# STUDIES IN THE PROTEINS OF INDIAN FOODSTUFFS.

## PART VII. THE GLOBULINS OF THE ACONITE BEAN. (*P. ACONITIFOLIUS* JACQ.)

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*P. aconitifolius* known as aconite bean or moth bean is a perennial or annual herb, found throughout India. It is cultivated as a hot-weather crop and repeated in the autumn. In the United Provinces and Bombay, it forms an important crop. The pulse is split and eaten as dal and also cooked in various ways. The pulse is known to be one of the most easily digestible of pulses in India. Hence it was of great interest to find out its composition and investigate its proteins.

Niyogi, Narayana and Desai (*Indian J. Med. Res.*, 1931–32, **19**, 859) isolated the globulins from the pulse, conducted a Van Slyke analysis of the protein and determined its nutritive value by feeding experiments. They did not make any attempt in fractionating the total globulins of aconite bean. In view of the easy digestibility ascribed to this pulse, it appeared desirable to extend the investigation with a view to purify and fractionate the total globulins into its individual proteins.

### EXPERIMENTAL.

The pulse was obtained from the Bombay market, sun-dried, powdered to pass through a 40-mesh sieve and preserved in glass stoppered bottles for the subsequent investigation.

20 gms. of meal was successively subjected to extraction with ether and then with alcohol. Separate batches (20 gms.) were also extracted with petrol ether and chloroform and the total nitrogen and total phosphorus values of the extract were determined with a view to find out the percentage of the lecithins. Table I gives the results of these analyses.

TABLE I.

| Solvent | Percentage of the extract | Phosphorus per cent. | Nitrogen per cent. | N/P |
|---|---|---|---|---|
| Ether | 0·85 | 0·42 | 7·82 | 18·3 |
| Petrol ether | 0·92 | 0·83 | 12·36 | 14·9 |
| Chloroform | 1·09 | 1·50 | 10·84 | 7·3 |
| Alcohol (after ether extraction) | 2·98 | Nil | 6·34 | .. |

It will be seen from the above table that chloroform appears to be the best solvent from the point of view of extracting the maximum amount of phosphorus containing extract. It is therefore possible that chloroform will extract lecithins more completely than any other solvent and for any detailed study of the lecithins, this solvent is recommended. Attention should also be drawn to the exceptionally high content of organic phosphorus in the aconite bean, which may be partly responsible for its reputed nutritive value.

## EXTRACTION AND FRACTIONATION OF GLOBULINS.

The seed meal was extracted with 5 per cent. saline and the total globulins obtained from the saline extract by dilution, were dissolved in 5% saline and the extract was subjected to fractionation by the following methods:—(1) gradual dilution with water and saturation with carbon dioxide; (2) graded additions of organic solvents like acetone: and (3) saturation with ammonium sulphate.

In the case of fractionation by additions of acetone, the crude saline extract of the aconite bean was used, since the author had in view the additional object of determining the success of the method for the isolation of the non-protein nitrogen.

*(i) Fractionation by Dilution and Carbon Dioxide Saturation.*—The concentration of the proteins at which their fractionation was conducted, was kept somewhere about 2.5 per cent. To determine the extent of dilution that was effective in causing precipitations of the fractions from the saline solution, a preliminary experiment was carried out. 5 c.c. of the solution was titrated against distilled water until

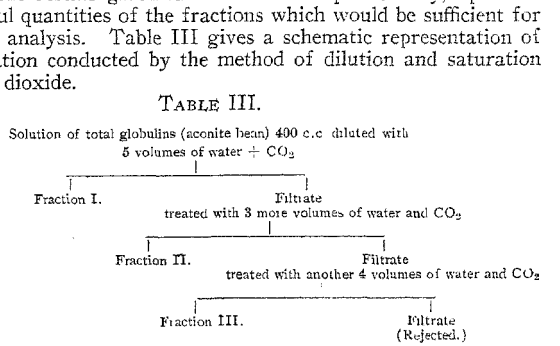
a definite turbidity appeared. The liquid was then centrifuged and the clear centrifugate was further titrated until the turbidity appeared a second time. The liquid was then centrifuged and the clear liquid titrated once again with further quantities of distilled water. The process was repeated until no more turbidity or precipitate appeared with further additions of water. Table II gives the results of such a preliminary experiment.

TABLE II.

| No. of titration | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Titre c.c. of $H_2O$ | 22·3 | 32·5 | 40·9 | 48·7 | 58·5 | 59·8 | Indefinitely large dilution |
| Amount of precipitate | ++ | +++ | +++ | +++ | +++ | + | — |

The extent of dilutions finally adopted for the experiment was based upon the results given in Table II and particularly, upon the yield of useful quantities of the fractions which would be sufficient for a subsequent analysis. Table III gives a schematic representation of the fractionation conducted by the method of dilution and saturation with carbon dioxide.

TABLE III.




The fractions, I, II, and III were washed on the centrifuge until free from chlorine, desiccated with graded strengths of acetone, then with absolute acetone and finally with ether. The preparations were dried *in vacuo* over sulphuric acid and the ground powders kept in glass stoppered bottles for analysis.

(*ii*) *Fractionation by Graded Additions of Acetone.*—In the course of these investigations, the crude saline extract was employed with a view to determine the concentrations of acetone

that are effective in yielding useful quantities of the precipitate. Different batches of the saline extract of the globulins were employed for each of the concentrations of the organic solvent used for precipitation. The quantity of the total and amino nitrogens were determined in the filtrate, which gives us an idea of the average complexity of the nitrogenous bodies left over in the filtrate. The reaction of the filtrate on the addition of trichloracetic acid, was tried and an aliquot of the filtrate after driving off the solvent on a water bath, was tested for tyrosine by reaction with tyrosinase. The precipitates obtained were tested for their solubility in saline solutions to determine roughly, the extent to which the preparations had suffered denaturation. Two series of experiments, one with alcohol and the other with acetone, were conducted. In the alcohol series, the amino nitrogen was determined by Foreman's titration method (*Biochem. J.*, 1928, **22**, 208), while in the case of the acetone series, Linderstrøm Lang's (*Compt. Rend. Lab. Carlsberg*, 1929, 17) method was adopted. The results are given in Tables IV and V.

TABLE IV.

*Aconite Bean Globulins (Alcohol Series).*

| Concentration | Saline Ext. c.c. | Alcohol c.c. | Percentage of total nitrogen in the filtrate | Percentage of amino nitrogen in the filtrate | Complexity total N/amino N | Trichloracetic acid | Tyrosinase Reaction | Solubility of the precipitate in saline |
|---|---|---|---|---|---|---|---|---|
| 0 | 10 | 0 | 100·00 | 100·00 | 3·25 | +++ | +++ | |
| 1 | 10 | 5 | 80·76 | 79·16 | 2·23 | + | + | |
| 2 | 10 | 10 | 55·04 | 78·38 | 2·18 | + | + | |
| 3 | 10 | 20 | 53·35 | 68·10 | 1·79 | — | + | All precipitates were found to be increasingly more and more insoluble as the concentration of alcohol increased. |
| 4 | 10 | 30 | 41·59 | 75·65 | 1·89 | — | + | |
| 5 | 10 | 40 | 38·90 | 65·49 | 2·02 | — | + | |
| 6 | 10 | 50 | 44·11 | 69·26 | 2·40 | — | + | |
| 7 | 10 | 60 | 44·11 | 58·33 | 1·78 | — | + | |
| 8 | 10 | 70 | 40·93 | 72·40 | 2·15 | — | + | |
| 9 | 10 | 80 | 38·06 | 56·11 | 2·14 | — | + | |
| 10 | 10 | 90 | 37·09 | 55·07 | 3·14 | — | — | |

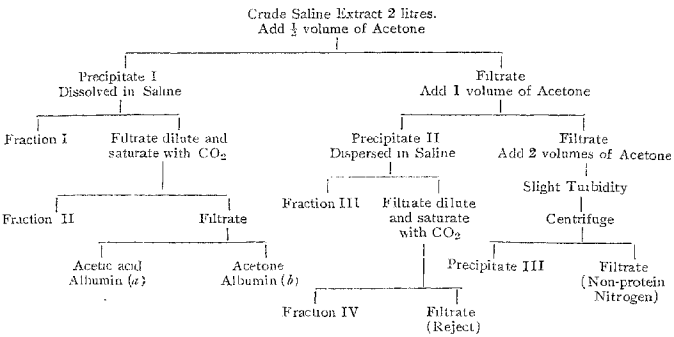
TABLE V.

*Aconite Bean Globulins (Acetone Series)*

| Concentration | Saline Ext c.c. | Acetone c.c | Percentage of N in the filtrate | Percentage of Amino N in the filtrate | Complexity | Trichloracetic acid | Tyrosinase Reaction | Solubility of the precipitate in saline |
|---|---|---|---|---|---|---|---|---|
| 0 | 10 | 0 | 100·00 | 100·00 | 2·08 | +++ | +++ | |
| 1 | 10 | 5 | 47·18 | 74·29 | 1·42 | + | ++ | |
| 2 | 10 | 10 | 41·52 | 62·00 | 1·30 | + | + | |
| 3 | 10 | 20 | 38·76 | 68·19 | 1·27 | — | + | All the precipitates were found to be easily and almost completely soluble in striking contrast to the insolubility of the precipitates obtained in the alcohol series. |
| 4 | 10 | 30 | 34·28 | 39·66 | 1·80 | — | · | |
| 5 | 10 | 40 | 31·45 | 35·05 | 1·87 | — | · | |
| 6 | 10 | 50 | 23·03 | 29·88 | 1·61 | — | · | |
| 7 | 10 | 60 | 18·79 | 26·87 | 1·46 | — | · | |
| 8 | 10 | 70 | 17·98 | 21·70 | 1·73 | — | — | |
| 9 | 10 | 80 | 17·66 | 19·54 | 1·80 | — | — | |
| 10 | 10 | 90 | 16·28 | 17·53 | 1·94 | — | — | |

A study of the two tables reveals many interesting facts. In the first place, acetone is a more efficient precipitant of the nitrogenous bodies than alcohol. At the final concentration of the solvent employed, about 37 per cent. of the total nitrogen remains over in the filtrate in the case of alcohol, while in the case of acetone only 16 per cent. is left over in the filtrate. Another important observation is that the precipitates obtained by acetone are more easily and more completely soluble than the precipitates obtained by the additions of alcohol. It is, therefore, to be concluded that both from the point of view of efficiency of precipitation and quality of the resulting precipitate, acetone commends itself and in the present investigation, it was accordingly employed for the fractionations. Table IV gives a schematic representation of the procedure employed for the fractionation of the globulins of the aconite bean with acetone.

TABLE VI.




The precipitates obtained were centrifuged and, after washing with water, were desiccated as described before. The residual filtrate was concentrated *in vacuo* and was treated for the non-protein nitrogen.

(*iii*) *Fractionation with Ammonium Sulphate.*—The globulin extract was treated with calculated amounts of finely ground ammonium sulphate raising the concentration of the salt, in stages to 1/3, ½, ¾, and full saturation. The globulins yielded fractions only at ¾ and full concentrations of the salt. The precipitates obtained at each stage were centrifuged, dissolved in water and dialysed in collodion bags against distilled water for about 3 weeks till they were free from ammonia. The preparations were then desiccated in the usual manner.

## ANALYSIS OF THE FRACTIONS AND DISCUSSION.

The next step in the investigation was to characterise these fractions. They were first analysed for their purity, moisture, ash, and total nitrogen contents. These results are given in Table VII.

TABLE VII.

*As percentages on the Weight of the Preparation.*

| | Fractions by | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dilution | | | Acetone | | | | $Am_2SO_4$ | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 4 | 1 | 2 |
| Moisture | 10·35 | 13·85 | 13·11 | 10·91 | 9·20 | 11·53 | 9·00 | 16·73 | 15·75 |
| Ash | 0·34 | 0·31 | 0·10 | 1·19 | 0·81 | 0·65 | 0·67 | — | 0·23 |
| Total nitrogen (Ash & moisture free) | 16·58 | 17·26 | 15·87 | 13·36 | 15·97 | 15·10 | 16·65 | 14·98 | 15·53 |
| Yield in gms | 1·20 | 6·50 | 1·70 | 1·00 | 6·50 | 0·70 | 1·50 | 6·0 | 5·0 |

The globulin fractions were then analysed for their essential amino acid (*e.g.*, cystine, tyrosine and tryptophane) content by the colorimetric methods of Folin and Merenzi (*J. Biol. Chem.*, 1929, **83**, 89, 103). Table VIII incorporates the results.

TABLE VIII.

*Essential Amino Acids of the Globulin Fractions of Aconite Bean (as percentages of total nitrogen).*

| | Fractions by the method of | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dilution | | | Acetone | | | | Ammonium sulphate | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 4 | 1 | 2 |
| Cystine | 0·60 | 0·34 | 0·40 | 1·28 | 0·69 | 2·59 | 0·74 | 0·54 | 0·44 |
| Tyrosine | 2·22 | 2·02 | 2·17 | 2·18 | 2·91 | 3·80 | 2·61 | 2·05 | 1·92 |
| Tryptophane | 0·42 | 0·39 | 0·35 | 0·43 | 0·36 | 0·71 | 0·33 | 0·42 | 0·33 |

It will be seen from Table VIII that if the values for cystine and tryptophane are taken into consideration, a definite fractionation has been obtained both by the method of dilution and saturation with ammonium sulphate. In the case of the acetone fractions, it will be observed that a more complete fractionation has been accomplished. These results require further confirmation and the work will have to be extended to the fraction of the purified total globulins. At present

the acetone method appears to offer the most efficient means of fractionating proteins.

## SUMMARY AND CONCLUSIONS.

1. The methods for fractionating globulins by dilution, salt saturation and precipitation with organic solvents, are described and a schematic representation of some of the methods is given in tables.

2. The globulins of the aconite bean have been subjected to three different methods of fractionation : (1) progressive salt saturation; (2) dilution; and (3) graded additions of acetone. The analytical data relating to these fractions obtained by all methods show that a definite separation has been accomplished. Of the three, the acetone method appears to offer the most efficient means of fractionation.

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