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BY

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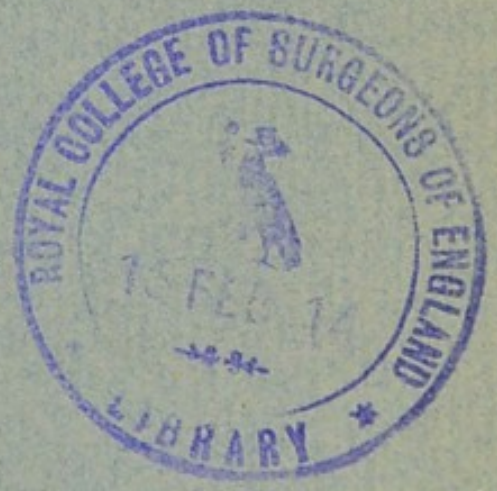

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By Frederick B. Power.

In a recent publication by Professor R. Kobert, of the University of Rostock, Germany, entitled: "Beiträge zur Kenntnis der vegetabilischen Haemagglutinins," which has been reprinted from a memorial volume of the *Landwirtschaftliche Versuchs-Stationen,* Band lxxix–lxxx, some very astonishing statements have been made respecting the protein of the bark of *Robinia Pseudacacia,* Linné. This protein substance was first obtained by me in the summer of 1889, and the fact that it possesses the well-known poisonous properties of the respective bark was conclusively shown in a paper read before the Wisconsin Academy of Sciences, Arts and Letters on December 27, 1889, which was also published in the *Pharm. Rundschau,* New York, 1890, **8,** 29–38. In a subsequent communication (*Pharm. Jour.,* London, 1901, **67,** 258) I had shown that this protein, to which in the meantime Kobert had assigned the name *robin,* possessed enzymic properties, and that it was capable of hydrolyzing both amygdalin and sinigrin (potassium myronate) with the production respectively of bitter almond oil and mustard oil, as also of clotting milk. In the last-mentioned paper consideration was taken of a statement in a dissertation by one of Professor Kobert's pupils, namely, Dr. Carl Lau (Rostock, 1901), whereby it was intimated that the toxic action of the Robinia protein had first been established by him. The exact statement by Dr. Lau (*loc, cit.,* p. 259) was as follows: "*Ich würde sehr gern noch eingehendere Versuche darüber eingestellt haben, ob die giftige Eiweisssubstanz der Robinienrinde ein Albumin, eine Albumose, oder ein Globulin, oder ein Gemisch zweier Substanzen ist. Zu derartigen Versuchen hätte ich jedoch viel grössere Mengen von Material gebraucht als sie mir zur Verfügung standen. Ich musste mich daher damit begnügen-festgestellt zu haben dass es sich thatsächlich um eine giftige Eiweisssubstanz handelt.*" In another place, with the incorrect assumption that I had assigned to the Robinia protein a name (*robinin*) which might cause it to be confused with the coloring matter of Robinia flowers, Lau remarked:

"Man wird daraus ersehen, wie zeitgemäss es war, unsern Giftstoff aus der Robinie in Robin unzubenennen."

It will be seen from the above quotations that in 1901 the poisonous action of the Robinia protein was recognized by Professor Kobert and his pupil, and in this connection it seems desirable to repeat what I had recorded in 1901 (*loc. cit.*, p. 259) that some time after having obtained the poisonous protein from Robinia bark I sent a specimen of it to the late Professor Flückiger, of Strassburg, and in a letter from him under the date of February 4, 1892, which is still in my possession, he wrote as follows: "I have to thank you for the poison of Robinia, which I sent finally to Prof. Kobert, Dorpat (Russia). He has also prepared the poison and states now that it nearly agrees with your preparation."

In view of all the well-known facts, which have been so completely substantiated, concerning the toxic action and other properties of the Robinia protein, it is difficult to understand how Professor Kobert could now have been led to make such obviously incorrect and misleading statements on this subject as are contained in the recent, above-mentioned publication. He there notes (*loc. cit.*, p. 82) that he has repeated his own experiments, and must withdraw the statements made together with Lau respecting the poisonous action of robin, those statements being now regarded by him as attributable to the impurity, imperfect solubility, or the immoderately large doses of the preparation used at that time. The preparation more recently employed by him, while acting energetically on some kinds of blood, was found not to be poisonous for rabbits when injected subcutaneously in amounts of 1 to 10 c.c. of a 0.4 per cent. solution. He therefore concludes that the symptoms of poisoning produced in man and animals by Robinia bark cannot be referred to robin, but presumes that the poisonous principle is the alkaloid or glucoside of the bark. Having thus inferred from the results of the above experiment that robin cannot be regarded as poisonous in small doses, he concludes that he must place it in the group of "phasins," or non-poisonous agglutinants.

Some still more surprising statements are made by Professor Kobert (*loc. cit.*, p. 83), which may literally be translated as follows: "For distinguishing the robin of Robinia bark from ricin the property of hydrolyzing sinigrin, as found by Power, would be admirably adapted, as this is not otherwise possessed by a single vegetable agglutinin. Experiments have shown, however, that

Power's statements are not valid for the robin of Robinia bark prepared by me (Kobert) and preserved in a dry state. It does not hydrolyze sinigrin even by its action for two days in an incubator and does not otherwise possess the property of hydrolyzing glucosides. It also has no coagulating effect on milk."

It is exceedingly unfortunate that Professor Kobert should have given such prominence to the results of experiments from which thoroughly incorrect inferences are liable to be drawn, especially by those who cannot conveniently repeat them, and he does not seem to have considered it necessary to ascertain the cause of his failure to obtain the results recorded by me. As the subject is one of considerable importance, I have deemed it desirable to present such facts as are believed to be sufficient to prove the incorrectness of Professor Kobert's conclusions, and to substantiate in every respect the accuracy of the statements previously recorded by me regarding the toxic action and other properties of the protein of Robinia bark.

In the first place it was noted in my paper on this subject in 1890 that a decoction made by boiling 100 grammes of the bark with water was taken without any ill effect or any perceptible action, whereas a cold infusion of about 5 grammes of the bark was in one instance so violent in its action as nearly to prove fatal. It was thus evident that the activity of the poisonous substance was destroyed at the temperature of boiling water, and this observation suggested not only the protein nature of the substance but also the method subsequently employed for its isolation. Moreover, the protein material, as precipitated by alcohol from the liquid obtained by macerating the ground bark with cold water, when collected, washed with alcohol, and dried in a vacuum or over sulphuric acid, possessed the same poisonous properties as the bark. When administered to a large dog in an amount representing about 30 grammes of the bark, it caused severe vomiting, which continued at intervals for several hours, and a considerably smaller quantity was not without effect. A solution of the same substance, when heated sufficiently to coagulate the protein, was quite devoid of activity. As the above experiments had been conducted with a bark collected by myself at Madison, Wisconsin, it may be noted that some years subsequently a quantity of protein material was prepared from Robinia bark collected in France. This protein material, when isolated by the simple method above described, pos-

sessed the same toxic properties as that previously obtained. It is well known that substances of this character lose their activity to a greater or less extent on keeping, even in a dry state, and that they also undergo change in this respect when their purification is attempted by methods of repeated solution and precipitation or by subjecting them to dialysis. Some change of this nature may have taken place in the material employed by Professor Kobert for his recent experiments, and this would appear to be the most probable explanation of the results now obtained by him, which, moreover, are so completely at variance with his own earlier observations.*

As a specimen of the Robinia protein which had been prepared by myself in 1904 was still available, it was deemed of interest to ascertain whether it still retained its original toxic properties. It was therefore kindly tested with respect to its activity by Dr. H. H. Dale, Director of the Wellcome Physiological Research Laboratories. An amount of 0.25 gramme was administered by the mouth to a dog, when, after an interval of about an hour, it produced two attacks of vomiting. This result, together with the observations previously recorded, as noted above, clearly demonstrate that *the poisonous constituent of Robinia bark is a protein.* They certainly lend no support to the statement of Professor Kobert that the respective protein, or robin, is a non-poisonous "phasin," or to his presumption that the activity of the bark is due either to an alkaloid or a glucoside.

There remains to be considered the statement of Professor Kobert (*loc. cit.*, p. 83) that the robin, or protein material prepared by him was not capable of hydrolyzing sinigrin, and possessed in fact no hydrolyzing action on glucosides, nor did it coagulate milk. His failure to obtain positive results in these experiments was certainly due to no inaccuracy in my observations, as would thereby be implied. Since the receipt of his publication I have again tested in this direction the above-mentioned specimen of

* Since writing this paper I have been favored with a private communication from Professor Kobert, in which he informs me that his method of testing the hydrolytic action of robin was by mixing a 1 per cent. solution of the protein with a 1 per cent. solution of sinigrin, and observing the result after keeping the mixture for some time, either at the room temperature or at a temperature of 38° C. It is not surprising that under these conditions no odor of mustard oil was perceptible. Apart from the extreme dilution of the robin solution employed, it is probable that in the preparation of the latter the active portion of the protein had been removed.

robin which was prepared in 1904, and had thus been kept, in well-stoppered bottles, for a period of nine years. This material was both in the form of dark brown scales, as originally obtained on drying the precipitated protein, and in the form of a lighter-colored powder, which was produced at the same time by triturating the first mentioned product. These two forms of the preparation were separately tested, both with amygdalin and with a well-crystallized specimen of sinigrin (potassium myronate) in the following manner: Into a small test-tube, provided with a well-fitting cork, was brought a small quantity of the respective glucoside, together with some of the dry protein, and a little water subsequently added. The tubes being then corked, and the mixtures vigorously shaken, they were set aside at the ordinary temperature (16–18° C.) and occasionally agitated. After a period of about 24 hours or less the tubes were opened, when in the one case there was a strong odor of bitter almond oil, and in the other an equally distinctive, sharp odor of mustard oil. The unmistakable results of these tests, which are doubtless obtained much more quickly with the fresh Robinia protein, thus not only confirmed my previous observations, but they have now also been confirmed independently by five chemists in these laboratories. It was not deemed necessary to again repeat the test with milk, the coagulation of which by the protein, or an enzyme therein contained, I had previously fully and accurately described.

The properties which the protein material designated as "robin" has been shown to possess renders it probable that, like other similar products, it is a mixture of substances, but no method is known to the present author by means of which a separation of its constituents could be effected without a corresponding loss of activity. It is also not known whether the toxic action of the protein is due to a substance which at the same time possesses enzymic properties, but as the last-mentioned properties are so varied in character, no doubt can be entertained respecting the presence of several enzymes. Apart from the frequently observed occurrence of enzymes, or mixtures of such, which effect the hydrolysis of amygdalin, it has been ascertained by Th. Bokorny (*Chem. Zeitung.*, 1900, 24, 771) that myrosin or a similar ferment is also widely distributed, having been found in plants of many different families besides the *Cruciferæ*, although the glucoside (sinigrin) which yields mustard oil has as yet only been found in the last-mentioned family.

A milk-clotting enzyme, or phytochymase, has also been stated to occur in various plants.

The confusion which is likely to be produced in the literature in consequence of the recent statements published by Professor Kobert is much to be regretted, especially as his conclusions, which appear to have been too hastily formed, are so obviously and demonstrably wrong. It is for this reason that I have deemed it my duty to again place on record the above-mentioned facts, as also to maintain that the observations noted in my previous publications (*Pharm. Rundschau*, 1890, **8**, 29, and *Pharm. Journ.*, 1901, **67**, 258) respecting both the toxic action and enzymic properties of the protein ("robin") of Robinia bark are perfectly correct.

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