THE EFFECT OF FREEZING LIVER ISSUE ON ITS THIAMINE PYROPHOSPHATE CONTENT

by

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In attempting to preserve, by freezing and storing at $-10°$ C, pigeon liver and brain tissue to await later analysis it was found that the thiamine pyrophosphate (TPP) content had appreciably decreased. Heart and breast muscle did not show this effect. This decrease, which is presumably caused by phosphatase action[1] was further investigated in liver tissue from pigeons and rats. TPP was determined by the manometric method[2].

It appeared that the decrease occurred only during the slow freezing and thawing periods, while the storing itself caused no detectable loss. Normal pigeon liver, which contains $4.0 \pm 0.14\ \gamma$ TPP per g of tissue, showed a decrease of $50 \pm 4\%$ when frozen and defrosted slowly by putting the glass tube containing the tissue in the refrigerator at $-10°$ and placing it at room temperature afterwards. In normal rat liver, containing $12.3 \pm 0.03\ \gamma$ TPP per g tissue, also a decline of from 40 to 60% occurred under these conditions. (Standard derivations of the mean).

It was found that repeated freezing and thawing carried out in a similar manner produced relatively small further decreases. The loss also appeared when liver minced first with Cooper scissors was used instead of whole tissue. This loss was completely prevented, however, by rapid freezing and thawing (freezing in dry ice-acetone bath; thawing by adding hot diluted HCl and placing immediately in the boiling waterbath).

A normal rat or pigeon liver mince showed no detectable TPP loss after standing at room temperature for approximately one hour. However, if a quantity of TPP was added to the mince, a considerable loss (80–90% of added TPP) occurred during this period. Even when the determination of TPP was carried out immediately after it had been added a loss of 15% occurred, which shows that the destruction of added TPP is a fairly rapid process. The destruction of added TPP was retarded, but not completely prevented, if the mince was allowed to stand at $0°$ C for the same length of time. Actual slow freezing and thawing caused less destruction of added TPP than standing at $0°$ C. This is, as appears above, in contrast with tissue TPP. Rapid freezing and thawing caused little decrease in added or tissue TPP. It thus appears that the loss of tissue TPP on freezing or thawing is due to the splitting off of TPP from protein by freezing, and not to increased phosphatase action.

As has been previously noted a significant residue is always found even after repeated freezing. This residue had also been found in work with normal liver homogenates which had suffered a TPP decline of about 60% after standing at $0°$ C for four hours, while with homogenates from thiamine-deficient liver tissue which had already lost more than 50% of its TPP no further loss occurred[3]. When liver tissue from thiamine-deficient pigeons and rats was subjected to freezing and thawing, again no loss was observed.

From these experiments it can be concluded that the TPP in liver tissue consists of at least two different fractions present in about equal amounts in normal tissue. One of these fractions is more easily split off from its protein bearers by freezing and homogenizing, while the other is rather more stably linked to protein. The easily split off fraction appears to be identical with the fraction which is rapidly lost when animals are placed on a thiamine-deficient diet.

This work forms part of investigations on the metabolism and physiological function of thiamine carried out by H. G. K. WESTENBRINK and collaborators.

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