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.