CRYSTALLINE EGG ALBUMIN

IV. THE RATE OF LIBERATION OF AMINO NITROGEN AND CYSTINE, TYROSINE, AND TRYPHTOPHANE COLORIGENIC VALUES DURING PEPTIC, ACID, AND ALKALINE HYDROLYSIS OF EGG ALBUMIN

BY HERBERT O. CALVERY, WALTER D. BLOCK, AND ELLEN D. SCHOCK

(From the Department of Physiological Chemistry, Medical School, University of Michigan, Ann Arbor)

(Received for publication, October 12, 1935)

In a recent investigation of the digestibility of proteins in vitro, Jones and Gersdorff (1) studied the rate of the liberation of cystine during peptic and acid digestion of casein. The Sullivan (2) method did not indicate any liberation of free cystine during peptic hydrolysis, while with acid hydrolysis it reached a maximum at 0.33 per cent at the end of 6 hours and remained constant during the extent of the experiment. Results by the Folin and Marenzi (3) method were quite different.

Jones and Gersdorff have further discussed at some length the liberation of amino acids during enzymic digestion of proteins, and they quote Abderhalden (4) as the only evidence that free amino acids are not liberated by the action of pepsin on protein. Since the work of Abderhalden several investigators have reported the isolation of free amino acids from peptic hydrolysates. Felix (5) has isolated free lysine from a peptic hydrolysate of histone, Northrop (6) isolated tyrosine from peptic self-digestion, Calvery and Schock (7) isolated tyrosine from a peptic hydrolysate of crystalline egg albumin, and Lieben and Lieber (8) have reported the presence of free arginine following peptic hydrolysis of several proteins. Although free amino acids may be liberated by peptic hydrolysis of protein, cystine does not seem to be one of them.

The present investigation is a study of crystalline egg albumin similar to that made by Jones and Gersdorff (1) of casein and includes the determination of tyrosine and tryptophane during
peptic and alkaline hydrolysis and the amino nitrogen during peptic hydrolysis.

EXPERIMENTAL

The albumin was prepared according to the method of Sörensen and Høyrup (9) and recrystallized twice. A final volume of 2000 cc. of protein solution was obtained containing 18.9 mg. of albumin per cc.

Peptic Hydrolysis of Albumin—1000 cc. of the above solution (18.9 gm. of protein) were taken, enough hydrochloric acid was added to make a 0.3 per cent solution, and 1.89 gm. of pepsin (Difco, 1:20,000) were added. A control containing only pepsin was run at the same time. Since, as pointed out in an earlier investigation, digestion is so rapid at the beginning of the experiment and initial values are so difficult to obtain, the first values recorded in Table I are the values at the end of 15 minutes in all cases. The digestion was carried out at 30° in a thermostat and samples were removed at definite intervals. The amino nitrogen was determined by the Van Slyke method and the cystine, tyrosine, and tryptophane were determined by the methods of Folin and Marenzi (3, 10).

Acid Hydrolysis of Albumin—To 320 cc. of the above albumin solution were added 180 cc. of concentrated sulfuric acid and the volume made to 500 cc. This made a solution of approximately 65 per cent sulfuric acid. In a more dilute solution a precipitate remained and the early determinations of cystine could not be made. Since the concentration of acid was so high, it was only heated to 85–95°. Samples were removed at the same intervals as during the peptic hydrolysis and only cystine was determined after the acid concentration was carefully adjusted to that of the Folin and Marenzi (3) determination.

Alkaline Hydrolysis of Albumin—This hydrolysis was carried out in large individual Pyrex test-tubes. 15 cc. of the albumin solution and 10 cc. of 50 per cent NaOH were thoroughly mixed and placed in a boiling water bath for the definite periods of time exactly comparable to those of the acid and peptic hydrolyses. The procedure was then exactly the same as that in the Folin and Marenzi (10) micromethod for tyrosine and tryptophane.

It has been previously definitely established that the nitrogen
content of crystalline egg albumin when dried at 105–110° and corrected for ash is 15.4 per cent (11). The total nitrogen was determined in the albumin solution used in this study and the albumin content calculated. The amino nitrogen is expressed as percentage of the total nitrogen, while the chromogenic values are expressed as percentages of tyrosine, tryptophane, and cystine in the albumin present. A summary of the results is incorporated in Table I.

### DISCUSSION

The results in Table I show that the Folin and Marenzi values for cystine in egg albumin as determined in this investigation differ...
quite markedly from those reported by Jones and Gersdorff (1) for casein. In the acid hydrolysate the values for cystine gradually rose to a maximum of 1.3 per cent, following which there was gradual destruction of the cystine by the strong acid used. This value of 1.3 per cent is the same as that previously reported by one of us and by other investigators. In the case of the peptic digest there was again no rapid rise at the beginning to a sharp peak but a slow rise to a value far above that found by acid hydrolysis. This has been previously found in other peptic digests (unpublished data). Also the final value after 36 days is greater than that found by acid hydrolysis.

In confirmation of the findings of Jones and Gersdorff that 0.1 N hydrochloric acid did not hydrolyze casein, it has been repeatedly demonstrated in this laboratory that 0.3 to 0.5 per cent hydrochloric acid has very little hydrolytic action on the native protein and none on the partial or final peptic hydrolysis products in the thermostat at 30° for long periods of time or when heated for 5 minutes at 85° (unpublished experiments of Miss Lila Miller and of ours).

In a very interesting paper published recently by Cohn and White (12) in an attempt to explain the early findings of Mendel and Lewis (13) it was found that cooked egg white was more readily digested than raw egg white. As shown by this present experiment, in which a pepsin to albumin ratio of 1:10 was used, and as pointed out by Calvery (14) in an earlier investigation, pepsin readily attacks uncoagulated crystalline egg albumin and the digestion is so rapid during the first few minutes that duplicate Van Slyke determinations for amino nitrogen cannot be made to check each other. It has further been often demonstrated in this laboratory that raw egg white as well as crystalline albumin can be readily digested by pepsin (unpublished data). This does not necessarily contradict the findings of Cohn and White (12), since the conditions of experimentation are somewhat different.

SUMMARY

Uncoagulated crystalline egg albumin is readily hydrolyzed when the pepsin (Difco, 1:20,000) to protein ratio is 1:10 and the acid concentration is approximately 0.3 per cent. During the digestion the amino nitrogen value gradually rises to a maximum
of 25.1 per cent in 36 days, while the chromogenic value obtained by the Folin and Marenzi method for cystine rises gradually to a peak of 2.1 per cent within 12 hours and then falls to a constant value between 1.6 and 1.7 per cent during the next 35 days. This is much higher than the highest value of 1.3 per cent obtained by acid hydrolysis in this experiment and the same value obtained previously by other investigators. The acid peak was reached after a gradual rise and did not follow the same curve at all as that obtained by Jones and Gersdorff (1) for the cystine content of casein.

The tyrosine and tryptophane colorigenic values obtained by the Folin and Marenzi micromethod during both peptic and alkaline hydrolysis rose gradually until they reached maximum values. The values were not the same during peptic hydrolysis as those obtained during alkaline hydrolysis. A discussion of the values obtained and their significance is included.

BIBLIOGRAPHY

CRYSTALLINE EGG ALBUMIN: IV.
THE RATE OF LIBERATION OF AMINO
NITROGEN AND CYSTINE, TYROSINE,
AND TRYPTOPHANE COLORIGENIC
VALUES DURING PEPTIC, ACID, AND
ALKALINE HYDROLYSIS OF EGG
ALBUMIN
Herbert O. Calvery, Walter D. Block and Ellen
D. Schock


Access the most updated version of this article at
http://www.jbc.org/content/113/1/21.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail
alerts

This article cites 0 references, 0 of which can be
accessed free at
http://www.jbc.org/content/113/1/21.citation.full.html#ref-list-1