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.

malian forms, particularly the rat, to respond differentially to auditory stimuli. Still greater difficulty has been encountered in the attempts made to train these animals to localize the direction of sounds and to discriminate pitches and loudnesses. Experimentalists are in general agreement that in problems related either to the peripheral or to the central aspects of the auditory modality one of the major problems is the development of a methodology for training the infra-primate subject. Two major difficulties relative to the available procedures in this field of investigation can be found in the literature. First,[1] certain methods require prolonged training periods and so preclude the formulation of any very detailed operative program. Second,[2] other methods of training eventuate in such decidedly abbreviated responses to auditory stimulation that it is difficult, if not upon occasion impossible in the later stages of learning, to determine whether the animal has or has not responded.

The apparatus and training procedure described at this time, it is believed, overcomes to a certain degree these difficulties. The apparatus consists of a modified living cage, the floor of which is an electric grid. Located within this cage and resting on the grid is a small platform which may become if need be a second grid. Acclimatization of the animal, in our experiments the rat, is permitted by placing it in the cage some hours before beginning the preliminary training. During this initial training period the animal is given a series of twenty shocks per day and is required to jump upon or down from the secondary grid-platform. Following the acquisition of this shock-escape response, a buzzer or a thousand-cycle tone is presented and is followed by the shock. All animals so far tested learn rapidly within the range of individual differences to escape the shock by making the appropriate shock-avoidance response. Thus in this experimental arrangement only one response can be made, namely, movement away from the present position. The rapid learning of the rat which has thus far been observed permits in terms of the time element the initiation of a more detailed operative program involving a study of cerebral mechanisms in audition.

The modified cage arrangement described above permits numerous modifications. It is believed this apparatus could be enlarged and consequently utilized for larger mammals, such as the cat, the dog and the guinea pig. Certain evidence already available shows the possibility as well as the plausibility of training animals to discriminate between tones on the basis of pitch and loudness differentia. A further modification for the study of auditory localizing behavior is in progress. Obviously, however, the size of the cage floor, the size of the second grid and the construction of the walls of the cage will depend upon the nature of the study as well as upon the size of the animal subject. In this particular arrangement the walls have been constructed of galvanized iron. A screened top with mirror arrangements obviates certain recording difficulties. The use of the solid walls thus makes impossible an alternative wall-clinging response. From one point of view, however, it is suggested that this response itself might be found sufficient for the determination of a statistically reliable response to sound stimuli.

The values of this modified living cage method in training the rat to respond to sound stimuli are numerous. First, the method is sufficiently simple to make unnecessary the cradle arrangements use by Britt[3] and Schlosberg.[4] Second, the experimental arrangement is comparatively close to the normal living conditions of the animal. The artificiality of the Y-maze arrangement[5] in relation to sound stimuli is somewhat obviated. Third, as a corollary of the above, the rapidity with which modifications in behavior occur makes possible the study of certain little understood brain mechanisms in at least one sense modality. Fourth, the response of the animal under the conditions of these arrangements is always clear-cut and observable. Fifth, the use of shock as an incentive seems from several points of view to be superior as a motivating agent over food. Shock can be controlled and measured in more rigorous fashion. Sixth, the possibility of the study of certain other sense modes in relation to cerebral function is suggested by the method of training just outlined.

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[2] W. R. Brogden and E. Culler, SCIENCE, 83: 269, 1936.
[3] S. H. Britt, *Jour. Comp. Psychol.*, 19: 243, 1935.
[4] H. Schlosberg, *Jour. Genet. Psychol.*, 45: 303, 1934.
[5] L. A. Pennington, *Jour. Comp. Psychol.*, 25: 195, 1938.

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