Letters to the Editor.

Dilatometric Investigations of the Tryptic Digestion of Proteins.

THE kinetics of the tryptic digestion of proteins can be conveniently followed in the two bulb dilatometer (*J. Indian Inst. Sci.*, 15A, 17, 1932) from the very commencement of the reaction. The hydrolysis is generally accompanied by a considerable contraction in volume which, in the case of

per milli mol. release of NH_2 appears to be characteristic of the structure and amino-acid make up of the protein.

The initial stages of tryptic digestion (during the first 30 mins.) appear to be accompanied by an interesting set of changes which are registered by the dilatometer but are not shown by a corresponding increase either in carboxyl or amino groups. There is, therefore, no linear relationship

TABLE.

Substrate concentration, 1 per cent.; P_H of reaction mixture 7.7 ; Concentration of enzyme in reaction mixture, 0.09 per cent.

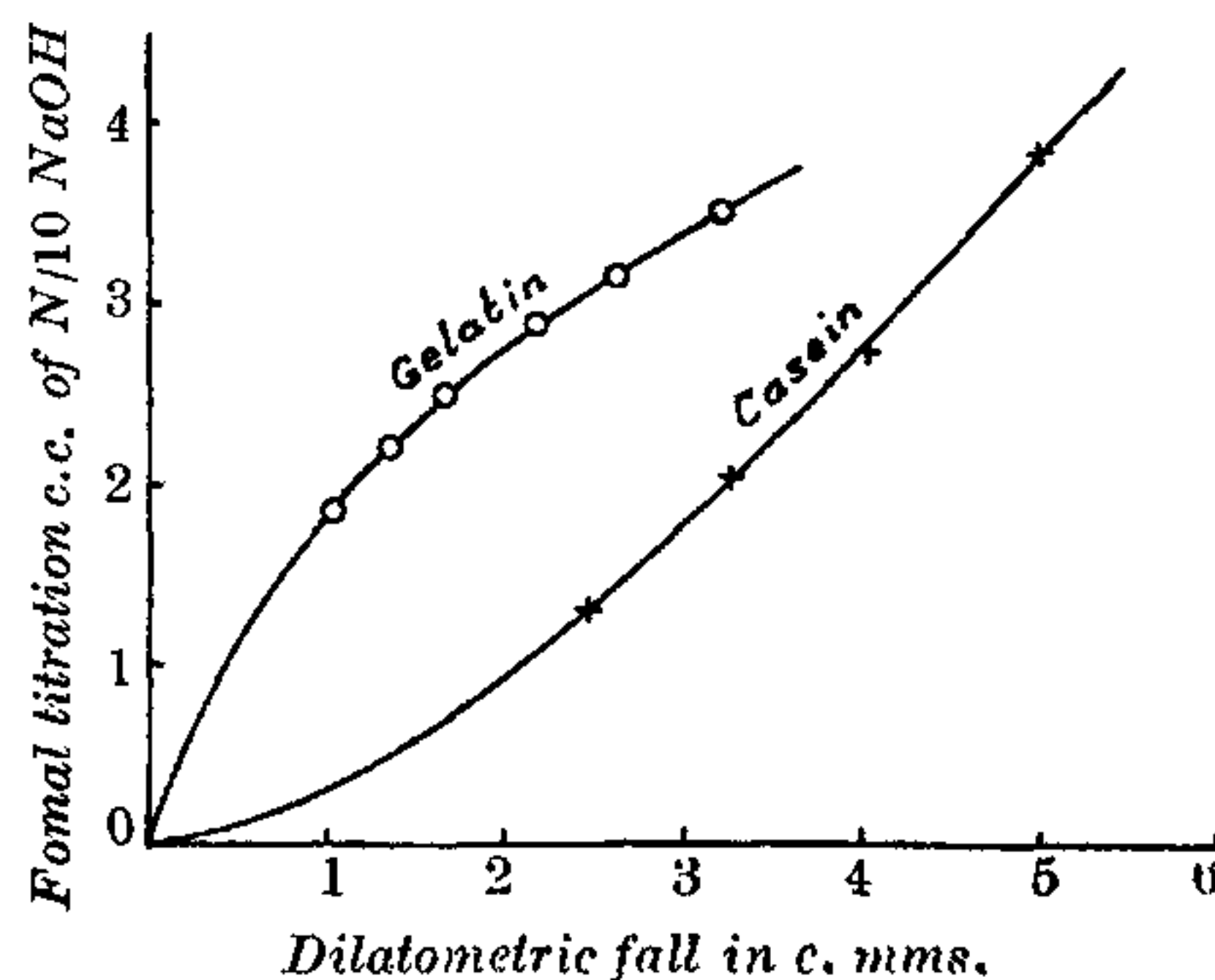|	Casein				Gelatin			
Time in mins.	30	60	90	120	30	60	90	120
Dilatometric depression in c.mm. ..	4·8	6·6	7·7	8·3	2·2	3·2	3·8	4·3
Amino nitrogen increase in mgms. ..	5·4	7·5	8·7	10·0	3·2	4·6	5·6	6·2

casein, amounts to nearly 21·5 c.mm. per gram of material digested in 1 per cent. concentration for 24 hours at 30°C. The volume change is proportional to the release of carboxyl or amino groups (see table and graph I) which are respectively estimated by Willstätter's titration and Van Slyke's gasometric method.

The tryptic hydrolysis of a 1 per cent. casein solution at P_H 7·7 gives a depression of about 12·3 c.mm. per milli mol. of NH_2 released during the hydrolysis, while gelatin under the same set of conditions, gives a contraction of about 9.7 c.mm. per milli mol. of NH_2. The volume change

between the volume change and carboxyl release (see graph II) during the period.

Dilatometric fall in c. mms.
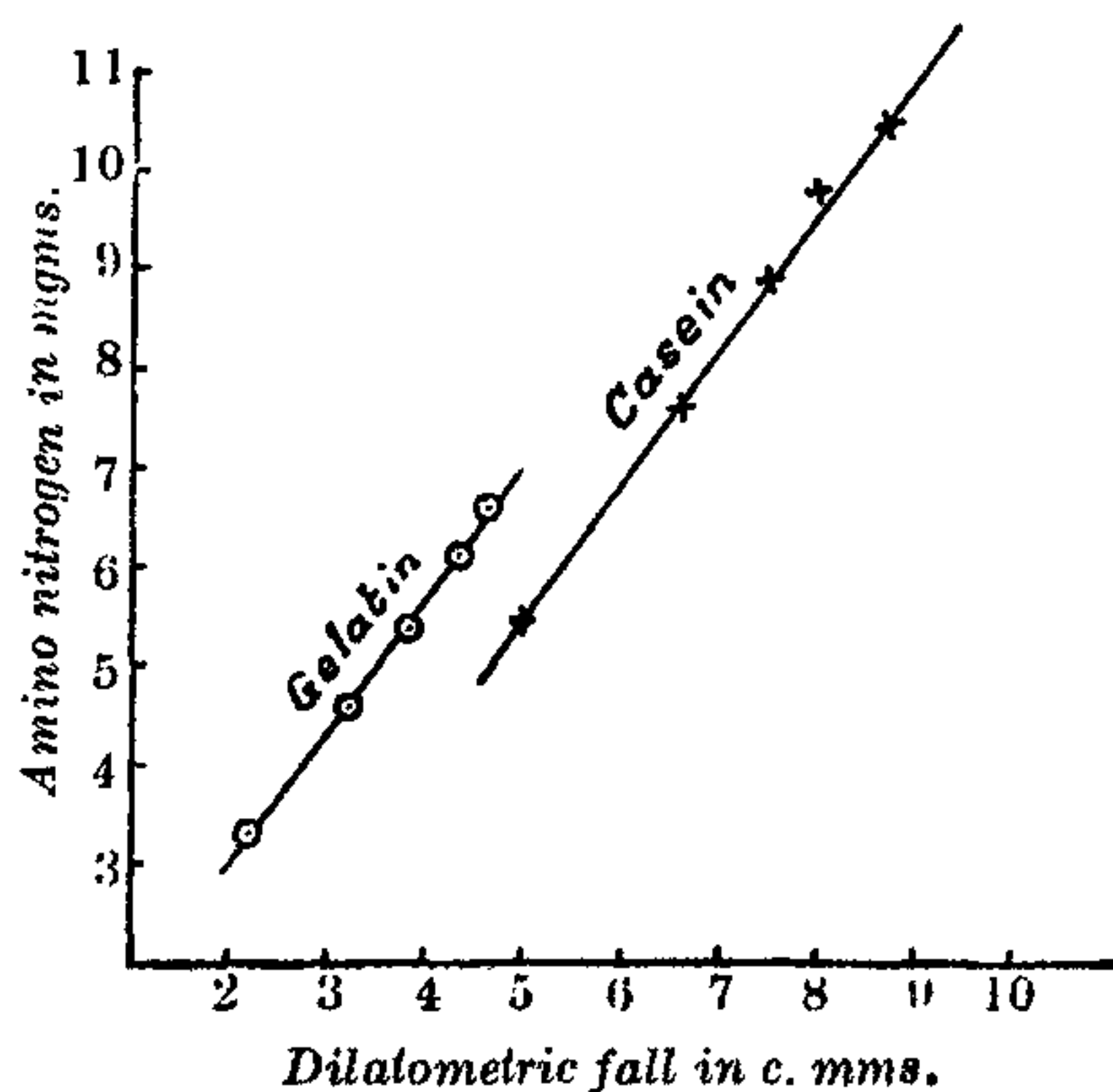
Graph II.

A study of the kinetics of the tryptic digestion of casein and gelatin reveals that casein is split up at approximately double the rate at which gelatin hydrolysis proceeds under comparable conditions. The dilatometer offers a very convenient method of studying the *in vitro* digestibility of various proteins.

By reason of the large change of volume accompanying the hydrolysis, the accuracy attainable by the dilatometric method is much greater than that usually obtained by the chemical methods. As the dilatometric column can be read with an accuracy of 0·5 mm. the error in measurement does not usually exceed 1·5 per cent. while

Dilatometric fall in c. mms.

Graph I.

that involved in Van Slyke analysis is about 4 per cent.

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The Ground Terms and Ionisation Potential of Br II.

IN the course of investigation of the spark spectra of Bromine,* a careful examination of the plates taken with an amount of inductance just sufficient to elicit the lines of Br II and Br III strongly (while there is considerable suppression of the lines due to higher stages) revealed the following combinations between the deepest $4p\ ^3P$ and $5p\ ^5S$, $5p\ ^3S$ of Br II.

	$5p\ ^5S_2$	$5p\ ^3S_1$
	6908	
$4p\ ^3P_2$	101529 (20)	108436 (10)
3058		
$^3P_1$	98470 (15)	105378 (10)
1251		
$^3P_0$		104127 (10)

Besides the identical behaviour of the five lines under different experimental conditions, the following progression of the interval of the ground term convinces one of the correctness of the identification.

$$mp(^3P_2 - {}^3P_1)$$

O I 158   F II 344   F II/O I = 2·18
S I 398   Cl II 696   Cl II/S I = 1·75
Se I 1988   Br II 3058   Br II/Se I = 1·54

The value of 3058 cm⁻¹ obtained for the interval $4p(^3P_2 - {}^3P_1)$ of Br II is thus quite satisfactory. An interesting feature to be noticed is that the intercombination lines $4p\ ^3P-5s\ ^5S$ are much stronger than the triplet–triplet combinations. This feature might be expected as these intercombination lines essentially form what are usually called the resonance lines. It is significant to observe here that the corresponding intercombination lines in

* *Nature*, **131**, 170, 1933.

the arc spectrum of selenium of the same row of the periodic table have been found in absorption by a column of the non-luminous vapour.†

From the quintet system $5p\ ^3P-ms\ ^5S$, identified by Bloch, Bloch and Lacroute,‡ it has now been possible to obtain the deepest term $4p\ ^3P_2 = 183280$ cm⁻¹, yielding a value of $22 \cdot 6v$ for the second ionisation potential of Bromine. The triplets identified by the above authors seem to be rather uncertain, due to the comparatively low intensity of the combination $5s\ ^3S_1-5p\ ^3P_1$. The author is indebted to Dr. K. R. Rao for placing the plates in the Schuman region, taken with the Siegbahn Vacuum Spectrograph at his disposal.

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The Action of Aluminium Chloride on Polymethoxyflavones.

A HYDROXYL group in the 5-position in a flavone resembles the *ortho*-hydroxyl in a phenolic ketone in its resistance to methylation under ordinary conditions. It appeared probable that, conversely, a 5-methoxyl of a flavone may be as easy to demethylate as an *ortho*-methoxyl of a ketone. Phloracetophenone trimethyl ether (I) is converted into the dimethyl ether (II)

MeO / OMe   MeO / OH
$CO.CH_3$ $CO.CH_3$
MeO   MeO
(I)   (II)
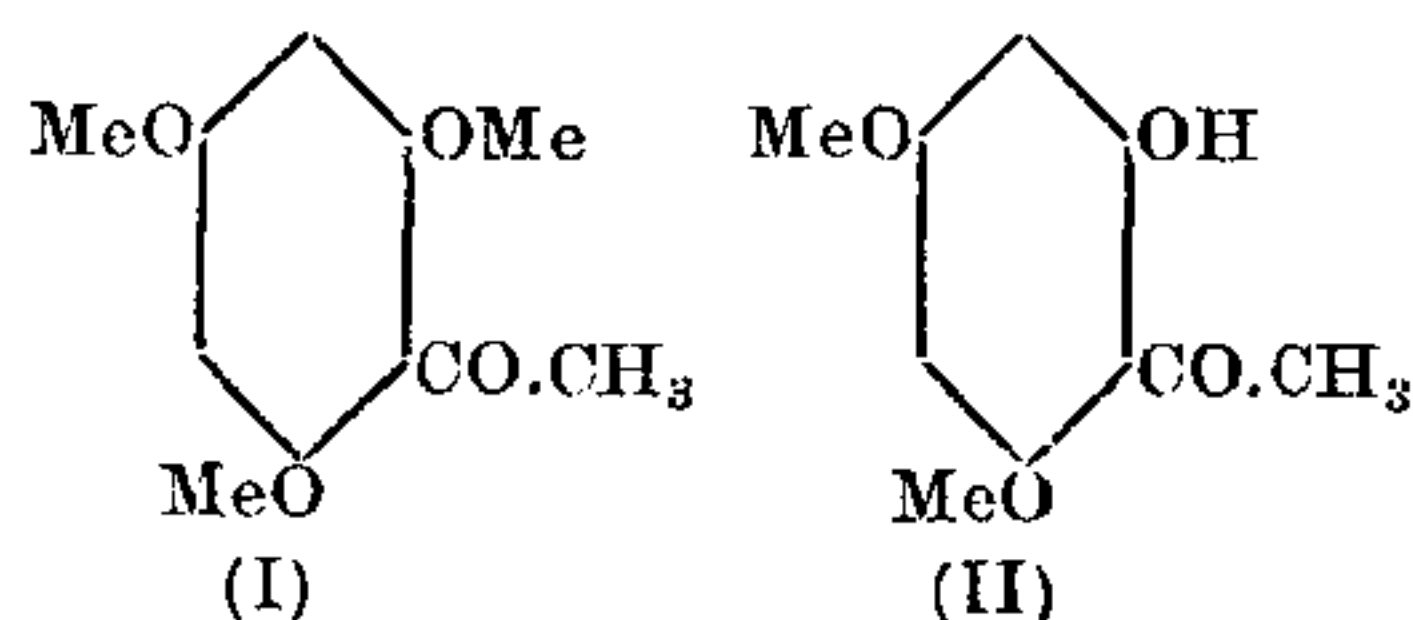
by heating with aluminium chloride at 110°.§ We have now found that, when a polymethoxyflavone is submitted to the action of aluminium chloride under specified conditions, demethylation takes place only in the 5-position and we are utilizing this observation for the synthesis of naturally occurring, partially methylated polyhydroxyflavones, such as wogonin, rhamnetin and rhamnazin.

† Kimura, *Jap. J. Phy.*, p. 81, 1926-27.
‡ *Compt. Rend.*, **193**, 232, 1931.
§ Kostanecki and Tambor, *Ber.*, **32**, 2260, 1899.