

In conclusion, the mechanism of enhanced diabetes with inflammation seems to be referable to an increased proteolysis in the inflamed area. The rise in protein breakdown processes is correlated both with gluconeogenesis, and with increased severity of the inflammatory reaction. The surplus glucose formed locally gradually diffuses into the circulation thus elevating markedly the blood sugar level. These studies will form the subject of a detailed forthcoming report.

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### THE ACTION OF CRYSTALLINE PEPSIN ON HORSE ANTI-PNEUMOCOCCUS ANTIBODY

We have recently shown that when diphtheria antitoxic pseudoglobulin is treated with crystalline pepsin, a cleavage of the antitoxin molecule occurs in a plane normal to the long axis and that all the reactivity remains with one fragment only.<sup>1</sup> We have now extended this study to horse anti-pneumococcus antibody digested with pepsin in acetate buffer at pH 4.5 according to the method of Grabar.<sup>2</sup>

Euglobulin fractions from several different horses containing Type I and Type II antibody<sup>3</sup> have been examined in the standard Svedberg oil-turbine ultracentrifuge. The different serums showed wide variations in the sedimentation constants observed in agreement with the experience of other workers.<sup>4</sup> Thus, for example, most of the antibody euglobulin from Type I serum No. 721-34 was concentrated in a component with  $s_{20} = 7 \times 10^{-13}$  cm/sec/dyne, and relatively little heavier material was present. On the other hand, a euglobulin fraction from Type II antiserum No. 513-50 showed major components with  $s_{20} = 7, 11, 18$  and  $30 \times 10^{-13}$  cm/sec/dyne; and specific absorption of the latter with SII indicated that the antibody was concentrated mainly in the three heaviest components. Both these preparations were among those digested with pepsin. The digested antibody was precipitated with specific polysaccharide and the specific precipitate dissociated with sodium chloride.<sup>5</sup> The purified, digested and dissociated antibody combines with twice as much polysaccharide per milligram antibody nitro-

gen as the normal antibody, in confirmation of Grabar<sup>2</sup> and Treffers and Heidelberg.<sup>6</sup> Its sedimentation constant is  $5.2 \times 10^{-13}$  cm/sec/dyne, suggesting a molecular weight of less than 100,000, and it is homogeneous as regards sedimentation behavior. The same sedimentation constant is obtained after pepsin treatment, regardless of the molecular weight of the antibody in the original starting material. The digested horse pneumococcus antibody combines with as much carbohydrate per milligram antibody nitrogen as normal rabbit pneumococcus antibody, and it is smaller in size.

Finally, it may be noted that digested horse pneumococcus antibody is more soluble in distilled water than is the untreated antibody, and it has been possible to utilize this property to facilitate removal of undigested antibody from the neutralized reaction mixture by simple dialysis.

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### A BIOTIN-LIKE SUBSTANCE PRODUCED BY DIPLODIA ZEA

In the course of studies of the physiology of the two species of the genus *Diplodia* which most commonly cause ear rots of corn it has become apparent that at least one important difference between them is the ability of *D. zea* (Schw.) Lév. to produce in culture some substance, apparently found in many crop plants, which is necessary for the growth of both *D. zea* and *D. macrospora* Earle. Margolin<sup>1</sup> has recently reported that *D. macrospora* requires "biotin or a biotin-like substance" for growth.

That this substance may be biotin is indicated by a number of similarities between it and the biotin obtained from egg yolks by Kögl and Tönnis.<sup>2</sup> A concentrate was obtained by the method of Buston and Pramanik<sup>3</sup> from a medium which contained  $\text{KH}_2\text{PO}_4$  .3 g.,  $\text{MgSO}_4$  .25 g.,  $\text{KNO}_3$  2.0 g., dextrose 30.0 g., and distilled water to make 1 liter and which had been staled for 4 or 5 weeks by *D. zea*. This concentrate is soluble in water and alcohol but is only very slightly or not at all soluble in ether, petroleum ether or chloro-

<sup>1</sup> M. L. Petermann and A. M. Pappenheimer, Jr., *Jour. Phys. Chem.*, 45: 1, 1941.

<sup>2</sup> P. Grabar, *Compt. rend. Acad. Sci.*, 207: 807, 1938.

<sup>3</sup> We are indebted to Dr. Elliott S. Robinson, of the Antitoxin and Vaccine Laboratory, Jamaica Plain, Mass., and to Dr. Harold Lyall, of the New York State Laboratories, Albany, N. Y., for generous samples of pneumococcus horse serum used in this work.

<sup>4</sup> M. Heidelberg and K. O. Pedersen, *Jour. Exp. Med.*, 65: 393, 1937. E. A. Kabat, *Jour. Exp. Med.*, 69: 103, 1939.

<sup>5</sup> M. Heidelberg and F. Kendall, *Jour. Exp. Med.*, 64: 161, 1936.

<sup>6</sup> H. P. Treffers and M. Heidelberg, *Jour. Exp. Med.*, 73: 125, 1941.

<sup>1</sup> A. S. Margolin, *Proc. W. Va. Acad. Sci.*, 14 (Keyser), in *W. Va. Univ. Bul.*, Ser. 41, No. 4-II, 1940.

<sup>2</sup> F. Kögl and B. Tönnis, *Zeits. für Physiol. Chemie*, 242: 43-73, 1936.

<sup>3</sup> H. W. Buston and B. N. Pramanik, *Jour. Biochem.*, 25: 1656-70, 1931.