XII. THE NATURE OF PLASTEIN.

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PLASTEIN, which is precipitated from concentrated solutions of the products of peptic digestion of proteins by the action of pepsin at p_H 4, was discovered by Danilewski [1886] who considered it to be a product of synthesis. Numerous workers have since given the subject their attention, the most noteworthy being Wasteneys and Borsook [1924, 2]. These authors [1930] consider plastein to be a protein produced by the synthetic activity of a proteolytic enzyme.

The evidence in favour of the view that plastein is a synthetic product has been admirably brought together by Wasteneys and Borsook [1930] and need not be repeated here.

The weight that should be attached to this evidence may, however, be considered briefly.

The work of Okunew [quoted by Sawjalow, 1901] indicates anhydride formation, but in no way proves the formation of complex molecules from simpler ones, while that of Sawjalow [1908] concerning the order of the "Danilewski Reaction" is invalidated, as pointed out by Wasteneys and Borsook [1930], since the conditions were suboptimal. His titration experiments are valueless on account of many doubtful assumptions made as to the basicity of plastein and the fact that no steps appear to have been taken to mask the effect of the free amino-groups.

Comparisons drawn between plastein and native proteins in respect of such properties as solubility relations, precipitation reactions, colour reactions and digestibility by enzymes are suggestive rather than conclusive, and the same may be said of the biological evidence [*cf.* Beard, 1927; Hermann and Chain, 1912; von Knaff-Lenz and Pick, 1913].

The decrease in free amino-groups, while undoubtedly well substantiated, is susceptible of other interpretations than that of proof of synthesis. Further splitting of proteoses or peptones present in the substrate, accompanied by cyclic anhydride formation, might well lead to such a decrease, a product being formed which by reason of its structure is almost insoluble at neutrality. Such a substance would probably contain a smaller percentage of its nitrogen as amino-nitrogen than do proteoses or peptones.

Wasteneys and Borsook submitted samples of a digest in which different amounts of plastein had been formed to a process of fractional analysis devised by them [1924, 1]. The composition of the original digest was determined

by the author by extrapolation of curves obtained by plotting the experimental results of Wasteneys and Borsook against their experimentally determined percentages of plastein-N formed. The theoretical percentages of proteose-N, peptone-N and subpeptone-N corresponding to each experimentally determined percentage of plastein-N could then be calculated on the assumption that the statements of Wasteneys and Borsook were true. The values so calculated were plotted against percentages of plastein-N. In Figs. 1 and 2 the unbroken lines represent the theoretical values while the broken curves show the experimental results of Wasteneys and Borsook.

Fig. 1. Series I [Wasteneys and Borsook, 1924, 2].

Fig. 2. Series II [Wasteneys and Borsook, 1924, 2].

The wide divergence of the broken curves in Figs. 1 and 2 from the corresponding unbroken ones shows that the assumption made by Wasteneys and Borsook as to the value of their results as evidence for synthesis is not justifiable. All that can be concluded is that, as plastein formation proceeds, all three fractions progressively decrease in concentration, but the question as to whether any one of them enters into the composition of plastein or is decreased as a result of further splitting must remain unanswered.

The conclusion which emerges from a critical examination of the evidence put forward to show that plastein is a synthetic substance is that no unequivocal evidence on the matter has been obtained. The view which regards plastein as a protein is open to doubt.

PRESENT INVESTIGATION.

In the investigation about to be described the following two questions were kept in view.

1. Is plastein a synthetic product produced by enzyme action?
2. If not, what is plastein and how is it formed?

A definite answer to the first question is afforded if the molecular weight of plastein be known, and hence steps were taken to obtain this information.

The work undertaken in an attempt to answer the second question falls

under two heads. Firstly, a determination of the nitrogen distribution of plastein was carried out in order that the analysis might be compared with that of the protein from the digestion products of which the plastein was prepared. Secondly, an attempt was made to fractionate the phenyl isocyanate derivative of plastein in the hope that, should plastein be found to consist of a mixture of relatively simple substances, some information as to their nature could be obtained.

Preparation of plastein.

The protein from which the peptic digests necessary for plastein formation were prepared was B.D.H. egg-albumin powder. Merck's pepsin powder was added in 0.125 % concentration to 2.5 % albumin solutions in dilute hydrochloric acid at p_H 1.7. When digestion at 37° had proceeded to the point at which no material precipitable by trichloroacetic remained, the enzyme was inactivated by heating at 100° for some time and the digest filtered. The liquid was concentrated under reduced pressure at 50° to about one-twentieth of its original volume. The product obtained after filtering once more was a reddish-brown syrupy liquid, the p_H of which was adjusted to 4 by addition of sodium hydroxide.

In order to prepare specimens of plastein Merck's pepsin powder was added to 2 % concentration and the mixture incubated in presence of toluene for one week. The heavy suspension of plastein was then filtered off, and washed on the filter with hot chloroform water until the washings ran clear and colourless and gave no test for chlorine ions. After drying *in vacuo* over calcium chloride for two weeks, the product being ground in a mortar at intervals, a friable yellowish-white powder was obtained. Specimens prepared in this manner were designated by *A*.

Later, it was found that slightly better yields were obtained if the suspension of plastein first obtained were poured into a large volume of boiling distilled water, the resulting suspension being allowed to settle, the clear top-liquor syphoned off, the residue filtered and the precipitate treated as in the former method. Such specimens were designated by *B*.

One specimen of *A* was suspended successively in absolute alcohol and absolute ether and, after separating by centrifuging, was finally dried *in vacuo* for one week. This specimen was submitted to ultracentrifugal analysis.

The molecular weight of plastein.

Consideration of the problem of the determination of the molecular weight of plastein made it evident that the only practicable method of carrying out such a determination was by use of the ultracentrifugal method developed by Svedberg and applied by him during the last few years to the investigation of the molecular weights of the proteins [*cf.* Svedberg, 1930].

Accordingly a specimen of plastein prepared from a peptic digest of egg-albumin in the manner described above was submitted to Prof. Svedberg who

very kindly consented to carry out the determination. Prof. Svedberg's report is given verbatim:

"We have now run your plastein in the ultracentrifuge. It was dissolved in phosphate buffer of p_H 9.2 (0.032 M in Na_2HPO_4 and 0.001 M in $NaOH$) and centrifuged for $2\frac{1}{2}$ hours at a temperature of 21.1° and at a speed of 42,500 r.p.m. corresponding to a centrifugal force about 100,000 times gravity. No sedimentation took place, indicating that the molecular weight of plastein is low. As an upper limit 1000 can be given. Probably the molecular weight is only a few hundred."

The possibility that under these conditions plastein should undergo alkaline hydrolysis, resulting in the breakdown of originally large molecules into molecules which are not sedimented in the ultracentrifuge, is remote. This determination appears to afford conclusive evidence that plastein is not a protein but rather a substance of comparatively low molecular weight.

The nitrogen distribution of plastein.

The procedure used by Plimmer and Rosedale [1925] in a study of the nitrogen distribution of the proteins of hens' eggs was adopted in the present instance, since their modifications of the original Van Slyke technique considerably shorten the process. Their results indicate that under carefully controlled conditions concordant results can be obtained.

In Table I the mean results for plastein are expressed as percentages of total nitrogen, the results of Plimmer and Rosedale for egg-white being given for comparison. Cystine estimations were not made.

Table I.

	Plastein Mean g. N/100 cc.	Plastein % of total N	Egg-white % of total N
Amide-N	0.0147	4.1	9.2
Humin-N	0.0055	1.5	2.0
Diamino-N	0.0756	21.0	22.1
Total N	0.0409	11.3	13.1
Amino-N	0.0347	9.6	8.9
Non-amino-N	0.0404	11.2	11.7
Arginine-N	0.0066	1.8	0.2
Histidine-N	0.0286	7.9	10.1
Lysine-N	0.2702	74.9	67.6
Monoamino-N	0.2670	74.0	66.0
Total N	0.0032	0.9	1.6
Amino-N	0.3608	—	—
Non-amino-N			
Total N (Kjeldahl)			

The nitrogen distribution of plastein is in general similar to that of its parent substance egg-albumin as determined by Plimmer and Rosedale. The most important differences lie in the values for amide-N and total monoamino-acid-N. Possibly too much stress should not be laid on these differences since, as Gortner and Holm [1917] have shown, the determinations of amide-N are subject to error. If more deamination of monoamino-acids during hydrolysis

of the protein occurred in the experiments of Plimmer and Rosedale than in the case of the hydrolysis of plastein carried out by the author, the differences would be explained.

Nevertheless, support is given to the view that these differences are significant, by comparison with the mean results of analyses of plasteins from various sources carried out by Sawjalow [1908] using the Hausmann-Osborne method and the results obtained by Rosenfeld [1907] by means of the method of Kossel and Kutscher [1900]. These results are given in Table II together with the values for caseinogen after Hart [1901]. They are expressed in percentages of total nitrogen.

Table II.

	Plasteins from various sources (Sawjalow)	Plastein from caseinogen (Rosenfeld)	Plastein from egg- albumin (Folley)	Caseinogen (Hart)	Egg-white (Plimmer and Rosedale)
Amide-N	5.35	3.12	4.1	9.48	9.2
Diamino-N	18.32	20.09	21.0	20.53	22.1
Monoamino-N	76.33	76.79	74.9	69.99	67.6

It can be concluded that on acid hydrolysis plastein yields less amide-N and more monoamino-acid-N than native proteins.

Fractionation of the phenyl isocyanate derivative of plastein.

In the course of an investigation into the nature of serum-albumin peptone it was found by Raper [1907] that well-defined fractions could be separated from the phenyl isocyanate compound of the water-insoluble portion of that fraction of the peptone precipitated by K_2HgI_4 . A similar procedure was used in the case of plastein, in view of the possibility that plastein may consist of a mixture of substances of peptone nature.

Preparation of phenylureido-plastein. To a suspension of 10 g. of plastein *A* in 1500 cc. of toluene water were added 20 cc. of 40 % NaOH. After filtering and cooling to 0°, phenyl isocyanate was added in small quantities, each addition being accompanied by vigorous shaking. The temperature of the solution was never allowed to exceed 2°. On the appearance of a white precipitate of diphenylurea, indicating the end of the reaction, the liquid was filtered. The phenyl isocyanate derivative of plastein was precipitated by acidification with acetic acid, the precipitate being filtered off, well washed with cold distilled water and dried *in vacuo*. The finely powdered material was extracted with ether in a Soxhlet apparatus until all traces of diphenylurea had been removed and finally dried for several days in a vacuum desiccator.

A second specimen was prepared from plastein *B* in a similar manner with the difference that in this case a faintly alkaline solution of plastein was used.

Fractionation. A faintly alkaline solution of the sodium salt was prepared by stirring 3 g. of phenylureido-plastein (first preparation) into 300 cc. distilled water, sodium hydroxide being added drop by drop until all solid had

dissolved. Addition of 9 g. of sodium chloride, followed by saturation with carbon dioxide, precipitated a gelatinous substance *A* which was centrifuged out, washed with distilled water and dried *in vacuo*.

From the filtrate a second fraction *B* was precipitated by acidification with acetic acid, this substance being separated in the same manner. The remaining liquid was found to contain very little nitrogen and was not dealt with further.

Substance *A* was boiled for $4\frac{1}{2}$ hours with absolute alcohol and the liquid filtered while hot from the very considerable undissolved residue *C*. After standing in the ice-chest for some days a small quantity of a white powder *D* was deposited from the alcohol. The latter when evaporated to dryness over steam deposited a further small quantity of a white solid *E*. Substances *D* and *E* when dry were of similar appearance—white friable powders—and both melted with blackening over the temperature range 223–228°. It is probable that they were identical.

The substance *C*, which comprised the main portion of the fraction precipitated by carbon dioxide and of the residue *B* precipitable by acetic acid, was titrated with sodium hydroxide in alcoholic solution in presence of phenolphthalein, and, for comparison, the phenyl isocyanate derivative of proteose, the most complex component present in the digests from which the plastein was prepared, was titrated in the same way. The proteose was obtained from a peptic digest of egg-albumin by saturation with sodium sulphate at 37°. The solid was skimmed off and twice purified by solution in warm distilled water, followed by salting out with sodium sulphate. A solution in distilled water was finally dialysed against frequent changes of distilled water for 24 hours. The phenyl isocyanate compound was prepared in the same manner as in the case of plastein.

The equivalent weights obtained were as follows:

Phenylureido-derivative <i>C</i>	1025
Phenylureido-derivative <i>B</i>	468
Phenylureido-derivative of proteose	782

These results afford no evidence that plastein contains substances of molecular weight of a higher order than that of proteoses.

A similar series of experiments was carried out with precisely similar results, using 3 g. of the second preparation of phenylureido-plastein.

These two experiments indicate that plastein prepared from egg-albumin consists of at least two substances which can be separated by suitable technique.

SUMMARY.

1. The evidence in favour of the view that plastein is a synthetic product is regarded as inconclusive.
2. The molecular weight of plastein was found by the ultracentrifugal method to be 1000 or less.

3. A determination of the nitrogen distribution of plastein shows that this substance contains less amide-N and more monoamino-acid-N than egg-albumin, the protein from which it was derived. The significance of these differences is unknown.

4. The phenyl isocyanate derivative of plastein has been shown to consist of at least two fractions with equivalent weights of the same order as that of proteose. Plastein thus appears to consist of a mixture of substances.

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