CI. *POST MORTEM* BREAKDOWN OF GLYCOGEN AND ACCUMULATION OF LACTIC ACID IN FISH-MUSCLE AT LOW TEMPERATURES.

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The temperature of maximum rate of change.

IN a previous publication [Sharp, 1934] it was shown that the rate of glycolysis in fish-muscle after death decreased with temperature down to 0° . Lowering of temperature to -10° resulted first of all in an increased rate of change in the frozen tissue at -2° and secondly in a falling off to a barely perceptible rate at -10° . It must follow therefore that between 0° and -10° there is a temperature at which the maximum rate of breakdown occurs in the frozen muscle.

The first part of the present work shows that the maximum rate of change takes place in the temperature interval -3.2° to -3.7° . The sampling error of the technique previously given in detail is too large to permit of more accurate determination.

Experimental.

The method of experiment was slightly different from that employed previously, for no attempt was made to obtain complete curves over any period

Fig. 1. Amounts of lactic acid accumulation in six lots of mixed muscle samples over arbitrary periods of 9-12 hours' storage at different freezing temperatures.

of storage. The muscle was stored for an arbitrary period at different temperatures and the loss in glycogen and the gain in lactic acid were estimated over this period. Using ten similar fresh samples from five haddocks, two were retained for immediate analysis and the remainder stored in pairs in air-tight

tinned containers at four temperatures for a definite period (9–12 hours) after which glycogen and lactic acid estimations were carried out by the methods given before in detail. Duplicate samples at each temperature served to reduce the sampling error and in most cases repeat experiments were done on different batches of fish. The results of six experiments are plotted in Fig. 1, and since in all cases the glycogen loss was accounted for by a corresponding increase in lactic acid, the plotted values represent either lactic acid accumulation or glycogen loss over the period of storage.

Conclusion.

In the temperature interval -3.2° to -3.7° the maximum rate of glycogen breakdown with corresponding lactic acid accumulation occurs in the frozen muscle.

In frog-muscle, Smith and Moran [1930; Smith, 1931] have shown that the increased rate of change below -0.9° is not due to oxygen starvation, but can be directly related to the amount of available water withdrawn from the muscle plasma in the form of ice. Freezing of the tissue causes increased salt concentration in the plasma, and, on the assumption that the plasma behaves like an isotonic solution of sodium chloride, it is estimated that at -2.5° , 80–82 % of the available water is withdrawn from the plasma in the form of ice, resulting in a five-fold increase in salt concentration.

In minced frog-muscle suspended in phosphate solutions of different concentrations maximum formation of lactic acid occurs in solutions freezing at -3° . Further, the solubility of rabbit-muscle globulin reaches a maximum in phosphate solutions freezing at -2° and falls rapidly in solutions freezing below -2° [Smith, 1932].

It seems therefore that the maximum rate of change takes place in the frozen muscle when a definite salt concentration is attained in the plasma, coincident with maximum solubility of the muscle globulin. Further increase in salt concentration by lowering of temperature results in decreasing solubility of the protein and decreasing rate of change.

The salt concentration of fish-muscle plasma is such that the freezing-point is approximately 0.6° lower than that of frog-muscle plasma. It follows from this that the plasma salt concentration attained in fish-muscle frozen at -3.2° would approximate to the concentration attained in frog-muscle at about -2.5° . Considered from the standpoint of the maximum rate of change occurring on the attainment of a definite salt concentration in the plasma, the present results agree closely with those obtained in the case of frog-muscle.

The critical temperature of freezing.

In frog-muscle with certain degrees of freezing, irreversible changes take place in the colloidal structure of the tissue. That such changes occur is evident on thawing the frozen muscle when it is observed that the muscle has become non-irritable and has lost the power of synthesising both glycogen and phosphagen [Moran, 1929; Smith, 1929; Kreps, 1931]. According to the intensity of freezing, muscles on thawing either return completely to normal condition or are irretrievably altered. For the gastrocnemius of the frog, these irreversible changes occur on freezing at -1.6° and storing at this temperature for a period of 24 hours. For example, on thawing at 5° muscles which have undergone different degrees of freezing, it is found that in the muscles stored at temperatures above -1.6° for 24 hours the accumulated lactic acid is resynthesised to glycogen until the normal resting level (0.05 %) is attained, whereas in

muscles stored at -1.6° and lower temperatures, lactic acid continues to accumulate at a rapid rate until the rigor maximum is reached. In the latter case the synthetic mechanism for the formation of glycogen from lactic acid is irreparably damaged.

Since, in fish-muscle, there is no resting level but a comparatively rapid accumulation of lactic acid at 0° and higher temperatures, both the proof of the existence and the determination of a critical temperature of freezing present greater difficulty than in the case of frog-muscle. Experimental proof depends on comparison of the rates of formation of lactic acid on thawing muscle which has suffered different degrees of freezing. The rate of acid accumulation at temperatures in the region -2° to -4° is however so great that only short periods of storage are permissible, since an appreciable concentration of glycogen is necessary in the muscle, just before thawing, before any true comparison of rates can be made.

Experimental.

That freezing at -20° damages the lactic acid recovery mechanism in fish-muscle is evident by the results plotted in Fig. 2. Of ten similar mixed samples of muscle, five were frozen and stored separately at each of the chosen temperatures, $+5^{\circ}$, 0° , -2.5° , -3° and -4° . The remaining samples were rapidly

Fig. 2.

Fig. 2. Lactic acid and glycogen concentrations in mixed samples of muscle after storage for 10 hours at different temperatures.

Δ Lactic acid in samples frozen at -20° . $\circ$ Lactic acid in samples stored directly at temperature.
 $\times$ Glycogen " " " " $\bullet$ Glycogen " " " "

Fig. 3.

Fig. 3. Rates of lactic acid accumulation at 4° in muscle, thawed after a freezing period of 4 hours at -1.6° , -2° and -6° .

$\bullet$ — $\bullet$ -1.6° . $\times$ — $\times$ -2° . $\circ$ — $\circ$ -6° .

frozen at -20° , immediately raised to their respective storage temperatures and kept thereat, with the duplicate samples, for a period of 10 hours, after which glycogen and lactic acid concentrations were estimated. Thus, at each temperature there were two samples, one of which had been rapidly frozen to

-20° and immediately raised to the temperature of storage, while the other had never been subjected to temperatures below the storage temperature.

The middle curves illustrate the different rates of change in the temperature interval $+5^{\circ}$ to -4° , showing maximum change between -2.5° and -4° , but, owing to an almost total loss of glycogen in this region over the storage period, the peaks of the curves have become flattened out. The effect of preliminary freezing at -20° is apparent at 0° and $+5^{\circ}$, at which temperatures total loss of glycogen and maximum accumulation of lactic acid have occurred in the frozen muscle samples. Since only a fraction of this difference can be accounted for by the rapid double passage through the interval where maximum rate of change occurs, it appears that in freezing at -20° the synthetic mechanism in the lactic acid-glycogen cycle has been destroyed, resulting in an increased rate of lactic acid accumulation in the thawed muscle.

It remained to be determined what degree of freezing was necessary to damage this synthetic mechanism in the muscle, and a comparison was made of the rates of lactic acid formation in thawed muscle which had undergone different degrees of freezing.

Similar lots of samples were frozen at -1.6° , -2° and -6° , and after a period of 4 hours at the freezing temperature were thawed in circulating brine at 4° . Samples were removed at intervals for lactic acid estimation. The rates of lactic acid accumulation in the thawed muscle are shown in Fig. 3, the moment at the freezing temperature just before thawing being taken as zero time. On thawing muscle which has been frozen at -1.6° the normal rate of change at 4° is resumed, whereas on thawing muscle which has been frozen at either -2° or -6° a very rapid rate of change takes place, until the lactic acid maximum is attained. Between -1.6° and -2° therefore a critical temperature of freezing similar to that obtained in the case of frog-muscle must exist, for, in freezing below this temperature the muscle is irretrievably altered, in so far as the recovery mechanism for the removal of accumulated lactic acid is broken down.

SUMMARY.

1. In fish-muscle in the frozen state the maximum rate of glycogenolysis occurs in the interval -3.2° to -3.7° .

2. Freezing at -2° and lower temperatures for a period of 4 hours causes injury to the muscle, resulting in very rapid lactic acid formation on thawing. Freezing at -1.6° has no such effect, and on thawing the normal rate of lactic acid formation is resumed. Between -1.6° and -2° a "critical" temperature of freezing exists.

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