Biopsy of Liver, Adipose Tissue and Mammary Gland of Lactating Cows

Abstract
Concurrent biopsies of liver, subcutaneous or abdominal fat, and mammary gland from 11 lactating cows slightly reduced milk production (4.8%) 5 days after surgery. Repeated biopsies 30 days later reduced milk production 12.7%. The tissues were suitable for enzymic and chemical assays.

Introduction
Metabolic changes resulting from dietary changes can be detected by measuring enzyme activity. Liver, adipose, and mammary tissues are especially adaptive to dietary alterations (1-5). Several studies of various liver biopsy procedures to obtain tissues for enzymic and chemical assay have been reported (5, 6, 8, 10). Techniques for mammary biopsy have been reported by several groups (7, 9). However, the effect of simultaneous biopsies of liver, adipose, and mammary tissues in a serial fashion on dairy cow performance has not been reported. Successful biopsy reduces variation and costs by permitting the study of several treatments in the same animal. This note describes effects of repeated biopsies on milk production and on viability of tissue obtained.

Material and Methods
Eleven mature Holstein cows in various stages of lactation were fed and milked in stanchions at the dairy barn. Dietary changes in grain for other studies (1-4) were approximately 30 days prior to the biopsies. On the day of surgery, the animals were moved to the veterinary clinic at 7:00 AM and returned to the dairy barn by 1:00 PM.

Prior to surgery 100 USP units of oxytocin were injected into the jugular vein, and the cows were milked. Each cow was on a hydraulic table in lateral recumbency for mammary biopsy. The mammary gland was clipped, then scrubbed with a germicidal solution over the surgical area five times. Procaine1 was used to anesthetize the area selected (either right or left front quarter). Procaine was infused subcutaneously to make a line block which was extended 10 cm parallel to the abdominal wall at the dorsal margin of the mammary gland. Skin and subcutaneous tissues were incised with a scalpel 5 cm ventrally to the anesthetized line. A scissors was used to remove 3 to 5 g of mammary tissue. In some instances ligation of severed blood vessels was necessary. A Gelfoam2 sponge was pressed into the biopsy area, and subcutaneous tissues were closed with o chromic catgut. The skin incision was closed with 0.3 mm Vetafil3 and sprayed with 2% nitrofurazone. Each cow was then removed from the table and placed in a restraining stock for standing surgical procedures.

Liver biopsies were obtained by aspiration (6) or by removing 3 to 10 g from the margin of the liver with pressure from the thumb and fingers after entering the abdominal cavity as for adipose biopsies. Hemorrhage was controlled by pressing a Gelfoam sponge into the site from which the liver biopsy was removed. For adipose biopsies the right paralumbar fossa and either the right or left post scapular region or the posterior surface of the thigh were clipped and surgically scrubbed. Procaine was the local anesthetic. The block for the right paralumbar incision extended 10 cm parallel to the vertebral transverse process on the dorsal margin and 10 cm vertically on the anterior margin. A 16-cm incision was made in the abdominal wall in the anesthetized region. Adipose tissue was removed from the perirenal area by digital pressure. This adipose sample is termed abdominal rather than perirenal because its exact location could not be visually confirmed. The peritoneum, fascia layers, and subcutaneous tissues were closed with number 2 chromic catgut. Vetafil (0.6 mm) was used to close the skin.

For subcutaneous adipose samples, procaine was used to block a line 5 cm dorsal to the postscapular or thigh site. The skin was incised and 2 to 3 g subcutaneous fat were removed. The incision was closed with Vetafil (0.6 mm) and sprayed with 2% nitrofurazone. Tissues

1 Bio-ergetic Laboratories, St. Joseph, Missouri.
2 Gelfoam, Upjohn Company, Kalamazoo, Michigan.
3 Vetafil Beagan, Haver-Lockhart, Kansas City, Missouri.
were prepared and enzymes measured as previously described (1–4).

Penicillin (3,630 units/kg body weight per day) was prophylactic treatment for 2 to 3 days post-surgery. The quarter of the mammary gland that was biopsied was infused4 after the evening milking on the day of surgery and the day following, also as a prophylactic treatment. All skin sutures were removed on the seventh day after surgery. Seven cows had two surgeries at about 30 day intervals, and then tissue was obtained at slaughter, except from one of the seven cows which died following the second surgery from peritonitis caused by accidental puncture of the intestine during surgery. Two cows had three surgeries and were not slaughtered. Two other cows were biopsied only once; then tissue was obtained at slaughter.

Results and Discussion

The average daily milk production decreased following surgery probably due to the trauma and stress of surgery (Table 1). A larger decrease was evident following repeated biopsies. Lower producers appeared to decrease more in production due to surgery than higher producers. Cow 329, the highest milk producer yielding 26.4 and 25.0 kg per day two days prior to the first and second surgery, recovered the most rapidly. On the second day after the first and second surgeries milk yields were 22.3 and 25.4 kg per day, respectively. Another method of looking at recovery in production (Table 1) compares milk production on the fifth day after surgery with the daily average for the five days preceding surgery. A 4.8% decrease in daily milk production indicated rapid return in milk production in less than a week.

Hemorrhage control is a problem with the biopsy of mammary glands. Milk from the biopsied quarter contained blood for up to five days after surgery. Clotted blood was expressed from the gland by hand stripping following machine milking. If the subcutaneous closure is not tight, a subcutaneous hematoma will develop. Removing residual milk from the gland before surgery with oxytocin aids closing of the mammary gland incision. There were no cases of mastitic infection relating to surgeries in these cows.

Because enzyme activity in biopsy tissues was important, we used only local procaine anesthetic. General anesthetics can be used for surgery but their action on the various enzymes is unknown. Procaine blocks dorsal to the surgical area reduce procaine contamination of the biopsy tissue. Enzymic activity (glyceride synthesis, lipoprotein lipase) of mammary tissue was about 30% greater in slaughter than in biopsy tissue (Table 2). The difference among cows was much greater than sampling technique as shown by the magnitude of the standard errors in Table 2. Increased dietary grain increased glyceride synthesizing and lipoprotein lipase activity of adipose tissue. After correction for dietary effects, the enzymic activity was about 45% greater in slaughter than in biopsy adipose tissue. This might have been from greater contamination of biopsy tissue with blood and connective tissue. Glycerolipid synthesis by five liver samples obtained by aspiration biopsy was an average of only 14% less than that by paired slaughter samples while two samples obtained by laparotomy were 20% more active than the paired slaughter samples. The differences among cows were much greater than between the methods of obtaining liver tissue. Others have found differences in dry matter and nitrogen content of liver biopsies by aspiration and laparotomy (10). We con-

| Table 2. Statistics on biopsy versus slaughter mammary enzyme data. |
|-------------------------|----------------|-----------------|
|                        | Mean ±se (P)   |                |
| Glyceride synthesis (μmole palmitate/hr/μg protein) |                |                |
| Biopsy                 | 28.2 ± 3.6     | <0.02           |
| Slaughter              | 35.1 ± 2.3     |                |
| Lipoprotein lipase (μmole fatty acid/hr/mg protein) |                |                |
| Biopsy                 | 6.5 ± 1.5      | 0.16            |
| Slaughter              | 8.9 ± 0.9      |                |
| a Standard error of the mean for 8 cows in each group. |                |                |
| b Probability that the mean of paired biopsy versus slaughter differences for 8 cows was not > 0. |                |                |
clude that biopsy tissue is suitable for metabolic studies, but that corrections may be needed to compare data from biopsy and slaughter. The metabolic aspects of this study are published elsewhere (1-4).

If repeated surgical procedures are required, careful attention to suture removal is important. Suture holes in the skin can be the source of abscesses. Inflammatory reaction and scar tissue formation are minimal if the skin sutures are removed 7 to 10 days after surgery.

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References

Modified Procedure for Isolation of a Major Swine Whey Protein

Abstract

A salt fractionation procedure for the isolation of a polymorphic whey protein in sow's milk was modified to include anion exchange chromatography. The protein, homologous to ruminant β-lactoglobulin, was crystallized from pooled milk and its identity to the previously isolated polymorphic whey protein was verified by alkaline and acid gel electrophoresis.

Kraeling and Gerrits (5, 6) have described a polymorphic protein in sow's whey. These workers have reported the distribution in three swine breeds, one of which was a crossbreed, of the genetic variants AA and BB according to their mobilities in alkaline gel electrophoresis. Kalan et al. (1, 2, 3) isolated each of the polymorphs from milk of sows found to be homozygous for the individual polymorphs. They have partially characterized these proteins for amino acid composition, C-terminal and N-terminal amino acids, molecular weight and isoionic point. The authors made a case for homology of the proteins with ruminant β-lactoglobulin. More recently, Kessler and Brew (4) reported on the isolation and partial characterization of a swine whey protein similar to the AA variant reported by Kalan et al. (1, 2, 3). They also have concluded homology with ruminant β-lactoglobulin for the protein they described. Kessler and Brew (4) isolated their protein by column chromatography with G-100 Sephadex in 50 mM NH4HCO3, pH 8.8, and DEAE-cellulose with a linear NaCl gradient in Tris-HCl buffer, pH 7.8.

The original isolation by Kalan et al. (3) of the polymorphic swine whey proteins was based on salt fractionation and, finally, crystallization from 55% saturated (NH4)2SO4 solutions. The procedure for the isolation of these proteins has been modified to include column chromatography and our report is this modification.