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DISTRIBUTION OF ACID PHOSPHATASE DURING AUTOLYSIS IN BOVINE LIVER

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The most important of the hydrolytic enzymes in animal tissues are part of the inner organelles of the cell, i. e. lysosomes (*De Duve* 1963). The establishment of the lysosomal concept can be considered to date from 1955 (*Appelmans et al.* 1955, *De Duve et al.* 1955). The first general summary and coordination of knowledge associated with the question was done in Basle in 1963 (*De Duve* e. g.).

Lysosomal enzymes are of great importance in many of the hydrolytic reactions of meat, among which may be mentioned phenomena associated with postmortal autolysis such as tenderization and various flavouring processes (*Tappel et al.* 1965). Lysosomal enzymes may also have an important part to play in reactions taking place in animal products exposed to a mild heat treatment. The activity of lysosomal enzymes seems also to be closely associated with the events which result in or precede physiological or pathological cell death (*Anderson* 1965).

Lysosomes are intracellular organelles which are surrounded by a "unit membrane". They can be shown to contain a great amount of acid phosphatase as well as a number of other hydrolytic enzymes. The unit membrane consists of lipoprotein molecules placed side by side (*Green & Tzagoloff* 1966). All the other membrane structures in the cell, such as the plasma membrane,

the endoplasmatic reticulum and the nucleus membrane are probably also built up from the same kind of "unit membrane".

Korn (1966) and *Sjöstrand* (1968) have recently thoroughly criticized the unit membrane theory. According to these authors it seems likely that the structural arrangement of a biological membrane may not be static throughout all in vivo conditions. At least locally, the membrane might be in a dynamic state capable of reversibly changing its molecular arrangement in response to intra- or extracellular stimuli (*Benedetti & Emmellot* 1968).

Evidently the principal function of the lysosome membrane is to separate the intralysosomal enzymes from their extralysosomal substrates in the cytoplasm. Lysosomal enzymes cannot affect these substrates as long as the lysosome membrane remains intact and its function unimpaired (*Sawant et al.* 1964). Permeability changes taking place in the lysosome membrane and factors influencing its stability can be studied by recording the passage of a lysosomal enzyme from the lysosome to the cytoplasm. Acid phosphatase is particularly well suited for this purpose. It is possible to show the presence of acid phosphatase in the cell both by histochemical and by biochemical means (*Gomori* 1953, *Holt* 1959, *Ericsson et al.* 1967).

The purpose of this work was to study the passage of acid phosphatase out of lysosomes during the course of postmortal autolysis in bovine liver and also the thermal stability of this enzyme at 37°C. The aim is further to use the standard material thus obtained in studies on factors affecting the interaction between membrane stability and energy metabolism.

THE EXPERIMENTAL PROCEDURE

Livers of healthy cows (body weight 100—140 kg) were used in the experiments. The liver or parts of it was removed as aseptically as possible immediately after slaughter, after which the liver was handled at 0°—4°C. About 20 min. passed from the death of the animal to the removal of the liver.

The liver, from which the serosa had been removed, was minced, guarding the material against contamination, into pieces with a diameter of 1—3 mm. The pieces were then mixed thoroughly and put into sterilized test tubes, 2 g in each, and then incubated at 37°C for various lengths of time. The distribution

of acid phosphatase*) was determined 0, 6, 12 and 24 hrs. after commencement of incubation.

In the contamination tests the distribution was determined in the same way as in the uncontaminated liver specimens. In these experiments the minced liver slices were mixed before incubation with a few drops of a bacterial suspension. The bacteria were taken from a growth on blood agar cultured from the surface of fresh bovine liver. An exact determination of the species in the flora which by routine analysis was found to be composed mainly of micrococci was omitted. The total bacterial count of the contaminated and the uncontaminated liver specimens was determined before incubation and 6, 12 and 24 hrs. after the commencement of incubation.

To estimate the unsedimentable acid phosphatase activity**) the liver sample (2 g), which was incubated at 37°C, was mixed with 8 ml of 0.25 M saccarose and minced in a pestle-type tissue homogenizer (A. H. Thomas, Philadelphia, USA) with a clearance of 0.12—0.18 mm operating at 1200 r. p. m. Thus a 20 % (w/v) suspension was obtained. Eight similar up-and-down movements were performed with the pestle. The temperature of the homogenate did never exceed 4°C. After an 0.5 ml aliquot of the suspension had been taken for the determination of the total activity, the rest of the suspension was centrifuged for 60 min. at 120.000 × g at 4°C. From the supernatant 0.5 ml was measured into 20 ml of 0.25 M saccarose with 0.2 % of Triton X-100 detergent (Rohm and Haas, Philadelphia, USA). The unsedimentable acid phosphatase activity was obtained by acid phosphatase determination of this solution.

To determine the total acid phosphatase activity***) of the 0.5 ml aliquot of the suspension mentioned above, the aliquot was mixed with 20 ml of 0.25 M saccarose with 0.2 % Triton

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*) By the distribution of acid phosphatase is meant the unsedimentable activity of the specimen as a percentage of the total acid phosphatase of the same specimen.

**) By the unsedimentable acid phosphatase activity of the spemen is meant the acid phosphatase activity existing outside the lysosomal membrane.

***) By the total acid phosphatase activity of the specimen is meant the simultaneously determined sum of the lysosome bound and the unsedimentable activity.

X-100 detergent. The suspension thus obtained was minced again in the tissue homogenizer as before and centrifuged at 8000 × g at 4°C for 10 min. The total acid phosphatase activity was obtained by acid phosphatase determination of the supernatant.

In the chemical determination of the acid phosphatase, disodium-paranitrophenol was used as a substrate and paranitrophenol as a standard (Sigma Chemical Co., St. Louis, USA). The pH of the reaction mixture was 4.8, the incubation time 15 min. and the temperature 37°C. The reaction was stopped and the colour developed with 0.1 N-NaOH solution.

Optical density measurements were made with a spectrophotometer, using a wave lenght of 410 mμ.

At least three parallel determinations were made of both the total and the unsedimentable activity of each liver sample.

In the investigation of the thermal stability of the total acid phosphatase, pieces of liver were incubated in sterilized test tubes at temperatures of 4°C and 37°C.

RESULTS

Distribution of acid phosphatase

In Fig. 1 the relationship between unsedimentable and total acid phosphatase activity in bovine liver is shown during 24 hrs.' incubation at 37°C. The share of unsedimentable activity of the total acid phosphatase activity of the specimen increases during 24 hrs. from 28—42 % before incubation to 62—72 % after 24 hrs. at 37°C.

The effect of bacterial contamination on the distribution of acid phosphatase activity

The results are shown in Fig. 2. The share of the unsedimentable acid phosphatase activity of the total acid phosphatase activity increases more rapidly in contaminated than in uncontaminated liver during incubation at 37°C for 24 hrs.

In contaminated liver samples this share was found to be 78—84 % and in the uncontaminated samples of the same liver the share was 62—69 %. The difference between the mean percentages, 81.0 % and 65.3 %, is highly significant ($P < 0.001$)*). The total number of bacteria in the contaminated liver sample

*) The Students t-test used.

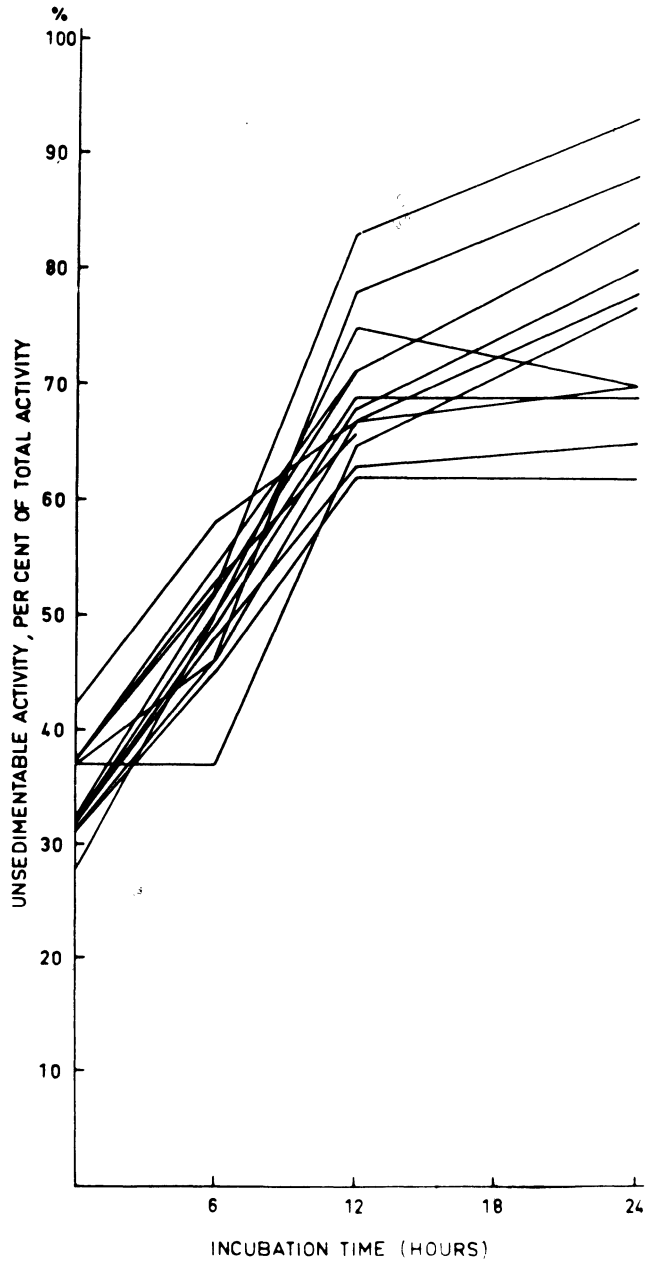

Figure 1. Distribution of acid phosphatase activity in bovine liver during 24 hrs.' incubation at 37°C.

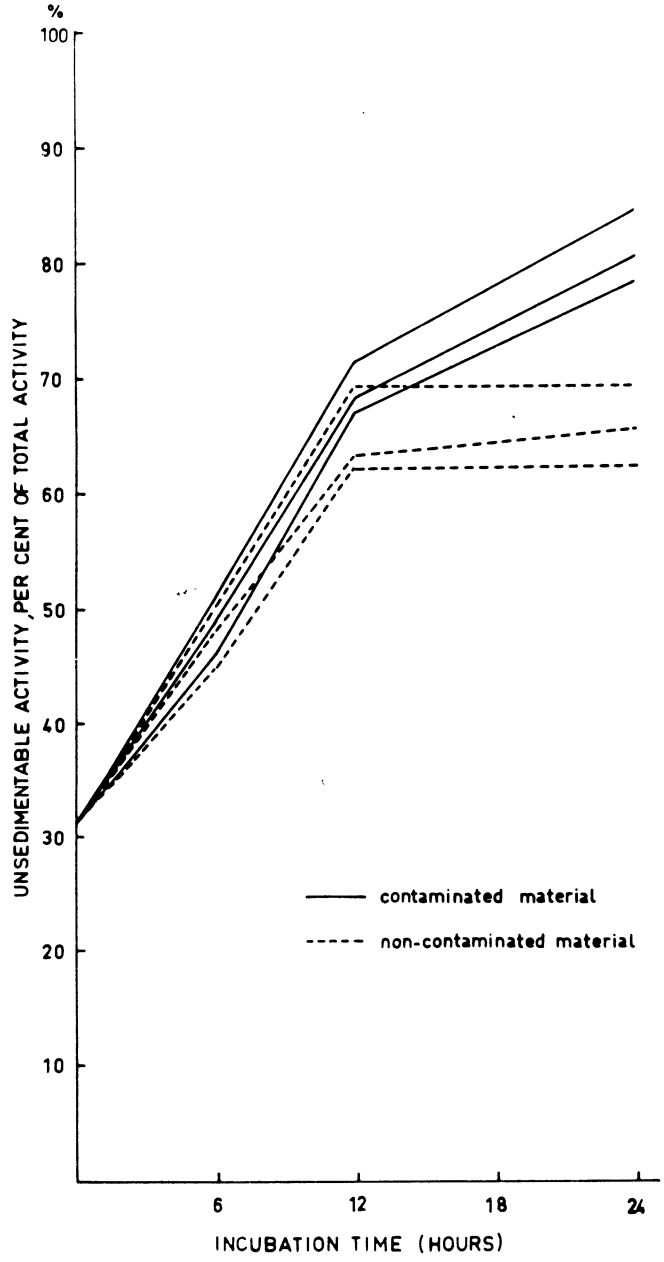

Figure 2. The effect of bacterial contamination on the distribution of acid phosphatase activity in bovine liver during incubation at 37°C.

before incubation was found to be 1.6×10^7/g, after 6 hrs. of incubation at 37°C 8×10^8/g, and after 24 hrs. 1.5×10^{10}/g. The corresponding total number in the uncontaminated liver was zero before incubation, 10^3/g after 6 hrs. at 37°C, 1.5×10^3/g after 12 hrs., and 10^4/g after 24 hrs. The pH of the contaminated liver was 6.5 after 24 hrs., that of the uncontaminated liver was 6.1. The results further showed that no significant difference was noted between the total acid phosphatase activity of the contaminated and the uncontaminated material after 24 hrs.' incubation at 37°C.

Heat stability of acid phosphatase total activity

Heat stability at 37°C is shown in Fig. 3. Incubation of bovine liver during 24 hrs. at 37°C decreased the total acid phosphatase activity to about one half of the amount before the incubation.

When bovine liver was incubated at 4°C, the total activity of acid phosphatase did not decrease significantly during 16 days (Fig. 4).

DISCUSSION

Earlier work on the distribution of acid phosphatase during autolysis of liver and other organs of various animal species (*Anderson* 1965 and *Tappel et al.* 1965) predicted that the progressive relative increase of unsedimentable acid phosphatase activity which was found would be associated with autolysis also in the liver of bovine animals.

To check the method, the amount of acid phosphatase activity was in a number of experiments also determined in the sediment obtained by ultracentrifuging. When the activity of acid phosphatase thus obtained was added to the corresponding activity in the supernatant, the sum was in most experiments almost the same as the acid phosphatase activity of the sample obtained after addition of Triton X-100 detergent.

Triton X-100 destroys the lysosomal membrane by breaking the bonds between its non-polar groups and liberates the lysosomal acid phosphatase (*Wattiaux & De Duve* 1956). According to the literature (e. g. *Anderson*) the Triton X-100 does not influence the enzyme activity. From these points of view the method can thus be considered suitable for the present investigation.

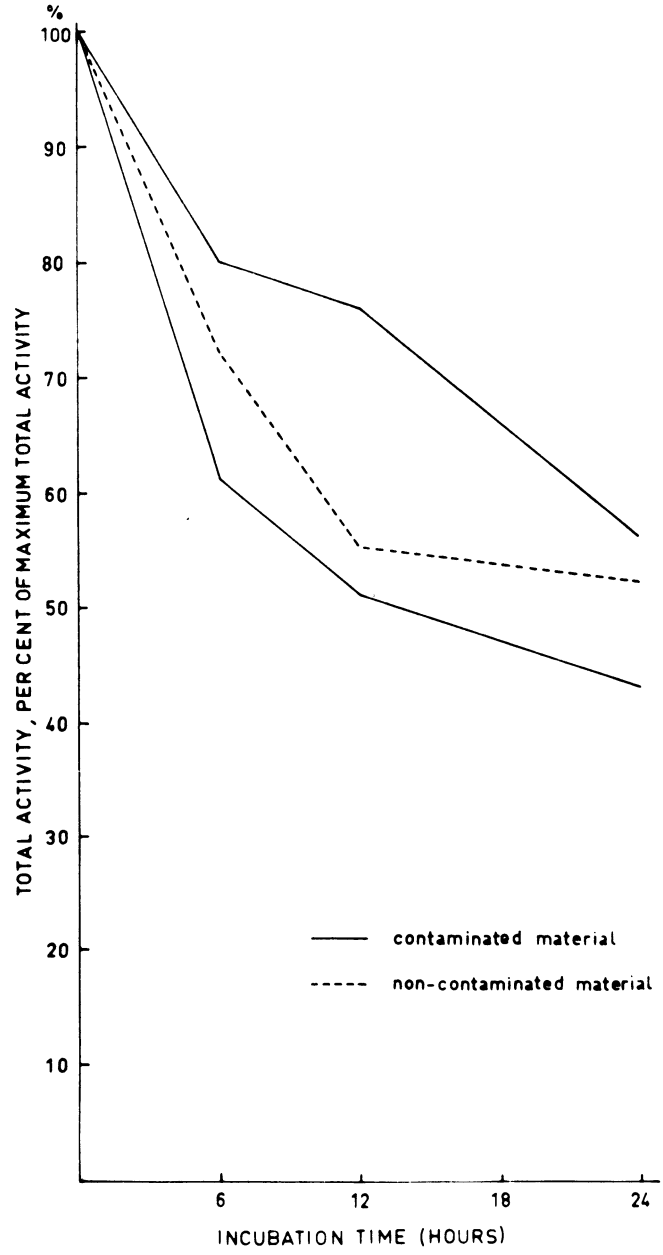

Figure 3. The total acid phosphatase activity in bovine liver during incubation at 37°C. Each curve represents separate liver samples and each point the mean of 9 parallel estimations.

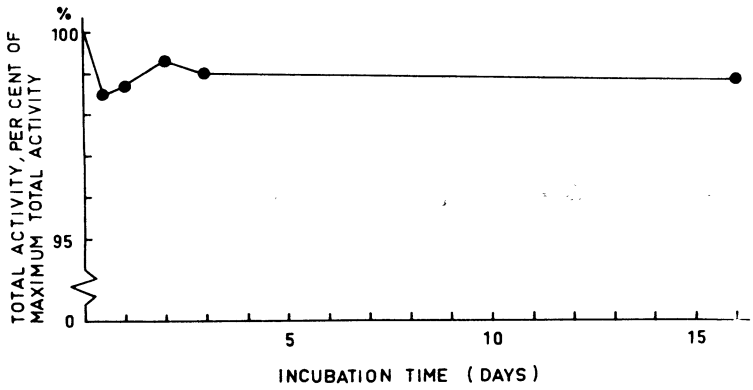

Figure 4. The effect of storage at 4°C on the total acid phosphatase activity of bovine liver.

As the acid phosphatase activity in different parts of the same liver could be expected to vary (*De Duve* 1963), the same part of the liver, namely the lobus caudatus, was used for sampling. In addition the minced liver slices were mixed thoroughly before the commencement of the determination procedure. The differences between the results of acid phosphatase activity determinations on various samples obtained from the same liver did not exceed 12 %. Because the animal's physiological state, dietary factors and possible pathological conditions might affect the lysosomal membrane and thus the liberation of acid phosphatase from the lysosomes (*Straus* 1967, *Tappel et al.*, *Weismann & Thomas* 1962), the liver samples were taken from animals differing from each other as little as possible. As the differences between the results of experiments on several liver samples of a single animal were comparatively small, the variations between the results from different livers, which appear in Fig. 1, cannot be inferred to be due to the methodical errors.

Bacterial contamination altered the distribution of acid phosphatase activity during incubation at 37°C so that the proportion of the unsedimentable activity increased significantly. A reliable explanation of this finding could not be given. Factors to be taken into account are probably the direct effect of bacterial enzymes on the membrane of the lysosomes and the indirect effect of bacteria and their enzymes on the postmortal reactions of liver cells. The effect of different species of bacteria on the stability of the lysosomal membrane may also vary. On the other hand the

effect of the same species of bacteria on the lysosomal membranes of liver cells taken from various animal individuals may differ, e. g. due to qualitative or quantitative variations in the contents of substances important for bacteria. The effect of bacterial contamination on the pH of the liver was negligible in this study.

The stability of the lysosomal membranes decreases when the pH of their surroundings deviates from the neutral towards either acidity or alkalinity (*Sawant et al.* 1964). The pH difference of 0.4 units between contaminated and uncontaminated liver samples measured after incubation would preferentially decrease the observed differences.

The author has observed a corresponding difference in the distribution of acid phosphatase between contaminated and uncontaminated cat liver after incubation at 37°C.

When bovine liver samples were incubated at 37°C during a 24 hr. period, a decrease in the phosphatase total activity was noted to the extent of about 50 % of the value obtained before the incubation period (Fig. 3). This decrease has not been described before. It remains to be seen whether the acid phosphatase of bovine liver can be separated into various fractions with regard to heat stability.

The heat stability of acid phosphatase of rat liver at 50°C has been studied before. In addition to the varying incubation temperature, differences also exist between animal species with regard to the changes of the activity levels of acid phosphatase (*Nelson* 1966), both of which facts decrease the possibility of applying the results obtained with rats to bovines or of comparing the rat results which those obtained in the present investigation.

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SUMMARY

The percentage distribution of acid phosphatase between lysosomes and cytoplasm in bovine liver during autolysis at 37°C was investigated. The share of the cytoplasmic acid phosphatase activity of the total acid phosphatase activity in liver tissue increased during

autolysis being before incubation 28 — 42 % and after 24 hrs.' incubation at 37°C 63 — 94 %. Microbiological contamination increased the proportion of cytoplasmic acid phosphatase.

When bovine liver was incubated at 37°C for 24 hrs., the activity of the total acid phosphatase decreased to about 50 % of the initial activity. During a 16 days' incubation at 4°C the total acid phosphatase activity of bovine liver, however, remained unchanged.

SAMMANFATTNING

Fördelning av surt fosfatas under autolys i nötlever.

Författaren har undersökt hur surt fosfatas, som huvudsakligen är ett lysosomalt enzym, fördelar sig mellan cellens cytoplasma och lysosomer i nötlever under olika stadier av autolys vid en temperatur av 37°C. Det lysosomala sura fosfatasets aktivitet före inkubationen vid 37° var mellan 28 och 42 % av det sura fosfatasets totalaktivitet, och efter 24 timmars inkubation mellan 63 och 94 %. En mikrobiell kontamination ökade under inkubationen det cytoplasmatiska sura fosfatasets relativa andel av totalaktiviteten.

Då nötlever inkuberades vid 37°C under 24 timmar, minskade den totala sura fosfatasets aktivitet till ungefär 50 % av den före inkubationen mätta aktiviteten. Under 16 dagars inkubation vid 4°C förblev däremot nötleverns totala sura fosfatasaktivitet oförändrad.

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