in .1 M. phosphate buffer at pH 7 (curve A) and in 6.6 M. urea + .1 M. phosphate buffer at pH 7 (curve B) are presented in Fig. 2. The solution yielding data

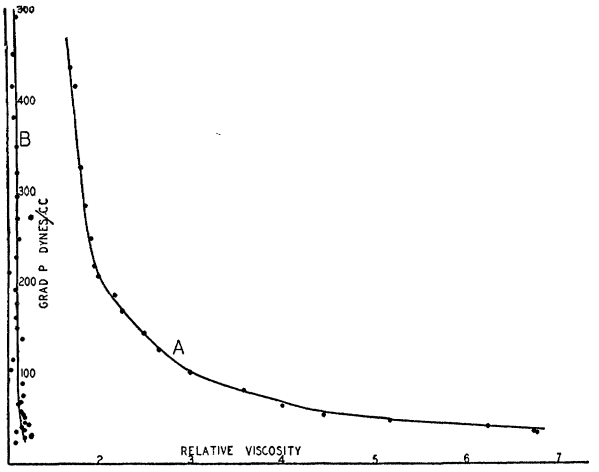

FIG. 2

for curve A contained 4.3 mg protein per cm³, whereas the solution yielding data for curve B contained 6.3 mg protein per cm³. The latter curve was obtained after the virus preparation had stood in the urea for 24 hours. Virus preparations prepared after the method of Stanley[9] show a comparable anomaly when dispersed in either water or in phosphate buffer.

In view of the anomalous viscosity shown by sols of the virus protein in phosphate buffer and the apparent deviation of the diffusion processes from those demanded by Fick's law, it is doubtful that unambiguous values for the particle weight of the chemically prepared virus protein may be obtained by methods involving diffusion in phosphate buffer. A probably less ambiguous value for the particle weight may be obtained from the data for the diffusion in urea solutions. On the basis of the observations presented in this communication, and recalling that a molecule is the smallest particle which can exist as an independent entity, would one not reason that a maximum value for the molecular weight that may be assigned to the virus protein is of the order of 10^5?

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RELATION BETWEEN THE INCREASE IN OPACITY OF YEAST SUSPENSIONS DURING GLUCOSE METABOLISM AND ASSIMILATION[1]

WHEN washed yeast cells are suspended in glucose-phosphate solutions, the opacity of the suspension, as measured by a photoelectric densitometer, increases with time.[2] The rate of change in opacity has been found to vary with the strain of yeast employed, the age of the culture and the experimental conditions. In a typical experiment with Fleischmann's GM strain of bakers' yeast (obtained 2 days after separation at the factory), the optical density (log opacity) changed from 0.170 to 0.417 within 6 hours after the addition of 5 per cent. glucose to cells previously washed and suspended in M/15 KH_2PO_4 (aerobic conditions, 25° C.). The observed change in optical density with time is entirely referable to the suspension and not to the instrument, since variation in the intensity of the light source or in the "constants" characteristic of the phototubes was eliminated by the type of circuit employed and the procedure used in obtaining the measurements.[3] From a consideration of the theory of densitometry as applied to the type of instrument employed,[4] it was apparent that the observed change in optical density resulted from an increase in cell number and from some progressive change in optical characteristics of the cells or from either of these factors operating alone.

We have found no significant increase in cell number under the experimental conditions employed above. This conclusion is based on 11 experiments on GM yeast and 4 experiments on Strain 4360 cultivated in this laboratory. The cell counts were made with a haemacytometer on at least 4 separate samples taken at various intervals during each experiment; 2,000 to 3,000 cells were counted in each sample. In the experiment on GM yeast cited above, the cell counts were as follows: immediately after adding the glucose, 49,640 cells/c.mm.; after 1 hour in glucose, 52,380; after 3 hours, 50,220; after 5 hours, 52,100; and after 6 hours, 52,350. A difference greater than 6 per cent. was considered significant for these suspensions.

Investigations of the changes which the cells of the GM strain undergo with time after suspension in a glucose solution show (a) that the opacity of the individual cells increases with time (evidence from direct microscopic observations and from photomicrographs); (b) that the dry weight per unit number of cells increases progressively with time, very nearly paralleling the increase in optical density of the suspension; (c) that the increase in weight results from the storage within the cells of substances syn-

[9] W. M. Stanley, *Jour. Biol. Chem.*, 115: 673, 1936.

[1] Support from the William F. Milton Fund of Harvard University is gratefully acknowledged.

[2] *Cf.* legend to Fig. 2 in T. J. B. Stier, W. Arnold and J. N. Stannard, *Jour. Gen. Physiol.*, 17: 383–392, 1934, where an increase in opacity under similar experimental conditions was reported.

[3] T. J. B. Stier, W. Arnold and J. N. Stannard, *ibid.*, p. 386.

[4] *Cf.* H. Mestre, *Jour. Bact.*, 30: 335, 1935; and L. G. Longsworth, *Jour. Bact.*, 32: 307–328, 1936.

thesized from the glucose (evidence obtained from chemical analyses of glycogen, total carbohydrate and total fat content); (d) that the cell volume, measured by a centrifuge method, increases with time.[5] The optical density of the suspending medium did not change during 6 hours at 25° C. The evidence obtained indicates, therefore, that the increase in optical density of the suspension is primarily due to progressive changes in optical characteristics of the cells and that these changes accompany the formation and storage of anabolic products within the cells.

On the basis of the above results it is proposed that the changes in optical characteristics of yeast cells during assimilation might be used as indicators of anabolic activity. Thus, the rate of increase in optical density of the cell suspension might be conveniently employed as a measure of the "rate of assimilation." A rapid, optical method for use in exploratory experiments dealing with the dynamic aspects of the assimilation process would obviously reduce the total time and effort demanded by a kinetic type of attack on the mechanism of carbohydrate and fat assimilation. The long, laborious chemical methods of analysis would still be employed, but only for each series of check experiments. Our present densitometer can be employed for this purpose within certain limits. It has been sufficiently reliable for the qualitative detection of whether or not assimilation is taking place in a given yeast suspension in which the total number of cells remains constant with time; it also has given a linear relation between the opacity of the suspension and the per cent. increase in dry weight of the cells during the early stages of carbohydrate assimilation (*i.e.*, during about the first 2 hours at 25° C.). We hope to develop a more satisfactory method of measuring specifically the change in cellular opacity in order to follow with greater accuracy the entire course of carbohydrate and fat storage.

Details of the experiments reported in this paper will be published shortly.

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THE USE OF PURIFIED THROMBIN AS AN HEMOSTATIC AGENT[1]

RECENTLY we have described a thrombin preparation which is far more powerful than any previously reported.[2] In a 1 per cent. solution, 1 cc will clot 1 cc of blood within 2 seconds. Animal studies show that such a solution is highly effective when applied locally to check hemorrhage. It has been found to be non-toxic when used to control the oozing of blood from operative surfaces. For this purpose it is applied by means of an atomizer. Under these circumstances a thin film of blood forms almost instantly, and this seals the finer vessels. The larger vessels are controlled by ligature as a preliminary measure.

With the thrombin spray, profuse bleeding from incised liver tissue can be checked completely in 5 seconds or less. Prolonged bleeding from bone is often very troublesome to the surgeon at the time of operation. With the application of thrombin we are able to stop this bleeding within 5 to 10 seconds. This obviates the need of bone wax, which is a foreign body. With the use of thrombin, we have been able to resect portions of brain and to check with ease the hemorrhage which is otherwise so difficult to control.

The same beneficial effects have been obtained in operations on dogs with a prothrombin deficiency which can be produced by chronic chloroform administration.[3] In such dogs, bleeding is very profuse at operation and may be fatal unless thrombin is applied.

The important applications to human surgery and to human bleeders await further study regarding the sterility of the thrombin preparations.

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ISOLATION OF CRYSTALLINE HETERO-TRYPSIN FROM BEEF PANCREAS

NORTHROP and Kunitz have prepared from beef pancreas the two crystalline enzymes, trypsin and chymotrypsin.[1] For both of these enzymes, specific substrates have been synthesized in this laboratory. Trypsin was shown to hydrolyze benzoyl arginine amide, while chymotrypsin was found to split benzoyl tyrosyl glycine amide. Another synthetic substrate, benzoyl glycyl lysine amide, was found to be hydrolyzed by crude pancreatic extracts, but not by trypsin or chymotrypsin.[2] Therefore, the hydrolysis of this lysine compound was attributed to an unknown enzyme

[5] *Cf.* O. W. Richards and T. L. Jahn, *Jour. Bact.*, 26: 385–391, 1933; and L. G. Longsworth, *ibid.*, for discussions of the effect of an increase in cellular opacity and volume during growth by cell division on measurements of population density by the photoelectric method.

[1] Aided by a grant from The John and Mary R. Markle Foundation. Funds for a technical assistant were supplied by the Graduate College, State University of Iowa.

[2] W. H. Seegers, H. P. Smith, E. D. Warner and K. M. Brinkhous, *Jour. Biol. Chem.*, 123: 751, 1938; *ibid.*, 126: 91, 1938.

[3] H. P. Smith, E. D. Warner and K. M. Brinkhous, *Jour. Exp. Med.*, 66: 801, 1937.

[1] M. Kunitz and J. H. Northrop, *Jour. Gen. Physiol.*, 19: 991, 1936.

[2] M. Bergmann and W. F. Ross, *Jour. Am. Chem. Soc.*, 58: 1503, 1936; M. Bergmann, J. S. Fruton and H. Pollok, SCIENCE, 85: 410, 1937.