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THE TOXIC FACTORS OF THE SEEDS OF *ABRUS PRECATORIUS*

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Abstract—Solvent extraction and precipitation, salting out, dialysis, ion exchange, gel filtration and electrophoresis have failed to separate the toxic component of the seeds of *Abrus precatorius* from the protein constituents. This supports the view that the toxicity of these seeds is due to one or more proteins.

INTRODUCTION

ABRIN, the toxic factor of the seeds of *Abrus precatorius* (jequirity, rosary bean) resembles ricin from *Ricinus communis* (castor oil plant). Both belong to the group of plant poisons known as phytotoxins, which also includes curcin, from *Jatropha curcas* (Barbados or purging nut) and robin, from the bark of *Robinia pseudaccacia* (black locust). Of these ricin has been most extensively studied, presumably because the seeds are readily available commercially.

Ricin is a protein[1,2] of known amino acids composition[3] and with a molecular weight variously reported as 77,000[1] and 36,000.[2] The work described here is concerned with establishing that abrin is also a protein.

RESULTS

Attempted Extraction of the Toxic Factor from the Seeds with Organic Solvents and its Behaviour on Dialysis

Exhaustive extraction of the ground seeds with diethyl ether, light petroleum, ethyl acetate, *n*-butanol, ethanol and methanol failed to remove the toxic factor. It was, however, extracted with aqueous solvents including acid and alkaline buffers, dilute acids and distilled water. Exhaustive dialysis of these aqueous extracts showed that the toxic factor was non-dialysable.

Elution of Abrin and Abrus precatorius *Proteins from Columns of G* 75, *G* 100 *and G* 200 *Sephadex*

In all these experiments the elution diagram for the toxic activity of the extracts was identical with that for protein and the point of maximum toxic activity on the elution curve coincided with the point of maximum protein concentration. The results with G 200 gel are shown in Fig. 1.

[1] E. A. KABAT, M. HEIDELBERGER and A. E. BEZER, *J. Biol. Chem.* **168**, 629 (1947).
[2] M. KUNITZ and M. R. MCDONALD, *J. Gen. Physiol.* **32**, 25 (1948).
[3] Y. MOULE, *Bull. Soc. Chim. Biol.* **33**, 1467 (1951).

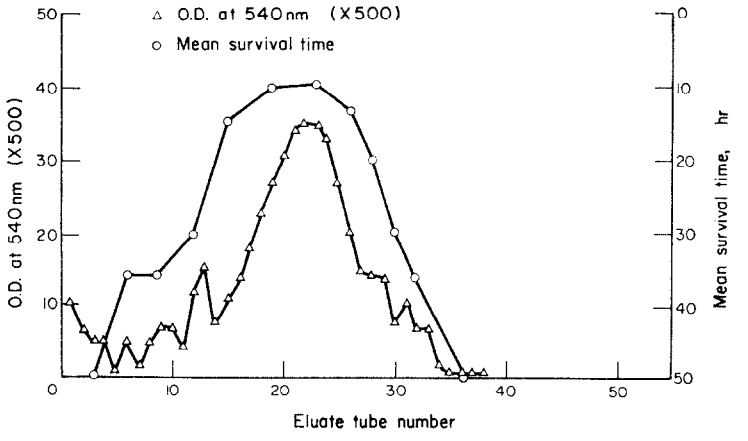

FIG. 1. ELUTION OF *Abrus precatorius* PROTEINS FROM COLUMNS OF SEPHADEX G 200.

Adsorption of Abrin and Abrus precatorius *Proteins on Ion-Exchange Resins*

Both the protein and the toxic activity were adsorbed on to columns of Amberlite IRC 50 (carboxylic acid) resin at pH 6 and could be eluted from it with 0·1 N HCl or with potassium chloride/HCl buffers with a pH of 1·8 or lower. The point of maximum protein concentration on the elution curve again coincided with that of maximum toxic activity (Fig. 2). It was also shown that neither the proteins nor the toxic activity could be adsorbed on Deacidite (anion) resins and that they were apparently irreversibly adsorbed on cellulose phosphate.

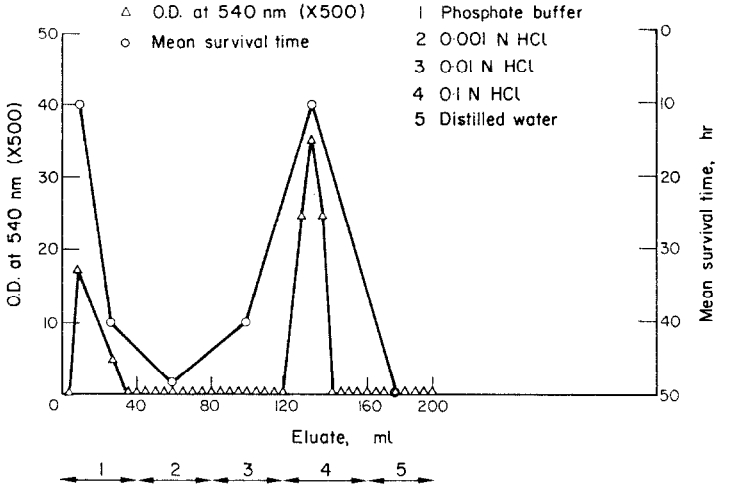

FIG. 2. ADSORPTION OF ABRIN AND *Abrus precatorius* PROTEINS ON AMBERLITE IRC 50.

The Electrophoretic Separation of Abrus Seed Proteins

Paper and starch block electrophoresis in acetate/acetic acid buffer at pH 4 and 5 resolved the abrus protein into three basic fractions. The toxicity of the extracts remained associated with each of these components, the areas of the electrophoretograms containing no protein being inactive.

DISCUSSION

Solvent extraction, qualitative Sephadex gel filtration, ion-exchange chromatography, electrophoresis, solvent and salt precipitation and dialysis have failed to separate the toxic activity of a saline extract of *Abrus precatorius* seeds from the proteins present in the extract.

Following the precipitation of the proteins with ammonium sulphate (50 per cent saturation), their separation from small molecular weight pigments by gel filtration, adsorption on to Amberlite IRC 50 and resolution into three basic components by electrophoresis, the toxic activity remains associated with each of these components. This supports the opinion[4,5] that the toxic activity of the seeds is attributable to the proteins present in them. It is also suggested that three basic albumins are involved, although further work is necessary to elucidate whether or not the toxicity associated with these is due to them or to a minor component which cannot be separated from them by the methods employed. It will be necessary to employ further different techniques to clarify this point.

EXPERIMENTAL

Attempted Extraction of the Toxic Factor from the Seeds with Organic Solvents and its Behaviour on Dialysis

10-g batches of *Abrus precatorius* seeds, freed from their seed coats, were individually extracted with 50-ml aliquots of diethyl ether, light petroleum, ethyl acetate, *n*-butanol, ethanol and methanol at room temperature until no further material could be removed. The organic solvents were then removed under reduced pressure and the individual residues dissolved in 10 ml of normal saline. Any insoluble material was removed by filtration. The same weight of seeds were also extracted with aqueous solvents (H_2O, dilute acids, acid and alkaline buffers) and, following standing at room temperature for 1 hr, the 10 ml of solvent employed was freed from insoluble material by centrifuging. A portion of each of these fractions was dialysed overnight against normal saline. For testing, 0·5 ml of each of the extracts were injected intraperitoneally into each of a group of four male, 15–20-g albino mice.

The Distribution of Abrin and Abrus precatorius *Proteins on Sephadex Gel Columns*

The G 75, G 100 and G 200 grades of the gel were employed and the method employed in all cases was the same.

A saline extract was prepared from 100 g of abrus seeds. It was mixed with an equal volume of saturated ammonium sulphate and allowed to stand at room temperature for 15 min. The precipitate was collected by centrifuging, dissolved in normal saline and freed from insoluble material by filtration. A dialysis sac containing polyethyleneglycol was then introduced in order to concentrate it[6] to 15 ml, this concentrated solution being applied to the columns.

50 × 1 cm columns of the gels (particle size 40–120 μ, Pharmacia, Uppsala, Sweden) were then prepared and, following a 3-day settling period, 3 ml of the abrus seed extract was introduced and the columns eluted with 1% NaCl with the collection of 2-ml fractions. The protein content of each of the fractions was estimated by using the Biuret reagent.[7] With the G 75, G 100 and G 200 gels a total of 45, 37 and 38 fractions respectively were collected. The tube contents were then combined as appropriate and assayed for toxicity by intraperitoneal administration of 0·5 ml into members of groups of four male, 15–20-g albino mice.

Absorption of Abrin and Abrus precatorius *Proteins on Ion Exchange Resins*

5 ml of the concentrated seed extract, mixed with 1 ml M/15, pH 6, KH_2PO_4/Na_2HPO_4 buffer was applied to a 10 × 1 cm column of analytical grade Amberlite IRC 50 previously converted to the H^+ form and equilibrated against the same phosphate buffer. The column was then washed with 40-ml volumes of the phosphate buffer, 0·001 N HCl, 0·01 N HCl, 0·1 N HCl and distilled water succesively in that order with the collection of 5-ml fractions of the effluent. The amount of protein present in each fraction was estimated from the absorbance at 280 nm.[8] The tube contents were then combined to give the following fractions: (i) the first three tubes of the buffer eluate, (ii) the remainder of the buffer eluate, (iii) the 0·001 N HCl eluate, (iv) the 0·01 N HCl

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[5] H. E. DURHAM, *Arch. Hyg.* **81**, 273 (1914).
[6] J. KOHN, *Nature* **183**, 1055 (1959).
[7] T. W. WEICHSELBAUM, *Am. J. Clin. Path.* **16**, 40 (1946).
[8] O. WARBURG and W. CHRISTIAN, *Biochem. Z.* **310**, 384 (1941).

eluate, (v) the 0·1 N HCl eluate, and (vi) the distilled water eluate. After mixing and neutralizing with NaOH, 0·5 ml aliquots of each fraction were assayed for toxicity as before.

Employing a series of KCl/HCl buffers, pH 2·2, 2·0, 1·8 etc. down to pH 1·0, it was shown that the pH of the eluting buffer had to be 1·8 or lower for the elution of the protein and the toxic activity which were removed together.

The Electrophoretic Separation of Abrus precatorius *Proteins*

The protein extracted from 100 g of abrus seeds with 1% NaCl, was precipitated with 50% ammonium sulphate, freed from small molecular weight contaminants by gel filtration and adsorbed on to Amberlite IRC 50. It was eluted with 0·1 N HCl and the eluate neutralized (40% NaOH) and concentrated by polyethylene glycol treatment until 0·02 ml gave a strongly staining spot when applied to paper and treated with Acid Green G. Lissamine green (0·25% in 3% TCA) and freed from background colour by washing with 5% acetic acid. 0·06 ml of the concentrate was then subjected to electrophoresis on three 3×20 cm strips of Whatman No. 1 chromatography paper in 0·2 M sodium acetate/acetic acid buffer at pH 4 and 5 at a constant current of 8 mA for 24 hr. Narrow strips were then cut along the whole length of each electrophoretogram and, after staining and washing as above, showed two protein bands.

Using the stained bands as markers the individual proteins were eluted with 2 ml of 1% NaCl and 0·5 ml aliquots assayed for toxicity. All the animals died within 19 hr. When this experiment was repeated with a new batch of seeds from Antigua, West Indies, which had not been stored for any length of time, three basic components were obtained on electrophoresis. Toxicity assay showed that all three proteins present in the Antigua batch of seeds were toxic and that the blank areas of the electrophoretograms between the protein bands showed no activity.

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