THE ENZYMATIC SYNTHESIS OF PROTEIN. I.

THE SYNTHESIZING ACTION OF PEPSIN.

By HARDOLPH WASTENEYS and HENRY BORSOOK.

(*From the Department of Biochemistry, University of Toronto, Toronto, Canada.*)

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I. HISTORICAL.

A condition in which a synthesis of protein-like material occurs, was first arranged in 1886 by Danilewski (1) who observed the formation of a precipitate when stomach extract was added to a concentrated solution of the products of peptic hydrolysis. He considered the causative agent to be an enzyme, because precipitation did not occur if the stomach extract had been previously heated to 100°C. This result was confirmed in 1895 by Okunew. Both Danilewski and Okunew concluded that the reaction involved synthesis of the products of protein decomposition into a more composite molecule approaching in complexity a native protein.

In 1901 Sawjalow (2, 3) investigated the phenomenon more extensively and named it plastein formation. He recognized the necessity for a suitable degree of acidity though he did not define it precisely.

Using Witte's peptone in a concentration of 27.2 per cent, Sawjalow obtained 13.6 per cent of the total dry weight in the precipitate. He attempted an estimation of the molecular weight of plastein by means of acid and alkali titration and arrived at an average value of 6,087.

Sawjalow and other workers, Lawrow and Salaskin (4), Kurajeff (5), and Bayer (6) sought to discover the source of plastein among the various fractions obtained by the method of Pick from a protein digest, but failed to reach any agreement.

Bayer reached the conclusion that proteoses took no part in the synthesis of plastein.

A. E. Taylor (7, 8) and T. B. Robertson (9, 10) have reported synthesis of protein by means of trypsin and pepsin.

In Taylor's experiments the synthesis was accomplished after 5 months standing and the yield was very small. In Robertson's experiments the yield was larger and more rapidly obtained. Robertson found indications which led him to assume the existence in pepsin of separate hydrolyzing and synthesizing components. Our experiments indicate that this assumption was possibly erroneous.

Bayliss (11) in a criticism of the results of Robertson considers that he was dealing with a colloidal precipitation phenomenon and not with an enzymatic synthesis.

The last contribution to the problem of the enzymatic synthesis of protein appeared in 1911 and 1912 (12, 13) by Henriques and Gjaldbäk. They obtained plasteins by means of trypsin and pepsin from partial acid and alkali hydrolysates and with trypsin from peptic digests. They made the important observation that neither enzyme was capable of producing plastein from any type of digest of gelatin; nor from any tryptic digest except of casein, and here only by pepsin. There is indicated in their work the possibility of the influence of dilution, of temperature, and of acidity on the plastein formation. In their final experiments they point out an anomalous simultaneous hydrolysis and synthesis. Their important contribution was the estimation of the ratio of the free amino to the total nitrogen in plastein, which they found to be lower for plastein than for any of its precursors. These results afforded the only evidence for assuming the complexity of the plastein molecule to be greater than that of the substances from which it is built; their additional observations of a decrease in formol titratable nitrogen, simultaneous with an increase in the tannic acid precipitable nitrogen, are not as significant, because the titration results do not parallel those from the tannic acid precipitation, and because they employed concentrations of tannic acid too dilute to give complete precipitation. The problem of the identity of the enzyme responsible for plastein formation was not attacked; nor are their results capable of any general kinetic interpretation, because it is probable from the high ratios of free amino nitrogen to total nitrogen in their peptic digests, 38 per cent, and the high ammonia liberation, 5 per cent, that their pepsin preparation contained in addition to pepsin, other enzymes of ereptic and autolytic types.

Summing up, the only unchallenged synthesis of protein is that of protamine by trypsin, attained by Taylor. The extreme slowness of this reaction and the paucity of the yield, despite the simplicity of the protein hydrolyzed, inspired little confidence of any probable physiological significance. The work on plastein, as a study of enzymatic synthesis, appears to have been virtually dismissed as an unphysiological and anomalous phenomenon. There have been no further contributions since the work of Henriques and Gjaldbäk in 1912. The mechanism by which the synthesis of protein is effected in the tissues is still only conjectural, despite the strong implications from mass law and thermodynamic reasoning that the conditions of plastein formation are the very conditions under which the synthesis occurs in the tissues.

II. EXPERIMENTAL.

In a concentrated solution, approximately 40 per cent, of the products of peptic hydrolysis of egg albumin (Merck, U. S. P.), whose reaction had been adjusted to pH 4.00,[1] pepsin brought about a precipitation of material which, on investigation, proved to be of the order of complexity of native protein.

Proof that this precipitate, which for the sake of historical continuity has been named plastein, is more complex than the substratum from which it is produced, is afforded by direct evidence from a number of sources.

First, the ratio of free amino to total nitrogen is lower in plastein than in the most complex product of the digest from which the plastein was synthesized. This was first observed by Henriques and Gjaldbäk, and has been here confirmed.

A suspension of plastein in distilled water was boiled for half an hour to destroy enzymes possibly carried out of the digest from which it was produced. It was then washed three times by decantation and three times on a filter with a stream of distilled water. A suspension of pure plastein was thus obtained, free of any adherent matter, which was very fine in texture, and grayish white in color. A suspension was made in 0.07 N HCl, and the amino nitrogen in 5 cc. determined in duplicate in the macro Van Slyke apparatus. The plastein was quite soluble in the nitrous acid; and the 5 cc. of suspension gave 1.12 mg. of free amino nitrogen. The total nitrogen of 5 cc., estimated by the macro Kjeldahl method, was 16.9 mg. The ratio, therefore, is

$$\frac{\text{Free amino N}}{\text{Total N}} = \frac{1.12}{16.9} = 6.6 \text{ per cent}$$

Proteose, obtained by salting-out with Na_2SO_4 from the same digest from which the plastein was synthesized, was twice reprecipitated and then redissolved. Assayed for free amino nitrogen, 5 cc. gave 4.39 mg. The total nitrogen of 5 cc. was 42.0 mg., therefore

$$\frac{\text{Free amino N}}{\text{Total N}} = \frac{4.39}{42.0} = 10.5 \text{ per cent}$$

[1] The experimental justification for this procedure will be given in a future paper.

Since the increase of the free amino nitrogen of a protein digest has often been taken as a measure of the degree and rate of hydrolysis, a decrease in the ratio of free amino to total nitrogen may justifiably be interpreted as proof of synthesis. With this criterion, therefore, plastein proves to be a synthetic product. The figures of Henriques and Gjaldbäk, obtained by the formol titration, indicate that the complexity of the plastein here obtained, is of approximately the same order as of many native proteins. These figures for a number of proteins, together with some determined by the authors, are given in Table I.

This criterion, however, cannot be trusted too far. The figures for casein would lead to the improbable conclusion that this protein is of lower complexity than a product of digestion of egg albumin; *viz.*, proteose. However, in the case of two substances

TABLE I.

Substance.	Free amino N / Total N
Edestin	2.8
Egg albumin	6.7
Casein	11.9
Fibrinogen	7.1
Serum globulin	6.0
Witte's peptone	14.4
Plastein*	6.6
Egg albumin*	3.5
Proteose*	10.5
Peptic digest of plastein*	17.5

* These values obtained by the authors with the Van Slyke method.

isolated from the same digest, differences in the value of this ratio must indicate corresponding differences in complexity.

Proof of an entirely different nature of the occurrence of synthesis here, is afforded by the results obtained from the fractional analysis of originally identical solutions, in which varying amounts of plastein had been formed. The analytical procedure has been given in detail in the previous paper. Regarding solely the results given in Table II, it is evident at once that all fractions of the peptic digest have contributed to the formation of plastein; and in approximately the same relative amounts as they are obtained in the peptic hydrolysis of albumin. These results show the fallacy of the search of Salaskin, Kurajeff, Bayer, Lawrow, and

others for the source of plastein in any one component of a digest, and afford striking proof of the synthetic nature of plastein.

The highest amount of plastein noted above, 27.3 per cent, falls considerably short of the amounts obtained later, when the optimum conditions in regard to temperature and reaction, enzyme and digest concentrations, had been located. Then, yields as high as 39 per cent were found repeatedly. A fuller description of the investigations by which these conditions were arrived at, will be published later.

TABLE II.

Plastein N in per cent of total N.	Proteose N in per cent of total N.	Peptone N in per cent of total N.	Subpeptone N in per cent of total N.
Series I.			
1.3	32.0	35.6	31.0
3.2	32.8	33.2	31.0
7.1	30.5	32.8	29.8
17.2	24.2	29.2	29.4
18.7	23.0	29.4	28.7
27.3	19.6	28.1	25.0
Series II.			
1.0	32.0	35.9	31.3
1.4	32.2	34.9	31.6
6.9	30.7	33.1	29.7
16.0	27.0	28.4	28.7
23.3	21.8	27.2	27.8
Series III.			
3.2	28.2	37.1	31.6
25.3	17.7	29.9	27.1

Proof in still another direction of the protein nature of plastein is obtained from the characteristic protein color of the biuret test given by this substance. Proteose (the most complex constituent of the digest), the whole digest, and a peptic hydrolysate of plastein itself all gave a color of distinctly different quality, the characteristic pink or rose shade. The color of the biuret gives a fair indication of the complexity of the substance causing the color. This pink nuance of the proteose is easily distinguishable from the mauve of protein. In addition, plastein

is precipitated from its solution in either concentrated or dilute solutions of strong acid, by trichloroacetic acid. In view of the absence of any effect of this reagent upon gelatin, or upon any of the proteoses, taken in conjunction with the other evidence quoted above, there can be little room for doubt that plastein is a product of a synthetic reaction, and that its complexity is of the order of a native protein. The similarity to native protein is further confirmed by the readiness with which it is digested by pepsin, at a similar hydrogen ion concentration, giving the same products, and with the same velocity.

A fairly heavy suspension of plastein was adjusted with 0.07 N HCl to a reaction where pH = 1.7. To a total volume of 250 cc., 0.5 gm. pepsin (Merck) was added, and the suspension was set away in the incubator at 37°C. In 1 hour all of the plastein had gone into solution. 22 hours later the pH had risen to 2.2, and on saturation with Na_2SO_4 at 33°C. a voluminous crop of proteose floated to the surface.

In another experiment, a freshly prepared plastein was washed on a filter, suspended in water, then boiled for half an hour; cooled, decanted three times with distilled water, and then washed three times on a filter with distilled water. A suspension was made in HCl, giving a final reaction where pH = 1.7. The free amino nitrogen of 5 cc. was found to be 1.29 mg. To 50 cc. of this suspension (*A*) 0.05 gm. of pepsin (Merck) was added.

To another 50 cc. (*B*) 0.05 gm. of boiled pepsin was added. Both were set away at 37°C. In 2½ hours *A* was clear and *B* was unchanged. 26 hours later the free amino nitrogen in 5 cc. of *A* was 2.8 mg.; and of *B* 1.37 mg. The slight rise in *B* was probably due to the commercial pepsin added, which is known to consist chiefly of the products of digestion of protein, and to the slight hydrolyzing effect of the acid. The ratio of free amino N to the total N in *A* was 17.1 per cent.

The ratio for proteose is 10.5 per cent; therefore, besides proteose, large amounts of even simpler products were produced on the hydrolysis of plastein, as is the case with protein.

No hydrolysis was obtained when proteose, salted-out from a digest from which plastein had been synthesized, was submitted to the action of pepsin.

Proteose from a peptic digest of egg albumin, which gave no precipitate with saturated copper acetate, was redissolved in water, boiled, filtered, and reprecipitated with Na_2SO_4. It was then redissolved in HCl, adjusted to pH 1.7, and 0.12 gm. of pepsin (Merck) was added. The free amino nitrogen of 5 cc. was 4.40 mg. 24 hours later this value for amino nitrogen

was unchanged. The total nitrogen was 42.0 mg., giving a value for the ratio of free amino to total nitrogen of 10.5 per cent.

The conclusion is, therefore, that it is possible for pepsin to synthesize a linkage, or linkages, which under other conditions it hydrolyzes.

Excessive quantities of enzyme are unnecessary for the synthesis of plastein. Pepsin in 8 per cent concentration gave rise to 33.2 per cent plastein; while in the same solution of products, the concentration of enzyme preparation usually employed in hydrolysis, *viz.* 0.1 per cent or 80 times less, gave 13.1 per cent plastein in the same length of time.

The properties of the plastein prepared resemble those of a protein denatured by dilute acid. It is only very faintly soluble in neutral water, but is quite soluble in dilute strong acids and alkalies, more so in the latter than in the former. A region of distinct solubility exists between pH 8.8 and 9.9, with a maximum solubility at pH 9.2. At hydrogen ion concentrations somewhat greater than pH 1.0, it is fairly soluble, but is quite insoluble at all pH values between 2.0 and 7.0. It is, therefore, insoluble in 0.07 N acetic acid. It is only slightly soluble in 10 per cent NaCl, but very soluble in strong HNO_3 and strong HCl. It is not soluble to any noticeable extent in 95 per cent alcohol.

It is incompletely precipitated from its solution in 0.025 N NaOH by the addition of NaCl to half saturation at room temperature; a much heavier precipitate is obtained on saturation. Boiling coagulates the plastein in alkaline, but not in acid solution. It is precipitated from its solution in either strong or weak HCl by trichloroacetic acid. It gives, as mentioned above, a characteristic protein biuret, quite distinct from a proteose biuret, and gives a characteristic, positive, Heller's test. If allowed to stand in concentrated HCl a purplish color develops.

It is rapidly digested by pepsin at pH 1.7.

A possible cause of the insolubility of plastein in neutral solutions is indicated by a phenomenon, which was encountered incidentally, and which demonstrates the inability of ordinarily soluble albumin to exist in a soluble form, under the conditions in which peptic synthesis occurs. To 4 cc. of a concentrated peptic digest of albumin at pH 4.0, 2 cc. of 5 per cent albumin solution

were added. A precipitate formed immediately, which increased on standing. After 1 hour at 38°C. the mixture was filtered; and on testing the filtrate with trichloroacetic acid, no precipitate was obtained, indicating the absence of any protein in solution. The total nitrogen of the precipitate was equal to the nitrogen of the protein added. The precipitate, after thorough washing, remained quite insoluble in water, and exhibited the same solubility properties as plastein. The digest contained no active enzymes, because it had previously been boiled for an hour. It seems that the soluble protein on coming into contact with the solution of products at pH 4.0 was immediately denatured, or at any rate was rendered insoluble.

The phenomenon is under thorough investigation.

The preliminary results of the first cursory examination are given below. At least two factors are of significance, the hydrogen ion concentration, and the concentration of products.

With acidities up to pH 5.4 the precipitation of protein went to completion. At hydrogen ion concentrations lower than this, a turbidity only was obtained, which did not increase on prolonged standing (1 week) at 38°C.

It was found that in solutions of products under 5 per cent protein is not precipitated. On the other hand albumin remains in solution in concentrations of products as high as 30 per cent, when the hydrogen ion concentration is at pH 7.0.

An experiment was carried out to ascertain if, with the precipitation of the albumin, the cause of the precipitation was removed. A mixture of albumin and digest was filtered after the first precipitation, and to the filtrate a second portion of albumin was added. Addition of trichloroacetic acid had shown that the first precipitation was complete. The precipitate formed again with the second portion of albumin, and no less quickly. This procedure was repeated twice more, and in both cases there again occurred the same speedy formation of the precipitate. After this fourth addition of albumin, the concentration of products, which originally had been 9 per cent, had now been reduced to 4 per cent. The experiment was, therefore, not carried any further because at 4 per cent the denaturation and precipitation of albumin no longer takes place. The results obtained, however, showed that in this precipitation of albumin there is no appreciable, if any, removal of the material which causes it.

In view of the well known exceptional behavior of gelatin in nutrition, an attempt was made to effect a synthesis of protein in

a concentrated peptic digest of gelatin. Henriques and Gjaldbäk have published the failure of this reaction in their experiments with every type of gelatin digest. But with our more definite knowledge regarding the optimal conditions for peptic synthesis, on the one hand, and the nutritional deficiencies of gelatin, on the other, it was thought that, despite their previous failure, a probability of success existed now, which warranted another attempt.

Accordingly a 6 per cent solution of gelatin was digested at pH 1.6, at 37°C. for 3 weeks. It was then filtered, heated at 80–90°C. for 1 hour to destroy the enzyme, and then concentrated *in vacuo* to a concentration of approximately 50 per cent. The resulting solution was clear, reddish brown, and somewhat viscous, but was pipetted without difficulty. It was adjusted to pH 4.0. To 10 cc. 0.5 gm. of Merck's pepsin was added, rubbed into solution, and the mixture set away with chloroform, tightly stoppered, at 37°C. At the end of a week the solution was still quite clear and did not give a precipitate either with trichloroacetic acid or on neutralization. Evidently synthesis of plastein, or of any other protein body had failed.

TABLE III.

No.	Gelatin digest. pH 4.0	Albumin digest. pH 4.0	Tyrosine.	Tryptophane.	Pepsin.	Plastein N.
	cc.	*cc.*	*gm.*	*gm.*	*gm.*	*mg.*
1	10		0.09		0.5	0
2	10		0.09	0.09	0.5	0
3	10			0.09	0.5	0
4	5	5			0.5	92
5	0	10			0.5	230

This, however, from the nutritional behavior of gelatin might have been predicted. Quite another result could be expected in the presence of tryptophane. With this in view five flasks were prepared. The materials were added to each, and the results are given in Table III. The amino acids were first dissolved in 0.07 N HCl, to which the enzyme was first added, and then the gelatin digest. All the mixtures were set away with chloroform, tightly stoppered, at 37°C. for 3 days. During this period tyrosine crystallized out of solution.

The analysis for plastein was carried out in the usual manner.

As the figures in Table III show, synthesis failed also in the presence of the amino acids. The results in Nos. 4 and 5 are of-

special interest. The experiment was carried out to test if in the synthesis of the products of the albumin digest, some of the hitherto unsynthesizable gelatin radicles could be carried into the plastein molecule. If this had occurred the plastein in No. 4 would have been more than half the amount in No. 5, because the synthesizable albumin material in the former was exactly half the amount of the latter. Less than half the plastein in No. 5 was found in No. 4.

The gelatin radicles were thus found to be incapable of taking part in the synthesis. Not only this, they exerted apparently a slight inhibitory influence on the synthesis of the albumin fractions.

Quite another conclusion is possible if it be assumed that the occurrence and extent of synthesis is dependent upon the existence in the digest of one essential substance, without which the reaction cannot occur. When this substance is completely utilized by its inclusion in the plastein molecule synthesis comes to a stop. In gelatin digests this substance is absent; and, moreover, it is neither tyrosine nor tryptophane. Beyond this superficial speculation it does not appear advisable to proceed; though most of the quantitative aspects of plastein synthesis are not incompatible with it. It was thought worth while only to point out this quite different possible explanation, which if true is of significance.

III. THEORETICAL.

In applying the mass law to the case of hydrolysis of protein the equation is written

$$\text{Protein} + H_2O \rightleftarrows \text{Products}$$

and from this equation it has generally been assumed that sufficient diminution of the concentration of water, in the absence of any great concentration of protein, would cause the reaction to proceed from right to left. The fact is usually overlooked that the molecular concentration of the water is enormous when compared with the concentration of the other substances and, therefore, even with tenfold concentration of the solution the ratio of the molecular concentrations is so little altered that reversion, by this mechanism alone, would be practically impossible *in vitro*.

The opinion is occasionally met with, that protein synthesis must be especially difficult, for reasons based on the fact that the hydrolysis of proteins goes to practical completion. The equilibrium point is apparently at 100 per cent decomposition, the catalyst is assumed not to influence the point of equilibrium and reversion of the reaction is consequently difficult of accomplishment.

To account for the fact of synthesis in the tissues, there have been postulated the influence of surfaces for the production of a concentration of water so low that molecularly it would be of the same order of magnitude as the concentration of the products and the protein. An example of this reasoning is found in Bayliss' "Principles of general physiology" (14). Beginning with an alcohol-water-acid-ester system, the usual $\frac{K_1}{K_2} = K$, the equilibrium constant, is deduced; where K_1 (ester) (water) = K_2 (alcohol) (acid). He then states:

"Put in this form, we see that if we increase one component, the result must be to decrease its fellow, since the value of the fraction *must remain unaltered.* Suppose we increase water, the value of this fraction can only be kept constant either by increasing (alcohol) (acid) or by decreasing (ester). In point of fact, of course, the two are identical, since one cannot take place without the other. The results of excess of water should be, therefore, to increase the hydrolytic reaction of the system, as found by experiment.

"The conclusion to be drawn from this fact is that, in order to obtain much indication of the synthetic aspect of enzyme action, the concentration of water must be diminished as far as possible.

"In the living cells, where synthetic processes readily take place, it seems that there must be some very effective means of doing this, perhaps by surface condensation or inbibition on the part of the colloids. But as yet we have no clear idea of the mechanism."

In the above example the molecular concentration of water is of the same order of magnitude as those of the other reacting components. In the protein systems usually encountered *in vitro*, however, and also in the tissues, if no special mechanisms are postulated, the molecular concentration of water must be enormously greater than that of the reacting components. If the essential conditions for synthesis were those demanded by the Bayliss example, reversion would, *in vitro*, be impossible of attainment,

because the enormous value of the molecular concentration of water would always drive the reaction from left to right.

To consider the 100 per cent decomposition of protein as "equilibrium" is incorrect, because one of the components on the left-hand side of the equation, *i.e.* water, is at relatively infinite concentration. In order to account for the occurrence of synthesis in the tissues it would, then, be obligatory to postulate, as Bayliss does, a special mechanism capable of effecting enormous diminution of the concentration of water, with the resulting enormous increase in concentration of the substances on the right-hand side of the equation.

But in the hydrolysis of protein, we have the formation of a number of components from 1 molecule of protein. This fact, overlooked in Bayliss' discussion, places the hydrolysis and synthesis of protein, and to a lesser degree of fats, in entirely another category.

Moore (15) by thermodynamic reasoning arrived at a conclusion, which can be paraphrased as follows: There is a constant relationship at equilibrium between the osmotic pressure of the substance undergoing decomposition, and the nth power of the osmotic pressure of any one of the products split off, where n is the number of molecules arising from 1 molecule of undecomposed substrate. The equation is $P_a = K.P_b^n$. The greater the value of n, the greater is the tendency for synthesis in concentrated solutions and for hydrolysis in dilute solutions. In confirmation of this general theorem Moore quoted experiments of Croft Hill, who has shown that the ferment of malt in concentrated solutions of glucose forms a disaccharide, and that if the solution contains less than 4 per cent of glucose the synthesis of the disaccharide does not occur. In quite dilute solutions of maltose, its hydrolysis is known to proceed to completion.

Since the heat of reaction of hydrolysis of proteins is very low, and the value of n is high, from thermodynamical reasoning reversibility is predictably possible in two ways, by increasing the concentration of the substances in solution (for our purposes the products of hydrolysis) or by raising the temperature. Thermodynamic possibility, however, except in an extremely rigid sense, does not insure the occurrence of a reaction. If, for instance, a dilute solution of protein is maintained at a reaction where pH

= 6.0, pepsin is unable to effect any hydrolysis (16); nor does the protein in the absence of pepsin suffer a considerable degree of hydrolysis, at low temperatures, merely as a result of acidity of this strength. At pH 1.7, however, in the presence of pepsin, the hydrolysis proceeds rapidly. Exactly the same necessity for suitable conditions, over and above the thermodynamic possibility, prevails in the case of reverse reaction; *i.e.*, synthesis.

The equation for the hydrolysis of protein may be written:

$$\text{Protein} + H_2O \rightleftharpoons Pd_1 \cdot OH + H \cdot Pd_2 + Pd_3 \cdot OH + H \cdot Pd_4 \ldots Pd_{n-1}OH + H \cdot Pd_n$$

where n is the number of molecules into which 1 molecule of protein is split, and Pd is the symbol for one of these products. The equilibrium equation becomes, if the molecular concentration of water may be considered constant,

$$\frac{(Pd_1 \cdot OH \times H \cdot Pd_2 \ldots \ldots \ldots Pd_{n-1}OH \times H \cdot Pd_n)}{(\text{Protein})} = K, \text{ or } (1)$$

$$\frac{(Pd)^n}{(\text{Protein})} = K \quad (2)$$

If the initial concentration of protein be denoted by C, the degree of hydrolysis by h, equation (2) may be written:

$$\frac{(h \cdot C)^n}{(1-h)C} = K \quad (3)$$

$$\frac{h^n C^{n-1}}{(1-h)} = K \quad (4)$$

$$C^{n-1} = K\frac{(1-h)}{h^n} \quad (5)$$

The first deduction from equation (5) is that the degree of hydrolysis, h, is dependent upon the initial concentration C of the protein in solution, and that, as it increases, h decreases; and, therefore, the possibility for synthesis increases. If the initial concentration is high, the degree of hydrolysis is small, and at equilibrium relatively little protein is hydrolyzed; this is especially marked for proteins, where n is high. If there is no protein at the outset of the

reaction, as is the case when a solution of the products of digestion is concentrated down, then sufficient protein is synthesized to provide the amount required to satisfy the equilibrium equation for the degree of hydrolysis existing at that concentration of substances in solution.

From equation (5) it is seen that as C increases in arithmetrical progression, as it does under experimental conditions, h decreases in geometrical progression, and hence the tendency for synthesis, which is a function of the reciprocal of h, increases in geometrical progression. Decreasing C in arithmetrical progression, the reverse holds true. Therefore, where n is high, in concentrated solutions, the tendency for synthesis is great and in dilute solutions the tendency for hydrolysis is great. The difficulties encountered in accounting on simple physicochemical grounds for synthesis *in vitro*, or in the tissues, thus vanish when the equilibrium equation for the hydrolysis of protein is properly stated.

SUMMARY.

1. Certain theoretical considerations are pointed out, which lead to the conclusion that the synthesis of protein from the products of hydrolysis should be possible *in vitro*, without difficulty, and without the interposition of any special mechanism for effecting abnormally high concentrations of products.

2. Experimental confirmation of this conclusion is demonstrated with pepsin in a concentrated solution of the products of peptic hydrolysis of egg albumin. Under optimal conditions 39 per cent of the nitrogen of the original digest was found in the synthesized protein.

3. Evidence is supplied that the substance synthesized is of the order of complexity of native protein.

4. The reaction is a reversible one, in that a linkage is synthesized in concentrated solution, which is hydrolyzed in dilute solution.

5. A phenomenon is described which may account for the solubility properties of the protein synthesized.

6. The products of the peptic hydrolysis of gelatin were found to be incapable of synthesis by means of pepsin. The presence

of tyrosine and tryptophane, or synthesizable albumin radicles, also failed to effect this synthesis.

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