the usual variables in bioassays by administering each form of the hormone by continuous injection. Nothing is known of the relative rates of absorption of the two forms of ACTH from the subcutaneous tissues into the blood and of their removal from the blood into the cells of the target organ nor is anything known about the relative proportions of the two forms of ACTH which are inactivated or otherwise wasted before they reach their target organ.

The results of these experiments support the conclusion of Reinhardt and Li(1) that the bioassay of ACTH by the ascorbic acid depletion test does not always correlate with other signs of adrenal cortex response.

Summary. A comparison was made of some of the metabolic and morphologic effects of ACTH protein and ACTH peptide given by continuous subcutaneous injection to normal (300 g) male rats for a period of 10 days. The signs of hypercorticalism were adrenal hyperplasia, thymus atrophy, loss in body-weight, rise in urinary NPN, glycosuria and pathologic changes in the stomach and kidneys. ACTH protein had about twice the biologic activity of ACTH peptide as measured by the above criteria, but was significantly less active than the peptide in causing depletion of adrenal ascorbic acid.

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Is Hypoprothrombinemia Caused by Deficiency of Vitamin K Different from that of Dicumarol?* (19296)

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It is generally accepted that vit. K deficiency causes a true hypoprothrombinemia, and until recently it was believed that Dicumarol likewise reduced only the prothrombin level of blood. One of us(1) obtained findings which were not in accord with this and postulated that the concentration of a factor other than prothrombin might be affected by Dicumarol. This was promptly questioned by Dam(2). Recently, however, he and his associates(3) have postulated that while a decrease of prothrombin occurs in both vit. K deficiency and after Dicumarol, an additional factor is diminished in the first condition, and a second hypothetical agent is decreased after giving Dicumarol. Other workers, likewise, have reached somewhat similar conclusions. MacMillan(4) postulated a decrease of an accelerating factor, while Lein and Lein(5) concluded that Dicumarol causes the formation of an altered prothrombin. Mann and his coworkers(6) recently reported findings which they interpret to mean that Dicumarol decreases a factor, designated "cothromboplastin," much more than prothrombin, while the reverse occurs in vit. K deficiency.

Obviously, there is a need to reinvestigate the problem of whether the hypoprothrombinemia of Dicumarol is essentially different from that of avitaminosis K. Dogs are especially well suited for this study since we(7)

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have shown that in this animal all of the prothrombin is in the active state, thus avoiding the complication of dealing with two types of prothrombin. Vit. K deficiency can readily be produced by a cholecystnephrostomy operation, since the resulting lack of bile in the intestine prevents the absorption of vit. K. That a true hypoprothrombinemia occurs is attested by the prompt restoration of the prothrombin level of the blood when such an animal is given vit. K(8).

Methods. The one-stage prothrombin test was employed. The prothrombin time of normal dog plasma was consistently 6 seconds. Dicumarol was given orally. A dose of 5 mg per kilo of body weight was administered daily until the prothrombin decreased to the desired level. The cholecystnephrostomy operation was done as described by Kapsinow, Engle, and Harvey(9). A dog with this internal biliary fistula remains remarkably well, provided vit. A is given parenterally and the animal is guarded against excessive hypoprothrombinemia. One dog has survived the operation for almost 2 years and has remained in an excellent state of nutrition.

Results. A simple means to determine whether the hypoprothrombinemia of vit. K deficiency is different from that induced by Dicumarol, is to mix the plasmas of both types and compare the prothrombin time of the mixture with that of the two plasmas. When plasma from a Dicumarolized dog having a prothrombin time of 20 seconds is mixed with an equal volume of plasma from a dog deprived of vit. K with a prothrombin time of 31 seconds, the mixed plasma had a prothrombin time of 22 seconds (Table I). The same value was obtained when the first plasma was mixed with a plasma from a Dicumarolized dog with a prothrombin time of 29½ seconds. On mixing the plasma having a prothrombin time of 31 seconds with the plasma having 29½ seconds, the resulting mixture had a prothrombin time of 30 seconds, even though one plasma was from a vit. K deficient dog while the other was from a dog that had received Dicumarol.

These data strongly suggest that Dicumarol, like vit. K deficiency, does not significantly affect any clotting factor other than prothrombin. This is in accordance with our recent studies(11) in which it was shown that Dicumarol acts as an antivitamin K. Since the concentration of the labile factor is not affected by vit. K deficiency, it is to be expected that if Dicumarol acts solely as an antivit. K, it, likewise, will not reduce the level of this clotting agent. This has been shown to be the case experimentally by Quick and Stefanini(12). Recently, Sørbye(3), Owen (13), and their associates have likewise concluded that the labile factor is not decreased by Dicumarol. It is unlikely that other hypothetical factors account for the prolonged prothrombin time resulting from either Dicumarol or lack of vit. K. It seems clear that in the dog the increase in the prothrombin time in both conditions is due to an actual decrease of prothrombin. It is likely that Dicumarol reduces only prothrombin in other higher mammals; but in man, for instance, the problem is complicated, since there is evidence that prothrombin occurs not only in the active form, but also in a precursor state(7,14,15). Preliminary studies indicate that Dicumarol reduces both the free and total prothrombin(16).

TABLE I. Effect of Mixing Plasmas of Dogs Given Dicumarol with Plasma from an Animal Deficient in Vitamin K.

Plasma of dog	Prothrombin time in sec — Observed	Calculated*
A (Dicumarol)	20	
B "	29½	
M (Vit. K deficiency)	31	
A 1 vol. M 1	22	23.5
A 1 B 1	22½	23.7
B 1 M 1	30	30

* On the basis of the standard prothrombin curve(10).

Summary. On mixing equal volumes of plasma obtained from a dog deprived of vit. K and plasma from a dog receiving Dicumarol, the mixture has a prothrombin time that corresponds to the expected average based on the standard prothrombin time curve. It is concluded that in the dog the increased prothrombin time in both avitaminosis

K and after Dicumarol is due to a decrease of prothrombin.

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Effect of Certain Enzyme Inhibitors on Hemolytic and Hemagglutinating Activity of Mumps Virus.* (19297)

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The hemolysin of mumps virus is a labile property with many of the characteristics of an enzyme(1-3). Studies of the effects of certain enzyme inhibitors on the hemolysin were undertaken to obtain further indications of its probable nature and of its similarities to various types of known enzymes. The action of these inhibitors on the hemagglutinating capacity of the virus was tested simultaneously to examine the relationships of the hemagglutinin and the hemolysin or their separate natures.

Materials and methods. A strain of egg-adapted mumps virus was used. 0.1 ml of dilutions of infected egg fluids were injected into the allantoic or amniotic sacs of 6-8-day-old, white, embryonated eggs. The eggs were incubated at 35°C and the infected fluids were harvested 4-6 days later. Allantoic fluids stored at −40°C were dialyzed for 24 hours at 4°C with phosphate buffered saline (pH 7.0-7.2) before use in these experiments. Tests for hemolytic and hemagglutinating activities of the virus were carried out with the methods previously described(2). The enzyme inhibitors were dissolved in phosphate-buffered saline and the pH was adjusted, as necessary, to 7.0-7.2 before addition to the diluents employed in the hemolysin and hemagglutinin titrations.

Results. Urethane(4-7) is a known inhibitor of a variety of enzymes, including several blood esterases. Its effect on the mumps hemolysin is shown in Table I. The inhibition of hemolysis noted with urethane is more marked with the higher concentrations. Using 10% hemolysis as the lowest significant degree of hemolytic activity, 0.1 M urethane decreases the hemolytic titer about 16 times. The 2-fold decrease in the hemagglutinin titer is not significant.

A variety of enzymes, including catalase, are inhibited by hydroxylamine(4-6). The effect of this substance on the hemolytic agent of mumps is shown in a typical experiment in Table I. Hydroxylamine, in a concentration of 0.01 M, was markedly inhibitory for the

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