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Amorphous Pepsin of Higher Activity Than Crystalline Pepsin from Commercial and Crystalline Pepsin.

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In this laboratory, very active amorphous pepsin fractions, approximately 2 to 4 times as active as crystalline pepsin, were obtained by McMeekin[1] and Freeland[2] when commercial pepsin solutions were adsorbed on specially prepared egg white at pH 3.0 and eluted at pH 6.1 with disodium phosphate. Recently by the salt fractionation of crystalline pepsin prepared from pepsinogen, Herriott, Desreux, and Northrop[3] obtained preparations with an activity 3 times that of crystalline pepsin.

It should be emphasized that the very active fractions of Herriott *et al.* were obtained from crystalline pepsin which they prepared from pepsinogen and not from the crystalline pepsin they prepared from commercial pepsin. In view of these findings it was thought desirable to apply the adsorption concentration method of McMeekin to crystalline pepsin obtained from commercial pepsin as well as to commercial pepsin.

Experimental. Protein nitrogen was determined by the Koch-McMeekin direct Nesslerization method[4] after the protein had been precipitated by 0.3 M trichloracetic acid. The colors were read in the Evelyn colorimeter with filter 520 and mg of nitrogen determined from a standard curve.

Pepsin activity determinations were made by Anson's hemoglobin method[5] and the colors developed were read in the Evelyn photoelectric colorimeter using filter 660. Values were read from a standard tyrosine curve. A hemoglobin blank, reagent blank, and a standard were always run with the samples.

The egg white substrate was prepared according to McMeekin.[1]

The adsorption and elution procedures were as follows: a solution of 10 g of Cudahy 1:10,000 USP pepsin (or varying amounts of crystalline pepsin) in 100 cc of 0.01N HCl was cooled in the ice box for 15 to 30 minutes, then adsorbed on 4 g of the specially treated egg white by shaking in a 100-cc Erlenmeyer flask for 2 hours at 7°C in a rotary shaker. The pepsin-egg white was filtered off and the pepsin eluted from the complex by the addition of 100 cc of 0.4% disodium phosphate, shaken in the cold as before, and filtered. The eluate was adjusted from pH 6.1 to pH 3.0. When ammonium sulfate was added slowly with constant stirring to half saturation, a soft cloudy precipitate appeared at once. The flask was left overnight at 8°C and the following morning a finely granular precipitate was present. Five g of Hi-Flo were added to the precipitate with stirring and the mixture filtered. The filter cake was transferred to a 250-cc centrifuge cup and stirred for about 15 minutes after the addition of 100 cc of 0.3% HCl. This was centrifuged and the supernatant poured off. Ninety cc thereof were transferred to a ¼-inch cellophane dialysis sac, dialyzed against 0.3% HCl for an hour in the ice box and then against frequently changed cold distilled water until the dialysate gave a negative Nessler's test. All solutions were kept in the ice box until ready for use.

Samples were removed for analysis and the solutions either frozen and evaporated in the frozen state immediately, or frozen with CO_2-acetone and stored until they could be dried in the frozen state. The assays were always done within 24 hours after the preparation of a fraction.

[1] McMeekin, Thomas L., Doctoral Dissertation, Department of Physiological Chemistry, University of Chicago, 1925.

[2] Freeland, Milnor, Doctoral Dissertation, Department of Physiological Chemistry, University of Chicago, 1931.

[3] Herriott, Roger M., Desreux, V., and Northrop, John H., *J. Gen. Physiol.*, 1940, **24**, 213.

[4] Koch, F. C., and McMeekin, Thomas L., *J. Am. Chem. Soc.*, 1924, **46**, 2066.

[5] Anson, M. L., *J. Gen. Physiol.*, 1938, **22**, 79.

TABLE I.
Specific Activities of Pepsin Preparations.

Preparations	No. 1	No. 2	No. 3	P-3B	P-3C
Original solution (specific activity)*	.04	.04	.04	.33	.30
Filtrate from egg white (specific activity)	—	.22	.26	.26	.24
Eluate (specific activity)	.31	.38	.63	.50	.33
After dialysis of ppt. by 1/2 sat. $(NH_4)_2SO_4$ (specific activity)	.31	.37	.66	.51	.22
After drying (specific activity)	—	.29	.11	.22	.20

*Specific activity is expressed as hemoglobin units per mg of protein nitrogen.

Crystalline pepsin was prepared according to Northrop[5] except in one case where a preparation just before final crystallization was taken up in a large volume of water, precipitated with magnesium sulfate, treated as before, and then crystallized. This product had a specific activity of 0.33 as compared with the usual specific activity of 0.21-0.23.

Results. The preparations obtained from commercial and crystalline pepsin by the adsorption technic are shown in Table I. Preparations No. 1, No. 2, and No. 3 are amorphous products prepared by the McMeekin procedure from 10% solutions of 10, 25, and 10 g respectively, of Cudahy 1:10,000 USP pepsin. Preparation No. 3 was dialyzed for a shorter period than No. 2, and No. 2 for a shorter time than No. 1. The activities are in the order $3>2>1$. These observations suggest that we may still have mixtures in each of these preparations and if so the mixtures might be made up of the more complex forms of pepsin which presumably are less dialyzable but more stable and less complex, possibly specific prosthetic groups, which probably would be lost by dialysis and are less stable.

P-3B is an amorphous preparation obtained by the McMeekin method from 13 g of freshly prepared crystal cake never dried or stored under magnesium sulfate.

P-3C is an amorphous preparation obtained by the McMeekin method from 4 g of an amorphous pepsin obtained by evaporation of a frozen solution of crystalline pepsin.

Summary. 1. Coagulated egg white removes peptic activity from concentrated solutions of commercial and crystalline pepsins. 2. The pepsins recovered by elution from the egg white are more active than crystalline pepsin. Preparations No. 1 and No. 2 from commercial pepsin and preparation P-3B from crystalline pepsin are respectively 1.5 to 1.7 and 2.5 to 3.0 times as active as crystalline pepsin. 3. Preparations No. 1 and No. 2 have about the same activity as the product Herriott *et al.* isolated from crystalline pepsin by salt fractionation while preparation P-3B has the same activity as the fraction isolated by the same workers by salt fractionation from (a) pepsinogen, and (b) crystalline pepsin prepared from pepsinogen.