A STUDY OF AN EXTREMELY PURE PREPARATION OF RICIN.[1]

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In September, 1905, Osborne, Mendel and Harris (1) published a paper entitled, "A Study of the Proteins of the Castor Bean, with Special Reference to the Isolation of Ricin." In their study they found that the toxic substance known as ricin was associated with the coagulable albumin of the castor bean, and that of this protein 0.0005 milligrams per kilo of body weight injected into a rabbit caused death on the seventh day, and a dose of 0.0032 milligrams per kilo injected into a guinea pig caused death in seven days. With their preparation (No. 15), the susceptibility of the rabbit and the cat were about equal, 0.1 milligrams per kilo causing death in two to three days in these two animals. In the guinea pig and dog, 0.5 milligrams per kilo caused death within forty-eight hours. They did not test in any more detail this preparation, which was about one hundred times more toxic than that of Cushny, who had up to this time prepared the most potent ricin. I shall not go into a further discussion of the historical aspect of this subject, but shall proceed to detail my results.

Preparation.—In the preparation of the ricin used in this work I followed exactly the method detailed by Osborne, Mendel and Harris. Although I followed their method in every step, I did not get the proportionate yield of ricin that they obtained, probably owing to their greater experience in the isolation of proteids. Roughly stated, the method consists of extracting the ground beans for forty-eight hours with 10 per cent. sodium chloride solution, two liters of solution to the kilo of meal.[2] This is filtered and

[2] All the oil has previously been removed from the ground beans by extraction with ether and pressure and then a further grinding.

then dialyzed for four or five days. It is again filtered and the precipitated globulin removed. To the filtrate is then added enough ammonium sulphate to bring it to a 45 per cent. concentration. After standing the precipitate is filtered off and dissolved in water, after which is added an equal quantity of the saturated ammonium sulphate solution. It is then allowed to stand over night and again the precipitate is removed by filtration.[3] The dried precipitate then contains less ammonium sulphate than it otherwise would. After this, the partially dried precipitate is dissolved in water, and enough saturated ammonium sulphate solution is added to bring it to a 33 per cent. concentration. When the precipitate that has been formed by this concentration of ammonium sulphate has settled, it is collected on a filter and then dissolved in water and dialyzed, first for seven days against running tap water and then against distilled water for another week. It is then carefully filtered and the filtrate evaporated as rapidly as possible to dryness *in vacuo*. The coagulable albumin so isolated is the most toxic fraction to be obtained from the proteins of the castor bean. Another fraction of coagulable albumin may be obtained by bringing the filtrate of the 33 per cent. ammonium sulphate concentration to a 50 per cent. concentration and this is also toxic but less so than the precipitate obtained from the 33 per cent. concentration, as the precipitate from the 50 per cent. concentration contains a greater quantity of proteose. All of the results detailed in this paper were obtained with the albumin precipitated by a 33 per cent. concentration of ammonium sulphate. Osborne, Mendel and Harris found in their preparation of this fraction coagulable albumin, 70.64, and proteose, 29.36.

To determine the amount of coagulable protein and proteose in the preparation used in this work, 0.827 gram, calculated as ash free,[4] were taken and dissolved in 10 per cent. sodium chloride solution. It was heated on a water bath for one and a half hours at 94° to 96° C. The coagulum was filtered off, washed, dried and weighed. It contained 0.524 gram, leaving .203 gram of proteose which

[3] These protein precipitates are best deprived of the excess fluid and salts by placing them between large quantities of filter paper and applying a gradually increasing pressure.

[4] Ash = 5.16 per cent.

gave the following result of percentages: coagulable albumin, 75.46; proteose, 24.54. It will be seen that this agrees fairly well with the figures obtained by Osborne, Mendel and Harris, except that the coagulable proteid is slightly higher.

The Toxicity of the Preparation.—Osborne, Mendel and Harris found that the minimal lethal dose of their most toxic preparation for various animals was as follows: for rabbits, 0.0005 milligram per kilo; for guinea pigs, 0.0032 milligram per kilo; for dogs, 0.5 milligram per kilo (death in forty-eight hours); for cats, .1 milligram per kilo.

The minimal lethal dose of the preparation under discussion was as follows: for rabbits, 0.0001 milligram per kilo; for guinea pigs, 0.0008 milligram per kilo; for dogs, 0.0006 milligram per kilo, for cats, 0.0002 milligram per kilo; for goats, 0.003 milligram per kilo (death in three days).

In the case of the goat this dose caused death on the third day. The autopsy findings in these various animals were similar. These doses are based on intramuscular inoculation. Intravenous injection gave a shorter incubation period, but no great difference in the minimal lethal dose. The time of death could be gauged within rather narrow limits by varying the size of the dose. In rabbits, 10 milligrams per kilo caused death in five to six hours. One hundred milligrams gave exactly the same result, showing that the minimal incubation period is between five and six hours. The pathological lesions were never as marked in these cases of short incubation periods as in those animals dying from the third to the sixth day. Death could be regularly produced up to the sixth day, but after this period, individual variations in the susceptibility of the animals became an extremely disturbing factor. Lesions caused by these extremely potent preparations are identical with those produced by the ordinary commercial preparations.

In 180 animals injected with fatal doses of ricin, the results were as follows:

The autopsy findings were intense hemorrhagic edema at the site of inoculation. The peritoneal cavity always contained a quantity of bloody fluid, varying in amount from 50 cubic centimeters to as much as 200 cubic centimeters. Nine per cent. of the ani-

mals showed the presence of a bloody fluid in one or both pleurae. Three per cent. showed the presence of this fluid in the pericardium. The heart and lungs were normal. About 5 per cent. of the animals showed a localized hemorrhagic area in the thymus; 2 per cent. in the thyroid gland; 15 per cent. in the pancreas; 45 per cent. in the adrenals. The pyloric portion of the stomach and the small intestine showed these lesions in every case. In the small intestines the lesions were more numerous in the duodenum. The lymph nodes of the peritoneal cavity were always enlarged and softened. The liver, as a rule, showed marked congestion and many areas of focal necrosis. The spleen was large, soft, and extremely engorged with blood. The microscopical examination of specimens from these various tissues showed exactly the same picture that Flexner (2) has described. The post-mortem picture is in some particulars similar to that caused by diphtheria toxin, especially the lesions at the site of injection in the adrenals and stomach.

The Hemagglutinin.—This function of the preparation was tested against the red blood cells of horses, rabbits, guinea pigs and dogs. One cubic centimeter of a 1 per cent. suspension of well-washed red blood cells, plus the ricin in one cubic centimeter of .9 per cent. sodium chloride solution. The amount of ricin required to bring about complete agglutination of the above quantity of red blood cells varied between 0.002 and 0.005 for all four species of red blood cells, showing that there was not any greater variation between the minimal agglutinal doses than between the minimal lethal doses.

Keeping Qualities.—At the end of two years and a half this preparation was tested for its toxicity in rabbits, dogs, and guinea pigs, and it was found to be absolutely inert, one-half gram producing no effect in a rabbit. On the other hand, the minimal agglutinating dose was only slightly raised. It now required between 0.005 to 0.009 gram to bring about complete agglutination of one cubic centimeter of a 1 per cent. red blood cell suspension.

Effects of Electric Current.—One gram of this protein preparation was dissolved in twenty-five cubic centimeters of distilled water and placed in a cell, similar to the one described by Field

and Teague (3), with agar-filled tubes as electrodes. A direct current was passed through this solution for five hours, the strength of the current being one hundred and ten volts, and from three to eight milliamperes. After a very short time a white flocculent precipitate appeared about the anode agar. At the end of this time, as no more precipitate was seen to be forming, this precipitate was collected on filter paper and washed thoroughly in distilled water, in which it was insoluble. It was then treated with water containing a slight trace of sodium carbonate, in which it was readily soluble. This preparation was far more active as a hemagglutinin than the original preparation. On the other hand, the minimal lethal dose was markedly decreased. The figures were as follows: .0001 milligram agglutinating dose, as against 0.002 to .005. The minimal lethal dose for rabbits was .002 milligram, as against 0.0001. The filtrate showed a very marked loss in agglutinating value per milligram of protein, but it also showed a loss in the minimal lethal dose as well.

CONCLUSIONS.

1. By following the method of Osborne, Mendel and Harris, we can obtain an extremely potent toxin from the castor bean.

2. It would appear, as a result of testing this preparation at the end of two and a half years, that the agglutinating function and the toxic function are two distinct properties.

3. This result is also borne out by the behavior under the electric current; either we are dealing with two different substances or else with a single substance with two distinct toxiphore groups, one of which is stable and the other labile.

BIBLIOGRAPHY.

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3. Field and Teague, *Jour. of Exper. Med.,* 1907, ix, 86.