XLVI. DILATOMETRIC STUDIES IN THE PROTEOCLASTIC DEGRADATION OF PROTEINS.

I. TRYPTIC HYDROLYSIS.

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The enzymic digestion of complex substances like proteins is attended by a series of physical and chemical changes each of which proceeds at varying rates at different stages of the reaction. The viscosity change of the protein-protease system, for example, proceeds rapidly in the early stages of the reaction, while the changes in optical rotation or in the concentration of amino-groups during the same period are not correspondingly large. Each of these estimations is thus predominantly a measure of the particular type of change occurring during a definite phase of the reaction.

For obtaining an intimate knowledge of the mechanism of protein hydrolysis, it is necessary not only to utilise all the methods that are now available but also to invoke the aid of new technique which would either reveal changes not recorded by other means or independently confirm those indicated by other methods. The dilatometer, therefore, offers a new method of attack in the investigation of the hydrolytic cleavage of complex bodies in general and proteins in particular.

From the colloid chemical point of view, the degradation of the protein molecule can be looked upon as a process in which the degree of aggregation of the substrate is being diminished, the few colloidal micellae giving rise to several crystalloidal molecules varying in magnitude and composition. This process is accompanied by a disturbance in the water equilibrium of the system. From the chemical point of view the hydrolysis can be considered as a reaction involving the progressive release of amino- and carboxyl-groups.

Each of the above changes brings about a positive or negative volume change, the combined result of which is registered by the dilatometer. In order to resolve and apportion the net dilatometric change to its constituent physical and chemical reactions, the volume change specific to each of them has to be determined. These relationships can be ascertained by following the reaction during that phase of hydrolysis at which the change in question proceeds either predominantly or exclusively. When such a choice of phase is not possible, the object can be achieved by adopting such experimental conditions that one definite reaction proceeds to the practical exclusion of others. One of the possible ways of accomplishing this is to fractionate the enzyme complex into simpler constituents and investigate their action on the protein.

Galeotti [1911] has conducted a dilatometric study of the tryptic hydrolysis of peptone and milk. The reacting fluids are subject to changes in surface tension which would affect the change in the capillary column of the dilatometer. The tryptic digestion of milk would really constitute at least two definite systems, *viz.* milk-fat-lipase and caseinogen-trypsin, so that the observed volume change would not measure any one specific reaction.

While the present investigation was in progress, Rona and Fischgold [1933] have published a paper on the peptic digestion of ovalbumin and caseinogen in which they have tried to correlate the NH_2 release with volume change. A linear relationship was found to exist between the release of amino-groups and the dilatometric depression in the case of ovalbumin; such a relationship, however, was not obtained in the case of caseinogen. The experimental procedure adopted by Rona and his collaborators does not lend itself to a study of the interesting changes which occur during the first thirty minutes of the reaction. With the new type of dilatometer described in one of our earlier communications [1932] it is possible to investigate these changes accompanying an enzymic hydrolysis from its very commencement.

The present communication deals with the dilatometric study of the tryptic hydrolysis of caseinogen and gelatin. The reaction has been followed by an entirely independent method, *viz.* determination of amino-nitrogen by Van Slyke's method.

Materials. Hammerstein's "casein," Gold label gelatin and Pfansteil's trypsin (1 : 75) were the materials employed in the present investigation. All the reactions were conducted at p_H 7·7, employing Sørensen's phosphate buffer, and at a temperature of 30°. Caseinogen and gelatin digestions have been carried out at two different concentrations, and the experimental procedure adopted is similar to that fully described in our previous communication [1932].

Discussion.

The dilatometric fall is expressed in mm.^{3} and the amino-nitrogen in mg. of nitrogen, and the data for each of the systems are graphically represented in Figs. 1 and 2, showing the course of hydrolysis of gelatin and caseinogen as followed by the dilatometer (Fig. 1) and the determinations of the amino-nitrogen (Fig. 2).

The general shape of the two corresponding sets of time-course curves, indicates that the kinetics of tryptic digestion can be followed in the dilatometer as well as by the determinations of amino-nitrogen. The dilatometric method offers a more elegant and less cumbersome technique for studying these reactions; by virtue of the ease and rapidity with which measurements can be conducted, the method possesses the additional advantage that, in the early stages, when enzymic reactions proceed rapidly, a greater number of readings could be obtained.

For the two systems investigated the relation between dilatometric depression and the corresponding increase in amino-nitrogen at different stages of the reaction (calculated from the graphs) is given in Fig. 3. The dilatometric fall in mm.^{3} per millimol release of amino-nitrogen at different time intervals is given in Table I.

A close study of the Table and graphs will reveal the existence of an interesting series of relationships between dilatometric change and release of amino-groups. During the first thirty minutes there is no proportionality between volume change and amino-nitrogen, the dilatometer registering in

a given time a greater or smaller amount of change in proportion to the amino-nitrogen released during the same interval. It will be observed from Table I that the dilatometric depression per millimol release of amino-nitrogen in the case of caseinogen is greater in the initial stages, decreases with

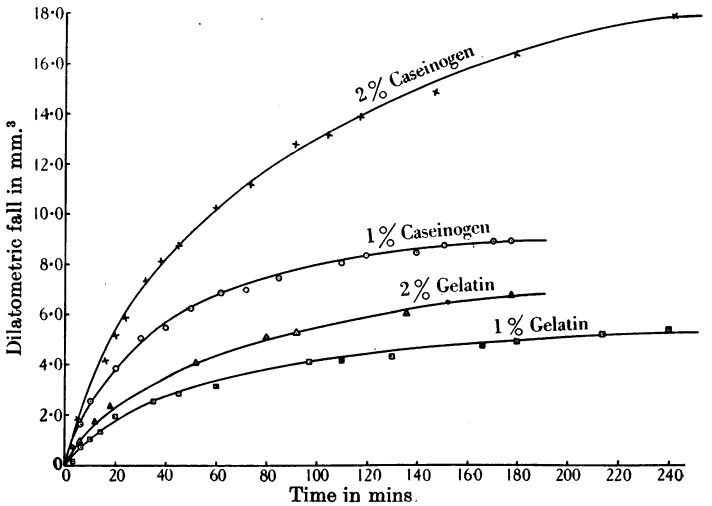

Fig. 1.

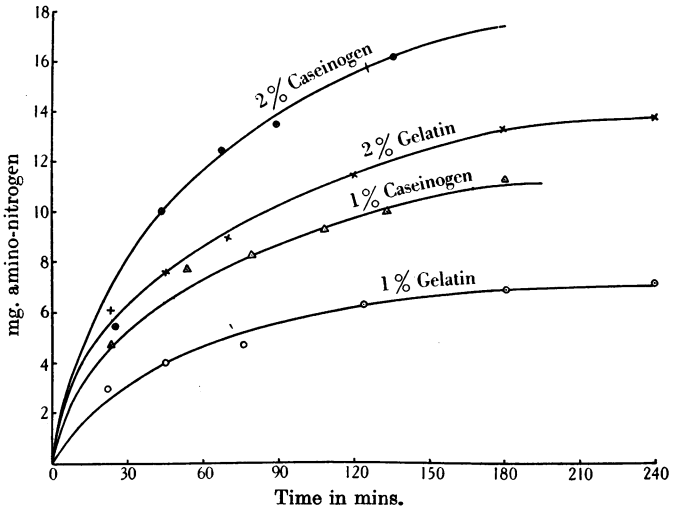

Fig. 2.

the progress of hydrolysis and attains practically a steady value after about 30–40 minutes. In the case of gelatin, however, the ratio increases to a steady value.

This fact is clearly borne out by the graphs (Fig. 3) where an attempt has been made to correlate volume changes with the formation of amino-nitrogen. It will be seen that a strictly linear relationship exists between them after about

Table I.

Time (mins.) ...	10	20	30	40	60	90	120
Dilatometric depression (mm.^{3}) per millimol amino-N.							
Gelatin 1 %	8·8	9·3	9·7	9·8	9·8	9·7	9·7
Gelatin 2 %	6·1	6·5	6·6	6·9	7·1	7·0	7·1
Caseinogen 1 %	13·4	12·6	12·4	12·4	12·3	12·4	11·6
Caseinogen 2 %	16·0	14·8	14·0	13·5	12·8	12·4	12·4

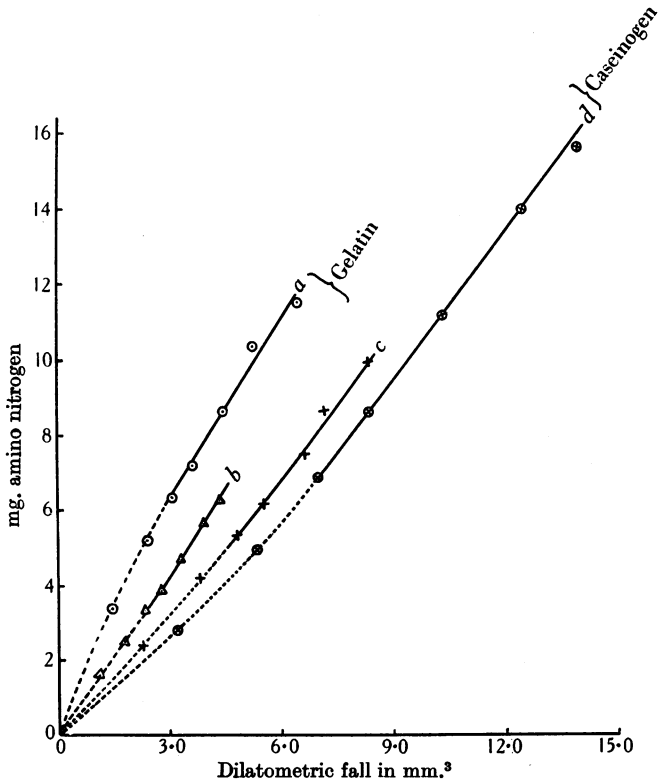

Fig. 3. *a*. 2 % gelatin. *b*. 1 % gelatin. *c*. 1 % caseinogen. *d*. 2 % caseinogen.

30 minutes in the cases both of gelatin and caseinogen. The magnitude of the initial change exclusively recorded by the dilatometer appears to be influenced by the relative concentrations of the enzyme and substrate and is closely connected with the process of liquefaction which generally precedes the rapid cleavage of proteins. A detailed study of this phenomenon is being made.

If the abnormal change occurring in the first stage of the reaction is ignored, a strict proportionality can be observed to exist between the dilatometric depression and the release of amino-nitrogen as shown by Fig. 3. The dilatometric depression per millimol release of amino-nitrogen, which is a constant for each protein, appears to be intimately associated with the structure and amino-acid make up of the protein. Gelatin has a constant of 8·7 while that of caseinogen is 10·8.

SUMMARY.

1. The course of the tryptic digestion of caseinogen and gelatin has been followed in the two-bulbed dilatometer which enables the reaction to be investigated from its very commencement.

2. The early stages of the reaction, during the first 30 or 40 minutes, are accompanied by changes registered by the dilatometer but not indicated by the determinations of amino-nitrogen. After this period, however, the dilatometric depression is proportional to the release of amino-nitrogen in the case of both the proteins.

3. The abnormality occurring in the initial stages of the digestion is closely connected with the process of liquefaction which generally precedes the rapid cleavage of proteins.

4. The dilatometric depression per millimol release of amino-nitrogen has been calculated and found to be 8·7 mm.^{3} in the case of gelatin and 10·8 in the case of caseinogen. This constant appears to be a function of the structure and amino-acid make up of the protein.

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