the cells are not known, but they without doubt form a most important class of agents, through which the chemical operations of life are accomplished. Many of the tissues are abundantly supplied with them. Twenty or more have been shown to be present in the liver. The kidney and muscle tissue are also widely supplied with them.

Among the more familiar processes in which enzymes take a prominent part might be mentioned those of digestion. Here a certain amount of adaptation of secretion to diet seems possible. Lactase, an enzyme present in the intestine of infants, is absent or present in negligible amounts in adults, but constant feeding of lactase results in a revival of its secretion. This adaptability of secretion to diet, may have an important bearing on the ill effects frequently produced by a sudden change in the character of the diet. The oxidizing enzymes have already been mentioned in connection with metabolism. There is one anomaly which finds a probable explanation in a defective enzyme action, namely that of alkaptonuria. Tyrosin and phenyl-alanin, products derivable from protein, are normally completely oxidized in the system, but in alkapton are oxidized only partially to homogentisinic acid. Diabetes may find an explanation in the failure of the glycolytic enzymes of the tissue to oxidize glucose. The clotting of blood, the softening of tissue by pus, the destruction of germs by the leucocytes, rigor mortis, and the softening of tissue by autolysis are familiar illustrations. The formation of urea, of dextrose from glycogen and of glycogen from dextrose, the breaking down of neucleic acid in purins, and the oxidation of the purins to uric acid are other cases, which could be greatly multiplied.

THE BEHAVIOR OF ENZYMES AT LOW TEMPERATURES.*

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The influence of low temperatures on enzymes is a subject of growing importance to the chemist, the biologist, and the bromatologist. Problems in this field may be studied from either the potential or the kinetic side; for either the resistance of an enzyme to, or its activity at, low temperatures may be investigated. Various researches, conducted during the last half century, have demonstrated that enzymes survive exposure to low temperatures and also act as catalysts at such temperatures. The reports of these researches are widely scattered in the literature; and frequently the original papers may be obtained for consultation only with difficulty. It is the purpose of this paper, which is based on primary sources, to give a *resume* of our present knowledge of this subject. One section is devoted to the resistance of enzymes to low temperatures, and one to their activity at such temperatures.

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In this paper full details will be given of the researches of various investigators on the influence of low temperatures on enzymes, and a complete bibliography appended.

THE RESISTANCE OF ENZYMES TO LOW TEMPERATURES.

The researches reviewed below demonstrate that the following enzymes survive exposure to low temperatures and again exert their usual catalytic power when brought into a suitable environment: diastase, inulinase, invertase, maltase, zymase, lipase, protease of plants, pepsin, trypsin, thrombin, rennin, oxidase, peroxidase, catalase, simple and aldehyde reductase. This order will be followed in presenting the data.

Solutions of *diatase, inulinase,* and *invertase,* after exposure for forty-five minutes to the temperature of liquid air (approximately —191° C.), retained unaltered their power to hydrolyze starch, inulin, and sucrose, respectively. The diastase was obtained from human saliva and from *Aspergillus niger;* the inulinase from the Aspergillus; and the invertase from both the Aspergillus and beer yeast.

After yeast press juice had been repeatedly cooled to —2° C., its *maltase* retained the power to invert maltose.

Zymase in yeast press juice survives complete freezing of the juice. The enzyme may be concentrated by cooling the juice to —2° C. and removing the ice crystals from the solution of enzyme in the mother liquor. Zymase has been prepared by trituration of a mixture of solid carbon dioxide (carbon dioxide snow)[1] and dehydrated yeast cells for a period of one-half hour. It is resistant to a temperature of —182° to —190° C., that of liquid air; yeast cell plasma, held at that temperature for twenty hours, retained unchanged its power to produce alcoholic fermentation.

The *lipase* of a pig pancreas, which had been kept in cold storage at 4° C. for seven days, retained about forty percent of its power to hydrolyze ethyl butyrate. Lipase was found in fresh eggs which had been held at 0° C. for sixty-six days. It was present in the crude abdominal fat of a chicken kept at 0° C. for twenty-four hours after death, in that of chickens of known history held hard frozen for periods of twelve and one-half, thirteen, sixteen, twenty-eight, twenty-nine, and forty-two months at a temperature of —9.4° to —12.2° C., and in that of birds, whose history prior to freezing was unknown, kept at that temperature for periods of fifty-four and eighty-nine months.

The *protease* of plants survives freezing temperatures. Sprouting wheat seedlings, excoriated peas, excoriated germinating peas, etiolated caulis tops, etiolated leaves and green leaves of the bean *Vicia faba* were frozen, usually for twenty-four hours. Their proteases were still able to produce autolysis of the tissue proteins at room temperature.

Solutions of *pepsin* and *trypsin,* after exposure to the temperature of liquid air (approximately —191° C.) for forty-five minutes, were practically unaltered in their ability to digest albumin.

The clotting enzymes *thrombin* and *rennin* likewise survive exposure to the temperature of liquid air. Thombin of dog's blood held at —180° C. for thirteen minutes retained in full its power to produce clotting of the blood.

Commercial rennet, kept at —180° C. for periods of one, five, ten, and thirty

[1] The temperature of solid carbon dioxide vaporizing at ordinary atmospheric pressure is approximately —75° C. (Richter *Inorganic Chemistry,* Fifth American Edition, p. 228.)

minutes, was entirely unchanged in its action on milk at 35° C. In another experiment a rennin solution, which was kept in boiling liquid air for over one hour, retained completely its power to produce clotting of milk.

Oxidase was present in milk held at 0° C. for as long as thirty-five days, and in cream held at that temperature for as long as twenty-eight days. Both milk and cream were rendered bacteriologically sterile by addition of 0.1 percent of formaldehyde before storage.

Both oxidase and *peroxidase* were found in the crude fat of a chicken kept at 0° C. for fifteen days after death, and in that of hard-frozen chickens, including birds of known history, kept in the freezer for nine months and birds, whose history prior to freezing was unknown, held in the freezer for periods of twenty-three and sixty-three months; the temperature of holding was —9.4° to 12.2° C.

Catalase has been found in milk and cream rendered sterile by addition of 0.1 per cent of formaldehyde and held at 0° C. for twenty-one days. It has also been detected in eggs, including both the white and the yolk, after the eggs had been kept at 0° C. for sixty-five days.

Catalase has also been demonstrated in the crude fat of chickens of known history held hard-frozen for nine months at a temperature of —9.4° to —12.2° C., in sole kept frozen for nineteen to twenty-one days at —2° to —9.5° C., and in cod kept frozen for thirty days at —2° to —6.5° C.

Simple reductase has been found in formalized, sterile milk and cream kept at 0° C. for twenty-eight days; the cream also contained *aldehyde reductase.*

Simple reductase occurred in the crude fat of chickens of known history held hard-frozen for nine months, and in that of chickens, whose history prior to freezing was unknown, kept in the freezer for twenty-three months. Aldehyde reductase was present in the birds of the latter class after periods of twenty-three and sixty-three months in the freezer. The temperature of holding was —9.4° to —12.2° C.

THE ACTIVITY OF ENZYMES AT LOW TEMPERATURES.

Studies have been made of the activity of the following enzymes at low temperatures: invertase, maltase, zymase, diastase, lipase, pepsin, trypsin, galactase, urease, rennin. This order will be followed in presenting the data. At times the enzyme studied was permitted to produce an autolysis, at times to act in solution on an artificial medium, at a given low temperature.

Invertase, maltase, and *zymase* are active at the temperature of an ice-box, for yeast press juice ferments saccharose, maltose, glucose, and fructose at that temperature.

The *diastase* of carp liver acts on starch at 0° C., converting it first into soluble starch, then into erythrodextrin, and finally destroying the latter compound.

The *lipase* of pig pancreas has been shown to hydrolyze ethyl butyrate at 0° C., and to hydrolyze neutral lard at —9° to —12° C. The lipase of pig liver splits ethyl butyrate at 0° C. and at —10° C. The lipase of crude chicken fat produces hydrolysis of ethyl acetate, butyrate, and benzoate and amyl salicylate at 0° C. and at a temperature of —6.7° to —9.4° C.

Pepsin.—The gastric protease of the frog, the pike, and the trout digests albumin at 0° C.; that of the frog also digests fibrin at 0° C., while that of the pike produces proteolysis of fibrin at temperatures as low as 0° C. Pepsin of pig's stomach converts coagulated ovalbumin into acid albumin, albumoses, and peptones at 0° C. Pepsin also digests ricin at that temperature.

Trypsin, derived from the intestinal tract of the carp, gave rise to proteolysis of gelatin and of fibrin at 0° C.

Galactase, the trypsin-like enzyme of milk, gives rise to a proteolysis in Cheddar cheese which is ripened at 15° F. (—9° C.), 25° to 30° F. (—1° to —4° C.), and 33° F. (+0.6° C.). Galactase also produces proteolysis in milk rendered sterile by addition of 0.1 percent of formaldehyde and held at 0° C. The digestion of the proteins observed in milk kept at —9° C. has been ascribed to the action of galactase.

Urease of the soy bean hydrolyzed urea at temperatures as low as 0° C.

When *rennin* acts on milk it first transforms the casein into paracasein and then precipitates the latter compound as a coagulum. The first stage of the reaction occurs at 0° C.; the second stage likewise takes place at that temperature, but the precipitate separates in a finely-divided condition without the formation of a distinct curd.

SUMMARY.

The power to survive prolonged exposure to low temperatures is possessed by various enzymes, including those producing hydrolysis of fats, of carbohydrates, and of proteins, those concerned in biochemical oxidations and reductions, the clotting enzymes and that of alcoholic fermentation. The enzymes retained their catalytic power after exposure, either *in situ* or in solution *in vitro,* to temperatures varying from a few degrees above 0° C. to the temperature of liquid air (—180° to —191° C.). The shortest periods of holding, invariably less than one day and usually less than one hour, were at the temperature of liquid air. The longest period of holding was eighty-nine months at a temperature of —9.4° to —12.2° C.

The activity of certain of these enzymes, including rennin, zymase, and those hydrolyzing fats, carbohydrates, and proteins, has been studied at low temperatures, varying from that of an ice-box to one of —9° to —12° C. While the enzymes produced autolytic digestion or acted on artificial media at these temperatures, the velocity of the reaction was always lessened to a considerable degree.