

DISCUSSION

THE SYNTHESIS OF PLASTEIN

CONTROVERSY still exists as to whether plastein¹ is protein. Chen² by chemical procedure has investigated the nature of plastein to the extent of determining whether the enzyme used in its formation constitutes part of the final product, because if so the reaction could scarcely be classed as enzymatic. This Chen did because he states he felt the immunological work of Flosdorf, Mudd and Flosdorf³ would lead one to suspect the enzyme to be a part of the plastein. The results of the latter workers showed that the antigenicity previously ascribed to plastein was in reality due to the enzyme used in "synthesis."

The purpose of the present communication is to point out that these immunological findings do not imply chemical combination on the part of the enzyme, as suggested by Chen. Small amounts of enzyme adsorbed or otherwise physically carried down with the precipitating plastein readily can account for the observed immunological specificity. It is not necessarily surprising that Chen was unable to detect, by the chemical procedures he used, amounts of material that are detectable by the sensitive immunological procedure of the precipitin reaction.

Concerning the controversy as to whether plastein is synthetic protein, Alcock⁴ states that "... if there is a synthesis, it can best be described as a polymerization, and the product has little relationship with the protein from which it ultimately derived." He is disposed to regard the reaction as resulting from a "condensation function" of the enzyme. Collier⁵ more recently has made further chemical investigation of the reaction and concludes that it is a true enzymatic synthesis of a protein. He does not maintain, however, that the substance is a typical protein or that the phenomenon explains protein synthesis *in vivo*. In explanation of Folley's results,⁶ Collier states that because the ultracentrifugal measurements of Folley were made at a pH of 9.2, which is far on the alkaline side of the isoelectric point, the sedimentation rate may have been much too low. Collier would feel, accordingly, that Folley's findings of weights on the order of a few hundred with a maximum of 1,000 are invalid.

Collier⁷ subsequently arranged for ultracentrifugal analysis in London, using isoelectric plastein dissolved in urea solution. The results showed "sedimentable material" to have been synthesized, but the material is completely inhomogeneous and is not a definite entity. Collier⁸ also attempted a determination of the

possible antigenicity using anaphylaxis in guinea pigs and found evidence for slight antigenicity. He did not, however, completely follow through a determination of the specificity. Although two animals given proteose plus enzyme as the test injection failed to react, two out of four animals tested with plastein also failed to react. Furthermore, 0.002 per cent. concentration of enzyme included in the test dose was far less proportionately than that which corresponds to the 4 per cent. optimal recommendation of Borsook and Wastenays for synthesis of plastein,⁹ and, in any event is of a lower order of magnitude than that which could adhere to precipitating plastein.

Formerly, the known antigenicity of plastein was a cardinal point offered in favor of its being protein. The work of Flosdorf, Mudd and Flosdorf would appear to invalidate such evidence, as pointed out originally by those authors, by showing that the specificity of the antigenic material could be accounted for as of the enzyme. It might well be, of course, that plastein could be a non-antigenic protein; the immunological procedure used would not distinguish between such material and substances of molecular weight of the order reported by Folley, or "peptone polymeres" as suggested by Alcock. Experiments concerning the antigenic nature of plastein without very careful regard for the specificity of that antigenicity should not be used as evidence in the controversy; in the instance where specificity was carefully determined, the results showed that the antigenicity does not constitute evidence in favor of the possible protein nature of plastein.

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THE USE OF PLASTIC AS A SUBSTITUTE FOR COVER GLASSES

IN SCIENCE, July 5, 1940, Vol. 92, pp. 17-18, we published a note concerning the use of plastic cover slips to take the place of glass cover slips, which can not be obtained at the present time, or can be obtained only at a very great expense.

While there were certain disadvantages connected with the substitution of this plastic material for glass, on the whole, it gave good service and the large majority of the sections stained with haematoxylin and eosin and mounted in Canada balsam were satisfactory during the first month or two. However, further experience has shown that after about four to five months many sections may become more or less decolorized. This method can therefore be used only if the sections are studied within the first two months and do not need to be preserved permanently. We are

¹ Wastenays and Borsook, *Physiol. Rev.*, 10: 110, 1930.

² Tung-Tou Chen, *Chin. Jour. Physiol.*, 15: 159, 1940.

³ Flosdorf, Mudd and Flosdorf, *Jour. Immunol.*, 32: 441, 1937.

⁴ R. S. Alcock, *Physiol. Rev.*, 16: 1-18, 1936.

⁵ H. B. Collier, *Can. Jour. Research*, 18B: 272, 1940.

⁶ S. J. Folley, *Biochem. Jour.*, 26: 99, 1932.

⁷ H. B. Collier, *Can. Jour. Research*, 18B: 305, 1940.

⁸ H. B. Collier, *Can. Jour. Research*, 18B: 305, 1940.

⁹ H. Borsook and H. Wastenays, *Jour. Biol. Chem.*, 63: 566, 1925.