryo neutralization test prior to the start of the experiment. Three hundred fifty-two chickens were vaccinated by injecting 0.2 cc of a 10% suspension of virus-bearing hamster brain into the base of the wattle. Forty infected hamster brains of the eighty-second subculture were used for preparation of the vaccine, with physiological saline as the diluent. One hundred thirty-four unvaccinated chickens were placed with the vaccinated birds as room contact controls. All of the chickens were checked carefully each day. Table I shows the observations during the 28-day period prior to challenge.

Three groups were then set up, each consisting of vaccinated birds, unvaccinated room control and normal susceptible birds of corresponding age. Group I was challenged with the virulent chick embryo propagated California strain, No. 11,914, subculture 18. Group II was challenged with the virulent chick embryo propagated Colorado strain, No. 8518, subculture 8. Group III was challenged with the virulent chick embryo propagated Connecticut strain, subculture 8. The results obtained after challenge are shown in

TABLE I.
Evaluation of Chicken Deaths Following Vaccination, Prior to Challenge.

| | Vaccinated (352) | Room controls (134) |
|---|---|---|
| Coccidiosis | 10 | 1 |
| Newcastle | 1 | 0 |
| Smothered | 1 | 2 |
| Miscellaneous | 15 | 2 |

[1] Reagan, Reginald, L., Lillie, Mary G., Poelma, Leo J., and Brueckner, A. L., *Am. J. Vet. Res.*, 1947, **8**, 136.

[2] Reagan, Reginald, L., Lillie, Mary, G., Poelma, Leo J., and Brueckner, A. L., to be published.