

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.

VALY MENKIN

DEPARTMENT OF PATHOLOGY,
HARVARD MEDICAL SCHOOL

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.¹ 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.²

Euglobulin fractions from several different horses containing Type I and Type II antibody³ 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.⁴ 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 30×10^{-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.⁵ The purified, digested and dissociated antibody combines with twice as much polysaccharide per milligram antibody nitro-

gen as the normal antibody, in confirmation of Grabar² and Treffers and Heidelberg.⁶ Its sedimentation constant is 5.2×10^{-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.

The authors are grateful to J. W. Williams for his continued interest in this work.

MARY L. PETERMANN

DEPARTMENT OF CHEMISTRY,
UNIVERSITY OF WISCONSIN

A. M. PAPPENHEIMER, JR.

DEPARTMENT OF BACTERIOLOGY,
SCHOOL OF MEDICINE,
UNIVERSITY OF PENNSYLVANIA

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. Margolin¹ 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.² A concentrate was obtained by the method of Buston and Pramanik³ from a medium which contained KH_2PO_4 .3 g., MgSO_4 .25 g., 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-

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

² P. Grabar, *Compt. rend. Acad. Sci.*, 207: 807, 1938.

³ 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.

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

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

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

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

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

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

form. It is not inactivated by refluxing with acidulated methanol, but this process gives an active compound which is chloroform soluble. It is stable under treatment by hydrochloric acid and sodium hydroxide, is readily adsorbed by animal char and dialyzes readily through a collodion membrane. It is thermostable but is rendered completely inactive by nitrous acid.

A culture of *Saccharomyces cerevisiae* obtained from Dr. L. H. Leonian grew rapidly in the "B medium" used by Robbins and Schmidt⁴ if a little of the staled medium was first added. Without the addition of the staled solution this "B solution" supported only a sparse development of the yeast.

It is known that vitamin H deficiency injury is induced in rats by a diet containing too much raw egg white.⁵ A recent report states that raw egg albumen will also inactivate biotin *in vitro*. A test somewhat similar to that of Eakin, Snell and Williams⁶ was ap-

plied to the staled medium. The white of a fresh egg and 10 ml of an aqueous solution of a concentrate of the staled medium were autoclaved for 20 minutes. The required aliquots of dextrose and minerals were then added and the solution re-autoclaved. Both *D. zeae* and *D. macrospora* grew well in this medium. Raw egg white added aseptically to a 500 ml of sterile medium containing 10 ml of staled solution completely inhibited growth of *D. macrospora* and markedly reduced growth by *D. zeae*.

These properties of the staled concentrate are very similar to many of those of vitamin H and coenzyme R which now are considered identical with biotin.^{7, 8}

The details of the work on which the foregoing statements are based will be contained in a thesis submitted by the junior author.

NEIL E. STEVENS
W. E. WILSON

UNIVERSITY OF ILLINOIS

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MODIFIED PHOTONREFLECTOMETER FOR USE WITH TEST-TUBES¹

THE substitution of test-tubes for refraction cells in photonreflectometric turbidity determinations has many desirable advantages, such as the simplification of technics, the elimination of the cost of the refraction cells and the availability of test-tubes.

One of the difficulties in adapting the use of test-tubes for photoelectric measurements has been the inherently poor optical qualities of the rounded sides of the tubes. This difficulty has been largely eliminated in the design of the "test-tube photonreflectometer," since a beam of light is directed up through the bottom of the test-tube instead of through the sides as in all conventional designs.

If an optically clear solution is placed in a test-tube and a beam of light is directed up through it the rounded bottom of the test-tube acts as a condensing lens and the light passes up through the solution. Since little or no light is reflected, the photronic cell, which is set at a 90° angle to the test-tube, is not activated and a zero reading is obtained on the galvanometer. If, however, a turbid solution is placed in the test-tube some of the light passing up through is reflected by the suspended particles and falls on the active surface of the photronic cell.

The amount of current generated by the photronic cell is dependent on the amount of light reaching it; this in turn is dependent on the number of particles in the suspension. This relationship holds over a range of turbidities—the lower limit of which is determined by the amount of light put through the system, the sensitivity of the photronic cell and the sensitivity of the recording galvanometer; the upper limit of which is determined by the factor of adsorption of light in too dense suspensions.

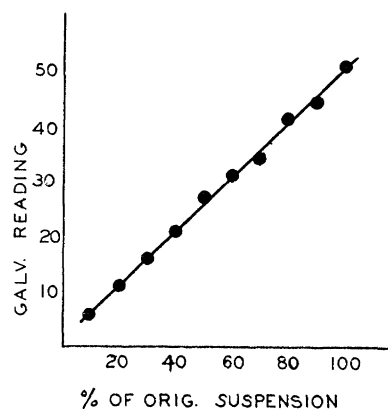


FIG. 1

Fig. 1 is a typical sample of a plot of galvanometer readings against known percentage dilutions of a bac-

⁴ W. J. Robbins and Mary B. Schmidt, *Bull. Torrey Bot. Club*, 66: 139-50, 1939.

⁵ Paul György, *Jour. Biol. Chem.*, 131: 733-44, 1939.

⁶ Robert E. Eakin, Esmond E. Snell and R. J. Williams, *Jour. Biol. Chem.*, 136: 801-02, 1940.

¹ R. L. Libby, *Jour. Immunol.*, 34: 71-73, 1938.

⁷ P. M. West and P. W. Wilson, *SCIENCE*, 89: 607-08, 1939.

⁸ Paul György, Catharine S. Rose, Klaus Hofmann, Donald B. Melville and Vincent du Vigneaud, *SCIENCE*, 92: 609, 1940.