CCXV. THE GLUTEN-DISSOLVING FERMENT OF WHEAT AND BARLEY SEEDS.

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IN the winter of 1931-32 Miss Kozmin drew our attention to the fact that gluten washed out from wheat grains damaged by the bites of the "tortoise bug" (*Eurygaster* species) within 2 hours at the most following the washing out turns into a liquid. This phenomenon was described by Kozmin [1929; 1930] and Berliner and Koopman [1929], both of whom have suggested that it was probably a case of the action of strong proteolytic ferments. However, testing by the usual method, *i.e.* by autolysis of 5 g. of flour from the affected grain for 6 hours at p_H 4.7 and a temperature of 34° showed only a slight increase of the free amino-groups in comparison with the autolysis of flour from normal grain; the normal grain gave an increase of 2.1 mg. and the damaged grain 2.44 mg. Amino-nitrogen was determined by the indirect method of titration of the carboxyl groups by the Willstätter method, and a difference of 0.34 mg. on a 5 g. aliquot could not be considered decisive. After 48 hours of autolysis there was hardly a change in the difference between normal and damaged flour. The total nitrogen content of the 5 g. of flour being about 120-130 mg. an increase of amino-nitrogen of about 0.3-0.6 mg. certainly could not cause such a liquefaction of the gluten as was mentioned above.

Our next hypothesis was to assume the existence of a specific ferment in the damaged grain dissolving only the proteins of the gluten without splitting the amino-linkages in the polypeptide chains. In order to verify this assumption we have prepared a water extract from the damaged grain by placing in a thermostat a mixture of 1 g. of flour, 5 cc. of water and 1 cc. of toluene, allowing it to stand there for 48 hours at a temperature of 33-35° and filtering. From the flour of the undamaged grain fresh gluten was washed off. Aliquots of 1 g. were weighed into Erlenmeyer flasks and to each were added 20 cc. of acetate buffer p_H 4.7, 5 cc. of the ferment extract and 1 cc. of toluene. One part of the flasks was placed for 10 minutes over a water-bath and then in a thermostat for 24 hours. The contents of the second part were analysed immediately and the third part was placed in a thermostat without heating. In the thermostat were also placed flasks containing only gluten without the extract and others containing only the extract without gluten. For analyses the contents of the flasks were filtered through an ashless filter directly into a Kjeldahl flask and carefully washed out. The residue on the filter was digested according to the Kjeldahl method and so was the filtrate mixed with the water used for the washing out. The analyses gave the following results.

	Nitrogen in mg.		
	In filtrate	In residue	Total
Without heating, 24 hours at 33-35°	20.13	50.16	70.29
Heated, 24 hours at 33-35°	15.63	53.56	69.19
Without heating, determined immediately	16.83	53.46	70.29
Gluten alone after 24 hours	6.58	50.37	56.95
Extract alone after 24 hours	13.31	—	13.31
			70.26

After 24 hours' digestion about 8 % of the nitrogen of the gluten passed into the filtrate.

An experiment was undertaken under the same conditions on the effect upon the gluten of a diastatic preparation ("maltin" of Kahlbaum, *viz.* a preparation from sprouted barley). The increase of amino-nitrogen titratable by the Sørensen method amounted after 24 hours only to 1.94 mg. The variation in solubility is expressed by the following values.

	Nitrogen in mg.		
	In filtrate	In residue	Total
Without heating, 24 hours at 33-35°	35.57	26.82	62.39
Without heating, determined immediately	4.28	55.87	60.15
Heated up to 100°, 24 hours at 33-35°	13.64	46.73	60.37
Gluten alone, 24 hours at 33-35°	12.18	44.22	56.40
Diastase alone, 24 hours at 33-35°	3.26	2.83	6.09
			62.49

The results obtained after 6 hours' treatment were also sufficiently definite, *viz.*:

	Nitrogen in mg.		
	In filtrate	In residue	Total
Without heating, 6 hours	16.57	43.50	60.07
Without heating, determined immediately	4.39	56.16	60.55
Heated up to 100°, 6 hours	4.53	55.31	59.84

A still more energetic action was observed in an alkaline medium at p_H 8.0. The results of a corresponding experiment carried on for 24 hours at a temperature of 33-35° with gluten and maltin were the following:

	Nitrogen in mg.		
	In filtrate	In residue	Total
Without heating, 24 hours	47.52	13.66	61.18
Without heating, immediately	5.82	54.49	60.31
Heated to 100°, 24 hours	6.90	55.07	61.97
Gluten alone, 24 hours	4.31	53.70	58.01

The effect upon the gluten at p_H 8.0 of the extract from the grain damaged by the bug gave on the whole the same results as at p_H 4.7.

	Nitrogen in mg.		
	In filtrate	In residue	Total
Without heating, 24 hours	9.16	53.77	62.93
Without heating, immediately	6.47	55.93	62.40
Heated to 100°, 24 hours	6.90	56.22	63.12

The next step was the study of the effect of the dissolving ferment upon the components of the gluten: glutenin and gliadin. The preparations of these proteins were made according to Kiesel *et al.* [1931] from normal wheat flour, p_H 4.7 (acetate buffer). Dry preparations of protein (0.2 g.) were used. The increase of amino-nitrogen titratable by Sørensen's method amounted in the

glutenin for the 24 hours to 3.24 mg. The effect of diastase ("maltin") gave the following results:

	Glutenin			Gliadin		
	Nitrogen in mg.			Nitrogen in mg.		
	In filtrate	In residue	Total	In filtrate	In residue	Total
Without heating, 24 hours	19.83 18.48	19.17	15.03 15.55	15.29	34.86 34.03	31.21 31.27
Without heating, immediately	14.53 14.93	14.73	20.14 19.45	19.29	34.67 34.48	20.09 21.41
Heated to 100°, 24 hours	8.07 7.30	7.69	26.45 27.11	26.28	34.52 34.41	*18.44 *20.08
Protein alone, 24 hours	3.21 2.98	3.09	28.76 28.60	0.68	31.97 31.58	19.54 19.05
Diastase alone, immediately	1.51 1.47	1.49	1.22 1.22	1.22	2.73 2.69	1.51 1.47
Diastase alone, 24 hours	1.83 1.83	1.83	0.81 0.88	0.85	2.64 2.71	1.83 1.83
						5.11 5.55 15.65 15.38 *15.24 *13.90 14.22 16.22 1.22 1.22 0.81 0.88
						5.33 5.33 15.52 15.52 14.57 15.22 15.22 — — 0.85 0.85
						36.22 36.82 35.74 36.79 *33.68 *33.98 33.76 35.27 2.78 2.69 2.64 2.71

* Gliadin without the ferment immediately after the experiment was started.

The dissolving effect upon gliadin is considerably greater than upon glutenin.

The enzymic nature of the disintegration of the protein without an increase of the free amino-groups or at least with a considerably smaller accumulation of amino-groups in comparison with the amounts of nitrogen rendered soluble is sufficiently clear from the above experiments. It is hard to imagine what linkages are really being split in the protein molecule during this reaction. It may be believed, however, as is thought by Astbury and Wood [1931], that the protein molecule consists of parallel polypeptide chains connected with each other by means of additional valencies into compound aggregates. Such connections may be formed between 2, 3 or 6 polypeptide chains, which is quite in agreement with the fact found by Svedberg [1931] that the molecular weight of the protein is either equal to 34,500 or 2, 3 or 6 times this. The breaking of the bond between the different polypeptide chains may cause a change of the aggregate state of the protein without freeing new amino-groups. The appearance of the latter is connected with the presence in the ferment preparations of real proteases.

SUMMARY.

1. The gluten of flour from grain damaged by the "tortoise bug" rapidly changes its aggregate condition without any noticeable increase of the free amino-groups.

2. The disintegration of the gluten and of the component parts of it, glutenin and gliadin, which is connected only with a change of the aggregate state of the protein, is a process of an enzymic nature.

3. The corresponding ferment, which is representative of an entirely new type of disaggregating ferments, acts at both acid and alkaline reactions and is found in the seeds of wheat damaged by the "tortoise bug" and in the diastatic preparation from malt.

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