STUDIES IN THE PROTEINS OF INDIAN FOODSTUFFS.

PART X. *'IN VITRO'* DIGESTIONS OF THE GLOBULINS FROM ACONITE BEAN *(P. ACONITIFOLIUS,* JACQ.) AND BENGAL GRAM *(CICER ARIETINIUM,* LINN.).

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In a previous communication (*J. Ind. Inst. Sci.*, 1936, 19A, 9), the relative digestibilities of the two globulins from *P. aconitifolius* and *Vigna catiang* as determined by the dilatometric method has been described. It has been shown that there is a proportionality between the dilatometric depressions and the corresponding values for amino nitrogen released during the digestion of these globulins. The present investigation relates to the comparative study of the *in vitro* digestions of the globulins from aconite bean and Bengal gram by enzymes (trypsin and pepsin) with a view to determining the rate of liberation of certain amino acids. Such studies provide us with valuable information with regard to the true availability of the amino acids from a particular protein in contrast to the purely chemical analysis which gives an idea of its total amino acid make up, all of which may not be available to the organism.

EXPERIMENTAL.

Tryptic digestion.—The globulins were prepared by the method of dilution and dissolved directly into Sorensen's phosphate buffer of pH 7.7. After a preliminary determination of the total nitrogen, the solutions were so diluted with the buffer, that 5 c.c. contained 11.961 mg. of nitrogen. Casein solution was prepared by dissolving casein (prepared by the author) in phosphate buffer of pH 7.7 and its nitrogen concentration adjusted to that of the globulin solutions.

The enzyme solution was prepared by dissolving 2.5 gm. of Pfansteihl's trypsin-kinase in 250 c.c. of phosphate buffer (pH 7.7). The solution was filtered and its nitrogen content determined.

The reaction mixture consisted of 700 c.c. of protein solution *plus* 70 c.c. of enzyme solution. The digestion was carried out at 30°C. Aliquots of 75 c.c. were pipetted at different intervals of time and treated with 15 c.c. of 10 per cent. trichloracetic acid (by preliminary trials, this amount was found to be sufficient) in order to precipitate the undigested protein. On filtering, clear filtrates were obtained. The filtrate at zero time was obtained by mixing the protein solution with pre-heated enzyme solution and by removing the proteins with trichloracetic acid.

Peptic digestion.—Globulins of aconite bean and Bengal gram prepared by the method of dilution, were dissolved in 1 : 14 phosphoric acid (87 per cent.) to yield a solution containing 11.961 mg. of nitrogen per 5 c.c. and the solution was found to have a pH of 2.0.

The enzyme solution was prepared by dissolving 2.0 gm. of Pfansteihl's pepsin in 200 c.c. of water. Nitrogen was determined on 5 c.c. aliquot.

75 c.c. of the protein solution was pipetted into 7 test-tubes and 7.5 c.c. of pepsin added. The digestion was allowed to proceed at 30°C. At different intervals of time, the contents of the test-tube were mixed with 10 c.c. 7.5 per cent. sodium hydroxide, in order to precipitate the undigested protein, warmed on water-bath for 10 minutes and filtered. Clear filtrates were obtained.

The clear filtrates obtained from tryptic and peptic digestions were employed for the determination of :—

(1) *Total nitrogen.*—Total nitrogen was determined on 5 c.c. aliquots (Table I). The percentage protein dissolved at various intervals of time was calculated. The results are represented graphically in Fig. I.

TABLE I.

Mgms. of nitrogen per 5 c.c. filtrate.

Time	Trypsin			Pepsin	
	Aconite bean	Bengal gram	Casein	Aconite bean	Bengal gram
10 mins.	0·4161	0·5210	2·1370	6·0950	6·5870
20 ,,	0·5199	0·6236	3·9370	6·3910	6·9310
40 ,,	0·7285	0·6754	6·6870	7·0780	7·0790
60 ,,	0·9369	0·7803	7·5560	7·2270	7·2250
2 hrs.	1·3010	0·8333	9·0020	7·3250	7·3240
4 ,,	1·8740	1·2490	9·8940	..	..
12 ,,	3·4360	1·7170	..	..	..
24 ,,	4·9960	2·3430	..	..	..
Total nitrogen in reaction mixture ..	11·38	11·38	11·42	11·29	11·29

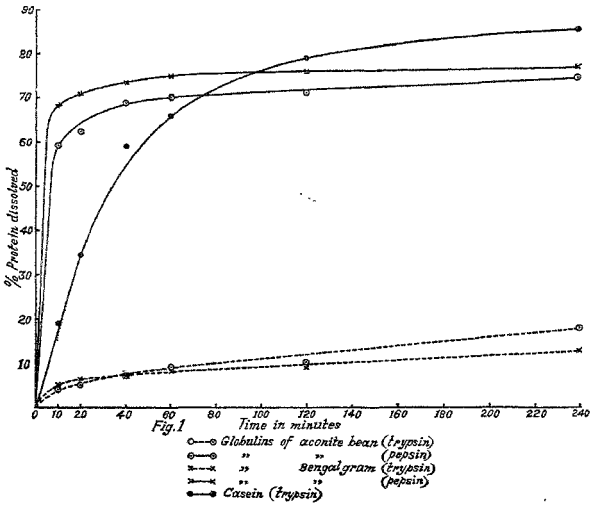

Fig. 1

(2) *Amino nitrogen and arginine.*—15 c.c. of the filtrates were neutralised with sodium hydroxide to the same tint to phenolphthalein and the solution was made up to 25 c.c. 5 c.c. each were employed for the determination of amino nitrogen and arginine.

(*a*) *Amino nitrogen.*—Amino nitrogen was determined by the method of Sorensen (*Biochem. Z.*, 1910, **25**, 1). Table II incorpo-

TABLE II.

Mgms. of NH_2 nitrogen released per 5 c.c. reaction mixture.

Time	Trypsin			Pepsin	
	Aconite bean	Bengal gram	Casein	Aconite bean	Bengal gram
10 mins.	0·0634	0·063	0·1839	0·2159	0·1918
20 ,,	0·0762	0·077	0·3787	0·4322	0·3359
40 ,,	0·1401	0·0896	0·7358	0·4679	0·3599
60 ,,	0·1652	0·1148	0·8606	0·4920	0·4081
2 hrs.	0·3049	0·1904	1·137	0·5040	0·5158
4 ,,	0·5210	0·2674	1·364	0·5040	0·5158
12 ,,	0·8004	0·3682	..	..	..
24 ,,	1·271	0·5461	..	..	..

rates the results of the amino nitrogen in mgm. per 5 c.c. reaction mixture. The experimental values are represented graphically (Fig. II) as c.c. of N/100 alkali.

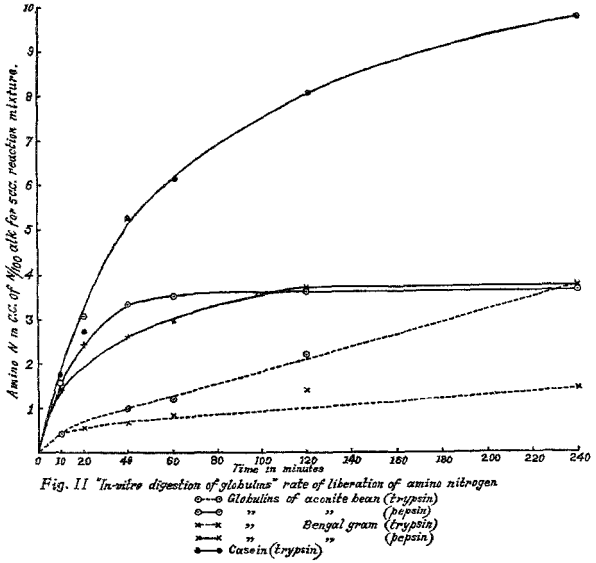

Fig. II "In-vitro digestion of globulins" rate of liberation of amino nitrogen

(*b*) *Arginine.*—Arginine was estimated by the enzyme method employing integrally pure arginase solution. To 5 c.c. of neutralised filtrate, 2 c.c. of phosphate buffer pH 8.0 and 1 c.c. of arginase solution was added, the reaction was allowed to proceed at room temperature for 24 hours. 2 c.c. of 20 per cent. neutral formalin was then added and the liberated ornithine was titrated against standard sodium hydroxide. It may be mentioned that for each filtrate a control was run in exactly similar manner, but with the difference, that the enzyme solution was added after the addition of formalin. The results calculated as the percentage of the total arginine (as determined by Van Slyke's method by Niyogi and others, *Ind. J. Med. Res.*, 1932, **19**, 1041) (for the two globulins and for casein the value obtained by the author) of the respective proteins, and they are graphically represented in Fig. III.

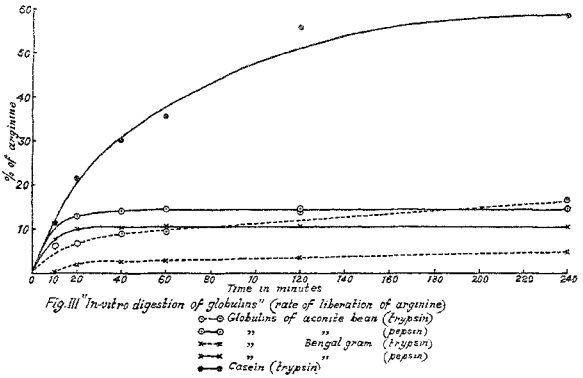

Fig. III "In-vitro digestion of globulins" (rate of liberation of arginine)

(3) Tyrosine and tryptophane could not be determined by the colorimetric method of Folin and Merenzi (*J. Biol. Chem.*, 1929, **87**, 83) owing to the presence of chlorine in the tryptic hydrolysates. A qualitative determination of tyrosine by tyrosinase in tryptic and peptic hydrolysates showed that the rate of liberation of tyrosine from the globulins of aconite bean is approximately twice that of globulins of Bengal gram.

(4) *Diketopiperizine rings.*—The filtrates from peptic and tryptic hydrolysates were qualitatively tested for the presence of diketopiperizine rings with *m*-dinitrobenzoic acid and slight warming on the water-bath (Abderhalden and Komm, *Hoppe-Seylers*, 1924, **140**, 99). Peptic hydrolysates of the two globulins developed an intense rose red colour on the addition of *m*-dinitrobenzoic acid; the tryptic hydrolysates of the globulins of aconite bean behaved in the same way, while the tryptic hydrolysate of Bengal gram globulins did not develop any colour, thus indicating (1) absence of diketopiperizines, (2) presence of resistant diketopiperizines. With a view to find out whether any resistant diketopiperizines are present, the solutions were heated on flame, no colour developed; an equal volume of 20 per cent. sodium hydroxide was added and again strongly heated: an intense rose red colour appeared, thus showing the presence of resistant diketopiperizines.

(5) *Optical rotations.*—Optical rotations of the filtrate were determined by employing a 20 cm. tube. Table III gives the fall in rotation per gram molecule of nitrogen dissolved.

TABLE III.

Fall in rotation per gram mol. of nitrogen dissolved.

Time	Trypsin			Pepsin	
	Aconite bean	Bengal gram	Casein	Aconite bean	Bengal gram
10 mins.	4·7	2·4	4·57	4·50	3·7
20 ,,	3·2	2·3	4·56	4·60	3·8
40 ,,	1·9	3·10	4·32	4·30	3·8
60 ,,	2·5	2·7	4·35	4·20	3·7
2 hrs.	2·2	..	4·18	4·10	3·6
4 ,,	1·9	2·1	3·88	3·90	3·6
12 ,,	1·4	2·0	..	..	..
24 ,,	1·4	2·0	..	..	..

SUMMARY AND CONCLUSION.

1. A close study of the graphs and tables reveals that casein shows a higher rate of digestibility by trypsin alone as compared with the two globulins.

2. The rate of digestion with pepsin appears to be very much greater than the rate at which the globulins are digested by trypsin.

3. The globulins of aconite bean are digested at a higher rate than the globulins of Bengal gram.

4. The products liberated during peptic digestion of the aconite bean globulins are simpler than those obtained from the globulins of Bengal gram (as determined by the ratio of total to amino nitrogen).

5. The globulins of Bengal gram contain a comparatively higher percentage of arginine (20.22 per cent.; aconite bean, 15.73 per cent., as determined by Niyogi and others by Van Slyke's method) than the globulins of aconite bean, the percentage of available arginine in the latter being much more than in the former. Casein (7.4 per cent. arginine as determined by the author) shows an exceptional character

with regard to rate of liberation of arginine—about 58 per cent. of its total arginine appears to be easily split off within four hours. These results are in confirmation with the findings of Dauphinee and Hunter (*Biochem. J.*, 1930, **24**, 1126).

6. The mode of attack of pepsin and trypsin on the globulins of Bengal gram appears to be different in that, the former gives rise to easily hydrolysable, while the latter to resistant diketopiperizines.

7. There is a steady fall in rotation in the case of aconite bean series, while in the case of Bengal gram, the corresponding values are practically steady, and this appears to suggest that the amino acid make up of the hydrolysates at different stages of reaction is the same in the case of Bengal gram.

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