XXXVI. DILATOMETRIC DETERMINATION OF THE RELATIVE DIGESTIBILITY OF PROTEINS.

By HEMMIGE BILIGIRIRANGACHAR SREERANGACHAR and MOTNAHALLI SREENIVASAYA.

From the Department of Biochemistry, Indian Institute of Science, Bangalore.

(*Received November 7th, 1934.*)

Digestibility of proteins is usually determined by feeding tests employing either the method of Mitchell [1924] or the alternative method of Osborne *et al.* [1919]. Experiments with animals are time consuming and cumbersome as compared with the *in vitro* studies carried out by Waterman and his colleagues [Waterman and Johns, 1921; Waterman and Jones, 1921; Jones and Waterman, 1923]. According to them the relative digestibilities of the proteins of a food material can be directly and satisfactorily determined by enzymic digestions *in vitro*.

In vitro studies offer a convenient method of investigating the digestion of proteins and starches as influenced by cooking, addition of salts and other activating or inhibiting agencies. The increased digestibility of phaseolin on cooking [Waterman and Jones, 1921], the relative indigestibility of arachin [Jones and Waterman, 1922], the inhibiting effect of gossypol on the digestibility of cotton seed proteins [Jones and Waterman, 1923], the influence of fats on protein digestion in general [Maughan, 1926] have all been studied by *in vitro* methods. These methods are particularly suitable for investigating the influence of active principles like glucosides and alkaloids on the digestion of proteins.

All *in vitro* experiments involve the enzymic digestion of the material under certain standardised conditions of reaction and temperature, so chosen that they approximate as closely as possible to the condition obtaining in the animal. The course of digestion is followed mostly by chemical methods involving a determination of either the amino-nitrogen released by van Slyke's method or the total nitrogen in the filtrate after removing the undigested protein by precipitants like trichloroacetic and phosphotungstic acids. In a few investigations the rate at which a particular amino-acid is liberated during digestion has been adopted as a measure of digestibility. Jones and Gersdorff [1933] have thus followed the peptic and acid digestions of caseinogen by estimation of the liberated cystine.

The relative digestibility of proteins can also be studied by any of the physical methods whose experimental values are proportional to the extent of digestion. Thus, the dilatometric estimation offers a convenient method of studying the relative digestibility of proteins, since it has been shown that the release of amino-nitrogen is proportional to the volume change accompanying the enzymic digestion [Sreenivasaya *et al.*, 1934]. The quantity of material required for an investigation of this type is comparatively small, the results are quickly and easily reproducible and the digestibility of a number of proteins with reference to that of a standard protein like caseinogen can be simultaneously determined.

Experimental.

The present communication relates to a study of the digestibility of three proteins, gelatin (Gold-label), and the globulins of *Phaseolus mungo* and *Dolichos lablab*, as compared with the digestibility of "Hammersten's casein." Since the principal object of the investigation was to demonstrate the adaptability of the dilatometric method for *in vitro* digestion studies, all the digestions were carried out with Pfanstiehl's trypsin at 30° and at p_H 7·7 employing Sørensen's phosphate buffer.

The globulins were extracted from the respective pulses with 5 % sodium chloride solution, the clear extract was dialysed against distilled water in cellophane bags, and the precipitated globulins were recovered by centrifuging. The wet precipitate was dissolved directly in the phosphate buffer and after a preliminary determination of the protein content (N × 6·25) it was adjusted to yield a 1 % protein solution. 50 ml. of this solution and 10 ml. of a 1 % trypsin solution were employed for the dilatometric determination. The reaction was conducted simultaneously in another flask and the digestion followed independently by a determination of the amino-nitrogen by van Slyke's method at definite intervals. The experimental procedure employed in this study is the differential method fully described in one of our earlier communications (1932).

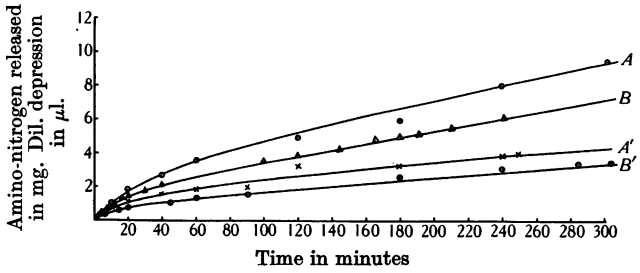

Fig. 1. *A*, *Phaseolus mungo* protein amino-nitrogen; *B*, *Phaseolus mungo* protein dil. depression; *A'*, *Dolichos lablab* protein amino-nitrogen; *B'*, *Dolichos lablab* protein dil. depression.

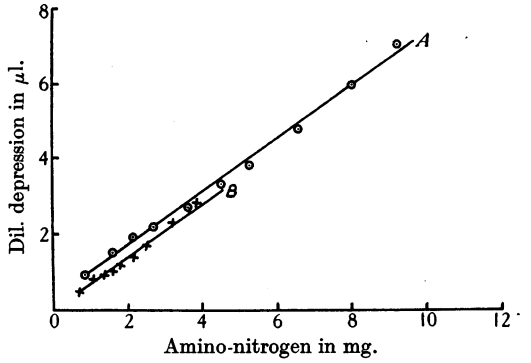

Fig. 2. *A*, *Phaseolus mungo* protein; *B*, *Dolichos lablab* protein.

The course of digestions of the globulins from *Phaseolus mungo* and *Dolichos lablab* has been followed both by the dilatometric and van Slyke's methods and the results are graphically represented in Fig. 1. Fig. 2 gives the correlation

between the dilatometric depression and amino-nitrogen indicating thereby that the dilatometric depressions are proportional to the release of amino-nitrogen during the digestion. That a similar proportionality also exists in the case of caseinogen and gelatin digestions has been established in one of our earlier communications.

Discussion.

If X is the amount of amino-nitrogen that is released during the digestion of a protein (expressed as A g. of total nitrogen) under certain standardised experimental conditions, then the digestibility K of the protein is given by $K = X \times 100$. The digestibilities of caseinogen, gelatin and the *Phaseolus mungo* and *Dolichos lablab* globulins for different intervals of time, are given in Table I.

Table I.

Time in hours ...	1	2	3	4	5
Caseinogen	11·1	14·8	16·9	18·0	18·6
Gelatin	6·7	9·1	10·4	10·9	11·3
P. mungo	5·7	8·3	10·3	12·6	14·5
D. lablab	2·9	4·0	5·1	6·1	6·7

The digestibility appears to increase with time (Table I) reaching a fairly steady value in about 4–5 hours. For comparative studies of digestibility a 4–5 hour period can therefore be fixed as a standard.

Since the dilatometric depressions V have been shown to be proportional to the amino-nitrogen release X during the digestion, the ratio between the digestibility of a protein and that of the caseinogen standard will be equal to the ratio of either the amounts of amino-nitrogen released or the depressions that accompany the digestion. The relative digestibility is therefore given by either

$$K_1 = \frac{\mathrm{NH_2\ (protein)}\ X_1}{\mathrm{NH_2\ (caseinogen)}\ X_2} \times 100$$

or

$$K_2 = \frac{\text{Dil. depression } V_1 \text{ (protein)}}{\text{Dil. depression } V_2 \text{ (caseinogen)}} \times 100.$$

The values of K_1 and K_2 for two periods of digestion are given in Table II and there is a fair agreement between the two values indicating the usefulness and reliability of the dilatometric method in assaying the relative digestibility of proteins.

Table II.

	Gelatin		*P. mungo*		*D. lablab*	
	K_1	K_2	K_1	K_2	K_1	K_2
4 hours	60	58	70	65	34	32
5 hours	60	59	78	74	36	35

The results indicate that *Phaseolus* protein is digested much more easily (about twice) than that of *Dolichos*, and are in agreement with the observations of Desai *et al.* [1931–32] made by feeding tests of these proteins.

Summary.

1. The globulins from *Phaseolus mungo* and *Dolichos lablab* have been subjected to tryptic digestion and the course of reaction followed both by the dilatometric and van Slyke's methods; it is found that the dilatometric depression obtained in each case is proportional to the release of amino-nitrogen.

2. It is shown that dilatometric depressions can be directly taken as a measure of digestibility and that the ratio of the depressions observed with the experimental protein and with the standard caseinogen represents the relative digestibility.

Our best thanks are due to Mr B. N. Sastri for his kind help in the preparation of the globulins and for his many helpful suggestions.

REFERENCES.

Desai, Narayana and Neogi (1931–32). *Indian J. Med. Res.* **19**, 1041.
Jones and Gersdorff (1933). *J. Biol. Chem.* **101**, 657.
Jones and Waterman (1922). *J. Biol. Chem.* **52**, 357.
—— —— (1923). *J. Biol. Chem.* **56**, 501.
Maughan (1926). *Biochem. J.* **20**, 1046.
Mitchell (1924). *J. Biol. Chem.* **58**, 873.
Osborne, Mendel and Ferry (1919). *J. Biol. Chem.* **37**, 223.
Sreenivasaya, Sastri and Sreerangachar (1934). *Biochem. J.* **28**, 351.
—— and Sreerangachar (1932). *J. Indian Inst. Sci.* **15A**, 17.
Waterman and Johns (1921). *J. Biol. Chem.* **46**, 9.
—— and Jones (1921). *J. Biol. Chem.* **47**, 285.