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.

in the pancreatic extract. This enzyme has been designated heterotrypsin.[3]

It has now been observed that heterotrypsin is contained in exudates from bovine pancreas. With the above-mentioned lysine-containing substrate as a guide, the task of isolating heterotrypsin from the pancreatic exudate was undertaken. This was easily accomplished by a process which consists essentially in a 0.4 saturation with ammonium sulfate at pH 7.0. The enzyme was thus obtained in the form of fibrous crystals which exhibited a very high activity towards benzoyl glycyl lysine amide. The crude crystals contained only traces of trypsin and chymotrypsin. A quantitative study of the action of pancreatic exudates on our synthetic substrates showed that the amount of heterotrypsin in the exudates is several times that of trypsin, while chymotrypsin is almost entirely absent.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

METHOD FOR "FIXING ICE CRYSTAL PATTERNS" IN FROZEN PRODUCTS

IN the course of microscopic studies of fruits and vegetables preserved by freezing, considerable difficulty was experienced in using the paraffin method for obtaining sections. Such tissues as ripe strawberry, peach and raspberry or young stems of asparagus, broccoli and spinach are almost without thickened cell walls; and after they are badly ruptured by ice crystals, become very flabby on thawing. It was found almost impossible to maintain the original structure throughout the long process of paraffin embedding.

Sectioning the thawed material with a sliding microtome was equally unsatisfactory.

After considerable experimenting, a method of sectioning the frozen material was developed which was very rapid and satisfactory.

In this case the microtome and all supplies were placed in a freezing room an hour in advance in order to become thoroughly chilled. Temperatures of the room were varied from 0°F. to above freezing, and it was decided that a temperature below 15° F. was uncomfortably cold and caused sections to be too brittle, while that above 25° F. was objectionable because it permitted partial thawing of the material from the warmth of the body. From 18° to 20° F. was decided to be the most suitable temperature for making frozen microtome sections. A refrigerated truck used for hauling frozen products made an ideal sectioning room.

Tissues of most fruits and vegetables were satisfactorily sectioned from ten to thirty microns thick provided the ice crystals were very small. Those frozen with solid carbon dioxide, by the immersion method or other methods producing a very quick freeze, sectioned as smooth as a block of green soft wood; while those containing large ice crystals could not be cut in sections less than thirty microns thick, and then both cell walls and cell contents were often fragmented. Frozen sections were floated in a chilled, killing and fixing fluid as they were cut and placed in very small petri dishes. Formic-acetic-alcohol (acetic acid 5 per cent., formalin 10 per cent., alcohol 70 per cent., water 15 per cent.) was satisfactory for this as it rendered the sections fairly stiff, which prevented sticking together and made handling much easier. Ice crystal patterns in vegetative tissue were very satisfactorily fixed.

For staining unmounted sections more than a dozen single and double stains were used with some degree of success. Best results were obtained with eosin, orange G and basic fuchsin for staining cell contents, and light green, basic fuchsin and methylene blue for cell walls. Due to lack of secondary thickening in cell walls of most of the material used, double staining was of limited value; however, safranin-Delafields haematoxylin was good for gross anatomical study of asparagus tips, and orange G, light green or safranin-light green was excellent for study of starch grains and cell walls of peas, beans and corn.

This method is very rapid, as the writer has, on numerous occasions, made sections and carried them through the process of dehydrating, staining and mounting in balsam, in one-half day. The adaptability of the method depends not so much on the material, nor on the temperature of the cutting room, as on the initial freezing temperature of the product.

In this way ice crystal patterns could be fixed, measured and photographed very accurately, and thereby establish a means of evaluating methods of freezing fruits and vegetables. The writer has made more than one thousand measurements of ice crystals in about a dozen products frozen by eight different methods.

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A NEW APPARATUS AND METHOD FOR TRAINING THE RAT IN AUDITORY DISCRIMINATION PROBLEMS

SEVERAL investigators have reported from time to time considerable difficulty in training lower mam-

[3] M. Bergmann and J. S. Fruton, *Jour. Biol. Chem.*, 118: 409, 1937.