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Dicoumarin Synthesized

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THE study of coumarin derivatives began with the identification of the causative agent in sweet clover responsible for the hemorrhagic disease of cattle now called “sweet clover disease.” The investigation of this disease and the isolation of the etiological factor has established both the chemical and physiological properties of dicoumarin.

“Sweet clover poisoning” was first reported in the literature as early as 1888 when European workers reported a disease in cattle which were being fed sweet clover, but they did not recognize the sweet clover as the cause of the disease. The disease was then considered to be of bacterial origin. In 1912, two Frenchmen, Gain and Brocq-Roussei, noted deaths in cattle which had been fed sweet clover, but again they did not correlate the sweet clover and the death of the animals.

In 1923, a Canadian named Schofield reported the discovery of a new specific disease in cattle resulting from the feeding of spoiled sweet clover. Practically simultaneously, Roderick at North Dakota State College began investigating the same disease. The work of both of these investigators was concerned mainly with the diagnosis, treatment, and prevention of the disease. They recognized sweet clover as the etiological agent, but did not know how it acted. Roderick did considerable work on the gross and microscopic pathology, together with a study of the blood changes, in an attempt to find the cause.

Roderick and Schalk, in the North Dakota Experiment Station Bulletin 250 (July 1931) described the disease quite well. This bulletin considered the diagnosis, etiology, symptoms, time required to produce the disease, and the hematology. They also thoroughly reviewed the early experimental work.

Sweet clover disease was first named “sweet clover poisoning” by Schofield in 1921. He definitely showed that spoiled sweet clover could cause fatal hemorrhage if fed to cattle or experimental animals, but that good quality sweet clover did not produce the condition and was safe if properly fed. He also correlated the presence of a mold (usually located within the clover stalk) with the occurrence of the disease. Therefore, he concluded that either the mold itself, or a decomposition product, caused the disease although he did not prove the correctness of his view.

Noninfectious

Roderick and Schalk in 1926 showed the disease was not transmitted from one animal to another, even by direct blood transfusion, and therefore was not infectious.

The earliest symptom of the disease was a failure of the blood to clot normally. All of these investigators found that in experimental animals fed spoiled sweet clover, the blood clotting time would increase from a normal of fifteen to thirty minutes to three to four hours, or often fail to clot at all. Non-crepitating subcutaneous swellings appeared which were round and firm. The mucous membranes appeared pale. Death from hemorrhage often followed an operation such as dehorning or castration. The time required for death to occur in the experimental
animals ranged from twenty-five to fifty-three days after being placed on sweet clover rations. Death occurred spontaneously in most of these cases. Young animals seemed to be more susceptible than older ones.

The hematology of the diseased animals was studied. Red blood cell counts made in the later stages of the disease showed a marked decrease in number. This was thought to be due to the internal hemorrhage. The hemoglobin was correspondingly lowered. There was a decrease in the blood platelets but this was not considered significant. The leucocyte count was not of pathological significance. Determination of the calcium content of the blood from these animals did not show sufficient variation from the normal to be considered significant in the slowed clotting time. It was thought for a while that the condition might be some form of a deficiency disease. However, it was shown that cattle contracted the disease even if an adequate ration was fed along with the sweet clover. The prothrombin content of the blood showed a decrease proportional to the coagulation time. This was one of the most important changes noted.

**Early Investigations**

Schofield, through reference to an article in "Manual of Poisonous Plants" by Pammel, and also Roderick and Schalk, recognized the coumarin content of sweet clover as a potential factor in the disease. These investigators independently carried out experiments in which coumarin was fed to rabbits in considerable amounts. The animals died but did not show any hemorrhagic lesions, so it was thought that if coumarin was responsible it must be a decomposition product that produced the lesions. This was as near as they came to discovering the actual cause of the disease.

Coumarin (C₈H₇O₄) occurs as colorless crystals, with a characteristic pleasant, fragrant odor and a bitter, aromatic, burning taste. It is the constituent responsible for the fragrant odor of new mown clover. Coumarin is often used as a flavoring agent for various food products and medicinal preparations.

In a short time following the first reports of this new disease, its occurrence was being recognized in many states. The outstanding feature always noted was a failure of the blood to clot. Blood coagulation is primarily a process involving the plasma. The process of coagulation is complex and the exact details are still somewhat uncertain.

It is generally accepted that plasma contains all of the essential elements necessary for complete coagulation, and that the combination of the blood protein, fibrinogen, and thrombin produces fibrin which is the essential element of the clot. Fibrin is an insoluble protein not found in normal blood. It is the manner in which thrombin is formed that is debated.

**Morawitz Theory**

As explained by the Morawitz theory, thrombin is formed by the reaction of thrombokinase (cephalin) and calcium with prothrombin, normally present in the plasma, whereby the prothrombin is converted to thrombin. The thrombin in turn unites with the fibrinogen to form fibrin. This theory suggests that calcium is not essential for the final stages of clotting, but that its presence is necessary primarily for the activation of prothrombin.

Howell’s theory assumes that prothrombin can be changed to thrombin by calcium alone, but this reaction is normally prevented by a substance, heparin, in the blood, which he designates as an antiprothrombin. When thrombokinase develops in sufficient amount, it neutralizes the heparin (antiprothrombin) and thus prothrombin is free to react with calcium to produce thrombin. Thrombin plus fibrinogen then yields the fibrin.

**Data Accumulated**

Between 1920 and 1933, data on sweet clover disease rapidly accumulated. Most of the work up to this time had been done by Schofield in Canada and Roderick in North Dakota. The most conclusive thing they discovered was the altered prothrombin content of the blood. This was also confirmed by several other workers. In

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1933, investigations were undertaken at the University of Wisconsin when Dr. R. A. Brink of the Genetics Department initiated some attempts to select a non-bitter strain of sweet clover. He suggested the problem to Dr. H. A. Campbell, Dr. K. P. Link and other biochemists there. This started the second phase of the investigation of sweet clover poisoning, which resulted in the discovery and isolation of the "hemorrhagic agent." It was this work that established Link with Schofield and Roderick as the pioneers in this field.

In 1937, Roberts and Link published a report dealing with the determination of coumarin, melilotic acid and coumaric acid in sweet clover. In 1940, Campbell, Roberts, Smith, and Link published the results of their research dealing with the preparation of what they called the "hemorrhagic concentrate" found in sweet clover.

Two problems confronted these workers. First, there were no chemical criteria available to establish the presence of such a factor, and second, bio-assay offered the only means of testing the concentrates and extracts which they obtained, so they used a method in which the prothrombin level of rabbit plasma was taken as the standard. In 1936-37 they developed a method for the preparation of the active concentrate. After several attempts they were able to obtain it in some quantity. There followed a long and tedious series of experiments through which, by the process of elimination, they were able to determine its chemical structure.

At the same time, work by other investigators was also being done upon the extraction and utilization of heparin as an anticoagulant. Work on the toxicity of dicoumarin on mice, rats, and guinea pigs was carried on by Rose et al of the Eli Lilly Research Laboratories. In 1942, Stats and Bullowa reported the effects of a single dose of dicoumarin upon blood coagulation in man. The dosage varied from 1 to 12 mg. per Kilo of body weight, the average dose used being 8 mg. per Kilo, given orally. They studied the prothrombin level and coagulation time before the administration and for several days following administration. A prolongation of coagulation time was reported in about six days beginning between twenty-four and forty-eight hours after the administration of the drug.

The human dosage as determined by Barker et al of the Mayo Foundation was 300 mg. orally the first day, 200 mg. the second day, and 200 mg. per day thereafter, for five to thirty-three days.

"hemorrhagic agent," the identification and the synthesis of the substance was accomplished. Up until then, they had always referred to the toxic factor as the "hemorrhagic agent" for want of a better name. Finally, it was shown that the toxic factor of spoiled sweet clover was chemically 3,3' methylene-bis (4-hydroxycoumarin). Studies in experimental feeding proved that the new substance synthesized by these workers was identical with that found in the spoiled sweet clover. It was also concluded that this new substance was not identical with any of the sixty or more naturally occurring coumarins previously reported, but was a newly found form which caused the disease.

Allen, Barker, and Waugh of the Mayo Foundation suggested this new substance be called "dicoumarin," as it is a family name and because the 3,3' methylene-bis (4-hydroxycoumarin) is a "di-" coumarin. The Wisconsin Alumni Research Foundation, which controls the distribution of this product, gave it the trade name "Dicoumarol." Either name, dicoumarin or dicoumarol, is now acceptable.

Clinical Application

Recently, extensive investigations have been carried on to determine the possible rational clinical applications of dicoumarin as an anticoagulant. Work on the toxicity of dicoumarin on mice, rats, and guinea pigs was carried on by Rose et al of the Eli Lilly Research Laboratories. In 1942, Stats and Bullowa reported the effects of a single dose of dicoumarin upon blood coagulation in man. The dosage varied from 1 to 12 mg. per Kilo of body weight, the average dose used being 8 mg. per Kilo, given orally. They studied the prothrombin level and coagulation time before the administration and for several days following administration. A prolongation of coagulation time was reported in about six days beginning between twenty-four and forty-eight hours after the administration of the drug.

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determinations of the prothrombin and coagulation times were made before, during, and after administration.

Butsch and Stewart suggested 300 mg. on two successive days as the basic dosage for prophylactic oral use. They reported the physiologic effect from this dosage should last about four weeks.

The Abbott Laboratories, Mayo Foundation, and Columbia University have been leaders in the investigation of dicoumarin and its clinical application. Either oral or intravenous methods of administration may be used. Latent periods of from twenty-four to forty-eight hours are always observed in oral administration. The advantages of administration directly into the circulatory system are the control of absorption and a more exact blood concentration.

Clinical case reports following the use of dicoumarin are favorable in such conditions as pulmonary thrombosis, thrombophlebitis, and postoperative embolism. Research by Allen, Barker, and Waugh indicates that heparin and dicoumarin can be successfully used together, giving a quick and more prolonged action. Vitamin K apparently has no effect on the lowered prothrombin level resulting from the administration of dicoumarin.

Doubtless there will be many case reports published in the near future dealing with the effects of this new therapeutic product. Present indications are that it has great clinical possibilities.

BIBLIOGRAPHY


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hog cholera. Foot and mouth disease, brucellosis, and tuberculosis cause the greatest losses at the present time in the cattle industry. With the foot and mouth disease vaccine, which is beginning to be manufactured in Chile, it is hoped that this disease can soon be eradicated. The vaccine was developed by Waldmann and Koebe in 1938, and its efficiency was partly proved in Europe before World War II. Brucellosis will also be reduced to some extent when calfhood vaccination has been more completely developed. Tuberculosis as well as infectious abortion has been eliminated at this time from all purebred stock by semi-annual testing and the elimination of the positive reactors from the herdbooks.

We feel justified in thinking that our veterinary medical school is one of the best in South America. Its influence has extended all along the Pacific coast and in these countries there are veterinarians who acquired their degrees in Chile and at the present time occupy important positions in the departments of hygiene and animal production.