STUDIES IN THE PROTEINS OF INDIAN FOODSTUFFS.

PART IX. DIGESTIBILITY OF THE GLOBULINS FROM COWPEA (*VIGNA CATIANG*, WALP.) AND ACONITE BEAN (*P. ACONITIFOLIUS*, JACQ.)

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In a previous communication (*Biochem. J.*, 1935, 29, 291), the relative digestibility of the two globulins from *Dolichos lablab* and *Phaseolus mungo*, has been determined by the dilatometric method which has now been extended to a more detailed study of the digestibility of the globulins of the cowpea and the aconite bean as compared with that of casein.

EXPERIMENTAL.

Globulins prepared by the method of dilution were directly dissolved in Sorensen's phosphate buffer, pH 7.7. After a preliminary determination of the total nitrogen, the nitrogen value of the solution was adjusted by dilution to yield a substrate containing 469.0 mgms. of nitrogen per 100 ml. The casein solution was prepared by dissolving Hammerstein's casein previously ground and wetted with water, in phosphate buffer of pH 7.7. Slight warming to 40°C. facilitated solution. An aliquot was employed for the determination of total nitrogen and the solution was then adjusted to the same concentration of nitrogen by adding the requisite amount of the phosphate buffer.

The enzyme solution was prepared by dissolving 2.5 gms. of a trypsin-kinase preparation (Pfansteihl's) in 100 ml. of phosphate buffer (pH 7.7), the solution was filtered and the nitrogen value of the filtrate adjusted to yield a solution containing 11.23 mgms. of nitrogen per 5 ml. of the enzyme extract, so as to agree with the concentration of enzyme employed in our previous experiments (*Proc. Ind. Acad. Sci.*, 1935, 2B, 316).

The reaction mixture for the dilatometer, consisted of 50 ml. of the substrate and 5 ml. of the enzyme and the reaction was independently carried out in a separate flask, from which aliquots, at definite intervals of time were drawn and the amino nitrogen estimated by Linderstrom Lang's acetone titration method. The experimental values are represented graphically in Figs. 1 and 2. Table I gives the values of the

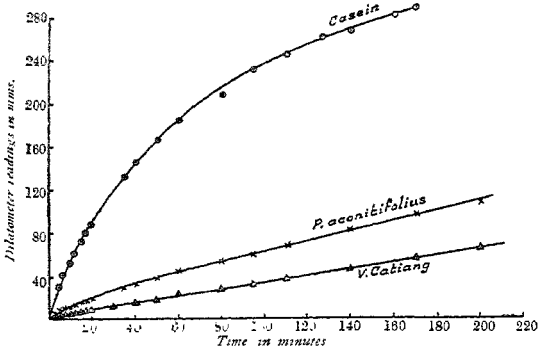

FIG. 1. "In-vitro" digestion.

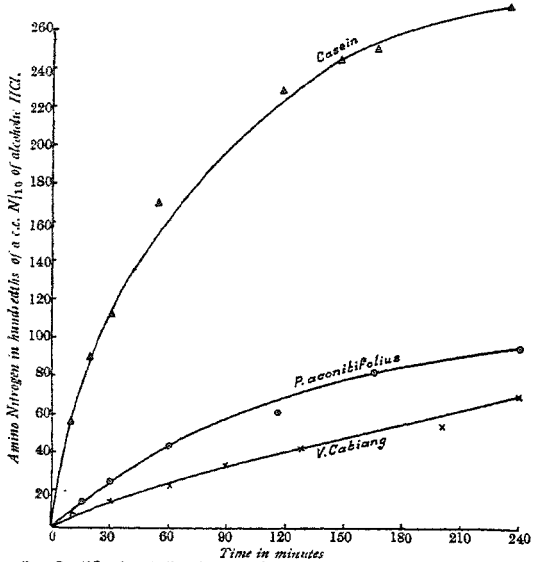

FIG. 2. "In-vitro" digestion of globulins of *P. aconitifolius* and *V. catiang*

dilatometric depressions in μl for casein, the globulins of cowpea and those of aconite bean, while Table II incorporates the results of the amino nitrogen in mgms. per equivalent amount of the reaction mixture as obtains in the dilatometer. The values for the dilatometric depressions given in Table I are, therefore, comparable with the values for the amino nitrogen given in Table II.

TABLE I.

Dilatometer readings in μl.

Time in minutes	10	20	30	60	90	120	150	180	210	240
Casein	6·05	10·86	14·60	21·91	26·29	29·68	33·71	34·19	.	..
Cowpea	0·50	1·10	1·60	2·70	3·70	4·60	5·66	6·60	7·60	12·90
Aconite bean	1·60	2·70	3·30	5·30	6·95	8·50	10·05	11·60	12·90	14·30

TABLE II.

In milligrams of amino nitrogen per 55 ml. of the reaction mixture.

Time in minutes	10	20	30	60	90	120	150	180	210	240
Casein	8·90	13·28	14·70	16·08	23·21	30·38	36·51	37·96	..	..
Cowpea	0·80	1·40	1·80	2·90	3·95	5·00	6·00	7·10	8·15	9·20
Aconite bean	1·80	3·00	3·95	5·85	7·50	8·75	10·20	11·60	12·95	14·30

DISCUSSION.

The correlation existing between the two methods of following the digestion by the *in vitro* method, is shown in Figs. 3 and 4, which clearly indicate that there is a strict proportionality between the two methods.

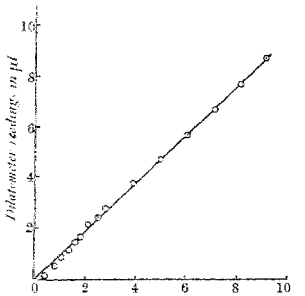

FIG. 3. Correlation graph for *V. catung.*

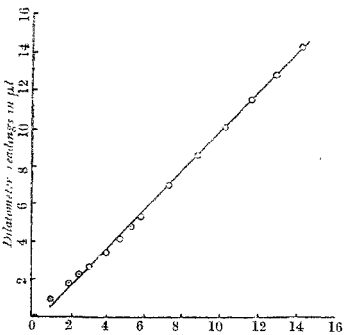

FIG. 4. Correlation graph for *P. aconitifolius.*

The relative digestibility of a given protein with reference to a standard like casein is given by either

$$(1)\quad K_1 = \frac{NH_2 \text{ in mgms. (Protein)}}{NH_2 \text{ in mgms. (Casein)}} \times 100.$$

or

$$(2)\quad K_2 = \frac{\text{Dilatometric depression in } \mu l \text{ (Protein)}}{\text{Dilatometric depression in } \mu l \text{ (Casein)}} \times 100.$$

Table III gives the values for K_1 and K_2 for different intervals of time.

TABLE III.

Time in hours		2	3	4
Aconite bean	K_1	28·5	33·7	33·8
	K_2	28·8	30·6	30·6
Cowpea	K_1	15·4	18·9	20·7
	K_2	16·5	18·7	22·9

A close study of the data, the graphs and the tables, reveals that the globulins of the aconite bean are digested at a much higher rate and in a much shorter time than the globulins of the cowpea. From Table III it will be seen that the proteins of the aconite bean are nearly 1.7 times more digestible than those of the cowpea.

PEPTIC DIGESTION OF THE GLOBULINS.

When the digestibility of these pulse globulins are, however, compared with casein, they are found to have very low values; it is, however, possible that when the proteins are first subjected to the action of pepsin, and then to tryptic digestion, the globulins may really be found to possess a higher digestibility. Although for purposes of comparison, the study of the action of any one enzyme on a particular protein, may be sufficient to evaluate its digestibility, for obtaining an idea strictly comparable with *in vivo* conditions, the protein has got to be subjected to the same sequence of enzyme action as obtains under natural conditions. The globulins were, therefore, subjected to the successive actions of pepsin and trypsin, to determine if a higher digestibility than that obtained by the direct action of trypsin, can be obtained.

Globulins of cowpea and aconite bean, prepared by the method of dilution, were dissolved in 1 : 14 phosphoric acid (87 per cent.) to yield a solution containing 469 mgms. of nitrogen per 100 ml. and the solution was found to have a pH of 2.0. The course of digestion was followed not only dilatometrically but also by titrations of the reaction mixture at definite intervals of time by the method of Linderstrom Lang. In a few cases, the course of digestion was followed viscosimetrically, employing Ostwald's (5 ml.) capillary Viscosimeter.

The peptic digest, after 4 hours of reaction, was neutralised with the requisite amount of sodium hydroxide so as to adjust the reaction of the mixture to a pH of 7.7. An aliquot, equivalent to the amount of protein employed for the peptic digestion, was subjected to the further action of trypsin. A control dilatometer experiment with the same amount of pepsin, buffer and trypsin, as was employed in the above series of digestion experiments, did not record any volume change, showing thereby that the protein constituting the pepsin does not vitiate the subsequent results of tryptic digestion.

DISCUSSION.

The experimental values for dilatometer are plotted in Fig. 5, while the values for the release of amino nitrogen are illustrated in Fig. 6.

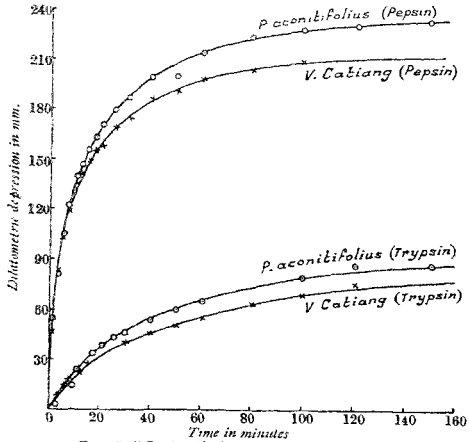

FIG. 5. " In-vitro " digestions of some globulins.

The titrations in the case of cowpea series during the peptic and tryptic digestions, could not be carried out since voluminous precipitates resulting on the addition of alcoholic hydrochloric acid and acetone, interfered with the attainment of any sharp end point. Table IV gives the dilatometric depressions in μl for the two globulins when subjected to peptic and tryptic digestions while Table V gives the titration values

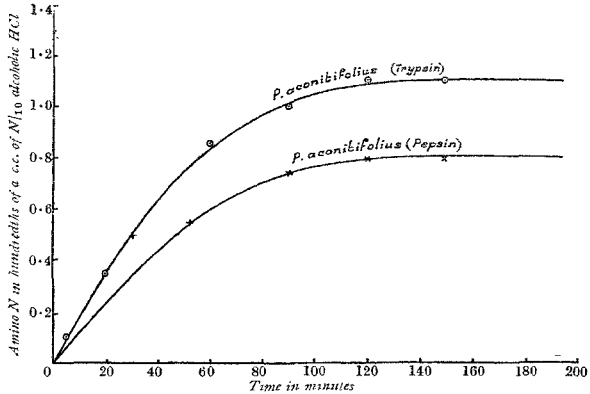

FIG. 6. "In-vitro" digestion of the globulins of *P. aconitifolius*.

for aconite bean digestions, calculated for the same amount of the reaction mixture (55 ml.) as obtains in the dilatometer.

TABLE IV.

Dilatometric Depression in μl.

Time in minutes		10	20	30	60	80	100	120	150
Pepsin	Cowpea	16·20	19·00	21·01	23·30	23·98	24·69	24·17	..
	Aconite bean	16·52	19·70	22·33	25·09	26·40	26·94	27·18	27·18
Trypsin	Cowpea	3·03	3·92	4·87	6·61	7·66	8·19	9·23	9·44
	Aconite bean	2·49	4·51	5·34	7·60	..	9·26	..	10·21

TABLE V.

(*Aconite Bean*)

Mgms. of NH_2 nitrogen released per 55 ml. of reaction mixture.

Time in minutes	30	60	90	120	150
Pepsin	5·90	8·80	10·92	11·65	11·65
Trypsin	7·79	13·55	16·35	17·28	17·28

It will be seen from the graphs and the Tables IV and V that when the globulins are subjected to the successive actions of pepsin and trypsin, higher digestibilities are recorded for both the proteins, those of aconite bean, however, still maintaining a higher value with regard to its digestibility. These results emphasise the importance of maintaining strict *in vitro* sequence of action, in the course of *in vitro* digestion studies, if one is to evaluate the true digestibility of proteins.

By this mode of successive action, the digestibility of the cowpea globulins is increased seven times, while that of aconite bean proteins is increased only about four times. This shows that the aconite bean globulins are capable of being degraded to a greater extent by the independent action of trypsin alone, as in the case of casein. The ease and completeness with which a given protein can be degraded by the independent and exclusive action of trypsin, is a valuable property and is useful in feeding invalids whose peptic activity is impaired for some reason or other.

VISCOSIMETRIC MEASUREMENTS.

The course of peptic digestion of the globulins of cowpea and aconite bean, has also been followed in the viscosimeter. The results are plotted in Fig. 7, which shows that in both cases, the earlier fall

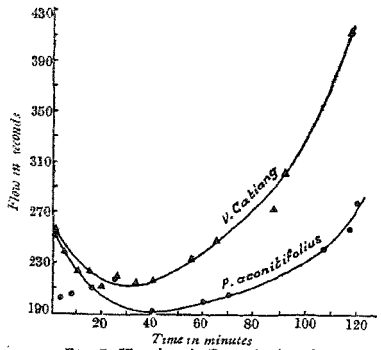

FIG. 7. Viscosimetric Determination of the digestibility of globulins by pepsin.

in viscosity is followed by a steeper rise in the later stages of the digestion. An explanation of this anomalous but consistent behaviour is

to be found in the fact that the peptic digestion first brings about a disaggregation of the globulins and, therefore effects a fall in viscosity. As the digestion proceeds, however, the reaction mixture can be observed to become more and more turbid, and finally an actual precipitate is formed in both cases. In the case of cowpea, however, the amount of the precipitate thus formed was very much larger and this fact is borne out by the higher rises of viscosity recorded (Fig. 7) for this protein during its peptic digestion.

If we compare the earlier part of the two curves (Fig. 7), we find that the aconite bean proteins record a higher fall in viscosity, showing thereby a higher digestibility. Even the later part of the curve which rises up again, points to the same conclusion. The digestibility curve for cowpea records a very much steeper rise, showing that the amount of the insoluble peptones, is very much higher in this case than in the case of the globulins of the aconite bean. The separation of heavy precipitates during the peptic digestion of vegetable proteins is a factor of great significance which should be taken into account in assaying the digestibility of proteins.

SUMMARY AND CONCLUSIONS.

1. The digestibilities of the total globulins of cowpea and aconite bean, have been studied by physical and chemical methods.

2. The experiments show that there is a proportionality between the dilatometric depressions and the corresponding values for amino nitrogen released during the digestion of these globulins.

3. The digestion experiments have been extended to a study of the successive action of pepsin and trypsin; and it is found that when the globulins are subjected to the natural sequence of enzyme action, they record higher digestibilities, than when they are subjected to the direct action of trypsin alone.

4. For obtaining a better idea of the digestibility of a particular protein, it is suggested that the latter method of subjecting the protein to the successive actions of pepsin and trypsin, be adopted.

5. The exceptional character of casein with regard to its high digestibility irrespective of the natural sequence of action of two enzymes or the direct action by trypsin, is noteworthy.

6. Experiments definitely and consistently record a higher digestibility for the globulins of aconite bean and the viscosimetric data

relating to the course of digestion of the two globulins, indicate that the cowpea globulins, during the peptic digestion, liberate an indigestible residue in a much greater proportion. It is suggested that in assaying the digestibility of proteins the amount of insoluble residues which result during their digestion may be taken into consideration.

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