radioactivity of the glycine in the supernatant and in the protein hydrolysate was determined by thin-layer chromatography of the DNP-amino acids (Stahl, 1965), and extraction of the glycine spot into methanol for measurement of the extinction and the radioactivity. The ratio of glycine in the free and protein-bound pools was determined for a few rats by using an automatic amino acid analyser. (I am grateful to Dr D. Picou for these analyses.)

Total glycine flux, and protein turnover rates in muscle and liver, were calculated by formulae derived by Waterlow & Stephen (1968).

The total flux of glycine entering and leaving the bloodstream was several times the value for lysine, but in view of the greater metabolic activity of glycine this was not surprising. Values of liver-protein half-life were much higher than those previously obtained, and this is thought to be caused by an error in the calculation due to loss of label through channels other than protein synthesis.

The calculated half-life of muscle protein (about 6 days) agrees very well with the values obtained by the lysine method and by a totally different method involving measurement of catabolic rates (. . . Millward, unpublished work). This suggests that side reactions of glycine in this tissue are quantitatively unimportant.

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Degradation of Human Serum Albumin by Human Pepsins

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Pepsin in gastric juice from man and other animals has been shown to produce non-symmetrical pH-activity curves with two maxima. The meaning of these curves was reviewed critically by Taylor (1962), who considered nine possible mechanisms. Only three can account adequately for all the observations: (1) the existence of more than one pepsin; (2) a single enzyme with two active centres; (3) a complex action of pepsin on proteins involving differences in pH optima for the initial and subsequent splitting of the substrate by the enzyme. Experiments by several workers have shown that at least two peptic enzymes are present in human gastric juice (Richmond, Tang, Wolf, Trucco & Caputto, 1958; Seiffers, Segal & Miller, 1963).

Our studies on the brief digestion of human serum albumin with whole gastric juice and three pepsin

fractions (A, B, C) separated by DEAE-Sephadex chromatography are reported. Gel-filtration and ultracentrifuge techniques were used to study the stability, molecular size and size homogeneity of the initial products of digestion, to determine the mode of enzyme action of the pepsin fractions.

Albumin solutions were digested at pH 2.0 or pH 3.4 at 20° or 37°. At suitable intervals samples of the digest mixtures were inactivated with alkali and filtered through Sephadex G-75. The gel-elution patterns indicated that albumin was rapidly split at both pH 2.0 and 3.4 by gastric juice and by pepsin fractions B and C to large intermediate fragments (mean mol. wt. 23 000), from which small peptides were only slowly formed. In contrast, pepsin fraction A appeared to degrade albumin directly to small peptides at pH 3.5.

Peptic digestion of albumin studied in the ultracentrifuge under diffusion conditions showed unchanged size heterogeneity of the initial reaction products over long periods of time. The characteristics of the intermediate fragments were consistent with the mixture of two types of fragments obtained by Weber & Young (1964a,b).

Our results suggest that the initial degradation of albumin by pepsin fractions B and C occurs by a 'multi-chain' action (cf. Whelan & Bailey, 1954), whereas digestion by fraction A may occur by a 'single-chain' action. Further, similar studies with bovine haemoglobin as substrate indicated that fraction A was less capable of digesting proteins that retain their tertiary structure in acid solution.

Studies of the initial reaction rates also indicate that the pepsin fractions B and C are similar to each other but different from fraction A in the range pH 2.0-3.9. Fraction A appears to function synergistically with fractions B and C in the initial digestion of albumin by gastric juice.

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