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Effect of Denaturation on Digestibility of Ovalbumin by Pepsin and Trypsin.

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The object of this investigation is to throw light on the fundamental mechanism underlying the process of denaturation, by comparing the rates of digestion by pepsin and trypsin of natural ovalbumin with those of the same protein denatured in various ways. Increase in the digestibility of denatured proteins would point to hydrolysis or some other kind of degradation, while decrease in digestibility would indicate a change in the opposite direction.

Crystalline ovalbumin was prepared from egg white and, after dialysis, was used either as such, or after denaturation. The denaturing agents used were acid, alkali, heat, alcohol and shaking. The protein solution was mixed with a calculated amount of acid or alkali, enzyme solution and enough water to make a 1% of protein. A series of such mixtures of varying pH was prepared and maintained at 37.5° C. for a definite length of time, generally 4 hours, at the end of which the enzyme action was quickly stopped, by making the solution either acid or alkali as the case may be. The undigested protein was precipitated with trichloracetic acid and the soluble nitrogen in the filtrate was determined. After making corrections for the nitrogen derived from enzymes the percentage of total nitrogen digested was calculated. The optimal pH and the maximal digestion of the different proteins are shown in Tables I and II.

TABLE I.

Relative rates of peptic digestion of natural and denatured ovalbumin at their respective optimal pH's.

Time of incubation, 4 hours. Concentration of enzyme 0.2%.

Protein	Percentage digestion	Optimal pH
Natural	51	1.03
Alkali denatured	56	1.22
Acid denatured	52	1.37
Acid heated	52	1.63
Shaking-denatured	51.5	1.56
Alkali-heated	48	1.6
Alcohol denatured	37.5	1.4

TABLE II.

Relative rates of tryptic digestion of natural and denatured ovalbumin at their respective optimal pH's. Arranged in the order of increasing digestibility.

Protein	Concentration of enzyme	Duration of incubation	Percentage digestion	Optimal pH
	%	hour		
Natural	5	4	43	10.7
Acid-heated	5	4	46.5	9.25
Shaking-denatured	5	4	92	10.5
Alkali-heated	5	4	96	9.10
Acid denatured	5	1	96	10.30
Alcohol denatured	2	1	99	10.10
Alkali denatured	2	2/3	89	9.40

Summary: (1) In the peptic series the rates of digestion of natural and different forms of denatured proteins are, with a few exceptions, practically the same within the limit of experimental error. This indicates that the fundamental changes underlying denaturation do not affect those linkages in the albumin molecule which are hydrolyzed in peptic digestion. (2) In the tryptic series the rate of digestion of natural albumin is exceeded by all forms of denatured proteins. This finding indicates that the changes produced by the denaturing are in the direction of degradation and probably of the same nature as tryptic digestion. (3) Natural and denatured proteins have different optimal pH's of digestion. There is a tendency for these optimal points to shift toward the neutral point when the protein is denatured. Northrup[1] showed that the rate of digestion of a protein is, in general, a function of the concentration of ionized protein, and that it is minimal at the isoelectric point of the protein, and maximal at that pH at which the protein is completely combined with acid or alkali to form salts. In the light of this hypothesis our findings seem to indicate that the isoelectric point and the maximal dissociation of the albumin are shifted toward the neutral point by denaturation.

[1] Northrup, J. H., *J. Gen. Physiol.*, 1922, v, 263.