

A Research Note

Changes in Connective Tissue Histology in Freeze-Thaw Cycled and Refrigerated Pork Liver

ABSTRACT

Alterations in the connective tissue of pork liver during storage were examined histologically. The connective tissue outlining the lobule of pork liver was apparently unaltered during refrigerated storage (R-S), but showed extensive crimping or waviness during freeze-thaw (F-T) cycling. Intralobular connective tissue (reticulin) disappeared during R-S but was still visible after six F-T cycles. The existence of a proteolytic enzyme active against Type III collagen at refrigerator temperatures but irreversibly inactivated by freezing was postulated.

INTRODUCTION

LIVER, a valuable meat by-product, is frozen soon after slaughter to prevent both bacterial and autolytic deterioration. Ambatzoglu (1972) and Partmann (1973) showed that short periods of freezing and slow thawing had little effect on the over-all histological appearance of the liver tissue. In these studies gross changes in the structure of the tissue attributable to ice crystal formation during freezing were sought. Recently, Strange et al. (1985) reported that liver cells were destroyed, and adhesion of liver cells to each other and to the tissue matrix was altered during refrigerated storage (R-S) and freeze-thaw (F-T) cycling. They stated that the collagen matrix in the liver tissue remained intact during freeze-thaw cycling.

This study examines by specific histological techniques the effects of refrigerated storage and freeze-thaw cycling on the collagen of pork liver.

MATERIALS & METHODS

TWO INSPECTED trimmed whole pork livers were obtained soon after slaughter and packed in ice during transportation. Each liver was divided into 100g samples, vacuum packaged and either frozen or refrigerated.

Storage conditions

Two different storage regimens were used: F-T and R-S. F-T cycling was carried out after an initial freezing time of 70 hr (at -20°C) by thawing the vacuum packaged liver samples at $+5^{\circ}\text{C}$ for 24 hr (F-T cycle 1). Liver samples were refrozen for 24 hr and thawed for 24 hr for each additional F-T cycle. R-S vacuum packaged liver samples were stored at $+5^{\circ}\text{C}$ for up to 6 days. Liver tissue was sampled fresh (within 3 hr of slaughter—no vacuum packaging), after 1, 4, and 6 F-T cycles and after 4 and 6 days R-S.

Sampling, fixing, sectioning, and staining

Liver pieces, 2.5 X 2.5 X 1.25 cm were cut, placed in pH 7, 0.08M phosphate buffer containing 10% formalin for at least 24 hr before 20 micron thick sections were cut on a freezing microtome. Sections were stained for collagen and reticulin using Foot's modification of Hor-

tega's silver carbonate method (Edwards, 1950). Photomicrographs were taken on a Zeiss Photomicroscope.

RESULTS & DISCUSSION

THE HEXAGONAL-SHAPED LOBULE is the major structural unit of pork liver and its outlined by a prominent connective tissue structure (perilobular collagen) (Fig. 1a). The lobule contains liver cells arranged around sinusoidal spaces that lead to the central vein. Veins, arteries, lymph vessels and bile ducts necessary for liver function are located at the vertices of the lobule. Within the lobule, a reticular network of connective tissue, called reticulin, outlines the sinusoidal spaces (Fig. 2a).

During storage changes occur in the perilobular collagen. In fresh liver (Fig. 1a) this collagen is straight and well defined with visible individual strands. Veins, arteries and ducts present in the perilobular tissue are clearly outlined. The lobule is filled with an orderly arrangement of cells, and the central vein is clearly defined. After 4 and 6 R-S days, respectively, the integrity of the central vein is destroyed, and small voids appear in the lobule (Fig. 1b, 1c). However, the perilobular collagen structure remains well defined.

The effects of freezing and thawing on the perilobular collagen after 1, 4 and 6 F-T cycles are shown in Fig. 1d, 1e, and 1f, respectively. Freezing and thawing cause crimping or waviness of this collagen with the waviness increasing with the number of F-T cycles. This crimping could result from destruction of liver cells within the lobule or from changes in hydration of collagen fibers caused by formation of ice crystals during freezing. Other phenomena noted in F-T liver were: decrease in size of lobule, appearance of large voids and disruption of the orderly arrangement of cells within the lobule.

Fig. 2a shows fresh liver at a higher magnification. The reticular fibers on the sinusoidal surfaces of the hepatocytes were well defined by the silver stain. Nuclei were spherical and many cells were binucleate. The cytoplasm appeared light gray.

Fig. 2b and 2c show the effect of refrigerated storage on the reticular fibers. The majority of these fibers, intensely stained in fresh liver, have disappeared.

Fig. 2d, 2e and 2f show effects of freeze-thaw cycling on the reticular network. These fibers stained clearly even after six F-T cycles, while the cells themselves underwent extensive destruction and distortion. The reticulin appeared less compact after freezing and thawing.

Electron microscopic observations and osmium-lead-uranyl stained fresh, R-S and F-T liver samples confirmed the results of the light microscopic observations. Fresh liver tissue preparations have fibrous structures between the hepatic and the sinusoidal lining cells while the R-S tissue did not. Retention of the reticular fibers in the F-T liver was not obvious because of distortion of the tissue, but some traces were noted.

Collagens comprise from 1 to 5% of the protein in the liver tissue with species variation. Information on the composition of liver collagen is available for rat and human liver only. Liver tissue contains types I, III, IV, and V collagens (Rojkind and Ponce-Noyola, 1982). Type I collagen is most abundant

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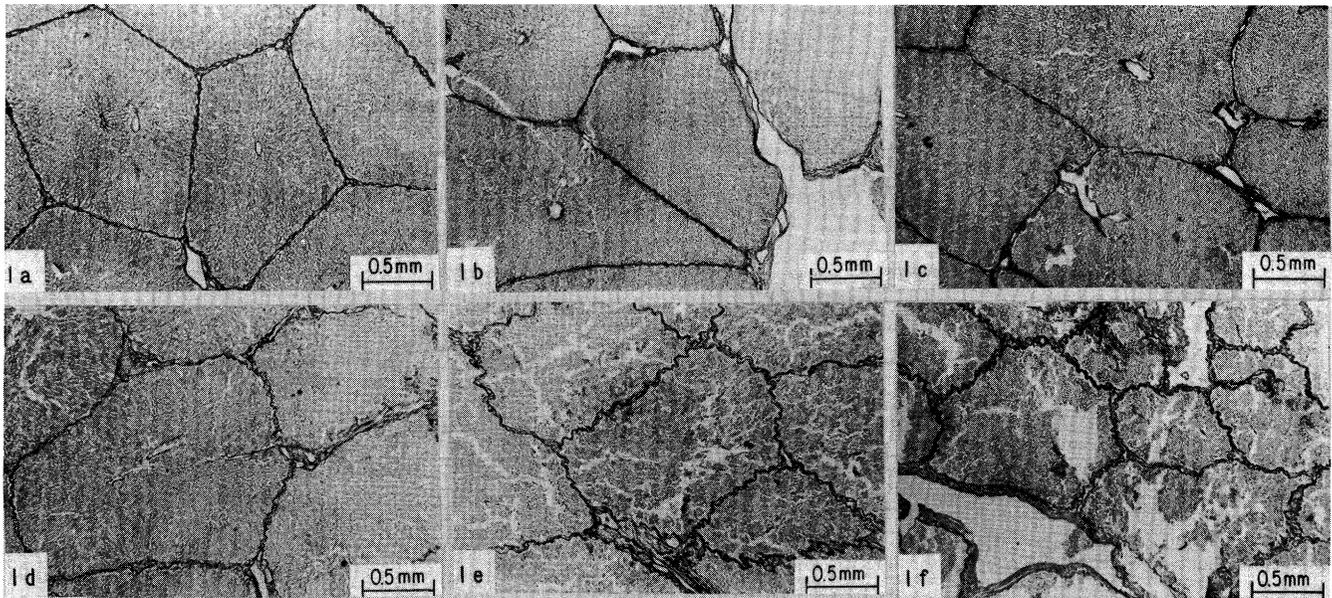


Fig. 1—Photomicrographs of silver carbonate stained pork liver sections: (a) 3-hr postmortem; (b) refrigerated for 4 days; (c) refrigerated for 6 days; (d) after one freeze-thaw cycle; (e) after four freeze-thaw cycles; (f) after six freeze-thaw cycles.

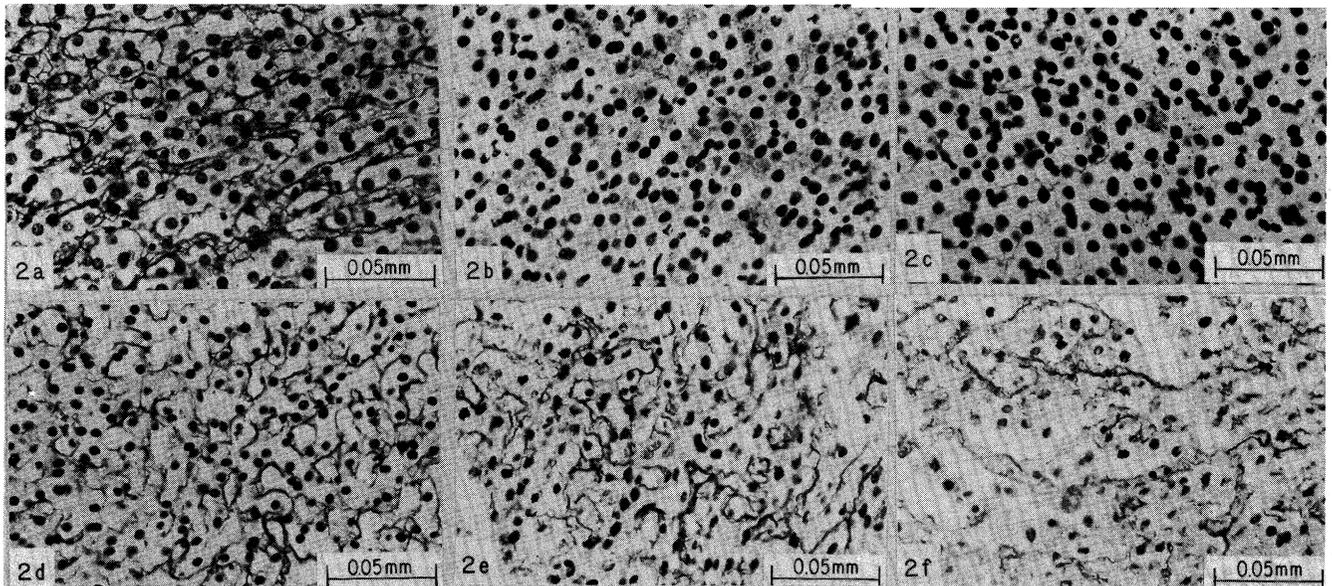


Fig. 2—Photomicrographs of silver carbonate stained pork liver sections: (a) 3-hr postmortem; (b) refrigerated for 4 days; (c) refrigerated for 6 days; (d) after one freeze-thaw cycle; (e) after four freeze-thaw cycles; (f) after six freeze-thaw cycles.

and is the major component of the extracellular matrix of the liver. Reticular fibers consist of type III collagen (Brodsky and Eikenberry, 1982) and are present in the intralobular area separating the hepatic cells from the sinusoidal lining cells (Dellmann, 1973). Type III collagen is degraded more readily by proteolysis than the other types (Harper, 1980). Types IV (component of basement membrane) and V (component of vascular membranes) collagens are nonfibrous in structure (Brodsky and Eikenberry, 1982).

Changes in the reticular network of liver observed during storage implies an endogenous proteolytic enzyme active against Type III collagen that is active at refrigerator temperatures (+5°C) but irreversibly inactivated by freezing. This proteo-

lytic enzyme probably is present in the extracellular fluid and may be inhibited by an excretion of viable cells. Strange et al. (1985) demonstrated the during refrigerated storage, cell destruction was not extensive until 6 days of storage but cell viability was reduced after only 1 day of storage.

REFERENCES

- Ambatzoglu, D. 1972. Sulle alterazioni del fegato di bovino refrigerato, congelato e liofilizzato. *Ann. della Facolta di Medicina Veterinaria di Torino*. 19: 227.
- Brodsky, B. and Eikenberry, E.F. 1982. Characterization of fibrous forms of collagen. Ch. 5. In "Methods in Enzymology," Vol. 82, (Ed.) L.W. Cunningham and D.W. Frederiksen, p. 127. Academic Press Inc., New York.

- Dellmann, H-D. 1971. "Veterinary Histology." Lea and Febiger, Philadelphia, PA.
- Edwards, J.E. 1950. Methods for the demonstration of intercellular substances of the connective tissues. In "Microscopical Technique," 3rd ed., (Ed.) R.M. Jones, p. 240. Paul B. Hoeber, Inc., New York.
- Harper, E. 1980. Collagenases. *Ann. Rev. Biochem.* 49: 1063.
- Partmann, W. 1973. Histologische veränderungen in rind — und schweinefleisch sowie schweineleber unter definierten gefrier — und auflaufbedingungen. *Die Fleischwirtschaft* 53(1): 65.
- Rojkind, M. and Ponce-Noyola, P. 1982. The extracellular matrix of the