

ASCORBIC ACID AND BLOOD COAGULATION*

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The hemorrhagic symptoms of scurvy have been known for centuries. In the 20th century, the isolation, synthesis, and development of quantitative methods of determination of ascorbic acid have stimulated a large number of workers to correlate the various symptoms that make up the nutritional disease, scurvy, with the *in vivo* fate of this vitamin. In spite of these studies the basic mechanisms responsible for the vascular fragility and hemorrhagic symptoms of scurvy remain unclear.

Our task is to review the possible influence of ascorbic acid on the clotting mechanism, which in turn may significantly affect a fundamental lesion of scurvy, that is, loss of vascular integrity. A recent review article, "Scurvy and Blood Coagulation,"¹ summarizes the literature in this field to date. Rather than repeat here the material covered in that report, we shall concern ourselves with the following questions: (1) May some of the hemorrhagic and coagulation phenomena seen in clinical and experimental scurvy relate to nutritional factors other than ascorbic acid? (2) What is the significance of altered sensitivity of scorbutic animals to drugs that influence coagulation? (3) What is the effect of stimulating the metabolic pathway of ascorbic acid synthesis on the clotting mechanism? (4) Can vascular integrity and coagulation factors be independently altered?

Soon after Link and his co-workers^{2,2a} isolated Dicumarol, the cause of the hypoprothrombinemia associated with hemorrhagic sweet clover disease of cattle, they undertook a study of the role of ascorbic acid in Dicumarol-induced hypoprothrombinemia.²⁻⁴ This was probably prompted by the similarity between the hemorrhagic diathesis of scurvy and that of hypoprothrombinemia. Their studies show that in certain species ascorbic acid, like vitamin K, can antagonize the action of Dicumarol and that in scurvy the hypoprothrombinemic effect of Dicumarol is enhanced. These observations are of particular interest since vitamin K deficiency itself results in frank hypoprothrombinemia while vitamin C deficiency does not.

It is known that some scorbutogenic diets supplemented with ascorbic acid do not produce perfectly normal guinea pigs. Constable⁵ has shown that the anemia seen with some scorbutogenic diets and attributed to ascorbic acid deficiency, is not seen with a more complete but still scorbutogenic diet. Could the observations of Link *et al.* be due in part to the nonspecific effects of scurvy, such as starvation secondary to loss of appetite,⁶ or to the diet he employed? To test these possibilities under standard conditions we studied,⁷ in collaboration with Chenkin and Weisberg the effect of starvation on coumarin-induced hypoprothrombinemia in guinea pigs. Acenocoumarin was used in these ex-

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periments because this drug, unlike Dicumarol, could be readily dissolved at neutral pH and given intraperitoneally, thus avoiding variations in absorption. No clotting abnormality was found in acutely starved (but ascorbic acid-supplemented) guinea pigs. Paired animals, treated in addition with acenocoumarin, showed an exaggerated hypoprothrombinemic response as compared to normally fed, acenocoumarin-treated guinea pigs. There was also suggestive evidence of the presence of a circulating heparinlike clotting inhibitor in the starved group.

The possibility that drug metabolism may be slower in starved animals than in normal animals, leading to an exaggerated drug action, has been pointed out by Dixon *et al.*,⁸ who attributed this effect to changes in liver microsomes. Conney reports elsewhere in this monograph that in mild scurvy, zoxazolamine is metabolized more slowly than in control guinea pigs. In the case of acenocoumarin we did not find differences in metabolic rate in starved guinea pigs as compared with fed guinea pigs. Ninety minutes after administration of 30 mg./kg. of acenocoumarin intraperitoneally, plasma concentrations of the drug ranged from 30 to 50 mg./l. in both groups. Thus an altered rate of coumarin metabolism in starvation appears not to be the explanation for the enhanced response to coumarin in the guinea pigs.

The capacity of some drugs to stimulate biosynthesis of ascorbic acid was suggested as early as 1940.⁹ Link *et al.*,¹⁰ aware of these reports, studied the effects of vitamin C-stimulating drugs on the prothrombin response to Dicumarol. Some "vitamin C stimulators," for example, Chloretone, antagonized the action of Dicumarol in the rat. Dicumarol itself had only a transient effect on ascorbic acid excretion. It was found^{10,11} that the "stimulators" did not act in the same manner as vitamin K. However, in the rat, in spite of the effectiveness of the stimulators, large doses of ascorbic acid did not antagonize the action of Dicumarol. In contrast, as stated before, large doses of ascorbic acid did antagonize Dicumarol in the rabbit and guinea pig. Because of this apparent paradox, Link and his co-workers¹⁰ suggested that the effects of the stimulators were related to the biosynthesis of ascorbic acid rather than to ascorbic acid itself. Today there is additional evidence that this conclusion was correct.¹²

Elsewhere in this monograph are presented details of the glucuronic acid pathway of glucose metabolism and its relationship to the biosynthesis of ascorbic acid. In effect, the vitamin C stimulators are better described as "glucuronic acid pathway stimulators." Often these stimulators induce an associated increase in rate of metabolism of a variety of drugs, sometimes including the stimulator itself. Although certain species (particularly man, monkey, and guinea pig) cannot synthesize ascorbic acid from gulonic acid,¹²⁻¹⁴ these species have the ability to perform the other steps of the glucuronic acid pathway. That the pathway can be stimulated in man and guinea pig has been demonstrated by isotopic and other experiments.¹²⁻¹⁴ Thus one may expect pretreatment with glucuronic acid pathway stimulators to affect the response to drugs such as the coumarins even in man and guinea pig. It was therefore of interest to us that Avellaneda¹⁵ had found that barbiturates (which are stimulators) antagonize the action of a coumarin (biscoumacetate) in man. Barbiturates themselves have no appreciable effect on coagulation.¹⁶

To test further whether glucuronic acid pathway stimulators would influence coumarin-induced hypoprothrombinemia in species unable to synthesize ascorbic acid, we chose the guinea pig¹⁷ as an experimental animal. We found that pretreatment with a stimulator (barbital) completely antagonized the action of acenocoumarin. We have also confirmed and extended similar observations in man. In human subjects the route of administration proved to be an important variable. We also investigated other known "stimulators"^{9,12} such as aminopyrine and derivatives for their ability to antagonize coumarins. Aminopyrine and particularly 4-aminoantipyrine affected the response to coumarins in quite a different manner than barbital.¹⁸ 4-Aminoantipyrine (a metabolite of aminopyrine¹⁹) induced a "hyperprothrombinemia," in untreated animals and a moderately reduced response in coumarin-treated animals. Our studies suggest that the normal guinea pig has less proconvertin activity (a component of the prothrombin complex) than other species and that this activity is increased by 4-aminoantipyrine and several other drugs. It is of interest that Link and his co-workers had also observed shortened prothrombin time after administration of caffeine and other xanthines^{2,2a,20,21} to dogs, rats, and rabbits. We have confirmed these results. Link and his associates attributed the xanthine-induced increase in coagulability to a concomitant increase of fibrinogen. They also reported the production of hyperprothrombinemia with vitamin K²² in dogs, rats, and rabbits. We were unable to produce hyperprothrombinemia in the guinea pig⁷ with vitamin K.

Recently Soviet workers observed that large doses of ascorbic acid resulted in a transient and slight "shortened coagulation time" in humans and rabbits.²³ Whether this is related to the ascorbic acid antagonism of coumarins as noted above is not known.

Our further studies in guinea pigs had then revealed that not all glucuronic acid pathway stimulators will block the prothrombin response to coumarins. Thus while barbiturates and Chloretone were effective, 3-methylcholanthrene by the same technique did not block response to coumarins. In fact 3-methylcholanthrene (administered intraperitoneally in oil) apparently causes release of a heparinlike substance, thus accentuating the effect of the coumarin. As noted previously, 4-aminoantipyrine also acts differently from barbital since 4-aminoantipyrine actually increased prothrombin activity (that is, shortened prothrombin time) in control animals. This latter effect is more reminiscent of the xanthine effect than it is of the glucuronic acid pathway stimulators.

From the preceding it is clear that alteration in a nutritional factor, such as ascorbic acid or its biosynthetic mechanism, may influence clotting factors in many different ways. Even when altered coagulability was not found, the possibility remained that a coagulation factor change at the tissue or cellular level was influencing vascular integrity.

Clotting abnormality is not generally described as an integral part of the syndrome of scurvy. Yet there are scattered reports that ascorbic acid shortens clotting time in man and rabbit,²³ and a number of studies¹ show that vitamin C deficiency can cause changes in platelets, prothrombin complex, and thromboplastin complex.^{24,25} On the other hand, hemorrhage into the extravascular spaces of any etiology may initiate a chain of reactions involving clotting fac-

tors, resulting in an alteration in the coagulation properties of circulating blood. This intimate relationship between the clotting mechanism and vascular integrity makes it very difficult to determine definitely whether the clotting system is a mediator of scorbutic hemorrhage.²⁶

In conclusion, the direct effects of ascorbic acid deficiency and those effects that are secondary to the deficiency or the diet limitations imposed in order to produce the deficiency must be differentiated. Reaction to certain drugs and the fate of the drugs themselves are influenced by the factors that influence the fate and concentration of ascorbic acid in the animal. Conversely, certain drugs can influence pathways leading to ascorbic acid. These effects are frequently reflected in the response of the animal to drugs that influence coagulation. The extent to which vascular integrity is related to altered coagulability remains undetermined.

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