7060 C

# Parallel Adsorption of Crystalline Pepsin and Peptic Activity Upon Casein and Ovalbumin.*

JAMES B. SUMNER.

*From the Department of Physiology and Biochemistry, Medical College, Cornell University.*

Waldschmidt-Leitz and Kofrányi[1] found that by repeatedly shaking a solution of crystalline pepsin with small quantities of suspension of cantaloupe seed globulin they could adsorb the peptic activity while the crystalline pepsin-protein was left in solution. They accordingly claim that Northrop's[2] crystalline pepsin is simply a case of an enzyme adsorbed upon a protein. Their evidence for the non-adsorption of the pepsin-protein was obtained by determining the weight of dry matter left after centrifuging off the suspended material and by blank determinations made with cantaloupe globulin alone.

It is unfortunate that these authors did not employ a specific test for the protein of crystalline pepsin, for their centrifuged solutions may have contained not only crystalline pepsin, but also cantaloupe globulin in quantity not indicated by blank determinations, as well as digestion products. By the employment of a test which is specific for crystalline pepsin-protein, provided other proteins are not present in the solution, I have been able to demonstrate that when the peptic activity of a solution of crystalline pepsin is completely adsorbed upon casein or coagulated ovalbumin the pepsin-protein is also completely adsorbed. Furthermore, I have found that the rates of adsorption of peptic activity and of pepsin-protein are the same. These findings conclusively disprove the work of Waldschmidt-Leitz and Kofrányi unless one is willing to make the highly improbable assumption that crystallized cantaloupe seed globulin possesses some peculiar action upon pepsin which is not exerted by casein or by denatured ovalbumin.

Casein and coagulated ovalbumin were chosen as adsorbents because of their insolubility at pH 4.8. Edestin could not be used since it was too soluble below pH 5 and it was not desirable to subject pepsin to solutions less acid than this. Coagulated edestin

* This work was aided by a grant from the Sage Research Fund of Cornell University.

[1] Waldschmidt-Leitz, E., and Kofrányi, E., *Naturwiss.*, 1933, **10**, 206.

[2] Northrop, J. H., *J. Gen. Physiol.*, 1930, **13**, 739.

formed large lumps which could not be pipetted in suspension. The casein was prepared by washing 10 gm. of Merck's casein repeatedly with dilute acetate buffer of pH 4.8. The casein was then mixed with 90 cc. of water and 10 cc. of 0.5 M acetate buffer of pH 4.8. The ovalbumin was prepared by heating 1000 mg. of recrystallized,

EXP. I.
Adsorption of pepsin and pepsin-protein on casein.

| Solution | cc. used for adsorption | $[PU]^{Hb}$ | $[PU]^{Hb}$ as % | pepsin-protein as % |
|---|---|---|---|---|
| Original | 100 | .0115 | 100 | 100 |
| Filtrate I | 91 | .0077 | 67 | 60 |
| " II | 75 | .0045 | 39 | 40 |
| " III | 72 | .0021 | 18 | 20 |
| " IV | | .00085 | 7.3 | 10 |

EXP. II
Adsorption of pepsin and pepsin-protein on ovalbumin

| Solution | cc. used for adsorption | $[PU]^{Hb}$ | $[PU]^{Hb}$ as % | pepsin-protein as % |
|---|---|---|---|---|
| Original | 100 | .0110 | 100 | 100 |
| Filtrate I | 91 | | | |
| " II | 77 | .0063 | 57 | 50 |
| " III | 72 | .0038 | 34 | 30 |
| " IV | | .0018 | 16 | 20 |

EXP. III.
Adsorption of pepsin and pepsin-protein on ovalbumin.

| Solution | cc. used for adsorption | $[PU]^{Hb}$ | $[PU]^{Hb}$ as % | pepsin-protein as % |
|---|---|---|---|---|
| Original | 100 | .0096 | 100 | 100 |
| Filtrate I | 95 | .0066 | 69 | 70 |
| " II | 89 | .0042 | 44 | 40 |
| " III | 82 | .0020 | 21 | 20 |
| " IV | | .00012 | 1.2 | 0.8 |

dialyzed ovalbumin in 90 cc. of water for 15 minutes, cooling and adding 10 cc. of acetate buffer. Both proteins were suspended by shaking just before pipetting. The casein was lumpy and difficult to pipette.

Crystalline pepsin solutions were made by dissolving pepsin crystals in a little water containing 10 cc. of acetate buffer, diluting to 200 cc. and filtering. The pepsin employed in Exp. I and II was once crystallized; the pepsin ued in Exp. III was twice crystallized. The pepsin concentrations (N x 6.54) were respectively 45, 48, and 37 mg. per 100 cc. in Exp. I, II, and III.

Adsorption was carried out by adding 5 cc. of suspended protein to 100 cc. of pepsin at room temperature. After rotating for

6 minutes the material was filtered and the filtrate tested for peptic activity and for pepsin-protein; the remaining filtrate was then used for a second adsorption. For determination of peptic activity the method of Anson and Mirsky[3] was employed and the values are expressed as their pepsin units, or $[PU]^{Hb}$. When solutions of low peptic activity were tested it was necessary to digest for intervals longer than the usual 5 minutes.

Pepsin-protein was estimated by adding one drop of 10% sodium hydroxide to 5 cc. of the clear pepsin solution, followed, after mixing, by 1 cc. of 0.5 N sulfuric acid. The suspension of denatured pepsin-protein was then compared with various dilutions of the original pepsin solution which had been coagulated in the same manner. With this test it was found possible to make approximate estimation of as little as one part of crystalline pepsin-protein in 300,000 of water, using a beam of light in a dark room. The results of 3 experiments are shown in the tabulation.

[3] Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1932, **16**, 59.