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**Title**

CHANGES IN THE HYDROGEN ION CONCENTRATION OF BEEF  
LIVER DURING STORAGE

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CHANGES IN THE HYDROGEN ION CONCENTRATION OF  
BEEF LIVER DURING STORAGE.

by

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Beef liver is used extensively for feeding fish fry in ponds. In many places it is difficult to obtain fresh liver and as a consequence it must be stored. It has been commonly noticed that when liver that is no longer fresh is fed to the young fish the mortality rate increases so it appeared necessary to investigate the changes that liver undergoes during storage. The following studies were designed to be preliminary to a more detailed investigation which would lead to (a) improved methods of storing liver, and (b) determination of the toxic substances formed during the decomposition.

The methods available for storage of liver vary according to the location of the feeding ponds. In some localities ice is available and the problem of storage is not serious but in many cases the best cooling is by means of the overflow water from the ponds. As a result of the varying conditions the changes undergone by the liver had to be considered under conditions representative of refrigeration temperatures to those of summer conditions. The temperatures chosen were 6°, 16°, 20° and 30° Centigrade.

The most convenient method for following the changes in the liver was by means of the hydrogen ion concentration or pH which decreased from a barely acidic condition of pH 6.6-6.3 to one that was definitely acidic, pH 4.6-4.9. The rate of change is shown in Fig. I for the chosen temperatures and it is seen that the changes take place slowly at first, then the pH falls rapidly to a low value where it remains fairly constant or undergoes only a very slow change downward. The interval between the death of the animal and the onset

of the rapid decomposition as indicated by the rapid fall in pH is dependent on the temperature of storage.

Fig. II shows the relationship existing between the time required for the liver to attain various pH values and the temperature of storage. If the pH at which the toxic effects appeared was known it would be possible to predict with reasonable accuracy how long the liver could be stored at any available temperature without danger of toxic decomposition. Further work is necessary along this line.

Fig. III shows the rate of decomposition of ground liver as compared with a block of liver and it is evident that the rate of decomposition in the pulp is greater than in unground material.

A breis was prepared from finely divided liver to which a few millilitres of toluene were added. This prevents bacterial action but permits normal enzyme action. When the changes undergone by this breis were compared with one which was not sterilized with toluene it was distinctly shown that the former did not have the sharp break in the pH curve which was characteristic of the unsterilized breis (Sevringhaus, Kroehler and Bradley). It is apparent that the rapid decomposition is bacterial rather than autolytic, while the slow initial and final reactions are probably true autolytic changes. A careful bacterial analysis should be made in order to throw further light on this point.

It was further shown that the increase in acidity was paralleled by the increase in titratable acid and inorganic phosphorus which probably occurs as phosphoric acid from the hydrolysis of the phospholipins or nucleic acids (Sevringhaus). There is also a sudden very large increase in the amino acids present which begins at the same point as the rapid increase in acidity, and it may be that it is at this point that the toxic substances are formed. The results of these determinations are shown in Fig. IV.

### Methods

The liver was obtained as soon as possible after death, cleaned of all fatty tissue and used in one of three ways; as a block of flesh, as a finely ground pulp or as a breis prepared by making 100 gms of the ground liver up to 250 mls total volume with boiled distilled water.

The samples were stored under conditions where the temperature was constant to within 1°C.

### Hydrogen ion concentration

A 10 gm sample was weighed out to the nearest 0.01 gm, thoroughly mixed with 50 mls. water, allowed to settle and the pH determined on the supernatant liquid by means of the quinhydrone electrode. With unground liver a sample was finely ground before weighing and mixing with water.

### Titrateable acid

The sample taken for the pH determination was centrifuged and 10 mls of the supernatant liquid pipetted out, diluted with 50 mls. distilled water, phenolphthalein added and titrated with 0.1N  $H_2SO_4$ . The results were reported as mls. 0.2N acid.

### Amino acids

The amino acids were determined on the centrifugate by the Sorensen formal titration.

### Inorganic phosphorus

The inorganic phosphorus was determined on the centrifugate by the method of Fiske and Subbarow.

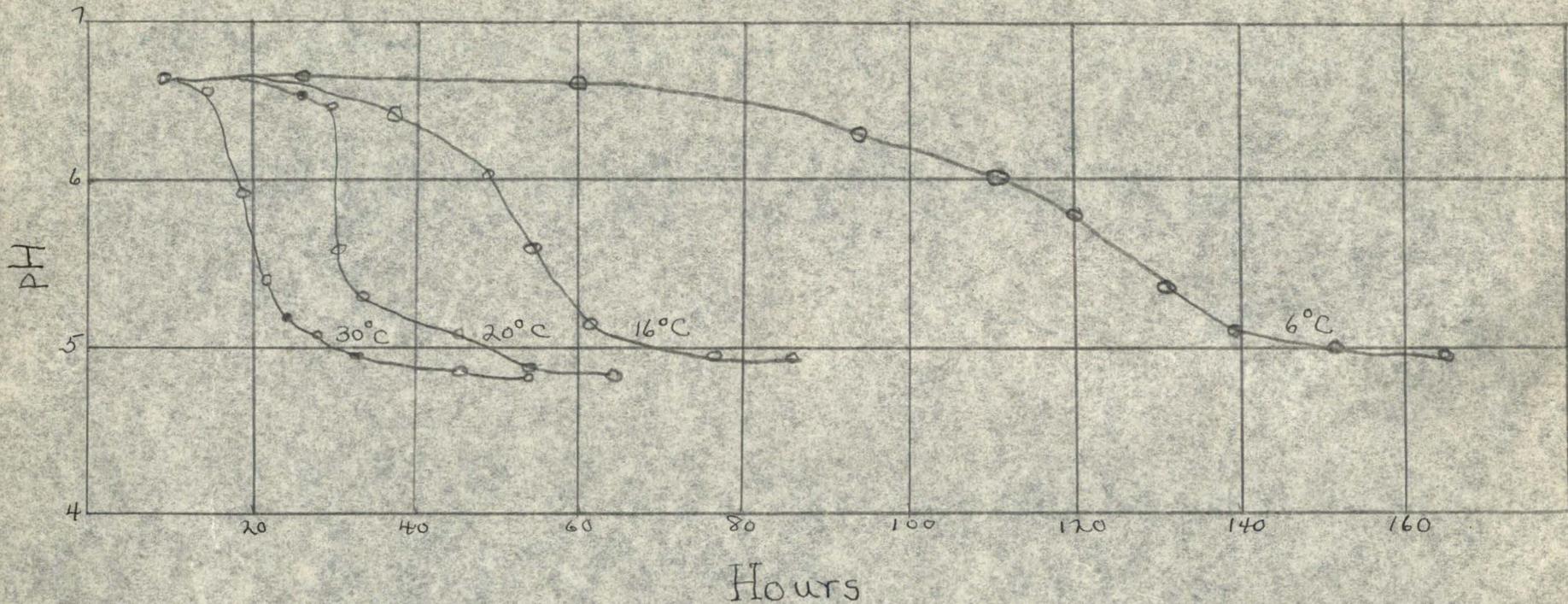
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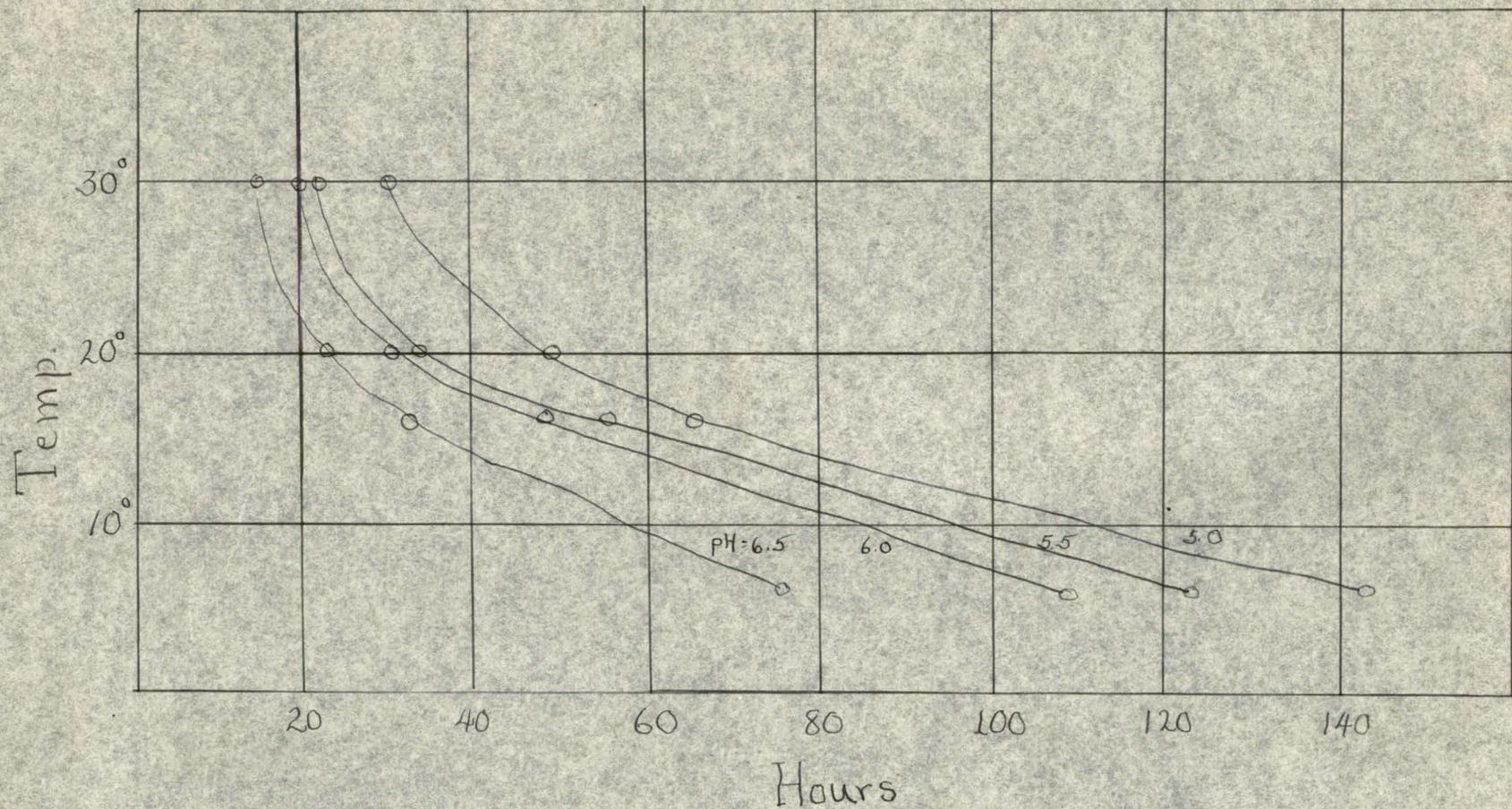
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Fig. I

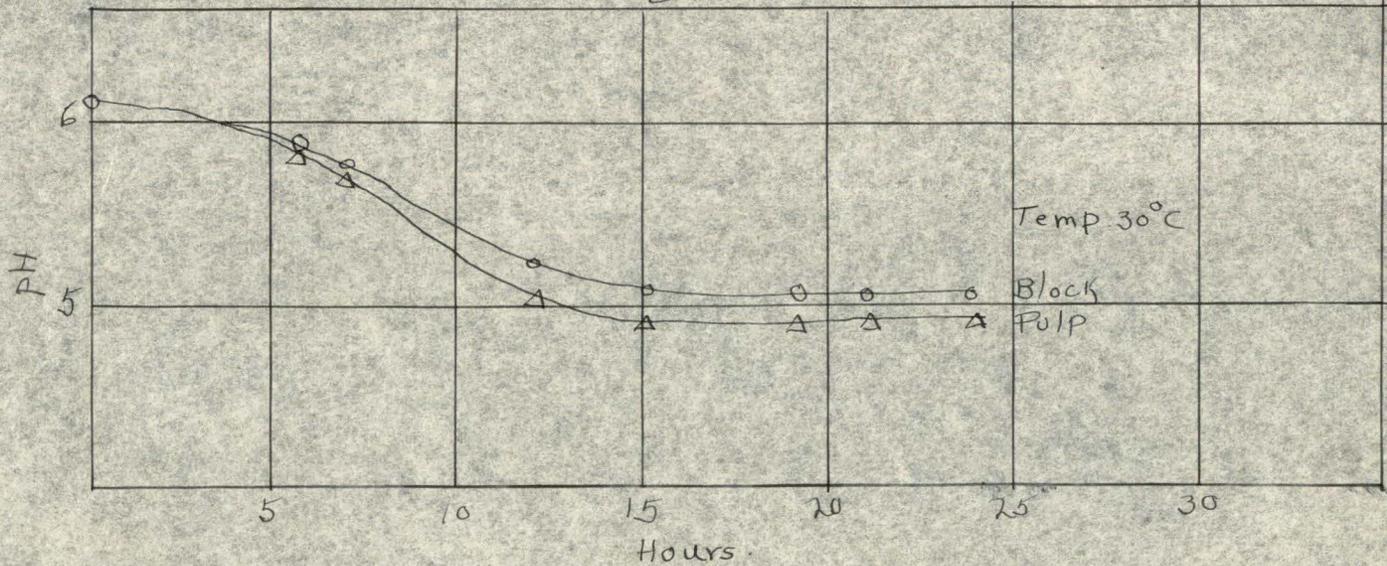
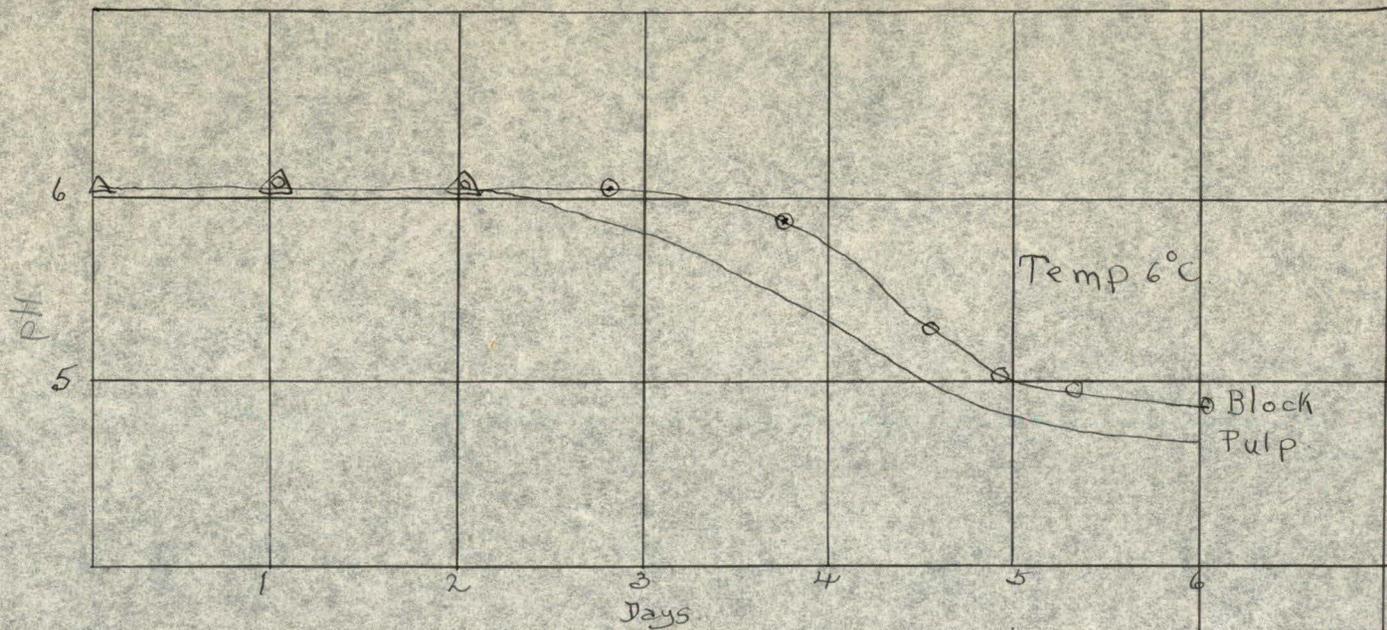


Relation of pH changes to time at various storage temperatures

Fig II

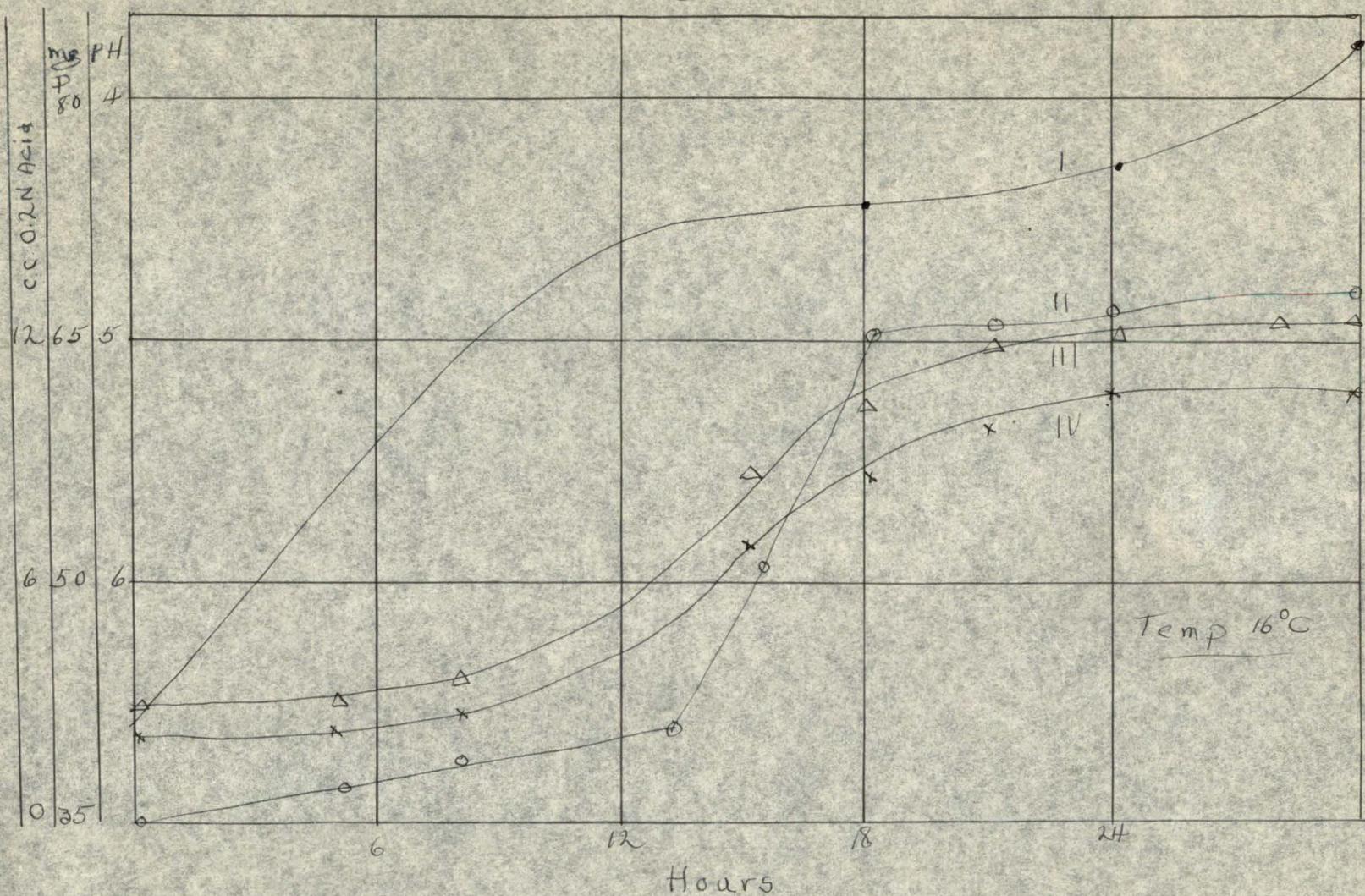


Time - temperature for attaining various pH values.



Comparison of rate of decomposition for block and pulped liver

Fig IV



Relationship of pH (III), P (I), titratable acid (IV) and amino acid (II) to time of storage at 16°C.