

Quantitation and Characterization of Drip from Frozen-Thawed and Refrigerated Pork Liver

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ABSTRACT

Drip from fresh (F), refrigerated (R-S), and frozen and thawed (F-T) vacuum packaged pork liver slices was characterized by blood or plasma content, enzymatic activity, protein content and SDS-PAGE patterns. Amount of drip from F and R-S liver slices was 2.2% and contained 40% blood. F-T increased drip but the amount depended on the number of F-T cycles. F-T drip contained 28% plasma. Enzymatic activity was higher for F-T drip but protein content was about the same. SDS-PAGE protein patterns of drip resembled patterns of model liver-blood mixtures but variations were evident due to storage conditions.

INTRODUCTION

THE VALUE OF LIVER as an export commodity is adversely affected by product deterioration during transportation and storage. Weight loss during refrigeration and/or freezing and thawing is of major concern. Sliced frozen beef livers lost between 13–18% of their weight during thawing and subsequent storage at 1–3°C (Smith et al., 1983a) and chilled whole beef livers lost 7% of their weight after 15.5 days at 1–3°C (Smith et al., 1983b). Strange et al. (1985a) reported losses of 11(±5) % for frozen-thawed and 6(±4.5) % for refrigerated pork livers. A substantial portion of these weight losses is due to formation of a fluid, reddish exudate called drip.

Drip is formed by muscle as well as liver tissues. Lawrie (1979), Anon and Calvelo (1980), Hamm et al. (1982) and Gonzalez-Sanguinetti et al. (1985) describe the formation of drip in frozen-thawed muscle tissue as movement of intracellular water and sarcoplasmic proteins to the extracellular space. Awad et al. (1968, 1969), Lawrie (1979) and Anon and Calvelo (1980) report that drip from muscle is composed of water, sarcoplasmic proteins and low molecular weight compounds.

Drip from liver and from muscle may differ because of differences in structure and function of striated muscle and liver. George et al. (1980) report that 4.2% muscle is extracellular space while James et al. (1983) find about 10%. Warriss (1977) reports a capillary volume of 1.6% for muscle. Liver has much larger extracellular spaces; about 22% of the liver volume consists of sinusoids or capillary bed and large blood vessels (Blouin et al., 1977). Liver and other edible offal contain a large portion of the residual blood in the carcass (Warriss, 1977; Warriss and Leach, 1978).

The drip from either fresh or frozen thawed liver is not well characterized. Lyon and Thomson (1984) measure % solids and % moisture in the drip from frozen-thawed poultry liver. Hamm and Masic (1975), Barbagli and Serlupi Crescenzi (1981) and Gottesmann and Hamm (1984) report the presence of mitochondrial enzymes in "press juice" or cytoplasm as diagnostic of previously frozen liver.

The objectives of this study were to develop a method for determining the amount of blood in the drip; to determine the relationship between % drip from liver and % blood in drip;

and to characterize the drip from fresh, refrigerated and frozen-thawed liver.

MATERIALS & METHODS

FIFTEEN whole pork livers were obtained from a local slaughterhouse immediately following federal inspection and removal of the bile duct and gall bladder. Three livers were used in preliminary experiments to establish optimum assay procedure. In the actual experiments, six livers were utilized for both the fresh and refrigerated storage (R-S) tests and six others for the freeze-thaw cycle series (F-T).

Liver and fresh citrated blood were obtained on the same day. The blood was collected directly from the bleeding animal in a plastic bucket coated with 4.4g citric acid monohydrate (Baker Chemical Co.). Two liters blood were transferred to a wide mouth plastic jar also coated with citric acid monohydrate, yielding a final concentration of 0.4% citric acid. Plasma was prepared by centrifuging the whole blood at 10,000 X g for 30 min in a Sorvall refrigerated centrifuge using the GSA head. Plasma was decanted from the packed cells.

Each liver was cut into 1.25 cm slices; six 125–150g samples of liver slices were weighed to the nearest 0.1g, vacuum packaged and sealed with a Smith SuperVac (Smith Equipment Co., Clifton, NJ) in 7 X 7 sized "IKD ALL-VAC 13 FBR" pouches. The remaining liver was macerated for 30 sec in a Cuisinart food processor and the resulting slurry was mixed with either whole citrated blood or plasma to contain 25%, 50% or 75% liver. Three samples of each mixture and of either blood or plasma were vacuum packaged and stored in the same manner as the matching liver slices.

In refrigerated storage (R-S) experiments, the macerated livers were mixed with whole blood and the liver slices and liver-blood mixtures were stored at +5°C. Samples were analyzed on day of slaughter (fresh) and after 2 and 4 days of storage.

In freeze-thaw (F-T) experiments the macerated liver was mixed with plasma, and the liver slices and the liver-plasma mixtures were frozen at -20°C for an average of 7 days before the initial thawing at +5°C for 24 hr (F-T cycle 1). Samples were then refrozen for 24 hr at -20°C and rethawed at +5°C for 24 hr (F-T cycle 2). This procedure was repeated for F-T cycle 3. The liver and liver-plasma mixtures were analyzed at the end of each thaw period.

After storage, drip was removed from the packages of sliced liver with a disposable pipette and weighed and percent drip was determined. The sliced liver was discarded. The drip was centrifuged at 50,000 X g for 30 min to remove all suspended solids and the pellet was examined by light microscopy, after staining with Wright Stain (Conn, 1940).

The liver-blood and liver-plasma mixtures were centrifuged at 10,000 X g for 30 min in the GSA head to remove most of the debris before final centrifuging for 30 min at 50,000 X g to prepare the supernate for further analysis. Samples of fresh liver and aliquots of drip, and liver-blood and liver-plasma mixture supernates were freeze-dried for electrophoretic examination. All other analyses were carried out on the supernate.

Assays

The following measurements were made on all supernates: % protein (%PRO) (biuret method), specific acid phosphatase activity (AP04) (Sigma Technical Bulletin 104, activity in Sigma Units per mg protein (from protein determination), and hemoglobin concentration in mg per mL supernate (Hb) [Drabkin's reagent Sigma Technical Bulletin 525, hemoglobin standard curve prepared with porcine hemoglobin (Sigma Chemical Co., St. Louis, MO)].

The following analyses were made on some of the supernates: % ash, sodium, potassium and iron, and specific glutamic dehydrogenase activity (GDH).

Ash was determined by weighing between 2 and 6g of supernate into dry, tared, acid washed glass crucibles. The samples were dried on a hot plate and ashed at 550°C for 54 hrs starting in a cold muffle furnace. The weight of ash was determined by difference and percent calculated.

Sodium, potassium and iron of the ash were determined using a Perkin-Elmer 5000 Atomic Absorption Spectrophotometer (Perkin-Elmer, 1976). Prior to analysis, all glass and plasticware were rinsed with 20% nitric acid followed by three rinsings with deionized water. Ash was dissolved in 20 mL 20% nitric acid and diluted to 25 or 100 mL, depending on sample size, with 0.5N hydrochloric acid. Dilutions of the dissolved ash of 1:50 and 1:10 were satisfactory for sodium and potassium determinations while undiluted samples were used for iron. Metal content is reported as millimoles per kilogram supernate.

GDH was measured according to a method adapted from Schmidt (1965). All reagents, incubations and assays were maintained at 25°C with thermostatically controlled water bath and a jacketted cuvette. Two mg NADH (preweighed vial containing the disodium salt of β nicotinamide adenine dinucleotide, reduced form, Sigma Grade III) were dissolved in 15 mL pH 8 buffer containing 930 mg triethanolamine hydrochloride, 100 mg disodium ethylenediaminetetraacetic acid (EDTA) and 1.127g ammonium acetate per 100 mL. For each GDH assay, 0.73 mL of buffer + NADH mixture was incubated with 0.25 mL of the appropriate dilution of sample for 15 min before addition of 0.02 mL solution of 0.106g freshly prepared α -ketoglutaric acid in 2 mL water. Nonspecific substrates for NADH-requiring reactions present in the sample were eliminated by the incubation step. Preliminary studies showed that sufficient NADH for the GDH assay was present in the reaction mixture after the incubation. The reaction assayed was α -ketoglutaric acid + NADH + NH_4^+ $\xrightarrow{\text{GDH}}$ NAD⁺ + glutamic acid. A Shimadzu (Shimadzu Corp., Kyoto, Japan) Graphi-cord UV-240 with optional program capability was used to monitor the change in absorbance at 366 nm of NADH. Activity of glutamic dehydrogenase was determined in IU units for 5 sec intervals. The mean activity of 10 intervals was used to calculate specific activity (glutamic dehydrogenase activity per mg protein).

SDS-polyacrylamide gel electrophoresis was performed on freeze-dried aliquots of the supernates from drip, liver-blood and liver-plasma mixtures, on a sample of freeze-dried fresh liver and on a high molecular weight standard mixture (Sigma SDS-6H) according to Basch's modification (Basch et al., 1985) of the Laemmli procedure (Laemmli, 1970). An E-C Vertical Gel electrophoresis unit (E-C Apparatus Corp., St. Petersburg, FL), and an E-C 500 power supply were used and 3mm thick gels were run. Plugging and running gels were 10% and the stacking gel was 4%; each gel contained 2.5% N,N'-methylene bis-acrylamide. The gels were stained with Coomassie Brilliant Blue 250.

Statistical analysis

Analysis of variance, linear correlations and multiple regression equations were determined using SAS (1979). Duncan's New Multiple Range test and Student's t test were conducted as described in Steel and Torrie (1980).

RESULTS & DISCUSSION

PRELIMINARY EXPERIMENTS, employing three livers, were used to optimize assay procedures, to determine if the assays reflected changes in liver-blood or -plasma mixtures, and to determine the suitability of liver mixtures as a model of liver drip. When liver-blood mixtures were frozen and thawed, red cells ruptured and the resultant extremely high levels of hemoglobin altered and interfered with most of the assays. However, excessively high hemoglobin was not noted in F-T drip. Therefore, liver-plasma mixtures were used as a model for drip in F-T studies rather than liver-blood mixtures.

Analysis of variance on data from the liver-blood or liver-plasma mixtures was used to select assays which were most affected by changes in % blood or % plasma in the mixtures and least affected by storage time or liver to liver variation. Multiple regression equations for predicting % blood and % plasma in liver mixtures were generated using data from mix-

tures for four livers and validated by data from the other two livers. These results are shown in Table 1. New multiple regression equations were then calculated using data from all livers.

The order of contribution of the different assays to the significance of the multiple regression fit for % blood was % PRO > AP04 > Hb^{1/2} and the multiple regression equation for predicting % blood using data from all liver-blood mixtures was:

$$\% \text{ Blood} = 151.2 - 5.8 (\% \text{ PRO}) - 13.3 (\text{AP04}) - 2.0 (\text{Hb}^{1/2})$$

R² = 0.9638; six livers; observations N = 63.

The order of contribution of the different assays to the significance of the multiple regression fit for % plasma was % PRO > AP04 > GDH > Hb^{1/2} and the regression equation for predicting % plasma using data from all liver-plasma mixtures was:

$$\% \text{ Plasma} = 141.5 - 4.4 (\% \text{ PRO}) - 27.7 (\text{AP04}) - 128.8 (\text{GDH}) - 1.65 (\text{Hb}^{1/2})$$

R² = 0.9649; six livers, observations N = 63.

These multiple regression equations were used to calculate the amount of blood in R-S liver drip and plasma in F-T liver drip. The results are shown in Tables 2 and 3. The amount of drip from both R-S and F-T stored livers increased significantly

Table 1—Calculated % blood and % plasma of known liver-blood and liver-plasma mixtures using regression equations generated from data on other mixtures

% Blood known	Refrigerated storage		Freeze-thaw storage	
	% Blood calculated ^a	% Plasma known	% Plasma calculated ^b	
25	21.8(±4.8)(N=6)	25	35.7(±7.9)(N=6)	
50	42.7(±4.0)(N=6)	50	55.7(±6.1)(N=6)	
75	68.6(±2.7)(N=6)	75	75.7(±3.6)(N=6)	
100	102.6(±0.3)(N=3)	100	97.7(±1.2)(N=3)	

^a Equation generated using data of liver-blood mixtures from four livers and used to calculate % blood in liver-blood mixtures from two other livers.

$$R^2 = 0.9690. \% \text{ Blood} = 144.5 - 4.9 (\% \text{ PRO}) - 13.7 (\text{AP04}) - 3.3 (\text{Hb}^{1/2})$$

^b Equation generated using data of liver-plasma mixtures from four livers and used to calculate % plasma in liver-plasma mixtures from two other livers.

$$\% \text{ Plasma} = 163.2 - 7.0 (\% \text{ PRO}) - 18.2 (\text{AP04}) - 173.6 (\text{GDH}) - 0.12 (\text{Hb}^{1/2})$$

R² = 0.9838.

Table 2—% Drip and calculated % blood from R-S livers

	Fresh		Stored 2 days		Stored 4 days	
	% Drip	% Blood ^c	% Drip	% Blood	% Drip	% Blood
	0.82	36.8	2.70	50.3	3.10	47.1
	1.06	37.4	2.30	51.6	3.03	41.6
	0.00		2.65	38.1	2.37	34.1
	0.00		2.65	37.5	3.23	35.4
	2.22	46.7	3.69	39.7	5.07	49.1
	2.27	40.0	4.52	47.7	4.37	38.3
	0.93	37.5	3.40	62.5	3.99	38.9
	0.46	33.8	4.12	47.6	2.70	39.3
			1.71	47.8	1.82	45.7
	0.24	34.3	1.43	44.4	1.01	39.9
			0.90	33.8	1.79	35.6
	0.18	20.0	1.21	37.3	2.27	35.7
Mean % Drip						
	0.72 ^a		2.61 ^b		2.90 ^b	
S.D. =	0.80		1.16		1.16	
N =	10		12		12	
Mean % Blood						
		35.8 ^a		44.9 ^b		40.1 ^{ab}
S.D. =		7.6		8.1		4.9
N =		8		12		12

^{a,b} Means in the same row having no superscripts in common are significantly different, p < 0.05. Duncan's new multiple range test.

^c Calculated using the equation

$$\% \text{ Blood} = 151.2 - 5.8 (\% \text{ PRO}) - 13.3 (\text{AP04}) - 2.0 (\text{Hb}^{1/2})$$

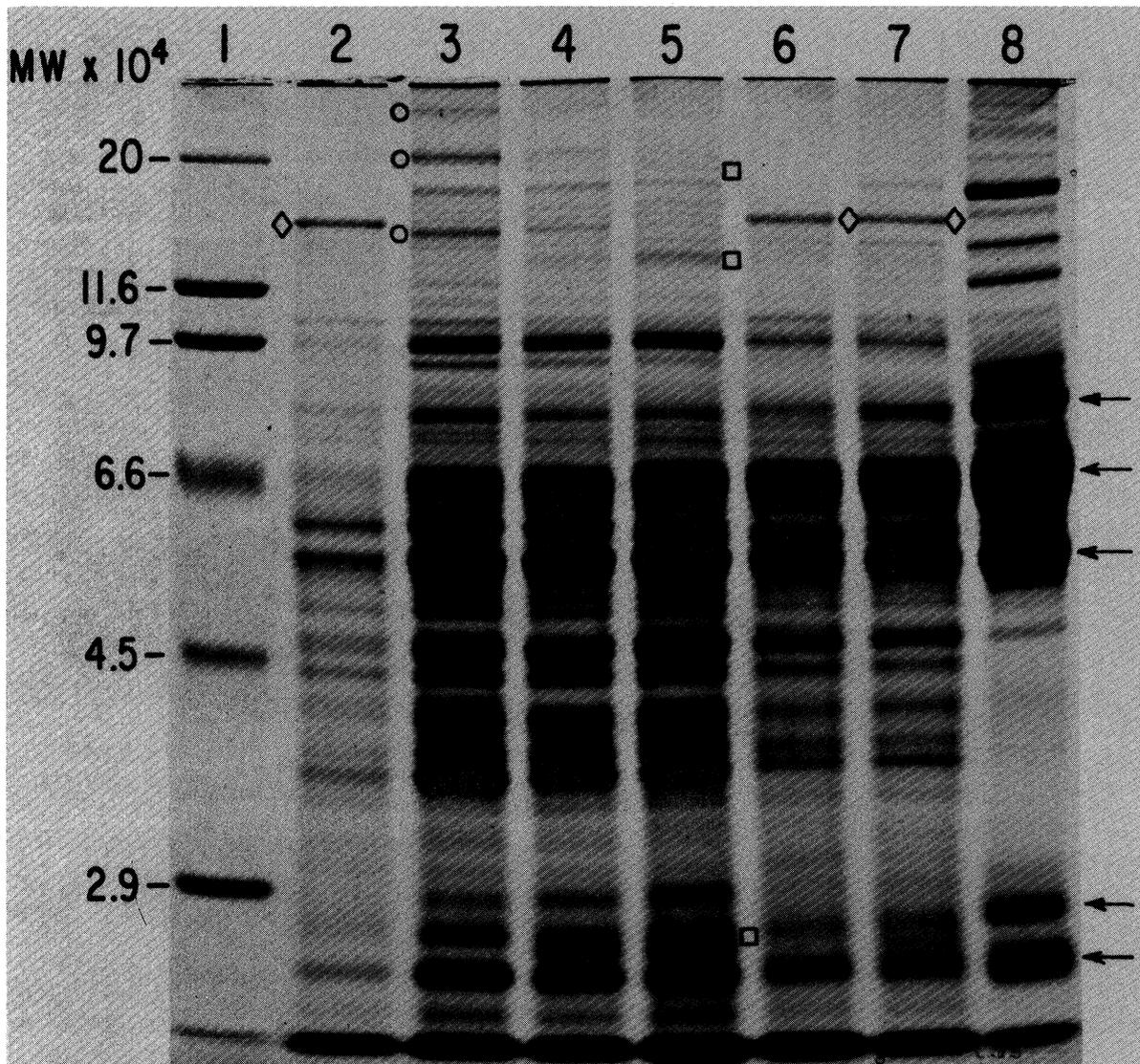


Fig. 1—Electrophoretic patterns of R-S drip and liver-blood mixtures; \diamond protein bands present in whole liver and supernate of liver-blood mixtures; \circ protein bands which disappear during storage; \square protein bands which appear during storage, \blacktriangleleft major blood protein bands. Lane 1—Molecular weight standards. Lane 2—Freeze-dried whole fresh liver. Lane 3—Freeze-dried supernate of drip from fresh liver. Lane 4—Freeze-dried supernate of drip from R-S liver after 2 days storage. Lane 5—Freeze-dried supernate of drip from R-S liver after 4 days storage. Lane 6—Freeze-dried supernate of a 75% liver — 25% blood mixture. Lane 7—Freeze-dried supernate of 50% liver — 50% blood mixture. Lane 8—Freeze-dried supernate of 100% blood.

Table 5—Means of assays on the drip from F-T cycled livers

	Cycle 1	Cycle 2	Cycle 3
% PRO ^c	16.73 ^a	15.40 ^b	15.14 ^b
S.D. ^g	1.28	1.08	1.10
APO4 ^d	1.11 ^a	1.14 ^a	1.25 ^b
S.D.	0.10	0.12	0.11
GDH ^e	0.020 ^a	0.027 ^a	0.033 ^a
S.D.	0.013	0.015	0.020
Hb ^f	30.82 ^a	23.26 ^b	22.86 ^b
S.D.	11.70	3.47	3.07

^{a-b} Means in the same row having no superscripts in common are significantly different, $p < 0.05$. Duncan's new multiple range test.

^c % protein in drip.

^d Specific acid phosphatase activity in Sigma units.

^e Specific glutamic dehydrogenase activity in I.U. units.

^f Hemoglobin concentration in mg/mL of drip.

^g Standard deviation N = 12.

dent's $t = 7.69$ ($p < 0.01$) for the difference. The higher Hb content in F-T liver drip presumably came from ruptured red cells even though intact red cells were observed in the drip pellet.

The amount of drip was considerably higher in F-T than in R-S livers, but the drip increased during both types of storage. For F-T livers drip was $8.45 \pm 3.67\%$ ($N = 36$) and for R-S livers drip was $2.18 \pm 1.38\%$ ($N = 32$); Student's $t = 9.32$ ($p < 0.01$). In both R-S and F-T the additional volume of drip came from the extracellular space of the liver which was initially filled with blood. In F-T storage, however, the damage to the cells releasing fluid into the extracellular space and the shrinking of the collagen which surrounded the liver parenchyma (Strange et al. 1985b) forced the blood out of the tissue forming the additional drip.

CONCLUSION

LIVER DRIP consisted of 40% blood with the rest of the fluids released by the cells during cell death and damage to the cell membranes, in contrast to muscle drip which was almost entirely sarcoplasmic proteins and water. The drip from R-S livers showed evidence of proteolytic breakdown while the drip from F-T livers did not. F-T liver drip had higher levels of

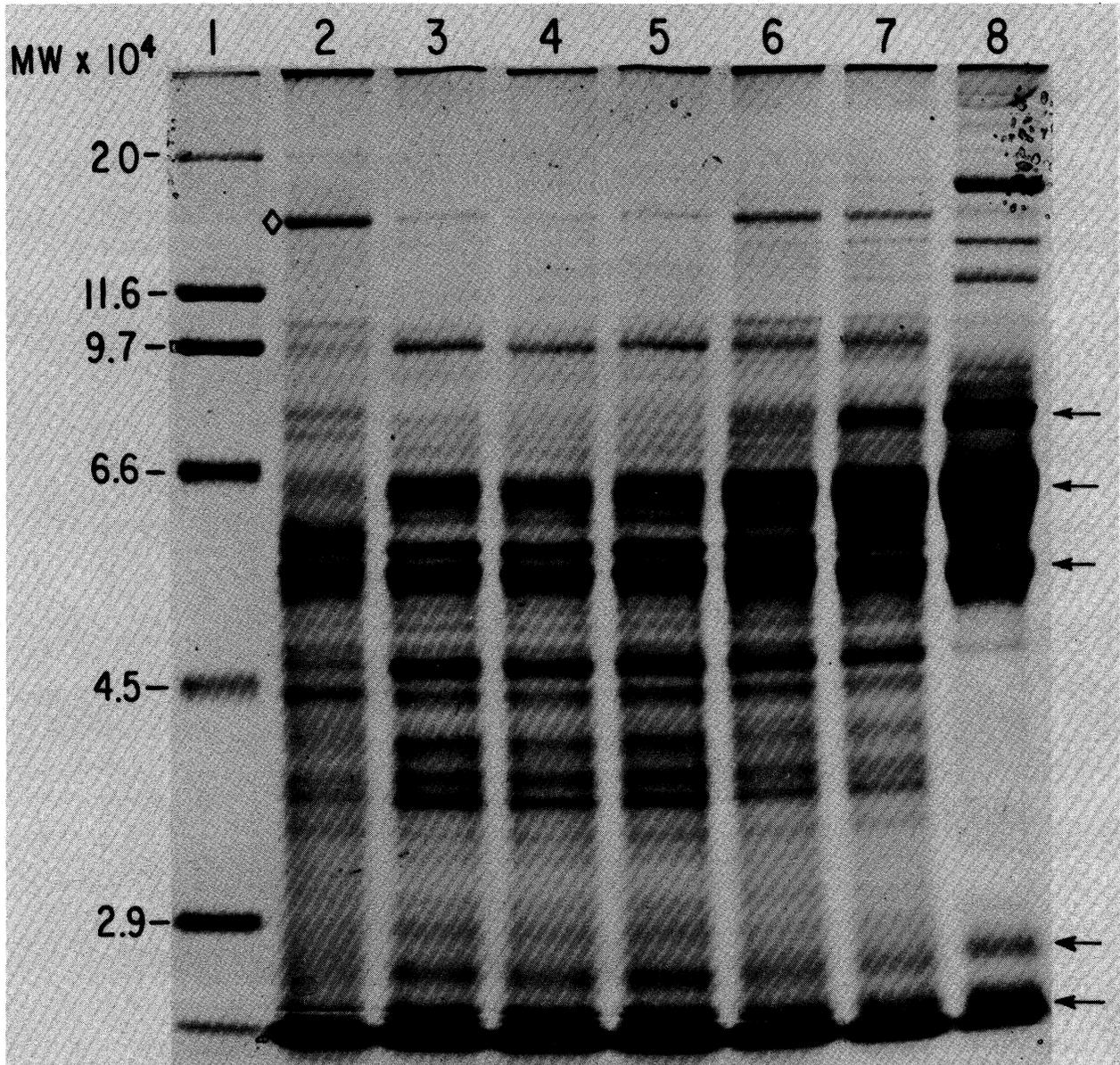


Fig. 2—Electrophoretic patterns of F-T drip and liver-plasma mixtures; ◊ protein band present in whole liver, F-T drip and liver-plasma mixtures; ← major plasma protein bands. Lane 1—Molecular weight standards. Lane 2—Freeze-dried whole fresh liver. Lane 3—Freeze-dried supernate of drip from F-T cycle 1 liver. Lane 4—Freeze-dried supernate of drip from F-T cycle 2 liver. Lane 5—Freeze-dried supernate of drip from F-T cycle 3 liver. Lane 6—Freeze-dried supernate of a 75% liver — 25% plasma mixture. Lane 7—Freeze-dried supernate of 50% liver — 50% plasma mixture. Lane 8—Freeze-dried supernate of 100% plasma.

GDH activity indicating destruction during freezing and thawing of subcellular organelles, particularly mitochondria.

The appearance of stored liver could be improved by the removal of blood immediately postmortem. This could be accomplished by pumping the liver clear of blood using its circulatory system and either water or a low salt solution. This treatment, which may or may not decrease weight loss, would give the liver a better appearance because the drip would then contain fewer dark colored components. This approach to liver processing would be of particular utility with export market livers because the reduction in the amount of blood in livers would reduce or eliminate package staining which lowered the value of the product.

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Reference to brand or firm names does not constitute endorsement by the U.S. Dept. of Agriculture over others of a similar nature not mentioned.

DRIP FROM PORK LIVER. . .

Table 3—% Drip and calculated % plasma from F-T livers

Cycle 1		Cycle 2		Cycle 3	
% Drip	% Plasma ^d	% Drip	% Plasma	% Drip	% Plasma
2.38	18.4	3.29	32.9	7.21	27.9
1.70	20.5	5.80	25.1	4.31	15.4
8.00	28.6	11.50	34.9	11.82	28.1
10.39	27.9	13.40	30.7	14.35	30.1
6.73	28.9	9.18	32.3	13.30	29.0
6.45	25.9	10.90	28.5	13.30	23.8
3.91	19.1	5.86	20.7	9.25	20.6
3.75	17.0	6.92	23.3	10.00	24.6
6.64	24.0	5.90	31.9	8.56	35.1
5.09	25.0	7.58	31.5	11.69	22.1
6.49	33.5	7.93	39.2	14.83	37.8
8.61	37.7	11.23	38.5	15.83	41.9
Mean % Drip					
5.84 ^a		8.29 ^b		11.20 ^c	
S.D. = 2.58					
2.58		2.98		3.43	
N = 12					
Mean % Plasma					
25.5 ^a		30.8 ^b		28.0 ^{ab}	
S.D. = 6.2					
6.2		5.6		7.5	
N = 12					

^{a-c} Means in the same row having no superscripts in common are significantly different $p < 0.05$. Duncan's new multiple range test.

^d Calculated using the equation

$$\% \text{ Plasma} = 141.5 - 4.4 (\% \text{ PRO}) - 27.7 (\text{APO4}) - 128.8 (\text{GDH}) - 1.65 (\text{Hb}^{1/2})$$

as time of storage increased. The portion of R-S drip assignable to blood ranged between 20-60% of the drip volume with a mean of 40% blood and the portion of the F-T drip assignable to plasma ranged between 15-40% of the drip volume with a mean of 28% plasma. Plasma was about 2/3 of the total volume of blood, and therefore, the amount of blood in F-T liver drip was between 20-60% with a mean of 40% in terms of blood. Correlation coefficients for % blood in drip versus % drip and % plasma in drip versus % drip were calculated. Significant positive correlations were noted between the amount of R-S drip and % blood ($r=0.5146$; $N=32$; $p < 0.01$) and between the amount of F-T drip and % plasma ($r=0.5265$; $N=36$; $p < 0.01$). As the volume of drip increased, its blood content also increased.

The parameters measured on the refrigerator stored (R-S) drip samples were % protein (% PRO), specific acid phosphatase activity (APO4) and hemoglobin concentration (Hb). The data from the R-S liver drip are shown in Table 4. Protein content of the drip showed significant differences related to the day of storage but no trend was noted with storage time. Specific acid phosphatase activity showed no significant changes with time and this indicated a lack of additional damage to lysosomal membranes during refrigerated storage. GDH, a measure of mitochondrial damage, was not detectable in refrigerated liver drip even after six days of storage.

The concentration of Hb showed a significant decrease with time. Ash, sodium and iron were not affected by storage time but potassium levels in the supernate increased as storage time increased indicating liver cell death and breakdown of the compartmentalization of ions by the living cell.

Examination of the pellet from the R-S drip by light microscopy with Wright Stain showed intact red blood cells, white blood cells, nuclei from hepatocytes, individual hepatocytes, clumps of hepatocytes and cells the size normally associated with bacteria. The pellet represented about 15% of the total weight of the drip formed during refrigerated storage.

The 10% SDS-PAGE patterns (Fig. 1) show the changes occurring to the drip proteins during R-S storage (lanes 3, 4 and 5). All samples had extremely complex protein patterns which were very similar. Changes due to storage occurred in the high molecular weight ($> 9.7 \times 10^4$) region. Several minor bands (circles) disappeared as storage time increased and other bands (squares) which had lower molecular weights appeared. The proteins in the region 4.5×10^4 to 2.9×10^4 M.W. were from liver while several major bands (lane 8 arrows) were from

Table 4—Means of assays on the drip from R-S livers

	Fresh	Stored for 2 days	Stored for 4 days
% PRO ^c	16.26 ^a	14.85 ^b	15.65 ^{ab}
S.D. ⁱ	1.34	1.36	0.90
N ^j	8	12	12
AP04 ^d	0.98 ^a	1.00 ^a	1.07 ^a
S.D.	0.09	0.08	0.10
N	8	12	12
Hb ^e	17.9 ^a	13.1 ^b	10.7 ^c
S.D.	5.5	2.8	2.1
N	8	12	12
% Ash	1.10 ^{ab}	1.10 ^a	1.17 ^b
S.D.	0.08	0.05	0.04
N	5	8	8
Na ^f	43 ^a	43 ^a	38 ^a
S.D.	9	15	6
N	5	8	8
K ^g	58 ^a	73 ^b	73 ^b
S.D.	3	10	10
N	5	8	8
Fe ^h	1.96 ^a	3.36 ^a	3.30 ^a
S.D.	0.85	1.60	1.47
N	5	8	8

^{a-b} Means in the same row having no superscripts in common are significantly different, $p < 0.05$. Duncan's new multiple range test.

^c % protein in drip.

^d Specific acid phosphatase activity in Sigma units.

^e Hemoglobin concentration in mg/ml of drip.

^f Millimoles sodium/kg of drip.

^g Millimoles potassium/kg of drip.

^h Millimoles iron/kg of drip.

ⁱ Standard deviation.

^j Number of assays run.

the plasma proteins. With a few minor differences the drip separation patterns resembled the blood mixture patterns (lanes 6 and 7). The band marked by a diamond was the most notable exception. This band also appeared in the protein pattern of freeze-dried whole liver (lane 2).

The parameters measured on the freeze-thaw samples were % protein (% PRO), specific acid phosphatase activity (APO4), specific glutamic dehydrogenase activity (GDH) and hemoglobin concentration (Hb). The data from F-T liver drip are shown in Table 5.

Percent protein and Hb concentrations decreased as storage time increased but specific enzyme activities increased. APO4 increased from 1.11 to 1.25 Sigma units indicating increasing breakdown of the lysosomes in the liver cell.

Examination of the pellet from the F-T drip by light microscopy with Wright Stain showed intact red blood cells (even after 3 F-T cycles) and cells and debris very similar to the pellet from the drip of R-S livers. The pellet represented about 10% of the total weight of drip formed during F-T cycling.

The 10% SDS-PAGE separations (Fig. 2, lanes 3, 4 and 5) show the distribution of proteins in drip during F-T cycling. All drip samples were very similar to each other and to the liver-plasma mixtures shown in lanes 6 and 7. A protein band, marked by a diamond on lane 2, was present in the F-T drip.

Differences in the characteristics of drip from R-S and F-T livers were subtle. They both contained about the same amount of blood or plasma. The drip from R-S livers showed some evidence of proteolysis, while the drip from F-T livers did not. There were changes in protein composition during refrigerated storage of liver that were not seen during repeated freezing and thawing of the liver.

F-T liver drip contained higher levels of enzymatic activity: specific acid phosphatase activity (APO4) for F-T drip was 1.16 ± 0.12 ($N=36$) and for R-S drip was 1.02 ± 0.10 ($N=32$); Student's $t = 5.39$ ($p < 0.01$) for the difference. F-T liver drip also contained glutamic dehydrogenase activity and presumably other enzymatic activities associated with the mitochondria. Hemoglobin concentration was higher in F-T than in R-S liver drips: for F-T drip it was 25.6 ± 8.0 mg/mL ($N=36$) and for R-S drip 13.4 ± 4.4 mg/mL ($N=32$); Stu-