STUDIES ON ABRUS PRECATORIUS LINN

Part II.—Biochemical and Pharmacological Studies on Abrulin

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(Received March 27, 1962)

Some of the biochemical and pharmacological properties of abrulin have been reported. The toxin is quite stable up to 80°C. and is not appreciably digested by trypsin and pepsin. Abrulin causes haemagglutination and is toxic in an amount of 50 mg./kg. when given orally. The toxicity of abrulin is enhanced through the addition of proteins.

Introduction

In their last communication Khan, Hashmi and Khan[1] described the isolation of a very toxic fraction from jequirity seeds. This fraction was designated by them as abrulin to distinguish it from abrine, a non-toxic methyl tryptophane compound isolated by Ghattak and Kaul.[2] The toxicity of abrulin against albino mice, rats, cats, rabbits and guinea pigs together with its general action on the body and the post-mortem findings were reported. The protein nature of abrulin was also discussed.

Work was further extended to study the biochemical and pharmacological properties of the toxin. In the present communication a few properties like haemagglutination, toxicity by mouth and the stability of the toxin towards heat have been discussed.

Experimental

Material and Method.—Jequirity seeds of the scarlet variety were decorticated and then ground to a 30 mesh powder. This powder was extracted with 10% sodium chloride solution and the extract was designated as the original solution (O.S.). Abrulin was prepared from the original solution by double precipitation with sodium chloride and saturated ammonium sulphate solution as reported earlier.[1] Both the original solution and abrulin were used throughout the study.

Stability of the Original Solution and Abrulin Towards Heat.—A solution of known concentration of the toxin solution was heated in a three-necked round bottom flask over a water bath. The centre neck carried a condenser while through one of the side necks was fitted a thermometer. The third neck was closed with a stopper and was opened only to remove the solution. Aliquot samples were removed at 60°, 70°, 75°, 80°, 85° and 90°C. After cooling, the aliquots were injected in rats. It was noted that the toxin was quite stable up to 70°C. The activity, however, diminished after 75°C. and completely disappeared at 80°C. This confirms the findings of Martin[3] whose globulin was also deactivated at 80°C. It was also observed by the present authors that the original solution did show slight activity even up to 85°C. This might be due to the protecting effect of the albumin rather than to the presence of another toxin.

Haemagglutination Action of Abrulin and the Original Solution.—The haemagglutinating power of the solution was measured as the minimum amount of protein giving definite agglutination of 0.2 ml. of the human Group "O" erythrocyte suspension prepared in the following manner: Five ml. of the citrated blood was centrifuged to settle down the red cells. The cells were washed thrice with normal saline and then suspended in 20 ml. of the same solution. For our experimental purposes, 1 ml. of the stock solution of the erythrocytes was diluted to 5 ml. with normal saline and 0.2 ml. of this solution was used for each concentration of the toxin. All tests were carried out in a total volume of 1 ml. and the mixture of the toxin and the erythrocytes was allowed to stand at room temperature. The concentration at which no button formation was noted was taken as the haemagglutination dose. It took about 3 to 4 hours to settle down all the cells. It was noted within experimental error, that 2 μg. of the pure abrulin and 4 μg. of the original solution showed agglutination.

Toxicity by Mouth.—In the course of certain experiments, which will be discussed in another paper, *Abrus precatorius* powder was fed by mouth.

In order to limit the oral dose and to avoid any untoward effects due to the toxin, it was considered necessary to test the toxicity of the original solution and also of abrulin by mouth. In the present experiments both the original solution and purified abrulin were used in a dosage of 25 mg., 50 mg., and 100 mg. protein per kg. body weight of the animal. Rats weighing between 150-200 g. were used throughout this study and the solutions were fed with the help of a stomach tube. The results are recorded in Table 1.

TABLE 1.—EFFECT OF THE ORIGINAL SOLUTION AND ABRULIN BY MOUTH.

Protein/kg. body weight	Original solution	Abrulin
25 mg.	No death	No death
50 mg.	in 1/5 in 10 days	3/5 in 10 days
100 mg.	5/5 in less than 72 hours.	5/5 in less than 48 hrs.

Autopsies were performed on all the dead animals. It was observed that:

(a) blood remained fluid; (b) distinct haemorrhages were seen in the liver, heart, lungs, spleen and the kidneys, and (c) stomach and intestines were seen distinctly unaffected, in the case of abrulin, however, slight haemorrhagic spots being observed (with heavy doses—100 mg./kg.) at the pyloric end of the stomach.

It appears, therefore, that the action of the toxin is systemic rather than local and the organ of the body most affected is the liver. It may, however, be recalled that abrulin produces practically the same pathological changes when injected subcutaneously.[1] Even in sublethal doses the original solution and the toxin produced similar pathological changes although to a lesser degree, so that after a certain period the animal survives.

One interesting observation was recorded when 25 mg. protein of the original solution and abrulin were fed to the rats. All the animals survived in this case but on killing them 24 hours after feeding the drugs and then performing an autopsy, it was noted that whereas no apparent pathological change was noted in the case of the original solution, distinct haemorrhagic spots were found to be present on the liver, and slightly also on the lungs in the case of abrulin.

Effect of Added Proteins.—While working with polyvalent antivenine (courtesy Bureau of Laboratories, Karachi) it was noted that the toxicity of abrulin is enhanced through the addition of the former. A series of experiments were carried out both on rats and pigeons, and the injections were given subcutaneously, intramuscularly and intravenously. Table 2 shows the effect of the added protein. It is clear from this table that antivenine appears to potentiate the toxicity of abrulin.

Effect of Trypsin and Pepsin on Abrulin.—The toxin was digested with trypsin and pepsin at pH 8.0 and 4.0 respectively for 48 hours at

TABLE 2.—EFFECT OF ANTIVENINE ON THE TOXICITY OF ABRULIN.

Wt. of the rat	Toxin injected	Wt. of antivenine	Route of injection	Results
110 and 115 g.	4 mg./kg.	—	Subcutaneously	2/2 in 24 to 26 hrs.
100 g.	,, ,, ,,	5 mg.	,,	2/2 in 18 to 22 hrs.
100 g.	,, ,, ,,	10 mg.	,,	2/2 in 18 to 22 hrs.
105 g.	,, ,, ,,	20 mg.	,,	One died in less than 12 hrs. other died in 18 to 20 hrs.
110 g.	,, ,, ,,	20 mg.	,,	Both alive.
110 g.	,, ,, ,,	120 mg.	,,	Both alive
		EXPERIMENTAL ANIMAL: PIGEON		
—	350 mg./kg.	—	Intravenously	Died after 7 hrs.
—	,, ,, ,,	50 mg.	,,	Died after 5 hrs. and 45 minutes.
—	,, ,, ,,	50 mg.	Intramuscularly	Died in less than 12 hrs.
—	,, ,, ,,	—	Subcutaneously	Died after 25 hrs.

37 °C. and the rate of digestion was followed by toxicity tests on rats. It was observed that, within experimental error, both the proteolytic enzymes have very little effect on the toxin. All the experimental animals died at about the same time as the controls which were given undigested toxin.

Discussion.—A comparison of the haemagglutinating action and toxicity by mouth of the original solution and abrulin shows that the latter is the active factor of the jequirity seeds. Martin[3] however, isolated a globulin and an albumin, both of which were found by him to be active, although the globulin was noted as comparatively more active. From the above-mentioned results, it appears that only the globulin is active and that any activity shown by the albumin may have been due to its contamination with the globulin. This is further confirmed by the fact that when the solution left after the precipitation of the toxin with ammonium sulphate solution, was dialysed till the non-dialysable portion showed no haemagglutinating property, the toxicity also disappeared.

The potentiating effect of antivenine is rather interesting. Further experiments in this connection are needed to confirm whether other proteins also affect the toxicity of abrulin or whether this characteristic is specific of antivenine. It will not be out of place to mention that antivenine is produced locally by the Bureau of Laboratories, Karachi and is used as an antidote to snake venom. Abrulin also produces symptoms very much similar to snake venom.

Acknowledgements.—The authors express their indebtedness to Dr. Salimuzzaman Siddiqui, F.R.S., Chairman, Pakistan Council of Scientific and Industrial Research, and Dr. S. Mahdihassan, Head of the Biochemical Research Division, for their valuable suggestions and encouragement throughout this work. Their thanks are also due to Dr. Mian Mohammed Siddique Hussain, Director, Bureau of Laboratories, Karachi, for a generous supply of polyvalent antivenine.

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