Researchers in the meat animal field are turning more and more to the effect of nutrition and breeding upon the carcass. Coupled with our interests as meats men this is encouraging, but leads us quickly to the conclusion that we need better techniques in approximating certain carcass characteristics in the live animal. At present it is difficult to estimate the lean to fat ratio in experimental animals prior to experimentation. In order to make critical studies of fat deposition we need to follow it closely during the fattening period. The technique does not exist that will answer all the problems of researchers, so we must be constantly in search of better methods of estimating the degree of fatness in live animals.

We are indeed fortunate to have had the probing technique perfected by the Iowa researchers in estimating fat back thickness of live hogs. Likewise the Antipyrine technique is one that we should find of considerable value in meat animal research.

My discussion today on biopsy techniques is nothing sensational, for it has been used by experimenters for many years for studies of one type or another.

Ewing and co-workers in 1918 (Texas Agr. Exp. Sta. Bul. 226) developed a technique employing a borer, consisting of a twisted clock spring inserted in a cannula, for removing fat samples from the back fat of hogs. Scott in 1920 (Fla. Agr. Exp. Sta. Bul. 157, pp. 67-68) reported a method for removal of fat samples from the ham. Scott and Black in 1951 (Jour. Agr. Res. 42: 47) advanced a biopsy technique for obtaining fat back samples. These techniques limit the procedure to extraction of subcutaneous deposits of adipose tissue.

At the Wisconsin Station we have been interested in a biopsy technique not only for subcutaneous fat deposition but for histological studies and intra-muscular fat deposition from animals while on a feeding trial. In other words, we would like to have each animal serve as his own control.

We have developed a method for such a study in swine and cattle. In case some of you are interested in this as yet unpublished work, an attempt will be made to outline each procedure.

The procedure for swine as described in the following was developed by R. A. Merkel and carried out on 60 hogs, 15 of which were selected at each of the following weights: 36, 80, 125, and 170 pounds respectively. All feed and water was withheld for approximately 12 hours prior to the operation. The two lighter weight groups were anesthetized by an intraperitoneal injection of a 10 percent aqueous solution of magnesium sulfate and chloral hydrate (5\% MgSO4 + 5\% chloral hydrate). However, for the heavier hogs an intravenous injection of the same anesthetic was administered via the posterior auricular vein or its branches.

The animals were restrained in lateral recumbency on the operating table. The operative area was shaved, scrubbed with soap and water, and 70
percent alcohol and tincture of iodine applied. An incision $1\frac{1}{2}$ to 3 inches in length was made parallel to and 1 to 2 inches (dependent on the weight of the hogs) laterally from the vertebral column just posterior to the last rib.

The incision was made through the subcutaneous tissue and fat to the epimysium of the longissimus dorsi at which time a mechanical fat back measurement was made with a sterilized tape measure. The adipose and connective tissue was separated from the muscle by blunt dissection and a rectangular sample approximately $1\frac{1}{2}$ inches long, $3/4$ inch wide, and $3/8$ inch thick was removed. This sample can be fixed for histological studies or prepared for chemical analysis, as desired.

Hemorrhage resulting from resection of the muscle was controlled by gauze tampons. Occasionally it was necessary to ligate larger vessels in order to arrest bleeding. Sulfonamide-urea wound powder was applied to the wound and 20-day chromic catgut No. 2 was used to suture the subcutaneous tissue and fat. The skin was sutured with $1/8$ inch umbilical suture tape, iodine applied, and covered with a collodion bandage.

Following the operation the hogs were penned separately and held off feed for approximately 12 hours before returning them to their respective lots. The sutures were removed 10 days later; any visible secondary complications were not apparent.

A procedure for securing muscular tissue from beef animals prior to slaughter was developed by W. Batterman and G. D. Wilson. The description of this biopsy technique which follows was applied to 16 aged cows and 16 yearling steers averaging 1100 and 750 lbs., respectively.

A dehorning chute was used to restrain the animals in a standing position. A small area of the tail head was shaven, tincture of iodine applied and 2.5 percent procaine was injected extradurally between the first and second coccygeal vertebra. The steers required 10-12 cc. of the procaine solution while the cows required 18-20 cc. to render them insensitive in the rear quarters. The animals remained standing during the operation.

The operative area was located in the posterior aspect of the thigh. The location of the incision was determined by palpating the thigh to locate the intermuscular septa between the semimembranosus and semitendinosus muscles. The operative area was shaved, scrubbed with soap and water and tincture of iodine applied.

A 5-6 inch incision through the skin and subcutaneous tissue was made directly over the intermuscular septa, 2-3 inches ventral to the tuber ischi (pin bone). This exposed the fleshy part of the semimembranosus muscle. Using a curved bistoury with a blunt point the semimembranosus muscle was separated medially from the intermuscular septa 2 inches. A rectangular portion of muscle approximately 2 inches in diameter and 4 inches long (150-400 bms.) was removed beveling the bottom of the incision to permit adequate drainage.

A sulfonamide-urea wound powder was dusted into the incision and the skin and subcutaneous tissue were put in a position with closely placed mattress sutures using $1/8$ inch linen suture tape.
The incision was painted with tincture of iodine and a collodion bandage applied.

The entire operation can be performed in 30-45 minutes provided the animal remains quiet for that period of time.

The animals were examined periodically for infection in the operative area. In some cases it was found necessary to open the ventral portion of the incision to allow drainage. The sutures were removed 7-10 days after the operation. There were no adverse effects of the operation and the incision was completely healed in 3-5 weeks.

These biopsy techniques may be used for the study of fat deposition, both subcutaneous and intramuscular. We have attempted to use them for a study of the pattern of marbling by using Sudan IV stain. The results were not satisfactory, but we consider the fault to lie in our staining method. In addition, we believe that biopsy techniques provide a means for studying many other muscle characteristics.

It should be pointed out that biopsy techniques have shortcomings, among which is the problem of getting a representative sample either of the entire animal or an individual muscle. This is a problem because certain muscles cannot be used without affecting the normal function of the animal. In addition, the site of biopsy is limited since it is difficult to provide natural drainage for many areas of the body.

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MR. KLINE: This is not directed to Bob. I believe the group at Oklahoma have a liver biopsy technique, they use on beef cattle.

MR. HILLIER: Yes, that technique is for obtaining samples of liver and studying vitamin A and carotene storage in cattle as they progress through feeding tests. I think they probably have entered some of those cattle at least a dozen times, once a month for about two years. They have done it on the calves just as soon as they were born and before they had nursed -- on tiny calves and animals of all ages.

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