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THE ORIGIN OF THE HUMIN FORMED BY THE ACID HYDROLYSIS OF PROTEINS. VII. HYDROLYSIS IN THE PRESENCE OF KETONES[1]

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In the earlier papers of this series[2] it has been shown that the formation of the black acid-insoluble humin of protein hydrolysis is dependent upon the presence of tryptophane in the protein molecule. The humin, however, is not formed solely by decomposition of the tryptophane molecule, but is produced by the interaction of tryptophane with some other, as yet unidentified, component accompanying the tryptophane in the protein.

Likewise, it has been shown that artificial "humins" can be produced by condensing tryptophane with aldehydes by means of 20% hydrochloric acid, and that when a protein is hydrolyzed in the presence of an appropriate aldehyde, it is possible to recover the tryptophane nitrogen practically quantitatively as the "acid-insoluble humin nitrogen." We have, therefore, stated[3] that "of all the known hydrolytic products of proteins, tryptophane alone is concerned in the reaction which produces black insoluble humin, but this reaction cannot take place without the presence of some as yet unidentified component of the protein molecule." This unknown component we have postulated to be either an aldehyde or a ketone.

Fürth and Lieben[4] have questioned our evidence that tryptophane can be estimated quantitatively by making use of the amount of nitrogen recovered as "insoluble humin." It is sufficient to point out that the experimental evidence presented by Fürth and Lieben does not have a bearing upon the question. We have repeatedly stated that *the recovery of the tryptophane nitrogen in the insoluble humin fraction is quantitative only when an exact equivalent of aldehyde is present* and that in order to estimate the tryptophane content of a protein it is necessary to plot a curve as is shown in Fig. 1 of an earlier paper.[5] Under such conditions we still believe that the tryptophane content of a protein can be determined fairly accurately. Fürth and Lieben point out that there is no correlation between the quantity of humin nitrogen formed in an ordinary pro-

[1] Presented before the Division of Biological Chemistry of the American Chemical Society, Pittsburgh, Sept. 7, 1922. Published with the approval of the Director as Paper No. 352, Journal Series of the Minnesota Agricultural Experiment Station.

[2] (a) Gortner and Blish, THIS JOURNAL, **37**, 1630 (1915). (b) Gortner, *J. Biol. Chem.*, **26**, 177 (1916). (c) Gortner and Holm, THIS JOURNAL, **39**, 2477 (1917); (d) **42**, 821 (1920). (e) Holm and Gortner, *ibid.*, **42**, 632 (1920); (f) **42**, 2378 (1920).

[3] Ref. 2c, p. 2498.

[4] Fürth and Lieben, *Biochem. Z.*, **116**, 224 (1921).

[5] Ref. 2c, p. 2481.

tein hydrolysis (no aldehyde added) and the tryptophane content of the protein. This we admit, nor have we ever contended that such a relationship existed. In all of the proteins which we have examined, we have not found a single instance in which the ratio of tryptophane and unknown (aldehydic?) component was such as to cause maximum insoluble humin formation. In every instance where tryptophane was present in appreciable amount we have found it necessary to add aldehyde in order that all of the tryptophane should be removed in the insoluble humin.

In view of the uncertainty regarding the nature of the unknown component which it has been suggested is either an aldehyde or a ketone and inasmuch as all of our previous studies have involved the use of aldehydes, we have in the present instance studied the effect upon humin formation of the addition of ketones to the hydrolysate.

Experimental Part

Experiments were carried out using as a protein the same sample of fibrin that we used in the earlier work. Acetone and acetophenone were selected as ketones representative of the aliphatic and aromatic series. The acetone was of the highest purity obtainable and was redistilled over calcium oxide. The effect of these ketones on the amino acids, tyrosine and tryptophane, was likewise studied.

Three-g. samples of fibrin were hydrolyzed for 24 hours with 75 cc. of 20% hydrochloric acid with and without the addition of ketone. The nitrogen distribution was then determined upon the hydrolysate according to Van Slyke's[6] method, modified by the separation of the humin nitrogen fraction into 3 portions, acid-insoluble, acid-soluble, and phosphotungstic acid humin.[7]

TABLE I

THE HUMIN NITROGEN FRACTIONS AND AMMONIA NITROGEN OBTAINED WHEN 3 GRAMS OF FIBRIN WAS HYDROLYZED FOR 24 HOURS IN THE PRESENCE OF INCREASING AMOUNTS OF ACETONE

Acetone added Cc.	Acid-insol. humin N Mg.	Acid-soluble humin N Mg.	Total humin N Mg.	Ammonia N Mg.
0.0	8.8	4.2	13.0	31.4
0.1	8.1	4.2	12.3	lost
0.5	9.1	3.3	12.4	31.2
1.0	8.9	lost	..	33.0
2.0	8.9	3.5	12.4	28.4
3.0	9.8	3.8	12.6	33.0
5.0	10.0	4.4	14.4	27.9

Table I shows the effect upon the acid-insoluble humin nitrogen, acid-soluble humin nitrogen, total humin nitrogen and ammonia nitrogen

[6] Van Slyke, *J. Biol. Chem.*, **10**, 15 (1911).

[7] Compare Ref. 2c, pp. 2486–7.

fractions when fibrin was hydrolyzed in the presence of increasing amounts of acetone.

Table II shows comparative analyses (the averages of duplicate determinations) of fibrin hydrolyzed alone and in the presence of 5 cc. of acetone.

TABLE II

COMPARATIVE NITROGEN DISTRIBUTION OF FIBRIN (3 GRAMS) HYDROLYZED ALONE AND IN THE PRESENCE OF 5 CC. OF ACETONE

	Fibrin alone		Fibrin + Acetone	
	Mg.	%	Mg.	%
Total N taken	424.6	...	424.6	...
Ammonia N	31.4	7.40	27.4	6.45
Humin N				
(a) Insoluble	8.8	2.07	9.0	2.12
(b) Soluble	4.2	0.99	4.3	1.01
(c) Phosphotungstic	3.6	0.85	3.6	0.85
Basic N	124.2	...	125.6	...
(a) Arginine N	54.0	12.72	54.4	12.81
(b) Histidine N	16.0	3.77	7.2	1.70
(c) Cystine N	2.1	0.49	2.0	0.47
(d) Lysine N	52.0	12.25	62.0	14.60
(e) Amino N	73.0	...	80.0	...
N in filt. from bases	250.4	...	242.7	...
(a) Amino N	230.8	54.36	229.6	54.07
(b) Non-amino N	19.6	4.62	13.1	3.08
Total recovered	422.6	99.53	412.6	97.17

Table III lists the humin and ammonia fractions obtained when tryptophane and tyrosine were boiled with acid in the presence of the ketones.

TABLE III

THE HUMIN NITROGEN FRACTIONS OBTAINED WHEN TRYPTOPHANE AND TYROSINE WERE BOILED FOR 24 HOURS WITH 20 PER CENT. HYDROCHLORIC ACID IN THE PRESENCE OF ACETONE AND ACETOPHENONE

Ketone added Cc.	Amino acid added Mg.	Insol. humin N Mg.	Sol. humin N Mg.	Total humin N Mg.
Acetone	Trytophane			
cc.	mg.			
0.10	100	0.4	3.8	4.2
4.00	100	0.5	6.7	7.2
Acetophenone				
0.10	100	0.2	1.5	1.7
3.00	100	1.1	4.4	5.5
	Tyrosine			
0.50	100	0.0	0.2	0.2
Acetone				
0.0	100	0.0	0.5	0.5
0.1	100	0.0	0.1	0.1
4.0	100	0.0	0.4	0.4

It is evident from the data presented that the action of ketones upon proteins or upon tryptophane is not similar to the action of the aldehydes which have been studied. Acetone at least produces no appreciable effect upon the nitrogen distribution among the products of hydrolyses of fibrin and there is no reason for believing that acetophenone would react differently. The humin nitrogen fractions of fibrin are unchanged by hydrolysis in the presence of acetone. Table III shows that neither tryptophane nor tyrosine unites with ketones to form an acid-insoluble humin. No black flecks of humin developed in these hydrolysates. There is some evidence that ketones may increase the acid-soluble humin from tryptophane, but we have already shown[8] that a part of the tryptophane nitrogen appears in this fraction when tryptophane is boiled with mineral acids, so that it remains to be proved whether or not ketones actually unite with the tryptophane or merely accelerate a reaction already in progress. At any rate, ketones can be eliminated as a factor in the normal black humin formation of protein hydrolysis. The effect of this conclusion is that the unknown component of proteins which is involved in humin formation is, in all probability, an aldehyde.

Summary

1. Fibrin was hydrolyzed alone and in the presence of acetone and acetophenone and various nitrogen distributions among the products were studied.

2 It is concluded that hydrolysis in the presence of ketones does not alter the nitrogen distribution of a protein as measured by Van Slyke's method.

3. The acid-insoluble humin of a protein hydrolysis is apparently not formed by the interaction of a ketone with tryptophane.

4. It is suggested that the humin formation in a normal protein hydrolysate is due to the interaction of tryptophane with some as yet unidentified aldehydic component of the protein molecule.

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[8] Ref. 2f, p. 2382.