Hemorrhagic Sweet Clover Disease, Dicumarol, and Warfarin: the Work of Karl Paul Link

Studies on the Hemorrhagic Sweet Clover Disease. IV. The Isolation and Crystallization of the Hemorrhagic Agent

Studies on the Hemorrhagic Sweet Clover Disease. V. Identification and Synthesis of the Hemorrhagic Agent

Studies on the Hemorrhagic Sweet Clover Disease. XIII. Anticoagulant Activity and Structure in the 4-Hydroxycoumarin Group

Karl Paul Link (1901–1978) received his Ph.D. in 1925 from the University of Wisconsin, working with plant biochemist William E. Tottingham. He spent the next 2 years in Europe studying carbohydrate chemistry with Sir James Irvine in Scotland, microchemistry with Fritz Pregl in Austria, and organic chemistry with Paul Karrer in Switzerland. He returned to the University of Wisconsin as an assistant professor in agricultural chemistry (now biochemistry) in 1927 and was promoted to associate professor in 1928.

Initially when he set up his laboratory, Link concentrated on plant carbohydrates and soon established himself as one of the outstanding carbohydrate chemists of his day. Using the microchemical techniques he learned with Pregl, he and his students were able to characterize carbohydrate derivatives that they had isolated and synthesized.

However, the direction of Link’s research changed drastically when he became involved in the isolation and characterization of the hemorrhagic factor produced in spoiled sweet clover hay. These experiments are the subject of the three *Journal of Biological Chemistry* (JBC) Classics reprinted here. Sweet clover was widely used as hay in the 1920s when a series of wet summers had led to an epidemic of “bleeding disease” in cattle. The cause of the disease was traced to sweet clover hay that had been improperly cured and infected with molds. There was also evidence that the defective coagulation in the cows was due to a deficiency in prothrombin.

Link became interested in the sweet clover problem in 1933 when a farmer came to his laboratory with about 100 lbs of spoiled sweet clover and blood from a cow that had died from hemorrhaging after eating the spoiled hay. Realizing that the farmer's dying cattle represented a huge loss in the depths of the great depression, Link and his students set out to isolate and characterize the hemorrhagic agent from the spoiled hay. It ended up taking 5 years for Link’s student Harold A. Campbell to recover 6 mg of crystalline anticoagulant. In the first JBC Classic reprinted here Campbell presents his isolation and crystallization of the hemorrhagic agent. To follow the progression of the fractionation he developed an assay in which he fed his concentrates to rabbits and tested their blood for changes in prothrombin levels (1). From his experiments, Campbell concluded that the hemorrhagic agent had the formula C₁₉H₁₂O₆ and that it represented a product that had never before been found in nature.

Next, Link’s student Mark A. Stahmann took over the project and initiated a large scale extraction of spoiled sweet clover hay, which is the subject of the second JBC Classic. In about 4 months he was able to isolate 1.8 g of recrystallized anticoagulant. This was enough material
for Stahmann and Charles F. Huebner to check their results against Campbell’s and to thoroughly characterize the compound. Through degradation experiments they established that the anticoagulant was 3,3'-methylenebis-(4-hydroxycoumarin), which they later named dicumarol. They confirmed their results by synthesizing dicumarol and proving that it was identical to the naturally occurring product.

Several years after the discovery of dicumarol, others showed that a deficiency of vitamin K (“Koagulations-Vitamin”) in experimental animals led to a severe tendency to bleed. Subsequently, it was shown that vitamin K-deficient animals were also hyperprothrombinemic. The structure of vitamin K was established in 1939, and Link soon recognized that dicumarol and vitamin K had similar structures. Administration of dicumarol inhibits the formation of normal amounts of prothrombin, and vitamin K counters its action. The exact biochemical basis for these actions has only been established more recently. After testing dicumarol on animals, Link collaborated with clinicians at the Wisconsin General Hospital and the Mayo Clinic to test the compound’s ability to control clotting in human patients. Dicumarol was eventually released into clinical medicine in 1941, and it has enjoyed widespread use as an anticoagulant ever since, gaining particular fame after it was used to treat President Eisenhower after his heart attack in 1955.

Link and his colleagues synthesized over 100 analogues of dicumarol and tested many of them for anticoagulant activity. In the final JBC Classic reprinted here Link reports on the anticoagulant activity of 106 synthetic compounds. Although none of the compounds screened in these experiments were more potent than dicumarol, Link would eventually discover that another analogue was many times more potent than dicumarol in animal tests. Link judged the compound to be so toxic that he didn’t bother to patent it. Stahmann, on the other hand, believed it had potential and wrote a patent with the aid of an attorney at the Wisconsin Alumni Research Foundation (WARF). The compound, which was later named warfarin (after WARF), became widely used as a rodenticide and a therapeutic agent.

Link was honored for his scientific accomplishments with his election to the National Academy of Sciences in 1946, and was awarded the Kovalenko medal of the Academy in 1967. He also won the Cameron Award in 1952 from the University of Edinburgh, the John Scott medal in 1959 from the city of Philadelphia, the Lasker Award in 1955 from the American Public Health Association, and the Lasker Award in 1960 from the American Heart Association.

Link was valued not only by the scientific community but also by the students at the University of Wisconsin. As well as being a brilliant lecturer he could always be counted on to back the students’ position in any issue with staff or administration. He eventually established a legal defense fund for students in trouble with the university or with the law because of their support of unpopular causes. Link was also a great liberal and defender of unpopular causes, serving as faculty sponsor for organizations such as the John Cookson Karl Marx Discussion Group and the Labor Youth League. But perhaps he was best known on campus for his “non-traditional” clothing including large bow ties, flannel shirts, work shoes, shorts, and sometimes even a cape and knickers.1

Nicole Kresge, Robert D. Simoni, and Robert L. Hill

REFERENCES

1 All biographical information on Karl Paul Link was taken from Refs. 2 and 3.
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